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Nondestructive rainbow trout (*Oncorhynchus mykiss*) freshness estimation by using an affordable open-ended coaxial technique

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Abstract: In the present work, a nondestructive device set up for a rapid and reliable freshness assessment of rainbow trout during 10 days of storage in ice was evaluated. The device was characterized by a vector network analyzer interfaced with an open coaxial probe to be placed in contact with the fish eye. The acquisition of the reflected scattering parameter (S11), which is the ratio between the amplitude of the reflected and the incident signal, was assessed in the 50 kHz–3 GHz spectral range. S11 is composed of a real part and an imaginary part, and both parts were used to predict quality index method for freshness evaluations. Partial least squares regression predictive models of the demerit scores related to fish eye attributes (eye pupil and eye shape) and the day of storage were set up. The main results showed that both the real and imaginary parts of the S11 decrease as a function of storage time. The combination with multivariate analysis allowed to set up predictive models of the storage time and the demerit scores with R^2 values up to 0.946 (root mean square error [RMSE] = 0.88 days) and 0.942 (RMSE = 3.17 demerit scores related to the fish eyes attributes), respectively (external validation). According to our results, the proposed cheap solution appears a useful tool for the freshness assessment of rainbow trout.

KEYWORDS
dielectric properties, principal component analysis, sensory analysis, shelf life

Practical Application: This work shows that dielectric properties have the potential to discriminate stored fish according to their freshness quality. A device based on this principle can play a significant role in the postharvest processes, contributing to higher product quality and safety and supporting producers and retailers during the qualitative inspections.

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1 INTRODUCTION

Fish products provide consumers with a variety of high-value components, such as essential amino acids, $n - 3$ fatty acids, and important micronutrients. Therefore their consumption, production, and commercialization are increasing worldwide (Calder, 2018; Chen et al., 2022; Hoffmire et al., 2012; Mohanty et al., 2019). Aquaculture production of rainbow trout (*Oncorhynchus mykiss*) in the EU reached about 210 thousand tons in 2019, being the most valuable freshwater species on the continent (FEAP, 2019). Rainbow trout can be found on European markets all year round, presented in several ways, whole or filleted, fresh or smoked. Whole fresh portion trout is by far the more commercialized; however, fresh fish is highly perishable product with a very short shelf-life.

Spoilage activities in fish start immediately after harvesting as a result of the presence of autolytic enzymes, high water activity, low pH, and high content of unsaturated fatty acids, which create the optimal conditions for bacterial growth (Alasalvar et al., 2010; Ghaly et al., 2010; Lougovois, 2005). In particular, autolytic enzymes that remain active after the death of the fish are responsible for several biochemical and physical changes resulting in the protein degradation and lipid oxidation (Ghaly et al., 2010). These processes involve also the formation of volatile organic compounds responsible for off-flavors and the deterioration of sensorial and nutritional quality (Whitfield, 1999). The above cited changes can be partially slowed down by the storage under controlled temperatures (e.g., melting ice) and the application of strict inspection controls (Badiani et al., 2013). According to the European Commission (EC) regulation No 853/2004 (European Parliament, 2004), “Fresh fishery products mean unprocessed fishery products, whether whole or prepared, including products packaged under vacuum or in modified atmosphere, that has not undergone any treatment to ensure preservation other than chilling.” As a result, freshness is one of the major contributors to the quality of fish as well as the major attribute which influence the market value and the consumers’ willingness to buy fresh fish (Freitas et al., 2021). In addition, freshness is associated with important food safety concerns as it is directly linked with several health risks for consumers (Olafsdóttir et al., 1997; Rocculi et al., 2019).

Traditional freshness analytical methods are based on quantitative chemical, microbiological, and physical techniques aimed at assessing different parameters recognized as indicators of fish postmortem changes. Among chemical indicators, trimethylamine (TMA), total volatile basic nitrogen, free fatty acids, thiobarbituric acid, and *K* value generated from the autolytic decomposition of adenosine triphosphate are well correlated with fish spoilage. Total viable bacteria count and the alterations in color and texture are respectively microbial and physical indicators of fish spoilage. Generally, these methods are time-consuming, expensive, and destructive (Prabhakar et al., 2020).

Sensory evaluation is a commonly used method for the assessment of fish freshness. In European Union, its application in fish inspection services is defined in the EC Regulation No 2406/96 (European Parliament, 1996) (García et al., 2017; Luten & Martinsdottir, 1997). This regulation lays down a freshness scheme, based on sensory evaluation, to distinguish fish products freshness during commercialization into three categories (i.e., EXTRA, A and B). More precise and worldwide recognized is the quality index method (QIM) sensory evaluation (Freitas et al., 2021). This method is based on a scheme developed by the Tasmanian Food Research Unit (Bremner, 1985) and is specifically designed for each fish species. Several QIM schemes have been developed for the main commercial fish species including Atlantic salmon (Sveinsdottir et al., 2003), cod (Cardenas Bonilla et al., 2007), and sea bream (Sant’Ana et al., 2011). This method assigns demerit scores (ranging from 0 for fresh products to 2 or 3 according to freshness) to several spoilage-related attributes such as color, smell, and texture changing of a particular fish species. The sum of all scores gives the quality index demerit score, the lower the score the fresher the fish. Developing a QIM for a particular fish species involves the selection of the more appropriate attributes in order to observe a linear increase in the quality index demerit score with the storage time. The application of this method is inexpensive, nondestructive, and rapid, but it requires a trained panel (Bernardi et al., 2013; Freitas et al., 2021).

