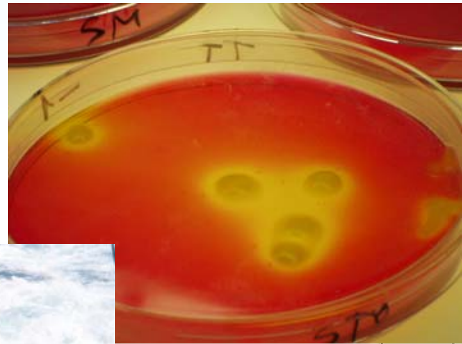
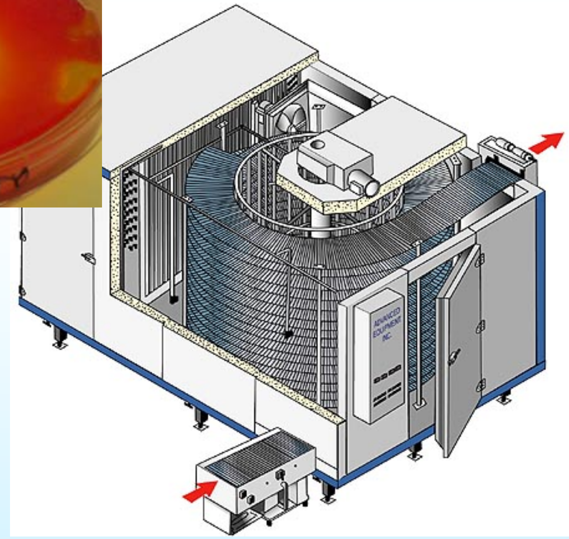
Effect of freezing on fish microbial flora





Goli, T.; Nguyen, T.T. 2004. Effect of freezing on fish microflora. Seminar on Food Safety and International Trade : the French-Thai approach and the EU Regulation, September 9-10, Bangkok, Thailand

Effect of freezing on food

process

Objectives of freezing process :

Bring the thermal centre of food < -10°C asap

Delay the consumption (storage < -20°C)

Cryogenic cold : spray or immersion in LIN

Small quantities

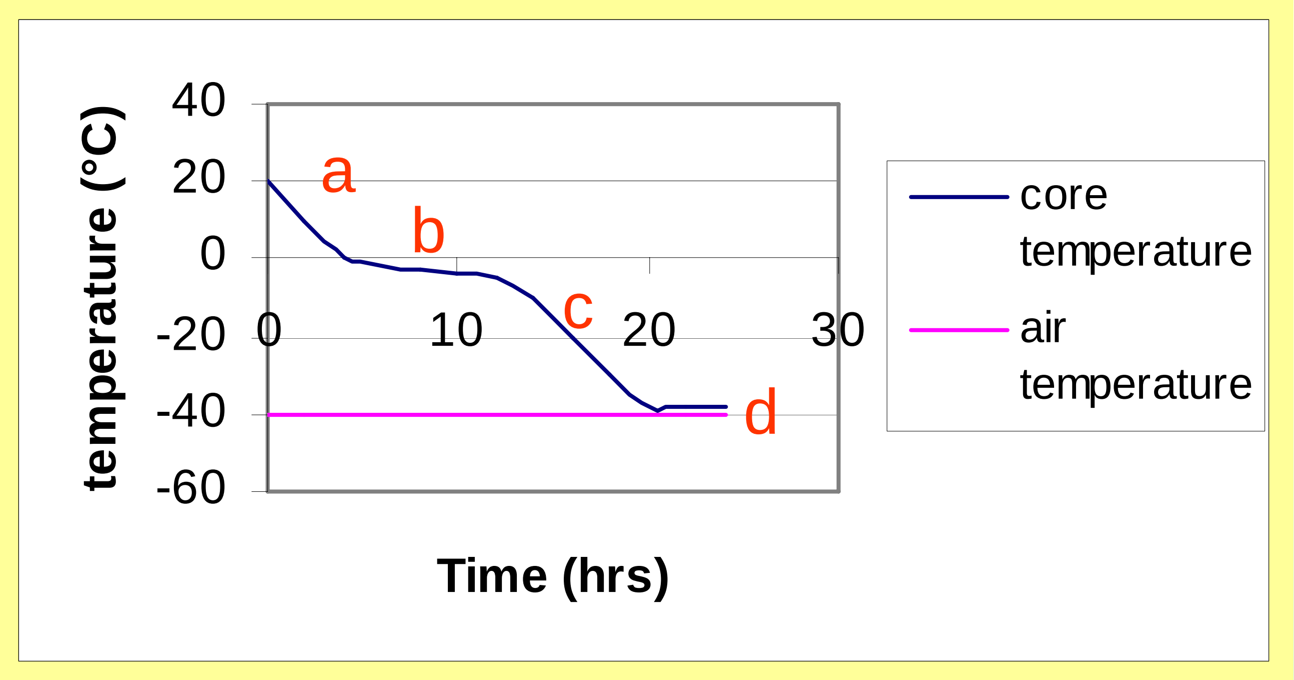
High added value food (IQF)

