



## *Prorocentrum minimum* (Pavillard) Schiller A review of a harmful algal bloom species of growing worldwide importance

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### Abstract

*Prorocentrum minimum* (Pavillard) Schiller, a common, neritic, bloom-forming dinoflagellate, is the cause of harmful blooms in many estuarine and coastal environments. Among harmful algal bloom species, *P. minimum* is important for the following reasons: it is widely distributed geographically in temperate and subtropical waters; it is potentially harmful to humans via shellfish poisoning; it has detrimental effects at both the organismal and environmental levels; blooms appear to be undergoing a geographical expansion over the past several decades; and, a relationship appears to exist between blooms of this species and increasing coastal eutrophication. Although shellfish toxicity with associated human impacts has been attributed to *P. minimum* blooms from a variety of coastal environments (Japan; France; Norway; Netherlands; New York, USA), only clones isolated from the Mediterranean coast of France, and shellfish exposed to *P. minimum* blooms in this area, have been shown to contain a water soluble neurotoxic component which killed mice. Detrimental ecosystem effects associated with blooms range from fish and zoobenthic mortalities to shellfish aquaculture mortalities, attributable to both indirect biomass effects (e.g., low dissolved oxygen) and toxic effects. *P. minimum* blooms generally occur under conditions of high temperatures and incident irradiances and low to moderate salinities in coastal and estuarine environments often characterized as eutrophic, although they have been found under widely varying salinities and temperatures if other factors are conducive for growth. The physiological flexibility of *P. minimum* in response to changing environmental parameters (e.g., light, temperature, salinity) as well as its ability to utilize both inorganic and organic nitrogen, phosphorus, and carbon nutrient sources, suggest that increasing blooms of this species are a response to increasing coastal eutrophication.

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## 1. Introduction

The apparent increase in the frequency, magnitude, and distribution of harmful phytoplankton species within the past decade (Anderson, 1989; Hallegraeff, 1993; Smayda, 1997; Anderson et al., 2002) has focused attention on the unique physiological, ecological, and toxicological aspects of the individual species involved. While blooms and the toxic effects associated with some harmful algal bloom (HAB) species are restricted to local environments, other species are appearing in areas where they were previously unknown, apparently 'spreading' in a geographical sense via a variety of possible mechanisms (e.g., ballast water transport, aquaculture development, transport of seed stock, and increasing eutrophication GEOHAB, 2001). Many HAB species are thus becoming endemic in regions where no previous records of their presence exist.

*Prorocentrum minimum* is a common, neritic, bloom-forming dinoflagellate with a pan-global distribution (Fig. 1). Its ecology and bloom dynamics have been well documented in selected environments (e.g., Chesapeake Bay, Baltic Sea) and its ease of culturing and the widespread availability of clones for experimental use have led to an extensive literature on this species. The apparent spreading of *P. minimum* to previously unreported areas as well as documentation

of its toxins and indirect harmful effects on ecosystems suggest that a review of the extant literature on this species is timely.

## 2. Taxonomy and systematics

*P. minimum* was first described by Pavillard (1916) as *Exuviaella minimum* from the Gulf of Lion. What is now considered to be *P. minimum* was subsequently described as *Exuviaella apora* Schiller (Lebour, 1925), *Prorocentrum triangulatum* (Martin, 1929), *Exuviaella mariae-lebouriae* (Parke and Ballantine, 1957), and *Prorocentrum cordiformis* (Bursa, 1959). The considerable confusion that has existed with regard to the identification of *P. minimum* since its initial description (Table 1) is in large part due to its extremely variable cell shape (Hulburt, 1965) and the presence of a small anterior spine which is not always recognizable under light microscopy. This variability in cell shape has often led to the description of local forms as new species (Fukuyo et al., 1990; Marasović et al., 1990), leading to the variety of synonyms for *P. minimum* in the literature (Table 1). Examples of the morphological variability of this species are given by Hulburt (1965), who examined clones isolated from blooms around Woods Hole, Massachusetts and Long Island, New York, and Hajdu et al. (2000) who

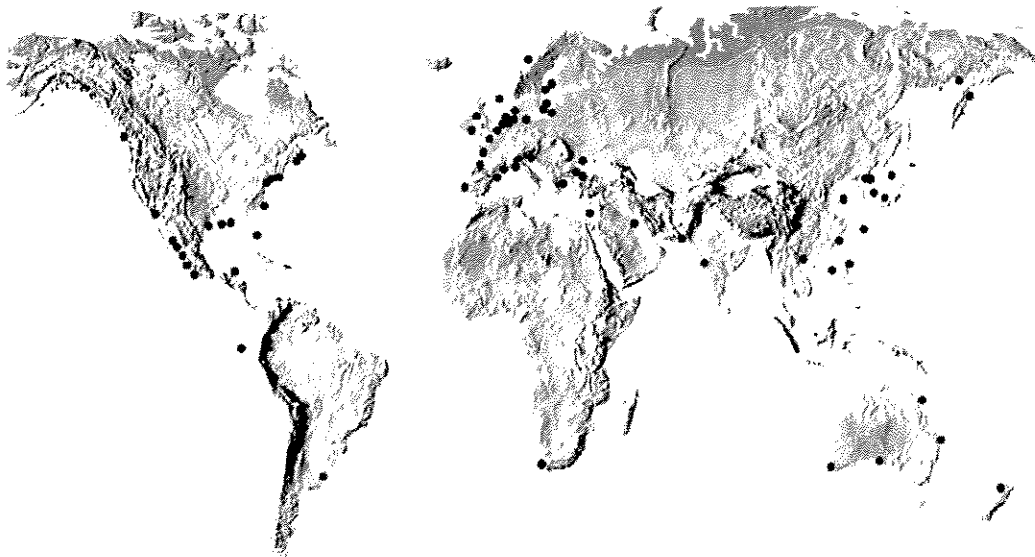


Fig. 1. Global distribution of *Prorocentrum minimum* based on the literature cited in this review.

Table 1  
Common taxonomic synonyms of the dinoflagellate *Prorocentrum minimum* from studies where morphological characteristics are described

Name	Reference
<i>Prorocentrum minimum</i> (Pavillard) Schiller, 1933	Schiller (1933); Campbell (1973); Taylor (1976); Loeblich et al. (1979); Toriumi (1980); Honsell and Talarico (1985); Kimor et al. (1985); Horiguchi (1990); Marasović et al. (1990); Hansen and Larsen (1992); Grzebyk et al. (1998); Hajdu et al. (2000); Pertola et al. (2003)
<i>Exuviaella minima</i> Pavillard, 1916	Pavillard (1916)
<i>Exuviaella apora</i> Schiller, 1933	Parke and Ballantine (1957)
<i>Exuviaella marie-lebouriae</i> Parke and Ballantine, 1957	Parke and Ballantine (1957); Dodge (1965)
<i>Prorocentrum triangulatum</i> Martin, 1929	Martin (1929)
<i>Prorocentrum cordiformis</i> Bursa, 1959	Bursa (1959)
<i>Prorocentrum minimum</i> var. <i>triangulatum</i> (Martin) Hulburt, 1965	Hulburt (1965)
<i>Prorocentrum minimum</i> var. <i>mariae-lebouriae</i> (Parke and Ballantine, 1957) Hulburt, 1965 Hulburt (1965) <i>Prorocentrum mariae-lebouriae</i> (Parke and Ballantine, 1957) Loeblich, 1970	Loeblich (1970)
<i>Prorocentrum mariae-lebouriae</i> (Parke and Ballantine, 1957) Dodge et Bibby	Dodge and Bibby (1973)
<i>Prorocentrum mariae-lebouriae</i> (Parke and Ballantine, 1957) Faust	Faust (1974)
<i>Prorocentrum cordatum</i> (Ostenfeld) Schiller, 1933	Velikova and Larsen (1999)
<i>Prorocentrum cordatum</i> (Ostenfeld) Dodge	Taylor et al. (2003)

examined variability in *P. minimum* cell size in different areas of the Baltic Sea. Hulburt (1965) reported a gradation in *P. minimum* shape from triangular to heart-shaped and oval, with a considerable overlap in size. Similarly, Lu et al. (2005) have reported on *Prorocentrum* spp. in Chinese blooms that vary in shape and other morphology as well. Hajdu et al. (2000) demonstrated that *P. minimum* in different areas of the Baltic exhibited different average cell sizes, with triangular shaped cells dominating in open sea populations and a round-oval form common in coastal zone populations. Different shaped *P. minimum* cells were often present in the same samples in both studies. Generalizations made concerning relationships between *P. minimum* morphology and geographical distributions and salinity must be regarded with caution, however, as Pertola et al. (2003, 2005) reported relationships between cell shape that are contrary to those reported by Hajdu et al. (2000).

