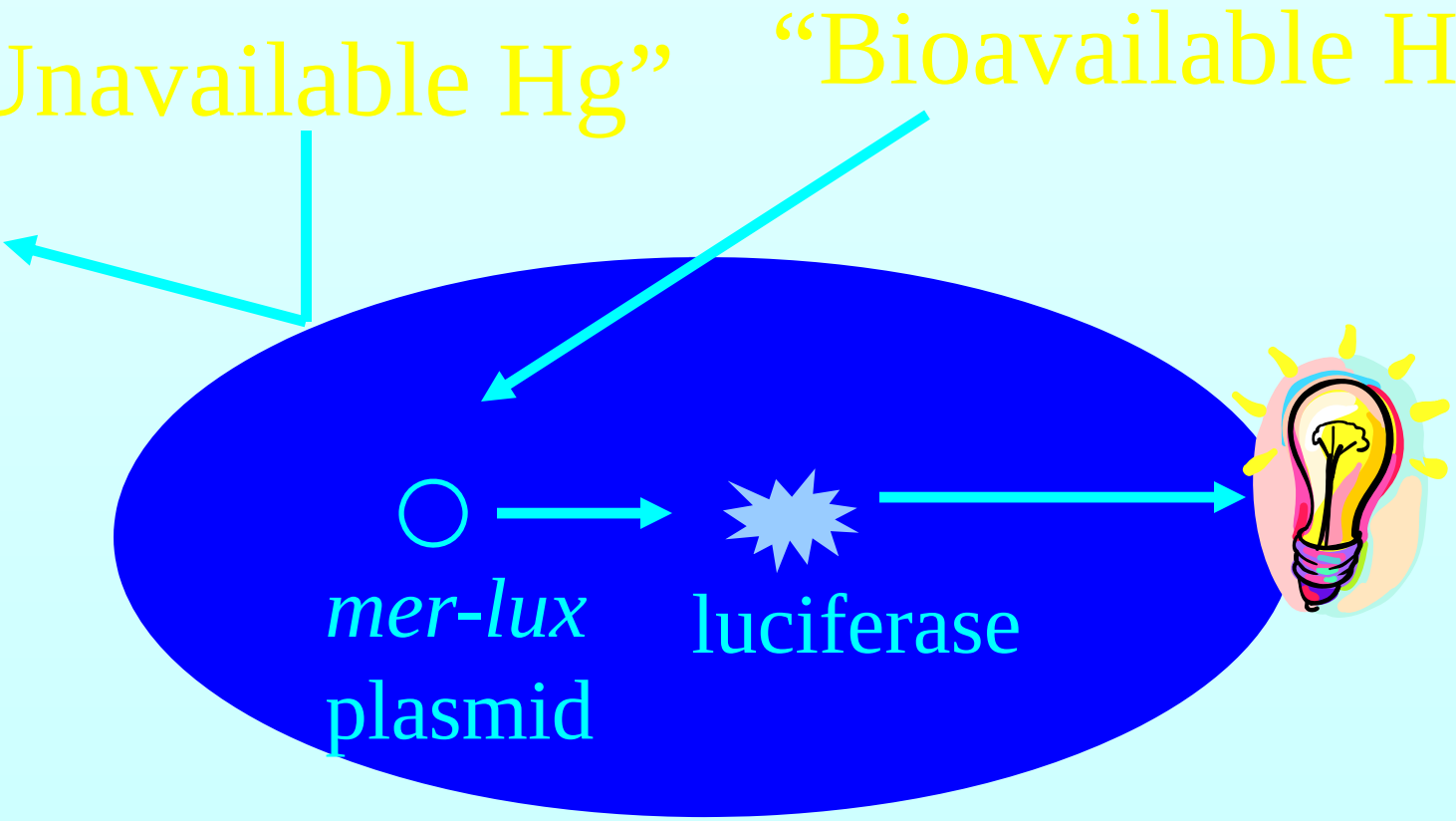
The use of a Genetically Engineered Bioreporter to Study Bioavailable Hg(II) in Natural Waters