Mechanical cold : air blast freezing, plate freezing and fluidized bed.

Big quantities, high initial investment.

Effect of freezing on food process

4 steps :



a. 1st T°. fall

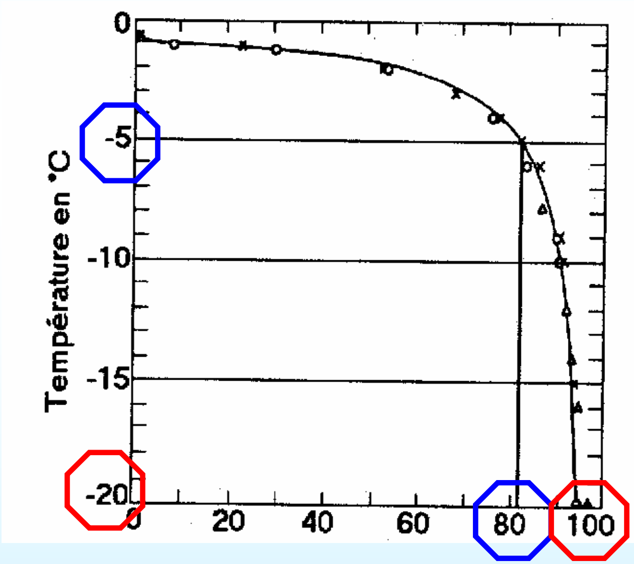
b. Ice formation

c. 2nd T°. fall

d. Thermal equilibrium

Effect of freezing on food

Ice crystallization



% of frozen water/total water
(lean fish)

