GENETIC IDENTIFICATION OF *BRACHIONUS* SPECIES USED IN DIFFERENT HATCHERIES

EU project (ROTIGEN, Q5RS-2002-01302)

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Aquaculture Europe 2004
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Rotifers and Aquaculture

- Important live feed for the larviculture of marine fish
- Improved culture methods have led to rotifer mass production (160,000/mL)

- Success of the industry depends on rotifer mass cultures (mainly belonging to *Brachionus*)
- Basic and applied research on rotifers are needed to improve mass culturing

Great confusion on the species status of *Brachionus*

MORPHOLOGY → 3 TYPES

L	SM	SS
B. plicatilis	*B. ibericus*	*B. rotundiformis*

DNA ANALYSES → 9 BIOTYPES

B. plicatilis	*B. ibericus*	
"Nevada"	"Tiscar"	
"Austria"	"Almenara"	*B. rotundiformis*
"Manjavacas"	"Cayman"	

Problems in Rotifer Cultures

Sudden + frequent crashes may be caused by:

- Existing contamination
- Uncontrolled exchange of "unidentified" strains among hatcheries → mixed populations
- Different biotypes REQUIRE different culture conditions

Aims of this Project

- What is the species status of strains used in European hatcheries?
- Do many different clones exist in a single culture?
- Are culture conditions and genetic make-up of hatchery strains linked to crashes?

Sampling

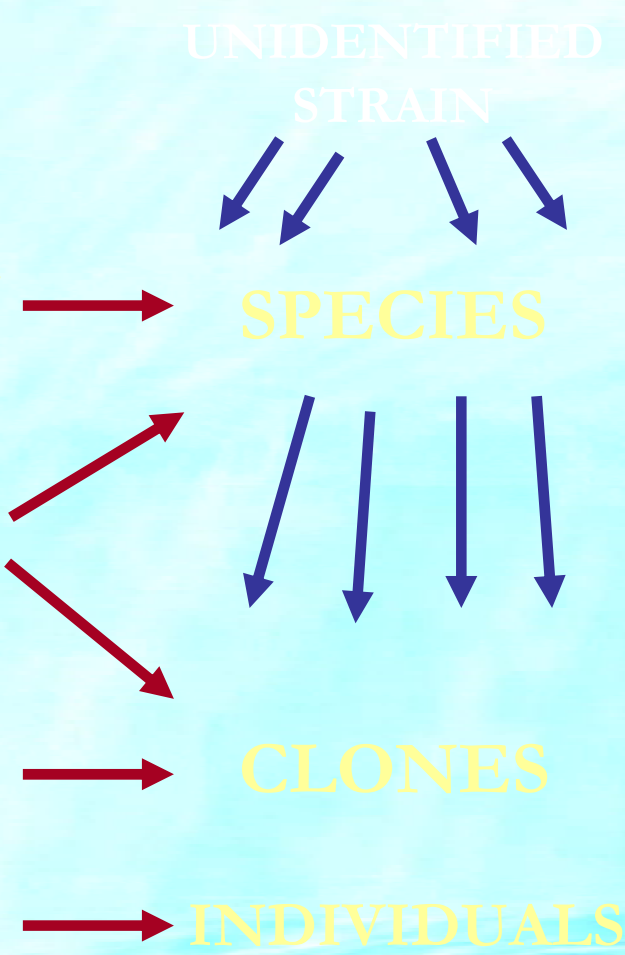
Origin	Hatchery	Strains
Italy	MRS	7
Norway	NTNU	2
Spain	PRODEMAR	2
Portugal	TiMar	2
Greece	FRI	2
	PLAGTON	2

DNA extraction

of live or ethanol preserved samples with Chelex & Wizard® Genomic DNA Purification Kit (PROMEGA)

