Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta)

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Over the last decade, certain coccolithophores have been the subject of extensive multidisciplinary research. Several species of coccolithophore, belonging mainly to the families Pleurochrysidaceae and Hymenomonadaceae, inhabit inshore coastal waters where they may occasionally bloom and hence impact aquaculture resources. The toxicity to Artemia salina larvae of 11 species of coccolithophore (nine coastal and two oceanic members of the order Coccolithales) was tested. For the nine coastal species, tests were conducted with rapidly growing and stationary phase cultures at a range of cellular concentrations and for two different exposure times (24 and 48 h). Five of the coastal species (four in the genus Pleurochrysis as well as Jomonlithus littoralis) were found to be toxic to A. salina nauplii. Allelopathic effects of a cell-free filtrate of a culture of a toxic coccolithophore were also tested on three flagellate microalgal species: Scrippsiella trochoidea, Tetraselmis sp. and Isochrysis galbana. Negative effects of the filtrate on growth rates and motility of S. trochoidea and Tetraselmis sp. were recorded, suggesting that the toxin of the coccolithophore tested could be an exotoxin similar to that produced by other non-calcifying members of the Prymnesiophyceae. The fact that certain coccolithophores were found to be toxic to invertebrates and were shown to exhibit allelopathic activity could imply negative effects at different trophic levels in coastal areas.

INTRODUCTION

Over the last few decades, the incidence of nuisance and toxic blooms of phytoplankton organisms has apparently been increasing in coastal waters worldwide (GEOHAB, 2001). Nuisance effects include the production of foam or mucilage, while water-soluble exotoxins excreted by microalgae may be the causative agents of mass mortalities of marine vertebrates or invertebrates (GEOHAB, 2001). Transfer through the food chain of potent microalgal endotoxins and accumulation in shellfish or finfish can also cause a variety of illnesses in humans (GEOHAB, 2001).

Several taxonomic groups of microalgae feature genera with known harmful marine species. These include dinoflagellates (Alexandrium, Dinophysis, Gymnodinium and Karenia being among the main toxic genera), diatoms (mainly Pseudo-nitzschia) and raphidophytes (Chattonella, Fibrocapsa and Heterosigma). Among haptophytes, toxic species are reported in the class Prymnesiophyceae. This class contains four orders (Edvardsen et al., 2000), two of which include known toxic species. The order Prymnesiales includes some notable toxic species in the genera Prymnesium Massart and Chrysochromulina Lackey, causative agents of large fish kills (Moestrup, 1994).

Four species of Prymnesium are reported to be toxic to vertebrates or invertebrates (Table I), producing potent haemolytic compounds termed ‘prymnesins’ (Yariv and Hestrin, 1961).

The chemical structure of prymnesins has not been fully characterized. Several haemolytic compounds have been found in Prymnesium parvum, one identified by Kozakai et al. as a mixture of two galactolipids (Kozakai et al., 1982). Igarashi et al. found two haemolytic compounds, termed prymnesins 1 and 2, which were identified as polyoxy-polyene-polyethers (Igarashi et al., 1995, 1996).

Five species of Chrysochromulina are also listed as toxic (Table I), notably Chrysochromulina polyplepis and C. leadbeateri, both of which are bloom-forming species responsible for massive fish kills in Scandinavian waters (Moestrup and Thomsen, 1995). Yasumoto et al. established that C. polyplepis produces two compounds, one haemolytic and one ichthyotoxic (Yasumoto et al., 1990). The major haemolytic compound was a galactolipid, 1-acyl-3-digalacto-glycerol. Small amounts of a polyunsaturated fatty acid, octadecapentaenoic acid, were also detected.

