Quantitative tools in microbial and chemical risk assessment

Deyan Stratev[1] and Vasilis P Valdramidis[2]

[1]Department of Food Quality and Safety and Veterinary Legislation, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
[2]Department of Food Chemistry, National and Kapodistrian University of Athens, Greece

Abstract

The EU-FORA programme 'Quantitative tools in microbial and chemical risk assessment' was dedicated to training on predictive microbiology fundamentals, implementation of different modelling strategies, design of experiments and software tools such as MATLAB, GInaFiT and DMFit. The fellow performed MATLAB training on maximum specific growth rate (μ_{max}) determination according to the Ratkowsky model. GInaFiT training on different models for bacterial inactivation and DMFit training on growth parameters of *Vibrio parahaemolyticus* were also carried out. Optical density measurements of *V. parahaemolyticus* bacterial cultures were performed. The obtained kinetics of optical density measurements were used to estimate μ_{max}. Hereafter, Minimum inhibitory concentrations and non-inhibitory concentrations of aminoglycoside antibiotics were estimated based on the quantification of the fractional areas of the optical density vs time. It can be concluded that the results of the quantitative characterisation of *V. parahaemolyticus* are reliable and can be used for exposure assessments. Also, the turbidimetric assay can be applied for successful estimation of minimum inhibitory concentrations and non-inhibitory concentrations.

Correspondence: eu-fora@efsa.europa.eu

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Summary

The final report of the EU-FORA fellowship programme 'Quantitative tools in microbial and chemical risk assessment' hosted by the National and Kapodistrian University of Athens, and supervised by Prof. Vasilis P. Valdramidis and Prof. Charalampos Proestos is dedicated to summarising the activities of the fellow Deyan Stratev during programme implementation. The fellow is currently working as an associate professor in food safety and his scientific background is related to seafood microbiology. The introduction describes an outline of the working programme and its aims which included training in predictive microbiology fundamentals, the implementation of different modelling strategies, the design of experiments, training with the use of three different software tools (MATLAB, GInaFiT and DMFit) selected according to fellow's research topic and provided with an overall understanding of the main differences between modelling growth or inactivation models. Conclusions provide the main achievements as a result of EU-FORA programme training while some recommendations are also suggested.

Table of contents

1. Introduction

1.1. Description of the work programme

The EU-FORA training programme 'Quantitative tools in microbial and chemical risk assessment' was designed to train fellow Deyan Stratev in the use and development of predictive models for assessing the bacterial responses for survival, growth and the probability of microbial recovery. The assessments included microbial responses under different food processing and preservation scenarios. Applications were performed by programming in MATLAB while analysis and interpretation of data were considered as inputs in microbial and chemical risk assessment studies. By the end of the activity, the trainee attained a fundamental understanding of the substantial body of applied modelling, statistics and recent developments in the field of predictive modelling and quantitative risk assessment of foods in the area of microbiology and chemistry. The trainee has also exercised personal responsibility and autonomous initiative in solving complex food safety, spoilage and chemical problems that are solved in a rigorous and sound approach. He has exploited available software packages and quantitative approaches for enriching current studies in the field in order to communicate results and innovations of research to peers.

1.2. Aims

The overall aim of the EU-FORA training programme 'Quantitative tools in microbial and chemical risk assessment' was to:

i) develop trainee's capacity to design and generate informative experimental data;
ii) understand model structure development and selection to describe quantitatively chemical, and microbiological phenomena and develop capabilities for quantifying accurately the sources of stochasticity;
iii) make trainee familiar with optimisation software and model simulation tools that can be exploited for developing decision-making and quantitative risk assessment in food microbiology and chemistry.

1.3. Additional activities for network widening

The fellow participated in various events to improve his multidisciplinary work and research activities. He also participated in several secondary activities to meet these goals during the EU-FORA programme, which are described in Appendix A.

2. Training

2.1. Training on software

There are two possible ways to organise data processing in scientific research. One of them is MATLAB software, which has a pre-made code and can be tailored to the needs of the project. Another option is to use Excel and possibly some additional features such as GInaFiT or DMFit. These give better support if users are very familiar with the predicative microbiology topic.

