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Ichthyotoxicity of the microalga *Pseudochattonella farcimen* under laboratory and field conditions in Danish waters

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ABSTRACT: Blooms of the marine dictyochophyte *Pseudochattonella farcimen* have been associated with fish kills, but attempts to verify ichthyotoxicity of this microalga under experimental conditions have not been successful. In the early spring of 2009 and 2011, *P. farcimen* bloomed in the inner Danish waters. The blooms occurred at a seawater temperature of ~2°C and correlated with extensive kills of farmed salmonid fish (2009) and wild populations (2011). Several strains of *P. farcimen* were isolated from the 2009 bloom. However, exposure of rainbow trout *Oncorhynchus mykiss* to laboratory-grown *P. farcimen* cultures did not reveal any toxic effects. During the 2011 bloom, fish were exposed to bloom water under both laboratory and field conditions. While no clinical effect was observed on fish incubated in bloom water in the laboratory trial, a remarkable difference was seen in the field trial between rainbow trout kept in tanks supplied with a continuous flow of filtered versus non-filtered bloom water. Histological examination of the gill tissue revealed karyorrhexis and epithelial loosening in the affected fish. Microscopy analysis of algal cell morphology suggested that mucocysts detected on the cell surface only in freshly sampled bloom water might be associated with ichtyotoxicity.

KEY WORDS: *Pseudochattonella farcimen* · Dictyochophytes · Ichthyotoxicity · Mucocysts · Harmful algae

INTRODUCTION

Until 2009, the microalga *Pseudochattonella farcimen* was known as *Chattonella* aff. *verruculosa* (Raphidophyceae) (Hara et al. 1994) and *Verrucophora farcimen* (Dictyochophyceae) (Edvardsen et al. 2007). However, Hosoi-Tanabe et al. (2007) proposed the genus *Pseudochattonella* (Dictyochophyceae) for *P. verruculosa,* a closely related species. For reasons of priority, *V. farcimen* was finally named *P. farcimen* (Dictyochophyceae) in 2009 (Eikrem et al. 2009). To our knowledge, the distribution of *P. farcimen* is so far limited to the North Sea and the interconnected Scandinavian waters around Denmark, including the southern part of the Kattegat and the Belt Sea. Since 1998, *Pseudochattonella* spp. have been found every year in Scandinavian waters, but they only seem to bloom in certain years. Some blooms have occurred in relatively warm waters, up to 18°C, while other blooms have occurred in colder waters around 2–5°C (Riisberg & Edvardsen 2008). To date, *P. farcimen* blooms have only been reported from cold water conditions.

Only some blooms of *Pseudochattonella* spp. in the North Sea and in Scandinavian waters have been recognized as ichthyotoxic, but historically all cold-water blooms of *Pseudochattonella* spp. in Scandina-

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vian waters have been ichthyotoxic. Warmer-water blooms (up to 18°C) caused by *Pseudochattonella* spp. generally do not lead to fish mortalities, with the exception of the bloom in 1998 (Riisberg & Edvardsen 2008). The ichthyotoxic mechanism of *Pseudochattonella* spp. is presently not known.

Pseudochattonella spp. have large amounts of mucocysts, giving these algae their characteristic warty appearance. Together with *Chattonella globosa* (Chang et al. 2012), *Pseudochattonella* spp. are the first dictyochophytes reported to have mucocysts, which are otherwise common among the raphidophytes (Edvardsen et al. 2007). Mucocysts are saccular or rod-shaped extrusomes, which are able to discharge a mucus-like mass. The function of mucocysts on microalgae is not clear; they have been suggested to act as grazer deterrents (Tillmann & Reckermann 2002), or as a mechanism to capture and eat bacteria (Jeong et al. 2010). Interestingly, some of the raphidophytes possessing mucocysts (Jeong et al. 2010) have also been related to fish-killing events, e.g. *Fibrocapsa japonica* (Pezzolesi et al. 2010), *Heterosigma akashiwo* (Twiner et al. 2005), and *C. ovata* (Hiroishi et al. 2005). Furthermore, the cell morphology of *Pseudochattonella* spp. can change considerably. While some cells are elongated carrot-like shaped, others are almost spherical; intermediate shapes can also be found (Edvardsen et al. 2007). The small spherical cells are seen in laboratory cultures during non-optimal growth conditions (Jakobsen et al. 2012).

