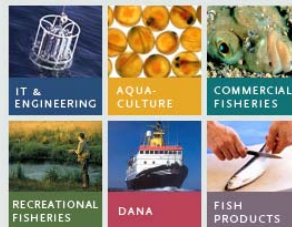


## Microbial safety and quality of aquatic muscle food

Paw Dalgaard

Microbiology and Hygiene, Department of Seafood Research,  
Danish Institute for Fisheries Research (DIFRES),  
Technical University of Denmark (DTU)  
[www.difres.dk/micro/pd.htm](http://www.difres.dk/micro/pd.htm) - [pad@difres.dk](mailto:pad@difres.dk)



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Lyngby

- Seafood-borne human disease
- Safety and risk management
  - Histamine and histamine fish poisoning
  - Viruses
  - Bacteria: *Vibrios* and *Listeria monocytogenes*
- Quality and shelf-life
  - Microbial spoilage
  - Shelf-life: Determine, predict and extend
- Perspectives and challenges

DIFRES-DTU

Lyngby

Seafood-borne human disease

- Hazards and risks: Probability and severity must be considered
- Hazards most often responsible for seafood-borne human disease:

Type of seafood	Primary "microbial" hazards responsible for human disease
Finfish products	Histamine and in some regions also salmonella
Bivalve molluscs	Viruses og <i>vibrio</i> bacteria

- Examples of rare but severe seafood-borne human diseases:
  - Listeriosis due to *Listeria monocytogenes* (20-30% mortality)
  - Botulism due to *Clostridium botulinum* (~ 10% mortality)

Seafood-borne human disease

USA (1990 – 2003)	Outbreaks	Cases
Virus/norovirus	82 (9%)	2860 (31%)
Histamine/HFP	341 (38%)	1651 (18%)
Salmonella	61	1244
<i>Vibrio</i> spp.	80	1180
Ciguatoxin	215	818
<i>Cl. perfringens</i>	11	427
<i>B. cereus</i>	12	401
<i>S. aureus</i>	18	197
Paralytic shellfish	21	137
Other agents	58	397
<b>Total</b>	<b>899</b>	<b>9312</b>

Histamine and histamine fish poisoning (HFP)

- Implicated products: Marine finfish with > 500 mg histamine/kg
- Symptoms: Flushing, rash, headache, diarrhoea and vomiting
- **Free histidine** → Histidine decarboxylase → **Histamine**
- Histidine decarboxylase producing bacteria is required
- Significant growth is required → more than 1-10 million bacteria/g
- Once formed histamine is stable (heat, freezing, salt, pressure)

**Legislation - critical concentrations of histamine:**

EU: 100-200 mg/kg (EC 2073/2005)

USA: 50 mg/kg (Defect action level, FDA/CFSAN 2001)

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Taylor 1986; Lehane & Olley 2000

Lyngby

Histamine and histamine fish poisoning (HFP)

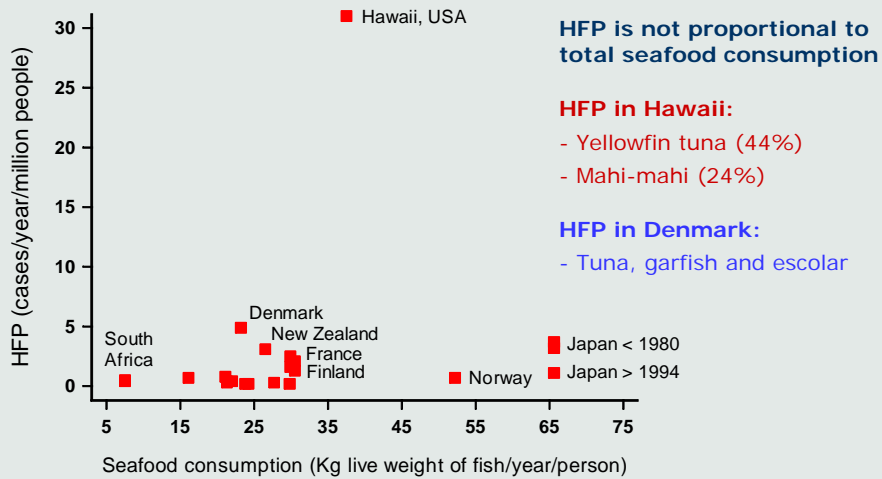
Country	Year	Incidents	Cases	
			Total	per year/million
<b>Hawaii, USA</b>	<b>1990-2003</b>	<b>111</b>	<b>526</b>	<b>31</b>
<b>Denmark</b>	<b>1986-2005</b>	<b>64</b>	<b>489</b>	<b>4.9</b>
New Zealand	2001-2005	11	62	3.1
<b>Japan</b>	<b>1970-1980</b>	<b>42</b>	<b>4122</b>	<b>3.2</b>
	<b>1994-2005</b>	<b>68</b>	<b>1523</b>	<b>1.1</b>
France	1987-2005	123	2635	2.5
Finland	1998-2005	15	89	2.1
Taiwan	1986-2001	8	535	1.5
UK	1976-2004	515	1300	0.8
Switzerland	1966-1991	76	111	0.7
South Africa	1992/2004	10/3	22/21	0.4
Australia	1995-2000	7	34	0.4
<b>USA</b>	<b>1990-2003</b>	<b>341</b>	<b>1651</b>	<b>0.3</b>
Canada	1975-1995	39	109	0.2

