

Characterization of the toxic principles from field collected and laboratory-cultured samples of the harmful alga, *Prymnesium parvum*

Matthew J. Bertin^{1,2}, Paul V. Zimba³, Kevin R. Beauchesne⁴ and Peter D.R. Moeller^{1,2,5}

¹Marine Biomedicine and Environmental Sciences Center, MUSC, Charleston, SC

²Hollings Marine Laboratory, Charleston, SC,

³Center for Coastal Studies, Texas A&M University, Corpus Christi, TX

⁴JHT in support of the Hollings Marine Laboratory, NOAA, Charleston, SC,

⁵Toxin/Natural Products Chemistry, NOAA National Ocean Service, Charleston, SC

The golden alga, *Prymnesium parvum*, has been implicated in fish and aquatic animal kills in the United States since 1985. This alga is most often associated with blooms in estuarine and marine waters, but has been observed to affect high conductivity inland waters and aquaculture facilities. In addition to widespread ecological impacts through the loss of entire fish populations within lakes, an economic burden is also felt by state and local agencies due to year class losses of fish for stocking lakes as well as loss of fishing and recreational use of the affected body of water. Two ichthyotoxic and hemolytic compounds have been described and structurally characterized from *P. parvum*, prymnesin -1 and -2. Data from both the Moeller lab and others suggest that undescribed and more ecologically relevant toxins are present in *P. parvum*. To identify novel toxins *P. parvum* was first cultured and field unialgal blooms were also collected; these were extracted with ethyl acetate followed by methanol. Filtrate from *P. parvum* cultures was similarly extracted. These crude extracts were tested for cytotoxicity against mammalian cell lines (N2A and GH4) with activity observed in both the methanol and ethyl acetate fraction from cultured cell mass and in the methanol fraction from *P. parvum* filtrate. Bioassay-guided fractionation using high pressure liquid chromatography (HPLC) and mass spectrometry (MS) identified multiple cytotoxic fractions. Further characterization of these fractions with nuclear magnetic resonance spectroscopy (NMR) indicates that lipids dominate the cytotoxicity observed in cultured cell mass, while free fatty acids, specifically oleic acid and elaidic acid dominate the cytotoxicity observed from filtrate samples. A cytotoxic non fatty acid sample from the filtrate with a nominal molecular ion at m/z 540 is currently being characterized by high resolution NMR and accurate mass spectrometry. Isolating and characterizing the important toxin(s) from *P. parvum* will allow relevant experimentation with field and culture populations to assess cues for toxin accumulation, lead to improved detection methods and further research avenues including toxin neutralization, characterization of toxin bioactivity, and possible anticancer properties.

This work is supported by the South Carolina Department of Health and Environmental Control and NOAA/NOS.