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Accumulation of iron in Dall's porpoise *Phocoenoides dalli* off the Sanriku coast of Japan

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Abstract: The 13 tissues (liver, kidney, muscle, bone, skin, heart, lung, intestine, blubber, spleen, pancreas, fore stomach and main stomach) of 22 Dall's porpoise (*Phocoenoides dalli*) (11 ♂ and 11 ♀) off Sanriku coast of northern Japan were measured for iron (Fe) bioavailability. Particularly higher Fe concentrations were found in the lung, liver and spleen than the remains. Lower Fe concentrations were recorded in skin and blubber. Hepatic Fe concentration positively correlated with maximum duration of dives among cetacean species ($y = 285.17x^{0.5267}$; $r^2 = 0.92$; y : average hepatic Fe concentrations in $\mu\text{g} \cdot \text{g}^{-1}$ dry weight; x : maximum diving duration in min). Using this correlation, the maximum duration of dives of Dall's porpoise was estimated as 11.85 min.

Key words: *Phocoenoides dalli*; iron; accumulation

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Iron (Fe) is an important essential element for all mammals. It is a component of many Fe-containing enzymes, as well as an integral constituent of hemoglobin and myoglobin^[1]. Thompson^[2] suggested that the iron levels in deep diving marine mammals be of interest and he considered that extremely high Fe concentrations in tissues of some seal species might be related to increased myoglobin levels associated with deep diving. Watanabe et al.^[3] found a positive correlation between hepatic Fe concentrations and maximum duration of dives of pinnipeds. However, similar research has been not yet been done on cetaceans. In the present study we investigated Fe bioavailability in Dall's porpoise *Phocoenoides dalli* True (1885), a small toothed whale, distributed in the cold waters of the northern North Pacific Ocean and adjoining seas^[4], with the objective of elucidating the tissular accumulation pattern and possible biological functions of Fe in this species, which may apply to other cetaceans as well.

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1 Materials and methods

Thirteen kinds of organ and tissue (forestomach, main stomach, blubber, bone, heart, intestine, kidney, liver, lung, muscle, pancreas, skin, spleen) samples of 22 Dall's porpoises (11 ♂ and 11 ♀, 1–6 years old), collected from the legally local dolphin harpoon-fishery in the Sanriku coast of northern Japan between January and April 2000, were used in the present study. No pathological symptoms were found in these animals through observations of behavior and anatomy. All samples were stored in polyethylene bags at -20°C until analysis. The background of analytical process are elaborated as follows: 1) A block of fresh sample was dried for 12 h at 80°C . 2) About 0.1 g of dried sample was weighed in a Teflon PTFE tube, and 1.5 mL of purified HNO_3 was added. 3) After pre-digestion at room temperature overnight, the sample was digested in a microwave oven for 6 minutes at 200W and this procedure was repeated twice. 4) The resultant solutions were diluted to a known volume with Milli-Q water (Milli-Pore Company) and transferred to acid-washed tube. 5) Iron was determined by a flame AAS (Shimadzu, AA-680). 6) The detectable limit of determination of the methods used was: $0.01\text{g}\cdot\text{g}^{-1}$ dry weight. 7) The standard reference material SRM1577b (National Institute of Standards and Technology, USA) was used to assess the Fe recovery rate and it was 111.4%.

2 Results and discussion

The concentrations of Fe in 13 organs and tissues are provided in Tab. 1. All Dall's porpoises studied were collected by the dolphin harpoon-fishery from free ranging animals without pathological symptoms. Therefore, the concentrations data in various organs and tissues could provide the information on normal requirement levels of Fe in nutrition. Fe levels were found highest in lung tissue, followed by the liver, spleen, muscle, kidney, heart, main stomach, pancreas, intestine, forestomach, bone, blubber, and skin. No gender differences of Fe concentration were found in all organs and tissues studied. It is interesting that hepatic, renal and muscular Fe in striped dolphins increased with age until 8 years and remained constant thereafter^[5]. But in Dall's porpoise no significant correlation was found between Fe concentrations in organs or tissues and age.

