Final Report to Suffolk County Department of Health Services'
Office of Ecology

Influence of Groundwater Constituents on Initiation of the Brown Tide in the Peconics Bay System

Contract No.: 525822801130000001

Principal Investigators: Profs. Gordon T. Taylor and Sergio Sañudo-Wilhelmy

Institution: Marine Sciences Research Center, SUNY, Stony Brook, NY 11794

Associate Investigator: Dr. Christopher Gobler, Southampton College, LIU

Period Included: 7 July 99 – 31 Dec 03

Submitted by: Prof. Gordon T. Taylor

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Date: _____

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Executive Summary

A combination of laboratory-based bioassays, manipulative field experiments and field reconnaissance were employed to evaluate the central hypothesis that chemical constituents introduced into coastal L.I. embayments by groundwater (GW) initiate Brown Tide outbreaks. These chemical constituents may act by directly stimulating growth of *Aureococcus anophagefferens*, the Brown Tide organism or by depressing growth of the Brown Tide's ecological competitors. Likely suspects investigated were GW, sediment porewater (PW), pesticides (Aldicarb, Aldicarb sulfoxide, Aldicarb sulfone, Alachlor and Metolachlor), salinity, metals (selenium, iron and copper) and nitrogen nutrient speciation (nitrate, ammonium, urea, amino acids).

Our laboratory studies directly assaying algal growth responses to additions of groundwater from monitoring wells or submarine groundwater discharge through the sediments of Peconic Bays yielded mixed results. Growth of *A. anophagefferens* was clearly suppressed with increasing amounts of Flanders Bay and West Neck Bay (WNB) porewater, but so was growth of two other algal species. Submarine groundwater discharge collected on other dates and at other sites yielded weak differential responses from the test species. The potential for submarine groundwater discharge to alter algal growth and community composition was clearly demonstrated in a few instances. However, we conclude that additions of GW or PW to nutrient-replete BT medium provided rather weak selective advantages to a subset of the species tested, if at all. In all instances where treatment effects were observed, there was a clear selection against *A. anophagefferens,* favoring growth of several other test species. We observed instances where PW and GW were inhibitory and instances where they were somewhat stimulatory to two or more species. The chemical source of these effects was unknown, so we proceeded to investigate pesticides, nutrient speciation and trace metal availability.

Results from our laboratory studies of the pesticides listed above did not support the hypothesis that the groundwater-borne pesticides select for growth of *A. anophagefferens* or select against other common phytoplankters. Inhibitory effects were usually apparent only at concentrations of about 1 ppm. The highest Aldicarb concentrations reported in eastern Suffolk County GW was 515 ppb in 1980 and concentrations have been declining since then (SCDHS). With dilutions into overlying baywater, maximum Aldicarb concentrations are estimated to be 45 to 129 ppb. These concentrations appear to be too low to significantly influence growth of the phytoplankton species tested. In no instance, was a clear selective advantage conferred to *A. anophagefferens* over other test species, a requirement for Brown Tide onset. Study of alachlor

and metolachlor represented an expansion of the original project's scope and was confined to the parent molecules. Of necessity, all bioassays tested for responses to a single pesticide or a single pesticide metabolite to uncover a "smoking gun". The environmental reality is that several pesticides and metabolites co-occur in GW because of multiple land applications and are carried into the bays by SGD. Therefore, additional investigations utilizing the more abundant and mobile oxanilic acid and ethane sulfonic acid metabolites of alachlor and metolachlor as well as environmentally-relevant combinations of pesticide and their degradates are necessary to conclusively rule out a link to pesticide inputs.

Our laboratory studies of nutrient speciation suggest that in the absence of other limitations, phytoplankton communities supported primarily by NO_3^-, will be dominated by diatoms and perhaps chlorophytes. Whereas phytoplankton communities supplied primarily with low-to-moderate concentrations of reduced N-species, such as NH_4^+ and dissolved organic nitrogen (DON), will be dominated by *A. anophagefferens* and to a lesser extent dinoflagellates. Results are completely consistent field observations of *A. anophagefferens* bloom dynamics in Long Island estuaries (LaRoche et al. 1997; Gobler and Sañudo-Wilhelmy 2001) and our PW and GW bioassays (discussed above).

While it was already known that *A. anophagefferens* has an absolute selenium (Se) requirement, we did not know that it was the only test species with this requirement until our laboratory studies. The other four species grew equally well with or without Se. In the field, low levels of bioavailable selenium and iron measured in the West Neck Bay waters during Brown Tide suggest that these essential trace elements could limit BT. However, we did not find any quantitative relationships between metal levels and the Brown Tide intensity. In fact, the range of bioavailable selenium (>0.4 nM) measured in the field was about twice the concentration needed to fulfill *A. anophagefferens'* uptake requirements (0.25 nM). Therefore, results suggest that the Se available in WNB did not limit Brown Tide during our sampling. In contrast, levels of low molecular weight iron were extremely low (<1.4 nM) and within iron-limitation thresholds. In fact, the highest levels of bioavailable iron were detected before or during the Brown Tide. Temporal distributions of total phytoplankton and metals suggest that high biomass only occurs when water concentrations of selenium and iron are relatively high. These results suggest that some phytoplankton species benefit from the availability of these important bioactive trace metals.

We conclude that submarine groundwater discharge can exert strong selective pressures on phytoplankton communities, depending upon its source and composition. We further conclude that the likelihood of Brown Tide being initiated by introduction of the pesticides tested via submarine groundwater discharge is not supported by these results. We note that additional investigations utilizing other pesticide metabolites and relevant combinations of pesticide degradates are warranted. Lab and field data support the hypothesis that seasonal changes in nitrogen speciation, and possibly elemental ratios (N:P:Si:Fe:Se), can alter competitive advantage between *A. anophagefferens* and its competitors. Confounding environmental factors in our field reconnaissance program may have masked the true importance of trace metal dynamics (concentrations and total vs bioavailable). We believe that a more detailed examination of a few key trace metals, like selenium and iron, under controlled conditions is also warranted for future studies. We posit that availability of other growth factors, such as B-vitamins, may be important in shaping phytoplankton community structure in BT-prone Long Island embayments.

Project's Objectives:

We originally hypothesized that some subtle and specific control confers competitive advantage to *A. anophagefferens* over co-occurring species to promote onset of the brown tide. We proposed that some factor(s), other than macronutrients, selects for proliferation of *A. anophagefferens* or selects against competing phytoplankton species. We speculated that biocides and their daughter products carried by groundwaters entering L.I. bays may be one possible mechanism by which low concentrations inhibit growth of sensitive microalgae, which compete with *A. anophagefferens,* while *A. anophagefferens* is insensitive to this material. Conversely, it is also possible that these products selectively stimulate growth of *A. anophagefferens.* The same sort of arguments can be made for constituents of septic tank and landfill leachate as well as materials applied to agricultural lands and golf courses. The bioactive constituent may simply be a required micronutrient, such as a metal, vitamin or cofactor, for which *A. anophagefferens* has high affinities and rapid uptake systems. It may be a particular chelator for essential trace elements, such as citrate or other organic ligands, for which *A. anophagefferens* possesses a high specificity. Lastly, it may be a molecule that affects cell regulation, analogous to hormones. Introduction of hormone-like pesticides into local bay systems may tip the ecological balance and select for *A. anophagefferens*. Many of the possibilities described above were considered in this investigation.

Research has tested the hypothesis that meteorologically-driven hydrologic pumping of groundwater constituents into the Peconics and Great South Bay systems, via submarine groundwater discharge (SGD), stimulates the onset of brown tide (BT).

We expanded the scope of our experiments to also examine the effects of macronutrient speciation and concentration as well as salinity and selenium availability in selecting the dominant phytoplankton species in L.I. embayments. We postulated that SGD influences interspecies competition among phytoplankters by means of one of the following mechanisms:

1. provision of an essential micronutrient (inorganic or organic), e.g., iron, selenium, vitamins, etc.
2. provision of a chelator which enhances bioavailability of essential trace metals
3. provision of an algal inhibitor to which *A. anophagefferens* is insensitive, conferring competitive advantage to the brown tide
4. provision of a growth-stimulating cofactor to which *A. anophagefferens* is responsive, i.e., dilute parent or breakdown product of agricultural biocide with nutrient or hormone-like activity
5. introduction of specific macronutrients in ratios or concentrations that favor *A. anophagefferens* due to its unique uptake affinities

Activities:

Note: There was a hiatus in funding from 6 July 01 to 10 January 2002 due to Suffolk County budget cuts. Funding for our project was restored to the County budget through the tireless efforts of Legislator Vivian Fisher, Dr. Vito Minei (SCDHS) and Dr. Robert Nuzzi (SCDHS). Monetary advances from the Research Foundation of SUNY were not permitted without a firm commitment for Year 3 funding, so we lost the technician who was performing most of the experiments and most tasks were suspended after 6 Jul 01. With restoration of funding, we were able to hire a new technician in February 2002 and partially support two graduate students (William Kentrup &Yoko Tsukamoto). We also note that at SCDHS's request, we expanded the project's original scope to include Alachlor and Metolachlor bioassays.

1. Acquisition of required equipment (incubators, rotary light table) and supplies
2. Acquisition of required algal cultures
3. Collection of groundwater from monitoring wells on Shelter Island and subtidal seepage from West Neck and north Flanders Bays.
4. Completed a series of competition bioassays with Aldicarb and Aldicarb derivatives (see Table 1).
5. Completed a series of competition bioassays with groundwater and subtidal seepage (see Table 1).
6. Completed a series of competition bioassays examining effects of nitrogen speciation with ammonium, nitrate, urea and glutamic acid (Table 1)
7. During summer 2001, completed groundwater and porewater enrichment experiments with natural phytoplankton communities from western Great South Bay.
8. Results from items #6 and 7 resulted in manuscript submitted to *Marine Ecology Progress Series* (Appendix I).
9. Completed a series of competition bioassays to examine salinity effects (see Table 1).
10. Completed a collaborative mesocosm study with Drs. Caron and Lonsdale during summer 2000 to examine influence of groundwater on succession of naturally occurring phytoplankton communities
11. Completed field study of dynamic relationship between the speciation of selenium, iron and copper and *A. anophagefferens* and all other phytoplankton abundances.
12. Completed field study on the importance of groundwater seepage on the cycling and budget of bioactive trace metals in West Neck Bay.
13. Collection of groundwater from monitoring wells on Shelter Island and subtidal seepage from West Neck on 22 May and 9 October 2002 to capture high and low flow conditions, respectively.
14. Performed 8 series of competition bioassays with Alachlor and Metolachlor (see Table 1).
15. Completed four series of experiments evaluating sensitivity of bioassay species to solvents (ethanol and methyl cellosolve) used to dissolve Alachlor and Metolachlor

16. Completed a series of competition bioassays with one groundwater sample from shoreline of West Neck Bay (see Table 1).
17. Completed experiments comparing growth responses of all 5 bioassay species to f/2 and BT base media and media with intermediate Se and Fe concentrations
18. Completed sample analysis and we are currently drafting a manuscript on selenium, iron and copper cycling in West Neck Bay with special consideration of SGD.
19. Acquisition of new algal cultures from CCMP (Bigelow Lab)
20. Student (William Kentrup) completed Masters Thesis on effects of plankton on metals concentrations and partitioning, entitled "Impact of Biological Activity on the Size-fractionation of Trace Metals in a Coastal Environment (West Neck Bay, Long Island)" (abstract attached and copy of thesis has been mailed to Dr. Nuzzi)
21. Draft of a manuscript from Kentrup's thesis is in preparation with Sañudo-Wilhelmy and Taylor as coauthors.
22. student (Yoko Tsukamoto) completed field collections and initiated sample analysis for her Master Thesis, entitled "The Importance Of Chemical Reactions In Subterranean Estuaries: The Concentrations of Trace Metals and Nutrients in Submarine Groundwater Seepage at West Neck Bay, Long Island, New York" (abstract attached)
23. Simultaneous analyses of *in vivo* fluorescence, extracted chlorophyll a concentrations, cell number, cell volume, and major and minor elemental compositions of all 5 test species. Measurements necessary for intercalibration of analyses and to assess differences in element requirements from the environment. First attempt failed when we learned that 2 cultures were contaminated and experiment was repeated.
24. High school intern (Kristin Goodrich) assisted in the nitrogen speciation experiments over two summers and submitted her project to the Intel, LISEF and Junior Science and Humanities Symposium competitions. Kristin was a semifinalist in the Intel competition and presented her work at the national finals of the Junior Science and Humanities Symposium in Louisville KY this past summer.

Significant Findings:

A. Laboratory Studies

1. One of our early objectives was to determine if groundwaters (GW) from monitoring wells and submarine groundwater discharge (sediment porewaters = PW) collected through piezometers selectively inhibited or stimulated *A. anophagefferens* and four co-occurring algal species under nutrient-replete conditions. To this end, we designed bioassays, exposing replicate cultures of each species to increasing amounts of GW or PW while holding salinity constant. Knowing the lowest optimal salinity for all assay species was critical, so we tested growth response to salinity while holding nutrient concentrations constant (standard BT medium additions) on two occasions. Atlantic seawater collected 8 km southeast of Shinnecock Inlet was diluted with tapwater to represent similar ionic composition of local GW. Tapwater was aged and aerated to dechlorinate.

- Fig. 1 presents growth curves of *A. anophagefferens* and *Prorocentrum minimum*, a dinoflagellate common to local waters, exposed to varying salinities. Three conclusions arise

from these results. First, *A. anophagefferens* appears to be stenohaline and its growth is significantly diminished at salinities below 25 psu. Secondly, *P. minimum* appears to be more euryhaline, showing little response to variation in salinity. Lastly, reproducibility within the bioassay is remarkably good. This experiments was carried out with triplicate incubation flasks for each treatment and species. Where there is little or no treatment effect (*P. minimum*), means and SD overlap and conversely where strong treatment effects exist, overlap in error bars is absent (e.g., 20 psu - *A. anophagefferens*). Triplication of incubation flasks was carried out in several experiments until we were convinced that variance within treatments was much smaller than among treatments. Triplication was then discontinued because of space and analytical time constraints.

- Exponential portions of the growth curves were used to calculate growth rate constants using regression analysis of ln (N/N_0) vs time on no fewer than five time points, where N and N_0 = in vivo fluorescence at times x and 0, respectively. The slope is equivalent to the first-order growth rate, μ, (d^{-1}) and the standard error of the slope is an estimate of the precision. Coefficients of determination (r^2) were always > 0.90. Results suggest that *A. anophagefferens* attained maximal growth at salinities above 25 psu (Fig. 2). Growth of *P. minimum* appeared relatively insensitive to salinity.
- In a repetition of this experiment with five microalgal species, 25 psu appeared to be the minimum salinity supporting near maximal growth rates for all species (Fig. 3). Growth of a cyanobacterium, *Synechococcus bacillaris,* diatom, *Thalassiosira pseudonana* and chlorophyte, *Nannochloris atomus* appeared to be unaffected over the salinity range tested. Based on results from both salinity experiments, a final salinity of 27 psu was used in all GW and PW amendment experiments.

