Pelagic and epiphytic summer distributions of *Prorocentrum lima* and *P. mexicanum* at two mussel farms in the Gulf of St. Lawrence, Canada

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ABSTRACT: The variations in abundance of *Prorocentrum lima* (Ehrenberg) Stein, an epiphytic species implicated in diarrhetic shellfish poisoning (DSP), were studied during the summer of 1999 in the Magdalen Islands, an archipelago located in the southern Gulf of St. Lawrence, eastern Canada. This investigation was initiated as a result of an incident in 1998, when 20 people became ill and exhibited DSP-like symptoms following the consumption of blue mussels collected from one of the Magdalen Islands lagoons. *P. lima* was regularly found as an epiphyte on the epibionts growing on the mussel socks at the 2 aquaculture sites investigated between June and October 1999. *P. lima* abundance on the epibionts varied from 100 to 9600 cells g⁻¹ dry wt epibiont, with maximum abundances observed in July and August. *P. lima* was found in low abundance in the water column as well as in the digestive glands of the mussels. There was no correlation between cell abundances in the digestive glands, the water column and the cells attached to the epibionts. At the same sites, *P. mexicanum* Tafall was identified for the first time in eastern Canada. *P. mexicanum* abundances were low throughout the summer and began to increase both in the water column and in mussel digestive glands by early September. A significant correlation was found between the *P. mexicanum* abundances in the water column and in the mussel digestive glands at both sampling sites. The identification of these 2 potentially toxic species in the St. Lawrence highlights the importance of *Prorocentrum* spp. as toxin producers in coastal waters, as well as the need for adequate monitoring of non-pelagic harmful algal bloom species (HABs).

KEY WORDS: *Prorocentrum lima* · *Prorocentrum mexicanum* · Diarrhetic shellfish poisoning · Epiphytes · Mussel culture · Toxic dinoflagellate · Biofouling community

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INTRODUCTION

Toxic phytoplankton species implicated in diarrhetic shellfish poisoning (DSP), particularly *Dinophysis* spp. and certain benthic/epiphytic *Prorocentrum* spp., commonly occur in Canadian waters (Cembella

et al. 1992). In several European countries, a link has been shown between *Dinophysis* spp. and DSP (as reviewed by Sournia et al. 1991). However, in eastern Canadian waters, *Dinophysis* blooms appear to be non-toxic or may be only facultatively toxic under certain environmental conditions since blooms of more than 50 000 cells l⁻¹ have not resulted in substantial DSP toxin accumulation (Cembella et al. 1992). In North America, the only confirmed case of DSP occurred in August 1990 when 13 people became ill

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after consuming cultured blue mussels from Mahone Bay, Nova Scotia (Quilliam et al. 1993). The DSP toxins found in the mussels were not associated with the *Dinophysis*-rich samples collected following the incident. However, another dinoflagellate, *Prorocentrum lima* (Ehrenberg) Stein, isolated from the same area, was found to be a producer of okadaic acid (OA) and dinophysistoxin-1 (DTX-1) in unialgal cultures (Marr et al. 1992). A study carried out later by Lawrence et al. (1998) suggested that *P. lima* could have been responsible for the accumulation of DSP toxins at this site. Similarly, on the east coast of Maine, USA, following several unexplained incidents of shellfish-related gastroenteritis, Morton et al. (1999) showed that DSP toxicity was found only in phytoplankton or mussel digestive gland samples containing *P. lima*. *P. lima* occurs worldwide in coastal areas, in temperate and tropical oceans and mostly in benthic and epiphytic habitats (Faust et al. 1999). In eastern Canada, this dinoflagellate has already been found in substantial concentrations in the water column and attached to vegetation at aquaculture sites in the Miramichi estuary, New Brunswick (Bates 1997), and near Indian Point, Nova Scotia (Lawrence et al. 2000).

In 1998, 20 people exhibited DSP-like symptoms following the consumption of cultured blue mussels from the Magdalen Islands in the Gulf of St.

