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# Investigating the compartmentalization and the activity of class 1 integron and IncP-1 plasmids in the Orne River, France



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## Objective.

To identify environmental reservoirs of integrons and to determine if these compartments support integron recombination activities.

## Abstract.

An increasing number of reports highlighted the compartmentalization of antibiotic resistance genes (ARG) and mobile genetic elements (MGE) in natural environments, especially aquatic ecosystems<sup>1</sup>. Because MGE such as class 1 integrons and IncP-1 plasmids are genetic structures that capture and/or mobilize ARG, they are now used as a proxy for tracking ARG abundance<sup>2,3</sup>. Recently, we determined the relative abundance of class 1 integrons and IncP-1 plasmids in various compartments of the Orne River (France), namely raw water, suspended materials and sediments. We have shown that the suspended materials are statically?? enriched in class 1 integrons and IncP-1 plasmids in comparison to raw water (Student t-test for paired values;  $p < 10^{-3}$ ). The analysis of core sediments demonstrated that the top layers (recent deposits) are similar to water suspended material while deeper layers exhibited an increase of the MGE relative abundance with depth. All together, this progressive enrichment of class 1 integrons and IncP-1 plasmids may suggest that attachment to suspended materials may act as a selector of MGE-carrying bacteria. On the other hand, if many studies focused on the abundance of MGE, only a few investigated their activity and the expression of their mobility functions. We addressed this question by developing a whole-cell biosensor in which the *lux* operon has been placed under the control of the class 1 integron-integrase promoter ( $P_{int1}$ ). Preliminary results ~~tend to~~ show that in some instances, the incubation of the whole-cell biosensor in particular Orne water samples could slightly induce the activity of the  $P_{int1}$  promoter, and in the mean time also potentiate the effect of SOS-inducing antibiotics at sub-inhibitory concentrations.

## 1. Abundance of class 1 integrons and IncP-1 plasmids in different river compartments.

Raw water, suspended material and top core sediment have been collected from different sampling sites along the Orne River. Fifteen pairs of raw water samples and suspended materials were collected during 4 sampling campaigns whereas 2 core sediments were sampled. Total genomic DNAs were extracted and quantitative PCR was used to determine the relative abundance of class 1 integrons and IncP-1 plasmids (Figure 1). Suspended material was shown to be statistically enriched?? in both MGE and ?? compared to raw water. Moreover, if the top core sediments were shown to be statistically-enriched-in IncP-1 plasmids in comparison to raw water, they did not differ from suspended material for both MGE and . These results demonstrate that bacteria harboring class 1 integrons or IncP-1 plasmids are statistically enriched ~~on~~ in suspended materials before settling to form sediment.

