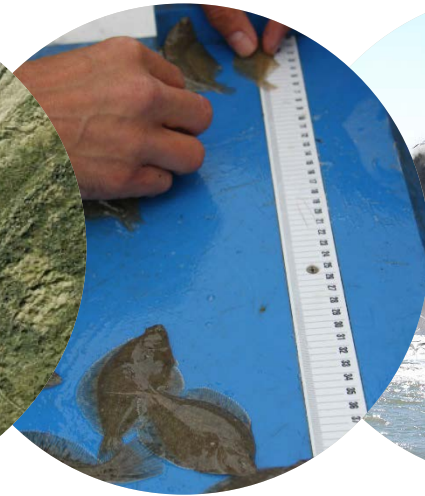


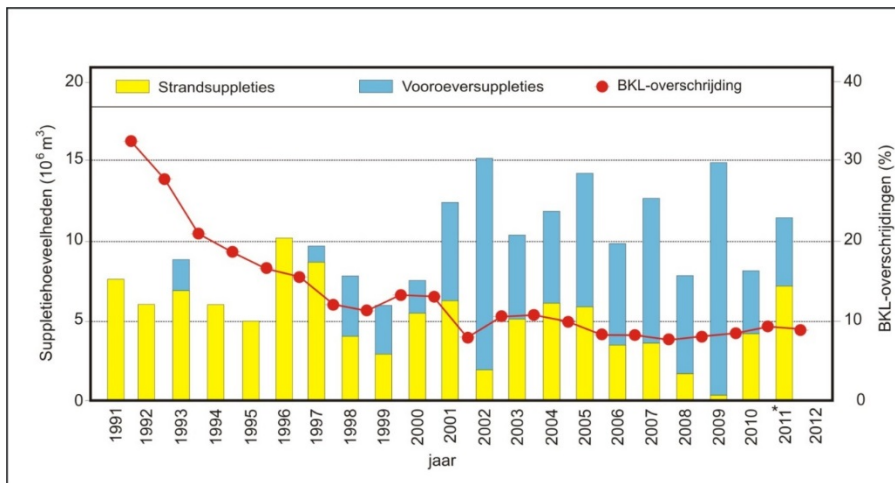
RNA/DNA ratios to study growth in coastal nursery areas

Comparison of methods and relation with environment

Maarten Rutting, Richard Crooijmans , Ralf van Hal & Ingrid Tulp



Regular nourishments since 1991



Impact on nursery function?

- Knowledge on the impact:
 - Benthic community restores
<3 year after sand
nourishment
 - Effects on fish community?
 - Effects on the nursery
function?
=> fish growth?



June 2017: MSC project

■ Animal Breeding & Genetics

Richard Crooijmans



- compare two methods RNA:DNA
- Investigate growth juvenile flatfish in June in nurseries along the Dutch coast
- Proxy: RNA:DNA metabolic activity => instantaneous growth

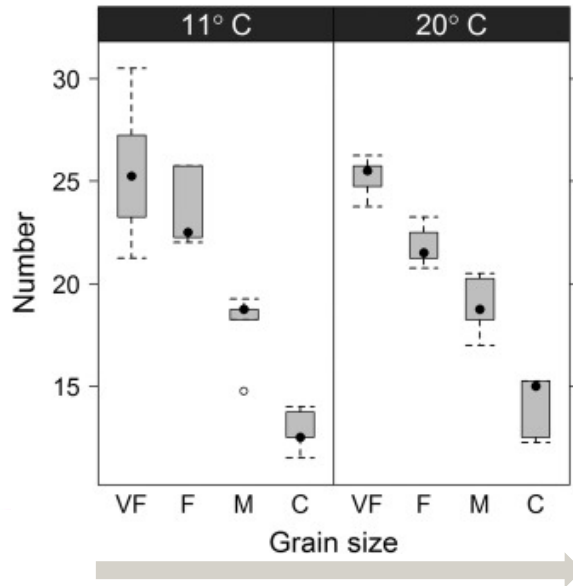


Hypothesis

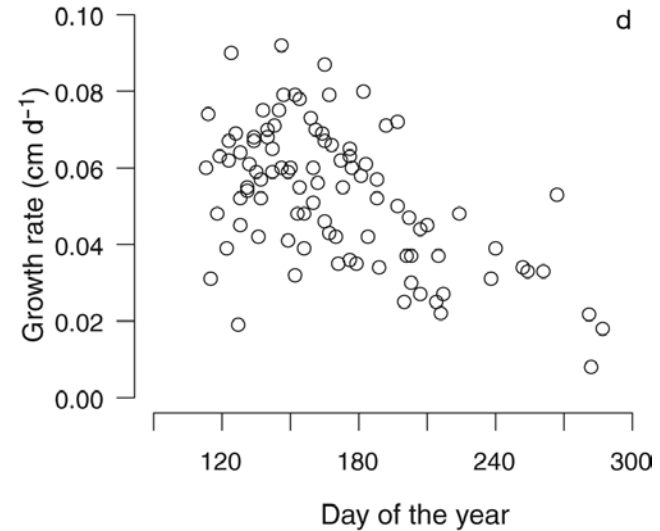
In June: growth not yet limited

Clear sediment preference (sole)