Lawrence, Canada. The analyses revealed that the mussels contained DTX-1 (no OA), but in too a low concentration to explain the symptoms ($0.1 \mu\text{g g}^{-1}$ digestive gland; regulatory limit $1.0 \mu\text{g g}^{-1}$). This prompted us to initiate a study to determine the source of DTX-1 in the Magdalen Islands, with a focus on *Prorocentrum lima* which has been previously detected in the lagoons. Our specific objectives were: (1) to determine the seasonal variations of *P. lima* concentrations on the epibionts fouling the mussel socks, in the water column and in mussel digestive glands; and (2) to measure the concentrations of OA and DTX-1 in mussel digestive glands. Our study also led to the discovery of *P. mexicanum* Tafall, another potentially toxic species (Tindall et al. 1984, 1989, Pearce & Hallegraeff 2001).

MATERIALS AND METHODS

Study site. The Magdalen Islands are located in the southern Gulf of St. Lawrence (Fig. 1). The 7 islands of this archipelago are connected by sand bars which form 3 shallow lagoons of 7 m maximum depth. The sampling was conducted at 2 mussel farming sites located in the Havre-aux-Maisons (HAM) and Grande-Entrée (GE) lagoons (Fig. 1). A distance of ca. 30 km separates the 2 sampling sites, and water circulation between the 2 lagoons is limited to a narrow channel. The mussel species cultured at these sites are *Mytilus edulis* and *Mytilus trossulus*.

Field sampling. The mussel farming sites were sampled weekly between 06:00 and 09:00 h from June 30 to October 11, 1999. Sampling consisted of collecting epibionts (composed of hydrozoans and macroalgae intertwined around a mussel sock), mussels, phytoplankton and seawater. Epibionts and mussels were collected by a diver at the bottom of a sock (4.5 m depth at HAM and 6 m depth at GE). To keep the depth of the sampled mussels and associated epibionts constant, successive socks suspended on a common horizontal line were sampled from week to week. First, the diver collected the epibionts growing on the bottom of the sock with an 8 l polyethylene bag. Thereafter, the last ca. 30 cm of the cleaned mussel sock was cut and put in a second 8 l polyethylene bag. Phytoplankton was then collected from a small boat using a 2 m conical Nytex® net (20 μm mesh size) installed on a 0.5 m diameter metal ring, and ending with a 0.3 l codend. A vertical tow was performed between 0–5 m at HAM and 0–6 m at GE. Phytoplankton samples were preserved with a Lugol's solution to a final concentration of 1% (v/v). Finally, a water sample from 4.5 m at HAM and 6 m at GE was collected with a 2 l Niskin bottle

Fig. 1. Sampling sites of Havre-aux-Maisons (HAM) and Grande-Entrée (GE) at the Magdalen Islands, eastern Canada. NB: New Brunswick; NFLD: Newfoundland

to measure salinity, water temperature (with a mercury thermometer) and nutrient concentrations (NO_2 , NO_3 and PO_4). Daily wind speed and direction were obtained from the meteorological station of the HAM airport.

Laboratory analyses. Salinity was measured with a SCT salinometer (YSI model 33). Samples for nutrient analysis were filtered using an Acrodisc 25 mm syringe filter with a 0.8 μm Versapor membrane and the filtrates frozen in polypropylene cryovials at -40°C for later analysis using a Technicon auto-analyzer[®] (Parsons et al. 1984). Epiphytic microalgae were quantified using a modified version of the method of Yasumoto et al. (1979). The bags containing the epibionts were vigorously shaken for 2 min to dislodge the epiphytic microalgae from the epibionts. All material was poured through stacked 350, 150, 100 and 20 μm sieves, and thoroughly rinsed with 0.7 μm filtered seawater. The macroalgae and hydrozoans collected on the 350, 150 and 100 μm sieves were identified and rinsed with distilled water to remove salts, freeze-dried and weighed. Microalgae, including *Prorocentrum* spp., were collected on the 20 μm sieve. The cells were resuspended in a known volume of filtered seawater and a subsample was preserved with a Lugol's solution to a final concentration of 1% (v/v). Epiphytic microalgae from the epibionts and phytoplankton from the vertical tows were enumerated in a 5 ml Palmer-Maloney counting chamber with an inverted microscope at 250 $\times$ magnification. The abundances of *Prorocentrum* cells are expressed respectively as cells g^{-1} dry wt epibiont and cells m^{-3} . It is important to note that the *Prorocentrum* abundances measured in the water column were very low and only semi-quantitative, since the volume of filtered seawater was an approximation due to the possibility of water overflowing the net as it was raised through the water column.

