# PUTATIVE METACASPASE ACTIVATION IN AGING *KARENIA BREVIS* CULTURES: PRELIMINARY INSIGHT INTO CELLULAR MECHANISMS REGULATING BLOOM TERMINATION

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*Karenia brevis*, a toxic dinoflagellate, is responsible for near annual harmful algal blooms (HABs) off the west coast of Florida. These blooms cause extensive ecological and economic losses due to massive fish kills, marine mammal mortalities, and human illness caused by neurotoxic shellfish poisoning and respiratory irritation. The development of successful management strategies for *K. brevis* blooms is contingent upon understanding the molecular mechanisms that govern bloom initiation, propagation, and termination. Molecular mechanisms regulating *K. brevis* bloom demise, in particular, have remained largely uninvestigated, although recent studies have discovered programmed cell death (PCD) pathways in several other bloom-forming phytoplankton species. We have identified in *K. brevis* putative metacaspases, known central mediators of PCD in plants, fungi, and protists, containing a well-conserved caspase catalytic diad domain. Western blot analysis of *K. brevis* protein extracts collected from an early stationary phase culture revealed immunohybridization of a distinct protein band to a polyclonal antibody raised against a recombinant *Emiliania huxleyi* metacaspase protein. Caspase-specific activity in aging cultures was examined by measuring specific cleavage of fluorogenic canonical caspase tetrapeptide substrates. Preliminary results from these studies indicate an increase in caspase-specific activity in stationary phase cultures when compared to early and mid-logarithmic cultures. Identification of putative metacaspases from our EST libraries, cross reactivity with an *E. huxleyi* metacaspase polyclonal antibody, as well as caspase-specific activities in aging cultures provide preliminary evidence that *Karenia brevis* contains PCD machinery and may utilize an apoptosis-like pathway during cell death. Further characterization of the involvement of metacaspases in cell death may lead to the identification of molecular biomarkers for bloom termination.

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