Various authors (Hulburt, 1965; Dodge, 1975; Fukuyo et al., 1990) have recommended that different forms of *P. minimum* be treated as variations (or morphotypes) within the species. Hulburt (1965) further suggested the retention of varietal status for three forms: *P. minimum* var. *minimum*, *P. minimum* var. *triangulatum*, and *P. minimum* var. *mariae-*

*lebouriae*. Steidinger and Tangen (1997), however, did not recognize these different varieties as separate taxa, and Hajdu et al. (2000) suggested that varietal distinctions are not meaningful for Baltic clones of *P. minimum*. Given the presence of blooms over a wide range of salinities and the large variability in cell shape, the effects of environmental factors (e.g., salinity) as well as physiological status and nutritional mode (e.g., autotrophic versus mixotrophic nutrition) on cell shape merit further investigation.

*P. minimum* is small (approximately 20  $\mu\text{m}$  in length and slightly smaller in width), variably heart shaped, triangular, or oval, flattened in side view, with a slight concavity at its wider end (Faust, 1974). Its surface is covered in small spines which display a variety of surface densities, and a single spine, not always recognizable under light microscopy, is located at its anterior end. Although frequently confused with *P. balticum*, *P. minimum* is larger, with a different shape and surface marking (Steidinger and Tangen, 1997). There is also considerable debate on the conspecificity of the older species *P. cordatum* (= *Exuviaella cordata*; Ostenfeld, 1901) and *P. minimum* (Marasović et al., 1990; Velikova and Larsen, 1999; Taylor et al., 2003). In a recent review of dinoflagellate taxonomy, *P. minimum* is considered synonymous with *P. cordatum* (Ostenfeld) Dodge

(Taylor et al., 2003). The original description of *P. cordatum* is from a Caspian Sea isolate, differing from *P. minimum* only in its lack of spine. It is considered endemic in the Caspian, Black, Azov, and Aral Seas while *P. minimum* had never been recorded in this area prior to the early 1980s (Marasović et al., 1990). After examination of samples of *E. cordata* from a June 1986, Black Sea bloom, Marasović et al. (1990) concluded that the causative species was actually *P. minimum* and recommended re-examination of samples previously identified as *E. cordata* from earlier blooms. Velikova and Larsen (1999) examined Caspian Sea samples with scanning electron microscopy and reported the presence of a small spine. They therefore concluded that these two species were conspecific and the name *P. cordatum* had precedent over *P. minimum*. Yet debate remains as others have found that Velikova and Larsen's micrographs do not clearly show the morphological details of the apical area and type specimens were not examined, only samples from the type locations. Velikova and Larsen's proposal for the conspecificity of these two species is based on the assumption that Ostenfeld's original description, which does not show a small spine in any of the four drawings, was incorrect and that the presence of a spine was missed due to the use of light microscopy. Thus, the conspecificity of *P. cordatum* and *P. minimum* is currently accepted by some researchers (Taylor et al., 2003), but not by others (Pertola et al., 2003). In light of the debate over its identity, the name *P. minimum* will continue to be used throughout this review, but we do so for convenience and not because of analysis of the micrographs in question. A study which combines light and electron micrographic examination of *P. minimum*, *P. balticum*, and *P. cordatum* isolates from a

variety of geographical distributions (including type locations) and salinities, in combination with genetic analyses, would help resolve the taxonomic confusion associated with *P. minimum*.

### 3. Toxicology and harmful effects

#### 3.1. *P. minimum* toxins and human effects

Human impacts from *P. minimum* outbreaks have been suggested from around the world (Table 2). The most significant human mortality attributed to *P. minimum* occurred in Lake Hamana, Japan in March, 1942, when 114 of 324 affected people died after consuming oysters (*Venerupis semidecussata*) and short-necked clams (*Tapes semidecussata*) (Akiba and Hattori, 1949). Seventy-one deaths were later attributed to ingestion of toxic oysters (*Crassostrea gigas*) from the same region in March 1943, and ingestion of toxic clams in March 1949. Symptoms included heavy liver injury (necrosis and fatty degeneration), hemorrhage diathesis with frenzy, unconsciousness and coma, and death occurring 24–48 h after symptoms appeared. Akiba and Hattori (1949) isolated a nitrogenous toxic substance from the mid-gut of the short necked clam which produced symptoms in mice similar to those observed in humans, which they named "venerupin" and the associated syndrome was named Venerupin Shellfish Poisoning (VSP). The source of the toxin was hypothesized to be *P. minimum* (as *E. mariae-lebouriae*) by Nakajima (1965a,b,c, 1968) based on a similarity between toxic constituents in *P. minimum* and venerupin. Two water soluble toxins were subsequently isolated from *P. minimum* (Okaichi and Imatomi, 1979) which caused symptoms

Table 2  
Human effects attributed to *P. minimum*

Location and year	Vector	Impact	Reference
Lake Hamana, Japan, 1942	Oysters ( <i>Venerupis semidecussata</i> ) Clams ( <i>Tapes japonica</i> )	114 deaths 342 humans effected	Akiba and Hattori (1949) Nakajima (1965a,b,c, 1968)
Obidas Lagoon, Portugal	Shellfish	Human poisoning	Silva (1963, 1985) Silva and Sousa (1981)
Norway <sup>a</sup> 1979, 1981	Mussels	Human poisoning	Tangen (1980, 1983) Langeland et al. (1984)
Wadden Sea <sup>a</sup>	Mussels	Human poisoning	Kat (1979)
Long Island, USA <sup>a</sup> , 1985	Clams, <i>Mercenaria mercenaria</i>	3 humans affected	Freudenthal and Jijina (1985)

<sup>a</sup> Toxicity in these events cannot be definitively attributed to *P. minimum* as *Dinophysis* spp. were present during the *P. minimum* bloom.

of prostration, dyspnea, and diarrhea in mice, with pathology involving liver necrosis. Shimizu (1987), in a review of the outbreak, suggested that the yield and toxicity of the two fractions isolated by Okaichi and Imatomi could not be definitively related to the dinoflagellate (or at least the clone examined) as the source of the toxin responsible for deaths in the original 1942 incident and Landsberg (2002) also concluded that definitive evidence that *P. minimum* was involved with VSP does not exist. Given the passage of time between the original incident and the attribution of the incident to *P. minimum*, the cause of the original outbreak may never be definitively established.

Other episodes of human poisoning, in which symptoms have been coincident with consumption of toxic shellfish associated with *P. minimum* blooms, have been reported from Norway (Tangen, 1983; Langeland et al., 1984), the Netherlands (Kat, 1979, 1983a,b, 1985), and Long Island, New York, USA (Freudenthal and Jijina, 1985; Table 1). In each of these cases, however, symptoms (e.g., vomiting, diarrhea, gastro-intestinal distress, and recovery within 2–4 days) bear a striking resemblance to symptoms associated with diarrhetic shellfish poisoning (DSP). An examination of phytoplankton community composition within the affected areas during or immediately prior to the outbreaks reveal the presence of *Dinophysis acuminata*; thus, attribution of toxic effects to *P. minimum* cannot be confirmed in these instances.

