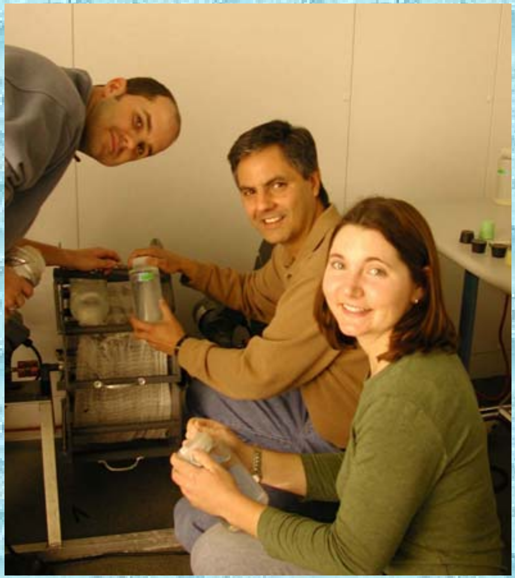
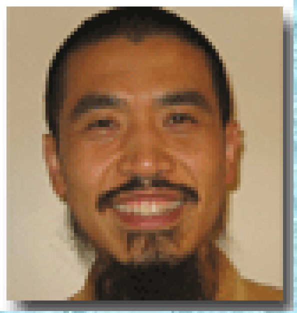
Copepod Resistance to Toxic Phytoplankton

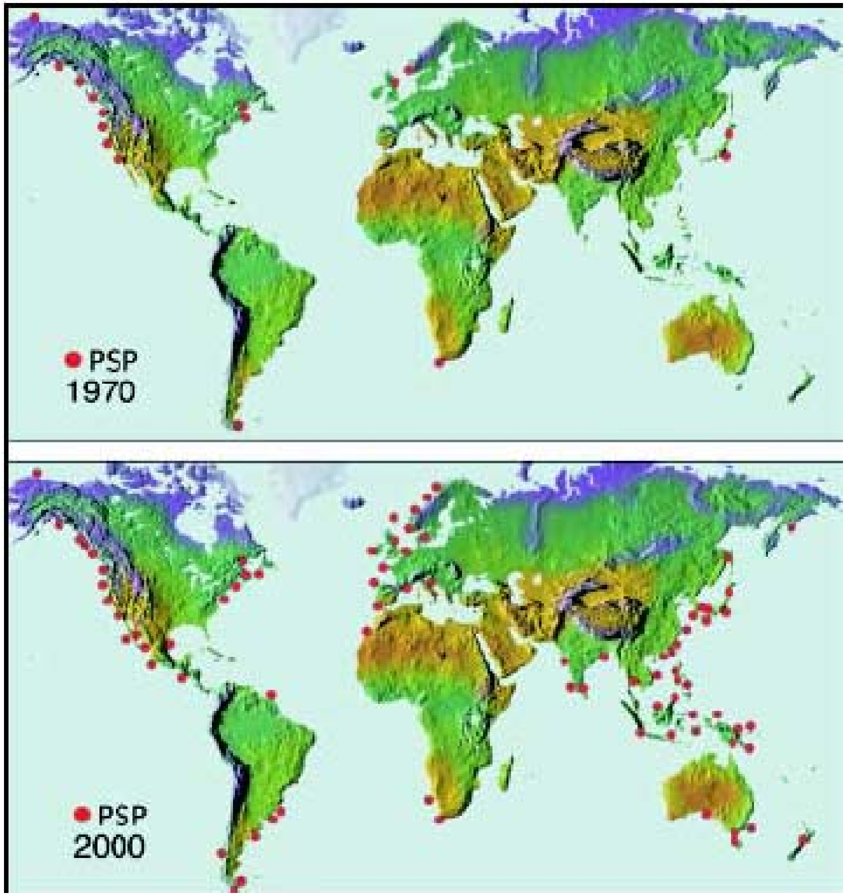
Hans Dam, Sean Colin, Sheean Haley, David Avery, Lihua Chen, Huang Zhang & Senjie Lin

www.marinesciences.uconn.edu





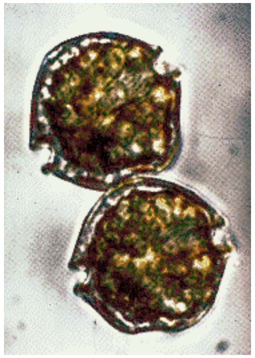
Dramatis Personae



Source: *GEOHAB Rep. 1* (2001)

