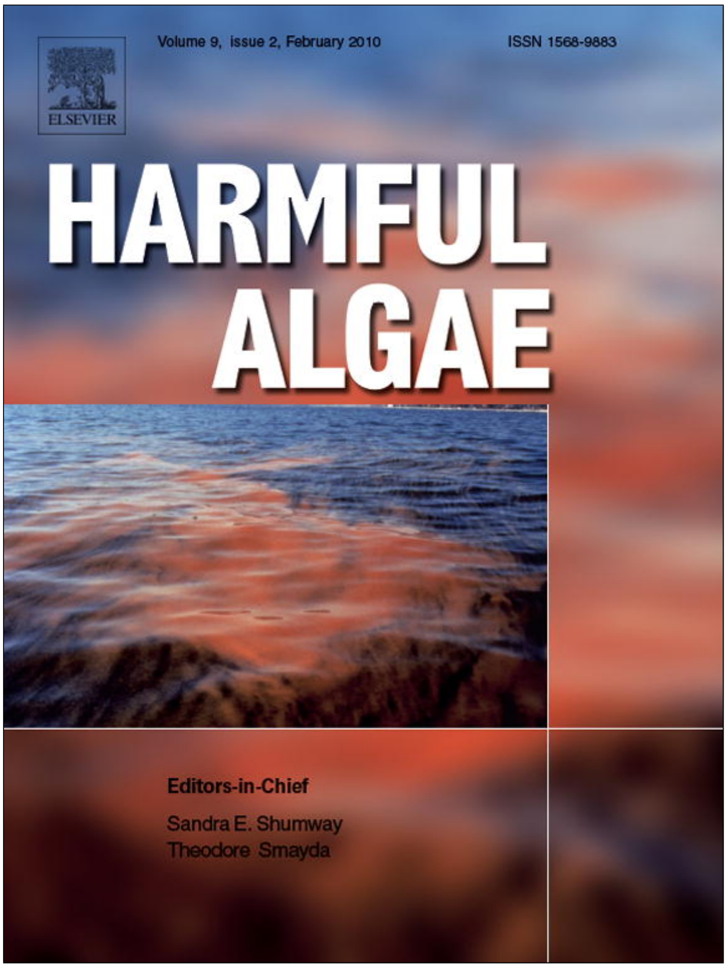
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The catastrophic 2008–2009 red tide in the Arabian gulf region, with observations on the identification and phylogeny of the fish-killing dinoflagellate *Cochlodinium polykrikoides*

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ABSTRACT

Harmful algal blooms (HABs) caused by the marine ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* Margalef are responsible for mass mortalities of wild and farmed fish worldwide, with catastrophic impacts to aquaculture and local economies. Here we report on the *Cochlodinium* species responsible for a severe and widespread HAB in the Arabian Gulf and Gulf of Oman that has lasted for more than eight months at this writing, killing thousands of tons of fish and limiting traditional fishery operations, damaging coral reefs, impacting coastal tourism, and forcing the closure of desalination plants in the region. To identify the causative organism, cultures were established from cells isolated along the Arabian Gulf shore of the United Arab Emirates. Taxonomic analyses using scanning and light microscopy, and partial analysis of the large subunit (LSU) ribosomal RNA (rRNA) gene confirmed the *C. polykrikoides* classification. rRNA gene sequences of *C. polykrikoides* isolates from the Arabian Gulf were identical to isolates from the northeastern USA, Puerto Rico, Mexico, and Malaysia, known as the "American/Malaysian" ribotype. To our knowledge, this is the first HAB event associated with *C. polykrikoides* in the Arabian Gulf or the Gulf of Oman. The sudden emergence of *C. polykrikoides* in these Gulfs coincides with an apparent global expansion of this taxon, as well as a recent increase in HAB impacts observed in this region. The mechanisms underlying this expansion require further investigation, and may include increased nutrient enrichment of coastal waters in the Arabian Gulf and Gulf of Oman from domestic and industrial inputs, natural meteorological and oceanographic forcings, and the recent introduction of this species through ballast water discharge. A pattern of subsequent recurrence of *C. polykrikoides* blooms following an initial outbreak has been observed in other parts of the world, suggesting that this species may become a persistent HAB problem in this region. As Arabian Gulf countries rely on desalination plants as the primary source of freshwater, the disruption of plant operations by recurring *Cochlodinium* blooms poses a serious threat to the drinking water supply in the region, and represents an unprecedented HAB impact.

1. Introduction

The marine ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* Margalef is one of several taxa responsible for the ecologically and economically important phenomenon known as harmful algal blooms (HABs), commonly referred to as "red tides". First described from Puerto Rico in the Caribbean by Margalef (1961), the geographic distribution of *C. polykrikoides* is widespread, and populations have been documented in tropical and warm-temperate waters around the world, including the Caribbean Sea, eastern and western Pacific Ocean, the eastern Atlantic Ocean, Indian Ocean, and Mediterranean Sea (see Kudela et al., 2008; Matsuoka et al., 2008). *C. polykrikoides* is notable for causing mass mortalities of wild and farmed fish, particularly in Japan and Korea, where these events are of national concern due to their catastrophic impacts to aquaculture industries and coastal economies. Fisheries impacts in a single bloom season can be devastating; in 1995, a particularly severe and widespread *C. polykrikoides* bloom persisted for nearly eight weeks along the entire south coast of Korea, ultimately resulting in economic losses of US$ 95 million (Kim, 1998).

In recent decades, the rapid emergence of *Cochlodinium* as a major HAB problem has attracted considerable attention from governmental and commercial organizations, and spurred

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Abbreviations: HABs, harmful algal blooms; LSU, large subunit; bp, base pair(s).

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research by the scientific community into the causes and management of these events. However, understanding certain basic biological aspects of this taxon such as its life cycle and mechanisms of toxicity has been challenging. The means by which *C. polykrikoides* kills finfish and shellfish has yet to be fully characterized and may involve a combination of unrelated factors, including: (1) production of ichthyotoxic substances, including neurotoxic, hemolytic, and hemagglutinating fractions (Onoue et al., 1985; Onoue and Nozawa, 1989b) and paralytic shellfish poisoning (PSP) toxins (Onoue and Nozawa, 1989a); (2) production of reactive oxygen species (ROS) (Kim et al., 1999); and (3) production of extracellular mucoid polysaccharide substances (Kim et al., 2002b). The roles of ichthyotoxic fractions and PSP toxins are not well substantiated, but field observations and additional experimental evidence lend support for the latter two mechanisms. During a severe *Cochlodinium catenatum* bloom in the eastern Pacific, Guzmán et al. (1990) observed copious amounts of viscous foam and mucus in the water column and documented the suffocation of scleractinian corals by these mucilagic substances, indicating that the copious production of polysaccharides may be an important indirect mechanism of mortality. Recent experiments by Tang and Gobler (2009) showed that ichthyotoxicity was dependent on cell viability but not on physical contact between cells and fish, suggesting that extracellular, ROS-like compounds may indeed play a role in bloom toxicity.

