Gene Transfer Agents in the Reef Environment

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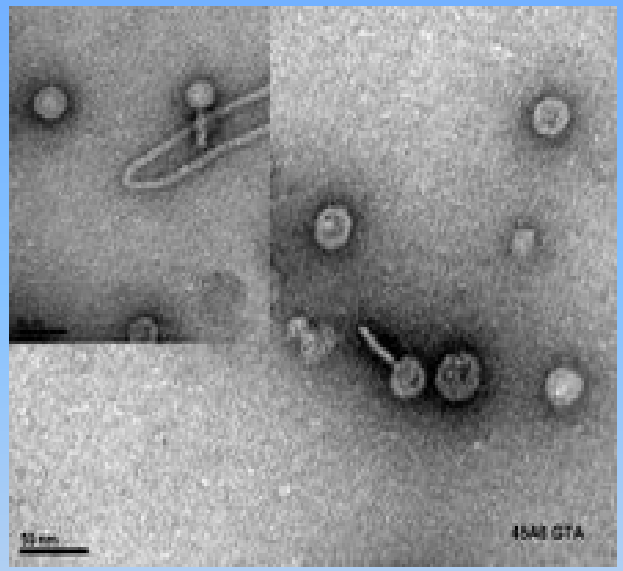
Today

- What are Gene Transfer Agents?
- What are they doing in the oceans?
- What might they be doing in the reef environment?

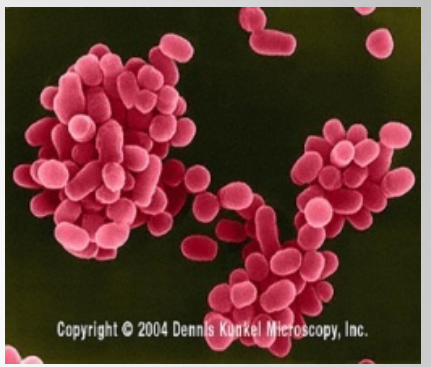
Gene Transfer Agents (GTAs)

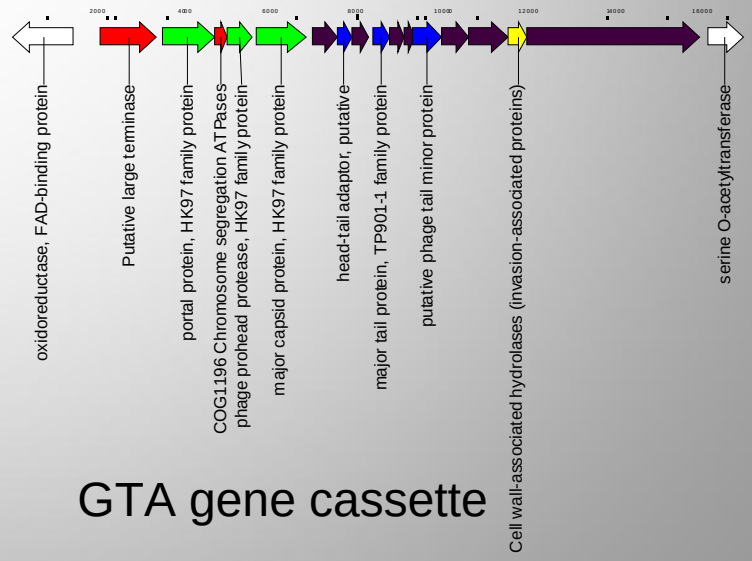
- First discovered in 1974 (*Rhodobacter capsulatus*)
- type of bacterial "sex"
- Package random chunks of DNA from the host-Led to high rates of gene-swapping
- "Little Genetic Escape Pods"



GTAs and Alpha Proteobacteria

- Most Bacteria of this type (Alphas) contain them
- Alphas commonly Associated with Reef Environments and *Symbiodinium* endosymbionts
- What are GTAs doing?





