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# Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA

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**ABSTRACT:** Two sets of observations were made on the distribution of *Pseudo-nitzschia* taxa in a fjord in the San Juan Islands, Washington, USA. From May 21 to 31, 1996, we observed the spatio-temporal distribution of a dense bloom of *P. fraudulenta*. Microscopic observations of live material were compared to physical-optical water-column structure, currents and wind. At the start of the study, dense concentrations of *Pseudo-nitzschia* spp. were observed directly at the surface. Optical profiles indicated that most cells were concentrated in a thin layer at ~5 m depth, which appeared to be contiguous throughout the sound. Several days later, sustained winds forced a plume of lighter water over the surface of the sound, displacing the original water mass, with its entrained flora, to depth. The resulting near-bottom thin layer persisted for several days, and contained  $>10^6$  *Pseudo-nitzschia* spp. cells  $\Gamma^{-1}$ . Microscopic examination of live cells from the deep layer revealed that colonies were alive and motile. In 1996 and again in 1998, we observed *P. pseudodelicatissima* living within colonies of *Chaetoceros socialis*. Water-column thin layers, near-bottom thin layers and populations of *Pseudo-nitzschia* spp. within *C. socialis* colonies could easily escape detection by routine monitoring procedures, and may be a potential source of unexplained toxicity events.

**KEY WORDS:** *Pseudo-nitzschia* · *Chaetoceros socialis* · Thin layers · Physical forcing

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## INTRODUCTION

*Pseudo-nitzschia* H. Peragallo in H. & M. Peragallo is a cosmopolitan genus of marine planktonic diatoms comprising approximately 20 species (Hasle 1994, Hasle & Fryxell 1995, Hasle & Syvertsen 1996). Although sometimes ranked as a subgeneric section of *Nitzschia* Hassall, the recent trend has been to recognize these taxa at the generic level on the basis of ultrastructural and ecological features (Hasle 1994, Hasle et al. 1996).

The systematics and ecology of *Pseudo-nitzschia* spp. are of special interest, since several species produce the neurotoxin domoic acid (reviewed by Bates et al. 1998, Bates 2001). Filter-feeding shellfish and finfish (e.g. anchovies) retain the toxin, which is then passed along the food chain and can result in amnesic shellfish poisoning (ASP) or domoic acid poisoning (DAP) of humans, sea birds and marine mammals (Bates et al. 1998, Scholin et al. 2000). In addition to its impact on the ecosystem, ASP has resulted in both human death and permanent neurological impairment (Bates et al. 1989, Bird & Wright 1989). Therefore, since some *Pseudo-nitzschia* species can cause harmful algal

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blooms (HABs), there is particular interest in understanding the population dynamics of both the genus as a whole, and of its individual species.

In order to develop numerical models which forecast blooms of *Pseudo-nitzschia* spp., and predict their toxicity, risk to public health, as well as economic and ecosystem impacts, we need to understand the factors that control species-specific distributions of phytoplankton in space and time. To date, numerous autecological studies have been conducted on *Pseudo-nitzschia* spp., summarizing specific responses to nutrients, temperature and salinity. However, very few studies have investigated the effects of light levels on growth dynamics (reviewed by Bates 1998 and Bates et al. 1998). Field studies have attempted to correlate the presence of blooms to regional conditions (e.g. prolonged dry sunny periods followed by rain), upwelling events, and global climatic patterns (e.g. El Niño Southern Oscillation). Many plausible scenarios have been proposed, yet despite this large body of information, there seems to have been little real progress in establishing clear patterns of the environmental conditions that will lead to a bloom, or in consistently identifying the source, or seed population which initiates a bloom.

Additionally, there have been numerous domoic acid events for which the source of the toxin could not be found. Horner et al. (1997) and Bates et al. (1998) reviewed reports of contaminated razor clams and Dungeness crabs from Pacific Northwest waters, sea scallops from Georges/Browns Bank in the North Atlantic, and cultured mussels from Newfoundland. In each case, the source of domoic acid was not determined. Since *Pseudo-nitzschia* spp. form needle-like cells approximately 50 to 150  $\mu\text{m}$  in length, with colonies sometimes exceeding 1 cm, they are an obvious member of the net phytoplankton, unlikely to be overlooked. Blooms must reach substantial concentrations ( $>10^5$  cells  $\text{l}^{-1}$ ) before shellfish accumulate enough toxin to result in closure of a fishery. Therefore, a *Pseudo-nitzschia* spp. bloom should be easy to detect by microscopic examination of the net-plankton flora. That it was not observed in the above cases implies that other kinds of organisms can produce domoic acid, that the ecology of *Pseudo-nitzschia* species may be more complex than predicted by autecological studies, or that the sampling strategy failed to locate the cells within the water column.

In addition to factors which regulate cell numbers or density of a bloom (in growth and loss terms), several other kinds of information may be important in understanding phytoplankton ecology and the dynamics of HABs at the species-specific level. Examples include behavior of the organism, the role of life-history stages, large and small-scale biological-physical interactions,

and the relative location of the population of toxic algae and the 'target' organism (e.g. filter-feeding fishes and shellfish) within the water column (Donaghay & Osborn 1997). Formation of thin layers of harmful algae — structures only centimeters to a few meters in vertical extent, with horizontal scales on the order of kilometers (Bjørnsen & Nielsen 1991, Donaghay et al. 1992, Cowles & Donaghay 1998, Dekshenieks et al. 2001) — may also be important, because the dynamics and impacts of a HAB population concentrated into a thin layer may be very different from that of a population spread throughout the water column.

We had the opportunity to make observations on thin layers of *Pseudo-nitzschia fraudulenta* (Cleve) Hasle and on behavioral/life history aspects of *P. pseudodelicatissima* (Hasle) Hasle during an ongoing oceanographic investigation on the coupling of physical, optical and biological processes in coastal waters. This study took place in East Sound, a small fjord in the San Juan Islands, in the southern part of the Strait of Georgia on the US-Canadian border. The genus *Pseudo-nitzschia* is common in East Sound, as it is throughout the Pacific Northwest region (Gran & Angst 1931, Forbes & Denman 1991, Taylor et al. 1994, Taylor & Haigh 1996, Horner et al. 1997, Trainer et al. 1998a).

## MATERIALS AND METHODS

**Field site.** East Sound is a fjord on the coast of Orcas Island, Washington, USA (Fig. 1). It is oriented NW to SE, and is ~12 km long and 1 to 2.5 km wide. For most of its extent, it is ~30 m deep, with a tidal range from 0.3 to 3.5 m. Circulation with contiguous waters is partially obstructed by a sill, which extends approximately half way across the western side at the mouth. Circulation within East Sound is driven by local winds, tides and bathymetry (Rattray 1964). It is also strongly influenced by regional-scale physical forcing events in the southern part of the Strait of Georgia, which affect the distribution of water mass types throughout the San Juan archipelago (Thomson 1981). Since Orcas Island has no major rivers, the primary source of freshwater to East Sound is the Fraser River, located ~23 km to the north, on the Canadian mainland. In early summer, after the mountain snows have melted, river outflow is at its maximum (Thomson 1981). During periods of strong winds from the north, the Fraser River plume is advected southward and can reach the mouth of East Sound. The warmer, fresher water from the Strait of Georgia is less dense and overrides the higher salinity water already present in East Sound, displacing it to depth (Twardowski & Donaghay 2001). This sequence of events is a common occurrence in this system.