On each sampling day, 50 to 60 mussels were dissected to extract the digestive glands. Half of the samples were frozen and sent to the Canadian Food Inspection Agency (CFIA) for DSP toxin analyses using the method of Van de Riet et al. (1995). The other half was used for the gut content analyses. The digestive glands were weighed and triturated into a 2 mm grid to release microalgal cells. The material was then poured onto successively stacked 100 and 20 μm sieves, and thoroughly rinsed with 0.7 μm filtered seawater. Material collected on the 20 μm sieve was resuspended in 400 ml filtered seawater. A 200 ml subsample was preserved with a Lugol's solution to a final concentration of 1% (v/v). All *Prorocentrum* spp. cells were enumerated, as described earlier, and the abundance was reported in cells g^{-1} wet wt digestive gland.

RESULTS

Havre-aux-Maisons (HAM)

Physico-chemical parameters

Water salinity varied between 27.5 and 31.5‰ from the end of June to mid-October (Fig. 2a). Water temperature varied between 17 and 21.5°C from late June to mid-September and decreased to 10.5°C thereafter (Fig. 2b). Inorganic nutrient concentrations were low, with nitrogen ($\text{NO}_3 + \text{NO}_2$) and phosphate (PO_4) concentrations remaining below 0.35 and 0.65 $\mu\text{mol l}^{-1}$, respectively (data not shown). Nutrient concentrations exhibited no particular temporal pattern.

Abundance and nature of the epibionts

The total biomass of the sampled epibionts ranged between 3 and 8 g dry wt between June 30 and September 15, increased sharply to 19 g dry epibiont wt on September 22, and remained above 15 g dry epibiont wt until the end of the sampling on October 11 (Fig. 2c). The sharp increase in biomass observed in September was principally due to the appearance of the rhodophyte *Trailliella intricata* which formed very dense clusters. The epibiontic assemblage was composed of hydrozoans and a succession of macroalgal species belonging to 3 divisions: Chlorophyta, Phaeophyta and Rhodophyta. From June 30 to August 11, the chlorophytes *Ulva lactuca*, *Rhizoclonium* spp. and *Chaetomorpha* spp. dominated the assemblage (Fig. 2c). During this period, *Polysiphonia* spp. were also present but in lower abundance. From August 11 to September 15, the phaeophytes *Ectocarpus* spp. gradually replaced the chlorophytes. From September 22, *Ectocarpus* spp. were replaced by the rhodophyte *T. intricata*. In October, *T. intricata* was responsible for ca. 50% of the total biomass, while the other half consisted of hydrozoans.

Prorocentrum lima distribution

Prorocentrum lima was found attached to the epibionts, free in the water column and in mussel digestive glands during the whole sampling period. The abundances of attached *P. lima* were low in late June, increased during July and reached a maximum of 9671 cells g^{-1} dry epibiont wt on August 4 (Fig. 2d). Thereafter, their abundances decreased and remained around 4000 cells g^{-1} dry epibiont wt up to September 15, and abruptly decreased again and remained under 1000 cells g^{-1} dry epibiont wt until the end of the sam-

Fig. 2. Temporal variations in (a) salinity, (b) water temperature, (c) epibiont biomass and major genus or species of macroalgae present, the abundance of *Prorocentrum lima* and *P. mexicanum* (d) on the epibionts, (e) in the water column, and (f) in mussel digestive glands (MDG) at Havre-aux-Maisons (HAM) during 1999. dw: dry weight; ww: wet weight

pling period (Fig. 2d). In the water column, *P. lima* abundances were low (<900 cells m⁻³) and often undetectable (Fig. 2e). *P. lima* was also found in low abundances in the digestive glands of the mussels, remaining below 170 cells g⁻¹ wet digestive gland wt between June 30 and October 11 (Fig. 2f).

