



## Data Article

# Bread wheat (*T. aestivum*) variability: Phenotypic and genotypic data from 75 varieties



Mélanie Lavoignat<sup>a,b</sup>, Emmanuelle Bancel<sup>a</sup>, Hélène Rimbert<sup>a</sup>,  
Sandrine Bagnon<sup>c</sup>, Michaël Benigna<sup>c</sup>, Alain Chassin<sup>d</sup>,  
Sandrine Berges<sup>d</sup>, Annie Faye<sup>a</sup>, Emmanuel Heumez<sup>e</sup>,  
Sibille Perrochon<sup>a</sup>, Larbi Rhazi<sup>f</sup>, Bernard Valluis<sup>g</sup>, Flavie Souply<sup>g</sup>,  
Marie Cécile Leroux<sup>g</sup>, Pascal Giraudeau<sup>h,i</sup>, Catherine Ravel<sup>a,\*</sup>

<sup>a</sup> Université Clermont Auvergne-INRAE, UMR1095 GDEC, Clermont-Ferrand, France

<sup>b</sup> AgroParisTech, Paris 75005, France

<sup>c</sup> QUALTECH, Vandoeuvre, France

<sup>d</sup> UE INRAE PHACC, Clermont-Ferrand, France

<sup>e</sup> UE INRAE GCIE, Estrées-Mons, France

<sup>f</sup> UniLaSalle, Beauvais, France

<sup>g</sup> Association Nationale de la Meunerie Française (ANMF), Paris, France

<sup>h</sup> SECOBRA Recherches, Maule, France

<sup>i</sup> Union Française des Semenciers (UFS), Paris, France

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## ABSTRACT

Most bread wheat is consumed after processing, which mainly depends on the quantity and quality of protein in the grain. Storage protein content and composition particularly influence the end use quality of milled grain products. Storage proteins are components of the gluten network that confer dough viscoelasticity, an essential property for processing. To explore grain storage protein diversity, 75 bread wheat accessions were grown with two replicates each at two locations. Grains were harvested at maturity and samples were phenotyped for each site and each

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\* Corresponding author.

E-mail address: [catherine.ravel@inrae.fr](mailto:catherine.ravel@inrae.fr) (C. Ravel).

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**Keywords:**

*Triticum aestivum* L.  
Wheat grain quality  
Storage protein composition  
Gluten polymers  
Rheological properties  
Genotypic markers

replicate plant. Grain hardness, thousand-kernel weight and grain nitrogen content were measured. The protein composition of flour from each replicate was characterised by reverse phase-high performance liquid chromatography (RP-HPLC). The molecular distribution of flour polymers was determined by asymmetric flow field-flow fractionation (AF4) and dough technological properties were assessed using a Glutomatic system and a Chopin alveograph. In addition, the 75 accessions were genotyped by the Breed-Wheat 35k genotyping array (Axiom TaBW35K) containing 34,746 single nucleotide polymorphism markers (SNPs). The dataset produced by this work includes six files with raw data, two files with protocols and figures. Data show the genotypic and phenotypic variabilities of the material used and can be used to explore genetic and environmental effects on traits involved in grain protein quality. This dataset is associated to the research article "Differences in bread protein digestibility traced to wheat cultivar traits" [1].

