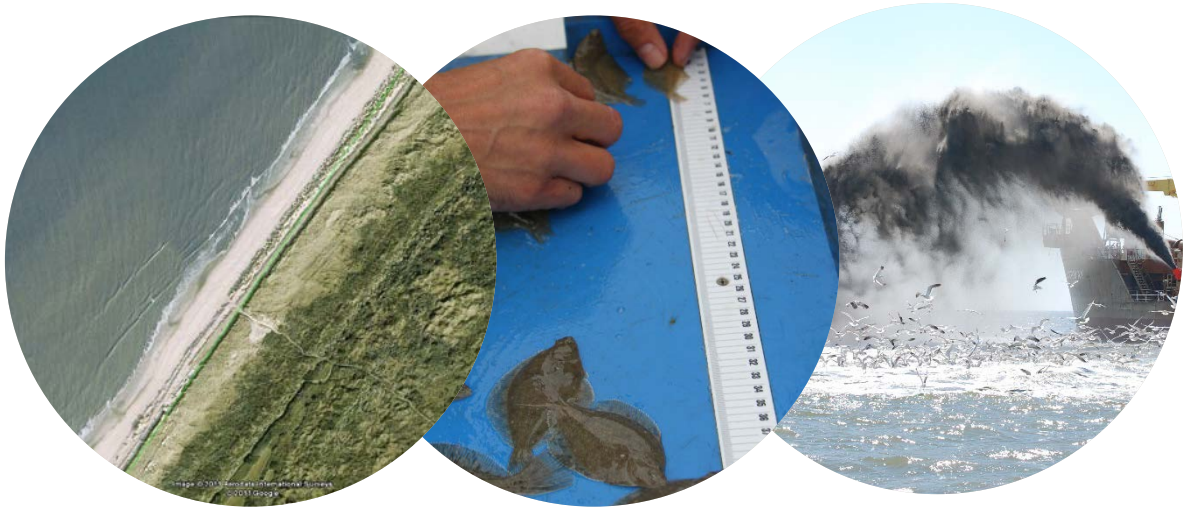
RNA/DNA ratios used to study growth in coastal nursery areas

Comparison of methods and relation with environment

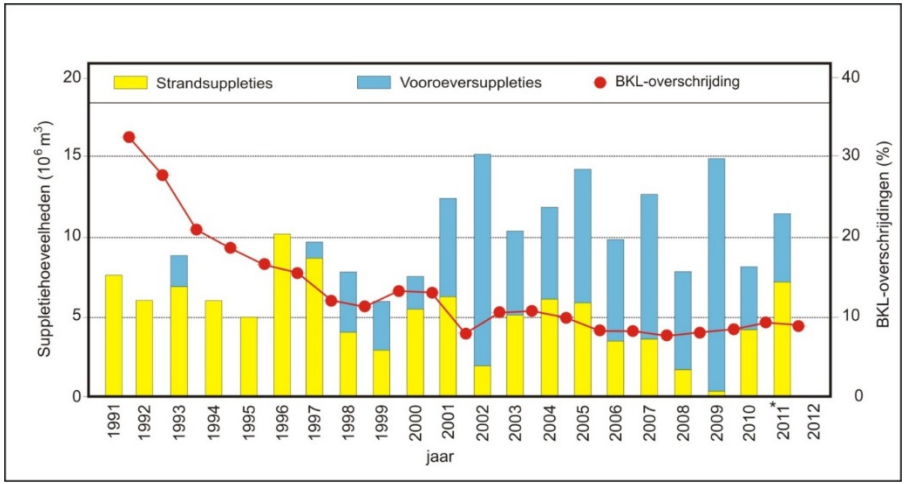
Maarten Rutting, Richard Crooijmans , Ralf van Hal & Ingrid Tulp



WAGENINGEN
UNIVERSITY & RESEARCH

Living below sea level…

Regular nourishments since 1991



Sand nourishments and nurseries

Impact on nursery function?

- Knowledge on the impact:
  - Benthic community restores within a year after sand nourishment
  - Effects on fish community?
  - Effects on the nursery function?

  =>fish growth?

June 2017: multidisciplinary survey

- Wageningen Marine Research
- Multidisciplinary survey

MSC Maarten Rutting:

Fish growth RNA/DNA

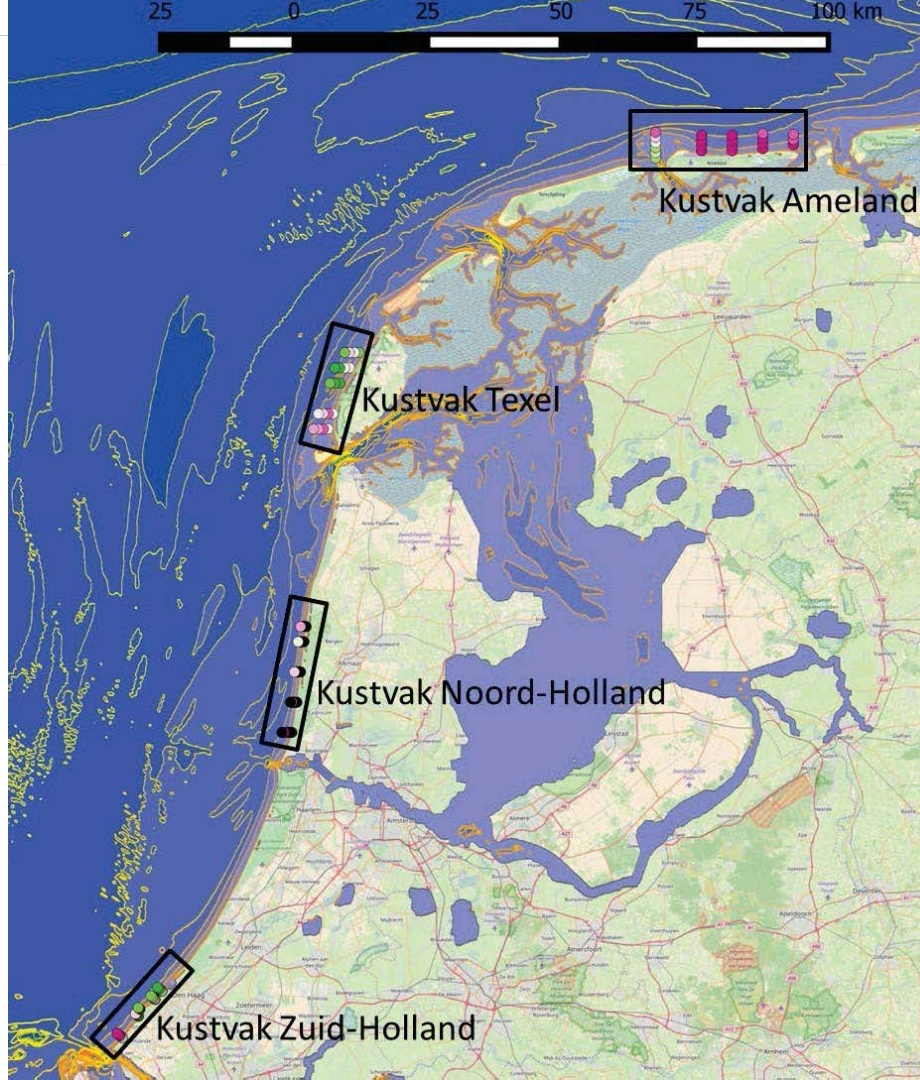
- Animal Breeding & Genetics

Richard Crooijmans

Locations

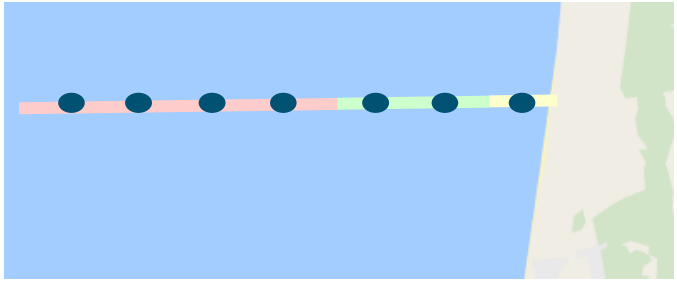
- Location 1: Zuid-Holland
- Location 2: Noord-Holland
- Location 3: Texel
- Location 4: Ameland

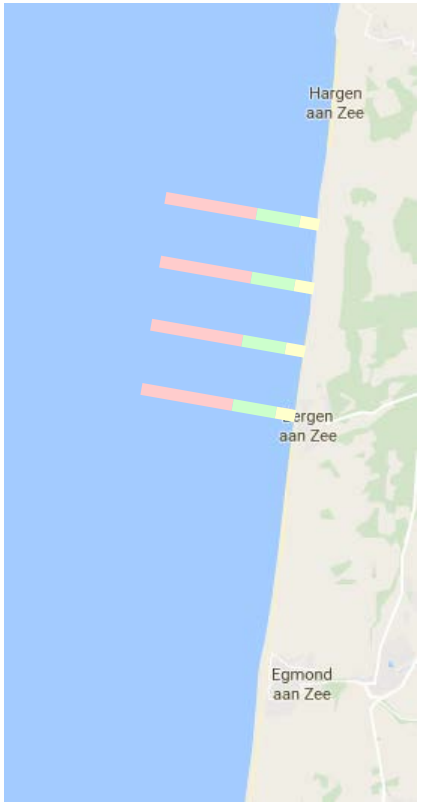
=>4 consecutive weeks from South to North



Transects: fish sampling

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





Aim MSc project

- compare methods to measure RNA:DNA ratio's
- Investigate growth juvenile flatfish in June in nurseries along the Dutch coast
- Growth ~ related to abiotic factors?
- Relation with sand nourishments: role of sediment?

