

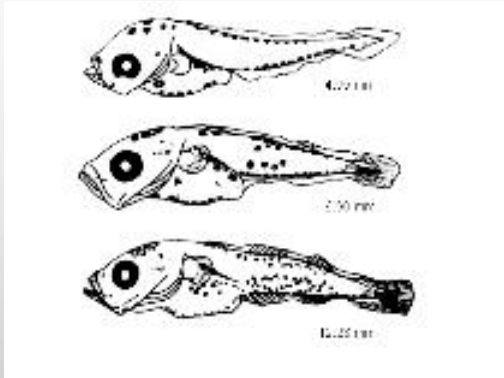


# On the Way to identify Fish Eggs and Larvae with the Help of Microarrays

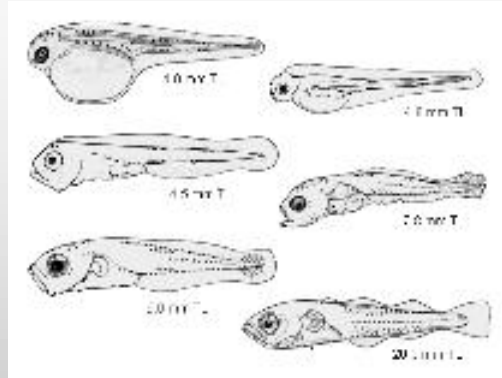
Nina Silkenbeumer,  
Kristina Kappel, Dietmar Blohm



# Identifying fish eggs and embryos is difficult and at some stages not possible

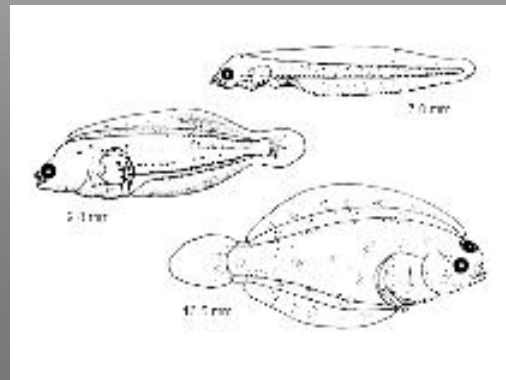


Whiting  
(*Merlangius merlangus*)

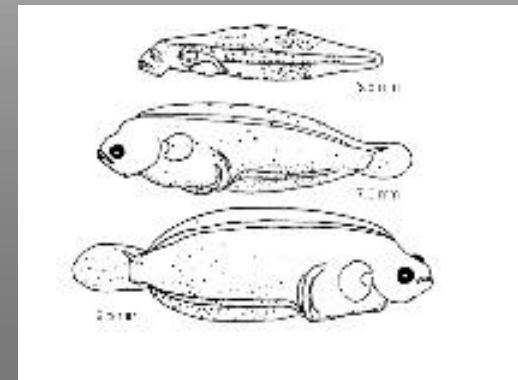


Atlantic cod  
(*Gadus morhua*)

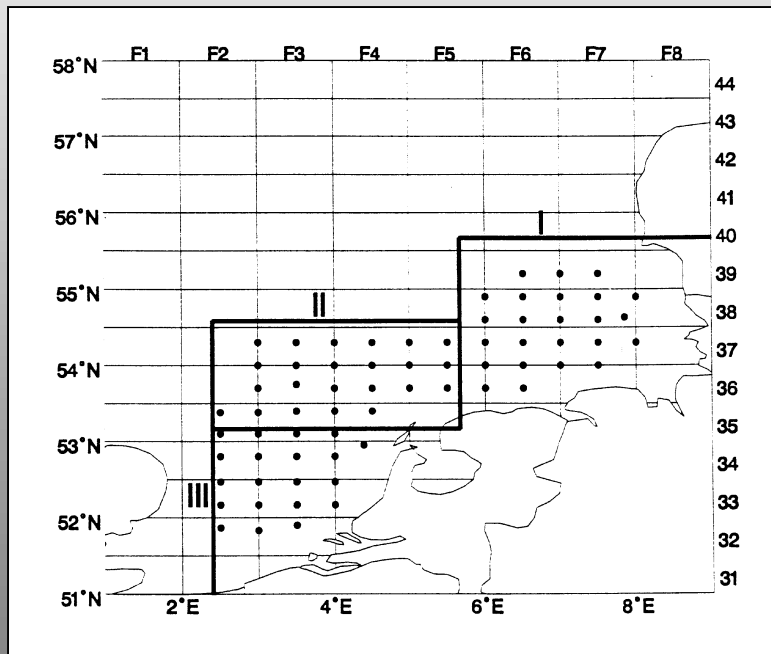
European plaice  
(*Pleuronectes platessa*)