2. BT medium was prepared in seawater diluted to 27 psu with a series of freshwaters containing between 0 and 100% submarine groundwater discharge from N. Flanders Bay mixed with aged tapwater. Equal nutrient additions were made to each and final PW volumetric contributions varied between 0 and 32.5%. This experimental design would test whether sample water contained selective inhibitors or stimulatory constituents in excess of that provided by complete BT medium.

- Growth of *A. anophagefferens* was clearly suppressed with increasing amounts of Flanders Bay PW (Fig. 4).
- Growth responses of the other microalgal test species were quite different. Only growth of *P. minimum* was inhibited at the highest PW additions (Fig. 5).
- Growth rates of *A. anophagefferens* are clearly compromised when PW contributes > 10% to seawater's dilution and appears to be toxic at 20% and above (Fig. 6). Growth of *S. bacillaris, P. minimum* and *N. atomus* appear to be unaffected. In contrast, growth of *T. pseudonana* appears to be stimulated by incremental additions of PW.

3. Using results from individual bioassays, growth performance simulations were run by arbitrarily assuming an initial condition of equivalent biomass for all five species tested (e.g., N_0 = 100 cells ml^{-1}), representing an early spring condition in the field. To predict how a fixed % of PW determines relative abundances of the five species through time within a simplified phytoplankton community, growth rates observed at 0 and 32.5% PW were applied to the logistic growth equation ($N = N_0e^{\mu t}$). Our model assumes no cell removal and

that nutrient supply keeps pace with plankton uptake, resulting in steady-state nutrient concentrations for the duration of the simulation (7 d)

- Figure 7 illustrates that in BT medium diluted with tapwater alone (0% Flanders PW), after 7 days phytoplankton communities would be dominated by *S. bacillaris* and *N. atomus,* followed by *A. anophagefferens* and *P. minimum,* while *T. pseudonana* would be the least abundant. If provided with high proportions of submarine groundwater discharge (32.5% Flanders PW), simulated phytoplankton community would be dominated by *N. atomus, T. pseudonana* and *S. bacillaris*, followed distantly by *P. minimum.* Cells of *A. anophagefferens* would be rare.

4. Similar experiments were performed with submarine groundwater discharge from West Neck Bay (WNB) and groundwater from nearby monitoring wells. Algal responses varied considerably depending on when and where samples were collected.

- Assays of submarine groundwater discharge collected from the shallow intertidal of WNB on 22 May 2002 revealed inhibitory effects on growth of *A. anophagefferens* and *S. bacillaris* and a small effect on *T. pseudonana* (Fig. 8). The other two species were not assayed on this occasion due to low available sample volume.

- Growth rates of *A. anophagefferens* and *S. bacillaris* in this experiment systematically decreased with increasing PW proportions. Negative effects on *T. pseudonana* are suggested but not statistically significant.

5. Submarine groundwater discharge collected at another time (June 2000) and site in WNB yielded entirely different responses from the test species.

- Growth rates for all but *T. pseudonana* were indistinguishable among treatments (Fig. 10). Growth of *T. pseudonana* appeared to be slightly inhibited at the highest PW exposure.

6. Groundwater from a monitoring well on the shores of WNB appeared to induce little inhibitory or stimulatory effect on the test species.

- Growth of *T. pseudonana* was slightly depressed at the highest GW proportion, while growth of the other four species was invariant (Fig. 11).

- A second trial with monitoring well GW produced growth rates that were virtually indistinguishable between treatments – no inhibition or stimulation for any species (Fig. 12).

7. Submarine groundwater discharge collected from the shallow intertidal of WNB on 22 May 2002 also produced growth rates that were virtually indistinguishable between treatments – no inhibition or stimulation for any species (Fig. 13). Any nutrients contained in this PW must have represented a trivial enrichment over that provided in BT medium, especially for the growth-limiting nutrient. Otherwise, stimulation would be observed at highest exposures. Furthermore, if inhibitory chemicals were present, they were too dilute to produce an effect.

8. Groundwater collected from a monitoring well 26' from the WNB shoreline on 22 May 2002 appeared to induce a small stimulatory effect for *A. anophagefferens, S. bacillaris* and *T. pseudonana* but only up to 10% contribution (Fig. 14). Higher proportions of GW did not yield higher growth rates. *P. minimum* and *N. atomus* were unaffected by GW additions.

9. In trial II of the experiment just described, results were nearly the same (Fig. 15). Most stimulatory responses were apparent between 0 and 10% additions of GW.

10. In trial II with PW collected from the shallow intertidal of WNB on 22 May 2002, no treatment effects whatsoever were evident (Fig. 16). This PW sample appeared to have no inhibitory nor stimulatory effects at all. Whatever nutrients it bore, must have been a small fraction of BT medium's content and inhibitory chemicals, if present, were too dilute.

11. PW collected from WNB's shallow intertidal through a piezometer on 22 May 2002 appeared to suppress growth rates of *A. anophagefferens, S. bacillaris* and *T. pseudonana* between 0 and 20% contribution (Fig. 17). These are growth rates calculated from growth curves presented in Fig. 8.

12. In the last field experiment to be presented, we examined whether the baywater itself from WNB after naturally mixing with submarine groundwater discharge had any effect on microalgal growth. Baywater was collected contemporaneously with GW and PW on 22 May 2002.

- Baywater had no conclusive effect on any test species at any exposure level (Fig. 18). Thus whatever nutrients or inhibitors submarine groundwater discharge may have added to the bay at that time were either consumed or inactivated.

13. **<u>To summarize the GW and PW studies</u>, we conclude that additions of GW or PW to nutrient-replete BT medium provided rather weak selective advantages to a subset of the species tested, if at all. In no instance was there a clear selection for *A. anophagefferens* to outgrow the other four test species. We observed instances where PW and GW were inhibitory and instances where they were somewhat stimulatory to two or more species. Clearly, GW and PW carry varying levels of macronutrients (nitrate, ammonium, phosphate, silicate) and micronutrients (metals and possibly vitamins) that potentially support growth in local bays. However, intentional addition of BT medium nutrients to these experiments precluded evaluating those effects. We explicitly evaluated those effects in later experiments (item #20 and the attached manuscript – Taylor et al. in review).**

14. We hypothesized that biocides and/or their metabolites carried in GW from the watershed to the bays are a potential source of selective inhibition. To test this, we used a similar experimental design in which we added increasing amounts of commonly used pesticides to BT medium prepared in full-strength seawater. We set the pesticide concentrations to encompass the range of possible exposures in our coastal embayments; from 0.1 ppb to 1.0 ppm. Most of the pesticides also have limited solubility in water, so standard solutions were prepared in 10% methyl cellosolve (EGME; ethylene glycol monomethyl ether) and diluted in the same solution. We performed separate bioassays with 10% EGME and 10% ethanol alone for all algae to assess solvent toxicity. Ethanol was slightly inhibitory and the 10% EGME treatment was indistinguishable from the control (water) (not presented). Thus, we used 10% EGME to prepare all stock pesticide solutions.

15. Aldicarb has been used extensively on Long Island and is commonly detected in monitoring wells. Surveys by SCDHS report a maximum aldicarb concentrations of 515 ppb in a monitoring well near Hallocks Bay in 1980 and concentrations declining to 181 ppb in 1985. Assuming that GW is diluted at least four-fold (based on salinity) when mixing with baywater, surface water concentrations of aldicarb may vary between 45 and 129 ppb. We also assayed its two common metabolic daughter products; aldicarb sulfoxide and aldicarb sulfone

- No statistically significant differences were observed between controls and exposures up to 1000 μg L^{-1} (1 ppm) for any of our five test species (Fig. 19). Aldicarb does not appear to interact with any of these microalgae.
- With the exception of *T. pseudonana*, aldicarb sulfoxide did not appear to have any effect on microalgal growth rates in Trial #1 (Fig.20). Results for *T. pseudonana* were ambiguous; growth sharply decreasing from control to 0.1 μg L^{-1} and increasing randomly at higher concentrations. We therefore repeated the experiment.
- In trial #2 with aldicarb sulfoxide, responses of *A. anophagefferens* and *P. minimum* were variable while growth of the other three species was relatively constant among treatments (Fig. 21). Again results are somewhat inconclusive and the experiment was repeated.
- In trial #3 with aldicarb sulfoxide, lower concentrations of the pesticide metabolite had no effect on any algae. At 1000 μg L^{-1}, however, it appears that growth of all but *N. atomus* was inhibited (Fig. 22).
- In trial #1 with aldicarb sulfone, *P. minimum* did not grow in any treatment (Fig. 23). In fact, our maintenance culture had failed to grow so a new culture was obtained from Bigelow Lab's culture collection (CCMP). In this experiment, growth of *A. anophagefferens* appears to be slightly inhibited at aldicarb sulfone concentrations $\geq$ 100 μg L^{-1}.
- In trial #2 with aldicarb sulfone, all 5 species of algae grew equally well at concentrations $\leq$ 100 μg L^{-1} (Fig. 24). At 1000 μg L^{-1}, however, all species appeared to be inhibited, especially *A. anophagefferens, P. minimum* and *T. pseudonana*.

16. Although we were not originally contracted to study alachlor or metolachlor, these pesticides have recently been identified in GW from monitoring wells from the east end of LI. At Dr. R. Nuzzi's request, we included these pesticides in our laboratory studies.

- The first two trials with alachlor were unsuccessful due to contamination problems in several of our maintenance cultures. Fresh cultures were obtained and experiments were repeated.
- A very clear dose response of *A. anophagefferens'* growth to alachlor concentration was apparent from Trial #3 with alachlor (Fig. 25); growing at a rate of 0.32 d^{-1} in controls and actually dying off in the 1000 μg L^{-1} treatment. *S. bacillaris, N. atomus* and *P. minimum* appear relatively insensitive to alachlor, growing at rates of about 0.40, 0.36 and 0.30 d^{-1}, respectively, except at 1000 μg L^{-1} where growth was slightly reduced. Response of *T. pseudonana* was intermediate with growth dropping off above a threshold concentration of 1 μg L^{-1}.
- Results from Trial #4 with alachlor were qualitatively similar to those of Trial #3 (Fig. 26), but the magnitude of the responses was less. Once again *S. bacillaris* and *N. atomus* appeared to be relatively insensitive to all exposures to alachlor.

17. Trials with metolachlor were problematic; several algal species failed to grow in Trials #1 and 4 and *T. pseudonana* failed to grow in any treatment in Trial #3.

- In Trial #2, metolachlor concentrations of $\geq$ 10 μg L^{-1} suppressed growth of *A. anophagefferens*, although it still grew after exposures to 1000 μg L^{-1} (Fig. 27). Growth of *S. bacillaris* and *N. atomus* were little affected by exposures to any concentrations of

metolachlor. Results for *P. minimum* were ambiguous; low growth at 10 μg L^{-1} and higher at 1 and 100 μg L^{-1}. Growth of *T. pseudonana* only appeared inhibited at exposures of > 10 μg L^{-1}.

- In Trial #3, all species appeared relatively unaffected by metolachlor exposures below 1000 μg L^{-1} and only slightly impaired at this highest exposure concentration (Fig. 28). *T. pseudonana* failed to thrive in any treatment of this experiment, so a new culture was obtained afterwards.
- Results from Trial #5 with metolachlor corroborated previous findings (Fig. 29). *S. bacillaris, N. atomus* and *P. minimum* were little affected by exposures to any concentrations of metolachlor. *A. anophagefferens* and *T. pseudonana* appeared to be sensitive only to the highest exposure (1000 μg L^{-1}).

18. **To summarize our laboratory studies of the pesticides, Aldicarb, Aldicarb Sulfoxide, Aldicarb Sulfone, Alachlor and Metolachlor, we find no compelling evidence to support the hypothesis that these groundwater-borne pesticides select for *A. anophagefferens* or select against other common phytoplankters. Inhibitory effects were usually apparent only at concentrations of about 1 ppm (1 mg L^{-1}). The highest Aldicarb concentrations reported in eastern Suffolk County GW is 515 μg L^{-1} (ppb) (SCDHS). With dilutions into overlying baywater, maximum Aldicarb concentrations in surface waters are estimated to be 45 - 129 ppb. These concentrations appear to be too low to significantly influence growth of the phytoplankton species tested.**

    The USEPA does report that some of these biocides are "highly toxic to aquatic plants". However, in reviewing the studies cited by the USEPA (PAN Pesticides Database – www.pesticideinfo.org/List_AquireAll.jsp?), we note that only freshwater macrophytes and phytoplankton are routinely assayed. For example, alachlor is reported to be highly toxic to the freshwater green microalga, *Selenastrum capricornutum*, yielding an EC_{50} between 1.64 and 8 ppb, depending on the study (EPA R.E.D. Facts EPA-738-F-98-018, Dec. 1998; PAN Pesticides Database). The EC_{50} represents a 50% reduction in photosynthetic rate, but not necessarily lethality. A total of 16 different test phytoplankters were screened for sensitivity to alachlor in 45 separate reports. In 18 instances toxic end points were not reported, even after exposures to as much as 200 ppm alachlor, meaning the pesticide had no detectable effect. In the remaining 20 trials (excluding *S. capricornutum*), the EC_{50}'s for these 15 species varied from 26 to 3000 ppb, averaging 480 ppb. From these findings, we conclude that a wide range of responses to alachlor is possible among freshwater phytoplankton species and among experimental trials for the same species. Results from the most sensitive species appears to be the one reported in the EPA R.E.D. Fact Sheet. Furthermore, the response of marine phytoplankton may be quite different as a result of varying physiologies and the difference between fresh and saline water chemistries.

    Our studies of alachlor and metolachlor represented an expansion of the original project's scope and were confined to the parent molecules. Further studies of their more abundant and mobile oxanilic acid and ethane sulfonic acid metabolites are warranted to evaluate their toxicity to marine phytoplankton. Of necessity, all bioassays tested for responses to a single pesticide or a single pesticide metabolite to uncover a "smoking gun". The environmental reality is that several pesticides and

metabolites co-occur in GW because of multiple land applications and are carried into the bays by SGD. To date, we can only say that the hypothesis that Brown Tide is initiated by the pesticides tested is not supported by our findings. <u>However, additional investigations utilizing alachlor and metolachlor metabolites and environmentally-relevant combinations of pesticide and their degradates are necessary to conclusively rule out a link to pesticide inputs.</u>

19. To put our field study of selenium dynamics into perspective (described below), we performed similar bioassays with our test species to examine responses to varying concentrations of Se and Fe. End-members in these experiments were f/2 and BT media. Other treatments had varying relative concentrations of these two metals.