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Dalgaard et al. 2008 – BIOCOM, SEAFOODplus

Lyngby

Histamine and histamine fish poisoning (HFP)



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Dalgaard et al. 2008 – BIOCUM, SEAFOODplus

Lyngby

Histamine and histamine fish poisoning (HFP)

- Data from 137 outbreaks with 1882 cases
- 500-5000 mg histamine/kg is common in finfish that cause HFP

Histamine (mg/kg)	Outbreaks		Cases		Seafood
	Number	%	Number	%	
> 5000	19	14	211	11	Escolar, kahawai, kingfish, marlin, saury, tuna, yellowfin tuna
1000 - 5000	61	45	977	52	Amberjack, anchovies, bluefish, cape yellowtail, castor oil fish/escolar, kahawai, mackerel, mahi-mahi, marlin, pilchard, red tuna, sailfish, sardines, swordfish, tuna
500 - 1000	24	18	520	28	Anchovies, garfish, kahawai, mahi-mahi, mackerel, marlin, sardines, tuna
< 500	33	24	174	9	Anchovies, bonito, escolar, mackerel, mahi-mahi, pilchard, red tuna, sardines, skipjack, salmon, tuna

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Dalgaard et al. 2008 – BIOCUM, SEAFOODplus

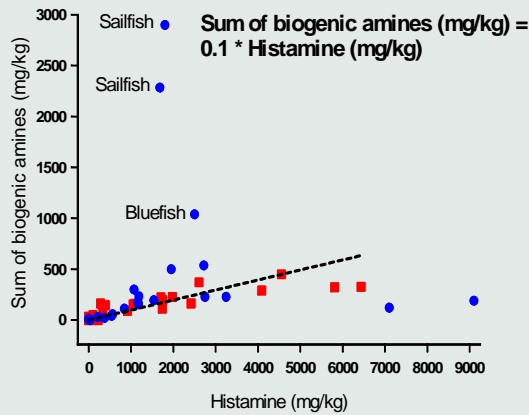
Lyngby

Histamine and histamine fish poisoning (HFP)

**Seafood from outbreaks of HFP**

Histamine more abundant than other biogenic amines

100 g portion: 50 - 500 mg histamine and 5 - 50 mg other biogenic amines

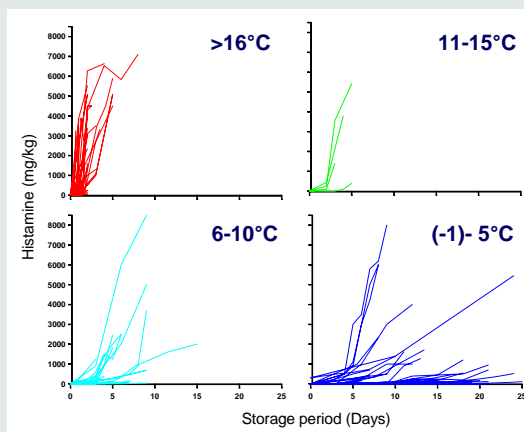


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Lyngby

Histamine and histamine fish poisoning (HFP)

Toxic concentrations of histamine can be formed in seafood at below 5°C



- EU : Fresh fish at 0-2°C (EC 853/2004)
- USA : Fresh fish at less than 4.4°C (FDA/CFSAN 2001)

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Lyngby

Histamine and histamine fish poisoning (HFP)

Both **mesophilic** and **psychrotolerant** bacteria can be responsible for histamine formation and thereby HFP

