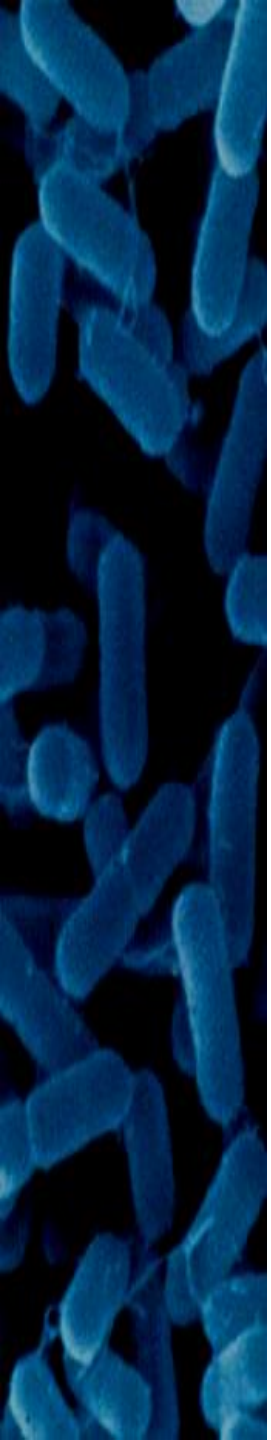

Predictive Microbiology (theory)

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efsa
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Summer School
"In Silico Methods for Food Safety"

Predictive Microbiology

THE CONCEPT

A detailed knowledge of microbial responses to environmental conditions, synthesized in a mathematical model, enables objective evaluation of processing, distribution and storage operations on the microbiological safety and quality of foods, by monitoring the environment without recourse to further microbiological analysis

Predictive Microbiology

THE PRINCIPLES

- Growth, survival and inactivation of microorganisms in foods are reproducible responses
- A limited number of environmental parameters in foods determine the kinetic responses of microorganisms (Temperature, Water activity/water phase salt, pH, Food preservatives
- A mathematical model that quantitatively describes the combined effect of the environmental parameters can be used to predict growth, survival or inactivation of a microorganism and thereby contribute important information about product safety and shelf-life

Roberts and Jarvis (1983)

Predictive Microbiology

APPLICATIONS

- Predict the effect of product characteristics and storage
- conditions on microbial responses (safety and shelf-life)
- Predict effect of changes in parameters (product development)
- HACCP plans – establish limits for CCP
- Food safety objectives – equivalence of processes
- Education – easy access to information
- **Quantitative microbiological risk assessment (QMRA)**
- (The concentration of microbial hazards in foods may increase or decrease substantially during processing and distribution)

Predictive Microbiology

TYPES OF MODELS

- **Primary models**: describing the microbial evolution (growth, inactivation, survival) as a function of time. Estimate kinetic parameters

- **Secondary models**: describing kinetic parameters as a function of influencing factors like pH, temperature, water activity, concentration of preservatives, …

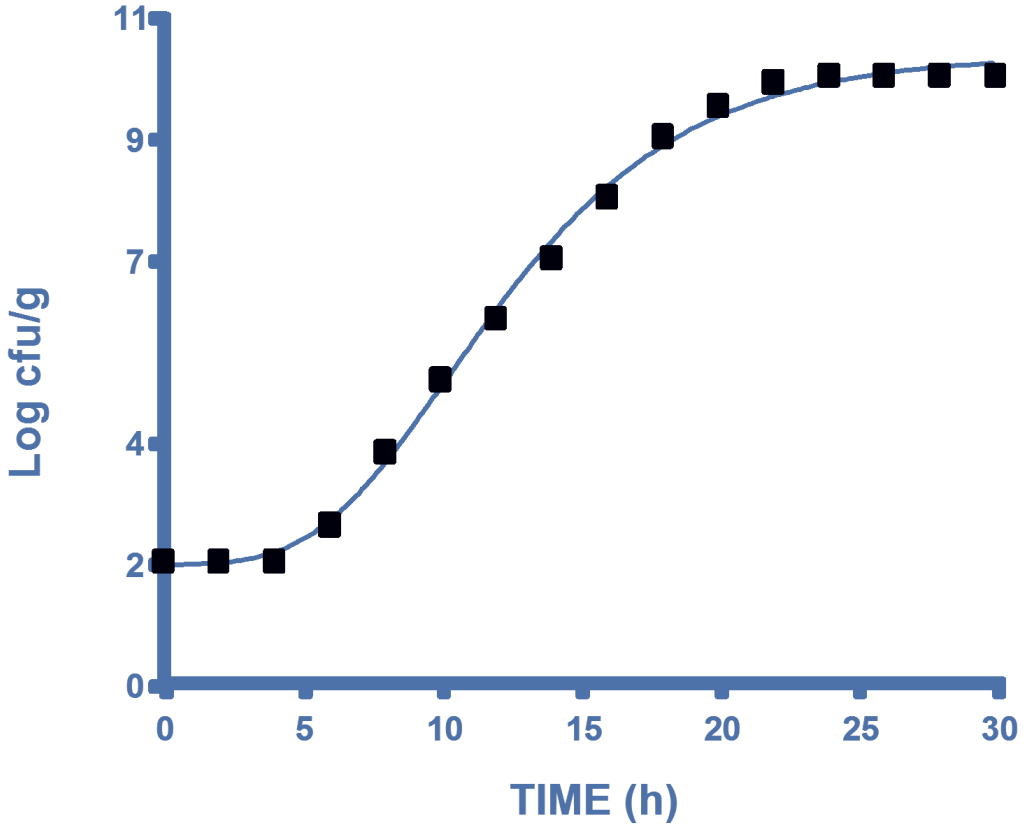
- **Tertiary models**: integrate primary and secondary models in a software tool

Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- Fitting data to primary model

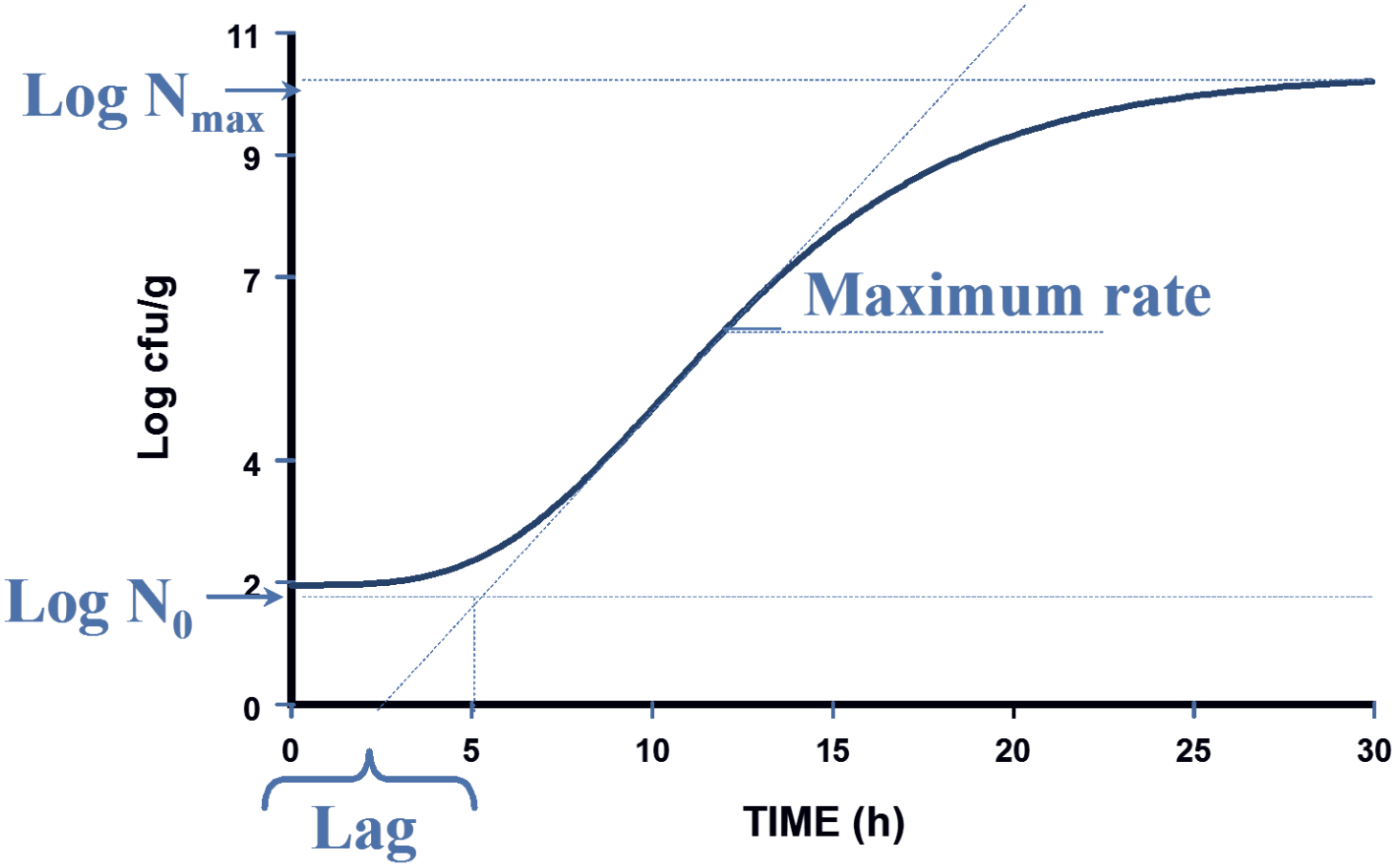
Translate growth curves to numbers



Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- Estimation of kinetic parameters



Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

➢ Primary models

Exponential model

$$Log(N_t) = Log[N_o \times \exp(\mu_{max} \times time)]$$

Logistic model without lag

$$Log(N_t) = Log\left(\frac{N_{max}}{1 + \left[\frac{N_{max}}{N_0} - 1\right] \times \exp(-\mu_{max} \times time)}\right)$$

Logistic model with lag

$$Log(N_t) = Log\left(N_{min} + \frac{N_{max} - N_{min}}{1 + \exp(-\mu_{max}(time - t_i))}\right)$$

Baranyi & Roberts (1994)

$$Log(N_t) = Log(N_0) + \frac{1}{\mu_{max}} \times \left[time + \frac{1}{\mu_{max}} \times Ln\left(\frac{\exp(-\mu_{max} \times time) + q_0}{1 + q_0}\right)\right] - \frac{1}{Log(10)} \times Ln\left(1 + \frac{\exp\left(\mu_{max} \times \left[time + \frac{1}{\mu_{max}} \times Ln\left(\frac{\exp(-\mu_{max} \times time) + q_0}{1 + q_0}\right)\right]\right) - 1}{\exp(Log(N_{max}) - Log(N_0))}\right)$$

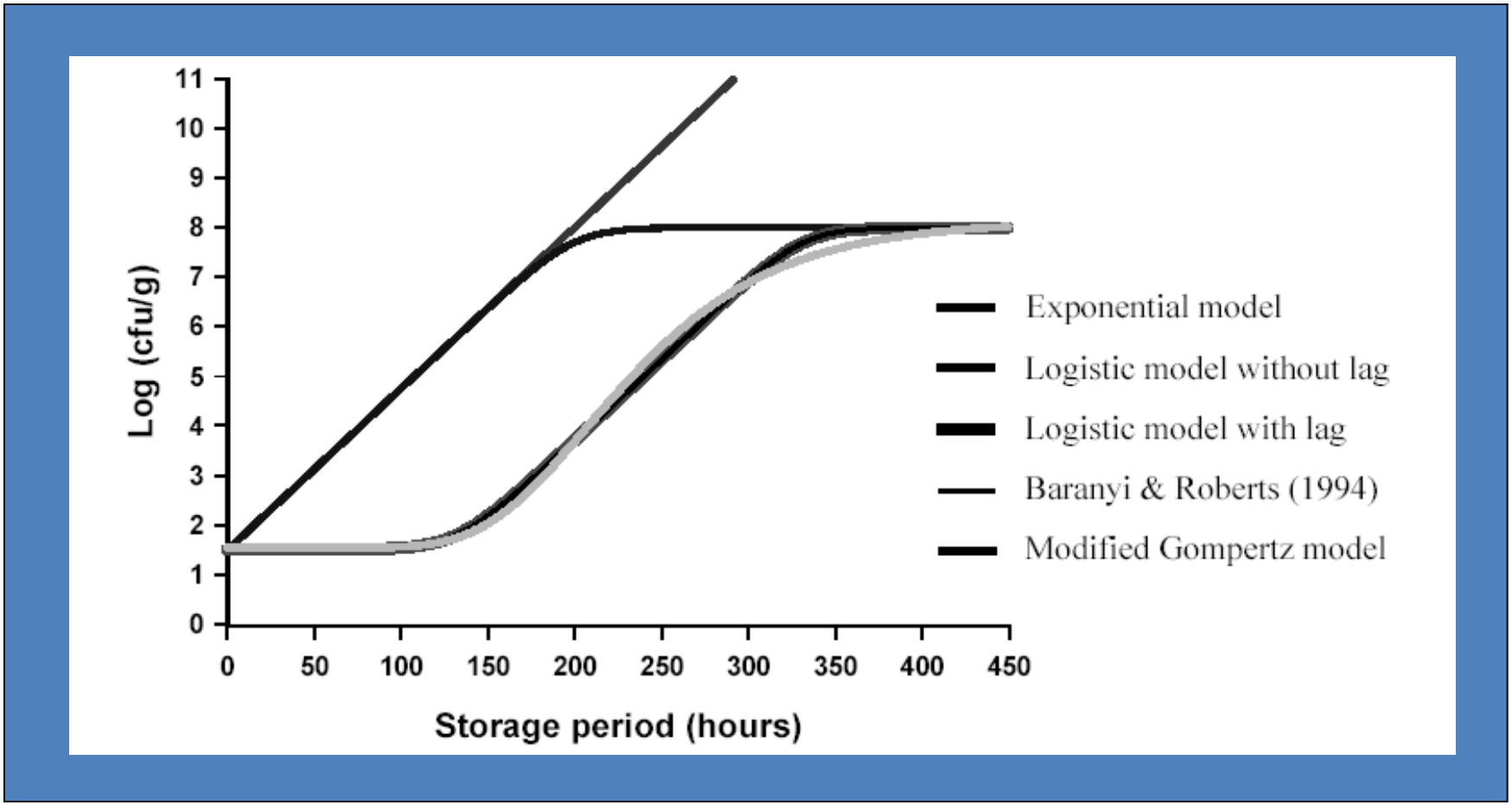
Modified Gompertz model

$$Log(N_t) = Log(N_0) + \left(A \times \exp\left(-\exp\left[\frac{\mu_{max} \times \exp(1)}{A} \times (lag - time) + 1\right]\right)\right) / Ln(10)$$

Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

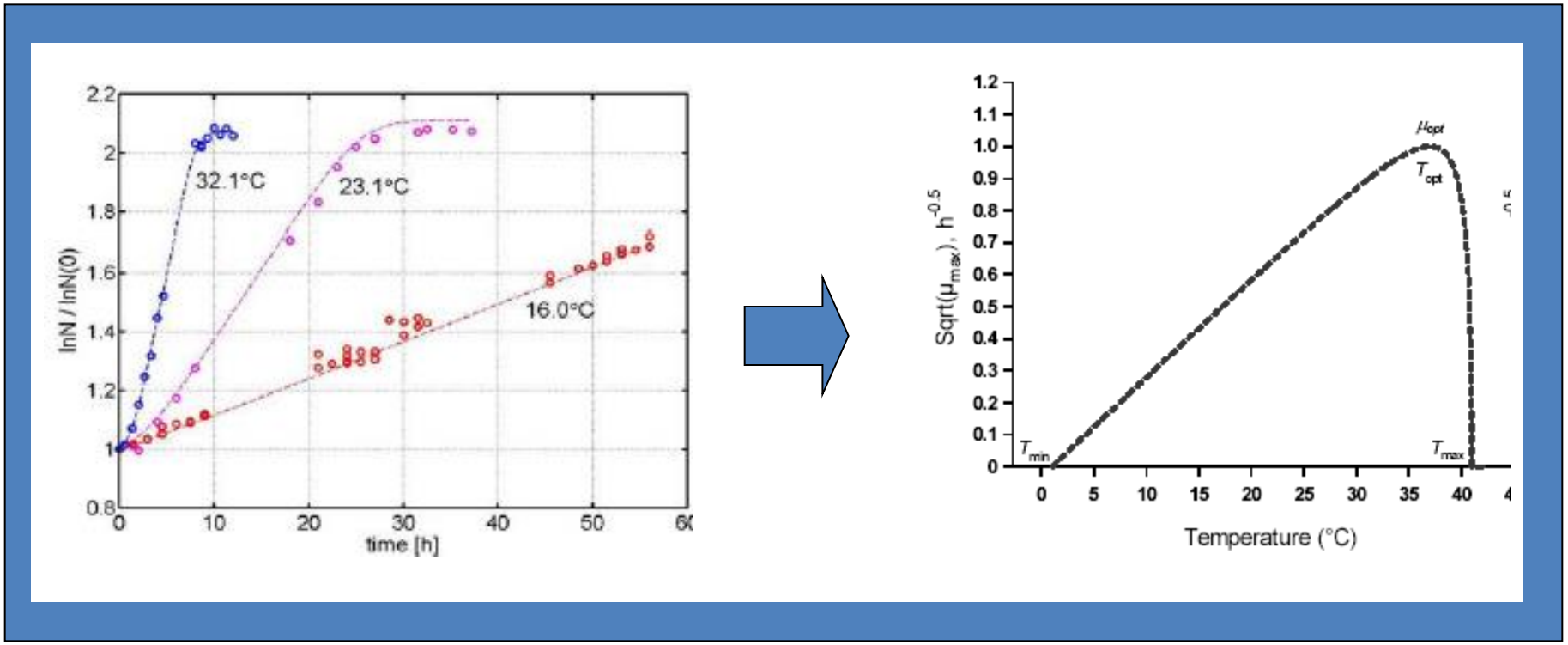
- **Primary models**



Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- **Mathematical description of the effect of the environmental factors to the kinetic parameters (secondary models)**

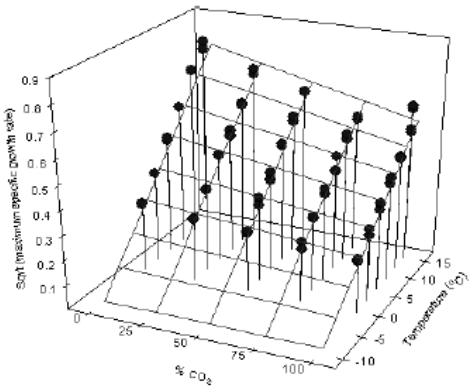


Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- Secondary models

- Kinetic models
  - Polynomial and constrained linear polynomial models
  - Square-root-type models
  - Arrhenius type models
  - Cardinal parameter models
  - Gamma concept models
  - Artificial neural networks
- Growth/no growth interface models (probabilistic models)

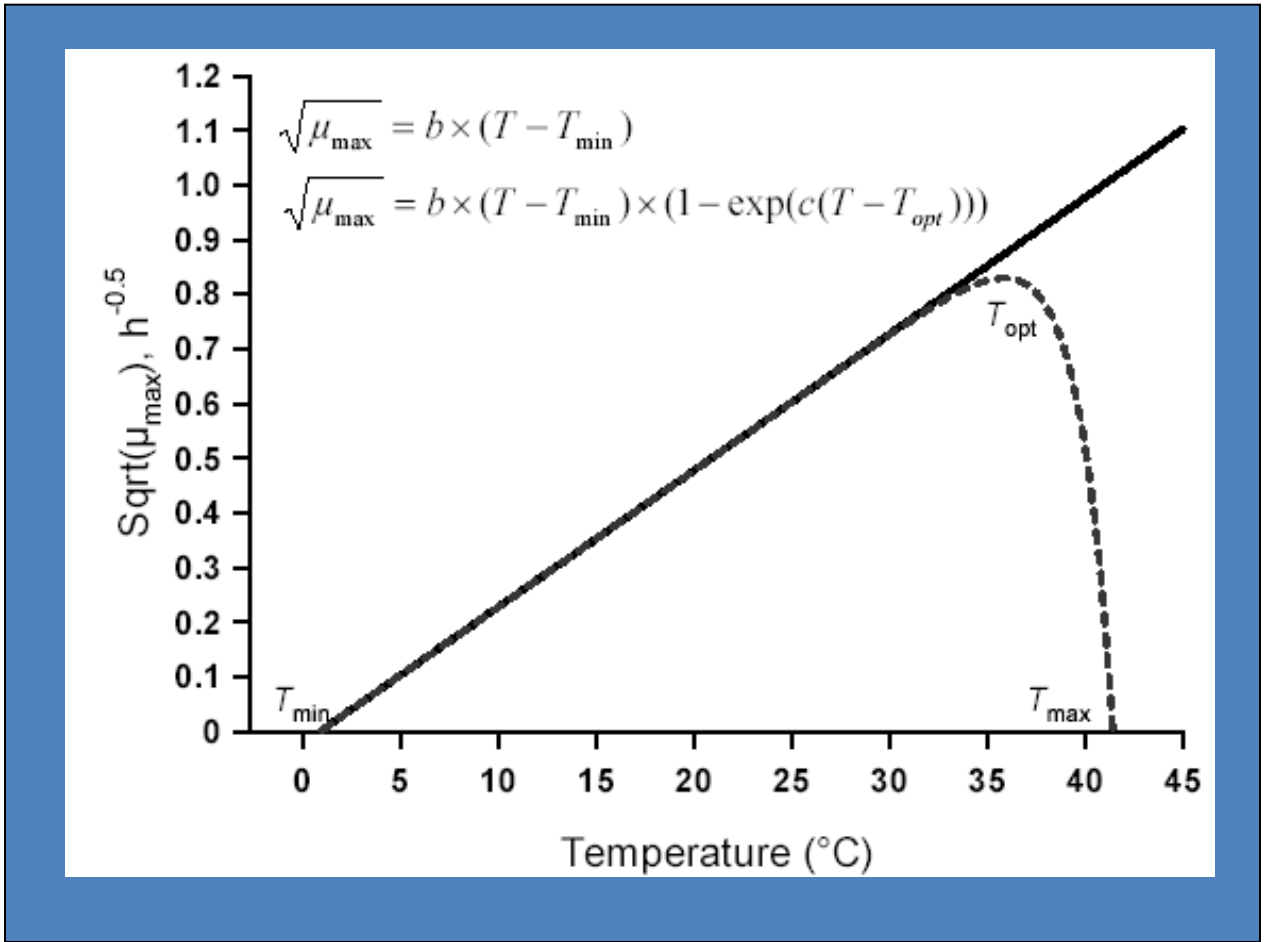


Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- **Secondary models**

Square root type model



Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- Secondary models

Square root type model

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot \sqrt{a_w - a_{w\,min}}$$

$$\sqrt{\mu_{max}} = b(T - T_{min}) \times \frac{(\%CO_{2\,max} - \%CO_2)}{\%CO_{2\,max}}$$

$$\mu = b \cdot (a_w - a_{w\,min}) \cdot (pH - pH_{min}) \cdot (pH - pH_{max}) \cdot (T - T_{min})^2$$

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot (1 - \exp(c(T - T_{max}))) \cdot$$

$$\sqrt{(a_w - a_{w\,min})(1 - \exp(d(a_w - a_{w\,max})))}$$

Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- **Secondary models**

Gamma concept models

many factors that affect microbial growth rate act independently, effect of each can be represented by a discrete term that is multiplied by terms for the effect of all other growth rate affecting factors

$$\mu = f(\text{temperature}) \times f(a_w) \times f(\text{pH}) \times f(\text{organic acid}) \times f(\text{other}_1) \times f(\text{other}_2) \times \ldots . f(\text{other}_n)$$

the effect on growth rate of any factor can be expressed as a fraction of the maximum growth rate (i.e., the rate when that environmental factor is at the optimum level)

$$\gamma = \frac{\text{Growth rate at actual environmental conditions}}{\text{Growth rate at optimal environmental conditions}}$$

Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- **Secondary models**

Gamma concept models

$$\mu_{max} = \mu_{max\ opt} \cdot \gamma(T) \cdot \gamma(a_w) \cdot \gamma(pH)$$

$$\gamma(T) = \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^2$$

$$\gamma(pH) = \frac{(pH - pH_{min}) \cdot ((pH_{max} - pH)}{(pH_{opt} - pH_{min}) \cdot (pH_{max} - pH_{opt})}$$

$$\gamma(a_w) = \frac{a_w - a_{w\,min}}{1 - a_{w\,min}}$$

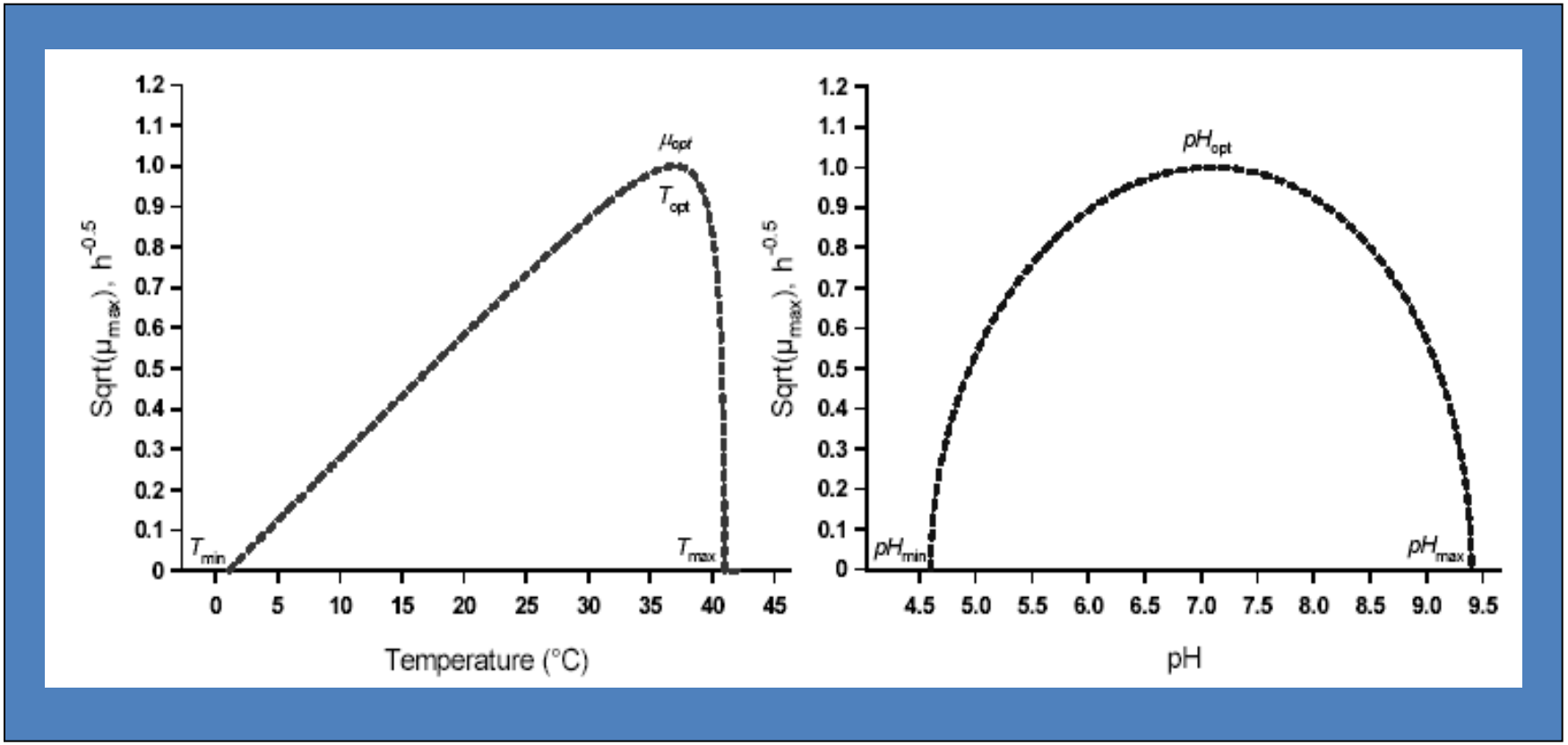
$$\gamma(CO_2) = \left(\frac{\%CO_{2\,max} - \%CO_2}{\%CO_{2\,max} - \%CO_{2\,opt}} \right)^2 = \left(\frac{\%CO_{2\,max} - \%CO_2}{\%CO_{2\,max}} \right)^2$$