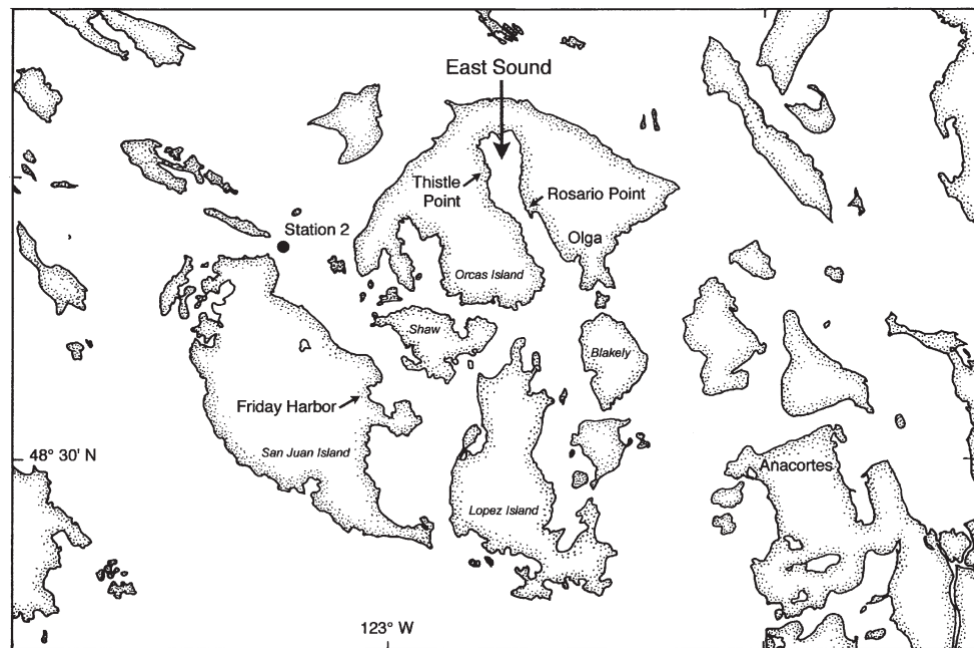


Fig. 1. Map of the San Juan Islands, with detail of East Sound. (d) Stn 2 in the Speiden Channel (from Gran & Thompson 1930)

The first field study took place from May 21 to June 1, 1996; additional observations were made in May and June 1998.

**Phytoplankton sampling.** The phytoplankton was sampled in 2 years.

**May and June 1996:** On May 21 and 23, dense concentrations of phytoplankton were visible at the surface near the mouth of the sound, and were collected by bucket. On May 25, 26 and 27, samples integrating the upper 10 m of the water column were collected via vertical net tows (64  $\mu\text{m}$  mesh). On May 28 and 30 and June 1, samples were collected from discrete depths between 0 and 30 m utilizing a siphon tube attached to the high-resolution profiling system, as detailed by Donaghay et al. (1992). All samples were immediately examined live, aboard ship, with phase-contrast microscopy. Archival images of each live sample were recorded on videotape. Whole water samples from May 28 and 30 and June 1 were preserved with formalin for subsequent enumeration of *Pseudo-nitzschia* spp. cells from inside and outside optical thin layers (described below). One ml aliquots were counted in a Sedgwick-Rafter chamber.

**May and June 1998:** Observations on the association between *Chaetoceros socialis* and *Pseudo-nitzschia pseudodelicatissima* were made in the field over the course of several weeks, using exclusively live material collected by surface bucket or subsurface siphon. Upon collection, all samples were immediately examined live, aboard ship, using phase contrast microscopy. Archival images of each live sample were recorded on 35 mm film and videotape, documenting

the association in live material throughout the period of study. Additionally, whole-water samples were preserved with formalin and returned to the laboratory. *C. socialis* and *P. pseudodelicatissima* cells were subsequently enumerated in a Sedgwick-Rafter chamber.

**Permanent slides and electron microscopy.** Net tows from May 25, 1996, and June 24, 1998, were prepared for species-level identification of *Pseudo-nitzschia*. Material for permanent slides was desalinated by dialysis through 15  $\mu\text{m}$  Nitex, dried on coverslips, heat-oxidized and mounted in Hyrax. Material for electron microscopy was desalinated, oxidized in  $\text{KMnO}_4$ , cleared with HCl (Simonsen 1974), rinsed in sterile, filtered, deionized water, and mounted on Formvar-coated grids. They were examined on a JEOL 1200EX STEM. Identifications were based on morphometric data and taxonomic descriptions in Hasle & Syvertsen (1996) and Hasle et al. (1996).

**Winds and tides.** Wind velocity and tidal height in East Sound were recorded for the duration of the study (Fig. 2). Wind speed and direction were recorded at a weather station (Davis Instruments) located on Thistle Point (Fig. 1). Tides were measured near Rosario Point with an Endeco Model 1029 tide gauge.

**Fine-scale physical, chemical and optical structure.** The physical, chemical and optical structure of the water column were simultaneously profiled at centimeter scales. Physical and chemical structure were measured by a Seabird SBE 911-plus CTD equipped with a ducted flow conductivity cell, a fast-response thermistor, a combination pH/eH sensor, an oxygen



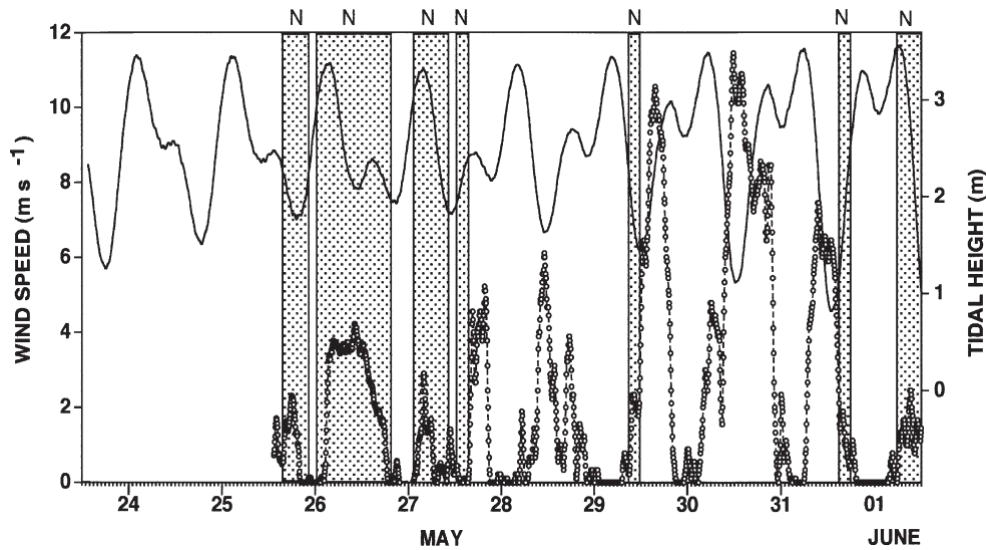


Fig. 2. Wind speed and direction and tidal height in East Sound for study period. (—○—) Average wind speed (5 min averages); stippling: periods of north winds; line: tidal height

sensor, and a Digiquartz pressure sensor capable of measuring depth changes of 1 cm. The optical structure was measured with WET Labs (Philomath, OR) absorption and attenuation meters (an ac-9 and aa-9). An ac-9 measures spectral absorption and attenuation at 9 wavelengths between 412 and 750 nm. The aa-9 was a modified ac-9, with the normal attenuation channel replaced by a second absorption channel. Using the methods described by Twardowski et al. (1999) and Twardowski & Donaghay (2001), the spectral absorption by dissolved substances was measured with an aa-9 equipped with a 0.2  $\mu\text{m}$  filter. At the same time, total spectral absorption and attenuation by dissolved and particulate material was measured with the ac-9. Spectral absorption by particulate material was then calculated as the difference between the two. Particulate absorption at 440 nm ( $a_p$  440), a primary wavelength of absorption by chlorophyll *a*, was used as an indicator of phytoplankton biomass. Vertical profiles of photosynthetically available radiation (PAR) were obtained with a Biospherical Instruments (San Diego, CA) PUV-500. Current magnitude and direction were continuously measured at 50 cm resolution with a downward-looking RDI 1200 kHz Acoustic Doppler Current Profiler (ADCP). Reported current velocities are based on 10 min averages of the ADCP data, which were taken at the same time as each of the profiles.

## OBSERVATIONS

### Taxonomy

In 1996, 3 species of *Pseudo-nitzschia* were identified from East Sound. Two occurred as planktonic colonies living freely within the water column (Fig. 3a),

and the third upon/within the spherical colonies of *Chaetoceros socialis* Lauder (Fig. 3b). In 1998, the *C. socialis*–*Pseudo-nitzschia* sp. association was observed again, this time in greater abundance, and persisting over the course of several weeks. Both single cells and colonies were seen within the *C. socialis* matrix. Using electron microscopy, the dominant taxon in 1996 material was identified as *P. fraudulenta* (Cleve) Hasle (Fig. 4a); small amounts of *P. pungens* (Grunow ex Cleve) Hasle were also present (Fig. 4b). The taxon living in association with *C. socialis* was identified as *P. pseudodelicatissima* (Hasle) Hasle (Fig. 4c,d).