Survey - Benthos

Survey - Sediment

Survey - Fish

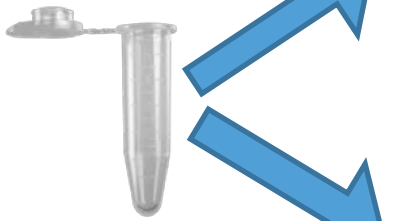
Tissue collection

- starting points
  - For tissue collection:
    - Directions from Benjamin Ciotti (thanks!)
  - For isolation:
    - Protocol and Guide for Estimating Nucleic Acids in Larval Fish Using a Fluorescence Microplate Reader (Caldarone, *et al.*, 2001)

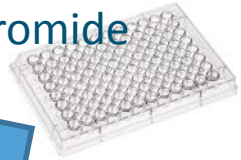
RNA/DNA Quantification: two methods

- Ethidium bromide
- Qubit Fluorometer
    - Already used before to analyse RNA:DNA ratio's
    - Using
        - RNA High Sensitivity Assay Kit (Invitrogen™)
        - dsDNA High Sensitivity Assay Kit (Invitrogen™)

RNA/DNA Quantification



Add Ethidium bromide



Measure #1

Add RNAse + Incubation 30 min

Measure #2

Calculate fluorescence for DNA

Quantify RNA and DNA

Add Qubit DNA- and RNA dye

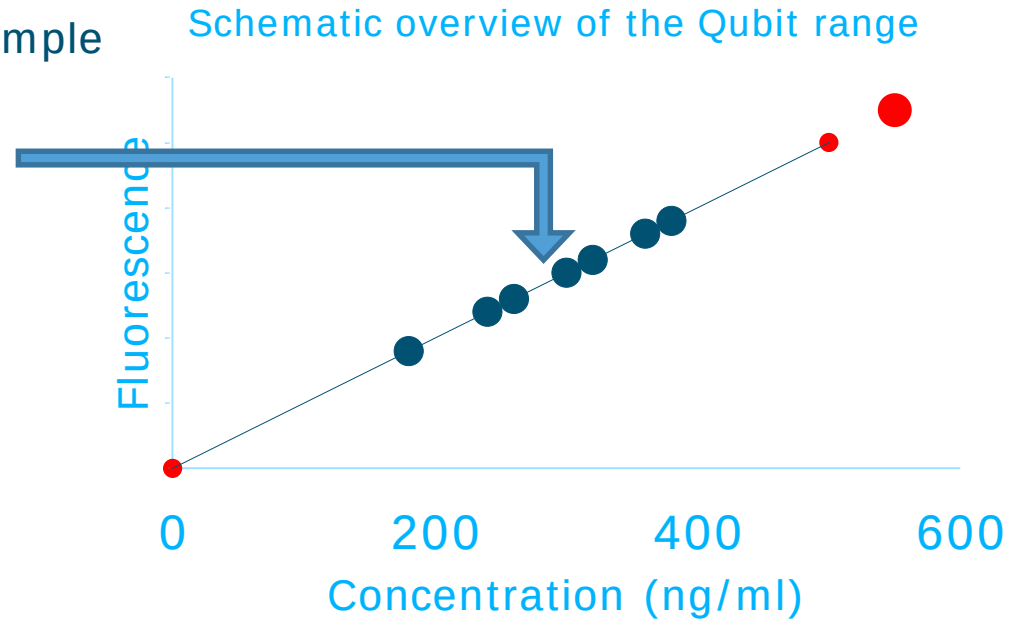
Incubation 2 min

Measure

Qubit Quantifies RNA and DNA
+
Correction for sample input

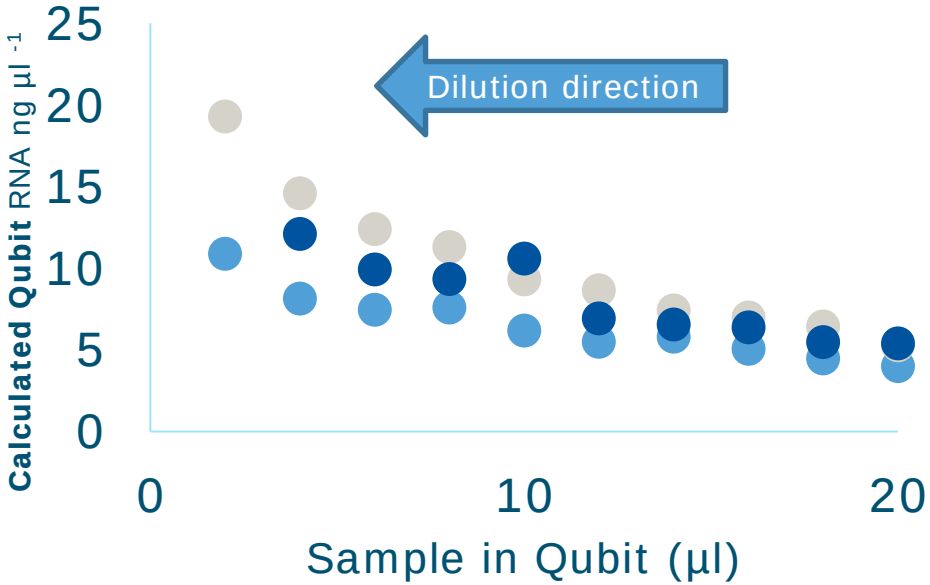
Qubit range

- Ideally
  - maximum amount of sample (20 µl)
  - measurements should fall in the middle of the range
- Possible for DNA
- Not possible for RNA
  - ➢ Dilution required