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## Specifications Table

|                                |   |
|--------------------------------|---|
| Subject                        | Agricultural Sciences: Agronomy and Crop science  |
| Specific subject area          | Wheat grain quality   |
| Type of data                   | Table<br>Figure   |
| How the data were acquired     | For phenotypic data: Grain hardness was measured with Near Infra-Red Spectrometry (NIRS). Wholemeal flour ground in a Falaise mill was used to characterize storage protein content (elemental analyser), composition (Reverse Phase High-Performance Liquid-Chromatography) and flour polymers (Asymmetric Flow Field Flow Fractionation). T55 flour was used to characterise dough rheological properties from Chopin Alveogrammes. The gluten, obtained by lixiviation from the T55 flour of each accession, was characterised by the Glutomatic system.<br>For genomic data: DNA was extracted from young leaves of each accession with a metabisulfite protocol. All accessions were genotyped with Axiom TaBW35K. |
| Data format                    | Raw<br>Analysed   |
| Description of data collection | Seventy-five bread wheat ( <i>T. aestivum</i> ) accessions representing modern, old varieties and landraces were field grown in 2016–2017 in two replicates at two locations in France, Clermont–Ferrand and Estrées–Mons. After harvest, two types of flour were milled from each sample (75 cultivars x 2 locations x 2 replicates).  |
| Data source location           | <ul style="list-style-type: none"> <li>• INRAE</li> <li>• Clermont–Ferrand (45°46′31.7″N 3°08′41.2″E, 358 m above sea level) and Estrées–Mons (49°52′36.6″N 3°01′53.6″E, 57 m above sea level)</li> <li>• Both locations are in France.</li> </ul>  |
| Data accessibility             | With the article and in public repositories<br>Repository name: (1) Recherche Data Gouv and (2) European Variation Archive (EVA)<br>Data identification number: (1) 10.15454/PIMPHZ and (2) accession number PRJEB56753   |

(continued on next page)

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Direct URL to data:

(1) <https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.15454/P1MPHZ>

(2) <https://www.ebi.ac.uk/eva/?eva-study=PRJEB56753>

Instructions for accessing these data:

All data are publicly accessible at Recherche Data Gov.

Markers anchored on the IWGCS reference sequence GCA\_900519105.1 (RefSeq v1.0 assembly for *Triticum aestivum*, cv Chinese Spring) are also publicly available at EVA.

Related research article

M. Lavoignat, S. Denis, A. Faye, L. Halupka, S. Perrochon, L. Rhazi, P. Giraudeau, S. Déjean, G. Branlard, E. Bancel, C. Ravel, Differences in bread protein digestibility traced to wheat cultivar traits, *J. Cereal Sci.* 107 (2022) 103533. <https://doi.org/10.1016/j.jcs.2022.103533>.

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## Value of the Data

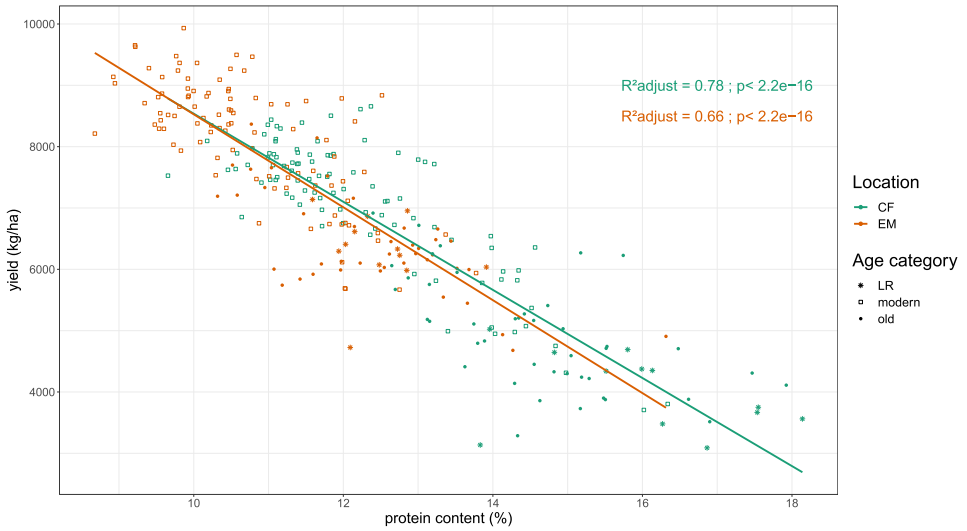
- Wheat is an economically important crop cultivated worldwide. Wheat storage protein content and composition are key parameters of flour end-use quality. Our data provide a phenotypic and genotypic description of 75 *T. aestivum* accessions cultivated in two locations. These accessions represent a large diversity of release years and technological characteristics. Our data are significant as they focus on storage proteins in grain and flour including information on protein polymers.
- These data are useful information for scientific communities dealing with wheat grain quality and use.
- These data allow investigation of genetic and environmental effects on several grain quality traits. They also allow comparison of quality traits between modern and older varieties.

