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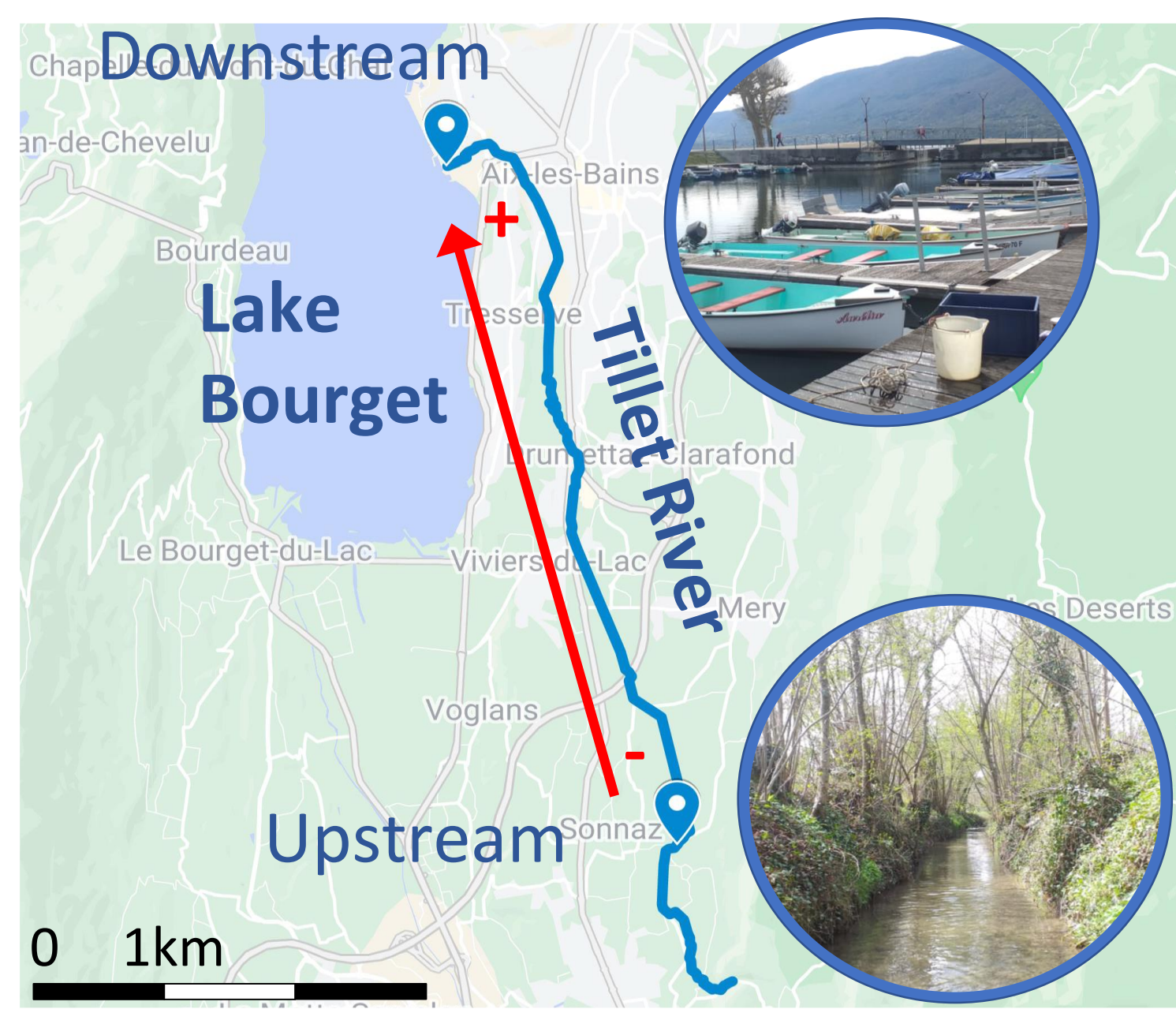
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In situ translocation experiment to assess the adaptation and resilience of periphytic communities to pharmaceutical substances using a PICT approach

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Introduction



→ Gradient of pharmaceutical concentrations
Map of the study site: the Tillet River

Aquatic environments are contaminated by pharmaceutical substances worldwide [1]. This pollution poses ecotoxicological risks to exposed organisms, including microbial communities, such as periphyton. Given the ecological role of periphytic communities (e.g. biomass production, nutrient recycling, etc.), preserving their integrity is essential for well-functioning aquatic ecosystems. However, knowledge about effects of pharmaceuticals on freshwater periphyton is still very limited.