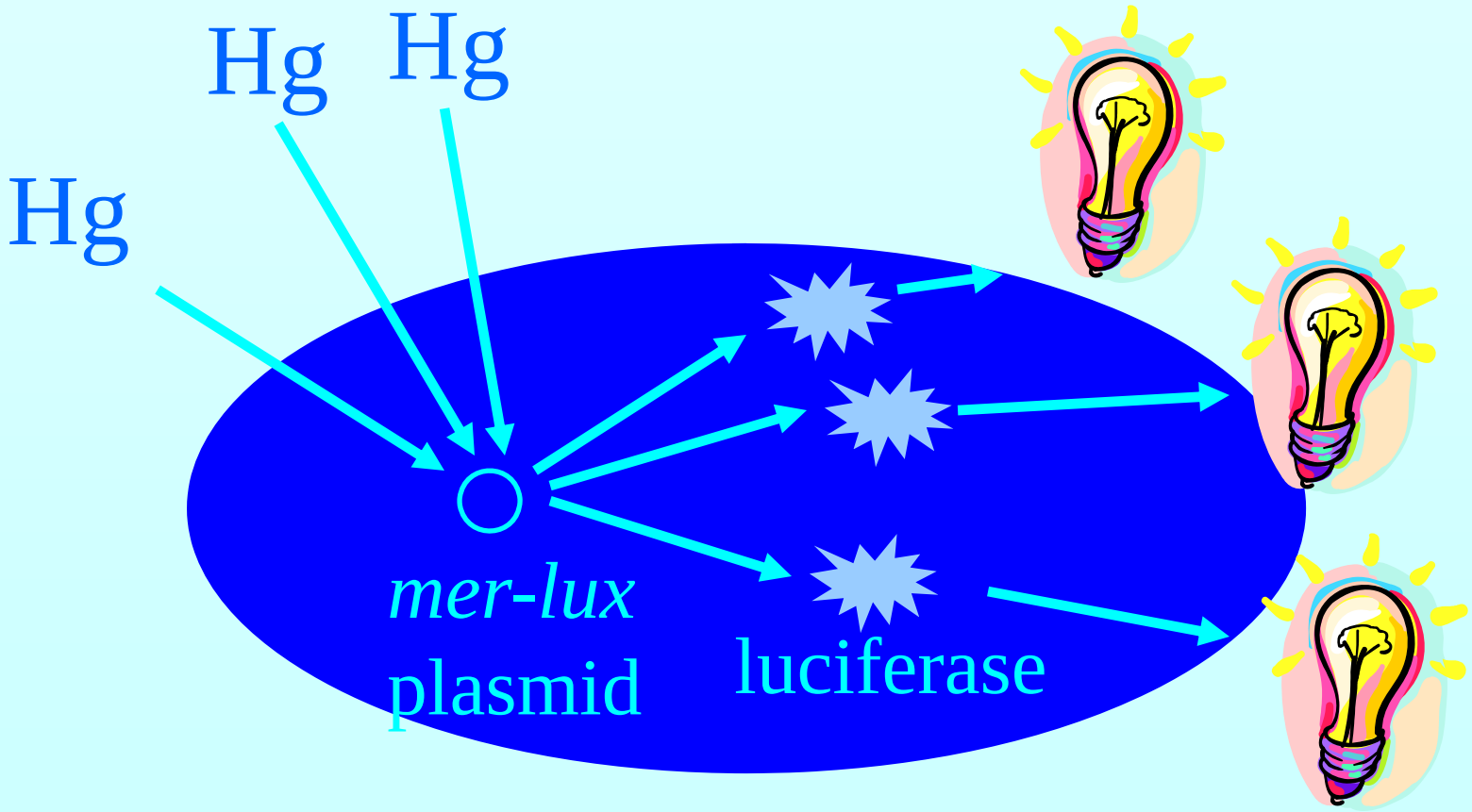
J.W.M. Rudd
C.A. Kelly
M. Holoka

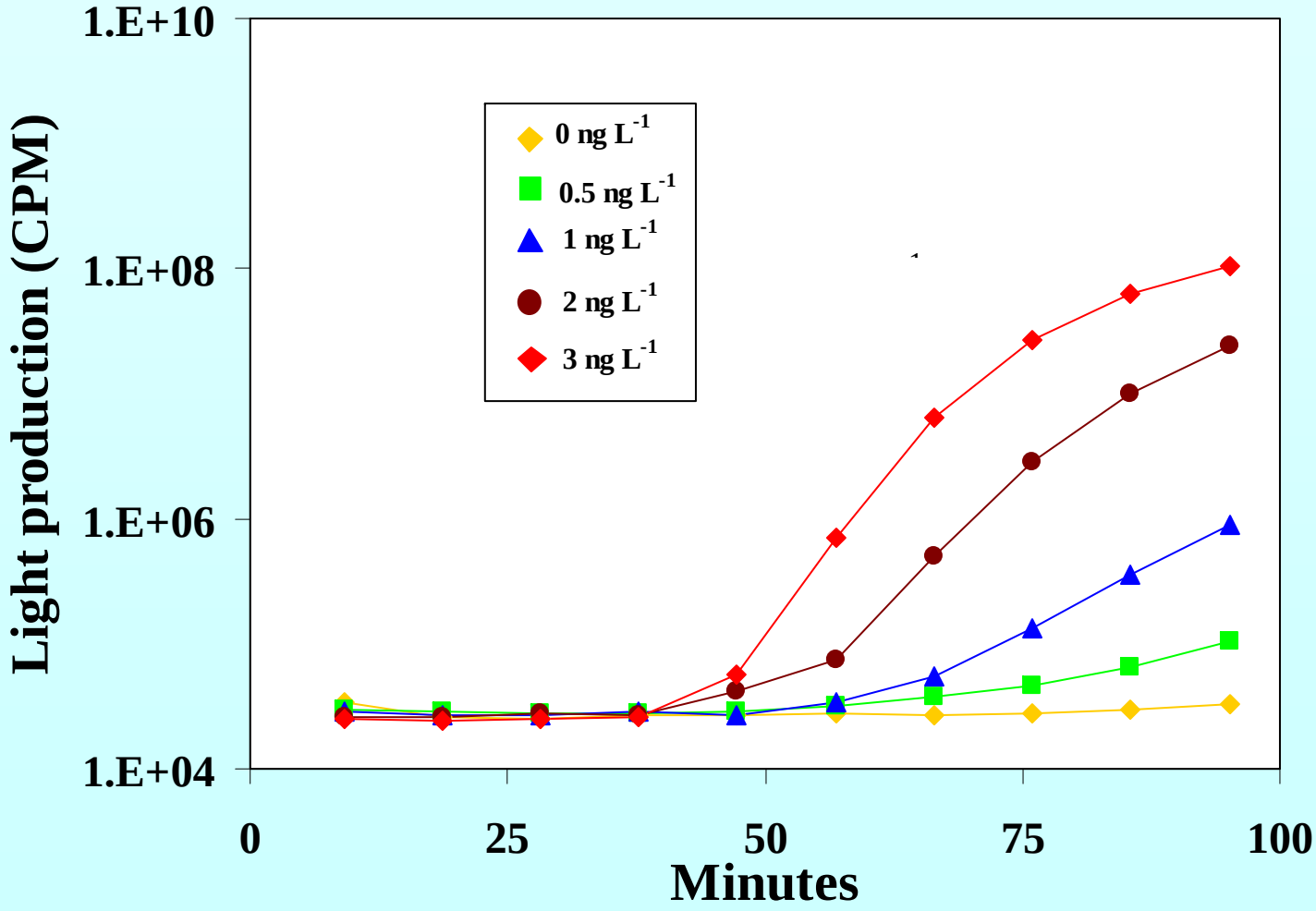
Hg(II) Induced Light Production

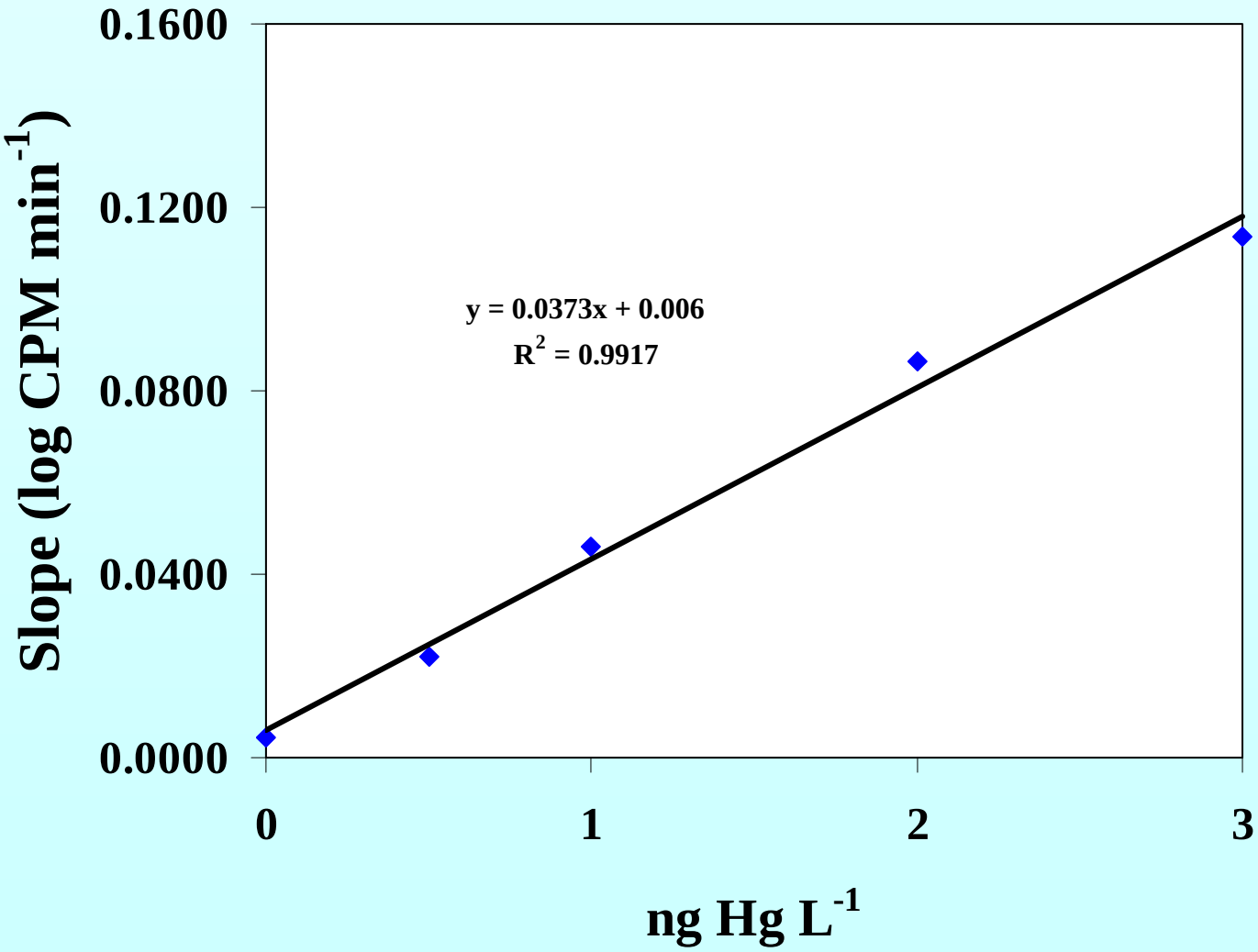


Vibrio anguillarum pRB28 "Bioreporter" Cell

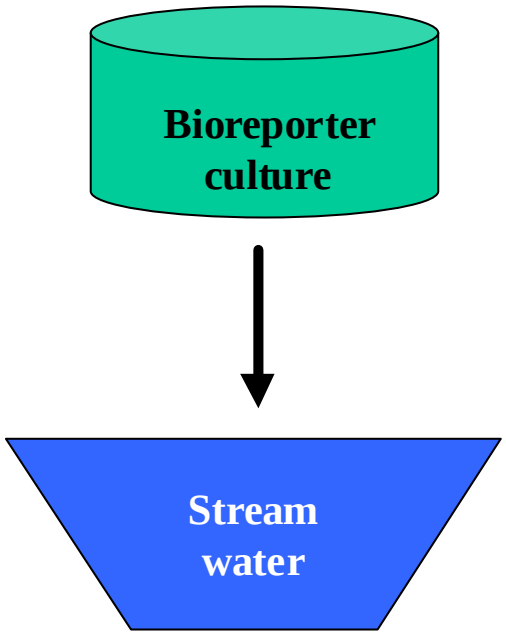