## 1. Objective

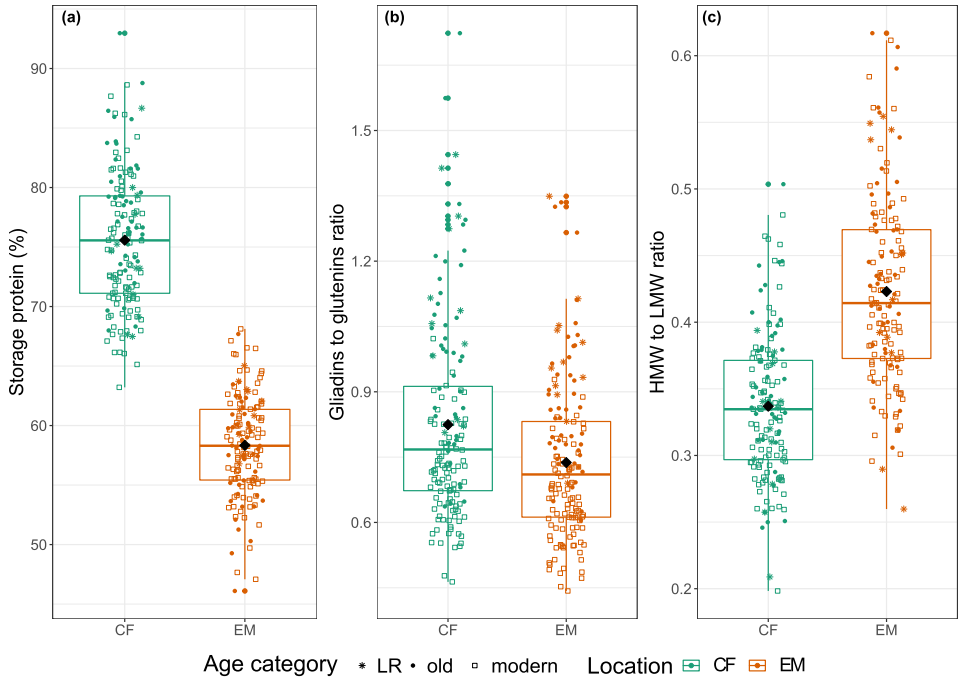
The dataset provides various types of information on traits related to bread wheat grain quality. The objective was to identify contrasted genotypes for storage protein content and composition, glutenin polymer characteristics and technological quality. Based on this screening, a subset of these 75 lines was further investigated in the research paper [1].

## 2. Data Description

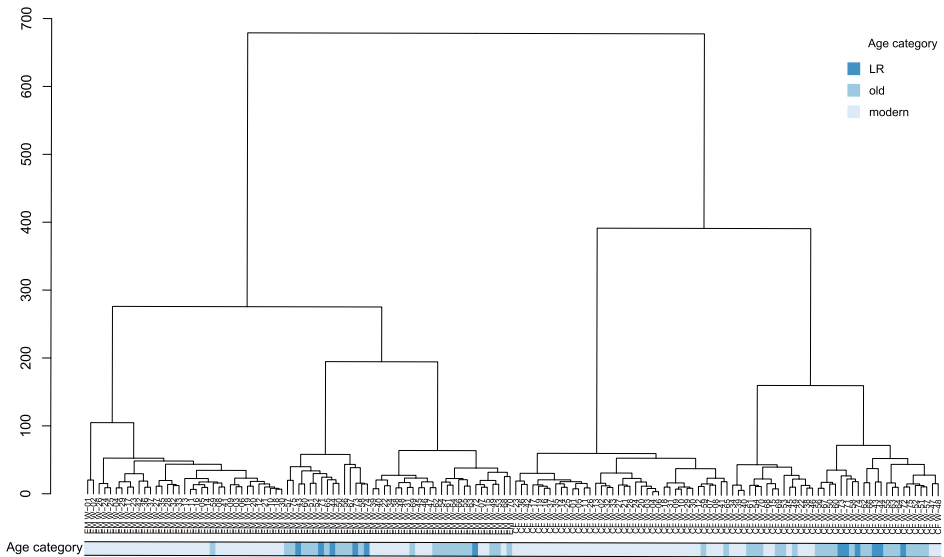
The dataset describes 75 *T. aestivum* accessions grown in two replicates at two locations in France, Estrées–Mons and Clermont–Ferrand. Accessions are classified into three categories depending on the year of their release for modern or old varieties or as landraces. Their geographical origin is specified as well (file named “Material”). In total, 300 samples are characterized. Data are organised into sixth files. The first file describes the field trials and some plant and grain traits. The relationship between yield and protein content is illustrated in Fig. 1. Protein composition of wholemeal flours expressed as dry weight is available in the second file and plotted in Fig. 2. The amount of nitrogen allocated to alpha/beta, gamma, omega and total gliadin fractions and to high molecular weight (HMW), low molecular weight (LMW) and total glutenin fractions is quantified. From this data, HMW to LMW and gliadin to glutenin ratios are calculated. Flour polymers are characterised in the third file. Polydispersity, conformation and quantity of polymers describe polymers as a whole. Mass, radius of gyration and hydrodynamic radius describe the average polymer in the sample. The fourth file gives technological data of gluten and dough made from flour derived from each accession sample. Hierarchical clustering based on phenotypic data provides information on the diversity of the genetic material for grain quality traits (Fig. 3). The fifth file details the 34,746 markers genotyped with Axiom TaBW35K.