Similarly, there are unanswered questions regarding the life stages of *Cochlodinium*, particularly regarding cyst formation and how it contributes to the initiation and spread of blooms. Kim et al. (2007) describe a life cycle comprising two different morphological stages—an armored and unarmored vegetative swimming stage, the latter forming the long chains of cells that are commonly observed in this taxon, and a hyaline cyst. Other workers describe several other morphotypes of resting cysts (Matsuoka and Fukuyo, 2000; Kim et al., 2002a, 2007). The cyst-motile stage relationship in *Cochlodinium* is thus not yet established.

Although *C. polykrikoides* is primarily recorded from tropical to warm-temperate areas, bloom populations have been documented in temperate coastal waters of the northeastern USA (Nuzzi and Waters, 2004; Gobler et al., 2008; Tomas and Smayda, 2008) and British Columbia, western Canada (Whyte et al., 2001); thus, the potential for these cysts to overwinter in sediments and initiate blooms when environmental conditions are suitable seems likely, given its seasonal occurrence in temperate areas. However, additional studies involving the collection and germination of *Cochlodinium* cysts from field sediments and experiments to characterize the temperature tolerances and maturation interval of these cysts are still needed to confirm the role of cyst formation in *Cochlodinium* bloom dynamics.

In some areas, *Cochlodinium* only recently emerged as a dominant HAB-causing species after persisting for years at low abundance as a minor component of the phytoplankton assemblage. In Korea, for example, blooms of *C. polykrikoides* were first documented in 1981; since then, outbreaks have occurred regularly and now affect most of the country's coastal areas (Kim, 1998; Ahn et al., 2006). Similarly, *Cochlodinium* was infrequently observed in the coastal waters of California, USA, which previously supported a diatom-dominated community; however, a large and sudden outbreak occurred in 2004, affecting over 800 km of coastline and causing mass dieoffs of mussels; in the years following this event, *Cochlodinium* has emerged as a common bloom-forming organism along the California coastline (Curtiss et al., 2008). Consistent with this trend, over the past decade fish kills associated with *Cochlodinium* blooms were documented for the first time from Canada (Whyte et al., 2001), the Philippines (Vicente et al., 2002), and most recently in Malaysia (Anton et al., 2008). Kudela et al. (2008) noted that *Cochlodinium* appears to be undergoing a global expansion of its geographic range and bloom frequency, in synchrony with other HAB phenomena (e.g., Anderson, 1989; Glibert et al., 2005). The dramatic rise in the global aquaculture industry, which is poised to overtake capture fisheries as a source of food fish (FAO, 2009), clearly increases the potential for economic losses from *Cochlodinium* blooms; thus, understanding the mechanisms underlying this recent expansion in the Arabian Gulf region and the environmental factors regulating bloom dynamics will become increasingly important to minimize mass mortalities and ensure the successful and sustainable management of farmed and wild fish stocks, as well as coral reef resources.

Here we report on the identification and phylogeny of *Cochlodinium* isolated from an extensive bloom that affected the Gulf of Oman and the Arabian Gulf from August 2008 to May 2009 (still continuing at this writing). The bloom is notable for its size and intensity. It caused massive fish kills, damaged coral reefs, restricted fishing activities, and forced desalination plants in Oman and the United Arab Emirates (UAE) to cease or modify operations due to clogging of intake filters or the fouling of reverse osmosis membranes. To our knowledge, this is the first observance of *Cochlodinium* blooms in this region and provides further evidence for the recent global expansion of this species. The pattern of subsequent recurrence of blooms following an initial outbreak (e.g., Curtiss et al., 2008; Kim et al., 2004) suggests that *Cochlodinium* may become a persistent HAB problem in this region.

2. Materials and methods

2.1. Description of study area

The Arabian Gulf is a semi-enclosed, shallow (average 30 m in depth), subtropical sea surrounded by a large, arid land mass. It is connected to the Gulf of Oman by the Strait of Hormuz, which restricts water exchange between these two water bodies (Fig. 1). Due to this unusual physical environment, the Arabian Gulf experiences marked temperature extremes atypical of seas at similar latitude, with hot, dry, tropical conditions during the summer and temperate conditions during the winter. In addition to extreme variations in temperature, the Arabian Gulf also experiences substantial fluctuations in salinity levels, which can exceed 70 psu in some embayments. High salinity levels are driven by strong evaporation, which exceeds combined rainfall and freshwater inputs by over a factor of ten (Sheppard, 1993). Circulation in the Gulf is in an anti-clockwise motion, driven primarily by density gradients (see Sheppard, 1993), creating a reverse estuarine flow similar to the circulation of the Mediterranean Sea (Reynolds, 1993). Water enters the Gulf through the Strait of Hormuz, moves northwards along the Iranian coast, while a secondary coastal current flows southerly along the northern Iranian coast, against the inflow water in the Strait of Hormuz, driven by density differences from river runoff in the northern Gulf (Reynolds, 1993). A southward coastal flow moves along the southwestern coastline of the Arabian Gulf, where stagnation and evaporation in the southern embayments increases salinities to ~40 psu. This denser water sinks and flows towards the entrance of the Gulf, where it lies beneath the incoming water.

Circulation in the Gulf of Oman is dominated by a clockwise gyre in the west and a counter-clockwise gyre in the east, creating a region of upwelling along the Iranian coast at the interface between the two (Reynolds, 1993).

