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Environmental optima for seven strains of *Pseudochattonella* (Dictyochophyceae, Heterokonta)[1]

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Abstract

The ichthyotoxic flagellate *Pseudochattonella* has formed recurrent blooms in the North Sea, Skagerrak and Kattegat since 1998. Five strains of *P. farcimen* and two strains of *P. verruculosa* were examined in an assay comparing the light response of specific growth rates over a range of temperatures and salinities to get further knowledge on the autecology of

members of this genus. Temperature optima were lower in *P. farcimen* (9 - 15 ºC) than in *P. verruculosa* (12 - 20 ºC). *P. farcimen* also showed a somewhat lower salinity optimum (18 - 26) than *P. verruculosa* (20 - 32). All strains showed light-dependent growth responses reaching saturation between 18 - 52 µmol photons $\cdot m^{-2} \cdot s^{-1}$ at optimal temperature and salinity conditions. Compensation point estimates ranged from 4.2 - 15 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Loss rates increased with temperature and were lowest at salinities close to optimal growth conditions. Blooms of *P. farcimen* have been recorded in nature under conditions more similar to those minimizing loss rates rather than those maximizing growth rates in our culture study.

Key index words: compensation irradiance; growth rates; light; loss rates; *Pseudochattonella*; salinity; saturating irradiance; temperature.

Introduction

The heterokontophyte flagellate *Pseudochattonella* (Y.Hara & Chihara) Hosoi-Tanabe, Honda, Fukaya, Inagaki & Sako has formed recurrent extensive blooms in the North Sea, Skagerrak and Kattegat since 1998 (Edvardsen et al. 2007, Riisberg and Edvardsen 2008). Strains isolated from the Skagerrak bloom in 2001 in 2006 were found to belong to a new species, *P. farcimen* (Eikrem, Edvardsen et Throndsen) Eikrem, Edvardsen et Throndsen. This species differs both genetically and morphologically from *P. verruculosa* (Y. Hara et Chihara) Tanabe-Hosoi, Honda, Fukaya, Inagaki et Sako (= *Chattonella verruculosa* Hara et Chihara) isolates from Japan and elsewhere (Edvardsen et al. 2007, Riisberg and Edvardsen 2008, Eikrem et al. 2009). *Pseudochattonella* has also been observed blooming in the Baltic Sea (Łotocka 2009) and has occurred yearly along the Swedish west coast since the first

recording in 1998 (Karlson and Andersson 2003, Håkansson et al. 2007), but was recorded for the first time in the Oslo Fjord as late as October 2008 (Berge et al. 2009). *P. verruculosa* is known to form recurrent harmful algal blooms (HAB) in Japan (Imai et al 1998, Hallegraeff and Hara 2003, Hosoi-Tanabe et al. 2007) and has also been observed blooming in New Zealand (MacKenzie et al. 2011). The genus *Pseudochattonella* has been shown to be toxic to fish, although the mechanism of toxicity is not known (Skjelbred et al. 2011). Based on molecular phylogenetic analyses and morphological and ultrastructural data, the genus *Pseudochattonella* was shown to belong to the Class Dictyochophyceae and not the Raphidophyceae as previously presumed (Hosoi-Tanabe et al. 2006, 2007, Edvardsen et al. 2007). Nuclear-, chloroplast- and mitochondrial-encoded DNA sequences identified one strain isolated from German North Sea in 2000 as *P. verruculosa*, while strains from Skagerrak isolated in 2001 and 2006 belonged to *P. farcimen* (Riisberg and Edvardsen 2008). Expressed sequence tags (EST) library has been analysed in strains from *P. farcimen* (Dittami et al. 2012).

The Skagerrak blooms of *Pseudochattonella* in 2001, 2006, 2007 and 2011 occurred early in the year at water temperatures 2 - 5ºC, overlapping with the spring bloom of diatoms (Edvardsen et al. 2007, Riisberg and Edvardsen 2008, SMHI; AlgAware http://www.smhi.se/publikationer/Algrapporter, IMR; Algeinfo http://algeinfo.imr.no/). The *Pseudochattonella* blooms off the coasts of Germany, Denmark, Sweden and/or Norway in 1998, 2000, 2002 and 2004 occurred later in the season (April – May) at higher water temperatures (Lu and Göbel 2000, Backe-Hansen et al. 2001, Riisberg and Edvardsen 2008). Whether these were *P. farcimen* or *P. verruculosa* has not been determined, but the presence of *P. verruculosa* in the considerably warmer water temperatures during these blooms in 2000 and the presence of *P. farcimen* in the winter blooms of 2001 and 2006, suggests that these blooms may be due to physiologically distinct species or strains. The salinity of surface water