Predictive Microbiology

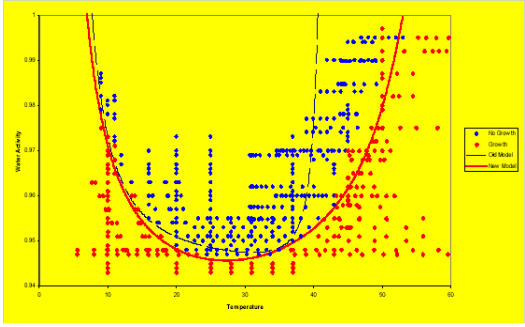
STEPS IN MODEL DEVELOPMENT

- **Secondary models**

Cardinal parameter model



Predictive Microbiology



STEPS IN MODEL DEVELOPMENT

- Growth no growth boundary models

Probability models for the ability of growth

The dependent variable is discrete (1 for growth and 0 for no growth)

Use of logistic regression with logitP transformation of the response variable

$$\text{logit } P = \log(P/(1-P))$$

$$\text{logit } P = Y$$

where P is the probability of the outcome of interest.
logit P is described as some function Y of the explanatory variables, i.e.:

$$e^{Y}/(1+e^{Y}) = P$$

Predictive Microbiology

STEPS IN MODEL DEVELOPMENT

- **Validation of models**

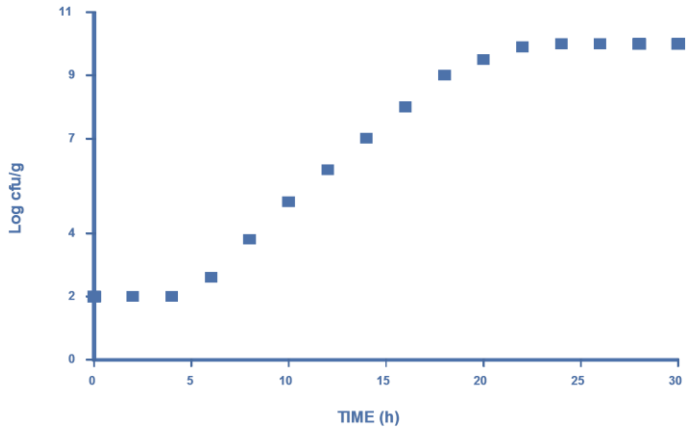
Most models are developed in laboratory media. There can be no guarantee that predicted values will match those that would occur in any specific food system. Before the models could be used in such a manner, the user would have to validate the models for each specific food of interest.

Internal validation: Comparison between predicted and observed values for data used for model development
External validation: Comparison between predicted and observed values for independent data

Predictive Microbiology

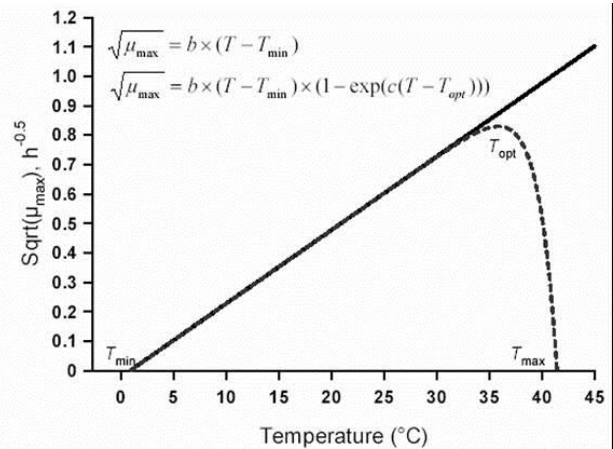
STEPS IN MODEL DEVELOPMENT

➢ **Prediction of microbial growth**



Primary model

Secondary model



Calculation of kinetic parameters for the environment of interest

Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Dynamic modeling of *Listeria monocytogenes* growth in pasteurized milk

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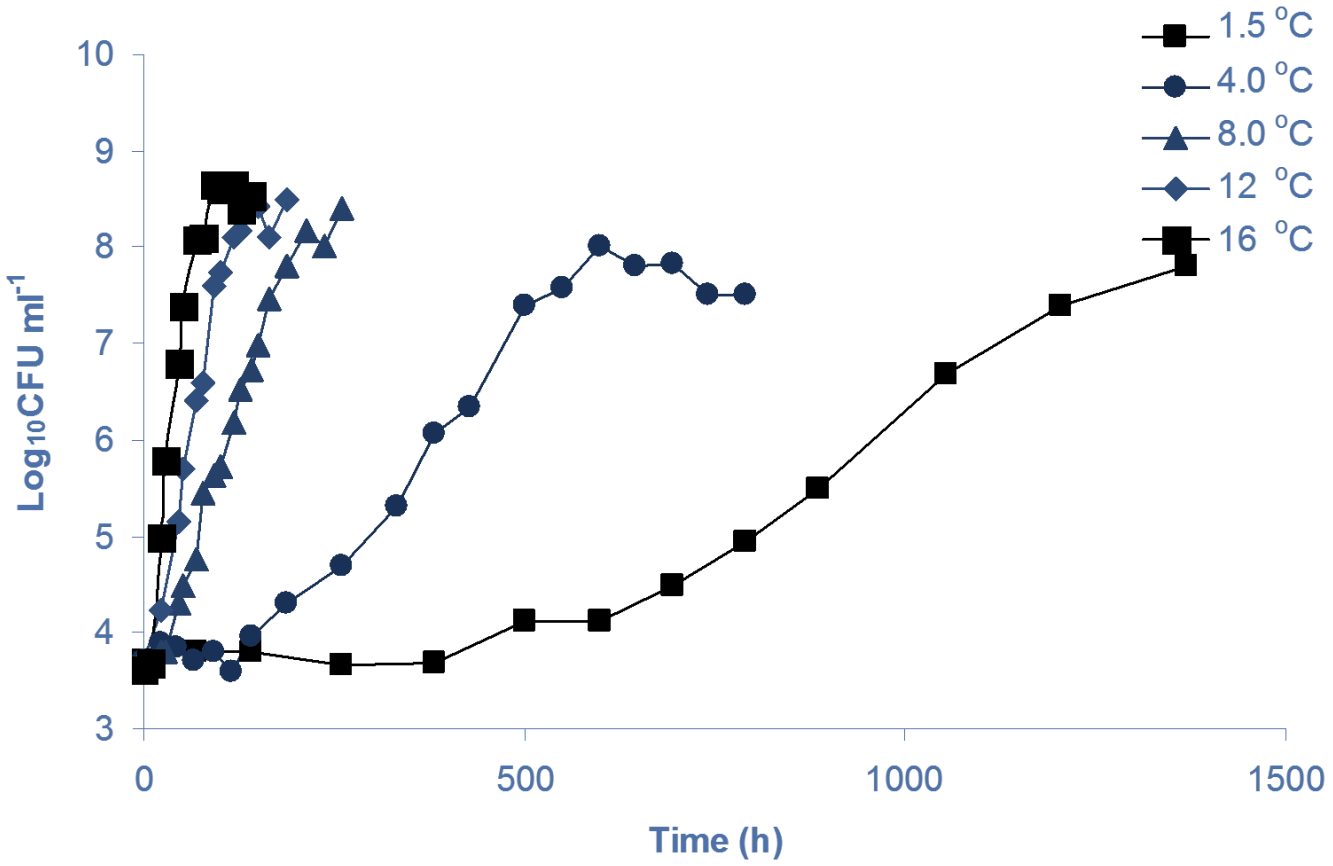
2 Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece

Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 1. Data collection

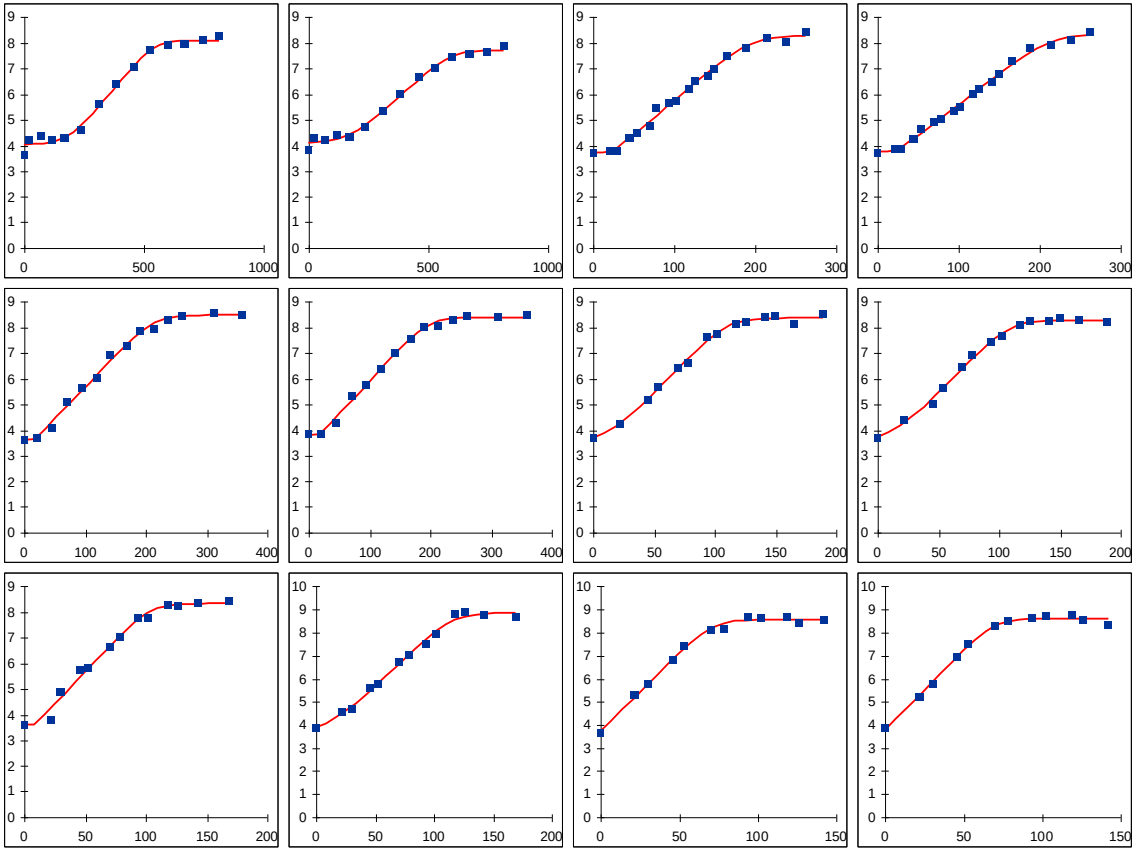


Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 2. Fitting growth data to a primary model



Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 2. Estimation of kinetic paramaters

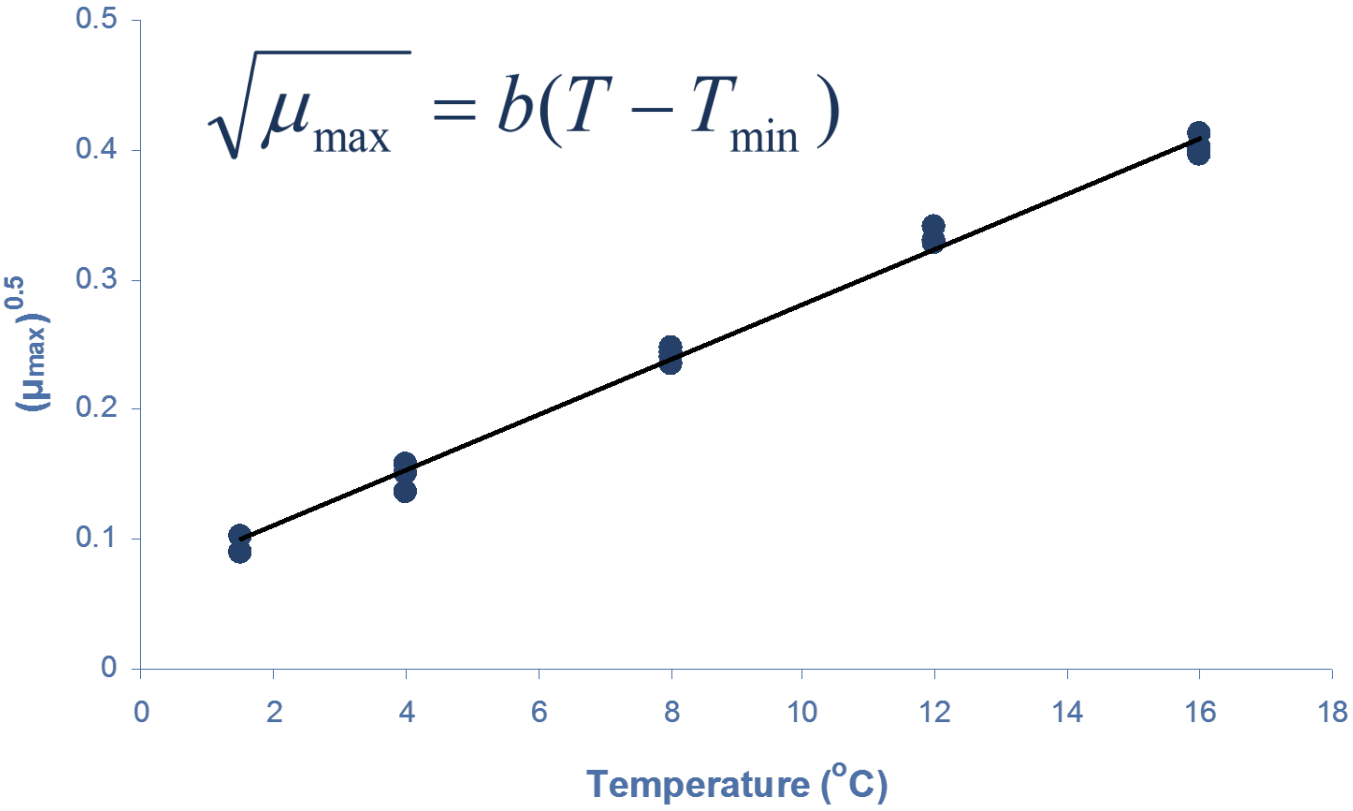
curve	rate	lag	y0	yEnd
1A	0,0046	502,45	3,70	7,74
1B	0,0035	460,90	3,80	7,74
4A	0,0121	199,31	3,79	7,74
4B	0,0110	164,03	3,60	7,77
4C	0,0111	181,19	4,03	8,07
4D	0,0083	164,91	4,11	7,68
8A	0,0267	16,40	3,50	8,26
8B	0,0252	25,24	3,66	8,29
8C	0,0250	17,10	3,59	8,46
8D	0,0257	19,04	3,80	8,37
12A	0,0476	13,26	3,68	8,35
12B	0,0476	14,26	3,74	8,25
12C	0,0502	9,29	3,60	8,31
12D	0,0478	12,79	3,89	8,83
16A	0,0773	3,98	3,57	8,51
16B	0,0804	5,88	3,71	8,56
16C	0,0829	8,81	3,44	8,23
16D	0,0832	5,99	3,30	8,15
16E	0,0967	10,24	3,60	8,45
16F	0,0973	7,83	3,63	8,62

Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 3. Fitting kinetic parameters data to a secondary model



Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 3. Fitting kinetic parameters data to a secondary model

$$\sqrt{\mu_{max}} = b(T - T_{min})$$

Table 3 Parameters and statistics of the square root-type model for the effect of temperature on the maximum specific growth rate (μ_{max}) of *Listeria monocytogenes* in pasteurized milk

Parameter	Estimated value μ_{max} (h^{-1})	Lower 95% CI	Upper 95% CI	r^2
b	0·024	0·023	0·025	0·988
T_{min} (°C)	−2·32	−3·02	−1·61	

Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 4. Validation under dynamic conditions

prediction based on the square root model for the estimation of the "momentary" rate and the differential equations of Baranyi and Roberts model which were numerically integrated with respect to time:

$$\frac{d}{dt}x = \left[b\left(T(t) - T_{\min}\right)\right]^2 \left(\frac{q}{q+1}\right)\left(1 - \frac{x}{x_{\max}}\right)^m x$$

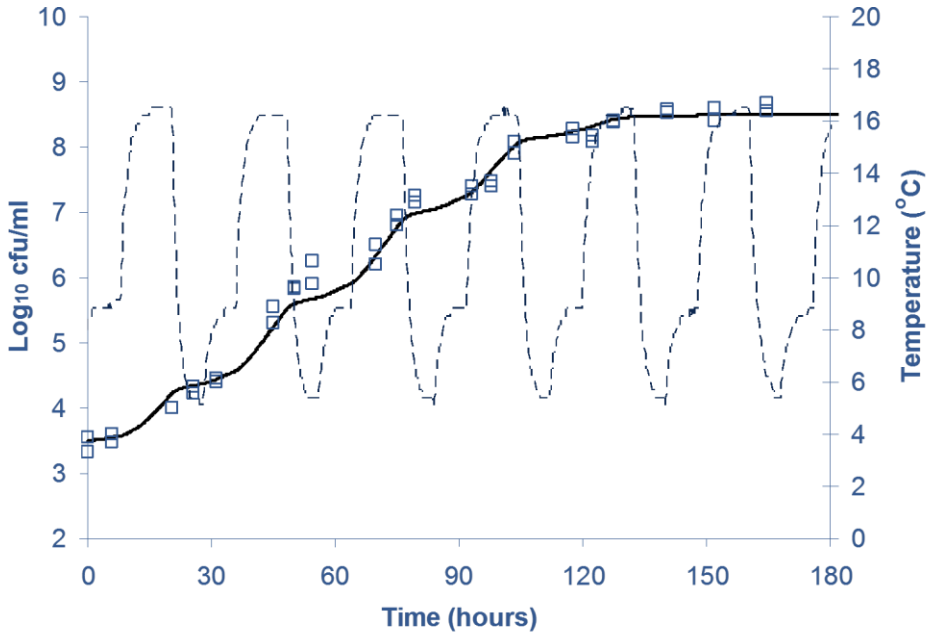
$$\frac{d}{dt}q = \left[b\left(T(t) - T_{\min}\right)\right]^2 q$$

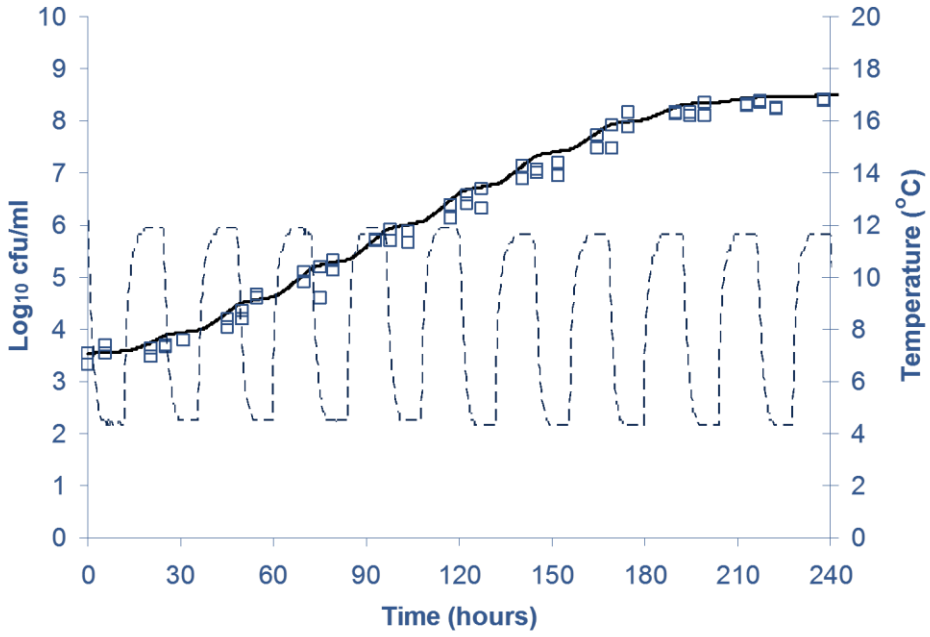
Predictive Microbiology

Example 1

Development of a model for *Listeria monocytogenes* growth in pasteurized milk

Step 4. Validation under dynamic conditions





Predictive Microbiology

Example 2

Modeling the Boundaries of Growth of Salmonella Typhimurium in Broth as a Function of Temperature, Water Activity, and pH

Journal of Food Protection, Vol. 67, No. 1, 2004, Pages 53–59

Modeling the Boundaries of Growth of *Salmonella* Typhimurium in Broth as a Function of Temperature, Water Activity, and pH

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MS 03-166: Received 22 April 2003/Accepted 17 August 2003

Predictive Microbiology

Example 2

Strains: ***S. Typhimurium (ATCC 70408, ATCC 14028, R-4, R-5, SF-530***

Growth medium: TSB

Method: Optical density in microtitre plates

Storage time: 60 ημέρες

Conditions:

pH (HCl 1N): 3.76, 3.94, 4.24, 4.45, 4.76, 4.96, 5.19, 5.47, 5.96, 6.44

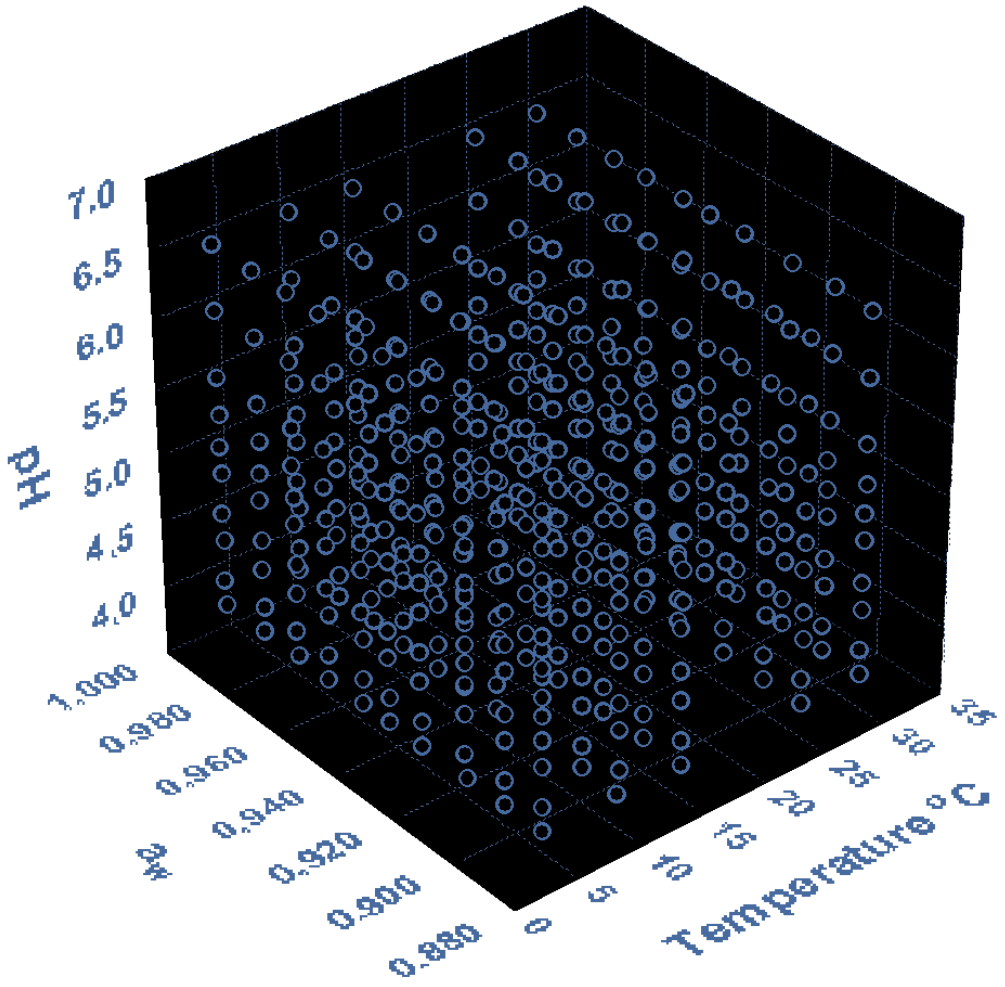
a_w (NaCl): 0.997, 0.983, 0.971, 0.960, 0.948, 0.939, 0,928, 0.913, 0.900

T: 10, 15, 25, 30, 35 ^oC

Predictive Microbiology

Example 2

Combination of conditions tested

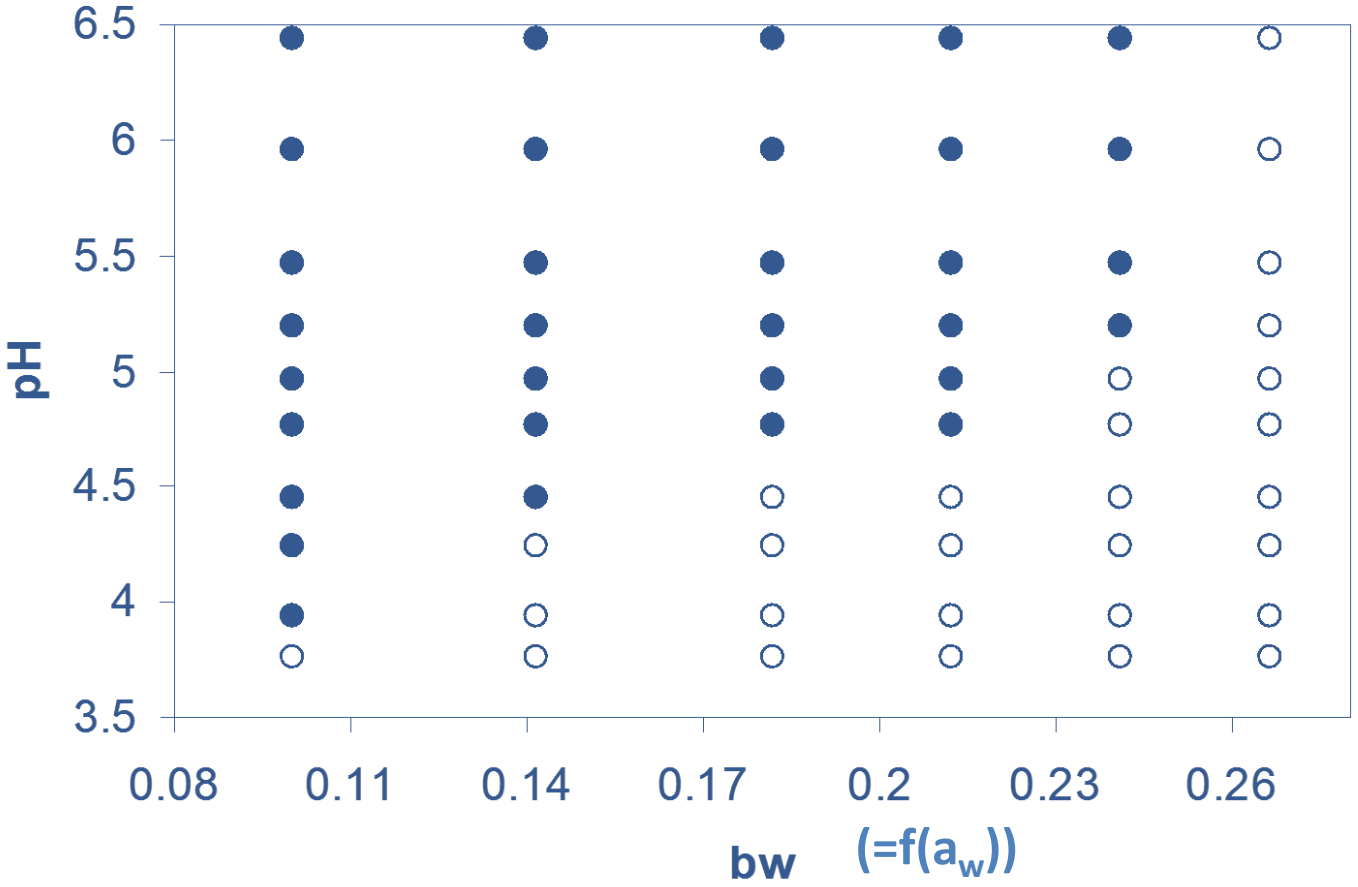


In each conditions we record growth (1) or no growth (0)