The need for a rapid, cheap, and nondestructive method for freshness evaluation of fish products is pushing research to find alternatives. The state of the art of indirect and noninvasive techniques applied to fish freshness assessment mainly covers e-sensing techniques, optical spectroscopic solutions, and nuclear magnetic resonance (Franceschelli et al., 2021). These approaches are commonly combined with statistical algorithms able to extrapolate qualitative and quantitative models describing the variability occurring in the fish during storage. E-sensing techniques, including electronic noses (El Barbri et al., 2008) and tongues (Ruiz-Rico et al., 2013), colorimetric sensor array (Morsy et al., 2016), and computer vision systems (Rocculi et al., 2019), are deeply investigated because of their ability to simulate the human sensorial perceptiveness. In addition, the physicochemical and structural properties of the fish samples have been researched by modeling information coming from fluorescence (Omwange et al., 2020), infrared (Tito et al., 2012),

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hyperspectral imaging (Shao et al., 2023), and Raman spectroscopy (Herrero, 2008). Indirect techniques cover also those based on fish dielectric properties explored by a few works conducted by using the time domain reflectometry technique (Kent et al., 2004), impedance analyzers (Wang et al., 2008), commercial freshness meters (Vaz-Pires et al., 2008), and open-ended coaxial probe (Iaccheri et al., 2022). As well known, dielectric properties such as dielectric constant and loss factor change according to the physical and chemical characteristics of the food product, being mainly influenced by the moisture content (Ragni et al., 2016, 2017). According to Iaccheri et al. (2022), dielectric constant and loss factor of anchovy eyes, acquired in the 250–2400 MHz spectral range, decrease as a function of storage time. Authors attribute this behavior, to a reduction of the moisture on the surface of the fish eyes and to other modifications occurring in fish postmortem. In addition, the observed slight decrease in the dielectric constant in relation to frequencies evidences the presence of γ dispersion related to dielectric relaxation of hydration (Gabriel et al., 1983). In this way, eye dehydration occurring during storage may be monitored by using dielectric spectroscopy and correlated with the freshness of the fish.

This study represents a preliminary step in the exploration of rainbow trout (*O. mykiss*) freshness through the application of a rapid and cheap spectroscopic technique based on the fish's dielectric properties. Measurements of real and imaginary parts of the reflection (S11 scattering parameter) acquired on fish eye at different storage times were combined with those obtained through the application of a specific QIM scheme by a trained sensory panel. Outcomes from this study can be useful for the setting up of a novel rapid and nondestructive method for fish freshness evaluation, which will support producers and retailers in the busy commercial setting.

2 | MATERIALS AND METHODS

2.1 | Storage condition and experimental plan

Fresh-farmed rainbow trout (*O. mykiss*) from a commercial producer located in the north of Italy (Trentino province, Madonna di Campiglio area, 46°14′00′N, 46°14′00′E) were delivered to the Laboratory of Fish Quality Evaluation at the University of Bologna (Cesenatico, Italy) for three distinct storage trials between April and June 2021, following the procedure described in detail by Luten et al. (2006) in "Seafood Research from Fish to Dish—Quality, Safety and Processing of Wild and Farmed Fish." The fish had been slaughtered by ice-killing (hypothermia), packed with flaked ice into polystyrene boxes and delivered to the laboratory within 3–4 h from harvesting. Upon arrival, the fish were randomly divided into batches of eight individuals, covered with ice, and stored gutted in a refrigerator set at 0 ± 1°C. On the day of analysis, one batch was removed from the refrigerator. Each fish was coded, its mass (g) and its length (mm) were measured and then placed randomly on white trays on the evaluation desks. The first two storage trials were conducted as preliminary tests with the aim of setting up the quality index scheme. In detail, for each storage trial, a total of 80 trout were considered and 8 fish for each day of storage (0–10 days) were assessed. For the third storage trial, a total of 48 trout were considered and 8 fish for each day of storage (0, 2, 4, 6, 8, and 10 days) were assessed. At each considered day of storage, sensory evaluation, Torrymeter assessment, and spectral acquisitions were carried out. The length of storage was chosen based on several research works, evidencing that, based on microbiological and sensorial data, the end point of whole rainbow trout edibility, whenever stored in ice, is between 9 and 12 days (Rezaei et al., 2008; Tavakoli et al., 2018). Moreover, as laid down by the trout producer, a maximum shelf-life of 7 days from harvesting is to be considered.

2.2 | Sensory evaluation

Sensory evaluation was performed by a trained panel of four assessors (sex ratio 1:1, 25:45 age range) according to the QIM. This sensory approach is based on the freshness assessment scheme proposed for rainbow trout by (Grigorakis et al., 2018) and previously developed for Atlantic salmon by Sveinsdottir et al. (2003). Some modifications to the original scheme were implemented during the two preliminary storage trials, to best match the spoilage features occurring in the rainbow trout used in this experiment. In the way it is structured, and provided that fish is continuously kept under melting ice, the QIM demerit score is linearly related to the storage time. Modifications were addressed to those attributes that least fit the linear model in terms of R^2. In detail, the attribute "texture," composed of two description points in the original scheme, was increased to four. The attribute "abdomen odor" was removed as redundant, because the panelists were not able to discriminate any differences from the general "skin odor." In addition, some minor adjustments were applied to the description of the attributes, to better match the external features of the rainbow trout used in this experiment. The so-developed QIM scheme is shown in Table 1. Sensory evaluation was conducted in a laboratory that complies with the general criteria for the design of sensory analysis rooms proposed by the International Standard Organization (ISO) (ISO 8589:2014, sensory analysis—general guidance for the design of test

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TABLE 1 Quality index method scheme developed for rainbow trout (*Oncorhynchus mykiss*).