in Skagerrak varied from 17 to 30 during the bloom in 1998 (Backe-Hansen et al. 2001) and from 22 to 28 with a drop down to 12 during the bloom in 2001 (Naustvoll et al. 2002) . Effects of temperature and salinity on growth responses, performed with a strain of *P. verruculosa* from Japan (Yamaguchi et al. 1997) and *P. farcimen* from Skagerrak (Skjelbred and Naustvoll 2006), indicated a difference in optimal temperature between these two species; different strains from each of these species might also differ in their growth optima. In this study, we used an assay of three independently varying environmental factors (irradiance, temperature and salinity) to assess their effects on specific growth and loss rates in strains from the two *Pseudochattonella* species.

Material and methods

Algal strains and culture conditions. Water samples were collected from Skagerrak by the Ferrybox system on the transect Oslo-Kiel (*www.iis.niva.no/Ferrybox/*), or off Flødevigen Biological Station, southern Norway, January - March 2006. Fifteen clonal, but non-axenic strains of *P. farcimen* were obtained in 2006 by a modified capillary isolation method, where single cells were transferred to 96-wells culture plates (Nunc AS, Denmark) containing algal medium. Three of these new strains, isolated from different samples, were used for this study (Table 1). *P. farcimen* strains isolated from Skagerrak in 2001 and *P. verruculosa* strains originating from Japan and Germany were also included. All strains were grown in IMR ½ medium (Eppley et al. 1967), supplemented with 10 nM selenium (Edvardsen et al.1990, Imai et al. 1996). Stock cultures were grown at a salinity of 25 and kept at temperatures 4 and 12ºC, at an irradiance approximately 50 µmol photons $\cdot m^{-2} \cdot s^{-1}$ and a 14:10 light:dark (L:D) cycle.

Assay for estimating growth and loss rates. The main experiments were carried out essentially as described by Skjelbred et al. (2012) in 96-well culture plates with white walls and optical bottoms (Nunc AS, Denmark). Each strain was exposed to a 3-way factorial design with six temperatures (4 - 21ºC), five salinities (15, 20, 25, 30, 35), and eight irradiance levels (21.7 - 96.7 µmol photons $\cdot m^{-2} \cdot s^{-1}$; 14:10 L:D). Each treatment combination was performed in four replicates, giving a total of 960 experimental units per experiment. Additional experiments to study the low-light response of growth and maintenance were carried out for four of the seven strains (UIO109, UIO113, UIO115 and NIES670) in 24-well culture plates with black walls and optical glass bottom (Greiner Bio-one, Germany) at six different temperatures (4 - 20ºC), five salinities (15, 20, 25, 30, 35) and five light levels (5.3 - 29.5 µmol photons $\cdot m^{-2} \cdot s^{-1}$; 14:10 L:D). Each treatment combination was replicated times, giving 600 experimental units per experiment. The salinity of the medium was adjusted with distilled H_2O before nutrient addition and controlled with a refractometer (S/Mill, ATAGO CO Ltd, Japan). Each plate was covered by a transparent sheet printed with a gray-scale gradient made in Adobe Photoshop (Adobe, San José, CA, USA) and laminated. Illumination was provided by Fluora L 18 W / 77 (OSRAM, Germany). Different temperature treatments were kept in thermostat-controlled rooms and placed upon a water bath containing 5 L of water as a heat reservoir against external temperature fluctuations. Temperature stability was verified by continuous monitoring with temperature recorders (LogTag Recorders Ltd, Hong Kong). Inoculum cultures were kept in exponential growth under the same light source as in the experiment at a salinity of 25 and temperature of 4 or 12ºC. For three strains (UIO113, UIO114 and UIO115) inoculum cultures from both temperatures were used. Concentration of inoculum for the experiments was approximately 1000 cells $\cdot mL^{-1}$.

Growth trajectories in individual wells were monitored by in vivo fluorescence (IVF) as a proxy for chlorophyll concentration. While cellular chlorophyll content is known to vary

strongly with light adaptation, one should expect a direct proportionality between biomass and chlorophyll in cells adapted to the same growth conditions. In vivo fluorescence was determined daily at the same time of day using a microplate reader (FLx 800, BioTek, Inc. Vermont, USA) with excitation at 460 nm (40 nm bandwidth) and emission at 680 nm (30 nm bandwidth).