In this context, we performed an *in situ* study by implementing a Pollution Induced Community Tolerance (PICT) approach, a relevant diagnostic tool to establish *in situ* direct causal relationships between toxicants and their effects [2].

Two main hypotheses were tested:

- 1) Chronic exposure to pharmaceuticals increases periphytic community tolerance to these substances
- 2) Tolerance dynamics (i.e. gain or loss of tolerance) reflect changes in exposure levels.

To this aim, we carried out a 10-week translocation study in the Tillet River (Savoie, France), considering an upstream reference station and a downstream station located in an urban area, and previously shown to be contaminated by different classes of pharmaceuticals. PICT measurements were combined with chemical analyses of pharmaceuticals in surface water using polar organic chemical integrative samplers (POCIS).

Materials and methods

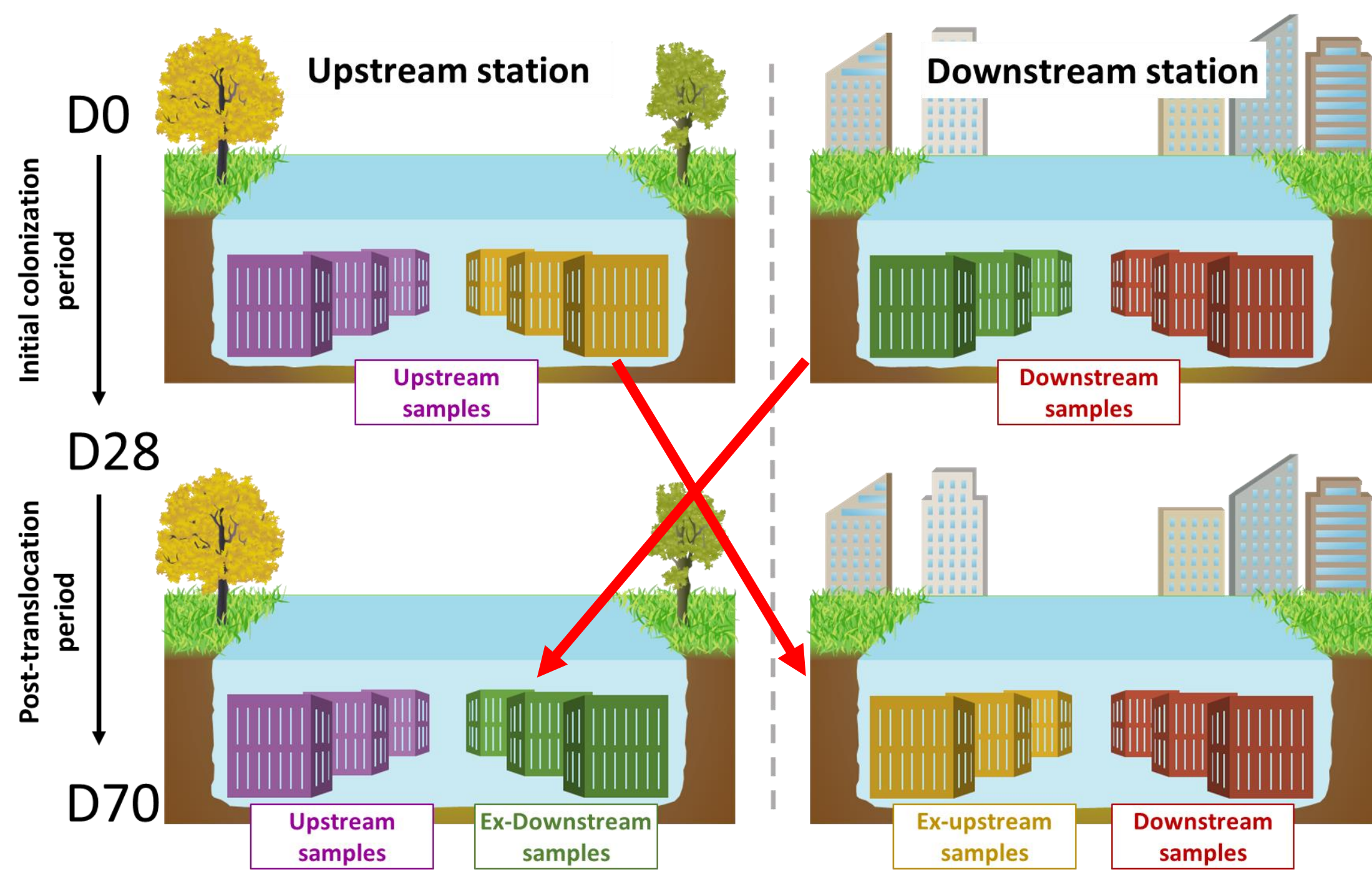


