Progress Report HISC contract 54962 – March 9, 2007

Are invasive species of dinoflagellates that cause harmful algal blooms entering Hawaii in the ballast water of commercial ships?

The introduction of invasive harmful and toxic dinoflagellates into Hawaii by ballast water discharge is highly possible given the historical evidence of introductions in this manner in other parts of the world. The threat is compounded by the fact that currently ballast water regulations only require effective discharge of 95% of the potentially infected ballast water, leaving 5% as an point of entry for invasive organisms. While the 95% exchange may effectively control macro-invertebrate and vertebrate invaders, the residual 5% is profoundly problematic for microorganisms such as harmful and/or toxic dinoflagellates. Complicating our ability to detect and interpret dinoflagellate invasions via ballast water is the fact that there is almost nothing known about the natural populations of dinoflagellates and other such microorganisms in Hawaiian waters. Without this baseline, it will be impossible to design a management strategy aimed at identifying and implementing policy to avoid introducing invasive dinoflagellates in ballast water. Further, in the event of invasions or introduction of non-native dinoflagellates it is not known how these invaders will interact with native dinoflagellates communities. For example, bleached corals must reacquire their dinoflagellate symbionts from the environment after a bleaching event. The introduction of large numbers of non-native or invasive dinoflagellates may disrupt the delicate balance of the native dinoflagellate communities through competition and reduce the abundance of coral symbionts. Such a scenario would ultimately have profound indirect ramifications for the future of Hawaii's valuable coral reef resources. And of course the the more direct effects of red tides and toxic dinoflagellate blooms have had dramatic impacts on human health and fisheries across the globe.

The HISC funded collaborative project between the Gates Lab at HIMB and DAR is focused on producing an effective baseline of native and previously introduced dinoflagellate communities in Hawaiian waters and evaluating the stringency and success of current ballast water exchange regulations in restricting the transport of invasive and potentially harmful dinoflagellates from ports and regions where harmful algal blooms are a problem.

To achieve this we are using a DNA bar-coding approach to characterize seawater samples collected from:

1) Various ports where ships traveling to Hawaii regularly take on ballast water and collected on our behalf by our MATSON (<50 samples in hand)
2) Samples taken from ballast tanks BEFORE and AFTER a deep ballast water exchange. This experiment was conducted at our request by MATSON and directly addresses the efficiency of current ballast regulations that discharge of 95% of potentially infected ballast water, leaving 5% as an entry-point for non-native organisms.
3) Honolulu harbor water (n=3)
4) One quarter mile outside Honolulu harbor (n = 2)
5) Johnston Atoll, affectionately known as the "toilet" in shipping circles because it is the site where ballast waters have historically been exchanged (n = 2).
6) French Frigate Shoals in the NWHI National Marine Monument, a pristine site (n = 2).
7) Kaneohe Bay (n = 1).

The samples are processed by:

1) Filtering the water samples through a filter with a 5 micron (0.005 mm) mesh size
2) Extracting the DNA of all biological materials on the filter
3) Amplifying dinoflagellate DNA sequences from the DNA extracts using dinoflagellate specific protocols
4) Cloning the amplified products to produce multiple copies of each dinoflagellate DNA sequence recovered.

5) Sequencing each of the clones
6) Identifying the type of dinoflagellate that each cloned sequence represents by comparison with the global databases.
7) Archiving each cloned sequence at -80oC.

Results to Date

To complete the laboratory analyses I have hired Mindy Mizobe as a technician. Mindy has a BS from UH Manoa and has performed outstandingly on the project. To date she has processed all samples to step 1 or 2 above and a subset of them all the way through to step 7 (Appendix 1 attached). To date we have obtained 134 DNA sequences representing a variety of dinoflagellate species, **many of which are harmful and/or toxic**. As anticipated, at Johnston Atoll where there is a deep history of ballast water dumping, 16 of the 18 different types of dinoflagellates detected are members of toxic or harmful groups. In contrast, of the 17 dinoflagellates types detected in Kaneohe Bay where there has been no ballast exchange activity, only 6 belong to toxic or potentially harmful groups. The comparison of these sites also suggests that the harmful and toxic members are able to out-compete the less toxic and or native varieties. We are currently analyzing samples taken from French Frigate Shoals (made possible by our collaboration with the NWHI Marine National Monument staff, DAR and NFWS) and those collected within and outside Honoulu Harbor (by DAR) to explore these findings in more detail.