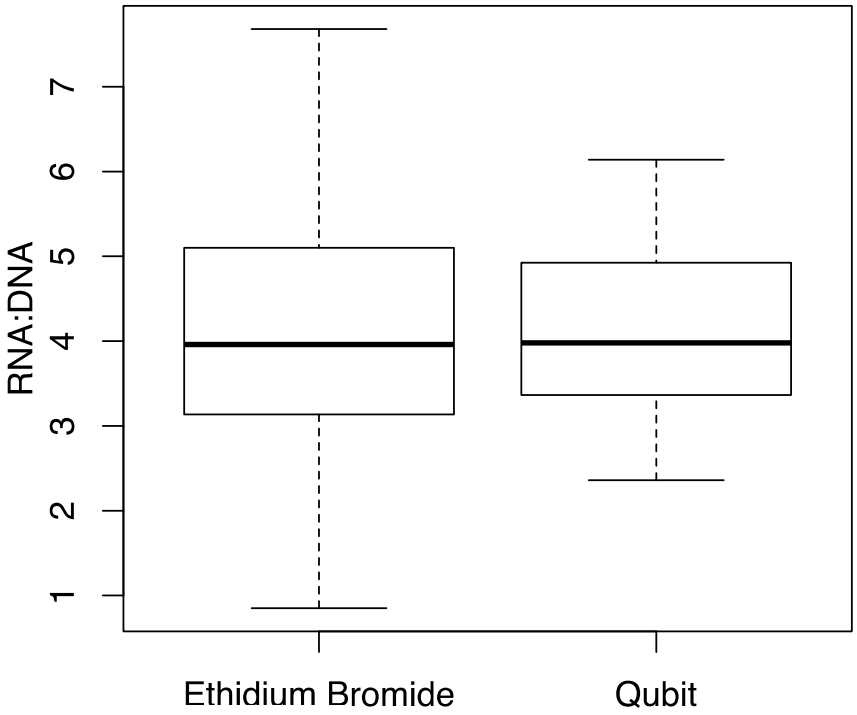


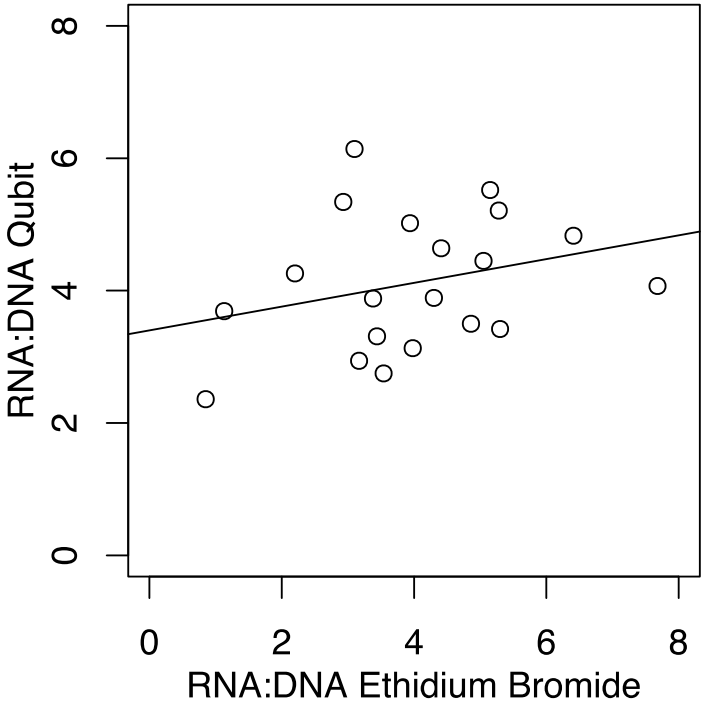
Dilution effect

- Using less sample volume:
  - bias in the result
- Correction needed for diluting
- Dilution series to produce corrections for diluting

