

## MYOGLOBIN IN PELAGIC SMALL CETACEANS

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### Summary

Although myoglobin (Mb) is considered to contribute significantly to the oxygen and diving capacity of marine mammals, few data are available for cetaceans. Cetacean by-catch in the tuna driftnet fisheries in the Sulu Sea, Philippines, afforded the opportunity to examine Mb content and distribution, and to determine muscle mass composition, in Fraser's (*Lagenodelphis hosei*) and spinner (*Stenella longirostris*) dolphins and a pygmy killer whale (*Feresa attenuata*). Age was estimated by body length determination. Stomach contents were analyzed for the presence or absence of milk and solid foods. It was hypothesized (a) that Mb concentration ([Mb]) would be higher in Fraser's and spinner dolphins than in other small cetaceans because of the known mesopelagic distribution of their prey, (b) that [Mb] would vary among different muscles according to function during diving, and (c) that [Mb] would increase with age during development. The results were as follows. (1) Myoglobin concentrations of the longissimus muscle in adult Fraser's (6.8–7.2 g 100 g<sup>-1</sup> muscle) and spinner (5–6 g 100 g<sup>-1</sup> muscle) dolphins and in an immature pygmy killer whale (5.7 g 100 g<sup>-1</sup> muscle) were higher than those reported

previously for small cetaceans. (2) [Mb] varied significantly among the different muscle types in adult dolphins but not in calves; in adults, swimming muscles had significantly higher [Mb] than did non-swimming muscles, contained 82–86% of total Mb, and constituted 75–80% of total muscle mass. (3) Myoglobin concentrations in Fraser's and spinner dolphins increased with size and age and were 3–4 times greater in adults than in calves. The high Mb concentrations measured in the primary locomotory muscles of these pelagic dolphins are consistent with the known mesopelagic foraging behaviour of Fraser's and spinner dolphins and suggest that the pygmy killer whale is also a deep-diving species. The high Mb concentrations in epaxial, hypaxial and abdominal muscle groups also support the primary locomotory functions suggested for these muscles in other anatomical studies. As in other species, the increase in [Mb] during development probably parallels the development of diving capacity.

Key words: myoglobin, cetacean, muscle, *Lagenodelphis hosei*, *Stenella longirostris*, *Feresa attenuata*, spinner dolphin, Fraser's dolphin, pygmy killer whale.

### Introduction

High myoglobin concentrations are considered to be one of the most important adaptations of diving vertebrates; elevated [Mb] serves as a major characteristic separating divers from their terrestrial counterparts (Kooyman and Ponganis, 1998). In these animals, Mb concentrations in skeletal muscles are 10–30 times greater than those in non-diving vertebrates (Kooyman, 1989), and the oxygen-storage function of Mb appears to be of greater importance than its role in facilitated diffusion (Castellini and Somero, 1981; Baldwin, 1988; Ponganis et al., 1997). In some diving animals, e.g. emperor penguins *Aptenodytes forsteri* and bottlenose dolphins *Tursiops truncatus*, the amount of oxygen stored in muscle Mb is greater than that stored in either the lungs or blood (Kooyman and Ponganis, 1998).

A correlation between body [Mb] and diving performance in diving birds and mammals has been demonstrated in various studies (Scholander et al., 1942; Ridgway and Johnston, 1966; Blessing 1972a,b; Keijer and Butler, 1982; Snyder, 1983;

Baldwin et al., 1984). This is consistent with the hypothesis that most dives performed by these animals are aerobic (Kooyman et al., 1980; MacArthur, 1990).

It has also been demonstrated for a number of species, including diving birds and the harbour porpoise *Phocoena phocoena*, that the concentration of Mb varies in different skeletal muscles and is closely related to muscle activity, the requirement for sustained work and contraction strength (Whipple, 1926; Shenk et al., 1934; Pattengale and Holloszy, 1967; Blessing, 1972a; Stephenson et al., 1989; Turner and Butler, 1988; Wittenberg and Wittenberg, 1989; Davis and Guderly, 1990; MacArthur, 1990; Ponganis et al., 1997). This is exemplified in the Baikal seal *Pusa siberica*, in which Mb concentrations vary twofold between swimming and non-swimming muscles and are highest in the spinal locomotory muscles (Neshumova and Cherepanova, 1984).

Because of these findings, we speculated (a) that [Mb] of the

primary locomotory muscles would be highest in species with deep, long-duration dives, (b) that [Mb] would vary among individual muscles and would be dependent on the function of a given muscle during diving, and (c) that [Mb] would increase during development as diving capacity increases. Cetacean by-catch in the tuna driftnet fisheries in the Sulu Sea, Philippines, afforded the opportunity to examine these hypotheses in cetacean species with known deep-water prey.

Our aims in this study were (1) to measure Mb concentrations in three species of pelagic cetaceans and to compare these with the values obtained for other cetaceans; (2) to compare Mb concentrations between and within various skeletal muscles of the three species of dolphins, to relate this variation to the known function of the muscle in question and to examine the implications of this variation for the estimation of muscle oxygen stores and capacities; and (3) to document changes in [Mb] with body size and age.

### Materials and methods

#### *Collection of muscle tissue*

Muscle samples were collected from dolphins accidentally caught in a tuna driftnet fishery operating at Siaton in the eastern Sulu Sea in the Philippines. Animals were obtained within 3–5 h after death; muscle samples (either 10 cm×10 cm×5 cm or 5 cm×5 cm×4 cm) were placed on ice and transported to the laboratory. If not analyzed immediately, samples were frozen in Ziploc plastic bags and stored in a freezer at –14 °C for up to 14 days. Analyses for seven out of 27 animals included such frozen samples.