### Thin layers of *Pseudo-nitzschia fraudulenta*

The 1996 *Pseudo-nitzschia fraudulenta* bloom was observed over a period of 12 d in conjunction with physically-forced hydrographic events in East Sound. At the start of the field study on May 21, weather conditions were sunny and very calm. Dense concentrations of phytoplankton were present at the sea surface near the sill. Ship-board microscopic examination of live material showed the sample to be dominated by needle-like colonies of *Pseudo-nitzschia* spp., *Rhizosolenia hebetata* forma *semispina* (Hensen) Gran and *R. pungens* Cleve-Euler. Also present were various *Chaetoceros* species, *Odontella longicruris* (Greville) Hoban, *Eucampia zodiacus* Ehrenberg, *Asterionellopsis glacialis* (Castracane) Round, *Alexandrium catenella* (Whedon & Kofoid) Balech and *Protoperdinium* spp. The weather pattern continued, and on May 23 the bloom was still present near the sill. Massive numbers of *Pseudo-nitzschia* spp. colonies >1 cm in length were visible directly at the surface.

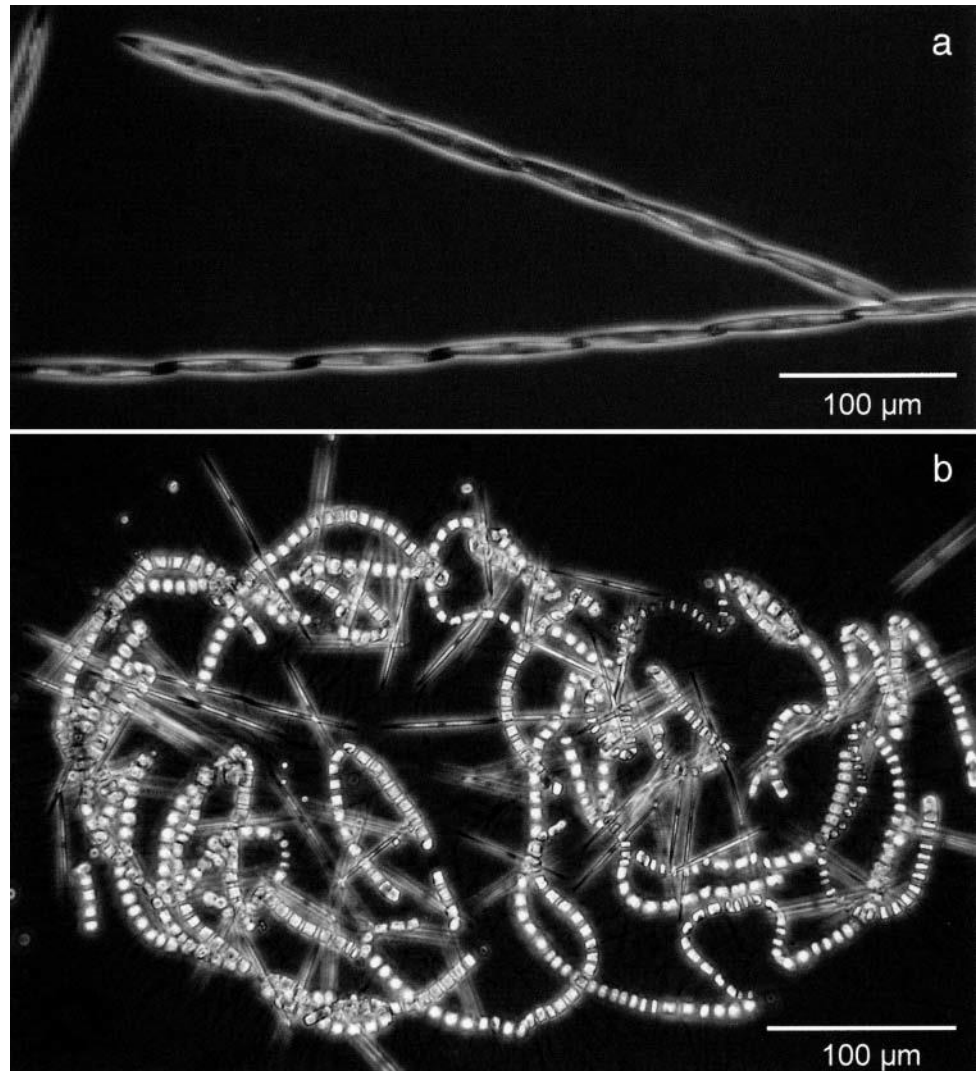


Fig. 3. Light micrographs (phase-contrast) of live diatom material. (a) *Pseudo-nitzschia fraudulenta*, showing stepped mode of colony formation. (b) *P. pseudodelicatissima*, showing single cells and short chains living within and upon a colony of *Chaetoceros socialis*, photographed in the field; this is a relatively small colony of *C. socialis*, collected early in the bloom; all cells of both species are highly pigmented and the assemblage is free of debris and other detritus

Profiles of physical and optical structure (Fig. 5) (which start about 1 m below the surface) showed that concentrations of phytoplankton (inferred from  $a_{p440}$  measurements) were not only high near the surface (1 to 4 m), but increased by a factor of 4 in a thin layer located near the bottom of the primary pycnocline at about 5 m depth (Fig. 5j). Physical and optical profiles at 4 additional stations along the longitudinal axis of the sound (Fig. 5k) indicated that a similar thin layer was located near the base of the primary pycnocline throughout the sound (Fig. 5f,i). Although  $a_{p440}$  at the surface declined by a factor of 2 along the axis of the sound (compare Fig. 5j,f), the maximum  $a_{p440}$  increased by more than 50% within the thin layer, reaching peak values of  $6.5 \text{ m}^{-1}$  (Fig. 5f) in the upper sound. This was more than 12 times the surface absorption at this northern sound station, and 6 times greater than near-surface absorption at the mouth of the sound.

These values were some of the highest we have ever observed in coastal waters, and they suggest that intense concentrations of phytoplankton were present.

On May 25, physical-optical profiles were taken at 9 stations, transecting north to south along the axis of the sound, and ending outside the sill in the channel between Orcas, Blakely, Lopez and Shaw Islands (Fig. 6). Temperature and salinity data indicated that stratification extended almost to the surface at all stations south of Rosario Point (mid-sound), with a gradual warming and freshening of surface waters along this segment of the transect (Fig. 6d–i). However, north of Rosario Point, surface waters were relatively cooler and saltier, and the surface mixed layer thickened to nearly 12 m at the northernmost station in the sound. Optical profiles at each station showed a low ( $a_{p440} \sim 0.5 \text{ m}^{-1}$ ), broad peak in particulate absorption outside the sound (Fig. 6r) that became thinner and

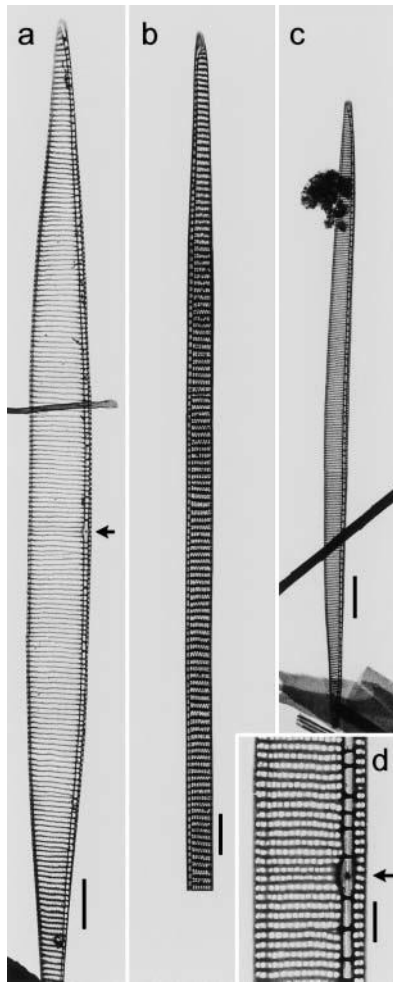


Fig. 4. Transmission electron micrographs. (a) *Pseudo-nitzschia fraudulenta*; characteristics: central interspace present (arrow), transapical axis 6  $\mu\text{m}$ , apical axis  $\sim 110 \mu\text{m}$ , 21 striae and 19 fibulae within 10  $\mu\text{m}$ , 2 rows of pores. (b) *P. pungens*; characteristics: central interspace absent, transapical axis 3.5  $\mu\text{m}$ , 11 striae and 12 fibulae within 10  $\mu\text{m}$ , 2 rows of pores. (c,d) *Pseudodelicatissima*; characteristics: central interspace present (arrow), transapical axis  $\sim 3 \mu\text{m}$ , apical axis  $\sim 80 \mu\text{m}$ , 34 to 40 striae and 14 to 16 fibulae in 10  $\mu\text{m}$ , single row of pores. Not revealed by Electron microscopy: overlap of cells in chains 1/9 to 1/10 of cell length. Scale bars in (a) (b) (c) = 5  $\mu\text{m}$ , (d) = 1  $\mu\text{m}$

more concentrated progressing northward along the axis of the sound, reaching maximum values 20-fold higher at mid-sound ( $a_{p440} \sim 4.0 \text{ m}^{-1}$ ; Fig. 6m). At the northern end, peak absorption values decreased, and the peak broadened (Fig. 6j–l). Collectively, this data suggests that a strong bloom was still in progress along the entire axis of the sound.

