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Non-destructive nuclear magnetic resonance image study of belly bursting in herring (*Clupea harengus*)

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Abstract

Herring (*Clupea harengus*) captured during the heavy feeding spring season were examined by magnetic resonance imaging (MRI) analysis. MRI was performed at room temperature on the frozen-thawed herring for approximately 50 h. The results showed that the stomachs were filled with prey and that they were very resistant to degradation. The ventral muscle, on the other hand, together with the upper part of the intestine (as confirmed during collection of samples on board fishing vessels), seemed to be the most sensible structures where the autolysis commenced and extended to the rest of the abdominal cavity.

Keywords: Belly bursting; Herring; *Clupea harengus*; Pelagic fish; Magnetic resonance imaging; Post-mortem degradation; Digestive enzymes; Heavy feeding

1. Introduction

"Belly bursting" refers to the fast post-mortem degradation of the abdominal wall in pelagic fish which may occur during the heavy feeding season, usually in spring. It may be so severe that a few hours after capture may be enough for the fish to become unsuitable for human consumption. Belly bursting is commonly attributed to the effect of proteases from the digestive system of the fish, the zooplankton, the intestinal flora or the fish muscle (Gildberg, 1982; Martinez & Gildberg, 1988; Huss, 1995), but exactly which of these activities are implicated in the phenomenon and their relevance in herring is not known. In capelin (*Mallotus villosus*) it has been attributed to weakening of the collagen due to gastric acid leakage and pepsin activities (Gildberg, 1982), while in anchovy (*Engraulis encrasicolus*) belly bursting was mostly attributed to tryptic and chymotryptic activities from the pyloric caeca (Martinez & Gildberg, 1988; Martinez, Olsen, & Serra, 1988; Martinez & Serra, 1989). The only mention we have found in the literature to this phenomenon in herring is by Almy (1926) who attributed it to trypsin-like activities.

The enzymes contained in the hepatopancreas of the zooplankton are also a potential source of activity contributing to the belly bursting of the fish: *Calanus finmarchicus* for example, a common prey for herring, may be autolyzed in a few hours (Overrein, Evjemo, Jørgensen, Olsen, & Rainuzzo, 1999; Overrein, Olsen, Evjemo, & Rainuzzo, 1999). The hepatopancreas of zooplankton is rich in protease, lipase and chitinolytic activities among others, able to degrade proteins relatively fast (Turkiewicz, 1995).

Finally, the fish muscle itself also contains a variety of proteases including serine peptidases, cysteine peptidases (calpains and cathepsins) (Delbarre-Ladrat, Cheret, Taylor, & Verrez-Bagnis, 2006), and metallopeptidases, of which matrix metalloproteinases have been described in fish (Bracho & Haard, 1995; Kinoshita et al., 2002; Kubota, Toyohara, & Sakaguchi, 1998; Lødemel & Olsen, 2003; Lødemel, Mæhre, Winberg, & Olsen, 2004; Saito, Sato, Kunisaki, & Kimura, 2000). Upon the death of the fish, the fine control of these activities is terminated and

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the enzymes start their contribution to the post-mortem muscle degradation. Matrix metalloproteinases in particular are calcium-dependant neutral/alkaline metalloendopeptidases that degrade practically all components of the extracellular matrix (cells, mainly fibroblasts, and macromolecules such as collagens, glycoproteins and proteoglycans). This type of enzymes have been found and characterized in several fish species (see the references above). Small pelagic species have not received so much attention, although Sato et al. (1997) found that solubilization of collagen in the muscle of sardine (*Sardinops melanosticta*) occurs during chilled storage and that it correlates well with muscle softening.