Concentration of Bioavailable Hg




1.E+10
1.E+08
1.E+06
1.E+04
Light production (CPM)
0 ng L-1
0.5 ng L-1
1 ng L-1
2 ng L-1
3 ng L-1
0
25
50
75
100
Minutes

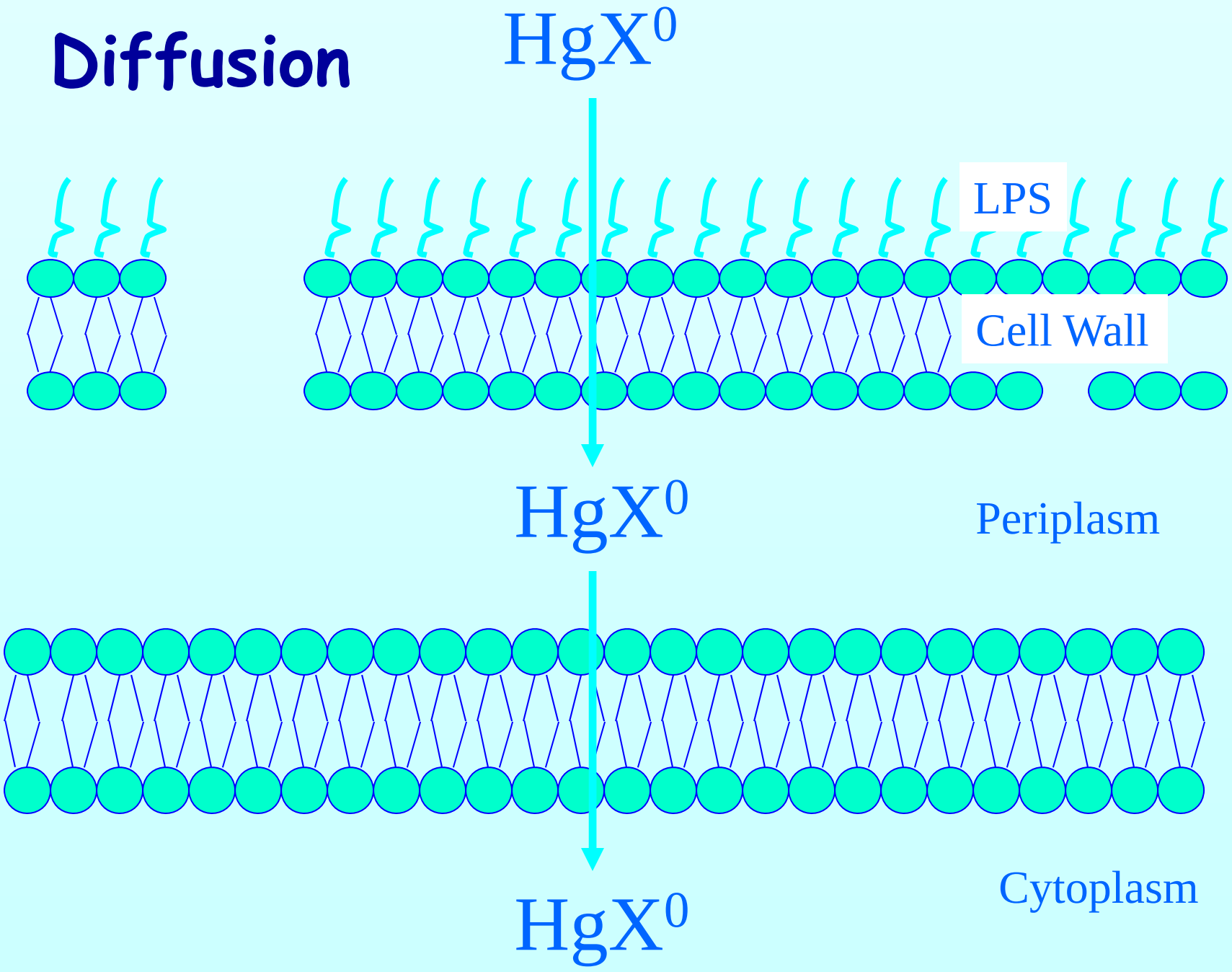

0.1600
0.1200
0.0800
0.0400
0.0000
Slope (log CPM min-1)
y = 0.0373x + 0.006
R2 = 0.9917
0
1
2
3
ng Hg L-1