Vertical net tows integrating the top 10 m of the water column at each station (encompassing most subsurface layers) showed that a similar flora was present in the upper 10 m throughout the sound, comprising *Pseudo-nitzschia* spp., *Rhizosolenia hebetata* forma

*semispina*, *R. pungens*, *Odontella longicruris* and *Thalassionema nitzschioides* (Grunow) Grunow ex Hustedt. By cell number, *Pseudo-nitzschia* spp. accounted for  $\sim 50$  to 55% of the net phytoplankton at the northern stations,  $\sim 40\%$  in the mid-sound, declining to  $\sim 25\%$  near the sill. This pattern of dominance of *Pseudo-nitzschia* spp. in the integrated tows strongly supports the hypothesis that the thin layer at the base of the pycnocline was a subsurface bloom dominated by this taxon.

On May 26, a 5  $\text{m s}^{-1}$  wind developed from the north, with gusts reaching 9 to 12  $\text{m s}^{-1}$ . Winds were sustained for approximately 16 h (Fig. 2), and triggered the advection of the Fraser River buoyant plume into East Sound. On May 27, salinity at the mouth of the sound had dropped from 29.2 to 27.0 psu. A thick lens of lower-salinity water extended from the mouth to Rosario Point. It thinned considerably toward the more northern part of the sound, indicating that a new, low-salinity water mass was flowing inward at the surface. Velocity profiles confirmed this, showing that surface waters were moving into the sound at 35  $\text{cm s}^{-1}$ , and deep waters (15 to 25 m) were moving out of the sound at 15 to 20  $\text{cm s}^{-1}$ . Optical profiles showed a dramatic drop in particulate absorption at the surface, with most  $a_{p440}$  values being less than 1  $\text{m}^{-1}$ . Although thin layers were still evident at some stations near mid-sound (Fig. 7c,d), their absorption signals were reduced, with peak values only 1.5 to 2.9 times greater than the particulate absorption of the surrounding water. The patterns of large-scale spatial coherence seen on May 23 (Fig. 5) and May 25 (Fig. 6) had clearly disappeared on May 27 (Fig. 7).

Representative profiles from each day of sampling were used to evaluate the sequential changes in salinity and optical structure from May 23 to 31 (Fig. 8). On May 28, profiles from the northern part of the sound (Fig. 8e) revealed the presence of a broad  $a_{p440}$  maximum at mid-depths, and a thin layer of  $a_{p440}$  at 24 to 24.5 m, near the base of the primary pycnocline (Fig. 8e). Prior to this date, near-bottom thin layers had not been observed. Water siphoned from within the deep layer (24 to 24.5 m) was immediately examined live, under the microscope. The  $> 20 \mu\text{m}$  phytoplankton was dominated by long, needle-like colonies of *Pseudo-nitzschia fraudulenta*, which appeared both healthy and free of surface bacteria. There were very few empty frustules, and many colonies were motile. They were not aggregated into flocs of marine snow. Subsequent enumeration of a preserved sample yielded a density of  $2 \times 10^6$  *Pseudo-nitzschia* spp. cells  $\text{l}^{-1}$ , comprising 86% of the  $> 20 \mu\text{m}$  phytoplankton by number. Other taxa present at this depth included *Rhizosolenia hebetata* forma *semispina*, *Thalassionema nitzschioides*, *Odontella longicruris* and *Asterionellopsis glacialis*,



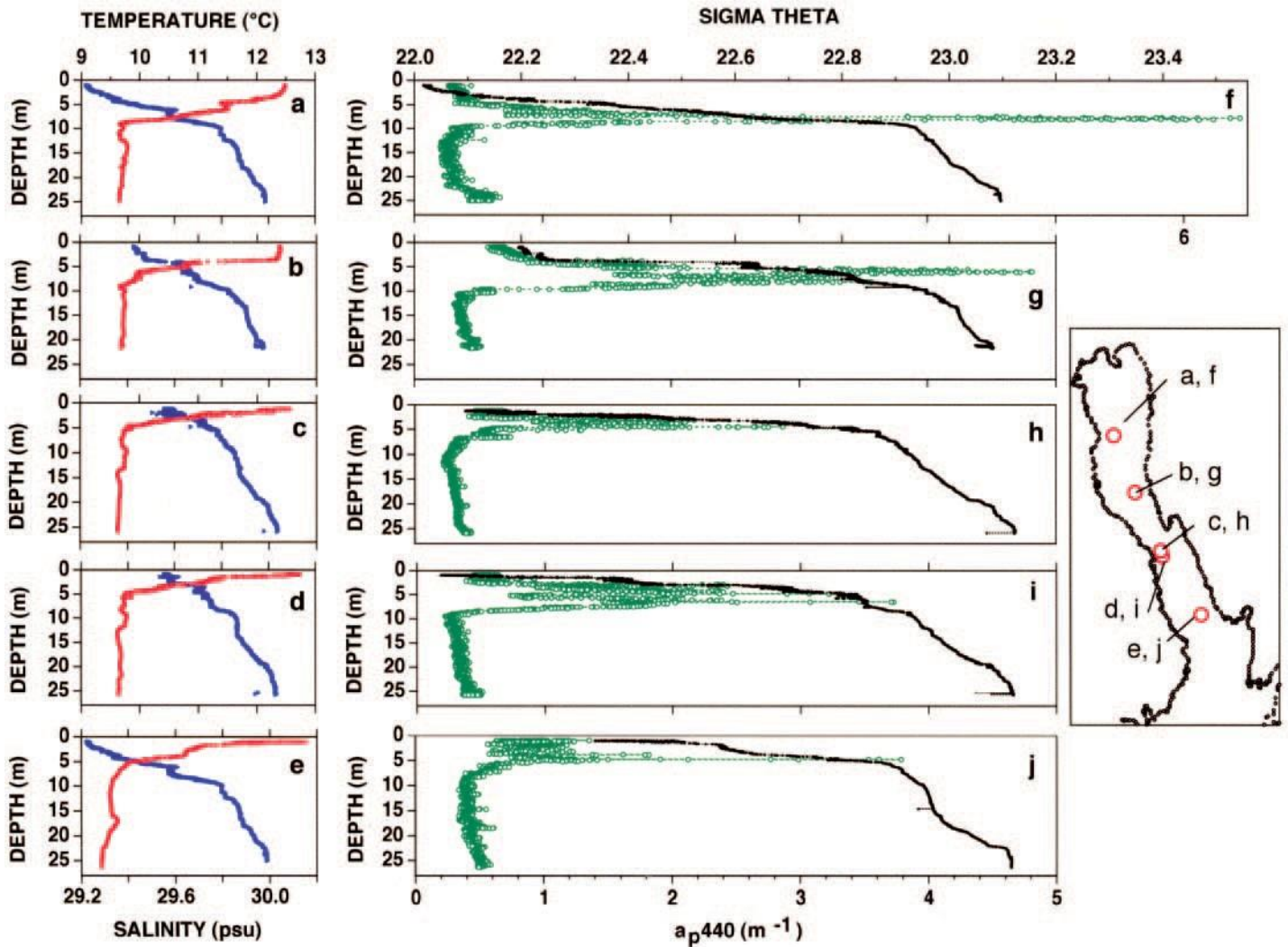


Fig. 5. Initial physical and optical structure in East Sound on May 23, 1996. (a–e) Temperature (red) and salinity (blue) profiles; (f–j) particulate absorption,  $a_{p440}$  (green) and sigma theta (black) profiles. Inset map shows stations where samples were collected

but unlike the *Pseudo-nitzschia* spp., these appeared to be in poor health. At a depth of 20.5 to 21 m, just above the layer, concentrations dropped to  $2 \times 10^5$  cells  $l^{-1}$  and the relative dominance of *Pseudo-nitzschia* spp. declined to 63% of the cell count.