A range of human impacts of *P. minimum* via various shellfish vectors have been reported (Table 2). In a review of the effects of algal blooms on shellfish and aquaculture, Shumway (1990) suggested that toxic effects were often associated with *P. minimum* blooms and the toxin that accumulated in shellfish could cause effects on both shellfish harvested from bloom water and human shellfish consumers. The only definitive link between *P. minimum* and toxic shellfish, however, has been made by Grzebyk et al. (1997), who isolated several axenic clones of *P. minimum* from a French Mediterranean site which produced a water soluble neurotoxic component that rapidly killed mice. Denardou-Queneherve et al. (1999) were able to document the same toxic fraction from shellfish during a large ( $56 \times 10^6$  cells  $L^{-1}$ ) *P. minimum* bloom in the Salses-Leucate lagoon on the French Medi-

terranean coast. Outbreaks of shellfish toxicity associated with *P. minimum* blooms in Portugal (Grzebyk et al., 1997) and China (Chen and Gu, 1993) are more problematic in definitively linking to *P. minimum* toxicity. In Portugal (Obidos Lagoon), *P. minimum* has been implicated in several human poisonings in which symptoms appear characteristic of Paralytic Shellfish Poisoning (PSP) rather than DSP or the hepatotoxic related symptoms similar to the Japanese outbreak (Silva, 1980, 1985; Silva and Sousa, 1981). PSP events were reportedly caused by *P. minimum* in the Zhengjiang and Fujiang provinces from 1978 to 1985, but details are scarce (Chen and Gu, 1993).

Although the majority of *P. minimum* clones in culture are non-toxic, the toxicity associated with clones isolated from Japanese (Nakajima, 1965a,b,c, 1968) and French (Grzebyk et al., 1997; Denardou-Queneherve et al., 1999) coastal environments confirms the potential risk for human health associated with *P. minimum* and/or its co-occurring bloom species. The neurotoxic components in axenic *P. minimum* clones isolated from the French Mediterranean coast (Denardou et al., 1995; Grzebyk et al., 1997) appear completely dissimilar to hepatotoxic components described from Japanese clones. In the French study, toxic components were water soluble, sodium channel blockers (Denardou-Queneherve et al., 1999) producing neurotoxic symptoms in mice (convulsions, spasms with pronounced palpitations, death within minutes) and no PSP or DSP toxins were detected. Denardou et al. (1995) and Grzebyk et al. (1997) concluded that the development of toxicity in *P. minimum* blooms was clone-related and furthermore, that stable environments favored toxin production. The varying reports of toxicity in *P. minimum* thus may be related to the examination of different clones, use of different experimental protocols (e.g., axenic versus non-axenic conditions), and/or different environmental conditions.

Thus, *P. minimum* has been considered potentially toxic to humans via ingestion of toxic shellfish, although reported association between *P. minimum* blooms and human toxicity are rare (Tangen, 1983; Taylor et al., 2003). Many HAB researchers consider this species benign and harmless to human health (Maestrini, 1990); however, in areas where clones

with demonstrative toxic components have been isolated (e.g., French Mediterranean coast) or in areas where toxicity associated with *P. minimum* blooms has not been fully explained or attributed to other causes, *P. minimum* must be considered potentially toxic. Moreover, the potential for harmful effects related to high algal biomass occurs in all areas where the dinoflagellate blooms.

### 3.2. Direct effects of *P. minimum* exposure

*P. minimum* is a commonly used algal food source in experimental studies due to the widespread availability of clones and ease of culturing. No adverse effects have been reported in a large variety of experiments conducted using this species as a representative dinoflagellate food for zooplankton (Iwasaki et al., 1984; Dam and Colin, 2005), ciliates (Rosetta and McManus, 2003), shellfish (Pillsbury, 1985; Denis et al., 1999; Colin and Dam, 2002), or in toxicity tests conducted on fauna (e.g., crabs, fish) collected from large *P. minimum* blooms (e.g., Kimor et al., 1985). This is possibly due to the common experimental use of non-toxic clones. It should be noted, however, that several studies do document the existence of subtle negative effects when *P. minimum* is used as a food source, most notably a reduction in zooplankton egg production when compared with diatom diets (Turner et al., 2001) and a reduction in oyster grazing rates compared to a unialgal diet of the diatom *Thalassiosira weissflogii* (Luckenbach et al., 1993). A histological study on four life-history stages of the eastern oyster (Wikfors and Smolowitz, 1995) reported that feeding larvae showed poor growth and development of the digestive system when fed on *P. minimum*, although there were no toxic effects on its embryonic development from living, heat-killed cells or from growth medium extracts from *P. minimum* cultures. Stoecker et al. (1981) found that *P. minimum* was a poor food source for a tintinnid ciliate, while Skovgaard (2000) found that the mixotrophic dinoflagellate *Gymnodinium resplendens* grew well with *P. minimum* in its diet. These reported differences in the food quality of *P. minimum* may be related to the lack of specific compounds (e.g., fatty acids, sterols, and/or essential amino acids) within cells. Given the variety of nutritional modes in *P. minimum* (see below), it is

possible that specific compounds acquired via mixotrophic feeding may be nutritionally essential for certain grazers of *P. minimum* and that these compounds are not available when the dinoflagellate derives its nutrition from photosynthesis. Essentially, the nutritional mode of the dinoflagellate may potentially influence its palatability to grazers.

Recently, *P. minimum* isolated from annually recurring blooms in Chesapeake Bay tributaries were shown to have negative impacts on oysters and scallops in laboratory exposures (Wikfors and Smolowitz, 1999; Hégaret and Wikfors, 2005). Wikfors and Smolowitz (1993) noted poor survival, development, and growth of oyster and clam larvae when *P. minimum* blooms were present and suggested that the high density of *P. minimum* in natural blooms could have detrimental effects on the filtering and feeding process of the oyster population. In bioassay experiments, 100% mortality of juvenile scallops was found when exposed to *P. minimum* isolates from Chesapeake Bay tributaries (Hégaret and Wikfors, 2005). Luckenbach et al. (1993) found that bloom levels of *P. minimum* caused 100% mortality of juvenile oysters with 14 days and at 33% bloom density caused 43% mortality over 22 days. This research, as well as observations of poor growth of caged northern quahogs, *Mercenaria mercenaria*, in Long Island Sound during *P. minimum* blooms (Wikfors and Smolowitz, 1993), suggests that the particular *P. minimum* clones which have been blooming in Long Island and annually in the Chesapeake Bay may potentially contain a toxic fraction. Observations that *P. minimum* systematically affected absorptive cells and produced thrombi throughout the vascular systems of bay scallops indicate that an enterotoxin may be involved in these particular cases (Wikfors and Smolowitz, 1993; Landsberg, 2002).

Organism effects related to *P. minimum* blooms may not necessarily be detrimental to human health. One such effect is that of 'pink oyster phenomena', which has in Galveston, Texas, been attributed to the indirect effects of eastern oysters feeding on *P. minimum* blooms (Pickney, personal communication). The coloration is derived from the pigments in the algal food, decreasing marketability and consumer acceptance of oysters due to loss of visual appeal.