-5°C : 80% of water frozen as pure water ice

-20°C : 90%

High increase in solutes concentration : osmolarity and ionic strength increase

Ice border moving from outside toward core

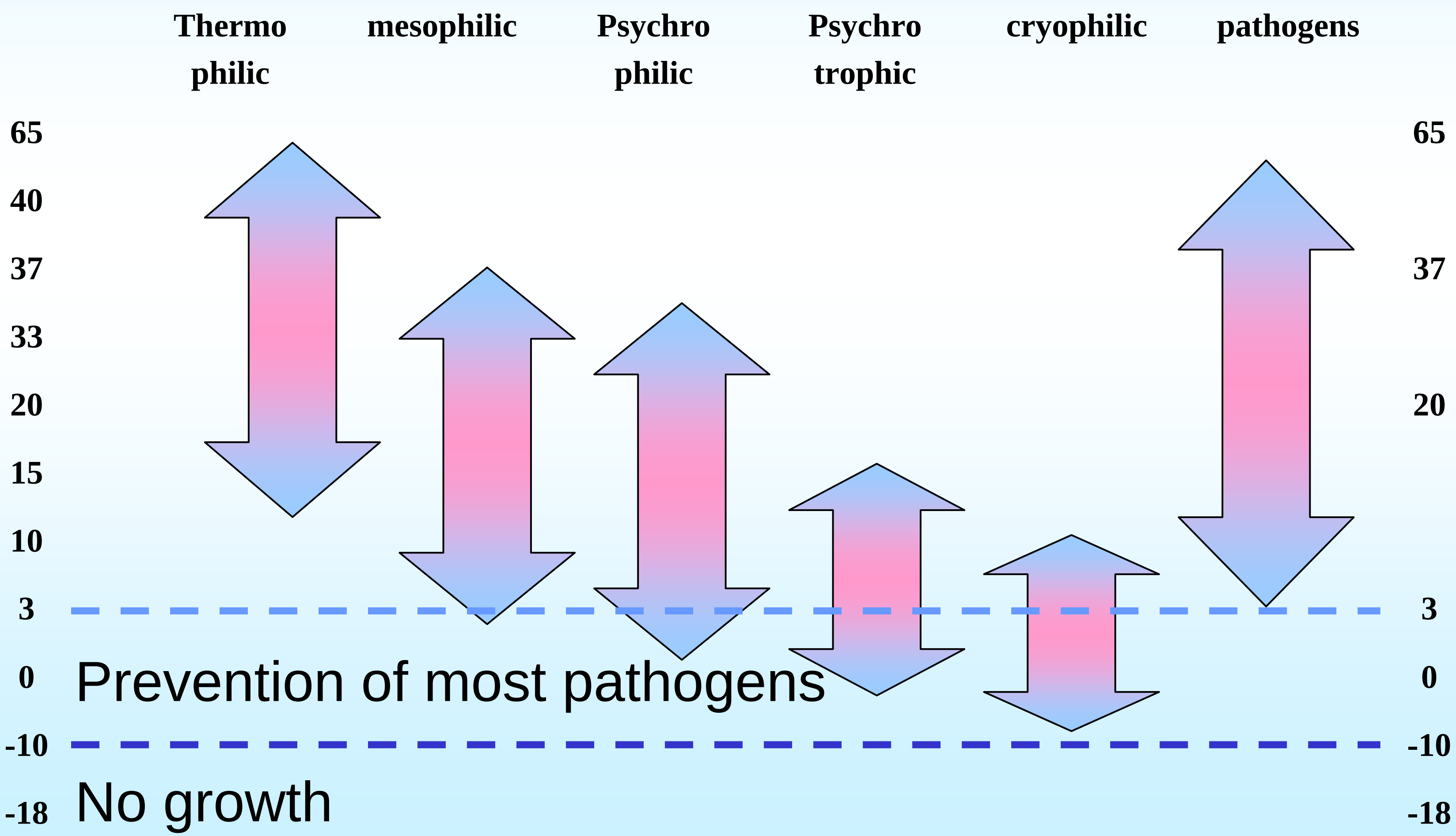
Effect of freezing on food

degradation

- ⇒ **Increase of ice crystals size, enhanced by time, increase and fluctuations in temperature (« Ostwald » maturation)**
- ⇒ **Disruption of cell walls (plants) and membranes (meat) under mechanic stress**
- ⇒ **Dehydration**
- ⇒ **Lipids oxidation : rancidity**
- ⇒ **Proteins denaturation (drip loss, freeze burn)**

Impact of Temperature drop on bacteria in fish

Bacteria growth temperature domains



Impact of Temperature fall on bacteria in food

- **Survival of lactic flora, > optimisation of industrial process for cheesemaking (lactic starters)**

- **Little interest in food safety :**
  No bacteria totally killed by freezing (1% – 90%)
  The remaining flora will quickly grow during thawing

- **Arrhenius :** *degradation rate = $a(T\text{-}To)^2$*
  Fall of rate of all chemical and enzymatic reactions

Impact of Temperature fall on bacteria in food

Mechanisms of freeze damage

Accumulation of cellular damages leading to stress and death, due to :

- Extracellular ice formation
- Intracellular ice formation
- Concentration of extracellular solutes
- Concentration of intracellular solutes
- Low temperature