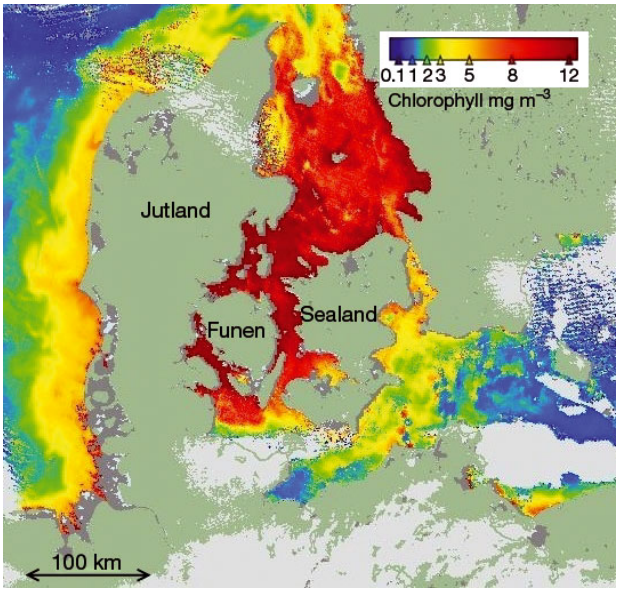

Fig. 1. Satellite image of chlorophyll concentration during the *Pseudochattonella farcimen* bloom on 16 March 2011 in the inner Danish waters. (Image provided by the Danish Meteorological Institute, http://marcoast.dmi.dk)

During February to March 2009 and 2011, extensive ichthyotoxic blooms of the dictyochophyte *P. farcimen* occurred in the inner Danish waters, including the Great Belt and the Little Belt areas. Both blooms started developing at temperatures of ~2°C and at salinities of 15–17. The blooms resulted in a noticeable brown discoloration of the water with very little transparency in the water column, particularly during the bloom of 2011. Based on satellite images, the 2011 bloom covered all waters around the island of Funen (Fig. 1).

Attempts to replicate the acute ichthyotoxic effect of *Pseudochattonella* spp. in the laboratory have so far been unsuccessful (Skjelbred et al. 2011). The aim of this study was to test the acute ichthyotoxic effect of *P. farcimen* in laboratory cultures and in mesocosm experiments using natural assemblages during bloom periods.

MATERIALS AND METHODS

Preparation of *Pseudochattonella farcimen* strains

Six strains of *P. farcimen* were established from the bloom in 2009 by single cell isolation as described by Jakobsen et al. (2012) (Table 1). The strains were kept at exponential growth in TPP® tissue culture flasks using pasteurized F/20 media (Guillard & Ryther 1962) at a salinity of 17 prepared from seawater diluted with tap water, a temperature of 15°C, and an irradiance of 120 µE m^{-2} s^{-1} provided by cool white fluorescent tubes (light:dark cycle 10:14). Four weeks after the cultures were established, the temperature was lowered to 4°C.

Laboratory experiment, 2009

Rainbow trout *Oncorhynchus mykiss* were exposed to established cultures of *P. farcimen* for 3 d. Experiments were carried out in triplicate 60 l glass aquaria with a water temperature of 4°C and a salinity of 17. Three acclimated fish (200–300 g) were added to each aquarium. Aquaria were provided with an irradiance of 120 µE m^{-2} s^{-1} and a light:dark cycle of 10:14 h. *P. farcimen* was grown in aerated F/20 media directly in the experimental aquaria to the desired cell concentration before the addition of fish. Oxygen content (WTW pH/Oxi 340i), temperature, and pH (WTW pH 3210) were recorded regularly. Concentrations of *P. farcimen* in the range of 1×10^3 to 50×10^3 cells ml^{-1} were tested for acute ichthyotoxic effects.