The following muscles or muscle groups were sampled from Fraser's dolphins *Lagenodelphis hosei* Fraser, spinner dolphins *Stenella longirostris* (Gray) and a pygmy killer whale *Feresa attenuata* Gray: epaxial (m. multifidus, m. longissimus, m. semispinalis), hypaxial (m. hypaxialis lumborum), abdominal (m. rectus abdominis), intercostals (intercostalis), m. sternohyoideus, scapular (infraspinatus) and diaphragm and dermal muscles (panniculus). Because of the marked variation in coloration of the longissimus muscle from anterior to posterior, three regions were sampled: the cervical and upper thoracic (or 'anterior' longissimus, sampled directly posterior to the flipper), the lower thoracic (or 'mid'-longissimus, sampled at the level of the dorsal fin) and the caudal extension, the extensor caudae lateralis (ECL) (or posterior longissimus), sampled half-way between the dorsal fin and flukes. The nomenclature of the axial muscles used in this study follows that used by Strickler (1980) and Pabst (1990).

To compare the myoglobin concentration of the spinner dolphin with that of Fraser's dolphin, samples from the left longissimus muscle at the level of the dorsal fin (referred to as mid-longissimus) immediately lateral to the neural spines were obtained from six adult Fraser's dolphins (body length 220–247 cm) and 13 adult spinner dolphins (body length 170–192 cm). Samples from the same area of the longissimus muscle were obtained from one immature pygmy killer whale

(body length 210 cm). To compare changes in [Mb] with body length and age, additional samples from mid-longissimus muscle were obtained from three calf and immature spinner dolphins (body length 115–123 cm) and four calf and immature Fraser's dolphins (body length 98–150 cm). Additional details such as sexual maturity and reproductive condition (e.g. pregnant or lactating) were noted. The stomach contents were analyzed for the presence/absence of milk or solid food such as fish (bones and/or otoliths), squid (beaks) and crustaceans.

Although we were able to take samples of longissimus muscles from a total of 16 spinner dolphins, 10 Fraser's dolphins and one pygmy killer whale, field conditions and competition with local market vendors, who bought the dolphins from the fishermen, prevented us from carrying out a more thorough sampling of various muscles in all the animals. We were able to collect multiple muscle samples from only five Fraser's dolphins (body length 98–247 cm), two spinner dolphins (body length 180 and 192 cm) and one pygmy killer whale (body length 210 cm).

#### *Myoglobin concentration determination*

Muscle sample homogenization and extraction, and measurement of [Mb] in Mb standards (Horse Mb, Sigma) and tissue samples utilized the method of Reynafarje (1963). Myoglobin concentration was expressed as g Mb 100 g<sup>-1</sup> muscle. Each Mb determination was the mean value from five adjacent 70 mg muscle samples at each site in each animal.

To verify that storage for 14 days at –14 °C did not affect [Mb], values from fresh and frozen samples of the same muscles of one dolphin were compared. Twenty subsamples obtained from four fresh samples (five subsamples each) and from four frozen samples (also five subsamples each) were compared using a two-factor analysis of variance (ANOVA); there was no significant difference ( $P=0.51$ ).

Muscle water content was determined by using lyophilization techniques (Rehunen and Harkonen, 1980) to verify that pathological *post-mortem* changes and frozen storage had not resulted in tissue edema or desiccation.

#### *Muscle mass*

The total body mass and muscle mass (kg) of five Fraser's dolphins (one calf, one juvenile and three adults), one spinner dolphin (adult) and one pygmy killer whale (immature) were measured using a beam balance. Although we were able to obtain mid-epaxial muscle samples from 16 spinner dolphins, owing to the reasons discussed above, we were able to determine the muscle mass of only one adult spinner dolphin. The muscle groups weighed were epaxial, hypaxial, abdominal, intercostals and pectoral, diaphragm, head and 'neck' muscles, scapular and dermal. The total muscle mass was the sum of these component muscle groups and is expressed as a percentage of total body mass. The m. semispinalis was not included with the epaxial locomotory

muscles because of its distinctive light coloration and its previous classification as a non-swimming muscle (Pabst, 1990); it was instead grouped with the head and facial muscles.

*Calculations*

Total Mb content of the body (in g) was estimated for two spinner and three Fraser's dolphins and one pygmy killer whale by summing the Mb content ([Mb] × muscle mass) of each muscle group (this value is referred to as the weighted sum). Unweighted total myoglobin content was also estimated using the [Mb] of the mid-longissimus muscle alone multiplied by the total muscle mass. Oxygen stores (ml) and capacity (ml O<sub>2</sub> kg<sup>-1</sup> muscle) of the different muscles were calculated by assuming 1.34 ml O<sub>2</sub> g<sup>-1</sup> Mb (Lenfant et al., 1970).

*Data analysis*

A single-factor ANOVA was used to test whether there was a significant difference in the [Mb] of different muscles sampled for each species, and a Tukey *post-hoc* comparison was employed to determine which particular muscles had significantly higher or lower [Mb] values. A *t*-test was used to compare the [Mb] of the mid-longissimus muscle of adult spinner dolphins with that of adult Fraser's dolphins, and a two-tailed *t*-test was used to compare Mb concentrations in adult male and female spinner dolphins (there were not enough samples of both sexes to test for differences between male and female Fraser's dolphins). To test whether there was a significant difference between the weighted and the unweighted total Mb contents, a one-tailed paired *t*-test was used. Correlation analysis was used to determine the strength of association between body length and [Mb] for spinner and Fraser's dolphins. Differences were accepted as significant at *P*<0.05. Results are expressed as means ± S.E.M.

**Results**

*Myoglobin concentrations*

As seen in Table 1, the Mb concentrations in the mid-longissimus muscle of adult spinner and Fraser's dolphins were higher than those observed in other small cetaceans (except for the false killer whale *Pseudorca crassidens*). The concentration in Fraser's dolphins (7.1 g 100 g<sup>-1</sup> muscle) was significantly higher (*P*<0.001) than that in spinner dolphins (5.5 g 100 g<sup>-1</sup> muscle), and the value for the immature pygmy killer whale was comparable with that of spinner dolphins. No significant difference was found in the [Mb] between male and female spinner dolphins (*P*=0.113). The mean coefficient of variation (CV) for the 97 sets of five subsamples was 6.5 %.