Seafood	Bacteria	References
Fresh tuna	<i>Morganella morganii</i>	Kawabata et al. 1956
Fresh tuna	<i>Hafnia</i> sp.	Havelka 1967
Fresh tuna	<i>Morganella morganii</i>	Sakabe 1973
Fresh tuna	<i>Raoultella planticola</i> ( <i>Klebsiella pneumoniae</i> )	Lerke et al. 1978
Dried sardines	<i>Photobacterium phosphoreum</i>	Kanki et al. 2004
Tuna in chilli sauce	<i>Morganella psychrotolerans</i> and/or <i>Photobacterium phosphoreum</i>	Emborg et al. 2005
Cold-smoked tuna	<i>Photobacterium phosphoreum</i>	Emborg & Dalgaard 2006
Cold-smoked tuna	<i>Morganella psychrotolerans</i>	
Tuna heated in flexible film	<i>Morganella morganii</i> subsp. <i>morganii</i>	BIOCOM
Fresh tuna	<i>Photobacterium phosphoreum</i>	BIOCOM

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Lyngby

Histamine and histamine fish poisoning (HFP)

'Toxic' histamine formation by bacteria from seafood

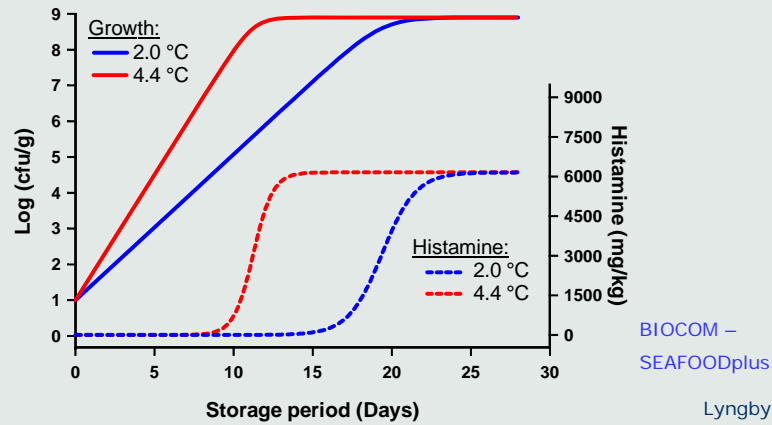
	0 - 5° C	Above ca. 10° C
<i>Enterobacteriaceae</i>		
<i>Morganella morganii</i>	-	+++
<i>Morganella psychrotolerans</i>	++	+++
<i>Raoultella planticola</i>	-	+++
<i>Enterobacter aerogenes</i>	-	+++
<i>Vibrionaceae</i>		
<i>Photobacterium phosphoreum</i>	++	++
<i>Photobacterium damsela</i>	-	+++
Lactic acid bacteria		
<i>Tetragenococcus myriaticus</i>	-	++
<i>Lactobacillus</i> spp.	-	++

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Histamine and histamine fish poisoning (HFP)

- Histamine formation can be predicted by mathematical models
- Efficient chilling is essential to delay histamine formation by psychrotolerant bacteria (*M. psychrotolerans* and *P. phosphoreum*)

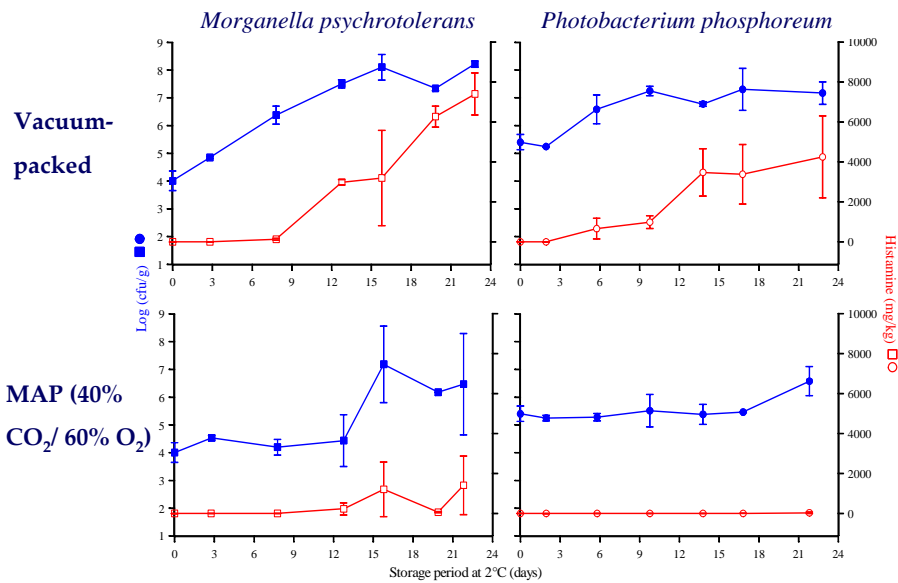


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BIOCOM –  
SEAFOODplus

Lyngby

Histamine formation in inoculated tuna steaks at 2° C



Emborg et al. 2005

Outline: Microbial safety and quality of aquatic muscle food

- Seafood-borne human disease
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  - Histamine and histamine fish poisoning
  - **Viruses**
  - Bacteria: *Vibrios* and *Listeria monocytogenes*
- Quality and shelf-life
  - Microbial spoilage
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Viruses