2.2. Field sampling and culture establishment

Field samples used in this study were collected in December 2008 from coastal waters near Ras Al-Khaimah, located in the

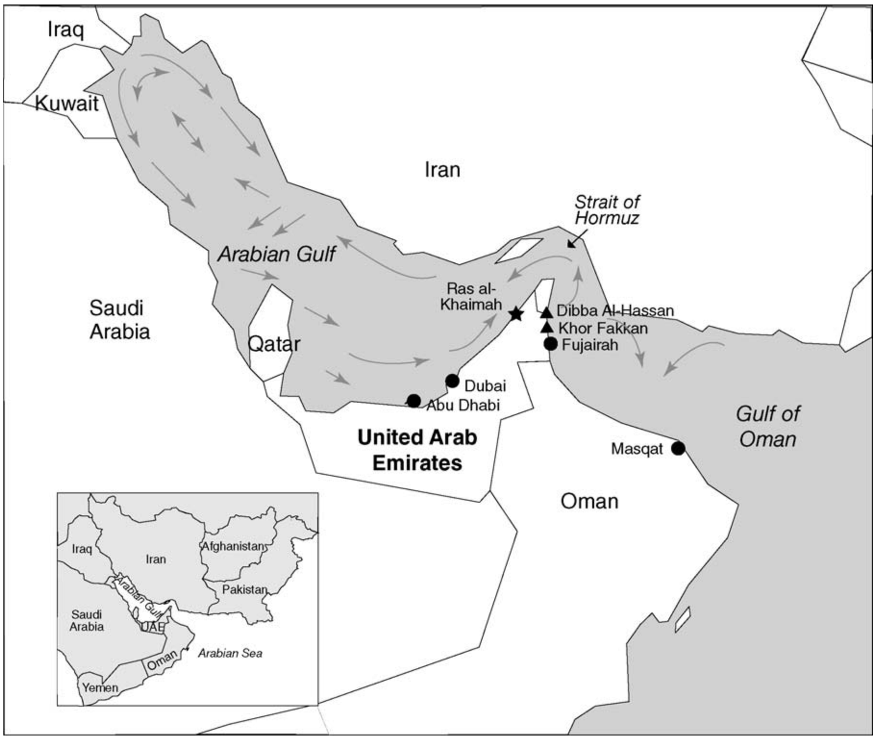

Fig. 1. Map of the Arabian Gulf showing location of field sites for samples collected in December 2008 from the coastal waters of Ras al-Khimah, UAE, near the Strait of Hormuz. Star symbol (★) indicates sample collection site, triangles (▲) indicate locations where fish kills and marine mammal mortalities were reported. Arrows indicate circulation patterns (from Reynolds, 1993).

northern United Arab Emirates (UAE) near the border with Oman (Fig. 1). Grab samples of surface water (approximately 12–15) were collected from boats with a bucket, transferred to plastic bottles, and allowed to sit at room temperature in indirect light on the laboratory bench. An aliquot of each sample was preserved with Lugol's iodine and used for preliminary identification. After approximately one week, aliquots of the living samples were placed in plastic vials, supplemented with filtered seawater, and returned to the USA. Clonal cultures were established from these subsamples by isolating motile cells using micropipetting and rinsing each individual cell in drops of sterile medium. Live cultures were maintained in tubes with 25 mL of *f*/2-Si sterile medium (Guillard and Ryther, 1962; Guillard, 1975) made with Vineyard Sound seawater, at 25 °C and at a photon flux density of ca. 160 μmol photons m^{-2} s^{-1} under a 14:10 light:dark cycle.

2.3. Morphological identification and taxonomic analysis

For the morphological analyses, cells of the *Cochlodinium* isolates were observed and photographed using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) at 400× using Nomarski differential interference contrast optics, and a JEOL5600LV scanning electron microscope (JOEL, Tokyo, Japan). For examination using scanning electron microscopy (SEM), approximately 1 mL of each culture was fixed with 1% osmium tetroxide for 24 h; desalted with a ten step gradient from 32 psu seawater to freshwater (90%, 80%, etc., to 100% freshwater), followed by dehydration using a ten step gradient from freshwater to 100% acetone (10%, 20%, etc., to 100% acetone), which was then followed by a gradient of HMDS. Samples were filter-mounted to a stub and sputter coated with 1.5 nm of gold–palladium (Denton Vacuum Desk II Sputter Unit, Moorestown, NJ, USA). To remove the polysaccharide mucilage, a second set of cultures were fixed in 2% glutaraldehyde, placed in a 2 mL microcentrifuge tube, vortexed briefly (~1–2 s) twice a day for three days, desalted, dehydrated and filter-mounted as described above. Measurements (length and width) of at least 25 cells observed with the BX51 were analyzed using MicroSuite Five (Olympus, Japan).

2.4. DNA analysis

Four isolates from the bloom were selected for DNA sequencing. DNA was extracted from live cells of each isolate using Chelex® (Bio-Rad) chelating resin (Walsh et al., 1991; Richlen and Barber, 2005). The highly variable D1–D2 domains of the large subunit ribosomal RNA gene (LSU rRNA) were amplified from all isolates using the polymerase chain reaction (PCR) and the previously reported primers "D1R" (5′-ACCCGCTGAATT TAAGCATA-3′) and "D2C" (5′-CCTTGGTCCGTGTTTCAAG) (Scholin et al., 1994). PCR reactions contained 1.0 μL template DNA, 14.5 μL sterile deionized water, 2.5 μL GeneAmp® 10× PCR buffer composed of 500 mM potassium chloride and 100 mM Tris–HCl (pH 8.3 at room temperature), 2.5 μL 8 μM dNTPs (2 μM each of dATP, dCTP, dGTP, dTTP), 2.0 MgCl$_2$ (25 mM), 1.25 μL of each primer (10 mM), and 0.125 units/μL AmpliTaq® DNA Polymerase. Amplifications were performed using an Applied Biosystems GeneAmp PCR system (Applied Biosytems Inc., Foster City, CA, USA) as follows: 94 °C for 4 min; then 35 cycles of 94 °C for 30 s, 57 °C for 1 min, 72 °C for 2 min; and a final extension of 72 °C for 10 min. PCR amplification products were visualized by electrophoresis on 1% TAE agarose gel adjacent to a 100 bp DNA ladder. Positive PCR products were purified using the Qiagen MinElute PCR purification kit (Valencia, CA, USA) following the manufacturer's instructions. Purified products were stored at −20 °C until needed for sequencing. An estimate of the concentration was determined relative to the 100 bp DNA ladder.

DNA sequencing was performed using ABI BigDye version 3.0 (Applied Biosytems Inc., Foster City, CA, USA). The reactions consisted of 0.5 μL BigDye, 0.6 μL of 10 μM forward or reverse PCR primer, 0.1 μL DMSO and 1–3 μL purified PCR product to a volume

Table 1
Strain identification number, collection location, geographic region, and GenBank accession number for *Cochlodinium polykrikoides* cultures and sequences used in this study.