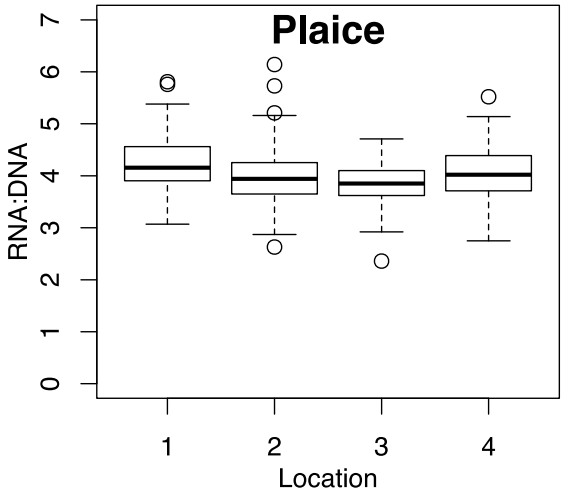


Method comparison

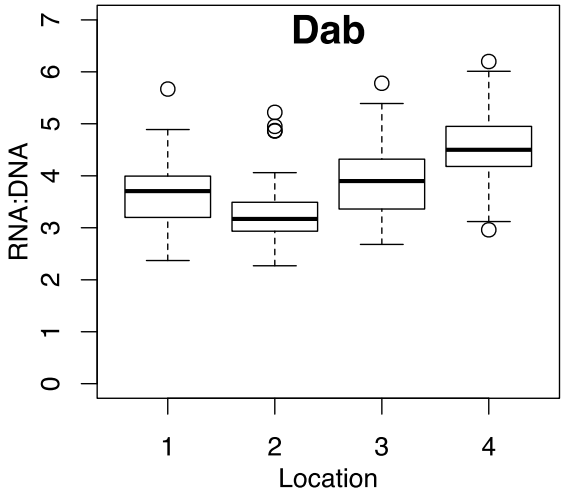




Location differences



N: 76 120 109 87



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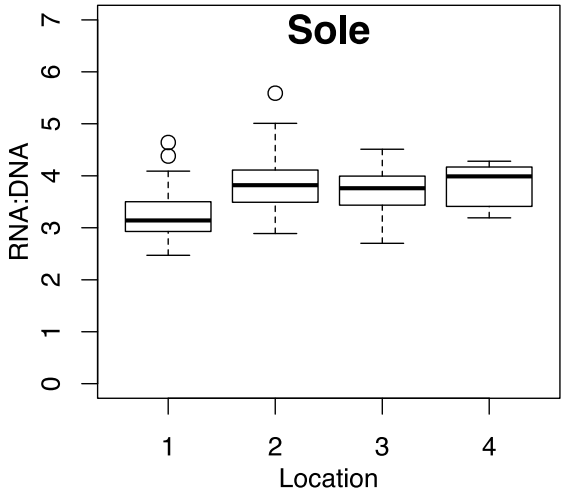
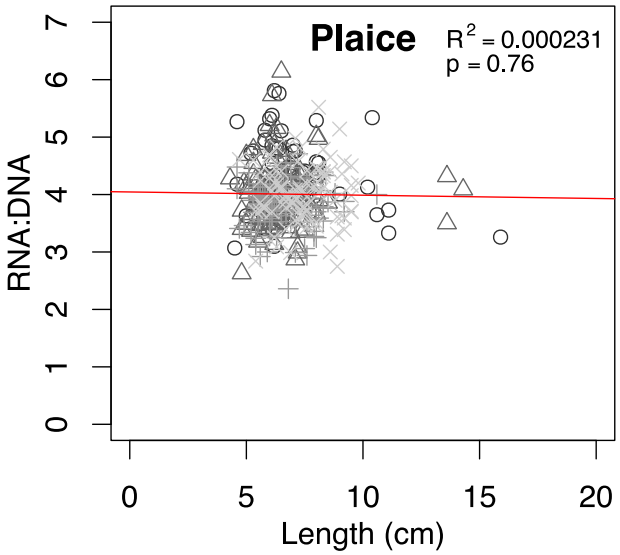