Effet of freezing on bacteria : influent factors

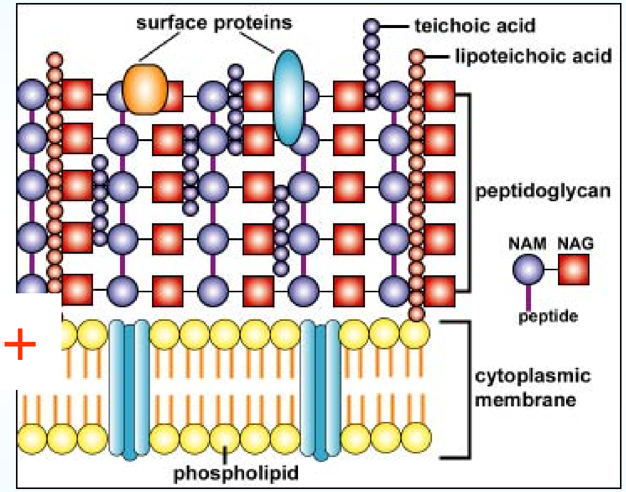
1. **Bacterial type and strain**
2. **Cell age**
3. **Growth conditions before freezing**
4. **Nature of suspending medium (fish/meat/vegetables)**
5. **Freezing and thawing conditions**
6. **Storage conditions**
7. **Culture medium**

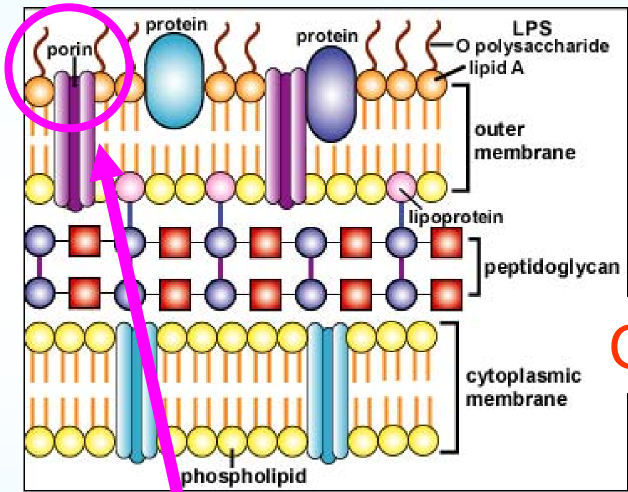
Effet of freezing on bacteria : influent factors

1. Bacterial type and strains

Cell Enveloppe composition

Gram +





Gram -

Gram + more resistant to freezing than Gram -

- **Gram - : the more resistant strains show OMPF porin protein, responsible of big pores in cell wall whereas less resistant show OMPC proteins (narrow pores) = less permeability**

- **Gram + : thiner enveloppe : more permeability at low temperatures**

Effet of freezing on bacteria : influent factors

1. Bacterial type and strains

Cryophilic and psychrophilic **more resistant, because of adaptation mechanisms (membrane fluidity and permeability)**

Spores **much more resistant than vegetative form**

Salmonella spp. **resistant among G-, but very susceptible to repeated freeze/thaw cycles.**

C. Jejuni **suscpetible to freeze/thaw cycle. In Danemark and Iceland, freeze thaw use to reduce the risk in positive tested poultry carcasses (25 fold)**

Effet of freezing on bacteria : influent factors

1. Bacterial type and strains

Vibrio spp. **known for sensitivity to freezing.**

- ***V. Vulnificus* in oysters : counts reduced by 2-5log By freezing**
- ***V. para*. In oyster homogenate : 5 log reduction**

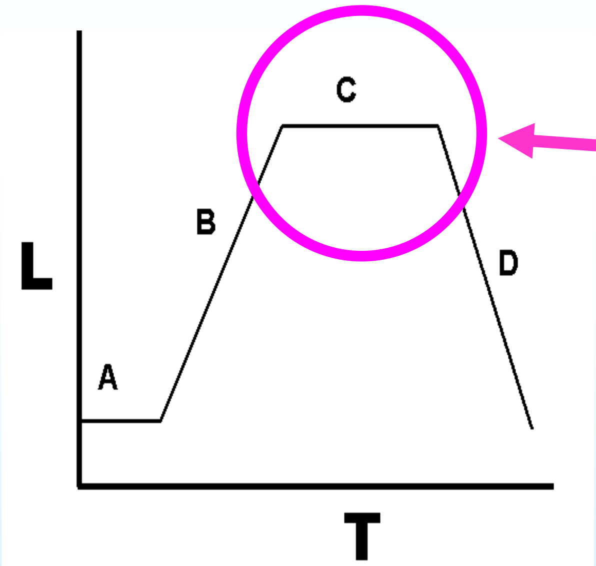
Pseudomonas, Micrococci, Staphylococci and Streptococci known to be resistant to freeze/thaw and storage.