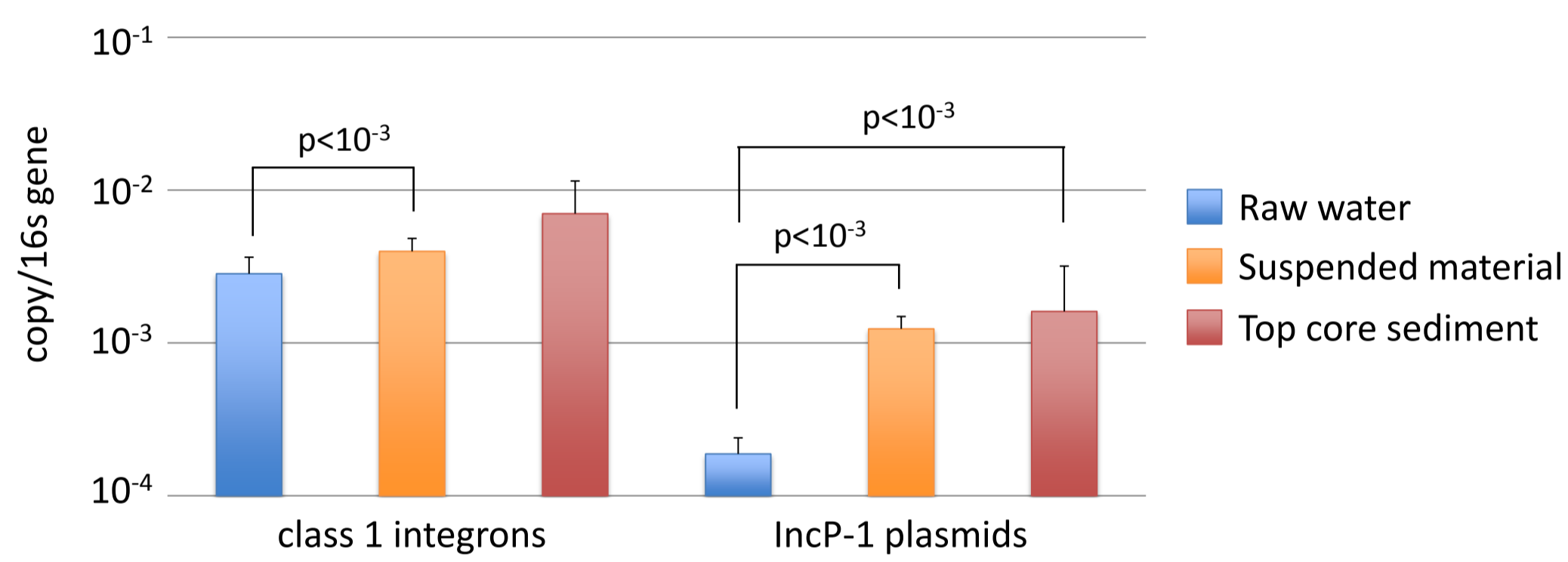


Figure 1: Relative abundance of class 1 integrons and IncP-1 plasmids in the water, suspended material and, top core sediment from the Orne River. The mean and standard error of the relative copy number of class 1 integrons and IncP-1 plasmids are presented. The p-values indicate statistical differences enrichments in MGE in either suspended material or top core sediment by comparison to raw water (Student t-test).

## 3. Development of a whole-cell biosensor strain to studying integron activity.

The whole-cell biosensor *Escherichia coli* MG1656(pSEVA236:: $P_{int1}$ ) was developed by cloning the promoter of the integrase gene from a class 1 integron in a plasmid containing a *lux* operon (Figure 3). This strain allows us to report the integron activity by monitoring light emission either in response to the SOS-inducing antibiotics or in river water.