Prorocentrum mexicanum distribution

Prorocentrum mexicanum was also present in our samples. To our knowledge, this is the first time that this potentially toxic species has been formally identified in eastern Canada. An empty theca resembling *P. mexicanum* was observed in a previous sample from this site, but could not be clearly identified (Bérard-Therriault et al. 1999). On the epibionts, *P. mexicanum* abundances were very low, reaching a maximum of 790 cells g⁻¹ dry epibiont wt on September 29 (Fig. 2d). In the water column, *P. mexicanum* abundances were below 1000 cells m⁻³ from July 22 to September 8, reached 9500 cells m⁻³ on September 29, and declined to between 1000 and 2500 cells m⁻³ in early October (Fig. 2e). The abundance of *P. mexicanum* in the digestive glands of mussels followed the same pattern as in the water column, remaining under 100 cells g⁻¹ wet digestive gland wt from the end of June to early September, and increasing above 100 cells g⁻¹ wet digestive gland wt by mid-September and reaching a maximum of 6092 cells g⁻¹ wet digestive gland wt on September 29 (Fig. 2f).

Grande-Entrée (GE)

Physico-chemical parameters

Water salinity ranged between 27.0 and 31.2‰ between July 1 and October 5 (Fig. 3a). Water temperature ranged between 17 and 21°C from July to mid-September and dropped to 13°C in October (Fig. 3b). As observed at the HAM site, nitrogen (NO₃ + NO₂) and phosphate (PO₄) concentrations were low, remaining under 0.26 and 0.62 μmol l⁻¹, respectively (data not shown).

Abundance and nature of the epibionts

The distribution of epibionts was similar to that observed at HAM (Fig. 3c). The biomass of epibionts oscillated between 4 and 10 g dry epibiont wt during the whole sampling period except in late September to early October when it reached 18 g dry epibiont wt (no epibionts were collected on August 16 and 23). Samples collected between July 1 and August 9 were mainly composed of hydrozoans, *Chaetomorpha* spp. as well as *Polysiphonia* spp. From August 30 to September 20, the assemblage was mostly composed of hydrozoans, *Rhizoclonium* spp. and *Bryopsis plumosa*. As observed at HAM, the increase in biomass at the end of September resulted from the proliferation of *Trailiella intricata*.

Prorocentrum lima distribution

The distributions of both *Prorocentrum* species at the GE site were remarkably similar to their distribution at the HAM site located ca. 30 km away. On the epibionts, *P. lima* abundances were low in early July, reached 6000 cells g⁻¹ dry epibiont wt on July 19 and 8087 cells g⁻¹ dry epibiont wt on September 6, and decreased abruptly thereafter (Fig. 3d). As at HAM, *P. lima* abundances were low in the water column, remaining below 300 cells m⁻³, and were often undetectable (Fig. 3e). *P. lima* was also found in very small quantities in the digestive glands of the mussels, remaining below 80 cells g⁻¹ wet digestive gland wt (Fig. 3f).

Prorocentrum mexicanum distribution

As observed at HAM, *Prorocentrum mexicanum* was mainly found in the water column but was also present, albeit in low abundances, on the epibionts, reaching a maximum of 507 cells g⁻¹ dry epibiont wt on September 27 (Fig. 3d). Otherwise, attached *P. mexicanum* cells did not exceed 300 cells g⁻¹ dry epibiont wt, and were often unde-

Fig. 3. Temporal variations in (a) salinity, (b) water temperature, (c) epibiont biomass and major genus or species of macroalgae present, the abundance of *Prorocentrum lima* and *P. mexicanum* (d) on the epibionts, (e) in the water column, and (f) in mussel digestive glands (MDG) at Grande-Entrée (GE) during 1999

tectable. In the water column, *P. mexicanum* abundances were low throughout the sampling period (<600 cells m^{-3}) with a small peak on August 16 (1000 cells m^{-3}) and a larger one at the end of our sampling season (1700 cells m^{-3}) (Fig. 3e). *P. mexicanum* abundances in mussel digestive glands followed the same pattern as in the water column with maximums in mid-August (129 cells g^{-1} wet digestive gland wt) and at the end of September to early October (242 cells g^{-1} wet digestive gland wt) (Fig. 3f).