Toxic Dinoflagellates

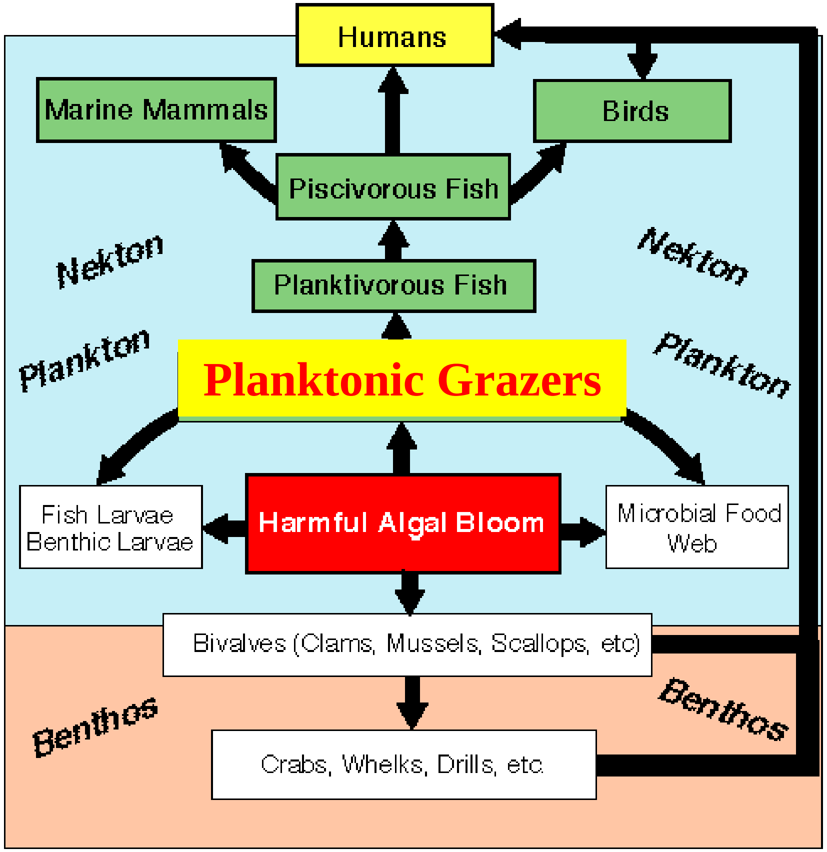
Alexandrium



Saxitoxins
- **Block Na^+ channels**
- **Cause Paralysis**

Acartia hudsonica

Grazer Toxin Resistance

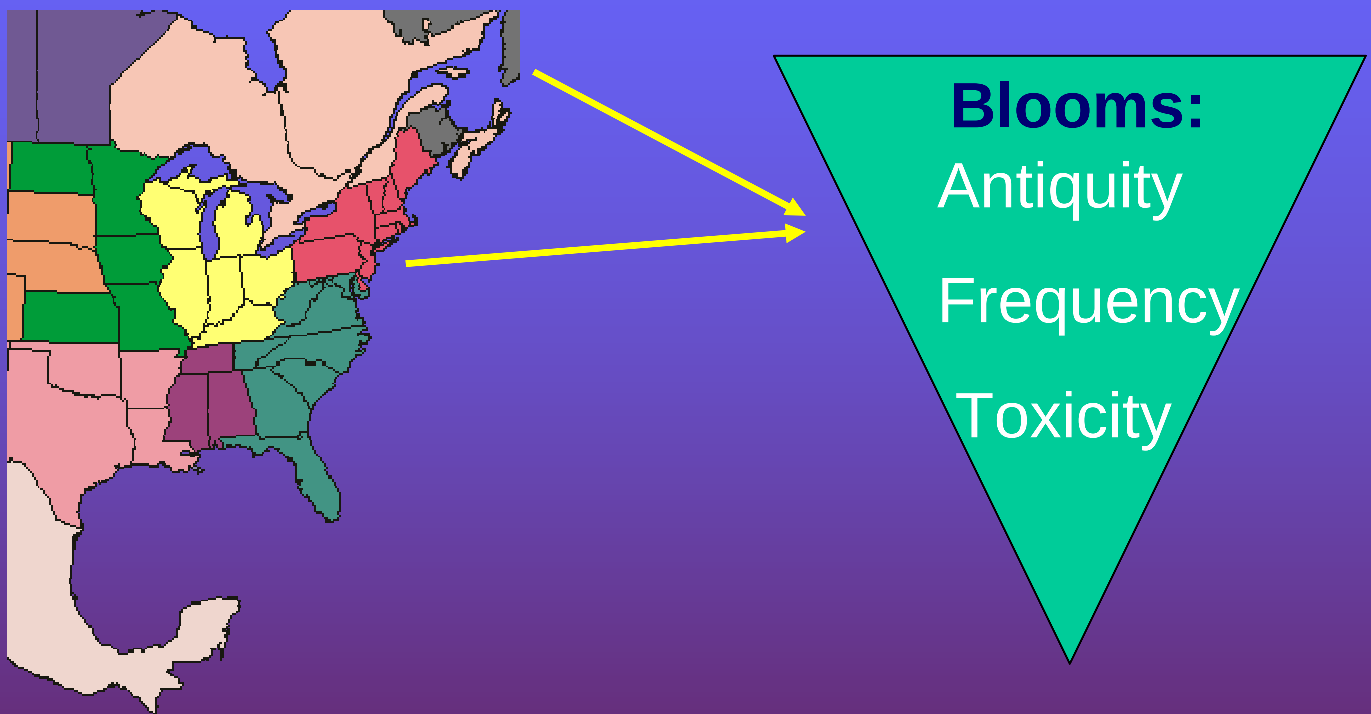


- Toxin biomagnification?
- Grazer control of HAB?

Sales Pitch !!!!

- Resistance:
  - Real
  - Important for bloom control and toxin transfer in food web
- But bloom control and toxin transfer tied both to genetics & mechanism(s) of resistance

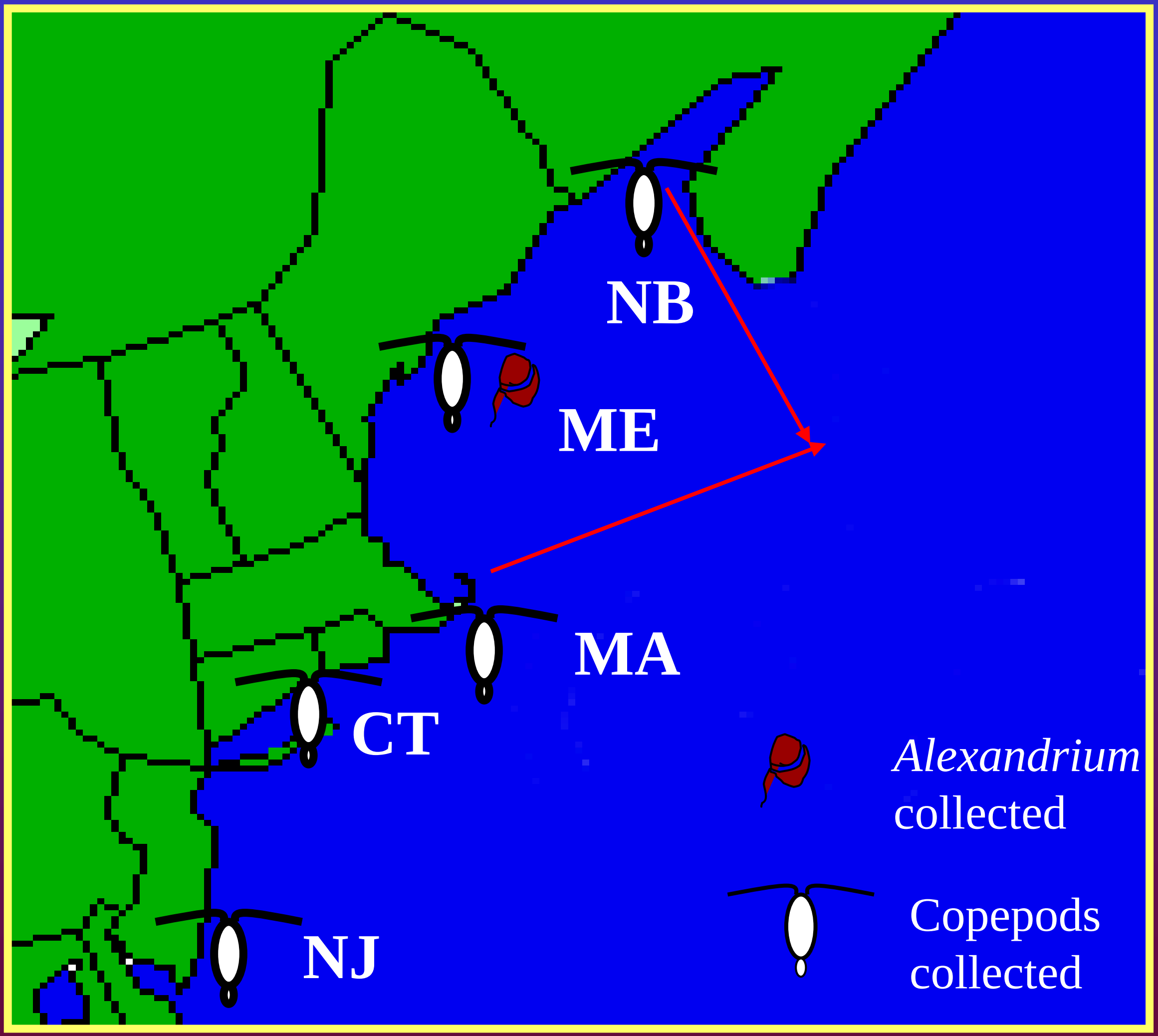
Hypothesis

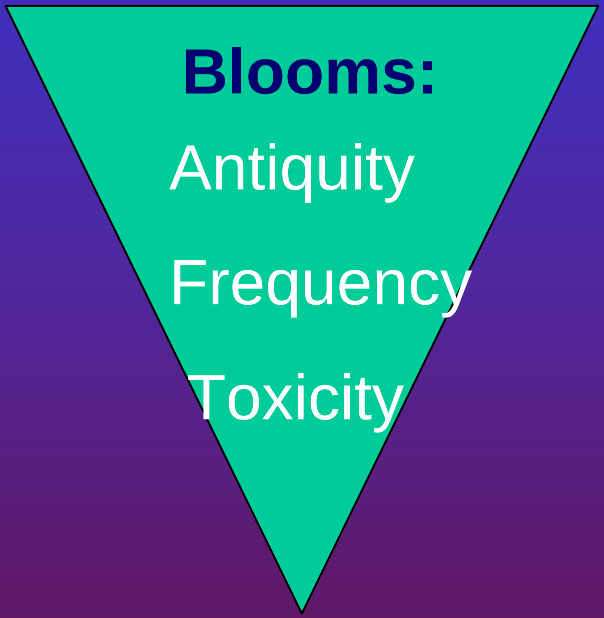


Prediction:

Natural Selection: Enhanced fitness in **historically exposed** grazer populations.