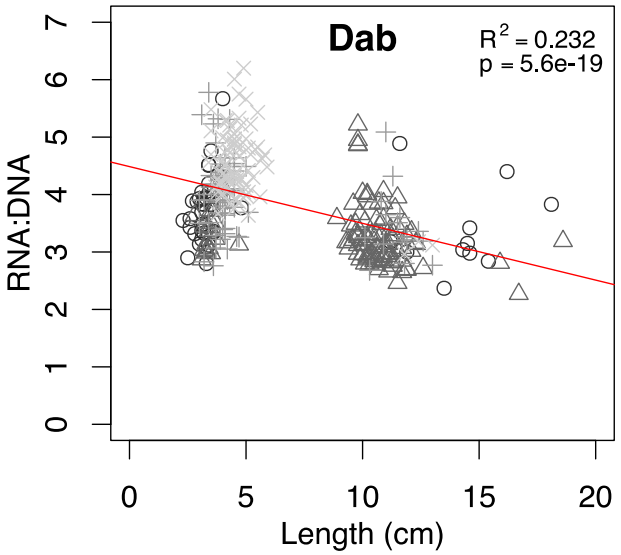
N: 17 33 23 5

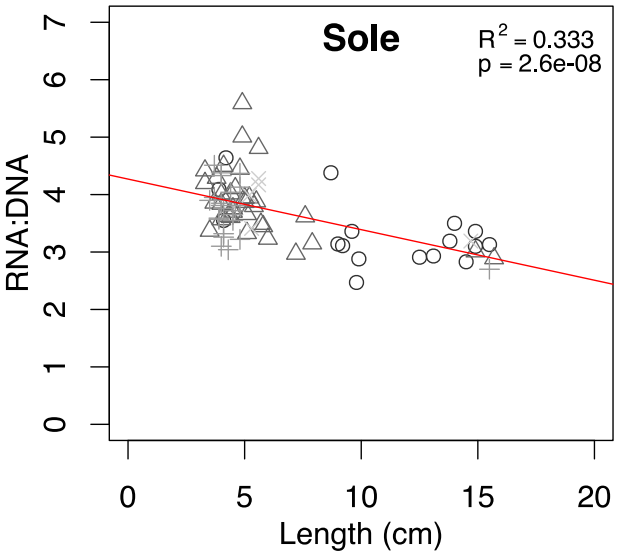
Factors considered

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

RNA/DNA~fish length

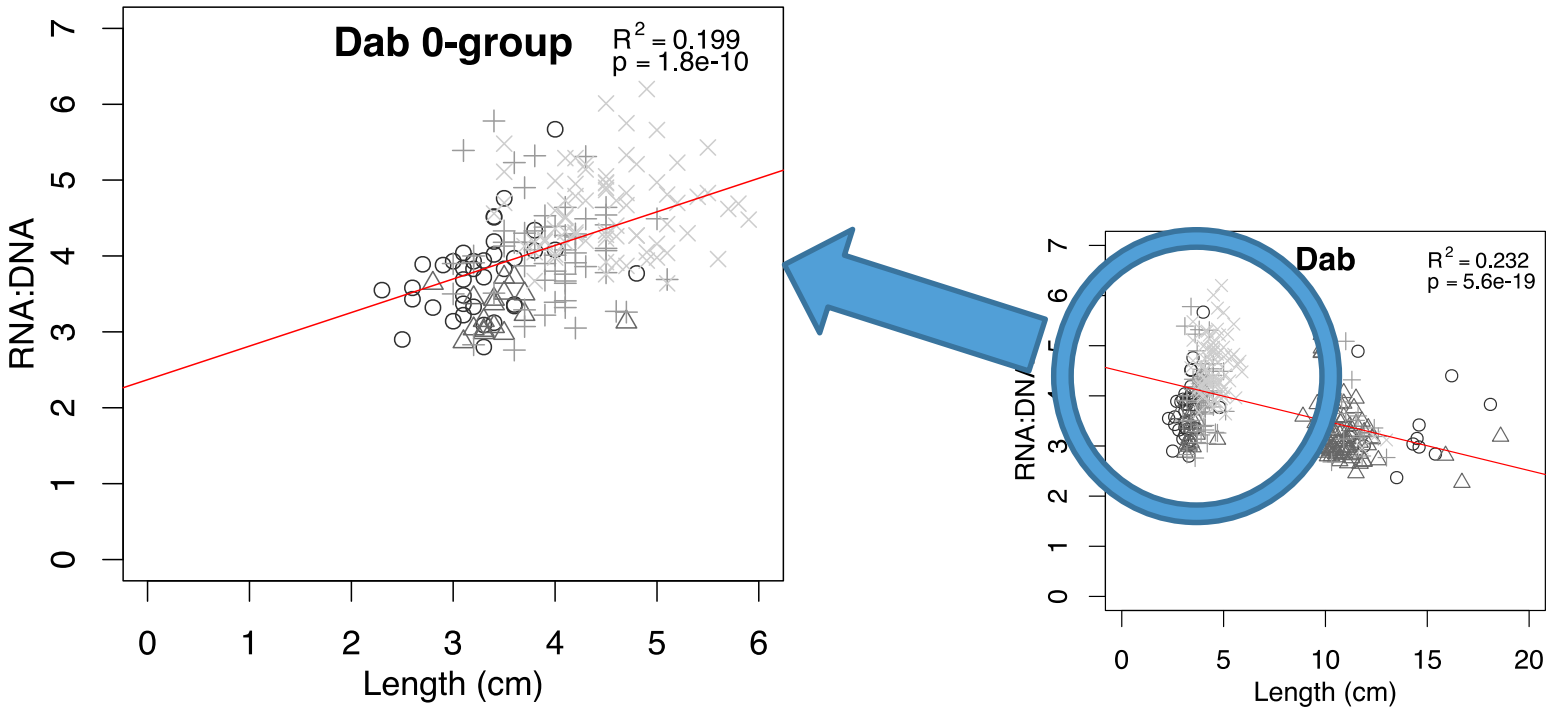






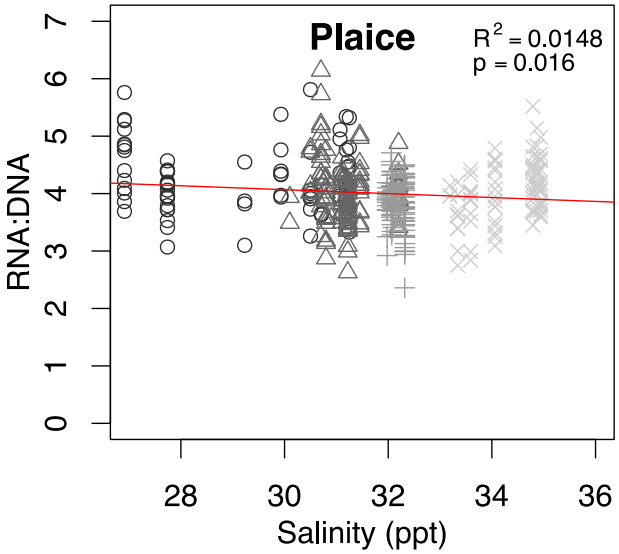
locations:	1 (o)	2 (Δ)	3 (+)	4 (×)