Calculations of specific growth and loss rates. Specific growth rate μ (d^{-1}) in each experimental unit was calculated as the slope of a linear regression for log-transformed in vivo fluorescence against time. Data from at least four consecutive days after d two were used in the analysis. All experiments were terminated before reaching stationary phase. This first level of data reduction gave a single specific growth rate estimate for each experimental unit. In the second level of data reduction, a light response curve was fitted to all pairs of specific growth rate (μ; d^{-1}) and irradiance (E; µmol photons $\cdot m^{-2} \cdot s^{-1}$) for each combination of temperature and salinity. The light response curve was based on a Poisson single-hit model (Dubinsky et al. 1986, Baumert 1996):

$$\mu = \mu_{\max}\left(1-\exp\left(-\frac{E-E_0}{E_k-E_0}\right)\right)$$

This model has three parameters (μ_{max} = the light-saturated maximal growth rate, E_0 = the compensation light level where growth becomes zero, and E_k = the saturating light level where the initial slope of the curve extrapolates to the asymptotic level), which are treated as random effects across the temperature and salinity treatment levels (Pinheiro and Bates 2009). Finally, the fitted values for the light response parameters μ_{max} and E_0 were analyzed as functions of temperature and salinity by generalized additive models (GAMs; Wood 2006). A GAM model was first fitted to show the overall temperature and salinity response pattern across all strains. GAM models for each strain were then used to estimate parameter values

and their associated standard errors at optimal growth conditions (maximal μ_{max} and minimal E_0). The locations of temperature and salinity optima were estimated by resampling with replacement within each strain/species and calculating the μ_{max} - or 1 / E_0 -weighted mean temperature and salinity of each bootstrap sample. This procedure yielded a cloud of bootstrapped optima which can be visualized as 95% confidence ellipses in temperature, salinity space. Loss rates were estimated as the negative of the net, specific growth rate extrapolated to zero light. The statistical computing environment R (www.r-project.org) was used for all analyses.

Comparison between IVF and cell counts. Subsamples for cell counting in a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) were fixed in 0.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) and 0.5% paraformaldehyde (Merck KGaA, Damstadt, Germany) final concentration, quickly frozen in liquid nitrogen, and stored at - 80ºC until analysis. TrueCount beads (Becton Dickinson, San Jose, CA, USA) and 0.5 µm latex beads (Polysciences Inc, USA) were used as internal standards for converting flow cytometry counts to absolute cell concentrations.

Results

Four strains of *Pseudochattonella* (UIO109, UIO113, UIO115 and NIES670) were incubated in both black and white microplates and thus exposed to 390 different combinations of light, salinity and temperature, while the remaining three strains (UIO114, UIO125 and JG8) were exposed to 240 different combinations in white microplates. All strains showed a positive growth rate response to light, especially at optimal temperature and salinity conditions, which saturated between 20 and 50 µmol photons $\cdot m^{-2} \cdot s^{-1}$. The strains of *P. farcimen* achieved positive, but low growth rates at the lowest temperature (4ºC), while none of these strains

grew at 20ºC. Both strains of *P. verruculosa* sustained positive growth at 20ºC. The strain JG8 failed to grow at 4ºC, except at 25. The strain NIES670 failed to grow at the lower temperatures (4 and 7ºC), except at 25.

Maximum growth rate estimates for *P. farcimen* were from 0.41 ± 0.04 d^{-1} (strain UIO109) to 0.52 ± 0.05 d^{-1} (strain UIO113; Fig. 1; Table 2). Corresponding estimates for the *P. verruculosa* strains were 0.51 ± 0.15 d^{-1} for JG8 and 0.61 ± 0.07 d^{-1} for NIES670. The optimal salinity and temperature conditions of the five strains of *P. farcimen* differed just slightly from each other with salinity optima in the range 19 - 25. However, the lower bound of the 95% confidence region for UIO113 went slightly lower (down to 18), while the upper bounds of UIO109 and UIO125 reached up to 26. Temperature optima for *P. farcimen* were generally in the range 10 - 14ºC, except for UIO125 and UIO113 where the confidence regions ranged 9 - 13ºC and 11 - 15ºC, respectively. The *P. verruculosa* strain JG8 had slightly higher salinity and temperature optima ranging from 20 - 27 and 12 - 16ºC. Salinity optima for NIES670 ranged from 24 - 32 and temperature optima from 14 - 20ºC.