=> Growth ~ sediment



Post et al 2017



Van der Veer et al 2010

Locations

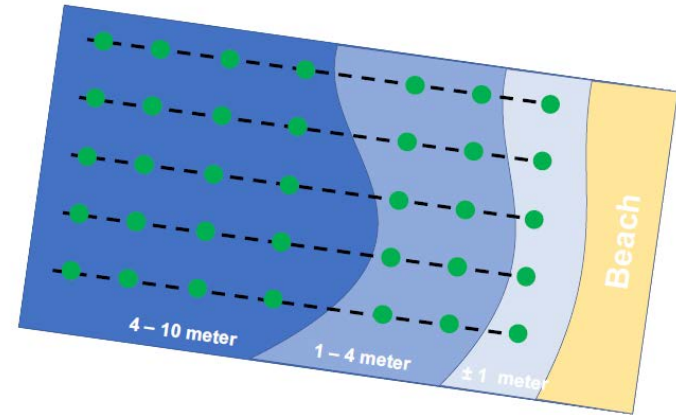
- Location 1: Zuid-Holland
- Location 2: Noord-Holland
- Location 3: Texel
- Location 4: Ameland

= > 4 consecutive weeks from
South to North

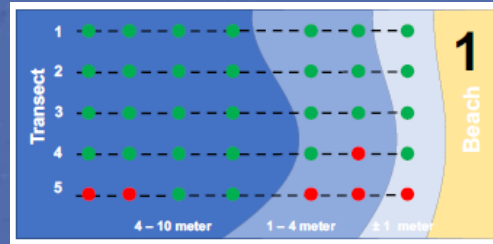
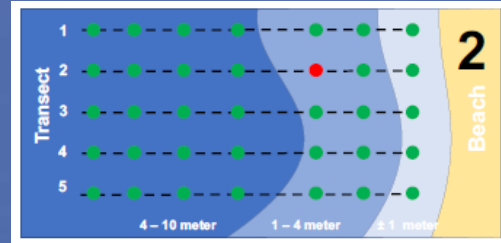
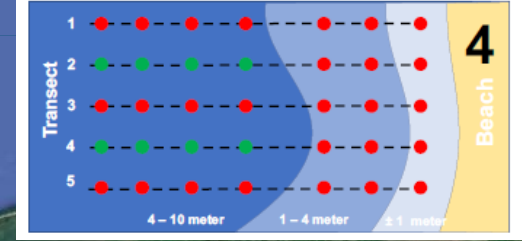
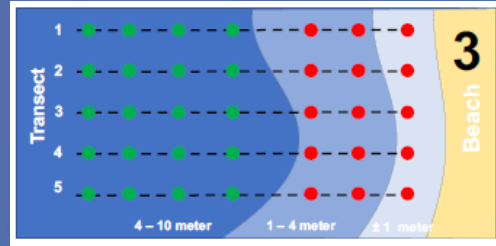


Transects: fish sampling

- Transects per location
- Fish sampling:
 - 0-1 m: walking push net
 - 1-4 m: dinghy: 2 m beam
 - 4-10 m: vessel 3 m beam
- Stratification based on sediment
- Continuous recording abiotics
- Benthos sampling