Predictive Microbiology

Example 2

Combination of conditions tested



Open symbols: **No Growth**

Closed symbols: **Growth**

Predictive Microbiology

Example 2

Model development

Method:Logistic Regression

Model:Polynomial

$$\text{Logit}(P) = a_0+a_1T+a_2\,pH+a_3b_w+a_4TpH+a_5Tb_w+a_6pHb_w+a_7T^2+a_8\,pH^2+a_9b_w^{\,2}$$

Logit (P): ln[P/(1-P)]

P: probability of growth (range 0-1)

a_i parameters to be estimated

$b_w=(1-a_w)^{0.5}$

Predictive Microbiology

Example 2

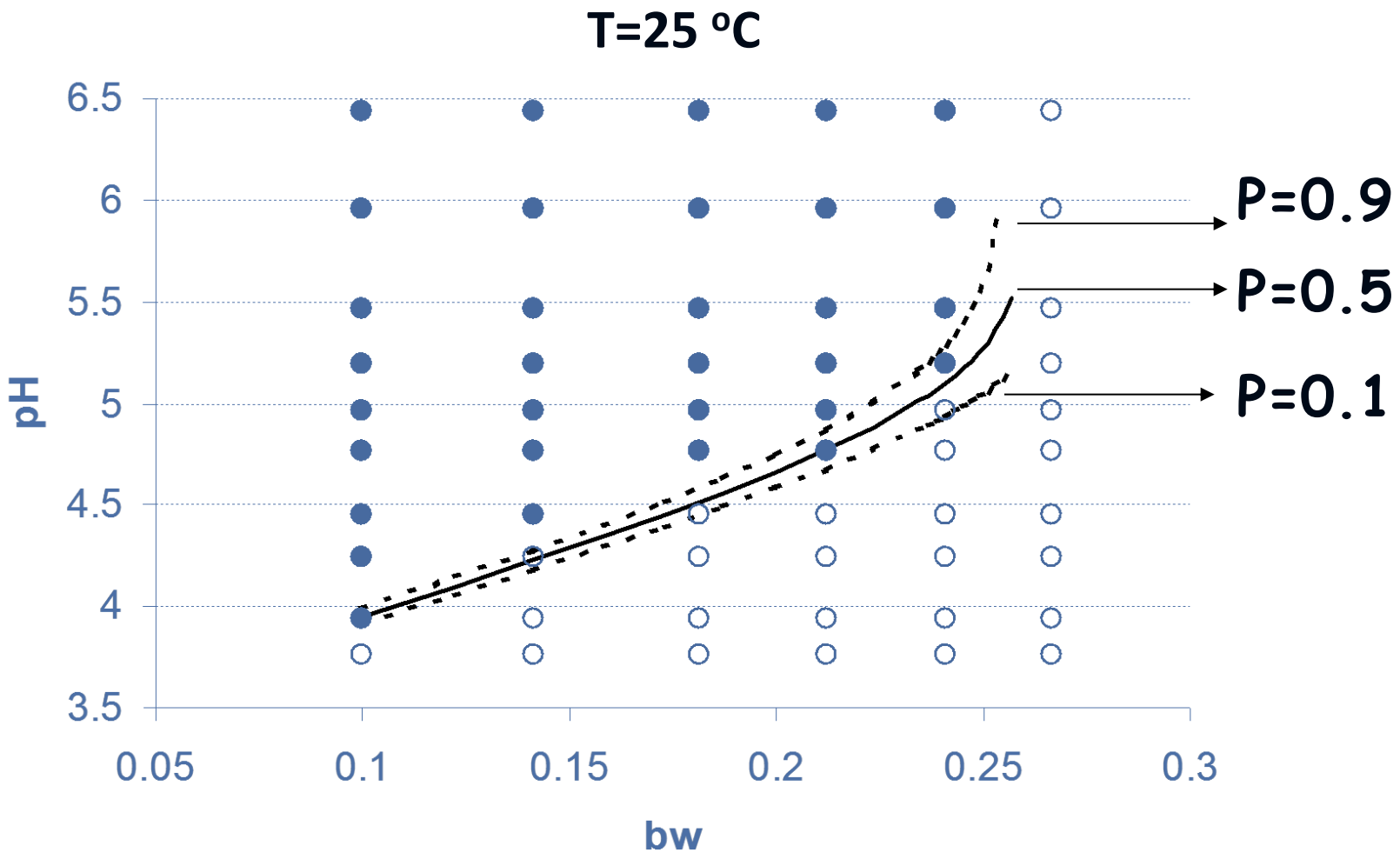
Model parameter estimation

Coefficient	DF	Estimate	St. error	Chi-square	P
Intercept	1	-438.1	65.7	44.4	<0.0001
Temperature	1	5.465	0.89	37.4	<0.0001
b_w	1	233.5	84.6	7.62	0.0058
pH	1	128.0	19.9	41.4	<0.0001
b_w x pH	1	-235.6	45.0	27.4	<0.0001
Temperature x pH	1	-0.236	0.06	14.3	0.0002
Temperature^2	1	-0.074	0.01	40.9	<0.0001
b_w^2	1	1606	329	23.8	<0.0001
pH^2	1	-5.186	1.25	17.1	<0.0001