Toxicological analyses performed on the mussels collected at the 2 sampling sites were negative for OA and DTX.

DISCUSSION

The results of this study establish the presence of 2 potentially toxic *Prorocentrum* species in the HAM and GE lagoons of the Magdalen Islands in the Gulf of St. Lawrence, Canada, i.e. *P. lima* and *P. mexicanum*. *P. lima* occurs in coastal areas worldwide, in temperate and tropical oceans (Taylor et al. 1995, Faust et al. 1999) and has also been observed at the Magdalen Islands in the past (Bérard-Therriault et al. 1999). *P. mexicanum* is generally described as a tropical or sub-tropical species (Steidinger & Tangen 1996, Tindall & Morton 1998). However, the geographic distribution of *P. mexicanum* is not restricted to these warm waters and remains obscure due to taxonomical problems. We regard *P. rathymum* Loeblich et al. as a taxonomic synonym of *P. mexicanum*, as proposed by Steidinger (1983). Dodge (1975) regarded *P. mexicanum*, together with other *Prorocentrum* species, as conspecific with *P. maximum* (Gourret) Schiller, but he depicted, on his Plate 3 of Fig. F, a specimen with a long apical spine quite different from *P. mexicanum*. A very similar and apparently conspecific specimen with that depicted by Dodge (1975) was published by Halim (1967) (his Plate 7, Fig. 96 and Plate 9, Fig. 134). Since then, there have been many records of *P. maximum* published in the literature, e.g. from the Mediterranean Sea and as far north as North Sutherland (Scotland) (Dodge 1989). The identity of these records remains unclear as no figures are given. However, since 1979, *P. mexicanum* has been reported in the Mediterranean Sea (e.g. Estrada 1979, as *P. rathymum*) and seems to be widespread there (M. El-brächter unpubl. data). *P. mexicanum* has also been found in the brackish waters of Hamana Lake on the Pacific coast of Japan (Okamoto 1992) and in the temperate waters of the Tasmanian east coast (Pearce et al. 2000). *P. mexicanum* has also been exceptionally reported on the French Atlantic coast (Nezan & Piclet 1996, Belin & Raffin 1998). To our knowledge, in North

America, *P. mexicanum* has never been recorded north of Florida waters (Bomber et al. 1985) in the Atlantic and north of the Gulf of California (Garate-Lizarraga & Martinez-Lopez 1997) in the Pacific. Our observations as well as previous observations clearly show that *P. mexicanum* can develop in northern latitudes. Furthermore, the Magdalen Islands lagoons seem to have appropriate physical conditions for *P. mexicanum*, which is known to thrive in protected shallow inshore areas (Carlson & Tindall 1985, Bomber et al. 1989, Morton & Faust 1997, Pearce et al. 2000).

The 2 *Prorocentrum* species were found in the water column, attached to the epibionts and in mussel digestive glands. However, they exhibited a clear difference in their habitat preference, with *P. lima* being more abundantly attached to the epibionts and *P. mexicanum* more abundant in the water column. This is in accordance with Taylor et al. (1995), who noted that both species are epibenthic on seaweeds and that *P. mexicanum* is also planktonic (Fukuyo 1981, Faust 1990, Taylor et al. 1995). Overall, the temporal variations in the abundance of the 2 species were remarkably similar at the 2 sampling sites located ca. 30 km apart, suggesting that our weekly sampling successfully captured large temporal patterns.