Strain	Collection location	Region	GenBank accession number
CCPk03	Narodo, South Korea	Eastern China Sea	DQ779985
CCPk05	Hakdong, South Korea	Eastern China Sea	DQ779986
CCPK06	Saryang-do, South Korea	South China Sea	AY347309
CP2002-1	Namhae, South Korea	South China Sea	EF506622
IN1-ND104	Oita, Inokushi Bay, Japan	NW Pacific Ocean	AB288383
IN5-ND81	Oita, Inokushi Bay, Japan	NW Pacific Ocean	AB295044
PP-6	Busan, South Korea	Eastern China Sea	EF506618
HK	Hong Kong	South China Sea	EF506623
C. poly	South Korea	Eastern China Sea	AF067861
C. poly	South Korea	Eastern China Sea	AY725423
PR107	Phosphorescence Bay, Puerto Rico	Caribbean Sea	AB295050
CPPV-1	Mexico	Tropical Eastern Pacific	EF506627
CP2	Sabah, Malaysia	South China Sea	AB295049
CPFB-06-1	Long Island, New York, USA	NW Atlantic	EF110556
CPDBC4*	United Arab Emirates	Arabian Gulf	GQ500117
CPCB10	Cotuit Bay, MA, USA	NW Atlantic	EF506625
MBCP	Manila Bay, Philippines	South China Sea	AB295046
OM7-ND59	Omura Bay, Japan	Eastern China Sea	AB295047

* Strain maintained in culture at the Woods Hole Oceanographic Institution, Woods Hole, MA.

of 6 μL with nuclease-free water. Thermocycling conditions were 60 cycles of 96 °C for 15 s, 50 °C for 5 s, and 60 °C for 4 min. Reactions were precipitated using isopropanol, air dried, and resuspended in Hi-Di Formamide before being analyzed on an ABI 3730xl capillary sequencer. Products were sequenced in both the forward and reverse direction.

2.5. DNA sequence analysis

Sequences were edited by eye for base-calling errors and consensus sequences assembled using Sequencher 4.2.2 (Gene Codes, Ann Arbor, MI, USA). Consensus sequences were aligned with LSU rRNA gene sequences of *C. polykrikoides* available in GenBank and imported into the software program, MacClade 3.06 (Maddison and Maddison, 2001), where alignments were checked by eye. The strain identification number, collection location, geographic region, and GenBank accession numbers for *C. polykrikoides* sequences used in this study are listed in Table 1.

Modeltest V. 3.7 (Posada and Crandall, 1998) was used to select the appropriate model of nucleotide substitution for phylogenetic analyses. Phylogenetic trees were constructed with PAUP* version 4.0b 10 (Swofford, 2000) using maximum parsimony (MP) and maximum likelihood (ML) analyses using *Heterocapsa rotundata* LSU rRNA gene (accession no. EU165312) as an outgroup. Parsimony analyses were conducted using the heuristic search, simple addition, with gaps treated as missing data. Heuristic searches using maximum likelihood employed the following parameters selected by the Akaike Information Criterion in Modeltest (AIC): The general-time-reversible model (GTR + G) (Rodriguez et al., 1990) with base frequencies (A = 0.2422, C = 0.2069, G = 0.3009, T = 0.2500), variable substitution rates (AC = 0.1755, AG = 1.4408, AT = 1.1258, CG = 0.7896, CT = 3.3422, GT = 1.0000), and gamma distribution = 0.2606. Bootstrap support values were determined for both parsimony and maximum likelihood using 1000 replicates.

3. Results

3.1. Description of event

Patches of red water (discoloration) were first observed in the port of Dibba Al-Hassan on the east coast of the UAE in late August 2008 (Fig. 1). Nearly two months later, red tide blooms and fish kills were observed there, from 21 to 23 October 2008. The bloom subsequently entered the Arabian Gulf through the Strait of Hormuz, spreading to coastal waters of the UAE, Qatar, and Iran. It also spread southwards along the east coast of the UAE to Fujairah and then south to Oman. The bloom was remarkably immense and severe, affecting more than 1200 km of coastline in the region and causing massive mortalities of wild and farmed fish as well as extensive coral reef damage. Near Fujairah, UAE, cell counts of 1.1–2.1 × 10^7 cells/L were recorded from surface waters during the bloom period (October, 2008); salinity at the time of highest cell counts was 39 psu and water temperature was 27 °C (Rajan, personal observation). The news media reported concentrations as high as 27 million cells/L in some areas (UAE-Interact, 2009). The bloom forced the closures of at least five seawater desalination plants in the UAE due to clogging of intake filters, concerns that the bloom would irreversibly foul reverse osmosis membranes, or other operational problems caused by the dense blooms (WDR, 2008; Nazzal, 2009). There was also a concern that red tide toxins might end up in the finished, drinking water. Impacts were felt by coastal recreation and tourism, due in part to an unpleasant odor associated with the bloom that permeated coastal communities, and in part because of fears regarding the potential risk to swimmers. Regional news agencies reported that thousands of tons of fish and marine mammals were killed; in Dibba Al-Hassan, over 650 tons of dead fish washed ashore and in Khor Fakkan more that 700 tons were reported (WDR, 2008). In the UAE, the bloom also restricted traditional fishing activities within eight miles of the eastern coast and three miles of the western coast. News reports described significant damage to coral reefs in the Dibba Marine Protected Zone, a marine protected area established by the UAE, and a scarcity of reef fish compared to population levels before the bloom (Landais, 2009).

In Iranian waters on the north side of the Gulf of Oman and Arabian Gulf, information regarding the bloom's extent and impacts is scant; however, media reports describe the spread of the bloom along the Arabian Gulf's eastern coast, to the western coast in Khuzestan Province, with impacts to farmed fish and shrimp (PressTV, 2009).

3.2. Morphological analysis

Morphological characters important in the identification and discrimination of *C. polykrikoides* include cell size and shape, the presence of rod-shaped chloroplasts, and the degree of rotation of the cingulum, which makes 1.8–1.9 turns around the cell (Faust and Gulledge, 2002; Matsuoka et al., 2008). The shape and size of the chloroplasts are important in differentiating *C. polykrikoides*