A time series of physical and optical profiles collected on May 29 revealed a similar near bottom phytoplankton layer at a mid-sound station (represented by Fig. 8f). Particulate absorption ( $a_{p440}$ ) in this layer was lower than at the upper-sound station on May 28 ( $0.75$  vs  $1.5$   $m^{-1}$ ), but was also reduced proportionally at shallower depths ( $a_{p440}$  values just above the thin layer dropped from  $0.5$  to  $0.2$   $m^{-1}$ ). Therefore, this layer remained the dominant fine-scale feature of the water column. It was detected in all 7 profiles collected at this station over a 6 h period, and was always located near the base of the primary pycnocline.

The near-bottom thin layer was still present 2 d later (May 31), with only a slight decline in  $a_{p440}$ , from  $0.78$  to  $0.68$   $m^{-1}$  (Fig. 8g). Siphon samples taken from within the layer indicated that it was still dominated by *Pseudo-nitzschia* spp., with concentrations at 26.9 m reaching  $8 \times 10^5$  cells  $l^{-1}$  (94% of  $> 20$   $\mu m$  phytoplankton). Colonies were very long (30+ cells) and appeared free of surface bacteria or other detritus. Colonies were separate from each other, and were not tangled into aggregates. There were very few dead (empty) cells.

On May 31, a second thin layer was detected at 3 to 4 m at the mid-sound station (Fig. 8g). It persisted throughout the 24 h sampling period. Phytoplankton siphoned from within this layer was dominated by *Odontella longicruris*, *Thalassiosira* spp., *Thalassionema nitzschiioides* and *Asterionellopsis glacialis*. *Pseudo-nitzschia* spp. concentrations in samples from

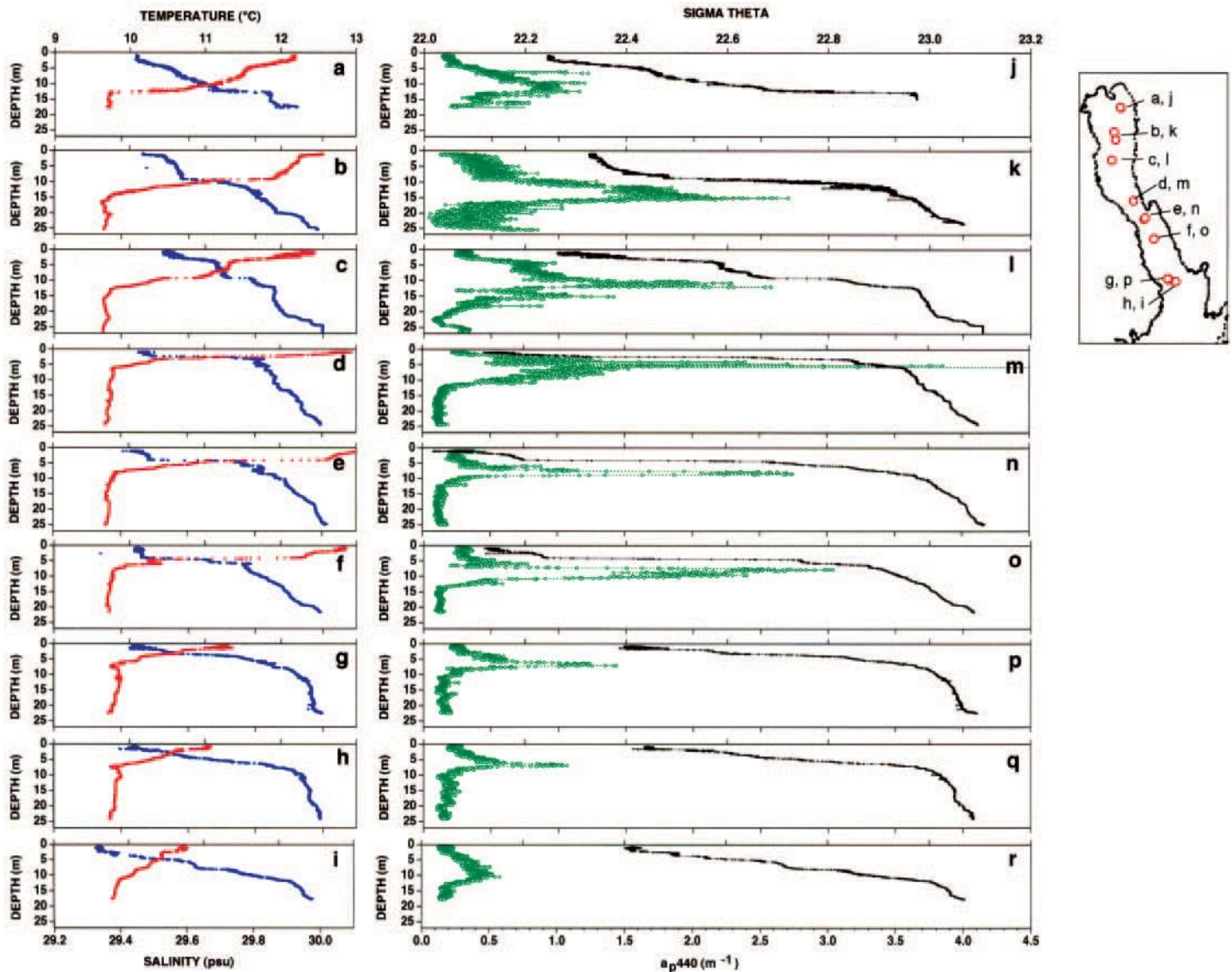


Fig. 6. Physical and optical structure in East Sound on May 25, 1996, prior to arrival of Fraser River plume. (a–i) temperature (red) and salinity (blue) profiles; (j–r)  $a_{p440}$  nm (green) and sigma theta (black) profiles. (Stns q and r were outside the sound)

the upper water column (2.5, 4.3, 5.2 and 7.5 m) ranged from 0 to  $5 \times 10^4$  cells  $l^{-1}$ , comprising only 0 to 7% of the  $>20 \mu m$  phytoplankton population. Thus, the thin layer situated at 3 to 4 m had a very different composition and history from the near-bottom layer (see Twardowski & Donaghay 2001 for discussion of the evolution of the 3 to 4 m layer).

#### Biological-physical interactions

The effect of regional-scale physical forcing events on the vertical distribution of *Pseudo-nitzschia* spp. in East Sound was examined by comparing the salinity structure of the water column to the location of thin

layers of high particulate absorption on each of the 7 d of profiles (Fig. 8). From May 23 to 25, before the buoyant plume reached East Sound, thin layers were observed in the upper water column in a water mass with a salinity of  $\sim 29.8$  psu (Fig. 8a–c). This relationship was consistent along the axis of the sound, suggesting that a single, coherent layer was present throughout the area. No near-bottom layers were observed during this period (Fig. 8a–c).

When the Fraser River plume first began to flow across the surface of the sound on May 27, it displaced the  $\sim 29.8$  salinity surface (with the entrained phytoplankton) downward to  $\sim 13$  m (Fig. 8d). Near-bottom layers were found only after a thick lens of plume water covered much of East Sound. Like the thin layer