High variability in susceptibility to cold injury between strains.

Understanding high sensitivity to freezing would be helpful i dealing with resistant micoorganisms

Effet of freezing on bacteria : influent factors

2. Cell age



Stationary phase : greater resistance to freezing than exponential phase.

Effet of freezing on bacteria : influent factors

3. Growth conditions before freezing

Cold storage before freezing > increased resistance

Vibrio spp. : non culturable state induced by storage (VNC) at 4°C > enhanced resistance to lethality caused by freezing.

Cold shock adaptation mechanisms :

Membrane lipids modifications:

- **maintain membrane fluidity for nutrient uptake**

E.coli **:**	**C16:0 ↘**	**C18:1 ↗**
L. monocytogenes **:**	**C17:0 ↘**	**C15:0 ↗**

Effet of freezing on bacteria : influent factors

3. Growth conditions before freezing

Production of cold-shock proteins/polypeptides

Production of new specific proteins, of activation of the production of existing proteins in the cytoplasme, or in the membrane. (16 in E.coli for a T° shift of 13°C or more.

Supposed to aid to resist to very low temperature (protection against recrystallisation?).

Uptake of compatible solutes (i.e. betaine, proline and carnitine –osmoprotective / cold adaptation)

Effet of freezing on bacteria : influent factors

4. Nature of food matrix

Fat content :

antagonisms between free fatty acids (antimicrobial, more active on Gram+) vs. glycerol/glycerides (protective)

	Fat level (%) (beef mince)	5	10	20	30	50
% surv.*						
Salm. kent.		13	18	20	25	10
S. aureus		22	24	75	55	28

***(storage 10 wks at -35°C)**

Effet of freezing on bacteria : influent factors

4. Nature of food matrix

Meat type :

Salmonella survival in:	chicken 26%	bacon 10%	lean beef 10%
S. Aureus	beef mince 96%	bacon 42%	pork mince 79%

Cold adaptation (E.coli 0157:H7) : non effect on beef and pork, but induces enhanced protection on milk, whole egg, sausage.

pH :

Susceptibility to freezing injury enhanced by acidity of food matrix. *(L. monocytogenes* for instance)

Not easy to compare (temperature, time, growth phase, strain…)

Effet of freezing on bacteria : influent factors

5. Freezing and thawing rates

Freezing rate :
fast rate > small crystals > increased survival

Thawing rate :
fast rate > small crystals > increased survival

large ice crystals formation > bacteria injuried

But!!!
Once the product has been thawed, bacteria begin to reproduce again

Effet of freezing on bacteria : influent factors

6. Storage conditions

Time :

Slow or no decrease with time at constant temperature.
In some cases most of the lethality occurs in the first 7 days of storage, then constant count. (C. jejuni, beef trimmings, -18°C)
In most cases, 0 – 99% destruction during 1 year.

Temperature :

Low storage temperature aid microbial survival.
Low survival between –5 and –10°C.
Counts virtually constant < -30°C
(Salmonella found to decrease more at -20° than -35°C on salmon)

Effet of freezing on bacteria : influent factors

6. Storage conditions

Temperature fluctuations :

Slow decrease with time because of Ostwald maturation. May occur in case of open display cabinets : slight decrease or constant.

Freezing/thawing cycles :

Slight decrease (1-2 log.) with cycles is a function of initial count.

But quality of the food is degradated a lot before sanitation effect is significant.