Light compensation point estimates for all strains of *Pseudochattonella* were in the range from 4.2 - 14.9 µmol photons $\cdot m^{-2} \cdot s^{-1}$with salinity and temperature optima in the range 20 - 30 and 8 - 18 ºC (Fig. 2; Table 2). *P. farcimen* strains UIO109, UIO113 and UIO114 and *P. verruculosa* strain JG8 had the lowest compensation points (range 4.2 ± 1.2 to 6.9 ± 3.0 µmol photons $\cdot m^{-2} \cdot s^{-1}$), which were significantly different from the other strains. Somewhat higher light demands for positive net growth were observed for the remaining three strains, UIO115 (11.4 ± 2.9 µmol photons $\cdot m^{-2} \cdot s^{-1}$), NIES670 (13.6 ± 2.9 µmol photons $\cdot m^{-2} \cdot s^{-1}$) and UIO125 (14.9 ± 0.4 µmol photons $\cdot m^{-2} \cdot s^{-1}$). Saturation light level estimates for all strains of *Pseudochattonella* were in the range from 18.3 - 51.8 µmol photons $\cdot m^{-2} \cdot s^{-1}$ with salinity and temperature optima similar to those for compensation light. Saturation and compensation light levels were very closely correlated ($R^2 = 0.99$; Fig. 3; Table 2).

Lowest rates of maintenance metabolism (or loss rates), estimated as the negative of the net, specific growth rate extrapolated to zero light, were observed at intermediate salinities and increased with temperatures (Fig. 4). Loss rates accounted for 5.4 - 59.7 % of daytime production for the different environmental conditions.

Cell counts were measured by flow cytometry at the end of some of the growth experiments (Fig. 5). Some strains had noticeably higher (UIO109b) or lower (UIO115c) IVF per cell, but on the average IVF explained 85% of the cell count variance.

Discussion

The first bloom of *Pseudochattonella* recorded in North Europe in 1998 occurred at temperatures increasing from 8 to 13ºC during the bloom and with salinity varying from 17 - 30 (Backe-Hansen et al. 2001). Since then, *Pseudochattonella* has also formed blooms in very cold water (2 - 5ºC), in the Skagerrak and Kattegat (Edvardsen et al. 2007), indicating a realized niche at lower temperatures as well. Some cold water species have shown higher temperature optima in cultures compared to the environment in which they occurred (Russell 1990, Reay et al. 2001). This was also found in studies with snow algae (Hoham 1975), algal isolates from Antarctica (Seaburg et al. 1981) and diatoms (Fiala and Oriol 1990, Suzuki and Takahashi 1995), as well as for *P. farcimen* in this study. Ability to maintain positive net growth at lower temperatures may have several competitive advantages such as monopolizing available nutrient sources prior to the appearance of competing taxa with higher temperature requirements (Russell 1990, Reay et al. 2001, Beman et al. 2005) and reduced grazing pressure from herbivores by blooming before vernal emergence of zooplankton (Wiltshire et al. 2008). However, infectious parasites like chytrids and other fungi (Wei et al. 2010) seem to coexist with hosts throughout the growing season (Correa and Sánchez 1996), giving cold-adapted species no advantage. Parasites are dispersed as propagules (Gleason et al. 2008) and

might affect the succession of phytoplankton populations (VanDonk and Ringelberg 1983, Kagami et al. 2007).

Temperature optima for the *P. farcimen* strains were overlapping and only small differences were observed in this study. Significant differences were observed for temperature optima between *P. verruculosa* strain NIES670 and the strains of *P. farcimen*. Maximum temperature optima for strain NIES670 were in the range 14 - 20ºC in this study and 15 - 21ºC in the study by Yamaguchi et al. (1997). Strain JG8 showed intermediate temperature optima between NIES670 and *P. farcimen* strains, but this strain also achieved positive growth rates at 20 ºC. NIES670 failed to show positive growth rates at 4ºC in this study, although stock cultures could be maintained for several months at this temperature (B. Skjelbred, unpublished data). The distantly related *Dictyocha speculum* Ehrenberg reached maximum growth rates at 11 - 15ºC and salinity 20 - 30; no growth were observed at 20ºC in that study (Henriksen et al. 1993).

Changes in salinity affect organisms in three ways: 1) osmotic stress with direct impact on the cellular water potential; 2) ionic stress caused by the inevitable uptake or loss of ions (this is dealt with by the acclimation); 3) change in the cellular ionic ratios due to the selective ion permeability of the membrane (Kirst 1990). Only slight differences in salinity optima were observed for the strains of *P. farcimen* while he strains of *P. verruculosa* showed somewhat higher salinity optima than those of *P. farcimen*.