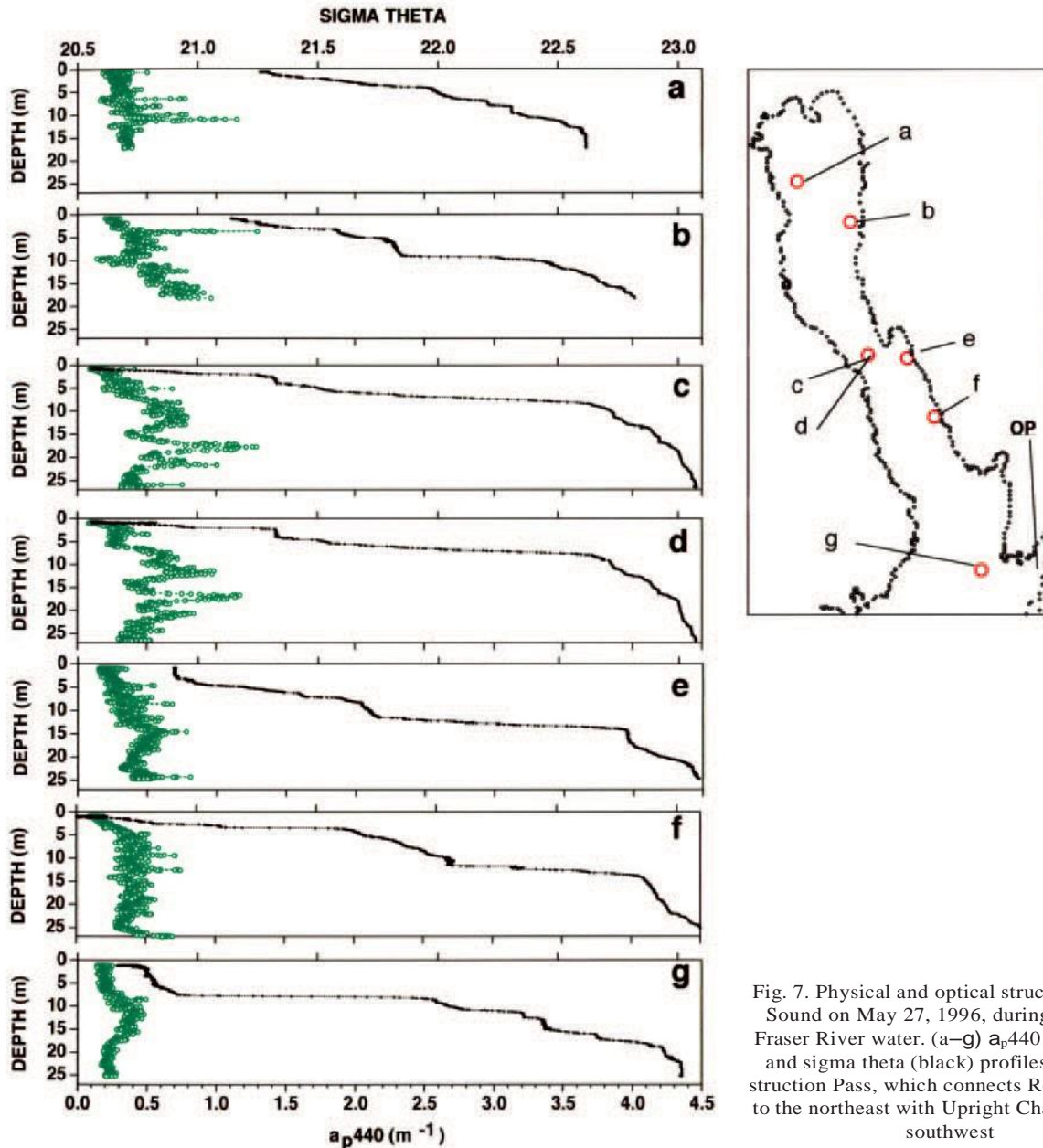


Fig. 7. Physical and optical structure in East Sound on May 27, 1996, during influx of Fraser River water. (a–g)  $a_{p440}$  nm (green) and sigma theta (black) profiles. OP: Obstruction Pass, which connects Rosario Strait to the northeast with Upright Channel to the southwest

of the upper water column, the near-bottom, *Pseudo-nitzschia*-dominated thin layer observed on May 28, 29 and 31 was also located in water with a salinity of  $\sim 29.8$  psu (Fig. 8e–g). In contrast, the upper water-column layer observed on May 31 was located in Fraser River plume water, identified by its different salinity signature of 27.4 psu (Fig. 8g).

#### *Chaetoceros socialis*–*Pseudo-nitzschia pseudodelicatissima* association

In 1996, spherical colonies of *Chaetoceros socialis* were first observed with *Pseudo-nitzschia pseudodeli-*

*catissima* living upon/within them. In May and June of 1998, *C. socialis* bloomed in East Sound, reaching densities of  $\sim 8 \times 10^6$  cells  $l^{-1}$ . Careful examination of live, gently collected material showed that in many samples, virtually 100% of the *C. socialis* colonies were colonized by *P. pseudodelicatissima*, (Fig. 3b), occurring both as single cells, and as short chains of up to about 5 cells. The *C. socialis*–*P. pseudodelicatissima* associations were generally free of entrained debris, other phytoplankton and heterotrophic flagellates (Fig. 3b), and were not aggregated into flocs of marine snow. Different sizes of *P. pseudodelicatissima* cells were noted (apical length of 48 to 102  $\mu m$ ). The association persisted throughout the 3 wk we spent in the



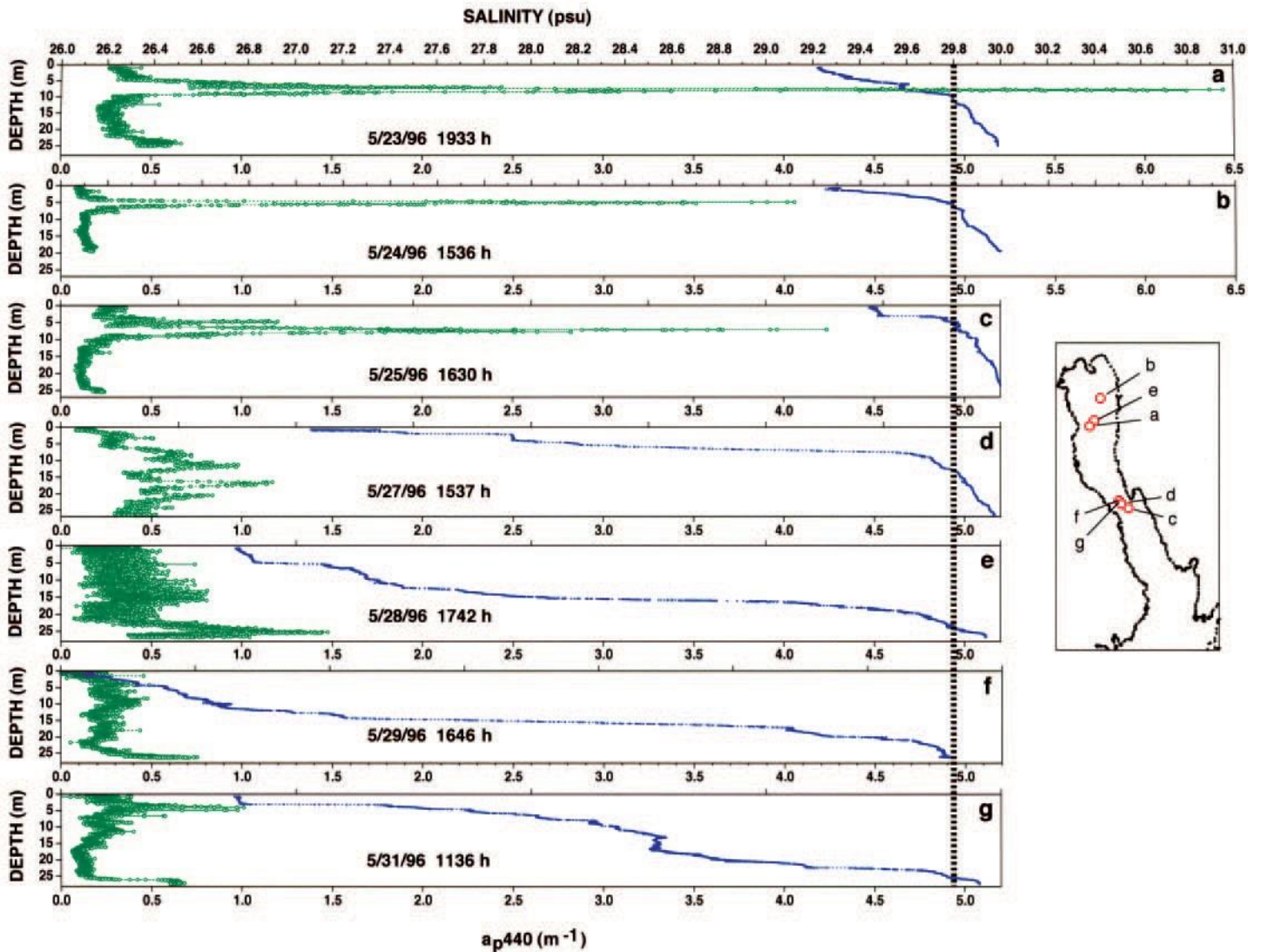


Fig. 8. Salinity structure associated with thin optical layers in East Sound, May 23 to 31, 1996. (a–g) Individual profiles of salinity (blue) and  $a_{p440}$  (green); each panel is labeled with date (mo/d/yr) and time of profile. Vertical dashed line traces 29.8 psu across all graphs

field. Only near the end of our study (mid-June) was *P. pseudodelicatissima* occasionally observed living freely in the water column, independent of *C. socialis*. Concentrations of *P. pseudodelicatissima* reached  $\sim 6 \times 10^4$  cells  $l^{-1}$ .