Hypothesis Test: Differences Among Copepod Populations





Common Garden Experiments

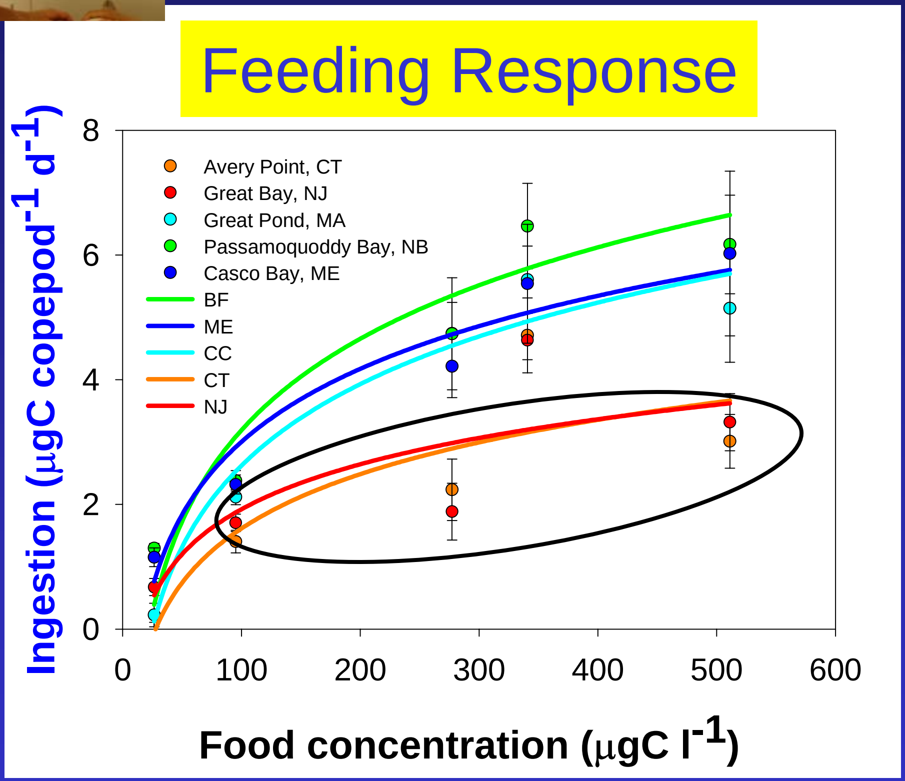
Same conditions, many generations
Non-toxic phytoplankton diet

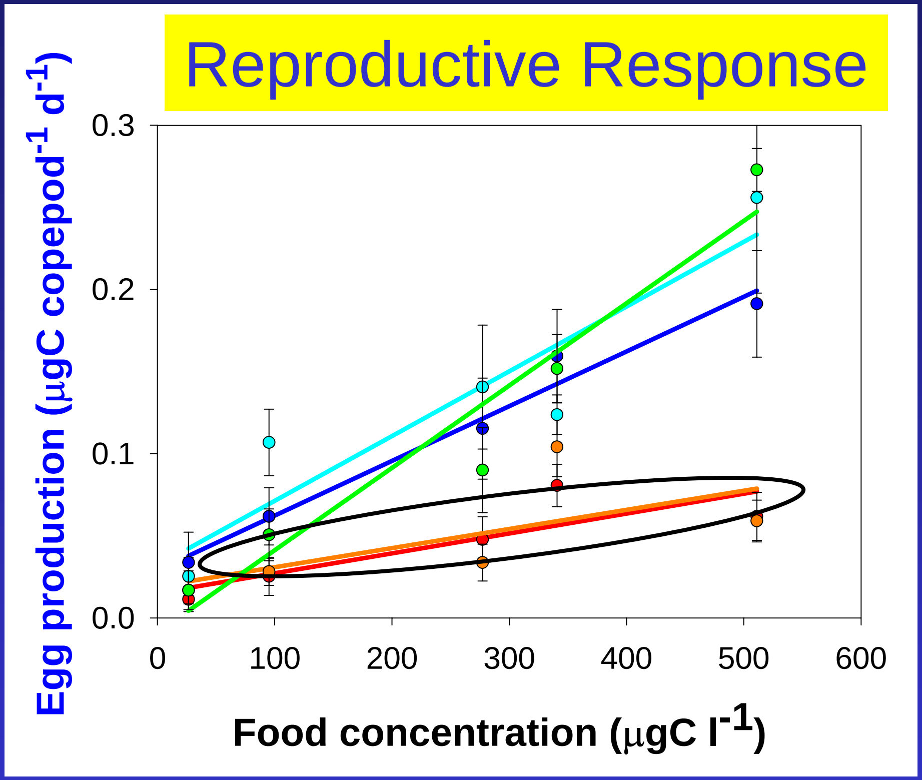
- **Phenotypic variation** = genetic (G) + environ(E) + G x E

Evidence for Resistance

- Enhanced performance (ingestion and egg production) in historically exposed copepod populations.
- No fitness (λ) penalty in historically exposed population, but significant penalty in unexposed population.
- Genetic selection experiments with unexposed populations.

Interpopulation Differences

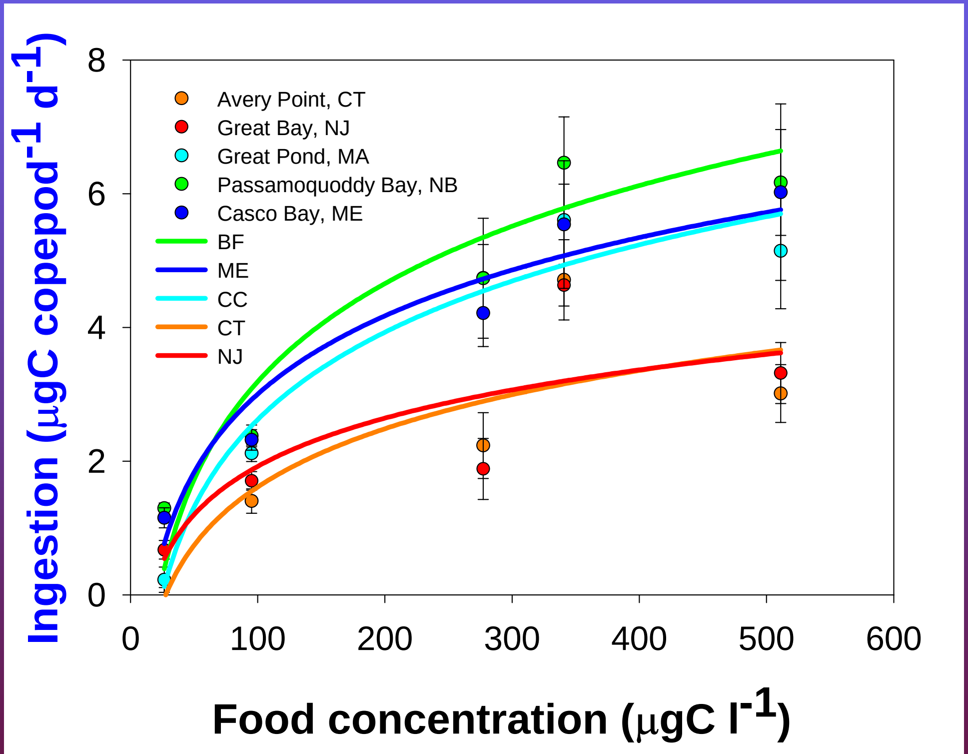




- Southern populations: Lower feeding and reproduction.
- **Results consistent with resistance hypothesis!**
- Not due to physiological compensation