With respect to the order Phaeocystales, large blooms of species belonging to the genus Phaeocystis Lagerheim...
The genera Ochrosphaera Schussnig and Phaeocystis Pringsheim belong to a small group of coccolithophores occurring consistently in nearshore marine waters, in contrast to the majority of the coccolithophores, which are open-ocean organisms (Heimdal, 1993). To our knowledge, the only other report of toxicity tests involving coccolithophores was that of Rhodes et al., who found that the common oceanic species Emiliania huxleyi Hay et Mohler and Gephyrocapsa oceanica Kampartner, both members of the Isochrysidales, were non-toxic to brine shrimp (Rhodes et al., 1995). Flynn et al. reported that Oxyrrhis marina avoided preying on I. galbana when the latter became nitrogen stressed (Flynn et al., 1996). These results may imply that some form of toxin could be present in this member of the Isochrysidales. However, the nature and mode of action of the potential toxic substance have not been elucidated.

The ALGOBANK culture collection at the University of Caen, France (http://www.unicaen.fr/algobank), contains unialgal cultures of a large number of haptophytes, including one of the most important collections of living coccolithophores, with over 30 species in culture (Probert and Houdan, 2004). We performed a preliminary screening using the brine shrimp lethality assay in order to evaluate the toxicity of coccolithophores. In view of the fact that natural toxic events linked to marine prymnesiophytes have most often been reported in nearshore areas (or in saline ponds/lakes), we focused on species from families typically found in inshore coastal waters (nine of the 11 species tested). In order to assess potential allelopathic activity of toxic coccolithophores, the effect of a cell-free filtrate of a culture of Phaeocystis roseoalveolata on the growth of three species of phytoplankton was tested.

**METHOD**

Cultures and culture conditions

Table II lists the 11 species of coccolithophore obtained from ALGOBANK and used in the toxicity tests. All algal strains were clonal and in the diploid, heterococcolith-bearing stage of the life cycle. The nine coastal species were grown in 1:1 K/2 (Keller et al., 1987) and ES Tris II

Table I: Non-calcifying marine prymnesiophytes reported to be toxic to vertebrates and invertebrates

<table>
<thead>
<tr>
<th>Species</th>
<th>Tested on</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysochromulina</td>
<td>Bryozoan</td>
<td>Jebram (1980)</td>
</tr>
<tr>
<td>brevifilum</td>
<td>E. pilosa</td>
<td></td>
</tr>
<tr>
<td>Chrysochromulina</td>
<td>Bryozoan</td>
<td>Jebram (1980)</td>
</tr>
<tr>
<td>kappa</td>
<td>E. pilosa</td>
<td></td>
</tr>
<tr>
<td>Chrysochromulina</td>
<td>Fish</td>
<td>Eikrem and Thvonsen (1993)</td>
</tr>
<tr>
<td>leadbeateri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysochromulina</td>
<td>Fish,</td>
<td>Yasumoto et al.</td>
</tr>
<tr>
<td>polyepis</td>
<td>invertibrates</td>
<td>(1990)</td>
</tr>
<tr>
<td>Chrysochromulina</td>
<td>Bryozoan</td>
<td>Jebram (1980)</td>
</tr>
<tr>
<td>strobius</td>
<td>E. pilosa</td>
<td></td>
</tr>
<tr>
<td>Prymnesium</td>
<td>Fish</td>
<td>Chang (1985)</td>
</tr>
<tr>
<td>calathiferum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prymnesium</td>
<td>Brine shrimp</td>
<td>Fresnel et al.</td>
</tr>
<tr>
<td>faveolatum</td>
<td>(A. salina)</td>
<td>(2001)</td>
</tr>
<tr>
<td>Prymnesium</td>
<td>Fish,</td>
<td>See Moestrup</td>
</tr>
<tr>
<td>parvum*</td>
<td>invertibrates</td>
<td>(1994)</td>
</tr>
<tr>
<td>Prymnesium</td>
<td>Brine shrimp</td>
<td>Fresnel et al.</td>
</tr>
<tr>
<td>zebrinum</td>
<td>(A. salina)</td>
<td>(2001)</td>
</tr>
<tr>
<td>Phaeocystis</td>
<td>Sea-urchin</td>
<td>Hansen et al.</td>
</tr>
<tr>
<td>pouchetii</td>
<td>embryos</td>
<td>(2003)</td>
</tr>
</tbody>
</table>