Temporal variations of *Prorocentrum lima* concentrations in the lagoons

In both lagoons, *Prorocentrum lima* was mainly found on the epibionts fouling the mussel socks. Throughout the sampling period, the average abundance of *P. lima* was 4060 cells g^{-1} dry epibiont wt at HAM and 2969 cells g^{-1} dry epibiont wt at GE. These abundances are slightly greater than the average 2500 cells g^{-1} macroalgae measured by Lawrence (1998) in Mahone Bay, Nova Scotia. *P. lima* abundance was always low (<1000 cells m^{-3}) in the water column, and we found no relationship (positive or negative) between the abundance of attached and free-living cells. The absence of a relationship is not surprising given the weekly frequency of our sampling. In spite of the low *P. lima* abundances in the water column, cells were frequently found in mussel digestive glands, suggesting that they were available for ingestion. It is also noteworthy that *P. lima* cells were sometimes found in mussel digestive glands even when they were not detected in the water column, highlighting the inability of standard pelagic monitoring programs to predict toxic outbreaks due to epiphytic species.

Temporal variations of epiphytic microalgae may result from widely different mechanisms. Increases in cell abundance may result from the colonization of the substrate by free-living cells and/or *in situ* growth of

already attached cells, while decreases in abundance may result from detachment or mortality of attached cells and/or loss of substrate. The temporal patterns presented in Figs. 2d & 3d should thus be interpreted with respect to the succession of epibionts. The abundances of attached *Prorocentrum lima* cells g^{-1} epibiont at both sites exhibited a strong seasonal pattern. This was characterized by a gradual increase in early July followed by variable but relatively high levels until early September at GE and mid-September at HAM when abundances started to decrease to reach minimum values in early October. Since no sampling was conducted prior to June 30, we may have missed an earlier bloom of *P. lima*. However, the coincidence between the colonization of the epibionts by *P. lima* at both sites is striking and suggests that we may have caught the beginning of the growing season. The appearance of *P. lima* on the epibionts in early summer was not associated with changes in water salinity or temperature, and the weight and taxonomic composition of the epibionts were also stable at both sites during this period. The colonization of the epibionts by *P. lima* in early July was thus not linked to any of these environmental parameters and may have resulted from a circannual internal rhythm. The sharp decrease

of attached *P. lima* cells in both lagoons in September appears to be related to vertical mixing caused by strong winds. It is important to point out that the total number of *P. lima* cells (not standardized by g epibiont) also decreased drastically during this period at both sites (data not shown). At HAM in mid-September, the decrease coincided with a shift in the composition of the epibiotic assemblage with the filamentous brown alga *Ectocarpus* spp. being replaced by the rhodophyte *Trailiella intricata*. The loss of *Ectocarpus* spp. (and the associated *P. lima* cells), which was loosely attached to the mussel socks, coincided with the passage of the tropical storm Floyd south of our study area on September 16 to 18 with persistent NNE winds of ca. 10 m s^{-1} (Fig. 4a,b). Since the Magdalen Islands lie on a NE-SW axis, NNE winds favor the development of waves within the lagoons and may affect the HAM sampling station, while the GE station remains protected by the coast (refer to Fig. 1). The physical removal of *Ectocarpus* spp. seems to have favored the proliferation of *T. intricata*, which remained poorly colonized by *P. lima* for the remainder of the season. During the post-Floyd period, the fouling biomass increased considerably at HAM but *P. lima* abundances remained low, possibly due to the lower water

Fig. 4. Temporal variations of *Prorocentrum lima* on the epibionts in relation to the daily wind speed. Abundance of *P. lima* at (a) Havre-aux-Maisons (HAM), and (c) Grande-Entrée (GE) during September and October 1999. The wind speed and direction are illustrated in (b)

temperatures. Jackson et al. (1993) showed that the maximal growth rate of *P. lima* strains isolated from Mahone Bay decreased markedly with decreasing temperature, falling from 0.5 divisions d^{-1} at 20°C to 0.1 divisions d^{-1} at 10°C. The temperature preferences of *P. lima* strains from the Gulf of St. Lawrence are not known, but water temperatures below 13°C in October at HAM may have limited the growth of this species. At GE, *P. lima* abundance on the epibionts was not affected by the NNE winds of September 16 to 18, but rather by strong S winds close to 10 m s^{-1} on September 10 to 11 (Fig. 4b,c). The entrance of the GE lagoon faces SW and is therefore influenced by S winds (refer to Fig. 1). Strong S winds on September 29 to 30 were also associated with a second decrease in *P. lima* abundance (Fig. 4b,c). Contrary to HAM, these events were not associated with the removal of macroalgae, possibly due to the difference in macroalgae species and the depth of the socks.

