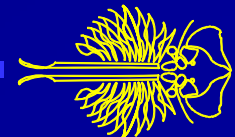


# Effect of feed type and probiotic bacteria on different rotifer biotypes and the possible relation to crashes

**EU funded project:** Genetic implications in the production of rotifers in commercial finfish hatcheries (Q5RS-2002-01302)

Rotifer workshop: 9 September 2005  
Ghent University



## Project partners:

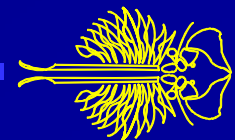
Laboratory for Aquaculture & Artemia Reference Center  
Ghent University, Belgium

Sea Fisheries Department, Ministry of Agriculture, Ostend,  
Belgium

Department of Genetics, Development and Molecular Biology  
Aristotle University of Thessaloniki, Greece

Norwegian University of Science and Technology, Trondheim,  
Norway

Maricoltura di Rosignano Solvay srl, Livorno, Italy



# Feeds currently used for rotifer production

Basically: split in 2 groups

## ALGAE

Fresh

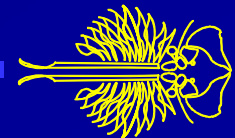
Paste's

Spray dried

## YEAST's

Fresh

Spray dried

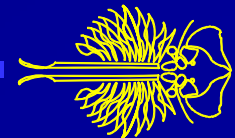


# Is one feed type better suited for a particular rotifer biotype?

3 rotifer biotypes: *B. plicatilis* s.s. } all 'L-type'  
B. p. 'Cayman' }  
B. p. 'Nevada' }

Feeds: *Nannochloropsis oculata* paste  
CS-3000

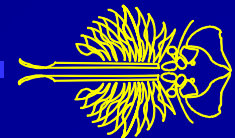
Batch cultures (3 days)  
25°C/25 ppt, continuous light



## Results: Daily growth rates

Batch	<i>N. oculata</i> paste			CS-3000		
	Cayman	B.p s.s.	Nevada	Cayman	B.p s.s.	Nevada
1	0.46 ± 0.06	0.53 ± 0.02	0.41 ± 0.03	0.20 ± 0.06	0.42 ± 0.04	0.21 ± 0.08
2	0.44 ± 0.03	0.43 ± 0.03	0.26 ± 0.05	0.28 ± 0.04	0.39 ± 0.10	0.15 ± 0.07
3	0.39 ± 0.06	0.39 ± 0.01	0.49 ± 0.08	0.22 ± 0.05	0.32 ± 0.00	-0.01 ± 0.03
4	0.44 ± 0.03	0.49 ± 0.10	0.50 ± 0.09	0.23 ± 0.05	0.20 ± 0.03	0.00 ± 0.25

Conclusions: Good growth of all biotypes on *Nannochloropsis paste*  
 Lower growth on CS-3000  
 B.p. s.s. : best growth on CS-3000 of the 3 biotypes  
 Nevada biotype: problems on CS-3000



# Does a variable feeding regime have an effect on rotifer growth rate and diversity in batch cultures?

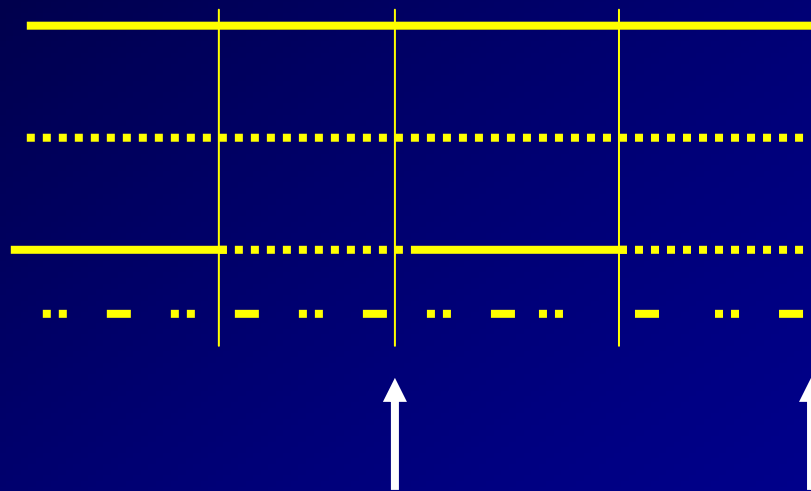
Experiment: Effect of feed type and feeding regime

Inoculation: 100 rotifers+eggs/ml (3 biotypes: *B. plicatilis* s.s., Cayman, Nevada)

Rotifers preconditioned to the feeds

→ A majority of comm. hatcheries provides a mixture of feeds (Questionnaire)

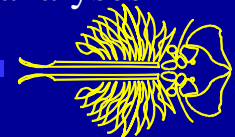
Experiment lay out:



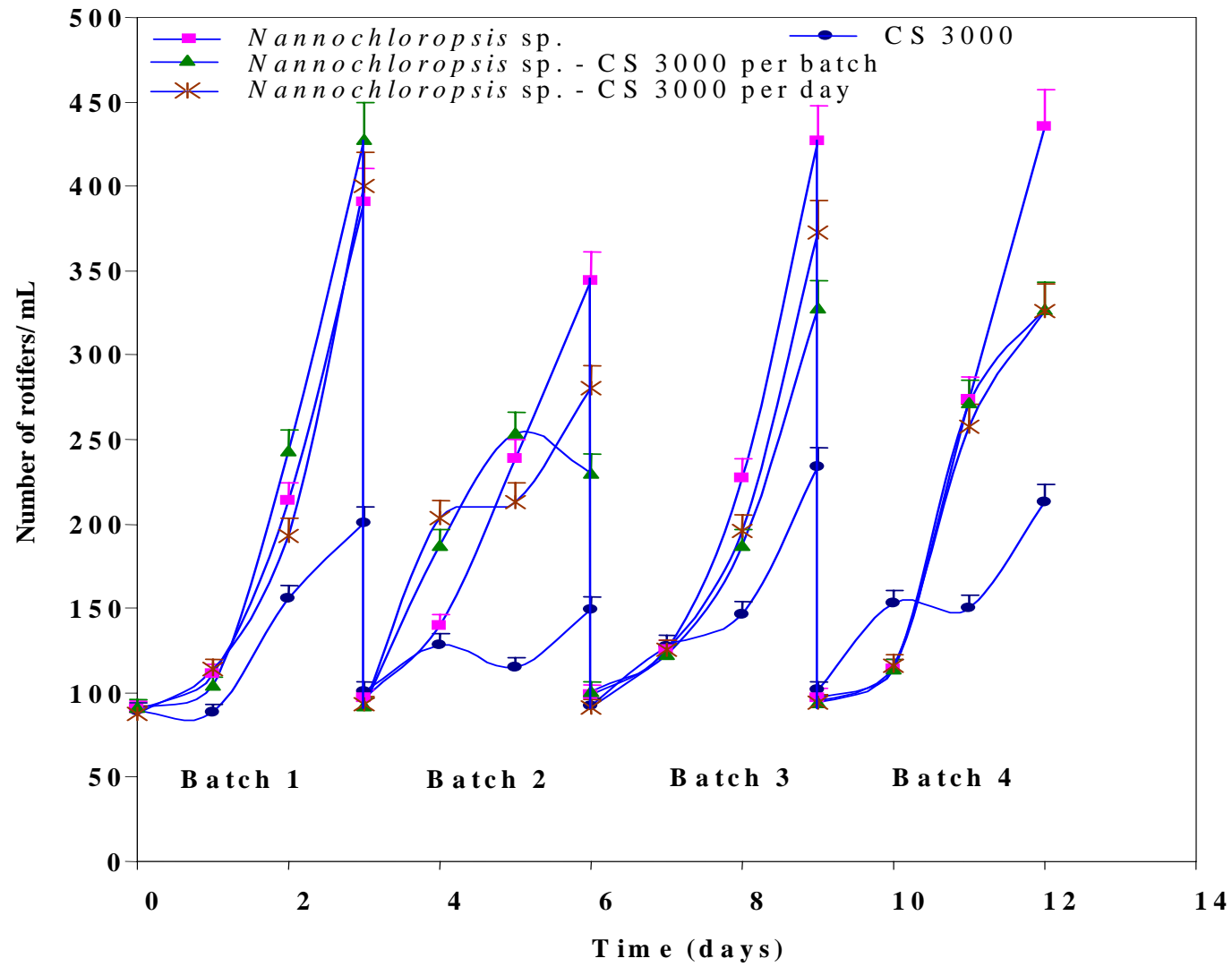
Legend:

- .. : algae
- : CS-3000
- | : start or end of batch

Sampling of individuals for genetic analyses



# Results:



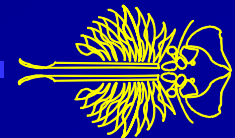
## Results: Daily growth rates

	CS-3000	<i>N. oculata</i> paste	<i>N. oculata</i> /CS-3000 per batch	<i>N. oculata</i> /CS- 3000 per day
Batch				
1	$0.41 \pm 0.18^b$	$0.63 \pm 0.08^b$	$0.70 \pm 0.14^b$	$0.63 \pm 0.13^b$
2	$0.02 \pm 0.39^a$	$0.45 \pm 0.11^b$	$0.12 \pm 0.29^a$	$0.19 \pm 0.37^a$
3	$0.24 \pm 0.39^a$	$0.61 \pm 0.05^b$	$0.49 \pm 0.05^b$	$0.55 \pm 0.02^b$
4	$0.20 \pm 0.18^a$	$0.67 \pm 0.08^b$	$0.52 \pm 0.12^b$	$0.52 \pm 0.19^b$

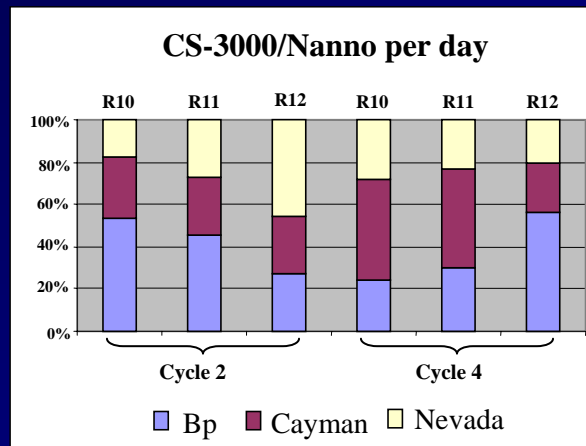
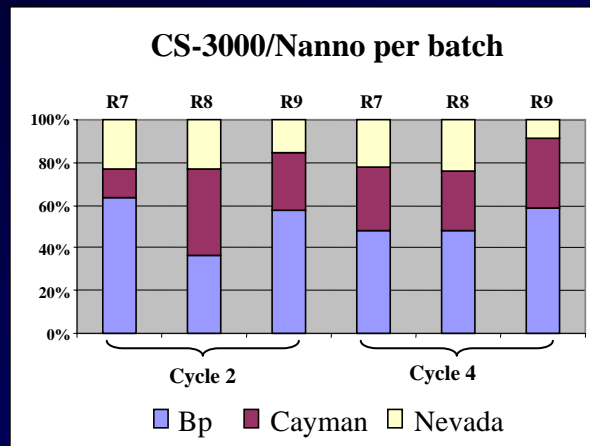
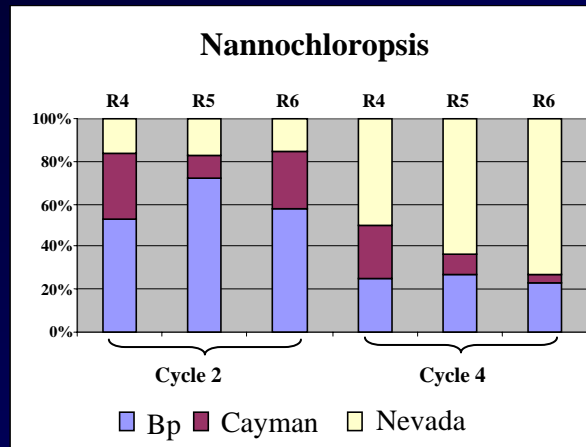
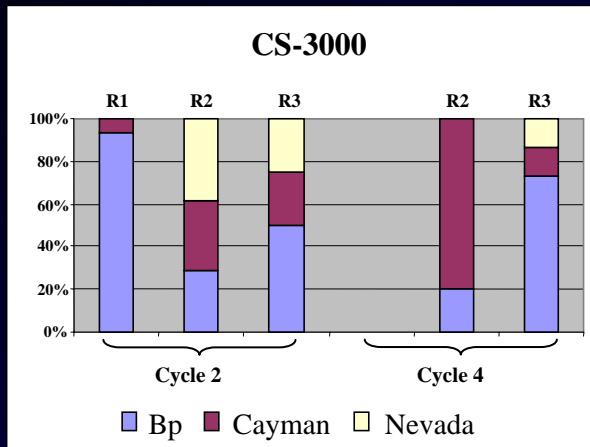
All cultures perform worse during the 2<sup>nd</sup> batch