Flounder  
(*Plathichthys flesus*)



# Pelagic Fish Eggs in the Southern North Sea are caught for several reasons



Back-calculation of spawning stock biomass



Detection of shifts in species dominance and regional distribution

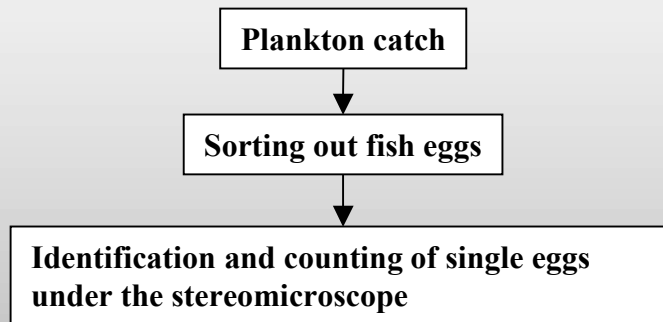


Evaluation of effects of human influence or climate changes

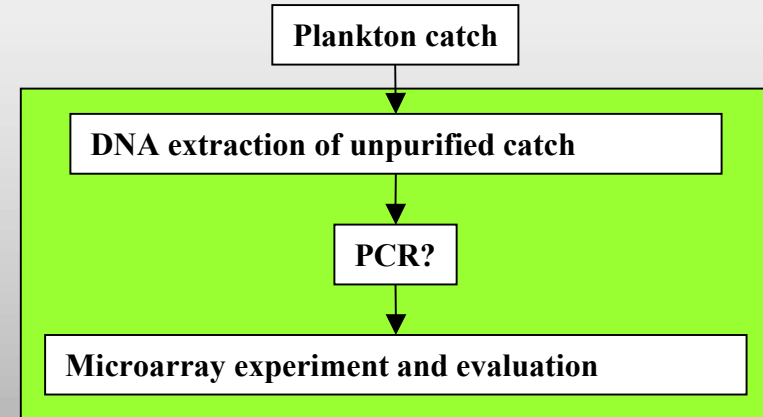
H.v. Westernhagen *et al.* (2002)

# Procedure of Fish Egg Measurements

## Conventional procedure



## Microarray driven analysis



### Advantages:

- quantification possible
- malformations detectable
- stage of development detectable
- automation possible (Gensensor)
- ⇒ routine application
- easily expandable

### Disadvantages:

- expertise needed
- no automation possible
- quantification difficult
- malformations and developmental stage not detectable