Lab assay of stream water bioavailable Hg

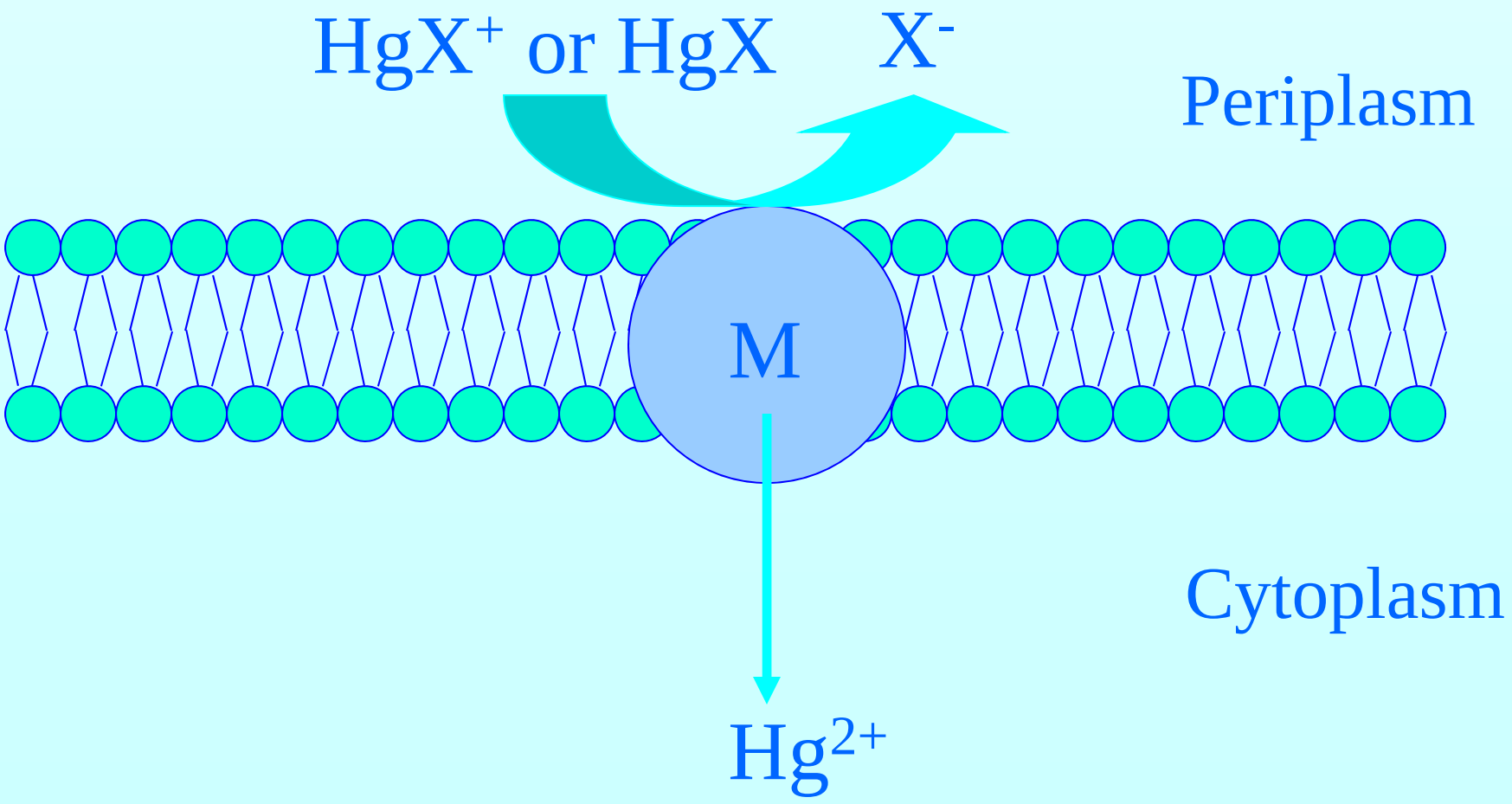


Facilitated uptake of Hg(II) by an aquatic bacterium and effects of changes in pH