Crash of 1 replicate at the end of batch 2 on CS-3000

Switching feed types per batch or per day gives similar growth rates



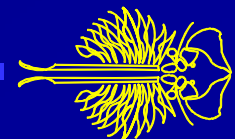
# Results: Genetic diversity change during 4 batch cultures (Uthess)



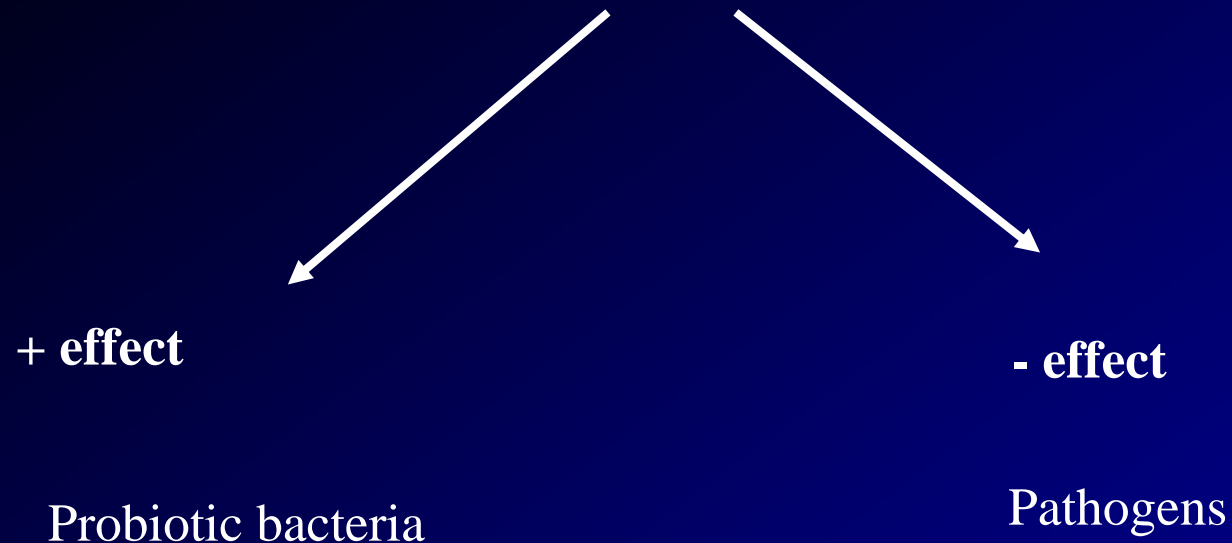
## Conclusions:

- Nevada kicked out with pure CS-3000
- Higher diversity with mixtures of feeds at reasonable growth rate

**Crash:** End batch 2, replicate 1  
 Lowest diversity at the end of batch 2



## Bacteria and rotifers

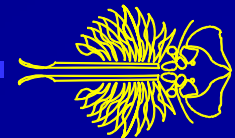


### However:

Literature on bacteria in rotifer cultures: contradiction?

Toxicity of *Vibrio alginolyticus* on the survival of the rotifer *Brachionus plicatilis* by Yu *et al.*, 1990.

‘Most bacteria detected in healthy rotifers and turbot were identified as *Vibrio alginolyticus*’ (Gatesoupe, 1990).



