In vivo MR Imaging and MR Spectroscopy in Marine Biology

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Introduction

In vivo MR imaging (MRI) and MR spectroscopy (MRS) have gained broad significance in experimental medical and pharmacological research. MR imaging and spectroscopy tools have been used with standard laboratory animals to answer numerous physiological questions. MR investigations have become routine for studying brain and cardiac (patho)physiology, and with the development of high-speed and high-resolution techniques the number of MRI and MRS applications continues to increase.

Recently, animal MR instrumentation have become available for MRI/MRS studies in the field of comparative physiology. Aquatic and especially marine organisms, with their fascinating physiological and biochemical characteristics and their extreme capability to adapt to environmental change, are most interesting research subjects in evolutionary, ecological and comparative physiology. In this article we will outline specific problems and solutions for studies in a seawater environment and present some of our applications in marine biology.

MR Hardware

The dielectric properties and conductivity of seawater lead to a significant decrease in the quality factor (Q) of conventional RF coils and a reduction in the signal-to-noise ratio (S/N).

Fig. 1: a) Animal chamber used for MRI experiments containing a specimen of the Antarctic eelpout *P. brachycephalum*. **b)** Coronal MR image of a pregnant eelpout and localized ^{1}H-MR spectra (PRESS) obtained from voxel **A**, the fish embryo, and **B**, the lumen of the uterus.

Bruker has developed special seawater-adapted birdcage resonators and surface coils, that can be adjusted over a wide conductivity range (up to 90 mS/cm) to minimize the negative effects of highly conductive media. The use of seawater probes and high-power transmitters (up to 2 kW) allows 90° pulse widths and amplitudes to be corrected for the effects of salinity. Thus, in seawater one can usually obtain >90% of the S/N that is achieved in fresh water [1].

Experimental Setup

Physiological investigations of marine organisms may require long-term experiments or time courses lasting up to several days under well-controlled conditions. Flow-through animal chambers of variable size are ideal for maintaining stable environmental conditions for water-breathing animals inside the MR magnet. Thermostatted reservoirs outside the magnet can provide recirculating water equilibrated with defined gas mixtures under specified physicochemical conditions. Parameters such as temperature, pH and nitrification can be controlled and monitored through the use of appropriate probes, and these data can be stored on the computer for later correlation with the MR data.

Benthic marine organisms (those living on the sea floor) are most suitable for MRI studies. The animals are relatively insensitive to handling stress and display little spontaneous activity inside the magnet. Therefore, anesthetics are not required, and the conscious animals can be studied under conditions close to those in their natural environment. **Fig. 1a** depicts a typical animal chamber containing an Antarctic fish of the eelpout family (*Pachycara brachycephalum*) under control conditions at a water temperature of 0°C. The sluggish life style and slow metabolism of these animals facilitate MRI studies of the unrestrained fish. **Fig. 1b** presents an MR image of a pregnant eelpout inside the chamber shown. The image was acquired with the standard Bruker RARE sequence and is undisturbed by movement artifacts. Localized in vivo ^{1}H-MR spectroscopy was applied to embryonic fish (Fig. 1b, voxel A) with the classical Bruker ^{1}H-PRESS sequence. Automation routines programmed using the macro manager within the ParaVision software package allowed us to combine MRI and MRS examinations over several days.