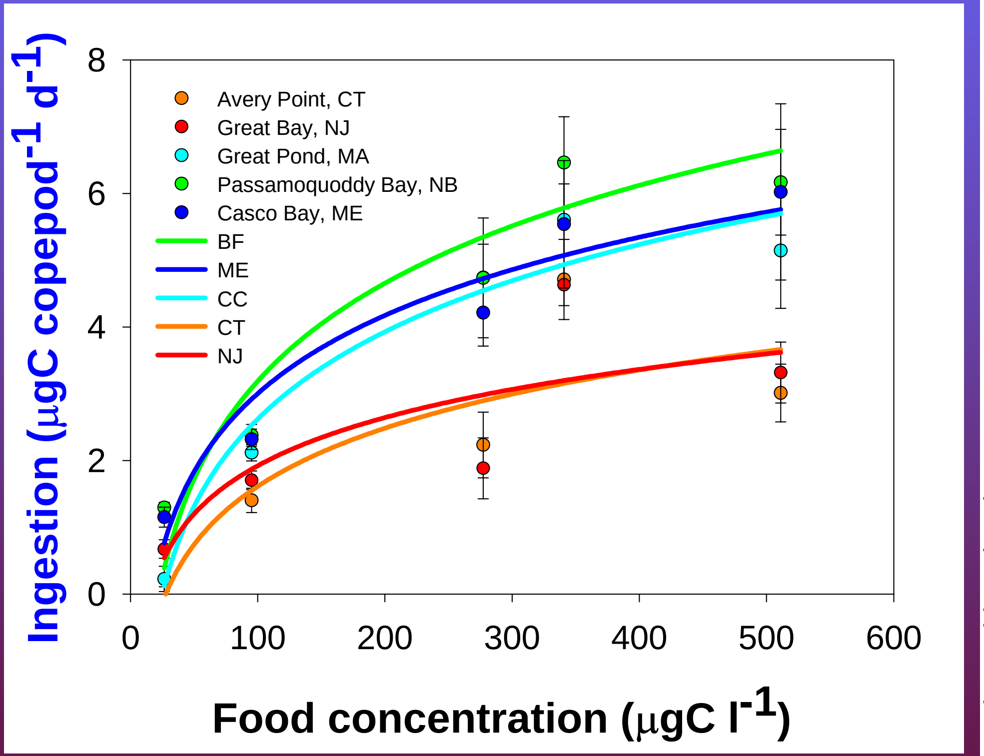
Grazer Control

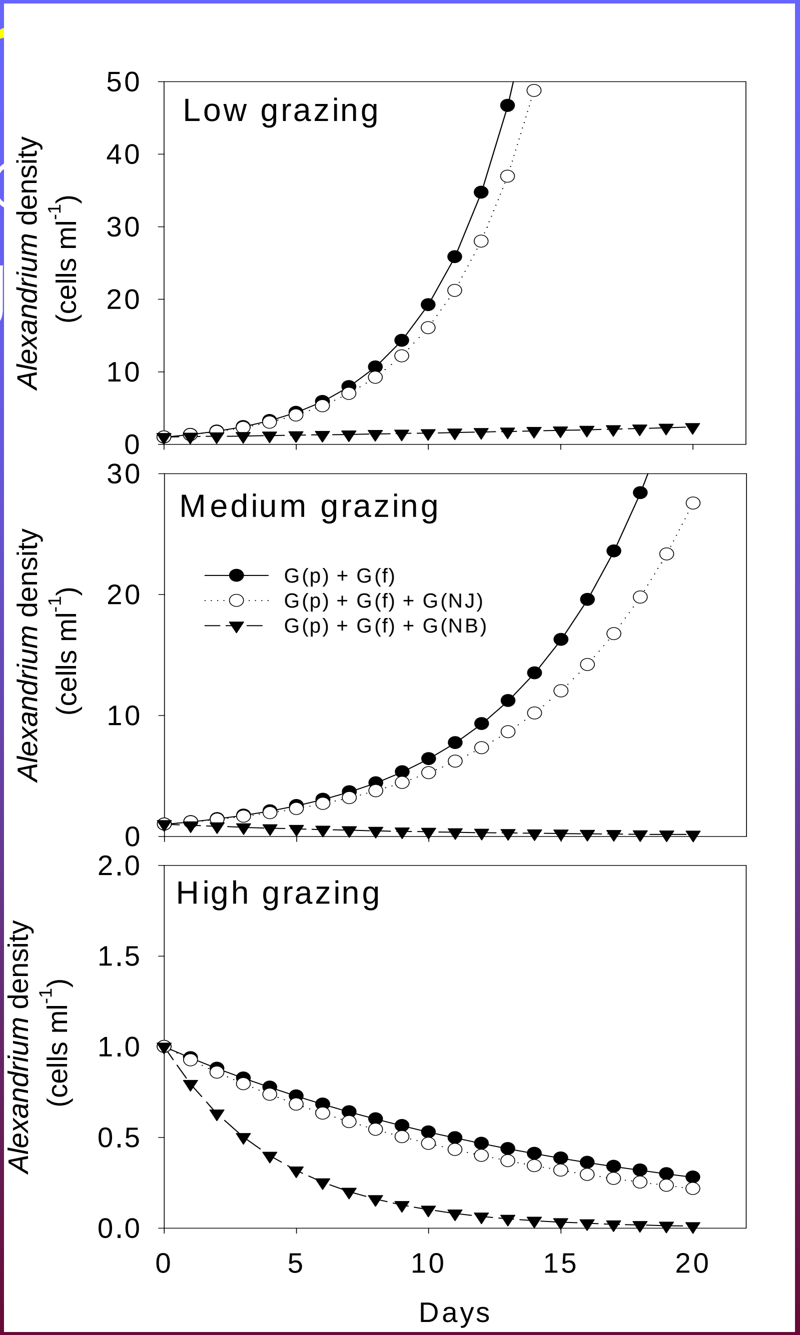
Resistant copepod population can keep *Alexandrium* growth in check



Grazer Con

Resistant copepod p
keep *Alexandrium* g





But Why Do Toxic Blooms Persist in the Presence of Resistant Populations?

Limnol. Oceanogr., 52(5), 2007, 000–000

Newly discovered reproductive phenotypes of a marine copepod reveal the costs and advantages of resistance to a toxic dinoflagellate

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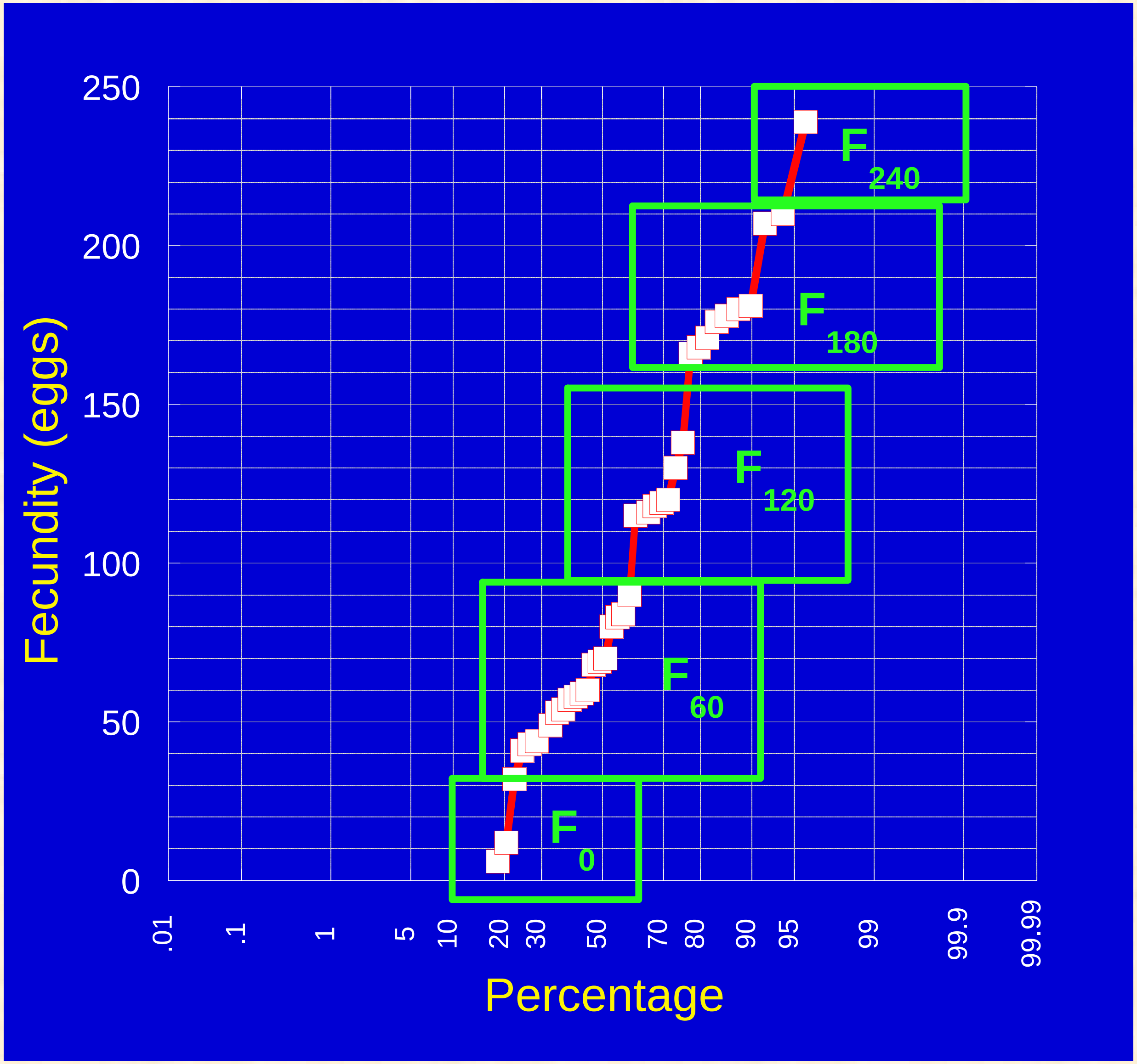
University of Connecticut Department of Marine Sciences, 1080 Shennecossett Rd., Groton, Connecticut 06340