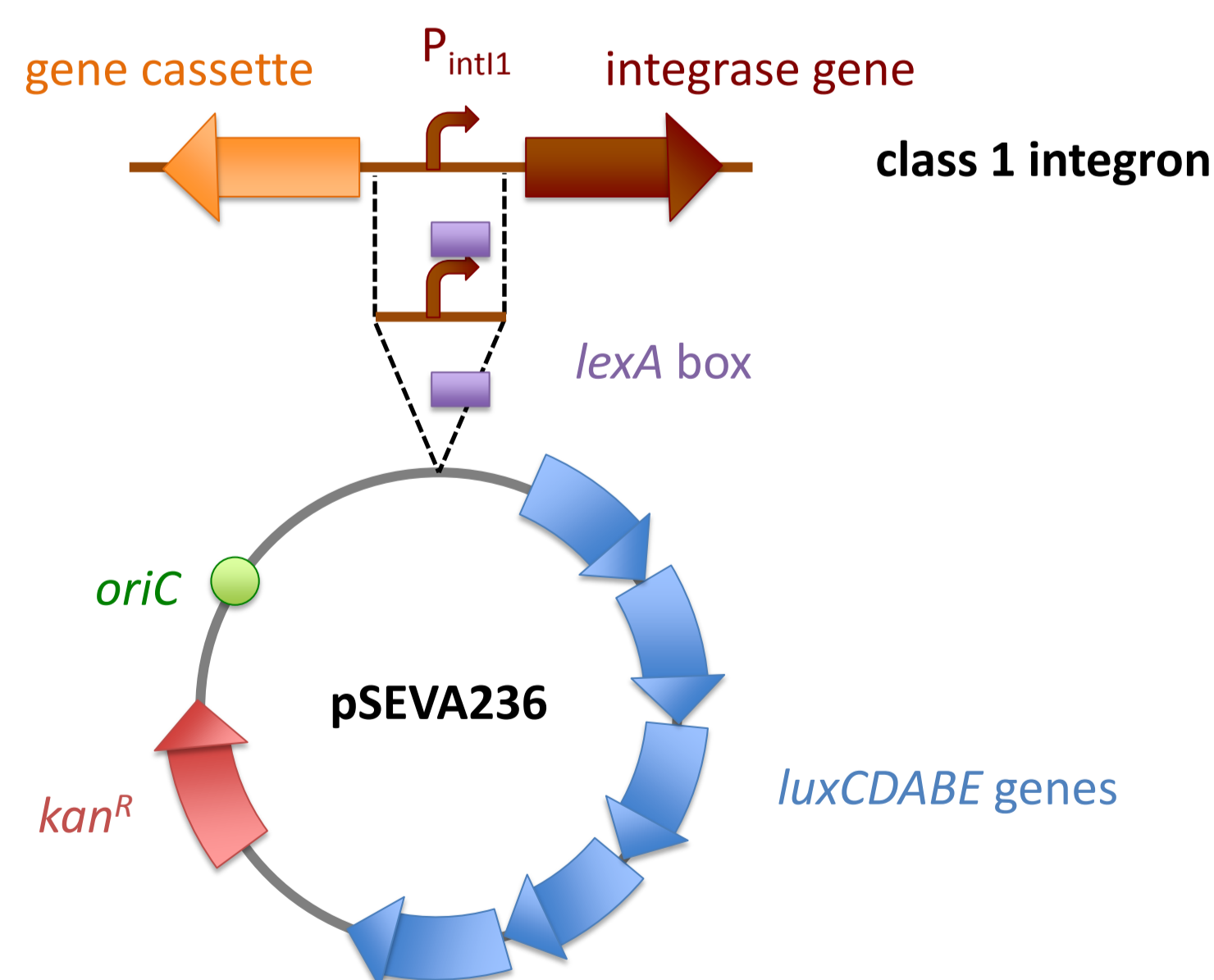


Figure 3: Construction of the reporter plasmid pSEVA236:: $P_{int1}$ .

## References.

- Baquero F, et al. (2008) Antibiotics and antibiotic resistance in water environments. *Curr Opin Biotechnol* 19, 260-265.
- Gillings MR, et al. (2015) Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J* 9, 1269-1279.
- Jechalke S, et al. (2015) Effects of 100 years wastewater irrigation on resistance genes, class 1 integrons and IncP-1 plasmids in Mexican soil. *Front Microbiol* 6.

## 2. Abundance of class 1 integrons and IncP-1 plasmids varies with sediment depth.

A 51 cm-long sediment core was collected from the Orne River and its content in eubacterial 16S rDNA genes, class 1 integrons and, IncP-1 plasmids was analyzed by quantitative PCR (Figure 2). First, the statistical analysis of the absolute amount of the 3 markers showed that the eubacterial 16S rDNA genes were the only one to correlate with sediment depth (Pearson's correlation coefficient: -0.80;  $p < 10^{-3}$ ). Second, the analysis of the relative abundance of class 1 integrons and IncP-1 plasmids in the sub-surface sediments shows that (i) their concentrations vary by a factor of 50 to 100 between the different layers, (ii) their presence increase with sediment depth, even by taking account of the values from depths 45 and 47 cm, since there are positive correlations between the relative abundance of class 1 integrons and depth (Pearson's correlation coefficient: 0.58;  $p = 0.02$ ) and between the relative abundance of IncP-1 plasmids and depth (Pearson's correlation coefficient: 0.68;  $p = 0.004$ ). These results can be explained by three hypotheses, either suspended material at the origin of the sediment come from different sources or bacteria carrying these two types of MGE would survive better than bacteria devoid of them, i.e. these elements protect bacteria against death, or the elements transfer in the bacterial population over time making the relative occurrences increase with aging.

