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In vivo assessment of fat composition in Senegalese sole (*Solea senegalensis*) by real-time ultrasonography and image analysis of subcutaneous fat

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ABSTRACT

An ultrasound and image analysis non-invasive and non-destructive method for predicting skin fat, filet muscle fat, and whole body fat depots in live Senegalese sole (*Sole senegalensis*) was evaluated. The experiment was conducted with Senegalese sole of commercial market size (n = 46, mean weight 278 ± 78 g). The fishes under a mild anesthesia were placed in a rectangular metallic container and were scanned with a real time ultrasound (RTU) scanner equipped with a linear array transducer of 7.5 MHz. For all fish, four cross-sectional RTU images of the dorsal anterior filet was obtained. The RTU images were analyzed using ImageJ software to determine the fish subcutaneous fat (SF) at each section. After this, fish were killed, and the right dorsal filet of each fish was dissected in muscle and skin. These two components and the remaining of the fish were ground and homogenized for chemical analysis. The skin fat, filet muscle fat and whole body chemical fat contents were obtained. The relationships between the SF obtained from image analysis and skin fat, filet muscle fat and whole body fat were computed using simple regression and partial least squares regression (PLSR). The skin and filet muscle had lower fat than the whole body (1.5 and 4.5 versus 6.6%, respectively). Filet muscle presents the lowest fat depot (1.5%). The SF obtained from RTU and image analysis shows a mean value between 2.8 and 3.5%. All fat depots were significantly correlated with SF obtained by RTU and image analysis, with R^2 values ranging from 0.707 to 0.851 for skin fat, 0.725 to 0.802 for filet muscle fat and 0.664 to 0.760 for whole body fat. The PLSR models explained accurately the skin fat ($R^2 = 0.875$ and RMSEP = 0.506), filet muscle fat ($R^2 = 0.800$ and RSMEP = 0.117) and whole body fat ($R^2 = 0.785$ and RSMEP = 0.586). Our results suggest that RTU and image analysis is a practical and feasible non-invasive and non-destructive technique for in vivo fat depots prediction in Senegalese sole fish.

Statement of relevance: In aquaculture, the knowledge of the body composition in live fish, particularly fat content, is important for feeding, reproduction and genetics programs. However, all those programs depend critically on quick, accurate, and above all, non-invasive methods to predict body composition in live fish. Traditionally fish body composition was determined by comparative slaughtering followed by chemical analysis. Comprehensive studies using image techniques such as real time ultrasonography (RTU) show the ability of this technique to predict fish body composition traits. This technique is non-invasive and non-destructive and therefore is very useful for use in vivo for fish body composition examinations. Our present work investigated the ability of RTU and image analysis to predict in vivo fat composition of *Solea senegalensis*. Through this work monitoring fat variations with time and the possibility of repeated measurements on the same individuals and on a high number of live fish throughout the life-cycle is possible. Therefore our present study has close relevance to aquaculture particularly to nutrition status of farmed fishes.

1. Introduction

Sole (*Solea solea* and *Solea senegalensis*) have been considered as promising new flatfish species for Mediterranean marine fish farming due to the need to develop profitable markets (Dinis et al., 1999, Imsland et al., 2003). *S. senegalensis* is usually raised in extensive polyculture (in earth ponds) in the south of the Iberian Peninsula, where it can achieve higher growth rates than European seabass, being second only to gilthead seabream (Dinis et al., 1999). Its high price and market demand have triggered the interest in producing this species under more intensive conditions. In fact, Senegalese sole production increased from 110 to 500 tons from 2008 to 2011, especially in Portugal and Spain. The knowledge of the body composition in live fish, particularly fat content, is important for feeding, reproduction and genetic programs (Probert and Shannon, 2002; Romvári

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et al., 2002, Veliyulin et al., 2005). Moreover, it has been well documented that fat contributes to the nutritional and organoleptic characteristics of fish flesh (Izquierdo et al., 2005; Cardinal et al., 2011) and is decisive to meet the consumer acceptance (Probert and Shannon, 2002). However, feeding, reproduction, and genetic programs depend critically on quick, accurate, and above all, non-invasive methods to predict body composition in live fish (Veliyulin et al., 2005; Haffray et al., 2013). Traditionally fish body composition was determined by comparative slaughtering followed by chemical analysis (Oberle et al., 1997, Valente et al., 2011). Other methods such as ultrasound velocity (Ghaedian et al., 1997; Suvanich et al., 1998, Sigfusson et al., 2000) or spectral techniques (Wu et al., 2014) are based on filet samples or dead fish. Comprehensive studies using image techniques such as computer tomography (CT) (Rye, 1991; Romvári et al., 2002; Hancz et al., 2003a; Kolstad et al., 2004), magnetic resonance imaging (MRI) (Collewet et al., 2001, 2013) and real time ultrasonography (RTU) (Bosworth et al., 2001; Silva et al., 2010) show the ability of these techniques to predict fish body composition traits. From a practical point of view, the latter shows characteristics which suggest a future potential for this technique for use in vivo in fish body composition examinations. In fact, this is a simple, rapid and reasonably priced technique (Stouffer, 2004). Additionally, as water is an excellent coupling medium between transducer and fish, the RTU image acquisition with fishes in water is simplified because the stress is reduced and the time available for the ultrasound examination increases (Silva et al., 2010; Haffray et al., 2013). For other species, this technique has proven to be sufficiently precise and accurate to be used as a tool for body composition studies (for reviews see Stouffer (2004), Silva and Cadavez (2012)). Despite these attributes, little information is available about RTU to predict fish composition and to our knowledge, this is the first study conducted for fat fish assessment using RTU and image analysis. The objective of the current study was to investigate the ability of RTU and image analysis to predict in vivo fat composition of *S. senegalensis*.