- While we knew that *A. anophagefferens* has a Se requirement, we did not know that it was the only one of the test species with this absolute requirement until this experiment (Fig. 30). The other four species grew equally well with or without Se in the medium.
- Selenium concentrations as low as 0.25 nM were sufficient to saturate *A. anophagefferens'* uptake requirements.
- Iron concentrations as low as 11 μM were sufficient to saturate uptake requirements for all five microalgal species.

20. Results from our comparisons of phytoplankter response to nitrogen concentration and speciation (Marine Ecology Progress Series, in final review) are summarized as follows. Presented with NO_3^- only, rank order of cell production was *Thalassiosira pseudonana* > *Nannochloris atomus* > *Synechococcus bacillaris* > *Prorocentrum minimum* > *A. anophagefferens* at all concentrations (10 – 1500 μM N). In contrast, communities developing under low to intermediate concentrations of NH_4^+ or glutamate were dominated by *A. anophagefferens* and *P. minimum*, while *T. pseudonana* comprised a minor fraction of the simulation community. At higher concentrations, *A. anophagefferens* lost its competitive advantage and simulated communities were dominated by *N. atomus* and *S. bacillaris*. At low to intermediate urea concentrations (1-100 μM N), *T. pseudonana* was most abundant, followed by *A. anophagefferens*. At higher urea concentrations, *A. anophagefferens* became rare in simulated communities, being displaced by *N. atomus* and *S. bacillaris*. Extrapolation of experimental and simulation results to local field conditions suggests that in the absence of other limitations phytoplankton communities supported primarily by NO_3^- will be dominated by diatoms, like *T. pseudonana* and perhaps chlorophytes, like *N. atomus*. Whereas phytoplankton communities supplied primarily with low-to-moderate concentrations of reduced N-species, such as NH_4^+ and dissolved organic nitrogen (DON), will be dominated by *A. anophagefferens* and to a lesser extent dinoflagellates, like *P. minimum*. **<u>In summary,</u> results are completely consistent field observations of *A. anophagefferens* bloom dynamics in Long Island estuaries (LaRoche et al. 1997; Gobler and Sañudo-Wilhelmy 2001)**.

B. Field Studies

21. Concentrations of bioactive trace metals (selenium, iron and copper) measured in the dissolved (filterable) pool in WNB during different stages of a brown tide bloom (Fig. 31) were consistent with values reported for other coastal embayments that do not experience similar blooms.
22. While levels of low molecular weight (<1 kDa) bioavailable copper were similar to those measured in the dissolved phase, concentrations of bioavailable selenium and iron were significantly lower than the levels measured in the filterable fraction (Figure 31).
23. Although low levels of bioavailable selenium and iron measured in the water column of WNB during the Brown Tide bloom suggest that these essential trace elements could be limiting the bloom, we did not find any quantitative relationships between metal levels and the Brown Tide bloom (Fig. 32).
24. The range of bioavailable selenium (>0.4 nM) measured in the field was about two times the concentration needed to fulfill *A. anophagefferens'* uptake requirements (0.25 nM). Therefore these results suggest that the amount of selenium available in WNB did not limit Brown Tide during our sampling. In contrast, the levels of low molecular weight iron were extremely low (<1.4 nM) and within the region of iron-limitation. In fact, the highest levels of bioavailable iron were detected before or during the Brown Tide bloom (Figure 31).
25. Although no significant relationships among the different metal pools measured in the water column of WNB and total chlorophyll were evident (Fig. 33), the temporal distribution of metals suggests that high biomass only occurs in periods when water concentrations of selenium and iron are relatively high (Fig. 34). These results suggest that some phytoplankton species benefit from the availability of these important bioactive trace metals.
26. Summaries of findings from our field campaigns are presented in the attached abstracts prepared by the graduate students partially supported on this contract.

Abstract of Completed Masters Thesis

Impact of Biological Activity on the Size-fractionation of Trace Metals in a Coastal Environment (West Neck Bay, Long Island)

William Kentrup (partially supported by SCDHS contract)

The partitioning of Cu and Fe between particulate, colloidal and soluble pools was measured in the water column of West Neck Bay, Long Island over the course of a phytoplankton bloom followed by a bacterial bloom. Samples were collected on ~5 day intervals from a depth of 1 meter below the surface between April 9 and October 5, 1998. Particulate metal fractions were separated into labile and refractory pools by acid digestion and dissolved fractions were separated into colloidal (1000 NMW to < 0.2 um) and soluble (< 1000 NMW) fractions by cross-flow ultrafiltration. Fluctuations in these pools were compared to changes in chlorophyll a, particulate and dissolved organic matter, and suspended particulate matter concentrations.

The net affect of the bloom on total metal concentration was a significant buildup of Cu associated with DOM accumulation and a removal of Fe via particle stripping. During the phytoplankton bloom, total Cu concentrations increased from 22 nM to 37 nM and total Fe concentrations decreased from 4841 nM to 2523 nM. Decreased levels for both metals were associated with bacterial activity.

Colloidal Cu represented between 61% and 80% of the total Cu pool, and particulate Fe represented between 79% and 99% of total Fe. Changes in fraction-specific metal concentrations, metal accumulation and removal rates, and solid-solution distribution coefficients (log Kd, Kp and Kc) were found to be significantly correlated with chl a concentrations, % POM and with bloom products (especially DON). Fractionation changes led to decreases in all Cu log K values over the course of the bloom, log Kc values being both highest and least affected. For Fe, log Kc values showed the largest drop during the bloom, while log Kp values were most stable.

These findings show how Cu, with low nutrient value and a high affinity for dissolved constituents and Fe, with much higher cellular requirements and high particle reactivity, can exhibit differential fractionation during biological events that are typical of many coastal regions. This, in turn, implies distinct fates for metals that preferentially associate with particulate or dissolved water column constituents. Furthermore, this study points out that the biological condition of an aquatic environment should be considered in models that attempt to characterize trace metal behavior on time scales of weeks to months.

Masters Thesis Abstract

THE IMPORTANCE OF CHEMICAL REACTIONS IN SUBTERRANEAN ESTUARIES: THE CONCENTRATIONS OF TRACE METALS AND NUTRIENTS IN SUBMARINE GROUNDWATER SEEPAGE AT WEST NECK BAY, LONG ISLAND, NEW YORK

Yoko Tsukamoto (partially supported by SCDHS contract)

Recent studies have shown that some coastal aquifers need to be classified as "subterranean estuaries," where seawater mixes with fresh groundwater. Within the estuary, similar chemical reactions observed in surface estuaries (e.g., desorption process) are expected to occur. Although groundwater has been recognized to have a significant impact on coastal environments, most past studies only measured nutrients and trace metal concentrations in inland groundwater, and the chemical reactions in subterranean estuary were not considered. Thus, in order to establish the relative importance of in situ chemical reactions in subterranean estuary, this study has measured dissolved organic matter, inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}), and trace metals (Fe, Mn, Si, Ag, Cd, Cu, Ni, and Zn) in two inland groundwater (26 ft and 8-12 ft deep), intertidal porewater, and seawater.

West Neck Bay, located within the Peconic Estuary at the eastern end of Long Island, was chosen to be the site of study due to the absence of river discharge, its restricted water exchange with the estuary and the occurrence of Brown Tide. Samples were collected using trace metal clean technique in May (the period of high groundwater flow) and October, 2002. Our preliminary results show that conservative mixing between the Bay's water and the groundwater could not account for the organic carbon measured in the intertidal porewater. We are measured the rest of trace metals and inorganic nutrients.

Table 1. Brown Tide Bioassays

Exposures		Time conducted	Success?
• Aldicarb Sulfoxide	***Run I***	5/08/00 - 5/25/00	Y
• Aldicarb Sulfoxide	***Run II***	7/06/00 - 7/23/00	Y
• Aldicarb Sulfone		8/11/00 - 8/30/00	Y
• Aldicarb Sulfone & Sulfoxide		9/22/00 - 10/10/00	Y
• Aldicarb		7/20/01-8/10/01	Y
• WNB Underflow		10/20/00 - 11/04/00	Y
(Groundwater > sediments; "June 2000 West Neck Bay GW")			
• North Flanders Bay Underflow		11/24/00 - 12/11/00	Y
(Groundwater > sediments; "June 2000 Flanders GW")			
• W. Neck Bay Ground Water (Monitoring Well)		1/05/01 - 1/22/01	Y
• Salinity Gradient	*Run I*	2/09/01 - 2/26/01	Y
• Salinity Gradient	*Run II*	3/02/01 – 3/22/01	Y
• N speciation - ammonium		4/20/01-5/3/01	Y
• N speciation – nitrate		6/11/01-6/22/01	Y
• N speciation - urea		7/15/01-8/2/01	Y
• N speciation – glutamic acid		8/3/01 – 8/21/01	N
• Alachlor	***Run I***	6/10/02-6/14/02	N
• Alachlor	*Run II*	6/17/02 - 6/20/02	N
• Alachlor	*Run III*	7/8/02 - 7/20/02	Y
• Alachlor	*Run IV*	8/16/02 - 8/30/02	Y
• Metolachlor	*Run I*	6/21/02 - 7/11/02	N
• Metolachlor	*Run II*	7/12/02 - /24/02	Y
• Metolachlor	*Run III*	9/06/02 - 9/17/02	Y
• Metolachlor	*Run IV*	10/01/02 - 10/09/02	N
• WNB Groundwater	*Run II*	10/18/02 - 11/01/02	Y
• Solvent evaluation	*Run I*	6/28/02 - 7/07/02	Y
• Solvent evaluation	*Run II*	10/15/02 – 10/23/02	Y

Table 1. Brown Tide Bioassays (cont'd.)

Exposures		Time conducted	Success?
• Solvent evaluation	*Run III*	10/28/02 – 11/06/02	Y
• Solvent evaluation	*Run IV*	11/08/02 – 11/18/02	Y
• N speciation – glutamic acid	*Run II*	8/3/01 – 8/21/01	Y
• Groundwater - WNB	*Run I*	6/24/03-6/3-03	Y
("22 May 2002 WNB-Med/land (26ft)")			
• Salinity/nutrient/light testing		7/01/03-07/31/03	Y
• Groundwater - WNB	*RunII*	08/01/03-08/11/03	Y
("22 May 2002 WNB-Med/land (26ft)")			
• Porewater - WNB	*Run I*	05-22-03—6-05-03	Y
("22 May 2002 WNB/Shallow Intertidal")			
• Porewater - WNB	*Run II*	08-16-03—8-29-03	Y
("22 May 2002 WNB/Shallow Intertidal")			
• Porewater - WNB	*Run I*	09-03-03-09-16-03	Y
("22 May 2002 WNB/Shallow Intertidal Piezom")			
(poss. contamination/Repeat in 3 species only and 4 percent GW/not enough GW sample)			
• Porewater - WNB	*Run II*	12-03-03-12-15-03	Y
("22 May 2002 WNB/Shallow Intertidal Piezom")			
• Seawater – WNB	*Run I*	10-24-03-11-06-03	Y
("22 May 2002 WNB/Seawater")			
• Particulate Phosphate		11-24-03	N
• In vivo fluorescence/extracted chlorophyll a		10-24-03	Y

Archived samples that have not been tested:

5-22-00 Shinnecock Seawater

11-2-00 West Neck Bay GW

11-4-00 Fish Cove Seawater

10-9-02 GW SI 26ft

10-09-02 GW 8-12ft

10-09-02 SW WNB

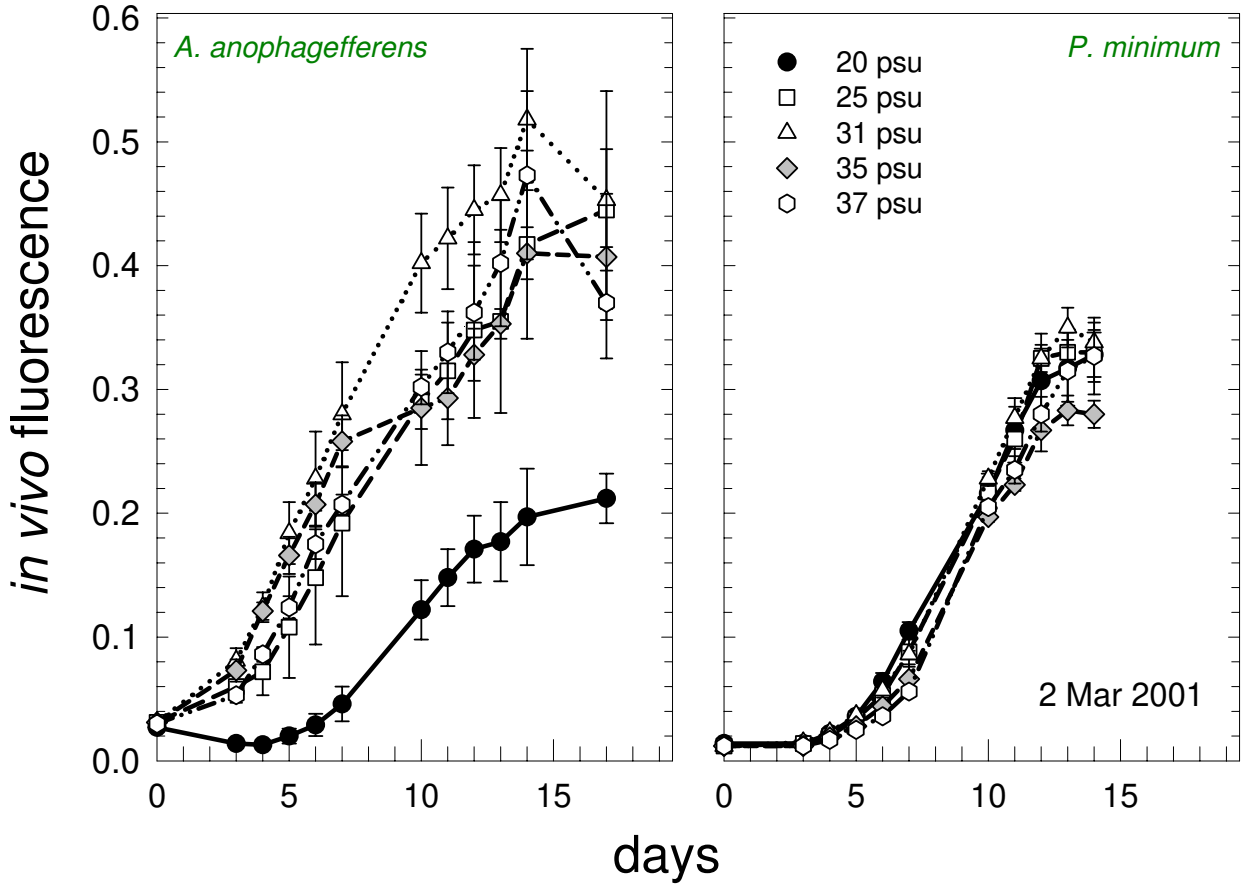

Fig. 1. Growth curves of *Aureococcus anophagefferens* and *Prorocentrum minimum* exposed to varying salinities under nutrient-replete conditions in BT medium. Cultures were maintained at 20° C on a 14:10 light:dark cycle, illuminated by a bank of 6 x 20W fluorescent lights that provided 48-63 µmol quanta m^{-2} sec^{-1} to culture flasks.