2.1.1. MATLAB

MATLAB is a versatile tool used in numerous scientific fields, e.g. engineering, data analysis, algorithm development, etc. With it, one can analyse data, visualise it and create scripts. When used with a pre-written code, MATLAB can simulate different scenarios in real cases. An example of MATLAB implementation was how to determine the maximum specific growth rate (μ_{max}) according to the Ratkowsky model (Figure 1).
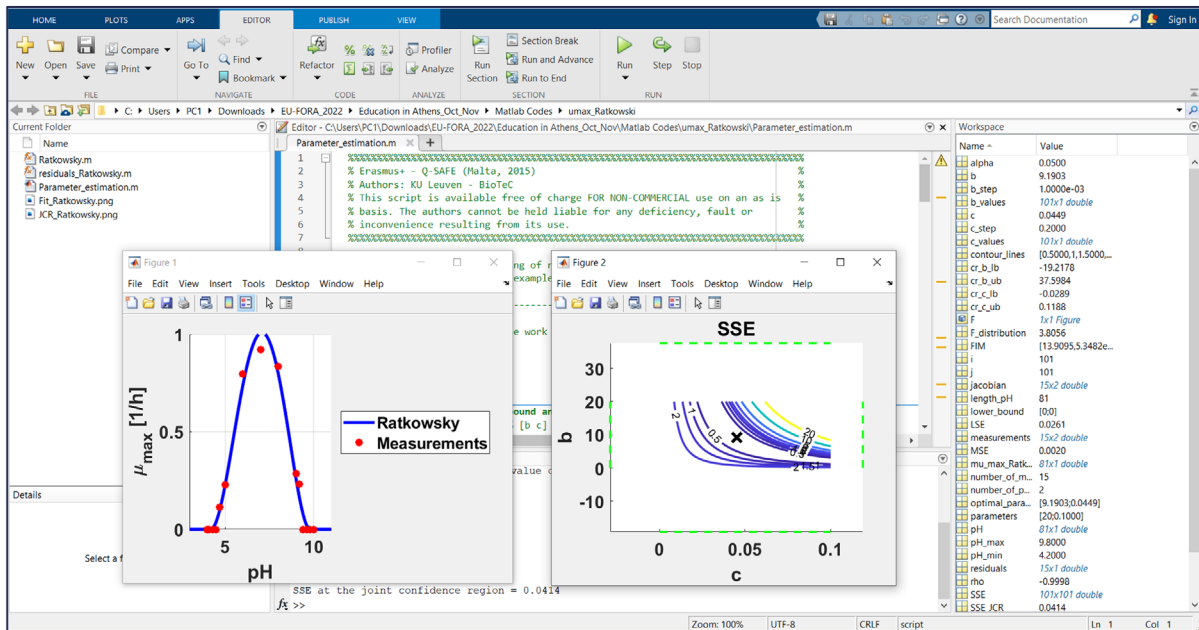
Figure 1: MATLAB training example on maximum specific growth rate (μ_{max}) determination according to the Ratkowsky model by the use of Q-Safe book (Valdramidis et al., 2017)

2.1.2.   Microsoft® Excel add-ins – GInaFiT

Excel can help with many calculations and GInaFiT can help to simplify some of the calculations and even prevent typical mistakes in data processing (Figure 2). The tool is efficient in testing 10 types of microbial survival models relating to the growth of the microbial population with time (Geeraerd et al., 2005). These are:

1) classical log-linear curves,
2) curves displaying a shoulder before a log-linear decrease,
3) curves displaying a tail after a log-linear decrease,
4) survival curves displaying both shoulder and tail,
5) concave curves,
6) convex curves,
7) convex/concave curves followed by tailing,
8) biphasic inactivation kinetics,
9) biphasic inactivation kinetics preceded by a shoulder,
10) curves with a double concave/convex shape.