Predictive Microbiology

Example 2

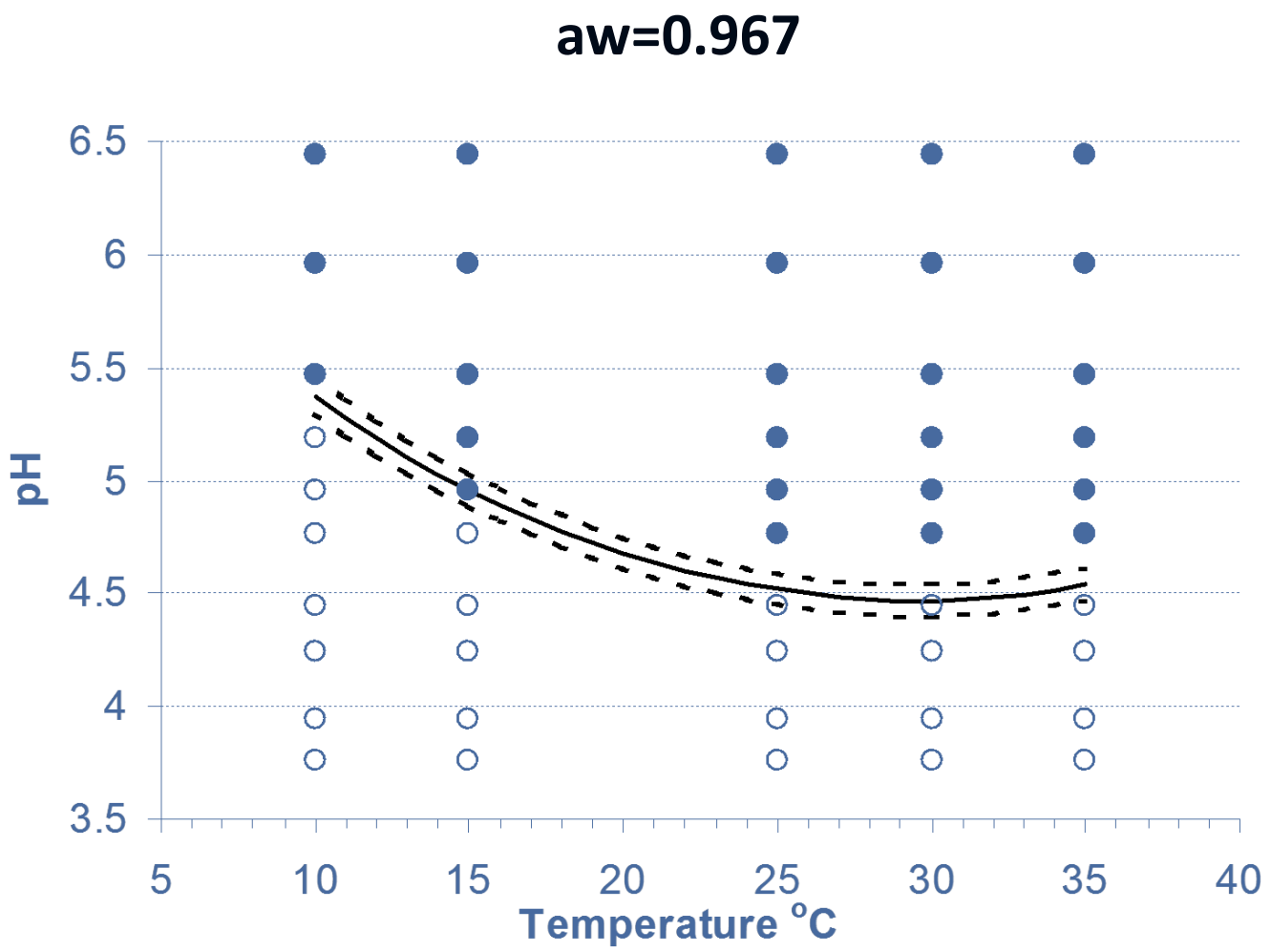
Growth boundaries prediction



Predictive Microbiology

Example 2

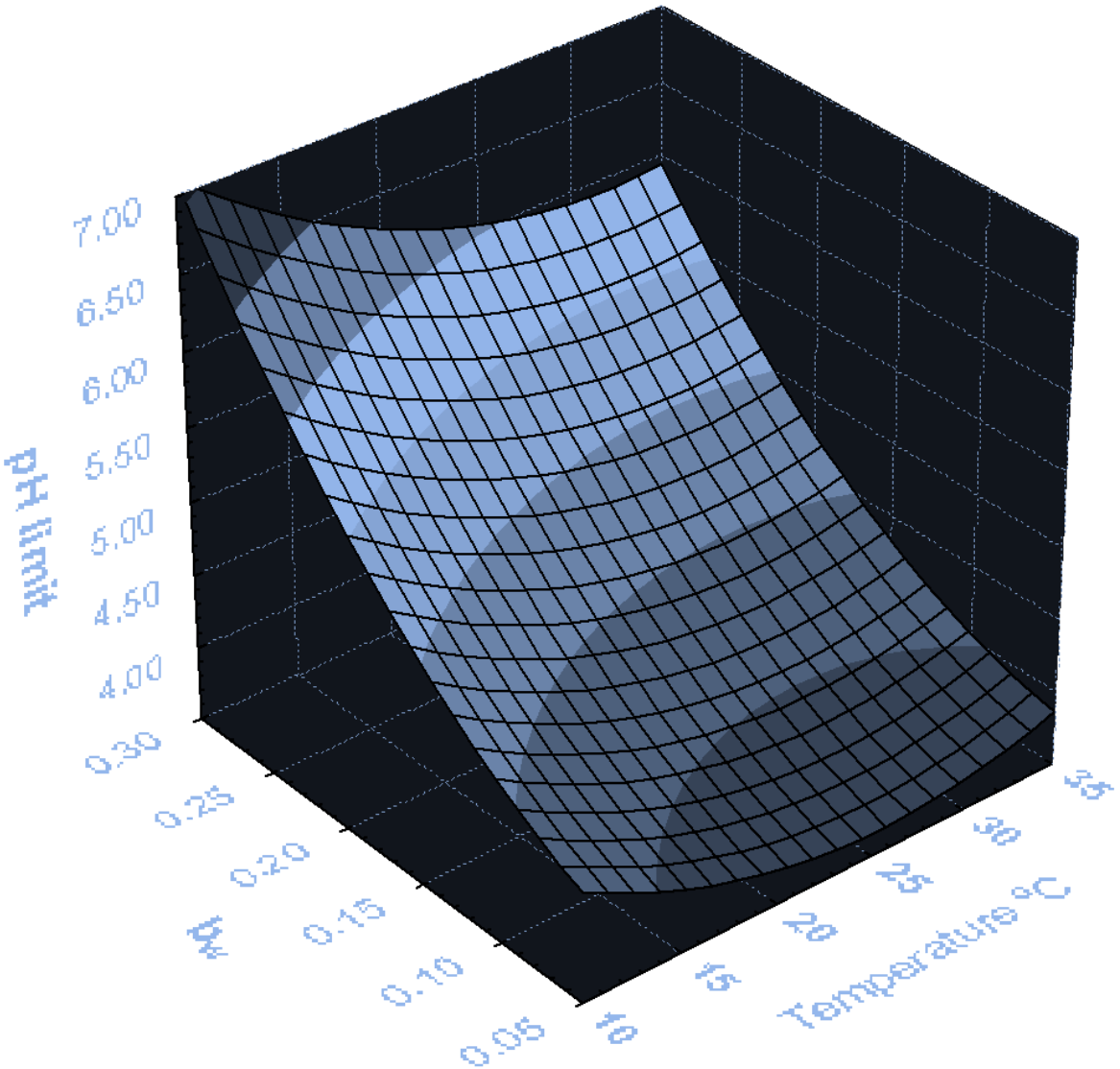
Growth boundaries prediction



Predictive Microbiology

Example 2

Growth boundaries prediction



Risk Assessment

Risk Assessment Stages

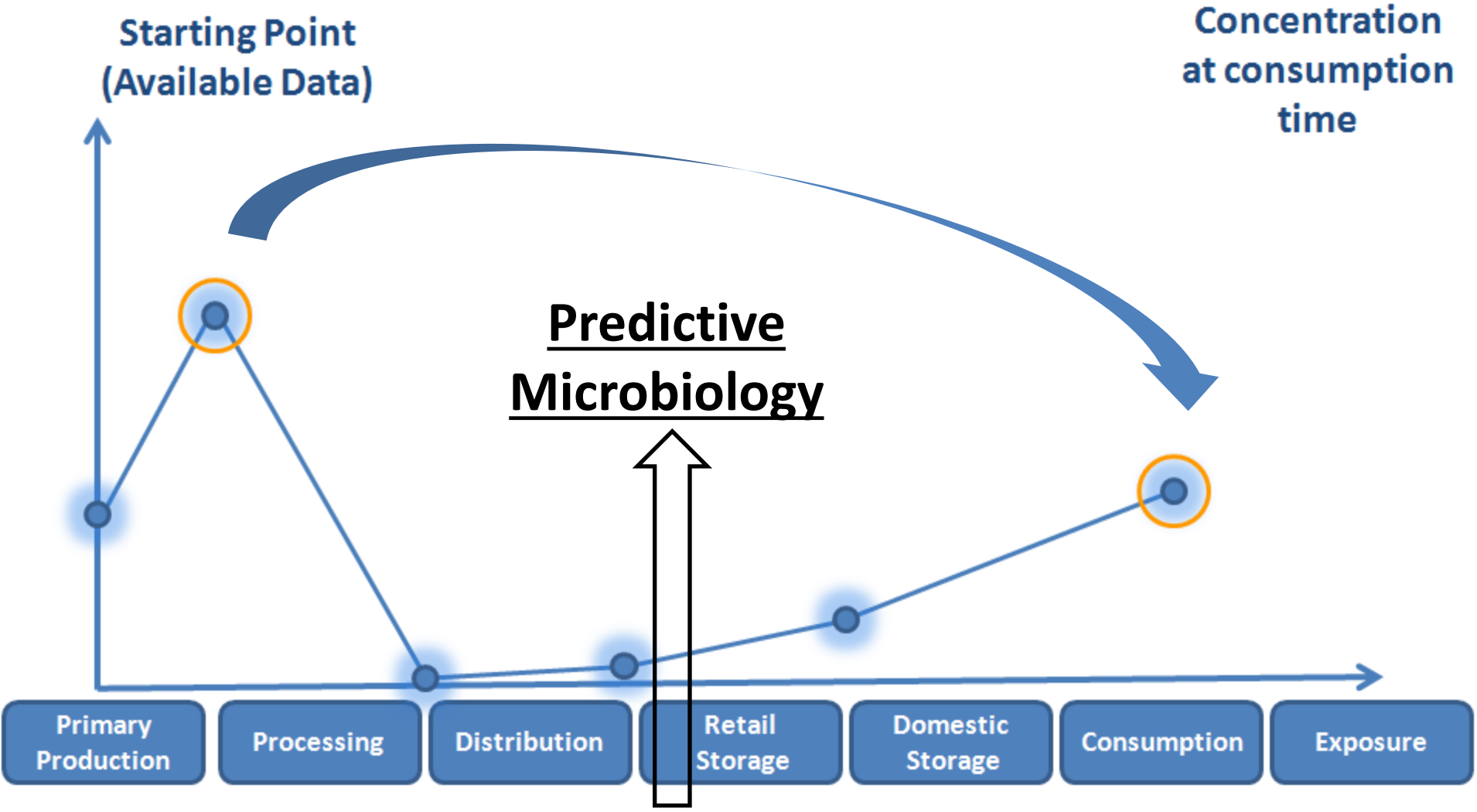
Hazard Identification: what biological, chemical and physical agents are we dealing with and with which foods is it associated?

Hazard Characterization: what illness can be caused, associated in relation to dose and population?

Exposure Assessment: how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of organisms are likely to be ingested?

Risk Characterization: the integration of the above resulting in the probabilities of illness

Exposure Assessment



Microbial Risk Assessment

Important Aspects of Risk Assessment

Variability represents a true heterogeneity of the population that is a consequence of the physical system and irreducible (but better characterized) by further measurements

Uncertainty represents the lack of perfect knowledge of a parameter value, which may be reduced by further measurements

Risk Assessment

Variability (Example)

We all want to move to the 5th floor using the elevator in groups of 5 (randomly selected) people
The weight limit of the elevator is 480 kg

Estimate the chance of exceeding the weight limit

Deterministic method (variability is not taken into account)

Average individual weight=70 kg

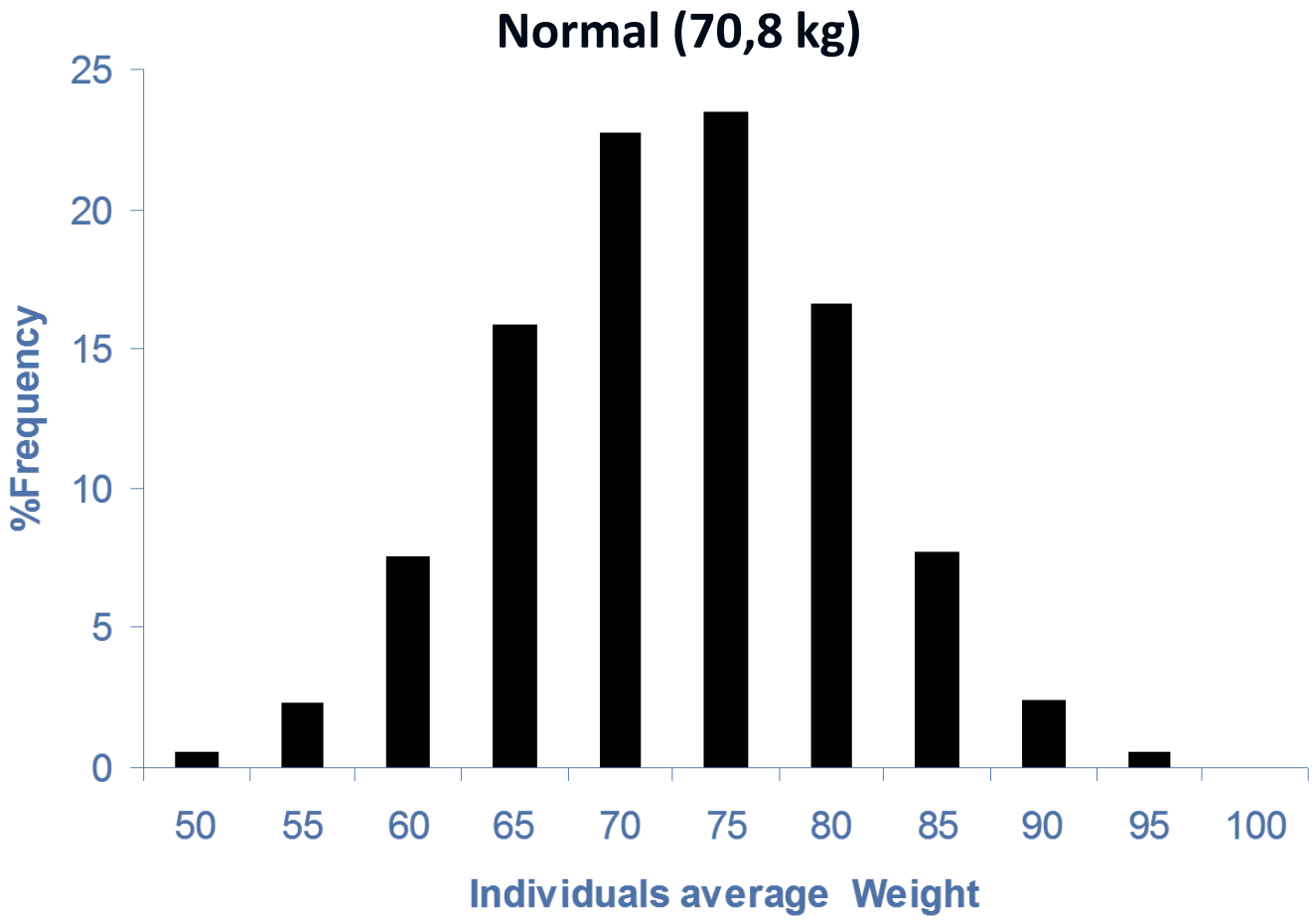
5 persons x 70 =350 kg<480 kg

The weight limit is not exceeded

Exposure Assessment

Variability (Example)

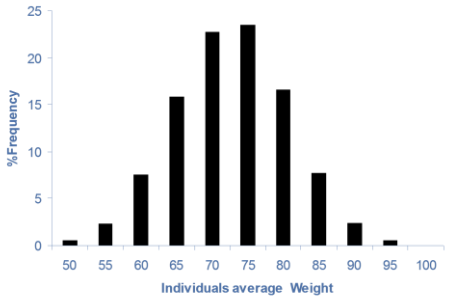
Stochastic method (variability is taken into account)



Exposure Assessment

Variability (Example)

Stochastic method (variability is taken into account)



Random selection of 5 values

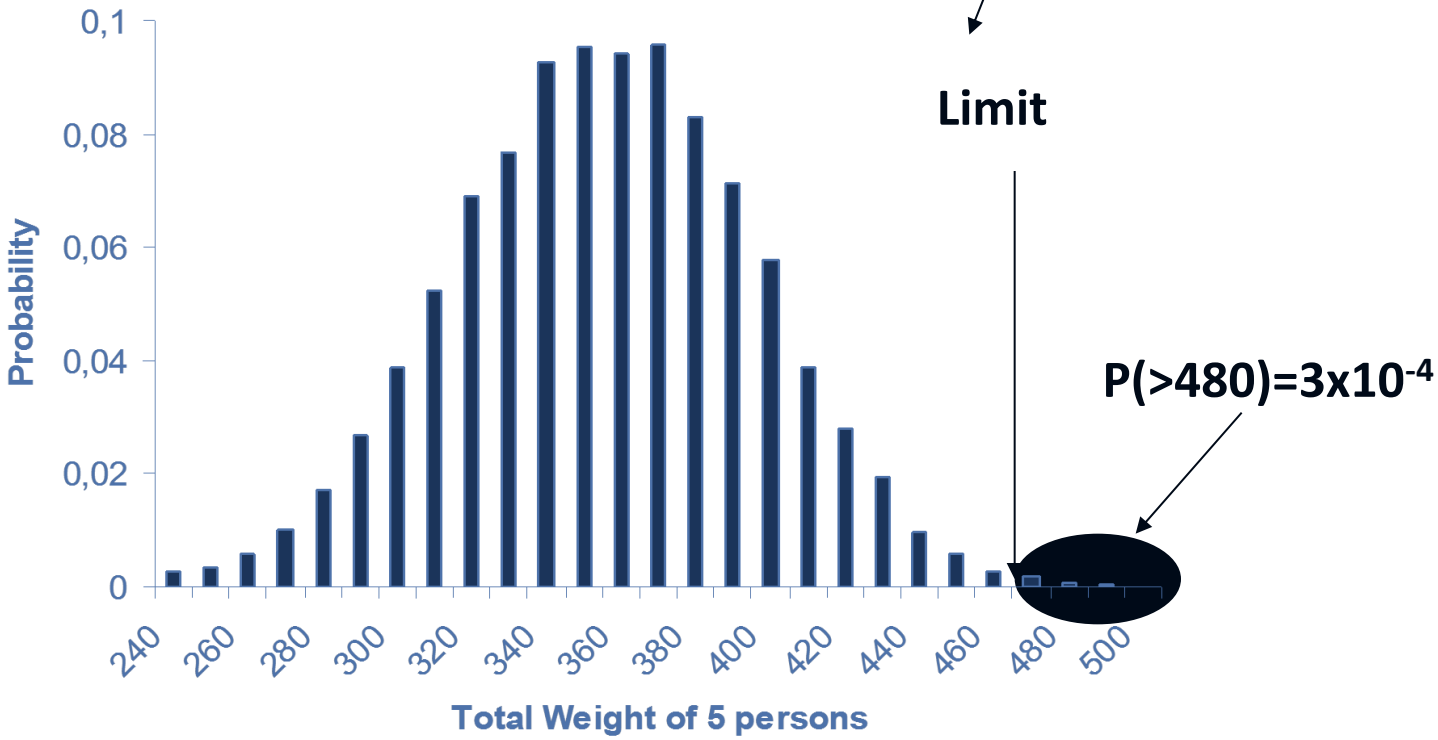
Repeat 100000 (iterations)

Sum the 5 values

Limit

P(>480)=3x10^{-4}

Monte Carlo Simulation



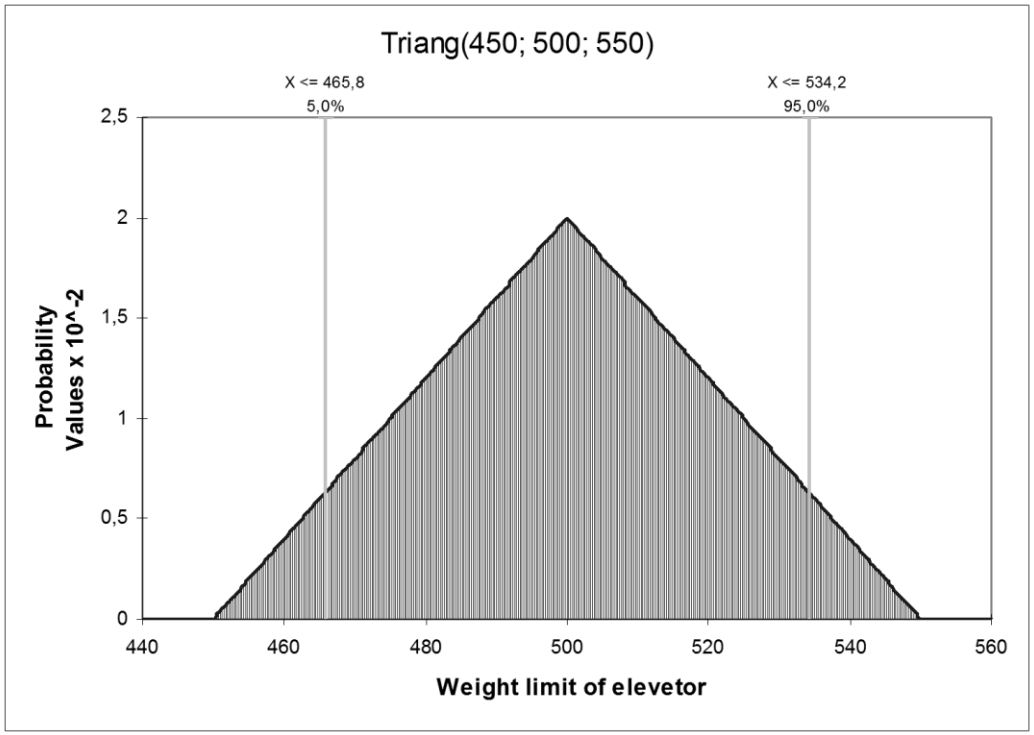
Exposure Assessment

Uncertainty (Example)