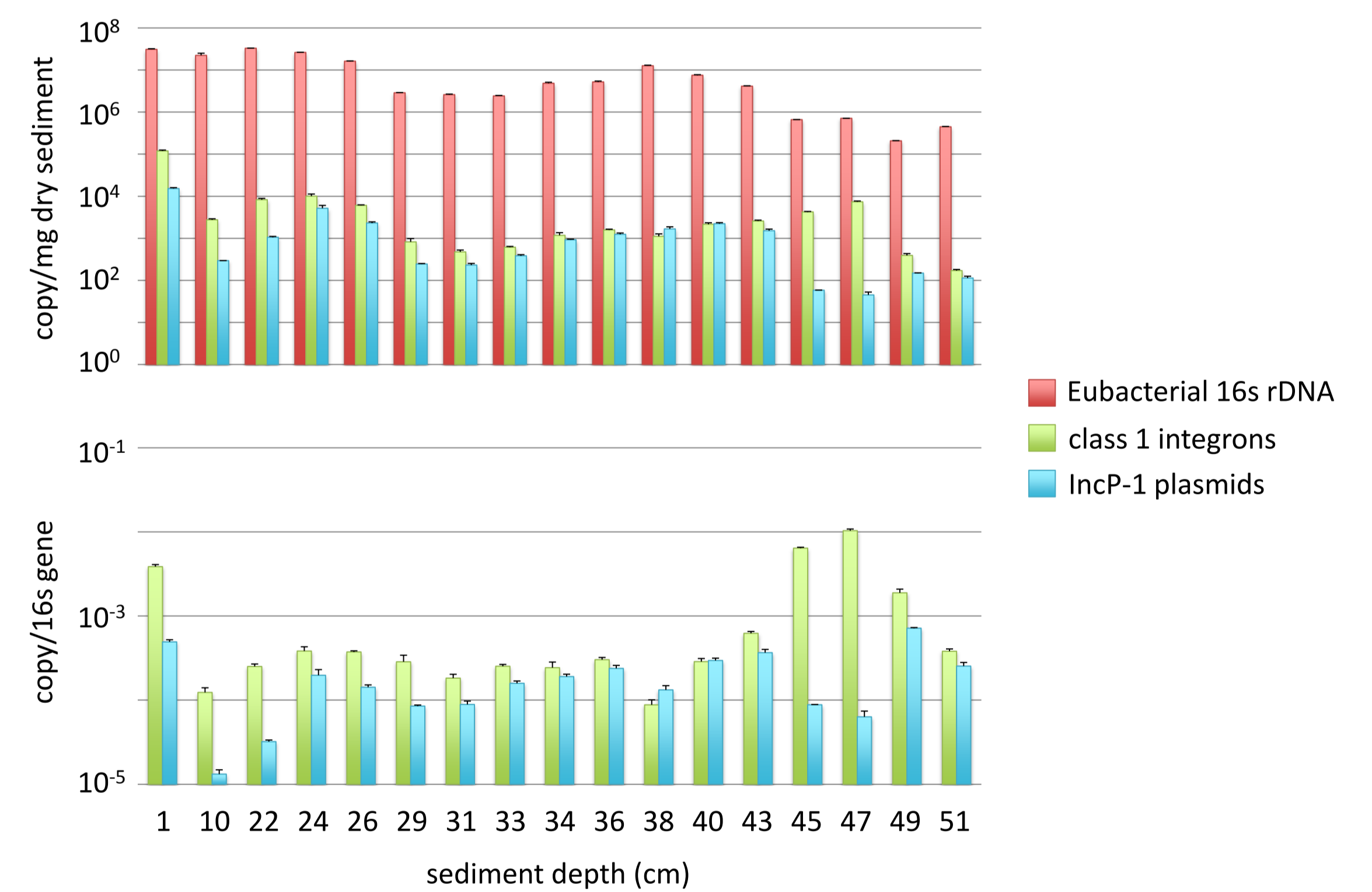


Figure 2: Absolute and relative abundance of class 1 integrons and IncP-1 plasmids in different layers of a sediment core from the Orne River. The mean and standard error of the copy number of eubacterial 16S rDNA genes, class 1 integrons and, IncP-1 plasmids are presented.

## 4. The Orne water potentiated the effect of ciprofloxacin on integron activity.

The whole-cell biosensor *Escherichia coli* MG1656(pSEVA236:: $P_{int1}$ ) was exposed to either mitomycin C or ciprofloxacin prepared in pure or Orne water. Whatever the water, the antibiotics highly induce the integrase promoter and the light emission (Student t-test,  $p < 10^{-3}$ ) (Figure 4). If the responses of the whole-cell biosensor were similar for mitomycin C prepared in pure and Orne water, the relative light emission was significantly higher for ciprofloxacin when prepared in Orne water. This result suggests that some natural environments could promote the activity of class 1 integrons and/or their response to DNA damage-induced stress.