The models were originally published by Bigelow and Esty (1920), Cerf (1977), Geeraerd et al. (2000, 2005), Mafart et al. (2002), Albert and Mafart (2005), and Coroller et al. (2006). Along with the obtained parameter values, some statistical measures are automatically given: standard errors of the parameter values, the sum of squared errors, the (root) mean sum of squared errors, the R2 and the adjusted R2. In addition, t4D, the time needed for a 4 log reduction of the initial microbial population, as originally proposed by Buchanan et al. (1993), is also automatically reported (for data sets covering at least 4 decimal reductions).
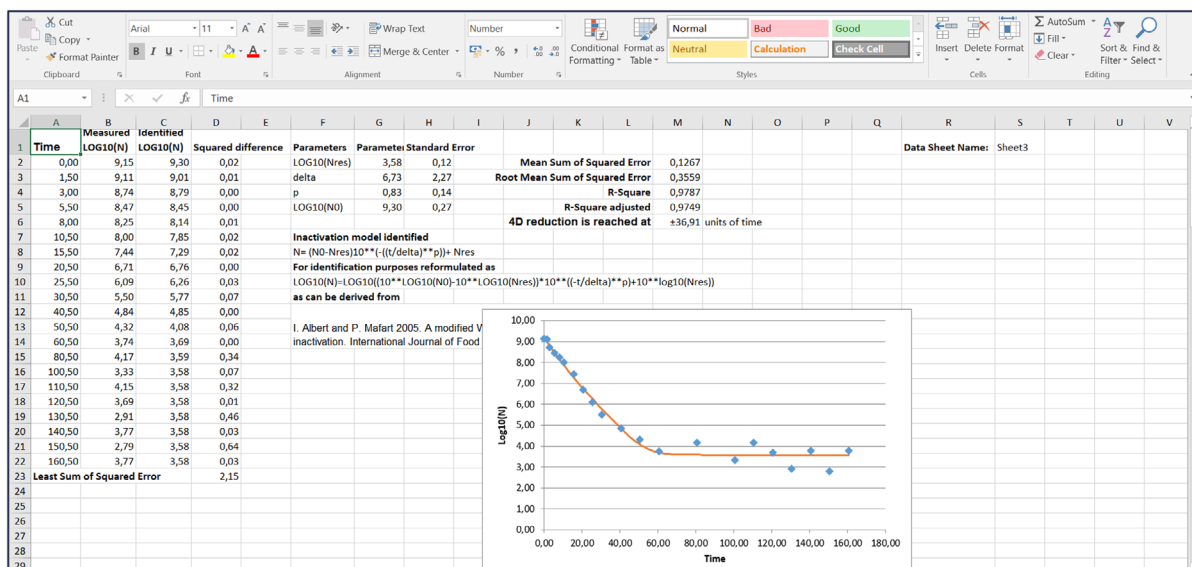
Figure 2: GInaFiT example on a modified Weibull model for bacterial inactivation

There are two approaches in using the tool depending on whether users have or do not have an idea of the general shape of their survival curves. The time for a 4-decimal reduction can be useful to summarise the information present in a data set, for example if a common survivor curve shape cannot be selected for a range of different conditions tested (Geeraerd et al., 2000).

The tool is also equipped with some built-in features testing for misuse, e.g. when the number of data points (observations) used is too limited in comparison with the number of parameters in the model type chosen (the number of parameters ranges from 2 to 5 for the 10 model types available).

2.1.3. Microsoft® Excel add-ins – DMFit

DMFit is a tool that can be found online at https://browser.combase.cc/DMFit.aspx or added as a Microsoft® Excel add-in. The web edition is a web-based application compliant with bacterial curves. In it, a linear phase is preceded and followed by a stationary phase. The desktop version is used to model the time variation of the logarithm of cell concentrations of bacterial batch cultures. DMFit can be used to present in a visual mode growth or survival data, obtain parameters estimates from data consistent with growth or survival models, calculate growth or death rate, lag phase time, initial or final cell count and estimate standard errors on these parameters (Figure 3).

Data input is fairly easy since it is compatible with Excel spreadsheets or text files (in online and desktop versions). Data can be inserted in two different types of models:

a) Model of Baranyi and Roberts (1994) – describes a sigmoid bacterial curve. This model, unlike other sigmoid curves, has very close to linear mid-phase. It has four main parameters (initial value, lag, maximum rate and final value) and two curvature parameters: mCurv and nCurv which describe the curvature of the sigmoid curve, respectively, at the beginning and at the end of the growth phase. This tool allows to describe only the growth or death at the stationary phase, if mCurv parameter is set to zero and 'no lag' model option is selected. The model can also describe only the lag phase and growth or death phase if nCurv is set to zero and 'no asymtot' model option is selected.

b) Trilinear, biphasic or linear models. The trilinear model describes a bacterial growth curve with three straight lines: the lag phase and the stationary phase are presented by two horizontal straight lines. The slope of the third straight line describing the growth/death phase is called the 'maximum rate'. The biphasic model must be used when a data-based curve has no lag phase or no stationary phase. The linear model can be used in case bacterial counts describe only growth or death phase.