MRI/MRS Applications

All MR experiments were carried out at 4.7 T on a Bruker BioSpec DBX system with actively shielded gradient coils (50 mT/m). A cylindrical ^{1}H resonator (i.d.: 20 cm), adapted for high-conductivity samples in cross-coil operation mode, was used in combination with various surface coils for signal excitation and reception.
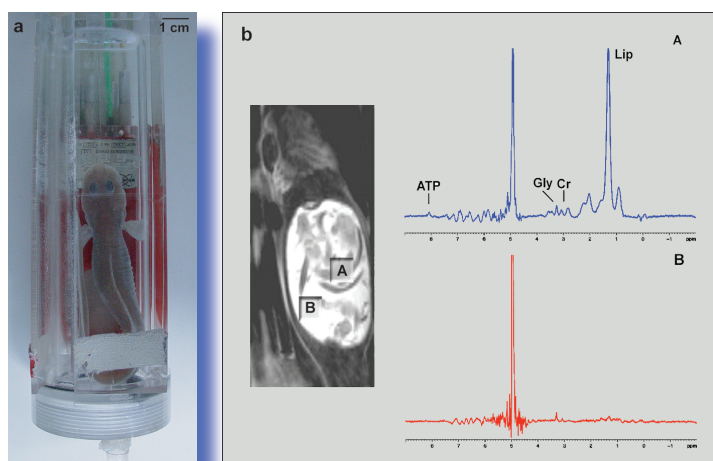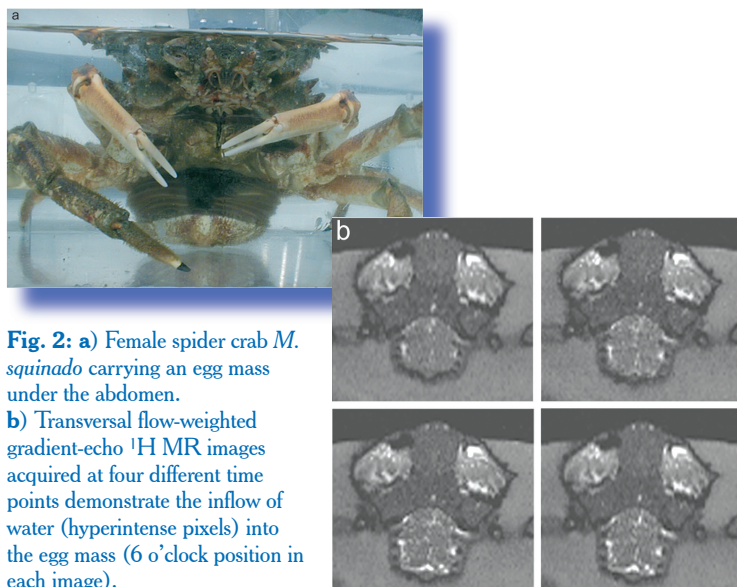
Fig. 2: a) Female spider crab *M. squinado* carrying an egg mass under the abdomen.
b) Transversal flow-weighted gradient-echo ¹H MR images acquired at four different time points demonstrate the inflow of water (hyperintense pixels) into the egg mass (6 o'clock position in each image).

1. Marine ecology: brooding behavior of crabs

In one of our first MRI investigations of marine animals, we analyzed the energetic cost of reproduction in marine invertebrates. We posed the question: Do brachyuran crabs display active brooding behavior, and, if so, what are the corresponding costs of parental care? Reproduction is one important ecological criterion which defines the fitness of an individual species in its ecosystem. Brooding female brachyuran crabs such as *Cancer pagurus* and the marine spider crab *Maja squinado* carry large egg masses under their abdomen for a period of 80 to more than 250 days. It has been suggested that oxygen availability is limiting in crab egg masses and that females may be able to monitor and actively adjust oxygen levels in the egg mass by ventilatory activity [2].

In this study we used MRI to observe patterns of flowing water actively pumped by the brooding female through the egg mass and also monitored oxygen concentration and the crab's behavior. The egg mass was monitored using a standard flow-sensitive gradient-echo (GE) sequence with the following parameters: FOV = 11.75×13 cm, four slices with thickness = 2 mm, matrix size = 256×192, TR = 76.5 ms, TE = 6 ms, flip angle α = 45°, total measurement time = 2 min. Crabs were fixed with their carapace to the top of the animal chamber, but were allowed to move their legs and abdomen. **Fig. 2a** shows a female marine spider crab *Maja squinado* inside the experiment chamber. The egg mass under the abdomen is clearly visible. **Fig. 2b** shows the time course of a flow-weighted MR image in a transversal plane through the animal and the egg mass. Flow weighting results in enhanced image intensity due to inflow of fully relaxed water into the imaged slice. The results show that at all times water entered (was pumped into) the egg mass from all sides, giving evidence for an active brooding behavior of the female crab.