Table 1. Overview of the 6 *Pseudochattonella farcimen* strains isolated from Danish waters during an ichthyotoxic bloom in the spring of 2009

Strain name	Place of isolation N	E	Month of isolation	Temp. (°C)	Salinity
K-1808	55° 49′ 22″	10° 03′ 06″	February	2	15
K-1809	55° 49′ 22″	10° 03′ 06″	February	2	15
K-1804	55° 28′ 50″	11° 05′ 25″	March	4	20
K-1805	55° 28′ 50″	11° 05′ 25″	March	4	20
K-1806	55° 28′ 50″	11° 05′ 25″	March	4	20
K-1807	55° 28′ 50″	11° 05′ 25″	March	4	20

Laboratory exposure trial with bloom water, 2011

Fifty liters of water (salinity 15, temperature 2.5°C) were collected at the Hjarnø fish farm (55° 49′ 17″ N, 10° 03′ 10″ E) on 10 March during the peak of the *P. farcimen* bloom in 2011 and brought back to the laboratory. The transport of water took ~1 h, and efforts were made to maintain the temperature at 2.5°C during transportation. In the laboratory, *P. farcimen* was identified microscopically. Twenty rainbow trout (0.5 g) were transferred to a number of duplicate 2 l glass bottles (Duran®) and exposed to the following treatments: (1) bloom water, (2) bloom water slowly heated over 1 h to 10°C and subsequently lowered over 1 h to 2.5°C before use, (3) supernatant of 2 l centrifuged (1500 × *g*, 15 min) bloom water, and (4) North Sea bottom water (salinity 30) without algae, mixed with tap water to reach a salinity of 15 (negative control). The oxygen content was kept above 95%, with a low amount of aeration in all treatments. The bottles were kept in a cooling water bath to keep a constant temperature during the 12 h experimental period. In treatments 2 and 3, fish were added immediately after the preparation of the water.

Field experiment, 2011

A mesocosm experiment over a time period of 2 wk was carried out at Snaptun Harbor (55° 49′ 17″ N, 10° 03′ 10″ E) in March 2011, during the stationary growth phase of the *P. farcimen* bloom. The stationary growth phase of the bloom was indicated by no further increase in cell numbers and the observation of multinucleate stages. The multinucleate stages started to develop at high cell concentrations when growth became limited (Jakobsen et al. 2012).

The aim of the experiment was 2-fold. One was to establish a controlled experimental setup, in which the clinical observations observed on farmed fish and wild fish could be reproduced. The second was to determine whether the toxic effect of the bloom water was associated with direct contact between the fish and the algal cells.

Bloom water was pumped using immersion pumps from the harbor into 3 parallel filter tanks: (1) an empty tank, (2) a tank containing blue mussels *Mytilus edulis* acting as a biological filter, and (3) a sand filter with sand grain sizes in the range 0.09 to 0.25 mm (Dansand$^{A/S}$ no. 18; Fig. 2). From each filter tank, water was siphoned (~700 l h^{-1}) to duplicate fish tanks (Promens, product code 001000) and from the fish tanks back into the harbor. The water was not recycled through the filters. All filter and fish tanks had a volume of 1 m^3. The 6 fish tanks were stocked with rainbow trout, European whitefish *Coregonus lavaretus*, and flounder *Platichthys flesus* as listed in Table 2. Rainbow trout were provided from a disease-free freshwater facility producing fish for stocking of marine fish farms (Aarup Moelle Dambrug ApS, Denmark); whitefish and flounder were provided from a land-based marine facility producing fish for restocking of wild populations (Fishlab, Højbjerg, Denmark). Physico-chemical parameters (temperature, pH, and oxygen concentration) were recorded twice a day. Water samples for the microscopical enumeration of *P. farcimen* and morphology analyses were collected daily. The enumeration was done using a Sedgewick Rafter Counting Cell Slide with a total of 400 cells counted. The fish were monitored at least twice a day, and their behavior was recorded. When abnormal behavior was observed, individuals were sampled for microscopical examination.

