Characterizing muscle tissue in humpback whale calves

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Introduction

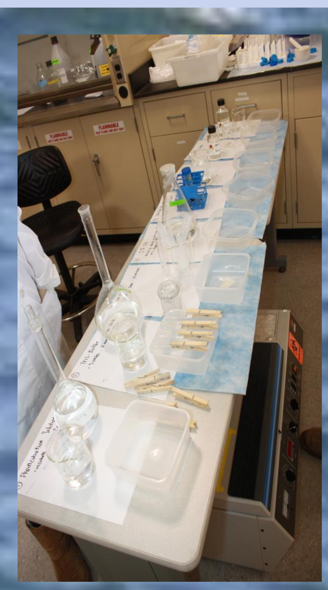
Over 65 million years ago the early ancestors of whales and dolphin evolved from terrestrial hoofed mammals like cows and camels. Today they are perfectly adapted to the ocean; their bodies have become streamlined for efficient movement through the water, their forelimbs are now modified to flippers that enhance maneuverability and their hind limbs have disappeared completely.

Beyond these morphological adaptations, whales have also evolved a suite of physiological adaptations to their marine environment. These adaptations include the ability to breathe hold. Even though, as air breathing mammals, whales are dependent on surface supplies of oxygen, some whales can stay submerged for more than 60 minutes.

Mature humpback whales typically dive for up to 10-15 minutes when feeding however for humpback whale calves, previous research shows that their breath holding capacity is extremely limited in the first months of life. In this study we explore the development of their breath holding capacity. Specifically, our aim is to determine the nature of their muscle tissue, as this may explain why their respiratory capacity is so limited in their early months of life.

Hypothesis

Ha: There is a significant difference in distribution of fast and slow twitch muscle fibers of humpback whale calves compared to other neonate marine mammals, such as odontocetes and pinnipeds.



Methodology

We used humpback whale calf tissue from two recently stranded animals and applied two different methods of tissue staining .

Method 1: Metachromatic ATPase staining (Ogilvie and Feeback 1990)

Step 1: We cut 10µm cross sections of muscle tissue, using the cryostat.
Step 2: Slides were pre-incubated at pH 4.5
Step 2: Slides were then rinsed in the Tris Buffer.
Step 3: Slides were incubated in a solution of ATP for 25 minutes.
Step 4: Slides were rinsed again with 3 changes of 1% calcium chloride dihydrate
Step 5: Slides were stained with 1% aqueous toluidine blue, dehydrated in ethanol and cleared in xylene.

Method 2: Immuno-histochemical staining (Dearolf 2003)

Step 1: Again we used 10µm cross sections of muscle tissue, mounted on poly-L-lysine coated slides.
Step 2: Sections were quenched with peroxidase quenching solution, and then blocked using a proteinaceous blocking agent.
Step 3: Primary antibodies for either slow and fast myosin were applied to alternate sections and slides were incubated for 1 hour in a water bath at 25° C.
Step 4: We applied a secondary antibody to amplify the reaction, washed the slides then stained them with DAB chromagen.

The stained slides were carefully examined, cells were counted and for each stain the darkest stained cells were taken as our positive result.

Results

Humpback whale calves have both Type I (slow twitch) and Type II (fast twitch) muscle fibers.