Abstract

We document for the first time toxin-resistant reproductive phenotypes of copepods and we describe a novel procedure to identify these phenotypes. Individual copepods of the species *Acartia hudsonica* were raised on two diets: a standard nontoxic diet and a diet containing the toxic dinoflagellate *Alexandrium fundyense*, both offered at nonlimiting concentrations. Resistant individuals were defined as those that survived on the toxic diet. We examined several life-history characters, including survivorship, age at metamorphosis, age at maturity, fecundity, and fitness. During this study, we discovered five resistance-related reproductive phenotypes that appeared as discrete classes in a frequency distribution of fecundity. After grouping the data according to these phenotypes, we calculated the fitness of each phenotype on each diet. We also calculated the cost and advantage associated with resistance. On the standard diet, one phenotype had 46% lower fitness than the phenotype with the highest fitness, indicating that possessing resistance alleles can carry a substantial cost. A different phenotype showed maximum relative fitness on the toxic diet and reduced relative fitness on the standard diet. From these results, we argue that resistance is conferred by a simple genetic system showing heterozygote advantage and leading to a polymorphism for resistance. Such a polymorphism will prevent the fixation of resistance alleles in natural populations. It may also confound the interpretation of typical experiments that measure average population responses.

Resistant Reproductive Phenotypes

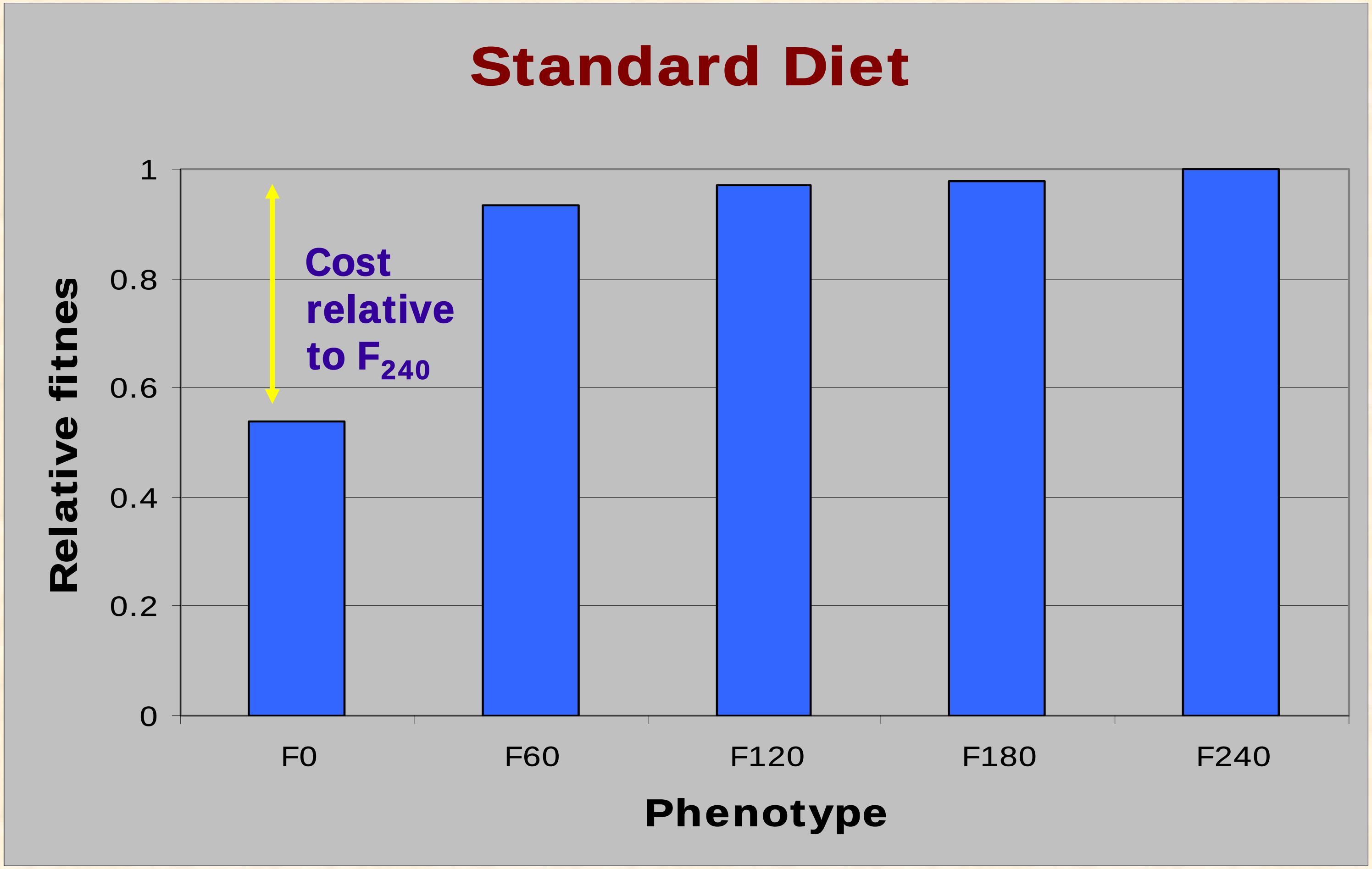


Phenotype Fitness

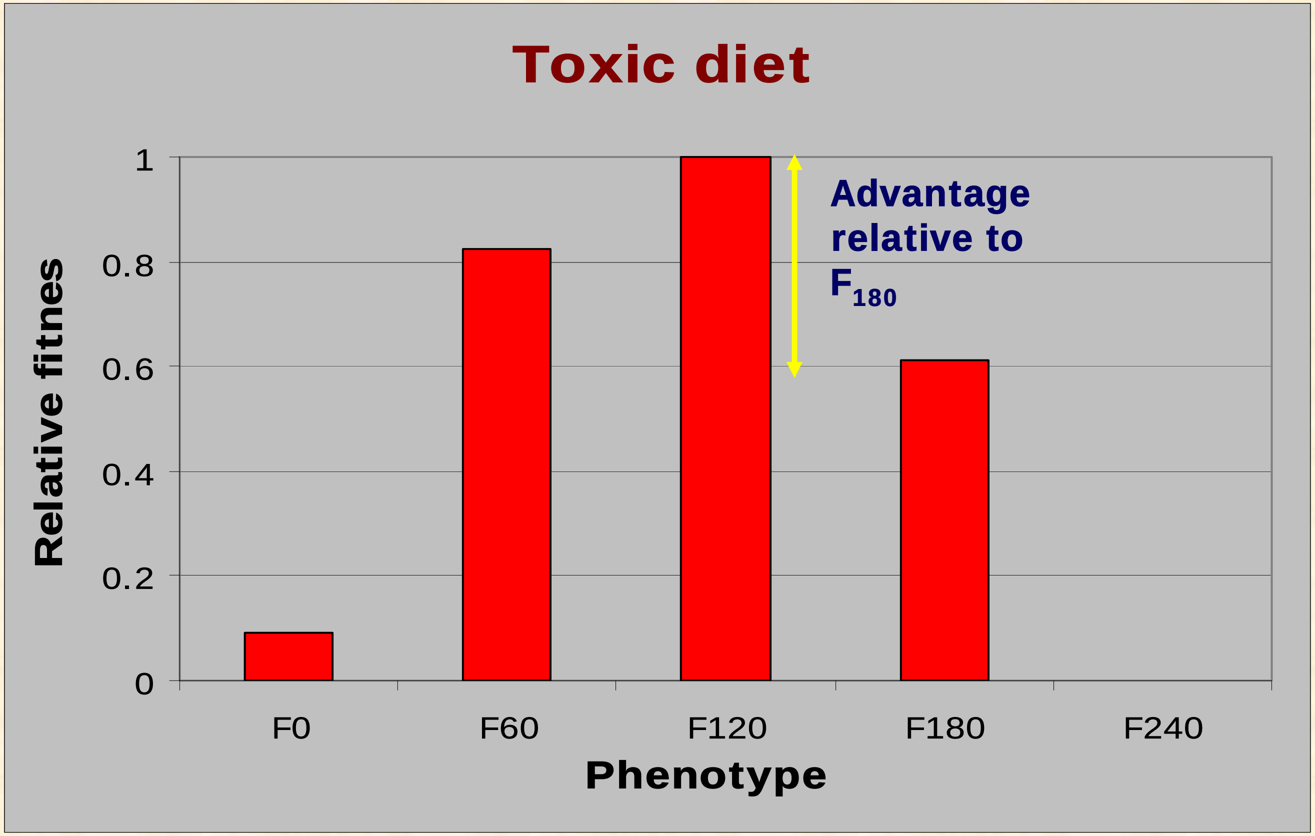
Differential survivorship between diets included by assuming that each cohort started with identical frequency of phenotypes and weighting phenotype fitness accordingly.

			Fitness			Weighted Fitness	
Phenotype			Standard	Toxic		Standard	Toxic
F0	Mean		0.658	0.210		0.658	0.066
	n		16	5			
	S.D.		0.527	0.469			
[illegible]	[illegible]		[illegible]	[illegible]		[illegible]	[illegible]
[illegible]	[illegible]		[illegible]	[illegible]			
	S.D.		0.013	0.016			
F120	Mean		1.181	1.167		1.181	0.729
	n		8	5			
	S.D.		0.011	0.008			
F180	Mean		1.192	1.188		1.192	0.446
	n		8	3			
	S.D.		0.01	0.01			
F240	Mean		1.219	0.000		1.219	0.000
	n		1	0			
	S.D.		.	.			

Relative Fitness Phenotype Cost



Relative **Phenotype** Fitness Advantage

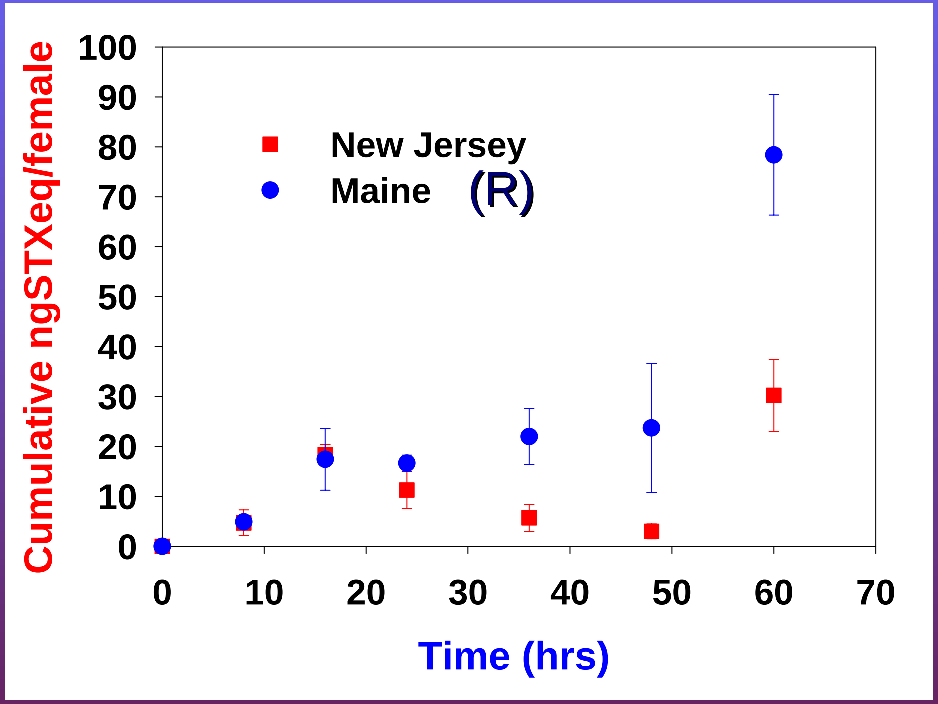


Hypothetical

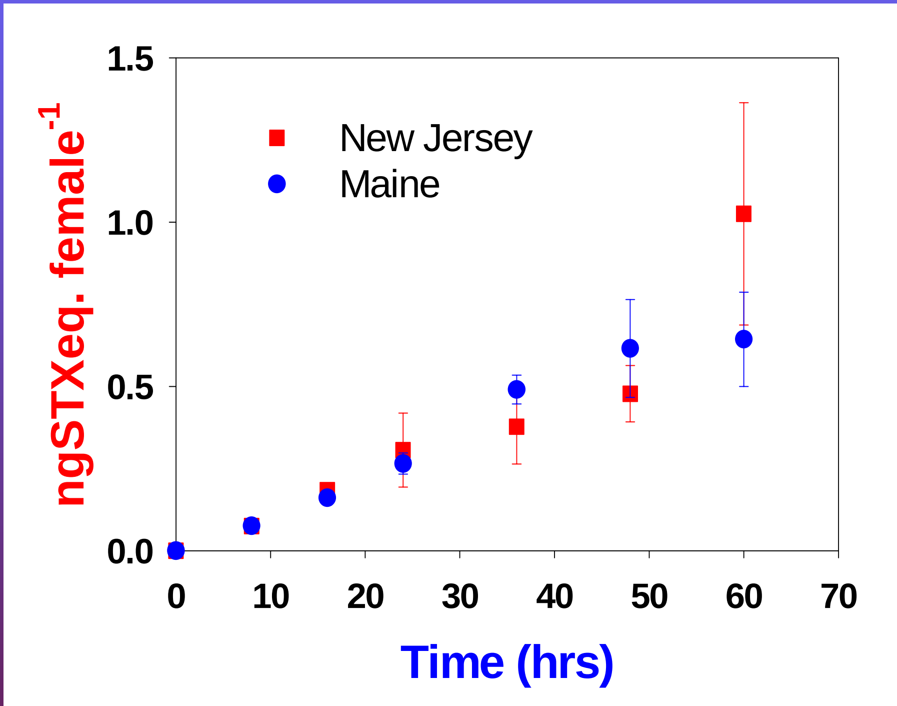
RRRR RSRR RSRS RSSS SSSS

Resistance: Higher Toxin Transfer?