The comparison of fresh and frozen muscle revealed no significant difference (two-factor ANOVA, *P*=0.51). In addition, mean muscle water content was 73.2±0.55 % for Fraser's dolphin and 72.4±0.05 % for spinner dolphin. These

values are close to the 75 % water content of most mammalian muscle (Reynafarje, 1963) and demonstrate that Mb concentrations are not elevated by either tissue edema or tissue desiccation in the samples.

*Muscle mass*

Relative muscle mass as a percentage of body mass was greater in adult Fraser's dolphins (56–59 %) than in the adult spinner dolphin (52 %) and immature pygmy killer whale (44 %). Relative muscle mass of calf and juvenile Fraser's dolphins was less (41–46 % of body mass) than that of the adults. In adults, muscle mass distribution was similar in all three species (Fig. 1). Epaxial locomotory, hypaxial and

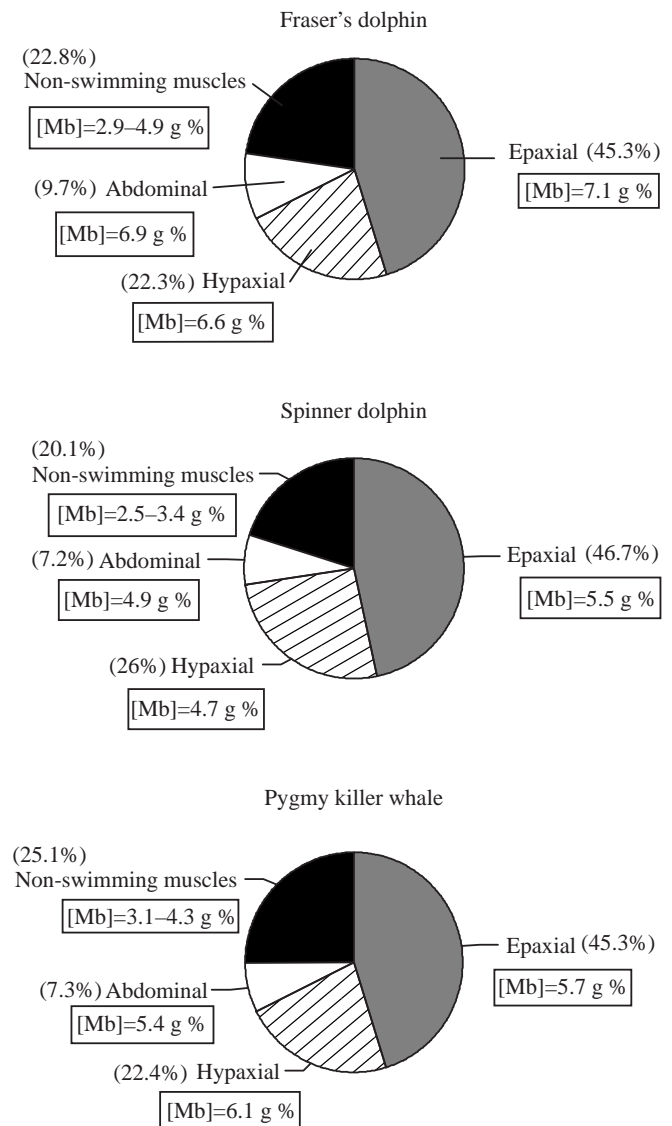


Fig. 1. Percentage of total muscle mass made up by individual muscle groups in Fraser's and spinner dolphins and pygmy killer whale. The myoglobin concentrations [Mb] of the muscles are also shown; units are g Mb 100 g<sup>-1</sup> muscle, or g %.

Table 1. *Myoglobin concentrations and oxygen capacities of cetaceans*

Diving species	<i>N</i>	[Mb] (g 100 g <sup>-1</sup> muscle)	Muscle	Oxygen capacity (ml kg <sup>-1</sup> muscle)	Source
Large cetaceans					
<i>Hyperoodon rostratus</i> Bottlenose whale	1	6.3*	—	85.0	Scholander (1940)
<i>Physeter macrocephalus</i> Sperm whale	—	5.7*	—	76.0	Scholander (1940)‡
<i>Balaenoptera physalus</i> Minke whale	1	3.7*	—	50.0	Scholander (1940)
Small cetaceans					
<i>Tursiops truncatus</i> Bottlenose dolphin	1	3.5	Sacrospinal	44*	Eichelberger et al. (1939)
	—	3.2	—	43*	Blessing and Hartschen-Nieyemeyer (1969)
	—	3.4	Deep muscles	46*	Man'kovskaya (1975)
	—	2.5	Mid-longissimus	34*	Harrison and Davis (1998)
<i>Phocoena phocoena</i> Harbour porpoise	2	4.1	Tail extensor	56*	Blessing (1972a)
<i>Stenella attenuata</i> Spotted dolphin	5	2.5	—	34*	Castellini and Somero (1981)
<i>Platanista indi</i> Indus river dolphin	1	2.6	Lumbar muscle	34*	Blessing (1972b)
<i>Delphinus delphis</i> Common dolphin	3	3.4	Dorsal and ventral muscles	46*	Korzhev and Glazova (1971)
<i>Pseudorca crassidens</i> False killer whale	—	6.3	Mid-longissimus	84*	Harrison and Davis (1998)
<i>Sousa chinensis</i> Humpback dolphin	—	2.5	Mid-longissimus	34*	Harrison and Davis (1998)
<i>Lagenodelphis hosei</i> Fraser's dolphin (sexually mature; body length 220–286 cm)	6	7.1±0.06	Mid-longissimus	84*	This study, O <sub>2</sub> capacity is weighted value from three animals
<i>Stenella longirostris</i> Spinner dolphin (adults, body length 170–192 cm)	13	5.5±0.10	Mid-longissimus	54*	This study, O <sub>2</sub> capacity is weighted value from one animal
<i>Feresa attenuata</i> Pygmy killer whale (sexually immature; body length 210 cm)	1	5.7	Mid-longissimus	66*	This study, O <sub>2</sub> capacity is weighted value

Muscles used for the analysis of myoglobin concentration are indicated when available.