*The alternate phase of this species (P. patelliferum) is also toxic (Larsen et al., 1993).
(Cosson, 1987) medium under controlled conditions of 17°C, a light:dark (L:D) cycle of 16:8 h and a salinity of 33. For each of the coastal species, two series of cultures were conducted under identical conditions in order to compare the effect of culture age (rapid growth and stationary stages). Cell density was measured by manual counting using a Malassez counting slide [cells were fixed with a nicotine solution according to Zirbel et al. (Zirbel et al., 2000)]. For the two oceanic species tested, cultures were grown in K/5 medium (Keller et al., 1987) with other culture conditions being identical, and toxicity tests were conducted at the end of the growth stage (~100 000 cells mL⁻¹). The growth rate k was calculated following the formula: \( k = \ln(C_t/C_0)/t \), where t is the duration of experiments, \( C_t \) is the fluorescence reading at time t and \( C_0 \) is initial fluorescence.

Two non-calculating prymnesiophytes, *I. galbana* (non-toxic control) and *P. parvum* (toxic control), were used as controls in the *Artemia* test (see below) and grown in exactly the same conditions as the coastal coccolithophores. *Isochrysis galbana* (strain AC34), along with *Tetraselmis* sp. (AC260) and *Scrisspsiella trochoidea* (AC219), were used in the allelopathy tests and culture conditions are defined below.

### Artemia bioassay

*Artemia* tests were performed following the protocol of the *Artemia* Reference Centre (Vanhaecke and Persoone, 1981) with some minor modifications. *Artemia salina* cysts (Hobby, Bonn, Germany) were incubated for 48 h in 2 L of sterile seawater under a 16:8 h L:D photoperiod at room temperature with moderate aeration to achieve continuous suspension. After 48 h, an aliquot of hatched nauplii was collected and five individuals were transferred with ~50 µL of medium into each of 250 (5 mL) tubes. Control tubes were filled with 5 mL of sterile culture medium, and experimental tubes were each completed with 5 mL of varying algal cultures and concentrations. Either three or four concentrations were tested for each algal culture (concentrations from 1 × 10⁵ to 5 × 10⁶ cells mL⁻¹ depending on the species) in triplicate. Living and dead (non-motile after 5 min of observation) larvae were counted in each tube after 24 and 48 h of exposure in the dark at 17°C.

### Allelopathy test

A stationary phase culture of *P. rossosensis* (250 mL, 400 000 cells mL⁻¹) was gently filtered onto Whatman GF/C glass fibre filters (nominal pore size 1.2 µm) to eliminate cells and subsequently filter sterilized through 0.22 µm pore size cellulose acetate membrane filters. The filtrate was enriched with sterile K/5 medium supplements (Keller et al., 1987). Three species, the prymnesiophyte *I. galbana*, a prasinophyte *Tetraselmis* sp. and the dinoflagellate *S. trochoidea*, were cultured in this enriched *P. rossosensis* filtrate in triplicate in 15 mL borosilicate tubes (all three species were grown in K/5 medium prior to the experiment). All of the cultures for the allelopathy tests were grown in the conditions described above. Each species was also cultured in both K/5 and K/2 medium as controls, also in triplicate. Growth was monitored by recording *in vivo* chlorophyll a fluorescence daily (at the same point in the L:D cycle) using a Turner Designs 700 fluorimeter. The growth rate k was calculated with \( t = 5 \) days, equivalent to the end of the exponential phase of *I. galbana*.