MRI proved to be useful for comparing the brooding behavior of different female crab species. The sagittal MR images shown in **Fig. 3** show that the water flow through the egg mass of *Maja squinado* was higher and more continuous than the flow generated by *Cancer pagurus*. This indicates a higher

energy investment of *M. squinado* for brooding and consequently a better oxygen supply to the eggs. This difference in behavior may explain why egg hatching in *C. pagurus* is delayed compared to *M. squinado* [3].

2. Marine ecophysiology: thermal tolerance

The flow-weighted MR images discussed above not only revealed patterns of water supply to the crustacean egg mass but also depicted haemolymph flow through the circulatory system. This observation suggested that MR technology may provide an elegant way to study the role of the cardiovascular system in thermal tolerance. We were interested in understanding why the marine spider crab *Maja squinado*, in its natural environment in the British channel, is usually found at summer water temperatures of 12 - 16°C and avoids temperatures below 8°C.

Multi-slice flow-weighted GE ¹H MR images were collected in coronal and transversal planes at water temperatures between 2°C and 12°C [FOV = 12 cm, 4 to 6 slices with thickness = 2.2 mm, matrix size = 256×192, TR = 100 ms, TE = 10 ms, flip angle = 80° (4 ms Hermite pulse), 4 averages in 2.5 - 3.8 min]. In some cases, additional multi-slice flow-weighted images (30 slices) were collected at 12°C and at 5°C (recording time 20 min). Water flow through the system was reduced during data acquisition to minimize signal intensity originating from seawater flow surrounding the animal. Three-dimensional maximum intensity projection maps (MIPs) and surface projection maps were created from the multi-slice data sets with the 3D graphic routine Xtip in Bruker's ParaVision software.

For anatomical studies multi-slice RARE images were collected in coronal planes [FOV = 12 cm, 10 slices with thickness = 2 mm, slice interval = 2.5 mm, matrix = 256×192, TR = 3 s, TE = 18.4 ms, RARE factor 16, 8 averages in 10.5 min].

