

Coping with a changing ocean: Real-time cardiac MRI on an animal model with a natural cardiovascular disorder

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Introduction

The changing climate is already affecting the world oceans. Marine animals with body temperature set by those of the environment are more and more confronted with warming temperatures and increasing CO₂ concentrations, known as ocean warming and acidification. Currently a pCO₂ of 380 ppm can be observed in the upper ocean layers, which represent a significant increase from pre-industrial values of 280 ppm.

Marine crustaceans such as the edible crab *Cancer pagurus* belong to calcifying invertebrates, which are believed to be most susceptible to ocean acidification. Crustaceans have a semi-closed cardiovascular system with a very basic one-chambered heart. Cardiovascular performance is usually not very regular and often interrupted by phases of bradycardia. Nevertheless, the regulation and effectiveness of cardiovascular performance is believed to be comparable to vertebrate systems (McMahon & Burnett, 1990).

Recent investigations on the effects of ocean warming and acidification have shown that marine crustaceans respond to increased CO₂ concentration with an elevation of heart rate, limiting their residual scope for performance and thus shifting their heat tolerance limits to cooler temperatures (Walther et al. 2009). Aim of this study was therefore to develop applications of real-time cardiac MRI to heart functioning in a marine crustacean for future monitoring of heart functioning and blood flow under conditions of ocean warming and acidification.

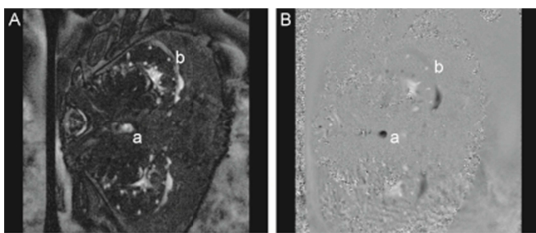
Material and Methods

Animal model: Marine crustaceans *Cancer pagurus* were sampled by dredging in the German bight in spring 2011 at water depths of 10-25 m and a water temperature of 15°C using the research vessel Uthörn (Alfred Wegener Institute). Water salinity was 30.5 ppt; pH value was around 8.1 pH units. Animals were kept in aerated re-circulated seawater aquaria at constant temperatures of 15°C and a constant pCO₂ of 380 ppm at the AWI until experimentation. Animals were fed twice a week with frozen mussels; water parameters and quality were checked regularly.

Experimental protocol: MRI experiments were performed in a Bruker 4.7T magnet equipped with a 50mT/m gradient coil and Avance III electronics using a sea water adapted 20 cm 1H birdcage resonator. Animals used had a 13 cm diameter carapace and around 300 g body weight. Animals were kept in rectangular plastic chambers (size: 20,5 x 17.5 x 9 cm, volume of 2 l) re-circulated with seawater. The back of the carapace was glued with dental wax to a Perspex bar fixed with plastic screws to the lid of the chamber, while the legs were free to move, as described in Bock et al. 2001. After positioning of the chamber using pilot scans and an acclimation period of 2-3 hours inside the magnet, velocity maps and real-time cardiac MRIs were performed regularly under control conditions (sea water temperature 15°C, pCO₂ 380 ppm). Ocean acidification was simulated by changing the seawater pCO₂ values of 1200 ppm, a value within the range expected by the end of this century.

Measurement parameters for velocity mapping were as follows: TE: 12ms, TR: 25ms, FOV: 14x14cm, matrix: 512x256, 1 slice, thickness: 3mm, flip angle: 60°, Venc_{max}: 12cm/s, 4 averages, resulting scan time 38.5s.

Real-time cardiac MRIs were acquired using Bruker's Intragate[®] protocol for cine imaging: TE: 3.6ms, TR: 6.8ms, FOV: 6.42x6.42cm, matrix: 128x128, slice thickness: 6mm, flip angle: 60°, expected heart rate: 40bpm, cardiac frames: 25, respiration rate: 5bpm, NR: 250 resulting scan time 1m54s.



Results & discussion

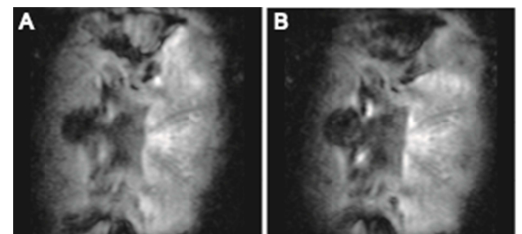
Velocity maps and real-time cardiac images were recorded in sufficient quality, despite of surrounding seawater and ventilatory activity of the animal. Phase of bradycardia induced a distinct but still detectable reduction in flow rates. Figure 1 presents a coronal view of *Cancer pagurus* under control conditions recorded with a flow weighted Flash MRI sequence (A) in comparison to the corresponding velocity map (B). Velocity maps revealed blood flow of around 8 cm/s for the *Aorta sternalis* (a) and 1.8-3.0 cm/s for gill arteries (b). The aortic flow rates correspond very well with previous results

from ultra-sonic Doppler measurements on lobster.

Figure 2 presents a real-time MR image from the cancer heart under control conditions (A) and elevated pCO₂ levels (B). The shape of the heart and the ligands fixing it to the carapace are clearly visible in the center of the MRIs. Oxygenated blood coming from the gills enters the heart from the posterior (left side on the image). A cardiac ganglion regulates heart rate. Interestingly, heart performance increased under elevated CO₂ conditions visible in brighter areas inside the heart in figure 2B, whereas blood flow in the *Aorta sternalis* from the velocity maps remains more or less unchanged (not shown), indicating a limitation in cardiovascular performance under elevated CO₂ concentrations.

Conclusions

Velocity mapping and real-time cardiac MRI were successfully applied to a marine crustacean and used to investigate the effect of elevated CO₂ partial pressures according to ocean acidification scenarios. High-resolution recordings demonstrated the cardio circulatory limitations, which set the limits to heat tolerance, and are exacerbated by ocean acidification.



Literature

Bock C, Frederich M, Wittig RM, Pörtner HO (2001). Simultaneous observation of haemolymph flow and ventilation in marine spider crabs at different temperatures: a flow weighted MRI study. *Magn Reson Imag* 19:1113-1124
 McMahon BR, Burnett LE (1990). The crustacean open circulatory system: a reexamination. *Physiol Zool* 63, 35-71
 Walther K, Sartoris FJ, Bock C, Pörtner HO (2009). Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. *Biogeosciences* 6, 2207-2215