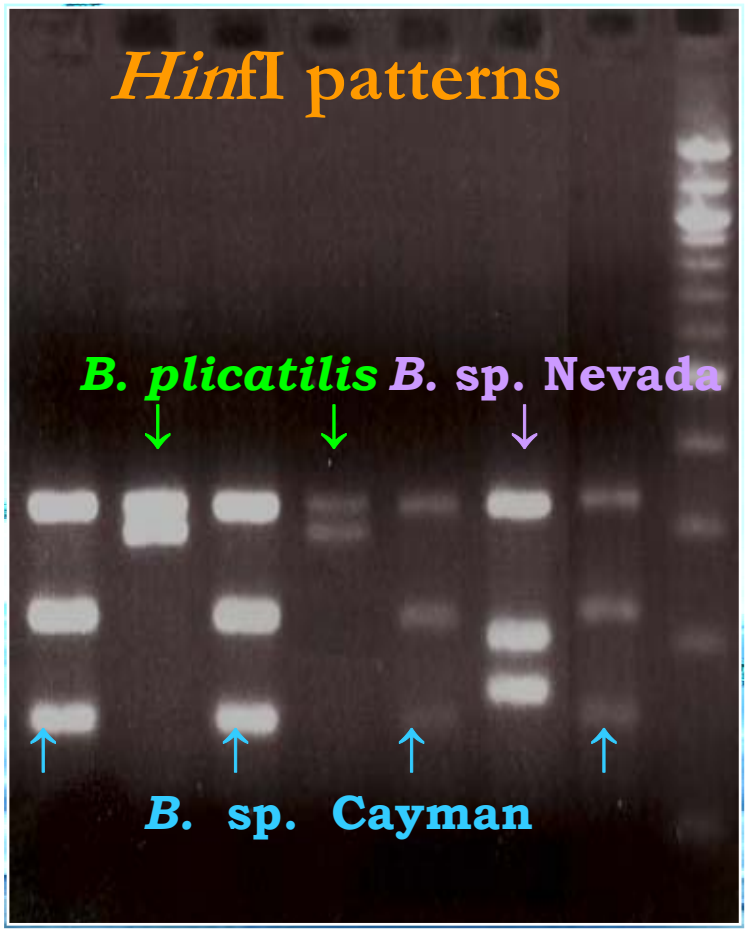
Genetic Markers & Levels of Sensitivity

- RFLP analysis of COI mtDNA region with appropriate endonucleases
- SSCP analyses of 16S rDNA
- DGGE analysis of 16S rDNA
- Microsatellite analysis of 7 loci

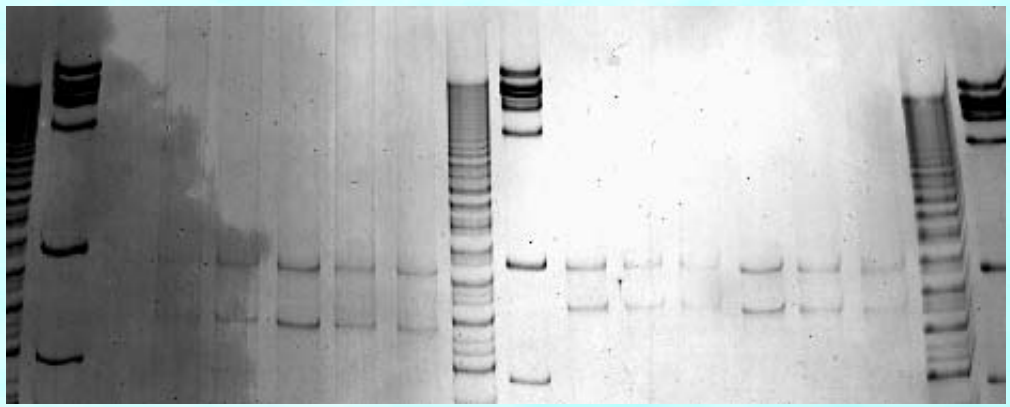


Results on Species/Biotypes Identification

RFLP analysis



SSCP analysis



B. sp. Cayman *B.* sp. Nevada

Genetic Identification of European Hatchery Strains

Origin	Hatchery	Samples	RFLP Biotypes	SSCP Haplotypes
Italy	MRS	7 strains	(6) Cay (1) Aus	H1 H6
Norway	SINTEF	1 strain (30 clones)	Nev	H2, H3
	TF	1 strain (3 clones)	(1) Cay (2) Nev	H4 H5
Spain	PRODEMAR	Small strain (30 clones)	Cay	H1
		Large strain (30 clones)	Nev	H2
Portugal	TiMar	2 strains	Cay	H4
Greece	FRI	2 strains	Cay, Plic	-
	PLAGTON	Strain 1	-	H4
		Strain 2	Plic	-

