C. Abstract/Project Summary

Title: OHHI 2005 Sea scallops as sentinels of deepwater pollution
Institutions: University of Wisconsin-Milwaukee; Marine Biological Laboratory; Texas A&M; Northeast Fisheries Science Center; Northwest Fisheries Science Center
Investigators: Frederick Goetz; Terry Wade; Steven Roberts; Paul Rago; Deborah Hart; Tracy Collier
Total Cost and Budget Period: $274,876; 10/01/2005-09/30/2007

Oceans make-up approximately 72% of the surface of the earth and contain 97% of the world's water supply. It is now recognized that, even in their vastness, oceans are significantly polluted. Pollution has been portrayed to be more dramatic within inshore shallow coastal regions because of the proximity to urban centers and large watersheds. However, recent estimates of global organic contaminants have indicated that urban coastal sediments in fact constitute only 0.2-0.5% of the total global PCB inventory, whereas 87-97% is located in the sediments of remote (relative to urban areas) and deeper continental shelf regions. In the past, large-scale monitoring programs, such as "Mussel Watch," have been conducted to determine the degree of contaminant loading in organisms in shallow in-shore, coastal regions. **In contrast, the contaminant burden and effect in organisms living in deeper off-shore regions of the coastal shelf have received very little attention.** This may be a result of the effort and expense that would be required to sample organisms in deeper waters. **However, a unique opportunity already exists to conduct this type of sampling at essentially no cost. It is the Northeast Fisheries Science Center (NEFSC) annual sea scallop survey.** In the proposed study, the NEFSC sea scallop survey will be used as a platform to obtain samples of sea scallops for contaminant analysis and for determining possible effects on the expression of environmentally relevant genes. In the future, contaminant loading and transcriptomics can be correlated to use gene profiling as an inexpensive means to determine contaminant effects in the biota of deeper water. This study is the first of its type to explore contaminant loading in a resident organism of the deeper continental shelf.

In the proposed research, samples of soft body parts of sea scallop will be collected and freeze archived by the principal investigator on the NFESC scallop survey from stratified sites extending from Georges Bank to Cape Hatteras, NC. Because of the cost involved with complete contaminant analysis, two prescreening assays will be conducted on a subsample of the scallops that are collected at each stratified site: 1. Hemocytes will be prescreened onboard using a lysosomal destabilization assay; and 2. The expression of a "Multixenobiotic resistance gene" (P-glycoprotein) will also be assayed in gills, digestive glands and gonads using quantitative PCR (QPCR). The determination of which sea scallop samples should eventually be used for complete contaminant and gene analysis, will be based on the prescreening assays and also the location of sampling sites relative to potential areas of pollution (e.g., Hudson Canyon). Contaminant analysis will include pesticides, polycyclic aromatic hydrocarbons (PAH), polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB). Gene analysis will consist of the expression of 12 pollution-relevant genes that will be assayed by QPCR in tissues from scallops sampled at the same sites as those sent for contaminant analysis. Results of this project will provide important data on contaminant levels and correlative gene expression in an organism inhabiting a deeper (40-100 m) region of the shelf.

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D. PROJECT DESCRIPTION

I. Statement of Problem

Introduction: Oceans make up approximately 72% of the surface of the earth and contain 97% of the world's water supply. It is now recognized that, even in their vastness, oceans are significantly polluted, threatening the health and future of the organisms within the ocean and ultimately the human populations that depend on them. Ocean contaminants are diverse, including fertilizers, pesticides, heavy metals, organochlorines, organophosphates, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, oil, sewage and solids such as plastics (Shahidul Islam and Tanaka, 2004). Contaminants have been reported from the Arctic to the Antarctic, and significant contaminant loads have even been reported in deepsea fish inhabiting regions >1000 m (Borghi and Porte, 2002). Pollution has been portrayed to be more dramatic within inshore coastal regions because of the proximity to industrial outflows and large watersheds. While contaminants may certainly be concentrated in urbanized coastal regions, recent estimates of global organic contaminants based on the analysis of >2,000 PCB datasets from marine continental margin sediments, have indicated that urban coastal sediments in fact constitute only 0.2-0.5% of the total global PCB inventory (Jonsson et al., 2003). In contrast, 87-97% of the PCB inventory is located in the sediments of remote (relative to urban areas) and deeper continental shelf regions. Approximately half of that amount resides in North Atlantic shelf sediments. Calculations based on the amounts of PCBs in highly contaminated areas like superfund sites, indicate that the very large inventories of contaminants in the remote sediments of the shelf could never have come from urbanized sites but are more likely to have been deposited there over time via the atmosphere and total river discharge (Jonsson et al., 2003). Given that there is an estimated mixing of sediments on average down to approximately 10 cm (Boudreau, 1998), there is the potential for a very large amount of persistent contaminants to be continually made available to organisms that live on the bottom in off-shore, deeper regions of the shelf. Yet these areas are rarely assessed for biological contamination.

In 1986 the NOAA National Status and Trends Program established the "Mussel Watch" project to monitor spatial and temporal trends of chemical contaminants in bays and estuaries of coastal regions. In Mussel Watch, selected contaminants including heavy metals, PCBs, DDT, chlordane, dieldrin, butyltins and PAHs were analyzed from the tissues of various mollusks collected at specific sites along the Atlantic coast (and other nonAtlantic sites as well). The rationale was that bivalves were relatively stationary residents and, being filter feeders, would concentrate contaminants and be a more consistent source to measure contaminants from. This program has given a very good profile over time of the contaminant loads in inshore coastal regions (O'Connor, 2002). **What this program has not be able to do is determine the amount of contaminants that are present in organisms in deeper off-shore regions of the coastal shelf.** In addition, since only levels were analyzed, whether or not the contaminants had a biological impact on the organisms sampled for Mussel Watch is unknown. Programs that are being developed in Europe through the International Council for the Exploration of the Sea (ICES), are now using fish and bivalves to assess pollution in deeper sea regions in the North Sea, and to determine the effect that these levels are having on key cellular and genomic responses (e.g., ICES CM 2004/E:04 Ref. ACME). A goal of that project is to use biological and gene markers to assess contaminant loading and environmental impact.

A critical limiting factor for sampling deeper water sites off-shore is the expense and effort involved. In addition, in deepwater sites it is difficult to consistently sample the same organism across great distances. **However, a unique opportunity already exists to do exactly this type of sampling. It is the Northeast Fisheries Science Center (NEFSC) sea scallop survey.** This is an annual survey of sea scallop populations conducted in the summer that extends from the Georges Bank to Cape Hatteras, NC (Hart and Chute, 2004). With the exception of providing a research investigator, there are no costs to do sampling on this platform. In addition, given that the Atlantic sea scallop population is the most valuable wild scallop fishery in the world and one of the most economically important fishery in the U.S., it is a sampling platform that will continue to exist in the future for long-term analyses if desired. Sampling of this population would fill a niche that has been largely ignored.

We propose a small but highly focused study that will use the NEFSC scallop survey to obtain sea scallop samples from selected sites along the Atlantic coast for both contaminant analysis and screening by contaminant-relevant genes. Our goal is to provide initial data on the contaminant burden in an organism inhabiting a deeper (40-100 m) region of the shelf and to correlate this data with expression levels of candidate genes that have been associated with contaminant burdens in other bivalves. While these data can stand by themselves in terms of publication, we intend to use them to determine whether more extensive sampling should be completed in the future.

The use of bivalves for contaminant screening: Mollusks have become one the most important and frequently used models for environmental toxicological research (review: Rittschoff and McClellan-Green, 2005). There are over 250 citations for papers on the environmental toxicology of *Mytilus* species alone. However, their most valuable use has been as sentinel monitors of contaminants. **The attributes that make mollusks exceptional as environmental monitors are 1) they are common; 2) widely distributed; 3) fairly immobile with high site fidelity; 4) reasonably resistant to contaminants; and 5) continuously filter the water that they live in.** This last point means that they can concentrate contaminants that are in the water or in the microorganisms contained within the water. As discussed above, one of the largest and longest continual use of mollusks for monitoring has been in the Mussel Watch program in the U.S. (O'Connor, 2002). There was also an International Mussel Watch that was under the auspices of the UNESCO Intergovernmental Oceanographic Commission, and the United Nations Environmental Program (UNEP). It assessed the extent of chemical contamination of international coastal areas. In the study proposed here we will use the sea scallop (*Placopecten magellanicus*), a bivalve with several of the most unique characteristics of mollusks: 1) they are long-lived (10-15 years) and thus can concentrate even more contaminants; and 2) they live in deeper waters (30-110 m) that are infrequently sampled.

II. Scientific Objectives of Study

The specific research objectives of this study are:

1) Using the NEFSC survey, sample sea scallops from locations along the Atlantic coast and archive (freeze) soft body parts from samples for potential contaminant and gene expression analysis.

2) Prescreen the hemocytes of representative sea scallops from sampling sites (#1) using a lysosomal destabilization assay.

3) Prescreen gills, digestive glands and gonads of representative sea scallops from sampling sites (#1) using the expression of a "Multixenobiotic Resistance" gene.

4) Based on the results of objectives #2 and #3, and on the location of sampling sites, choose sea scallop samples for full contaminant and gene analysis.

5) Perform contaminant analysis on composite samples from selected sampling sites (#4), assaying pesticides, PAHs, PCBs, and PBDEs.

6) Perform genomic expression analysis for 12 predetermined genes and differential display PCR on gills, digestive glands and gonads from scallops collected at sites identical to those being used for contaminant analysis (#5).

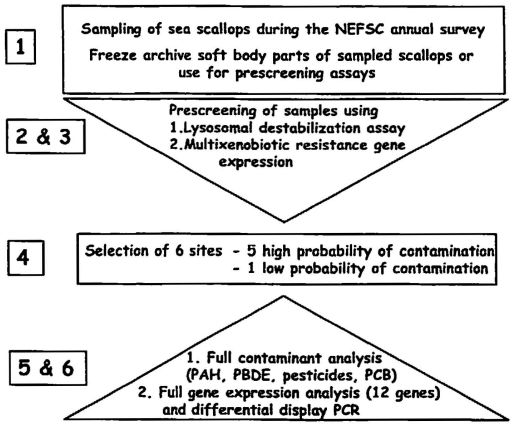


Figure 1: Workflow for sampling, prescreening, contaminant and gene expression analysis. #s on left indicate research objectives. See figure 3 for detailed sampling scheme and use of samples

III. Statement of Work: including experimental design and methods

The overall workflow for the completion of the research objectives is given in figure 1 and discussed in detail below.

Objective 1: Using the NEFSC survey, sample sea scallops from locations along the Atlantic coast and archive (freeze) soft body parts from samples for potential contaminant and gene expression analysis.

Sea Scallop Sampling Platform: Sea scallops occur from the North Shore of the Gulf of St. Lawrence south to Cape Hatteras, at depths ranging from 18-110 m. Beds or aggregations of sea scallops are frequently found that may be related to temperature, food availability substrate and currents. Sexes are separate in sea scallops and gonads are generally mature in the summer with spawning occurring in September/October, though there can be spring and fall spawning in some populations. The early life history is similar to other bivalves with pelagic trochophore and veliger stages, followed by settling in the pediveliger stage to juvenile stage. While scallops are the most mobile of bivalves there is no evidence of mass migrations for long distances. Movements are usually localized and related to currents. Therefore, there is generally strong site fidelity. Compared with many other bivalves, sea scallops are relatively long-lived (10-15 years).

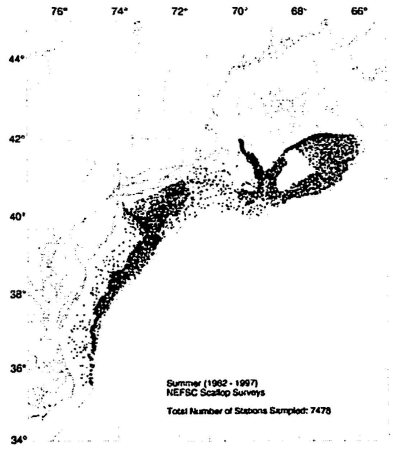


Figure 2: Sea scallop sampling sites and densities derived from NEFSC surveys conducted from 1982-1997 (from Reid et al., 1999). Note: surveys started in 1977 and have continued annually. Data is presented only for a portion of those surveys

Since 1977, the NEFSC of the National Marine Fisheries Service has conducted yearly surveys of sea scallop populations along the Atlantic coast of the United States (Reid et al., 1999). These surveys monitor the distribution, abundance and recruitment patterns of sea scallops from the Georges Bank to Cape Hatteras, North Carolina (figure 2; Hart and Chute, 2004) and are critical for stock assessment (e.g., Northeast Fisheries Science Center Reference Document 04-10b: 39TH SAW ASSESSMENT REPORT). Beginning in 1979, NEFSC sea scallop surveys used a

Year	Season	Vessel	Start Date	End Date	No. of Stations	Study Area
1982	Summer	Albatross IV	1-Jun-82	11-Jun-82	420	Mid Atlantic Bight - Cape Hatteras
1982	Summer	Albatross IV	12-Jul-82	6-Aug-82	205	Gulf of Maine - Mid Atlantic Bight
1983	Summer	Albatross IV	26-Jul-83	2-Sep-83	615	George's Bank - Cape Hatteras
1984	Summer	Albatross IV	24-Jul-84	31-Aug-84	699	George's Bank - Cape Hatteras
1985	Summer	Albatross IV	22-Jul-85	31-Aug-85	571	Georges Bank - Cape Hatteras
1986	Summer	Albatross IV	29-Jul-86	29-Aug-86	501	Georges Bank - Cape Hatteras
1987	Summer	Albatross IV	6-Jul-87	13-Aug-87	641	Georges Bank - Cape Hatteras
1988	Summer	Albatross IV	7-Jul-88	16-Aug-88	619	Georges Bank - Mid Atlantic Bight
1989	Summer	Alb CH OR	9-Jun-89	9-Aug-89	435	Georges Bank - Mid Atlantic Bight
1990	Summer	Oregon II	26-Jul-90	20-Aug-90	409	Georges Bank - Cape Hatteras
1991	Summer	Oregon II	28-Jul-91	21-Aug-91	437	Georges Bank - Cape Hatteras
1992	Summer	Oregon II	1-Aug-92	22-Aug-92	426	Georges Bank - Cape Hatteras
1993	Summer	Oregon II	31-Jul-93	25-Aug-93	446	Georges Bank - Cape Hatteras
1994	Summer	Albatross IV	22-Jun-94	18-Jul-94	192	Georges Bank - Cape Hatteras
1995	Summer	Albatross IV	19-Jun-95	30-Jun-95	217	Mid Atlantic Bight - Cape Hatteras
1995	Summer	Albatross IV	25-Jul-95	6-Aug-95	314	Long Island
1996	Summer	Albatross IV	29-Jul-96	26-Aug-96	153	Georges Bank - Cape Hatteras
1997	Summer	Albatross IV	21-Jul-97	17-Aug-97	496	Georges Bank - Cape Hatteras

Table 1: Example of sampling times, locations and numbers of sites for sea scallop surveys from 1982-1987 (from Reid et al., 1999). Note: surveys started in 1977 and have continued annually. Data is presented only for a portion of those surveys

2.44-m (8-ft) wide dredge equipped with 5.1-cm (2-in) rings and a 3.8-cm (1.5 in) plastic mesh liner. Sampling is conducted using a stratified random design where strata are designated based on latitude and water depth. Within each strata, sampling stations are randomly designated. In addition, in locations where there is either significant commercial fishing or where there are recognized concentrations of scallops, additional sampling stations are randomly assigned. Examples of surveys conducted from 1982 through 1997 are given in Table 1. On average, 470 individual stations are sampled/year and sampling sites range from approximately 30 to100 meters in depth. Hydrographic data has also been collected during these surveys (Bascunan et al., 2004).

Proposed Sampling: In the summer of 2006 and 2007 (follow-up), the PI (Goetz) will go on the NEFSC sea scallop survey to work with the NOAA research group to sample scallops and obtain samples for the proposed study. As sampling is normally random, scallops will be collected from all major stratified survey sites (approximately 40 strata). There is virtually no cost in sampling sea scallops once you are on the survey so many more samples can be taken and freeze archived then would actually be analyzed. However, this approach will allow us to prescreen

samples in several ways (see below, objectives 2 & 3) to determine which sites potentially have scallops with the highest contaminant burdens. **In addition, this sampling will provide a valuable sample repository for any other researchers interested in surveying sea scallops across diverse habitats for other purposes.** For example, there is an interest in understanding a possible relationship between paralytic shellfish poisoning and sea scallops. This might be pursued in the future using these samples. There are several specific sites in the survey that have a very high potential to yield scallops with measurable contaminant loads. For example, sediments in the Hudson Canyon area have been surveyed by the USGS (http://pubs.usgs.gov/of/2003/of03-241/htmldocs/data_files.html) and there are significant levels of various contaminants including PCBs, dieldrin, phenanthrene and DDD extending out along the canyon from the coastline. This may also be true for the area located adjacent to Montauk Pt (Long Island)/ Block Island/Narragansett Bay and sampling sites adjacent to the Chesapeake Bay. The survey for sea scallop population structure is conducted with a randomized stratified design and would include some sites within the Hudson Canyon. Our NOAA collaborators (Paul Rago and Deborah Hart) have indicated that within certain areas, some additional site-specific sampling may be possible if work schedules and conditions permit (see letter of collaboration: Paul Rago). For comparison, we would also want to ensure that we obtained samples from sites that would have little on no contaminants. Based on their remote location, these samples would most likely be obtained on the Georges Bank but these would be obtained in any case as part of the randomized sample design.

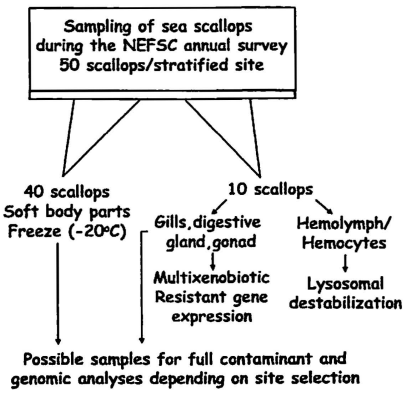


Figure 3: Overview of sampling scheme and use

The soft body portion of at least 50 sea scallops will be taken within sampling sites at each stratified or preselected site (sampling details outlined in Figure 3). Each scallop will be separately bagged and frozen for the possible later determination of contaminants after screening. Of those sea scallops sampled within each strata, a subset (precise number to be empirically determined but no less than 10) will also be prescreened on board using a lysosomal stabilization assay and later in the lab by the expression of a "multixenobiotic resistance gene."

Objective 2: Prescreen the hemocytes of representative sea scallops from sampling sites (#1) using a lysosomal destabilization assay.

Objective 3: Prescreen gills, digestive glands and gonads of representative sea scallops from sampling sites (#1) using the expression of a "Multixenobiotic Resistance" gene.

The original intent of the PI and his collaborators was to conduct contaminant and genomic analyses on scallops sampled at many sites from the Georges Bank to Cape Hatteras similar to what had been completed in Mussel Watch to observe the trends along the coast. However, the cost for these types of analyses on many samples would be very high. Given that it is unknown at this point what the contaminant burdens would be at these sites, we propose instead to do a limited but very focused analysis on samples selected on the basis of sites neighboring areas with sediment contamination (http://pubs.usgs.gov/of/2003/of03-241/htmldocs/data_files.html) and on samples that give high values on generalized cellular (lysosomal destabilization assay) and genomic (multixenobiotic resistance gene expression) assays. Depending on the results of these assays, future studies could then survey more sites, or survey scallops at specific sites in different ways (e.g., tissue specific).