**Fig. 1.** Grain yield plotted against flour protein content for two replicates of 75 accessions grown at two locations. Protein content is expressed as a percentage of grain dry weight. Accessions are classified into three age categories depending on the year they were released for old and modern cultivars, both predated by landraces. Adjusted R squared and *p*-values result from the linear regression model of yield versus protein content in Clermont-Ferrand (CF) and Estrées-Mons (EM).



**Fig. 2.** Boxplots of protein composition of wholemeal flour from the three age categories of varieties cultivated at two locations, Clermont-Ferrand (CF) and Estrées-Mons (EM). (a) Storage proteins as a percent of total proteins. (b) Gliadin to glutenin ratio. (c) HMW to LMW glutenin ratio. The function `geom_boxplot` from the `ggplot2` R package was used to draw the boxplots. Default parameters were used. Mean values are plotted (♦).



**Fig. 3.** Clustering of the 75 varieties cultivated at Clermont–Ferrand (CF) and Estrées–Mons (EM) based on the mean of the two replicates for each location. Manhattan distance was used to calculate dissimilarities among varieties for grain hardness, thousand-kernel weight, protein composition, polymer characteristics and technological properties. The Ward D agglomeration method was used for clustering. The shaded horizontal bar indicates the age category of each variety.

The sixth file uploaded onto EVA correspond to the 18,117 markers out of these 34,746 anchored on the IWGCS reference sequence GCA\_900519105.1 (RefSeq v1.0 assembly for *Triticum aestivum*, cv Chinese Spring). All markers (projected on GCA-900519105.1 or not) are available at Recherche Data Gouv. Moreover, all variables are summarised and explained in a file named “Variables”. Two laboratory protocols on wheat grain storage protein extraction and quantification are also available in files named “Protocol1” and “Protocol2”.

### 3. Experimental Design, Materials and Methods

#### 3.1. Experimental Trials

Grains of 75 *T. aestivum* accessions were obtained from French breeders or INRAE CRB ([http://www6.clermont.inra.fr/umr1095\\_eng/Teams/Experimental-Infrastructure/Biological-Resources-Centre](http://www6.clermont.inra.fr/umr1095_eng/Teams/Experimental-Infrastructure/Biological-Resources-Centre)). These cultivars were chosen to reflect modern varieties released after 1960 and old varieties released before 1960. It must be emphasised that W-39 and W-40 are two isogenic lines diverging for a transcriptional factor (NAMB1) that improves protein content without reducing yield potential. The field experiment was conducted with 75 bread wheat accessions grown in 2016–2017 in two replicates at Estrées–Mons (EM, northern France) and Clermont–Ferrand (CF, central France). At sowing, the plot size was  $7 \times 1.3 \text{ m}^2$  and  $7.5 \times 1.5 \text{ m}^2$ , respectively. To avoid varietal mixture, the central part of the plots ( $5.5 \times 1.3 \text{ m}^2$  and  $5 \times 1.5 \text{ m}^2$ , respectively) was harvested. Usual farming methods for those areas were used which included applying appropriate fertilizers and pesticides. More precisely, fungicides were applied at heading and end of flowering at CF and during development and heading at EM. Herbicides were applied at pre-emergence and during development at both locations. Based on the remaining total nitrogen in the field at the end of winter, an application of 50 (on 04/14/2017) and three applications of 40 (on 03/20/2017, 03/27/2017 and 05/04/2017) nitrogen units per hectare were applied at EM and CF, respectively.