In **Fig. 4** anatomical MR images are depicted together with the corresponding flow-weighted images obtained at a temperature of 12°C. The anatomical images show excellent
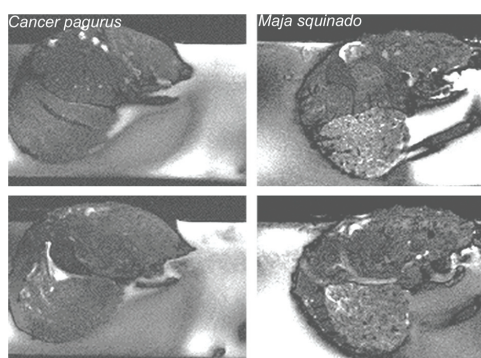
Fig. 3: Sagittal flow-weighted GE MR images obtained for the female crabs C. *pagurus* (left) and *M. squinado* (right) demonstrate that water inflow into the egg mass under the abdomen of the crab is higher for *M. squinado* (higher pixel intensities).
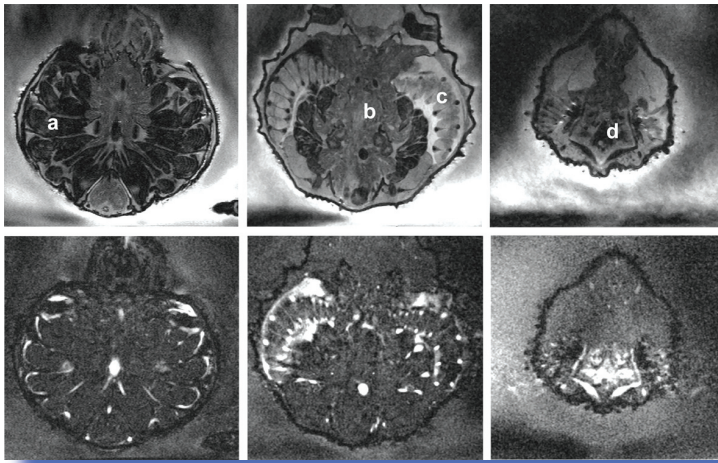
Fig. 4: Three coronal 2-mm slices showing anatomical MR images (RARE, top row) and the corresponding flow-weighted GE images (bottom row) acquired from the marine spider crab *M. squinado* under control conditions at 12°C (**a:** leg muscle, **b:** hepatopancreas and gonadal tissue, **c:** gills, **d:** heart).
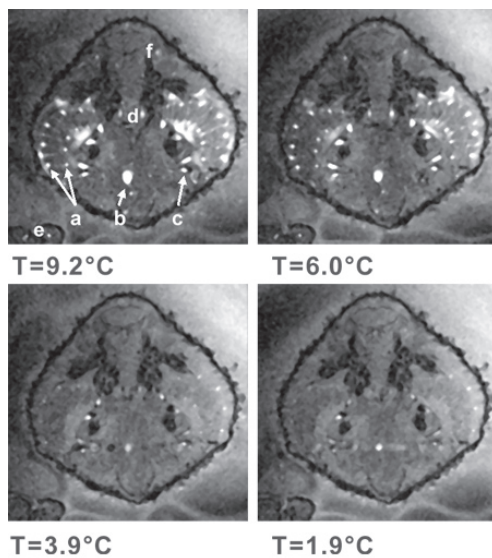
resolution, and even the smaller vessels such as the hepatic arteries are visualized.

Fig. 5 shows flow-weighted images obtained at four different water temperatures from the same coronal slice positioned in the center of the crab body. Haemolymph inflow causes vessels to appear very bright in the image for T = 9.2°C. The decrease in vessel brightness with temperature represents a decrease in flow, and the effect is most prominent for T < 6°C. Detailed ROI analysis of the MRI data revealed that the various types of vessel exhibit different thermal sensitivities (**Fig. 6**). The pattern observed indicates that the maintenance of flow through gill or leg arteries is most critical for resistance to cold in these animals [4].

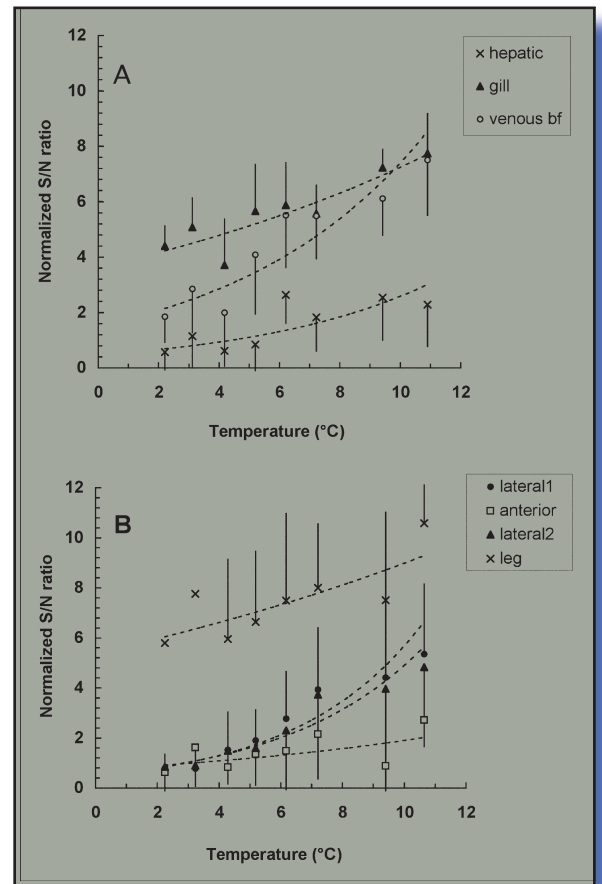### 3. Evolutionary marine physiology: adaptation to cold

