More Realistic Projections of Population Responses to Stressors: Incorporating Genetics (SP2 MYP)

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Agency Problem

Current population risk assessment methods do not take into account population genetics. However, it is widely acknowledged that chemical stressors can alter the genetic structure of wildlife populations, and that the genetic structure of wildlife populations can affect stressor responses.

- Guidance is needed on how to take advantage of increasingly accessible molecular genetic information to improve projections of wildlife population responses to chemical and non-chemical stressors.
- Major ecological research questions as to how to integrate population genetics and dynamics need advancement.
- Finer scale issues specific to Agency needs, such applicability and logistics, are also required for risk assessments supporting the regulation of chemicals by the US EPA's Office of Pesticide Programs.

A research program integrating ecologists, geneticists, and toxicologists across US EPA's Office of Research and Development research laboratories has been developed to advance understanding of population genetic-dynamic interactions in populations under stress. We designed research to take advantage of complementary expertise and capabilities, and used theoretical as well as experimental approaches, which include aquatic species of ecological importance and regulatory usefulness.

Research Goals

The goal of this program is to demonstrate and test techniques to better understand how genetic alterations modify risks to wildlife populations from chemical and non-chemical stressors. We have focused on two areas, one addressing generalized stress responses and the other specific to our concerns with chemical stressors:

(1) Characterizing predictive relationships between reduced genetic diversity and population sustainability, and evaluating tools (markers and models) for incorporating genetics into population dynamic projections.

(2) Characterizing genetic responses to multi-generational exposures to toxic chemicals, identifying the genetic basis for chemical tolerance, and evaluating its broader applicability in understanding species variation in chemical sensitivities.

Conclusions and Future Directions

Conclusions representing Office of Research and Development research laboratory collaborative research and graduate and post-doctoral training are reported in key publications (including several that received the Agency's Science and Technology Achievement Awards), and include:

- Genetic diversity reduction exacerbates stress responses. Additional research is needed to evaluate molecular genetic markers for their usefulness, and validate and generalize relationships predicting stress responses in low diversity populations.
- For some species, responses to multi-generational chemical exposures include genetic changes that mitigate chemical toxicity. The molecular basis for chemical tolerance and its applicability for predicting variation among species in chemical sensitivity is currently under investigation.

Impact and Outcomes

This research expands and refines current population risk assessment capabilities for aquatic and wildlife species, including (but not limited to) threatened and endangered species, as needed by US EPA's Offices of Pesticides Programs and Water. Research reduces uncertainty in ecological risk assessment by providing tools and methods to estimate the magnitude and outcome of genetic and evolutionary changes produced by anthropogenic stressors with respect to population viability, including:

- Molecular genetic markers to acquire genetic information for aquatic species of interest; molecular approaches (i.e., quantitative trait loci, QTL) to identify genetic loci related to fitness under conditions including chronic chemical exposure.
- Population genetic methods that provide complementary information on the effects of chemical stressors, including long-term exposures, on biological integrity and ecological condition
- Improved predictive and diagnostic population modeling approaches that incorporate genetic alterations as a key determinant of population viability.

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Methods, Models, Approach, and Results-to-Date

Model Species: amenable to laboratory and field studies

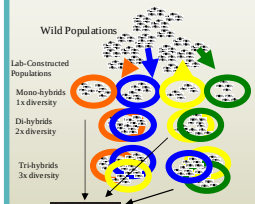
Americamysis bahia

1) Characterizing predictive relationships between reduced genetic diversity and population sustainability, and evaluating tools (markers and models) for incorporating genetics into population dynamic projections

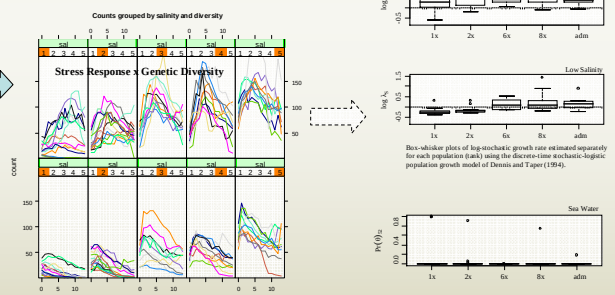
- Evolutionary and population genetic theory predict that populations with low levels of genetic diversity are more likely to lack the allelic resources necessary to adapt and persist in novel environments.
- Defining the strength and shape of the relationship between genetic diversity and population fitness will improve predictions of adaptive capacity, i.e., persistence in response to stressors.
- A marine toxicity test species (*Americamysis bahia*) provides a tractable biological model to construct populations that vary in their genetic diversity.
- Genetically constructed populations provide opportunity to test genetic tools and statistical methods for estimating genetic risk, and determine empirically the relationship between genetic diversity and adaptive responses to stress.