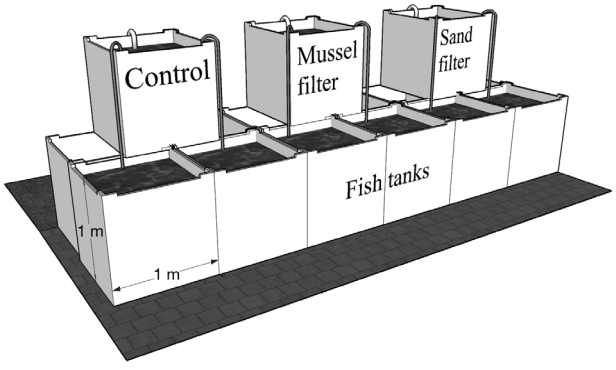

Fig. 2. Experimental setup established in Snaptun Harbor during the ichthyotoxic bloom of *Pseudochattonella farcimen* in 2011. Natural bloom water was pumped from the harbor and passed through a mussel filter, a sand filter, or a control tank not containing any filter material. Fish were contained in duplicate fish tanks below the filter tanks

Table 2. Number and weight of fish in each of the 6 experimental fish tanks in the field experiment during the 2011 ichthyotoxic bloom of the microalga *Pseudochattonella farcimen*. Clinical effects are indicated by plus or minus symbols

Common name	Scientific name	Weight (g)	No. of fish	Clinical effects observed
Flounder	*Platichthys flesus*	5	30	–
Rainbow trout	*Oncorhynchus mykiss*	500	10	+
Rainbow trout	*Oncorhynchus mykiss*	100	50	–
European whitefish	*Coregonus lavaretus*	500	10	–
European whitefish	*Coregonus lavaretus*	100	50	–

Histology of rainbow trout gill tissue

Gill tissue samples were fixed in phosphate-buffered formalin and embedded in paraffin, and 3 µm sections were cut and stained in hematoxylin and eosin (Evensen & Lorenzen 1997). Microscopical analyses focused on samples from fish exposed to non-filtered seawater and seawater that had passed through the sand filter.

RESULTS

Field observations

During the 2009 bloom, *Pseudochattonella farcimen* reached a concentration of 10×10^3 cells ml^{-1}. There were no reports of affected wild fish, but mortality of farmed fish was high. In fact, 1 marine farm, situated near the site of the field trials in 2011, lost 100% of its Atlantic salmon *Salmo salar* stock of fish sized 1–2 kg, and close to 50% of its rainbow trout stock of fish sized 150–500 g. While the mortality was rather acute in the case of the Atlantic salmon, taking place within 2 d, the mortality among rainbow trout was more protracted, with a duration of approximately 2 wk and a tendency for larger individuals to be the most affected. Clinical examination revealed no signs of infectious diseases. Caged cod *Gadus morhua* and European whitefish at the same farm site were apparently not affected.

In 2011, the bloom concentration was 10-fold higher, reaching cell concentrations of 100×10^3 cells ml^{-1}. *P. farcimen* made up 95% of the total algal assemblage by cell numbers both in 2009 and 2011. Due to the ichthyotoxic bloom of *P. farcimen* in 2009, fish farmers postponed the release of fish in 2011 until the end of March, and hence there was no recorded mortality of caged fish in 2011. However, in contrast to 2009, the bloom in 2011 had a severe impact on the wild sea trout *Salmo trutta* population. Several anglers reported dead *S. trutta* along the coastlines in combination with the absence of live fish. The 2011 bloom was also the determinant factor for the postponement of angler unions to release young *S. trutta* to the sea, which normally occurs in the early spring.