Specific accumulation patterns were observed in the lung, liver and spleen for Fe, suggesting these as the three target organs for storage, utilization or metabolism of Fe. Muscular Fe can be listed in higher concentration rank. Average Fe level in lung and liver was found $1224\mu\text{g}\cdot\text{g}^{-1}$ (range: 734~1890) dry weight and $1049\mu\text{g}\cdot\text{g}^{-1}$ (range: 444~1990), respectively. High lung Fe might contribute to rapid turnover of oxygen for Dall's porpoise, as Fe plays a crucial role in oxygen transport^[6]. Cetacean lungs have a powerful ventilation capacity, and the animals inhale air rapidly, and retain that air for prolonged periods of apnea before exhaling. Some species are able to remove nearly 90 per cent of the oxygen contained in each breath much faster and more efficiently than in humans and most of terrestrial mammals^[7]. Both suckling young and adult striped dolphin also showed higher Fe level in lung than other tissues^[8].

It is well known that liver is one of the most important Fe storage sites in mammals^[9]. Positive correlation between hepatic Fe concentration and maximum duration of dives of pinnipeds and sea birds was reported by Honda et al.^[10] and Watanabe et al.^[3]. A similar correlation was found in the present study (Fig. 1). The mode can be shown by the following equation: $y = 285.17x^{0.5267}$ ($r^2 = 0.92$, y : average hepatic Fe concentrations in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight; x : maximum duration of dives in min).

Tab. 1 Iron concentrations in various tissues of Dall's porpoise from Sanriku coast, Japan
(mean \pm s. d., range on $\mu\text{g g}^{-1}$ dry weight and sample numbers)

tissue	female	male	total
forestomach	143 \pm 57 100~ 291(11)	171 \pm 127 87~ 454(10)	156 \pm 95 87~ 454(21)
main stomach	303 \pm 68 238~ 446(10)	334 \pm 56 263~ 445(11)	319 \pm 62 238~ 446(21)
blubber	46 \pm 16 15~ 72(11)	42 \pm 35 1.2~ 134(11)	44 \pm 27 1.2~ 134(22)
bone	134 \pm 88 56~ 257(7)	89 \pm 65 38~ 246(10)	108 \pm 76 38~ 257(17)
heart	491 \pm 48 423~ 571(10)	508 \pm 64 454~ 661(10)	499 \pm 56 423~ 661(20)
intestine	147 \pm 36 90~ 202(11)	180 \pm 70 116~ 333(10)	163 \pm 56 90~ 333(21)
kidney	595 \pm 127 402~ 777(11)	612 \pm 291 334~ 1392(11)	604 \pm 219 334~ 1392(22)
liver	1114 \pm 447 506~ 1964(11)	983 \pm 498 444~ 1994(11)	1049 \pm 466 444~ 1994(22)
lung	1175 \pm 361 799~ 1797(11)	1273 \pm 351 734~ 1887(11)	1224 \pm 351 734~ 1887(22)
muscle	781 \pm 56 685~ 887(11)	722 \pm 73 618~ 816(11)	752 \pm 70 618~ 887(22)
pancreas	176 \pm 42 120~ 276(11)	207 \pm 58 132~ 302(11)	191 \pm 52 120~ 302(22)
skin	56 \pm 27 3.4~ 88(11)	43 \pm 23 6.2~ 80(11)	49 \pm 25 3.4~ 88(22)
spleen	821 \pm 234 610~ 1340(11)	988 \pm 223 602~ 1342(11)	905 \pm 239 601~ 1342(22)

Storage of oxygen in the skeletal muscles of diving mammals in which myoglobin acts as the primary oxygen carrier, is an important adaptation to aquatic life^[16, 17]. High myoglobin content in the skeletal muscles enhances diving capacity. Average muscular Fe concentrations of Dall's porpoise are more than $700\mu\text{g}\cdot\text{g}^{-1}$ dry weight. In cetaceans the higher storage of hepatic Fe might be related to the synthesis of muscular hemoglobin.