Ingestion



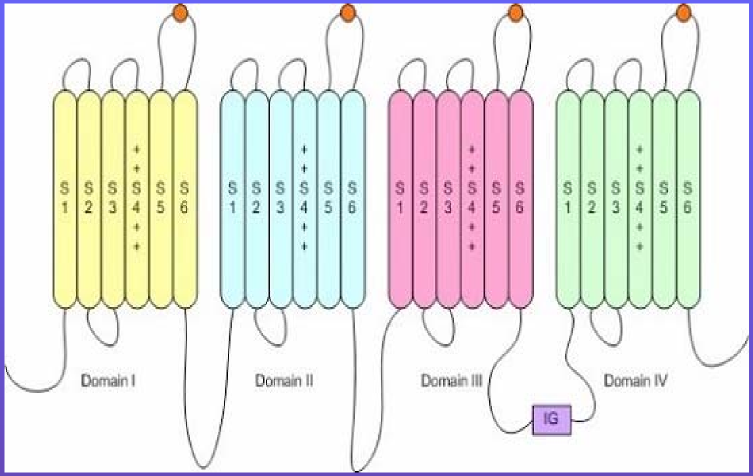
Accumulation

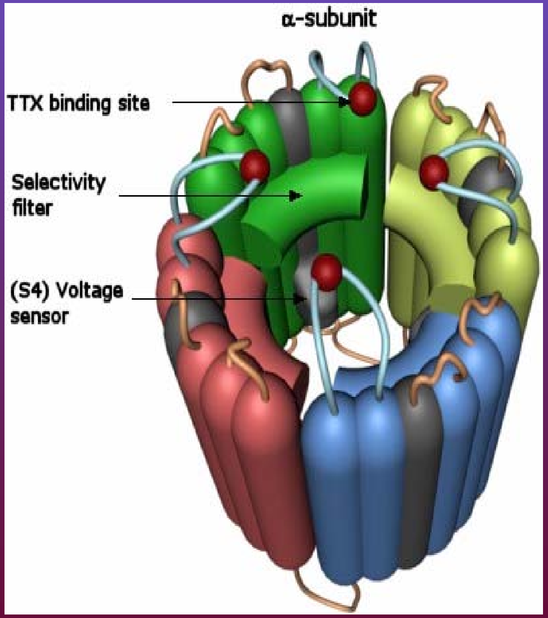


Greater toxin ingestion, but not accumulation in resistant population !

Resistance Mechanism

- Saxitoxin binds and blocks sodium ions from flowing into cell
- Nerve signal prevented
- Paralysis ensues

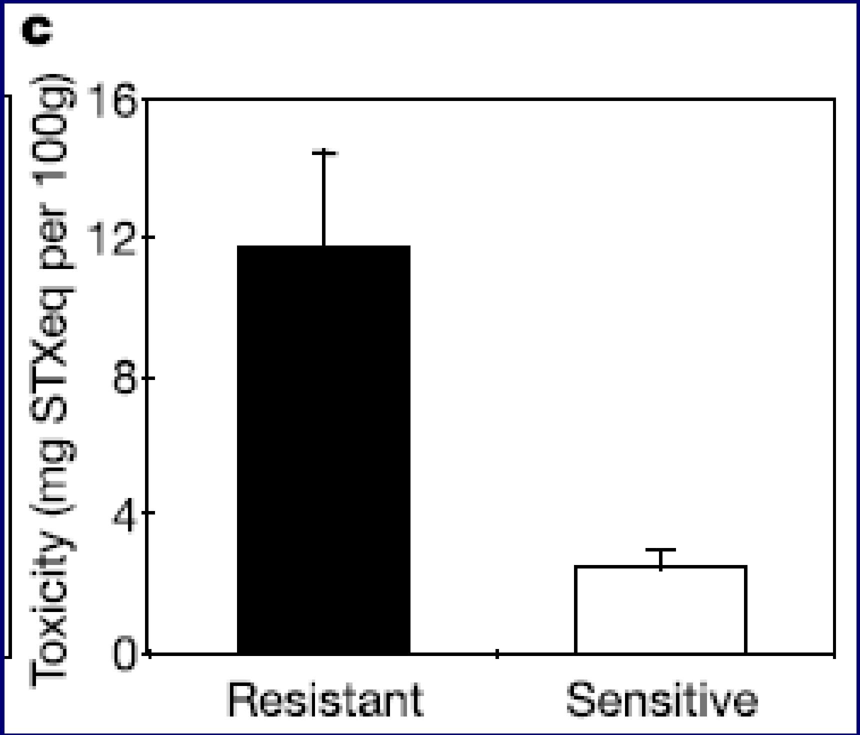


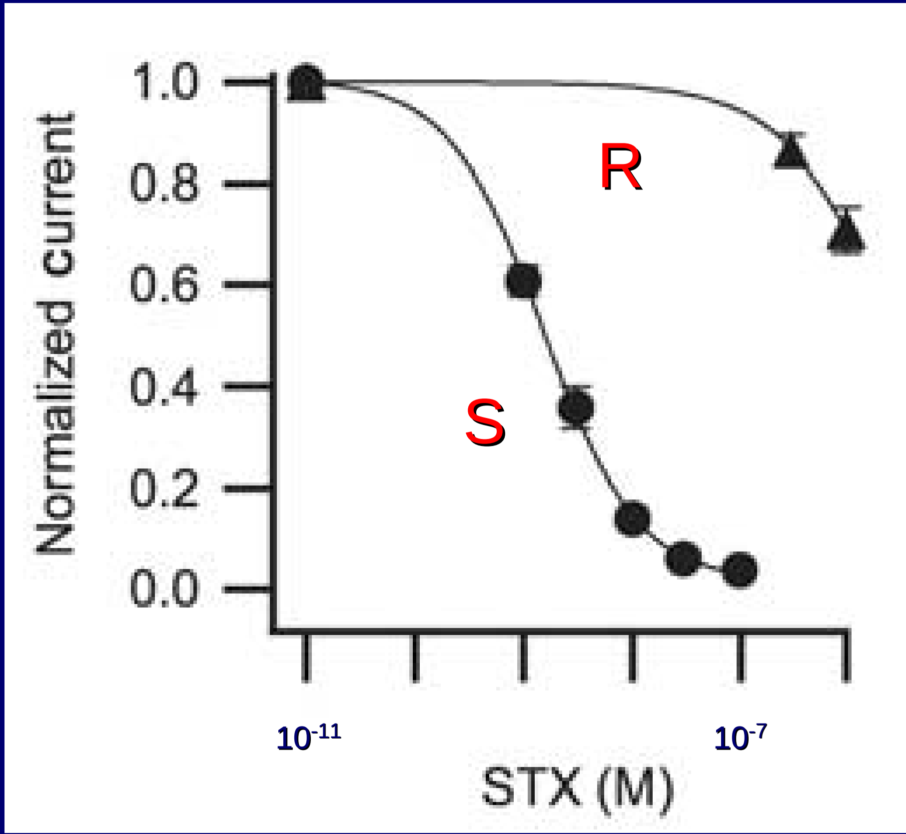


Source: Tim smith@ http://www.chemsoc.org/

Saxitoxin Resistance in Clams

Bricelj et al. 2005

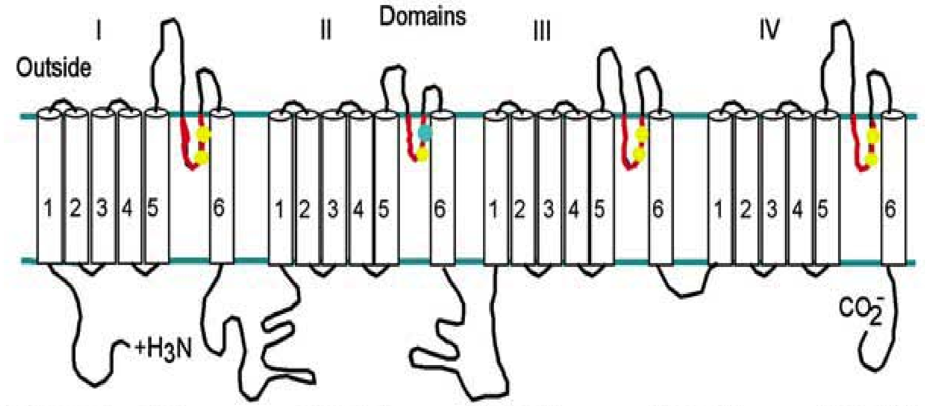




Saxitoxin Resistance in Clams

Bricelj et al. 2005

- Single nucleotide substitution at STX receptor site
- Similar mutations cause resistance in insects

