

Magic solutions to bacterial problems in early life stages: Do they exist?

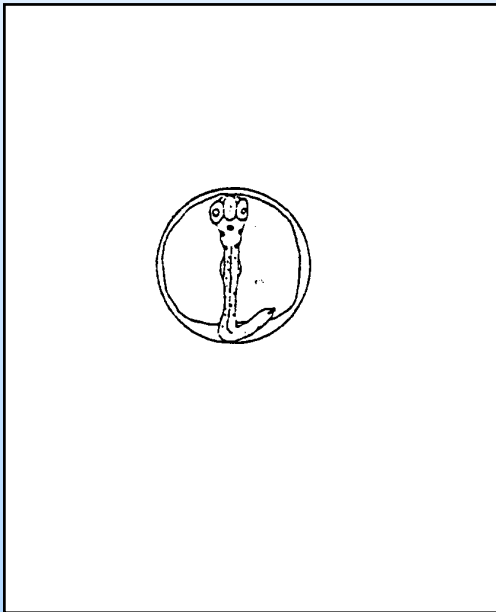
Ingrid Salvesen, Jorunn Skjermo and Olav Vadstein

SINTEF Fisheries and Aquaculture, Norway

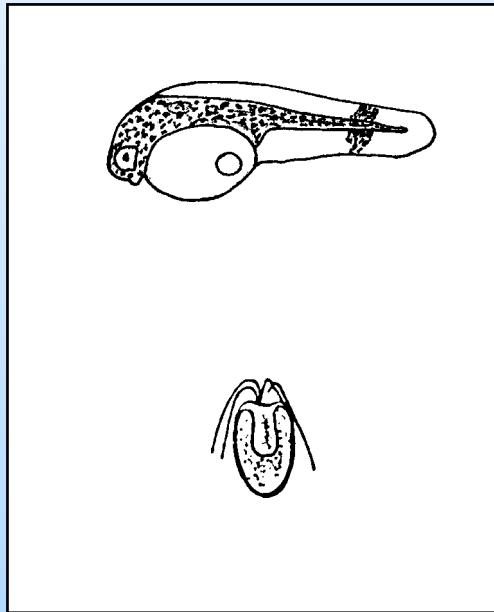
NTNU, Trondhjem Biological Station, Norway

MICROBIAL CONDITIONS

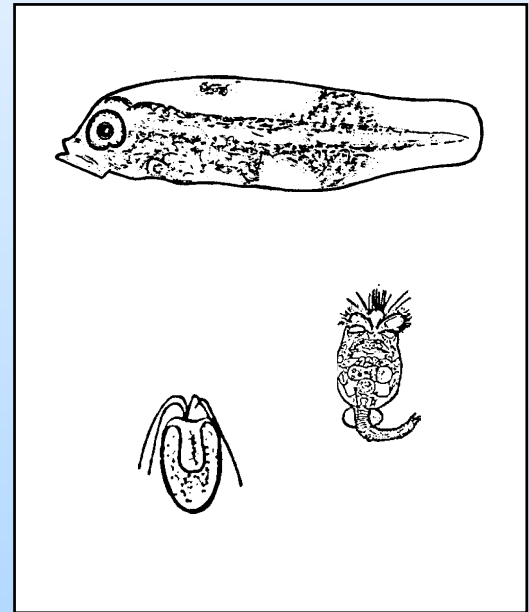
EGG



YOLK SAC



FIRST FEEDING



two-step

three-step

Physical, chemical factors: temp., exchange rates, water quality...

MICROBIAL CONTROL??

- It is a **dynamic** system!
- High reproductive rates and metabolic versatility - high speed of adaptive changes
- Short generation times - large population fluctuations
- Capacity for dormancy
- Beneficial mutations can be rapidly established by natural selection
- Various strategies for survival:
 - Not resource limited - high growth rates
 - Resource limited - many competitive adaptations