Phototaxis was observed for *Pseudochattonella* during 1998 cruise (Backe-Hansen et al. 2001) and this behaviour was also observed for stock cultures in Erlenmeyer flasks (B. Skjelbred, unpublished data). Growth rates increased linearly with light at low irradiance and levelled off asymptotically to saturation with increasing irradiance. Saturation irradiances estimate ranged from 18.3 - 51.8 µmol photons $\cdot m^{-2} \cdot s^{-1}$, which was similar to previous studies (Zondervan et al. 2002, Magaña and Villareal 2006, Aydin et al. 2009). The estimated

compensation points ranged from 4.2 - 14.9 µmol photons $\cdot m^{-2} \cdot s^{-1}$ and were also in accordance with previous work (Laws and Bannister 1980, Duarte and Ferreira 1995, Lewitus and Kana 1995, Magaña and Villareal 2006). It should be noted that the validity of these compensation point estimates apply to the spectral composition and photoperiod used in our assays, while they may be different under other environmental conditions (Hobson and Guest 1983). Our observations support the generalization by Kirst (1990) that growth primarily depends on light and temperature, with the tolerated salinity range becoming broader as these parameters approach optimality.

While respiration accounts for 30 - 50% of daytime primary production in plants, only 10% of light-saturated rates keep utilized for respiration in microalgae (Geider and Osborne 1989). In our study the loss rates accounted for 5.4 - 59.7% of daytime production for the different environmental conditions (e.g., salinity and temperature). For *Pseudochattonella* strains, loss rates increased with temperature, in accordance with previous results (Staehr and Sand-Jensen 2006). Lowest loss rates were observed at intermediate salinities, less energy was used for osmotic adjustment (Kirst 1990).

Blooms of *P. farcimen* have been recorded in nature under conditions similar to lowest loss rates rather than optimal growth rates in this study (Lu and Göbel 2000, Backe-Hansen et al. 2001, Edvardsen et al. 2007, Riisberg and Edvardsen 2008). Minimalizing loss rates are contributing to maintenance of the population, despite lower growth rates.

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References

Aydin, G.Ş., Kocataş, A. & Büyükişik, B. 2009. Effect of light and temperature on the growth rate of potentially harmful marine diatom: *Thalassiosira allenii* (Bacillariophyceae). *Afr. J. Biotechnol*. 8:4983-4990.

Backe-Hansen P., Dahl E. & Danielssen D. S. 2001. On the bloom of the *Chattonella* in the North Sea/Skagerrak in April-May 1998. In Hallegraeff, G. M., Blackburn, S. I., Bolch, C. J. S. & Lewis, R. (Eds.) *Harmful Algal Blooms 2000*. Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 78-81.

Baumert, H. 1996. On the theory of photosynthesis and growth in phytoplankton. 1: Light limitation and constant temperature. *Int. Revue Ges. Hydrobiol*. 81:109-139.

Beman, J. M., Arrigo, K. R. & Matson, P. A. 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *Nature* 434:211-214.

Berge, J., Andersen, T., Amundsen, R., Bjerkeng, B., Bjerknes, E., Gitmark, J., Gjøsæter, J., Johnsen, T., Lømsland, E., Magnusson, J., Nilsson, H., Paulsen, Ø., Rohrlack, T., Sørensen, J. & Walday, M. 2009. *Overvåking av forurensingssituasjonen i indre Oslofjord 2008*. Report, NIVA (In Norwegian) ISBN 978-82-577-5549-2, pp 144.

Correa, J. A. & Sánchez, P. A. 1996. Ecological aspects of algal infectious diseases. *Hydrobiologia* 326/327:89-95.

Dittami, S. M., Riisberg, I., John, U., Orr, R. J. S., Jakobsen, K. S. & Edvardsen, B. 2012. Analysis of expressed sequence tags from the marine microalgae *Pseudochattonella farcimen* (Dictyochophyceae). *Protist* 163:143-161.

Duarte, P. & Ferreira, J. G. 1995. Seasonal adaption and short-term metabolic responses of *Gelidium sesquipedale* to varying light and temperature. *Mar. Ecol. Prog. Ser.* 121:289-300.