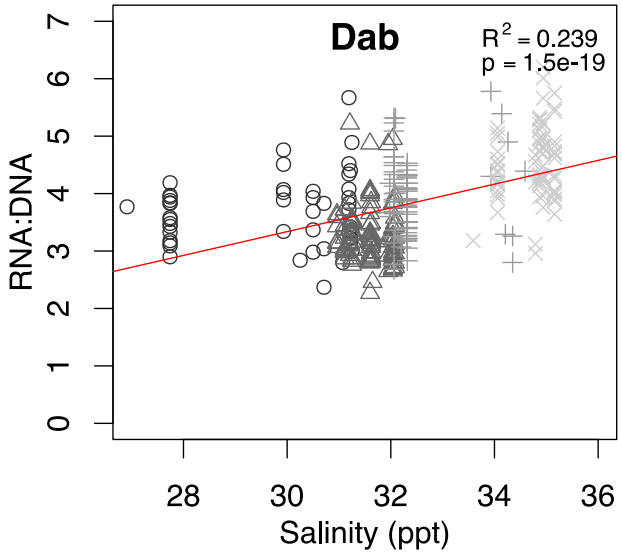
RNA/DNA~fish length

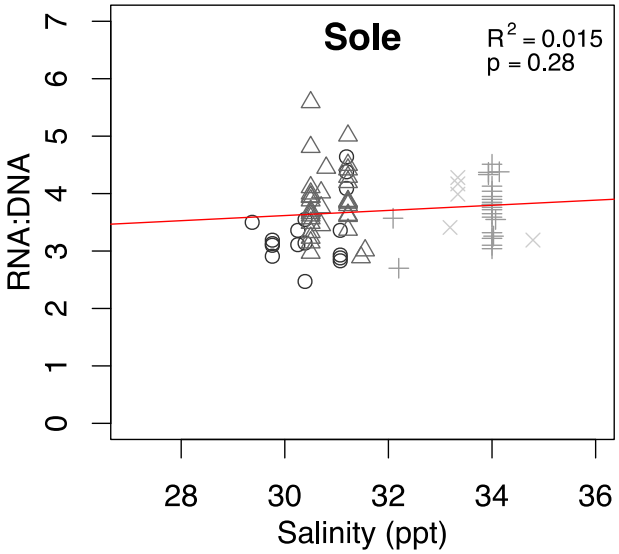


locations:	1 (o)	2 (Δ)	3 (+)	4 (×)

RNA/DNA~salinity







locations:	1 (o)	2 (Δ)	3 (+)	4 (×)

Preliminary analysis (lme)

factor	plaice	dab	dab 0 group	sole
fish length	ns	-	+	-
temperature	-	-	ns	ns
salinity	-	+	+	ns
water visibility	-	ns	ns	ns
density shore crab	-	ns	ns	ns
density large brown shrimp	ns	-	ns	ns
Locations (factor)	ns	s	s	ns

Discussion

- Qubit suitable to measure RNA/DNA
- range RNA high sensitivity kit too limited to accurately quantify RNA in fastest growing juveniles

  =>Solution: Qubit™ RNA Broad Range Assay Kit

- Seasonal effect ~ location effect
- Variation in RNA/DNA related to several (a)biotic factors
- Negative effect epibenthic predators
- Location and salinity confounding
- *Relation with sediment: still too be included in analysis*

Future work

- Include sediment data
- Refine Qubit method
- Next step: collecting fish later in the year, when food becomes limiting and growth is reduced

Thanks for listening

Questions (not too technical ☺)

Discussion: Qubit vs Ethidium bromide

Pros:

- Measure both DNA and RNA
- Easy to use, less steps involved that influence outcome
- No enzymatic steps required
- safer to use and requires less training
- possible to use the kits with a Fluorometric plate reader