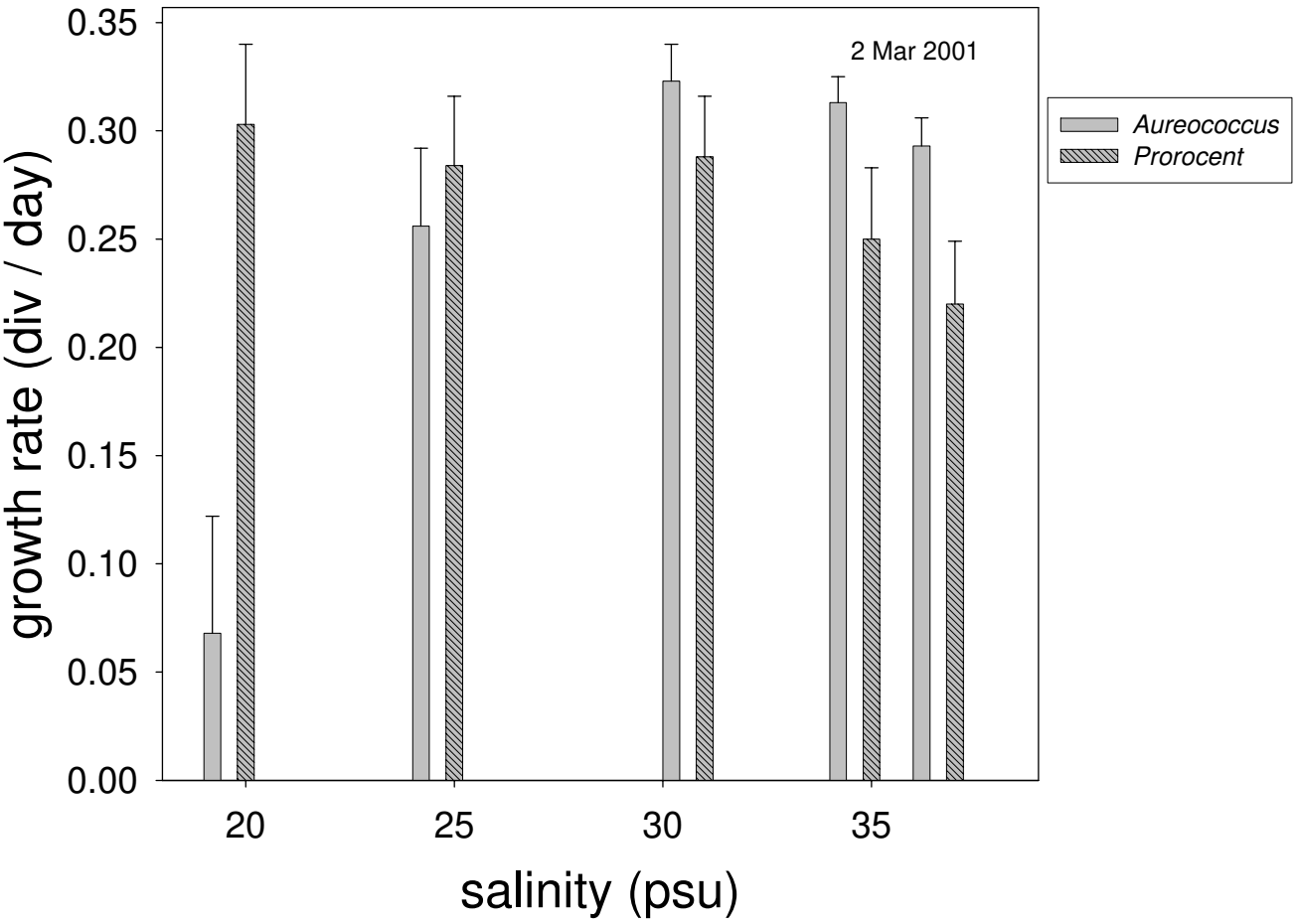

Fig. 2. Growth rate constants, μ in $N = N_0 e^{\mu t}$, calculated from linear regressions of exponential phase of transformed growth curves from Fig. 1 [ln (N/N_0) vs time] for *A. anophagefferens* and *P. minimum*. Growth rate is estimated from regression slope of $\geq$ 5 points and error bars are standard error of the slope (SE). In all cases, r2 $\geq$ 0.90

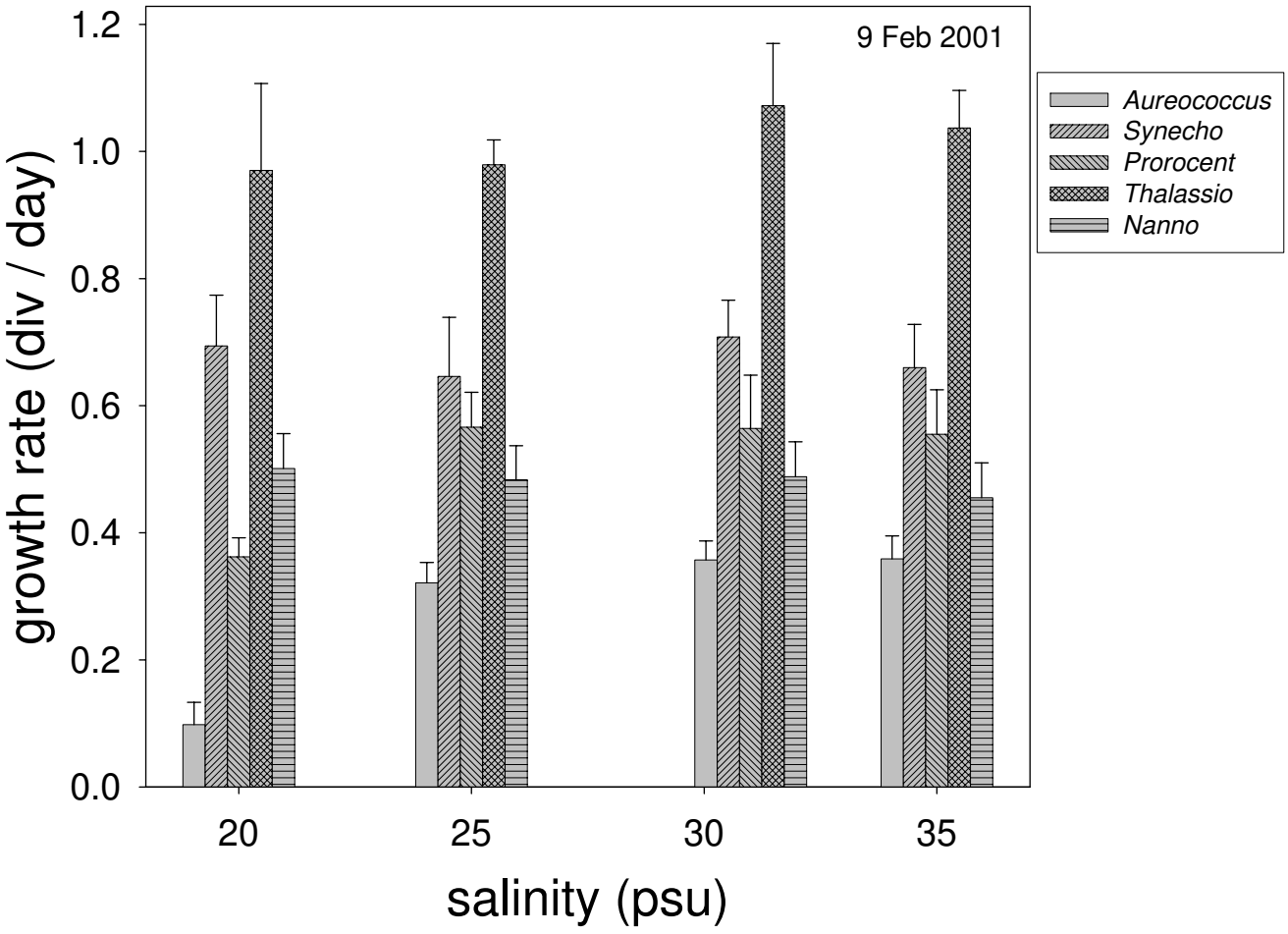

Fig. 3. Response of growth rate constants of all five microalgal species to variations in salinity.

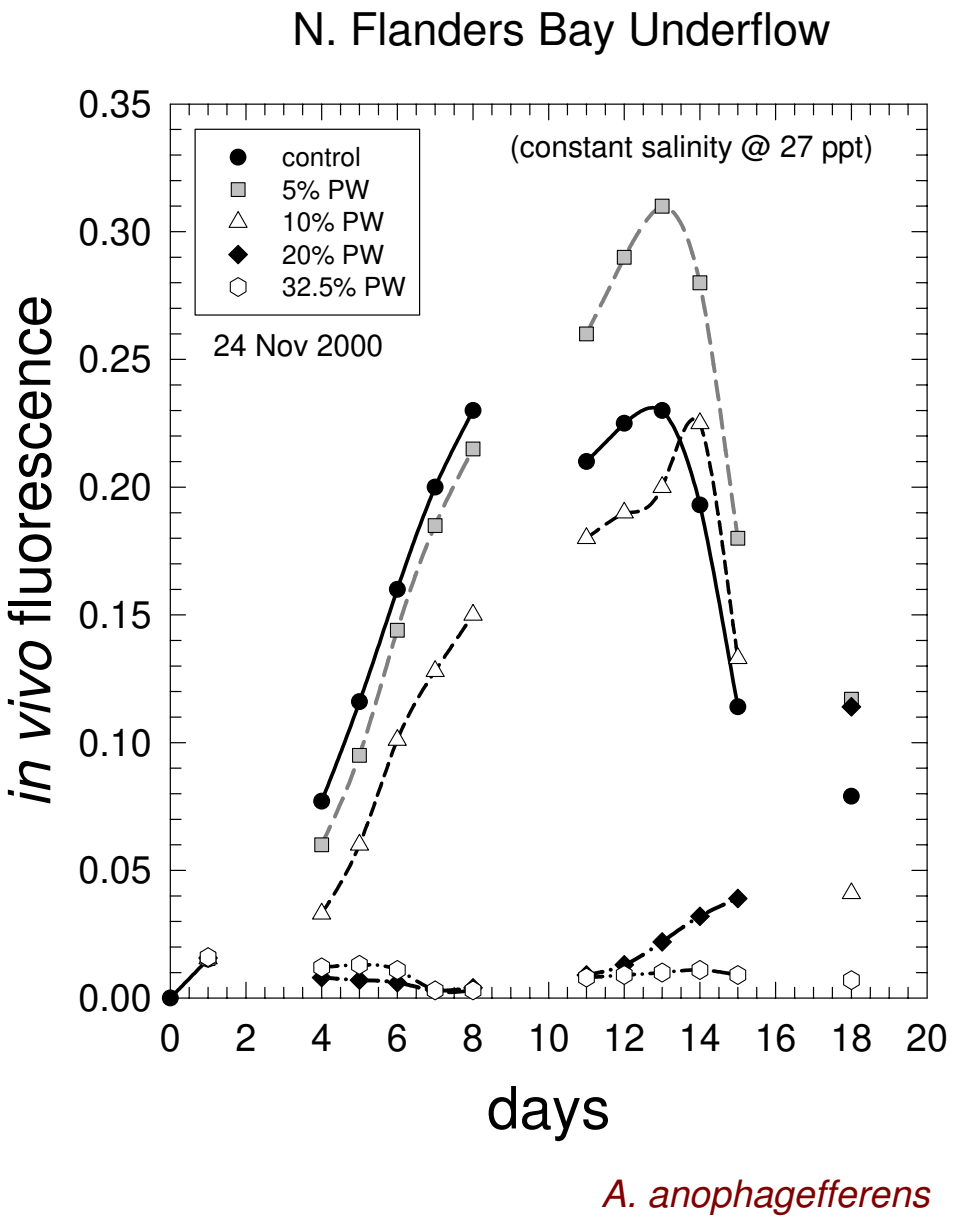

Fig. 4. Growth curves of *Aureococcus anophagefferens* exposed to varying proportions of N. Flanders Bay submarine groundwater discharge under nutrient-replete conditions in BT medium. All treatments exposed to equivalent salinities, added nutrients and light regimes. Conditions same as described in Fig.1.

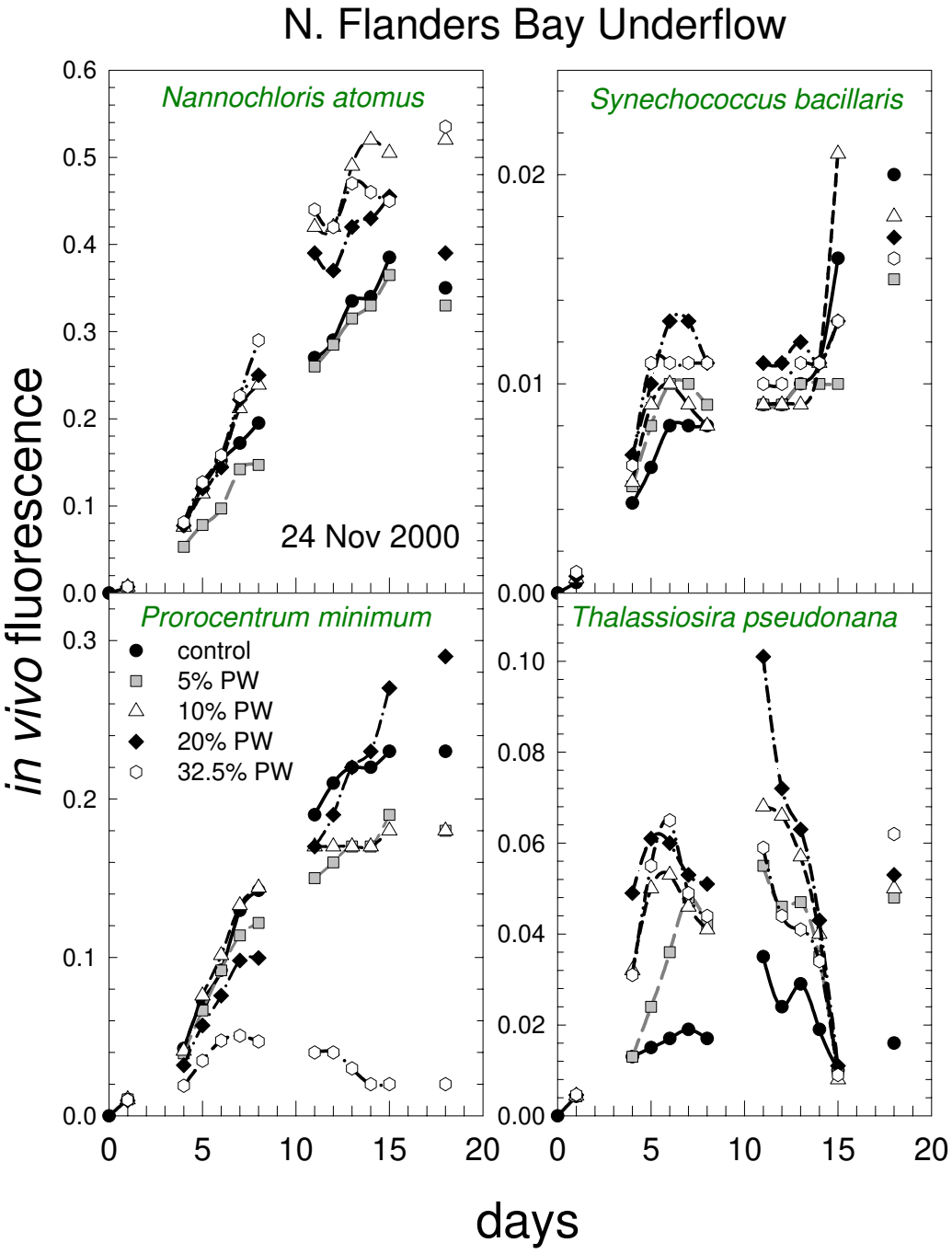

Fig. 5. Growth curves of *Nannochloris atomus, Synechococcus bacillaris, Prorocentrum minimum and Thalassiosira pseudonana* exposed to varying proportions of N. Flanders Bay submarine groundwater discharge under nutrient-replete conditions in BT medium.