Quality parameter	Description	Demerit
Skin		
Color	Pearl-shiny, green glass spots on the back	0
	Less shiny, slight metallic-pink discoloration on the side	1
	Greenish color spread, pink–orange discoloration on the side and gill cover	2
	Green–gray discoloration and attenuation of the pink–orange nuance on the lateral side and gill cover	3
Mucus	Clear, shiny, and abundant	0
	Less clear, more viscous, and less abundant	1
	Milky, coagulated, and scarce	2
Odor	Fresh grass, river weed, cucumber	0
	Neutral to metallic smell	1
	Metallic, damp cellar and slight musty smell	2
	Oxidized blood smell, sour and decaying vegetation	3
Texture	Rigor	0
	Post rigor, firm, elastic	1
	Less firm, less elastic	2
	Soft	3
Abdomen		
Color	Pure white/silver flecks, solid	0
	Whitish/grayish discoloration, slight longitudinal depression	1
	Yellowish/dark gray discoloration, evident longitudinal depression	2
Eyes		
Pupil	Limpid, black with metal shiny	0
	Lightly opaque, grayish discoloration	1
	Mat, gray	2
Shape	Convex shape	0
	Flat	1
Gills		
Color	Slightly sunken	2
	Bright red, burgundy (Pantone 19-1617)	0
	Marsala red (Pantone 18-1438) and apical discoloration	1
	Totally discolored, gray–yellowish	2
Mucus	Glossy, clear	0
	Filamentous, milky	1
	Yellow, clotted, and filamentous	2
Odor	Fresh grass, river weed	0
	Neutral to metallic smell	1
	Oxidized blood, fish bowels, mushroom smell	2
	Rotten, sulfurous, decaying vegetation	3
Total demerit points		0–24

rooms). Evaluations were conducted at room temperature under a white fluorescent light (Philips Master TL-D 36 W/65, average intensity of 1200 lux on the working area, color temperature of 6500 K). Assessors kept a 1-m distance from each other while working.

2.3 | Torrymeter freshness assessment

Changes in chemical and physical properties occurring during storage as a consequence of spoilage were assessed by using a commercial Fish Freshness Meter "295—Torrymeter" (Distell, Fauldhouse, West Lothian, UK), according to the procedure described by Lougovois et al. (2003). By means of two pairs of concentrically arranged electrodes, the meter converts the electronically measured phase angle between the current (A) and voltage (V) on a 0–18 scale that increases with fish freshness. Before sensory evaluation, two measurements were taken on the left and the right side of the fish by applying the Fish Freshness Meter probe directly in contact with the portion of the skin just behind the gill cover, above the lateral line. Before each measurement, the electrodes were cleaned to remove debris (scales and mucus) that could compromise the measurement. The meter readings were read on the digital display.

2.4 | Spectral acquisition

Spectral acquisitions were conducted immediately after the sensory evaluation procedure by placing a coaxial probe in contact with the fish eyes. For each fish, both right and left eyes were acquired. The layout of the instrumental chain is shown in Figure 1. The vector network analyzer (VNA Nano V2, HCXQS in collaboration with OwOComm, China) is interfaced with an open coaxial probe connected to the instrument via port CH0 through a semirigid coaxial cable for high frequencies (50 Ω). The cable instrument and probe were assembled using specially made supports. The open coaxial probe was obtained starting from a female SMA connector, for the electronic boards, by turning and subsequent surface gilding of the brass. The VNA selected for the present research is quite a cheap portable instrument available on the market (about 100€). Nano-VNA V2 is a two-port VNA, CH0 and CH1 (reflection/transmission), designed for frequencies from 50 kHz to 3 GHz. Antenna analyzers, such as Nano-VNA, are designed to measure impedance (in ohms) and "standing wave ratio" (SWR). In a transmission line, the so-called VSWR (voltage standing wave ratio), also referred to simply as SWR is a parameter that indicates the ratio between the maximum value and the minimum value of the magnitude of the voltage along the line. At a fixed frequency, SWR is a measure of the impedance mismatch between the transmission line and its load. The higher the SWR, the greater the mismatch. The minimum value of the SWR, which corresponds to the condition of perfect adaptation for which the impedances of the transmission line and the load are equal to the absence of a reflected wave, is equal to 1. Finite values greater than 1 indicate a mismatch with the presence of a standing wave due to partial reflection from the load. SWR, therefore, is a measure of the impedance mismatch between the antenna and the receiver. Reflected spectra are reported as the input port voltage reflection coefficient (S11). The relationship between (S11) and SWR is previously reported by Franceschelli et al. (2023) and it is given by:

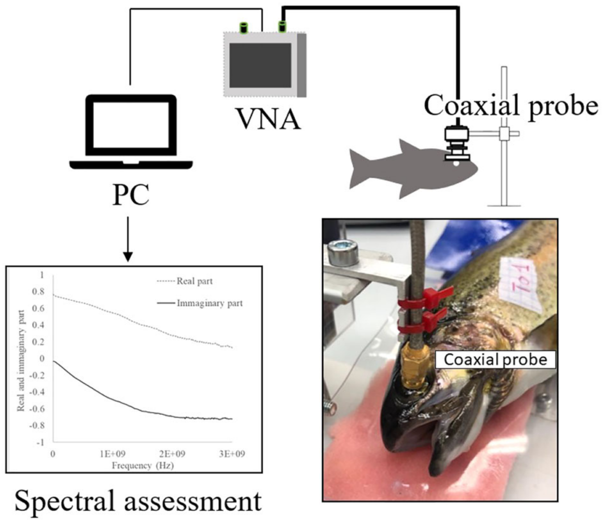

FIGURE 1 Setup of the instrumental chain with typical signal output and a picture of the contact between fish eye and the coaxial probe.

$$SWR = \frac{1 + |S11|}{1 - |S11|}$$

$$|S11| = \sqrt{(\mathrm{Re}\,(S11))^2 + (\mathrm{Im}\,(S11))^2}.$$

The scattering parameter S11 is composed of real (Re(S11)) and imaginary (Im(S11)) parts. In the present research, only the reflection port, CH0, was used and the scattering parameters S11 (reflection), real and imaginary, were measured. The assembled instrumental chain was calibrated to remove the three typical systematic errors in one-port measurements: directivity, source match, and reflection tracking (Chen et al., 2004). For the purpose, its own commercial calibration kit (SMA-type 50 Ω, HCXQS in collaboration with OwOComm, China) was

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used, accounting for open, short, and load calibration acquisitions and corrections. The spectra were acquired with the Nano-VNA-saver software (GNU, General Public License, version 0.3.8, Rune Broberg) for the whole frequency range 50 kHz–3 GHz, averaging three consecutive acquisitions of 301 spectral points.

