3. ABSTRACT/PROJECT SUMMARY

a) Project Title: **ECOHAB: Integrating Cell and Toxin Cycles of *Karlodinium veneficum* with Key Environmental Regulators: *In Situ* Studies of Predictive Determinants for Bloom Toxicity**

b) Institutions/Investigators: University of North Carolina at Charlotte
Dr. Matthew W. Parrow – Lead PI
University of Maryland Center for Environmental Science
Dr. Allen R. Place – Co PI

c) Total Proposed Cost: **$385,225** (no ship costs)

d) Budget Period: 09/01/2015 – 08/31/2018 **(3 Years)**

e) Project Summary: The dinoflagellate *Karlodinium veneficum* blooms along Mid-Atlantic coasts and produces structurally characterized, quantifiable toxins called karlotoxins (KmTX) that kill fish and exhibit widespread toxicity to other organisms. However, toxicity varies widely among different bloom populations, and within populations over time. Coastal management requires knowledge and tools to better predict how, when, and why *K. veneficum* cells (and thus blooms) become highly toxic. Our recent research has demonstrated that karlotoxins increase significantly in stationary phase cultures, particularly under growth-limiting conditions caused by low N, P, or selenium (Se). These and other results strongly suggest a simple but strongly predictive inverse relationship between the rate of cell proliferation and cellular toxin quotas in *K. veneficum* populations. Furthermore, it has been recently found that karlotoxin production is light-dependant with a diel biosynthetic cycle that closely corresponds to the cell cycle.

The **objective** of this project is to quantify the relationship between *K. veneficum* cell proliferation rates and toxicity in cultures and natural blooms by correlating the diel cell cycle, *in situ* growth rates, and cellular karlotoxin accumulation in relation to key environmental factors identified as primary growth/toxicity determinants of *K. veneficum*. The overall **hypothesis** is that growth-limited (slowly or non-proliferating) *K. veneficum* cells in both culture and field blooms will arrest in G1 for multiple L/D cycles, undergo several cycles of karlotoxin synthesis, and become significantly more toxic. Put simply, rapidly proliferating cells have a low toxin content whereas slowly or non-proliferating cells accumulate toxin and become more toxic.

The **experimental approach** will be to examine cultures (Year 1) and natural blooms (Years 2 & 3) of *K. veneficum* for cell cycle progression, *in situ* growth, and karlotoxin accumulation over time and in relation to key environmental factors using digital microfluorometry and LC-mass spectrometry. Year 1 experiments will consist of laboratory range-finding studies and Year 2 & 3 experiments will consist of intensive bloom sampling and mesocosm experiments in the Baltimore Harbor where blooms of toxic *K. veneficum* are an annual phenomenon. This research will provide novel information on how *K. veneficum in situ* growth rates and toxicity are related and integrated with environmental factors, concepts which are critically needed to validate predictive models to forecast bloom growth and toxicity.

Expected outcomes include adoption by area managers of information and techniques leading to better prediction of bloom toxicity, and improved quantitative understanding of the role of *in situ* population dynamics in *K. veneficum* bloom toxicity and ecology.