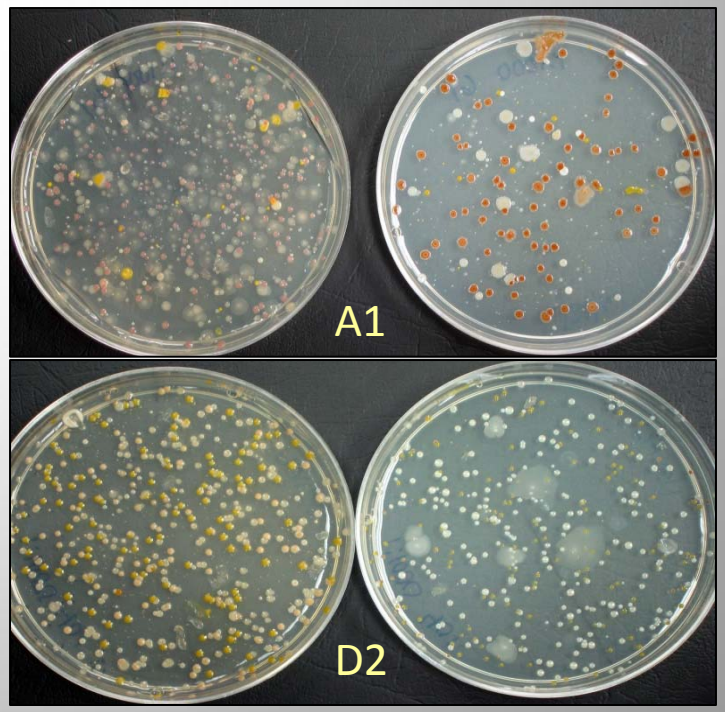
GTA gene cassette

GTAs and Alpha Proteobacteria

- Obtained Several Strains with Known GTAs from Culture Collections
- Isolated Several Strains From *Symbiodinium* endosymbionts
- Screened them for GTA Production (*Roseovarius nubinhibens, Ruegeria mobilis* 45A6)

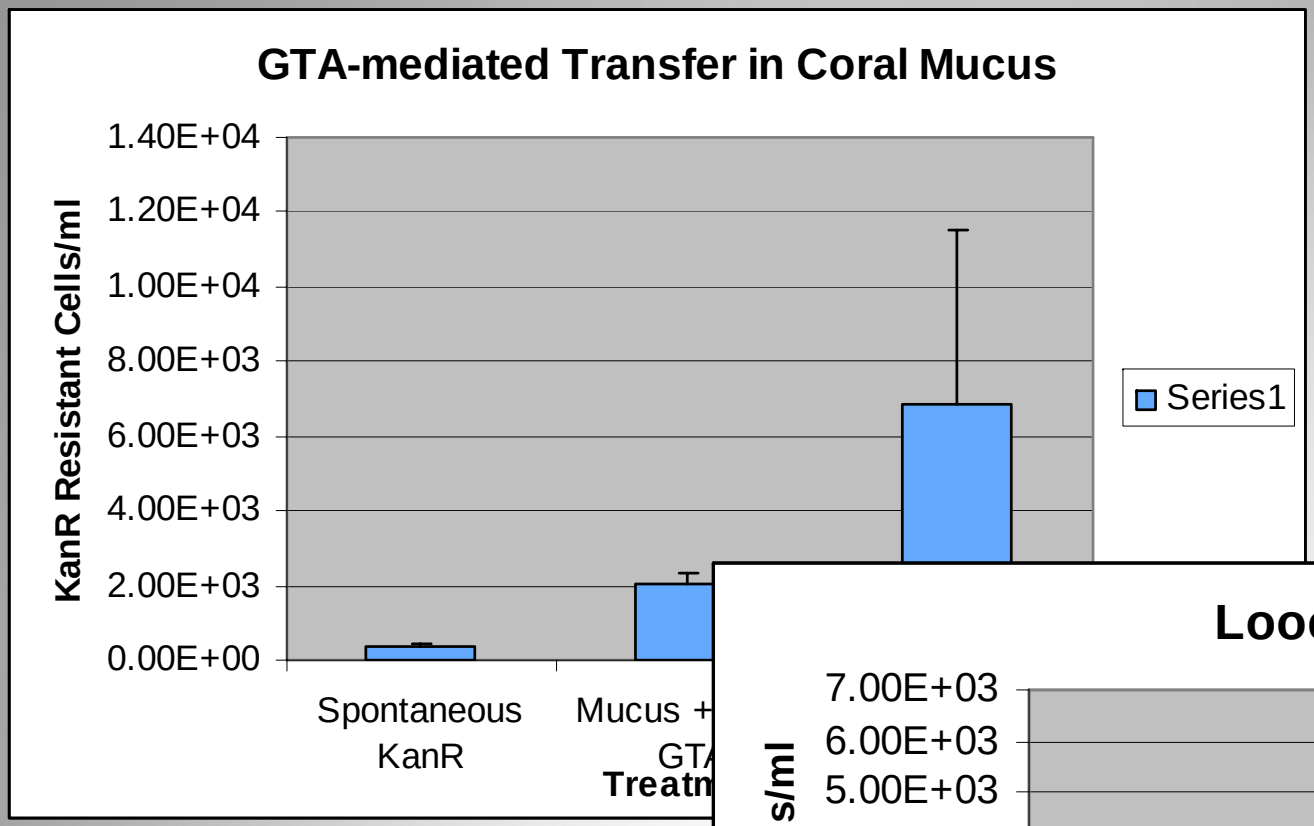
Culturable Bacteria Associated with *Symbiodinium*