2.5 | Data analysis

Significant differences in mass (g), length (mm), QIM, and Torrymeter scores acquired during the third storage trial were explored between means during the storage by using a one-way analysis of variance (ANOVA) and Tukey's post hoc test ($p < 0.05$). The assumptions related to data normal distribution and homogeneity of variances were explored through Anderson Darling's test and Levene's test, respectively. The coefficient of determination R^2 obtained from a linear regression between QIM attributes, QIM scores, and days of storage was explored and discussed. In addition, QIM scheme attributes were analyzed by principal component analysis (PCA) to better evidence the role of each sensorial attribute in the discrimination of the samples according to the storage time and their relationships. ANOVA, linear regression, and PCA analyses were performed with Minitab statistical analysis platform (Minitab 19.0.1, Pennsylvania State University, State College, PA, USA).

For both real and imaginary parts of S11, average values of measurement conducted on right and left eyes were used as independent variables for partial least squares regression analysis (PLS) with the aim of setting up predictive models of the demerit scores related to the fish eyes attributes (as a sum of the total demerit points) and of the day of storage. Data were arranged in a 48 (samples) × 301 (spectral variables) matrix. Loading weights were exploited to select the spectral range, considering that higher values related to the frequency portion greatly contribute to explaining the variability. In this way, the lower frequencies were not considered for the analysis, and the selected frequency range was 1.2–3 GHz. The calibration sample set was used for computing the calibration models. Validation was then performed to well understand how the developed model would perform with unknown samples. Test set validation was applied. Seven samples that were not included in the training stage were randomly selected and used to validate the models. The procedure was repeated ten times and results in terms of coefficient of determination (R^2), root mean square error (RMSE), and significative PLS components (PCs) both for calibration and validation were reported and discussed. PLS analysis was performed with statistical analysis software (Unscrambler software, version 9.7, CAMO, Oslo, Norway).

3 | RESULTS AND DISCUSSION

3.1 | External appearance

Examples of pictures of rainbow trout (*O. mykiss*) captured during the storage trial and after spectral assessments are shown in Figure 2. Ice chilling is the most popular storage method used in the fishery and aquaculture industry to delay the onset of spoilage, which is related to oxidative, enzymatic, and microbial degradation (Badiani et al., 2013; Kontominas et al., 2021). As expected, during the analyzed 10 days of ice storage, changes in the eye, skin, and gill appearance were observed. The discoloration of the rainbow trout skin that occurred over the storage time can be explained by the oxidation of the carotenoid pigments. Astaxanthin, an abundant carotenoid pigment in fish, is converted into colorless carbonyl compounds by a lipoxygenase enzyme present in the skin tissue and atmospheric oxygen (Lougovois, 2005). Color fading is amplified by the coagulation of mucus, due to the dehydration of the mucin glycoprotein, which modifies the reflection of the light on the fish skin (Lougovois, 2005). More generally, oxidative process occurring in fish during spoilage involves the reaction of unsaturated fatty acids with atmospheric oxygen to form hydroperoxides. These are decomposed to carbonyl compounds (e.g., aldehydes and ketones), which are responsible also for the off-flavor appearance (Kontominas et al., 2021; Nie et al., 2022). Off-flavors are intensified by the autolytic degradation of nucleotides, the bacterial reduction of TMA oxide to TMA (Barrett & Kwan, 1985) and other microbial spoilage process (e.g., ammonia and hydrogen sulfide producing bacteria) (Gram, 1992; Lougovois et al., 2003). The odor of rainbow trout assessed during the storage trial was of fresh vegetables notes (e.g., cucumber and river weeds) until 4 days of storage. Following 2 days where the odor appeared as neutral or slightly metallic until reaching the characteristic off-flavors (e.g., oxidized blood, decaying vegetation) after 6 days of storage. The color of the gills assessed in rainbow trout appeared bright red or burgundy (Pantone 19-1617) with a glossy and clear mucus until 2 days of storage. As time increased, gill the color passed from Marsala red (Pantone 18-1438) with apical discoloration and a filamentous and milky mucus on day 4. After 8 days of storage, mainly because of microbial growth and blood oxidation, gills acquired a gray-yellowish with yellow coagulated mucus. The eyes pupil tended to be gray and opaque over the period of storage. This could be attributed to the internal chemical changes which lead to external changes such as color and appearance in fish post mortem (Dowlati et al., 2013). Moreover, with increasing storage time, the shape of the eye gradually passed from being flat to concave (sunken), it is reported in the literature (Lougovois, 2005). Regarding the texture, rainbow trout presented a firm and hard consistency, often in rigor