Most of these activities are localized in different anatomical structures in the fish (some are colocalized, for example pepsin and zooplankton are both in the stomach). Therefore it seemed interesting to be able to examine the different organs of the fish during belly bursting in a non-destructive manner. That would permit to identify the origin, or origins, of degradation and/or eliminate some anatomical structures as source of enzymatic activities. High resolution nuclear magnetic resonance imaging (MRI) seemed to be the best suitable technique: it is non-invasive and non-destructive and has already proven its value in medical research and diagnostics and contributed to increase our understanding of the causes and effects of many diseases, playing nowadays a major role in the diagnosis of some tumors (Delorme & Knopp, 1998; Hayes, Padhani, & Leach, 2002). Also important information about cartilage, soft tissue surrounding the skeletal structure, the spinal cord, various organs and other structures in human beings and animals can be obtained in detail using various MRI techniques by making images of high resolution and contrast in any plane (Collins & Ehman, 2001). The feasibility of using MRI for anatomical studies of aquatic organisms was first demonstrated by Blackband and Stoskopf (1990) and it has latter been applied by Bock, Sartoris, and Pörtner (2002) to study in vivo non-anaesthetized marine fish by MRI using a flow-through animal chamber. The latter authors were able to distinguish different anatomical structures in the fish and record localized ^{1}H NMR spectra in different organs. MRI combined with high resolution ^{1}H NMR made it possible to examine the composition and structure of muscle tissue in Atlantic salmon (*Salmo salar*) (Gribbestad, Aursand, & Martinez, 2005). In fish processing, MRI has found application as a tool for the optimization of various unit operations such as salting and desalting in cod (*Gadus morhua*) (Erikson, Veliyulin, Singstad, & Aursand, 2004), distribution of fat and water content in Atlantic salmon (*Salmo salar*) (Veliyulin, Aursand, & Erikson, 2005), and in the study of the spatial distributions of lipid- and collagen-rich tissues in freeze-thawed rainbow trout (*Salmo gairdneri*) (Collewet et al., 2001). Veliyulin, Borge, Singstad, Gribbestad, and Erikson (2006) applied MRI to calculate the spatial distribution of water and salt contents in salted cod and performed dynamic and constant temperature MRI studies of the freezing of Atlantic salmon and cod, including, in the case of cod, the estimation of the relative amount of unfrozen water. The same authors used MRI to detect backbone deformations in Atlantic salmon. Hills (1995) has published a very good review on the applications of MRI to food processing.

We decided to examine belly bursting in herring by using MRI studies as reported here. In addition, we have conducted complementary enzymatic analyses, which will be reported elsewhere (Felberg & Martinez, 2006), on fish captured at the same time than the ones used here. This is the first time that the internal organs of the fish are being visualized in a non-destructive manner and in real-time while the belly bursting phenomenon is taking place.

2. Materials and methods

2.1. Fish samples

Herring (*Clupea harengus*) of average weight of 150 ± 20 g had been captured by purse seiner in the North Sea in the spring of 2005. The fish had been feeding heavily on zooplankton, mostly *Calanus finmarchicus*. Immediately after capture, 20 fish were frozen onboard at −20 °C, the only temperature available for freezing and transport, and the rest of the fish was stored in ice until it started to show signs of belly bursting, at which point they were also frozen stored at −20 °C. All fish were sent frozen to our lab and stored also frozen until the MRI analyses were performed.

For MRI examination, one herring with and another without signs of belly bursting were taken out of the freezer, thawed at room temperature (approximately 20 °C for 3 h), individually wrapped in plastic foil, placed side by side in the magnet, and examined as described below. A total of 6 fish, 3 from each group, were examined.

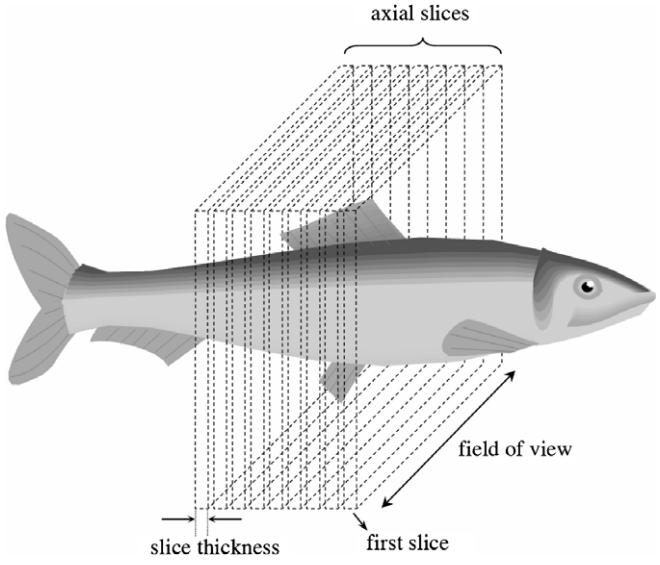

Fig. 1. Placement of the MRI axial slices.