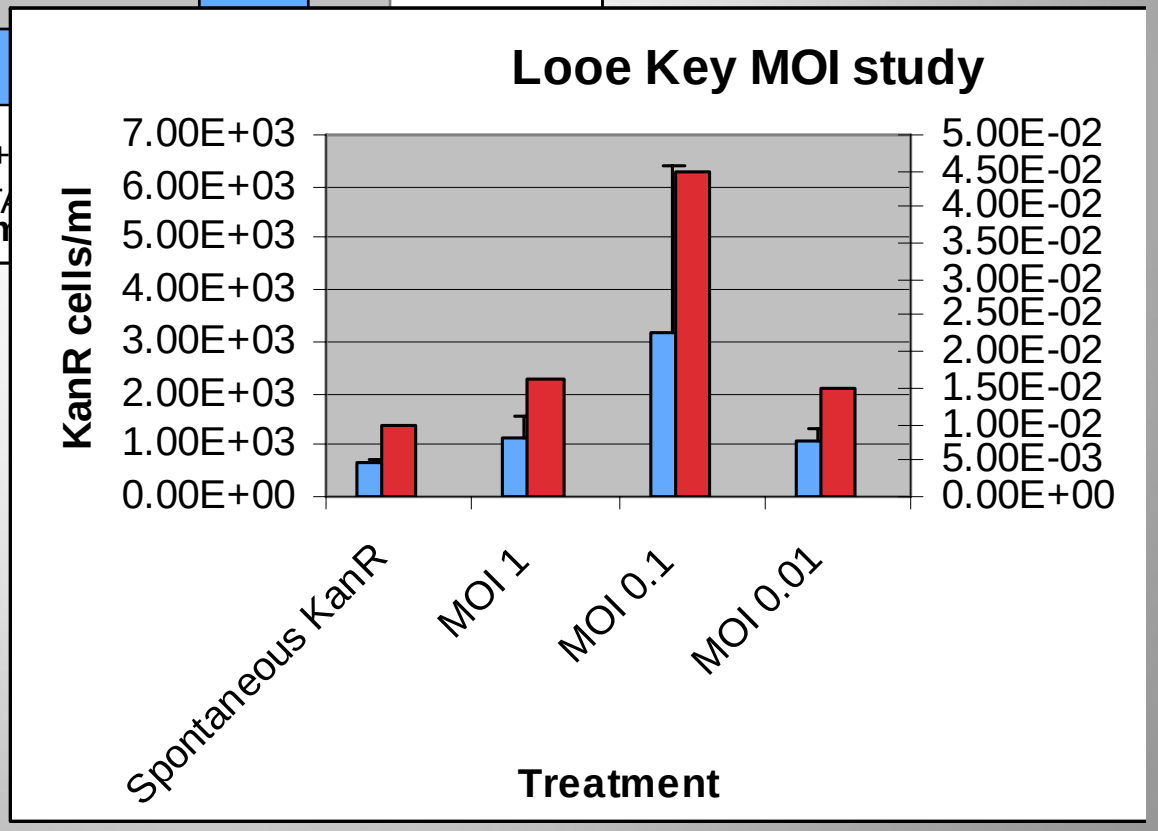
Marine Agar **GASWA**



Can GTAs transfer Genes to Ambient

- *R. mobilis* a re genetically "marked" w ce genes
- GTAs purifi s
- Added to re seawater community
- Incubated c


GTA-mediated Transfer in Coral Mucus
KanR Resistant Cells/ml
1.40E+04
1.20E+04
1.00E+04
8.00E+03
6.00E+03
4.00E+03
2.00E+03
0.00E+00
Spontaneous KanR
Mucus +
GTA
Treatm
Series1


Looe Key MOI study
KanR cells/ml
7.00E+03
6.00E+03
5.00E+03
4.00E+03
3.00E+03
2.00E+03
1.00E+03
0.00E+00
5.00E-02
4.50E-02
4.00E-02
3.50E-02
3.00E-02
2.50E-02
2.00E-02
1.50E-02
1.00E-02
5.00E-03
0.00E+00
Spontaneous KanR
MOI 1
MOI 0.1
MOI 0.01
Treatment

GTA-mediated transfer of marker genes documented to both cultured and natural populations.