# 1. Species selected



Whiting  
*Merlangius merlangus*



Atlantic cod  
*Gadus morhua*



Flounder  
*Plathichthys  
flesus*



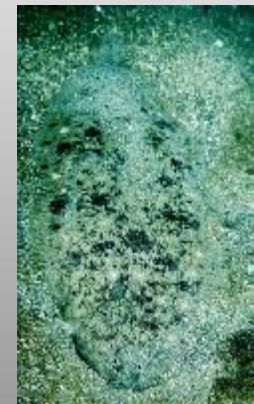
Dab  
*Limanda  
limanda*



American plaice  
*Hippoglossoides platessoides*

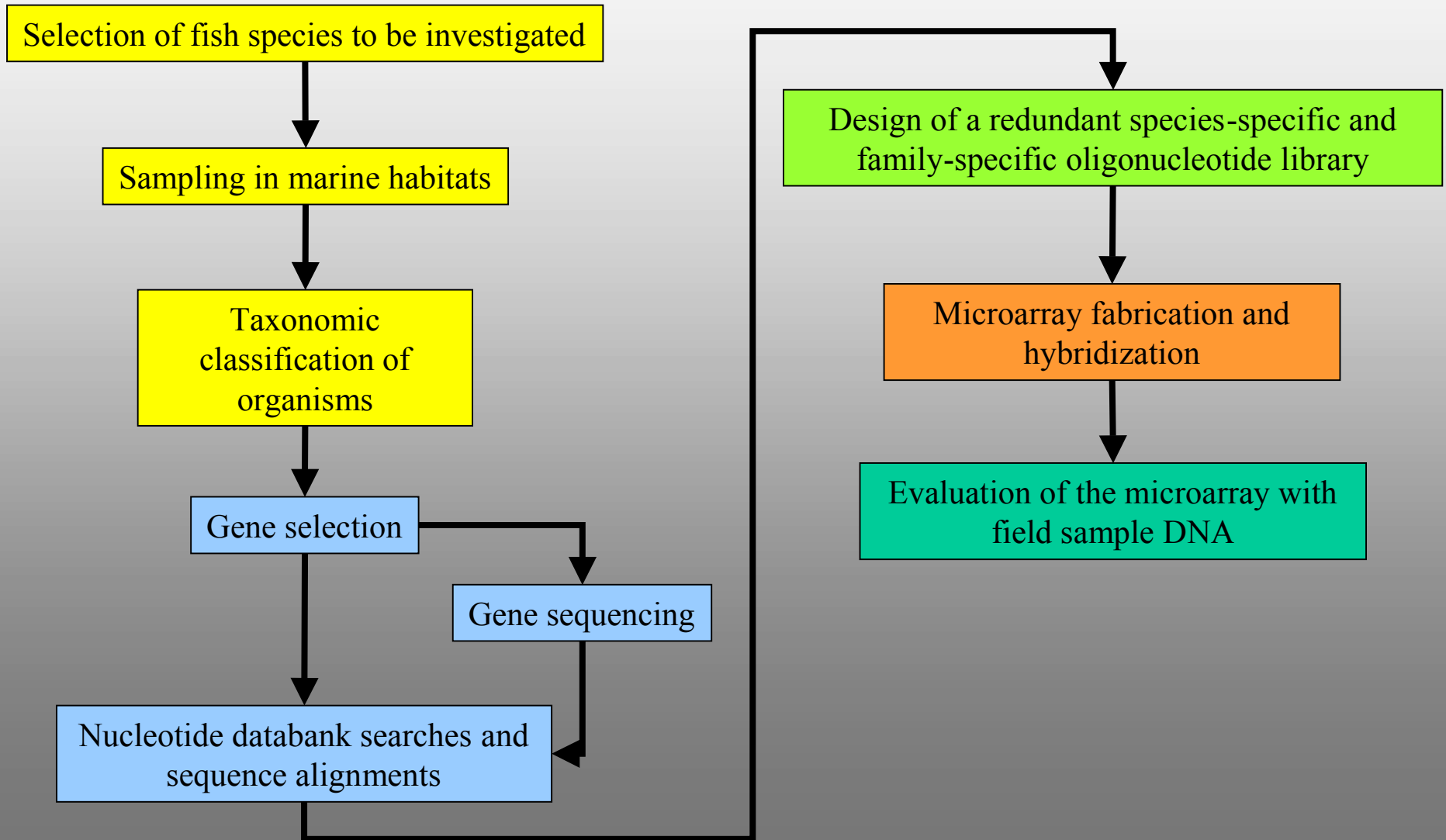


Plaice  
*Pleuronectes  
platessa*



Common sole  
*Solea solea*

# Work Concept



## 2. Gene Selection

Genes which are (often) used for phylogenetic analyses of fish species

### mitochondrial genes

- cytochrome b
- **16S rRNA**
- 12S rRNA
- **cytochrome oxidase I, II, III**
- **ATPase 6, 8**

### nuclear genes

- 18S rRNA
- 28S rRNA

Sequences in GenBank (10.09.2003)

	mito	16S	12S	cyt b	COI	ATPase 6	ATPase 8	28S	18S
Gadidae	3	7	7	132	15	1	1	11	6
Gadus morhua	1	1	1	94	2	1	1	6	5
Merlangius merlangus	0	0	0	1	1	0	0	0	0
Pleuronectidae	3	36	24	44	0	0	0	3	0
Limanda limanda	0	0	0	0	0	0	0	3	0
Platichthys flesus	0	1	0	2	0	0	0	0	0
Pleuronectes platessa	0	0	0	1	0	0	0	0	0

### 3. Design of COI Capture Probes

Several probes for each species:

- oligonucleotides of 18-22nt
- as many mismatches as possible to any other fish to be represented on the FishChip
- at least one of the mismatches central
- melting temperature around 60°C (Wallace)

## 4. Spotting and Hybridisation

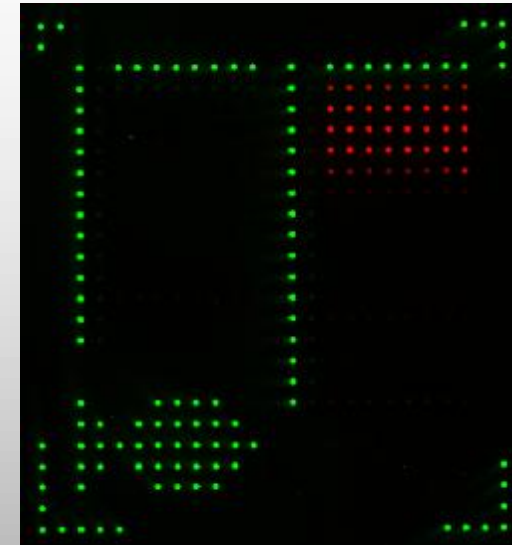
Spotting of 5'-amino-modified oligonucleotides onto activated glass slides