### Table II: Origin of the strains used in the toxicity tests

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Strain</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prymnesiaceae</td>
<td><em>Prymnesium parvum</em> N. Carter</td>
<td>AC 45</td>
<td>Bretagne (Fr)</td>
</tr>
<tr>
<td>Prymnesiaceae</td>
<td><em>Prymnesium parvum</em> N. Carter</td>
<td>AC 45</td>
<td>Bretagne (Fr)</td>
</tr>
<tr>
<td>Pachydiscaceae</td>
<td><em>Pleurochrysis carterae</em> (Braarud et Fagerland Christensen)</td>
<td>AC 1</td>
<td>Morocco</td>
</tr>
<tr>
<td>Hymenomonadaceae</td>
<td><em>Hymenomonas coronata</em> Mills</td>
<td>AC 115</td>
<td>Guadeloupe</td>
</tr>
<tr>
<td>Hymenomonadaceae</td>
<td><em>Hymenomonas globosa</em> (Magne) Gayral et Fresnel</td>
<td>AC 30</td>
<td>Iles Chausey (Fr)</td>
</tr>
<tr>
<td>Hymenomonadaceae</td>
<td><em>Ochrosphaera neapolitana</em> Schussnig</td>
<td>AC 94</td>
<td>Roscoff (Fr)</td>
</tr>
<tr>
<td>Hymenomonadaceae</td>
<td><em>Hymenomonas coronata</em> Mills</td>
<td>AC 115</td>
<td>Guadeloupe</td>
</tr>
<tr>
<td>Hymenomonadaceae</td>
<td><em>Hymenomonas globosa</em> (Magne) Gayral et Fresnel</td>
<td>AC 30</td>
<td>Iles Chausey (Fr)</td>
</tr>
<tr>
<td>Coccolithaceae</td>
<td><em>Coccolithus braarudi</em> (Gaarder) Baumann et al.</td>
<td>AC 392</td>
<td>Arcachon (Fr)</td>
</tr>
<tr>
<td>Coccolithaceae</td>
<td><em>Calcidiscus leptoporus</em> (Murray et Blackman) Loeblich et Tappan</td>
<td>AC 370</td>
<td>South Atlantic (Sa)</td>
</tr>
</tbody>
</table>

Fr, France; Sp, Spain; Sa, South Africa.
Statistical analyses and LD\textsubscript{50}

Statistical analyses were conducted with Statgraphs software, using the Kruskal–Wallis test with $P = 0.05$. The LD\textsubscript{50} was obtained by applying a linear regression between cell concentration and percentage mortality of *Artemia* nauplii. This index represents the microalgal cell concentration that leads to 50% mortality of *Artemia*.

**RESULTS**

**Toxicity**

Among the 11 species of coccolithophores tested, six were non-toxic to *A. salina* larvae: *Hymenomonas coronata, Hymenomonas globosa, O. neapolitana* and *Pleurochrysis dentata* (coastal species), *Calcidiiscus leptoporus* and *Coccolithus braarudii* (oceanic species). For all of these species, as for the negative (non-toxic) control species *I. galbana*, there was 0% mortality of *A. salina* both in rapid growth and stationary phases of algal growth, and at all of the cell concentrations tested. There was also 0% mortality after 48 h in the control tubes with sterile medium only.

Five species of coastal coccolithophores belonging to the genera *Pleurochrysis* or *Jomonolithus* showed some degree of toxicity to brine shrimp with up to 100% mortality of *Artemia* nauplii (Figure 1; Table III).

*Pleurochrysis carterae* caused 100% mortality of *Artemia* nauplii at the highest cell concentration in rapid growth phase after 48 h of exposure (Figure 1a and b). In the stationary phase culture, 100% mortality was obtained at 160 000 cells mL\textsuperscript{-1} for 24 h of exposure and at 800 000 cells mL\textsuperscript{-1} after 48 h. The LD\textsubscript{50} for *P. carterae* was similar in growth and stationary phases, but variable with the time of exposure (Table III).