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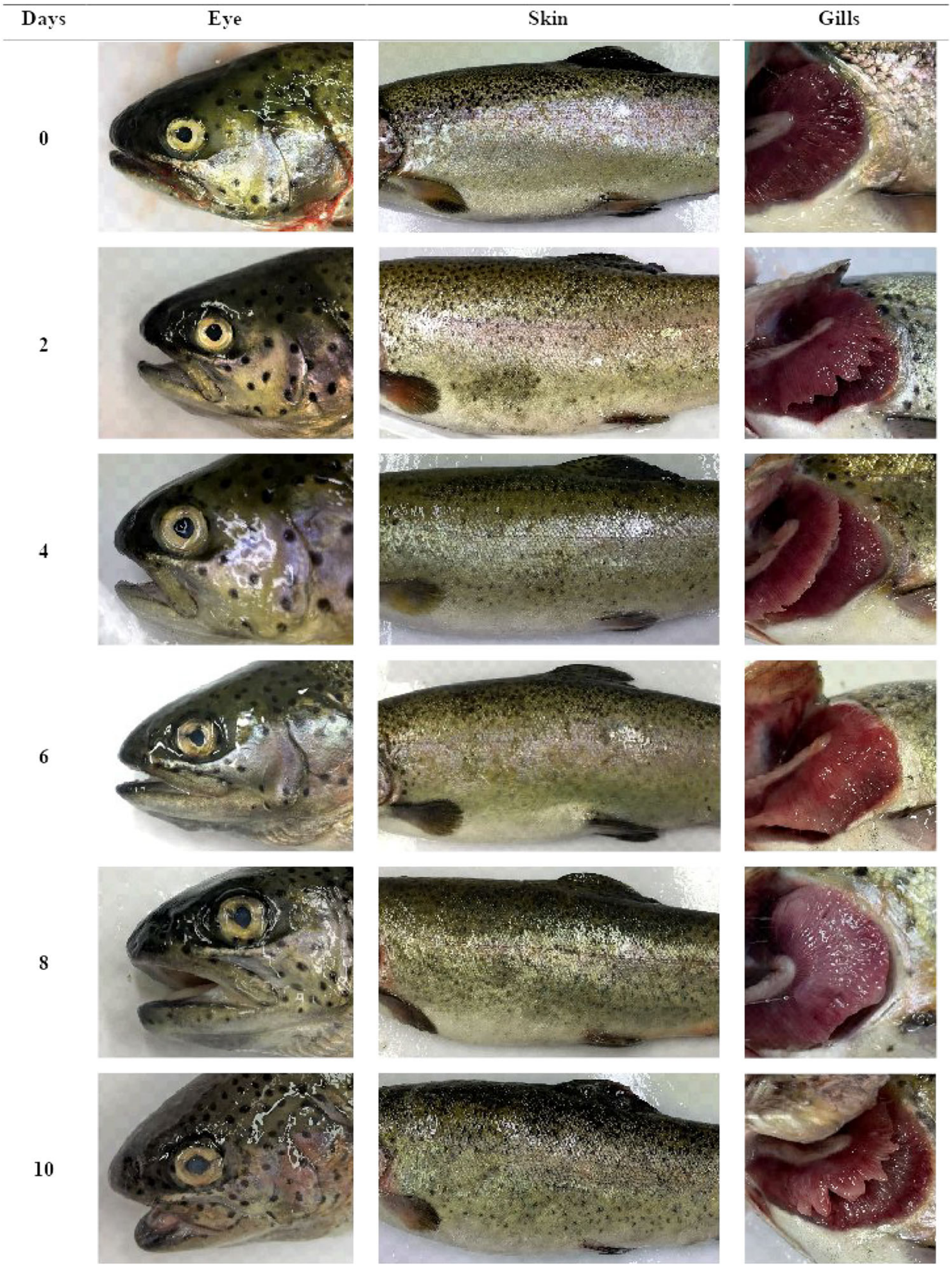

FIGURE 2 Examples of rainbow trout (*Oncorhynchus mykiss*) appearance during 10 days of ice storage acquired after spectral assessments.

mortis, until 2 days of storage. A progressive postmortem softening of the fish muscle was observed during the storage, as a result of enzymatic degradation generated by the activity of endogenous proteases on myofibrillar proteins (Cheng et al., 2014).

3.2 | Sensorial evaluation and Torrymeter freshness assessment

Mean values of the mass (g), length (mm), QIM, and Torrymeter scores acquired during the third storage trial are summarized in Table 2. The table also shows the results of the ANOVA conducted within the same analyzed parameter according to the storage time. As expected, storage time significantly influences both QIM ($p < 0.01$) and Torrymeter scores ($p < 0.01$). After post hoc test, nonsignificant differences in QIM mean values emerged between samples stored for 4 and 6 days. The QIM scheme developed in this work, shown in Table 1, reached its highest score after 10 days of storage and consisted of ten attributes for a total of 24 demerit scores. Other QIM schemes developed for rainbow trout focused on the degradation of the sensorial characteristic on longer storage periods such as

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TABLE 2 Mass, length, quality index method (QIM), and Torrymeter scores of rainbow trout (*Oncorhynchus mykiss*) during 10 days of storage in ice.

Storage (days)	Mass (g)	Length (mm)	QIM	Torrymeter
0	307.4 (23.6)	299 (6)	0.4^{a} (0.5)	13.1^{a} (0.8)
2	332.4 (62.5)	314 (17)	5.0^{b} (1.5)	13.1^{a} (0.7)
4	356.3 (43.5)	312 (6)	11.4^{c} (2.3)	12.6^{ab} (0.8)
6	361.6 (61.2)	312 (16)	13.9^{c} (2.4)	11.6^{bc} (1.3)
8	338.3 (51.8)	307 (14)	19.5^{d} (1.6)	10.5^{c} (1.1)
10	328.0 (313)	300 (5)	22.5^{e} (1.0)	11.0^{c} (1.0)

Note: Data are given as the mean ($n = 8$) ± SD (in brackets). Different superscript letters, within column, indicate significant differences among samples during storage ($p < 0.05$).

17 days (Grigorakis et al., 2018) and 12 days (Diler & Genç, 2018). However, the shelf-life of rainbow trout is considered to be somewhat shorter (Rezaei et al., 2008; Tavakoli et al., 2018). This is confirmed in this experiment by the onset of an unpleasant smell, which appeared after 6 days of storage, and become unacceptable on day 10. Significant differences were observed in Torrymeter mean values between samples at 0, 2, and 4 and samples at 8 and 10 days of storage. Differently from the QIM, Torrymeter assessment appears to discriminate trout samples only into two clusters of freshness; respect to day 0, the first significant differences between means appeared only starting from 6 days of storage. Torrymeter is a useful tool to evaluate fish freshness over several days, although less discriminative on shorter storage time (Cheyne, 1975). According to the manufacturer's with regard to salmon (*Atlantic salmon*) stored in ice, a Torrymeter score above 12 corresponds to a fish harvested for less than 3 days and to an "Extra" grade in the EC Regulation No 2406/96 (European Parliament, 1996). A Torrymeter score between 9 and 10 corresponds to a 5–8 days fish and to an "A" grade in the EC Regulation No 2406/96 (European Parliament, 1996). Results of the coefficient of determination R^2 obtained from linear regression models between each QIM quality parameter, QIM total, and days of storage are shown in Table 3. The results refer to both models calculated starting from all the considered samples or the mean values. By considering linear models obtained by using all samples, the gill color/appearance and gill mucus attributes were characterized by the lowest R^2 values. As evidenced in Table 3 and Figure 3, a coefficient of determination R^2 of 0. 948 was observed for QIM total demerit point. Passing to the models obtained from mean values, the R^2 values increased as expected.