Hybridization with Cy5-endlabelled PCR products of DNA extracts of single species individuals



Detection of hybridization signals with a microarray laser scanner



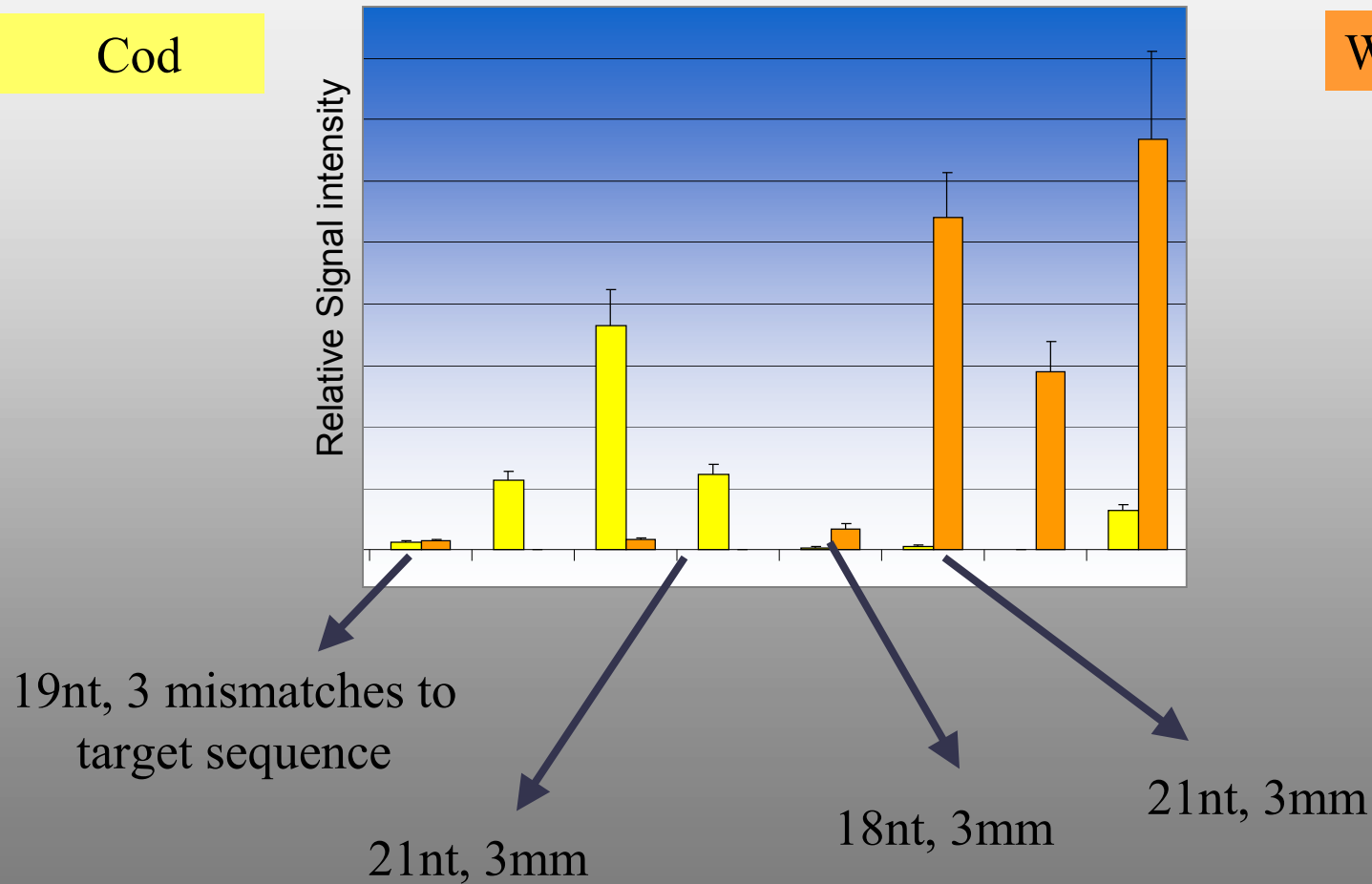
Evaluation of hybridization sensitivity and specificity of capture probes

Optimization of procedure: DNA-fragmentation, other labelling methods...