# Are bacteria associated with rotifer crashes able to induce another crash?

Need for several separated cultures of similar or identical clones

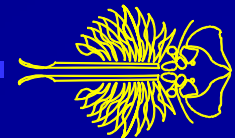
1 culture should crash

Be stand by all the time

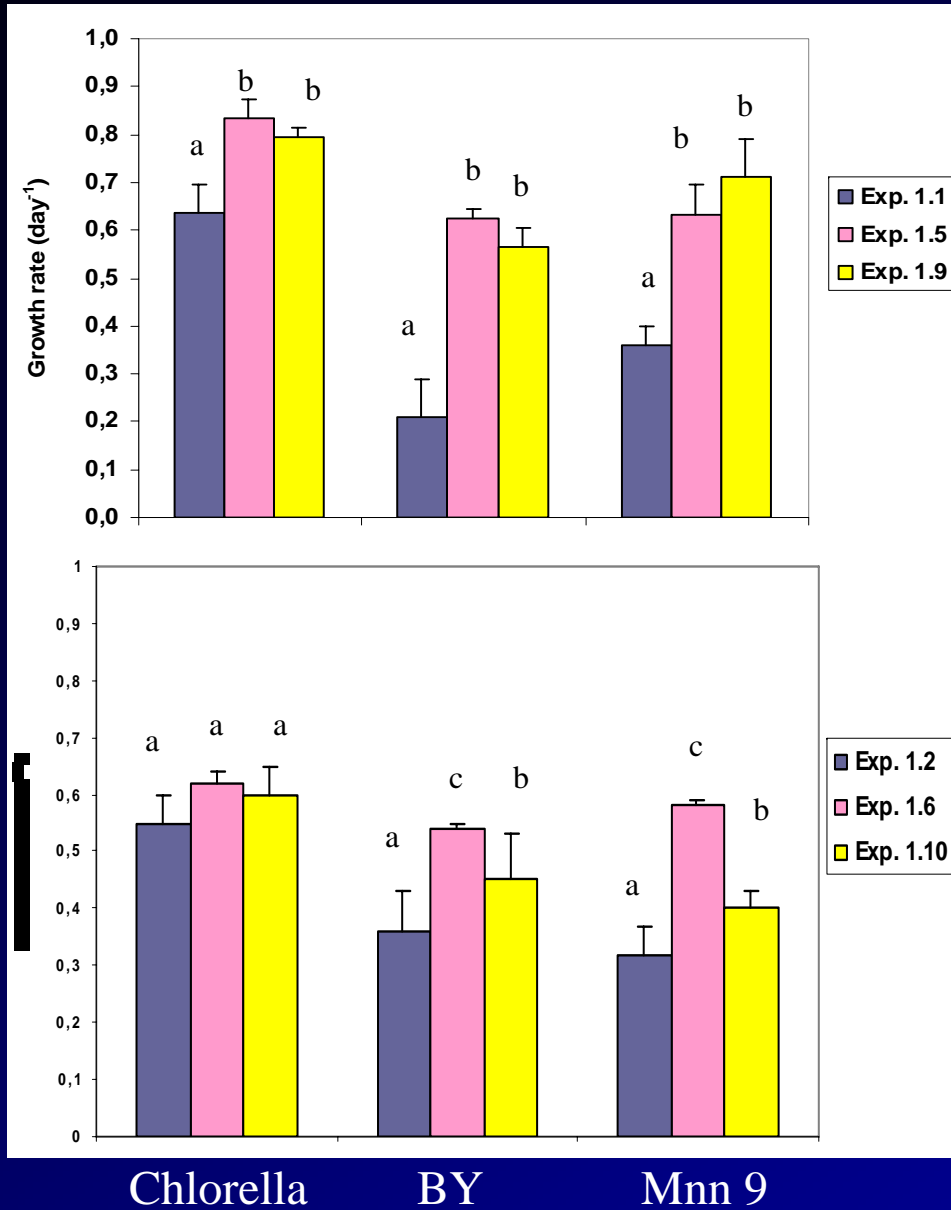
Axenic rotifers to avoid interaction of the rotifer microbial flora.

Bacterial communities isolated from a crashed rotifer culture or  
from good performing rotifer cultures:  $10^5$ CFU/ml

Different axenic feed types: *Chlorella*, fresh Baker yeast, Yeast mutant



## Results



## Conclusions

Rotifers **need** bacteria

*Chlorella* **compensates** for bad bacteria



composition+ 'Green water effect?'

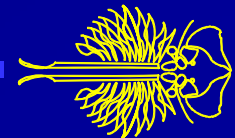
Yeast no help

A lot of hatcheries use algae during mass culture. (Questionnaire)

Axenic culture

Addition of bacteria from good cultures

Addition of bacteria from crashed cultures



Do the **same bacteria** have the **same effects** on **different rotifers**?

Feed: CS-3000

Bacteria added at start of each batch:  $10^6$  CFU/ml

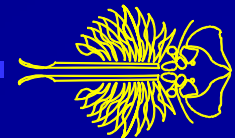
Bact. community added at start of each batch:  $10^5$  CFU/ml: Control 1

$10^6$  CFU/ml: Control 2

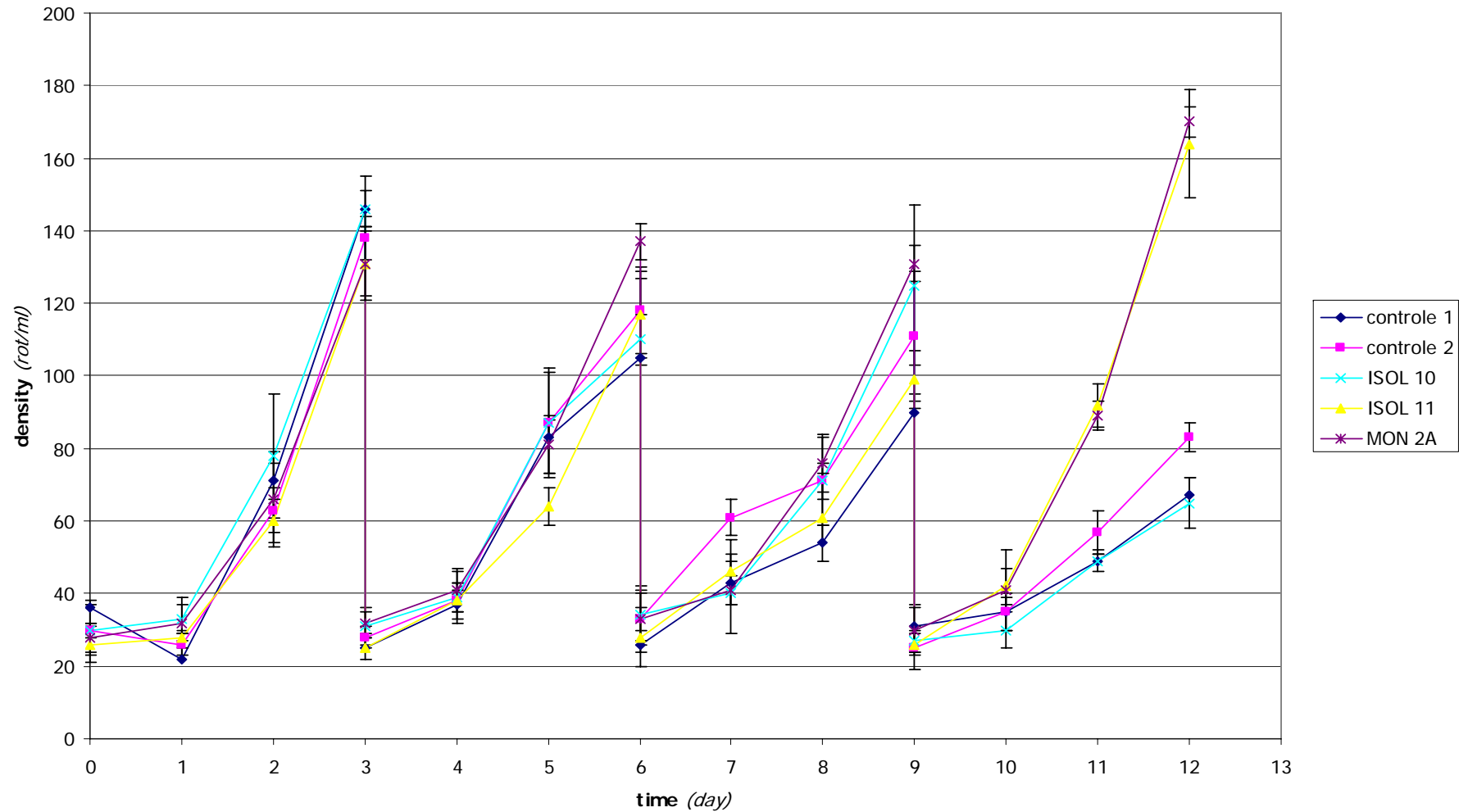
ISOL 10: *Phenylobacterium* sp.