- Seafood-borne human disease is primarily due to:
  - Noroviruses (Norwalk-like; winter vomiting disease)
  - Hepatitis A virus
- Other viruses are abundant in seawater but do not cause seafood-borne human disease
- Viruses are accumulated by live bivalve molluscs (filter feeders)
- Viruses do not grow in bivalve molluscs or seafood during storage
- Consumption of raw (or insufficiently cooked) contaminated bivalve molluscs is the primary reason for virus-related seafood-borne disease

### Control of viruses - prevention of seafood-borne disease:

- Consumer education: Avoid consumption of raw bivalve molluscs
- Avoid harvest of bivalve molluscs in polluted water
  - Virus survive very long time in cold seawater
- Use depuration of bivalve molluscs
- Heating (90°C) is efficient and high pressure processing (250-300 MPa) can reduce levels of noroviruses
- Effects of processing on low concentrations of viruses in seafood deserves further study
- Sensitive and specific (molecular) detection methods recently became available

- Seafood-borne human disease
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*Vibrio* species

- *Vibrio*-related seafood-borne disease is rare in Europe, but more common in Japan, Asia and USA
- Disease is often due to raw or insufficiently cooked bivalve molluscs – *Vibrio* bacteria are heat sensitive (and also killed by freezing)
- Disease is typically due to storage of products at high temperatures – rapid growth of the pathogenic *Vibrio* bacteria
- *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* are the most important species but *V. alginolyticus*, *V. fluvialis*, *V. hollisae* and *V. mimicus* have also caused gastrointestinal disease
- *V. vulnificus* infections can be fatal and individuals with liver disorder should never consume raw seafood (oysters and sushi)

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Huss et al. 2004; Dalgaard 2006

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*Listeria monocytogenes*

- Can be isolated (low concentration) from fresh and lightly preserved seafood
- Psychrotolerant (1-45°C) and halotolerant (0 – 10 % NaCl) but killed by cooking and hot smoking (ca. 75°C).
- Cause listeriosis and risk groups include children (< 1 year), elderly/old and persons with reduced immune defence (e.g. AIDS, pregnant)
- Mortality high (20-40%)
- A few listeriosis cases have been related to marinated trout, smoked fish, smoked mussels ("ready-to-eat" RTE products)
- Listeriosis is caused by high concentrations of *L. monocytogenes* and growth of the bacterium in seafood is more problematic than its occurrence.

DIFRES-DTU

Lyngby



*Listeria monocytogenes*

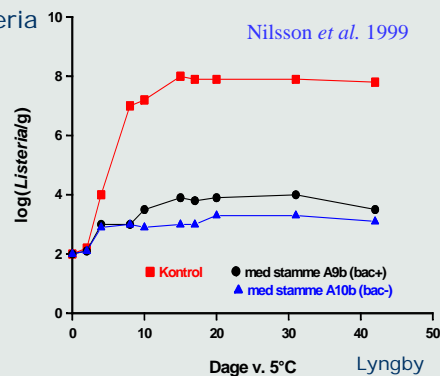
EU-regulation (EC 2073/2005) distinguish between ready-to-eat foods able or unable to support growth of *Listeria monocytogenes*

Ready-to-eat foods	Critical limit	Comments
Support growth	None in 25 g	- When produced
Support growth	100 cfu/g	- It must be <u>documented</u> that 100 cfu/g is not exceeded within the storage period
Unable to support growth	100 cfu/g	- <u>Documentation</u> - pH ≤ 4,4 or a <sub>w</sub> ≤ 0,92 - pH ≤ 5,0 and a <sub>w</sub> ≤ 0,94 - Shelf-life below 5 days