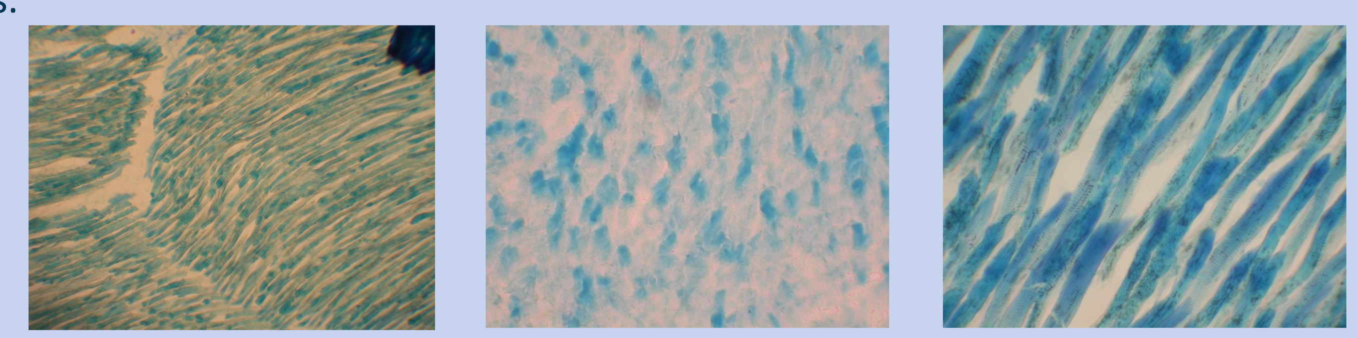


Figure 1: Metachromatic staining technique - Type I cells stained darkest

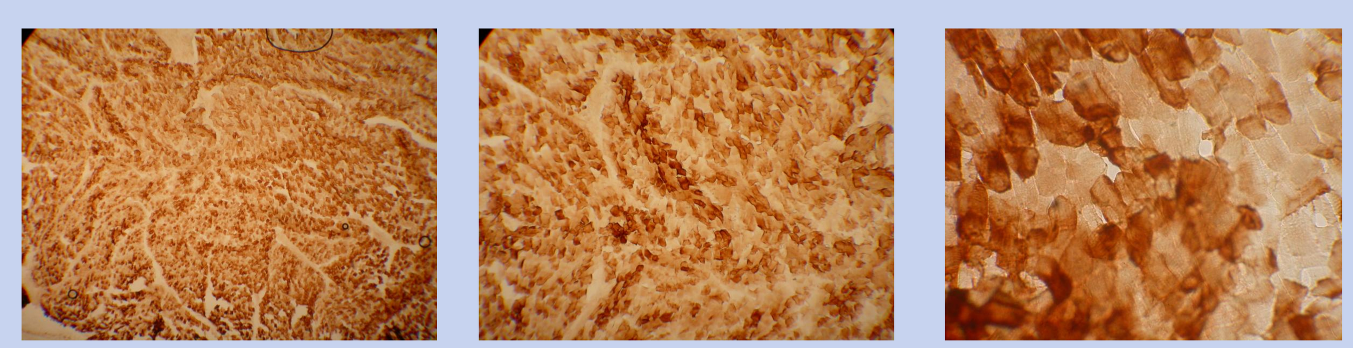


Figure 2: Immuno-histochemical staining technique - Type II cells stained darkest

Humpback whale calves were found to have 37.6 (s.d. 3) % type I muscle fiber and 45.9 (s.d. 3.8)% type II muscle fibers.

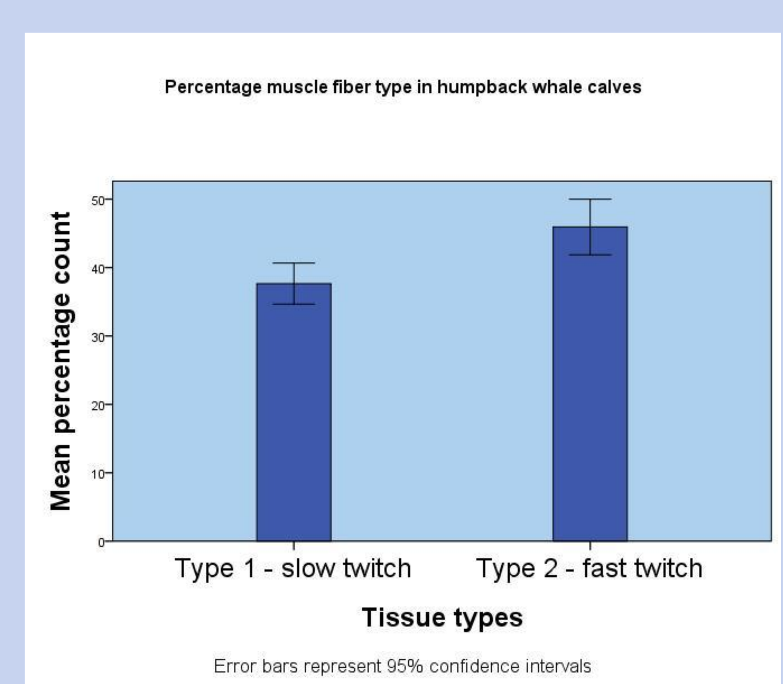


Figure 3: Proportions of muscle fiber types in humpback whale calf muscle tissue

Muscle Tissue Types

Type 1 tissue – for stamina

Slow Tissue

Oxidative tissue

Type 2 tissue – for sprinting

Fast Tissue

A- fast oxidative
B- fast glycolitic

Conclusions

- There is a significant difference in both the percentage of Type I and Type II muscle fibers of neonate humpback whales compared to neonate Weddell seals (Type I: t=-48.209, d.f.=5, p<0.001; Type II: t=25.272, d.f.=5, p<0.001).
- There is no significant difference in the percentage of Type I and Type II muscle fibers of neonate humpback whales and neonate bottlenose dolphins (t=-0.471, d.f.=5, p=0.658).
- Neonate Weddell seals have primarily Type I muscle fibers (Kanatous et. al., 2000), while neonate bottlenose dolphin (Dearolf, 2003) and humpback calves (our data) have almost equal proportions of Type I and Type II muscle fibers.

Neonate Weddell seals

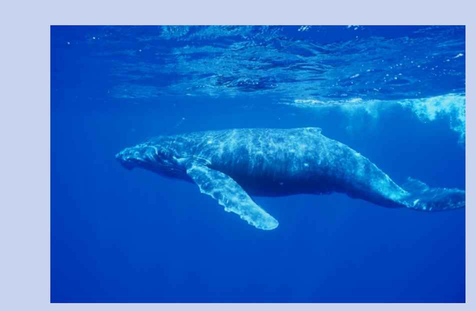
~94% Type 1 fibers

Mean myoglobin levels – 35.5 g/100g

Neonate bottlenose dolphin

~40% Type 1 fibers

Mean myoglobin levels – 0.7 g/100g



Neonate humpback whales

~ 38% Type 1 fibers

Mean myoglobin levels – 0.28 g/100g

Literature Cited

Dearolf, J. L. "Diaphragm muscle development in bottlenose dolphins (Tursiops truncatus)." *Journal of Morphology* 256.1 (2003): 79-88.

Kanatous, S. B., et al. "The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals." *Journal of Experimental Biology* 211.16 (2008).

Ogilvie R, W, and L Feeback D. "A metachromatic dye ATPase method for the simultaneous identification of skeletal muscle fiber types I IIa IIb and IIc." *Stain Technology* 65.5 (1990): 231-242.