Lysosomal Assay: Lysosomes are membrane-limited organelles and their role is the intracellular waste removal and digestion and recycling of macromolecules (e.g., proteins, carbohydrates and lipids) in the cell. As such, they routinely accumulate toxic compounds such as environmental contaminants but, if overloaded, they become destabilized resulting in the loss of membrane integrity and enlargement. As a result, relatively uncomplicated assays based on this "destabilization" have been used as a general cellular level biomarker for chemical exposure (review: Au, 2004; ICES CM 2004/E:04 Ref. ACME). Destabilization is well correlated with contaminant loading in bivalves (e.g., Hwang et al., 2004). While lysosomal destabilization assays are good as nonspecific markers for screening large numbers of samples, they would not provide information concerning types of contaminant exposure and specific biological effects.

On the NEFSC sea scallop survey, hemolymph samples will be drawn with a 1.0 ml syringe from the pericardial sac of 10 of the 50 scallops sampled/site prior to removing the soft body parts (see figure 3 for overall sampling scheme and use). Lysosomal destablization in the hemocytes in the hemolymph will be assayed using a red dye retention methods as described in Hwang et al., 2004. A 50 ul aliquot of a hemolymph/physiological saline mix (1:1) will be incubated on a slide in a light-proof, humidity chamber for 30 minutes to allow cells to adhere. Overlying fluid will be removed and 40 ul of a neutral red solution will be added, and the cells incubated for an additional hour. Cells will be scored for destabilization under a compound microscope. In normal cells, the neutral red dye is retained within the lysosome, whereas dye will leak into the cytoplasm from lysosomes that are destabilized. A subsample of cells will be assessed for the percentage of cells with stained/unstained cytosol.

Multixenobiotic resistance gene expression: Cellular efflux of anthropogenic toxins is a biochemical response of marine organisms exposed to pollution. This efflux, known as multixenobiotic resistance (MXR), is carried out by transport proteins located in cell membranes that belong to the ATP binding cassette proteins and is often the first line of defense against foreign chemical compounds. Two important efflux proteins found in marine organisms include P-glycoprotein and multi-drug resistance associated protein. Numerous contaminants induce

MXR proteins to remove compounds including pesticides, metals, endocrine disruptors, and pharmaceuticals and personal care products, and studies examining P-glycoprotein in mussels and oyster found animals from polluted sites had increased levels of expression (Minier et al. 1993; Kurelec et al. 1996). As discussed later in the proposal (Objective 6, below), the expression of certain genes in an organism can be directly correlated with a specific type of environmental contaminant exposure. While induced expression of MXR genes alone lacks the ability to identify specific xenobiotics in the environment, they are, as with lysosomal destabilization, good general indicators of contaminant exposure.

On board the survey ship, samples of the gills, digestive gland and gonads will be removed from the same scallops used for hemolymph sampling (above) and will be held in RNAlater (Ambion) at -20°C (see figure 3 for overall sampling scheme and use). **After the survey, these samples will be individually extracted for total RNA in the laboratory of S. Roberts (Co-PI) at the Marine Biological Laboratory (Woods Hole, MA) and will be analyzed by the Co-PI for MXR gene expression.** First-strand complimentary DNA (cDNA) will be produced from total RNA and the expression of the MXR gene (P-glycoprotein) will be assayed using quantitative PCR (QPCR) in a cDNA mixture derived from the 3 tissues. Performing the assay on mixed tissue cDNA will decrease the cost of analyzing a large number of samples, but for this phase of the study will give us the desired result: to see if there is any increase in MXR gene expression. Since we will have originally extracted the RNA on a tissue-specific basis (i.e., gills, digestive gland and gonad), we can go directly back to those RNAs to use them for the analysis of multiple gene expression on a tissue-specific basis if necessary after we have chosen the sites for the larger genomic analysis (i.e., for Objective 6).

Objective 4: Based on the results of objectives #2 and #3, and on the location of sampling sites, choose sea scallop samples for full contaminant and gene analysis.

As discussed earlier, based on the location of a sampling site(s) in relation to certain urban areas and/or river outflows, we would expect to find sea scallops that have measurable contaminant loads in areas in the Hudson Canyon and possibly off the eastern end of Long Island (Montauk Pt). The lysosomal destabilization assay and MXR gene expression can be used to confirm this and, of course, these prescreening assay may find other areas that appear to have significant contaminant loads. Based on location and prescreening, we will choose 5 locations for which complete contaminant analysis will be conducted on the sea scallop body parts archived in the freezer from the survey. In addition, we will conduct complete analysis on one site that should theoretically have no or very low contamination. This will act as a reference site. The selection of this site will also depend on the prescreening assays, but based on the remote location, we might expect that this would be a site in the Georges Bank area.

Objective 5: Perform contaminant analysis on composite samples from selected sampling sites (#4), assaying pesticides, PAHs, PCBs, and PBDEs.

Parameters to be Measured: Following the selection of sites in objective 4, soft body parts of at least 40 sea scallops/selected sites will be analyzed in 4 composite replicates of 10 scallops each (5 female, 5 male). **Contaminate analyses and interpretation will be conducted under the direction of Terry Wade (Co-PI) at the Geochemical and Environmental Research Group (GERG) at Texas A&M and will include pesticides, PCB, PAH and brominated**

diphenyl ethers (Table 2 for specific analytes). GERG has previously measured all of these contaminants for the NOAA National Status and Trends (NS&T) program by validated methods. GERG contaminant analysis will therefore be comparable to previous NS&T and International Mussel Watch (IMW) data collection. All the proposed methods are detailed in GERG Standard Operating Procedures (SOPs). Analysis of total PCB, organochlorine pesticides, PAH and PBDE will be provided. The tissue extraction, clean-up, and analytical methodologies are those developed and improved over the years for the NOAA NS&T Program and US Fish and Wildlife Service contracts. Sample batches will include, at most, 20 samples, a procedural blank, a duplicate, a matrix spike, and a certified reference material. Surrogates (internal standards) are added prior to extraction. Tissue samples will be ground with sodium sulfate, surrogates added, and the samples extracted using a tissuemizer with methylene chloride. Sample clean-up includes silica/alumina column chromatography and high performance liquid chromatography (HPLC) with Phenolgel columns (size exclusion). Organochlorine pesticides, PAH, PBDE and PCB concentrations in sample extracts will be determined using fused silica capillary columns

Table 2: Chemicals to be measured in Scallops.

Polynuclear Aromatic Hydrocarbons (PAHs)

Acenaphthene	1-methylnaphthalene	
Anthracene	1-methylphenanthrene	
Benz[a]anthracene	2,6-dimethylnaphtalene	
Benzo[a]pyrene	Naphthalene	
Biphenyl	Pyrene	
Chrysene	Benzo(b)fluoranthene	
Dibenz[a,h]anthracene	Acenaphthylene	
Dibenzothiophene	Benzo[k]fluoranthene	
2,6-dimethylnaphthalene	Benzo[g,h,i]perylene	
Fluoranthene	Indeno[1,2,3-c,d]pyrene	
Fluorene	2,3,5-trimethylnaphthalene	
2-methylnaphthalene	Phenanthrene	
	Perylene	
	Dibenzothiophene	

21 PCB Congeners:

PCB No.	Compound Name
8	2,4'-dichlorobiphenyl
18	2,2',5-trichlorobiphenyl
28	2,4,4'-trichlorobiphenyl
44	2,2',3,5'-tetrachlorobiphenyl
52	2,2',5,5'-tetrachlorobiphenyl
66	2,3',4,4'-tetrachlorobiphenyl
101	2,2',4,5,5'-pentachlorobiphenyl
105	2,3,3',4,4'-pentachlorobiphenyl
110/77	2,3,3',4',6-pentachlorobiphenyl 3,3',4,4'-tetrachlorobiphenyl
118	2,3,4,4',5-pentachlorobiphenyl
126	3,3,4,4',5-pentachlorobiphenyl
128	2,2',3,3',4,4'-hexachlorobiphenyl
138	2,2',3,4,4',5'-hexachlorobiphenyl
153	2,2',4,4',5,5'-hexachlorobiphenyl
170	2,2',3,3',4,4',5-heptachlorobiphenyl
180	2,2',3,4,4',5,5'-heptachlorobiphenyl
187	2,2',3,4',5,5',6-heptachlorobiphenyl
195	2,2',3,3',4,4',5,6-octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl

DDT and its metabolites

2,4'-DDD
4,4'-DDD
2,4'-DDE
4,4'-DDE
2,4'-DDT
4,4'-DDT

Chlorinated pesticides other than DDT

Aldrin
Alpha-Chlordane
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Lindane (gamma-BHC)
Mirex
Toxaphene
Trans-Nonachlor

Trace Elements (Selected Research Samples)

Aluminum
Antimony (sediment, only)
Arsenic
Cadmium
Chromium
Copper
Iron
Lead
Manganese (sediment, only)
Mercury
Nickel
Selenium
Silver
Tin
Zinc

Other Measurements

% Lipids

with mass selective detectors (MSD). Quantification will be based on the surrogates added prior to sample extraction. GERG will use an adaptation of U.S. EPA Method 1668A PCB analysis by GC/MS. PCBs will be identified and measured as individual congeners as well as a total for each homologue group (*i.e.*, by level of chlorination). Total PCBs would be determined by summing the homologue groups. Additional variables that will be measured include percent lipid and percent moisture. Determination of percent lipids will be made using a gravimetric method on an aliquot of extract (GERG SOP-9727). In order to determine moisture content, an aliquot of each sample, taken from the organic analyses fraction, will be dried at 105°C until constant weight is obtained using a balance of appropriate sensitivity (GERG SOP-9415).

In the NS&T Program trace organic analytical procedures, internal standards (also called surrogates) are added at the start of the analytical procedure and carried through the extraction process, cleanup, and instrumental analysis. The NS&T data internally compensate for recovery-rate. Acceptable recovery rates are ±50% or less. It is the analyst's responsibility to monitor recovery rates and to determine acceptability based on variation of these rates. Recovery rates will be reported. For the purpose of Quality Control, Certified Reference Materials (which includes Standard Reference Materials) of marine bivalves will be analyzed as part of each sample batch. Method Detection Limits (MDL) will be calculated and reported(EPA, 40 CFR, Ch. 1, Part 136, Appendix B), 1990. Sample extracts will be concentrated to 100 ul final volume when necessary to decrease the MDL.

There is an inverse relationship between sensitivity and precision. In general, the precision, as a function of concentration, appears to be independent of the nature of the analyte or the analytical technique. The interlaboratory coefficient of variation at the 10 ppb level is expected to be approximately 30%, and attainment of this level of precision will require the best possible effort on the part of the analyst. Thus the acceptable limits of precision for organic control materials for NS&T analyses have been ±30% on average for all analytes, and ±35% for individual analytes.

A minimum of 15% of the typical organic sample batch will consist of blanks, reference or control materials, duplicates and spike matrix samples. The use of control materials does not replace the use of duplicates and spiked matrix samples. In particular, the use of spiked matrix samples may demonstrate poor recovery of specific analytes. To assure interlaboratory data comparability and to assure continued analytical control, GERG will participating in intercomparison exercises. **In fact, as indicated in the letter of support from Dr. Usha Varanasi, if the proposal is funded then the Northwest Fisheries Science Center in Seattle, WA will also analyze the same samples for PBDE.** The PAH, pesticide and PCB analyses are standard analytical techniques that have been rigorously tested by interlaboratory intercalibrations, and standard reference materials are available for these analytes. In contrast, because PBDEs have only recently emerged as contaminants of concern, there have been very few interlaboratory intercalibrations for this technique, and no certified standard reference materials are available. Thus to increase reliability of analytical results, PBDE analyses will be done at both GERG (Terry Wade) and at the NWFSC (Tracy Collier).

Quality Control: It is the policy of GERG to conduct and carry-out its activities in accordance with a formal Quality Assurance Management Plan (QAMP) and a Generic Quality Assurance Manual (GQAM). The QAMP and GQAM are available on request. Quality Assurance (QA) involves all of the planned and systematic actions necessary to provide adequate confidence that the work performed by GERG conforms to the applicable contract specifications, regulatory

requirements, and state/national codes. The QAMP adopted by GERG serves as guidance to produce specific written Quality Assurance Project Plans (QAPP), implementation procedures, and a management philosophy which encourages, supports, and emphasizes the importance of Quality Assurance in carrying out work activities. Quality Assurance encompasses Quality Control (QC) which involves the examination of work performed versus the acceptance standards associated with those activities. In addition to its internal use, GERG provides their Quality Assurance Management Plan as an integral part of any proposal/scope of work which demonstrates the need for a systematic QA Program. This document adds another level of confidence that the activity performed meets the established requirements.

GERG management provides an environment which encourages and requires employees to strictly adhere to the Quality Assurance Project Plan and to carry-out their assigned tasks in a consistent and professional manner. GERG management also ensures that adequate resources are available to implement the QA Project Plan. This policy ensures that the requirements imposed by our clients and regulatory agencies are met and that both management and staff of GERG are committed to a high quality product and foster excellence in the work place.

GERG's will ensure consistency and minimize potential sample contamination during sample preparation. For all laboratory activities, GERG protects against cross-contamination and contamination of laboratory surfaces. Homogenization procedures at GERG are specified in GERG standard operating procedures SOP-9705 and SOP-9711. These tissues will be homogenized using a Tekmar™ Tissumizer, lab blender, hand blender or grinder. Between all samples all pieces of equipment are cleaned with Micro-all purpose liquid cleaner, rinsed with water, and then rinsed with methanol followed by methylene chloride. Samples are homogenized, when possible, to the point where there are no lumps or chunks visible and the tissue sample appears homogeneous. Homogenates will be stored frozen in certified clean glass jars with PTFE lids (PTFE [polytetrafluoroethylene] in order to prevent contamination of the sample from materials in the lid.

Objective 6: Perform genomic expression analysis for 12 predetermined genes and differential display PCR on gills, digestive glands and gonads from scallops collected at sites identical to those being used for contaminant analysis (#5).

The RNA that was extracted and reverse transcribed earlier for initial prescreening of samples by MXR genes (obj. 3), will be used for two types of more complete gene analysis (see Figure 3 for complete sample details). This RNA will have been derived from scallops obtained from the same site as those being analyzed for full contaminant work-up (obj. 5). The genomic analysis will be completed by the CoPI (S. Roberts) and the PI (F. Goetz).

<u>Multiple Gene Expression Analysis:</u>

The first gene analysis will be the screening, using QPCR, of the expression of 12 predetermined genes (Table 2) that have already been associated with contaminant burdens in the literature (see below). While the sea scallop homologs of these genes have not yet been cloned, they are all present, as indicated in Table 2, in other bivalves. In many cases these genes were already cloned by the PI (Goetz) and CoPI (Roberts) in the closely related bay scallop (manganese-containing superoxide dismutase, glutathione peroxidase, cytochrome P450 1A, multixenobiotic resistance protein) and, therefore, will be easily obtained in sea scallops in the first year of the grant (see Method below).

Selected Genes for Expression Analysis	Bivalve Species in Which Homolog is Identified	Genbank Accession #
Cu/Zn-superoxide dismutase	Mussel	MED581746
	Oyster	BQ426796
Manganese-containing superoxide dismutase	Bay Scallop	CN782370
Catalase	Mussel	AY580271
	Clam	*166314_O23.F
Glutathione Peroxidase	Oyster	CD650160
	Bay Scallop	CN782407
	Clam	*178914_IO4.F
Glutathione Réductase	Clam	*167214_M18.F
	Oyster	CD647589
Aryl Hydrocarbon Recptor (AhR)	Mussel	AF261769
Cytochrome P450 1A (CYP1A)	Mussel	AJ625323
	Bay Scallop	CK484494
	Oyster	CB617386
Glutathione-S-Transferase	Oyster	AJ558252
	Clam	*167614_F07.F
	Mussel	AY557404
	Bay Scallop	CN782430
Glutamine synthetase	Oyster	AJ558239
Metallothioneins [b]	Mussel	AJ005452
	Oyster	AY331696
Multixenobiotic resistance protein	Oyster	AY319412
	Mussel	AF159717
	Clam	*101_N07.F
Vitellogenin [c]	Yesso Scallop	AB055960
	Oyster	AB084783

* designates sequence number at TIGR database

[a] E values represent sequence comparison to human sequences

[b] designation of sequence as a metallothionein based on metal-induced expression of transcript not on sequence homology which is generally low

[c] since humans do not have vitellogenin, E value is to nearest vertebrate sequence (sturgeon-scallop; mummichog-oyster)

Table 3: Selected genes for full expression analysis and their most similar bivalve homologs

Cellular processes that have evolved to deal with environmental pollution include antioxidant defense systems, detoxification metabolism, and contaminant exclusion. In addition, certain physiological processes are abnormally altered in response to exposure. Collectively, monitoring expression profiles of multiple genes regulated in response to contaminant exposure provides valuable information regarding the source of contamination and the specific effects that a contaminant(s) may have on the physiology of an organism. In fact, the most inclusive way to monitor contaminant exposure would be to use a gene array containing hundreds to thousands of genes involved in pivotal cellular pathways. However, as a starting point, we are proposing to use a set of genes that have strong published relationships to pollution. Their specific relationships are described below.

Antioxidant Defense: Oxidative stress refers to the adverse effects of reactive oxygen species (ROS) on an organism's health. Reactive oxygen species (i.e. superoxide anion radical, hydrogen peroxide, hydroxl radical, singlet oxygen, and nitric oxide) are very reactive and are non specific in their targets. Targets include lipid membranes, DNA, and proteins resulting in lipid peroxidation, instable nucleotide sequences, and alteration of enzyme function, respectively. Endogenous sources of ROS include cellular electron transport chains that are involved in oxygen metabolism. In addition, environmental contaminants can participate in redox cycling resulting in excessive ROS. Xenobiotic compounds that have been shown to contribute to oxidative stress include quinines, diols, nitroaromatics, aromatic azo dyes, bipryridyls, transition metals, and perfluorinated fatty acids (PFFAs) (Schlenk and Di Giulio, 2002). A specific example involves perflourinated compounds, commonly used in corrosion inhibitors, stain resistant treatments and foam fire extinguishers. Researchers have shown that these compounds alter fatty acid oxidation which produces excess hydrogen peroxide (Sohlenius *et al.* 1994). Other compounds can contribute to oxidative stress such as coplanar PCBs and PAHs via binding and inactivation targets such as cytochrome P450 1A and glutathione, respectively.