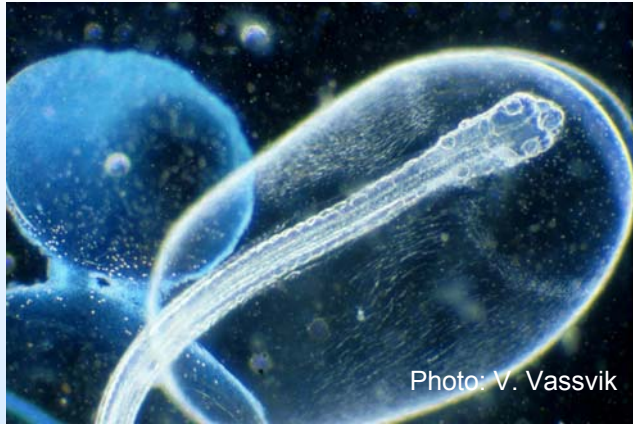
MICROBIAL CONDITIONS



Photo: V. Vassvik

Early life stages

- Sterile at hatching - the importance of the bacterial load and the composition of the ambient water for the establishment of a primary protective microflora
- Bacteria and microalgae ingested at the yolk sac stage
- Differences between marine fish species (short vs long yolk sac period, clear vs green water)
- Exposure to stressors in early life stages - adverse effects later in development



HATCHING

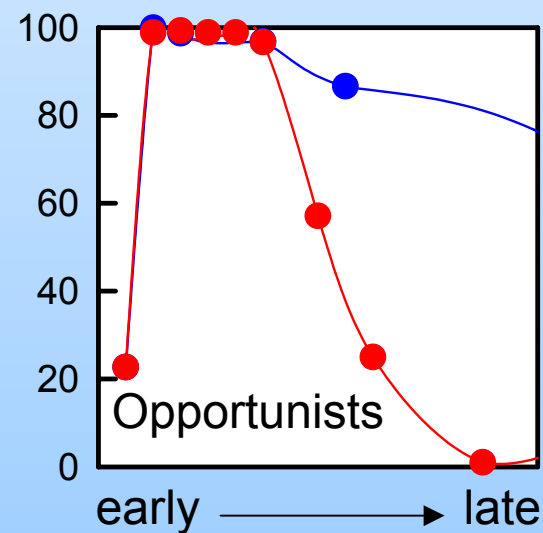
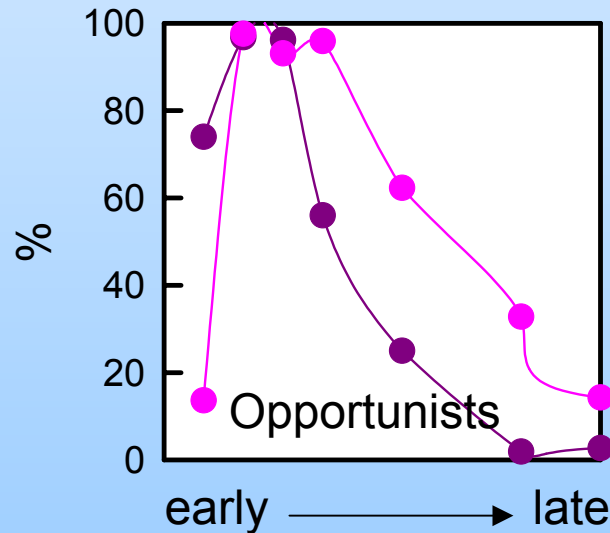
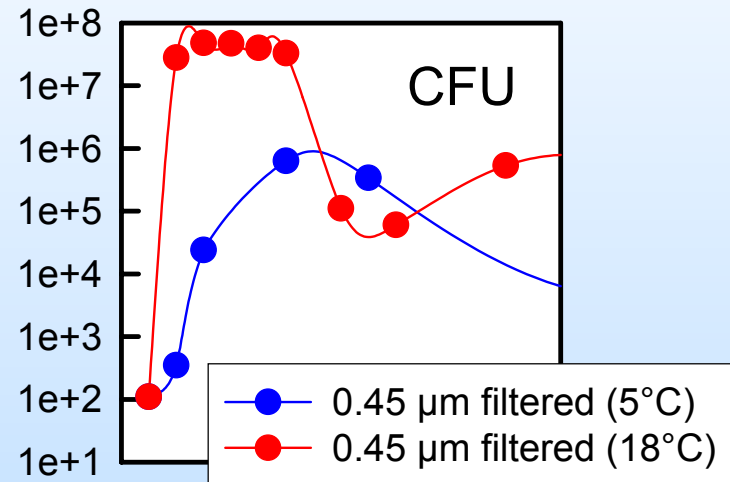
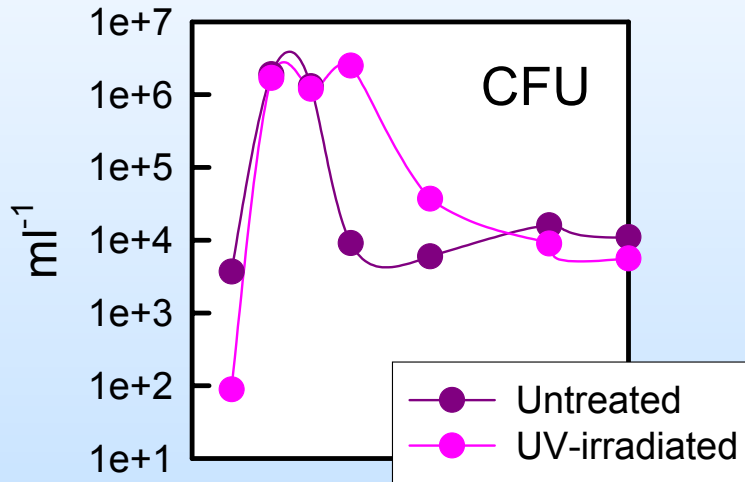
Enhanced organic loading