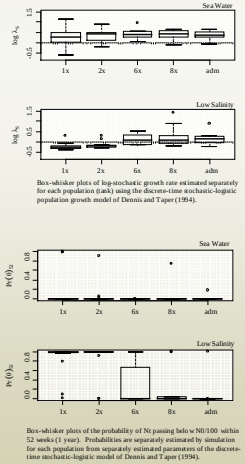
Linking theoretical and empirical approaches

Experimental System



Genetic Diversity Affects Population Growth Rates and Extinction Probabilities





Box-whisker plots of log-stochastic growth rate estimated separately for each population (rank) using the discrete-time stochastic-logistic population growth model of Dennis and Taper (1994).

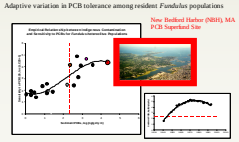
Box-whisker plots of the probability of Nt passing below N0/100 within 52 weeks (1 year). Probabilities are separately estimated by simulation for each population from separately estimated parameters of the discrete-time stochastic-logistic model of Dennis and Taper (1994).

Fundulus heteroclitus

2) Characterizing genetic responses to multi-generational exposures to toxic chemicals, identifying the genetic basis for chemical tolerance and evaluating its broader applicability in understanding variation among species in chemical sensitivities

- Evolutionary and population theory predicts that given sufficient genetic diversity, species/populations may adapt to multigenerational stressors, including chemical stressors.
- Although infrequently observed in the wild, 'evolved chemical tolerance' can reveal biochemical and genetic adaptive strategies, and suggest mechanisms underlying inter-species variation in chemical sensitivities.
- Wild populations of a non-migratory marine fish species (*Fundulus heteroclitus*, Atlantic killifish) vary extremely in their sensitivity to PCBs and other dioxin-like compounds (e.g., Van Veld and Nacci 2007). Some chronically-exposed *Fundulus* populations display profound adaptive variation in chemical sensitivity, or rapidly evolved chemical tolerance (Nacci *et al.* 1999, 2002a).
- A *Fundulus* population resident to an estuarine PCB superfund site, New Bedford Harbor, MA (USA) , provides a useful model to study genetic changes and ecological implications associated with multi-generational chemical exposures.

Adaptive variation in PCB tolerance among resident *Fundulus* populations

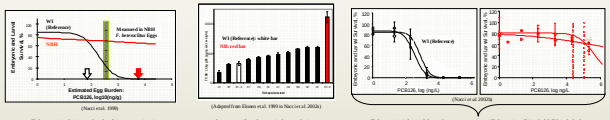


(Nacci et al. 2002a, b)

Magnitude and duration of NBH contamination, including empirical benchmark (red dash) for adverse ecological effects

Genetic Responses: Rapid adaptation and its ecological implications

Adaptive, profound inherited chemical tolerance in NBH *Fundulus*



(Nacci et al. 1999)

Tolerance is adaptive to levels of site-contamination

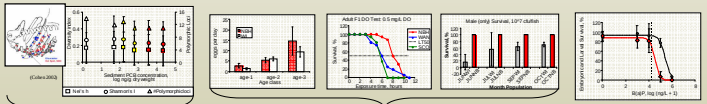
(Adapted from Elsasser et al. 1989 in Nacci et al. 2002a)

Intra- specific tolerance is large relative to inter-specific tolerance

(Nacci et al. 2002b)

Tolerance is inherited through two generations of lab-rearing, F1 in bold, F2 in light blue

Few general but some specific, predicted trade-offs in NBH *Fundulus*



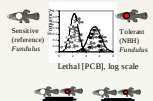
Neither AFLPs (above right, adapted from McMillan et al. 2006) nor allozymes (Roark et al. 2005) nor MHB regs (Cohen 2002) show reduced genetic diversity despite strong phenotypic divergence.

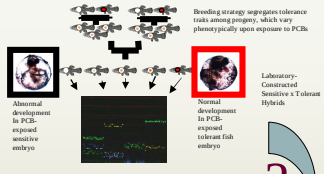
Chemically-adapted *Fundulus* are relatively fit under unstressed conditions (e.g., normal reproductive output, Nacci et al. 2002b) and stressed conditions (low oxygen or pathogen exposures, Nacci *et al.* in preparation).

Some chemical exposures are more toxic to PCB-tolerant fish, (Nacci *et al.* 2002c)

Genetic Basis: Molecular approaches to characterize chemical tolerance

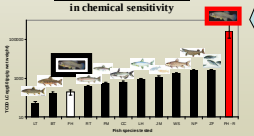
- Altered biochemical pathways in NBH *Fundulus* correlates of chemical tolerance (*e.g.*, Van Veld and Nacci 2007). However, target genes have not been identified.
- A quantitative trait locus (QTL) approach uses classic breeding strategies, taking advantage of variation among *Fundulus* populations in PCB tolerance, and provides unique opportunity to characterize biochemical, genetic and genomic mechanisms for PCB tolerance in this (and other?) fish species.
- *Fundulus* is becoming an important model species for ecological genomics (Burnett *et al.*, 2007)





Genetic Characterization of Embryonic DNA in *F. heteroclitus*: Quantitative Trait Locus molecular/statistical approach associating molecular genetic markers with tolerance trait

Variation among fish species in chemical sensitivity



(Adapted from Elsasser et al. 1998, in Van Veld and Nacci 2007)

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