Laboratory exposure trials with the laboratory-grown algal strain

Experimental exposure of rainbow trout to laboratory-grown algal cells revealed no abnormal behavior, no clinical signs, or mortality of the fish. The standard setup included aeration with atmospheric air to avoid effects of low O_2 and/or high CO_2 concentrations. During this experiment, the oxygen content was kept above 90% and pH changed from 7.9 ± 0.0 to 7.7 ± 0.06. Since the mechanical effect of aeration was suspected to affect the toxicity of the algae, the same setup was tested without aeration. The experiment without aeration was terminated after 12 h when the oxygen content was ~20% and pH had dropped from 7.9 ± 0 to 6.7 ± 0.06. However, we observed no difference in the performance of the fish exposed to *P. farcimen* and the controls without *P. farcimen* in any of the setups.

Laboratory exposure trial with bloom water

Experimental exposure of small rainbow trout was limited to 24 h, as the water conditions themselves with the combination of the low temperature (2.5°C) and a salinity of 15 were expected to stress the fish. However, temporary treatment with a similar concentration of NaCl is routinely used as a preventive measure against parasite and fungal infections under farming conditions, and no abnormal behavior was observed in the experiment. The fish showed no signs of being affected by the *P. farcimen* bloom water that was brought to the laboratory. During the 24 h experiment, only 3 fish died in random bottles, including the control bottles, probably because fish of this small size (0.5 g) are relatively fragile and susceptible to mechanical handling. During the first 12 h, the pH (mean of all experimental bottles ± SD) dropped significantly from 8.1 ± 0.2 to 7.7 ± 0.2 (*t*-test: $p = 0.002$), despite the

aeration. Initially, all *P. farcimen* cells in the treatment with bloom water were elongated in shape and displayed visible mucocysts. Water samples taken after 12 h of exposure revealed that the algal cells had changed shape from elongated to spherical and mucocysts were not visible on the cells. The algal cells that had been heated to 10°C and subsequently cooled to 2.5°C were all spherical and displayed no visible mucocysts at the start of the experiment.

Exposure of fish under field conditions

The mussel and the sand filters continuously removed 35 and 80%, respectively, of the *P. farcimen* cells, compared to the untreated bloom water (Table 3). One of the duplicate fish tanks receiving water from the sand filter contained a 3-fold higher algal concentration than the other fish tank receiving water from the sand filter (Table 3). This was due to a clogging of the sand filter and overflow of bloom water to this fish tank at the time the algal samples were taken. The true efficiency of the sand filter was thus higher than the 80%. The high amount of *P. farcimen* cells apparently exceeded the capacity of the mussel filter. The difference in efficiency of the filters was visible to the naked eye. The water that had passed the sand filter appeared clear compared to the brownish water that had passed the mussel filter and the dark brown water from the control filter tank. Microscopical examination revealed some cells left in the water that had passed the sand filter, but the majority of these cells were either small and spherical and without mucocysts, or damaged.

During the initial adjustment period of the filters, the grain size in the sand filter was too large to efficiently retain the algae, and high mortality amongst the rainbow trout was observed in all tanks while the other fish species remained unaffected (data not shown). When, after the ~2 wk adjustment period, the sand filter retained 80% of the algae, the experiment was initiated with new batches of fish. However, the *P. farcimen* bloom had in the meantime reached an early stationary growth phase, and we did not observe any fish mortality, although during the first 24 h of the experiment, large (500 g) rainbow trout were heavily affected in the tanks with bloom water and in bloom water treated with the mussel filter. The toxic effects observed on the fish included irregular swimming, occasionally upside-down, and some individuals hanging just below the surface without any normal escape reaction when caretakers approached the tanks. In the containers with water from the sand filter, the fish were not affected and showed normal swimming behavior. After the first 24 h, no effects on any of the fish species in any of the tanks were observed. The fish that had been affected previously recovered completely. No abnormal behavior was observed among the other fish species (Table 2).

Histology of rainbow trout gill tissue

Compared to the non-exposed fish (Fig. 3A), the gills of fish exposed to non-filtered seawater (Fig. 3B) were seriously affected: the nuclei of most of the epithelial cells were disintegrated (karyorrhexis), and the epithelium was loosened from the pillar-cells ('lifting'), most likely due to edema. Some epithelial cells appeared swollen. By contrast, the gills of fish exposed to water that had passed through the sand filter, which cleared the water by 80% (Table 3), were much less affected, with only few epithelial cells showing signs of karyorrhexis or having a swollen appearance, and without or with only minimal epithelial lifting (Fig. 3C).