Table 3  
Detrimental organismal and ecosystem effects attributed to *P. minimum* in culture and in situ

Location	Organism affected	Effect	Reference
Laboratory	Zooplankton	Decrease in egg production and hatching rates	Lacoste et al. (2001)
Laboratory	<i>Temora</i> spp.	Lower egg production compared to diatom diet	Turner et al. (2001)
Laboratory	Eastern Oyster ( <i>Crassostrea virginica</i> )	Poor growth and development of digestive system Impairment of oyster life stages Mortality (45%) of juvenile oysters Grazing rate inversely related to <i>P. minimum</i> cell density Immunological effects	Wikfors and Smolowitz (1995) Luckenbach et al. (1993) Luckenbach et al. (1993) Luckenbach et al. (1993) Hégaret and Wikfors (2005)
Obidos Lagoon, Portugal	Fish	Death	Silva (1980)
Bohai Bay, China	Fish	Death	Yu (1987); cited in Tseng et al. (1993)
Gwadar Bay, SW Pakistan	Fish	Death from O <sub>2</sub> depletion	Rabbani et al. (1990)
Bulgarian Black Sea	Fish and zoobenthos	Death from O <sub>2</sub> depletion	Moncheva et al. (1995)
Philippines	Fish	Death from O <sub>2</sub> depletion	Azanza et al. (2005)
French Mediterranean	Mussels	Toxicity	Grzebyk et al. (1997)
Dutch coastal area	Shellfish	Toxicity <sup>a</sup>	Kat (1985)
German Wadden Sea	Shellfish	Toxicity <sup>a</sup>	Kat (1985)
Gulf of Trieste	Mussels	Toxicity <sup>a</sup>	Tubaro et al. (1995)

<sup>a</sup> Toxicity in these events cannot be definitively attributed to *P. minimum* as *Dinophysis* spp. were present during the *P. minimum* blooms.

### 3.3. Ecosystem effects of *P. minimum* blooms

Although *P. minimum* is considered to be non-toxic to marine invertebrates in general, larger blooms have been reported to cause environmental damage due to high algal biomass and related effects (Tables 2 and 3). These effects include localized oxygen depletion, pH change, and significant light attenuation in bloom regions. Large *P. minimum* blooms in Gwadar Bay, SW Pakistan, the Bulgarian Black Sea, and the Philippines have resulted in fish and zoobenthos death (Rabbani et al., 1990; Moncheva et al., 1995; Azanza et al., 2005). Although oxygen depletion is the suggested mode of action in the Black Sea and Philippines mortalities, *P. minimum* was considered to have a toxic effect at high concentrations during a Pakistan bloom (Rabbani et al., 1990). Large changes in pH can also accompany bloom development. Increases in pH to 9.75 have been reported for dense *P. minimum* cultures and natural populations (Hansen, 2002; Fan and Glibert, 2005) and have been related to the death of a variety of phytoplankton as well as protists (e.g., ciliates and heterotrophic dinoflagellates) and copepods (Pedersen and Hansen, 2003a,b). These examples illustrate the difficulty often encountered in attributing observed detrimental effects associated with harmful algal blooms to specific causes.

Reductions in light attenuation coincident with high biomass *P. minimum* blooms can also result in the loss of seagrasses and shellfish (Shumway, 1990; Luckenbach et al., 1993; Wikfors and Smolowitz, 1993; Gallegos and Jordan, 2002). In a Chesapeake Bay study, Gallegos and Jordan (2002) found that losses of submerged grasses mirrored the distributional extent of *P. minimum* blooms. The impacts of one particularly large *P. minimum* bloom on submerged aquatic vegetation in the Chesapeake Bay are described and modeled by Gallegos and Bergstrom (2005).

## 4. Biogeography

### 4.1. Temperature and salinity relationships

Blooms of *P. minimum* have been reported in many coastal waters around the world (Fig. 1) including tropical, subtropical, and temperate climates. While there is a noticeable lack of reports of *P. minimum* blooms from Africa and South America, it is possible, given the apparent global distribution of this species, that limited monitoring activities within these regions rather than fewer actual blooms are responsible for the sparse observations.

*P. minimum* has been described as a eurythermal and euryhaline species and blooms can occur under a wide range of environmental conditions (Tango et al., 2005). In many cases, blooms occur in relatively warm, low turbulence environments during periods of high irradiance. In Kiel Fjord, Germany, *P. minimum* bloom has been associated with calm and warm (20–22 °C) weather conditions and high incident irradiances ( $0.49\text{--}2.62 \text{ kJ m}^{-2} \times 10^4$ ; Kimor et al., 1985). A massive *P. minimum* bloom along the Pakistan coast was also associated with warm temperatures (27–29 °C), high light levels, and moderate winds (Rabbani et al., 1990). In Chesapeake Bay, high density blooms ( $>3 \times 10^6 \text{ cells L}^{-1}$ ) are found when temperatures are between 12–22 °C and salinity is 5–10 PSU (Tango et al., 2005). However, temperature may not be a limiting factor for the development of *P. minimum* blooms. Blooms in Lake Nakanoumi, Japan, often develop in winter and spring when temperatures range from 3 to 12 °C. Springer et al. (2005) have followed the progression of a *P. minimum* bloom in the Neuse Estuary, North Carolina, and found it to persist for several months during the winter. Also, in the Mediterranean, *P. minimum* can be a prominent species at temperatures from 4 to 27 °C (Grzebyk and Berland, 1996). There are, however, virtually no reports of blooms in waters exceeding 30 °C for extended periods.

Experimental studies have shown that *P. minimum* can grow over a broad salinity range. Hajdu et al. (2000) reported that Baltic clones grew between 2 and 35 PSU, but exhibited optimal growth at 15–17 PSU. Natural blooms of *P. minimum* also occur across a wide range as well (Tango et al., 2005), but, in many studies, bloom outbreaks have been correlated with declining salinities (Mendez, 1993) usually associated with freshwater inputs, which, coincidentally, result in increased nutrient loading (Silva, 1985; Granéli and Moreira, 1990; Grzebyk and Berland, 1996). In Amurskii Bay, Japan, in 1991, a massive *P. minimum* bloom was stimulated by nutrient loading after heavy rains (Stonik, 1995). Similarly, in the Gulf of Trieste in the northern Adriatic Sea, *Prorocentrum* sp. outbreaks were found to correspond to low salinity and high  $\text{NO}_3^-$  availability (Cabrini et al., 1995). Furthermore, *P. minimum* blooms in Chesapeake Bay are typically observed in late spring and early summer. In one particularly large 1998 bloom in Chesapeake Bay, the

winter and spring prior to the blooms was characterized by higher than average precipitation, yielding higher than average river flow and nutrient loading (Glibert et al., 2001; Fan et al., 2003a).

Tyler and Seliger (1981) demonstrated an interdependency of temperature and salinity effects upon growth rate with a *P. minimum* clone isolated from the Chesapeake Bay. At higher summer temperatures, growth rates were independent of salinity, allowing this species to bloom in lower salinity environments, while a combination of lower salinity and temperature inhibited growth rates. Tyler and Seliger used this relationship to explain the subsurface transport of *P. minimum* from southern Chesapeake Bay to tributaries within the northern Bay where it blooms in late spring through summer months (Tyler and Seliger, 1978, 1981). The origin of *P. minimum* blooms in the Kiel Fjord was also thought to have the same subsurface process of advection (Kimor et al., 1985).

#### 4.2. Succession and allelochemic interactions

Blooms of *P. minimum* commonly occur following diatom blooms (Kondo et al., 1990a,b,c,d; Borkman et al., 1993) from the late spring throughout the summer (Kimor et al., 1985). In particular, an association with blooms of the diatom *Skeletonema costatum* has been described from a variety of locations. In European waters, Silva (1985) described harmful outbreaks of *P. minimum* in Obidos Lagoon, Portugal, in summer 1973 and winter 1983, both immediately preceded by dense *S. costatum* blooms. Bodeanu and Usurelu (1979) also reported a large bloom of *P. minimum* (as *P. cordata*) in the Romanian Black Sea, preceded by ‘massive’ growth of *S. costatum*. Kondo et al. (1990a,b) described in detail the relationship between *P. minimum* and *S. costatum* in Lake Nakanoumi, Japan, over a 10 year period; *S. costatum* blooms preceded by one month eight major *P. minimum* blooms. These two species also frequently co-occur in Narragansett Bay, Rhode Island (Karentz and Smayda, 1984). Near-annual *P. minimum* blooms in the Chesapeake are also preceded by spring diatom blooms, including the co-dominant *S. costatum* (Sellner, 1987). Burford (1997) also showed a succession from diatoms (including *S. costatum*) to *P. minimum*-dominated flagellate blooms in hypereutrophic shrimp ponds.