Studies by Bomber et al. (1985) and Morton & Faust (1997) on the epiphytism of dinoflagellates have shown that *Prorocentrum lima* was widely distributed among macroalgal substrates. *P. lima* cells occurred on chlorophytes, phaeophytes, rhodophytes and vascular plants although highest densities were observed on chlorophytes, such as *Penicillus capitatus* and *Avrainvillea nigricans* (Bomber et al. 1985), as well as on the rhodophyte *Acanthophora spicifera* (Morton & Faust 1997). We also found *P. lima* cells in association with epibion-

tic assemblages dominated by chlorophytes, phaeophytes and rhodophytes. Furthermore, preliminary laboratory experiments showed that *P. lima* strains isolated from the lagoons also attach to members of these 3 algal divisions (Réal Gagnon, Institut Maurice-Lamontagne, Ministère des Pêches et Océans Mont-Joli, Québec, pers. comm.). Like Faust et al. (1999), we also observed that *P. lima* adheres to the walls of culture flasks. The mucus produced by *P. lima* appears to allow cells to adhere to the substratum (Réal Gagnon pers. comm.).

Prorocentrum lima is known to produce OA (Mura-kami et al. 1982, Marr et al. 1992) and its derivative, methyl-OA (DTX-1) (Lee et al. 1989, Marr et al. 1992). A recent laboratory study has demonstrated that these DSP toxins accumulate in the digestive glands of the mussel *Mytilus edulis* when toxic *P. lima* cells are ingested (Pillet et al. 1995). However, in this study, we did not detect OA or DTX-1 in our samples even at the highest *P. lima* abundance of 167 cells g^{-1} wet digestive gland wt, suggesting that the magnitude of the *P. lima* population in 1999 was insufficient to result in the accumulation of toxins in the mussels. In 1997, in Mahone Bay, the *P. lima* abundances did not exceed 47 cells g^{-1} wet digestive gland wt and the toxins were also undetectable (Lawrence 1998).

Temporal variations of *Prorocentrum mexicanum* in the lagoons

In the lagoons, *Prorocentrum mexicanum* was principally found in the water column, but was also attached to the epibionts in very small numbers. At both sites, *P. mexicanum* abundance was generally low in the water column in July and August, but increased substantially in September (Figs. 2e & 3e). The most significant increase was observed at HAM between September 21 and 29 (9500 cells m^{-3}), a calm period with winds below 5 m s^{-1} . At GE, *P. mexicanum* was found in lower abundance than at HAM, but also increased in late September to early October. *In situ* growth could explain the temporal changes in *P. mexicanum* abundance observed in the lagoons. To explore whether the weekly changes in *P. mexicanum* abundance could be explained solely by *in situ* growth (instead of advection), we calculated the net population growth rate from the changes in cell abundance between consecutive sampling days (Fig. 5). Net population growth rates varied between 0.05 and 0.33 divisions d^{-1} . These rates are generally comparable with those of 0.1 and 0.2 divisions d^{-1} obtained by Morton et al. (1992) with *P. mexicanum* strains isolated from Knight Key, Florida, grown at salinities comparable to those measured in the lagoons. However, these Florida strains could not grow below 21°C. The optimum growth temperature for the strains isolated from the Magdalen

Fig. 5. Summer variations of *Prorocentrum mexicanum* abundance in the water column and net growth rates of this species (only positive rates are represented) in the lagoons of (a) Havre-aux-Maisons (HAM) and (b) Grande-Entrée (GE) in 1999