Missing hauls



Survey - Fish



Survey - Benthos



Survey - Sediment



Plaice

392

Dab

302

Sole

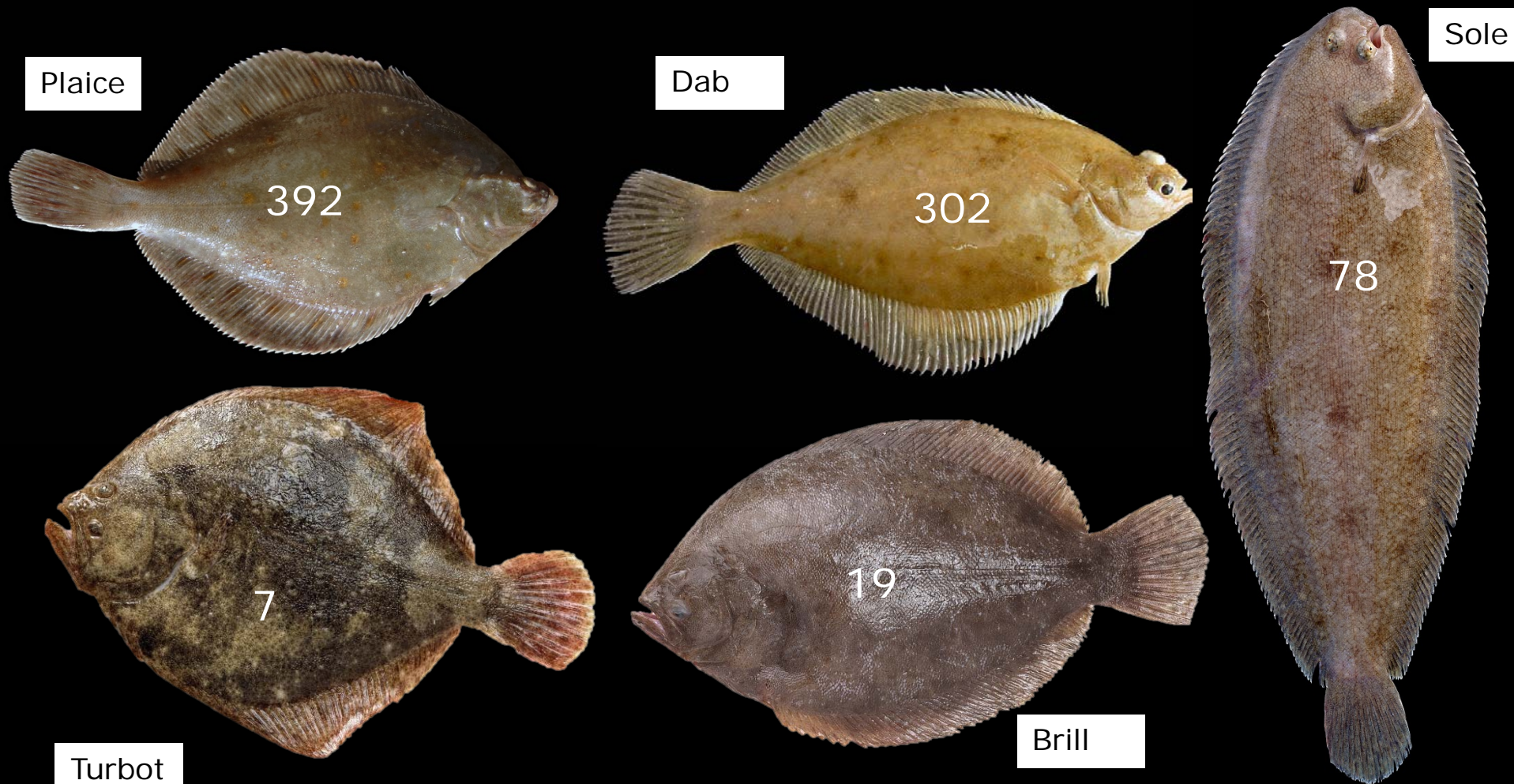
78

7

Turbot

19

Brill

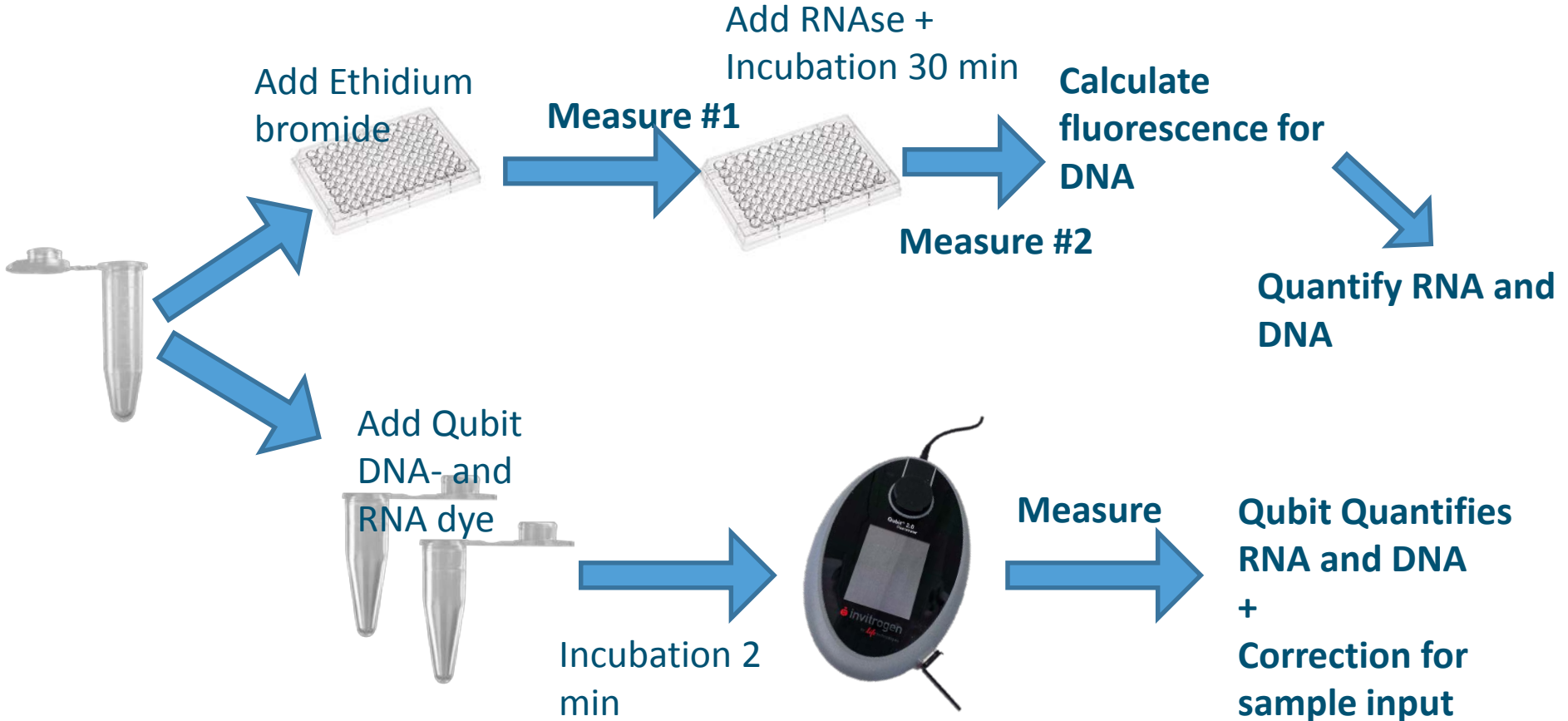


RNA/DNA Quantification: two methods

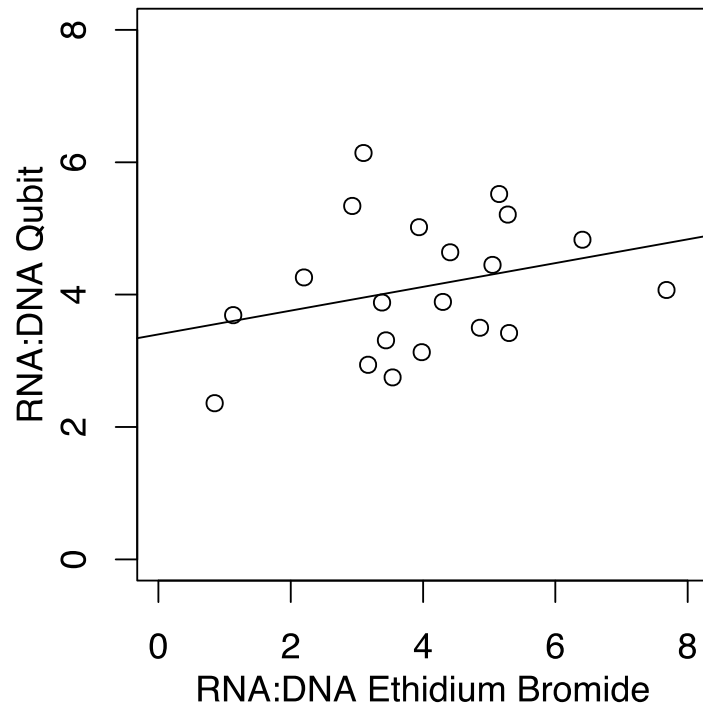