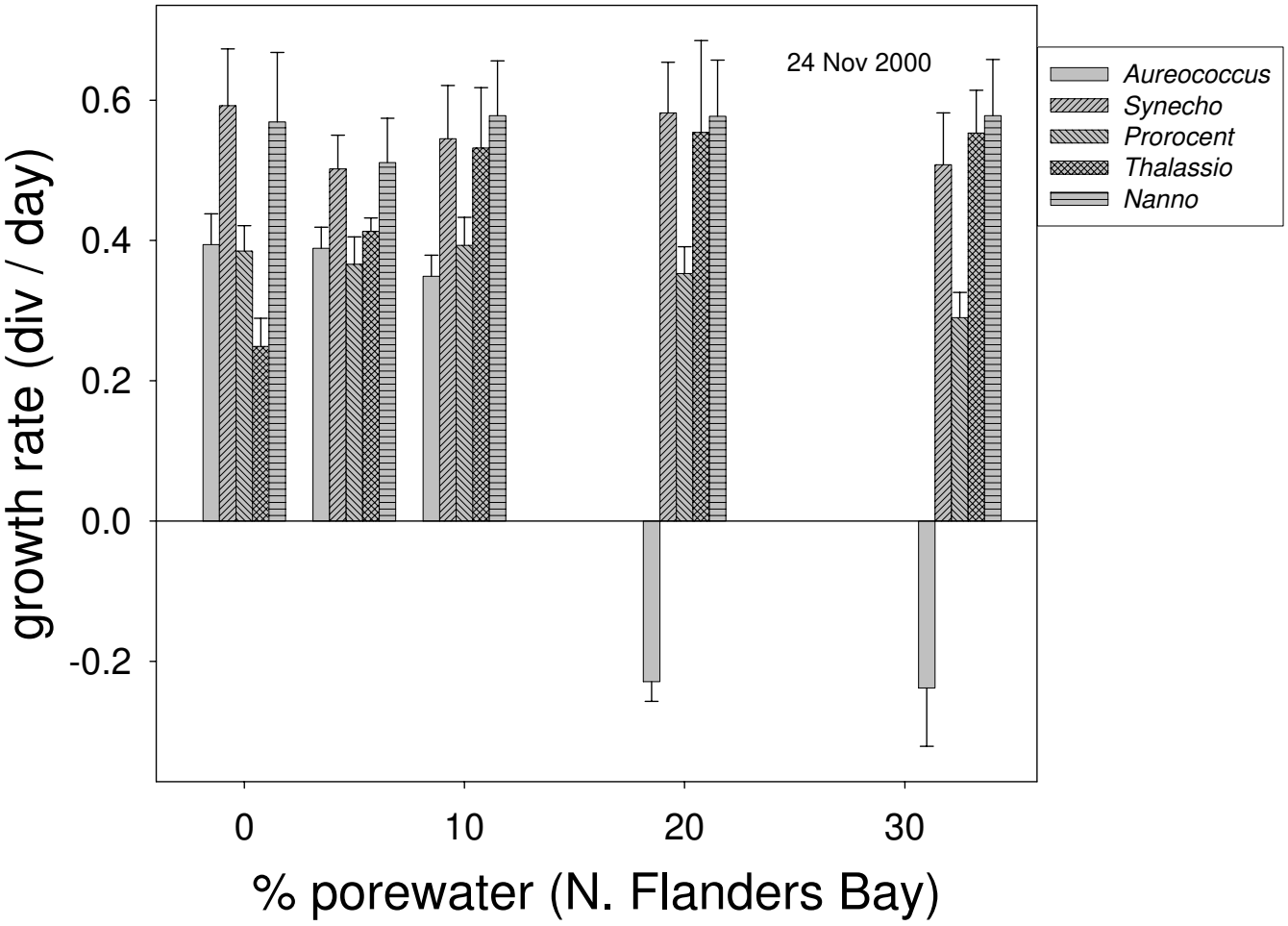

Fig. 6. Growth responses of all five microalgal species to variations in proportions of N. Flanders Bay submarine groundwater discharge. Rates calculated from Figs. 4 & 5.

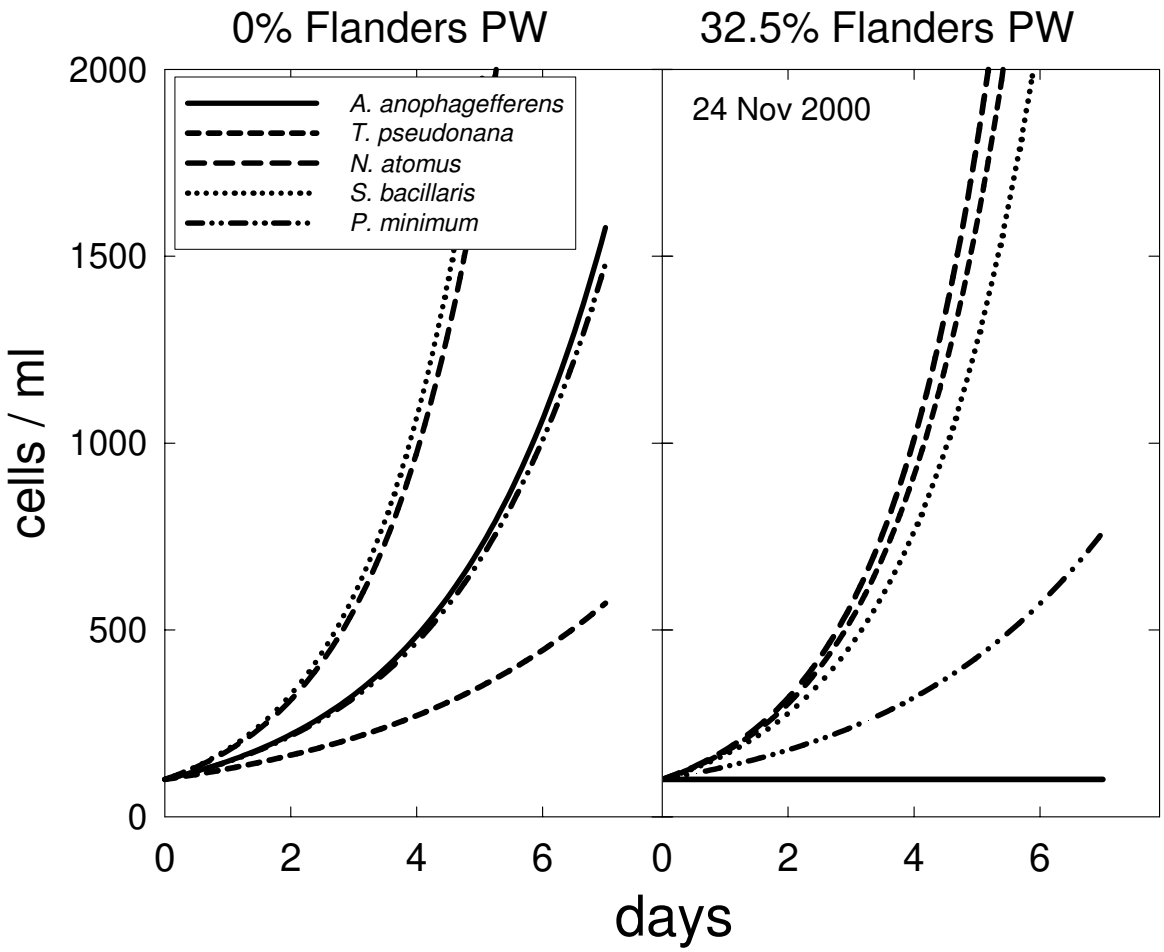

Fig. 7. Growth performance simulations for five species exposed to seawater diluted to 27 psu with aged tapwater only (0% Flanders PW) or completely with submarine groundwater discharge (32.5% Flanders PW). Model assumes that concentrations of each species is 100 cells ml^{-1} at day 0 and nutrient concentrations remain constant. Growth rates are applied to the logistic equation.

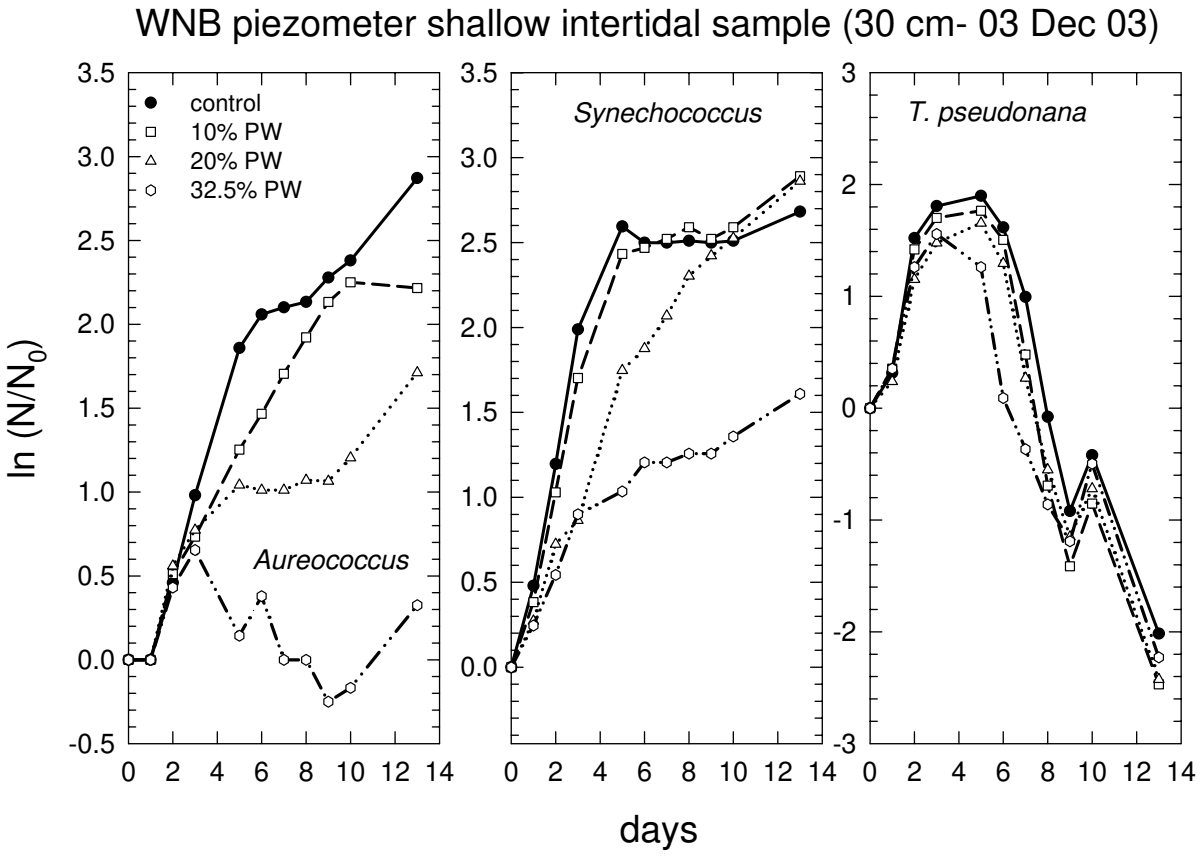

Fig. 8. Natural log-transformed growth curves of *A. anophagefferens*, *S. bacillaris and Thalassiosira pseudonana* exposed to varying proportions of submarine groundwater underflow from the shallow intertidal of West Neck Bay. Experimental conditions same as those described for Figs. 4-6.