2.2. MRI investigations

The MRI studies were carried out using the Bruker Avance AV300 small animal magnet (Bruker Optik GmbH, Germany) equipped with a 72 mm ^{1}H volume probe at room temperature (approximately 20 °C). The strength of the magnetic field in the instrument is 7 T corresponding to ^{1}H resonance frequency of 300 MHz. The system has a horizontal bore opening with the imaging area of sizes up to 6 cm in diameter and 10 cm in length.

Study of belly bursting in herring was carried out by monitoring changes in the anatomical structure of the fish as deterioration of the internal structures progressed. This was accomplished by taking a time series of 40 axial MRI slice images of the whole herring (perpendicular to the fish length) to cover most of the area of interest (from the gills to the anal opening as shown in Fig. 1). In this time study, 26 identical MRI measurements were performed with a 120 min delay between them. The whole time study took about 52 h.

The axial MRI images were acquired using a Multi Spin Multi Echo (MSME) protocol with the following parameters: echo time (TE) = 10.2 ms, relaxation delay (TR) = 620 ms, matrix size = 256 × 256 pixels, field of view

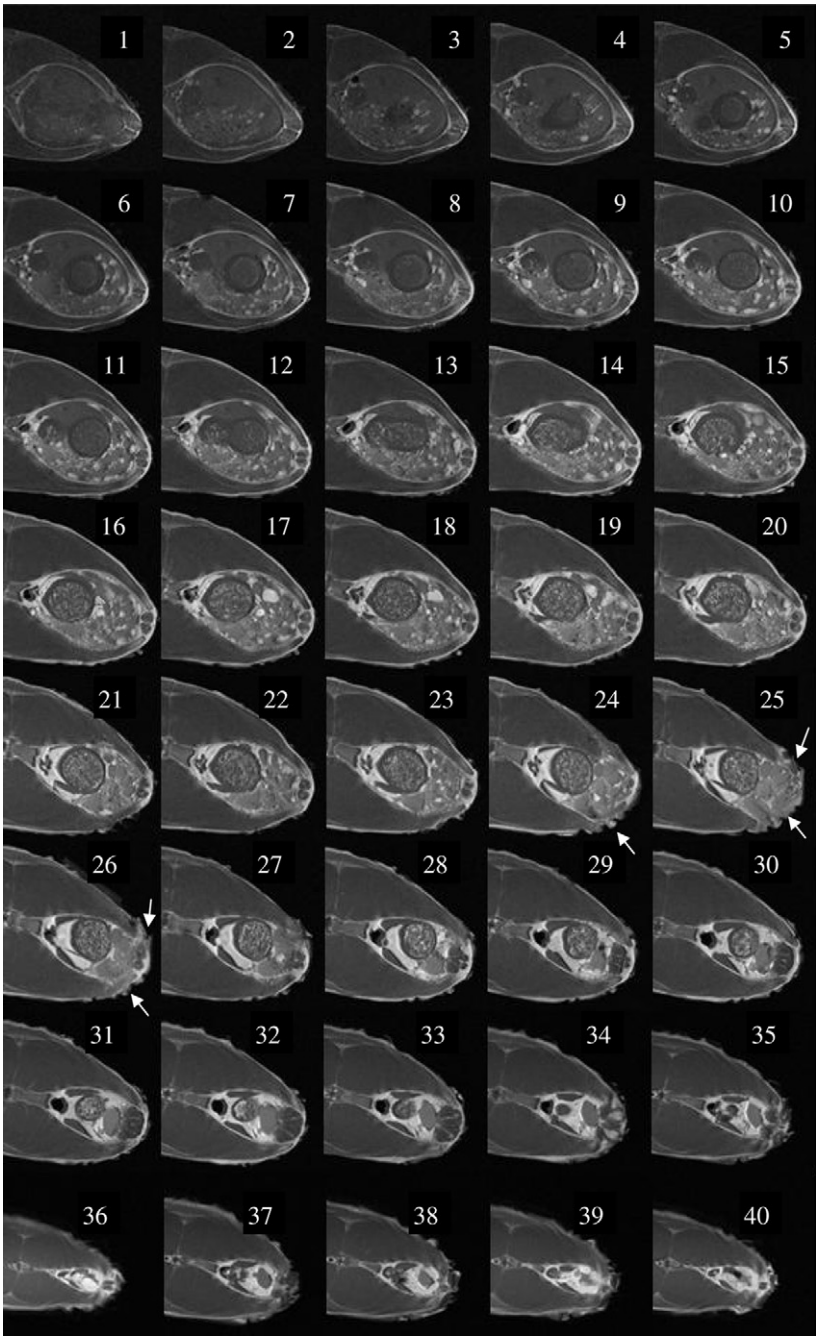

Fig. 2. MR images of all slices at the beginning of the MRI examination. Slice number 1 is closest to the head. The herring had been frozen immediately after capture. Damaged parts of the skin in slices 24–26 are indicated with arrows.

(FOV) = 32 mm, slice thickness = 2 mm and number of averages (NA) = 6. The total acquisition time of each examination was about 16 min. Use of TR shorter than $5 \cdot T_1$, where T_1 is the mean relaxation time in herring (about 50 ms as measured in our lab at 20 MHz proton resonance frequency), ensured that the images were T_1-weighted, which is a common way to obtain better intensity contrast between connective tissue or fat and muscle tissues in biological systems (Nott, Evans, & Hall, 1999).

3. Results and discussion

Fig. 2 shows all 40 axial slice images of the region schematically represented in Fig. 1. The feasibility of using MRI for anatomical studies of aquatic organisms, first demonstrated by Blackband and Stoskopf (1990) and latter applied by Bock et al. (2002) is confirmed by the present work. Like the latter authors, who were able to distinguished muscle tissues, gills, spine and stomach in the eelpout (*Zoarces viviparus*), we have been also able to identify several relevant structures in the present work, including the stomach, pyloric caeca, fat depots, intestines, white and red muscle, the backbone and the swim bladder (Fig. 3).

The time series of the study corresponding to the first slice is shown in Fig. 4. The herring to the left, which showed some signs of bursting already when frozen, displayed a hollow space in less than 13 h at 20 °C, while