CARBON BUDGET

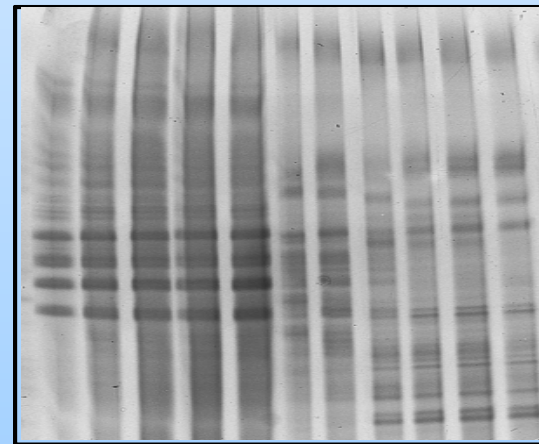
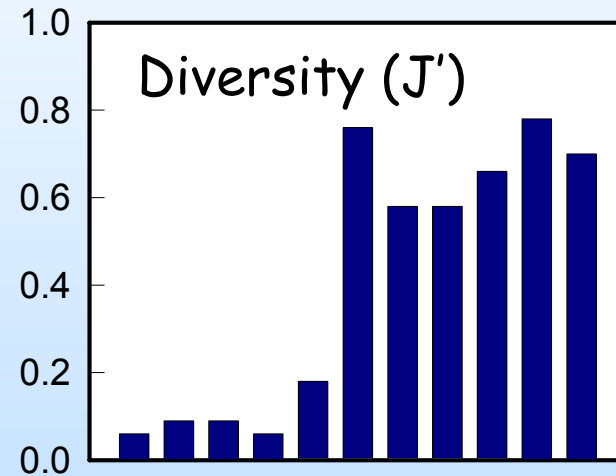
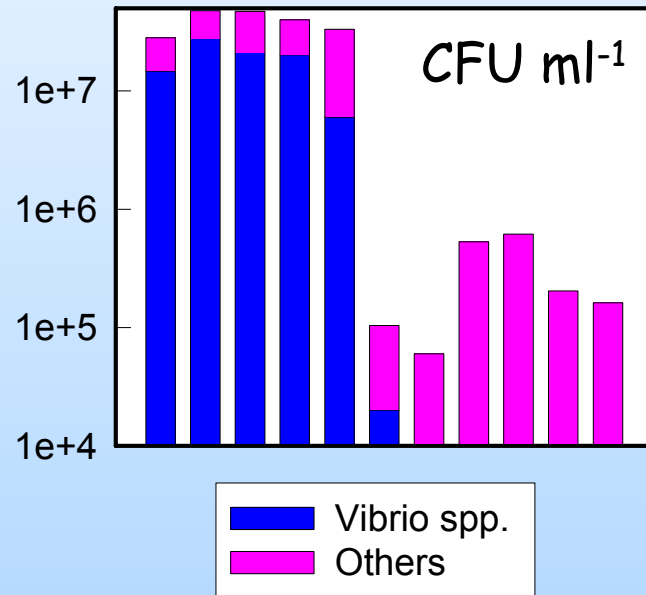
| | $\mu\text{g C egg}^{-1}$ | % |
|--------------------|--------------------------|------|
| Larvae | 552 | 65.0 |
| Chorion | 12 | 1.4 |
| Estimated DOC loss | 286 | 33.7 |

Dahlø et al., unpublished results

No bacteria introduced, only substrate...



No bacteria introduced, only substrate...