Antioxidant defense mechanisms defend cellular components from negative effects of ROS. Importantly, conditions that influence oxidative stress activate expression of genes involved in antioxidant defense. Examples of non-enzymatic antioxidants include vitamin E and asorbic acid. Enzymatic antioxidants catalyze ROS to less reactive compounds and include **superoxide dismutases (SOD), catalases, glutathione peroxidases, and glutathione reductases.**

Superoxide dismutases catalyze the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide. Manuzio *et al.* (2004) have demonstrated elevated Cu/Zn SOD (SOD3) expression in gill tissue of blue mussels is correlated with the habitat pollution. In addition, using suppression subtraction hybridization (SSH), Boutet *et al.* (2004), showed that Cu/Zn SOD was upregulated in oysters experimentally exposed to hydrocarbon contamination. The ability of SODs to be successful antioxidants is associated the activity of other enzymes such as glutathione peroxidases (GPx) that catalyze the reduction of hydrogen peroxide into water or organic peroxides into stable alcohols by oxidizing reduced glutathione into glutathione disulfide. Glutathione reductase then reduces glutathione disulfide to glutathione.

Metabolism: Detoxification metabolism is another important biochemical response to pollutant exposure. Cytochrome P450 monooxygenases (CYP) are a large family of enzymes that have received significant attention in this regard. One specific subfamily of CYP that has selectivity

for planar aromatic hydrocarbons is **cytochrome P450 1A** (CYP1A). Cytochrome P450 1A catalyzes the monooxygenation of compounds such as dioxins, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and is highly inducible by these pollutants (Stegeman and Hahn, 1994). CYP1A is regulated by an **aryl hydrocarbon receptor** (AhR) that once activated, initiates transcription of CYP1A. There has been extensive research related to CYP1A expression and activity in marine organisms exposed to pollution, particular fish. Researchers have shown that in fish, CYP1A is significantly induced by relavent levels of xenobiotics and gene expression is induced in fish from contaminated sites (Collier *et al.* 1992, Stegeman and Hahn 1994, Wirgin and Waldmain 1998). While protein expression, enzyme activity, and mRNA levels can all be measured, mRNA expression has its advantages due to the fact that transcriptional activation is an early event in induction and because substrate inhibition has the ability to complicate interpretation of enzyme activity and protein expression. Many studies have focused on natural populations of bottom dwelling fishes. One example is Atlantic tomcod from the Hudson River, New York that had high levels of hepatic DNA adducts and significantly increased prevalence of hepatocellular carcinomas (Wirgin *et al.* 1994, 1989; Dey *et al.* 1993). CYP1A mRNA differed 28-fold between the Hudson River population and cleaner sites, generally correlating with the sediment contamination (Kreamer *et al* 1991, Wirgin *et al* 1994). In other studies focusing on English sole from the Puget Sound, Washington, levels of CYP1A expression were also found to correlate with sediment concentrations of PAHs, but not other AHs (Collier *et al.* 1992, 1995). While there has been less research on the identification of CYP1A homologs in bivalves, researchers have identified multiple CYP P450 homologs in the oysters, upregulated in response to hydrocarbon exposure (Boutet *et al.* 2004). In addition, as part of a bay scallop EST study by one of the collaborators on the current proposal, a gene that is most similar to human CYP1A was identified.

Phase II enzymes often work in conjunction with phase I enzymes such as CYP1A by conjugating pollutants with hydrophilic compounds to increase exclusion rates. Catalyzed products of CYP1A are often good substrates for phase II enzymes but are still able to damage molecular targets. Three major groups of phase II enzymes include **glutathione S-transferases** (GST), uridinediphosphate glucuronosyltransferases (UDPGT), and the sulfotransferases (ST). The enzymes are classified based on the endogenous water soluble conjugant. GST is considered one of the most important phase II enzymes and also has a role in oxidative stress defense by catalyzing selenium independent glutathione peroxidase activity (Prohaska, 1980). When comparing GST activity in blue mussels from polluted and controlled sites, Manduzio *et al.* (2004) observered increased activity in gills tissue from mussels in close proximity to a cooling system of a power plant. Interestingly, the GST gene in oysters is upregulated in response to hydrocarbon exposure (Boutet *et al.* 2004) and following exposure to the parasite *P. marinus* (Tanguy *et al.* 2004).

Glutamine synthetase (GS) is involved in several cellular functions including nitrogen metabolism and catalyzes the conversion of glutamate to glutamine (Glu+NH3+ATP⇔Gln+ADP+Pi). It has been suggested that GS plays important role in ammonia detoxification following xenobiotic exposure. Most studies related to anthropogenic pollution and GS involve pesticides and plants as GS inhibition is a common mechanism of action for herbicides. For example in *Arabidopsis*, GS was induced under amino acid starvation and exposure to the herbicide acifluorfen (Zhao *et al.* 1998). In two recent studies using suppression subtraction hybridization (SSH), exposure to hydrocarbons as well as pesticides upregulated GS expression (Boutet *et al.* 2004, Tanguy *et al.* 2005a). Using RT-PCR to study GS

expression in oysters, Tanguy and colleagues (2005b) also demonstrated that hypoxia, pesticides and hydrocarbon exposure significantly regulates GS expression.

Metallothioneins (MT) are proteins that are involved in metal homeostasis, detoxification of metals, and scavenging of ROS. Therefore MTs play an important role in an organism's biochemical response to excessive metal exposure and oxidative stress. Expression of MT is transcriptionally controlled by intracellular metal concentration therefore several research groups have used gene expression as an indicator of metal exposure in aquatic organisms (for review see Viarengo *et al* 1999). Just as with CYP1A, analysis of MT expression has an advantage over residual metal analysis since it is a better indicator of metal bioavailability. Numerous field studies have demonstrated a relationship with metal exposure and MT expression. For example in channel catfish, MT induction was observed following arsenical exposure (Schlenk, *et al* 1997a). Levels of gene regulation has been shown to vary with exposure to different metals. For instance, Zn and Cd generally increase expression 10-20 fold compared to Cu (Schlenk et al. 1997b, Olsson and Kille 1997). Studies comparing fish MT expression with metal concentration in sediment and tissue generally have found a positive correlation (for review see Wirgin and Theodorakis 2002) MT homologs have been cloned in several bivalve species including oysters and clams (Tanguy et al 2001), however there have been few comprehensive studies to the relationship to contaminant exposure, bioaccumulation and mRNA expression.

Exclusion: Cellular efflux of anthropogenic toxins is another effective biochemical response of marine organisms exposed to pollution. This efflux, known as **multixenobiotic resistance** (MXR), is carried out by transport proteins located in cell membranes that belong to the ATP binding cassette proteins and is often the first line of defense against foreign chemical compounds. This mechanism was first described in drug-resistant tumor cell lines because of their ability to pump out hydrophobic drugs. In aquatic invertebrates and fish MXR has been shown to function in gill, mantle, and liver tissues. Numerous contaminants activate MXR including pesticides, metals, endocrine disruptors, and pharmaceuticals and personal care products (PPCPs). Studies examining P-glycoprotein, a MXR protein in mussels and oyster found animals from polluted sites had increased levels of expression (Minier et al. 1993; Kurelec et al. 1996). Recently researchers using a gene array approach to characterize gene responsiveness to perfluorooctane sulfonic acid (the most commonly found perfluorinated compound found in tissues of wildlife) have shown that multidrug resistance associated protein is significantly upregulated in rat hepatoma cells (Hu *et al.* 2005). While induced expression of MXR proteins alone lack to ability to identify specific xenobiotic or stressors in the environment, when analyzed in conjunction in with other indicators MXRs can provide reliable information regarding the contaminant exposure. Additionally, the low specificity of MXR activity allows homologs in bivalves to be excellent candidate genes for high-throughput analysis.

Enodcrine Disruption: **Vitellogenin** (VTG) is a yolk precursor glycophospholipoprotein this is synthesized in the liver, transported to developing oocytes and used for nutrition in developing fish. VTG transcription is induced by the sex steroid, estradiol-17β (E2) and E2 mimics (e.g. alkylphenol polyethoxylates, pharmaceuticals, HAHs, PAHs, PCBs, dioxins, furans, pesticides, heavy metals). Unlike all of the genes described previously, VTG gene is not involved in organism protection or defense, but rather is a downstream response to xenobiotic exposure. In

studies monitoring VTG expression in fish elevated levels have been reported in several populations including a largemouth bass from two rivers in northwestern Florida (Orlando *et al* 1999) and in carp downstream of a sewer plant in Minnesota (Folmar *et al.* 1996). Only recently have VTG homologs have been identified in bivalves including a scallop (Osada et al 2004).

Methods for Multiple Gene Expression Analysis:

Cloning sea scallop homogs: Initially, transcripts that will be targeted for expression profiling will have to be cloned and identified in the sea scallop. The first gene to be targeted will be the P-glycoprotein and/or the multi-drug resistance associated protein cDNA, both part of the multixenbiotic resistance genes. These will be necessary for the prescreening of samples as discussed in Objective 3, however we will have more than enough time (at least 6 months) to get them before they are needed. In order to isolate the sea scallop homologs of all genes listed in Table 3, a reverse transcription – polymerase chain reaction (RT-PCR) approach will be used. The PI and CoPI have extensive experience using this technique for gene discovery (e.g., Roberts and Goetz, 2001), including identifying novel gene homologs in scallops (Kim et al., 2004). For each gene listed in Table 2, nucleotide sequences from the listed bivalves (and other species as needed) will be aligned using MacVector 7.2 to identify regions of high homology for primer design. Primers will be used to amplify the target genes in sea scallop tissues. PCR products will be cloned into TOPO TA pCR 2.1 (Invitrogen) and positive colonies grown for plasmid DNA. Templates will be prepared in a Rev Prep Orbit (GeneMachines) and the resulting cDNAs sequenced using a modified dideoxy chain termination method with Big Dye Terminator (Applied Biosystems). Sequencing reactions will be precipitated and pellets resuspended in Hi-Di Formamide with EDTA (Applied Biosystems) and analyzed using a 3730 Sequencer (Applied Biosystems). All sequences will be analyzed by NCBI Blast programs to verify desired homologues are obtained (Altschul, 1997).

Quantitative RT-PCR: Once gene homologs from the sea scallop have been identified, quantitative RT-PCR (QPCR) will be used to quantify transcript levels of all 12 genes in gills, digestive glands and gonads from scallops in the populations selected after prescreening (objective 4). As discussed in objective 3 (prescreening for MXR gene expression) at sea, tissues from scallops will be preserved in RNAlater and frozen at -20°C for later RNA extraction in the laboratory. Total RNA will be extracted by homogenization of tissue in Tri-reagent as previously described (Chomczynski, 1993, Chomczynski, 1987). The mRNA isolation will be performed using the Poly-A-Tract mRNA isolation Sytem (Promega). Messenger RNA will be spectrophotometrically quantified and diluted to equal concentrations. Samples will be analyzed quantitatively using real-time RT-PCR (Brilliant SY BR Green QRT-PCR Master Mix Kit, 1-Step – Stratagene) in an Opticon2 Continuous Fluorescence Detection System (MJ Research). Reverse transcription (RT) and polymerase chain reaction (PCR) will be performed consecutively in the same reaction wells as follows: 30·min RT at 50°C, 10·min initial denaturation at 95°C, 40 cycles of 30·s denaturation at 95°C, 1·min annealing at 60–65°C, and 30·s extension at 72°C, with fluorescence measured at the end of every annealing and extension step. Specific primers will be designed for each gene based on the sequence information obtained after the initial cloning (previous section). Additionally, primers for sea scallop 18s RNA will be designed and used to normalize results. Each reaction will be performed in a separate well of a 96-well plate with a final volume of 25·ul. Immediately after each PCR, a melting curve analysis will be performed to determine if the desired product was amplified by increasing the

temperature from 55°C to 95°C at a rate of 0.2°C·s–1, and measuring fluorescence at every 0.5°C step. CT values will be converted to relative mRNA abundance levels based on their respective standard curves and normalized with 18S RNA values.

Differential Display Gene Analysis:

The second type of genomic analysis that will be carried out is the use of differential display PCR between tissue samples (gills, digestive glands and gonads) taken from sea scallops at contaminated and noncontaminated sites. Analysis of candidate gene expression as described above for the 12 genes in Table 3 is valuable and has a high probability of success since these specific genes have been linked to contaminant loading in the literature. However, the disadvantage of this approach is that it is based on the preconceived notion that these are the important genes in relation to contamination and/or in relation to sea scallops exposed to contaminants. There may be genes that we do not know about that are as important if not more important than the 12 listed in Table 3. To try and address this and to identify additional genes that could be used as biomarkers, a differential display approach will be performed. This technique requires no prior knowledge of sequence information and therefore will facilitate identification of novel candidate genes that can be used in the future.

Methods for Differential Display Gene Analysis:

Specifically, the recently developed GeneFishing DEG System (Seegene) will be used to isolate genes that are differentially expressed at various collection sites. The PIs have experience using this approach in several marine organisms including scallops (Roberts, 2004). This system is based on Annealing Control Primers (ACP) technology. The reason this approach is being used is that it is economical, fast, and can easily compare a large number of different samples simultaneously. The principle of ACP technology is based on the tripartite structure of a specific oligonucleotide primer (ACP) having distinct portions on the 3'- and 5'- end separated by a regulator and the interaction of each portion during two-stage PCR. The resulting PCR products will be run on an agarose gel, and differentially expressed bands removed. PCR products will be cloned into TOPO TA pCR 2.1 (Invitrogen) and positive colonies grown for plasmid DNA and sequenced as discussed above for the cloning of sea scallop gene homologs. All sequences will be analyzed by NCBI Blast programs for similarity to known genes (Altschul, 1997). ClustalW (MacVector 7.2) analysis will be used for sequence pair-wise and multiple protein alignments. Once gene products have been identified through DNA sequencing, quantititative RT-PCR will be used to confirm differential expression between tissues obtained from contaminated versus noncontaminated sea scallops.

IV. Relevance to the Oceans and Human Health Initiative (OHHI) Goals

One of the priorities of the OHHI is to use sentinel and model species to better inform our understanding of risks to human health or inform our understanding of ocean health as it relates directly or indirectly to changes in risk for human or public health. Specifically, within that priority; "2) whether sentinel species can be used to indicate future threats to, or improvements in, ecosystem integrity that are linked to public or human health." There is no larger issue linked to public and human health than environmental contamination and its impact on the ecosystem. This ultimately impacts human health by threatening the food we consume, the environment we recreate in and, in the case of the oceans, a potential source for novel drug discovery. In this regard, Mussel Watch which has utilized bivalves as sentinel species, has been a very successful

way to follow the contaminant load in shallow in-shore coastal regions (O'Connor, 2002). It has been a means to follow the integrity of the ecosystem over time and to determine if it is improving or getting worse. Contaminant loads that have been measured in that program can be correlated to the proximity of urbanization along the coast. **But what about off-shore and deeper regions of our coastal shelf ? What is the extent of pollution in these areas and how has that impacted the organisms that live in those regions ?** It might be hypothesized that since these areas are far from urban centers that there is no need to consider pollution in them. However, contaminants in biological residents of these regions have never been sampled. Therefore, it is impossible to say what type of contaminants are present and at what levels. Our proposal to utilize the sea scallop, a sentinel species that is long-lived, that continually filters the water that it lives in, and has high site fidelity should give us an accurate reading of exactly what is present in an organism inhabiting this area of the shelf. Admittedly, this study is small in scope with complete contaminant and genomic analyses being conducted on a relatively small set of samples. However, the study is very focused, and the results will provide the first information concerning biological contaminant loads in deeper regions of the shelf that have been previously ignored. As such, these data can be used to make predictions concerning a part of the ecological integrity of this region. Depending on the results, the sampling platform and tools that we develop and use for this study can be followed in the future to determine changes in pollution in this region over time. In addition, the samples that we take will provide a valuable repository for any other researchers interested in surveying sea scallops across diverse habitats for other purposes (e.g., paralytic shellfish poisoning).

One of the experimental approaches of this proposal is to develop and test the use of genomic tools as predictors of contaminant loading and biological effects in sea scallops, and to correlate this with actual contaminant amounts. This objective is in direct line with the goal of the OHHI in "Developing or utilizing new technologies that permit more rapid and accurate detection of ocean health threats (e.g. via observing systems) or that lead to improved understanding (including predictive capabilities) of processes leading to ocean-related health threat." Specific analytical contaminant analysis is an important initial approach to determine the type and amounts of contaminants present in an organism. However, for continued routine monitoring, contaminant analysis can be extremely expensive, labor intensive and time consuming. In contrast, molecular based techniques are rapid, require very little in the way of tissue collection, can be noninvasive, and can be predictive of specific classes of contaminants if the proper gene(s) are followed. They are also less costly than full contaminant analysis.

The Atlantic sea scallop is one of the most important fisheries in the U.S. with 2003 landings of 25,476 MT (meats) at a value of approximately $220,000,000 (Hart and Chute, 2004). It is also the most valuable wild scallop fishery in the world. Thus, humans consume scallops in large quantities and will continue to do so. There is now a move to commercially market the roe (gonad) of sea scallops, a tissue that is high in lipid and therefore has the potential for much greater burdens of persistent contaminants. For the protection of this fishery and for the protection of the health of humans that consume this fishery, it is important to know precisely what is in them and what may be impacting them in their environment. The results of our study will provide this information.

V. Project Management

There are 6 investigators working on this project: **Frederick Goetz** (Great Lakes WATER Institute, University of Wisconsin-Milwaukee); **Terry Wade** (Geochemical and

Environmental Research Group, Texas A&M); **Steven Roberts** (Marine Biological Laboratory); **Paul Rago** (National Marine Fisheries Service, Northeast Fisheries Science Center- Woods Hole); and **Deborah Hart** (National Marine Fisheries Service, Northeast Fisheries Science Center-Woods Hole) and **Tracy Collier** (National Marine Fisheries Service, Northwest Fisheries Science Center,- Seattle). Each participant has a very important and specific role in this project.

Frederick Goetz: Will serve as the Principal Investigator of the project and will be the overall project manager. He will be responsible to make sure that milestones are met and accomplishments as detailed in section "L" are attained. In addition, he will be the investigator that goes on the sea scallop survey to collect samples for the project and will conduct the differential display PCR analysis in year 2 of the grant.

The PI has had greater than 25 years of continuous research experience on various biological aspects of fish and bivalves. He has procured and administered over 2.5 million dollars in external research funds from many federal and state agencies including NSF, NIH, USDA, NOAA and state DNRs. He has published more than 100 papers concerning growth, reproduction, immunity and health of aquatic organisms. In his past position at the Marine Biological Laboratory in Woods Hole, he worked on various marine species including bivalves, and held research grants (in collaboration with Paul Parker) from the Cooperative Research Partners Initiative, NMFS NE Regional Office and the Massachusetts Environmental Trust to study cod and dogfish, respectively.

Terry Wade: Will be in charge of contaminant analysis and interpretation of the contaminant data. He will work with the PI to assimilate contaminant data for publication and to submit to the appropriate NCCOS center (National Oceanographic Data Center).

Wade served as Project Manager for GERG's NOAA NS&T Mussel Watch Project contract and has been active in the field of marine organic environmental chemistry for more than 30 years. He is a respected international expert in environmental chemistry. He has developed and validated methods for analysis of trace organics in environmental samples including aliphatic hydrocarbons, aromatic hydrocarbons, biomarkers, chlorinated hydrocarbons (including DDTs, Chlordane and dieldrin), PCB (including congeners, WHO, planar, aroclors and total), coprostanol, toxaphene, dioxins/furans, PBB, PBDE and butyltins. He has applied these methods to research projects involving air, rain, fresh water, seawater, soils, SPMDs, sediments, plant tissues and animal tissues. He has over 180 publications, 117 platform presentations and 31 posters which deal with method development, intercalibrations, and sources and fate of contaminants in the environment. He was GERG's Project Manager for U.S. Fish and Wildlife trace organic contaminant analysis contract that was responsible for the analyses of over 7000 samples resulting from the *Exxon Valdez* oil spill NRDA.