The toxic effect of *Pleurochrysis elongata* was similar to that of *P. carterae* in the growth phase, but 100% mortality of nauplii was recorded at the lowest cell concentration after 48 h of exposure (Figure 1c and d). The LD\textsubscript{50} differed between growth and stationary phase, and between 24 h and 48 h of exposure for the growth phase culture (Table III).

For *Pleurochrysis placolithoides*, mortality of *Artemia* was very different between growing and stationary phase cultures. In the growing phase, none of the cell concentrations led to 100% mortality of *Artemia* (Figure 1e and f). However, in the stationary phase culture, there was 80% mortality at the lowest cell concentration tested after 24 h of exposure, and 100% mortality for all other concentrations and at all concentrations after 48 h of exposure. The LD\textsubscript{50} differed between the growth and the stationary phases (Table III).

*Pleurochrysis roscoffensis* caused >60% mortality of *Artemia* after 24 h of exposure in the growth phase and >80% at 48 h, with 100% at the highest cell concentration (Figure 1g and h). In the stationary phase culture, after 24 h of exposure, the percentages of mortality were similar to those of the growth phase culture, but after 48 h, 100% mortality was reached for all concentrations tested. The LD\textsubscript{50} was inferior at the lowest concentration tested in both growing and stationary phases (Table III).

After 24 h of exposure to *Jomonolithus littoralis*, mortality of *Artemia* was 85%. This species caused 100% mortality of *Artemia* at and above 800 000 cells mL\textsuperscript{-1} in the growth phase after 48 h of exposure (Figure 1i and j). In the stationary phase culture, mortality was maximal (100%) at the highest concentration tested after 48 h of exposure. The LD\textsubscript{50} was similar between growth and stationary phases at between 250 000 and 300 000 cells mL\textsuperscript{-1} after 24 h, and <200 000 cells mL\textsuperscript{-1} after 48 h of exposure (Table III).

*Prymnesium parvum* (Figure 1k and l) did not cause 100% mortality when supplied in the growing phase, but reached this percentage at the highest cell concentration tested in the stationary phase culture after both 24 and 48 h of exposure. The LD\textsubscript{50} differed between 24 and 48 h of exposure in the growth phase, with 500 000 cells mL\textsuperscript{-1} for 24 h and <100 000 for 48 h. In the stationary phase, the LD\textsubscript{50} was in both cases <100 000 cells mL\textsuperscript{-1} (Table III).

**Effect of culture age**

Statistically, there were no differences ($P > 0.05$) in toxicity between rapid growth (growth rates between 0.3 and 0.5 day\textsuperscript{-1}) and stationary phase (growth rates between 0.1 and 0 day\textsuperscript{-1}) cultures for three of the five toxic species tested. Cultures of *P. elongata* and *P. placolithoides* were significantly more toxic ($P < 0.05$) during the stationary phase than during the growing phase.

**Effect of algal cell concentration**

While the five toxic species were typically more toxic at higher cell concentrations (Figure 1), for only three species, *P. carterae, P. elongata* and *P. placolithoides*, the concentration presented statistically positive ($P < 0.05$) effects on toxicity. For *P. roscoffensis* and *J. littoralis*, the negative effect of cell concentration on mortality was a result of high mortality of *A. salina* nauplii at low cell concentrations of these coccolithophores.

**Effect of exposure time**

The time of exposure (24 or 48 h) had an effect on mortality of *Artemia* for three of the coccolithophores tested. *Pleurochrysis carterae* and *P. elongata* were significantly more toxic ($P < 0.05$) after 48 h of exposure, in both growing and stationary phase cultures (Table III). The
The effect of *P. placolithoides* was similar to that of *P. carterae* in rapid growth phase, but for the stationary phase culture all of the *Artemia* died after 24 h, precluding statistical tests. *Pleurochrysis roscoffensis* and *J. littoralis* also induced a high mortality rate (100%) after 24 h, preventing the testing of the effect of exposure time.