Up to now, no information has been available about the maximum duration of dives of Dall's porpoise. In the present study, through regression analysis, maximum duration of dives of Dall's porpoise was estimated using hepatic Fe concentration. With an average Fe concentration in liver of $1049\mu\text{g}\cdot\text{g}^{-1}$ dry weight, the duration of Dall's porpoise dives was estimated at a

maximum of 11.85 min. Using remotely-deployed suction-cup tag, Baird and Hanson^[18] found that in Dall's porpoise a maximum dive depth of 94 m in 2.78 min. Therefore, if half of the above-calculated maximum duration of dives of Dall's porpoise, or 5.92 min, is spent diving, Dall's porpoise could be estimated to dive as deep as about 200m. This result was very near to the reported maximum diving depth of this species (180m) by

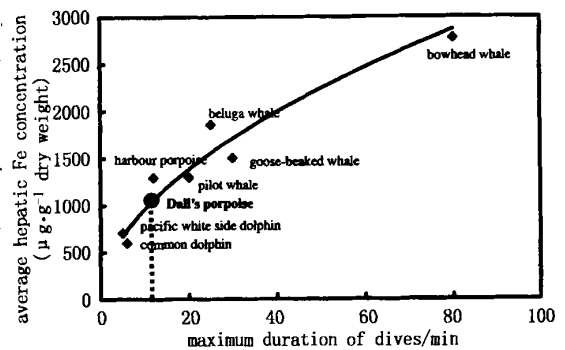


Fig. 1 Relationship between the maximum duration of dives and mean hepatic Fe concentration in cetaceans

Values on maximum duration of dives were from Schreier and Kovacs^[11] and mean hepatic Fe concentration data were from Knap and Jickells^[12], Becker et al.^[13], Krone et al.^[14] and Das et al.^[15]

Morejohn^[19]. Consequently, the value of 11.85 min seems to be an acceptable maximum duration of dives of Dall's porpoise.

High Fe level is also observed in the spleen ($905 \pm 239 \mu\text{g} \cdot \text{g}^{-1}$ dry weight, range: 7601~ 1342) for unknown reasons, though the spleen was reported as an important storage pool for oxygenated red blood in pinnipeds^[7]. Few data are available about spleen Fe level in cetaceans. A very high mean spleen Fe concentration ($3\ 238 \pm 3\ 719 \mu\text{g} \cdot \text{g}^{-1}$ dry weight) was found in striped dolphins from the coast of Italy. Spleen Fe concentration of one adult male was even recorded as high as $11\ 723 \mu\text{g} \cdot \text{g}^{-1}$ dry weight^[20].

Mean Fe concentrations of the kidney, bone, heart, intestine, pancreas, fore stomach and main stomach are between 100 and $600 \mu\text{g} \cdot \text{g}^{-1}$ dry weight. Those in blubber and skin are lowest and averaged as 44 and $49 \mu\text{g} \cdot \text{g}^{-1}$ dry weight, respectively. Fujise et al.^[21] reported similar data in one fetus, one adult female and one adult male of Dall's porpoise. Trace elements deposition in tissues is considered to be related to their bound proteins with sulphidric and hydroxylic groups^[8]. Therefore, it is likely that the proteins in skin do not promote Fe deposition and in blubber Fe has a low affinity for lipids.

3 Conclusions

Particularly high Fe concentrations suggest that the lung, liver and spleen seem to be "target organs or tissues" for Fe specific accumulation in Dall's porpoise and high pulmonary and hepatic Fe concentrations be related to the diving life. Skin and blubber are not good sites for deposition of Fe. A positive correlation between hepatic Fe concentrations and maximum duration of dives of cetaceans might therefore be a general characteristic among diving animals (at least pinnipeds, cetaceans and diving sea birds), which may be useful in estimating dive time duration. Use of this relationship, the maximum duration of dives of Dall's porpoise was estimated to be as long as 11.85 min.

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日本三陆沿岸白胸拟鼠海豚体内铁的蓄积

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摘要: 根据 22 头日本三陆沿岸白胸拟鼠海豚的检测结果, 研究了必需元素铁在 13 种组织和器官中的蓄积特征。肺、肝脏和脾脏中铁的浓度显著高于其它脏器, 铁在皮肤和鲸脂中的浓度却非常低。研究发现, 鲸类肝脏中铁的平均浓度与其最大潜水持续时间有着极其显著的正相关($y = 285.17x^{0.5267}$; $r^2 = 0.92$; y : 肝脏中铁的平均浓度, 单位为 $\mu\text{g} \cdot \text{g}^{-1}$ 干重; x : 最大潜水持续时间, 单位为 min)。利用该关系式, 首次推测白胸拟鼠海豚的最大潜水持续时间为 11.85min。

关键词: 白胸拟鼠海豚; 铁; 蓄积

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