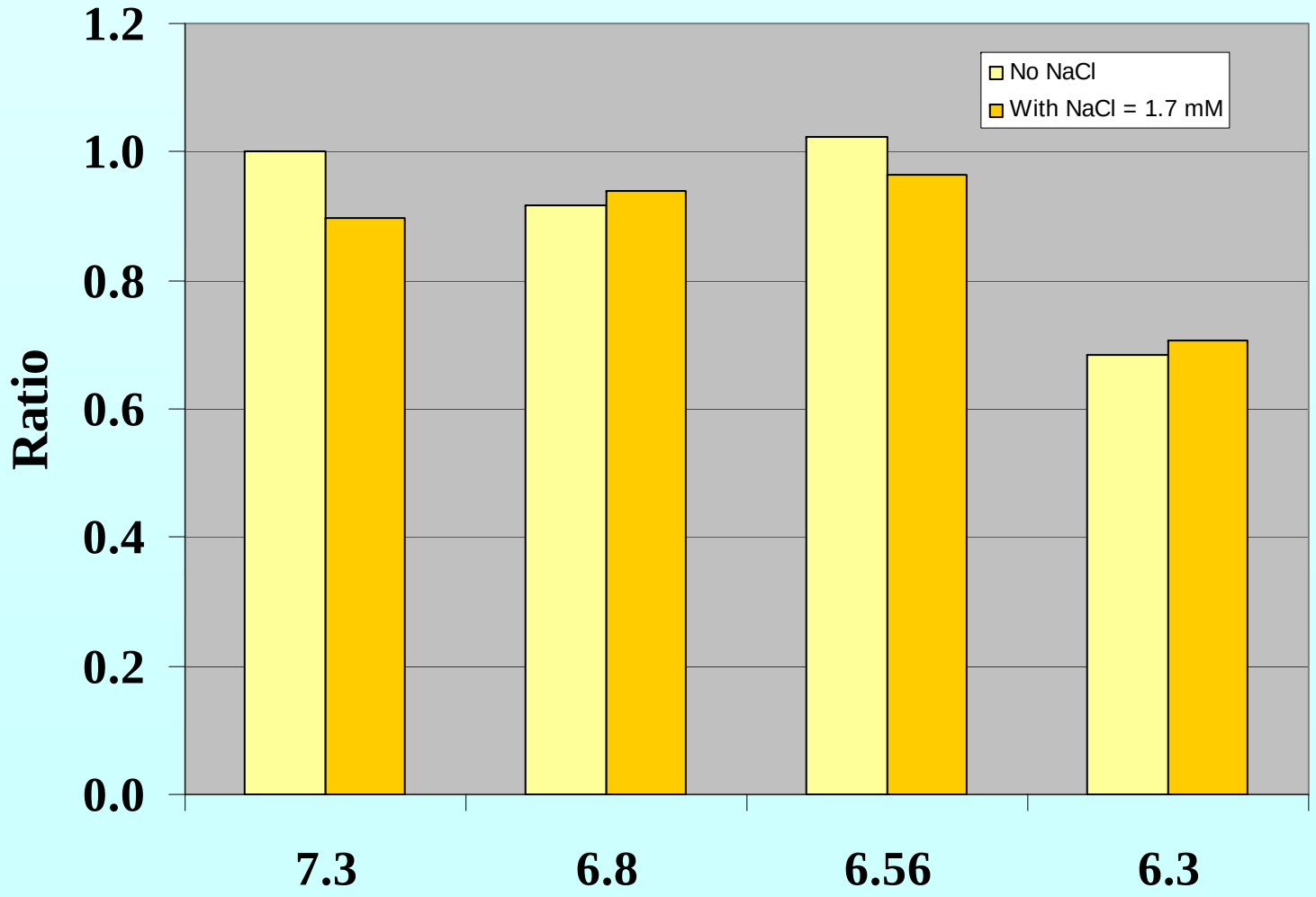
Diffusion



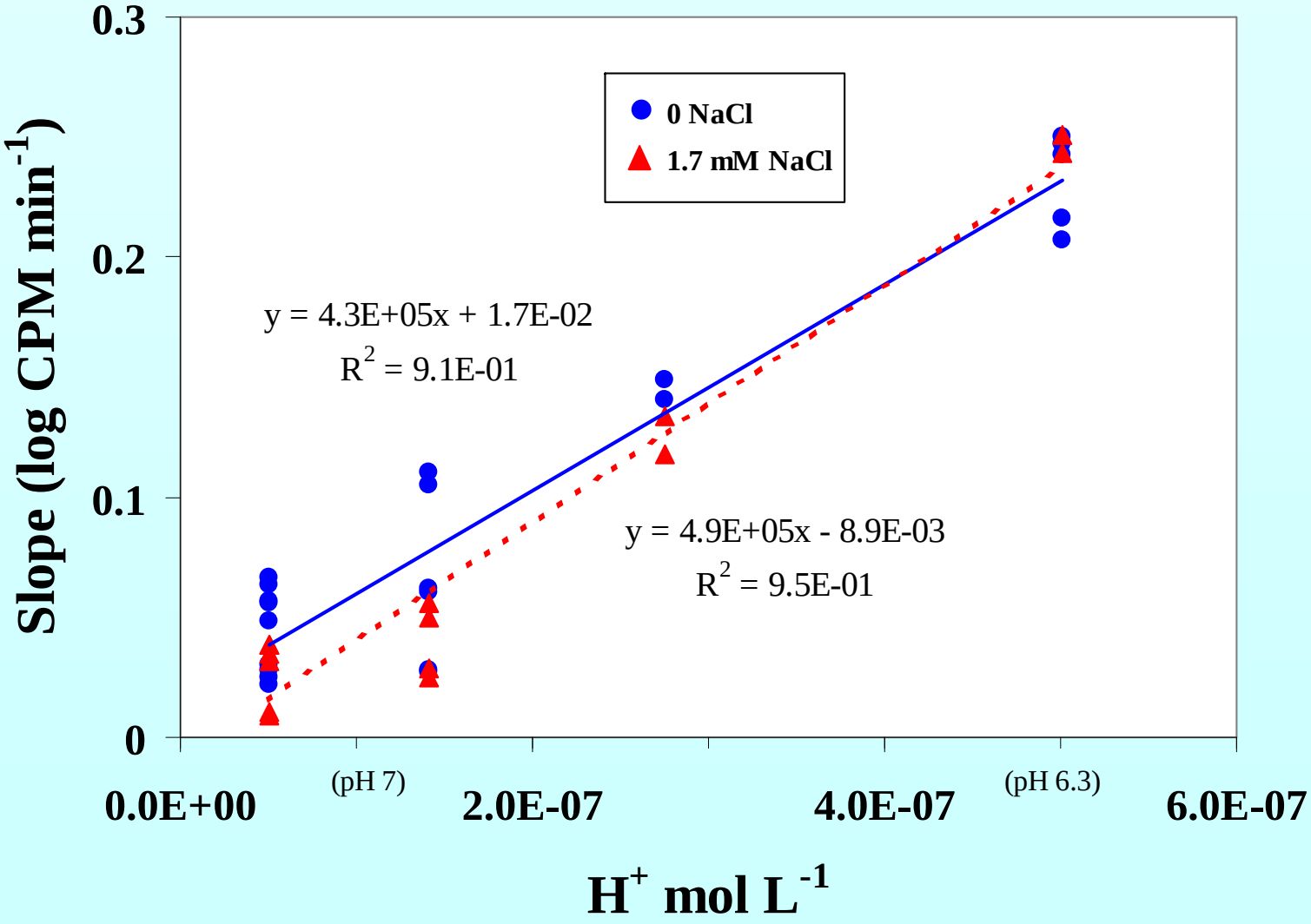
Facilitated Uptake

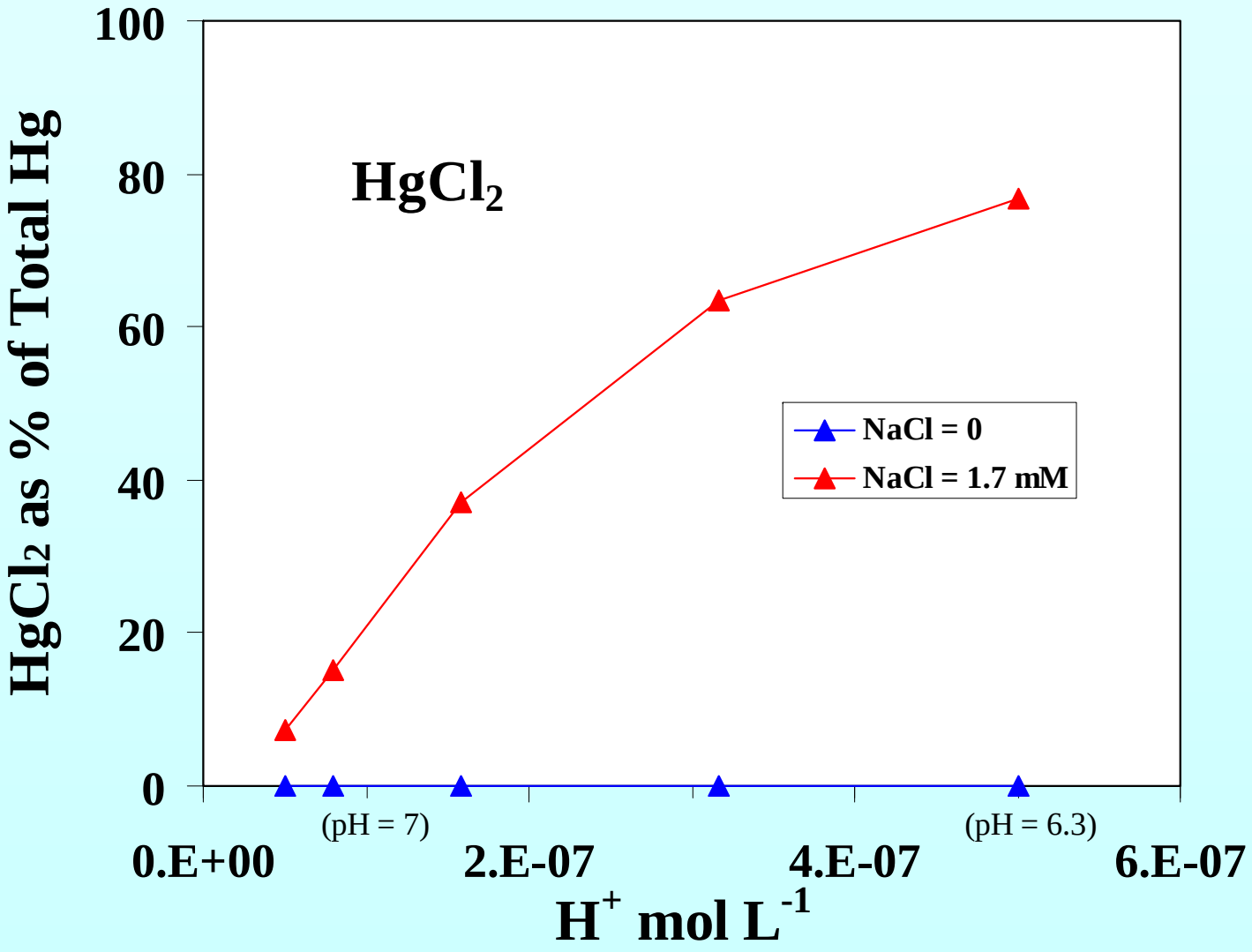


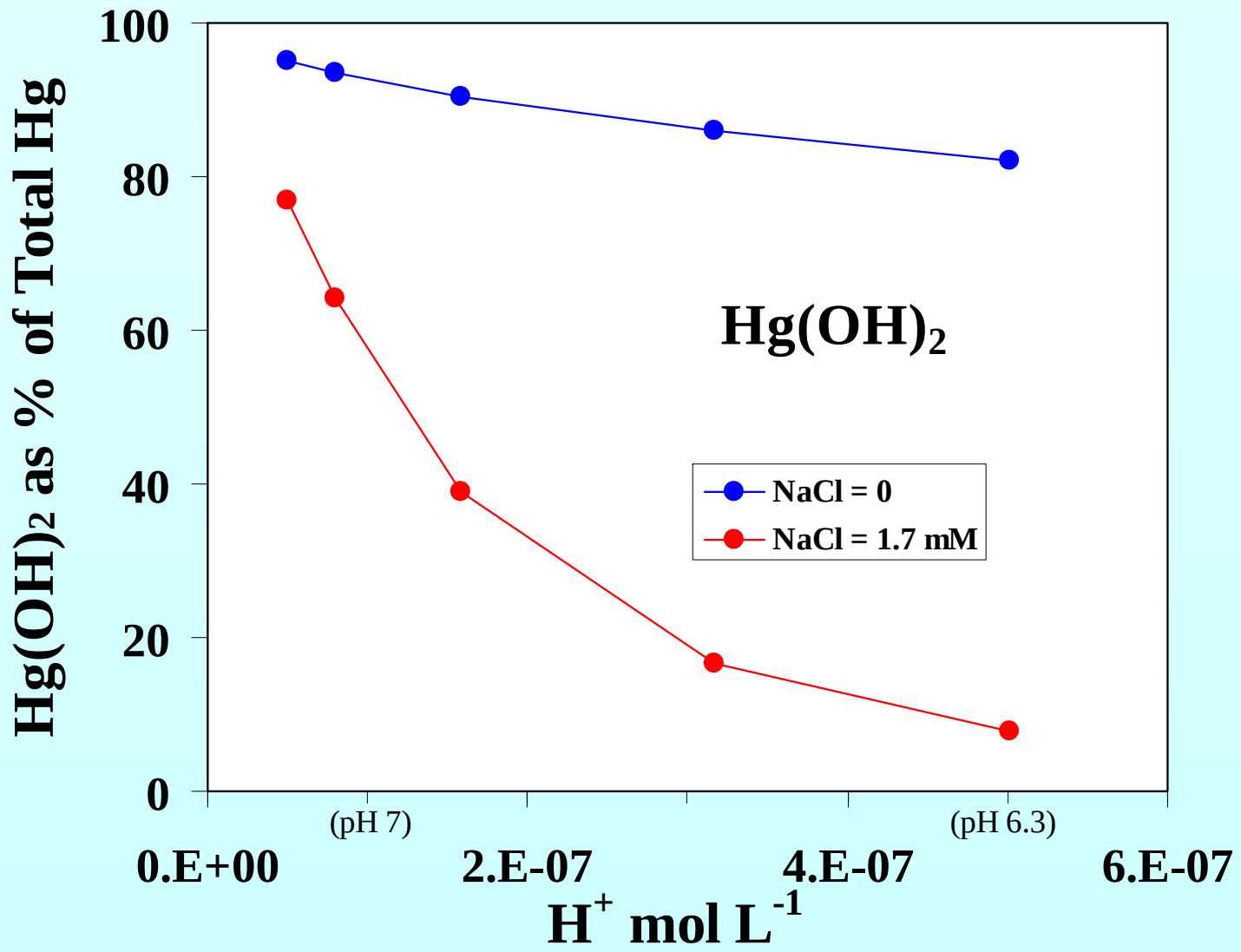
Effect of pH on Light production (Constitutive *lux* strain)