Stochastic method

Uncertainty: We don't know the weight limit of the elevator

Expert Opinion: Min:450, Max:550 Most likely:500

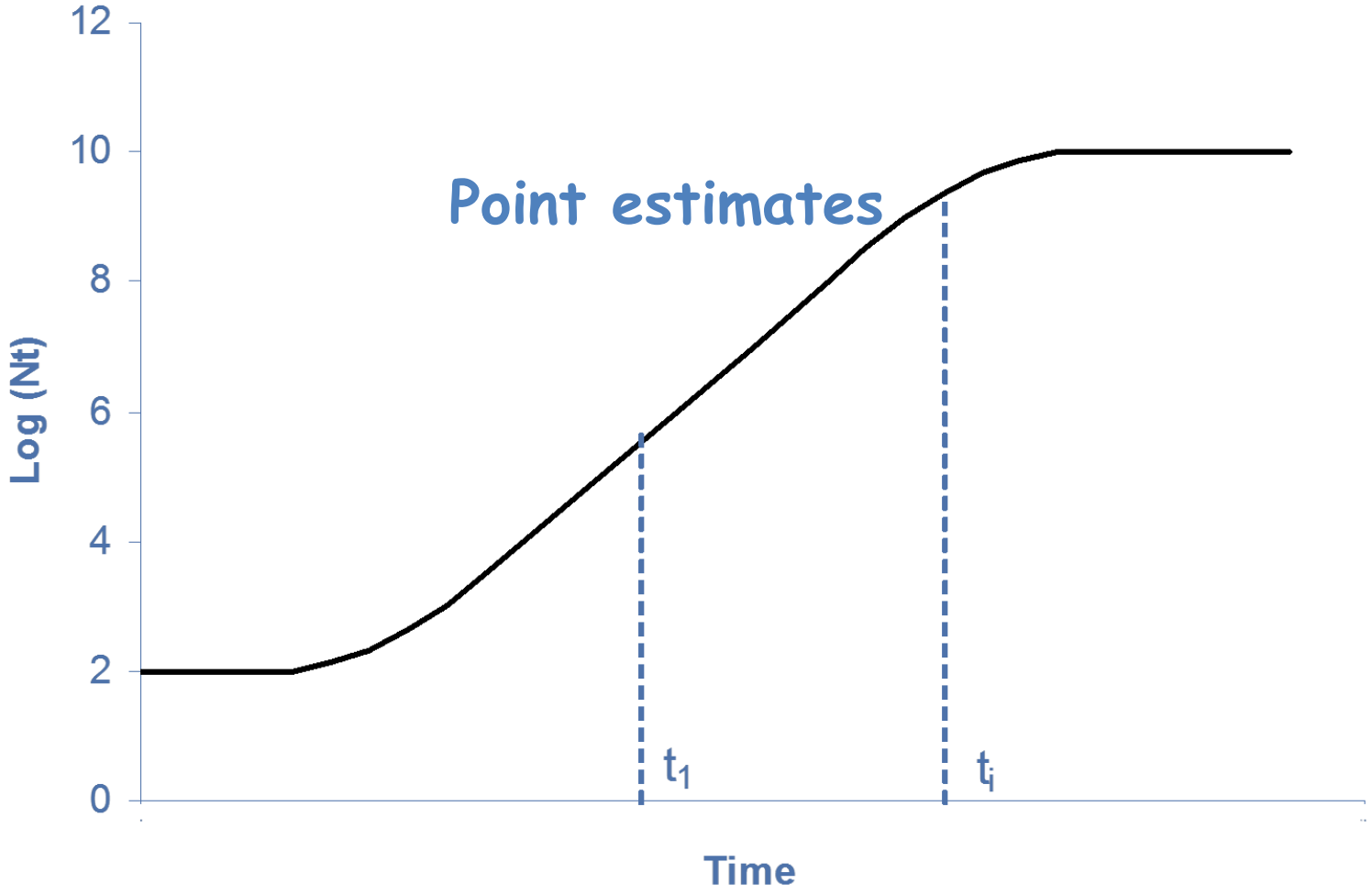


Predictive Microbiology in a Risk-based approach

- The use of predictive microbiology in a Risk-based approach has different demands than “traditional” predictive microbiology

- “Traditional” predictive models are developed and validated to produce point estimates of microbial population level

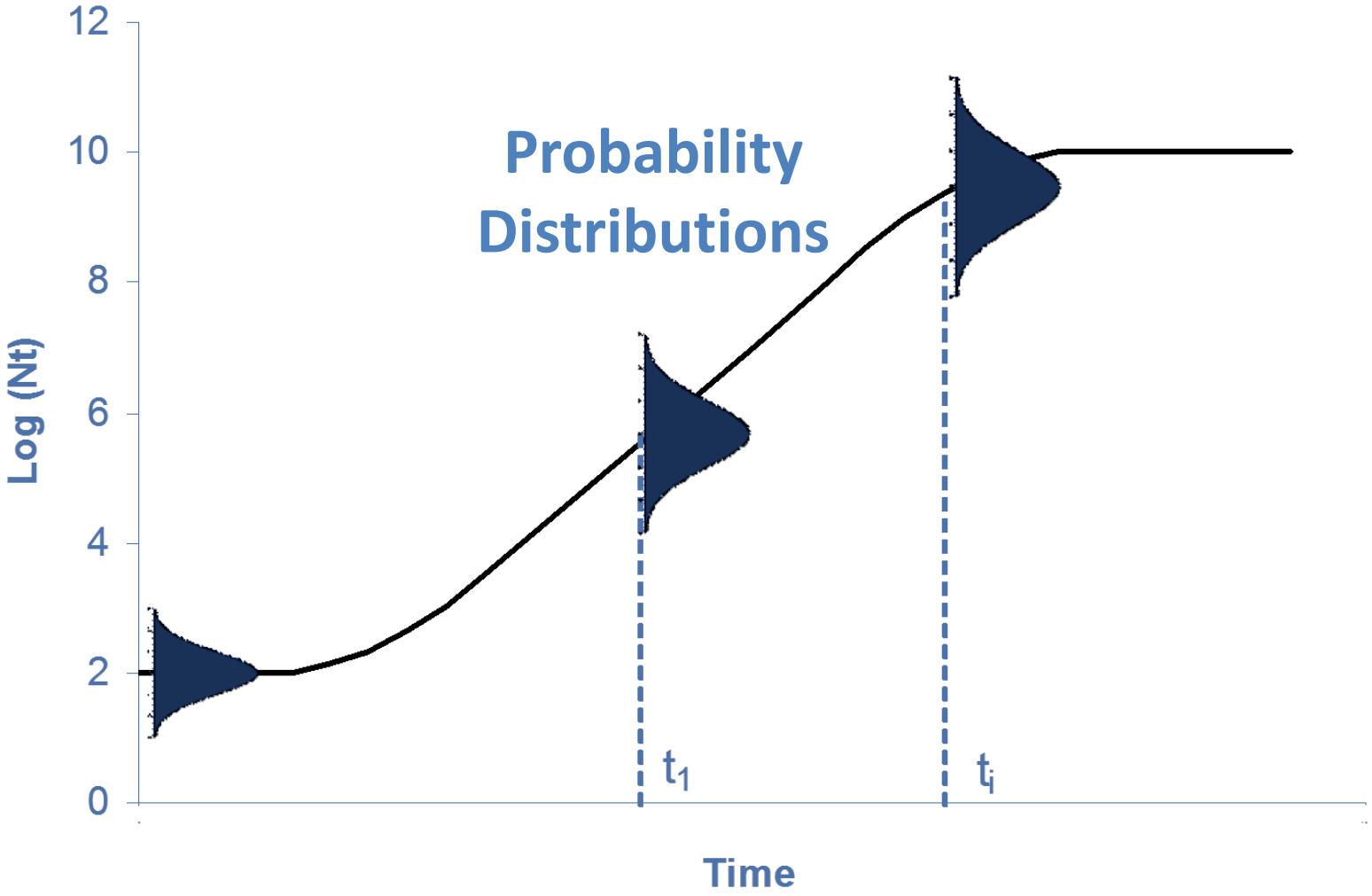
Traditional Predictive Microbiology



Predictive Microbiology in a Risk-based approach

- In a Risk-based approach however, microbial populations should be expressed in terms of probability (for example to predict the probability distribution of the microbial concentration at the time of consumption)

Predictive Microbiology in a Risk-based approach

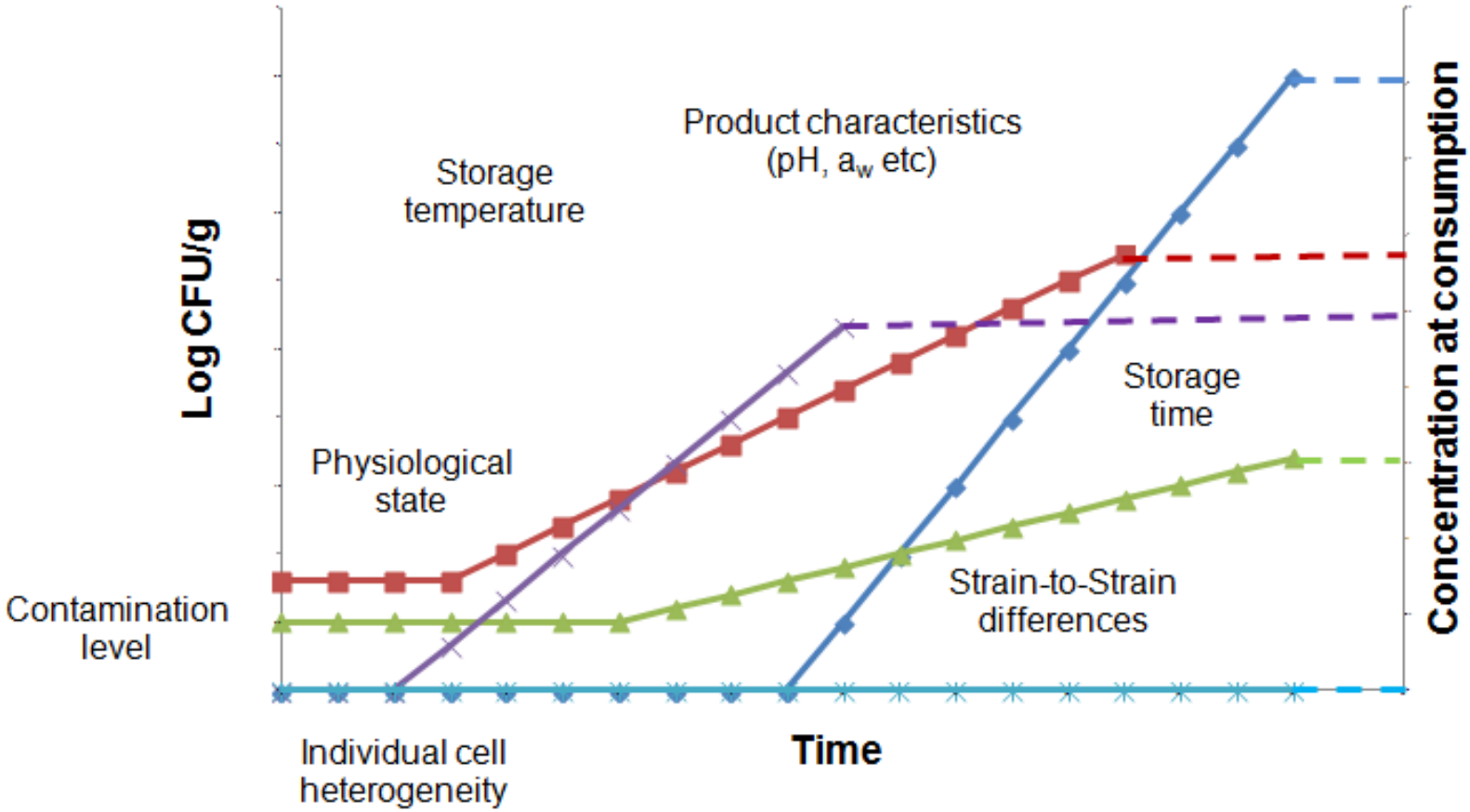


Predictive Microbiology in a Risk-based approach

Applications of predictive models used in a Risk-based approach should take into account both uncertainty and variability.