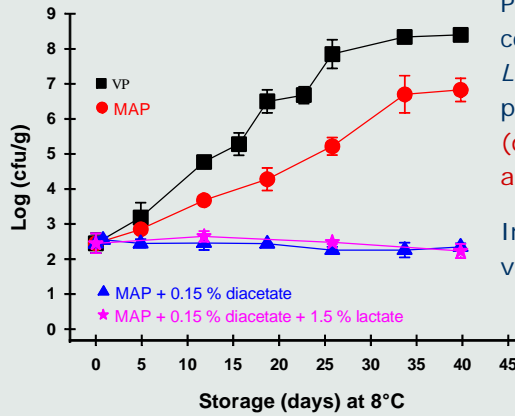
*Listeria monocytogenes*

- Salt, pH, smoke components, atmosphere and storage temperature for many fresh and lightly preserved seafoods are not sufficient to prevent growth of *L. monocytogenes*
- Growth in lightly preserved seafood can be limited by naturally occurring or added lactic acid bacteria
- Biopreservation with added lactic acid bacteria is possibly but not yet applied in the seafood sector





*Listeria monocytogenes*



Product characteristics and storage conditions determine if growth of *L. monocytogenes* can be prevented by addition of (di)acetate or if both (di)acetate and lactate are required

Important to prevent growth in various lightly preserved seafoods



DIFRES-DTU

Mejlholm and Dalgaard (2007) - J. Food Prot. 70, 70-84

Lyngby



*Listeria monocytogenes*

$$\mu_{max} = \mu_{max-ref} \cdot \left( \frac{(T - T_{min})}{(T_{ref} - T_{min})} \right)^2 \cdot \left( \frac{a_w - a_{wmin}}{a_{wopt} - a_{wmin}} \right) \cdot 1 - 10^{(pH_{min} - pH)} \cdot 1 - \sqrt{\frac{[LAC]_U}{[MIC]_{ULAC}}} \cdot \frac{(P_{max} - P)}{(P_{max} - P_{opt})} \cdot \frac{(CO_{2max} - CO_{2dissolved})}{(CO_{2max} - CO_{2opt})} \cdot 1 - \sqrt{\frac{[AC]_U}{[MIC]_{UAC}}} \cdot \xi$$

**Growth can be predicted**

Extensive growth and growth boundary models are developed for the effect of temperature, NaCl/a<sub>w</sub>, pH, lactate, phenol (smoke), CO<sub>2</sub>, diacetate and interactions between the parameters (ξ)

Each term results in a value between 0 and 1

$$\xi(\varphi(T, a_w, pH, [LAC], NIT, P, CO_2, [AC])) = \begin{cases} 1 & , \psi \leq 0.5 \\ 2(1 - \psi) & , 0.5 < \psi < 1 \\ 0 & , \psi \geq 1 \end{cases}$$

Interaction term (Le Marc et al. 2002)

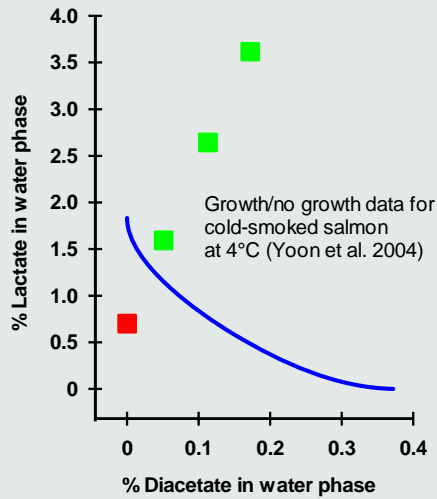
$$\psi = \sum_i \frac{\varphi_i}{2 \prod_{j=1}^i (1 - \varphi_j)}$$

DIFRES-DTU

Lyngby



*Listeria monocytogenes*



**Growth can be predicted**

Correct prediction of growth/  
no growth in 73 of 76 experiments  
(96%)

Both inoculated and naturally  
contaminated lightly preserved  
seafoods have been evaluated

New study, n = 29

Literature, n = 47

The new model perform markedly  
better than existing growth  
boundary models (less than 70 %)

DIFRES-DTU

Mejlholm and Dalgaard (2007) - J. Food Prot. 70, 70-84

Lyngby



*Listeria monocytogenes*

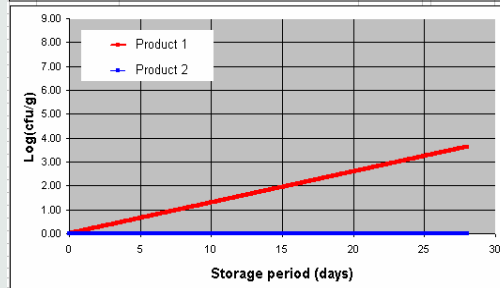
**Growth can be predicted**

- Cold-smoked salmon can allow growth of *L. monocytogenes* (Product 1)
- Growth is prevented by diacetate and MAP (Product 2)
- Product 2 comply with new EU regulation