The mode of interaction underlying the apparent temporal relationship between these two species is unknown. *S. costatum* is a ubiquitous coastal diatom with a global distribution. The relationship between these two species may simply result from the frequent proximity of two species with similar temperature and salinity preferences. Several laboratory studies suggest direct chemical mediation, however. Kondo et al. (1990c) showed that organic substances excreted by *S. costatum* in culture increased *P. minimum* cell yield and that this stimulation was up to three times greater than with other species examined (Iwasaki, 1979). Heil (1996) observed a stimulation of the dinoflagellate by *S. costatum* that was related to the growth stage of the diatom. The mechanism responsible for the stimulation in both of these studies was not determined. The stimulatory effects of organic compounds may not be exclusively algal related, however, as Kondo et al. (1990a,b) reported that lower molecular weight organic fractions isolated from bottom sediments of Lake Nakanoumi, Japan, were also stimulatory to *P. minimum* growth in culture, and Terlizzi et al. (2002), and Brownlee et al. (2003) reported that *P. minimum* growth was stimulated by extracts of barley straw. As described below, there is growing evidence that *P. minimum* may use organic as well as inorganic nutrients which may be an additional factor in the association with *S. costatum*.

Smayda and Borkman (2003) recently suggested that blooms of *P. minimum* in Narragansett Bay may also be related to blooms of *Heterosigma akashiwo*. By examining a 38-year record of the dynamics of these blooms, they found that years with high *P. minimum* abundance were followed by years with lower abundance and that the magnitude of the *P. minimum* blooms was related not only to the intensity of *S. costatum* blooms but also to that of *H. akashiwo*.

Culture experiments suggest that allelochemical interactions may play a significant role in promoting *P. minimum* blooms. Okamoto and Hirano (1987) reported that in laboratory cultures of *Nitzschia closterium*, the lag phase was prolonged by water taken from late phases of a *P. minimum* bloom on Lake Hamana-ko, and that *P. minimum* also inhibited growth of *P. micans* (Iwasaki, 1979). The biologically active extracellular metabolite, 1-(2,6,6-trimethyl-4-hydroxyxylohexanyl)-1,3-butanedione, a norcarotenoid referred to as beta-diketone, has been reported for

*P. minimum*, and found to be released in a single pulse during stationary phase (Andersen et al., 1980; Trick et al., 1981, 1984). Maximum release is related to light, temperature, and phosphorus-limited conditions. Trick et al. (1983) also demonstrated that *P. minimum* can produce procoentrin, an extracellular siderophore, under iron-limiting conditions, perhaps important in its competition for iron in natural communities. That *P. minimum* may produce extracellular exudates is also supported by the observations of Friedland et al. (1989) who found that menhaden abundance was positively correlated with abundance of *P. minimum* and speculated chemical cues given off by the phytoplankton attracted the fish.

While negative effects upon *P. minimum* growth have been reported after exposure to some compounds, these commonly involve non-algal sources. Both inhibition of growth by alkaloid degradation products of the marine sponge *Verongia aerophoba* (Weis et al., 1996) and cell death upon exposure to growth inhibitors secreted by the bacteria *Vibrio alginifestus* (Ishio et al., 1989) have been demonstrated for *P. minimum*.

Allelopathic interactions of phytoplankton are notoriously difficult to confirm experimentally or to attribute to a specific underlying physiological mechanism. Bacterial involvement, culture effects, and varying environmental conditions may all complicate data interpretation. The variety of literature reports identifying unique compounds produced by *P. minimum* and demonstrating temporal relationships between *P. minimum* blooms and other phytoplankton species do support the hypothesis that allelochemical interactions are a potentially important factor in the success of *P. minimum* blooms.

#### 4.3. Evidence for geographical spreading and an increase in bloom occurrence

Persuasive evidence has been presented in a series of review papers over the past decade that there has been a worldwide increase in the frequency, magnitude, and extent of many HAB species (Anderson, 1989; Smayda, 1989, 1990; Hallegraeff, 1993; GEOHAB, 2001; Anderson et al., 2002). The definitive introduction and spreading of a harmful species into a new region is difficult to document, however, due to the frequent lack of long-term data

sets on phytoplankton community composition and the possible previous presence of these species as part of the 'hidden flora' within the introduced region. While a novel HAB event may be immediately apparent within an ecosystem, the prior presence of the species responsible for that event at background concentrations may easily have gone undetected. Only for those species with a cyst stage that can be preserved in the sedimentary record can introductions be definitively documented (e.g., Dale et al., 1993).

Reports of *P. minimum* from the literature describe a species which has apparently undergone a rapid expansion in its geographical distribution in the last 30–40 years, and large blooms have been reported from many coastal areas around the world (Fig. 1). The geographical expansion of *P. minimum* blooms is substantiated by the existence of several long term data sets and its easy recognition in plankton samples (although it is sometimes misidentified). It lacks a recognized cyst stage (Matsuoka and Fukuyo, 1995), so it cannot be documented in sediment records.

The expansion into, and subsequent blooming of, *P. minimum* in three geographical regions – the Black Sea, the Baltic Sea, and the saline Lake Nakanoumi in Japan – are particularly well documented. Strong evidence is presented for the Black and Baltic Seas, areas for which long-term records of phytoplankton community composition are available. If one accepts the identification of *E. cordata* (= *P. cordatum*) as synonymous with *P. minimum* in the Black Sea prior to 1990 (Marasović et al., 1990; see also below), this species appears to have been well established along the Romanian coast of the Black Sea since the mid-1950s, with an increase in the incidence of blooms apparent in the 1970s and 1980s (Petrova-Karadjova, 1984; Marasović et al., 1990; Moncheva et al., 1995). In 1983, the species first appeared in the eastern Adriatic Sea (Sibenik Bay), with subsequently larger blooms in 1984, 1985, and 1986, which were attributed to increasing eutrophication (Marasović, 1986; Marasović et al., 1990).

Although Tangen (1980) did not include *P. minimum* in a list of dinoflagellates which bloomed in Norwegian waters from 1935 to 1978 (cited in Smayda, 1990), its presence was noted in conjunction with other dinoflagellate species in Oslo Fjord from August to September 1979 (Tangen, 1980), and it is now annually recorded in the fjord. It was noted in the

Kattegat in 1981 (Edler et al., 1982; Pingree et al., 1982; Pedersen, 1983; Granéli et al., 1984; Nielsen and Aertebjerg-Nielsen, 1984; cited in Kimor et al., 1985), and in the west coast of Sweden (Granéli and Granéli, 1982) and the southern Baltic (Hajdu et al., 2000) in 1982. By 1983, it was reported for the first time in the western Baltic, Danish coastal waters, Kiel Bay (Granéli, 1987), and Kiel Fjord (Kimor et al., 1985; Kimor, 1991). *P. minimum* is now widespread throughout the entire Baltic, forming dense, almost monospecific blooms in fjords (Bjergskov et al., 1990; Olesen, 2001; Hansen, 2002), and has penetrated into low salinity regions of the central Gulf of Finland (Hajdu et al., 2000, 2005). It should be noted that not all scientists are in agreement on the introduction of *P. minimum* into the Baltic; its presence within the English Channel had been recorded previous to its spread to the Baltic Sea (Dodge, 1982; Elbrächter, 1999), and Elbrächter (1999) does not consider it an alien species in the Baltic.