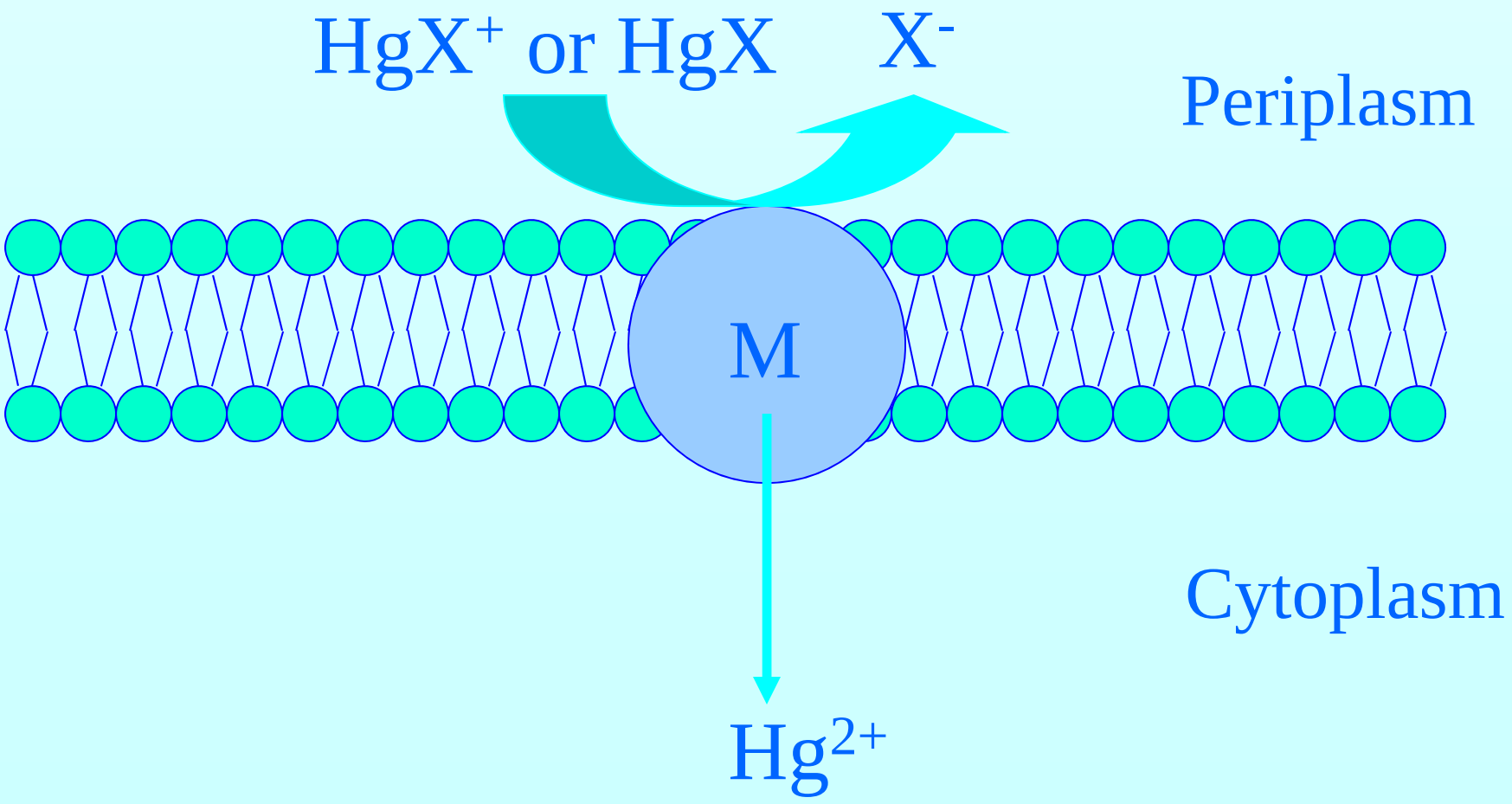
All assays 1 ng Hg/L




100
80
60
40
20
0
HgCl2 as % of Total Hg
HgCl2
NaCl = 0
NaCl = 1.7 mM
(pH = 7)
(pH = 6.3)
0.E+00
2.E-07
4.E-07
6.E-07
H+ mol L-1


100
80
60
40
20
0
Hg(OH)2 as % of Total Hg
Hg(OH)2
NaCl = 0
NaCl = 1.7 mM
(pH 7)
(pH 6.3)
0.E+00
2.E-07
4.E-07
6.E-07
H+ mol L-1