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High Frequency of Horizontal Gene Transfer in the Oceans

Lauren D. McDaniel,[1]* Elizabeth Young,[1] Jennifer Delaney,[1] Fabian Ruhnau,[2] Kim B. Ritchie,[3] John H. Paul[1]

Microbes rely on mutation and the processes of horizontal gene transfer (HGT; conjugation, transformation, and transduction) to acquire new traits. Gene transfer agents (GTAs) discovered in the purple nonsulfur bacterium *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) are host-encoded viruslike elements that package random fragments of the host chromosome and are found in the genome of almost every sequenced member of the α-Proteobacteria order Rhodobacterales (*1*). To test whether GTAs are natural vectors of gene transfer, we grew nine strains of marine α-proteobacteria containing putative GTA cassettes (table S1) and screened them for the production of GTA-like particles.

Both *Roseovarius nubinhibens* ISM and the isolate *Reugeria mobilis* 45A6 reproducibly produced putative GTA particles during stationary phase growth. We then generated genetically marked donor strains of *R. nubinhibens* and *R. mobilis* containing the transposon Tn5. GTA production in these marked donor strains was equivalent to that of the wild-type strains. To document gene transfer frequencies, we subjected wild-type strains or natural communities from a range of environments to treatment with donor strain GTAs and documented the rates of GTA-mediated gene transfer of kanamycin resistance (fig. S1). In the coral reef environment, spontaneous kanamycin resistance was 4.6×10^{-4}, whereas the GTA-mediated frequency was significantly higher at 2.5×10^{-2} ($P = 0.028$, Student's t test).

For this experiment, both spontaneous mutants and GTA treatments were examined for the presence of the Tn5 streptomycin kinase gene. A total of 47% of the GTA-treated viable colonies but none of the spontaneous revertants contained the gene. That 53% of the putative transductants did not contain the gene is not suprising because these may have contained only the kanamycin resistance gene (*nptII*) and not the flanking streptomycin kinase gene.

The recovery of the streptomycin kinase sequence, which is ~1000 base pairs (1 kbp) from the active site of the kanamycin resistance gene, suggested that up to 1 kbp of the central region of Tn5 was transferred. This is consistent with extracted DNA from the GTAs, which ranged from about 500 to 1000 bp in length (fig. S3). No spontaneous double antibiotic (kanamycin and streptomycin) resistance was detected, and the GTA-mediated frequency of 1.06×10^{-4} was significantly higher ($P = 0.023$). The Tn5 streptomycin kinase sequence was recovered in 1 in 10 viable double antibiotic–resistant strains, suggesting that modifications, truncations, or rearrangements may have occurred, as in natural transformation (*2*).

Similar frequencies of transfer were observed among differing environments (Table 1), demonstrating that cultivated GTAs transduce natural communities of marine bacteria. The 16*S* ribosomal RNA sequences examined showed that the majority of natural GTA recipients were most similar to marine *Flavobacterium* or *Flexibacter* strains (table S2), consistent with the prior reports of abundant *Flavobacterium* in marine systems (*3*).

R. nubinhibens contains both a GTA and an inducible prophage (*4*). Transmission electron microscopy (TEM) demonstrated that *R. nubinhibens*–induced prophage preparations contained tailed phage (*4*), whereas GTA particles were nontailed (fig. S2A), resembling the GTA of *Silicibacter pomeroyi* (*5*). In contrast to the GTA particles, the purified prophages of *R. nubinhibens* had no gene transfer activity. Additionally, maximal expression of the *R. nubinhibens* GTA terminase gene cooccurred with maximal GTA production (fig. S4). TEM of GTAs of *R. mobilis* revealed tailed viral particles (fig. S2B).

GTA dose, or multiplicity of infection (MOI), was linearly correlated with increased resistance to antibiotics (MOI range from 0.01 to 10, $R^2 = 0.9593$), which enabled extrapolations of gene transfer frequencies to natural systems (*6*).

GTAs from *R. nubinhibens* ISM show a wide host range and interspecific gene transfer under ecologically relevant conditions. Environmental gene transfer frequencies ranging from 6.7×10^{-3} to 4.7×10^{-1} (Table 1) are 1900 to 459 million times the frequency for transformation (*2*) and 650,000 to 31 million times the frequency of transduction previously measured in the marine environment (*7*). These results suggest a genomic flexibility in marine microbial populations that facilitates their adaptation to changing environmental conditions.

Table 1. Frequencies of transfer of marker genes to both cultured and natural communities. N/A indicates not applicable; BDL, below detection limit.