Mejlholm and Dalgaard (2007)  
J. food Prot, 70, 70-84

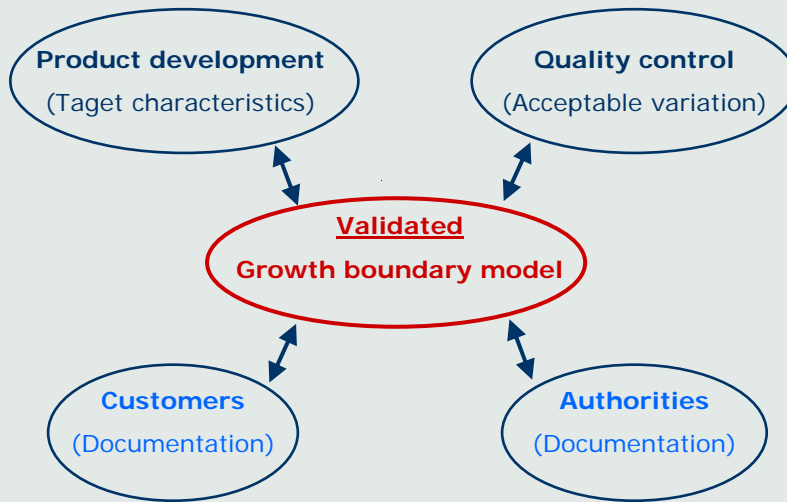
DIFRES-DTU

Product characteristics and storage conditions	Product 1	Product 2
<i>Listeria monocytogenes</i> , cfu/g	1	1
Storage period, days	28	28
Temperature, °C	5.00	5.00
Water phase salt, %	4.00	4.00
pH	6.10	6.00
Water phase lactate, mg/l	8000	8000
Smoke components (phenol, mg/l)	8.0	8.0
% CO <sub>2</sub> in headspace at equilibrium	0.0	25.0
Water phase diacetate, mg/l	0	1200
Nitrite, mg/kg	0	0





*Listeria monocytogenes*



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*Listeria monocytogenes*

**Ready-to-eat seafood:**

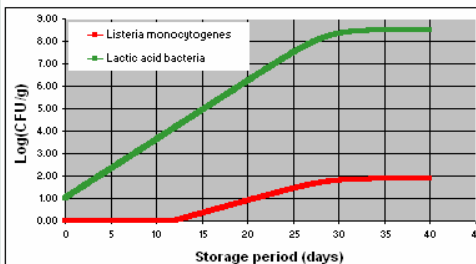
- Lactic acid bacteria has an important inhibiting effect on growth of *L. monocytogenes* (the Jameson effect)
- The anti-listerial effect of LAB can be predicted for many lightly preserved seafoods

Mejlholm and Dalgaard (2007)  
*J. Food Prot.* 70, 2485-2497

DIFRES-DTU

**Product characteristics and storage conditions**

<i>Listeria monocytogenes</i> , cfu/g	1
Lactic acid bacteria, cfu/g	10
Storage period, days	40
Temperature, °C	5.0
Salt in water phase of product, %	4.00
pH	6.10
Lactic acid i water phase of product, mg/l	7000
Smoke intensity (phenol, mg/kg)	8.0
% CO <sub>2</sub> in headspace gas at equilibrium	25.0
Diacetate in water phase of product, mg/l	0
Nitrite, mg/kg	0



Outline: Microbial safety and quality of aquatic muscle food

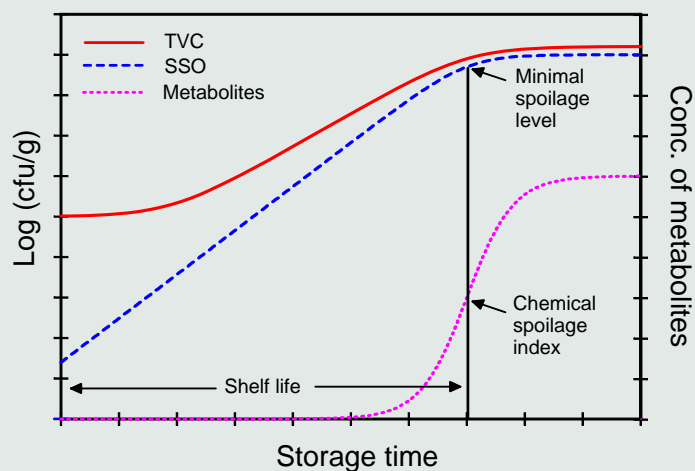
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Microbial spoilage