In the tool time is given in hours, bacteria count in $\log_{10}$ cfu/g and maximum growth or death rate is given as $\log_{10}$ cfu/g/h. After loading data, it must be ensured that units are aligned accordingly.
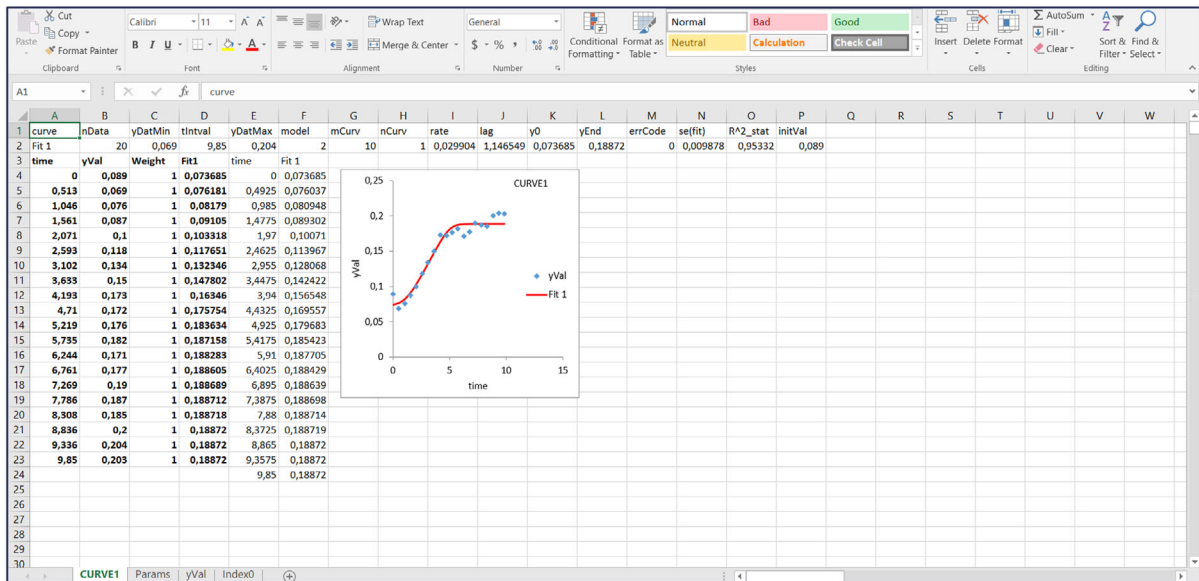
Figure 3: DMFit training example on growth parameters of *V. parahaemolyticus*

3. Case study

3.1. Data collection

OD measurements of *Vibrio parahaemolyticus* bacterial cultures were performed by means of a microplate reader at 630 nm. The obtained kinetics of OD measurements were used to estimate the growth rate of the cells, i.e. μ_{max}. Hereafter, MIC and NIC of aminoglycoside antibiotics (i.e. gentamicin and tobramycin) for the slowest and highest growers were estimated based on the quantification of the fractional areas of the optical density versus time.