The initiation, development, and maintenance of *P. minimum* blooms have been especially well documented in Lake Nakanoumi, Japan (Kondo et al., 1990a,b,c). *P. minimum* never caused a red tide in Lake Nakanoumi before 1973, when it formed a red tide in Yonago Embayment in a span of several days. After this event, there have been yearly winter-spring blooms within this general area, with twenty blooms of *P. minimum* observed between 1974 and 1984 (Kondo et al., 1990b).

Evidence for expansion elsewhere in the world is also clear. The increase in blooms along the Mexican coast (Cortés-Altamirano and Agraz-Hernández, 1994; Sierra Beltran et al., 2005) and in the Phillipines (Azanza et al., 2005) may be related to increases in aquaculture activities.

#### 4.4. Potential mechanisms for expansion, including eutrophication

Possible mechanisms responsible for this expansion and increase in bloom frequency include transport in ballast water, stimulation from aquaculture effluent, increased monitoring activities leading to increased observations, and eutrophication (GEOHAB, 2001). Vegetative cells of *P. minimum* have been collected from container ship ballast tanks from the Pacific Ocean (Yoshida et al., 1996) and the northern Baltic

Sea (Olenin et al., 2000). No data are available on its survival in ballast tanks over extended distances; however, Manoharan et al. (1999) examined survival of *P. minimum* in darkness for 10 days and demonstrated that cells were able to maintain metabolic integrity without significant cell death over this period by utilizing stored carbon sources (e.g., triacylglycerides, galactolipids). Tyler and Seliger (1981) demonstrated that *P. minimum* can survive between 20 and 35 days in total darkness, depending upon temperature. This species also has been shown to be photosynthetically flexible, increasing pigment concentrations and altering photosynthetic physiology to survive extremely low light levels for extended periods (Tyler and Seliger, 1981; Harding et al., 1983; Harding, 1988). This suggests that *P. minimum* would be capable of surviving in ballast water for extended periods.

An expansion in the geographical range and the establishment and reoccurrence of blooms of a phytoplankton species cannot occur without a hospitable environment (Omori et al., 1994). The occurrence of large blooms of *P. minimum* has often been linked to eutrophication in coastal environments (e.g., Black Sea: Marasović, 1986, and Marasović et al., 1990; Lake Nakanouri: Kondo et al., 1990b; Chesapeake Bay and tributaries: Glibert et al., 2001). Although blooms of *P. minimum* have a wide geographical distribution, they generally occur in waters influenced by freshwater inputs (coastal waters and estuaries) and/or anthropogenic loads (Silva, 1985; Stonik, 1995; Grzebyk and Berland, 1996; Glibert et al., 2001). *P. minimum* is capable of rapid growth which, combined with its ability to utilize both organic and inorganic nutrient sources (Granéli et al., 1989; Granéli and Moreira, 1990; Glibert et al., 2001; Fan et al., 2003a,b), its small size, and potential allelopathic properties (see above; Iwasaki, 1979; Okamoto and Hirano, 1987), may confer a competitive advantage over local phytoplankton populations upon its introduction in nutrient-enriched environments. Reports of associations between the spread of *P. minimum* and nutrient pollution are common and have been made in the Oslo fjord (Paasche et al., 1984) and the coast of China where such blooms are increasing in magnitude and frequency (Qi et al., 1993; Anderson et al., 2002). In China, along with other HAB species,

*P. minimum* and *Prorocentrum* spp. blooms have been spreading along the coast during the last few decades, causing damage to fisheries and human health (Zou et al., 1985; Qi et al., 1993; Lu et al., 2005). In the Bohai Sea, China, Zou et al. (1985) found that *P. minimum* developed following rainfall and wastewater discharge. It has been suggested that these events are due to progressive eutrophication along the China coast as the result of economic development, and the relationship between the growth of fertilizer use and red tide outbreaks in China support this idea (Anderson et al., 2002). There are numerous other examples as well. Cannon (1990) reported that *P. minimum* blooms often developed close to a sewage outfall area in the Port River, South Australia. Within the Black Sea, an increase in the occurrence of *P. minimum* (as *E. cordata*) blooms in the 1960s through 1980s was significantly correlated with increasing  $\text{PO}_4^{3-}$  concentrations (Marasović et al., 1990; Smayda, 1990). Kondo et al. (1990a) concluded that blooms of *P. minimum* in the brackish Lake Nakanoumi, Japan, were stimulated by high  $\text{NO}_3^-$  concentrations discharged from local rivers. In Chesapeake Bay tributaries, large *P. minimum* blooms can be preceded by high urea concentrations (Glibert et al., 2001), possibly related to seasonal agricultural urea fertilizer applications. Conversely, in Swedish coastal waters, Granéli and Moreira (1990) demonstrated stimulation of *P. minimum* growth by river waters from forested areas while diatoms were stimulated by river water from agricultural sources. Differences in the composition of agricultural fertilizers could help to explain the different responses in the Chesapeake and Swedish coastal waters.

## 5. Ecophysiology

### 5.1. Growth rates

Dinoflagellates have several significant ecophysiological differences when contrasted to diatoms. These differences include a lower affinity for nutrients, considerable nutritional diversity involving mixotrophic nutrition, and motility (Smayda, 1997). These ecophysiological characteristics are important in

Table 4  
Representative growth rates of *Prorocentrum minimum*

$\mu$ (d <sup>-1</sup> )	$\mu_{max}$	Conditions	Clone ID and origin	Reference
0.26–0.46		F/2, 15‰, variable light, 15 °C	Chesapeake Bay isolate	Harding et al. (1983)
0.24–0.62		Enriched seawater, 20‰, 150 $\mu\text{E m}^{-2} \text{s}^{-1}$	N.D.	Granéli et al. (1985)
0.12–0.36		F/2, 15‰, 258 $\mu\text{E m}^{-2} \text{s}^{-1}$ , 15 °C	N.D.	Coats and Harding (1988)
0.25–0.74		F/2, 187 $\mu\text{E m}^{-2} \text{s}^{-1}$ , 20 °C	Clone 1PM	Antia et al. (1990)
0.38		F/2, 19 $\text{W m}^{-2}$ , 19 °C	LAC6KA83 (Kattegat clone)	Nielsen et al. (1995)
0.37		F/2, 19 $\text{W m}^{-2}$ , 19 °C	LAC4LI (Atlantic clone)	Nielsen et al. (1995)
2.84	3.54 ( $\pm 0.21$ )	F/2-Si, in situ light and temp	Clone EX	Smayda (1996)
1.13		33 psu, 475 $\text{W m}^{-2}$ , 26.5 °C		Grzebyk and Berland (1996)
0.24–1.03		F/2-Si, in situ light and temp	Clone S1-25-6, Rhode Island	Heil (1996)
0.24–0.48		F/2-Si, 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ , 15 °C	Clone S1-25-6, Rhode Island	Heil (1996, 2005)
0.23–0.43		F/2, 80 $\mu\text{E m}^{-2} \text{s}^{-1}$	Gulf of Trieste, Italy	Micheli et al. (1996)
0.26–0.81		L1, 175 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ , 18 °C	Clone LAC5 ME66	Pan and Cembella (1998)
0.25–0.98		F/2, 180 $\mu\text{E m}^{-2} \text{s}^{-1}$ , 4–20 °C	Chesapeake Bay isolate <sup>a</sup>	Lomas and Glibert (1999a,b)
0.47–0.98		ASW, F/2 vitamin, trace metal and N additions, 100 $\mu\text{M photon m}^{-2} \text{s}^{-1}$	Chesapeake Bay isolate <sup>a</sup>	Fan et al. (2003a,b)

N.D.: not determined.

<sup>a</sup> Isolated from Chesapeake Bay by A. Lewitus and maintained in Horn Point Culture Collection.

bloom regulation and dynamics, and may directly influence growth rates and competitive ability.