Facilitated Uptake



All samples 2 ng Hg L^{-1}

Response with no DOC = 100%

pH	Response with 85 umol C L^{-1} added as DOC
7.3	29 %
6.84	43 %
6.53	77 %

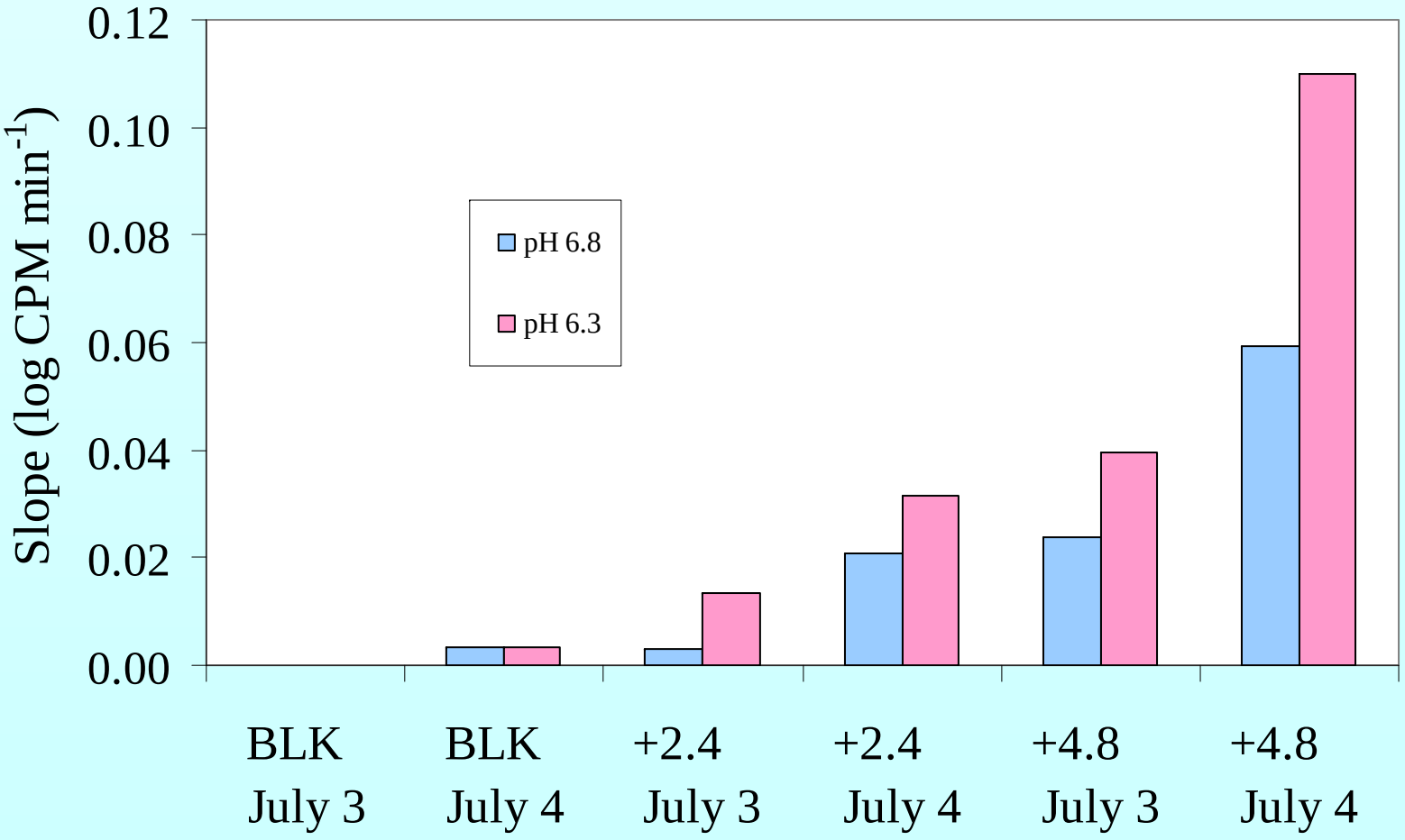
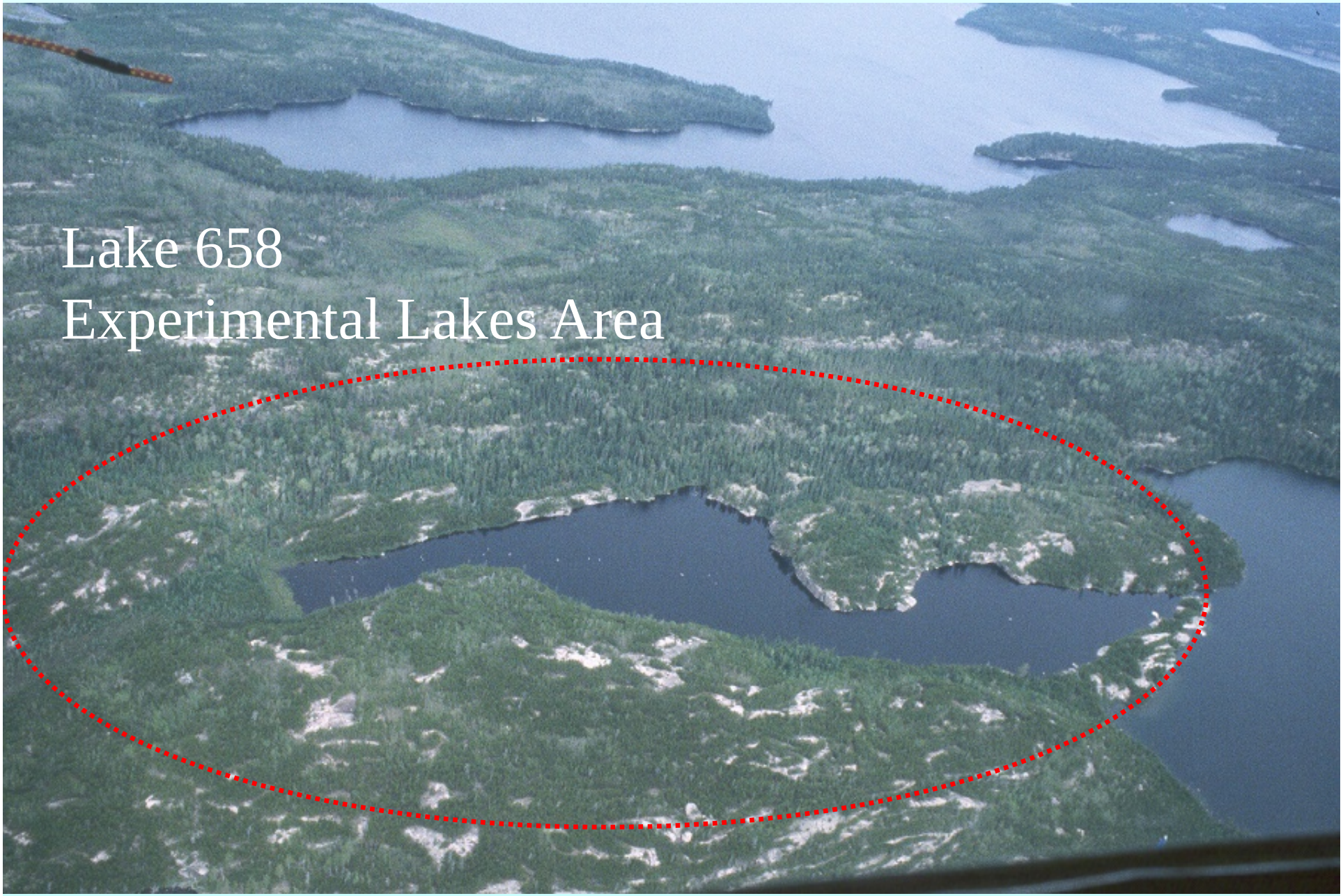
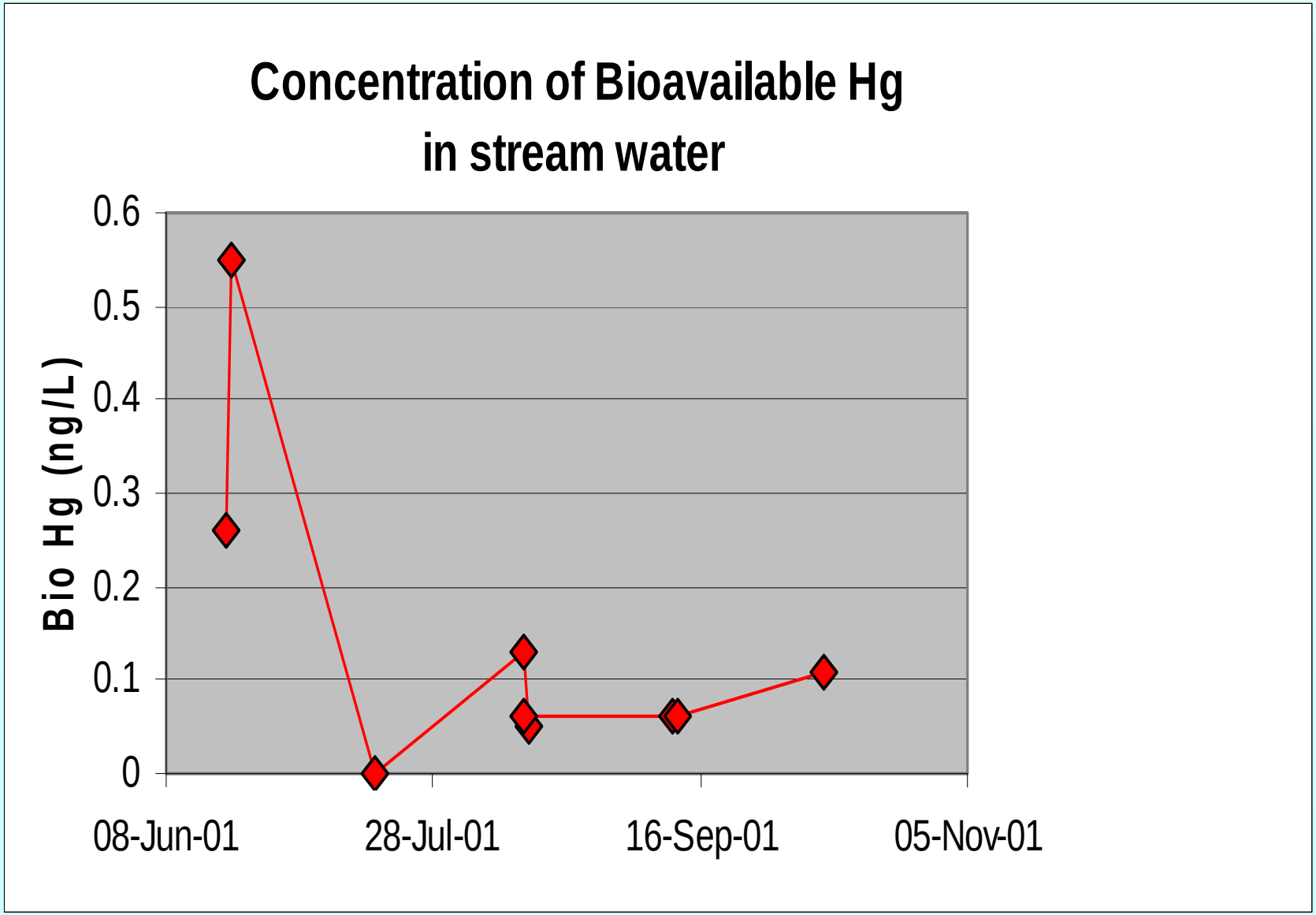
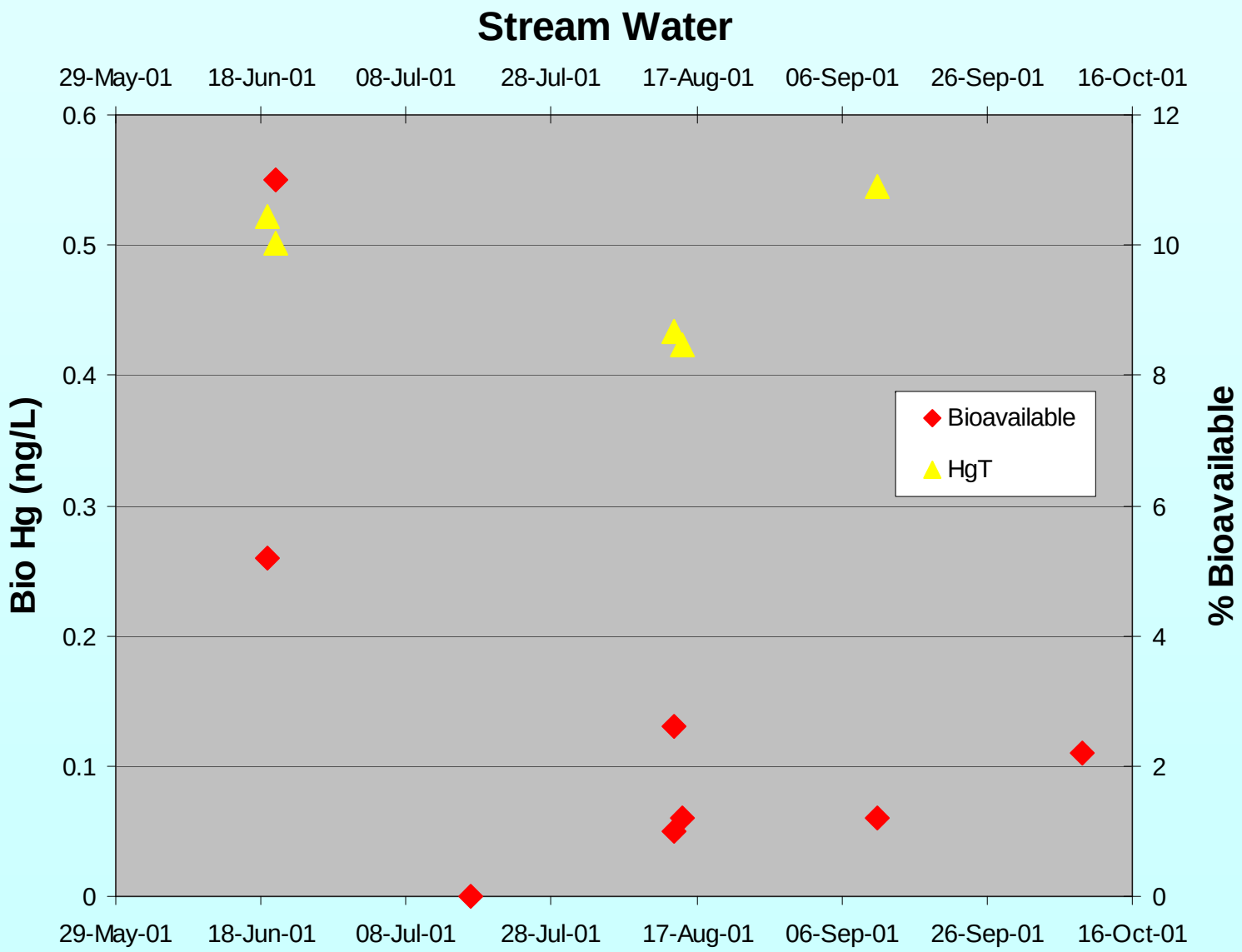