Specific spoilage organisms (SSO) and indices of quality/spoilage



DIFRES-DTU

Dalgaard, 1993

Lyngby

## Spoilage microflora for groups of fish and seafood products

Products	Spoilage bacteria	Metabolites
<b>Fresh, chilled and aerobic storage:</b>		
High TMAO conc. and pH >6	<i>Shewanella</i> spp.	TMA, H <sub>2</sub> S, sulphur compounds, hypoxanthine
Low TMAO conc. or low pH	<i>Pseudomonas</i> spp.	NH <sub>3</sub> , estere, sulphur compounds but not H <sub>2</sub> S
<b>Fresh, chilled and modified atmosphere packed:</b>		
Marine products with TMAO	<i>Photobacterium phosphoreum</i>	TMA, biogenic amines, hypoxanthine
Varmwater, low TMAO conc.	Lactic acid bacteria and <i>Brochothrix thermosphacta</i>	NH <sub>3</sub> , acetic acid, tyramine, diacetyl, acetoin, H <sub>2</sub> S
Tropical freshwater fish	<i>Aeromonas</i> spp. ?	?
<b>Fresh and lightly preserved seafood at ambient temperature</b>		
	<i>Aeromonas</i> spp, <i>Vibrio</i> spp./ <i>Photobacterium</i> spp and Enterobacteriaceae	TMA, sulphur compounds, biogenic amines
	<i>Enterococcus faecalis</i>	NH <sub>3</sub> , acetic acid, tyramine, diacetyl, acetoin, sulphur compounds?
<b>Lightly preserved and chilled products</b>		
	Lactic acid bacteria and <i>Brochothrix thermosphacta</i>	NH <sub>3</sub> , acetic acid, tyramine, diacetyl, acetoin, H <sub>2</sub> S
	<i>Photobacterium phosphoreum</i> , <i>Vibrio</i> spp.and Enterobacteriaceae	TMA, biogenic amines, alcohols, aldehydes and sulphur compounds

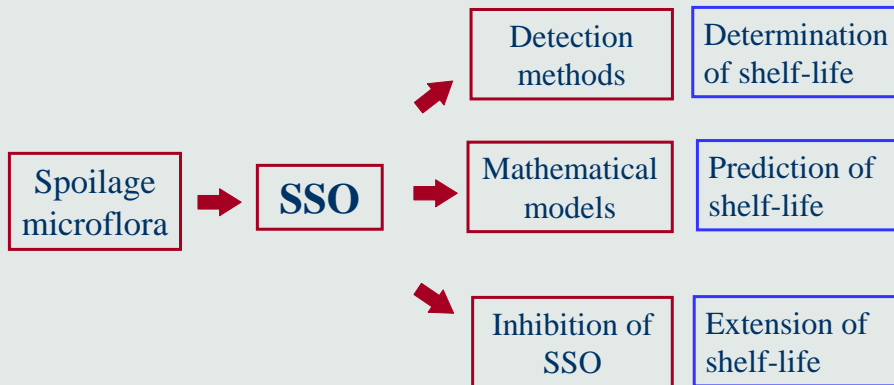
### Black colonies in Iron Agar

#### H<sub>2</sub>S-producing *Shewanella* from chilled fresh fish: *S. baltica*

Name of bacterium	Period	References
<i>Achromobacter</i> spp.	Before 1960	
<i>Pseudomonas</i> group IV or <i>Pseudomonas putrefaciens</i>	1960-1977	Derby and Hammer 1931, Shewan <i>et al.</i> (1960)
<i>Alteromonas putrefaciens</i>	1977-1985	Lee <i>et al.</i> (1977)
<i>Shewanella putrefaciens</i>	1985-1998	MacDonnel & Colwell (1985)
<b><i>S. baltica</i></b>	<b>After 1998</b>	<b>Ziemke <i>et al.</i> (1998)</b> <b>Fonnesbeck Vogel <i>et al.</i> (2005)</b>
<i>S. hafniensis</i>	2005-	Satomi <i>et al.</i> (2006)
<i>S. morhuae</i>	2005-	Satomi <i>et al.</i> (2006)
<i>S. glacialipiscicola</i>	2007-	Satomi <i>et al.</i> (2007)
<i>S. algidipiscicola</i>	2007-	Satomi <i>et al.</i> (2007)

Shelf-life: Determine, predict and extend

The concept of specific spoilage organisms (SSO) –  
potential practical applications