## DISCUSSION

A major characteristic of both the *Pseudo-nitzschia fraudulenta* and *P. pseudodelicatissima* blooms in East Sound is that each had highly non-homogeneous patterns of distribution which resulted in 'hidden' (*P. fraudulenta*), or 'camouflaged' (*P. pseudodelicatissima*) populations that could easily have been missed by routine monitoring techniques. In the first case their loca-

tion within the water column was closely tied to physical oceanographic processes, and in the second to the distribution of *Chaetoceros socialis* colonies. Further consideration of these issues can contribute to an increased understanding of the ecology of *Pseudo-nitzschia* spp.

### Critical-scale sampling

In order to conduct field studies on the dynamics of a taxon, one has to be able to effectively locate the population of interest within the water column. To do so, samples must be collected at a critical scale, where sampling resolution is matched to the scale of the structure. If the vertical distribution of a taxon spans

many meters, it can be detected by sampling at a fairly coarse resolution (e.g. every 5 or 10 m). However, when the majority of cells are concentrated into a thin layer, they are likely to escape detection because they are distributed at a finer scale than that of most traditional sampling grids (Donaghay et al. 1992). The thin layers of *Pseudo-nitzschia fraudulenta* observed in East Sound had half-heights of  $\leq 0.7$  m. Had we relied on bottle samples to detect this structure and to resolve the maximum concentration of cells at its peak, it would have required sampling at 0.2 m intervals, for a total of 600 samples for a single 30 m profile! We were able to detect the upper water column and near-bottom thin layers of *P. fraudulenta* in East Sound, and estimate their horizontal extent using a combination of high-resolution physical-optical profiles and microscopic examination of discrete samples collected from within the optically detected thin layers. If *Pseudo-nitzschia* spp. form similar thin layers in other coastal systems, it is not surprising that conventional bottle sampling does not reliably result in a correlation between the observed distribution of *Pseudo-nitzschia* spp. and the toxicity of fishes and shellfish.

### Physical forcing

The near-surface bloom of *Pseudo-nitzschia fraudulenta* observed from May 21 to 25, 1996, abruptly disappeared from the upper water column following a north wind event. Our sampling protocol allowed us to determine that the population was still in East Sound, and was now concentrated in a live, and apparently healthy near-bottom thin layer. Our observations are consistent with the hypothesis that less dense, low-salinity Fraser River water flowed in over the surface of East Sound. This, in turn drove near-bottom (but not intermediate) water out of the sound, until the water (with its entrained flora) that had been at the depth of the thin layer of the upper water column was now just above the sea floor. Since the *P. fraudulenta* cells remained within a water mass of  $\sim 29.8$  psu throughout the entire period, we conclude that the deep *P. fraudulenta* layer resulted from physical transport of water masses, rather than the sinking of a senescent population. Since buoyant plumes are a common feature in coastal waters, it seems likely that similar events in other systems could rapidly transport *Pseudo-nitzschia* species to depth, where, if toxic, domoic acid could quickly accumulate in benthic filter-feeders. In such cases, attempts to predict toxicity based on purely physiological considerations (e.g. sinking of a surface bloom following nutrient depletion) would fail.

Rapid displacement of surface water (with its entrained flora) to depth was the result of regional scale

physical forcing in the southern part of the Strait of Georgia (Thomson 1981). The reported sequence of events is common in this region, and the resulting near-bottom optical thin layers are a recurrent phenomenon in East Sound (Deksheniaks et al. 2001). In our study, the near-bottom layer was dominated by *Pseudo-nitzschia fraudulenta*, which appeared to remain healthy and motile despite the very low light (e.g. 1 to 2  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at this depth. Very few studies have sampled at the critical scales required to find thin layers, but there is repeated documentation in the literature that *Pseudo-nitzschia* spp. cells may often reside deep in the water column (e.g. Gran & Thompson 1930, Forbes & Denman 1991, Allen in Fryxell et al. 1997, Trainer et al. 1998a).

### Deep layers of *Pseudo-nitzschia* spp.

Our observations are concordant with the earlier work of Gran & Thompson (1930). Their study examined the distribution of diatoms in the San Juan archipelago with respect to general circulation patterns and water masses in the region. They discussed the distribution of '*Nitzschia seriata*', a name which at that time was used for all *Nitzschia* species that formed 'stepped' colonies (now called *Pseudo-nitzschia*). Gran & Thompson depict the transitory passing of a large population of '*N. seriata*', at depth, through the Spieden Channel (Fig. 1) from July 11 to 22, 1928. On July 11, diatoms were limited to the surface layer and the maximum recorded abundance of '*N. seriata*' was  $5 \times 10^3$  cells  $\text{l}^{-1}$ . Two days later,  $1.6 \times 10^5$  cells  $\text{l}^{-1}$  of '*N. seriata*' were recorded at 20 m depth, but only 0 to  $10^3$  cells  $\text{l}^{-1}$  at all other depths (1, 5, 10, 35, 50, 100 and 225 m). Their sampling scale was too coarse to determine whether this was a thin layer sampled only by chance, or part of a broad, subsurface *Pseudo-nitzschia* spp. maximum. The surface water was several degrees warmer, indicating the presence of a different water mass, interpreted as Fraser River-diluted seawater from the Strait of Georgia. Nearby at Olga, near the mouth of East Sound, '*N. seriata*' numbers were also greatest at depth: 2.9 and  $1.9 \times 10^5$  cells  $\text{l}^{-1}$  at 10 and 20 m, respectively. A week later, the bloom was not detected at either location. Collectively, this suggests that deep populations of *Pseudo-nitzschia* spp. are a recurrent phenomenon in the San Juan Islands, perhaps occurring annually in response to hydrographic conditions in early summer related to the seasonal outflow of the Fraser River. Regional hydrography can also influence blooms which are spread throughout much of the water column: the vertical position of a dense, mixed-species *Pseudo-nitzschia* bloom in Penn Cove, Whidbey Island (Puget Sound) was strongly influenced by



the position of buoyancy fronts of less saline water originating from the Skagit River outflow (Trainer et al. 1998a). Forbes & Denman (1991), examining an extensive data set from British Columbian shelf waters, noted that all but 1 sample in which *Pseudo-nitzschia* spp. concentrations exceeded  $10^5$  cells  $l^{-1}$  were collected at greater than 20 m depth. In Barkley Sound, on the west coast of Vancouver Island, Taylor & Haigh (1996) reported intrusions from offshore of *Pseudo-nitzschia* spp. at depth, as well as a deep, resident population, which coincided with toxicity of mussels. In the 1920s and 1930s, W. E. Allen collected an extraordinary phytoplankton data set along the US west coast. Fryxell et al. (1997) reviewed his *Pseudo-nitzschia* spp. data, which provided further evidence that healthy *Pseudo-nitzschia* spp. cells occur in deep layers. Their Fig. 4 depicts Allen's counts at 5 m intervals at 2 stations off La Jolla, California, in July and August 1927. Concentrations exceeding  $10^5$  cells  $l^{-1}$  were observed as deep as 60 m. On 2 occasions, peak concentrations occurred in a narrow, 5 to 10 m band. Allen did not have the hydrographic data that would have enabled him to trace water masses. He therefore reached the conclusion that the *Pseudo-nitzschia* spp. cells were controlling their vertical position in the water column, and unlike a *Chaetoceros* bloom, which deteriorated at depth, the *Pseudo-nitzschia* spp. cells maintained their health.

The above observations of deep layers of healthy, non-aggregated colonies are in contrast with those of Dortch et al. (1997), who also noted that *Pseudo-nitzschia* spp. could be abundant in bottom waters. These authors documented the sedimentation of *Pseudo-nitzschia* spp. blooms in the Gulf of Mexico in 1990 and 1991. In these cases, long colonies dissociated into single cells and doublets and sank, with up to 90% of the material collected in traps being composed of dead cells. The observations of Dortch et al. are consistent with those of Fryxell et al. (1990), who noted that in old cultures, colonies hundreds of cells in length broke apart simultaneously and settled to the bottom as single cells and doublets, which rapidly lost color.