### 3.2. Phenotypic Characterisation

Analysis of wheat grains, determination of protein composition and HMW glutenin alleles, characterisation of flour polymers and measurements of dough technological properties were performed for all varieties.

#### 3.2.1. Analysis of Wheat Grains

Thousand-kernel weight was measured with an Opto-Agri (Optomachine, Riom, France). Grain hardness was measured with Near InfraRed Spectroscopy (NIRS) and the grain nitrogen content was determined using an elemental analyser as described below.

#### 3.2.2. Quantification of Total Protein

After grinding grains using a Falaise ball mill (Falaise, France), aliquots of 5 mg of wholemeal flour were weighed in tin capsules. The total nitrogen in the sample was determined by the Dumas combustion method using a gas chromatography Thermo Flash EA 1112 Series elemental analyser (Thermo Finnigan, USA). Calibration was done using standards ranging from 5 to 250 µg of nitrogen following the manufacturer's recommendations. Total nitrogen content of flour is converted into protein content using the 5.7 Jones factor [2].

#### 3.2.3. Determination of Protein Composition by High-Performance Liquid Chromatography (RP-HPLC)

Protein composition was determined after sequential extraction according to protocols available at the URL provided in the specifications table, section Data Accessibility, and as described in Ref. [3] with modifications. Briefly, the non-prolamin fraction was extracted from 66.6 mg of flour with 50 mM phosphate buffer (pH 7.8) containing 100 mM NaCl. After centrifugation (10 min, 18,000 × g, 4 °C), the pellet was used to extract gliadins with 70% ethanol. Finally, glutenins were extracted at 50 °C from the resulting pellet using a 24 mM borate buffer (pH 9.8) containing 50% propanol-2 and 1% DTT.

Gliadins and glutenins were quantified by reverse phase high performance liquid chromatography (RP-HPLC) as described in Ref. [4].

#### 3.2.4. Determination of Glutenin Alleles by SDS-PAGE

For each accession, proteins were extracted from 10 mg of wholemeal flour. HMW-GS were fractionated in vertical slabs using SDS-PAGE with a modified protocol based on that of Singh et al. [5]. HMW-GS were identified according to the numbering system developed by Payne and Lawrence [6] with the names available at <https://shigen.nig.ac.jp/wheat/komugi/>.

#### 3.2.5. Determination of the Molecular Distribution of Wheat Flour Proteins by Asymmetrical Flow Field-Flow Fractionation (AF4)

Wheat protein molecular weight distribution was performed as reported previously with some modifications [7,8]. About 30 mg of wholemeal flour were dispersed in sodium phosphate buffer (1 mL, pH 6.9, 50 mM) containing SDS (2%, w/v) and incubated for 2 h at 60 °C with vortexing every 10 min. Samples were then sonicated (20 s at 30% of maximum power) with a 3 mm microtip probe before being centrifuged (12,500 × g) for 15 min at 25 °C, then filtered through a syringe filter of regenerated cellulose (0.45 µm porosity) and then injected (30 µL) into the AF4/MALLS system. The AF4 machine used was an Eclipse3 F System (Wyatt Technology, Santa Barbara, CA, USA) combined with a multi-angle light scattering (MALS) detector (Dawn® multi-angle Heleos TM, Wyatt Technology, Santa Barbara, CA, USA) and an Opti-lab® T-rEX™ refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). In addition, the Agilent HPLC 1200 Series (Agilent Technologies, Germany) was used in tandem with the AF4 system. The trapezoidal channel was 286 mm in length and the spacer used was 350 µm. The ultrafiltration wall consisted of regenerated cellulose membrane (10 kDa cutoff). The mobile phase consisting of the sodium phosphate buffer solution (pH 6.9, 50 mM) and SDS (0.1%, w/v) passed continuously through a 0.1 µm regenerated cellulose filter (Gelman Sciences, France). Absorbance was recorded at 214 nm. The cross-flow used for fractionation was focused at 0.5 min