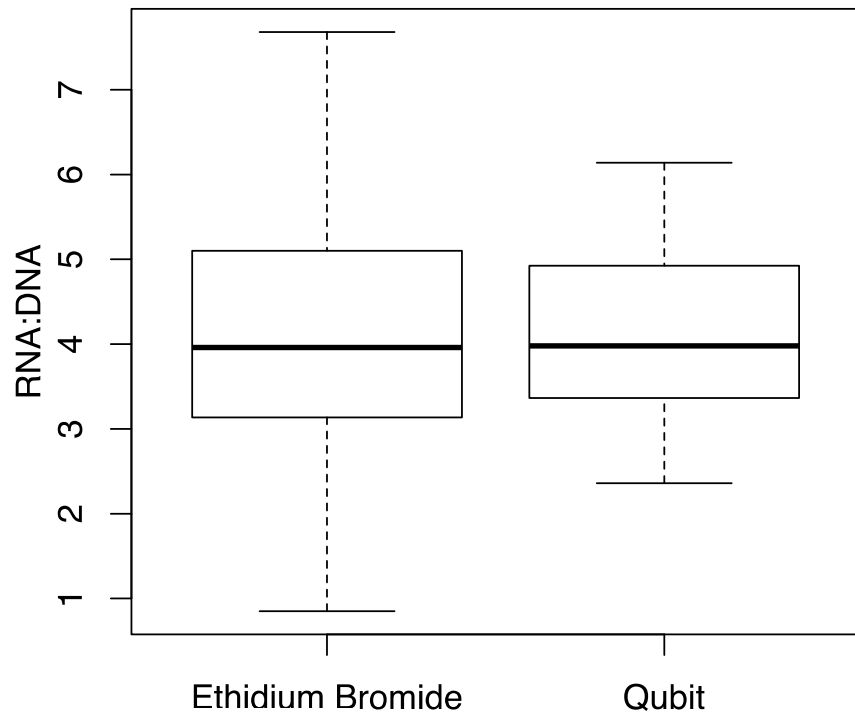
- Fish collection in field: -80°C
- Tissue collection in lab: 5 species
- Ethidium bromide
- Qubit Fluorometer
 - Already used before to analyse RNA:DNA ratio's
 - RNA High Sensitivity Assay Kit (Invitrogen™)
 - dsDNA High Sensitivity Assay Kit (Invitrogen™)