\* indicates a calculated value, i.e. that [Mb] is calculated from measured oxygen capacity or that oxygen capacity is calculated from measured [Mb] assuming a conversion factor of 1.34 ml O<sub>2</sub> kg<sup>-1</sup> Mb.

‡These data were reported to be possibly low and may have been almost twice as high (Scholander et al., 1942).

Values of [Mb] for *L. hosei* and *S. longirostris* are means ± S.E.M.

abdominal muscles constituted 45–47%, 22–26% and 7–10% of total muscle mass, respectively. The three swimming muscle groups together constituted 75–80% of total muscle mass. The body masses of the adult Fraser's dolphins, adult spinner dolphin and immature pygmy killer whale were 122–136 kg, 58 kg and 93 kg, respectively. The calf and juvenile Fraser's dolphin body masses were 9 and 58 kg, respectively.

#### *Variation in myoglobin concentration*

Skeletal muscles were divided into two major functional groups: the primary swimming muscles (the m. longissimus, m. multifidus, m. hypaxialis lumborum and m. rectus abdominis) and the non-swimming muscles (the rest of the skeletal muscles). Table 2 shows the [Mb] value for each muscle in the three species studied, with the results of statistical comparisons (single-factor ANOVA, and Tukey

*post-hoc* comparisons). The muscles were grouped into four categories (A, B, C or D) on the basis of the results of the Tukey test. Myoglobin concentrations in the group were as follows:  $A \geq B > C > D$ . Overall, [Mb] in the swimming muscles (groups A and B) of Fraser's and spinner dolphins (body lengths: Fraser's 228–247 cm; spinner 180 and 192 cm) and the pygmy killer whale (210 cm) was significantly higher than in the non-swimming muscles (groups C and D). Swimming muscle Mb concentrations were  $6.0\text{--}7.1 \text{ g } 100 \text{ g}^{-1}$  muscle in Fraser's dolphins,  $4.6\text{--}5.5 \text{ g } 100 \text{ g}^{-1}$  muscle in the spinner dolphins and  $4.9\text{--}6.1 \text{ g } 100 \text{ g}^{-1}$  muscle in the pygmy killer whale. Although there were no significant differences in [Hb] among the swimming muscles in any of the three species, the anterior portion of the longissimus muscle was found to have a significantly lower [Mb] than the other segments of this muscle in two of the three Fraser's dolphins. Significant variation was observed among the Mb concentrations of the non-swimming muscles of the Fraser's dolphin (note that the diaphragm, scapular and dermal muscles were not sampled in the spinner dolphins and pygmy killer whale). Thus, the non-swimming muscles in Fraser's dolphins can be divided into two groups on the basis of myoglobin concentration: group C, consisting of the m. semispinalis, m. intercostalis and m. sternohyoideus ([Mb]= $4.2\text{--}4.9 \text{ g } 100 \text{ g}^{-1}$  muscle), and group D, consisting of the diaphragm, scapular (m. infraspinatus) and dermal muscles ([Mb]= $2.9\text{--}3.5 \text{ g } 100 \text{ g}^{-1}$  muscle) (Table 2).

Myoglobin concentration analysis of the muscles of a 98 cm Fraser's dolphin calf yielded a different result; there was no significant difference observed among the various muscles sampled (single-factor ANOVA,  $P=0.700$ ). The mean [Mb] of all muscles was  $1.8 \pm 0.2 \text{ g } 100 \text{ g}^{-1}$  muscle. The stomach of this calf contained milk and no other food material.

*Distribution of myoglobin, muscle oxygen stores and muscle oxygen capacity*

Myoglobin content ([Mb]  $\times$  muscle mass) and muscle oxygen store had a similar pattern of distribution in all three species (Fig. 2). The epaxial locomotory muscles contained half the total Mb content, followed by the hypaxial muscles (25–28%) and abdominal muscles (6–12%). The rest of the skeletal muscles contained the remaining 13–16%. Overall, the swimming muscles (epaxial locomotory, hypaxial and abdominal combined) contained 82–86% of the total amount of myoglobin (or muscle  $O_2$  store) in all three species. Owing to the large muscle mass and high [Mb] in adult Fraser's dolphins, muscle  $O_2$  stores per kilogram body mass were nearly twice those of spinner dolphin and pygmy killer whale. Muscle oxygen stores in adult Fraser's and spinner dolphins and the pygmy killer whale were  $48 \pm 1.21 \text{ ml kg}^{-1}$  body mass ( $N=3$ ),  $28 \text{ ml kg}^{-1}$  body mass and  $29 \text{ ml kg}^{-1}$  body mass, respectively.