2. Material and methods

2.1. Fish and experimental procedures

The experiment was conducted with Senegalese sole (*S. senegalensis*) of commercial market size (n = 46, mean weight 278 ± 78 g, range from 153 to 401 g). The fishes were chosen randomly from the research facilities of the University of Trás-os-Montes and Alto Douro (Vila Real, Portugal). All animals were kept under the same management conditions, stocked in a rectangular PVC tank (bottom area 0.75 m^2, water column 50 cm, volume: 500 L, water-flow rate: 5.5 L min^{-1}), supplied with re-circulated seawater (18.7 ± 1.6 °C, salinity: 35.2 ± 1 ‰), and were fed ad libitum an extruded commercial diet (3 mm) for marine fish supplied by Sorgal S.A. Animal handling followed the guidelines of EU directive number 2010/63/EU concerning animal care and all RTU examinations were done by an experienced scientist with this technique and with accreditation by FELASA Category C.

2.2. Ultrasound procedure and image acquisition

Fishes were scanned with an Aloka SSD 500V real-time scanner (Tokyo, Japan) equipped with a linear array transducer of 7.5 MHz (UST-5512U-7.5, 38 mm, Tokyo, Japan). Fishes were placed in a rectangular metallic container with 3 cm depth saltwater, and the RTU scans were taken with fishes under to mild anesthesia with ethylenglycol monophenyl ether (0.3 mL L − 1). The RTU images were taken over the dorsal anterior filet at cross-sectional slices (S1, S2, S3 and S4) from the end of the operculum (Fig. 1).

For each fish, four cross-sectional RTU images (Fig. 2) were obtained. To achieve this, the transducer was placed perpendicular to the fish major axis and displaced along the fish in a cranial-caudally movement from the end of the operculum. To allow that, the transducer was always placed in the same relative position the length of the fish was considered. With this, a cross-sectional location from one individual matched the same location in another individual. To ensure near-field visualization and to prevent tissue distortions due to transducer pressure a gel standoff was placed between transducer and fish. The gel standoff also has the merit to reflect the curved shape of the fish in the RTU images. This aspect facilitates the tissue identification which improves the measurement process during image analysis.

To perform the RTU image acquisition, the RTU scanner was connected to a video camera (Sony DCR-HC96E, Tokyo, Japan), which grabbed in real-time the videos with the RTU images. At the laboratory, the videos were displayed, and the images of interest were saved in TIFF image format (Tag Image File Format) with a resolution of 720 × 480 pixels and stored for later image analysis.

2.3. Ultrasound image analysis

To determine fish subcutaneous fat (SF) the RTU images were analyzed with Image J (http://rsb.info.nih.gov/ij/) software. The determination of the SF was performed after several steps. In the first step, the original image (Fig. 3A) was converted into a grayscale 8-bit image. Then a gray level threshold for the SF was selected. This threshold represents the average gray level, for each RTU section image, at five different positions in the SF layer. In a second step, the selected SF threshold was applied in the 8-bit image (Fig. 3B) and the SF segmentation was performed (Fig. 3C). A region of interest (ROI) for SF was defined (Fig. 3D). For the ROI selection, care was taken to include all pixels related with SF. Based on this ROI, numerical data was extracted from the image. To eliminate subjective operator-to-operator differences, image acquisition and measurements were done by only one operator having large experience in ultrasound technology and interpretations of images.

2.4. Calculation of subcutaneous fat percentage

From each RTU section image the dorsal anterior filet section area was calculated using the area tool of the Image J. The percentage of SF was calculated as a surface ratio between the SF area obtained as explained previously, and the dorsal anterior filet section area. The SF percentage was determined for each section (SF1, SF2, SF3, and SF4). The combination of SF of two, three or all sections was also considered. For this, the SF average of the sections in the different combinations was considered. As a result, the combinations of S1 and S2 (SF1_2), S2 and S3 (SF2_3), S3 and S4 (SF3_4), S1, S2 and S3 (SF1_3), S2, S3 and S4 (SF2_4) and for all sections (SF1_4) were obtained.

2.5. Dissection and sample preparation

After ultrasound images had been recorded fish were killed by overdose with phenoxyethanol (500 mg L^{-1}) and, the right dorsal filet of each fish was dissected. Special care was used during dissection to have a standardized filet extract procedure. The right dorsal filet was

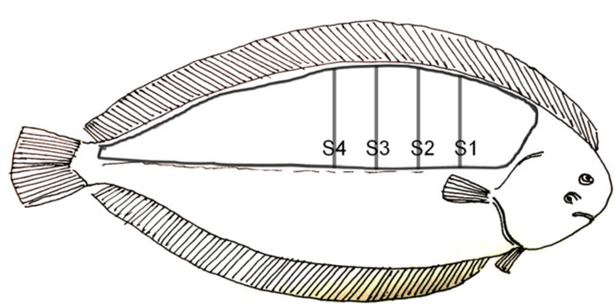

Fig. 1. Representation of the cross-sectional slices sites (S1, S2, S3 and S4) where the RTU images were obtained.