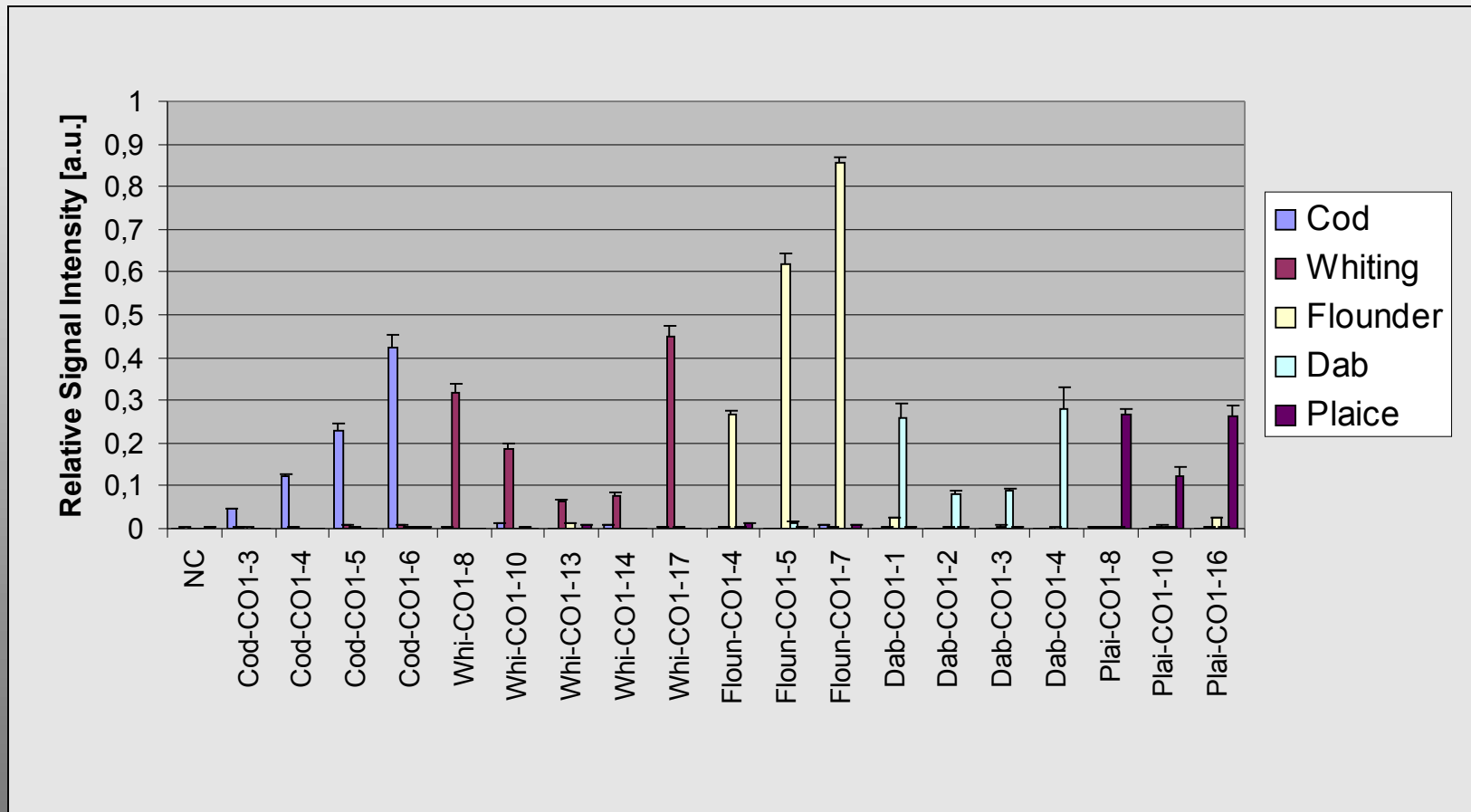
# Example for the problem of finding „good captures“: Discriminating Cod and Whiting

Cod

Whiting



# Single-target hybridization experiments with COI PCR-products from five different fish species



## Summary

- What makes a good capture probe?
- Through combination of probes, we can identify  
5 fish species from 2 families
- with capture probes for COI- and 16s DNA
- Can we identify every individual from each species?

## Outlook

- Increase of hybridization efficiency, equalize hybridisation
- Evaluation of other genes (e.g. nuclear genes) as possible targets.
- Design of capture oligonucleotides for other fish species and hybridisation evaluation.
- Increase the number of individuals.



Thanks to Dr. H.v. Westernhagen, Dr. R. Knust (AWI) and their group