Genetic Identification of Laboratory Strains

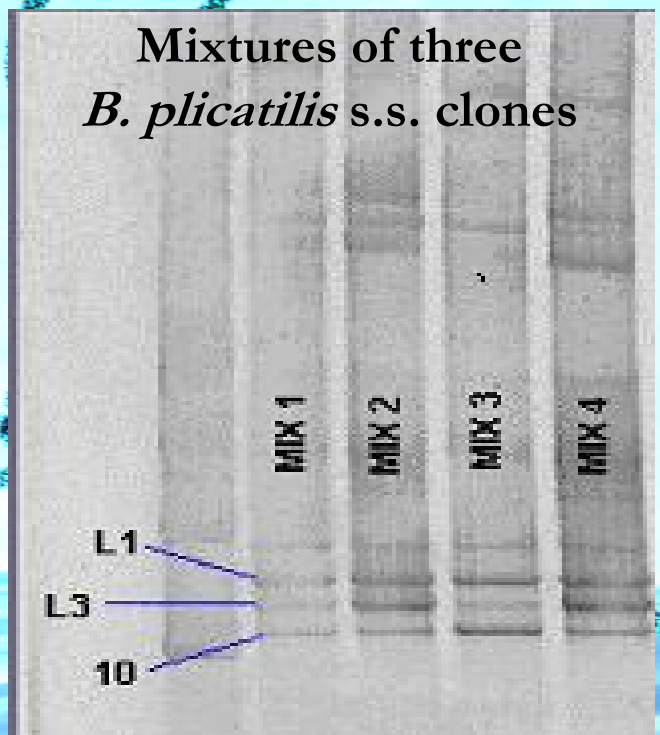
Origin	Samples	Biotypes RFLP-SSCP
Mexico	4 clones	(3) Cay (1) Pli
Japan	2 clones	Cay
Vietnam	1 clone	Cay
Ecuador	2 clones	Cay
Spain1	5 clones	(4) Cay (1) Mnj
Spain2	4 clones	Mnj

Overall Representation of *Brachionus* Biotypes in Hatcheries and Laboratories

Biotypes	European Hatcheries	Laboratory Strains
B. plicatilis s.s. (L)	2	1
B. sp. Nevada (L)	3	-
B. sp. Austria (L)	1	-
B. sp. Manjavacas (L)	-	2
B. sp. Cayman (SM)	12	7

Results: Intra-population level

DGGE analysis



- more sensitive than SSCP
- able to distinguish different clones of the same biotype
- enables mixed sample analysis
- more laborious than RFLPs & SSCP

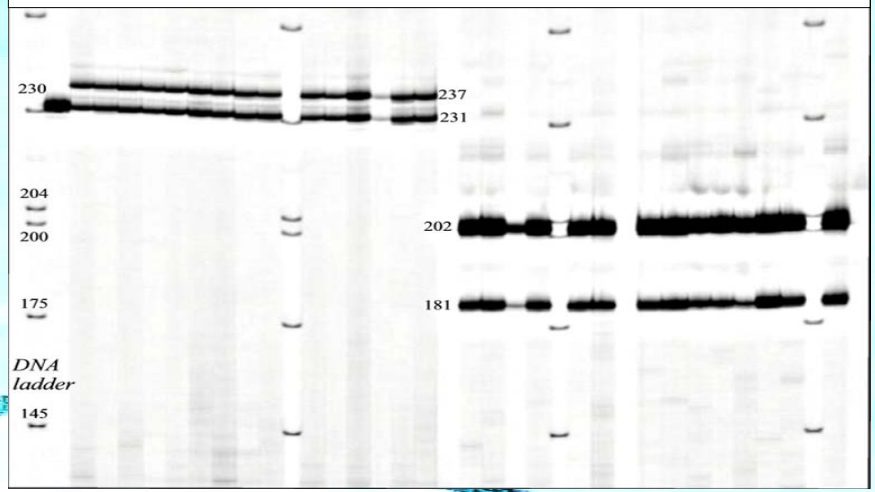
Results: Intra-population level

Microsatellite analysis

Genotyping of *B. plicatilis* s.s. hatchery individuals at 2 loci

Bp 5 locus

Bp 7 locus



- amplification at individual level
- highly sensitive
- discrimination of clones within strains
- primers available only for *B. plicatilis* s.s.
- primer pairs cross-amplify phylogenetically close species

- Microsatellite data on 2 strains have shown that individuals of each strain share the same genotype, i.e. they are clones

Discussion

A. Species identification in hatcheries

- One or maximum two species/biotypes present in each strain
- Could this be due to culture conditions?