DISCUSSION

To our knowledge, this is the first report showing acute clinical effects of *Pseudochattonella* sp. under

Table 3. Cell counts of *Pseudochattonella farcimen* 16 h after fish were added to the tanks. The clinical effects and histological changes in gills of larger rainbow trout *Oncorhynchus mykiss* are indicated by plus or minus. na: not assessed

Filtration	*P. farcimen* (cells ml^{-1})	Removal of cells (%)	Frequency of cells with mucocysts	Clinical effects	Histological changes
None	60600	–	High	+++	+++
None	49400			+++	+++
Mussel filter	40000	35	High	++	na
Mussel filter	31857			++	na
Sand filter	5900	80	Absent	–	+
Sand filter	16550			–	+

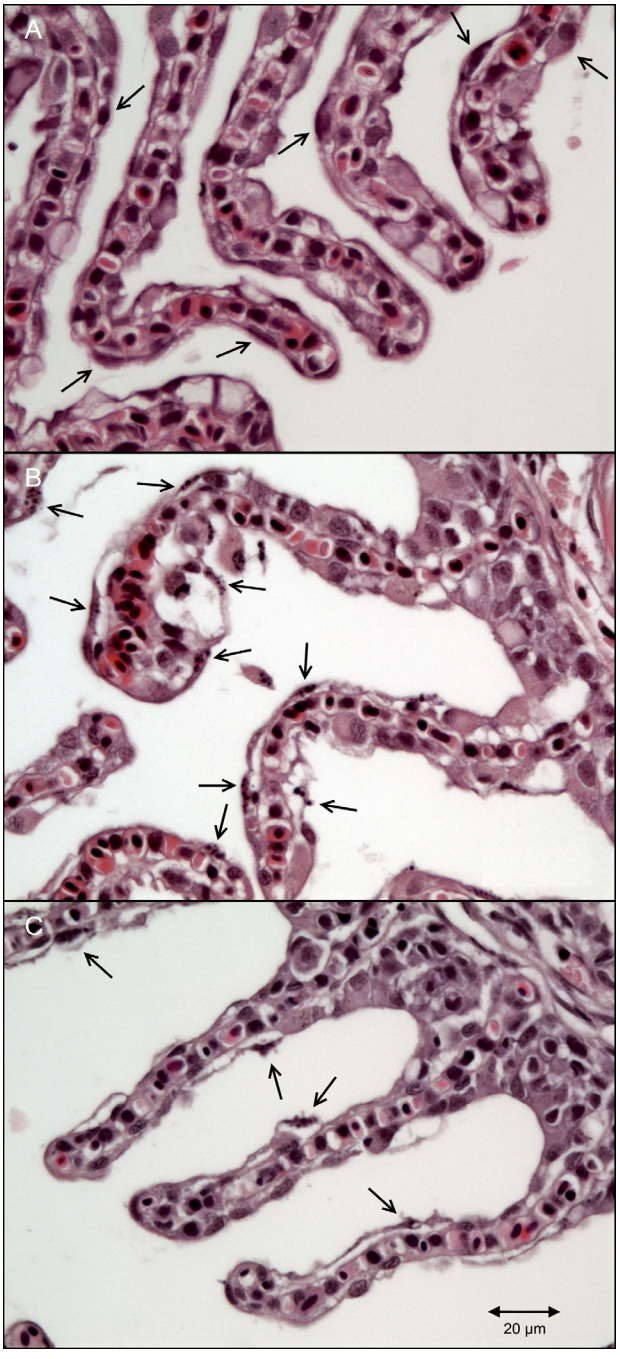

Fig. 3. Hematoxylin and eosin staining of gill tissue. Arrows indicate swollen cells, karyorrhexis, and loosening of epithelial cells. (A) Small naive rainbow trout *Oncorhynchus mykiss* not exposed to water containing *Pseudochattonella farcimen*. Epithelial cells are intact, as are the nuclei of these cells (bright holes are artifacts due to fixation in formalin). (B) Small rainbow trout, kept in water that was not filtered. Most of the epithelial cells are affected and show pronounced karyorrhexis and epithelial loosening. (C) Small rainbow trout, kept in water that was passed through a sand filter with grain sizes in the range of 0.09–0.25 mm. Some/few of the epithelial cells have disintegrated nuclei (karyorrhexis). Some areas of the lamellae show loosening of epithelial cells