ISOL 11: *Gluconobacter* sp.

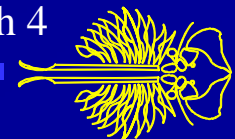
MON 2A: *Paracoccus denitrificans*



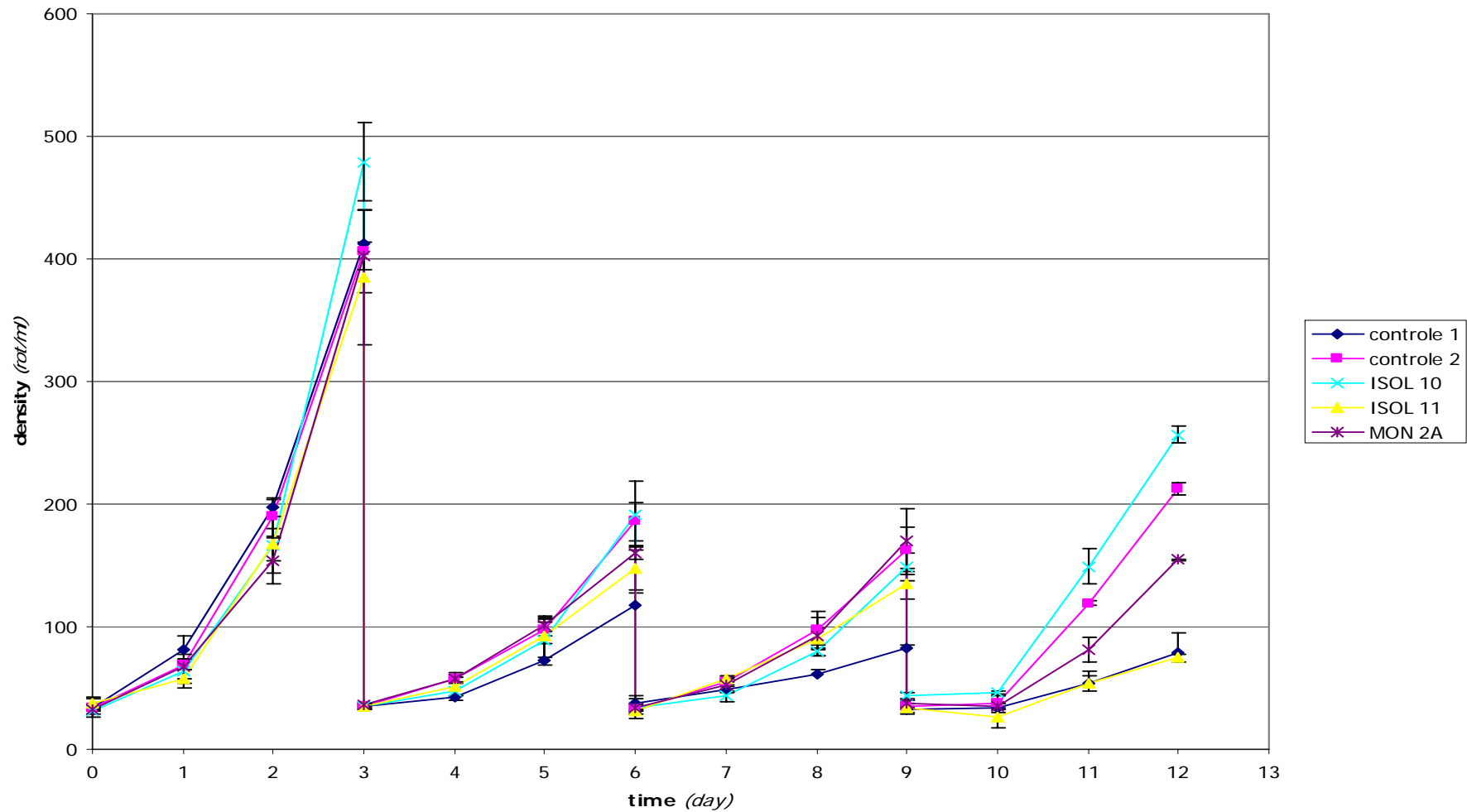
# Results: Density of Nevada



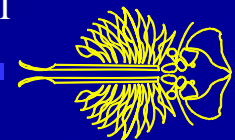
**Conclusions:** Addition of Isol 11 and MON 2A results in a higher growth rate in batch 4  
Addition of Isol 10 results in a lower growth rate than Control 2 in batch 4