3.3 | Principal component analysis

The biplot obtained from PCA, conducted by using the QIM quality parameters measured during the third storage trial and the storage time, is shown in Figure 4. The first

TABLE 3 Coefficient of determination (R^2) for each quality parameter assessed during 10 days of ice storage with the quality index method (QIM) scheme developed for rainbow trout (*Oncorhynchus mykiss*).

Quality attribute	R^2 (all samples)	R^2 (mean values)
Skin color	0.788	0.942
Skin mucus	0.821	0.942
Skin odor	0.809	0.936
Texture	0.819	0.936
Abdomen color	0.751	0.961
Eyes pupil	0.816	0.941
Eyes shape	0.819	0.979
Gills color	0.506	0.982
Gills mucus	0.592	0.876
Gills odor	0.848	0.979
QIM total	0.948	0.988

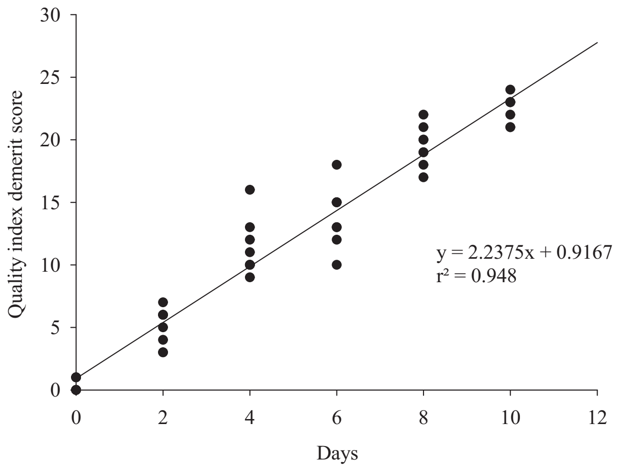

FIGURE 3 Linear regression between quality index demerit score and storage time assessed during the third storage trial with the quality index method (QIM) scheme developed for rainbow trout (*Oncorhynchus mykiss*).

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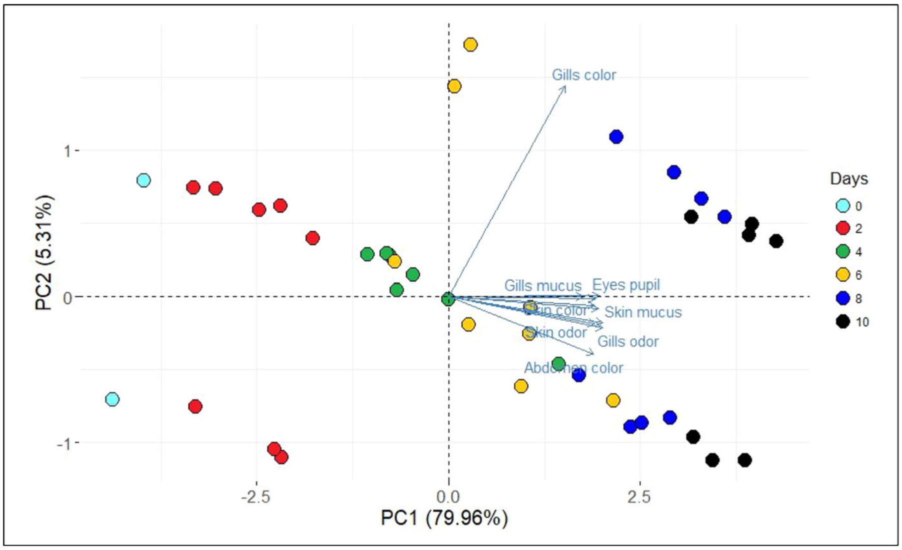

FIGURE 4 Principal component analysis (PCA) biplot including all quality parameters evaluated in the quality index method (QIM) scheme developed for rainbow trout (*Oncorhynchus mykiss*) during the storage time.

component (PC1) accounted for 79.96% of the variability and appeared to model differences related to the storage time while the second component (PC2), accounting for 5.31%, seems to explain the variability between samples within the same storage time. As indicated in Figure 4, PC1, associated with storage time, appears less influenced by the gill color/appearance and gill mucus quality attributes compared to the other analyzed attributes. On the contrary, gill color/appearance is the quality attribute that affect PC2 and therefore the variability between samples on the same day of storage.