DGGE

early → late in succession

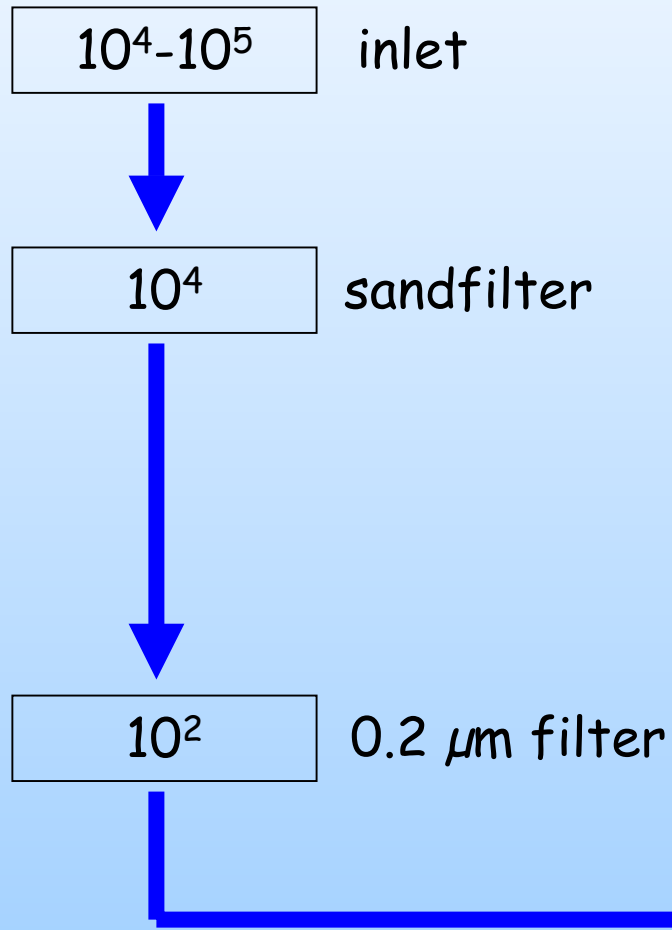
Beat them
or
join them?

The Unknown Enemy

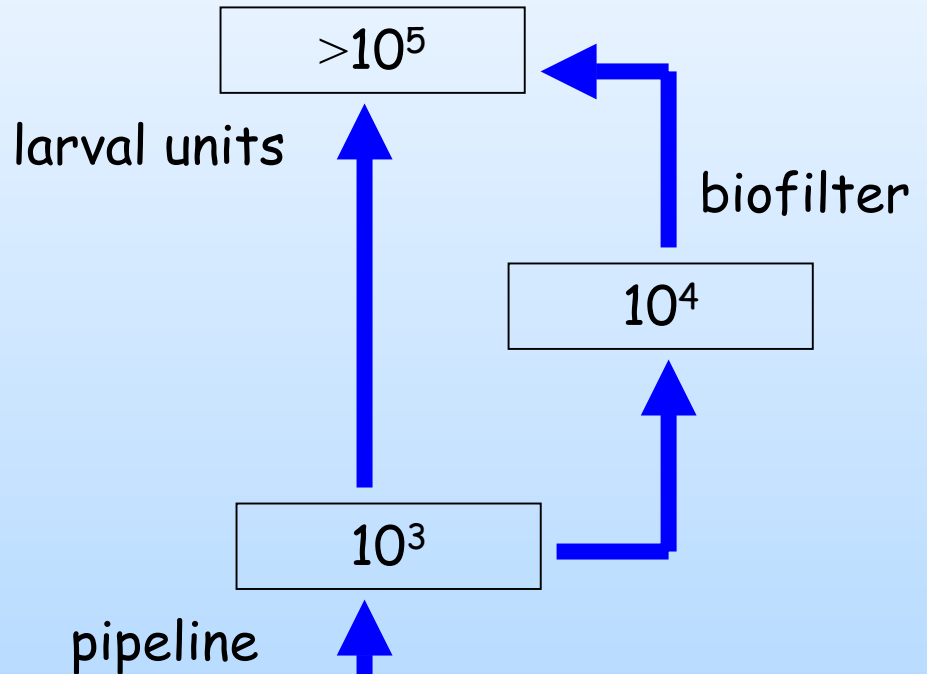


Fig. 2. Protective clothing of the kind worn by plague doctors during the seventeenth century.