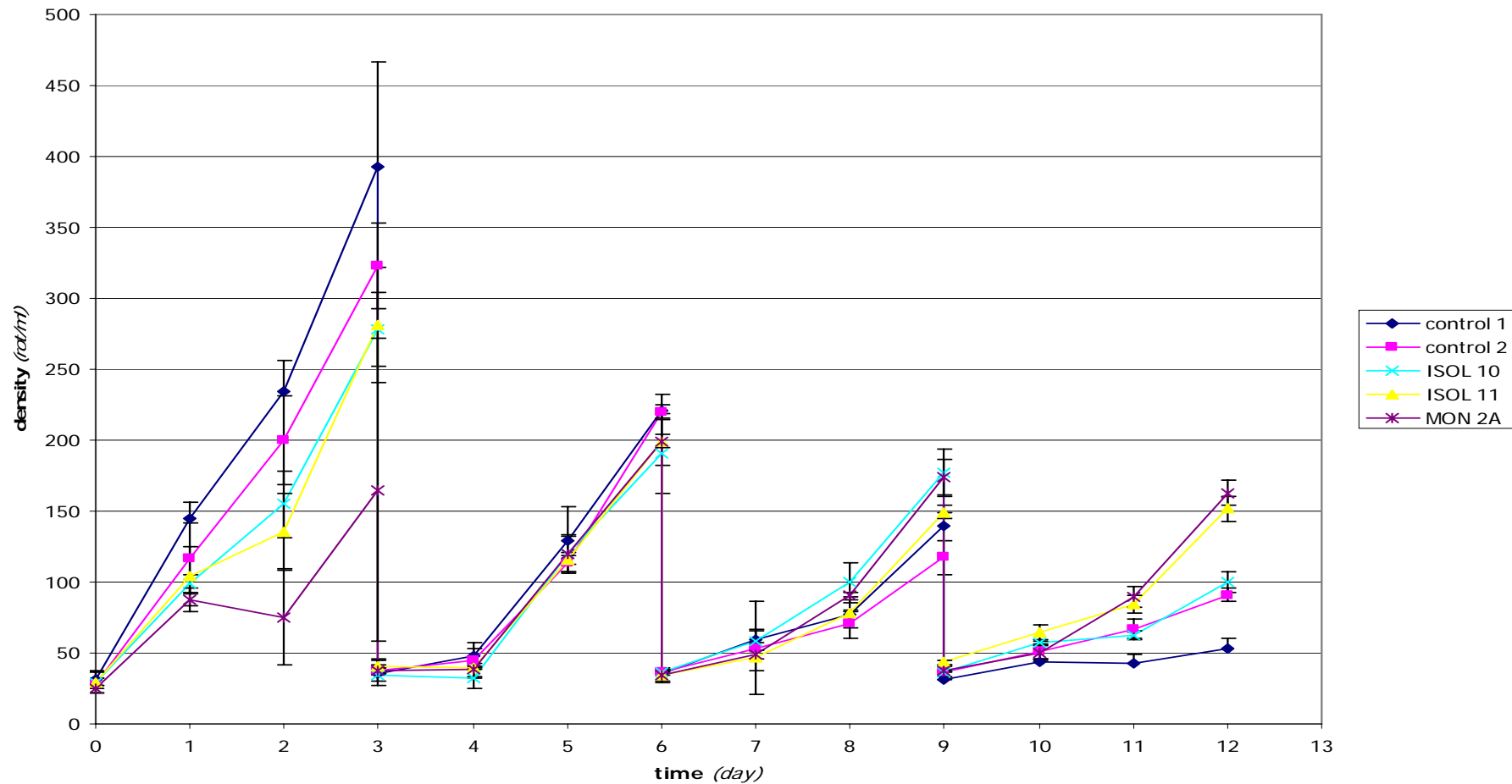
# Results: Density of Cayman



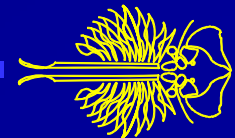
**Conclusions:** Addition of Isol 10 results in a higher growth rate  
Addition of Isol 11 results in a growth rate comparable to the lowest control



## Results: Density of B.p. s.s.



**Conclusions:** Addition of MON 2A + Isol 11 result in a higher growth rate  
Addition of Isol 10 results in a growth rate comparable to the controls



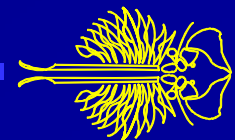
Biotype	Positive	Neutral	Negative
Cayman	<b>Isol 10</b>	MON 2A	Isol 11
B.p. s.s.	MON 2A Isol 11	<b>Isol 10</b>	
Nevada	MON 2A Isol 11		<b>Isol 10</b>

ISOL 10: *Phenylobacterium* sp.

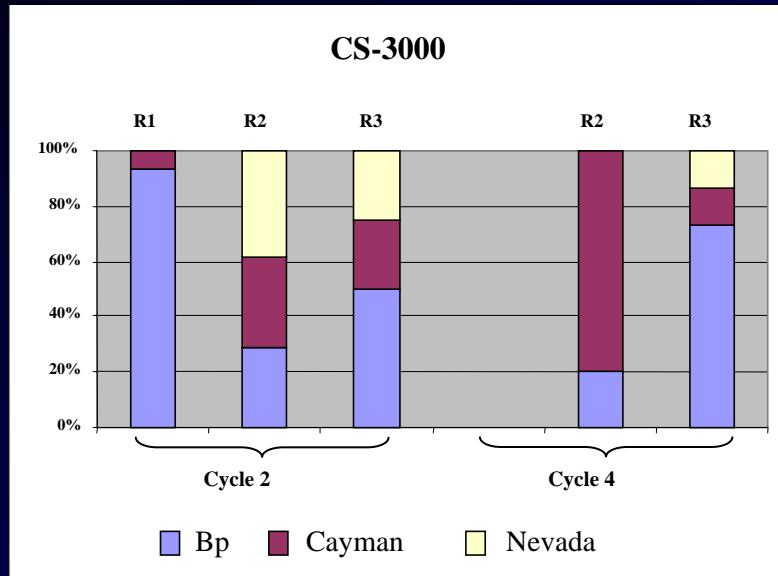
ISOL 11: *Gluconobacter* sp.