This can be achieved by the use of **stochastic modeling** where the parameters affecting microbial growth can be introduced as distributions

Sources of variability in microbial behaviour

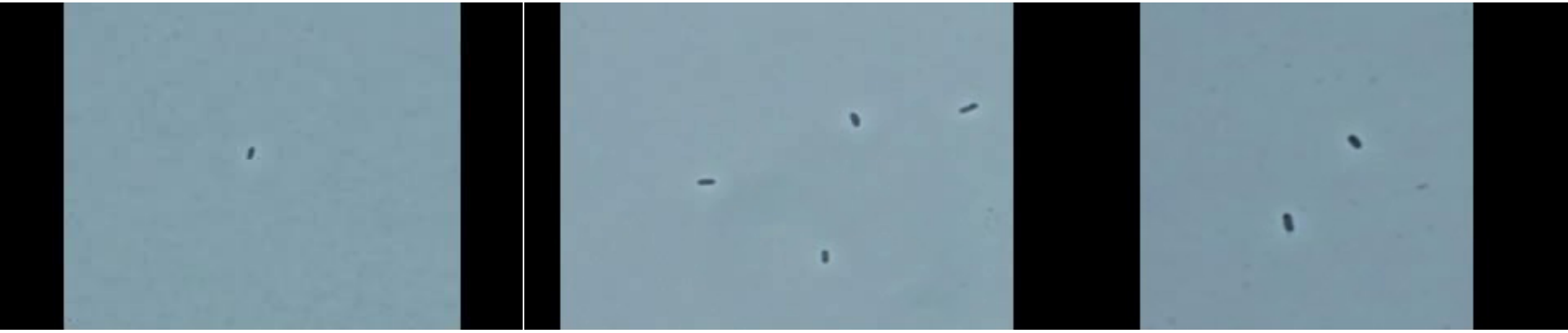


Individual cell heterogeneity (Noise) in microbial growth

Modeling Colonial Growth of Single Bacterial Cells:

The output of the method is a video for each individual cell

Colonial growth of Salmonella single cell at 25 ^{o}C



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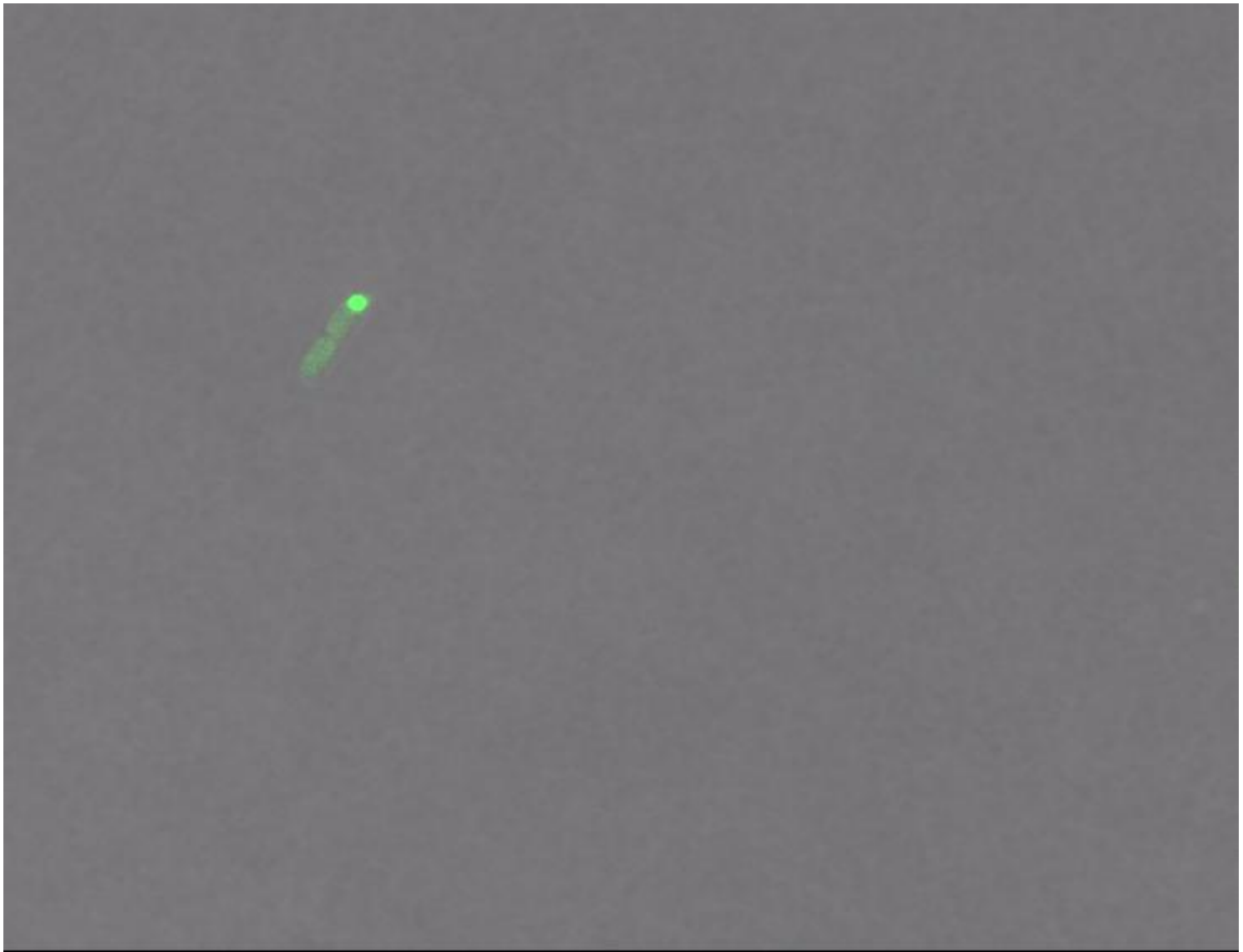
Stochasticity in Colonial Growth Dynamics of Individual Bacterial Cells

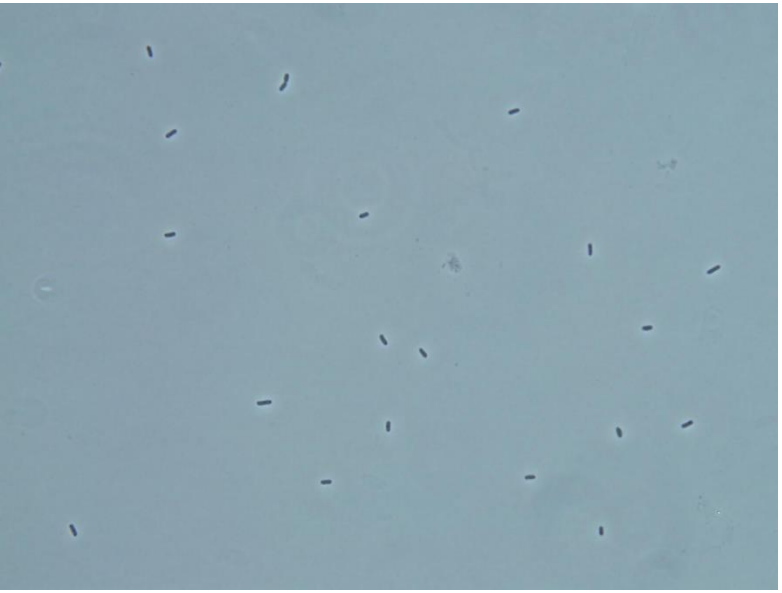
Konstantinos P. Koutsoumanis, Alexandra Lianou
Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

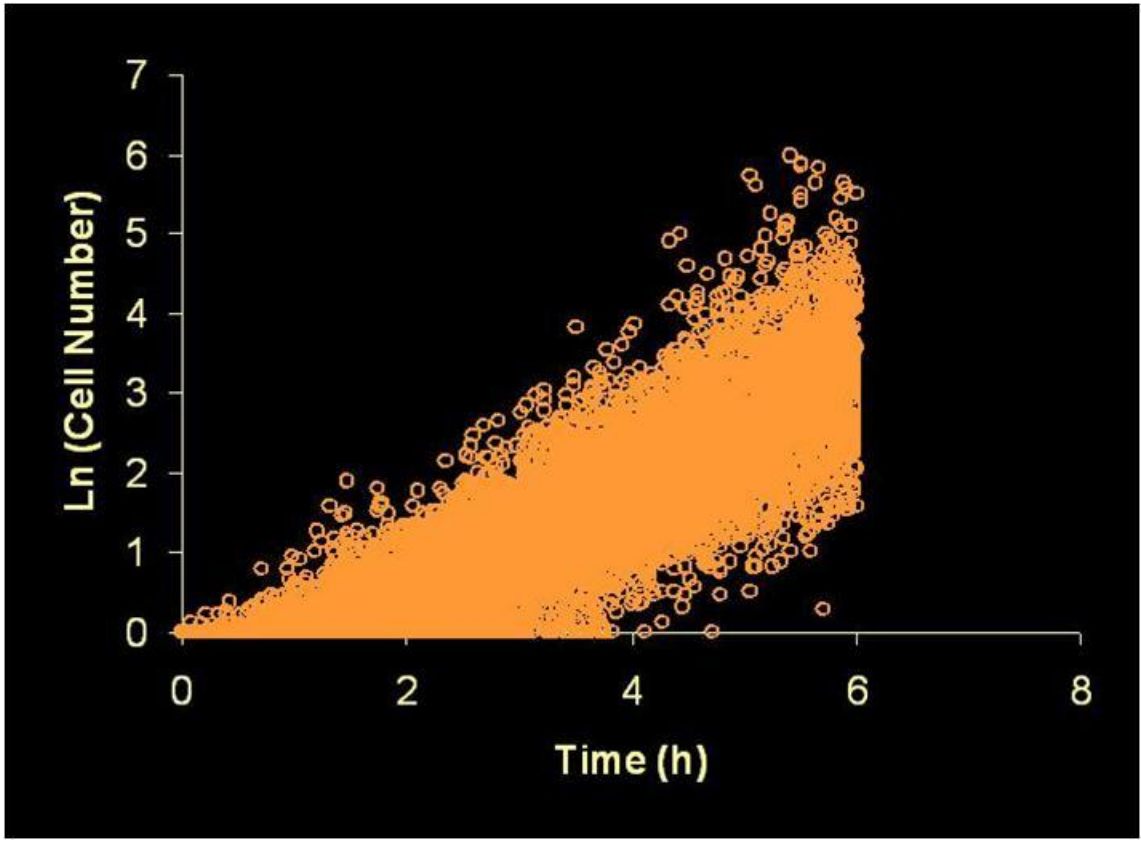
The reason for Individual cell heterogeneity (Noise) in microbial growth is the stochastic gene expression



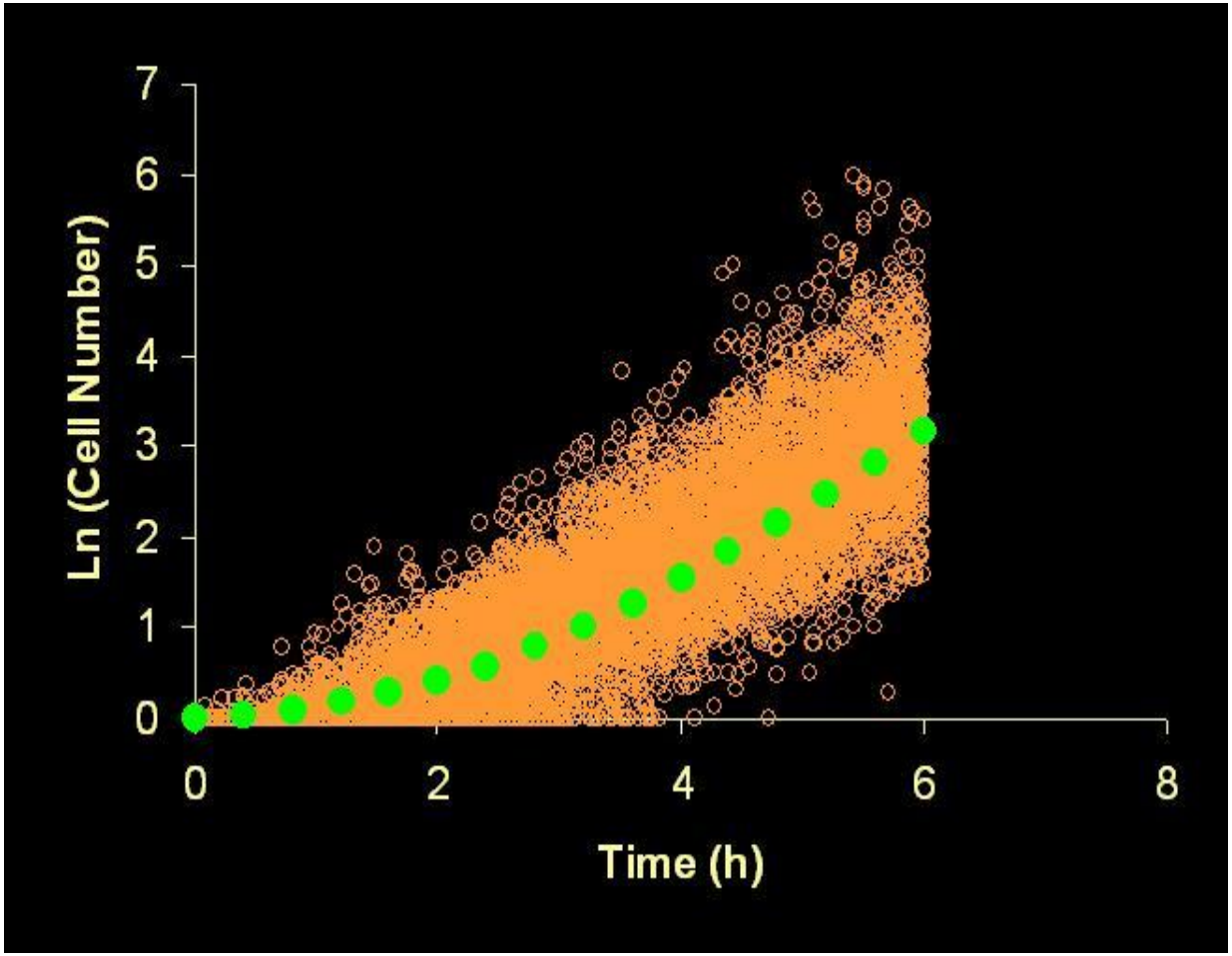
Individual cell heterogeneity (Noise) in microbial growth

Colonial growth of Salmonella single cell at 25 °C





Sources of variability in microbial behaviour



variability is extremely important in risk assessment

Predictive Microbiology (theory)

Questions?

For future questions you can contact me
kkoutsou@agro.auth.gr

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Summer School
"In Silico Methods for Food Safety"