**Fig. 1.** Graphical representation of the percentage mortality of *A. salina* nauplii as a function of algal cell concentration with standard error of the mean for the following species: (a) *P. carterae* growth phase; (b) *P. carterae* stationary phase; (c) *P. elongata* growth phase; (d) *P. elongata* stationary phase; (e) *P. placolithoides* growth phase; (f) *P. placolithoides* stationary phase; (g) *P. roscoffensis* growth phase; (h) *P. roscoffensis* stationary phase; (i) *J. littoralis* growth phase; (j) *J. littoralis* stationary phase; (k) *P. parvum* growth phase; (l) *P. parvum* stationary phase. Black, 24 h of exposure; grey, 48 h of exposure.
Prymnesium, Pleurochrysis, and Pleurochrysis P. placolithoides and P. parvum species to demonstrated to be toxic using the brine shrimp (P. carterae). DISCUSSION

The growth response in the control media (K/5 and K/2) of two of the test species, S. trochoidea K/2, was similar, but growth of both of these species was inhibited by the cell-free filtrate of P. roscoffensis (10 and 45% inhibition, respectively). Moreover, the cells of these flagellates lost their ability to swim. In contrast, there was no alteration of growth rate or motility for the prymnesiophyte I. galbana when grown in the cell-free filtrate of P. roscoffensis (Figure 2).

Allopathy

The growth response in the control media (K/5 and K/2) of two of the test species, S. trochoidea and Tetraselmis sp. (Figure 2), was similar, but growth of both of these species was inhibited by the cell-free filtrate of P. roscoffensis (10 and 45% inhibition, respectively). Moreover, the cells of these flagellates lost their ability to swim. In contrast, there was no alteration of growth rate or motility for the prymnesiophyte I. galbana when grown in the cell-free filtrate of P. roscoffensis (Figure 2).

Table III: LD50 of the five toxic coccolithophore species and P. parvum (positive control) in cells mL–1

<table>
<thead>
<tr>
<th></th>
<th>Growth phase (10^3 cells mL–1)</th>
<th>Stationary phase (10^3 cells mL–1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleurochrysis</td>
<td>750</td>
<td>&lt;200</td>
</tr>
<tr>
<td>carterae</td>
<td>500</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Pleurochrysis</td>
<td>650</td>
<td>&lt;100</td>
</tr>
<tr>
<td>elongata</td>
<td>100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Pleurochrysis</td>
<td>200</td>
<td>&lt;100</td>
</tr>
<tr>
<td>placolithoides</td>
<td>200</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Pleurochrysis</td>
<td>250</td>
<td>300</td>
</tr>
<tr>
<td>roscoffensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jomonolithus</td>
<td>500</td>
<td>&lt;100</td>
</tr>
<tr>
<td>littoralis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prymnesium</td>
<td>500</td>
<td>&lt;100</td>
</tr>
<tr>
<td>parvum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Time of exposure.

Fig. 2. Growth rates of S. trochoidea, Tetraselmis sp. and I. galbana in an enriched cell-free filtrate of P. roscoffensis compared with control cultures (light grey, Tetraselmis sp.; dark grey, S. trochoidea; black, I. galbana).

DISCUSSION

In this study, five coastal coccolithophores, P. carterae, P. elongata, P. placolithoides, P. roscoffensis and J. littoralis, were demonstrated to be toxic using the brine shrimp (A. salina) lethality assay. This is the first report of toxicity of these species to Artemia. Three of these species, P. elongata, P. placolithoides and P. roscoffensis, were more toxic than P. parvum, the toxic control (LD50; Table III). Jomonolithus littoralis was more toxic than P. parvum during the growth phase, but equally or less toxic in the stationary phase. Pleurochrysis carterae had a toxic effect similar to that of the positive control in the growth phase, but lower in the stationary phase. (NB. The use of chemically undefined soil extract in the medium means that the factor responsible for growth limitation in these cultures cannot reasonably be deduced.) The other coastal coccolithophores examined in this study, H. coronata, H. globosa, O. neapolitana and P. dentata, were non-toxic to A. salina (0% mortality).