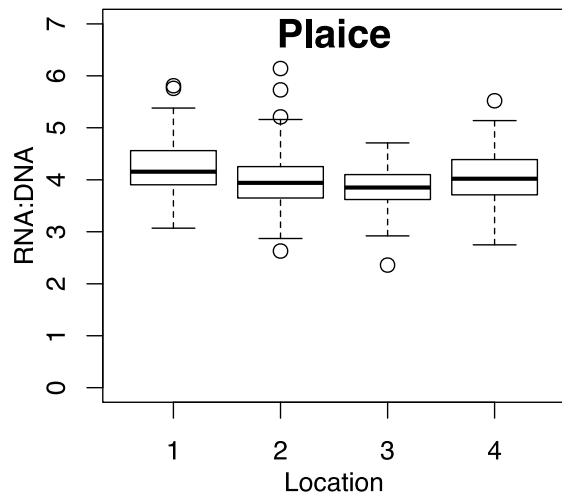
RNA/DNA Quantification



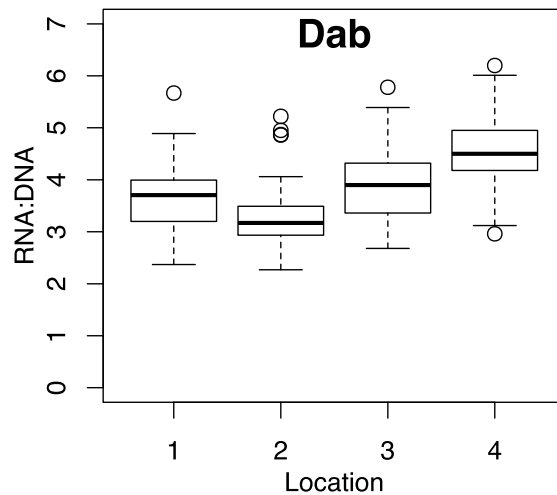
Method comparison



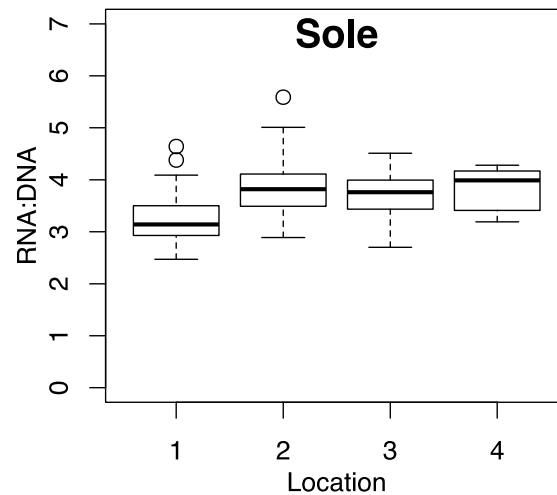
Location differences in RNA/DNA



N: 76 120 109 87



N: 48 103 74 77



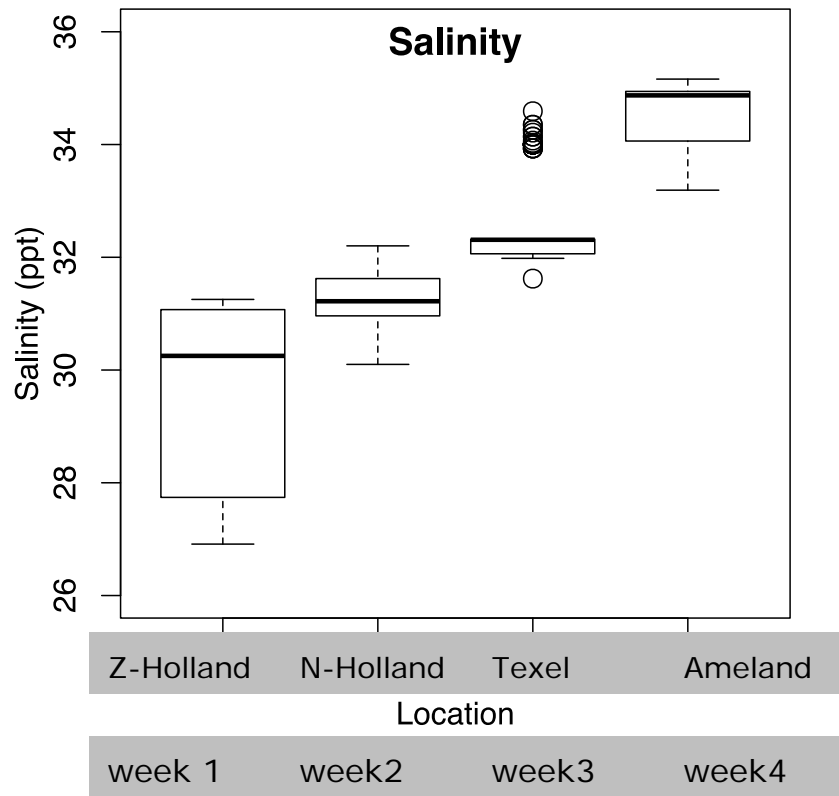
N: 17 33 23 5

Factors considered

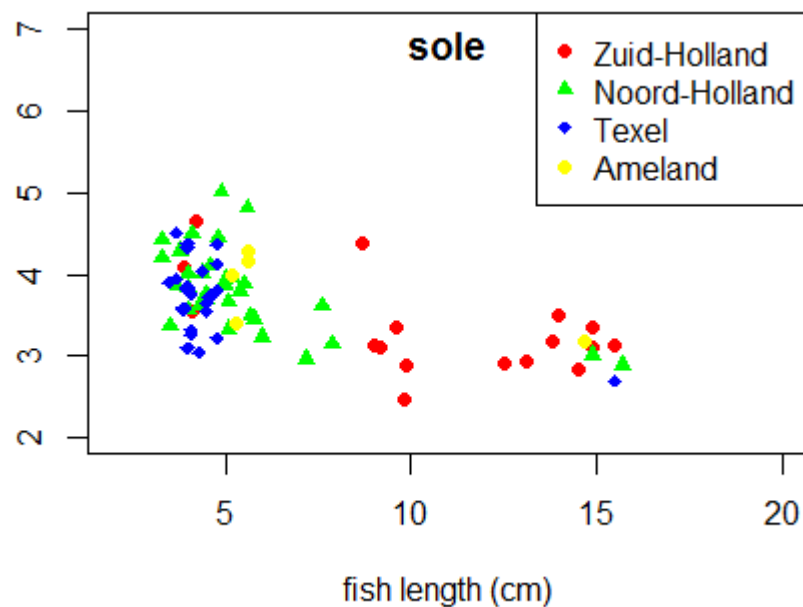
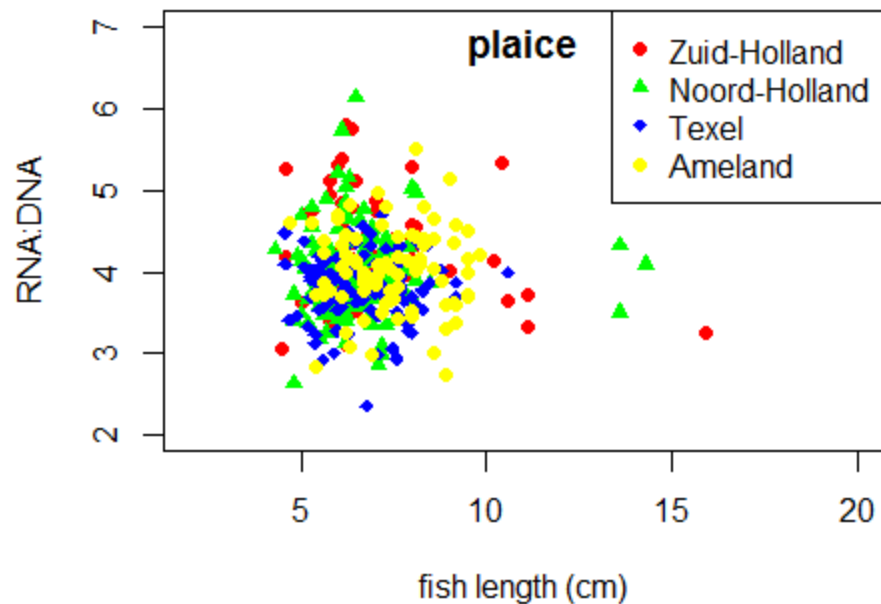