Dubinsky, Z., Falkowski, P. G. & Wyman, K. 1986. Light harvesting and utilisation by phytoplankton. *Plant Cell Physiol.* 27:1335-1349

Edvardsen, B., Eikrem, W., Shalchian-Tabrizi, K., Riisberg, I., Johnsen, G., Naustvoll, L. & Throndsen, J. 2007. *Verrucophora farcimen* Gen. et sp. nov. (Dictyochophyceae, Heterokonta) – A bloom forming ichthyotoxic flagellate from Skagerrak, Norway. *J. Phycol.* 43:1054-1070.

Edvardsen, B., Moy, F. & Paasche, E. 1990. Hemolytic avtivity in extracts of *Chrysochromulina polylepis* at different levels of selenite and phosphate. *In* Granéli, E., Sundström, B., Edler, L. and Anderson, D. M. (Eds.) *Toxic Marine Phytoplankton*, Elsvier, New York, pp. 284-289.

Eikrem, W., Edvardsen, B. & Throndsen, J. 2009. Renaming *Verrucuphora farcimen* Eikrem, Edvardsen et Throndsen. *Phycol. Res*. 57:170.

Eppley, R. W., Holmes, R. W. & Strickland, J. D. H. 1967. Sinking rates of marine phytoplankton measured with a fluorometer. *J. Exp. Mar. Biol. Ecol*. 1:191-208.

Fiala, M. & Oriol, L. 1990. Light-temperature interactions of the growth of Antarctic diatoms. *Polar Biol.* 10:629-636.

Geider R. J. & Osborne B.A. 1989. Respiration and algal growth: a review of the quantitative relationship between dark respiration and growth. *New Phytol.* 112:327-341.

Gleason, F. H., Kagami, M., Lefevre, E. & Sime-Ngando, T. 2008. The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. *Fungal Biol. Rev*. 22:17-25.

Hallegraeff, G. M., Hara, Y. 2003. Taxonomy of harmful marine raphidophytes. *In* Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. (Eds.) *Manual on Harmful Marine Algae*, UNESCO, pp. 511-522.

Henriksen, P., Knipschildt, F., Moestrup, Ø. & Thomsen, H. A. 1993. Autecology, life history and toxicology of the silicoflagellate *Dictyocha speculum* (Silicoflagellata, Dictyochophyceae). *Phycologia* 32:29-39.

Hobson, L.A. & Guest, K.P. 1983. Values of net compensation irradiation and their dependence on photosynthesis efficiency and respiration in marine unicellular algae. *Mar. Biol.* 74:1-7.

Hoham, R. W. 1975. Optimum temperatures and temperature ranges for growth of snow algae. *Arctic Alpine Res*. 7:13-24.

Hosoi-Tanabe, S., Otake, I. & Sako, Y. 2006. Phylogenetic analysis of the red tide flagellates *Chattonella antique*, *C. marina*, *C. ovata* and *C. verruculosa* (Raphidophyceae) based on the rRNA gene family. *Fisheries Sci*. 72:1200-1208.

Hosoi-Tanabe, S., Honda, D., Fukaya, S., Otake, I., Inagaki, Y. & Sako, Y. 2007. Proposal of *Pseudochattonella verruculosa* gen. nov., comb. Nov. (Dictyochophyceae) for a former raphidophycean alga *Chattonella verruculosa*, based on 18S rDNA phylogeny and ultrastructural characteristics. *Phycol. Res*. 55:285-192.

Håkansson, B., Lindahl, O., Rosenberg, R., Axe, P., Eilola, K. & Karlson, B. 2007. Swedish national report on eutrophication status in the Katteat and the Skagerrak. *Reports Oceanography*, SMHI, No 36. pp. 103.

Imai, I., Yamaguchi, M. & Watanabe, M. 1998. Ecophysiology, life cycle and bloom dynamics of *Chattonella* in the Seto Inland Sea, Japan. *In* Anderson, D. M., Cembella, A. D. & Hallegraeff, G. M. (Eds.) *Physiological Ecology of Harmful Algae Blooms*. Springer, Berlin (Heidelberg/ New York), pp. 95-112.

Imai, I., Itakura, S., Matsuyama, Y. & Yamaguchi, M. 1996. Selenium requirement for growth of a novel red tide flagellate *Chattonella verruculosa* (Raphidophyceae) in culture. *Fisheries Sci*. 62:834-835.

Kagami, M., de Bruin, A., Ibelings, B. W., Van Donk, E. 2007. Parasitic chytrids: their effects on phytoplankton communities and food web dynamics. *Hydrobiologia* 578:113-129.

Karlson, B. & Andersson, L. 2003. The *Chattonella*-bloom in year 2001 and effects of high freshwater input from river Göta Älv to the Kattegat-Skagerrak area. *Reports Oceanography*, SMHI, No 32 pp. 44.

