APPLICATION OF SYNTHETIC DNA IN STREAM TRACER INJECTION EXPERIMENTS

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Their **conclusions** after a number of field tests with DNA:

- Sensitive detection method;
- Availability of different sizes with variable valences;
- Infinite number of tracers;
- Cost effective;
- Harmless to the environment;
- Good alternative to organic, inorganic or radioactive tracers;
- Factors influencing transport of DNA tracers (e.g. degradation, sorption) need research;
DNA labeled montmorillonite (to trace particle transport in rivers);
Methods: jars with DNA labeled clay and various ‘waters’; PCR, gel-electrophoresis;
Result: DNA-montmorillonite surface complexes are stable in spring waters for periods of up to 18 days and only slowly desorb to the aqueous phase;
Work of Sabir and Mahler had one major disadvantage:

- **QUALITATIVE:** determining presence/absence;
Example of measured laboratory column breakthrough curves for bromide and DNA tracer.
Foppen et al., 2011: multiple artificial DNA tracers
• **Proof of concept:** Poly lactic acid (PLA) microspheres with a core of paramagnetic iron and a short strand of DNA;
  
  • PLA => protects DNA from decay;
  
  • Paramagnetic iron => to facilitate magnetic concentration;
Sharma et al., 2012: nanoparticles DNA tracers

Plot experiment:
- Asphalt surface;
- Simultaneous and instantaneous application of 400 mg tracer 1 and 2;
Objective

Quantitative comparison of the behaviour of one synthetic DNA tracer and dissolved NaCl in stream tracer injection experiments
Dimensions DNA tracer

length: ~ 0.33 nm/base

sugar phosphate backbone

bases

80 nt ssDNA: ~ 26 x 1.2 nm
Detection principle
Detection principle

In one qPCR well:

4.0 µl of sample from the injection experiment (with unknown DNA concentration)

2.0 µl 10X PCR buffer (100 mM Trizma®-HCl, pH 8.3 at 25 C, and 500 mM KCl),
0.8 µl of PCR grade MgCl₂ solution (25 mM),
0.125 µl of forward primer (62.5 nM final concentration),
0.125 µl of reverse primer (62.5 nM final concentration),
0.3 µl probe (150 nM final concentration)
1.0 µl Taq polymerase (final enzyme concentration of 0.5 U per PCR well)
0.25 µl dATP, 0.25 µl dGTP, 0.25 µl dCTP, 0.25 µl dTTP (all: 0.2 mM final concentration)
10.65 µl DEPC treated PCR grade water

20 µl total
Detection principle

40 cycles of denaturation (95 °C for 15 seconds) and annealing/extension (60 °C for 60 seconds)
Detection principle

Sample RFU is compared with a standard curve of known DNA tracer concentrations.

\[ y = -0.3115x + 10.445 \]

\[ R^2 = 0.9995 \]
Design considerations for a good DNA tracer and primers:

- 80 nucleotides (trade-off between costs and uniqueness, although DNA > 30 bp is already quite unique);

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tracer sequence:</th>
<th>Primer(s):</th>
<th>Probe:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>80 nucleotides</td>
<td>20 nucleotides</td>
<td>24 nucleotides</td>
</tr>
<tr>
<td>GC-content:</td>
<td>40-60%</td>
<td>40-60%</td>
<td>40-60%</td>
</tr>
<tr>
<td>Annealing temp:</td>
<td>-</td>
<td>55-60 °C</td>
<td>55-60 + 8-10 °C</td>
</tr>
<tr>
<td>3'-end:</td>
<td>-</td>
<td>Not GGG, not T</td>
<td>-</td>
</tr>
<tr>
<td>5'-end:</td>
<td>-</td>
<td>-</td>
<td>Not G</td>
</tr>
<tr>
<td>Complementarities:</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>BLAST search (nr/nt):</td>
<td>No similarity found</td>
<td>No similarity found</td>
<td>No similarity found</td>
</tr>
<tr>
<td>BLAST with primers:</td>
<td>No template found</td>
<td>No template found</td>
<td>No similarity found</td>
</tr>
<tr>
<td>BLAST with primer(s) and probe:</td>
<td>No template found</td>
<td>No template found</td>
<td>No template found</td>
</tr>
</tbody>
</table>