- Temperature
- Salinity
- Depth
- Date
- Tidal phase
- Location
- Sediment grain size
- Density of benthic prey <- *No data yet*
- Density of shore crab
- Density of large common shrimp (+30 mm)
- Density of flatfish (highly correlated with shore crab)



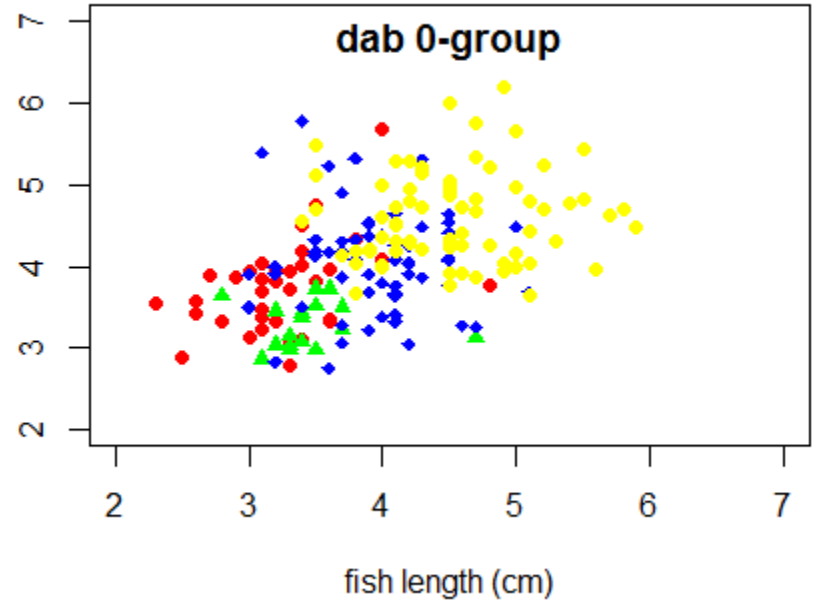
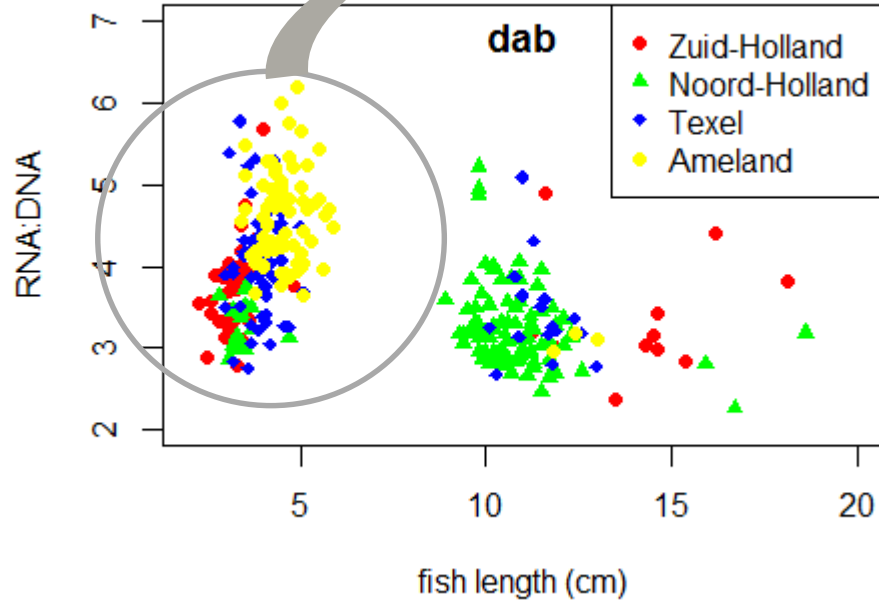
Location confounded with salinity and week