- *B. plicatilis* s.s. (Type L) in few strains
- Other *Brachionus* type L sp. were found instead

- *B.* sp. Cayman (Type SM) occurred in most strains

- *B. rotundiformis* (Type SS) never found

Discussion

A. Species identification in hatcheries

- Lack of knowledge regarding species/biotype status of hatchery strains is evident
- Morphologically identical samples may be genetically different (cryptic diversity)!
- Each biotype has different temperature and salinity preferences
- Caution is needed regarding frequent exchange of samples among hatcheries
- Caution is also needed to avoid contamination within hatcheries

Discussion

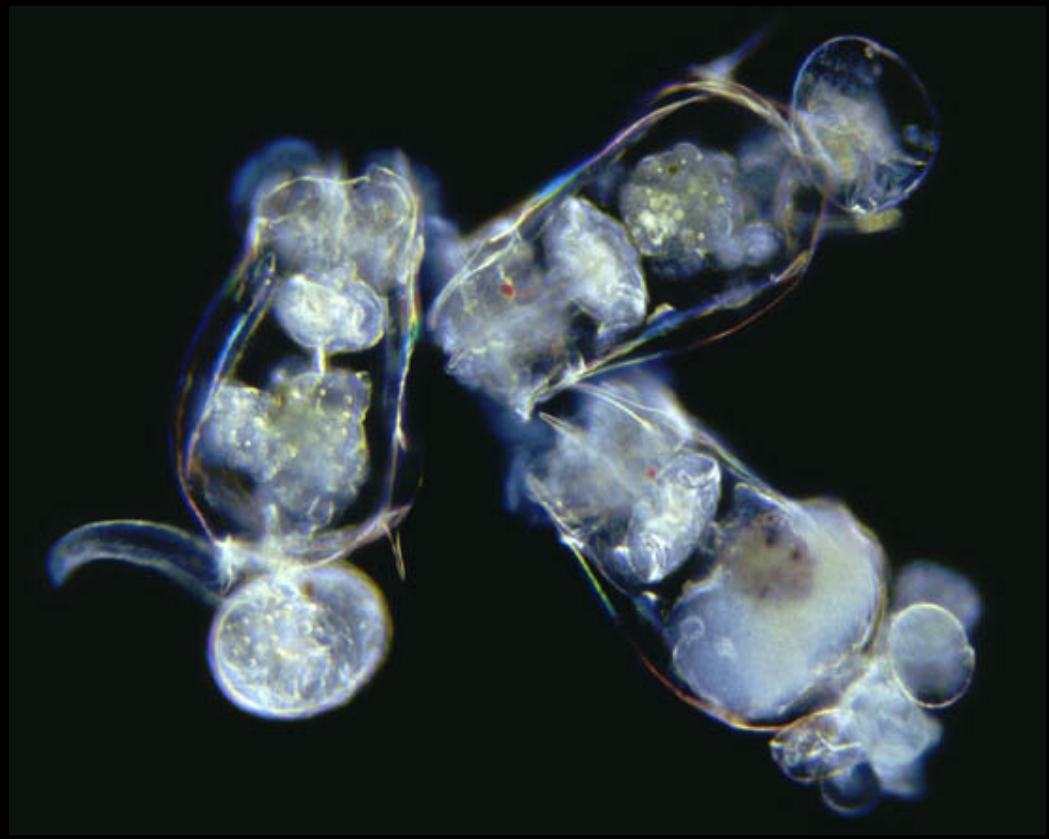
B. Variability among hatchery strains

- Polymorphism was detected within certain biotypes
- Molecular markers used so far cannot efficaciously detect the levels of polymorphism
- Rotifers are cultured in huge quantities
- Methods are required to obtain results at quantitative and/or qualitative level
- DGGE and Real Time PCR analyses are possible candidates

Discussion

C. Variability within Hatchery Strains

- Microsatellite analysis is the most informative technique at individual level (where applicable)
- Microsatellite data have revealed the absence of polymorphism within cultures
- Polymorphism is possibly lacking prior, than being depleted during rotifer cultures



Thank you

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