Emiliania huxleyi and G. oceanica, two species commonly found in open-ocean environments, were previously shown to be non-toxic using the same brine shrimp lethality assay by Rhodes et al. (Rhodes et al., 1995). Our results demonstrate that cultures of two other oceanic species, C. leptopora and C. braarudii, are also non-toxic to brine shrimp.

Of the range of coccolithophores tested so far, two major groups featuring potentially toxic species may be defined, corresponding to the families Pleurochrysidaceae and Hymenomonadaceae. Previous studies suggested that P. pseudoroscoffensis was toxic to Artemia (Reifel et al., 2001) and therefore all members of the Pleurochrysidaceae which have been tested, with the exception of P. dentata, are toxic to Artemia. Toxicity of microalgae being dependent on culture conditions (Johansson and Granéli, 1999), all of our experiments were undertaken in the same conditions and the lack of toxicity of P. dentata is significant. This organism, originally described as P. carterae var. dentata, was discovered in an inland saline pond in New Mexico (USA), far removed from coastal waters (Johansen et al., 1986), in contrast to other members of the genus Pleurochrysis, which are typically found in coastal marine environments. Formally considered as a simple variety of P. carterae, this taxon has recently been raised to the species level as P. dentata following molecular genetic studies (Sáez et al., 2003). Whereas P. carterae is toxic, P. dentata is not, and our results provide further support for the conclusion that this coccolithophore is a distinct species that occupies an isolated position within the Pleurochrysidaceae.

All of the species of Hymenomonadaceae tested in our study were not toxic to A. salina. Previously, O. neapolitana and some other haptophytes (e.g. Chrysochromulina brevisulm Parke et Manton, Chrysochromulina kapp Parke et Manton, Chrysochromulina strobilus Parke et Manton) were found to be toxic to the bryozoan E. pilosa (Jebram,
1980). However, the toxicity of these species to *A. salina* has not been demonstrated (Edvardsen, 1993; this study).

*Jomonolithus littoralis* is the only member of this genus with distinctive heterococcoliths (Inouye and Chihara, 1983) which was classified as *Incertae sedis* by Jordan and Green (Jordan and Green, 1994). Based on its internal cellular organization, this coastal species shows affinities with both the Pleurochrysidaceae and the Hymenomonadaceae (Inouye and Chihara, 1983). At present, the family Pleurochrysidaceae contains the single genus *Pleurochrysis* in which coccolithogenesis is distinctive, involving coccolithosomes (Outka and Williams, 1971). Because of the absence of coccolithosomes in *J. littoralis*, Billard and Inouye tentatively placed this species in the Hymenomonadaceae (Inouye and Chihara, 1983). At present, the family Pleurochrysidaceae contains the single genus *Pleurochrysis* in which coccolithogenesis is distinctive, involving coccolithosomes (Outka and Williams, 1971). Because of the absence of coccolithosomes in *J. littoralis*, Billard and Inouye tentatively placed this species in the Hymenomonadaceae (Billard and Inouye, 2004). However, with respect to toxicity, *J. littoralis* shows more affinity with the Pleurochrysidaceae. Clearly, more data are needed to clarify the taxonomic position of this enigmatic species, which may in fact belong to a family of its own.

The toxins of two non-calcifying members of the Prymnesiophyceae, *P. parvum* (Igarashi *et al.*, 1993) and *C. polypleis* (Yasumoto *et al.*, 1990), have been studied in recent years, and in both cases it appears that these toxins are haemolytic compounds. Allelopathic tests were carried out with *C. polypleis* by Myklestad *et al.*, who showed that addition of dense suspensions or filtrates of this flagellate to cultures of the diatom *Skeletonema costatum* resulted in growth inhibition (Myklestad *et al.*, 1995). The fact that filtrates could inhibit growth was interpreted as indicating that the *C. polypleis* toxin is an exotoxin. Schmidt and Hansen showed that dinoflagellates exposed to suspensions of *C. polypleis* lost their motility and growth rates were also decreased (Schmidt and Hansen, 2001). Allelopathy of *P. parvum* was demonstrated in cell-free filtrates by Granelli and Johansson, who studied the effect of nutrient conditions on this ability (Granelli and Johansson, 2003).