The oxygen capacity of the swimming muscles

Table 2. Myoglobin concentration in various muscles of adult Fraser's and spinner dolphins and a pygmy killer whale

Muscle	Myoglobin concentration ( $\text{g Mb } 100 \text{ g}^{-1}$ muscle tissue)		
	Fraser's dolphin ( $N=3$ ) (body lengths 228, 236, 247 cm)	Spinner dolphin ( $N=2$ ) (body lengths 180, 192 cm)	Pygmy killer whale ( $N=1$ ) (body length 210 cm)
Group A (Swimming)			
M. longissimus (Mid-portion)	$7.1 \pm 0.06$	$5.5 \pm 0.5$	5.7
M. multifidus (Caudal extension of ECM)	$6.9 \pm 0.12$	$5.2 \pm 0.8$	No sample
M. rectus abdominis longissimus (Caudal extension or ECL)	$6.9 \pm 0.16$ $6.7 \pm 0.25$	$4.9 \pm 1.1$ $5.2 \pm 0.4$	5.4 5.4
M. hypaxialis lumborum	$6.6 \pm 0.18$	$4.7 \pm 0.7$	6.1
Group B (Partly swimming)			
M. longissimus (Anterior)	$6.0 \pm 0.32$	$4.6 \pm 0.6$	4.9
Group C (Non-swimming)			
M. semispinalis	$4.9 \pm 0.73$	$2.5 \pm 0.3$	4.3
M. intercostalis	$4.4 \pm 0.13$	$3.4 \pm 0.4$	3.5
M. sternohyoideus	$4.2 \pm 0.18$	No sample	3.1
Group D (Non-swimming)			
Diaphragm	$3.5 \pm 0.21$	No sample	No sample
M. infraspinatus	$3.4 \pm 0.10$	No sample	No sample
Dermal (panniculus)	$2.9 \pm 0.23$	No sample	No sample

There was a significant difference in [Mb] between the various muscle groups labeled A–D (one-factor ANOVA:  $P \ll 0.001$  in three species); [Mb] in  $A \geq B > C > D$  (Tukey test).

There was no significant difference in [Mb] in muscles within a group.

Values are means  $\pm$  S.E.M.

ECL, extensor caudae lateralis; ECM, extensor caudae medialis.

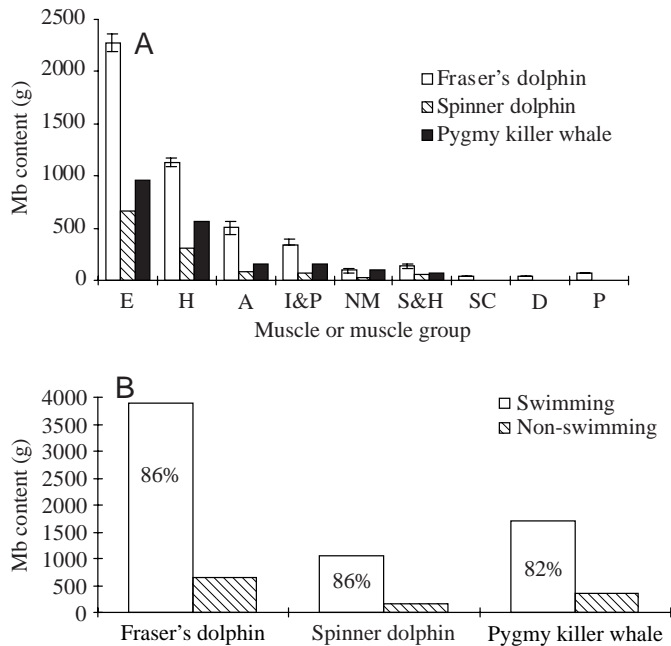


Fig. 2. (A) Myoglobin (Mb) content ( $[Mb] \times$  muscle mass) of the skeletal muscles of Fraser's ( $N=3$ ) and spinner's ( $N=1$ ) dolphins and a pygmy killer whale ( $N=1$ ). Values are means  $\pm$  S.E.M. for Fraser's dolphins. (B) Mb content of swimming versus non-swimming muscles. Mb content is expressed as a percentage of total Mb content of all muscles in the three cetacean species. E, epaxial locomotory muscles; H, hypaxial; A, abdominal; I&P, intercostals and pectorals; NM, 'neck' muscles; S&H, semispinalis and head muscles; SC, scapular muscles; D, diaphragm; P, panniculus.

averaged  $90 \text{ ml O}_2 \text{ kg}^{-1} \text{ muscle}$  in the Fraser's dolphins,  $58 \text{ ml O}_2 \text{ kg}^{-1} \text{ muscle}$  in the spinner dolphin and  $76 \text{ ml O}_2 \text{ kg}^{-1} \text{ muscle}$  in the pygmy killer whale (Fig. 3). The non-swimming muscles had a significantly lower oxygen capacity than the swimming muscles in all three species (paired  $t$ -test,  $P=0.0001$ ).

The total Mb content, calculated using the  $[Mb]$  of the epaxial muscle alone, was 4.6–13.7% greater than the weighted value obtained using  $[Mb]$  and mass of each muscle (Table 3).

*Developmental changes in myoglobin concentrations*

The color of the mid-longissimus muscle of spinner and Fraser's dolphins varied greatly from light pink in calves to dark or almost black in the adults. Fig. 4 illustrates the relationship between mid-longissimus  $[Mb]$  and body length in Fraser's and spinner dolphins. A strong positive correlation was observed between  $[Mb]$  and body length in both species (Fraser's dolphin,  $r=0.96$ ,  $P<0.05$ ; spinner dolphin,  $r=0.95$ ,  $P<0.05$ ). A simple linear regression also showed a significant relationship for both species (Fraser's dolphin,  $r^2=0.92$ ; spinner dolphin,  $r^2=0.62$ ). Myoglobin concentration also increased with body length in a non-swimming muscle, the intercostalis, in Fraser's dolphin ( $r=0.95$ ;  $r^2=0.89$ ,  $P=0.010$ ) (Fig. 5).