Effet of freezing on bacteria : influent factors

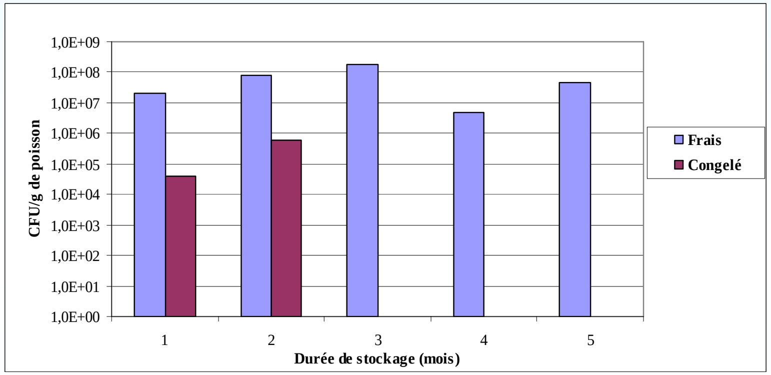
7. Culture medium

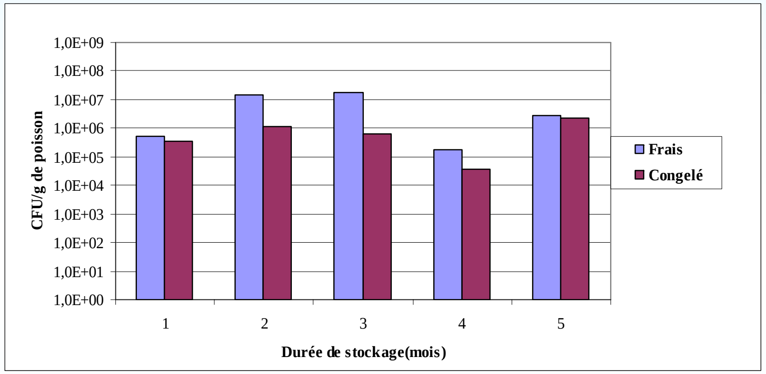
Selective media : under-estimation of count

- 3 E.coli strains in beef trimmings, -18°C, 12 wks
  - Constant count on non selective media (TSA)
  - 2 log. Decrease on Mc Conkey (selective)
- bacteria highly stressed, viable, but not culturable state (between death and life = VNC)
- Necessity of resuscitation step before culture, or detection techniques based on DNA/RNA (PCR-DGGE)

Example on enterobacteria (XLD medium)

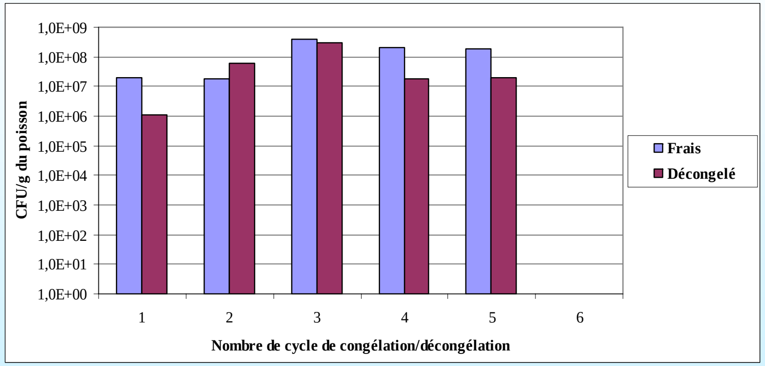
Effect of frozen conditions on enteric bacteria (/XLD)





daily
Fluctuations
-5 to -20°C
1 to 5 months

Time duration
1 to 5 months



Repetition of freezing/thawing cycles

Initial count

After process

CONCLUSION

Bacteria are not totally killed by freezing, whatever the strain, the type, etc…

- **Oustanding initial quality of fish is essential**
- **Freezing cannot be considered separately of thawing**
- **Thawing and storage conditions may lead to high loads within a short while**
- **Food must be processed as soon as possible after thawing**

Reduction of pathogens and spoilage bacteria in fish by freezing is not of practical importance

CONCLUSION

Future research needs

- Better understanding of interactions food - microoganisms
- Optimal conditions for major pathogens destruction
- Relationship growthphase – freeze/susceptibility
- Understanding VNC state and adaptation proteins formation
- Understanding the genetics of stress response
- Extracellular sensing components and intercellular signaling molecules

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