experimental conditions. Our results further demonstrated the requirement of the physical presence of the algal cells in the water for observation of these effects, suggesting that a direct interaction between the algae and the fish is needed to induce toxicity. Exposure time exceeding 24 h may be required in order to observe an effect on fish by *P. farcimen* (Skjelbred et al. 2011). However, our study showed that an acute effect on the fish caused by *P. farcimen* can be obtained within 24 h under controlled experimental conditions. Since the clinical effects were obtained with bloom water, we cannot fully exclude that the sand filter could have removed toxic particulate components other than the *P. farcimen* algae. However, the microscopical examination of the water before and after sand filtration strongly suggested removal of the highly prevalent mucocyst-bearing *P. farcimen* cells to be the main effect of the filtration. In our report, the frequencies of *P. farcimen* bearing mucocysts are estimated values from live algal samples (Table 3), since *P. farcimen* seems to discharge its mucocysts during fixation (Edvardsen et al. 2007).

In the laboratory, cultures of *P. farcimen* and *P. verruculosa* have been tested against salmon smolts *Salmo salar* and cod fry *Gadus morhua*, and changes in gill epithelial cells were observed in both species, but no acute toxic effects and no fish kills were observed, even when the fish were exposed to very dense algal cultures (Skjelbred et al. 2011). These results are in accordance with our observations using a stationary exposure setup with either laboratory-grown algal cultures or bloom water brought into the lab from the field. The setup with a continuous flow of bloom water under field conditions is believed to be essential for the acute clinical effects reported here, although we cannot rule out that the lack of effect of bloom water in our stationary experiment could be due to low susceptibility of the small sized fish that were used due to practical limitations. In the field trial, we thus only observed effects on the larger rainbow trout. This was in accordance with the field observations in 2009, where rainbow trout of ~0.5 kg and salmon of 1–2 kg showed more acute responses compared to rainbow trout of 150 g. Similar observations were reported by Backe-Hansen et al. (2001), who only observed effects of *Pseudochattonella* sp. on large Atlantic salmon around 2 kg, whereas smaller fish were not affected. Whether these size-related differences in susceptibility could be due to larger fish being more easily affected by reduced efficacy of the gills in terms of gas exchange remains to be determined. In our experiment, whitefish of

similar size as the larger rainbow trout displayed no abnormal behavior. Again, this was in accordance with the field observations in 2009 where cod and whitefish remained unaffected. Why some fish species are affected by *P. farcimen*, while others are not, is presently unknown. The farm site area affected in 2009 was 4–8 m deep, and the algae were equally distributed throughout the water column. Fish at the bottom and at the surface would thus be expected to be equally exposed to the algae, and it was not possible for the fish to move to any bottom water that was potentially free of algae. Species-related differences in metabolism rate/susceptibility to reduced functionality of the gills could be involved.

The histological effects that we found on the gill of the affected rainbow trout do support the assumption that *P. farcimen* affects the performance of the gills. Extension of such studies to fish of different sizes and species will contribute to pinpointing whether the histological changes correlate with the clinical effects or whether there is a size/species-related difference in the ability of the fish to cope with the tissue damage caused by the algae.