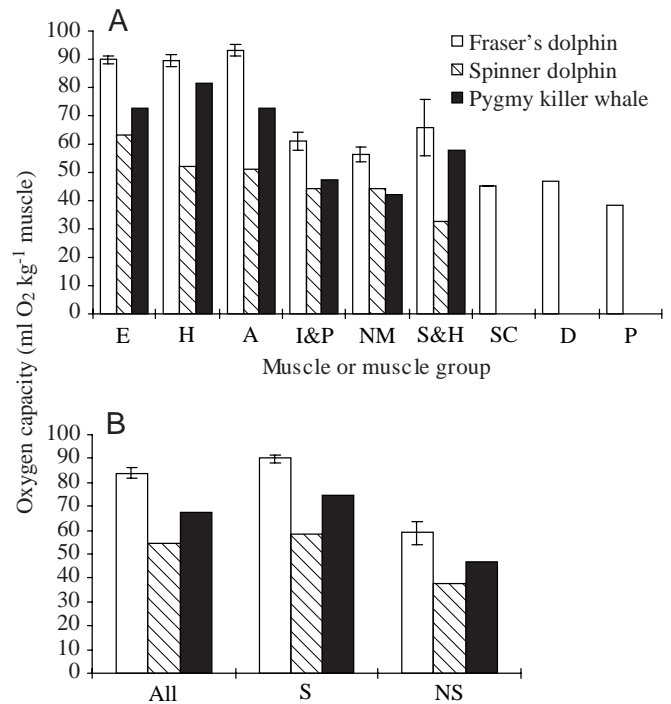


Fig. 3. (A) Muscle-mass-specific oxygen capacities ( $\text{ml O}_2 \text{ kg}^{-1} \text{ muscle}$ ) of Fraser's ( $N=3$ ) and spinner ( $N=1$ ) dolphins and a pygmy killer whale ( $N=1$ ). (B) Oxygen capacities of all muscles combined (All), swimming (S) and non-swimming (NS) muscles. Values are means  $\pm$  S.E.M. for Fraser's dolphins. E, epaxial locomotory muscles; H, hypaxial; A, abdominal; I&P, intercostals and pectorals; NM, 'neck' muscles; S&H, semispinalis and head muscles; SC, scapular muscles; D, diaphragm; P, panniculus.

Table 3. Total myoglobin content of three species of cetacean

Species (individual body length)	Total myoglobin content (g)		% Difference
	Weighted value	Using epaxial muscle $[Mb]$ value	
Fraser's dolphin			
Body length 228 cm	4574	4797	4.6
Body length 236 cm	4354	4675	6.9
Body length 247 cm	4724	5121	7.8
Spinner dolphin			
Body length 180 cm	1225	1419	13.7
Pygmy killer whale			
Body length 210 cm	2053	2161	5.0

Contents were either calculated as a weighted value (i.e. sum of  $[Mb] \times$  mass of particular muscle, and therefore taking into account variation in  $[Mb]$  among different muscle groups), or calculated using the  $[Mb]$  of the epaxial muscle alone (i.e. total muscle mass  $\times$   $[Mb]$  of epaxial muscle).

Weighted values are significantly lower than those calculated using epaxial muscle  $[Mb]$  ( $P=0.004$ , one-tailed paired  $t$ -test).

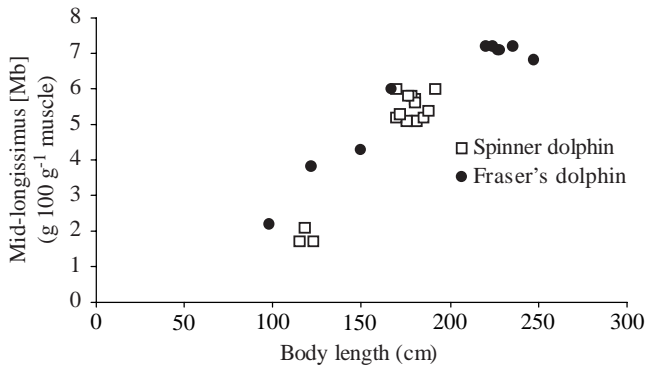


Fig. 4. Relationship between myoglobin content in mid-longissimus muscle and body length in Fraser's and spinner dolphins.

## Discussion

### Myoglobin concentration and muscle mass

The [Mb] measured here in Fraser's dolphins is among the highest reported values in diving mammals and birds (Snyder, 1983; Ponganis et al., 1997). Similar or higher values have been recorded only for sperm whales *Physeter macrocephalus* (Scholander, 1940; Scholander et al., 1942), false killer whales *Pseudorca crassidens* (Harrison and Davis, 1998) and ribbon seals *Histiophoca fasciata* (Lenfant et al., 1970).

The relative muscle mass of the adult Fraser's dolphins (mean 57%) was greater than that measured for the spinner dolphin and the pygmy killer whale and is among the highest reported in cetaceans; this is reflected in this dolphin's bulky and robust appearance (Perrin et al., 1994). Muscle mass in other dolphins is approximately 50% (Slijper, 1961); in the harbour porpoise *Phocoena phocoena*, it is 25% (Scholander, 1940) and in large whales it is between 31 and 62% (Lockyer, 1976).

Within a particular size range, a close correspondence between diving capacity (dive depth and duration) and [Mb] or oxygen

capacity has been shown in cetaceans (Snyder, 1983). The large muscle mass coupled with high [Mb] results in the body-mass-specific muscle oxygen stores in adult Fraser's dolphin being much greater than those of the spinner dolphin ( $48 \pm 1.21 \text{ ml kg}^{-1}$  body mass versus  $28 \text{ ml kg}^{-1}$  body mass, respectively), the pygmy killer whale ( $29 \text{ ml kg}^{-1}$  body mass) and the bottlenose dolphin *Tursiops truncatus* ( $14 \text{ ml kg}^{-1}$  body mass; Kooyman, 1989). The mean muscle oxygen capacity of Fraser's dolphins,  $84 \text{ ml O}_2 \text{ kg}^{-1}$  muscle (calculated for all muscles using [Mb], see Table 1) was also higher than that of the pygmy killer whale ( $66 \text{ ml O}_2 \text{ kg}^{-1}$  muscle), the spinner dolphin ( $54 \text{ ml O}_2 \text{ kg}^{-1}$  muscle) and other cetaceans. From these results, we suggest that the routine dives by Fraser's dolphins are of longer duration than are those of other small cetaceans.

