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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.

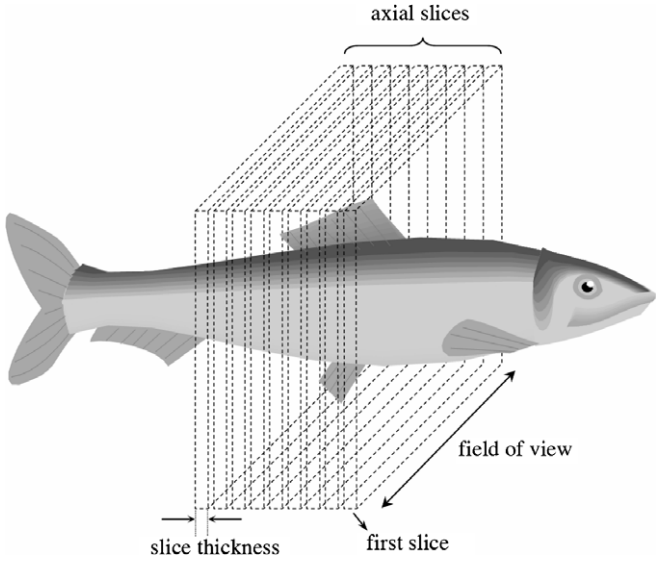

Fig. 1. Placement of the MRI axial slices.