Growth ~ fish length



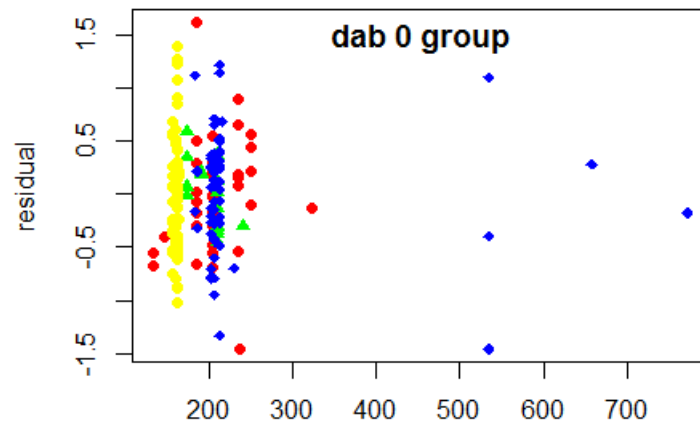
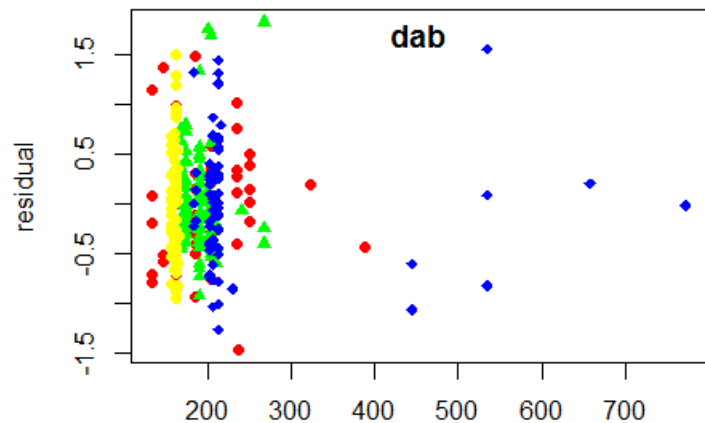
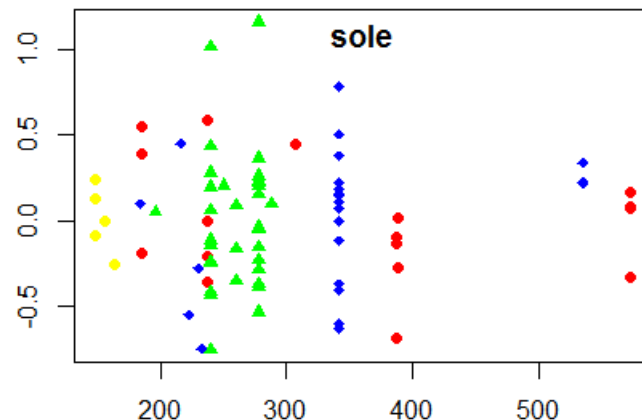
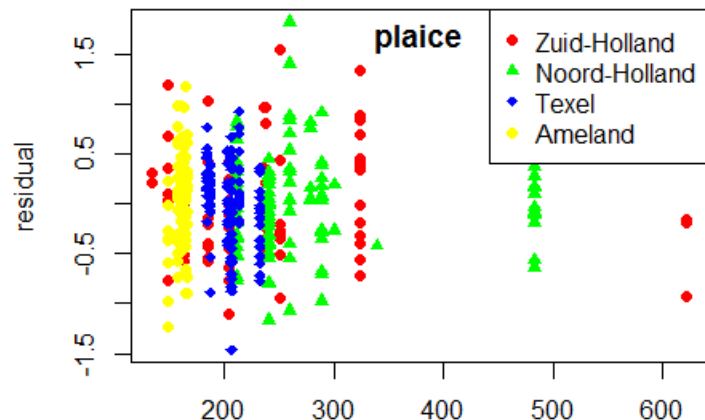
Growth ~ fish length