The prevalence of mesopelagic food items in the stomach contents of both Fraser's and spinner dolphins (Perrin et al., 1973, 1994; Robison and Craddock, 1983; M. L. L. Dolar, unpublished data) also suggests that both are deep-diving species. Examination of the stomach contents of Fraser's dolphins showed the presence of mesopelagic fish and crustaceans in size ranges uncommon in mid-water trawling collections and not known to be regular vertical migrators (Robison and Craddock, 1983). Some of these fish prey species, such as *Lampanyctus* spp., *Lampadena* spp. and *Scopelagadus* spp., are known to occupy the lower mesopelagic depths between 500 and 750 m (Robison and Craddock, 1983). The spinner dolphin's mesopelagic prey species, in contrast, are known to be vertical migrators, moving near the surface at night (Perrin et al., 1973). On the basis of an analysis of stomach contents, it has been suggested that spinner dolphins dive to depths of 200–300 m (Perrin et al., 1973). Although only one pygmy killer whale was sampled in the present study, the high [Mb] and oxygen capacity calculated for this species suggest that it may also be a good diver. Little is known about the prey of pygmy killer whales, although it has been suggested that they feed mostly on fish and squid (Jefferson et al., 1993). Lower Mb concentrations in other species of small cetaceans are consistent with their known foraging behavior. For example, the river dolphin *Platanista indi* is a shallow diver feeding on riverine organisms ([Mb]= $2.6 \text{ g } 100 \text{ g}^{-1}$  muscle, Table 1) and the bottlenose dolphin *Tursiops truncatus* ([Mb]= $2.5\text{--}3.5 \text{ g } 100 \text{ g}^{-1}$  muscle) feeds on schooling fish in waters approximately 40 m deep (Würsig and Würsig, 1979).

### Variation in myoglobin concentration: swimming versus non-swimming muscles

Myoglobin concentration varied significantly within the same animal depending on the muscle sampled. In general, the axial muscles (m. longissimus, m. multifidus and m. hypaxialis lumborum) contained significantly higher Mb concentrations (Table 2) than the muscles found in other parts of the body (e.g. m. intercostalis, m. infraspinatus and panniculus). This pattern may be explained by the fact that the axial muscles are used by cetaceans for locomotion (Parry, 1949; Slijper, 1961; Smith et al., 1976; Bello et al., 1985; Arkowitz and Rommel,

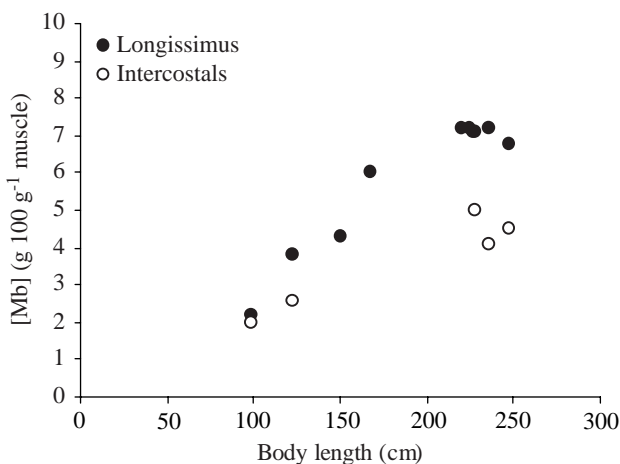


Fig. 5. Comparison of the increase in myoglobin concentration between the swimming (mid-longissimus) and non-swimming (intercostal) muscles as a function of body length in Fraser's dolphins.

1985; Hui, 1987; Pabst, 1990, 1993). With the exception of the m. semispinalis, the Mb concentrations of all axial muscles examined in this study were high, and all except the m. semispinalis are actively involved in the flexion of the body during swimming. Although part of the axial musculature, the m. semispinalis does not participate in locomotion. Instead, it is involved in changing the position of the skull relative to the vertebral column (Pabst, 1993). The [Mb] in this muscle is comparable with those found in non-swimming muscles such as the intercostal and the sternohyoid muscles (Table 2).

We found that the m. rectus abdominis, a non-axial muscle, has a [Mb] comparable with those of the mid- and posterior m. longissimus and m. multifidus (Table 2). This muscle in terrestrial mammals such as the cat *Felis domesticus* is long and slender and is positioned on each side of the linea alba. It originates at the pubic symphysis and inserts into the sternum and costal cartilages, acting to retract the ribs and sternum and to compress the abdomen; it has no apparent role in locomotion (Hyman, 1942). In adult dogs, this muscle was observed to have a [Mb] approximately 30% less than that of the leg muscles (Whipple, 1926). In cetaceans, however, this muscle appears to have a different function. In the three species examined, it is broad, thick and robust (9.7% of muscle mass in Fraser's dolphin, 7.2% in spinner dolphin and 7.3% in pygmy killer whale, Fig. 1) and its appearance is as dark as the axial musculature. A similar morphology was observed in *Platanista indi*, and this led to the suggestion that this muscle has an important role in swimming (Pilleri, 1976). Slijper (1961), Arkowitz and Rommel (1985), Pabst (1989), Pabst et al. (1995) and Rommel et al. (1992) also suggested the active involvement of this muscle in swimming; together with the m. hypaxialis, it may be involved in flexing the flukes. The high [Mb] in this muscle together with its large size strongly support an active role for this muscle in locomotion in these species.