Kirst, G. O. 1990. Salinity tolerance of eukaryotic marine algae. *Annu. Rev. Plant. Phys*. 41:21-53.

Laws, E. A. & Bannister, T. T. 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr*. 25:457-473.

Lewitus, A. J. & Kana, T. M. 1995. Light respiration in six estuarine phytoplankton species: contrasts under photoautotrophic and mixotrophic growth conditions. *J. Phycol.* 31:754-761.

Łotocka, M. 2009. The first recorded bloom of *Pseudochattonella farcimen* (Dictyochophyceae, Heterokonta), (Riisberg I., 2008) in the Gulf of Gdansk. *Oceanologia* 51:139-143.

Lu, D. & Göbel, J. 2000. *Chattonella* sp. bloom in North Sea, Spring 2000. *Harmful Algae News* 21:10-11.

MacKenzie, L. A., Smith, K. F., Rhodes, L. L., Brown, A., Langi, V., Edgar, M., Lovell, G. & Preece, M. 2011. Mortalities of sea-cage salmon (*Oncorhynchus tshawytscha*) due to a bloom of *Pseudochattonella verruculosa* (Dictyochophyceae) in Queen Charlotte Sound, New Zealand. *Harmful Algae* 11:45-53.

Magaña, H. A. & Villareal, T. A. 2006. The effect of environmental factors on the growth rate of *Karenia brevis* (Davis) G. Hansen and Moestrup. *Harmful Algae* 5:192-198.

Naustvoll, L-J., Dahl, E., Danielssen, D., Aure, J., Skogen, M. & Budgell, P. 2002. Chattonella i Skagerrak – en ny trussel for oppdrettsnæringen. Report IMR (In Norwegian) pp 4.

Pinheiro J. C. & Bates D. M. 2002. Mixed Effects Models in S and S-Plus. Springer, Berlin pp. 528.

Reay, D. S., Priddle, J., Nedwell, D. B., Whitehouse, M. J., Ellis-Evans, J. C., Deubert, C. & Connelly, D. P. 2001. Regulation by low temperature of phytoplankton growth and nutrient uptake in the Southern Ocean. *Mar. Ecol. Prog. Ser.* 219:51-64.

Riisberg, I. & Edvardsen, B. 2008. Genetic variation in bloom-forming ichthyotoxic *Pseudochattonella* species (Dictyochophyceae, Heterokonta) using nuclear, mitochondrial and plastid DNA sequence data. *Eur. J. Phycol*. 43:413-422.

Russell, N. J. 1990. Cold adaption of microorganisms. *Phil. Trans. R. Soc. Lond. B*. 326:595-611.

Seaburg, K. G., Parker, B. C., Wharton jr., R. A. & Simmons jr., G. M. 1981. Temperature-growth responses of algal isolates from Antarctic oases. *J. Phycol.* 17:353-360.

Skjelbred, B., Edvardsen, B. & Andersen, T. 2012. A high-throughput method for measuring growth and respiration in microalgal cultures. *J. Appl. Phycol*. DOI 10.1007/s10811-012-9819-z.

Skjelbred, B., Horsberg, T. E., Tollefsen, K. E., Andersen, T. & Edvardsen, B. 2011. Toxicity of the ichthyotoxic marine flagellate *Pseudochattonella* (Dictyochophyceae, Heterokonta) assessed by six bioassays. *Harmful Algae* 10:144-154.

Skjelbred, B. & Naustvoll, L. 2006. Growth preferences and toxicity of *Chattonella* aff. *verruculosa* (Heterokontophyta). *In 12th International Conference on Harmful Algae, Programme and Abstracts, Copenhagen, Denmark, 4-8 September, 2006*. International Society for the Study of Harmful Algae and the Intergovernmental Oceanographic Commission, Paris 281-282.

Staehr, P. A. & Sand-Jensen, K. 2006. Seasonal changes in temperature and nutrient control of photosynthesis, respiration and growth of natural phytoplankton communities. *Freshwater Biol*. 51:249-262.

Suzuki, Y. & Takahashi, M. 1995. Growth responses of several diatom species isolated from various environments to temperature. *J. Phycol.* 31:880-888.

Van Donk, E. & Ringelberg, J. 1983. The effect of fungal parasitism on the succession of diatoms in Lake Maarssveen I (Netherlands). *Freshwater Biol*. 13:241-251.