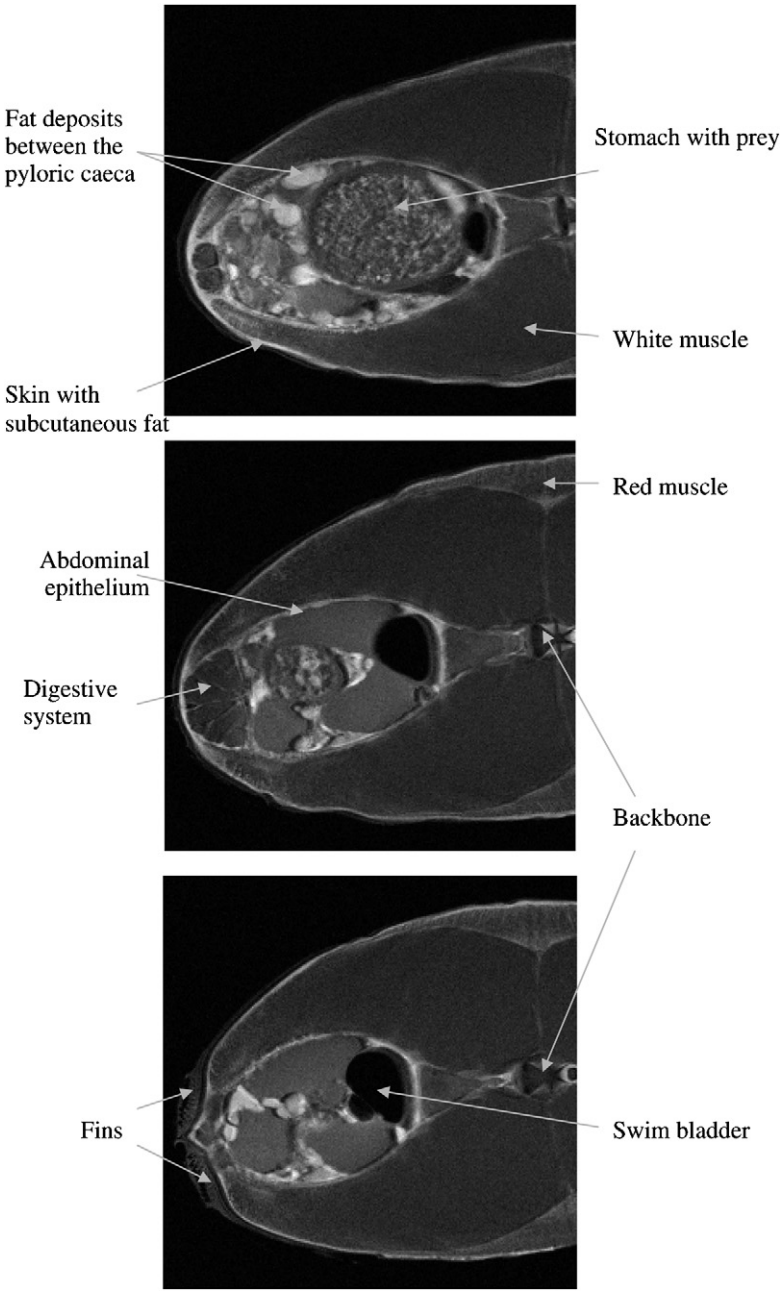

Fig. 3. Three MRI slices of herring showing several anatomical structures.

the herring frozen immediately after capture needed about 18 h to show a similar empty space. These black spaces originated because the fish organs liquefied and run out of the ventral cavity. Interestingly, neither the stomach nor its contents seemed to be degraded during the over 50 h that this study took. The results were similar for the 6 fish examined.

Unlike herring in the fishing vessels, which are cooled down immediately after capture to about −1.5 °C using refrigerated sea water (RSW), the fish examined here had to be frozen and thawed and examined at a high temperature. Consequently one would expect that belly bursting proceeded faster in this study than in the fishing vessels, but the observations from this study, as well as the examination of fish stored in ice immediately after capture and the results of enzymatic analyses reported by Felberg and Martinez (2006) are consistent: during the collection of samples, fish stored in ice started to show some signs of belly bursting (softening of the ventral muscle and visualization of the spines protecting the ventral cavity) after 24 h, but the stomachs and their contents were apparently intact even after 5 days of ice storage. Analysis of pepsin activity prepared from gastric mucosa of these herrings was also low (Felberg & Martinez, 2006) although it is obvious that these fish were producing and secreting active pepsin, since they were in an active process of digesting prey. An explanation for this lack of degradation in the stomach may be pepsin inhibition by excess of substrate (Felberg & Martinez, 2006). Interestingly, the results of this MRI study also exonerate the zooplankton contained in the stomach as the source of enzymes responsible for belly bursting, since they also remained intact during the over 50 h of examination.

The appearance of the empty space in the ventral cavity (Fig. 4) as well as the earlier weakening and degradation of the abdominal wall (indicated with arrows in Fig. 1) indicated that the source of belly bursting should be located in the intestine and the fish ventral muscle. Again, enzymatic analysis have demonstrated relatively high levels