In addition to our own physical collections, we have been able to obtain samples through a very fruitful collaborative relationship with the shipping company MATSON. During normal operations, and unlike most shipping companies frequenting Honolulu harbor, MATSON tries to keep the movement of water in their ballast tanks to a minimum and takes up and releases ballast water only as a last resort. However, due to the nature of our study, MATSON has agreed to conduct experiments on our behalf to examine the efficiency of the current ballast exchange regulations. Water is first taken into the tanks at a port of origin (ie China, Marshall Islands, Guam) and a 6L water sample is taken from the ballast tanks and frozen. This represents the pre-exchange sample. Then at a location approximately 200 miles offshore of Hawaii, the ballast tank is emptied (95%) and refilled, the ship comes into port and a 6L sample of this water is again taken– this represented the post-exchange sample.

For experiment 1, which originated in Shanghai Anchorage (L31-07.°N^122-32°E), the average concentration of DNA in the pre-and post-exchange sample was 3.08 ng/ul and10.68 ng/ul. Preliminary characterization reveals that the pre-exchange sample contains *Pfiesteria piscicida* (Appendix 1). *P. piscicida* is a dinoflagellate that has an infamous reputation for causing fish kills and adverse human health effects such as impaired concentration and learning. Our preliminary screens of the post-exchange ballast sample have not detected *Pfiesteria piscicida* however we are currently analyzing these samples in much more depth and so this absence remains to be confirmed. The post-exchange sample does however contain *Prorocentrum micans* and *Prorocentrum mexicanum*, which are both dinoflagellates associated with red tides. *Prorocentrum micans* blooms have been associated with shellfish kills due to oxygen depletion. *Prorocentrum mexicanum* produces fast-acting toxin (FAT) and the diarhetic shellfish poison (DSP) toxin and is very dangerous. In addition, the post-exchange sample contains both *Alexandium minutum* and *Peridinium quinquecorne*. *Peridinium quinquecorne* is a dinoflagellate that is associated with red tides and *Alexandrium minutum* causes dense reddish-brown tides that are associated with paralytic shellfish poisoning.

Our data clearly suggest that members of harmful and toxic dinoflagellate groups are entering Hawaiian waters in the ballast water of ships. We are currently working with MATSON to conduct more experiments for us so that we can determine the extent of the problem. In addition, we are continuing to collect baseline data for coastal waters in Hawaii. Our work to date **confirms the utility of a DNA sequence based approach** in the **early detection** of harmful and toxic dinoflagellates in Hawaii water.

SAMPLE LOCATION	TYPE	SAMPLE NAME	GENE	GENERA/SPECIES	Number of Sequences	HARMFUL	NON-TOXIC	SYMPTOMS CAUSED
Oakland, CA	PORT	E1	ITS2	*Labyrinthulid quahog parasite*	1	X		Protist causes high mortalities of wild and cultured hard clams.
	PORT	E1	ITS2	*Heterocapsa sp.-2*	2		?	Some spp. Cause red tides and human skin irritation
	PORT	E1	ITS2	*Cyclotella ct. scaldensis*	1		X	
				TOTAL	4			
	PORT	E1	18s ssu rRNA	*Heterocapsa rotundata-2*	2	X		Blooms cause mahogany tides
	PORT	E1	18s ssu rRNA	*Prorocentrum micans*	1	X		Blooms cause shellfish kills due to O2 depletion
	PORT	E1	18s ssu rRNA	*Pentapharsodinium tyrrhenicum*	1		X	
	PORT	E1	18s ssu rRNA	*Pfiesteria-like sp.*	1	X		Blooms associated with fish kills, neurotoxic, skin, and respiratory effects in humans.
					5			
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1A (3#	ITS2	*UNKNOWN*	1		?	
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1A (3#	ITS2	*soil fungus*	1		?	
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1A (3#	ITS2	*Symbiodinium sp. D1a*	1		X	
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST			TOTAL	3			
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1A (3#	18s ssu rRNA	*Pfiesteria piscicida-5*	5	X		Blooms associated with fish kills, neurotoxic, skin, and respiratory effects in humans.
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1A (3#	18s ssu rRNA	*Paramecium duboscqui/ Colpodella sp./ Adelina grylii*	1		X	
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST			TOTAL	6			
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1B (4#	ITS2	*Uncultured Glomus*	1		X	soil bacteria
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1B (4#	ITS2	UNKNOWN	1			
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1B (4#	ITS2	*Platyamoeba sp.*	1	X-?		possible vector for bacterial pathogens
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST			TOTAL	3			
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST	RJ 1B (4#	18s ssu rRNA	*Pfiesteria piscicida*	1	X		Blooms can cause major fish kills, studies pending on neurotoxic, skin, and respiratory effects in humans.
Shanghai anchorage L31-07.oN ^122-32.oE	PRE-Exchange BALLAST			TOTAL	1			

Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2A (1#	ITS2	*Vannella sp.-2*	2		x	Infected with numerous bacterial species. Causes taste and odor problem in water due to production of geosmin.
				TOTAL	2			
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2A (1#	EUK	*Gymnodinium/ Prorocentrum micans/ Prorocentrum mexicanum*	1	x		6 species of gymnodinium considered a HAB; P. micans cause red tides, fish kills due to anoxia; P. mexicanum produces Fast-Acting Toxin and might be the cause of Diarhettic Shellfish Poisoning.
				TOTAL	1			
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	ITS2	*H. catenoides-2*	2		x	
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	ITS2	*Grateloupia doryphora*	1	x		very invasive, out competes native alga
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	ITS2	*UNKNOWN*	1			
				TOTAL	4			
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	18s ssu rRNA	*Protoperidinium pentagonum/ Peridinium quinquecorne*	1	x-?		Peridinium quinquecorne blooms cause red tides. P. pentagonum is not harmful.
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	18s ssu rRNA	*Roscoffia sp.*	1		x	
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	18s ssu rRNA	*Thalassomyces fagei*	1		x	ellobiopsid parasite on protists
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	18s ssu rRNA	*Gymnodinium/ Prorocentrum micans/ Prorocentrum mexicanum*	1	x		6 species of gymnodinium considered a HAB; P. micans cause red tides, fish kills due to anoxia; P. mexicanum produces Fast-Acting Toxin and might be the cause of Diarhettic Shellfish Poisoning.
Forepeak (Deep water) L47-27oN ^174-36oE	POST-Exchange BALLAST	RJ 2B (2#	18s ssu rRNA	*Alexandrium spp.*	1	x-?		some spp. Are toxic (I.e., Alexandrium minutum)
				TOTAL	5			

NWHI-Johnston Atoll	COASTAL	6/7/06 #	18s ssu rRNA	*Dinophyceae sp.*	2	x-?		the family that encompasses many dinoflagellates including Pfiesteria piscicida and toxin-producing spp of Alexandrium.
		6/7/06 #	18s ssu rRNA	*Heterocapsa sp.*	2	x-?		some spp. Cause red tides and shellfish mortality. (I.e., H. circularisquama and H. rotundata)
		6/7/06 #	18s ssu rRNA	*Kryptoperidinium foliaceum/Peridinium foliaceum*	1	x-?		K.foliaceum causes the S. Carolina red tide and metabolic stress in oysters.
		6/7/06 #	18s ssu rRNA	*Alexandrium insuetum/ Scrippsiella trochoidea/ Alexandrium minutum*	1	x-?		S. trochoidea causes a non-toxic brown tide, A. insuetum causes a non-toxic red tide. A. minutum causes a red tide and PSP.
		6/7/06 #	18s ssu rRNA	*Kryptoperidinium foliaceum/Peridinium foliaceum*	2	x-?		K.foliaceum causes the S. Carolina red tide and metabolic stress in oysters.
		6/7/06 #	18s ssu rRNA	*Symbiodinium spp.*	1		x	
		6/7/06 #	18s ssu rRNA	*Heterocapsa sp.*	1	x-?		some spp. Cause red tides and shellfish mortality. (I.e., H. circularisquama and H. rotundata)
		JHN 23	18s ssu rRNA	*Kryptoperidinium foliaceum/Peridinium foliaceum*	1	x-?		K.foliaceum causes the S. Carolina red tide and metabolic stress in oysters.
		JHN 23	18s ssu rRNA	*Heterocapsa sp.*	4	x-?		some spp. Cause red tides and shellfish mortality. (I.e., H. circularisquama and H. rotundata)
		JHN 5P	18s ssu rRNA	*Dinophyceae sp.*	3	x-?		the family that encompasses many dinoflagellates including Pfiesteria piscicida and toxin-producing spp of Alexandrium.
		JHN 5P	18s ssu rRNA	*Kryptoperidinium foliaceum/Peridinium foliaceum*	2	x-?		K.foliaceum causes the S. Carolina red tide and metabolic stress in oysters.
		JHN 6P	18s ssu rRNA	*Alexandrium insuetum/ Scrippsiella trochoidea/ Alexandrium minutum*	1	x-?		S. trochoidea causes a non-toxic brown tide, A. insuetum causes a non-toxic red tide. A. minutum causes a red tide and PSP.
		JHN 6P	18s ssu rRNA	*Dinophyceae sp.*	2	x-?		the family that encompasses many dinoflagellates including Pfiesteria piscicida and toxin-producing spp of Alexandrium.
		JHN 6P	18s ssu rRNA	*Heterocapsa sp.*	1	x-?		some spp. Cause red tides and shellfish mortality. (I.e., H. circularisquama and H. rotundata)
		JHN 6P	18s ssu rRNA	*Uncult. Marine picoplankton*	1			