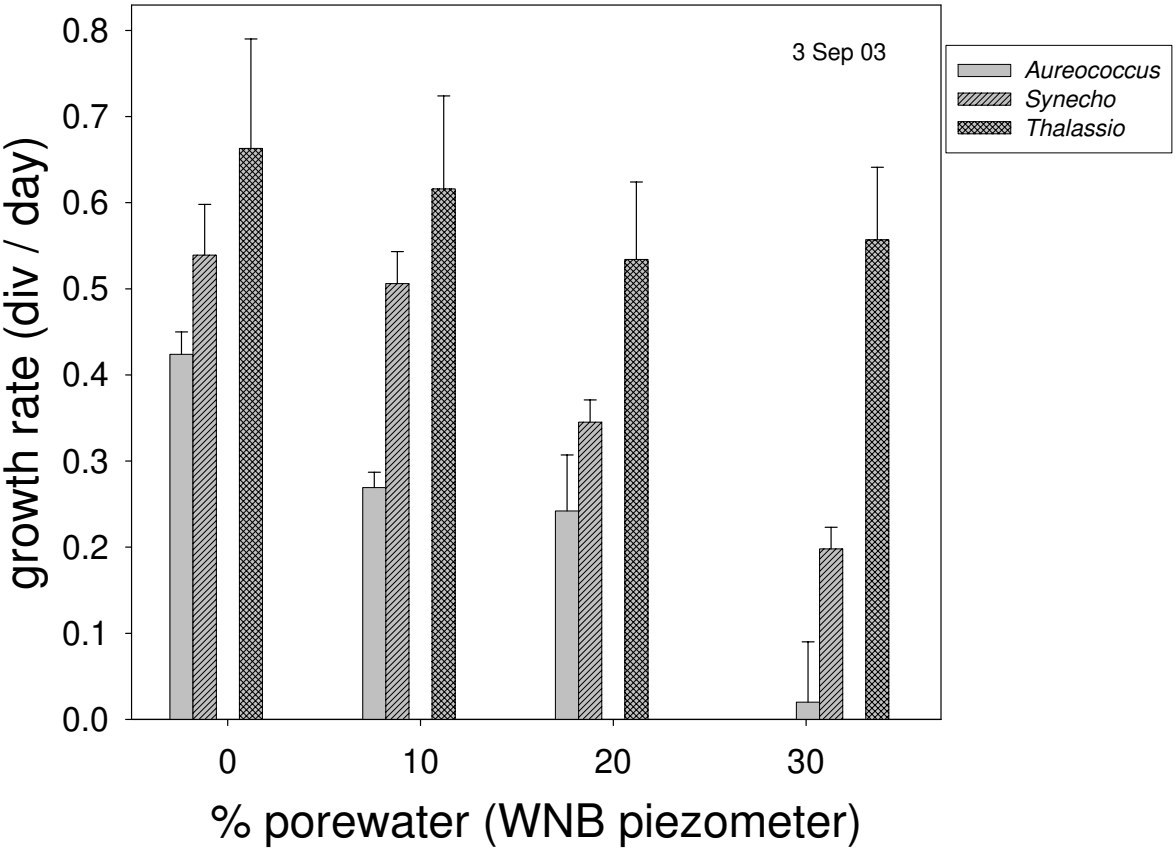

Fig. 9. Growth responses of three microalgal species to variations in proportions of West Neck Bay submarine groundwater discharge held at constant salinity (27 psu). Rates calculated from data comparable to Fig. 8. Sample water collected on 22 May 2002.

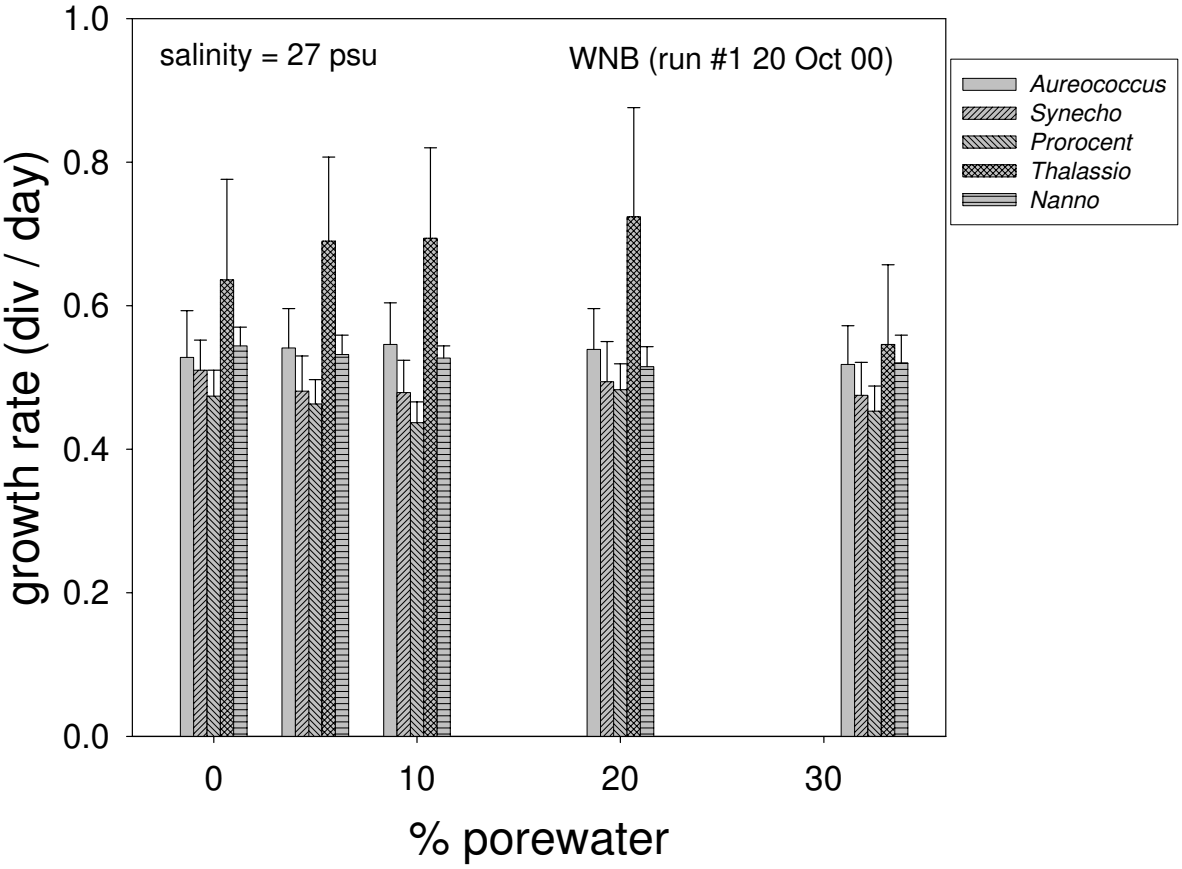

Fig. 10. Growth responses of all microalgal species to variations in proportions of West Neck Bay submarine groundwater discharge held at constant salinity (27 psu). Sample water collected in June 2000.

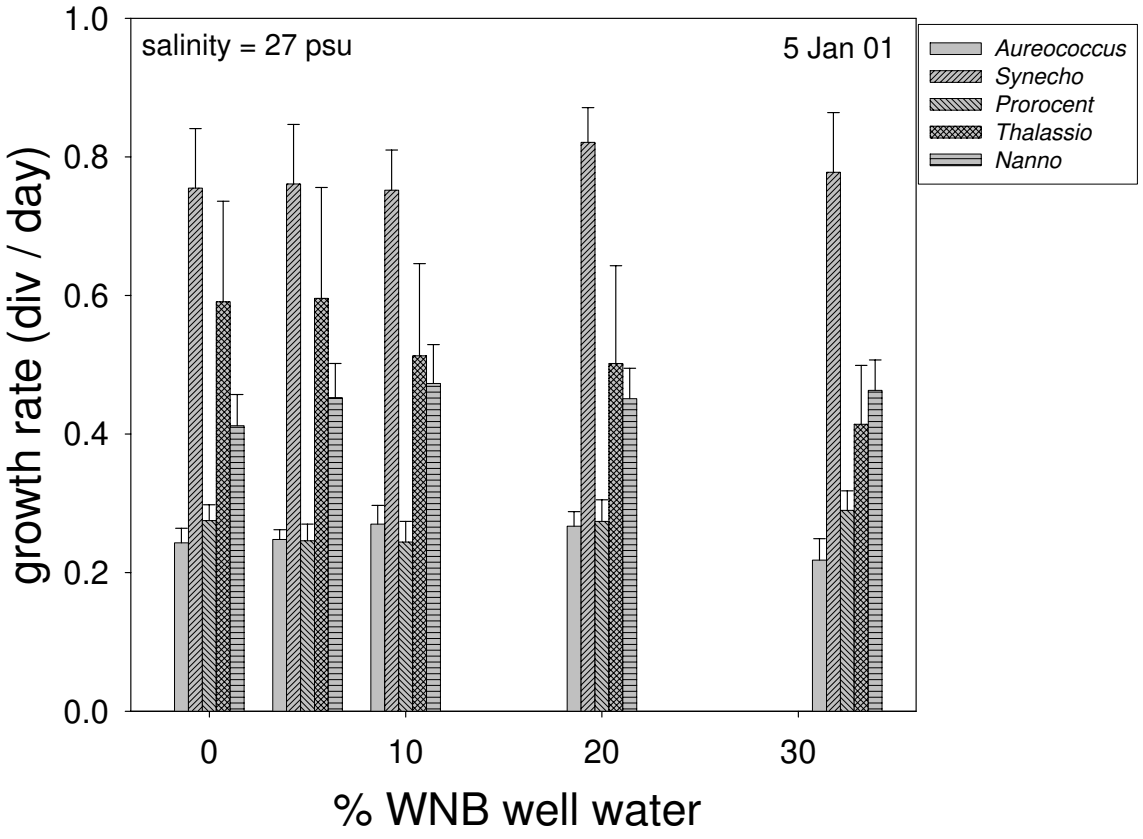

Fig. 11. Growth responses of all microalgal species to variations in proportions of groundwater collected from monitoring well close to West Neck Bay. Sample water collected in June 2000.

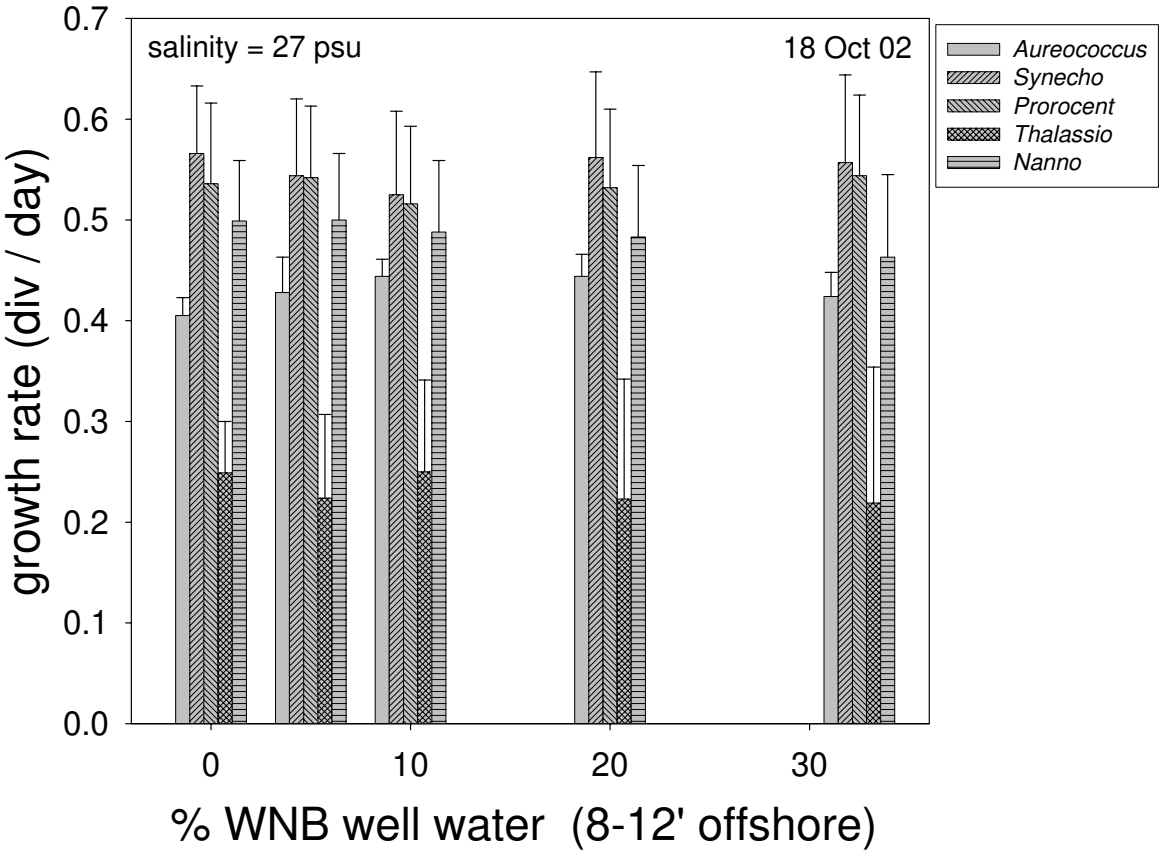

Fig. 12. Growth responses of all microalgal species to variations in proportions of groundwater collected from monitoring well close to West Neck Bay. Sample water collected in June 2000.

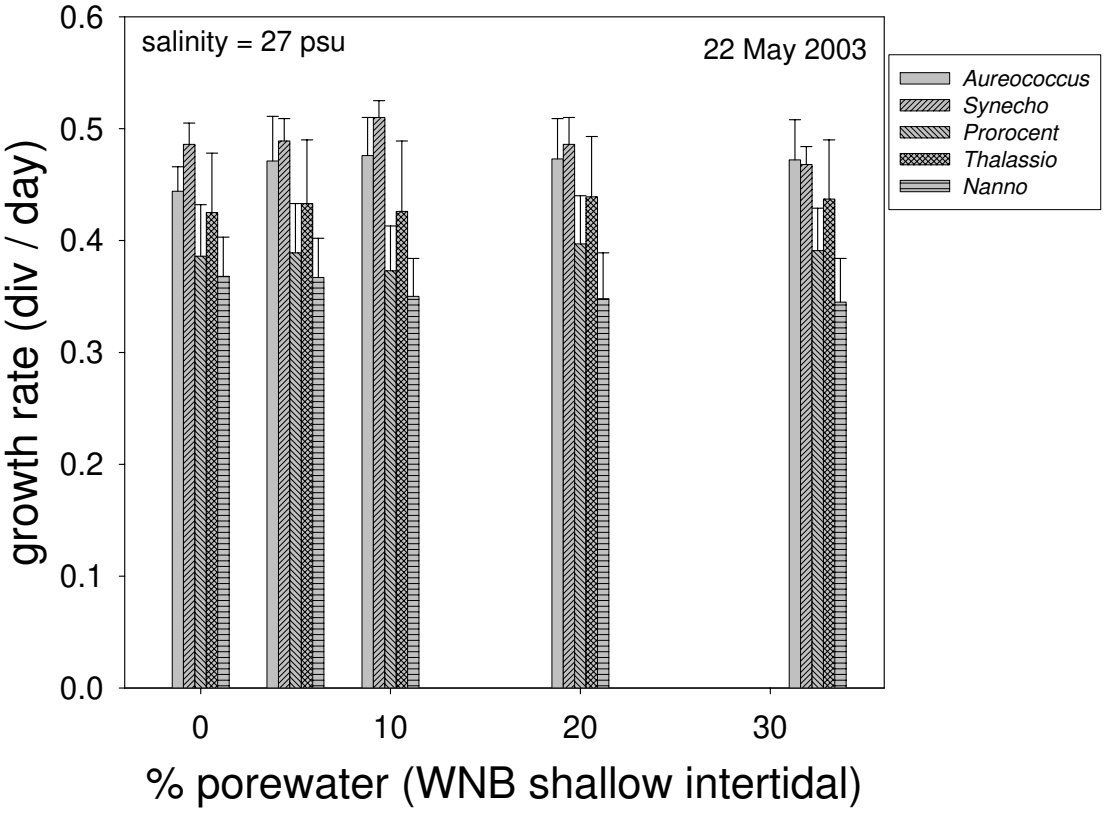

Fig. 13. Growth responses of all microalgal species to variations in proportions of porewater collected from shallow intertidal of West Neck Bay. Sample water collected in 22 May 2002.