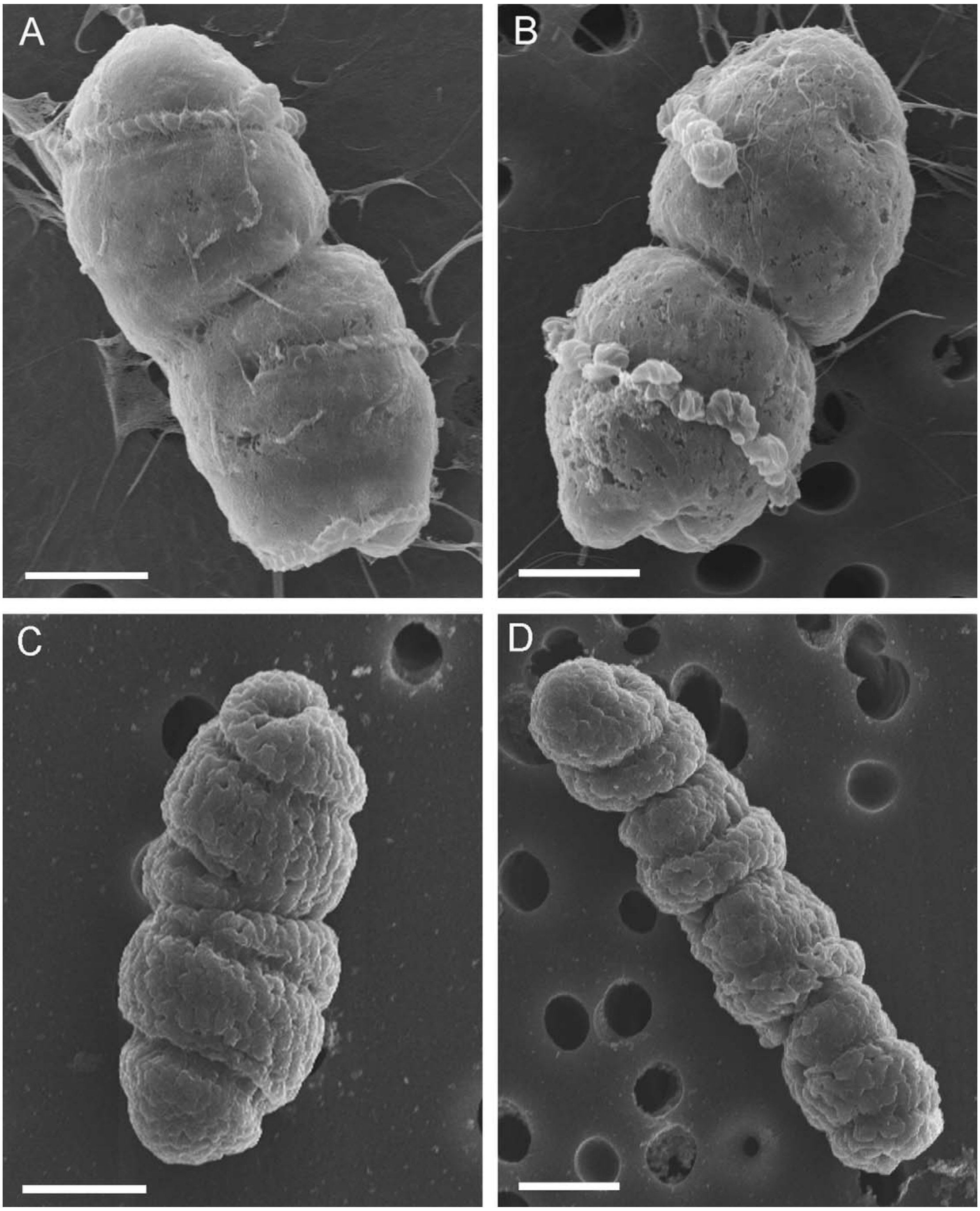

Fig. 2. SEM micrographs of *Cochlodinium polykrikoides* from the Arabian Gulf. Cells are oriented apically. (A) Two-celled chain enmeshed in a mucoid matrix; sulcus can be observed in the top cell, just below the cingulum in the anterior portion of the cell. (B) Two-celled chain showing transverse flagellum of each cell; the excavated cingulum of upper cell is clearly visible. (C) Two-celled chain following treatment to remove polysaccharide mucilage, revealing excavated cingulum of both cells. (D) Four-celled chain. Scale bars: 10 μm.

(rod-shaped) from *C. fulvescens* (granular shape) and from *C. convolutum* (reticulate shape). The holotype of *C. polykrikoides* is 50 μm in length, as were specimens from Malaysia, while isolates from coastal waters of Japan, Korea, and the northeastern USA are significantly smaller (30–40 μm) (Matsuoka et al., 2008). The cell sizes of Arabian Gulf cells ($n = 25$) were 38–41 μm in length and 33–36 μm in width, similar to the cells from Asia and the USA. The majority of cells observed in cultured material were in two-celled chains (Fig. 2A–C); rarely, four-celled chains were observed (Fig. 2D); single ellipsoidal cells were occasionally observed (e.g., Fig. 3A and B). Cells displayed a conical epitheca featuring a reddish-orange stigma and a bilobed hypotheca (Fig. 3B). The cingulum was excavated and displaced 1.6–1.9 body turns, and the sulcus was located anteriorly, just below the cingulum (Fig. 2A, B and D). The nucleus was clearly visible in the anterior of the cell (Fig. 3D), and many rod-shaped chloroplasts were present (Fig. 3E and F).

3.3. Phylogenetic analysis

The D1–D2 sequences of the LSU rRNA gene from 18 *C. polykrikoides* strains were included in the alignment (ca. 750 base pairs). Uncorrected pair-wise sequence divergence among isolates ranged from 0% to 13%. Maximum likelihood and parsimony