NWHI-Johnston Atoll Continued	COASTAL	JOH	18s ssu rRNA	*Kryptoperidinium foliaceum/Peridinium foliaceum*	3	x-?		K.foliaceum causes the S. Carolina red tide and metabolic stress in oysters.
		JOH	18s ssu rRNA	*Scrippsiella spp./ Prorocentrum micans/ Prorocentrum mexicanum*	1	x-?		some Scrippsiella spp. (I.e., S. trochoidea) cause red tide. P. micans cause red tides, fish kills due to anoxia; P. mexicanum produces Fast-Acting Toxin and might be the cause of Diarhettic Shellfish Poisoning.
		JOH	18s ssu rRNA	*Heterocapsa sp.*	1	x-?		some spp. Cause red tides and shellfish mortality. (I.e., H. circularisquama and H. rotundata)
				TOTAL	30			
KANEOHE BAY- Coconut Island Lagoon	COASTAL	B	ITS2	*Gymnodinium simplex*	4		x	
				Gymnodinium beii	3		x	
				Skeletonema sp.	6		x	
				Skeletonema costatum	2		x	
				Sellaphora laevissima	3		x	
				Cyclotella cf. Scaldensis	1		x	
				UNKNOWN	7			
				Karena brevis	1	x		Blooms cause red tide, NSP, respiratory infection, marine animal mortalities.
				Takayama cf. Pulchellum	1		x	
				uncult. Plasmodiophorid	1		x	
				Prorocentrum micans	1	x		Blooms cause shellfish kills due to O2 depletion
				Prorocentrum mexicanum	1	x		P. mexicanum produces Fast-Acting Toxin and might be the cause of Diarhettic Shellfish Poisoning.
				Ensiculifera spp/ Pentapharsodinium tyrrhenicum/ Prorocentrum mexicanum	2	x-?		P. mexicanum produces Fast-Acting Toxin and might be the cause of Diarhettic Shellfish Poisoning.
				TOTAL	33			
			18s ssu rRNA	*Peridinium quinquecorne*	14	x		Blooms cause red tides and anoxia that cause fish kills.
				Roscoffia capitata	1		x	

KANEOHE BAY- Coconut Island Lagoon Continued	COASTAL			*Gymnodinium simplex*	9		X	
				Cryptoperidiniopsoid sp.	1		?	unknown toxins, but tend to coexist with Pfiesteria spp.
				Symbiodinium sp.	1		X	
				Prorocentrum micans	6	X		Blooms cause shellfish kills due to O2 depletion
				Gymnodinium sp.	1	X-?		6 species of gymnodinium considered a HAB
				Lepidodinium viride	4		X	
				Peridinium polonicum	1	X		Blooms produce glenodine which is icthyotoxic.
				Gymnodinium beii	1		X	
					39			