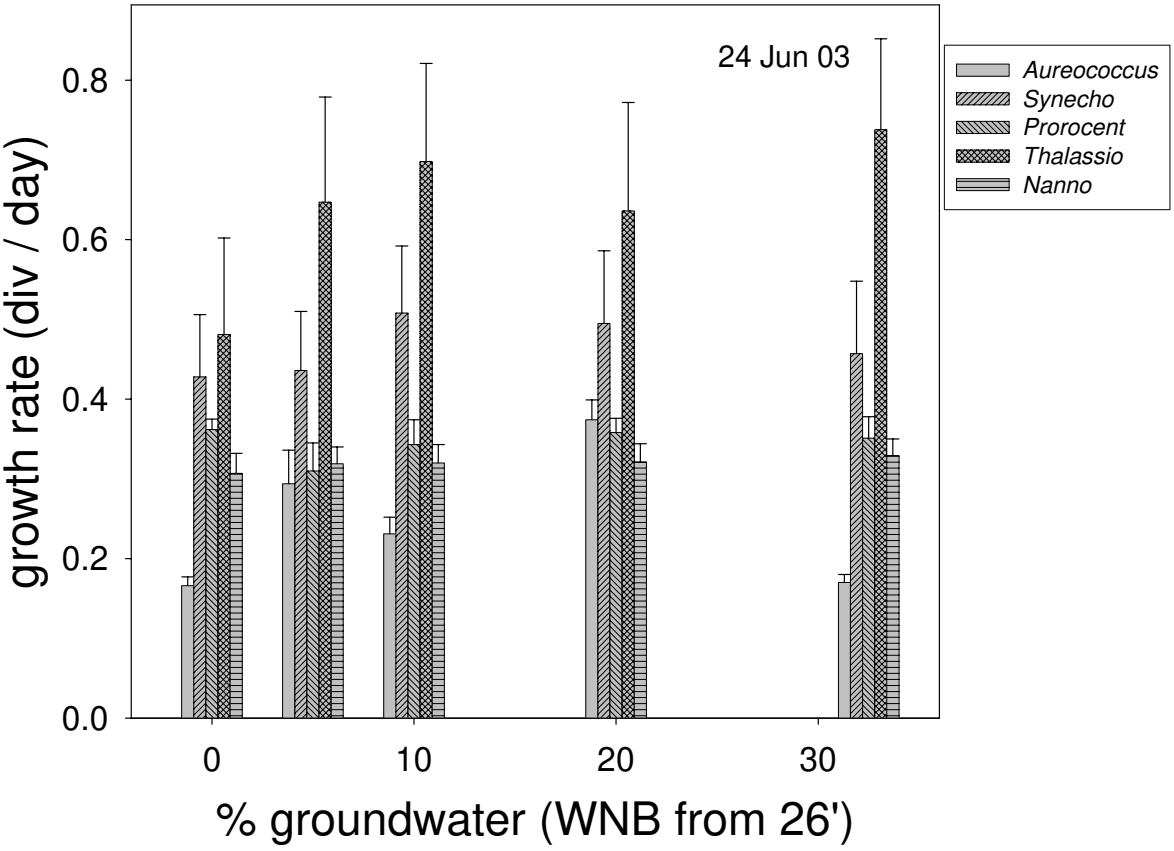

Fig. 14. Growth responses of all microalgal species to variations in proportions of groundwater collected close to West Neck Bay. Sample water collected in 22 May 2002.

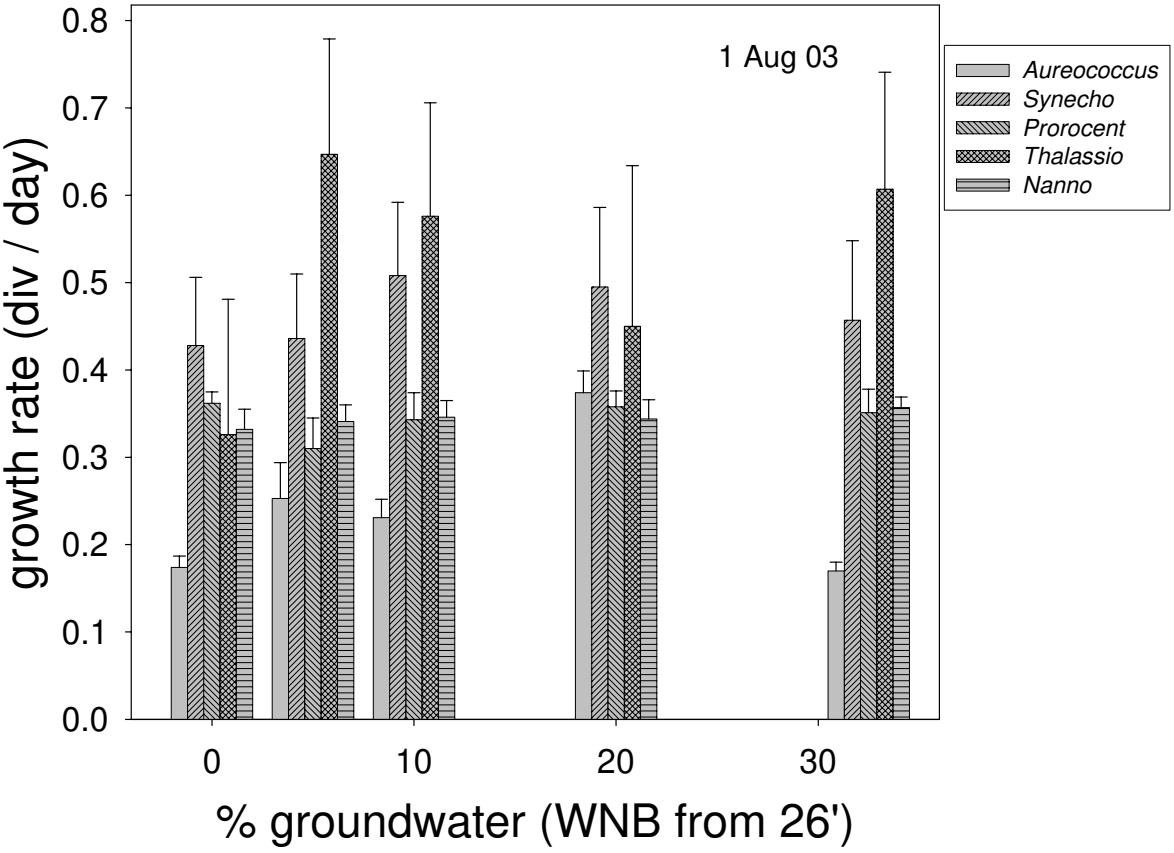

Fig. 15. Growth responses of all microalgal species to variations in proportions of groundwater collected close to West Neck Bay. Run II. Sample water collected in 22 May 2002.

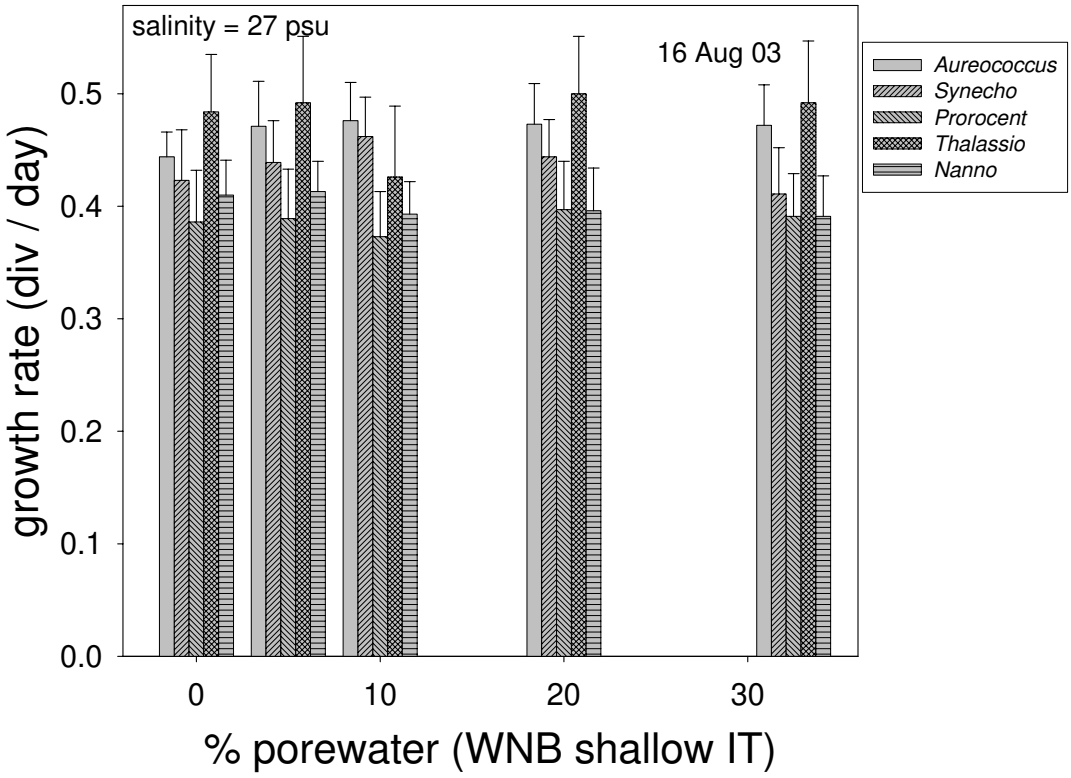

Fig. 16. Growth responses of all microalgal species to variations in proportions of porewater collected in shallow intertidal of West Neck Bay. Run II. Sample water collected in 22 May 2002.

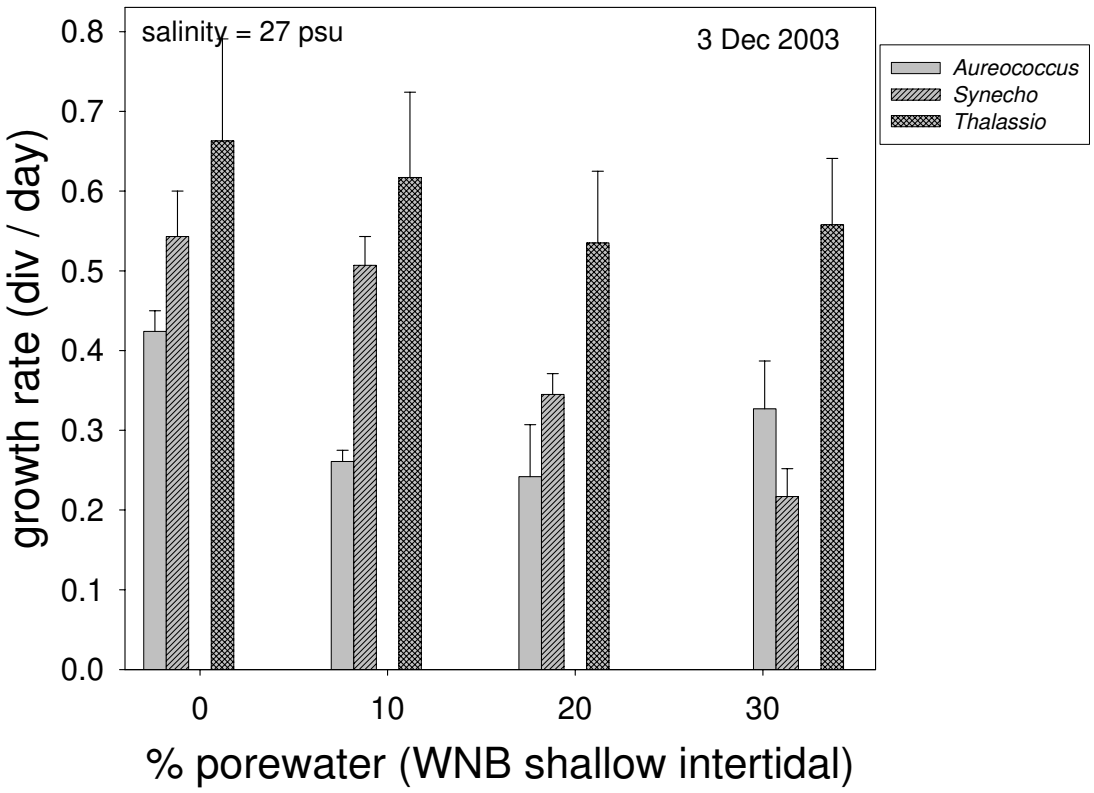

Fig. 17. Growth responses of all microalgal species to variations in proportions of porewater collected in shallow intertidal of West Neck Bay. Run II. Sample water collected in 22 May 2002.

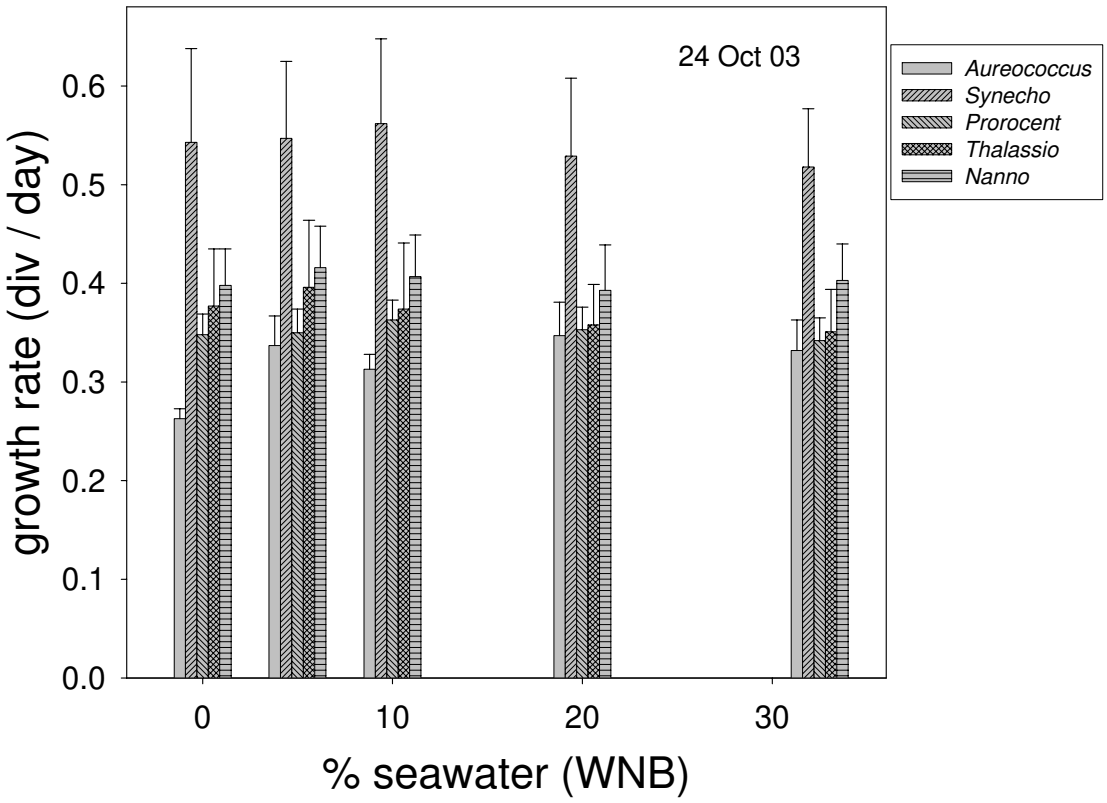

Fig. 18. Growth responses of all microalgal species to variations in proportions of baywater collected from West Neck Bay. Sample water collected in 22 May 2002.