Concerning *P. pouchetii*, Hansen *et al.* concluded that senescent colonial cells excreted an anti-mitotic compound into their surroundings (Hansen *et al.*, 2003). Our study has shown that cell-free filtrates of a *P. roscoffensis* culture have a negative effect on growth rates and motility of *Tetraselmis* sp. and *S. trochoidea*, while the prymnesiophyte *I. galbana* was unaffected. These results indicate that this coccolithophore produces an extracellular substance with allelopathic effects on other, unrelated, flagellate microalgal species. Consequently, we suggest that the toxin of *P. roscoffensis* is an exotoxin, which could be similar to that produced by other non-calcifying members of the Prymnesiophyceae, namely *C. polypleis* and *P. parvum*. Both of these flagellates belong to the Prymnesiales, an order which is phylogenetically closer to the Coccolithales (containing the toxic coccolithophores) than the Phaeocystales in which *P. pouchetii* is classified (Edvardsen *et al.*, 2000). To further test the hypothesis that *Pleurochrysis* toxins are similar to those of members of the Prymnesiales, it would be interesting to screen the toxic coccolithophore species for haemolytic activity.

Allelopathy seems to be a significant factor affecting the interaction between different species of phytoplankton and consequently phytoplankton succession (Keating, 1978; Legrand *et al.*, 2003). Production of allelochemicals could be an important ecological strategy for species with a moderate growth rate, such as *P. parvum* (Brand, 1984; Fistarol *et al.*, 2003). In this study, the growth rate of the *Pleurochrysis* species tested was inferior to that of *P. parvum*. Fresnel *et al.* reported that the slow-growing *Pyrmenesium zebrinum* Billard was toxic, while *Pyrmenesium annuliferum* Billard, which grows very rapidly and produces dense cultures, showed no signs of toxicity (Fresnel *et al.*, 2001). The release of toxins to the surrounding water may explain how slow-growing species can occasionally bloom.

Certain ubiquitous coccolithophores, such as *E. huxleyi*, *G. oceanica* and *Coccolithus pelagicus* (Wallich) Schiller, are well-known bloom-forming organisms (Rhodes *et al.*, 1995; Paasche, 2002), but these are all oceanic species which are apparently non-toxic. In recent years, a number of localized blooms of coastal coccolithophores have been observed. In France, for example, *P. roscoffensis* or *P. pseudoroscoffensis* may proliferate, generally in the autumn, in oyster ponds or water reservoirs for aquaculture in various localities along the coast (C. Billard, unpublished observations, with records since 1993). A bloom of *Pleurochrysis* sp. was recorded in August 1995 in Malmö (Sweden) and mortality of ducks was noted (C. Legrand, personal communication). Reifel *et al.* reported dense populations of *P. pseudoroscoffensis* in the Salton Sea (California) during February–August 1999, but concluded that such blooms were probably not toxic to vertebrates using the mouse bioassay (Reifel *et al.*, 2001).

According to the present study, certain coccolithophores are suspected to be toxic to invertebrates and further tests should be performed to assess their haemolytic capacities. It should be stressed that *Artemia* is not wholly representative of the natural predators of coccolithophores and future research should include a variety of potential predators. In view of the fact that certain coccolithophores are able to bloom in secluded coastal environments (e.g. oyster ponds), and are capable of excreting toxic allelochemicals, the possible impact on aquaculture of these coccolithophores, and particularly *Pleurochrysis* species, should be considered in the future.
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