Tracy Collier: Will analyze samples for contaminant loading as an independent laboratory for verification and for interlaboratory intercalibrations, especially for PBDE measurements.

Tracy Collier is the Program Manager for the Ecotoxicology and Environmental Fish Health program at the Northwest Fisheries Science Center. His research has covered some of the first work on metabolism of PAHs by fish, studies of the impacts of oil spills on marine fish, the enzymology of carcinogen activation and detoxication, and assessing overall effects of contaminants on fish populations through the use of field investigations. Currently he is working with the assessment of remediation efficacy and approaches for determining causality. He has

served on the Essential Fish Habitat Team for NMFS for which he received a Commerce Bronze Medal in 2000. He also is the Habitat Science Coordinator for the Science Center, and recently was part of the US delegation to Spain to assist in responding to the Prestige oil spill off the coast of Galicia.

Steven Roberts: Will be in charge of cloning and characterizing the 12 environmentally relevant genes in sea scallops. He will be in charge of processing all sea scallop tissues to obtain RNA and for QPCR prescreening for expression of the Multixenobiotic resistant gene. He will be in charge of QPCR analysis of the 12 environmental relevant genes in tissues from sea scallops selected for contaminant analysis.

Roberts is a young independent investigator who has focused on the physiology and corresponding genetic expression patterns in aquatic organisms. His research has involved finfish reproduction and growth, and at the Marine Biological Laboratory he has concentrated on the molecular genetics of shellfish. This has included overseeing a large bay scallop (*Argopecten irradians*) expressed sequence tag (EST) project that has identified thousands of transcripts in the scallop genome and several novel genetic markers. In addition, he is currently the principal investigator on a USDA funded project using quantitative gene expression profiling to characterize differences in Eastern oyster (*Crassostrea virginica*) populations in the Northeast US

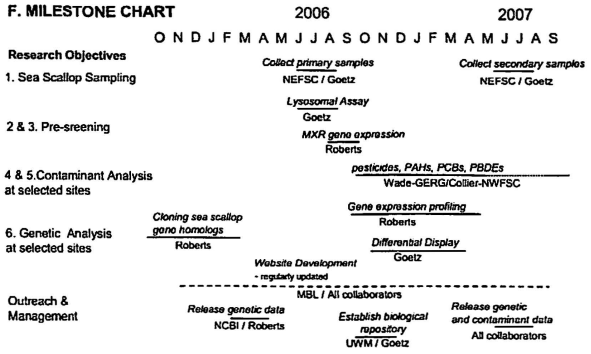
Paul Rago and Deborah Hart: Will consult with the PI on the selection of station locations during the 2006 and 2007 sea scallop dredge surveys conducted by the Northeast Fishery Science Center. In collaboration with the PI, they will facilitate the collection of biological material at appropriate sites.

Paul Rago is a Research Fishery Biologist at the Northeast Fishery Science Center of the National Marine Fisheries Service in Woods Hole. He is currently leader of the Stock Assessment Methods Group. In addition to spiny dogfish, he has worked on a wide variety of stock assessments, including scallops, lobsters, surf clams, ocean quahogs, monkfish, and yellowtail flounder. He has been active in a number of cooperative experiments with the fishing industry and the incorporation of vessel monitoring system data into stock assessments. Prior to working with National Marine Fisheries Service, Rago spent 15 years with the US Fish and Wildlife Service where he served as coordinator of the Emergency Striped Bass Study and a variety of Atlantic salmon studies. Deborah Hart is a Operational Research Analyst at the Northeast Fishery Science Center of the National Marine Fisheries Service in Woods Hole working on sea scallop population assessment.

VI. Project Timeline

The specific timeline and "Milestones" of the project are indicated in the Milestone Chart (Figure 4). It should be noted that the primary collection of sea scallop samples for the prescreening and later contaminant analyses would occur on the survey conducted in the summer of 2006 given the proposed October start date suggested by the OHHI. The sea scallop survey is actually conducted in 2 - two week portions. Therefore, screening for the MXR gene can occur even during the time that the survey is being conducted since samples can be off-loaded at Woods Hole for laboratory analysis by the CoPI (Roberts). Depending on the results of the initial year, then additional collections can be made by the PI on the 2007 survey at specific sites to broaden the survey, and for other reasons (specific tissues, etc.). However, given the start date

F. MILESTONE CHART



of October, this would not allow for very much further contaminant analysis prior to the end of the grant. If possible, it would be desirable to start the grant later and, therefore, have more time to analyze secondary samples in the second year. Alternatively, if a no-cost extension can be arranged, then funds can merely be held for this purpose.

VII. Facilities and Equipment

GERG Facilities for Contaminant Analysis: GERG has developed the facilities, possesses the knowledge and has the tools necessary to conduct complex projects. Through the Texas A&M Research Foundation, GERG owns ~15 acres of land in an industrial park in the southern part of College Station. The facility includes ~25,000 sq. ft. of air-conditioned office and laboratory space; 3,000 sq. ft. of high-ceiling; enclosed staging and storage space; and 2,000 sq. ft. of partially enclosed, roofed space for open storage, refrigerated storage, and shops. A 2-acre concrete apron connects the buildings and provides space for assemblage and storage of field equipment. GERG occupies several buildings including:

- Main Building: 14,495 sq. ft. heated
- East Building: 4,434 sq. ft. heated
- Portable Building: 712 sq. ft. heated
- Ocean Sciences Laboratory: 3,000 sq. ft. heated
- Warehouse: 2,045 sq. ft. enclosed
- Machine Shop: 2,305 sq. ft. open-bay

In addition to the heated space normally occupied by researchers and staff, GERG has over 2,000 square feet of enclosed warehouse space; 2,300 square feet of covered, open-bay machine shop; over 1,400 square feet of a secured "walk-in" type refrigerator and three secured

"walk-in" type freezers; and over 6,000 square feet of concrete staging and maintenance area for its field equipment, including storage for trucks, laboratory and shop vans, and freezer vans.

GERG Laboratory - GERG and Texas A&M University have established an equipment base for coastal marine research and environmental and analytical analyses. GERG's infrastructure of analytical instrumentation, laboratory facilities, and computers provides the tools needed to satisfy the requirements of our clients. GERG offers complete sample preparation laboratories as well as instrumentation for the analysis of trace elements, aliphatic and aromatic hydrocarbons, pesticides, polychlorinated biphenyls (PCBs), planar PCB, PBDEs, dioxin/furans, butyltins, semivolatile organic compounds, volatile organic compounds, and other organic compounds. A variety of digestion, extraction and purification techniques are available to suit each project's specific objectives. Once organics are isolated and purified, sample extracts are analyzed for molecular compositions using high resolution fused silica capillary gas chromatography. A multitude of gas chromatographic detectors are available including flame ionization, electron capture, flame photometric, photo ionization, low resolution mass spectrometers, and a high resolution mass spectrometer. Inorganic digests are analyzed by graphite furnace atomic absorption, cold vapor atomic absorption or inductively coupled plasma mass spectrophotometer (ICP-MS). All analytical instrumentation is fully computerized and automated for rapid and efficient sample throughput.

Organic analyses are provided by three gas chromatograph-low resolution mass spectrometers (GC/MS) or two dual-channel gas chromatographs with electron capture (ECD) detectors. GERG has two gas chromatographs with flame ionization detectors (FID) for general chromatographic analysis, one gas chromatograph with both a flame photometric detector for butyltin analysis, and a Nitrogen Phosphorus Detector (NPD) detector for organophosphorus pesticide analysis. GERG has a GC with a high resolution MS (GC/HRMS) for the analysis of dioxin/furan, dioxin-like PCBs and PBDEs. For trace metal analyses, GERG has graphite furnace, and cold vapor atomic absorption instruments. GERG has access to a Perkin Elmer ICP-MS ELAN 9000 through the Texas A&M Trace Characterization Laboratory. For other trace organic analyses, GERG has four high performance liquid chromatographs (HPLC) and one TOC analyzer. All gas chromatographs (GC) are fully automated with injectors and computers for data acquisition. A list of major analytical and sample preparation instruments available for use on the proposed work is provided in Table 2.1.

GERG's main laboratories occupy more than 6,950 square feet of space. This provides more than 250 square feet of laboratory space per research staff. Access to all areas within GERG is controlled to ensure confidentiality of samples and data.

Sample Control Laboratory - The Sample Control Laboratory is isolated from the main laboratories and is a controlled access area. This facility and the procedures used provide for the receipt, safe handling, and secure processing of environmental samples. The sample receipt area is isolated from the main laboratories and includes approximately 500 square feet of space. This area is lined with benches with chemically resistant bench tops and isolated room ventilation providing adequate space to safely process samples in a contaminant-free environment. A sample receipt area provides for the safe, controlled receipt and processing of samples under chain-of-custody SOPs. This area is partitioned from the sample preparation area where sample homogenization and aliquoting are performed. GERG has a Class II Type B2 biological safety cabinet which allows for preparation of potentially contagious samples including human tissues.

Sample Storage Area - The cold storage, controlled access areas occupy more than 1,400 square feet of space. These "walk-in" freezers and refrigerators are secured and the temperatures are monitored to detect system failures. Active and archived samples are stored in these areas

Table 2.1. Major Equipment Available at GERG.

Description	Number/Model Available
Gas Chromatography-High Resolution Mass Spectrometry	One Hewlett-Packard (HP) 5890 Series II GC-Fisons VG AutoSpec 5500 MS, Fisons CTC A200s autosampler)
Gas Chromatography with Electron Capture Detector	Two HP5890 Series II GCs equipped with dual column, dual autosamplers, and dual ECD
Gas Chromatography-Mass Spectrometry	One HP 5890 Series II GC/5972 MS with FID, equipped with OI 4560 Purge and Trpa and Multple Purging Module (MPM-16) Two HP 6890 GC/5973 MS, one with micro ECD Equipped with autosamplers, ChemStation data software.
Gas Chromatography with Flame Photometric Detector	One Fisons 8000 GC with Fisons AS200 autosampler
Gas Chromatography with Nitrogen-Phosphorus Detector	See Above for GC
Gas Chromatography with Flame Ionization Detector	Three HP 5890 GCs with autosamplers, one with dual column/dual FID, equipped with ChemStation data software
High Performance Liquid Chromatography	Two HP 1050 HPLC system (HP 1050 pump, 1050 autosampler, 1050 variable wavelength detector); One system consists of Spectra-Physics SP8800 ternary pump, HP1050 autosampler, Perkin-Elmer LC 40 fluorescence detector; One system consists of Spectra-Physics 8000 pump, ThermoQuest AS100 autosampler, Shimadzu SPD-10A UV detector, and LKB Superrac fraction collector.
Inductively Coupled Plasma (ICP) Spectrometer	Perkin Elmer DRCII ICP-MS with autosampler (available at Center for Trace Characterization, Department of Chemistry, Texas A&M University)
Atomic adsorption spectrometer	One Perkin-Elmer Zeeman 4100ZL, equipped with autosampler
Cold Vapor Mercury Analyzer	One Perkin-Elmer FIMS FIAS 400, equipped with autosampler
Total Scanning Fluorescence Spectrophotometer	Two Perkin-Elmer 650-40 Spectrophotometers One Perkin-Elmer LS50B Spectrophotometer
Accelerated Solvent Extractors	Two Dionex ASE 200 solvent extractors

Tissue grinders, homogenizers, ball mills, etc.	Numerous
TOC Analyzer	One Leco TOC analyzer
Computer facilities	Laboratory computers: 22 IBM compatible, Pentium CPU, Microsoft Windows NT, Windows 98, and Windows XP-based systems, 4 DEC VAX minicomputers

under chain-of-custody SOPs. Samples may be stored in this area for periods of 90 days or greater. Sample extracts and standards are stored in secured, dedicated refrigerators apart from the sample storage area.

Sample Preparation Laboratory - GERG's main sample preparation laboratories comprise more than 2,000 square feet of space. The laboratory staff each has more than six linear feet of unencumbered work space. All of the laboratories are fitted with exhaust hoods with chemically resistant tops on the bench surfaces.

Metal Analytical Laboratory - GERG's main laboratory building and presently occupies over 2,000 square feet of space. All of the laboratories are fitted with exhaust hoods with chemically resistant tops on the bench surfaces. Two (2) forced air hoods and one (1) perchloric acid hood are housed in an isolated controlled access area where samples are digested. Approximately eighty (80) teflon bombs are used in this area for the digestion of tissue samples and sufficient glassware is available for sample processing of sediment. The inorganic sample preparation room is adjacent to the inorganic instrument room and both rooms have a heating and ventilation system isolated from the remainder of the GERG laboratory. The laboratory uses a microwave digestion system from Milestone.

Data Control Area - Approximately 2,500 square feet of office space exists for the compiling, packaging, and shipping of analytical data and other deliverables as required by the client. The workspace is fitted with computers interlinked on a network, desks and work surfaces, photocopiers and binders.

Facilities for Genomic Analysis:

CoPI's (S. Roberts) laboratory: The Co-PD has an 800 sq.ft. research laboratory in the Marine Resources Center (MRC) at the Marine Biological Laboratory (MBL). The lab is equipped with routine laboratory furniture, plumbing, chemical and biohazard hoods, and other utilities. In the laboratory are 2 Power Mac G4s and 1 Power Mac G5 for data analysis. There is a single copy license for MacVector that can be run on all Macs. A local "Blast" on the Mac G5 along with complete NCBI nonredundant protein, nucleotide and EST databases is maintained. Programs for "phred" and "crossmatch" are maintained locally on the G5 for EST analysis. There are also 3 Dell Optiplex PCs for analysis and word processing. All computers in the lab are directly connected to the internet and they are also networked to one another and to computers running several pieces of laboratory equipment (e.g., ChemiImager 5500 and Opticon). The library also provides comprehensive and up-to-date database searching and electronic journals via the MBL internet site.

Equipment already present in the CoPI's lab that will be used for the proposed molecular research includes: ALF Express Sequencer; Alpha Innotech ChemiImager 5500; MJ Research Opticon 2 (includes a DNA thermal gradient PCR engine); Sorval Super T21 highspeed centrifuge; Perkin-Elmer EZ201 spectrophotometer; Lab-Line benchtop orbital bacterial shaking

incubator; Fisher Biotech hybridization incubator; 2-Boekel incubator shakers; 3 thermocyclers (MJ-PTC-200, PTC-100, PTC-150); 2-Road Runner Owl electrophoretic mingel systems for SDS PAGE; Trans-Blot SD-Biorad transfer system; 2- EC 600 and 1-EC 452 power supplies; Fisher 550 ultrasonic tissue processor; Various horizontal gel systems for RNA/DNA gels; TL-2000 translinker; Thelco high performance above ambient incubators for bacteria and hybridization; Balances - Cahn C-30 microbalance; top loading balances (O-Haus); semi-analytical balance Mettler AC-100); microcentrifuges; So-Low ultralow freezer (U85-22); 2 - 45.0 ft^3 sliding glass door refrigerators

At MBL there is a genome facility in the "Bay Paul" Center that has an ABI 3700 sequencer and automated plasmid DNA preparation system that can be used on a fee for sequence basis.

PI's (F. Goetz) Laboratory: The PI has a 800 sq.ft. research laboratory in the Great Lakes WATER Institute (GLWI) of the University of Wisconsin-Milwaukee. The lab is equipped with routine laboratory furniture, plumbing, chemical and biohazard hoods, refrigerators and other utilities. In the PI's laboratory is a Power Mac G5 for data analysis. There are also be 3 Dell PCs for analysis and word processing. All computers in the lab including those running equipment (e.g. NanoDrop and INGENIUS) are directly connected to the internet and are also networked to one another with a Snap Server.

Equipment already present in the PI's lab that will be used for the proposed molecular research includes: Syngene INGENIUS and GeneGnome gel and blot imaging systems; Stratagene Mx3000P Real time; Sorval Super T21 highspeed centrifuge; NanoDrop spectrophotometer; New Brunswick benchtop orbital bacterial shaking incubator; Fisher Biotech hybridization incubator and Boekel incubator shakers; DNA Engine Dyad Thermal Cycler (PTC-0220); power supplies for RNA/DNA and protein gels; various horizontal gel systems (Owl) for RNA/DNA gels; TL-2000 translinker; Precision Scientific 30M bacterial incubator; Balances - Cahn C-30 microbalance and O'Haus top loading and analytical balances; Microcentrifuges; Revco ultralow -80 freezer; Harris ultralow freezer (SLT-10LS)

The Great Lakes WATER Institute maintains a genomics core sequencing facility that has a new ABI 3730 sequencer and ancillary equipment (e.g., centrifuges and thermal cyclers) for in-house, high-throughput plasmid preparation and nucleic acid sequencing on a fee basis. "Standalone BLAST" with complete NCBI protein, nucleotide and EST databases, together with investigator-derived sequence databases, are maintained on a 4-node *Apple Workgroup Bioinformatics Cluster* (Apple G5 head node and cluster nodes) for intensive data processing and *Xserve RAID* (4x400GB Ultra ATA) for storage and backup. The Cluster also includes Bioteam's *iNquiry* software for standalone blast and 200 other in-house genomic applications. Custom programs run on the Cluster for automated sequence analysis including base-calling (phred), vector screen (cross-match), annotation of sequences (BLAST) and assembly of sequences (phrap). The program then stores all the data in an online database (postgresql) that is accessed through the web.

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ILESTONE CHART

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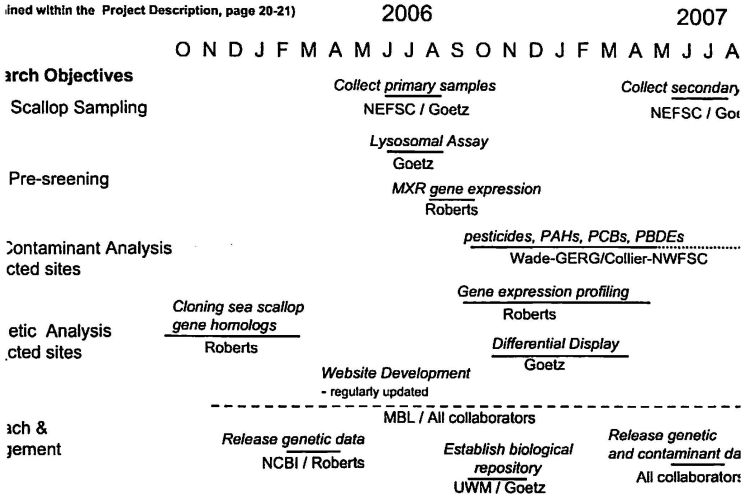
to start dates and research objectives
ined within the Project Description, page 20-21)



GEOCHEMICAL & ENVIRONMENTAL RESEARCH GROUP

GERG

College of Geosciences
Texas A&M University

April 22, 2005

Dr. Frederick Goetz
Senior Scientist
Great Lakes WATER Institute
University of Wisconsin-Milwaukee
600 East Greenfield Avenue
Milwaukee, Wisconsin 53204

Dear Rick:

I welcome the opportunity to collaborate with you on the research proposal "Sea scallops as sentinels of deepwater pollution" to be submitted to the NOAA Oceans and Human Health Initiative. The research approach and the team of experts that you have assembled for this cooperative research proposal are excellent. I am excited to be part of the team.