REDUCTION



RECOLONIZATION



'To culture fish
one has to
culture the water'
Chinese saying

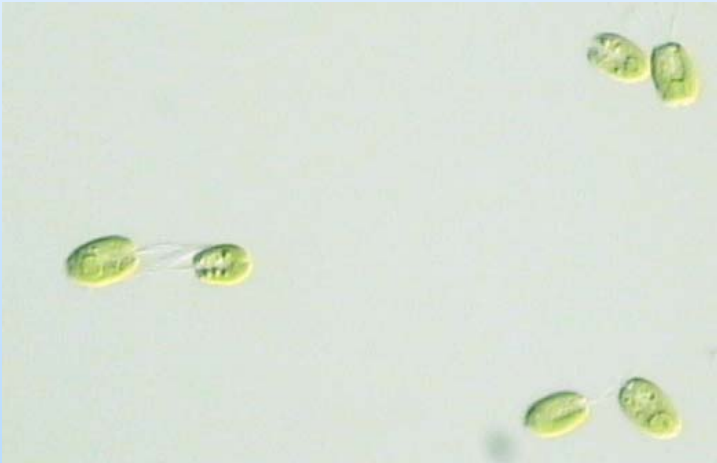
RECOLONIZATION

A probiotic approach?

MICROALGAE - green water technique

ALGAE

Interactions with bacteria



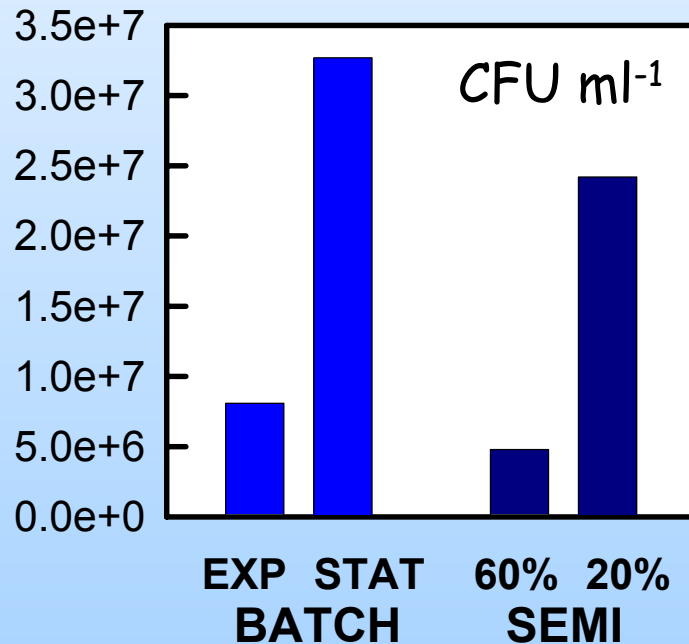
- stimulate growth by release of dissolved organic carbon
- inhibit growth through production of toxic metabolites
- interfere with the expression of traits important for virulence, colonization