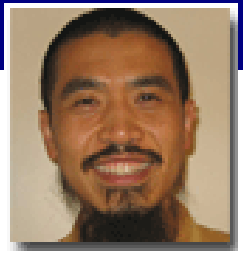


Species (common name)	Domain I	Domain II	Domain III	Domain IV
Rattus norvegicus (rat)	RLMTQDFWEN	RVLCGEWIET	VATFKGWMDI	ITTSAGWDGL
Takifugu pardalis (puffer fish)	RLMTQDNWES	RILCGEWIET	IATFKGWMDI	ITTSAGWDTL
Drosophila melanogaster (fly)	RLMTQDFWED	RVLCGEWIES	VATFKGWIQI	MSTSAGWDGV
Loligo opalescens (squid)	RLMTQDYWEN	RVLCGEWIES	VATFKGWINI	MSTSAGWDGV
Aplysia californica (sea hare)	RLMTQDFWEW	RVLCGEWIES	VATYKGWIDI	MCTSAGWSDV
Bdelloura candida (flatworm)	RLMTQDFWED	RVLCGEYIES	VATFKGWTDI	ISTTGGWHSV
Cyanea capillata (jellyfish)	QVCTLDYWES	RILCGKWIEP	TATLEGWFEE	ISTAAGWNGV
Mya arenaria sensitive	RLMTQDYWEN	RVLCGEWIES	VATYKGWIDI	MCTSAGWDGV
Mya arenaria resistant	RLMTQDYWEN	RVLCGEWIDS	VATYKGWIDI	MCTSAGWDGV

Acartia hudsonica Sodium Channel Gene Sequence

Multiple Amino Acid Sequence Alignment

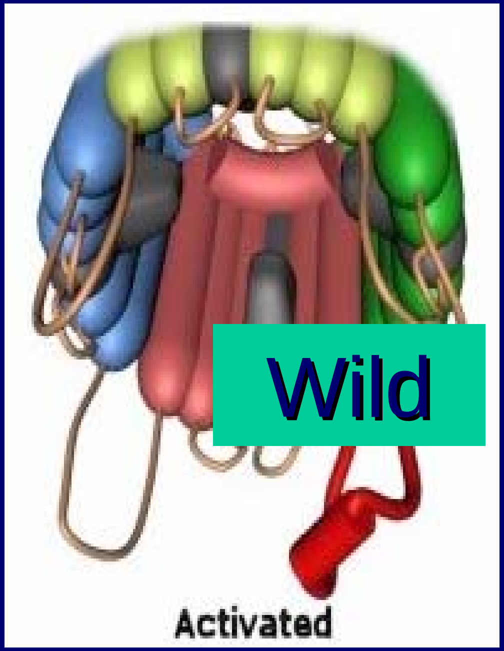
Bivalvia_AAX14719	IDNFNKQKKK---AG-SLEMFMTEDQKKYY
Sea_hare_AAC47457	IENFNSQKKK---AGGSLEMFMTEDQKKYY
house_fly_AAB47605	IDNFNEQKKK---AGGSLEMFMTEDQKKYY
fruit_fly_AAB59195	IDNFNEQKKK---AGGSLEMFMTEDQKKYY
Acartia_hudsonica type 1	IDNFNEQKKK ***GRDV***GGSLEMFMTEDQKKYY
Acartia_hudsonica type 2	IDNFNEQKKK---AGGSLEMFMTEDQKKYY
German_cockroach_AAC47484	IDNFNEQKKK---AGGSLEMFMTEDQKKYY
house_mouse_CAA70325	IDNFNQQKKK---LGGQ-DIFMTEEQKKYY
human_BAA78033	IDNFNQQKKK---FGGQ-DIFMTEEQKKYY
flatworm_BCU93074	IDNFNVQKKK---VGGSLEMFMTDDQKKYY
Squid_AAA16202	IDNFNQQKKG---AGGSLEVFMTDDQKKYY

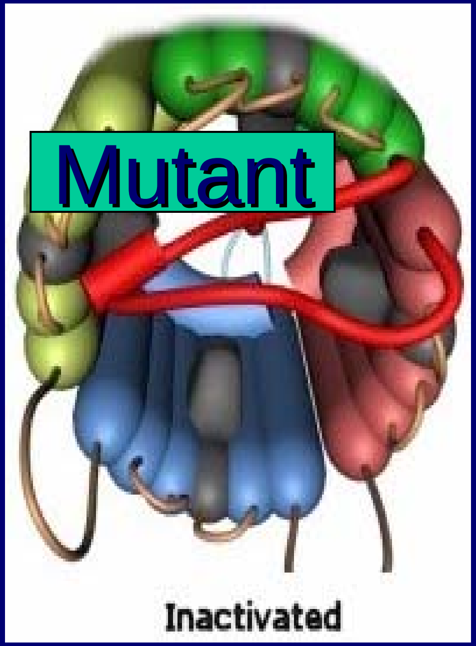


Work by Lihua Chen

Novel Mutation

- Mutation near inactivation gate
- **Hypothesis**: added length to arm of gate may cause a leaky channel (fitness advantage)





Source: Tim Smith@ www.chemosense.org

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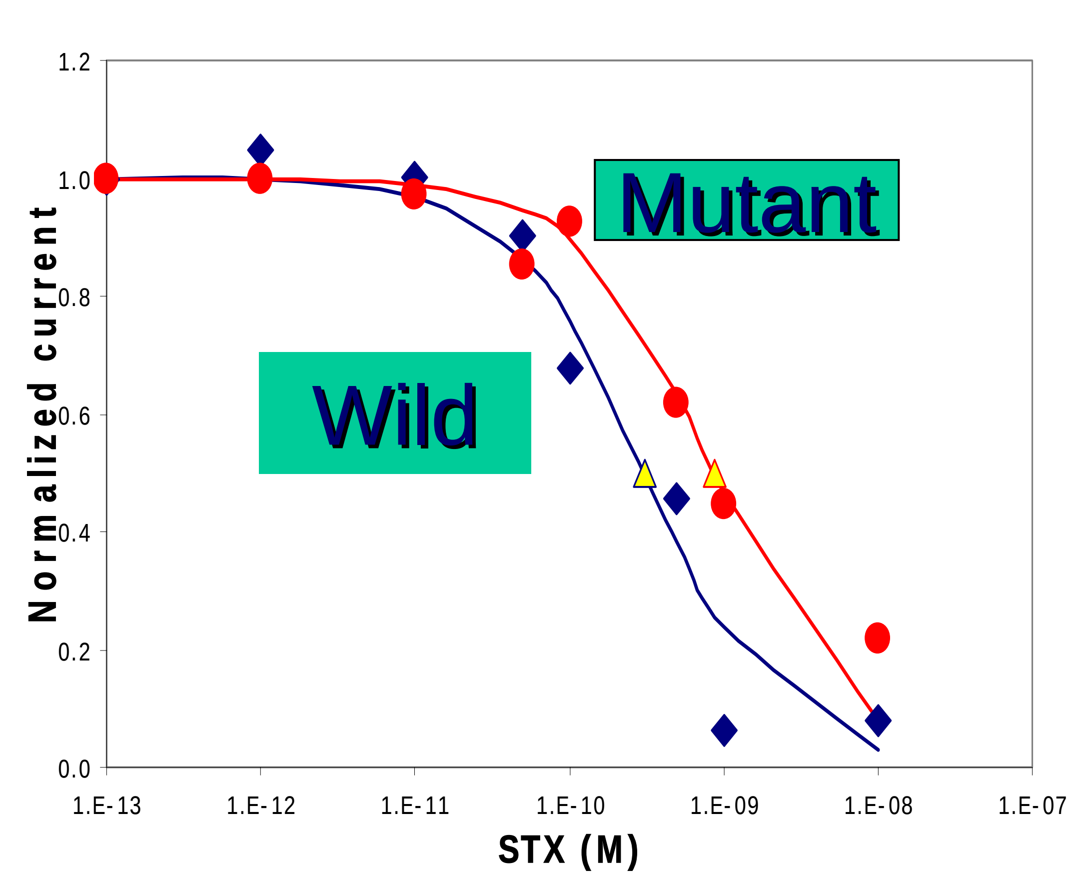

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Gozaimas