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Investigation of marine bivalve morphology by in vivo MR imaging: First anatomical results of a promising technique

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Abstract

Classically, investigation of the internal morphology and composition of molluscs and especially bivalves relies on destructive method (biometry, biochemistry and histology). These techniques have given essential information, but in contrast are time consuming and lead to the irreversible loss of the animal while they don't allow integration of the various levels of molecular-to-organism functioning. The aim of this study is to analyze for the first time the potential of NMR (nuclear magnetic resonance) imaging (MRI) to depict, with sufficient resolution and satisfactory contrast, the anatomy of a bivalve model, the Pacific oyster, *Crassostrea gigas*, without opening it.

MRI experiments were carried out at 19 °C in several non-anaesthetized adult Pacific oysters, analyzed individually in a standard General Electric Signa 1.5T (whole body) instrument with actively shielded gradient coils (23 mT/m). To enhance signal detection, the oyster was centered in the middle of a 12-cm diameter Helmholtz-like radio-frequency coil (medical wrist coil). After several trials, the best MRI acquisition sequence retained was a T1-weighted procedure (spoiled gradient echo sequence) through two orthogonal directions (transversal and sagittal sections). According to direction, MR parameters were as follows: TR = 200–400 ms, TE = 4–5 ms, FOV = 120 × 90 mm, matrix = 512 × 256 units, 6 signal averages per echo, spatial resolution = 230 μm, total scan time = 3–6 min.

The MR images obtained have satisfactory contrast-to-noise levels, and depict with a sufficient resolution all the main organs in the soft tissues of the oyster. Comparison with histology-based anatomical information shows that the MR images faithfully represent some detailed anatomical structures of Pacific oysters. Potential applications in shellfish aquaculture are reported, and perspectives are given which constitute starting point from furthers studies.

Keywords: Pacific oyster; MRI; Anatomy; Aquaculture; In vivo physiology

1. Introduction

Marine bivalves and especially the Pacific oyster, *Crassostrea gigas*, are economically important in the French aquaculture. Its production has become a major industry with a production above 100,000 metric tons in 2002. A quarter of this production relies on spat

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produced in hatcheries. Nevertheless, the physiology of this bivalve is not well known and control of its reproduction in hatcheries is empirically based. To increase knowledge on this species and improve hatchery techniques, it appears obvious that new tools and/or approaches need to be developed.

Classically, the investigation of soft tissue in marine molluscs and especially in marine bivalves relies on destructive methods, since a shell completely encloses the animal. Anatomical structures are generally studied after removing the shell, followed by dissection by mean of histologic serial sections generally with a resolution of 4–5 μm (e.g. Chavez-Villalba et al., 2002). The evolution of gametogenesis can be quantitatively assessed with the help of image analysis (e.g. Enriquez-Diaz et al., 2003; Fabioux et al., 2005). Analysis of physiological or biochemical changes within tissues necessarily involves the sacrifice of specimens as well as the preparation and analysis of numerous samples. These standard techniques give valuable information but are inconvenient for two main reasons that may be limiting in specific studies: these methods are (1) very time consuming and (2) necessarily destructive. Therefore, the development of noninvasive and quantitative procedures is needed to achieve a deeper understanding of biochemical and physiological adaptations in shelled animals.

Non-invasive methods can also be applied for production applications. For oyster farming and especially hatchery production, the potential applications of non-invasive procedures could be numerous, for example: (1) to identify the meat quality of broodstock when they enter the hatchery (e.g. Toussaint et al., 2005 for fishes); (2) to follow very easily gonadal evolution during broodstock conditioning; (3) to identify the sex of broodstock oysters before fertilization and cross-breeding; and (4) to provide a quality indication for oocytes and spermatozoa (see Hagedorn et al., 1997). Among the various non-invasive imaging methods and after preliminary trials, NMR (nuclear magnetic resonance) imaging (MRI) appears to be a promising way to reach such objectives.

There are several studies dealing with investigation by MRI for land or freshwater invertebrates (e.g. Jasanoff and Sun, 2002; Wecker et al., 2002), and a few reports concerning marine fishes and invertebrates (e.g. Bock et al., 2001; Bock et al., 2002; Mark et al., 2002; Toussaint et al., 2005), but no work on marine bivalves to our knowledge.

To complement these studies, the aim of the present work was (1) to provide the first NMR imaging results for Pacific oysters, (2) to depict their anatomy with optimal MR parameters and (3) to show the feasibility and the potentialities of this technique for further investigations in marine biology and as a practical hatchery tool.

2. Materials and methods

2.1. Animal origin and preparation

Adult Pacific oysters (shell length ca. 11–12 cm, approx. 150 g, 3 years old) came from Aber Benoît River (North Brittany, France). They were collected during spring 2001 and kept in aquaculture facilities of the IFREMER shellfish laboratory located in Argenton near Brest (France) under controlled conditions (food level, temperature, salinity) similar to their natural habitat. They were periodically brought to the department of radiology and biomedical imaging (Rennes South Hospital, France) for the NMR imaging procedure. No anaesthetics were employed and all animals survived normally after the procedure.

2.2. Nuclear magnetic resonance imaging procedure

All MR images were performed on a standard General Electric Signa 1.5T (whole body) imager with actively shielded gradient coils (23 mT/m). To enhance signal detection, the oyster was centered in the middle of a 12-cm diameter Helmholtz-like radio-frequency coil (medical wrist coil).

The images described here were acquired at ambient temperature (ca. 18–20 °C) using a spoiled gradient echo sequence (SPGR-sequence) manipulated to produce intensity maps of water and lipid proton densities (T1-weighted procedure) through selected planes of the specimen with an optimal signal-to-noise ratio (SNR). Slices were selected in two orthogonal directions (transversal and sagittal) and the slice thickness was 3 mm without slice separation. According to direction, MR parameters were as follows: echo time (TE) was around 4–5 ms, relaxation delays (TR) varied between 200 and 400 ms, the bandwidth was set to 15.6 kHz, the flip angle around 80°. Matrix and field of view (FOV) were equal to 512×256 units and 120×90 mm, respectively, leading to a minimal resolution of 230 μm. The number of sections imaged per oyster varied between 10 and 22 according to the direction. The total scan time varied from 3 to 6 min according to the number of slices. The NMR images were actively contrasted during this procedure. The degree of brightness in each image was proportional to the concentration of protons and their respective relaxation