MON 2A: *Paracoccus denitrificans*

**Conclusion:** There is possibly a link between certain bacteria and rotifer biotypes



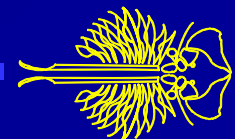
# Is there a possibility to maintain rotifer diversity by adding specific bacteria?



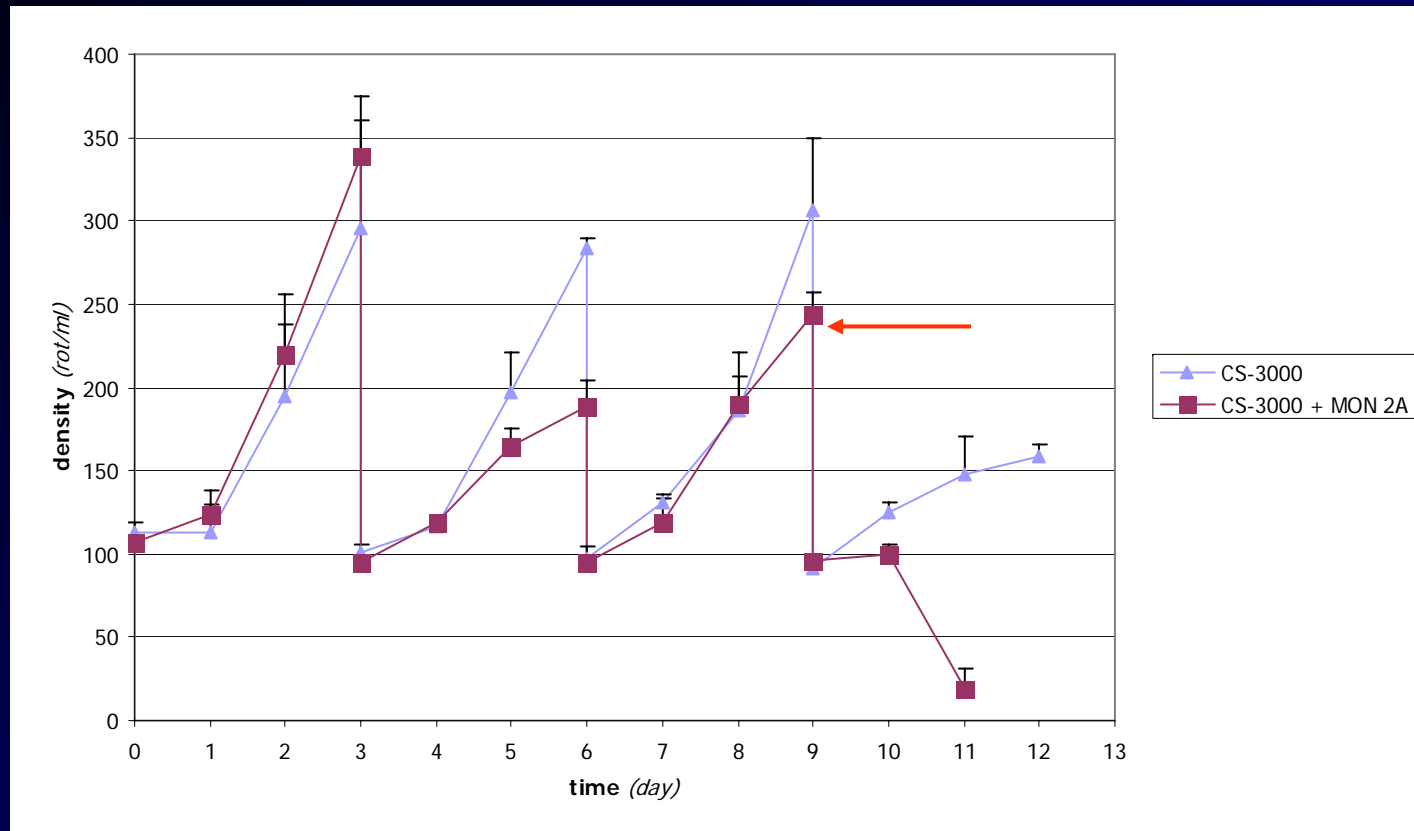
Nevada dropped out when fed solely CS-3000