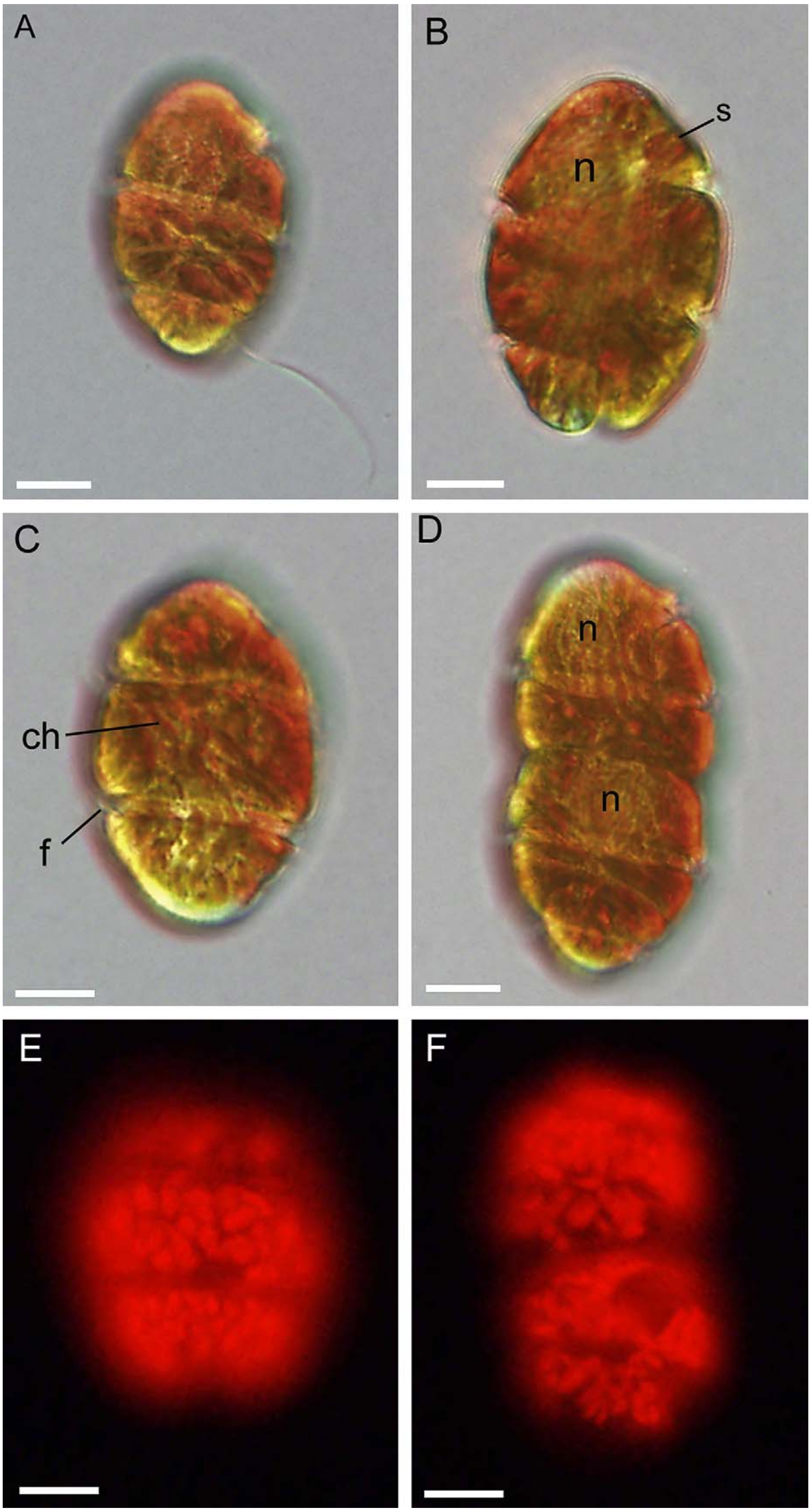

Fig. 3. Light and fluorescent micrographs of *Cochlodinium polykrikoides* from the Arabian Gulf. Cells are oriented apically. (A) Dorsal view showing longitudinal flagellum. (B) Dorsal view of cell showing the rounded epitheca and bilobed hypotheca; nucleus (n) and stigma (s) are visible in the anterior of the cell. (C) Dorsal view showing the rod-shaped chloroplasts (ch); transverse flagellum (f) is visible in the top cingulum. (D) Two-celled chain with nuclei (n) located in the anterior of the cell. (E) Fluorescent micrograph showing many rod-shaped chloroplasts. (F) Fluorescent micrograph of two-celled chain. Scale bars: 10 μm.

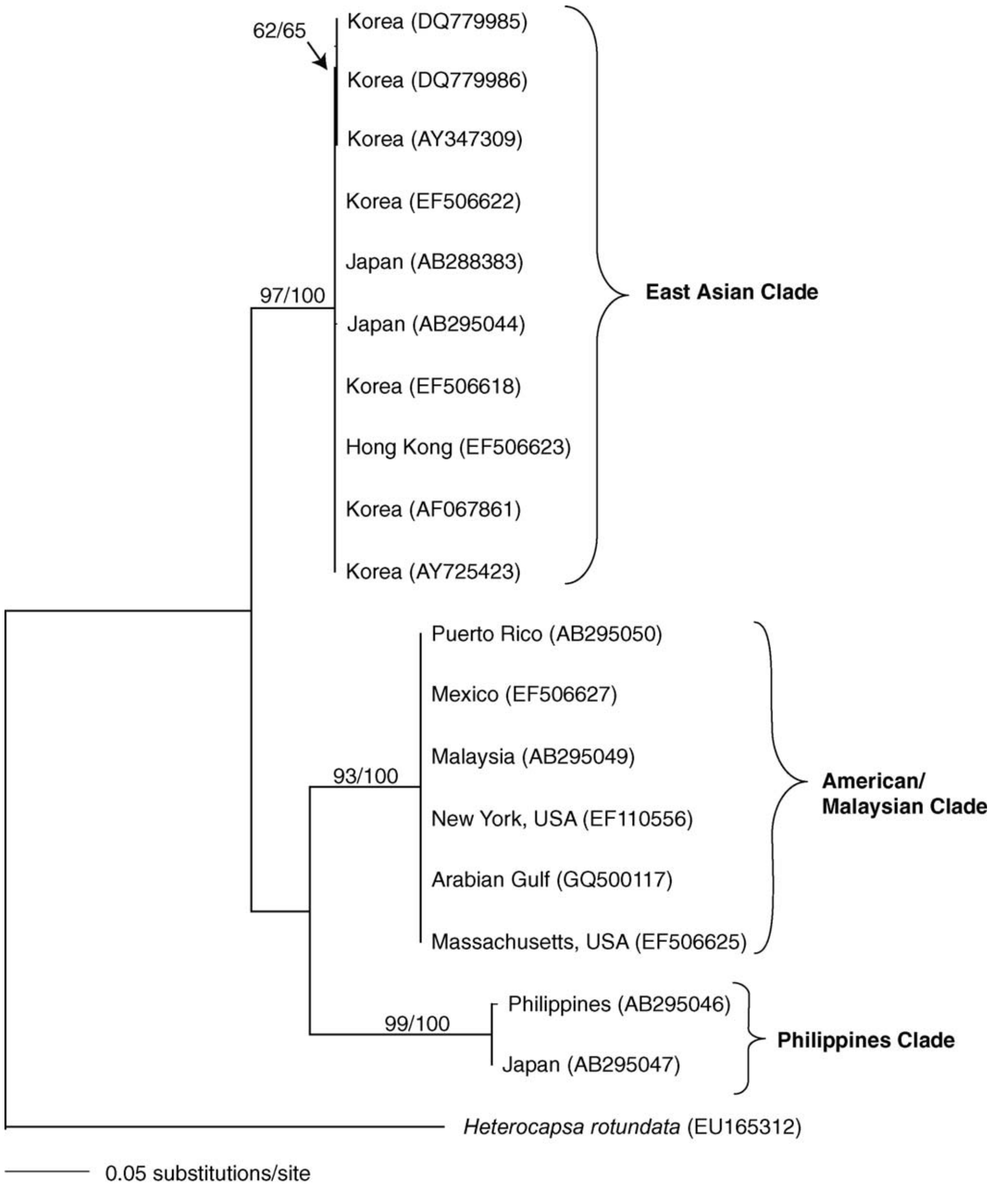

Fig. 4. Maximum likelihood tree using the GTR + G model on the LSU rRNA gene alignment (752 bp). Bootstrap values (1000) greater than 50% for maximum likelihood and maximum parsimony are shown above the branches. Clades are labeled according to designations by Iwataki et al. (2008). *Heterocapsa rotundata* was used as an outgroup.