The toxic mechanism of *P. farcimen* was previously associated with the unusual polyunsaturated fatty acid (PUFA) profile of this species (Giner et al. 2008, Dittami et al. 2012). However, the release of PUFAs has never been shown experimentally to be involved in the mechanism of ichthyotoxicity. In our study, the difference between fish supplied with filtered and non-filtered water suggests that a direct interaction between the algal cells and the fish is required to induce the toxic effect. Why a direct cell to gill contact seems to be needed is still unknown. Our results indicate that the mucocysts present on the cell surface of the *Pseudochattonella* spp. could play an important role. The actual number of *P. farcimen* cells needed to induce fish kills is presently unknown. Observations on *P. verruculosa* from New Zealand (MacKenzie et al. 2011) indicate that around 10 cells ml^{-1} can kill salmon, and our own observations from the fish farms in Denmark indicate that rainbow trout will start to be affected at around 500 cells ml^{-1} of *P. farcimen*. A reason that the rainbow trout were not affected in the fish tank receiving water from the sand filter with cell counts of several thousands (Table 3) could be due to the fact that only small spherical cells and not the larger elongated cells were allowed to pass the sand filter.

In the study of Skjelbred et al. (2011), swelling of the nuclei and hypertrophy of the epithelial cells was a common finding in the gills of salmon exposed to *P. verruculosa.* In the present study with *P. farcimen*, some degree of swelling of epithelial cells was likewise found in fish exposed to non-filtered sea water, but in the main part of the epithelium, karyorrhexis was a common finding, most likely because the exposure was rather intense due to the huge amounts of *P. farcimen.* We did not observe any mucus on the fish gills of dead and affected fish, which possibly could have led to suffocation during exposure to *P. farcimen*. Earlier exposure trials using *Pseudochattonella* spp. also did not lead to mucus on the gills (Skjelbred et al. 2011).

During the isolation of the *P. farcimen* strains in the spring of 2009, the algae were slowly acclimated from 4 to 15°C. The algae survived this acclimation and even showed a higher growth rate at 10°C than at 1 and 3°C (Jakobsen et al. 2012). (Note that the Cr_4 strain used by Jakobsen et al. 2012 was later named K-1807.) This observation is also consistent with Skjelbred et al. (2013). However, the cells lost the presence of visible mucocysts and changed shape from elongated to spherical during this acclimation, i.e. the laboratory-grown *P. farcimen* never displayed mucocysts during the exposure trials. Despite the use of relatively large rainbow trout (200–300 g) and prolonged exposure, no clinical effects were seen on the fish. In 2011, clonal strains were not established. Instead, natural bloom water containing *P. farcimen* was brought to the laboratory with the purpose of inducing an acute ichthyotoxic effect under experimental conditions. However, during this experiment, the algal cells again lost their visible mucocysts and changed shape from elongated to spherical. Why the cells change shape within 12 h is not known, but a significant drop in pH might be the explanation. No matter the explanation for the loss of visible mucocysts, we only observed ichthyotoxic effects of *P. farcimen* when the alga possessed mucocysts, i.e. during field sampling and in the field experiment in 2011. Little information about ichthyotoxicity and mucocysts is available in the literature. However, the raphidophyte *Chattonella antiqua* releases a large amount of its mucocysts when the algal cells come into contact with mucus from fish gills (Shimada et al. 1991). The observed epithelial lifting and nuclear disintegration in the gill tissue is expected to seriously compromise the oxygen uptake due to increased distance between the water and the pillar cells and dysfunction of the epithelial cells. The pillar cells have a dual function, as they constitute the backbone of the secondary lamellae and also act as endothelial cells. Pillar cell damage will thus reduce efficacy of gas exchange between the external water and the fish blood cells.

In conclusion, our results suggest that the ichthyotoxic effects of *P. farcimen* are related to the morphological stage of the algal cells. When the algae are elongated and display mucocysts, they are presumably most toxic. Future laboratory experiments to study the ichthyotoxicity of *P. farcimen* should thus aim at establishing culture and/or incubation conditions under which the algae display/retain mucocysts. Important parameters are believed to be the low and constant temperature around 3–4°C and likely a stable pH. The practical implications of our results depend on the rearing conditions of the fish. For fish kept in net pens, the only options appear to be to avoid stocking under blooms or moving/placing of the pens to/at sites with less risk of algal blooms. For land-based facilities, filtration and/or other physical treatment reducing the abundance of mucocyst-bearing algae could be an option.

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