3.4 | Spectral assessments

The open-ended coaxial technique measures the scattering parameters S11 as a function of sample response. In particular, the reflected signal is caused by an impedance mismatch, a discontinuity at the end of the cable. The dielectric properties of the sample are derived from this discontinuity. As the dielectric permittivity, S11 is a complex number composed of a real and an imaginary part. Both real and imaginary parts are related to the magnitude and the phase of the reflected electromagnetic wave. The real and imaginary parts of the scattering parameter S11 (reflection) are shown in Figure 5 for trout eyes samples during storage time. Averaged spectra of trout eyes were shown as a function of the sum of QIM total demerit points related to trout eyes, such as shape and pupils' attributes. Figure 5 shows spectra variability also as a function of storage time. Particularly, the low portion of the frequency range shows less spectral variability than that of high frequencies, both for the real and imaginary part of reflection. Physical-chemical modification induced by postmortem mechanisms influences the spectral response, as also previously reported for anchovy eyes by Iaccheri et al. (2022). According to the authors, a dehydration of fish eyes during storage time leads to a decrease in dielectric constant and loss factor, which is evidenced by a decrement in both real and imaginary parts of permittivity. In our trial, the real and imaginary parts of S11 spectra were used as predictors (x-variables) for PLS regression for QIM total demerit point of eyes and storage time estimation, the responses (y-variables). Results in terms of R^2, RMSE, and PCs for both calibration and test set validation models are reported in Tables 4, and 5. PLS-validated regression model gave considerable results in terms of the coefficient of determination and related errors. Particularly, PLS models demonstrated very good prediction ability in terms of R^2 0.946, 0.945 and RMSE 0.88, 0.79 (mean values for the real and imaginary part respectively), and R^2 0.942, 0.940 and RMSE 3.17, 3.03 (mean values for the real and imaginary part respectively), for storage time and QIM total demerit point of eyes respectively. As an example, predicted versus observed values of one PLS-validated model for both estimations of QIM total demerit point of eyes and storage time were shown in Figures 6 and 7. Predicted versus observed values confirm the ability of the proposed instrumental chain based on a coaxial probe to estimate both storage time and QIM total demerit point of eyes. The spectral results obtained in our trial can be compared with the relationship evidenced in the above cited previous research work between anchovy eyes freshness and changes in the dielectric properties (Iaccheri et al., 2022), where a discrimination of fish as a function of freshness was evidenced after a cluster analysis.

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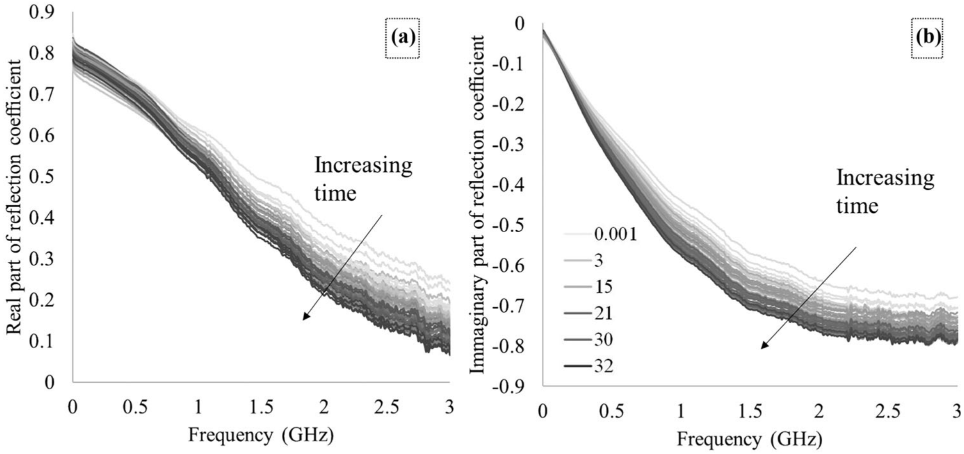

FIGURE 5 Real (a) and imaginary (b) part of S11 as a function of different storage time. The legend reports the sum of quality index method (QIM) total demerit point related to trout eyes. Validation procedure number 7 for R and 9 for I, see Table 4.

TABLE 4 Partial least squares (PLS) results both for real and imaginary part of the reflection scattering signal S11, used as predictors in multivariate model for quality index method (QIM) estimation.

	Total QIM estimation	R^2 calibration	RMSE calibration	R^2 validation	RMSE validation	PCs
Real part of S11	1	0.993	1.09	0.912	3.64	6
	2	0.997	1.41	0.914	4.43	6
	3	0.997	0.72	0.956	2.83	6
	4	0.994	0.97	0.945	3.32	6
	5	0.993	1.09	0.910	3.77	6
	6	0.991	1.15	0.981	2.11	6
	7	0.992	1.11	0.977	1.94	6
	8	0.989	1.36	0.950	2.92	6
	9	0.996	0.84	0.928	3.40	6
	10	0.997	0.71	0.943	3.36	6
Imaginary part of S11	1	0.998	0.54	0.911	3.18	9
	2	0.996	0.82	0.936	3.04	8
	3	0.996	0.78	0.960	3.01	8
	4	0.996	0.85	0.949	2.26	8
	5	0.993	1.05	0.939	3.17	7
	6	0.987	1.45	0.969	2.18	6
	7	0.997	0.57	0.900	4.57	9
	8	0.985	1.52	0.962	2.72	6
	9	0.997	0.71	0.931	3.20	8
	10	0.990	1.25	0.946	3.02	7

Note: From 1 to 10: validation procedures (each validation was conducted by considering a combination of different seven fish).
Abbreviation: RMSE, root mean square error.

Moreover, as shown in Table 4, the storage time estimation of the present device appears to produce a maximum RMSE value of 1.1 and 1.03 days for real and imaginary part, respectively. As a comparison, the discrimination capability of Torrymeter observed in our trial could be as high as 4 days, as shown in Table 2. This freshness assessment technique demonstrated its non-inferiority when compared to other sensors assessing the interaction between electromagnetic waves and fish eyes during storage. As an examples Shao et al. (2023), investigated the potentiality of

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TABLE 5 Partial least squares (PLS) results both for real and imaginary part of the reflection scattering signal S11, used as predictors in multivariate model for storage time estimation.

	Storage time estimation	R^2 calibration	RMSE calibration	R^2 validation	RMSE validation	PCs
Real part of S11	1	0.996	0.23	0.961	0.81	7
	2	0.980	0.47	0.947	1.10	7
	3	0.928	0.96	0.938	0.98	5
	4	0.994	0.28	0.945	0.79	6
	5	0.996	0.22	0.960	0.82	7
	6	0.993	0.30	0.912	1.05	6
	7	0.936	0.93	0.945	0.78	6
	8	0.982	0.47	0.957	0.85	5
	9	0.991	0.34	0.934	0.90	6
	10	0.995	0.24	0.956	0.73	7
Imaginary part of S11	1	0.991	0.32	0.967	0.76	7
	2	0.999	0.11	0.918	1.00	10
	3	0.994	0.27	0.970	0.61	7
	4	0.997	0.21	0.953	0.73	8
	5	0.993	0.28	0.947	0.85	7
	6	0.995	0.25	0.917	0.87	7
	7	0.989	0.37	0.920	0.96	6
	8	0.986	0.43	0.983	0.37	6
	9	0.982	0.47	0.964	0.73	6
	10	0.991	0.32	0.906	1.03	7

Note: From 1 to 10: validation procedures (each validation was conducted by considering a combination of different seven fish).
Abbreviations: PCs, partial least squares components; RMSE, root mean square error.