phylogenetic analyses recovered three major clades supported by high bootstrap values (Fig. 4), known as the "American/Malaysian", "Philippines", and "East Asian" clades (Iwataki et al., 2008). The DNA sequences of all four cultures isolated from the Arabian Gulf bloom displayed 100% identity with each other and also to isolates from Puerto Rico (GenBank accession no. AB295050), Mexico (GenBank accession no. EF506627), Malaysia (GenBank accession no. AB295049), Long Island, NY, USA (GenBank accession no. EF110556), and Cotuit Bay, MA, USA (GenBank accession no. EF506625). These isolates comprised the "American/Malaysian" clade, supported by high bootstrap values (ML = 93%). The second clade, known as the "East Asian" clade, included isolates from Korea, Japan, and Hong Kong and was also supported by high bootstrap values (ML = 97%). The third clade included isolates from Japan and the Philippines, comprising the "Philippines" clade, which formed a sister group to the "American/Malaysian" clade. Genetic distances within the East Asian and Philippines clades were extremely low ($<0.05\%$) and the few sequences' differences were distributed throughout the gene regions examined.

4. Discussion

Historical reports on the occurrence and frequency of HABs in the Arabian Gulf and Gulf of Oman are scarce; however, several taxa of potentially toxic phytoplankton have been documented in the Arabian Gulf and nearby coastal waters, as have red tides resulting in significant fish kills and aquaculture losses. In a review of phytoplankton ecology in the Arabian Gulf, Subba-Rao and Al-Yamani (1998) describe a north to south gradient in phytoplankton species diversity, characterizing the northern gulf as an area of low diversity, dominated by diatoms, and the open Arabian Gulf and the Gulf of Oman as an area of high phytoplankton biodiversity, comprising primarily tropical species. The presence of 38 potentially bloom-forming or harmful algal taxa were reported, including *Dinophysis caudata*, *D. miles*, *Prorocentrum minimum*, *P. compressum*, *Ceratium furca*, and *Pyrodinium bahamense* (Subba-Rao and Al-Yamani, 1998; Rajan and Al Abdessalaam, 2008). To our knowledge, there are no published reports of *Cochlodinium* in the Arabian Gulf (Rajan and Al Abdessalaam, 2008); however, it apparently was observed previously in Kuwaiti coastal waters (Husain, 2003).

In 1999 a major fish kill occurred in Iranian coastal waters, which was immediately followed by fish kills in Kuwait Bay, prompting the Kuwait EPA to issue an advisory against consumption of shellfish and finfish (Heil et al., 2001). Although the cause of mortality in Iranian waters was not confirmed, fish kills in Kuwait Bay were attributed to a massive bloom of *Karenia* sp., thought to be the first such event leading to fish kills in the Arabian Gulf (Heil et al., 2001). In 2001 massive fish kills were again reported in Kuwait Bay, however, mortality was primarily associated with an epizootic caused by a bacterial pathogen (*Streptococcus*) (Evans et al., 2002; Glibert et al., 2002).

In contrast with the Arabian Gulf, HAB occurrences have been more frequently reported in the Gulf of Oman, although this difference is likely at least partially attributable to increased awareness and detection capabilities provided by Oman's phytoplankton monitoring program, which was initiated in 1988 following a massive fish kill attributed to a *Noctiluca* red tide (Thangaraja et al., 2007). A review of HAB occurrences in the coastal waters of Oman between 1976 and 2004 showed that about 66 red tide events have been recorded, 25 of which resulted in mass mortality of fish and marine organisms (Al-Gheilani et al., in preparation). The dominant HAB species in the Gulf of Oman is the dinoflagellate *Noctiluca scintillans*, which forms blooms annually and occasionally causing fish kills (Thangaraja, 1990; Al-Gheilani et al., in preparation). Other taxa that have been associated with mortalities of fish and other marine animals in the Gulf of Oman and outer Arabian Sea include *Karenia selliformis*, *Prorocentrum* spp., *Ceratium* sp., *Pleurosigma* sp., *Dinophysis* sp., *Gonyaulax* sp., and unspecified diatom and dinoflagellate species (Thangaraja et al., 2007; Al-Gheilani et al., in preparation).

In both the Arabian Gulf and the Gulf of Oman, there is evidence that HABs and their impacts are increasing. Subba-Rao and Al-Yamani (1998) describe a steady increase in the number of dinoflagellate events in the Arabian Gulf during the past 60 years; significantly, the recent fish kills from HABs (Heil et al., 2001; Glibert et al., 2002) are the first recorded. Similarly, in the Gulf of Oman HABs and their impacts have become more widespread and persistent (Al-Azri et al., 2007; Thangaraja et al., 2007; Al-Gheilani et al., in preparation). These events are notable in that these increasing HAB impacts are not associated with the expansion of one particular species in this region but with multiple taxa.

The increased frequency of HABs in the Gulf of Oman and the first occurrence of fish kills into the Arabian Gulf are synchronous with a well-documented global expansion and intensification of HABs in general. The mechanisms driving the expansion of HABs are varied and additive, and include (1) improved scientific awareness and analytical capabilities; (2) increased use of coastal waters for aquaculture; (3) geographic expansion via natural dispersal and human-aided transport; and (4) long-term increases in nutrient loading of coastal waters and unusual climatological conditions (Anderson, 1989; Hallegraeff, 1993). Several of the aforementioned mechanisms are likely responsible for the increase in HAB events observed in the Arabian Gulf and Gulf of Oman. For example, this increase is certainly partially attributable to increased awareness and monitoring and likely reflects improvements in the breadth of data acquired in recent studies compared with the limited historic data. However, several recent bloom events (e.g., Heil et al., 2001), including the 2008–2009 *Cochlodinium* bloom, are unprecedented and may represent true HAB expansion events.