Sincerely,

Terry L. Wade, Ph.D.
Deputy Director for Environmental Sciences

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MARINE BIOLOGICAL LABORATORY

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April 21, 2005

Dr. Frederick W. Goetz
Great Lakes WATER Institute
University of Wisconsin-Milwaukee
600 East Greenfield Avenue
Milwaukee, Wisconsin 53204

Dear Rick,

This letter is to confirm that I will be a collaborator for the study we are proposing to NOAA's Oceans and Human Health Initiative entitled "*Sea scallops as sentinels of deepwater pollution.*" As a collaborator, I understand that I will be responsible for the completion of objectives directed at the characterizing transcript expression genes indicative of contaminant exposure in sea scallop. This will include cloning targeted genes from the sea scallop and using real-time quantitative RT-PCR to evaluate differences in transcript expression.

In the form of salary and benefits, I will receive a total of $22,389 over the two years. Including the salary of a technician, and supplies a total of $112,193 will go to the MBL to support the objectives of this project.

Sincerely,

Steven Roberts
Assistant Research Scientist

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
166 Water Street
Woods Hole, MA 02543-1026

April 24, 2005

Oceans and Human Health Initiative

To Whom It May Concern,

The purpose of this letter is to affirm our willingness to participate in the research project entitled "Sea scallops as sentinels of deepwater pollution". Our participation will involve consultations with the research team on the selection of station locations during the 2006 and 2007 sea scallop dredge surveys conducted by the Northeast Fishery Science Center. In collaboration with the principal investigator, we will facilitate the collection of biological material at appropriate sites. Pending coordination with senior scientific staff, and subject to an analysis of primary mission objectives, we will attempt to sample stations relevant to potential pollution gradients.

Thank you for the opportunity to participate.

Sincerely,

Paul J. Rago, Ph.D. /s/
Leader, Stock Assessment Task
Acting Chief, Population Dynamics Branch

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Northwest Fisheries Science Center
2725 Montlake Boulevard East
Seattle, WA 98112-2097

April 22, 2005

Dr. Frederick Goetz
Senior Scientist
Great Lakes WATER Institute
University of Wisconsin-Milwaukee
600 East Greenfield Avenue
Milwaukee, Wisconsin 53204

Dear Dr. Goetz,

This letter is written to confirm our interest in collaborating with you on your proposal titled "Sea scallops as sentinels of deepwater pollution". We find your proposal to be both novel as well as highly consistent with the aims of our West Coast Center for Oceans and Human Health, of which I am the Director. Your approach, which would examine a largely ignored section of our Nation's coastal ecosystem, utilizing a species with long life span, high site fidelity, and consumed in large quantities by humans, is one that has high merit in our view.

If your proposal is selected for funding by the NOAA's OHH Program Office, our laboratory would perform analyses of up to 25 environmental samples (plus the appropriate quality assurance samples), for a suite of aromatic hydrocarbons (AHs), as well as polybrominated diphenyl ethers (PBDEs). Depending on the availability of internal NOAA OHH funds, we would conduct these analyses with NOAA funds (estimated at $21,630) separate from the external grants program, since monies allocated to that program cannot be used to support NOAA activities. Your primary contact for this work would be Dr. Tracy Collier, who is the Principal Investigator for our Sentinel Species projects within our OHH Center.

We look forward to collaborating with you on this study if funded.

Sincerely,

Usha Varanasi

Usha Varanasi, Ph.D.
Science and Research Director

CURRICULUM VITAE - FREDERICK WILLIAM GOETZ

Academic Experience

B.A. - Colgate University (Hamilton, N.Y.) - 1972
Biology and German.

Ph.D. - University of Wyoming (Laramie, Wyoming) - 1976 - Zoology - Dissertation title: "The *in vitro* induction of final maturation and ovulation in brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*) ova and the hormonal control of final maturation in ova of other fish species" Advisor: Dr. Harold Bergman.

Professional Experience

2004 - present: Senior Scientist – Great Lakes WATER Institute, University of Wisconsin Milwaukee, Milwaukee, WI.

2004 - Present: Adjunct Professor – University of Wisconsin – Milwaukee - Biology Department.

2001 - 2003: Senior Scientist and Director of Program in Scientific Aquaculture - Marine Biological Laboratory - Woods Hole, MA

2001 - Present: Adjunct Professor - Boston University - Biology Department and Marine Biology Program

1989 - 2001: Professor and Director of Graduate Studies - Department of Biological Sciences University of Notre Dame

1984 - 1989: Associate Professor and Director of Graduate Studies - Department of Biological Sciences - University of Notre Dame.

1977 - 1984: Assistant Professor - Department of Biological Sciences - University of Notre Dame.

1976 - 1977: Postdoctoral Fellow - Fisheries and Marine Service, West Vancouver, British Columbia, Canada.

1974 - 1976: Research Assistant - University of Wyoming.

1972 - 1974: Teaching Assistant - University of Wyoming - Instruction of laboratories in physiology, embryology and ichthyology and occasional lecturer.

Editorial Board Member

"ZEBRAFISH" – Paul Collodi (Chief Editor). Mary Ann Liebert, Inc. (to begin publication in 2004).

"Reproductive Biology and Endocrinology" - Antonin Bukovsky (Chief Editor) http://www.rbej.com/home/ (acting as Advisory Editor)

Outside Research Support - Current

USDA - "Production of myostatin gene knockouts in zebrafish, and the effects of myostatin interacting proteins on salmonid muscle growth" Principal Investigator, submitted with S. Roberts and P. Collodi. 12/04-12/07. $426,000.

USDA - "Isolation and characterization of differentially regulated genes in activated trout macrophages." Principal Investigator, 12/01/03-12/01/06. $280,000.

USDA – "The zebrafish and giant danio as models for studying determinant and indeterminant growth in fish." Postdoc grant for Peggy Biga, 12/03-12/05. $89,968.

Cooperative Research Partners Initiative, NMFS NE Regional Office – "Reproductive Life History and Essential Fish Habitat Mapping of Western Georges Bank Cod: GIS mapping of eggs, larvae and juvenile cod." submitted with P. Parker, 6/1/2004-5/1/2005. $257,167. (transferred to MBL after moving to GLWI).

Massachusetts Environmental Trust - "Population genetics of the spiny dogfish in the Western Atlantic." submitted with P. Parker, G. Gerlach. 2003-2004. $25,288. (transferred to MBL after moving to GLWI).

NRAC - "Development of diagnostic and management techniques to select cod broodstocks and hatchery stocks free from nodavirus." Principal Investigator, 11/2003-11/2005. $124,612. (transferred to MBL after moving to GLWI).

Outside Research Support - Past funded projects

SEMAC - "Developing a cost-effective method to raise the banded sunfish in underutilized or seasonally fallow cranberry bogs." Principal Investigator, 03/2003-03/2004.

USDA – "Isolation and characterization of factors regulated during larval competence and metamorphosis in the bay scallop, *Argopecten irradians*" Postdoc grant for Steven Roberts, 8/02-8/04. $89,934.

USDA - "Characterization of myostatin expression in fish" Principal Investigator, 9/1/01-9/1/03. $200,000.

SEMAC - "The development of a recirculating system for the accelerated growth of quahog seed stock." Principal Investigator, 03/01/02-12/31/02. $10,508.

Illinois-Indiana Sea Grant - "The development of molecular and biochemical tools to assess changes in yellow perch growth hormone" Principal Investigator, 2/2000-3/2002. $79,295.

USDA - "Zebrafish mutagenesis: Isolation and characterization of reproductive mutants" Principal Investigator, 8/99-8/2001. $210,000.

Fulbright-US Spain Science and Technology Program Grant - "Impact of the immune response to a bacterial pathogen on the reproductive performance of female salmonid fish." Co-Principal Investigator with Dr. Jose Planas (U. Barcelona). 6/2000-6/2001. $18,800.

USDA - "Characterization of a family of ovarian and ovulation specific proteins in trout" Principal Investigator, 8/97-8/2000. $190,000.

NSF - "Isolation and characterization of progestin specific messenger RNAs from the yellow perch ovary." 8/94-8/97. Principal Investigator, $160,000.

USDA - "Characterization of an ovulation and ovarian specific mRNA from brook trout" Principal Investigator, 9/95-8/97. $164,000.

Notre Dame (Equipment Restoration and Renewal Program) - "Molecular phosphorimaging system." 11/95. Principal Investigator, $62,900.

USDA - "The 5th International Symposium on Reproductive Physiology of Fish." 3/95-2/96. Co-$7,500. (to support meeting in July, 1995).

NSF - "The 5th International Symposium on Reproductive Physiology of Fish." 3/95-2/96. $3,000. (to support meeting in July, 1995).

NIH-NICHD - "Involvement of PI cycling/protein kinase C in ovulation." Principal Investigator, 1990-1994, $293,000/3 years.

NSF - "Establishing the chemical identity, hormonal origins and biological and neural functions of the prostaglandin-derived sex pheromone in goldfish." 8/91-8/94. I was a contractor for this grant, $2,974.

NSF - "The mechanism and regulation of ovulation in fish." Principal Investigator, 1988-1989, $50,000.

NSF - Biological Instrumentation Program Grant. Co-Principal Investigator with Drs. Carpenter and Duman, 1987, $47,540.

NSF - "Mechanism and endocrine regulation of ovulation in teleosts." Principal Investigator, 1986-1988, $125,000.

Wisconsin Department of Natural Resources - "Histological preparation of chinook gonads." Principal Investigator, 1986, $9,987.

NSF - "Endocrine regulation of oocyte final maturation and ovulation in teleost fish." Principal Investigator, 1983-1986, $96,000.

NSF International (US-JAPAN Cooperative Sciences Program) - "Studies on the mechanism of ovulation in teleost fish." Principal Investigator, 1984, $19,334

Wisconsin Department of Natural Resources - "Histological preparation of sterile chinook salmon samples." Principal Investigator, 1984, $9,185.

Sport Fishery Research Foundation - "The hormonal induction of egg maturation and ovulation in marlin and tuna." Principal Investigator, 1983, $1,030.

NSF - "Endocrine control of oocyte final maturation and ovulation in yellow perch (*Perca flavescens*) and brook trout (*Salvelinus fontinalis*)." Principal Investigator, 1980-1982, $43,000.

Publications (2003-present)

McKenzie, S., Planas, J. and F.W. Goetz. (2003). LPS-stimulated expression of a tumor necrosis factor-like mRNA in primary monocytes and *in vitro* differentiated macrophages. Developmental and Comparative Immunology 27:393-400.

Roberts, S. and F.W. Goetz. (2003). Myostatin protein and RNA transcript levels in adult and developing brook trout. Molecular and Cellular Endocrinology 210:9-20.

Roberts, S. and F.W. Goetz (2003). Expressed sequence tag analysis of genes expressed in the bay scallop, Argopecten irradians. Biol Bull. 205(2):227-228.

Roberts, S., Barry, T., Malison, J. and F.W. Goetz. (2004). Production of a recombinantly-derived growth hormone antibody and the characterization of growth hormone levels in yellow perch. Aquaculture 232 (1-4): 591-602.

Goetz, F.W., Planas, J. and S. McKenzie. (2004). Tumour necrosis factors. Developmental and Comparative Immunology 28(5): 487-497.

Goetz, F.W., Norberg, B., McCauley, L. and D. Iliev. (2004). Isolation and characterization of the cod (*Gadus morhua*) steroidogenic acute regulatory (StAR) protein. Comparative Biochemistry and Physiology 137:351-362.

Goetz, F.W. 2004. The "ups" and "downs" in using subtractive cloning techniques to isolate regulated genes. Integrative and Comparative Biology 43, 786-793.

PR Biga, KD Cain, RW Hardy, GT Schelling, K Overturf, SB Roberts, FW Goetz, and TL Ott (2004). Growth hormone differentially regulates muscle myostatin1 and -2 and increases circulating cortisol in rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology 138, 32-41

Hollis, D.M., Roberts, S.B., Goetz, F.W., and Boyd, S.K. (2004). Acute neurosteroid modulation and subunit isolation of the $GABA_A$ receptor in the bullfrog, *Rana catesbeiana*. J. Mol. Endoc. 32, 921-934.

McCauley, L., Goecker, C., Parker, P., Rudolph, T., Goetz, F., Gerlach, G. (2004). Characterization and isolation of DNA microsatellite primers in the spiny dogfish (*Squalus acanthias*). Mol. Ecol. Notes 4: 494–496.

Young, G., Lokman, P.M, Kusakabe, M., Nakamura, I., and Goetz, F.W. (2004). Gonadal steroidogenesis in teleost fish. In: "Hormones and their receptors in fish reproduction; Molecular aspects of fish and marine biology" series. (ed. Choy Hew). World Scientific Press (in press).

Roberts SB, McCauley LAR, Devlin RH, Goetz FW. (2004). Myostatin expression in growth enhanced transgenic coho salmon (*Oncorhynchus kisutch*). J. Experimental Biology 207: 3741-3748.

Goetz, FW., Iliev, D., Liarte, C., McKenzie, S., Planas, J., McCauley, L. (2004). Analysis of genes isolated from lipopolysaccharide-stimulated rainbow trout (*Oncorhynchus mykiss*) macrophages. Molecular Immunology 41:1199-1210.

Hyun-Woo Kim, DL Mykles, FW Goetz, SB Roberts. (2004). Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians*. Biochim. Biophy. Res. Comm.1679:174–179.

MacKenzie, S., Liarte, L., Iliev, D., Planas, P., Tort L. and F.W. Goetz (2004). Characterization of a highly inducible novel CC chemokine from differentiated rainbow trout (*Oncorhynchus mykiss*) macrophages. Immunogenetics 56:611-615.

Goetz, F.W., McCauley, L. and Norberg, B. (2005). Using global genome approaches to address problems in cod mariculture. ICES Journal of Marine Sciences (in press).

Iliev, D., Liarte, C., MacKenzie, S., Goetz, F.W. (2005). Activation of rainbow trout (*Oncorhynchus mykiss*) mononuclear phagocytes by different pathogen associated molecular pattern (PAMP) bearing agents. Molecular Immunology (in press).

Biga, P., Roberts, S., Iliev, D., McCauley, L., Moon, J., Collodi, P., Goetz, F. (2005). The isolation, characterization, and expression of a novel Gdf11 gene and a second myostatin form in zebrafish, *Danio rerio*. Comp. Bio. Physiol, Part B (in press).

Jentoft, S., Topp, N., Seeliger, M., Malison, J., Barry, T., Held, J., Roberts, S. and Goetz, F. (2005). Lack of growth enhancement by exogenous growth hormone treatment in yellow perch (Perca flavescens) in four separate experiments. Aquaculture (in press).

Recent Invited Addresses

2004: "Analysis Of Expressed Sequence Tags Obtained From In Vitro Differentiated Rainbow Trout Macrophages Stimulated By Bacterial Lipopolysaccharides" World Aquaculture Society Meeting. Invited oral presentation in special session on "Application of Biotechnology and Molecular Tools in Aquaculture" Hawaii, March 1-4, 2004.

2004: "Transcriptomic analysis of gene expression – tools for advancing cod aquaculture" ICES symposium - Gadoid Mariculture: Development and Future Challenges. Plenary talk. Bergen, Norway 13-16 June 2004.

2004: "Using transcriptomic analysis to understand the innate immune system of fish" 5th International Symposium on Fish Endocrinology. "State of the Art" presentation. Campus of the University Jaume I of Castellon, Castellon, Spain, September 5-9, 2004.

Terry L. Wade

Deputy Director of Environmental Sciences

Specialties: Environmental Chemistry
Chemical Oceanography

Other Expertise: Methods Development
Atmospheric Deposition
Project Management
Marine Organic Geochemistry

Education: B.A. (Chemistry), Hartwick College, 1971; M.S. 1974, Ph.D. 1978 (Chemical Oceanography), University of Rhode Island.

Professional Experience: Adjunct Professor, Department of Oceanography, Texas A&M University, 2001-Present; Deputy Director of Environmental Sciences, Geochemical and Environmental Research Group, Texas A&M University, 1998-Present; Associate Director and Research Scientist, Geochemical and Environmental Research Group, Texas A&M University, 1992-present; Associate Research Scientist, Geochemical and Environmental Research Group, Texas A&M University, 1986-1992; Member, Graduate Faculty, Texas A&M University, 1986-Present; Assistant Research Scientist, Dept. of Oceanography, Texas A&M University, 1984-1986; Adjunct Assistant Professor, Dept. of Oceanography, Old Dominion University, 1984-Present; NASA-ASEE Summer Faculty Fellowship Program, NASA Langley Research, 1982; Joint Appointment, Dept. of Chemical Science, Old Dominion University 1979-1986; Assistant Professor of Oceanography, Dept. of Oceanography, Old Dominion University, 1978-1984; Research Assistant and Graduate Student, Graduate School of Oceanography, University of Rhode Island, 1971-1978; National Science Foundation Summer Research Assistant, Hamilton College, 1970.

Selected Publications (>180 Publications):

Gardinali, P.R., J.L. Sericano and T.L. Wade. 2004. Uptake and depuration of toxic aromatic halogenated hydrocarbons by the American Oyster (*Crassostrea virginica*): A field study. Chemosphere 54: 61-70.

Wade, T.L., S.T. Sweet, J.G. Quinn, R.W. Cairns, and J.W. King. 2004. Tributyltin in environmental samples from the Former Derecktor Shipyard, Coddington Cove, Newport, RI. Environmental Pollution 129: 315-320.

Mostafa, A.R., A.O. Barakat, T.L. Wade, Y. Qian, and D.X. Yuan. 2004. An Overview of Metal Pollution in the Western Harbor of Alexandria Egypt, Soil and Sediment Contamination 13: 299-311.

Hwang, H.-M., T.L. Wade, and J.L. Sericano. 2004. Destabilized lysosomes and elimination of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls in eastern oysters (*Crassostrea virginica*) Environmental Toxicology and Chemistry 23: 1991-1995.

Mostafa, A.R., A.O. Barakat, Y. Qian, and T.L. Wade. 2003. Composition, Distribution and Sources of Polycyclic Aromatic Hydrocarbons in Sediments of the Western Harbor of Alexandria, Egypt. J. Soils and Sediments (http://dx.doi.org/10.1065/jss2003.02.069): 3: 173-179.

Hwang, H.-M., T.L. Wade, and J.L. Sericano. 2003. Concentration and source characterization of polycyclic aromatic hydrocarbons in pine needles from Korea, Mexico and United States. Atmospheric Environment 37: 2259-2267.

Wade, T.L. and S.T. Sweet. 2003. Two to Five Ring Petroleum Polycyclic Aromatic Hydrocarbons: Biological Effects. In: M. McKay and J. Nides, eds., Proceedings: Twenty-first annual Gulf of Mexico information transfer meeting, January 2002. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2003-005, pp. 241-249.