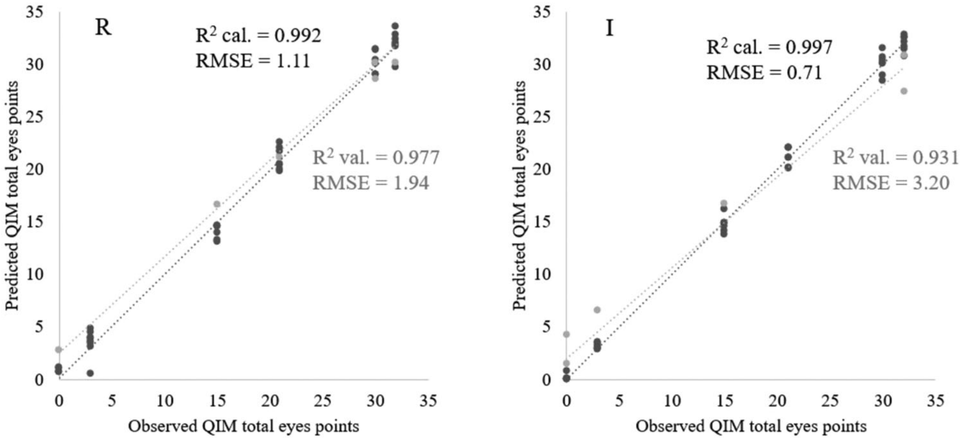

FIGURE 6 Predicted versus observed values for quality index method (QIM) estimation by using the real (R) and imaginary (I) part as predictors. Validation procedure number 1 for R and 6 for I, see Table 5.

a 400–1000 nm hyperspectral imaging technique in combination with multivariate data analysis for the prediction of freshness of yellow croaker and showed an $R^2 = 0.90$ after PLS validation. Furthermore, good correlations ($R^2 > 0.99$ in calibration) between fish eyes colorimetric attributes and storage time were observed also in several research works aimed at setting up methods based on machine vision systems (Rocculi et al., 2019; Wu et al., 2019).

Overall, the presented results show that the technique is promising for the freshness assessment of rainbow trout, even though some possible pitfalls can be verified. Attention should be paid when the coaxial probe is put in

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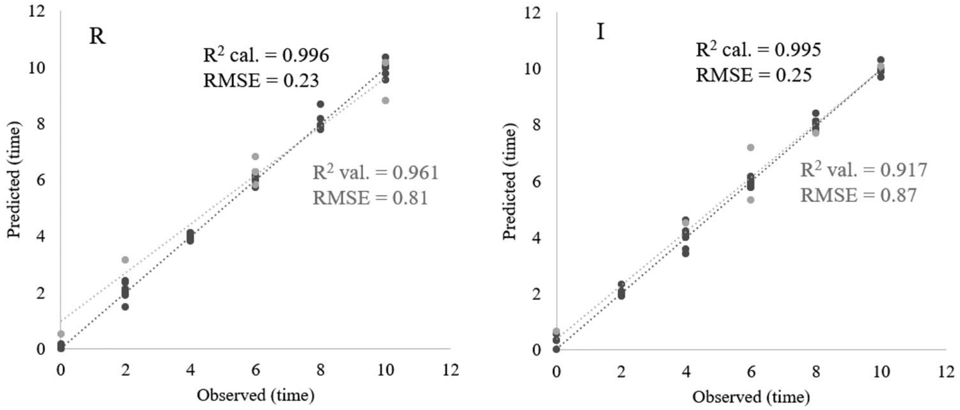

FIGURE 7 Predicted versus observed values for storage time by using the real (R) and imaginary (I) part as predictors.

contact with fish eyes, only a gentle pressure should be applied to prevent damages. In addition, being the dielectric responses matrix dependent, the so-developed model is intrinsically species specific being as much reliable only on rainbow trout. As such, dedicated measures should be developed to build new model for each species of fish. Despite this, the instrumental method is affordable, rapid, simple, and nondestructive avoiding the possible personal impact that sensory evaluation could subjected.

4 | CONCLUSION

A cheap and nondestructive device combined with multivariate statistical tools was set up aiming at exploring its ability in predicting the freshness of rainbow trout during storage of 10 days in ice. The proposed solution was based on PLS-validated regression models obtained by considering both real and imaginary part spectra of scattering parameter S11 acquired in the 50 kHz–3 GHz frequency range as independent variables. The acquisitions were conducted by placing a coaxial probe connected with VNA in contact with the fish eye. The day of storage and the demerit point calculated from a specific QIM scheme was chosen as dependent variables. According to the results, apart from gill color/appearance and gill mucus, all the considered sensorial attributes well describe the degradative changes occurring in the fish during the storage in ice. Both real and imaginary part spectra also contain information related to these changes. The tested device appears to be able to predict the storage time with an R^2 value of up to 0.946 and RMSE of 0.88 days and the total demerit scores associated with fish eye with an R^2 value of 0.946 and RMSE of 3.17. This simple solution can play a big role in the postharvest processes, contributing to higher product quality and safety and supporting producers and retailers during the qualitative inspections.

AUTHOR CONTRIBUTIONS

Andrea Bertini, Eleonora Iaccheri: Conceptualization; data curation; methodology; writing—original draft. **Martina Magnani**: Data curation; methodology; writing—original draft. **Anna Badiani, Alessio Bonaldo**: Conceptualization; supervision. **Luigi Ragni**: Conceptualization; resources. **Annachiara Berardinelli**: Conceptualization; methodology; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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