A wide range of mechanisms by which algae may regulate bacterial communities

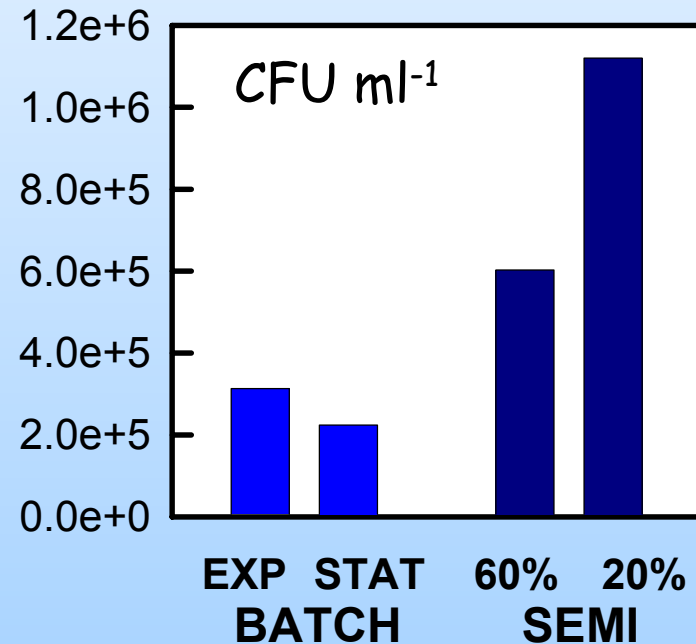
BACTERIAL LOAD

Isochrysis galbana

Associated with algal culture



Added to obtain a algal density of 100 cells μ l⁻¹ in larval culture



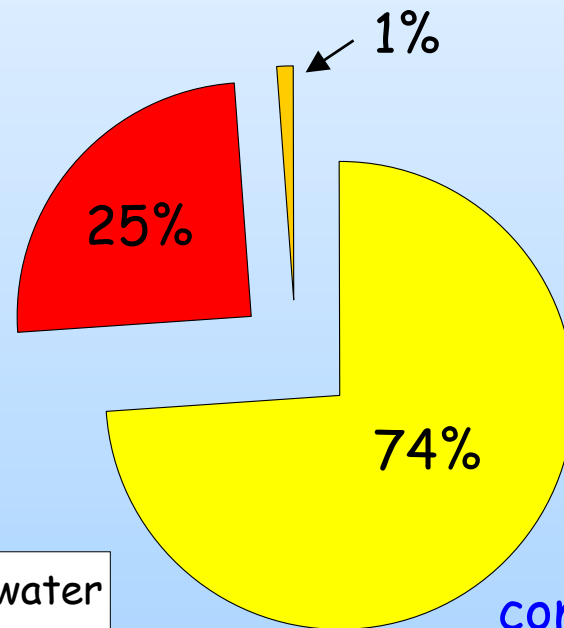
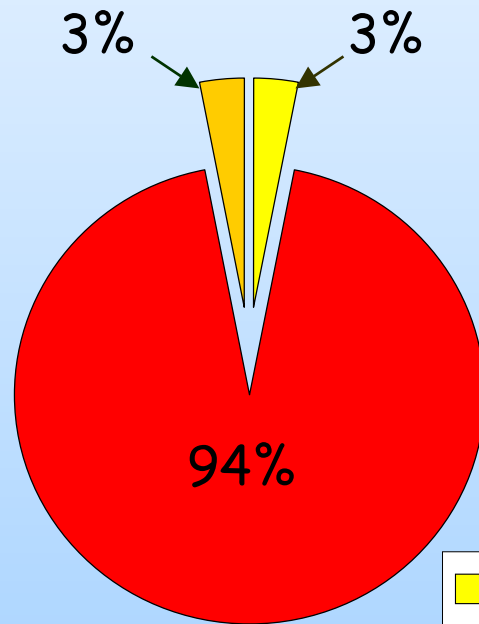
No (batch) or very low levels (< 0.01%, semi-continuous) of *Vibrio* spp.

Contribution of bacteria

bacterial level
in inlet water:

1×10^3

1×10^5



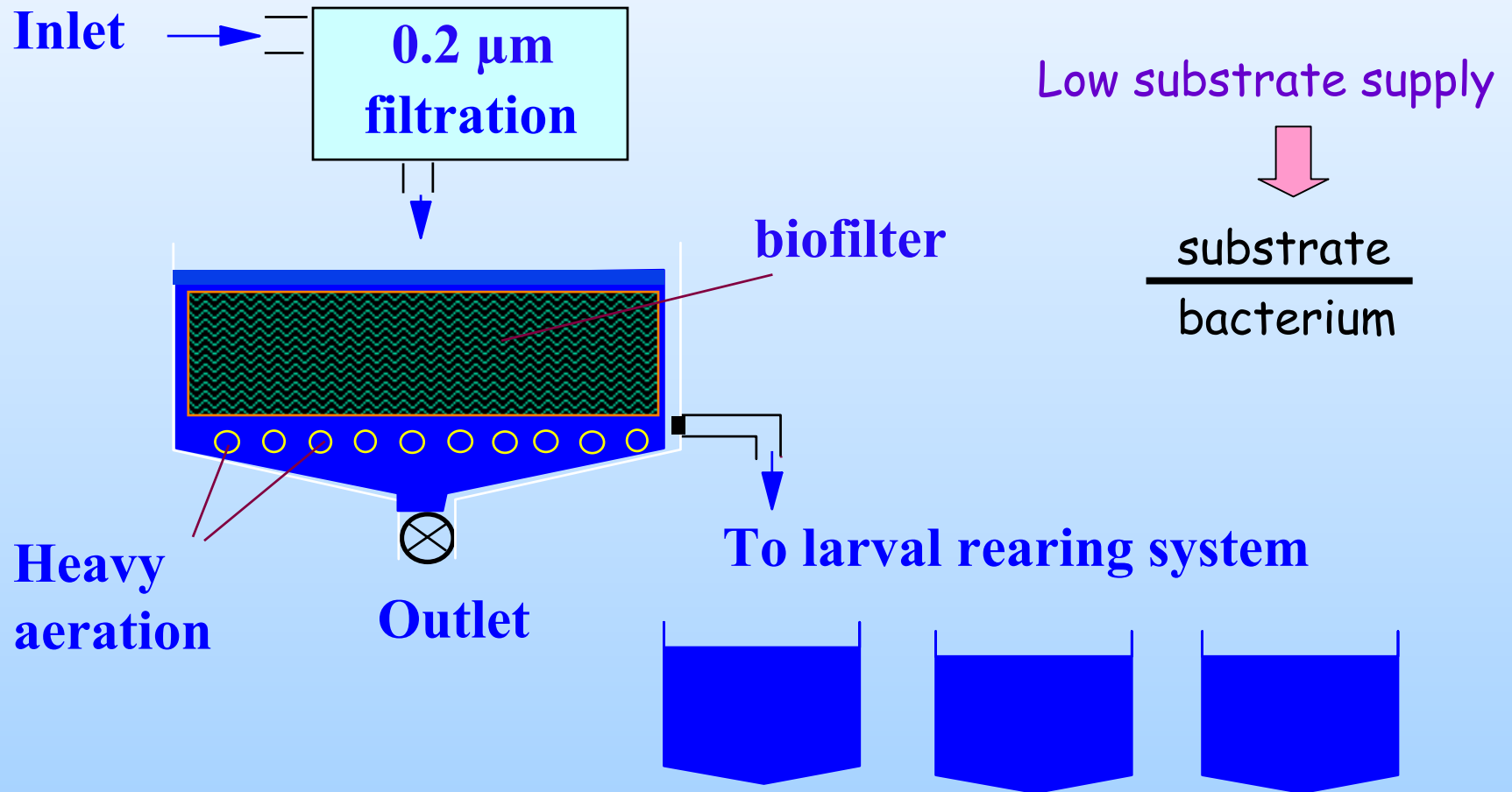
contribution
from different
sources into the
system

RECOLONIZATION

A probiotic approach

WATER TREATMENT - biofilter technology

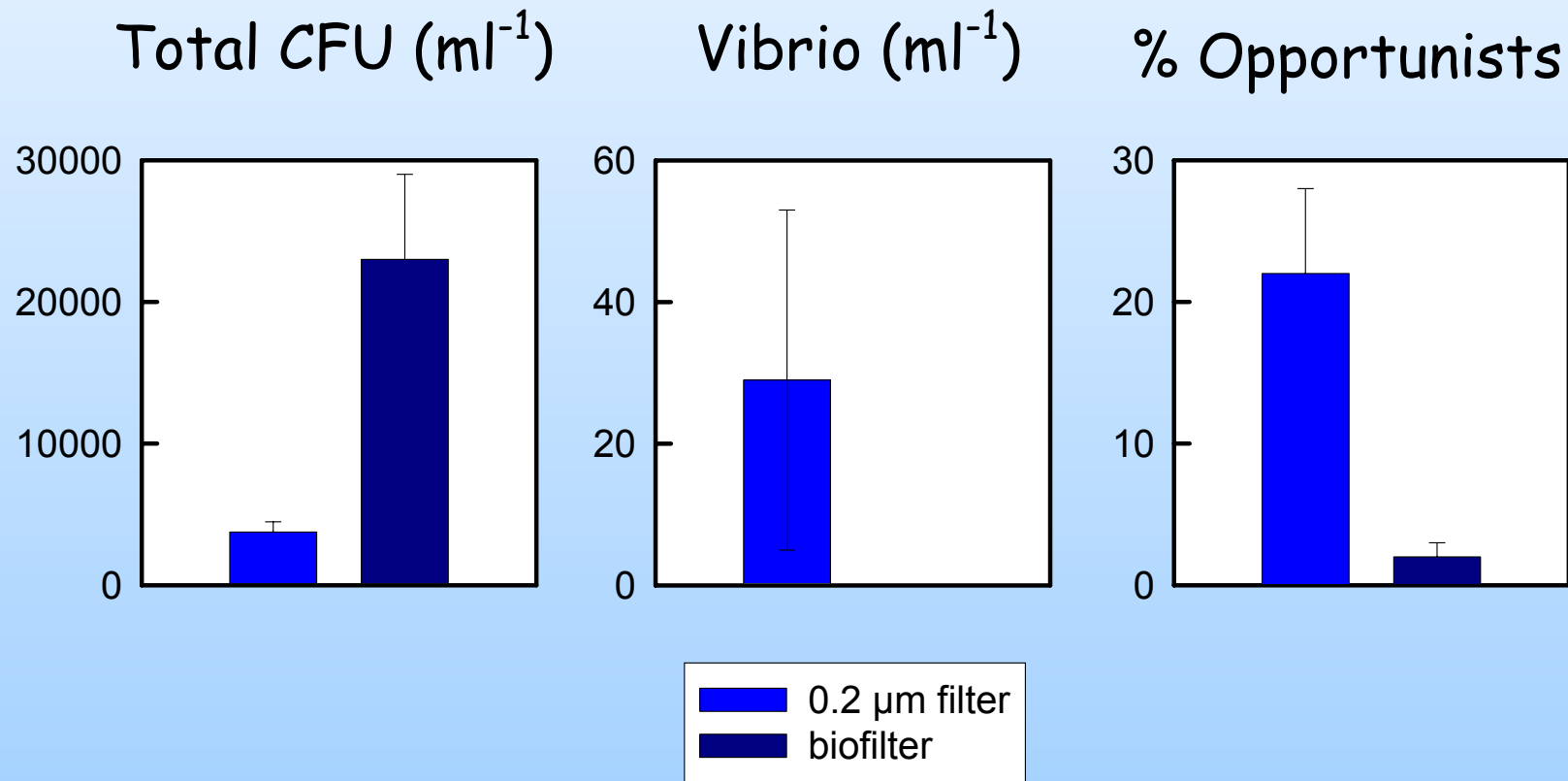
BIOFILTER (flow-through)