According to a recent hypothesis, thermally induced restriction of oxygen supply may be the prime reason why the habitats of aquatic animals are limited to specific temperature

windows [5]. In order to test this hypothesis in fish, a multi-parameter MRI/MRS study of thermal tolerance was carried out with an unrestrained and non-anaesthetized Antarctic eelpout *P. brachycephalum*. In vivo ^{31}P-MRS was used to investigate energy metabolism and acid-base regulation while flow-weighted MRI was used to monitor blood flow and T_2*-weighted MRI was used to visualize tissue oxygenation via BOLD contrast. The surrounding water temperature was changed in 1°C steps every 12 h over the range 0 - 14°C. The entire experiment required 9 days (!) of monitoring with inter-leaved acquisition of ^{31}P-MR spectra and axial flow-weighted and T_2*-weighted MR images (**Fig. 7**) from the middle section of the fish. Classical ventilation and oxygen consumption measurements were combined with the MR studies.

In order to test the hypothesis of thermally induced oxygen limitation, a second set of experiments used water equilibrated with excess oxygen, thereby exposing the fish to hyperoxic conditions. **Fig. 8** summarizes some of the results obtained for the two experiments. Under normoxia, ventilation and oxygen consumption displayed an exponential increase as

Fig. 5: Coronal flow-weighted GE images of *M. squinado* at different temperatures. Haemolymph flow in vessels (bright pixels) declined dramatically at temperatures below 6°C. Major vessels are:
a) gill arteries,
b) Arteria sternalis,
c) venous return,
d) hepatic arteries,
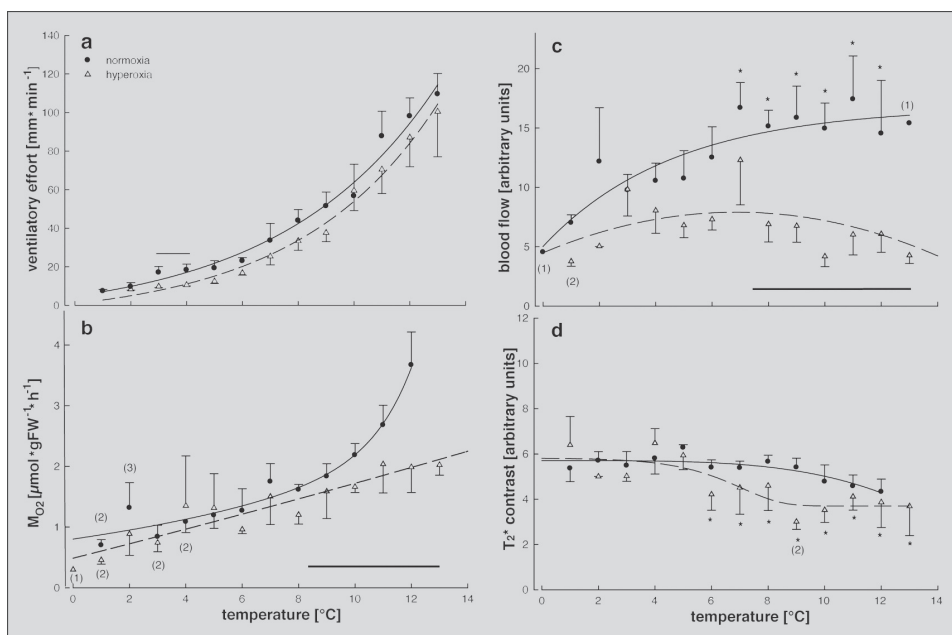**e)** leg artery,
f) Arteriae lateralis.