Dokken, Q.R., J.W. Tunnell, C.R. Beaver, S.A. Childs, K. Withers, T.W. Bates, I.R. MacDonald, and T. Wade. 2003. Long-Term Monitoring at the East and West Flower Garden Banks National marine Sanctuary 1998-1999. In: M. McKay and J. Nides, eds., Proceedings: Twenty-first annual Gulf of Mexico information transfer meeting, January 2002. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2003-005, pp. 293-295.

Kannan, K., K.J. Hansen, T.L. Wade and J.P. Giesy. 2002. Perufluorooctane sulfonate in oysters, *Crassostrea virginica*, from the Gulf of Mexico and Chesapeake Bay, USA. Arch. Environmental Contaminant and Toxicology 42:313-318.

Hwang, H.-M., T.L. Wade, and J.L. Sericano. 2002. Relationship between lysosomal membrane destabilization and chemical body burden in eastern

oysters (*Crassostrea virginica*) from Galveston Bay, Texas. 2001. Environmental Toxicology and Chemistry 21: 1268-1271.

Park, J.-S., T.L. Wade, and S. Sweet. 2002. Atmospheric Deposition of PAHs, PCBs, and Organochlorine pesticides to Corpus Christi Bay, Texas: Role of Air-Water Gas Exchange. Atmospheric Environment 36:1707-1720.

Farrington, J.W., T.L. Wade, C.M. Reddy, and G.L. Mills. 2002. Quinn, "Quinnones", and 34 Years of Progress in the Biogeochemistry of Organic Contaminants in Marine Ecosystem. Biogeochemistry of Organic Contaminants in Aquatic Ecosystems: Honoring Dr. James G. Quinn. ACS Division of Environmental Chemistry, Preprints of Extended Abstracts 42(2):183-189.

Wade, T. L, S. T. Sweet, J. G. Quinn, R. W. Cairns, and J. W. King. 2002. The Anti-Fouling Tributyltin in Environmental Samples from the Former Dereckter Shipyard, Coddington Cove, Newport, RI. Biogeochemistry of Organic Contaminants in Aquatic Ecosystems: Honoring Dr. James G. Quinn. ACS Division of Environmental Chemistry, Preprints of Extended Abstracts 42(2):194-198.

Golomb, D., E. F. Barry, D. K. Ryan, and T. L. Wade. 2002. Wet and Dry Atmospheric Deposition of Toxic Metals and PAHs near New England Coastal Water. Biogeochemistry of Organic Contaminants in Aquatic Ecosystems: Honoring Dr. James G. Quinn. ACS Division of Environmental Chemistry Preprints of Extended Abstracts 42(2):259-261.

Wade, Terry L., Jose L. Sericano, Yaorong Qian, Gary Wolff, and Guy Denoux. 2002. Method Detection Limits: Application to Organic Environmental Chemistry Data. Principles of Environmental Sampling and Analysis -- Two Decades Later. ACS Division of Environmental Chemistry, Preprints of Extended Abstracts 42(2):658-662.

Barakat, A.O., M. Kim, Y. Qian, and T.L. Wade. 2002. Organochlorine Pesticides and PCBs Residues in Sediments of Alexandria Harbor, Egypt. Marine Pollution Bulletin 44: 1421-1434.

Santschi, P.H., B.J. Presley, T.L. Wade, B. Garcia-Romero, and M. Baskaran. 2001. Historical Contamination of PAHs, PCBs, DDTs, and Heavy Metals from Mississippi River Delta, Galveston Bay and Tampa Bay Sediment Cores. Marine Environmental Research 52:51-79.

Wade, T.L., H.-M. Hwang, Y. Qian, S.T. Sweet, and J.L. Sericano. 2001. Biomarkers of Environmental Degradation Applied to Galveston Bay, Texas. State of the Bay Symposium V, 21 January-2 February 2001, Galveston, Texas 82-92.

Wade, T.L. 2001. Sustainable Development and Environmental Chemistry. ACS Division of Environmental Chemistry, Preprints of Extended Abstracts 41. 882-886.

Wade, T.L. P.H. Santschi, B.J. Presley, B. Garcia-Romero and M. Baskaran. 2001. Environmental Trends of Contaminants from Mississippi River Delta, Tampa Bay, and Galveston Bay Sediments Cores. ACS Division of Environmental Chemistry, Preprints of Extended Abstracts 41. 890-893.

Moles, A. and T.L. Wade. 2001. Parasitsm and phagocytic function among sand lance *Ammodytes hexapterus Pallas* exposed to crude oil-laden sediments. Bulletin of Environmental Contamination and Toxicology 66(4): 528-535.

Yuan, Dongxing, Dongning Yang, Terry L. Wade, and Yaorong Qian. 2001. Status of persistent organic pollutants in the sediment from several estuaries in China. Environmental Pollution 114:101-111.

Park, J.-S., T.L. Wade, and S. Sweet. 2001. Atmospheric distribution of polycyclic aromatic hydrocarbons and deposition to Galveston Bay, Texas. Atmospheric Environment 35(19):3241-3249.

Park, J.-S., T.L. Wade, and S. Sweet. 2001. Atmospheric deposition of organochlorine contaminants to Galveston Bay, Texas. Atmospheric Environment 35(19):3315-3324.

Kim, Y., E.N. Powell, T.L. Wade, B.J. Presley, and J.M. Brooks. 2001. The Geographic Distribution of Population Health and Contaminant Body Burden in Gulf of Mexico Oysters. Archives of Environmental Contamination and Toxicology 4:30-46.

Barakat, A.O., M. Kim, Y. Qian, and T.L. Wade. 2001. Butyltin compounds in sediments from the commercial harbor of Alexandria City, Egypt. Environmental Toxicology and Chemistry 20:2744-2448.

Qian, Y., T.L. Wade, and J.L. Sericano. 2001. The Distribution and Source of Polynuclear Aromatic Hydrocarbons in Galveston Bay, TX. Estuaries 24:817-827.

CURRICULUM VITAE – STEVEN BEYER ROBERTS

Academic Experience

B.S. – North Carolina State University (Raleigh, NC) – 1997
Natural Resources - Concentration in Marine and Coastal Resources
Minor in Zoology - Honors Program - Magna Cum Laude

Ph.D. – University of Notre Dame (Notre Dame, IN) – 2002
Integrative Cell and Molecular Physiology
"Characterization of Growth Hormone in Yellow Perch and Myostatin in Several Teleost Species"
Ph.D. Advisor: Dr. Frederick Goetz

Professional Experience

2003-Present · Assistant Research Scientist
Marine Biological Laboratory, Woods Hole, MA

2002-2003 · Postdoctoral Scientist
Program in Scientific Aquaculture
Marine Biological Laboratory, Woods Hole, MA

2000-2002 · Research Assistant and Graduate Student
University of Notre Dame

1998-2000 · Teaching Assistant and Graduate Student
General Biology Laboratories - University of Notre Dame

1997-1998 · Research Assistant – Center for Marine Science Research
University of North Carolina at Wilmington

1994-1997 · Lab Technician – Zoology Department
North Carolina State University

1994-1995 · Field Technician – NOAA / EPA
Environmental Monitoring and Assessment Program of Estuaries
University of North Carolina at Wilmington

Refereed Publications

Roberts SB, Jackson LF, King WK, Taylor RG, Grier HJ, Sullivan CV. (1999) Annual reproductive cycle of the common snook: Endocrine correlates of maturation. Transactions of the American Fisheries Society. 128:426-445.

Langenau DM, Goetz FW, Roberts SB. (1999) The upregulation of messenger ribonucleic acids during 17a, 20b-dihydroxy-4-pregnen-3-one-induced ovulation in the perch ovary. Journal of Molecular Endocrinology. 23(2):137-52.

Roberts SB, Langenau DM, Goetz FW. (2000) Isolation through cloning of fish prostaglandin endoperoxide synthase (cyclooxygenase) in Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish; B Norberg, OS Kjesbu, GL Taranger, E Andersson, and SO Stefansson, editors. Bergen, Norway. July 4-9, 1999. p 197.

Moser ML, Roberts SB. (2000) Effects of nonindigenous ictalurids and recreational electrofishing on the ictalurid community of the Cape Fear River drainage, North Carolina. in Catfish 2000: Proceedings of the

International Ictalurid Symposium; ER Irwin, WA Hubert, CF Rabeni, HL Schramm, Jr., and T Coon, editors. Davenport, IA. June 23-25, 1998. pp 479-485.

Roberts SB, Langenau DM, Goetz FW. (2000) Cloning and characterization of prostaglandin endoperoxide synthase-1 and -2 from the brook trout ovary. Molecular and Cellular Endocrinology.160(1-2):89-97.

Roberts SB, Goetz FW. (2001) Differential skeletal muscle expression of myostatin across teleost species, and the isolation of multiple myostatin isoforms. FEBS Letters. Vol 491, No. 3, pp. 212-216.

Roberts SB, Goetz FW. (2003) Myostatin protein and mRNA transcript levels in adult and developing brook trout. Molecular and Cellular Endocrinology. 210 (1-2): 9-20.

Roberts SB, Goetz FW. (2003) Expressed sequence tag analysis of genes expressed in the bay scallop, *Argopecten irradians*. Biological Bulletin. 205: 227-228.

Roberts SB, Barry T, Malison J, Goetz FW. (2004) Production of a recombinantly-derived growth hormone antibody and the characterization of growth hormone levels in yellow perch. Aquaculture. Vol. 232/1-4: 591-602

Hollis DM, Goetz FW, Roberts SB, Boyd SK. (2004) Acute neurosteroid modulation and subunit isolation of the GABAa receptor in the bullfrog, *Rana catesbeiana*. Journal of Molecular Endocrinology. 32(3):921-34

Biga PR, Cain KD, Hardy RW, Schelling GT, Overturf K, Roberts SB, Goetz FW, Ott TL. (2004) Growth hormone differentially regulates muscle myostatin1 and -2 and increases circulating cortisol in rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology. Vol 138(1):32-41

Kim H-W, Mykles DL, Goetz FW, Roberts SB. (2004) Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians*. BBA – Gene Structure and Expression. 1679(2):174-9

Roberts SB, McCauley LAR, Devlin RH, Goetz FW. (2004) Transgenic salmon over-expressing growth hormone exhibit decreased myostatin transcript and protein expression. Journal of Experimental Biology. 207(Pt 21):3741-8

Jentoft S, Topp N, Seeliger M, Malison JA, Barry TP, Held JA, Roberts SB, Goetz FW. (2005) Lack of growth enhancement by exogenous growth hormone treatment in yellow perch (*Perca flavescens*) in four separate experiments. Aquaculture. *In press*

Roberts SB, Romano C, Gerlach G. (2005) Characterization of EST derived SSRs from the bay scallop, *Argopectens irradians*. Molecular Ecology Notes. *In press*

Biga PR, Roberts SB, Iliev DB, McCauley LAR, Goetz FW. (2005) The isolation, characterization, and expression profile of a novel GDF11 gene in zebrafish. BBA – Gene Structure and Expression. *In review*

Roberts SB. (2005) Developmental stage-specific gene expression in the bay scallop. *In preparation*

Selected Non-refereed Publications

Moser ML, Bichy JB, Roberts, SB. (1998) Sturgeon distribution in North Carolina. Center for Marine Science Research, Wilmington, North Carolina. Final report to the United States Army Corps of Engineers, Wilmington District. 88 pp.

Roberts SB, Goetz FW. (2003) Genes involved with growth and development in the bay scallop (Extended Abstract) Proceedings of the 14th International Pectinid Workshop, April 23-29, St. Petersburg, FL, USA. Pg 137

Ettensohn K, Biga PR, Romano C, Devlin RH, and Roberts SB. (2004) Genes Differentially Expressed in Growth Hormone Transgenic Salmon (Abstract). Biological Bulletin 2004 207: 168

Roberts SB, Goetz FW. (2004) Quantitative real-time PCR methods for disease detection in marine fish. Proceedings of Aquaculture 2004, March 1-5, Honolulu, HI. pg 503 (Abstract)

Roberts SB (2004) Lab Studies: Genes Involved with Growth, Development of Bay Scallops. Global Aquaculture Advocate. 7(3): 55-56

Ongoing Research Support

03-17/556808 11/01/03 – 10/31/05
Northeast Regional Aquaculture Center
Development of diagnostic and management techniques to select cod broodstocks and hatchery stocks free from nodavirus.
$124,612

2004-04533 01/01/05 – 01/01/08
United States Department of Agriculture
Production of myostatin gene knockouts in zebrafish, and the effects of specific myostatin interacting proteins on salmonid muscle growth.
$426,000

04-1-3 02/01/05 – 01/01/07
Northeast Regional Aquaculture Center
Development of genetic markers to assess disease resistance in the Eastern oyster
$125,486

Past Research Support

2003-35206-12834 11/01/02 – 10/31/04
United States Department of Agriculture
Isolation and characterization of factors regulated during larval competence and metamorphosis in the bay scallop, *Argopecten irradians*.
$89,934

Barnstable County, Massachusetts 06/31/04 – 12/31/04
The use of microsatellite markers to improve bay scallop stock enhancement efforts
$20,000

Professional Activities

Pan American Marine Biotechnology Association
World Aquaculture Society
National Shellfish Association
Sigma Xi Scientific Research Society
American Fisheries Society

Reviewer for:
BARD, the United States - Israel Binational Agricultural Research & Development Fund
Maryland Sea Grant
National Sea Grant College Program: Oyster Disease Program
USDA National Research Initiative: Animal Growth and Nutrient Utilization
USDA National Research Initiative: Animal Reproduction

Undergraduate Students Mentored:
Eric Pilsmaker; Massachusetts Maritime Academy
Michelle Short; Hamilton College
Patrice Pazar, University of Colorado
Phoenix Becker; University of Maine
Adam Bisonette; St. Anselm College
Kristen Ettensohn; Dartmouth College
Carly Allen; University of Hawaii
Angela Sampson; Massachusetts Maritime Academy
Chris Dickson; University of Colorado

CURRICULUM VITAE - Paul J. Rago

Leader, Stock Assessment Methods Task
Northeast Fisheries Science Center
National Marine Fisheries Service
166 Water St.
Woods Hole, Massachusetts

Education

Ph.D. University of Michigan
1986 Rackham Graduate School (Natural Resources) Chairmen: J.T. Lehman, P.W. Webb

M.S. Colorado State University
1978 College of Forestry and Natural Resources (Fisheries Biology) Chairman: G.M. Van Dyne

B. S. University of Michigan , 1974 School of Natural Resources(Quantitative Methods)

Research Interests

- Dynamics of exploited fish and shellfish populations, especially non-aged based approaches.
- Estimation of abundance and mortality rates using modern statistical procedures.
- Graphical methods for Exploratory Data Analysis esp. non-parametric smoothing and spatial statistics.
- Simulation models for testing statistical models, forecasting, and evaluation of harvest policies.
- Application of tagging models.

Biographical Sketch

Paul Rago is a Research Fishery Biologist at the Northeast Fishery Science Center of the National Marine Fisheries Service in Woods Hole. He is currently leader of the Stock Assessment Methods Group. In addition to spiny dogfish, he has worked on a wide variety of stock assessments, including scallops, lobsters, surf clams, ocean quahogs, monkfish, and yellowtail flounder. He has been active in a number of cooperative experiments with the fishing industry and the incorporation of vessel monitoring system data into stock assessments. Prior to working with National Marine Fisheries Service, Rago spent 15 years with the US Fish and Wildlife Service where he served as coordinator of the Emergency Striped Bass Study and a variety of Atlantic salmon studies.

Relevant Publications

Rago, 2004 Fishery independent sampling: survey techniques and data analyses. Chapter 12 in J. Musick and R. Bonfil, ed. Elasmobranch Fisheries Management

Techniques. Asia-Pacific Economic Cooperation Fisheries Working Group. International Union of Concerned Scientists.

Murawski, S., P. Rago, and M. Fogarty. 2004. Spillover effects from temperate marine protected areas. American Fisheries Society Symposium 42:167-184.

Smith, S. J. and P. Rago 2004. Review of biological reference points for sea scallops: the benefits and costs of being nearly sessile. Canadian Journal of Fisheries and Aquatic Sciences 61:1338-1354.

Weinberg, J. R., P. J. Rago, W. W. Wakefield, C. Keith. 2002. Estimation of tow distance and spatial heterogeneity using data from inclinometer sensors: an example using a clam survey dredge. Fisheries Research 55:49-61.

Rago, P. J. 2001. Use of index measures for assessing stock status in Atlantic salmon. pp. 137-176. in E. Prevost and G. Chaput ed. Stock, recruitment and reference points: assessment and management of Atlantic salmon. INRA Editions, Paris. Book chapter.

O'Brien, L. , P. J. Rago, R. G. Lough, and P. Berrien. 2003. Incorporating early-life history parameters in the estimation of the stock-recruit relationship of Georges Bank Atlantic cod (*Gadus morhua*). J. Northw. Atl. Fish. Sci. 33:191-205.

Murawski, S. A., P. J. Rago, and E. A. Trippel. 2001. Impacts of demographic variation in spawning characteristics on reference points for fishery management. ICES Journal of Marine Science 58:1002-1014.

Brodziak, J. K. T., W. J. Overholtz, and P. J. Rago. 2001. Does spawning stock affect recruitment of New England groundfish? CJFAS 58:306-318.

Rago, P. J., K.A. Sosebee, J. K. T. Brodziak, S. A. Murawski, and E. D. Anderson. 1998. Implications of recent catches on the dynamics of Northwest Atlantic spiny dogfish (Squalus acanthias). Fisheries Research 39:165-181.

Brodziak, J., P. Rago, and R. Conser. 1998. A general approach for making short-term stochastic projections from an age-structured fisheries assessment model. pp. 933-954. Fishery Stock Assessment Models, Alaska Sea Grant College Program-AK-SG-98-01.

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Education

Ph.D. (Mathematics), California Institute of Technology, 1983.

S.B. (Mathematics), University of Chicago, 1978.

Positions Held

Operations Research Analyst, Population Dynamics Branch, Northeast Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Woods Hole MA, March 1999 - present. Chief stock assessment scientist for U.S. Georges Bank and Mid-Atlantic sea scallop (*Placopecten magellanicus*) stocks. Member, New England Fishery Management Council Sea Scallop Plan and Development Team, NEFSC Invertebrate Subcommittee.

Research Scientist, Faculty of Life Sciences, Tel Aviv University, April 1995 - Feb. 1999.

Assistant Professor of Mathematics, Knox College, Sept. 1988 - March 1995.

Visiting Scientist, Environmental Sciences Division, Oak Ridge National Laboratory, July 1992 - June 1993; Resident Faculty, ACM/GLCA Oak Ridge Science Semester, 1992.

Assistant Professor of Mathematics, Rhodes College, Sept. 1985 - May 1988.

Visiting Assistant Professor of Mathematics, Texas A&M University, Sept. 1983 - May 1985.

Current professional interests

Spatial dynamics of fisheries

Rotational fishery management strategies

Population dynamics and fishery management for the sea scallop *Placopecten magellanicus*

Food web and predator-prey theory

Intraguild predation

Benthic marine ecology

L. C. Hendrickson and D. R. Hart. 2000. An age-based cohort model for estimating the spawning mortality of semelparous cephalopods with an application to per-recruit calculations for the northern shortfin squid, *Illex illecebrosus*. Accepted to *Fisheries Research*, subject to minor revisions.