Illustration of the *in situ* experimental strategy

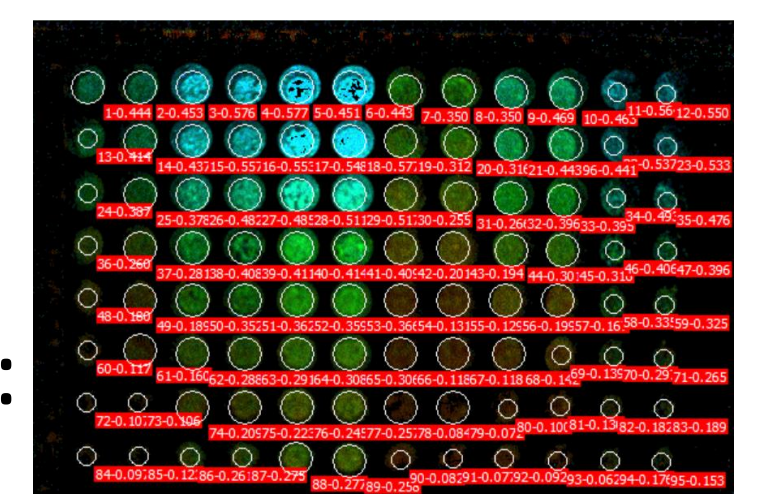
In Situ experimental strategy:

- After a 28-day initial colonization period, some periphytic biofilms were translocated (from up- to downstream and from down- to upstream).
⇒ Samplings were performed at days 28, 42, 56, and 70.
- Time averaged concentrations of selected pharmaceuticals ($n=42$) in surface water were estimated using POCIS deployed during successive 2-week periods at the two sampling stations throughout the study.



PICT measurements and tolerance comparisons:

- Acute toxicity tests on biofilms were performed with 7 pharmaceuticals: *atenolol*, *diclofenac*, *erythromycin*, *paracetamol*, *ofloxacin*, *sulfamethazine*, and *sulfamethoxazole*
- One of the three following activities was used as toxicity endpoint: β -glucosidase (4 hours), photosynthesis (4 h) or growth (72 h)
- Tolerance comparisons were based on estimated median effective concentrations (EC_{50}).