A unique characteristic of *P. minimum* is the large range of growth rates reported for this species (Table 4), from 0.12 to 3.54 day<sup>-1</sup>. Lowest growth rates are associated with culture studies in which *P. minimum* was grown under low to moderate light intensities (Table 4). Interestingly, the highest growth rates reported for *P. minimum* have been achieved under either high light conditions in culture or under in situ light conditions and warm temperatures. Antia et al. (1990) found that methodology also impacts growth rate estimates; in three out of four experiments with *P. minimum*, higher growth rate estimates were achieved from cell cycle analysis than from direct counts. As the three studies reporting the highest growth rates for *P. minimum* in Table 4 all utilized the direct count method, the association between high growth rates and high light conditions seems clear for this species. Furthermore, culture experiments conducted under low to moderate light conditions may significantly underestimate the growth potential of *P. minimum*.

Although culture studies have suggested that *P. minimum* is photosynthetically flexible and capable of adapting to a variety of light intensities (Faust et al., 1982; Harding et al., 1983, 1989; Coats and Harding, 1988; Fan and Glibert, 2005), the association of high growth rates with high light intensities suggests that

blooms of *P. minimum* may be favored by relatively extended periods of higher light intensities. Temperature is another important factor in determining the growth rate of *P. minimum*. Lomas and Glibert (1999a,b) showed that growth rates ranged from 0.25 day<sup>-1</sup> at 4 °C to 0.98 day<sup>-1</sup> at 20 °C; the  $Q_{10}$  for the growth rates in that study was 2.3. It was also observed that cell diameter increased from 12.8 to 17.2  $\mu\text{m}$  as growth temperature was reduced from 20 to 4 °C.

## 5.2. Nitrogen dynamics

The strategies by which *P. minimum* acquires its nitrogen have been studied in both laboratory and field conditions. In culture, *P. minimum* can grow on a range of nitrogen substrates, including  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and urea (e.g., Fan et al., 2003b). In that study, growth rates on urea and  $\text{NH}_4^+$  were found to be slightly higher than those on  $\text{NO}_3^-$  but this was not the case in a different laboratory study (Burns et al., 2000). Of interest is the extent any particular form of nitrogen may contribute preferentially to the growth of *P. minimum*, as this may be important in assessing whether eutrophication by nitrogen is contributing to the support of a bloom.

Both the relative proportion of different nitrogen substrates available to the cells and the growth temperature appear to be factors in determining the extent to which a particular nitrogen substrate is used

by this dinoflagellate. Lomas and Glibert (1999a,b) found that the uptake of  $\text{NO}_3^-$  was inhibited by  $\text{NH}_4^+$  but the strength of the inhibition was greater when *P. minimum* was growing at 20 °C than at 4 °C. This may be related to differences in temperature optima of the enzymes associated with  $\text{NO}_3^-$  reduction and  $\text{NH}_4^+$  assimilation. A similar pattern was found in a natural bloom study in the Chesapeake Bay: the affinity for different nitrogen substrates was found to vary both as a function of temperature and the natural nitrogen substrate on which the bloom was growing (Fan et al., 2003a). The parameter  $\alpha$ , the initial slope of the curve relating uptake rate to substrate concentration, varied linearly with temperature for all nitrogen substrates as a natural bloom progressed, but both the direction and slope for each nitrogen substrate differed: the relationship between  $\alpha$  and temperature for  $\text{NO}_3^-$  decreased with increasing temperature whereas that of  $\text{NH}_4^+$  and amino acid uptake increased (Fan et al., 2003a), indicating increasing affinity for the reduced nitrogen substrates and reduced affinity for  $\text{NO}_3^-$  as temperatures warmed. For urea, the slope of this curve was relatively flat, suggesting that the affinity for this substrate was not highly dependent on temperature. The affinity was also found to vary with the percentage of  $\text{NO}_3^-$  in the media or natural sample. As the relative contribution of  $\text{NO}_3^-$  increased, the affinity for other individual N forms decreased. These results suggest that *P. minimum* can take up a range of different nitrogen substrates and the relative rate of their use will depend on the ambient temperature, the relative proportion of each nitrogen substrate in the nutrient pool, and the nutritional state of the cells at the time the nutrient is supplied (Lomas and Glibert, 1999a,b; Fan et al., 2003a).

The importance of intracellular nitrogen pools cannot be overlooked as well. In one culture study, growth rate and  $\text{NO}_3^-$  uptake were studied when  $\text{NO}_3^-$  was supplied in the same amounts every 1, 2, or 3 days (Sciandra, 1991). Steady state growth could be maintained with the nitrogen source added every 1 or 2 days; with delivery every 3 days, growth rates declined but cells took up the  $\text{NO}_3^-$  rapidly when resupplied. The ability to form large internal pools was suggested as a mechanism providing a potential competitive advantage.

Enzymes involved in nitrogen acquisition have also been examined. The half-saturation constant for the

enzyme nitrate reductase is much higher than that for the enzyme urease in this dinoflagellate (Lomas and Glibert, 2000; Fan et al., 2003b), suggesting much higher affinity for urea than  $\text{NO}_3^-$ . The activity of urease was also found not to vary with nitrogen growth source but was observed to be higher during exponential growth than during early exponential or early stationary phase (Fan et al., 2003b). Furthermore, urease activity was maintained both day and night. This finding can be compared to results from Paasche et al. (1984) who compared day/night differences in nitrogen uptake by seven dinoflagellate species grown under laboratory conditions. Of those species, *P. minimum* and *Karenia mikimotoi* (as *Gyrodinium aureolum*) displayed extreme responses. *P. minimum* continued to take up nitrogen in the dark under all physiological states while *K. mikimotoi* had a nearly absolute light requirement for nitrogen uptake.

When the activity of nitrate reductase and urease were compared to nitrogen demand, urease was roughly equivalent to the nitrogen demand whereas the activity of  $\text{NO}_3^-$  reductase was not sufficient to meet demand, implying that urea may be an important nitrogen substrate for these cells when it is available (Fan et al., 2003b). The activity of these enzymes is consistent with the observation that large increases in urea (up to 15  $\mu\text{M}$  N) were found to precede a large outbreak of *P. minimum* in several tributaries of Chesapeake Bay (Glibert et al., 2001).

There are several other enzymes involved in nitrogen metabolism that have been examined in *P. minimum*. One is leucine aminopeptidase (LAP), one of the proteases that liberates amino acids. Although only one study has assessed this enzyme in *P. minimum* (Stoecker and Gustafson, 2003), the activity appears to be comparable to that of other dinoflagellate species (Berges and Falkowski, 1996; Stoecker and Gustafson, 2003), but such comparisons are made difficult by the use of different substrates (i.e., different assay methodologies) by different investigators. Whether or not the activity of this enzyme varies with nutrient status, growth rate, or other environmental factors is not known.

### 5.3. Phosphorus dynamics

Relative to the number of nitrogen uptake studies, there are far fewer studies of phosphorus uptake with

this dinoflagellate. Although *P. minimum* grows well on inorganic phosphorus sources in culture, it is capable of organic phosphorus utilization and has been shown to possess an inducible alkaline phosphatase enzyme in both cultures (Dyhrman and Palenik, 1997) and field populations from Narragansett Bay (Dyhrman and Palenik, 1999). Dyhrman (2005) provides a review of alkaline phosphatases: the enzyme appears to be located on the cell surface, regulated by the phosphorus state of the cells, and can be induced. An alkaline phosphatase of 200,000 Da and another protein of 130,000 Da were induced in *P. minimum* when cells experienced inorganic phosphorus stress (Dyhrman and Palenik, 1997). Further, Dyhrman and Palenik (1999) observed alkaline phosphatase activity during a natural *P. minimum* bloom in Narragansett Bay and, using a whole-cell immunolabeling technique, were able to show that enzyme activity in the field was inversely related to water column phosphate concentration.