3.2. Quantitative evaluations

The μ_{max} (h^{-1}) of *V. parahaemolyticus* isolated from Mediterranean mussels, veined rapa whelk, Mediterranean horse mackerel, oysters, Gilthead bream, Atlantic salmon, bluefish, European seabass ranged from 0.73 to 2.33. The highest grower was isolated from Atlantic salmon while the slowest one was recovered from Mediterranean mussels (Figure 4).
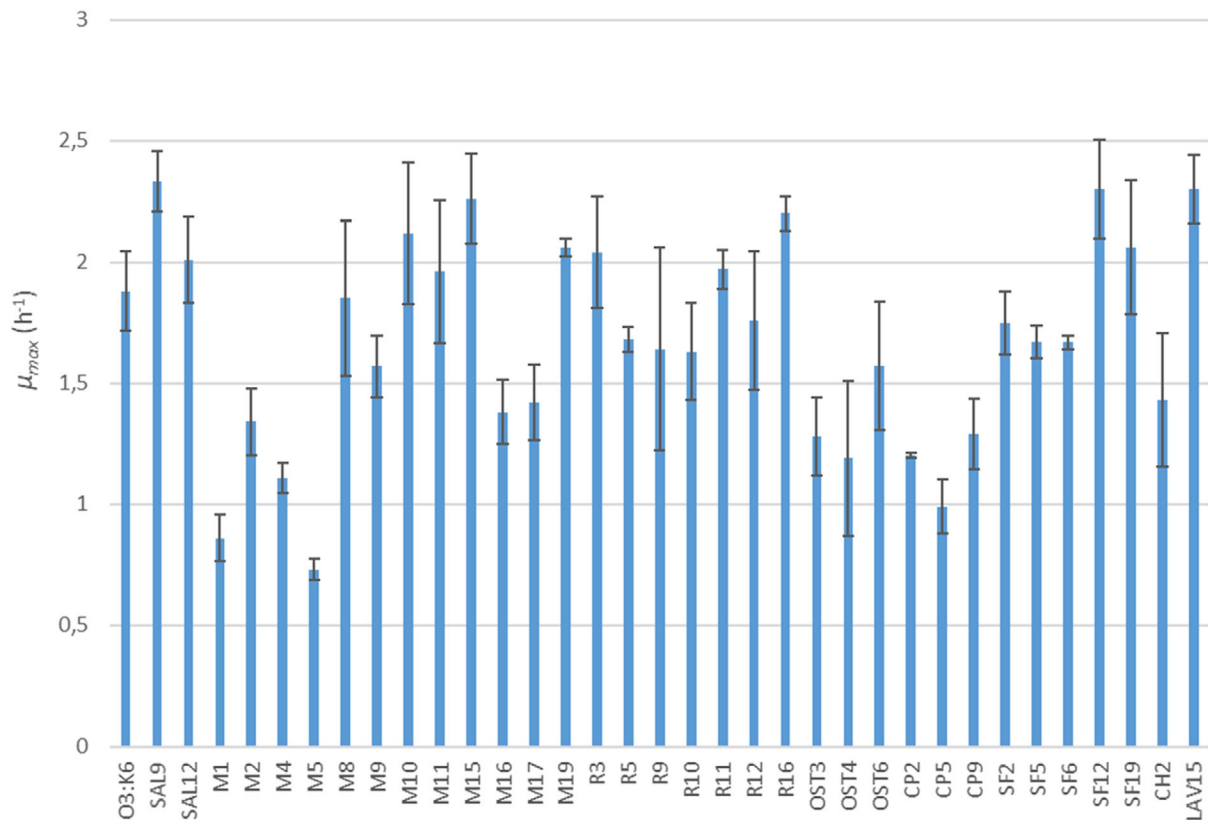
Figure 4: Growth variability of *Vibrio parahaemolyticus* strains isolated from seafood

MIC and NIC of the slowest grower of *V. parahaemolyticus* for gentamicin were found to be below 35 mg/mL, while for tobramycin they were found to be below 0.10 mg/mL. The highest grower showed a lower value of MIC for gentamicin, but a higher value for tobramycin. Also, the highest grower showed lower values of NIC for both gentamicin and tobramycin.

4. Conclusion

It can be concluded that all planned activities of the EU-FORA programme were carried out successfully. The hosting site provided the required material basis and full implementation of the fellow to the current work of the Department of Food Chemistry. The fellow developed skills to design and generate experimental data and apply software tools related to quantitative risk assessment and food microbiology. The hosting and sending organisations have already developed a collaboration and they are going to continue future work by staff exchange and training. Moreover, the methods for determination of maximum specific growth rate (μ_{max}) and MIC and NIC for aminoglycoside antibiotics were fully implemented in the microbiological laboratory of the sending organisation. It is worth mentioning that the staff of this sending laboratory has already applied these methods that the fellow transferred to them. The completed case study provided useful information for the quantitative characterisation of *V. parahaemolyticus* growth, which can be a main input for microbial exposure assessments as part of risk analysis of food-borne pathogens. It was demonstrated that the turbidimetric assay was useful for the successful estimation of MIC and NIC for antibiotics. This method can be applied in order to obtain reliable results for MIC and NIC of *V. parahaemolyticus* and address current needs for identifying the antibiotic resistance of food-borne pathogens.