Relative to phytoplankton, the concept of 'deep' depends not so much on the actual water depth as on the depth of light penetration — which will be entirely different in coastal and offshore waters. Nevertheless, there is repeated evidence of *Pseudo-nitzschia* spp. being found 'deep' in the water column, at very low light levels. If these are indeed viable populations, how are they surviving? The colonies we observed at depth were long and highly motile; hence they were unlikely to have been physiologically resting cells (e.g. Fryxell 1989, McQuoid & Hobson 1996). Could they have been heterotrophic? *Pseudo-nitzschia* spp. are raphid, pen-

nate diatoms (family Bacillariaceae Ehrenberg 1831). Facultative, and even obligate heterotrophy is well documented (Hellebust & Lewin 1977, Li & Volcani 1987) amongst pennates. However, although heterotrophy has been anecdotally reported for *Pseudo-nitzschia* spp. (Pan et al. 1996, Stewart et al. 1997), there has been no thorough evaluation of the ability of *Pseudo-nitzschia* species to persist in low light, or to live heterotrophically in the dark.

#### Association between *Chaetoceros* spp. and *Pseudo-nitzschia* spp.

*Chaetoceros* spp. and *Pseudo-nitzschia* spp. have often been observed to bloom at the same time (W. E. Allen in Fryxell et al. 1997). Aggregates of marine snow co-dominated by *Chaetoceros* spp. and *Pseudo-nitzschia* spp. have been reported by Alldredge & Gotschalk (1989). In contrast to *Chaetoceros/Pseudo-nitzschia* aggregates, which are passively formed by the mass flocculation and settling of a diatom bloom (Alldredge & Gotschalk 1989), our observations show that these genera may actively live in close association with each other. Since *Pseudo-nitzschia* spp. are pennate diatoms, this is not surprising: Although *Pseudo-nitzschia* spp. are considered planktonic, most pennate diatoms live on or in the sediments or attached to a substrate such as another organism, rock, sand grains or pilings (e.g. Round et al. 1990). Other pennate diatoms, especially *Nitzschia americana*, are commonly observed living on the cell bodies and setae of many *Chaetoceros* species (Taylor 1980, Rines & Hargraves 1988, 1990, Fryxell et al. 1990, Hasle & Syvertsen 1996, Rines: <http://thalassa.gso.uri.edu/rines/ecology/epipred.htm>).

*Chaetoceros socialis* provides a unique micro-environment for this kind of association because it has an unusually complex morphological structure. Individual cells are joined by fusion of the setae of adjacent cells into flexible colonies with specialized terminal valves. Three-quarters of the colony's setae are short and curved to one side of the chain; the remainder are extremely elongate, and are directed in the opposite direction. The commonly observed spherical colony is actually a 'super-colony', composed of numerous individual linear colonies, which are arrayed on the surface of the sphere, much like stitches on a baseball. The elongate setae traverse the interior of the sphere and meet at the center, while the short setae are directed outward, rendering the sphere 'fuzzy' in outline. *C. socialis* colonies are frequently colonized by many types of protists (Rines & Hargraves 1988, Sieracki et al. 1998, Rines unpubl. obs.). Long, needle-like '*Nitzschia*' living on *C. socialis* have been reported

by Margalef et al. (1955) and by Taylor (1982). Our repeated observations of *P. pseudodelicatissima* living upon/within *C. socialis* colonies are thus consistent with the biology of raphid, pennate diatoms.

The *Chaetoceros socialis*–*Pseudonitzschia pseudodelicatissima* association is of interest for several reasons: (1) By definition, *Pseudo-nitzschia* spp. are pelagic, colonial diatoms (Hasle 1994, Hasle & Syvertsen 1996). However, both single cells and chains of *P. pseudodelicatissima* were found living within the *C. socialis* colonies, suggesting that this taxon may have both unicellular and colonial life-history stages as has previously been suggested for *P. multiseriis* and *P. subcurvata* (Fryxell et al. 1990, 1991). Thus, there is the potential for a *Pseudo-nitzschia* spp. bloom to develop unnoticed both because it originated as single cells, easily misidentified as non-toxic '*Nitzschia* spp.', and because the developing population was camouflaged by the more obvious *C. socialis* colonies. (2) The interior of *C. socialis* colonies may constitute a micro-environment with conditions different from those in the water column, such that the growth dynamics of *Pseudo-nitzschia* spp. cells are decoupled from average water column processes. (3) *Pseudo-nitzschia* spp. are motile only when they have a substrate to glide on. Motility is important for sexual reproduction in *Pseudo-nitzschia* species, because colonies of different mating types must line up with each other, or clump together (Fryxell et al. 1991, Davidovich & Bates 1998), and the gametangia require a substrate against which to glide. *C. socialis* provides a substrate within the water column, which may provide a setting for completion of the life cycle.

### Implications

In order to predict the onset and consequences of *Pseudo-nitzschia* spp. blooms, we must understand which factors are most important in regulating their distribution through space and over time. To achieve this goal, the correct set of questions must be asked. However, scientific research is often guided by preconceptions and reigning paradigms — we ask questions leading us to look for what we expect to see. It is generally expected that, along with other phytoplankton, *Pseudo-nitzschia* spp. colonies will be found floating freely in the water column, that they will be located in the 'euphotic zone', and that they will be distributed throughout the surface 'mixed' layer. We questioned several common assumptions, viz (1) that field sampling strategies commonly employed to study phytoplankton in the mixed layer are effective in revealing the distributional patterns of *Pseudo-nitzschia* spp. populations; (2) that distributional patterns are strictly

the result of *in situ* processes in the water column; and (3) that *Pseudo-nitzschia* species have life histories typical of other planktonic diatoms. In doing so, we found populations of *Pseudo-nitzschia* spp. in 3 places in which one would not normally look for them: in an upper water-column thin layer; concentrated in a dense, apparently healthy thin layer, persisting at very low light levels just above the sea floor; and living in association with *Chaetoceros socialis* colonies. Although growth in response to the nutrient, temperature, light and salinity fields in the upper mixed layer is undoubtedly important in regulating the biomass of a bloom, we found that the vertical distribution of cells was not simply a function of *in situ* growth processes in the euphotic zone. The deep, dense layer of *Pseudo-nitzschia* spp. cells did not result from the sinking of a senescent and/or aggregating population: cells were rapidly advected downward by physical oceanographic events. Although hidden from routine sampling procedures, the *Pseudo-nitzschia* spp. cells were neither dead nor destroyed, nor had they left the sound. Long, motile colonies suggest a viable population, now in close proximity to filter-feeding shellfish. Might some of the unexplained domoic acid events be related to similar, near bottom thin layers of high cell density?

*Pseudo-nitzschia fraudulentata*, *P. pungens* and *P. pseudodelicatissima* have been reported to produce low levels of domoic acid (Martin et al. 1990, Rhodes et al. 1996, 1998b, Trainer et al. 1998b, Wekell et al. 1998). Although these taxa do not share the reputation of *P. australis* and *P. multiseriis* for causing large-scale domoic acid poisoning events, all 5 are reported from the Puget Sound region. They frequently co-occur, suggesting that species within the genus *Pseudo-nitzschia* are ecologically similar. Blooms hidden in thin layers or camouflaged within *Chaetoceros socialis* colonies pose an additional challenge to domoic acid monitoring efforts, because they imply that (1) a toxicity event can occur, even if *Pseudo-nitzschia* spp. have not been detected in the water column, and (2) that the toxicity within a thin layer may exceed a threshold value which would not be met if cell concentrations were integrated throughout the water column (e.g. by a net tow).

The extensive coastline of Puget Sound and British Columbia is dominated by fjords and islands. Different water masses, tidal currents and episodic weather systems interact to create complex hydrographic regimes, resulting in the type of physical forcing events that led to the observed *Pseudo-nitzschia fraudulentata* distributions in East Sound. Similar processes are likely to occur throughout this region as well as along the fjord-rich coastlines of Norway, Sweden, Finland, Scotland, Chile and New Zealand. Thus, *Pseudo-nitzschia* taxa may routinely occur in undetected thin layers, in a

suite of geographic areas which are extensively utilized by commercial and recreational fisheries as well as by the aquaculture industry. Dense, toxic thin layers have the potential to directly impact shellfish toxicity, to be advected at depth and unnoticed into a new region, or, when mixed back into the euphotic zone, to serve as a large inoculum initiating a sudden surface bloom. Consideration of alternative conceptual frameworks may be critical to the successful development of models predicting the dynamics and impacts of *Pseudo-nitzschia* spp. blooms.

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