Environment	Avg. spontaneous frequency	Range	Avg. GTA-mediated rate	Range	Number of trials
		Roseovarius nubinhibens GTA filter matings			
Culture	6×10^{-7}	5.2×10^{-8} – 2.0×10^{-6}	1.7×10^{-5}	7.5×10^{-8} – 7.9×10^{-5}	$n = 5$
Estuary	1.6×10^{-4}	2.8×10^{-5} – 3.0×10^{-4}	8.9×10^{-4}	6.2×10^{-5} – 1.1×10^{-3}	$n = 3$
		Roseovarius nubinhibens GTA liquid matings			
Estuary	1.2×10^{-3}	N/A	3.1×10^{-2}	1.2×10^{-2} – 5.0×10^{-2}	$n = 2$
Coastal	4.3×10^{-2}	N/A	2.8×10^{-1}	N/A	$n = 2$
Open ocean	2.5×10^{-2}	6.7×10^{-3} – 4.3×10^{-2}	3.9×10^{-1}	2.8×10^{-1} – 4.0×10^{-1}	$n = 3$
Reef	4.6×10^{-4}	N/A	2.5×10^{-2}	N/A	$n = 1$
Reef (double antibiotic)	BDL ($<10^{-6}$)	N/A	1.06×10^{-4}	N/A	$n = 1$
		Reugeria mobilis (45A6) GTA liquid matings			
Estuary	1.2×10^{-3}	0–2.1×10^{-2}	2.4×10^{-2}	4.2×10^{-2} – 1.1×10^{0}	$n = 1$
Coastal	4.3×10^{-2}	3.0×10^{-2} – 5.6×10^{-2}	2.8×10^{-1}	1.6×10^{-1} – 6.4×10^{-1}	$n = 1$
Open ocean	3.3×10^{-3}	$0 - 1 \times 10^{-2}$	4.7×10^{-1}	$0 - 3.6 \times 10^{0}$	$n = 2$
Reef	4.6×10^{-4}	N/A	1.1×10^{-1}	N/A	$n = 1$

References and Notes

1. A. S. Lang, J. T. Beatty, *Trends Microbiol.* **15**, 54 (2007).
2. H. G. Williams, J. Benstead, M. E. Frischer, J. H. Paul, *Mol. Mar. Biol. Biotechnol.* **6**, 238 (1997).
3. T. Woyke *et al.*, *PLoS ONE* **4**, e5299 (2009).
4. Y. L. Zhao *et al.*, *Appl. Environ. Microbiol.* **76**, 589 (2010).
5. E. J. Biers *et al.*, *Appl. Environ. Microbiol.* **74**, 2933 (2008).
6. Materials and methods are available as supporting material on *Science* Online.
7. S. C. Jiang, J. H. Paul, *Appl. Environ. Microbiol.* **64**, 2780 (1998).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/50/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References

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What else can GTAs Do?

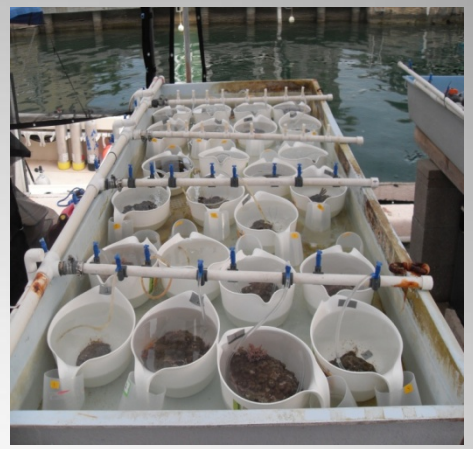
- *Roseobacters* and *Marinobacters* have been shown to increase growth rate of *Symbiodinium* (Ritchie, in prep)
- Since GTAs are from these kinds of bacteria, could GTAs alone have an effect on *Symbiodinum*?
- Maybe on the corals themselves?

Coral Reef Settlement Hypothesis

Ho: GTAs have no effect on coral larval settlement.
Ha: GTAs have an effect on coral larval settlement.

Methods Overview

1)Coral larvae collected and counted.
2) Larvae incubated with GTAs and settling surface.
3) Settling surface scored for settling and metamorphosis.