D. R. Hart and S. X. Cadrin. 2004. Yellowtail flounder (*Limanda ferruginea*) off the northeastern United States: Implications for movement among stocks. Pages 230-244 in: H. R. Akakaya, M.A. Burgman, O. Kindvall, C. Wood, P. Sjgren-Gulve, J. Hatfield, and M.A. McCarthy, eds. *Species Conservation and Management: Case Studies*. Oxford University Press.

D. R. Hart and A. S. Chute. 2004. Essential fish habitat source document: Sea scallop, *Placopecten magellanicus*, life history and habitat characteristics, 2nd ed. NOAA Technical Memorandium NMFS NE-189.

D. R. Hart. 2003. Yield- and biomass-per-recruit analysis for rotational fisheries, with an application to Atlantic sea scallop (*Placopecten magellanicus*). *Fishery Bulletin*, **101**:44-57.

D. R. Hart. 2002. Intraguild predation, invertebrate predators, and trophic cascades in lake food webs. *Journal of Theoretical Biology*, **218**:111-128.

D. R. Hart. 2001. Individual-based yield-per-recruit analysis, with an application to the sea scallop *Placopecten magellanicus*. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**:2351-2358.

D. R. Hart, L. Stone and T. Berman. 2000. Seasonal dynamics of the Lake Kinneret food web: the importance of the microbial loop. *Limnology and Oceanography*, **45**:350-361.

D. R. Hart, P.J. Mulholland, E.R. Marzolf, D.L. DeAngelis and S.P. Hendricks. 1999. Relationships between stream hydraulic parameters under varying flow and seasonal conditions. *Hydrological Processes*, **13**:1497-1510.

L. Stone and D. Hart. 1999. Effects of immigration on the dynamics of simple population models. *Theoretical Population Biology*, **55**:227-234.

E. Shochat, D. Hart and Z. Agur. 1999. Using computer simulations for evaluating the efficacy of breast cancer chemotherapy protocols. *Mathematical Models and Methods in Applied Science*, **9**:599-615.

D. Hart, E. Shochat and Z. Agur. 1998. Use of mammography screening trials data for inferring the growth law of primary breast cancer. *British Journal of Cancer* **78**:382-387.

D. Hart, L. Stone, A. Stern, D. Straile and U. Gaedke. 1997. Methods for constructing and balancing ecosystem flux charts: new techniques and software. *Environmental Modelling and Assessment*, **2**:23-28.

P. J. Mulholland, E. R. Marzolf, J. R. Webster, D. R. Hart and S. P. Hendricks. 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forested streams. *Limnology and Oceanography*, **42**:443-451.

D. R. Hart. 1995. Parameter estimation and stochastic interpretation of the transient storage model for solute transport in streams. *Water Resources Research*, **31**:323-328.

P. J. Mulholland, A. D. Steinman, E. R. Marzolf, D. R. Hart and D. L. DeAngelis. 1994. Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. *Oecologia*, **98**:40-47.

R. Fraga (editor), D. Hart, E. Herman, J. Stein and L. Welch. 1993. *Calculus Problems for a New Century*. Mathematical Association of America, Washington.

A. Gutek, D. Hart, J. Jamison and M. Rajagopalan. 1991. Shift operators on Banach spaces. *Journal of Functional Analysis*, **101**:97-119.

W. Arendt and D. R. Hart. 1986. The spectrum of quasi-invertible disjointness preserving operators. *Journal of Functional Analysis*, **68**:149-167.

D. R. Hart. 1985. Some properties of disjointness preserving operators. *Proc. Nederl. Acad. Wetensch.*, Series A **88** (also published as *Indagationes Mathematicae* **47**):183-197.

Submitted or in preparation

D. R. Hart and P. J. Rago. Long-term dynamics of U.S. sea scallop (*Placopecten magellanicus*) populations. Submitted to *North American Journal of Fishery Management*.

D. R. Hart. Effects of sea star and crab predation on sea scallop (*Placopecten magellanicus*) recruitment in the Mid-Atlantic Bight. Submitted to *Marine Ecology Progress Series*

S. Murawski, J. Brodziak, C. Legault, R. Brown, S. Cadrin, D. Hart, R. Mayo, L. O'Brien, P. Rago and M. Sissenwine. Re-shifting baselines in natural resource management: goals for rebuilding chronically overfished stocks. Submitted to: *Canadian Journal of Fisheries and Aquatic Sciences*.

D. R. Hart. Modelling adaptive rotational fishing strategies for the sea scallop *Placopecten magellanicus*.

Reports and Published Abstracts

D. R. Hart, and P. J. Rago. 2003. Rebuilding and management of sedentary stocks using area closures and rotational fishing: The Atlantic sea scallop example. Pg. 11 in: P.M. Mace (ed) Proceedings of the seventh NMFS national stock assessment workshop: Rebuilding sustainable fisheries and marine ecosystems. NOAA Tech. Memo. NMFS-F/SPO-62.

Hart, D.R. 2003. Overfishing definitions for sedentary stocks with rotational fishing or area closures. Pg. 54 in: S.J. Smith (ed.) Workshop on Reference Points for Invertebrate Fisheries held in Halifax, NS, 2-5 December 2002: Abstracts and Proceedings. *Can. Tech. Rep. Fish. Aquat. Sci.* **2448** viii+ 62 p.

J. S. Link and J. K. T. Brodziak (ed), with contributions by: J.K.T. Brodziak, D. D. Dow, S. F. Edwards, M. C. Fabrizio, M. J. Fogarty, D. Hart, J. W. Jossi, J. Kane, K. L. Lang, C. M. Legault, J. S. Link, S. A. MacLean, D. G. Mountain, J. Olson, W. J. Overholtz, D. L. Palka, and T. D. Smith. 2002. Status of the Northeast U.S. Continental Shelf Ecosystem: A Report of the Northeast Fisheries Science Center's Ecosystem Status Working Group. *Northeast Fisheries Science Center Reference Document* **02-11.**

D. Hart and P. Rago. 2002. Rebuilding sea scallop (*Placotopecten magellanicus*) stocks using area closures and rotational fishing. *Journal of Shellfish Research* **21**:416.

P. J. Rago and D. R. Hart. 2002. Fleet dynamics of the Atlantic sea scallop fishery. *Journal of Shellfish Research* **21**:416-7.

D. Hart. 2001. Sea scallops, In: *Status of the fishery resources off the Northeastern United States*, available at http://www.nefsc.nmfs.gov/sos/spsyn/iv/scallop/

D. Hart. 2000. Biological Projections, pg. 159-186 in: New England Fishery Management Council Sea Scallop Plan and Development Team, 2000 Scallop Stock Assessment and Fishery Evaluation Report. New England Fishery Management Council, Newburyport MA.

D. Hart. 2000. Trophic Cascades and Intraguild Predation in Aquatic Food Webs. In: P. Mace (ed) Proceedings of the 6th NMFS National Stock Assessment Workshop: Incorporating ecosystem considerations into stock assessments and management advice. What are the pros and cons of going beyond a single species?? *NOAA Technical Memorandum NMFS-F/SPO-46*

D. Hart. 1999. Biological Projections, pg. 81-112 in: New England Fishery Management Council Sea Scallop Plan and Development Team, 1999 Scallop Stock Assessment and Fishery Evaluation Report. New England Fishery Management Council, Newburyport MA.

D. Hart. 1997. "Food Web Explorer" users manual.

Software development

L. D. Jacobson and D. R. Hart. 2004. CASA-Nuevo, a length-structured stock assessment model (based on the CASA model of P.J. Sullivan et al.).

D. Hart. 1999-2003. Sea scallop area management simulator (SAMS). Projects future catches, abundance and size of sea scallops based on area specific mortality, growth, and recruitment.

A. Stern, G. Landan, S. Barry, D. Hart, L. Stone and S. Hochstäder. 1997. Food Web Explorer. A program for creating food web flux charts that are mass-balanced both in energy and nutrient flow. Algorithms discussed in Hart et al. (1997) *Envir. Model. Assess.* **2**:23-28.

D. Hart. 1993. Solute-fit. A program that models solute transport in streams including storage zones, using the stochastic model described in Hart (1995) *Wat. Resor. Res.* **31**:323-328. It includes a fitting algorithm that finds the parameters that best fit the results of solute tracer experiments.

The above programs were developed in AD-Model Builder/C++, Fortran-90 and Pascal. Also have knowledge of Basic, VBA, SQL-Plus, Matlab, Maple, Mathematica, Excel, Statistica, SAS, Minitab.

Grants and Awards

CMER Grant 2003-2005 (with J. Quinlan and J. Wilkin, Rutgers University), "Investigating Sea Scallop (*Placopecten magellanicus*) Population Dynamics Under A Management Strategy Featuring Closed Areas"

Gileadi Fellowship, 1998-1999.

Karen Doron Equipment Grant, 1997.

Howard Hughes Medical Institute summer faculty fellow grant, 1993.

Pew Foundation visiting scholar grants, 1991 and 1994.

Bohenblust graduate student prize, Dept. of Mathematics, California Institute of Technology, 1982.

Stock Assessments and Fisheries Management

Chief stock assessment scientist for sea scallop (*Placopecten magellanicus*) assessments for U.S. Georges Bank and the Mid-Atlantic Bight (SARC-32, 2000, and SARC-39, 2004). Developed new version of CASA dynamic length-based model for sea scallops (with L. Jacobson).

I. Biographical Sketch

FREDERICK W. GOETZ
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University of Wisconsin-Milwaukee
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Academic Experience

Ph.D.	University of Wyoming Laramie, Wyoming	1976 (Zoology)
B.A.	Colgate University Hamilton, N.Y	1972 (Biology and German)

Advisor: Dr. Harold Bergman.

Professional Experience

2004 - present	Great Lakes WATER Institute University of Wisconsin Milwaukee	Senior Scientist
2004 - present	University of Wisconsin Milwaukee Biology Department.	Adjunct Professor
2001 - 2003	Marine Biological Laboratory Program in Scientific Aquaculture Woods Hole, MA	Senior Scientist/Director
2001 - present	Boston University Marine Biology Program	Adjunct Professor
1989 – 2001	University of Notre Dame Department of Biological Sciences	Professor/ Director of Graduate Studies
1984 - 1989	University of Notre Dame Department of Biological Sciences	Associate Professor/ Director of Graduate Studies
1977 - 1984	University of Notre Dame Department of Biological Sciences	Assistant Professor
1976 – 1977	Fisheries and Marine Service West Vancouver, British Columbia Canada.	Postdoctoral Fellow
1974 - 1976	University of Wyoming	Research Assistant
1972 - 1974	University of Wyoming	Teaching Assistant

Publications

Goetz, F.W., McCauley, L. and Norberg, B. (2005). Using global genome approaches to address problems in cod mariculture. ICES Journal of Marine Sciences (in press).

Goetz, F.W. 2004. The "ups" and "downs" in using subtractive cloning techniques to isolate regulated genes. Integrative and Comparative Biology 43, 786-793.

Goetz, FW., Iliev, D., Llarte, C., McKenzie, S., Planas, J., McCauley, L. (2004). Analysis of genes isolated from lipopolysaccharide-stimulated rainbow trout (*Oncorhynchus mykiss*) macrophages. Molecular Immunology 41:1199-1210.

Hyun-Woo Kim, DL Mykles, **FW Goetz**, SB Roberts. (2004). Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians*. Biochim. Biophy. Res. Comm.1679:174–179.

Bobe, J. and **F.W. Goetz**, (2000). A S100 homologue mRNA isolated by differential display PCR is down regulated in the brook trout (*Salvelinus fontinalis*) postovulatory ovary. Gene 257:187-194.

Roberts, S. and **F.W. Goetz** (2003). Expressed sequence tag analysis of genes expressed in the bay scallop, *Argopecten irradians*. Biol Bull. 205(2):227-228.

Fullerton, A., G.A. Lamberti, D.M. Lodge and **F.W. Goetz**. (2001). Resource competition between eurasian ruffe and yellow perch: Growth and RNA responses in laboratory experiments. Transactions of the American Fisheries Society 129:1331-1339.

Goetz, F.W., Planas, J. and S. McKenzie. (2004). Tumour necrosis factors. Developmental and Comparative Immunology 28(5): 487-497.

Goetz, F.W., Norberg, B., McCauley, L. and D. Iliev. (2004). Isolation and characterization of the cod (*Gadus morhua*) steroidogenic acute regulatory (StAR) protein. Comparative Biochemistry and Physiology 137:351-362.

McKenzie, S., Planas, J. and **F.W. Goetz**. (2003). LPS-stimulated expression of a tumor necrosis factor-like mRNA in primary monocytes and *in vitro* differentiated macrophages. Developmental and Comparative Immunology 27:393-400.

Collaborators

Biga, Peggy (Great Lakes WATER Institute)
Binkowski, Fred (Great Lakes WATER Institute)
Bobe, Julien (Institut Nationale Research Agronomique, France)
Collodi, Paul (Purdue University)
Gerlach, Gabi (Marine Biological Laboratory)
Hollis, David (Great Lakes WATER Institute)
Iliev, Dimitar (Great Lakes WATER Institute)
Klapper, Rebecca (Great Lakes WATER Institute)
Llarte, Cristina (Universitat Autonoma de Barcelona, Spain)
Lindel, Scott (Marine Biological Laboratory)
Malison, Jeff (University of Wisconsin)
McCauley, Linda (WHOI)
MacKenzie, Simon (Universitat Autonoma de Barcelona, Spain)
Norberg, Birgitta (Austevoll Aquaculture Research Station)
Planas, Josep (Universitat de Barcelona, Spain)
Rise, Matt (Great Lakes WATER Institute)
Roberts, Steven (Marine Biological Laboratory)
Swanson, Penny (Northwest Fisheries Science Center, NOAA)
Winton, Jim (Western Fisheries Research Center, USGS)
Young, Graham (University of Washington)

Theses Directed

DeNeff, S.J. (1980-MS)	Cetta, F. (1983-MS)	Li, Z. (1993-MS)
Hajnik, C. (1998-MS)	Langenau, D. (1998-MS)	Theofan, G. (1981-PhD)
DeManno, D. (1987-PhD)	Berndtson, A. (1988-PhD)	Ranjan, M. (1989-PhD)
Hsu, S-Y (1993-PhD)	Coffman, M. (1999-PhD)	Bobe, J. (2001-PhD)
Bauer, M. (2001-PhD)	Roberts, S. (2002)	Iliev, D. (PhD ongoing)

Postdocs Advised

Steven Roberts (Marine Biological Laboratory)
Peggy Biga (Great Lakes Water Institute)

I. Biographical Sketch

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Professional Preparation

Ph.D. University of Rhode Island, Kingston, Rhode Island, 1978 (Chemical Oceanography)
M.S. University of Rhode Island, Kingston, Rhode Island, 1974 (Chemical Oceanography)
B.A. Hartwick College, Oneonta, New York, 1971 (Chemistry)

Appointments

1998-Present	Texas A&M University, College of Geosciences Geochemical and Environmental Research Group	Deputy Director
2001-Present	Texas A&M University, Department of Oceanography	Adjunct Professor
1993-1998	Texas A&M University, College of Geosciences Geochemical and Environmental Research Group	Associate Director
1992-Present	Texas A&M University, College of Geosciences Geochemical and Environmental Research Group	Research Scientist
1986-1992	Texas A&M University, College of Geosciences Geochemical and Environmental Research Group	Associate Research Scientist
1986-Present	Texas A&M University, Graduate Faculty	Member
1984-1986	Texas A&M University, Dept. of Oceanography	Assistant Research Scientist
1984-1986	Old Dominion University, Norfolk, VA Dept. of Oceanography	Adjunct Assistant Professor
1979-1984	Old Dominion University, Norfolk, VA Dept. of Chemical Science,	Joint Appointment
1978-1984	Old Dominion University, Norfolk, VA Dept. of Oceanography	Assistant Professor
1971-1978	University of Rhode Island, Kingston, RI School of Oceanography	Research Assistant/ Graduate Student

Publications

Wade, T.L. and J.G. Quinn. 1979. Geochemical distribution of hydrocarbons in sediments from Mid-Narragansett Bay, Rhode Island. Org. Geochem., 1:157-167.

Summers, J.K., **T.L. Wade**, V.D. Engle, and Z.A. Malaeb. 1996. Normalization of Elemental Concentrations of Contaminants in Estuarine Sediments of the Gulf of Mexico. Estuaries. 19: 581-594.

Park, J.-S., **T.L. Wade**, and S. Sweet. 2001. Atmospheric distribution of polycyclic aromatic hydrocarbons and deposition to Galveston Bay, Texas. Atmospheric Environment 35(19):3241-3249.

Park, J.-S., **T.L. Wade**, and S. Sweet. 2001. Atmospheric deposition of organochlorine contaminants to Galveston Bay, Texas. Atmospheric Environment 35(19):3315-3324.

Barakat, A.O., M. Kim, Y. Qian, and **T.L. Wade**. 2001. Butyltin compounds in sediments from the commercial harbor of Alexandria City, Egypt. Environmental Toxicology and Chemistry 20:2744-2448.

Qian, Y., **T.L. Wade**, and J.L. Sericano. 2001. The Distribution and Source of Polynuclear Aromatic Hydrocarbons in Galveston Bay, TX. Estuaries 24:817-827.

Kannan, K., K.J. Hansen, **T.L. Wade** and J.P. Giesy. 2002. Perufluorooctane sulfonate in oysters, Crassostrea virginica, from the Gulf of Mexico and Chesapeake Bay, USA. Arch. Environmental Contaminant and Toxicology 42:313-318.

Hwang, H.-M., **T.L. Wade**, and J.L. Sericano. 2002. Relationship between lysosomal membrane destabilization and chemical body burden in eastern oysters (Crassostrea virginica) from Galveston Bay, Texas. 2001. Environmental Toxicology and Chemistry 21: 1268-1271.

Mostafa, A.R., A.O. Barakat, **T.L. Wade**, Y. Qian, and D.X. Yuan. 2004. An Overview of Metal Pollution in the Western Harbor of Alexandria Egypt, Soil and Sediment Contamination 13:299-311.

Santschi, P.H., B.J. Presley, **T.L. Wade**, B. Garcia-Romero, and M. Baskaran. 2001. Historical Contamination of PAHs, PCBs, DDTs, and Heavy Metals from Mississippi River Delta, Galveston Bay and Tampa Bay Sediment Cores. Marine Environmental Research 52:51-79.

Moore, M.N., D.M. Lowe, **T. Wade**, R.J. Wedderburn, M.H. Depledge, G. Balashov, H. Büyükgüngör, H. Özkoc, Y. Daurova, Y. Denga, E. Kostylev, P. Mihnea, C. Ciocanu, S. Moncheva, and S. Tabagari. 1999. The UNESCO/IOC "Black Sea Mussel Watch Pilot Study": Biological Effects and Contaminant Residues. In: L.D. Mee and G. Topping, eds. Black Sea Pollution Assessment. Black Sea Environmental Series Vol. 10. United Nations Publications. New York. pp. 279-299.