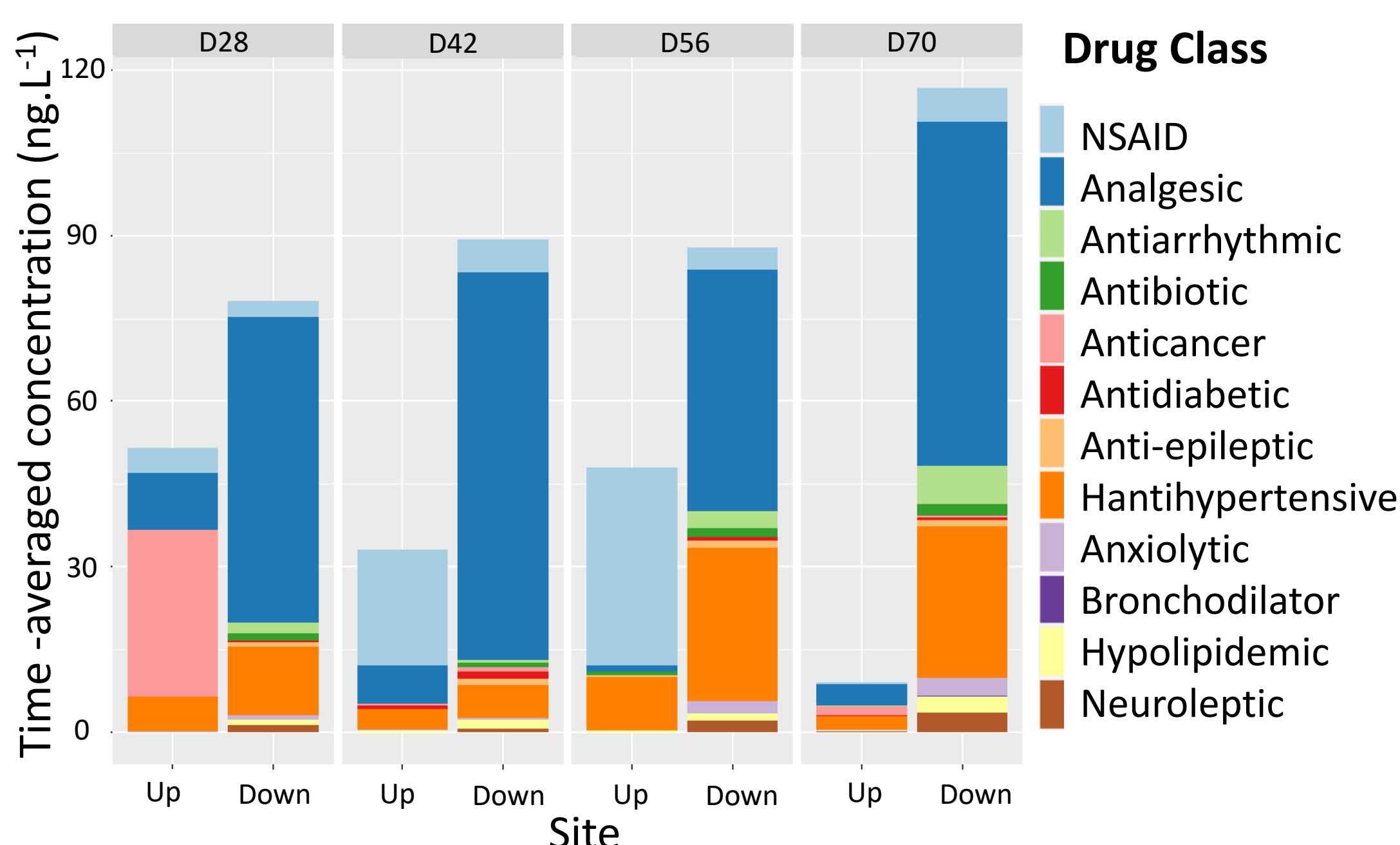


Ex. Algal fluorescence after 4h exposure to a gradient of diclofenac measured via Imaging-PAM

Results

Pharmaceuticals concentrations in surface water:

Number and concentrations of pharmaceuticals were higher downstream than upstream.



Pharmaceuticals concentrations in surface water in upstream (Up) and downstream (Down) sites

PICT responses:

- At day 28 the main differences in tolerance between Upstream and Downstream biofilms were observed with **ofloxacin** (toxicity tests on β -glucosidase, data not shown), **diclofenac** and **atenolol** (toxicity tests on photosynthesis)