Media reports on the chronology of the 2008–2009 bloom suggest that the *Cochlodinium* bloom affecting the Arabian Gulf may have originated in the Gulf of Oman and was subsequently transported into the Arabian Gulf though the Strait of Hormuz. As noted by Dorgham et al. (1987), regional circulation regimes clearly suggest an allochthonous origin of at least some taxa that originate in the Gulf of Oman and are advected into the Arabian Gulf. The mechanistic processes that were involved in the *Cochlodinium* bloom initiation and spread are not known, but may relate to physical forcing factors such as the Arabian Sea's reversing monsoon system that drive convective mixing, resulting in the upward transport of nutrients in the northern Arabian Sea. During the SW Monsoon season (summer; June–September), circulation patterns take the form of a gyre in the Arabian Sea composed of the Somali current flowing northeast along the east African coast, the broad eastward Southwest Monsoon Current, and the westward South Equatorial Current (Wyrtki, 1973). Wind-driven coastal currents drive a broad (~1000 km) region of strong and continuous coastal upwelling, with highest nutrient concentrations close to the Omani coast (Brock and McClain, 1992; Barber et al., 2001). The biological response includes an exceptionally high and sustained rate of primary productivity in coastal waters as well as the open-ocean regions during the SW Monsoon, yielding high concentrations of phytoplankton biomass (Ryther and Menzel, 1965; Brock and McClain, 1992; Barber et al., 2001). A gyre system in the Gulf of Oman (Reynolds, 1993) may have provided an offshore refuge or reservoir of cells that episodically were released into coastal currents that carried them north and west into the Arabian Sea, or south along the coast of Oman and the east coast of the UAE. Indeed, the pulsed nature of the outbreaks at different locations along the coast suggests an offshore source with wind-driven delivery patterns.

Flushing times in the Arabian Gulf are estimated to be approximately 3–5.5 years (Sheppard, 1993); thus, once they enter the system, organisms able to survive the salinity changes and adapt to the Gulf's unusual climatic and environmental conditions may become established. *Cochlodinium* is described as a euryhaline and eurythermal species with wide temperature and salinity tolerances, well-adapted to warm (25 °C) offshore waters (Kudela et al., 2008). Laboratory experiments examining the effects of temperature, salinity and irradiance on the growth of cultured *C. polykrikoides* from Korea found that the organism was able to grow in temperatures ranging from 21 to 26 °C and at salinities from 15 to 50 psu (Kim et al., 2001, 2004; Lee et al., 2001). Data are not yet available on the temperature and salinity tolerances of *Cochlodinium* isolates from the Arabian Gulf, but the general ability of *Cochlodinium* to sustain growth at a wide range of temperatures and its preference for high salinities likely contributes to the ability of this dinoflagellate to proliferate in the unusual environmental conditions in the Arabian Gulf.

The role that local influences such as nutrient loading may have had in supporting the growth of *Cochlodinium* during the course of the bloom is not known; however, nutrient measurements made prior to and during the 1999 red tide in Kuwait Bay indicated that elevated nutrients, potentially from aquaculture activities as well as industrial and sewage inputs, may have played a role in the initiation and maintenance of this bloom responsible for fish kills in that bay (Heil et al., 2001). Nutrient enrichment was also suggested as an important factor that contributed to the 2001 epizootic in Kuwait Bay (Glibert et al., 2002). The coastal waters of the Arabian Gulf have been exposed to a variety of environmental pressures and pollutants, including pollution from oil operations; industrial and domestic wastewater inputs such as treated and untreated sewage, desalination effluents, wastewater from fertilizer plants (e.g., urea), oil refinery outfalls; and land runoff from agricultural operations (Sheppard et al., 1992). The recently developed fish aquaculture industry in the region represents an additional source of nutrient enrichment to coastal waters, though the number of farms is presently quite small. In addition to anthropogenic eutrophication, nutrient regeneration from the decomposition of the algal bloom likely contributed to the size of the available nutrient pool; given the immense size and duration of the bloom coupled with low flushing rates in the Gulf, this nutrient flux may have helped to maintain and propagate the bloom. As eutrophic conditions have been implicated in *Cochlodinium* blooms elsewhere (e.g., Ahn et al., 2006; Anton et al., 2008), the potential stimulation of the 2008–2009 Arabian Gulf bloom by anthropogenic nutrient enrichment and/or nutrient regeneration clearly warrants further investigation.

To our knowledge, this is the first HAB event associated with *C. polykrikoides* in this region. Our phylogenetic analyses of isolates collected during the bloom show that partial LSU rRNA gene sequences of *C. polykrikoides* from the Arabian Gulf were identical to sequences comprising the "American/Malaysian" ribotype, and are genetically distinct from the "East Asian" and "Philippines" ribotypes responsible for red tides in Korea, Japan, and the Philippines (Iwataki et al., 2008). While geographic distribution of the "East Asian" and "Philippines" ribotypes are regionally restricted, the American/Malaysian ribotype is widespread and thus far includes isolates from the Caribbean, western Atlantic, Gulf of California (Mexico), and the South China Sea; here we show that this clade also includes populations present in the Arabian Gulf. With the exception of Japan, thus far it appears that there is little overlap in the geographic ranges of ribotypes; however, expanded geographic sampling and intensive local sampling may help determine if bloom populations and non-bloom "background" populations comprise a single ribotype or a combination of ribotypes, and may help resolve the perplexing phylogeographic distribution of the American/Malaysian ribotype. Such studies will benefit from the recent development of microsatellite markers for this species (Nishitani et al., 2007), which may reveal finer-scale genetic structuring among isolates within each clade and enable investigations regarding the origin and mechanisms of transport of *Cochlodinium* populations in the Arabian Gulf. Similarly, the examination of Arabian Gulf and Arabian Sea sediments for *Cochlodinium* cysts might help determine the origin of *C. polykrikoides* populations responsible for the bloom and help gauge the potential for future blooms in the region. These questions are particularly salient given the apparent global expansion of this species (Kudela et al., 2008) and attendant economic and ecological impacts. Given the long residence time of water in the Arabian Gulf, the existence of a cyst stage in the *Cochlodinium* life cycle, and the pattern of this organism's blooms in other regions, it seems likely that there will be future blooms of *C. polykrikoides* in the Arabian Gulf. Recurrent blooms are also likely in the Gulf of Oman and western Arabian Sea, for many of the same reasons. As Arabian Gulf countries rely on desalination plants as the primary source of freshwater for populations, agriculture, and industry, the disruption of plant operations by recurring *Cochlodinium* blooms poses a serious threat to the drinking water supply in the region and represents an unprecedented HAB impact. The sudden emergence of *C. polykrikoides* in the Arabian Gulf and the catastrophic impacts of the 2008–2009 bloom clearly illustrate the need for coordinated monitoring programs for HAB species, as well as development and testing of protocols and/or technology to prevent desalination plant closures during severe HAB blooms.

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