Hg additions to L. 658 water
Slope (log CPM min-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
pH 6.8
pH 6.3
BLK July 3
BLK July 4
+2.4 July 3
+2.4 July 4
+4.8 July 3
+4.8 July 4

Figure 4

Study of bioavailability of Hg in natural waters


Lake 658
Experimental Lakes Area


Concentration of Bioavailable Hg
in stream water
Bio Hg (ng/L)
0.6
0.5
0.4
0.3
0.2
0.1
0
08-Jun-01
28-Jul-01
16-Sep-01
05-Nov-01


Stream Water
29-May-01
18-Jun-01
08-Jul-01
28-Jul-01
17-Aug-01
06-Sep-01
26-Sep-01
16-Oct-01
0.6
0.5
0.4
0.3
0.2
0.1
0
12
10
8
6
4
2
0
Bio Hg (ng/L)
% Bioavailable
Bioavailable
HgT

L. 658 Stream and Lake surface
July-Oct. 2001

	rain water	stream water	Lake 658
Bioavailable Hg(II), ng/L	0.22	0.06	ND
Total Hg ng/L	10.0	8-10	1.8-3
% Bioavailable	2.2	0.67	ND
pH	6.0	5.5	6.8
DOC (umol/L)	<100	1500	750

Conclusions

- Hg(II) uptake by these bacteria is facilitated, i.e., not by diffusion of neutral species

- "Bioavailable" Hg(II) has two components 1) the concentration of available chemical species and 2) the rate of the *bacterial* uptake process itself

- Important parameters in determining bioavailability of Hg in natural waters are [DOC], pH and [THg]

These results may explain why:

- At lower pH, methylation is higher in surface sediments (Xun et al. 1987) and in DOC amended lake water (Miskimmin et al. 1992)
- Fish MeHg is higher in lakes with lower pH (Wiener et al. 2002)

Assay Components

Vibrio anguillarum **pRB28 Cells**
(10^5 cells/mL)

5 mM glucose
0.09 mM $(NH_4)_2SO_4$
6.7 mM Phosphate buffer

Water:
Milli-Q
Milli-Q + $Hg(NO_3)_2$
Milli-Q + $Hg(NO_3)_2$ + NaCl
Milli-Q +DOC + Hg
Whole Lake water + Hg