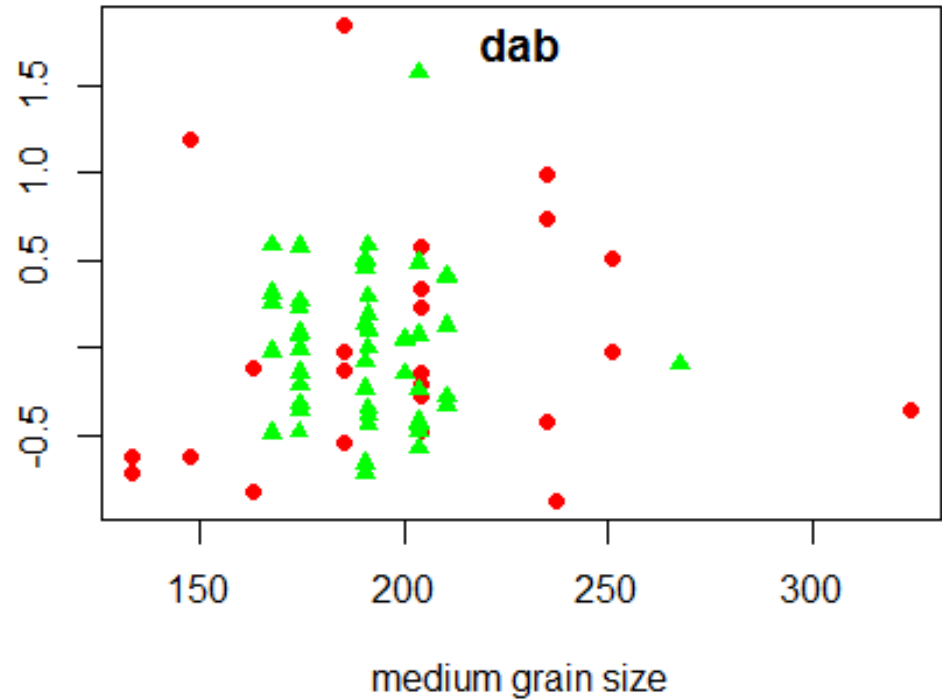
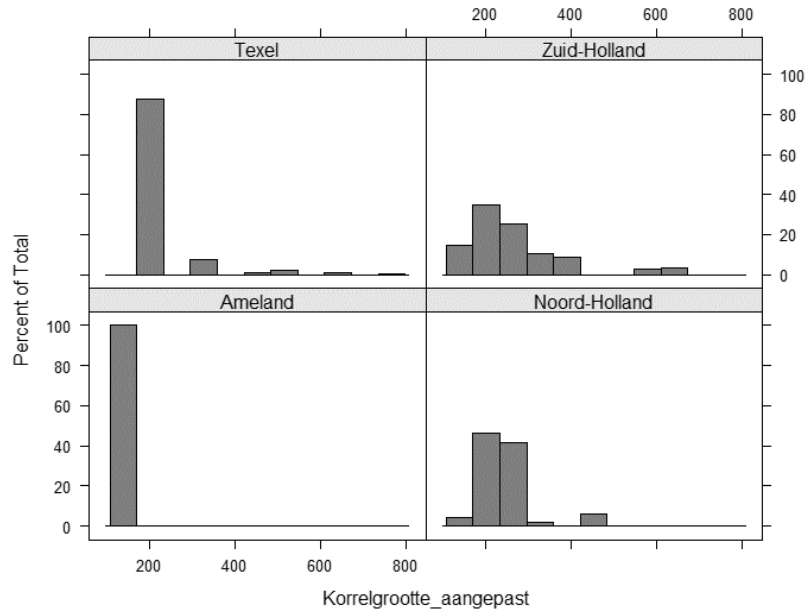
Preliminary analysis (lme)

factor	plaice	dab	dab 0 group	sole
fish length		-		-
time of day	-	-		-
date			+	
temperature		-	-	
water depth		-	-	+
salinity	-			
water visibility	-		-	-
Log(density shore crab)	-		+	+
Log(density large brown shrimp)		-	-	
Log(density conspecific)	+	+		-
Locations (factor)	S	S		

Relationship with sediment (medium grain size)?



only Z- and N- Holland: slight positive trend



Discussion

- Qubit suitable to measure RNA/DNA
- Effect of tissue weight: keep constant as possible
- range RNA high sensitivity kit too limited to accurately quantify RNA in fastest growing juveniles

=>Solution: Qubit™ RNA Broad Range Assay Kit

- Confounding: Seasonal effect ~ location effect ~ salinity effect
- Variation in RNA/DNA related to several (a)biotic factors
- variable effects epibenthic predators for growth plaice and dab
- No relation with sediment (apart from dab), but enough resolution?

Future work

- Next step: collecting fish later in the year, when food becomes limiting and growth is reduced