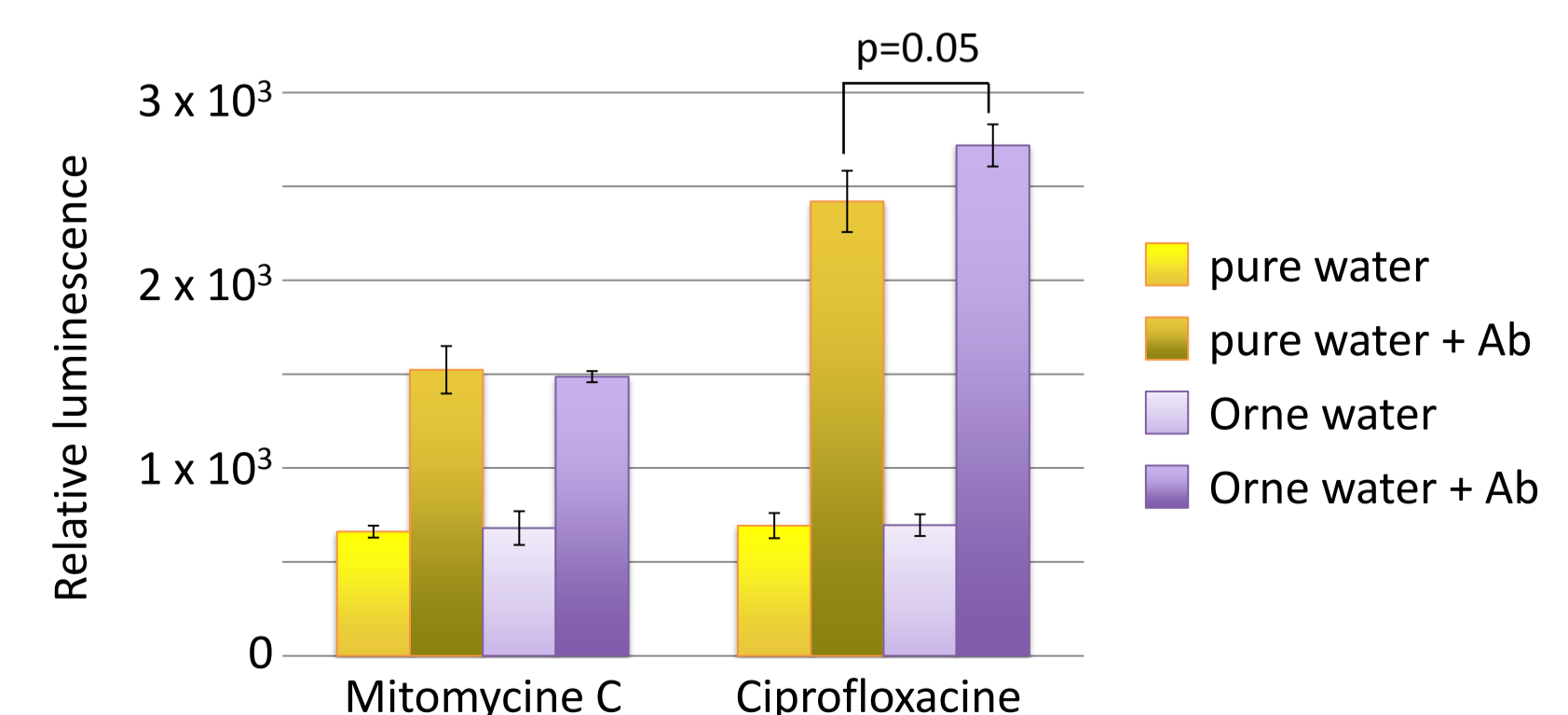


Figure 4: Activity of the promoter isolated from a class 1 integrons in either pure or Orne water. The mean and standard error of the relative light emission (count per second normalized to  $OD_{600nm}$ ) are presented. The whole-cell biosensor was exposed to mitomycin C (10 ng/mL) or ciprofloxacin (100 ng/mL). The p-value indicates statistical difference in light emission (Student t-test).