DIFRES-DTU

Dalgaard 2000 and 2006

Lyngby



Shelf-life: Determine, predict and extend

## Time-Temperature Integration Software

- [-] Seafood Spoilage and Safety Predictor (SSSP)
  - [+] Relative rate of spoilage (RRS) models
  - [-] Microbial spoilage models (MSM)
    - [-] Photobacterium phosphoreum
      - ... Fresh MAP cod fillets
      - ... Fresh MAP plaice fillets
      - ... Fresh MAP salmon steaks
    - [+] Shewanella putrefaciens
    - [+] MS models with user-defined parameter values
    - [+] Comparison of observed and predicted data
  - [+] Listeria monocytogenes

DIFRES-DTU

[Http://www.difres.dk/micro/sssp/](http://www.difres.dk/micro/sssp/)

Lyngby



Shelf-life: Determine, predict and extend

Product characteristics

Initial cell density (cfu/g):  Shell-life (days):

Temperature (°C):  Growth rate (µmax, 1/h):

Percentage CO2:



Shell-life prediction for:

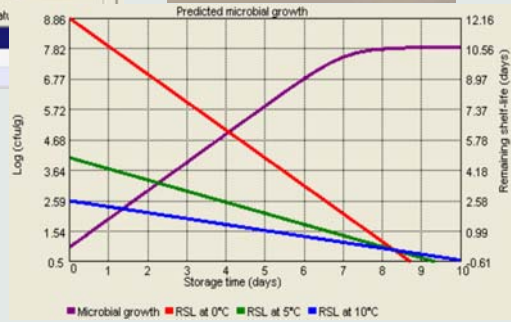
Series of constant temperatures | Temperature profiles from logger data | Calculation of % CO2 i

Remaining shelf-life (hours)

Temperature (°C):  Storage time (hours):

Prediction of remaining shelf-life for a series of constant storage temperatures

Temp. (°C)	Time (h)	0 °C (h)	5 °C (h)	10 °C (h)
0	0	291.8	116.25	62.05
1.8	240	-59.79	-23.82	-12.71



[www.difres.dk/micro/sssp/](http://www.difres.dk/micro/sssp/)

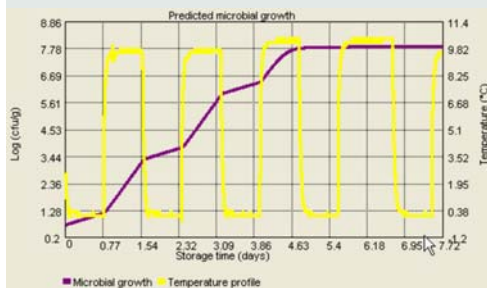
DIFRES-DTU



Shelf-life: Determine, predict and extend

SSSP predicts the effect of product temperature profiles:

- Series of constant temperatures
- Data recorded by temperature loggers



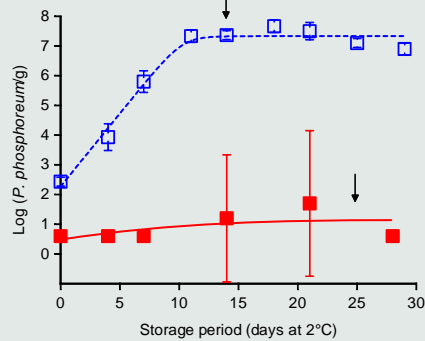
DIFRES-DTU

[Http://www.difres.dk/micro/sssp/](http://www.difres.dk/micro/sssp/)

Lyngby

Shelf-life: Determine, predict and extend

Extension of shelf-life – example: Shelf-life of MAP salmon is increased when *P. phosphoreum* is inactivated by freezing of the the fish raw material



- 14 days shelf-life for fresh MAP salmon at 2°C
- 21-28 days for frozen and thawed MAP salmon at 2°C

DIFRES-DTU

Emborg et al. 2002 Lyngby

Perspectives and challenges

- Importance of psychrotolerant bacteria
  - New studies in different countries
- Detection of pathogens and spoilage microorganisms
  - Molecular methods including real-time PCR
- Predictive microbiology models
  - New models and application software
  - Successful model validation - criteria accepted by authorities
- Reduce growth of microorganisms in aquatic muscle foods
  - Combinations of preserving parameters
  - New preservation technologies (high pressure, pulsed light, etc.) - it remains a challenge to identify optimal areas of application

DIFRES-DTU

Lyngby

Thanks

- Colleagues at DIFRES
- Financial support
  - SEAFOODplus
  - Directorate for Food, Fisheries and Agri Business
- Industry collaboration
  - Royal Greenland Seafood Ltd.
- I thank you for your attention