#### 5.4. Photosynthetic physiology

In Chesapeake Bay, USA, *P. minimum* exhibits annual, subsurface transport in the spring from the southern to the northern Bay, where it is upwelled and forms late spring blooms (Tyler and Seliger, 1978, 1981). Tyler and Seliger (1981) suggested that *P. minimum* could maintain its pigment content and photosynthetic capacity at extremely low light intensities and in nutrient-poor surface waters in summer, and could migrate to the higher nutrient pycnocline region to obtain its nutrients. These specific physiological characteristics may allow *P. minimum* to survive during subsurface transport and form and maintain blooms in higher temperature and high nutrient (because of the river runoff) conditions in the late spring and summer.

In another study (Harding and Coats, 1988), *P. minimum* cells collected from the subpycnocline did not show signs of deterioration or stress. After an initial bloom, *P. minimum* populations remained high under conditions of low light and high turbidity in surface water (Harding and Coats, 1988). The ability of *P. minimum* to maintain sufficient nitrogen and carbon uptake under low light conditions is related to its growth responses to spectral light quality, photoadaptation, and mixotrophic tendencies.

Faust et al. (1982) examined the growth and pigment response of *P. minimum* to varying quality and quantity of light. In batch culture experiments, growth rate decreased as light intensity decreased for white, green, and red light, but not for blue light. Highest growth rates were found under blue and red light. They further suggested that since proportionately more red light radiation is available in the natural estuaries where *P. minimum* blooms, levels may be high enough to sustain photosynthesis. Photoadaptation to spectral quality was also examined by Harding et al. (1989) in a natural bloom of *P. minimum*. High photosynthetic activity in the visible spectrum was noted where chlorophyll would absorb (430–460 nm and 660–680 nm); low light adapted cells had an enhanced photosynthetic activity in the 500–560 nm region due to photoadaptation increases via the carotenoid peridinin.

Furthermore, downward shifts in light intensity (Harding, 1988) in cultures at 10°, 15°, and 20 °C resulted in three- to four-fold increases in chlorophyll *a* cell<sup>-1</sup> within 3–7 days. As in other studies, the photosynthetic response depended on duration, magnitude, and frequency of the exposure to low light (Harding et al., 1987). In another study (Fan and Glibert, 2005), the rates of inorganic carbon acquisition (in the presence of nitrogen) throughout a *P. minimum* bloom were measured. As would be expected, carbon acquisition varied with irradiance, but this relationship changed throughout the progression of the bloom. On a cell basis, the maximum carbon uptake rates increased from the early stages of the bloom to mid-bloom, then declined again as the bloom aged. Consistent with the Harding (1988) and Harding and Coats (1988) findings, the data suggest that *P. minimum* displays considerable plasticity in its photosynthetic parameters and that adaptation to low light occurs when the adaptation period is sufficient.

#### 5.5. Organic carbon utilization

Mixotrophy is the ability of an organism to be both phototrophic and heterotrophic, in the latter case, utilizing either organic particles (phagotrophy) or dissolved organic substances (osmotrophy). Mixotrophy is a wide-spread phenomenon and recently, mixotrophic nutrition has been considered as a mechanism to supplement nitrogen, carbon, and

phosphorus requirements for *P. minimum* and other dinoflagellate species when inorganic nutrients or light are not sufficient to meet nitrogen or carbon demands (Bockstahler and Coats, 1993; Jacobson and Anderson, 1996). Using epifluorescent techniques, the presence of food vacuoles has been confirmed in *P. minimum* populations in Chesapeake waters, especially during summer nutrient limiting periods during which 45% of the cells were observed to ingest particles (Li et al., 1996). Further study suggested that this feeding process is primarily a mechanism for obtaining limiting nitrogen rather than a mechanism for supplementing carbon nutrition during light limitation (Stoecker et al., 1997). This feeding mechanism could contribute to the ability of *P. minimum* to dominate and form blooms under nutrient-limiting conditions.

The organic compound urea has long been recognized as an important nitrogen source for primary producers in marine environments (Antia et al., 1991). It was studied intensively during the last several decades (e.g., Remsen, 1971; McCarthy, 1972a,b; Price and Harrison, 1988; Lomstein et al., 1989; Therkildsen and Lomstein, 1994). More recently, urea has received increasing attention because it has become the most common nitrogen fertilizer used globally (Glibert et al., submitted for publication). Under some conditions, urea can serve as the sole source of nitrogen for many phytoplankton species in the field and in the laboratory (Thomas, 1968; Carpenter et al., 1972; Antia and Landymore, 1975; Bekheet and Syrett, 1977; McCarthy, 1980; Oliveira and Antia, 1986; Glibert, 1998). While urea can contribute both carbon and nitrogen to the cell, the contribution of urea-carbon is small. Using  $^{13}\text{C}$ -labeled urea, Fan and Glibert (2005) found, for natural *P. minimum* assemblages, that urea-carbon was <1% of the carbon uptake from  $\text{CO}_2$  at high light, but its relative contribution varied over the light–dark cycle. Even integrating over a 24-h period, urea-carbon could account for only a few percent of the total daily carbon required.

A large proportion of river nutrient loading is in the form of humic substances, high molecular weight, colored organic compounds which have been previously considered refractory in nature and not available for biological utilization (Geller, 1983). There is now considerable evidence to suggest that

organic substrates, including humics, may stimulate *P. minimum*. Stonik (1995) reported that *P. minimum* developed in Amurskii Bay, Sea of Japan following the discharge of organic material. Kondo et al. (1990a,b,c,d) showed that growth of *P. minimum* was stimulated by organic substrates extracted from sediments. In mesocosm experiments, Granéli et al. (1989) found that dinoflagellate populations, including *P. minimum*, were stimulated by inorganic nitrogen additions only when added in combination with humic acids. In a recent study, Carlsson et al. (1999) investigated the effect of humic substrates on the growth and nitrogen utilization of this dinoflagellate, finding that humic additions could stimulate its growth and after 1 week of incubation, used 35% of the organic nitrogen from the added humics. Heil (1996, 2005) also found that organic additions stimulated growth rates and cell yields of *P. minimum*, but the extent of stimulation varied with the organic fraction examined and its molecular weight. Greatest stimulation was observed with humic and fulvic acid additions, with a lesser stimulation observed with hydrophilic acid additions. More research is required to determine what fraction of the humic material *P. minimum* may be using.

*P. minimum* is thus capable of both autotrophic and mixotrophic nutrition in both laboratory and in situ conditions. Although much is known about the autotrophic nutrition of this species, considerable research remains to be done on its mixotrophic nutrition, including the environmental conditions which favor feeding, the range of both particulate and dissolved particles this species can consume, and the role that nutritional mode plays in bloom dynamics.

## 6. Conclusions

In summary, *P. minimum* is a widespread, bloom-forming dinoflagellate found in many coastal waters and estuaries around the world. Blooms of this species have increased in frequency and appear to have undergone a geographical expansion over the past several decades, possibly related to human activity and eutrophication within localized systems. This species has a range of physiological adaptations that make it responsive to eutrophication: it is capable of rapid

growth, it can use a range of inorganic and organic nutrient substrates, and it can grow under a range of photosynthetic irradiance conditions, salinities, and temperatures. Strains of *P. minimum* which contain a neurotoxic fraction resulting in rapid mouse death in assays have, to date, only been confirmed from clones isolated from the French Mediterranean coast. Yet, with *P. minimum* blooms increasing globally, their contribution to environmental effects will continue to expand. Much is yet to be learned about the range of environmental impacts of this species, its ability to adapt to widely varying natural conditions, as well as its potential to produce bioactive compounds that may be detrimental to fish, shellfish, or human health.

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