5. Recommendations

EU-FORA is an already recognised programme in training fellows in food risk assessment based on its 6 years of experience. It would be excellent if the programme continues training fellows and increases the pool of experts in food risk assessment.

References

Albert I and Mafart P, 2005. A modified Weibull model for bacterial inactivation. International Journal of Food Microbiology, 100, 197–211.

Baranyi J and Roberts TA, 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, 23, 277–294.

Bigelow WD and Esty JR, 1920. The thermal death point in relation to typical thermophylic organisms. Journal of Infectious Diseases, 27, 602–617.

Buchanan RL, Golden MH and Whiting RC, 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of Listeria monocytogenes inactivation, Journal of Food Protection, 56, 474–478, 484.

Cerf O, 1977. A review. Tailing of survival curves of bacterial spores. Journal of Applied Microbiology, 42, 1–19.

Coroller L, Leguerinel I, Mettler E, Savy N and Mafart P, 2006. General model, based on two mixed Weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. Applied and Environmental Microbiology, 72, 6493–6502.

Geeraerd AH, Herremans CH and Van Impe JF, 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. International Journal of Food Microbiology, 59, 185–209.

Geeraerd AH, Valdramidis VP and Van Impe JF, 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. International Journal of Food Microbiology, 102, 95–105.

Mafart P, Couvert O, Gaillard S and Leguerinel I, 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. International Journal of Food Microbiology, 72, 107–113.

Valdramidis VP, Cummins EJ and Van Impe JF, 2017. Textbook on Quantitative Tools for Sustainable Food and Energy in the Food Chain, 1st Edition. 331 pp. ISBN 978-90-77381-960.

Abbreviations

EU-FORA	The European Food Risk Assessment Fellowship Programme
MIC	minimum inhibitory concentrations
NIC	non-inhibitory concentrations
OD	optical density

Appendix A – Secondary activities

1) Lecture sessions provided by the hosting site

- Lecture – 'A History of Predictive Microbiology'
- Lecture – 'Predictive Microbiology Fundamentals'
- Lecture – 'Predictive Microbiology Fundamentals'
- Lecture – 'Experimental Design 1'
- Lecture – 'Kinetic Models'
- Lecture – 'Experimental Design 2'
- Lecture – 'QMRA Model - Scenario Analysis'
- Lecture – 'Parameter Estimation'
- Lecture – 'Applied Modelling'
- Lecture – 'Non-Linear Regression in Excel'
- Lecture – 'Predicting Microbial Behaviour during Food Storage in Risk Assessment'
- Lecture – 'Binomial Distribution'
- Lecture – 'Risk Assessment'
- Lecture – 'MATLAB - Getting Started'
- Lecture – 'MATLAB - Parameter Estimation'
- Lecture – 'MATLAB - Simulations'
- Lecture – 'Life Cycle Assessment'
- Lecture – 'Integrating Process Modelling approaches in Microbial Modelling'
- Lecture – 'Modelling Effect of Process and Formulation on Microbial Level in Food'
- Lecture – 'Thermal Inactivation: Application of Models'
- Lecture – 'Partitioning'
- Lecture – 'Implementation of Microbial Risk Assessment Model'
- Lecture – 'Building a Microbial Risk Assessment Model'
- Lecture – 'Basics of Multiscale Modelling in Predictive Microbiology'

2) Participation in IAFP's European Symposium on Food Safety with poster 'Growth variability of *Vibrio parahaemolyticus* strains isolated from seafood', Aberdeen, 3–5 May 2023.

3) 'FunShielf4Med' seminar 'Identification of Mycotoxins', Institute of Technology of Agricultural Products, Athens, 16 May 2023.

4) Visit at Laboratory of Food Quality Control and Hygiene of Agricultural University of Athens, 18 May 2023.

5) Participation in the 14th edition of the International Congress on Engineering and Food (ICEF14) with poster 'Quantitative assessments to characterise the antibiotic resistance of seafood microbial isolates', 19–23 June, Nantes, France.

6) Participation in working group meetings with PhD students of the National and Kapodistrian University of Athens and the research groups of Prof. Vasilis Valdramidis.