(Foppen et al., subm.)
Methods

- Improved protocol for taking DNA samples;
- Use of probe (more specific than the dye SYBR Green);
- Dilution error analysis of the ‘DNA method’;
- Mass balance of DNA injection experiments in selected streams in NL, Belgium and Lux.;
  - Instantaneous injection of known DNA and NaCl mass;
  - Determining BTCs at 2 locations downstream of point of injection;
- Separate:
  - Field batch experiment to determine DNA decay;
  - Batch experiment with river water and sediment to determine sorption;
Results: dilution error analysis

- Preparing a standard curve for qPCR analysis requires the preparation of 13 10-fold serial dilutions;
- Serial dilutions are prepared with Gilson Pipetman pipettes. In total 36 pipetting actions are required!

\[
y = -0.3115x + 10.445 \\
R^2 = 0.9995
\]
Results: dilution error analysis

- Dilution errors (Hedges, 2002):
  - Pipetting error: measured (in)accuracy and (im)precision of all pipetting equipment used in our laboratory was around 2% on a routine basis;
  - In a vial, DNA particles are randomly distributed, so counts of DNA follow the Poisson rule: 1) the variance is equal to the mean, 2) the standard deviation = square root of the mean. And, at each dilution: Poisson sampling error;
Results: dilution error analysis

Ratio of confidence interval (C.I.) at 95% confidence level and undiluted concentration (N*)

(Foppen et al., subm.)
Results: field batch experiments

(Foppen et al., subm.)
Results: batch experiments with (settled) sediment

Heuwelerbach

(Foppen et al., subm.)
Results: batch experiments

Observations:

1. DNA mass in the batch container was constant in time => for the duration of the experiments, decay rate coefficient was negligible;
2. Remarkable and statistically significant difference between the DNA mass injected and DNA mass recovered from the batch container => initial loss?
3. Differences between injected mass and recovered mass were not constant among the various brooks: 3-50% of injected mass was recovered;
4. (with sediment): In most batches and for short duration experiments, interaction of DNA with settled sediment was insignificant;

(Foppen et al., subm.)
Results

Maisbich

(Foppen et al., subm.)

\[ \begin{align*}
\text{NaCl tracer} : & \quad \text{T23 tracer} \\
\text{Added NaCl concentration (mg/L)} : & \quad \text{Time elapsed since injection (min)} \\
\text{T23 concentration (part/L)} : & \quad \text{Added NaCl concentration (mg/L)}
\end{align*} \]
Results

Heuwelerbach

\[\text{NaCl tracer} : \text{DNA T23 tracer}\]

(Foppen et al., subm.)
Results

Biezenloop

\[ \text{NaCl tracer}: \text{DNA T23 tracer} \]

\( \text{(Foppen et al., subm.)} \)
Results: injection experiments

Observations:

1. For NaCl and DNA: time of the first arrival (off-set on x-axis), shape of the rising limbs, and time to peak were identical;
2. Falling limbs of NaCl BTC were less steep than of DNA;
3. DNA covered a large concentration range of 4 to 5 logarithmic units;
4. In some samples: false positive. *Compounds present in river water have increased Taq polymerase activity?*

(Foppen et al., subm.)
## Results: DNA and NaCl mass balance injection experiments

### DNA

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Distance (m)</th>
<th>Q (l/s)</th>
<th>DNA mass injected (# of part.)</th>
<th>DNA mass recovered (# of part.)</th>
<th>DNA mass recovered (% of mass injected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maisbich</td>
<td>100</td>
<td></td>
<td>(1.32±0.55)·10^{15}</td>
<td>(4.31±1.81)·10^{14}</td>
<td>32.0±13.4</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>34</td>
<td></td>
<td>(4.34±1.82)·10^{14}</td>
<td>32.2±13.5</td>
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<tr>
<td>Heuwelerbach</td>
<td>300</td>
<td></td>
<td>(8.05±3.38)·10^{15}</td>
<td>(4.86±2.04)·10^{14}</td>
<td>6.0±2.5</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>15</td>
<td></td>
<td>(5.16±2.17)·10^{14}</td>
<td>6.4±2.7</td>
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<tr>
<td>Biezenloop</td>
<td>300</td>
<td></td>
<td>(1.00±0.42)·10^{16}</td>
<td>(4.64±1.95)·10^{15}</td>
<td>45.0±18.9</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>20</td>
<td></td>
<td>(5.26±2.21)·10^{15}</td>
<td>51.0±21.4</td>
</tr>
</tbody>
</table>