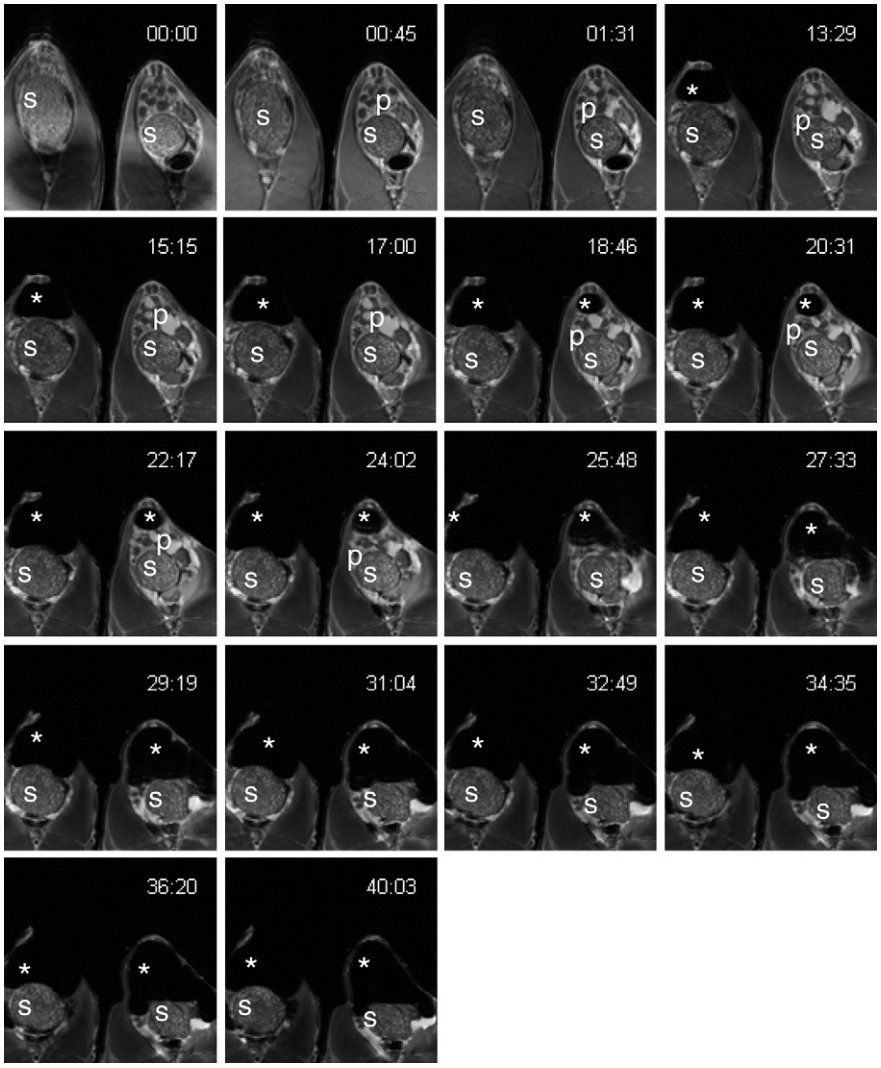

Fig. 4. Time series of the MRI study of the first slice (head side). The herring to the left had visible signs of belly bursting before freezing onboard, while the herring to the right was frozen undamaged. The time elapsed from the beginning of the MRI examination is indicated in each image. Labelled in the figure are: the stomach (s), pyloric caeca (p) wrapped around the stomach, and the hollow space formed as during belly bursting (∗).

(compared to extracts of dorsal muscle) of gelatin-degrading enzymes in extracts from ventral muscle already at the time of death of the fish, and that after 24 h of ice storage these activities were greatly increased (Felberg & Martinez, 2006). However, although not as resistant as the stomach, the pyloric caeca were more resistant to degradation than the intestine and ventral wall (Fig. 4). This result corroborates our observations during the collection of samples: although after 24 h of ice storage the pyloric caeca were intact and relatively resistant to manipulation, the upper intestine where the caeca secrete the enzymes, was weakened and broke easily upon manipulation. Thus, even if the source of digestive enzymes may be the pyloric caeca, the first internal structure that suffers degradation and from which active proteolytic enzymes may leak to the rest of the abdominal cavity, would be the intestine.

In summary, MRI has proven to be a useful tool to examine belly bursting in herring as it takes place and this is the first time that such a study has been undertaken. The results from this work clearly indicate that neither the stomach, nor the zooplankton it contains, are the source of enzymes responsible for belly bursting in this species. Together with enzymatic analysis (Felberg & Martinez, 2006) and the observations made during sample collection, we conclude that at least two anatomical structures are the origin of belly bursting in herring: the ventral muscle and the part of the intestine where the pyloric caeca empty their enzymes.

Although the potential of MRI is clearly significant (for example in the estimation of gastric filling and of how advanced belly bursting is, which are pieces of information of high value to fishermen and the industry buying the herring), its application is nowadays still limited to research activities due to the high investment and maintenance costs, the size of the equipment and the necessary infrastructure and highly skilled personnel.

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