Collaborators and Other Affiliations

Assem Barakat, Alexandria University, Egypt;
John Farrington; WHOI
Piero Gardinali, Florida International University
Frederick Goetz, Great Lakes WATER Institute
Hyun-Min Hwang, University of CA, Davis
K. Kannan, NY State Department of Health
Alla Mostafa, Alexandria University, Egypt
June Soo Park, University of CA, Davis
Eric Powell, Rutgers University
David Velinsky, Philadelphia Academy of Natural Sciences
Dongxing Yuan, Xiamen University, China

Advisor

Graduate Advisor: James G. Quinn, URI

I. Biographical Sketch

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Academic Experience

Ph.D. – University of Notre Dame Notre Dame, IN – 2002
(Integrative Cell and Molecular Physiology)

B.S. – North Carolina State University Raleigh, NC – 1997
(Natural Resources - Concentration in Marine and Coastal Resources)

Professional Experience

2003-Present	Marine Biological Laboratory Woods Hole, MA	Assistant Research Scientist
2002-2003 ·	Marine Biological Laboratory Program in Scientific Aquaculture	Postdoctoral Scientist
2000-2002 ·	University of Notre Dame	Research Assistant / Graduate Student
1998-2000 ·	University of Notre Dame General Biology	Teaching Assistant / Graduate Student
1997-1998 ·	University of North Carolina at Wilmington Center for Marine Science Research	Research Assistant
1994-1997 ·	North Carolina State University Zoology Department	Lab Technician
1994-1995 ·	University of North Carolina at Wilmington Environmental Monitoring and Assessment Program of Estuaries	Field Technician (NOAA / EPA)

Relevant publications

Roberts SB, Romano C, Gerlach G. (2005) Characterization of EST derived SSRs from the bay scallop, *Argopectens irradians*. Molecular Ecology Notes. *In press*
Roberts SB (2004) Lab Studies: Genes Involved with Growth, Development of Bay Scallops. Global Aquaculture Advocate. 7(3): 55-56
Ettensohn K, Biga PR, Romano C, Devlin RH, and **Roberts SB**. (2004) Genes Differentially Expressed in Growth Hormone Transgenic Salmon. Biological Bulletin 2004 207: 168
Kim H-W, Mykles DL, Goetz FW, **Roberts SB**. (2004) Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians*. BBA – Gene Structure and Expression. 1679(2):174-9
Roberts SB, Goetz FW. (2003) Expressed sequence tag analysis of genes expressed in the bay scallop, *Argopecten irradians*. Biological Bulletin. 205: 227-228.
Roberts SB, McCauley LAR, Devlin RH, Goetz FW. (2004) Transgenic salmon over-expressing growth hormone exhibit decreased myostatin transcript and protein expression. Journal of Experimental Biology. 207(Pt 21):3741-8
Hollis DM, Goetz FW, **Roberts SB**, Boyd SK. (2004) Acute neurosteroid modulation and subunit isolation of the GABAa receptor in the bullfrog, *Rana catesbeiana*. Journal of Molecular Endocrinology. 32(3):921-34
Roberts SB, Barry T, Malison J, Goetz FW. (2004) Production of a recombinantly-derived growth hormone antibody and the characterization of growth hormone levels in yellow perch. Aquaculture. Vol. 232/1-4: 591-602
Roberts SB, Goetz FW. (2003) Myostatin protein and mRNA transcript levels in adult and developing brook trout. Molecular and Cellular Endocrinology. 210 (1-2): 9-20.
Roberts SB, Goetz FW. (2001) Differential skeletal muscle expression of myostatin across teleost species, and the isolation of multiple myostatin isoforms. FEBS Letters. Vol 491, No. 3, pp. 212-216

Collaborators

Anderson, Robert (Chesapeake Biological Laboratory)
Barry, Terry (University of Wisconsin-Madison)
Biga, Peggy (Great Lakes WATER Research Institute)
Boyd, Sunny (University of Notre Dame)
Collier, Tracey (National Marine Fisheries Service)
Collodi, Paul (Purdue University)
Devlin, Robert (Fisheries and Oceans Canada)
Gerlach, Gabriele (Marine Biological Laboratory)
Goetz, Rick (Great Lakes WATER Research Institute)
Hollis, Dave (Great Lakes WATER Research Institute)
Iliev, Dimitar (Great Lakes WATER Research Institute)
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Leavitt, Dale (Roger Williams College)
Malison, Jeff (University of Wisconsin-Madison)
McCauley, Linda (Woods Hole Oceanographic Institute)
Mykles, Don (Colorado State University)
Rago, Paul (National Marine Fisheries Service)
Smolowitz, Roxanne (Marine Biological Laboratory)
Waite, Terry (Texas A & M University)

Students or Post-docs:
NONE

Thesis Advisor and Post-doc Sponsor:
Goetz, Frederick (Great Lakes WATER Research Institute)

I. Biographical Sketch

Paul J. Rago
Leader, Stock Assessment Methods Task
Northeast Fisheries Science Center
National Marine Fisheries Service
166 Water St.
Woods Hole, Massachusetts

Education

Ph.D.	University of Michigan Rackham Graduate School	1986 (Natural Resources)
M.S.	Colorado State University College of Forestry and Natural Resources	1978 (Fisheries Biology)
B. S.	University of Michigan School of Natural Resources	1974 (Quantitative Methods)

Biographical Sketch

Northeast Fishery Science Center National Marine Fisheries Service Woods Hole.	Research Fishery Biologist
US Fish and Wildlife Service Emergency Striped Bass Study/ A variety of Atlantic salmon studies.	Coordinator

Relevant Publications

Rago, P., 2004 Fishery independent sampling: survey techniques and data analyses. Chapter 12 in J. Musick and R. Bonfil, ed. Elasmobranch Fisheries Management Techniques. Asia-Pacific Economic Cooperation Fisheries Working Group. International Union of Concerned Scientists.

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Brodziak, **J.P. Rago**, and R. Conser. 1998. A general approach for making short-term stochastic projections from an age-structured fisheries assessment model. pp. 933-954. Fishery Stock Assessment Models, Alaska Sea Grant College Program- AK-SG-98-01.

Collaborators

I have had no collaboration with investigators working on marine pollution in the last 48 months. I have had extensive interactions with scientific staff within the Northeast Fisheries Science Center, state fisheries management staff, and fishery management Council staff over the past four years.

Thesis advisor or postdoctoral scholars sponsored:

Todd Gedamke, College of William and Mary.
Claire Murrin, Boston University
John Walter, College of William and Mary

Graduate and postgraduate advisors:

J. T. Lehman, University of Michigan
P.W. Webb, University of Michigan
J. Diana, University of Michigan

I. Biographical Sketch

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Education

Ph.D.	California Institute of Technology	1983 (Mathematics)
S.B.	University of Chicago	1978 (Mathematics)

Positions Held

1999 - present.	Northeast Fisheries ScienceCenter National Marine Fisheries Service NOAA Woods Hole MA	Operations Research Analyst
1995 – 1999	Tel Aviv University Faculty of Life Sciences	Research Scientist
1988 – 1995	Knox College Department of Mathematics	Assistant Professor
1992 – 1993	Oak Ridge National Laboratory Environmental Sciences Division	Visiting Scientist
1992	ACM/GLCA Oak Ridge	Resident Faculty,
1985 – 1988	Rhodes College Department of Mathematics	Assistant Professor
1983 – 1985	Texas A&M University Department of Mathematics	Visiting Assistant Professor

Publications

L. C. Hendrickson and **D. R. Hart**. 2005. An age-based cohort model for estimating the spawning mortality of semelparous cephalopods with an application to per-recruit calculations for the northern shortfin squid, *Illex illecebrosus*. Accepted to Fisheries Research, subject to minor revisions.

D. R. Hart and S. X. Cadrin. 2004. Yellowtail flounder (Limanda ferruginea) off the northeastern United States: Implications for movement among stocks. Pages 230-244 in: H. R. Akakaya, M.A. Burgman, O. Kindvall, C. Wood, P. Sjgren-Gulve, J. Hatfield, and M.A. McCarthy, eds. Species Conservation and Management: Case Studies. Oxford University Press.

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D. R. Hart, L. Stone and T. Berman. 2000. Seasonal dynamics of the Lake Kinneret food web: the importance of the microbial loop. Limnology and Oceanography, 45:350-361.

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L. Stone and **D. Hart**. 1999. Effects of immigration on the dynamics of simple population models. Theoretical Population Biology, 55:227-234.

E. Shochat, **D. Hart** and Z. Agur. 1999. Using computer simulations for evaluating the efficacy of breast cancer chemotherapy protocols. Mathematical Models and Methods in Applied Science, 9:599-615.

J. CURRENT AND PENDING SUPPORT

While all of the investigators on this proposal have a number of funded and pending projects, they all will have sufficient time available to commit the necessary time for the current study. It should be noted that some of the projects listed for some of the investigators will be completed by the start of the current project if it is funded (e.g., Roberts: *Development of diagnostic and management techniques to select cod broodstocks and hatchery stocks free from nodavirus)* and, therefore, time will become available for future projects. In addition, the primary commitments of all investigators on this proposal are research so they do not have strong commitments elsewwhere. Paul Rago and Deborah Hart are not included in current and pending since they are NOAA employees and since they are already committed to the Sea Scallop surveys in terms of time.

Frederick W. Goetz

Current

Isolation and characterization of differentially-regulated genes in activated rainbow trout macrophages. FW Goetz, PI. (20%) Funding period: 01/04 – 12/05. USDA ($280,000).

Production of myostatin gene knockouts in zebrafish, and the effects of specific myostatin interacting proteins on salmonid muscle growth. FW Goetz, PI (15)% , SB Roberts, Co-PI, P. Collodi, Co-PI. Funding period: 12/04 – 11/07. USDA ($426,000).

Pending

Assessing immunotoxicity of manufactured nanoparticles using cellular and genomic biomarker responses of the innate immune system. R. Klapper, PI, FW Goetz, Co-PI (10%) Funding period: 07/05 – 08/07. EPA (STAR) ($299,056).

Developing molecular biomarkers and anti-viral therapeutics for Infectious Salmon Anemia Virus (ISAV). ML Rise, PI, FW Goetz Co-PI (20%). Funding period: 08/05 – 07/08. USDA ($348,045).

Sea Scallops as Sentinels of Deepwater Pollution. FW Goetz, PI (20%) T Wade, S. Roberts, P. Rago, D. Hart (CoPIs). Funding period: 9/01/05 to 9/01/07. NOAA ($274,876).

Steven B. Roberts

Current

Development of diagnostic and management techniques to select cod broodstocks and hatchery stocks free from nodavirus. SB Roberts, PI (15%), S. Lindell, Co-PI, S. Johnson, Co-PI, D. Bouchard, Co-PI, G. Nardi, Co-PI, D.Berlinsky, Co-PI, N. Brown, Co-PI. Funding period: 10/03 – 10/05. NRAC-USDA ($124,612).

Development of genetic markers to assess disease resistance in the Eastern oyster. SB Roberts, PI (25%), R. Smolowitz, Co-PI, R. Karney, Co-PI, I. Sunila, Co-PI, D. Leavitt, Co-PI, W. Walton, Co-PI. Funding period: 02/05 – 02/07. NRAC-USDA ($128,486)

Production of myostatin gene knockouts in zebrafish, and the effects of specific myostatin interacting proteins on salmonid muscle growth. FW Goetz, PI , SB Roberts, Co-PI (33%), P. Collodi, Co-PI. Funding period: 12/04 – 11/07. USDA ($426,000).

Pending

Assessing resistance to QPX disease: development of cellular and molecular methods. R. Anderson, PI, R. Smolowitz, Co-PI, S. Roberts (17%), Co-PI, D. Leavitt, Co-PI. Funding period: 03/06 – 03/08. NRAC-USDA.

Sea Scallops as Sentinels of Deepwater Pollution. FW Goetz, PI, T Wade, S. Roberts (20%), P. Rago, D. Hart (CoPIs). Funding period: 9/01/05 to 9/01/07. NOAA ($112,193).

Terry L. Wade

Current

Analysis of Organic Contaminants. TL Wade, CoPI (2.5mo/yr). Funding period: 09/02-08/07. U.S. Fish and Wildlife Service ($3,000,000).

Atmospheric Deposition Study. TL Wade, PI (1.0mo/yr). Funding period: 3/1/03-8/30/05 EPA/CBBEP ($50,000).

City of Portland. TL Wade, PI (1.0mo/yr). Funding period: 3/1/04-3/31/06 City of Portland ($27,000)

Assessment of Sources, Levels, Trends and Effects of Persistent Organic Pollutants (POP) in the Mediterranean Coastal Environment. TL Wade, PI (1.0mo/yr) Funding period: 9/1/03-8/31/05. U.S./Egypt Joint Board on Scientific and Technical Cooperation ($31,700).

Various Analyses. TL Wade, PI (2.0mo/yr) Funding period: 11/1/04-1/15/05. Academy of Natural Sciences ($265,000).

Pending

Sea Scallops as Sentinels of Deepwater Pollution. TL Wade, PI (2.0mo/yr). Funding period: 9/01/05 to 9/01/07. NOAA ($32,000)

Analysis of Organic Contaminants. TL Wade, CoPI (2.0mo/yr). Funding period: 9/1/02-8/31/07. U.S. Fish and Wildlife Service ($3,000,000).

K. DATA MANAGMENT PLAN

Several forms of data will be generated in this project.

1. **Sequence information on sea scallop genes related to environmental contamination**: As they are sequenced, but no later than the completion of the project, the sequences will be submitted to Genbank at NCBI for public distribution. There is no cost to access this information and it can be obtained at: National Center for Biotechnology Information. The PI (Goetz) and the CoPI (Roberts) have been involved extensively in large scale sequencing projects involving fish and bivalves (e.g., Goetz, FW., Iliev, D., Liarte, C., McKenzie, S., Planas, J., McCauley, L. (2004). Analysis of genes isolated from lipopolysaccharide-stimulated rainbow trout (*Oncorhynchus mykiss*) macrophages. Molecular Immunology 41:1199-1210; Roberts, S. and F.W. Goetz (2003). Expressed sequence tag analysis of genes expressed in the bay scallop, *Argopecten irradians*. Biol Bull. 205(2):227-228) and are fully aware of the quality control necessary for generating sequences to be submitted to Genbank for public distribution. In our facilities, we only use NCBI acceptable programs and standards including: sequence analysis software utilizing base-calling (phred), vector screen (cross-match), annotation of sequences (BLAST) and assembly of sequences (phrap).

2. **Data on contaminant types and amounts**: By the completion of the project we will submit our data on contaminant analysis of sea scallops to the National Oceanographic Data Center (NODC). All contaminants for this project are being analyzed by the Geochemical and Environmental Research Group (GERG) at Texas A&M. GERG has previously measured contaminants for the NOAA National Status and Trends (NS&T) program by validated methods and thus, GERG contaminant analysis will be comparable to previous NS&T and International Mussel Watch data collection standards. GERG have previously submitted data to NODC and knows the format. GERG has an established quality control policy that is described in full in the Project Description.

3. **Outreach and Education:**

A major component of the proposed research will be the effective transfer of our research finding. While publication of results will be an important avenue to disseminate our results, a primary mechanism will be through a website dedicated to the continual progress of the project. The site will be hosted by the Marine Biological Laboratory in Woods Hole and will mimic the concept of a current site dedicated to the USDA funded research carried out by collaborators on a current project focused on Atlantic cod (*Gadus morhua*) stress and disease (**http://www.mbl.edu/aquaculture/nrac/**). Our website that we will develop for the sea scallop project will be more graphical and interactive given the broad geographic nature of the project. For example, we will have maps documenting the sampling cruises with updated information regarding conditions and sea scallop sampled. Once results concerning the differences in contaminant loading have been elucidated, these results will also be made available. We also plan to inform and interact with NOAA's Mussel Watch Program (Ocean Service, National Status and Trends Program, Center for Coastal Monitoring and Assessment) and to keep advisory groups such as the Sea Scallop Working Group (Massachusetts) informed of progress and results so that they can keep members up-to-date.

L. EVALUATION OF PROJECT

The project accomplishments will be evaluated in the following ways.

1. The completion and submission of progress and final reports as required by the NOAA OHHI program.

2. The following list of deliverables should be provided by the completion of the of the project.

   a. Frozen archived collection of sea scallop soft body parts from NEFSC survey arranged according to stratified collection - Maintained at the Great Lakes WATER Institute, Milwaukee, WI - Available for other researchers at cost for shipping.

   b. Gene sequences for sea scallop homologs of genes listed in Table 3 (Project Description) - Submitted to Genbank for public access.

   c. Full (PCBs, PBDEs, PAHs, pesticides) contaminant analysis including type and levels on sea scallops sampled from 5 sites collected during the NEFSC survey.

   d. Transcriptional profile of the expression of 12 genes presented in Table 3 (Project Description) for sea scallops sampled from 6 sites collected during the NEFSC survey.

   e. Gene sequences of novel contaminant related genes in sea scallops from differential display PCR - Submitted to Genbank for public access.

3. At least one publication to be completed and submitted to relevant peer-reviewed journal (e.g., Marine Environmental Research; Aquatic Toxicology) within 6 month of the end of the project that contains the elements of deliverables "c" and "d."

M. FEDERAL, STATE AND LOCAL GOVERNMENT ACTIVITIES

1. Mussel Watch: Program funded by NOAA through the National Status and Trends Program of the Center for Coastal Monitoring and Assessment. Mussel Watch is the longest continuous contaminant monitoring program in U.S. coastal waters. "The project analyzes chemical and biological contaminant trends in sediment and bivalve tissue collected at over 280 coastal sites from 1986 to present. The database includes: sediment and bivalve tissue chemistry for over 100 organic and inorganic contaminants; bivalve histology; and *Clostridium perfringens* data." A CoPI (T.Wade) of the current proposal was involved through GERG in doing some of the contaminant analysis from this project. Investigators in the Mussel Watch program were contacted by the PI (Goetz) by email concerning the submission of the current proposal on sea scallops.

2. International Mussel Watch: Program under the auspices of the United Nations Educational, Scientific and Cultural Organization (UNESCO) Intergovernmental Oceanographic Commission, and the United Nations Environment Programme (UNEP) Ocean and Coastal Areas Program. "Undertaken to assess the extent of chemical contamination, primarily in the equatorial and subequatorial areas of the southern hemisphere, with particular attention to coastal areas of developing countries." A CoPI (T.Wade) of the current grant was involved directly in IMW as the organization that did contaminant analyses.

3. Sea Scallop Working Group: " The Sea Scallop Working Group (SSWG) was organized in 1994 as a forum for discussion and action by a wide spectrum of stakeholders, state and federal officials, environmentalists, financial supporters, and scientists with the goal of supporting the development of sea scallop aquaculture in New England. Dr. H.O. Halvorson chairs SSWG meetings. SSWG meets bimonthly and involves all stakeholders. These meetings have concentrated on various topics; new cage technology, education, state aquaculture initiatives and review of state aquaculture projects, biological community associated with scallops, concerns of the investment community, how elected decision makers get technical information, right whale concerns, ownership and extension of demonstration lease project, exchanges with NOAA regulatory representatives and economic analysis of shellfish aquaculture. In addition, SSWG has provided input and evaluation to various federal initiatives, as the NOAA Policy Document on Aquaculture, and the White Paper on aquaculture by the State of Massachusetts." The PI (Goetz) was a member of the SSWG when he was employed at the MBL. H. Halvorson was contacted by the PI by email concerning the submission of the current proposal on sea scallops.

N. SUGGESTED REVIEWERS

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