Wei, L., Zhang, T., Tang, X. & Wang, B. 2010. Oomycetes and fungi: important parasites on marine algae. *Acta Oceanol. Sin.* 29:74-81.

Wiltshire, K. H., Malzahn, A. M., Wirtz, K., Greve, W., Manly, B. F. J. & Boersma, M. 2008. Resilience of North Sea phytoplankton spring bloom dynamics: An analysis of long-term data at Helgoland Roads. *Limnol. Oceanogr.* 53:1294-1302.

Wood, S. N. 2006. Generalized Additive Models: an introduction with R. CRC

Yamaguchi, M., Itakura, S., Nagasaki, K., Matsuyama, Y., Uchida, T. & Imai, I. 1997. Effects of temperature and salinity on the growth of the red tide flagellates *Heterocapsa circularisquama* (Dinophyceae) and *Chattonella verruculosa* (Raphidophyceae). *J. Plankton Res.* 19:1167-1174.

Zondervan, I., Rost, B. & Riebesell, U. 2002. Effect of CO2 concentration on the PIC/POC ratio in the coccolithophore *Emiliania huxleyi* grown under light-limiting conditions and different daylenghts. *J. Exp. Mar Biol. Ecol.* 272:55-70.

Figure Legends

Fig. 1. a) Contour lines for a generalized additive model of maximum growth rates as function of temperature and salinity, using data from all *Pseudochattonella* strains. b) Optimal conditions for light-saturated growth for each strain, expressed as 95% confidence regions calculated from the optima of 10000 bootstrap samples. *P. verruculosa* strain NIES670 has higher temperature optimum and somewhat higher salinity optimum than *P. farcimen* strains.

Fig. 2. a) Contour lines for a generalized additive model of compensation point as function of temperature and salinity, using compiled data from all *Pseudochattonella* strains (five *P. farcimen* and two *P. verruculosa* strains). b) Optimal conditions for light-use efficiency (i.e., minimal compensation point) for each strain, expressed as 95% confidence regions calculated from the optima of 10000 bootstrap samples.

Fig. 3. Relationship between saturation and compensation irradiances for all *Pseudochattonella* strains (five *P. farcimen* and two *P. verruculosa* strains) and temperature-salinity combinations; $R^2 = 0.99$.

Fig. 4. Contour lines for a generalized additive model of loss rates as function of temperature and salinity. Compiled data from four *Pseudochattonella* strains (three *P. farcimen* and one *P. verruculosa* strains); black microplates were used in these experiments.

Fig. 5. Comparison of in vivo fluorescence with cell counts measured by flow cytometry. Data from experiments in white microplates; R^2 is 0.85.

Table 1. Origin of the *Pseudochattonella* strains used in this study.

Species	Strain	Geographic origin	Date of isolation	Isolator
P. farcimen	UIO109	Skagerrak, Langesund	28.03.2001	Edvardsen B.
P. farcimen	UIO113	Skagerrak, Flødevigen	21.03.2001	Naustvoll L.
P. farcimen	UIO114	Skagerrak, Flødevigen	02.03.2006	Skjelbred B./ Riisberg I.
P. farcimen	UIO115	Skagerrak	30.01.2006	Skjelbred B./ Riisberg I.
P. farcimen	UIO125	Skagerrak, Flødevigen	09.03.2006	Skjelbred B.
P. verruculosa	JG8	Germany, North Sea	02.05.2000	Göbel J.
P. verruculosa	NIES670	Japan, Seto Inland Sea	16.07.1987	Yoshimatsu S.

Table 2. Maximum growth rates (μ_{max}; d^{-1}), compensation irradiances (E_0; µmol photons $\cdot m^{-2} \cdot s^{-1}$) and saturating irradiances (E_k; µmol photons $\cdot m^{-2} \cdot s^{-1}$) for five strains of *Pseudochattonella farcimen* and two strains of *P. verruculosa with* 2·SE corresponding to approximate ± 95% confidence intervals.

Strain	μ_{max}	2·SE	E_0	2·SE	E_k	2·SE
JG8	0.51	0.15	4.4	6.7	28.6	25.3
NIES670	0.61	0.07	13.6	2.9	51.8	5.7
UIO109	0.41	0.04	6.9	3.0	36.3	6.1
UIO113	0.52	0.05	4.2	1.2	34.1	2.4
UIO114	0.49	0.09	4.5	2.3	18.3	15.6
UIO115	0.48	0.05	11.4	2.9	51.0	7.7
UIO125	0.51	0.06	14.9	0.4	37.4	4.1