at a constant flow rate of 2 mL/min. The injection step was done over 1.0 min at a flow rate of 0.2 mL/min and a relaxation phase of 0.5 min. These steps were followed by elution at flow detector rate of 1.0 mL/min and cross-flow rate decreasing from 3.0 to 0.0 mL/min. After 14 min of elution, the cross-flow rate was stopped for 9 min. Calculation of the number-average ( $M_n$ ), the weight-average ( $M_w$ ) molar mass, the radii of gyration ( $R_z$ ) and hydrodynamic radii of gyration ( $R_h$ ) was performed using ASTRA 7.1.2 software.

### 3.2.6. Description of Dough Technological Properties

T55 flours were milled from each sample with the Laboratory Mill 3100 (Perten Instruments, Springfield, USA). These flours were used to measure (i) the dough technological properties with a Chopin Alveograph (Chopin Technologies, France) according to the norm NF EN-ISO 27971 and (ii) the gluten rheological properties using the Glutomatic 2200 (Perten Instruments, Springfield, USA) system after manual extraction of the gluten by lixiviation. The gluten index (GI) of wheat protein provides a simultaneous measurement of gluten quality and quantity (AACC, 2000). The GI value expresses the percentage weight of wet gluten remaining on a sieve after automatic washing with salt solution and centrifugation. It was determined according to NF EN ISO 21415-2-4-ICC 155.

### 3.3. Genotypic Characterisation

All varieties were genotyped with the Axiom TaBW35K array including 34,746 single nucleotide polymorphisms (SNPs). The fifth file lists the alleles for each accession except three (omitted due to poor DNA quality) for all markers. The TaBW35K array was designed in the scope of the BreedWheat Project (<https://breedwheat.fr/results/data-and-resources/?lang=en>) and consists in a representative subset of the larger TaBW280K genotyping array [9]. In the time of the BreedWheat project, markers were designed on the first *Triticum aestivum* draft sequence [10]. Once the IWGSC releases the *Triticum aestivum* Reference Sequence of *Chinese spring* [11], the TaBW35k SNPs data were anchored on the genomic sequences [9]. Not all the 35k SNP context sequences could be mapped on the new reference sequence. 18,117 markers only were kept in the VCF submitted in EVA with PRJEB56753 accession number. The full set of TaBW35k genotyping results are available in [entrepot.recherche.data.gouv.fr](http://entrepot.recherche.data.gouv.fr) portal with doi [10.15454/PIMPHZ](https://doi.org/10.15454/PIMPHZ).

### 3.4. Hierarchical Clustering

The means of the two replicates were calculated for all phenotypic data. Then the 75 cultivars grown at two locations were classified using hierarchical clustering based on all phenotypic data except yield and heading date. Data were scaled before calculating Manhattan distances. The Ward aggregation method was applied. The dendrogram (Fig. 3) was obtained using the dendextend package of R version 4.0.5 [12].

## Ethics Statement

The manuscript adheres to Ethics in publishing standards.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Towards wheat cultivars with a more digestible gluten (Original data) (European Variation Archive and Recherche Data Gouv).

## CRedit Author Statement

**Mélanie Lavoignat:** Formal analysis, Writing – original draft, Visualization; **Emmanuelle Bancel:** Conceptualization, Writing – review & editing, Supervision; **Hélène Rimbart:** Data curation, Writing – review & editing; **Sandrine Bagnon:** Investigation; **Michaël Benigna:** Investigation; **Alain Chassin:** Investigation; **Sandrine Berges:** Investigation; **Annie Faye:** Investigation, Data curation; **Emmanuel Heumez:** Investigation; **Sibille Perrochon:** Investigation; **Larbi Rhazi:** Investigation, Writing – original draft; **Bernard Valluis:** Investigation, Conceptualization; **Flavie Souply:** Investigation; **Marie Cécile Leroux:** Investigation; **Pascal Giraudeau:** Resources, Conceptualization; **Catherine Ravel:** Conceptualization, Writing – review & editing, Supervision.

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