Addition of *Paracoccus denitrificans*



## Rotifer density

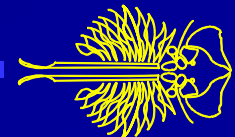


### Bacteria density:

End of batch 1-4 (CS-3000): log 6 – 7

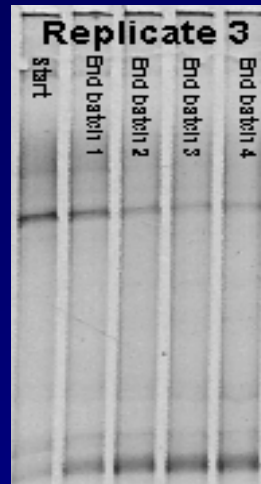
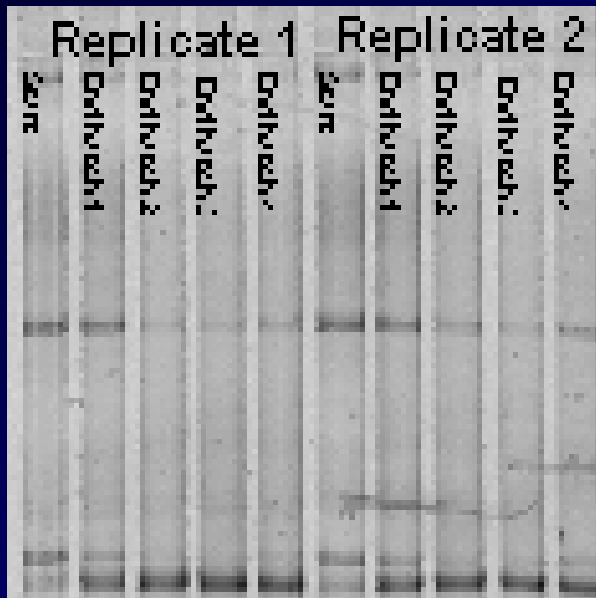
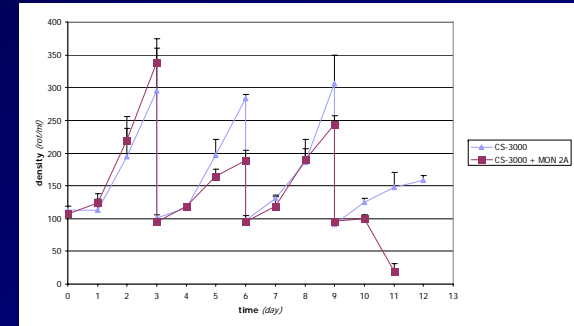
{ End of batch 1-2 (CS-3000 + MON 2A): log 6-7

{ End batch 3 (CS-3000 + MON 2A): log 8 or higher ←



# DGGE analyses of rotifer culture by DVZ

CS-3000

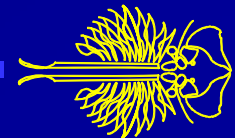


← 'Nevada' biotype

← *Brach. pl. s.s.*

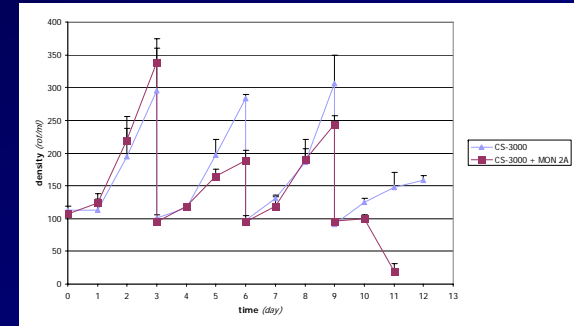
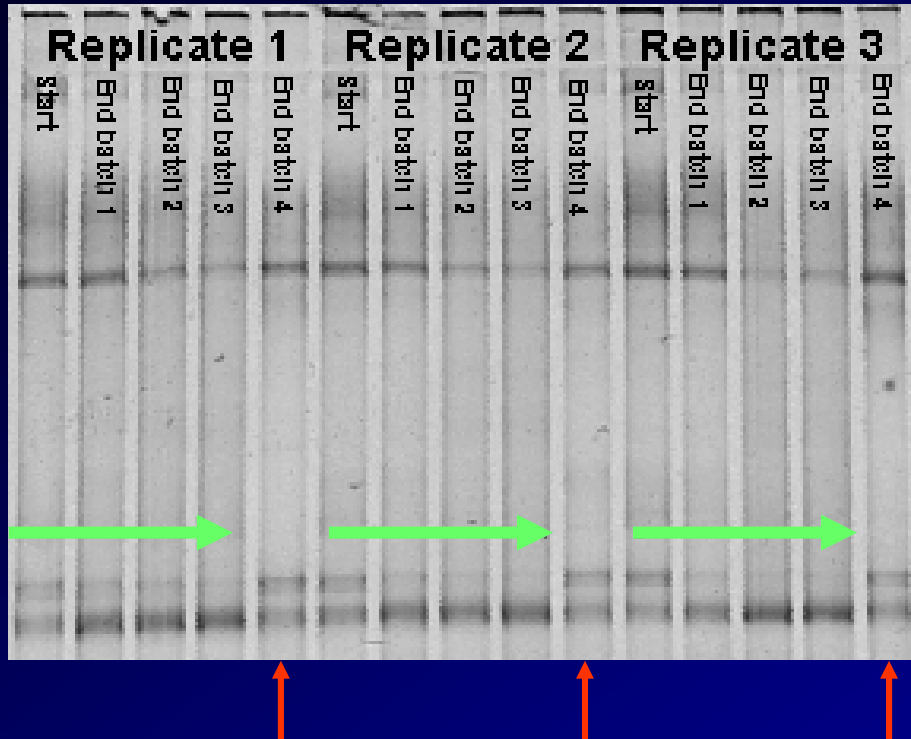
← 'Cayman' biotype

Cayman biotype takes over



# DGGE analyses of rotifer culture by DVZ

CS-3000 + *Paracoccus denitrificans*



← 'Nevada' biotype

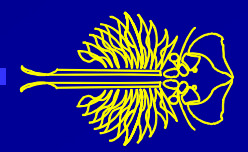
← *B. p. s.s.*

← 'Cayman' biotype

Cayman biotype takes over till batch 3

In batch 4: Cayman is a smaller proportion of the rotifer culture  
Nevada and *B.p. s.s.* increase their proportion

→ Is crash biotype specific?



## What did we learn from all this?

Not all **feeds** fit all rotifer biotypes

Diversity maintained longer when switching feed types

Rotifers need **bacteria**

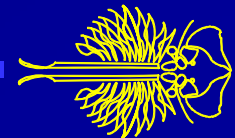
**Live algae** can compensate for unfavourable bacteria

Bacteria are not always/not only the reason for **crashes**

**Crashes** are possibly **biotype specific**



Better to switch feeds while culturing and to add of live algae



Thank you to :

ARC staff

Rotigen partners

European Community