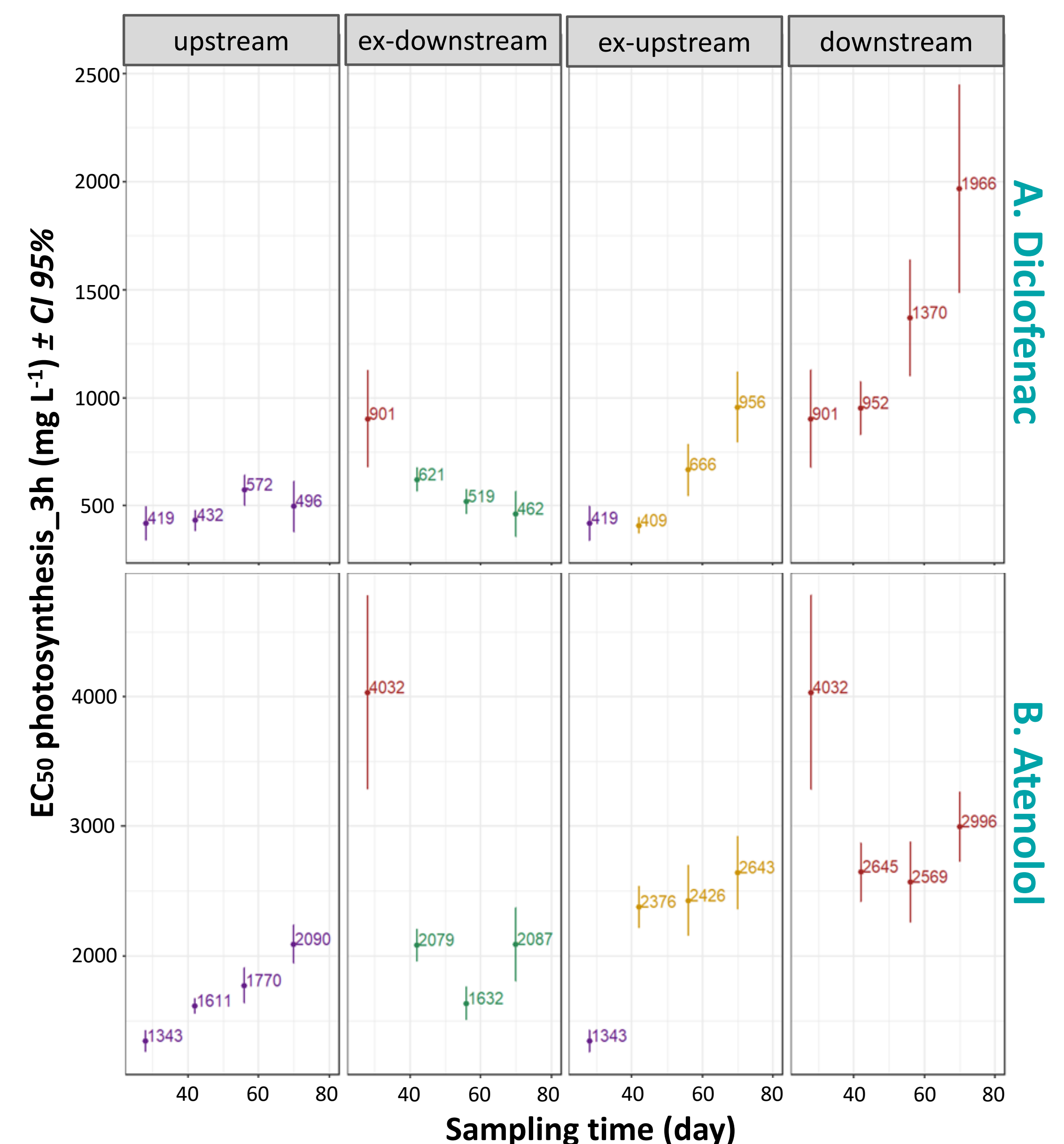
$EC_{50} \text{ upstream} < EC_{50} \text{ downstream}$

- Biofilms moved from upstream to downstream progressively increased their tolerance to both diclofenac and atenolol

$EC_{50} \text{ ex-upstream} \nearrow$

- Biofilms moved from downstream to upstream progressively decreased their tolerance to both diclofenac and atenolol

$EC_{50} \text{ ex-downstream} \searrow$



Conclusions

- As expected, pharmaceutical concentrations in river were significantly higher downstream than upstream.
- After the 28-Day colonization period, periphytic communities from the downstream site were more tolerant to 3 of the 7 tested substances (ofloxacin, diclofenac, atenolol), in accordance with our first hypothesis.
- The temporal evolution of tolerance levels to diclofenac and atenolol during the post-translocation period was also in line with our second hypothesis since biofilms moved from upstream to downstream progressively increased their tolerance while opposite changes were observed with biofilms moved from downstream to upstream.
- The results suggest that PICT can be a relevant *in situ* diagnostic tool to evaluate the chemical pressure by pharmaceuticals.

Further analyses will be conducted to

- 1) better characterize the *in situ* microbial exposure to pharmaceuticals (concentration measurements within periphytic biofilms)
- 2) evaluate whether temporal changes in tolerance reflect temporal changes in microbial diversity (bacterial & algal metabarcoding)