Skjermo et al., 1997

RECOLONIZATION IN BIOFILTER

A magic solution?



TWO WATER QUALITIES

0.2 μm filter and biofilter

How is the bacterial community of the water affected by enhanced organic loading in combination with microalgal addition?

ORGANIC LOADING

| | Total CFU | | Vibrio | | % Opport. | |
|-------------|------------------|------------------|------------------|------------------|-----------|-----------|
| | 0.2 µm | biofilter | 0.2 µm | biofilter | 0.2 µm | biofilter |
| Clear water | $2.1 \cdot 10^7$ | $1.3 \cdot 10^6$ | $4.0 \cdot 10^6$ | $9.5 \cdot 10^3$ | 100 | 99 |

Low levels of opportunistic bacteria (0.3-7%) and no *Vibrio* spp. observed in both water qualities before organic loading

Everything is everywhere,
the environment selects

M.W. Beijerinck

ORGANIC LOADING

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| Green water | | | | | | |
| <i>Isochrysis</i> | $2.5 \cdot 10^7$ | $9.8 \cdot 10^5$ | $5.4 \cdot 10^4$ | $1.2 \cdot 10^3$ | 98 | 69 |
| <i>Pavlova</i> | $2.0 \cdot 10^7$ | $5.4 \cdot 10^6$ | $2.0 \cdot 10^6$ | $3.0 \cdot 10^2$ | 88 | 8 |

ORGANIC LOADING

| | Total CFU | | Vibrio | | % Opport. | |
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| | 0.2 μ m | biofilter | 0.2 μ m | biofilter | 0.2 μ m | biofilter |
| Clear water | $2.1 \cdot 10^7$ | $1.3 \cdot 10^6$ | $4.0 \cdot 10^6$ | $9.5 \cdot 10^3$ | 100 | 99 |
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| <i>Chaetoceros</i> | $1.5 \cdot 10^7$ | $2.4 \cdot 10^7$ | $1.3 \cdot 10^6$ | $1.9 \cdot 10^5$ | 60 | 19 |
| Mix (1:1:1) | $1.4 \cdot 10^7$ | $1.3 \cdot 10^7$ | $8.2 \cdot 10^5$ | $5.9 \cdot 10^5$ | 83 | 44 |

Relatively high bacterial densities and large proportions of opportunistic bacteria are associated with cultures of Bacillariophyceae (e.g. *Chaetoceros* sp.)

CONCLUSIONS

- The significance of microbial conditions in early life stages
 - exploit growth potential
- Important differences in response to enhanced organic loading
- No magic solutions, but large potentials for microbial management
 - What is removed, will be replaced.....