### NaCl

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Distance (m)</th>
<th>Q (l/s)</th>
<th>NaCl mass injected (kg)</th>
<th>NaCl mass recovered (kg)</th>
<th>NaCl mass recovered (% of mass injected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mais-down</td>
<td>100</td>
<td></td>
<td>2.00</td>
<td>1.91</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>34</td>
<td></td>
<td>1.69</td>
<td>84.7</td>
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<tr>
<td>Heuwelerbach</td>
<td>300</td>
<td></td>
<td>0.75</td>
<td>0.73</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>15</td>
<td></td>
<td>0.71</td>
<td>94.3</td>
</tr>
<tr>
<td>Biezenloop</td>
<td>300</td>
<td></td>
<td>2.00</td>
<td>2.12</td>
<td>106.1</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>20</td>
<td></td>
<td>1.98</td>
<td>98.8</td>
</tr>
</tbody>
</table>
Results: DNA and NaCl mass balance injection experiments

Observations:

1. Likely, also in the injection experiments, an instantaneous loss of DNA mass occurred;
2. The loss was dependent on the brook in which the DNA mass was injected;
3. Per brook, the magnitude of DNA loss was similar in both injection and batch experiment;
4. For two consecutive measurement points downstream of the point of injection, DNA mass recoveries were identical;

(Foppen et al., subm.)
Results

Biezenloop – mass balance modeling with OTIS

\[ \text{\( \triangle \) : NaCl tracer} \]

\[ \text{\( \diamond \) : DNA T23 tracer} \]

(Foppen et al., subm.)
Results: Curve fitting-by-eye by OTIS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>x (m)</th>
<th>D (m²/s)</th>
<th>A (m²)</th>
<th>α (s⁻¹)</th>
<th>Aₛ (m²)</th>
<th>Init. loss (-)</th>
<th>λₛ̂ (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maisbich</td>
<td>100</td>
<td>0.10</td>
<td>0.21</td>
<td>5.0·10⁻⁴</td>
<td>1.5·10⁻³</td>
<td>0.72</td>
<td>1·10⁻²</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1.00</td>
<td>0.05</td>
<td>8.0·10⁻⁵</td>
<td>3.0·10⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heuweler-bach</td>
<td>300</td>
<td>0.10</td>
<td>0.11</td>
<td>0.0</td>
<td>n.a.</td>
<td>0.89</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>0.30</td>
<td>0.06</td>
<td>0.0</td>
<td>n.a.</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Bloop</td>
<td>550</td>
<td>0.10</td>
<td>0.23</td>
<td>1.0·10⁻³</td>
<td>4.0·10⁻²</td>
<td>0.50</td>
<td>1·10⁻³</td>
</tr>
</tbody>
</table>

(Foppen et al., subm.)
Conclusions

Reconfirming Foppen et al., 2011:

1. Synthetic DNA performed as well as salt with regard to the shape and timing of the BTCs;
2. We found that DNA tracers covered a large concentration range of 4 to 5 logarithmic units;

(Foppen et al., subm.)
Conclusions

New:

1. The use of a Taqman probe with a length of 25 nucleotides increased the accuracy to detect synthetic DNA during qPCR;
2. Step-wise identification and application of revised and improved protocols for sample taking, sample storage, and DNA tracer analysis further increased accuracy;

(Foppen et al., subm.)
As a result, we:

(a) quantified the error in the workflow;
(b) found that DNA, both in batch experiments and after injection into the brook, behaved in the same way. First, an instantaneous DNA tracer loss occurred, and then, the DNA mass became constant;
(c) found that for the duration of our experiments, DNA tracer decay and main channel sorption were insignificant;
(d) quantified longitudinal dispersion, transient storage zone dimensions and storage zone exchange rate, based on combined use of NaCl and DNA breakthrough curves;

(Foppen et al., subm.)
Improving the DNA-method

1. Causes and quantification of initial DNA tracer losses;
2. Reducing the occurrence of false positives in determining DNA tracer concentrations;
3. Long-term behavior of synthetic DNA tracer needs to be explored;
4. Use in DNA tagged nano-particles?

(Foppen et al., subm.)
Future applications

Current state:
1. Determining longitudinal and transversal dispersion in rivers with discharges $> 100$ m$^3$/s;
2. Determining source areas and analysing flow paths in multi-tracer applications in complex flow systems (e.g. karstic areas; flow in fractured aquifers);
3. Studying sediment transport processes (particle tracking velocimetry);

Future:
Quantitative multi-tracer applications in selected surface water and groundwater applications;

(Foppen et al., subm.)
THANKS