- ***Porites astreoides-*** Brooding Coral

- ***Montastraea faveolata-*** Broadcast Spawner

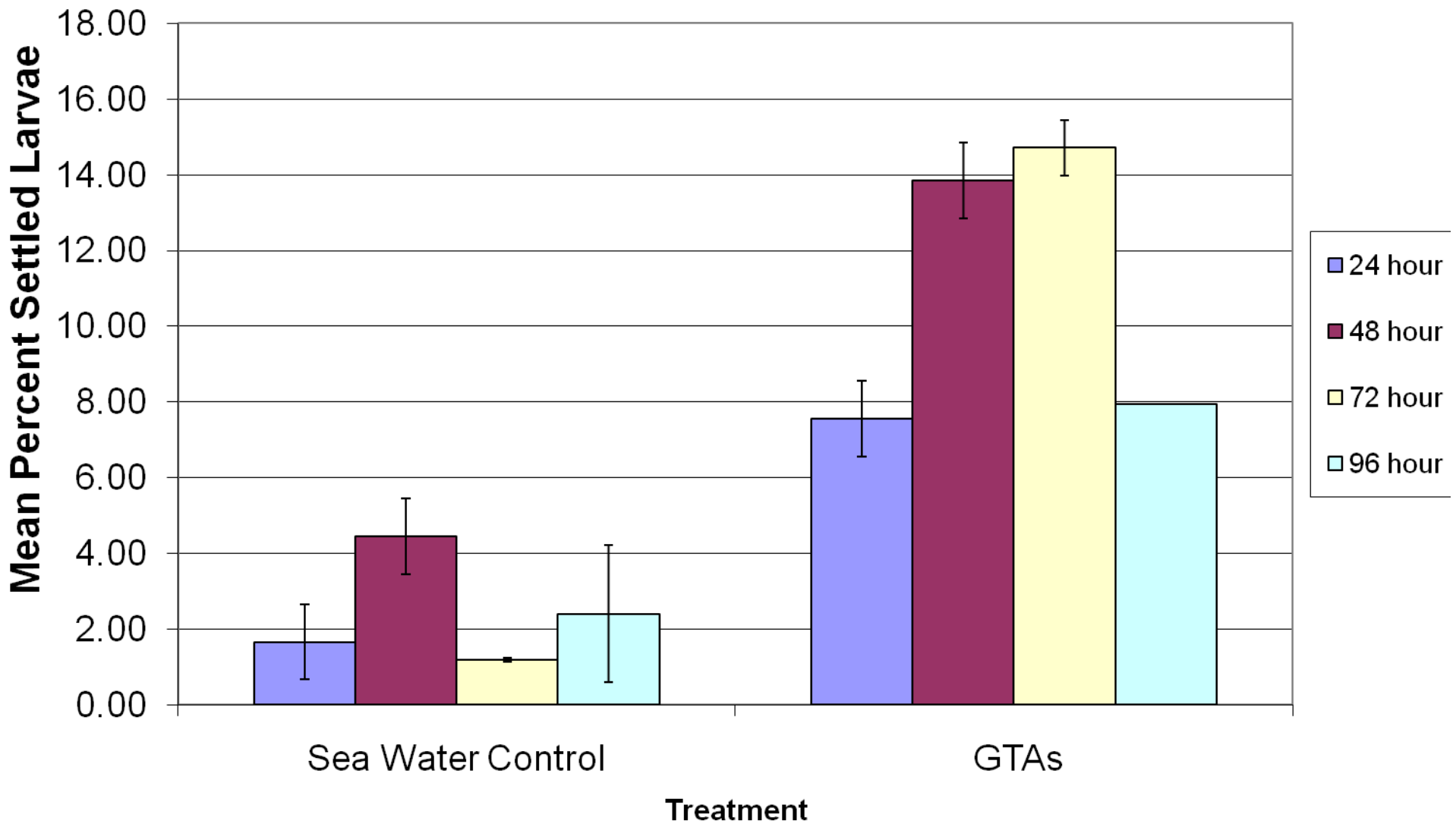
Experimental Set-up

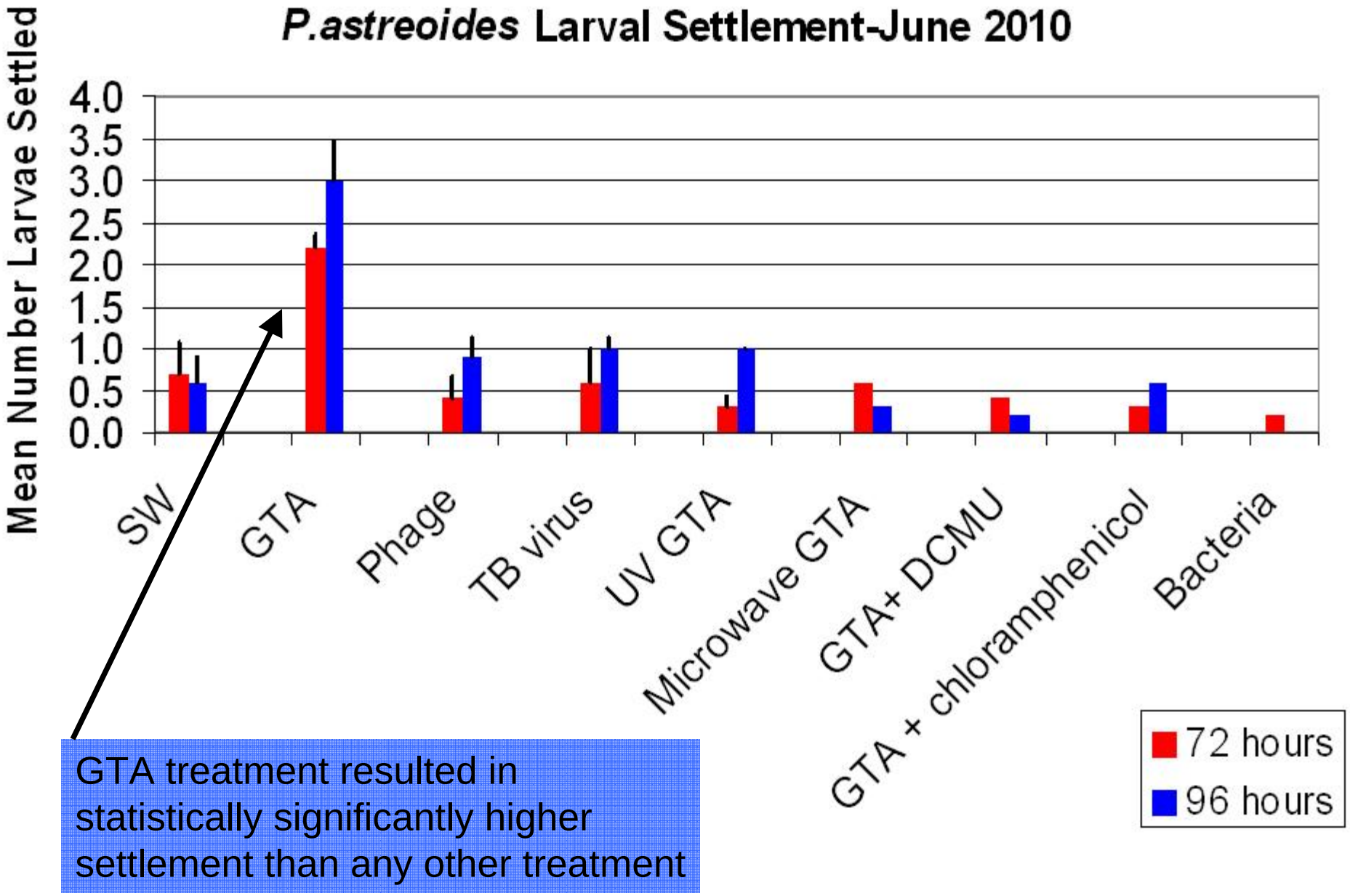
Each treatment replicated 10 times with 20 larvae/plate, plus 1 biofilm/plate, (provided on glass slides cured in the natural reef environment for 3 weeks) , plus 20 ml sterile seawater/plate.

Treatments: (Varied per Experiment)