Fig. 6. Relationship between *Prorocentrum mexicanum* abundance in the water column and in mussel digestive glands (MDG) for Havre-aux-Maisons (HAM) (●, dotted line) and Grande-Entrée (GE) (○, solid line) (a) with all data points except the highest value (Δ) at HAM and (b) within the 0 to 2000 cells m^{-3} range

Islands lagoons is not known. However, the relatively high net population growth rates measured in late September, when water temperature varied between 15 and 18°C, suggest that the St. Lawrence strains are acclimated to colder conditions. The temperature in September 1999 was clearly above the seasonal normal, reaching 16.9°C compared to the average 12.8°C. In addition, the daily wind speed of 5 $m s^{-1}$ was below the average 7.5 $m s^{-1}$ recorded by Environment Canada (1993) for the 1961 to 1990 period. Warmer waters and relatively calm conditions may thus have favored the growth and accumulation of *P. mexicanum* in the water column in September during this particular year.

In contrast to *Prorocentrum lima*, *P. mexicanum* abundances in the water column and in mussel digestive glands were positively correlated at HAM ($r^2 = 0.545$, $y = 0.064x + 27.87$, $n = 12$) and GE ($r^2 = 0.809$, $y = 0.11x + 3.27$, $n = 12$) when all data points were included in the regression except for the single highest value (Fig. 6a). Within the 0 to 2000 cells m^{-3} range, the 2 regression lines are similar: $y = 0.12x - 3.52$ ($r^2 = 0.72$, $n = 9$) for HAM, and $y = 0.11x + 3.27$ ($r^2 = 0.81$, $n = 12$)

for GE (Fig. 6b). This tight relationship suggests that our weekly sampling was adequate to capture most of the temporal variations in *P. mexicanum* abundance and that the distribution of this species was relatively homogeneous in the lagoons. In practice, this relationship indicates that *P. mexicanum* abundance in mussels (and probably *P. mexicanum*-derived toxins in mussels) could be predicted from cell abundances measured in the water column by regular monitoring programs. The chemical structure of the toxins produced by *P. mexicanum* are undefined, although to date, many toxic effects have been observed. For example, *P. mexicanum* extracts caused death in mice (Tindall et al. 1984, Carlson & Tindall 1985, Pearce & Hallegraeff 2001) and also have a toxic effect on guinea pig ileum preps (Bomber & Aikman 1991). A cytotoxic activity has also been observed with cultures of *P. mexicanum* (S. Morton pers. comm.). Nakajima (1981) and Yasumoto et al. (1987) showed hemolytic activity with extracts of *P. rhathymum* (synonym of *P. mexicanum*). One of the *P. mexicanum*-derived toxins is a water-soluble fast-acting toxin (FAT) which was isolated by Tindall et al. (1989). FAT include spirolides, pinnatoxins and gymnodimine; however, their significance for human health has not yet been determined (Anderson et al. 2001). The presence of *P. mexicanum* in our samples was unexpected, consequently, the toxins produced by this species were not targeted in the toxicity measurements during our study. As far as we know, *P. mexicanum* does not produce OA or DTX, but diarrhetic effects may also be caused by other toxins like azaspiracids (James et al. 2000). Thus, the possibility that *P. mexicanum* was implicated in the 1998 poisoning incident remains unclear but cannot be ruled out.

CONCLUSIONS

This research has not allowed us to draw conclusions about the source of DTX found in the farmed mussels of the Magdalen Islands in 1998. However, *Prorocentrum lima* and *P. mexicanum* were found living in very close proximity to the mussels and were part of their natural diet. Although no toxins were detected in shellfish tissue, this does not indicate that these species are harmless. Further studies are needed to determine the toxicity of these St. Lawrence strains. The unexpected discovery of *P. mexicanum* has introduced a new element into the shellfish poisoning problem at the Magdalen Islands and implies a wider distribution than previously believed for this species in North America. Finally, our results stress the importance of implementing monitoring programs allowing the detection of non-pelagic HABs.

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