Fig. 6: Temperature dependence of haemolymph flow through various vessels in *M. squinado*, as monitored by flow-weighted GE MRI. At low temperatures the highest flow is maintained in the gill and leg arteries.
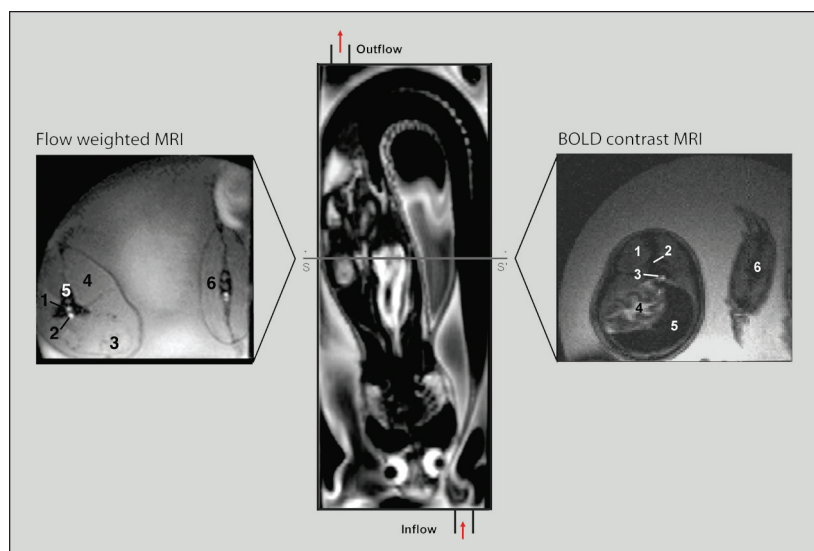
Figure 7: Typical transversal flow- and T_2*-weighted MR images obtained from an unrestrained and non-anaesthetized Antarctic eelpout *P. brachycephalum* under control conditions. The position of the transversal slices is indicated in the coronal overview (left image: 1, aorta dorsalis; 2, vena cava posterior; 3, stomach; 4, dorsal muscle; 5, spine; 6, tail; right image: 1, dorsal white muscle; 2, spine; 3, blood vessels; 4, stomach; 5, liver; 6, tail).

water temperature increased. Blood flow also increased but leveled off above 6°C. Interestingly, hyperoxia had little effect on ventilation and caused oxygen consumption to increase only linearly with temperature. Under hyperoxia blood flow showed little change with temperature and, in fact, decreased as temperature increased above 6°C. These findings are in line with the hypothesis that cardiovascular performance plays a role in thermal tolerance, as illustrated above for the spider crab. Hyperoxic conditions induced a significant decrease in the oxygenation of white muscle tissue for $T > 6°C$ (Fig. 8d), possibly as a consequence of the lower levels of blood flow and of whole-animal oxygen demand (Figs. 8a-c). The results indicate that, as a trade-off at higher temperatures, blood perfusion to muscle tissue may be reduced so that oxygen can be delivered to important aerobic organs such as the liver (data not shown) [6]. Altogether, the results of these experiments support the hypothesis of an oxygen-limited thermal tolerance in aquatic animals [5].

Summary

The present generation of MR instruments optimized for animal studies provides excellent and powerful tools for marine biology studies at all levels, ranging from ecology to physiology. The representative data presented in this article complement the data that can be obtained by conventional physiological and biochemical methods. The results illustrate the usefulness and potential of in vivo MR for research in the fields of comparative, ecological and evolutionary animal physiology.

Fig. 8: Ventilatory effort (**a**), oxygen consumption (M_{O2}) (**b**), arterial blood flow in the *aorta dorsalis* (**c**), and white muscle T_2*-contrast (tissue oxygenation) (**d**) were monitored in the eelpout as a function of temperature. Measurements were performed under normoxic (solid circles) and hyperoxic conditions (open triangles). The horizontal bar parallel to the x-axis marks temperatures where the differences between the two data sets reached statistical significance.

References

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