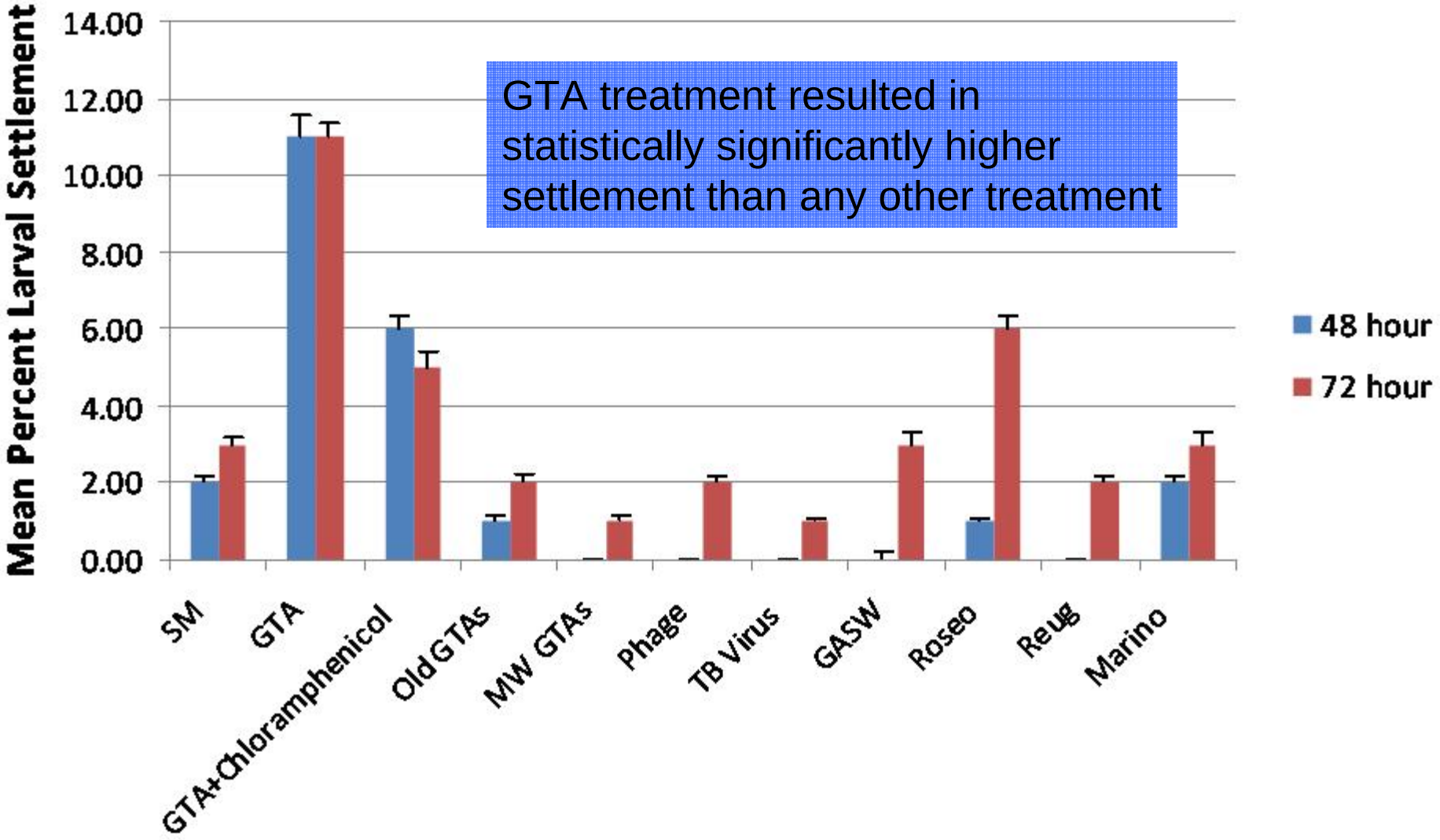
- Media Controls
- GTAs from *Reugeria* 45A6
- Inactivated GTAs from *Reugeria* 45A6
- Non-specific viruses
- chloramphenicol/GTAs (bacterial protein synthesis inhibitor)
- DCMU/ GTAs (photosystem II metabolic inhibitor)
- *Reugeria mobilis* bacterial strain

P. astreoides settlement- May 2010




P.astreoides Larval Settlement-June 2010
Mean Number Larvae Settled
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SW
GTA
Phage
TB virus
UV GTA
Microwave GTA
GTA+ DCMU
GTA + chloramphenicol
Bacteria
72 hours
96 hours
GTA treatment resulted in statistically significantly higher settlement than any other treatment

M. faveolata Larval Settlement September-2010

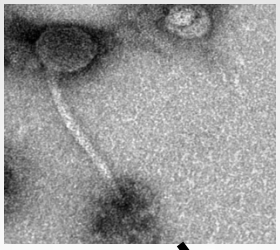


What's going on here?

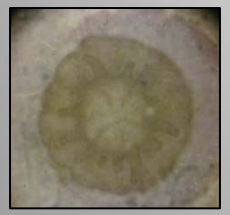
- Increased settlement requires procaryotic protein synthesis and photosynthetic energy
- Controls indicate that facilitated settlement is peculiar to GTAs and not viruses in general

Theories

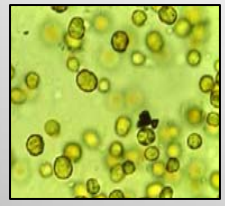
GTAs transfer genes from *Reugeria mobilis:*



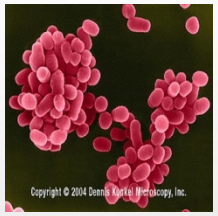
to α-proteo's associated with the coral larvae.

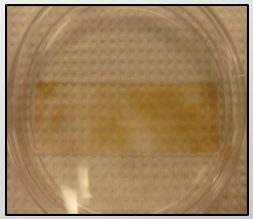


to α-proteo's associated with *Symbiodinium.*

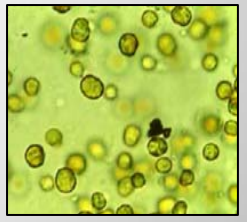


to α-proteo's already on the surface of the biofilm.





directly to the *Symbiodinium.*



directly to the larvae.

Applications to Protecting the Reef Environment:

- **Can GTAs be a sort of "Gene Therapy" for the coral reef environment???**
- **A possible way to deliver beneficial genes to the coral holobiont.**

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