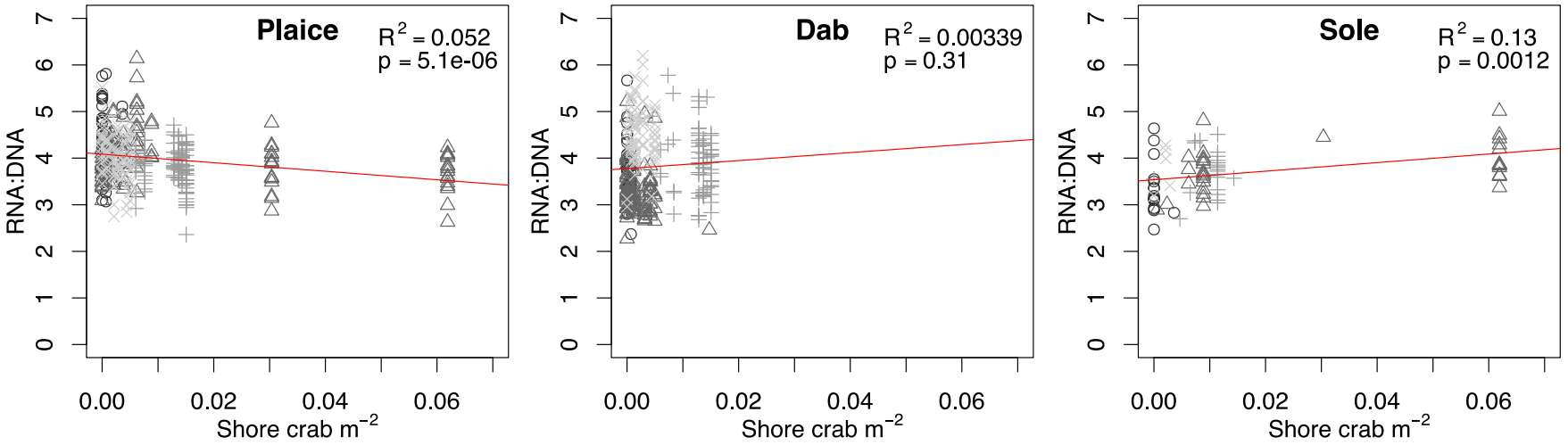
Cons:

- range RNA high sensitivity kit too limited to accurately quantify RNA in fastest growing juveniles

  =>Solution: Qubit™ RNA Broad Range Assay Kit

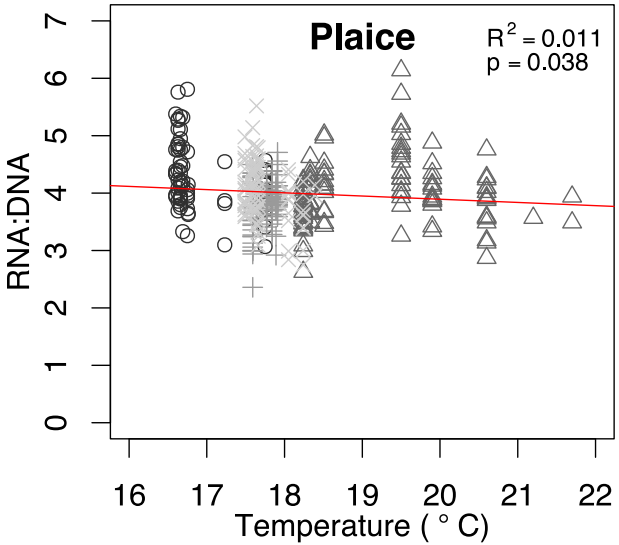
- RNA quantification is sensitive to dilution

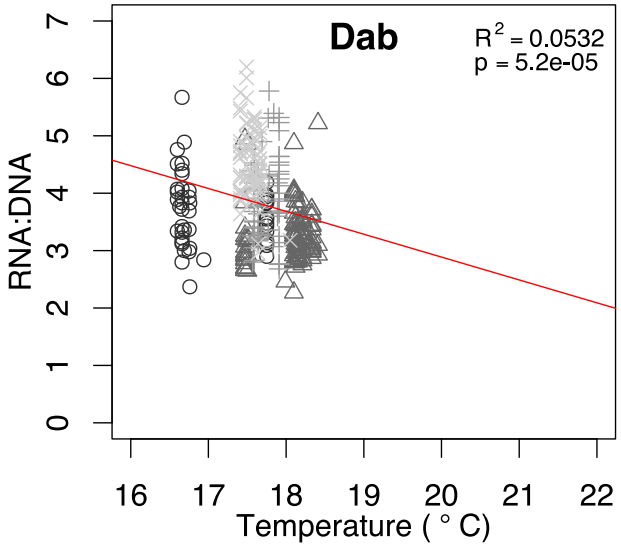
Shore crab Density

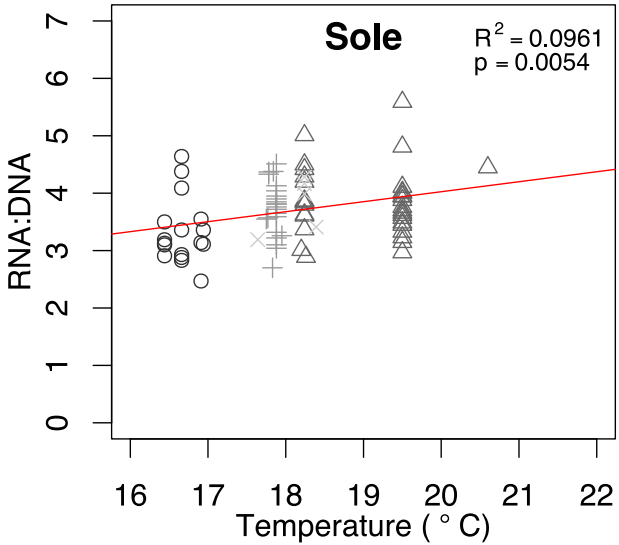


locations:	1 (o)	2 (Δ)	3 (+)	4 (×)

RNA/DNA~temperature







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