Our results also show that [Mb] may vary within a muscle possibly depending on the force- or work-generation requirements of a particular region. The long axial muscles of cetaceans are composed of many short muscle fibers that arise from several skeletal components and insert *via* a series of tendons along the length of the muscle (Pabst, 1990). Thus, different segments of a single muscle, for example the longissimus, may perform different actions or generate varying forces. In this study, we found that the [Mb] in the longissimus muscle varied slightly between the anterior (cervical and upper thoracic) and middle (lower thoracic and anterior lumbar) and caudal regions. The variation was only significant in the anterior region in two of the three Fraser's dolphins, where the [Mb] was significantly lower in the anterior region than in the thoracic and caudal regions (Table 2). The anterior region of the longissimus muscle does not appear to be involved in swimming to the same extent as the more posterior regions (Pabst, 1993). The lower thoracic region tended to have the highest [Mb] value, which may be related to the high forces generated by this part of the longissimus muscle. Pabst (1993) showed that, in the bottlenose dolphin *Tursiops truncatus*, the

longissimus muscle in the thoracic and anterior lumbar region develops large forces that are transmitted to the vertebrae anterior to the fluke and assist in the extension of the caudal peduncle. The posterior extension, the extensor caudae lateralis, appears to perform constant, sustained work (Pabst, 1993).

Intramuscular variation in [Mb] of the longissimus muscle has been reported previously by Harrison and Davis (1998) for three other cetacean species: the false killer whale *Pseudorca crassidens*, the humpbacked dolphin *Sousa chinensis* and the bottlenose dolphin *Tursiops truncatus*. Similar observations were also reported for the m. gracilis in the dog *Canis familiaris*, with the proximal portion having the highest value, followed by the middle and distal portions (Degens et al., 1994). Since muscle fiber type composition is uniformly distributed (approximately 50% fast twitch, 50% slow twitch) throughout the longissimus dorsi muscle, at least in the bottlenose dolphin *Tursiops truncatus* (Bello et al., 1985), the variation in [Mb] observed in the present study is unlikely to be dependent on variations in fiber type distribution. Our results suggest that [Mb] in the three cetacean species studied correlates with the activity of the muscle or the muscle segment and thus probably with the force or duration of work that it must generate during diving.

Because of the high affinity of Mb for O<sub>2</sub>, Mb-bound O<sub>2</sub> has long been considered an important store of O<sub>2</sub> for muscle metabolism during diving. The large proportion of total body O<sub>2</sub> which is bound to muscle Mb (48% in bottlenose dolphins; Kooyman, 1989) and the distribution of Mb primarily in swimming muscles (82–86% in the present study) suggest that Mb-bound O<sub>2</sub> in the swimming muscles may be a determinant of aerobic dive limit in these species.

#### *Variation of myoglobin concentrations: implications for the estimation of total muscle O<sub>2</sub> stores*

The disparity in the [Mb] between the swimming and the non-swimming muscles may affect the estimation of total myoglobin content and therefore total muscle oxygen stores. If only one muscle is sampled for [Mb], the total body muscle O<sub>2</sub> store may be over- or underestimated, depending upon whether the sampled muscle is a primary swimming muscle. In these three cetacean species, the use of the epaxial swimming muscle [Mb] resulted in significant, although only 5–14%, overestimates of the actual total Mb content. The swimming muscle group constituted 75–80% of total muscle mass and contained 82–86% of the total Mb in these species. Such constraints are relevant to the calculation and interpretation of total muscle O<sub>2</sub> stores in diving species. However, it would appear, at least in these species, that the epaxial muscle [Mb] yields a reasonable, if slightly high, estimate.

#### *Developmental changes in myoglobin concentrations*

Two conclusions can be drawn regarding the [Mb] in calf and juvenile Fraser's dolphins: (1) myoglobin concentration was significantly lower than in adults, and (2) there was no variation in [Mb] among different muscles in calves;



differentiation started to occur in juveniles. In addition, the muscles of the calves were also relatively less massive, constituting only 41 % of total body mass compared with 57 % in adults. The low [Mb] resulted in low muscle oxygen capacity of only 30 ml O<sub>2</sub> kg<sup>-1</sup> muscle, as opposed to 84 ml O<sub>2</sub> kg<sup>-1</sup> muscle in adults. Our data show that myoglobin concentrations increased as the dolphin increased in length (or age; a strong relationship between age and body length has been shown for both species: Amano et al., 1996; Perrin and Gilpatrick, 1994) up to a body length of 228 cm in Fraser's dolphin and 170 cm in the spinner dolphin (Fig. 4). In this study, Fraser's and spinner dolphins with body lengths at or exceeding these values were found to have reached sexual maturity. At these lengths, [Mb] reached a plateau at 6.8–7.2 g 100 g<sup>-1</sup> muscle in Fraser's dolphins and 5–6 g 100 g<sup>-1</sup> muscle in spinner dolphins (Fig. 4). Sexual maturity is reported to occur at 210–220 cm (5–6 years old) in female and 220–230 cm (7–10 years old) in male Fraser's dolphins (Amano et al., 1996) and at 165–170 cm (4–7 years old) in female and 160–180 cm (7–10 years old) in male spinner dolphins (Perrin and Gilpatrick, 1994).

The low [Mb] and lack of differentiation of [Mb] in the muscles of calves emphasize the role of Mb in diving since calves do not need to forage and probably do not dive as extensively as the adults. The presence of milk and the absence of solid food in the stomach of the 98 cm Fraser's dolphin calf support this assumption. However, differentiation in [Mb] between the swimming and non-swimming muscles appears to occur at an early stage: the [Mb] of the swimming muscles (mid-longissimus) increased rapidly with body size, while the limited data for the intercostals (non-swimming muscle) suggest a slower rate of change (Fig. 5). At approximately 122 cm body length, the [Mb] of the mid-longissimus was significantly higher than that of the intercostals (ANOVA,  $P < 0.001$ , Tukey test, mid-longissimus > intercostals). As the dolphins approached adult length, the difference between the Mb concentrations in the longissimus and intercostal muscles became greater. This developmental change in the concentration of Mb is consistent with observations for other diving mammals (Kooyman et al., 1983; Thorson and Le Bouef, 1994) and birds such as the pigeon guillemot *Cephus columba* (Haggblom et al., 1988) and penguins (Weber et al., 1974; P. J. Ponganis, unpublished observations).

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