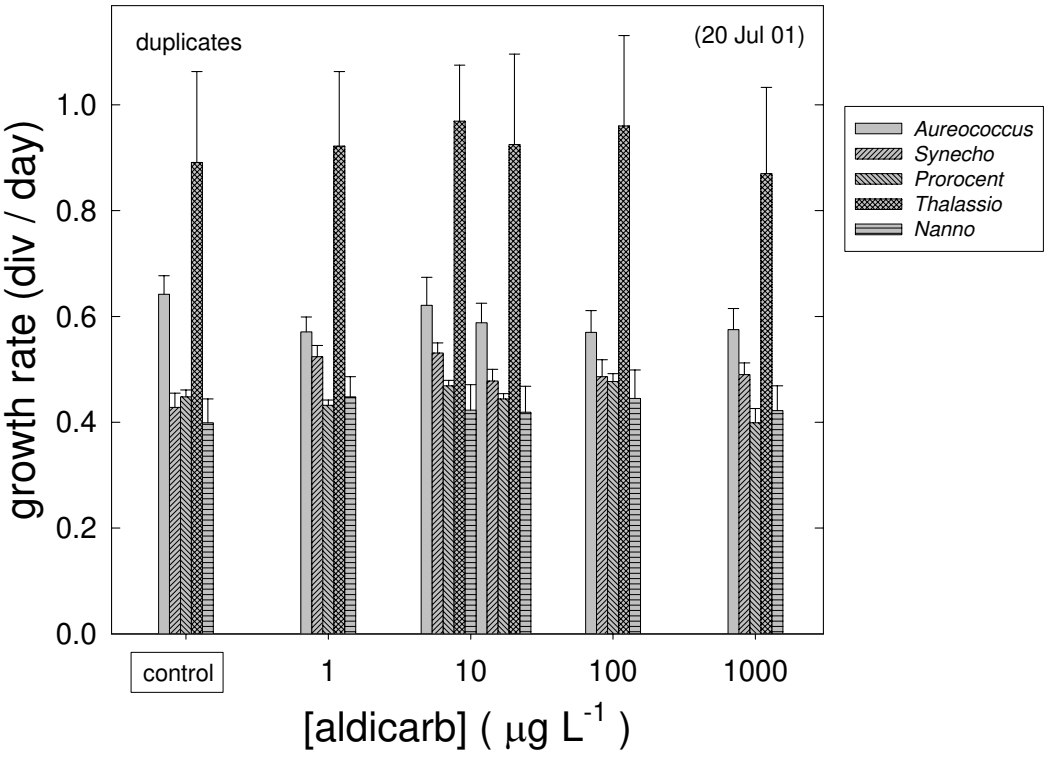

Fig. 19. Growth responses of all microalgal species to variations in exposures to the pesticide, Aldicarb. All incubations conducted in duplicate.

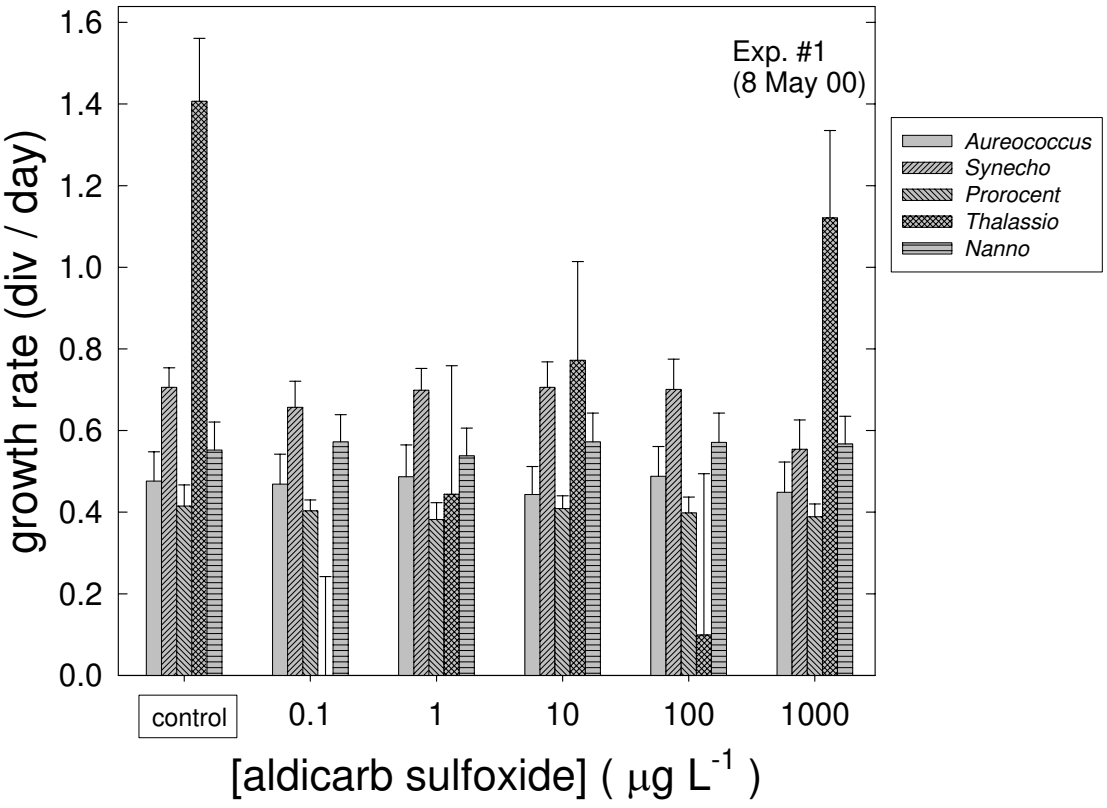

Fig. 20. Growth responses of all microalgal species to variations in exposures to the pesticide metabolite, Aldicarb sulfoxide, Trial #1.

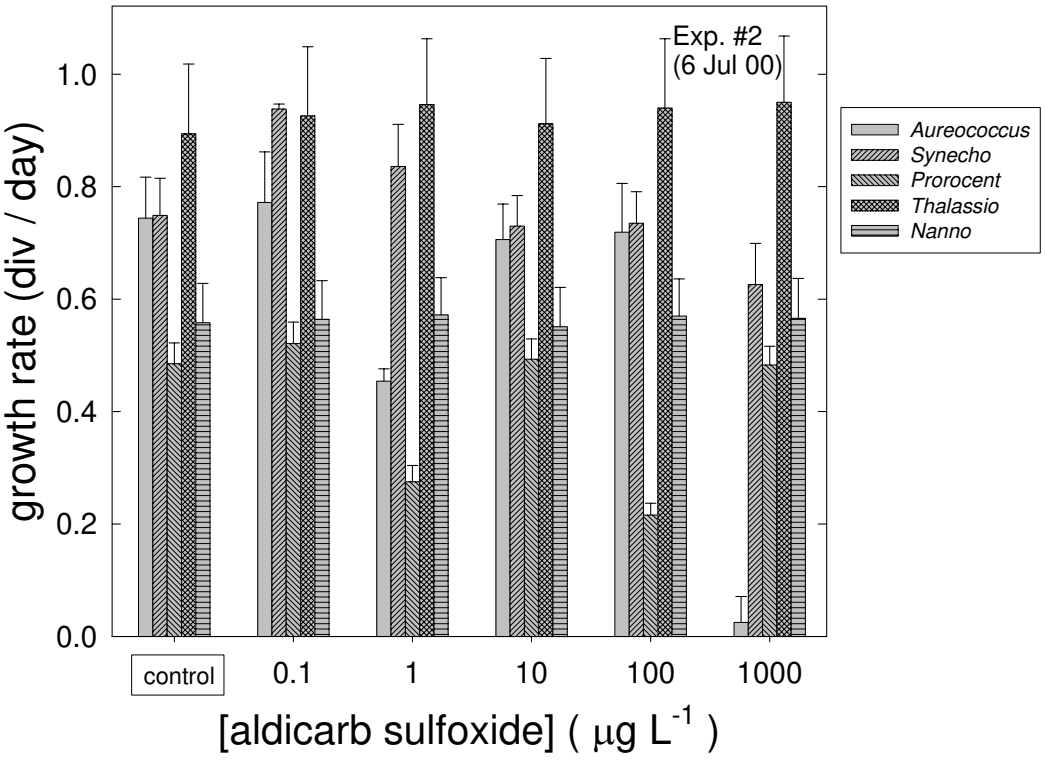

Fig. 21. Growth responses of all microalgal species to variations in exposures to the pesticide metabolite, Aldicarb sulfoxide, Trial #2.

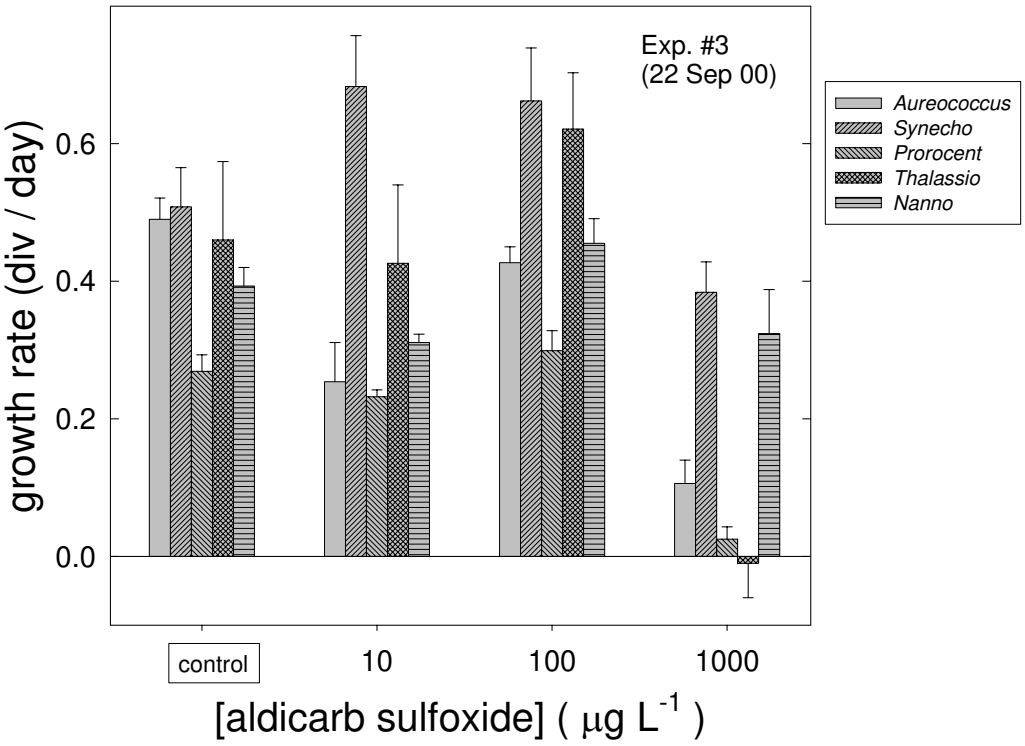

Fig. 22. Growth responses of all microalgal species to variations in exposures to the pesticide metabolite, Aldicarb sulfoxide, Trial #3.

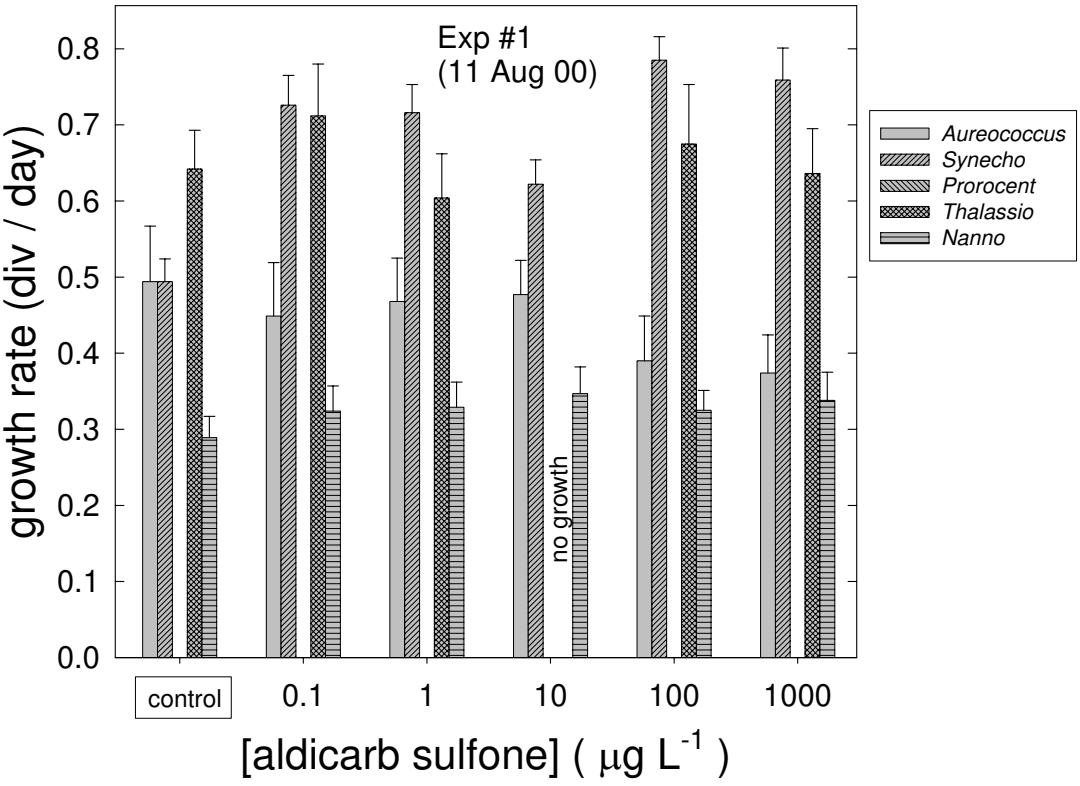

Fig. 23. Growth responses of all microalgal species to variations in exposures to the pesticide metabolite, Aldicarb sulfone, Trial #1.

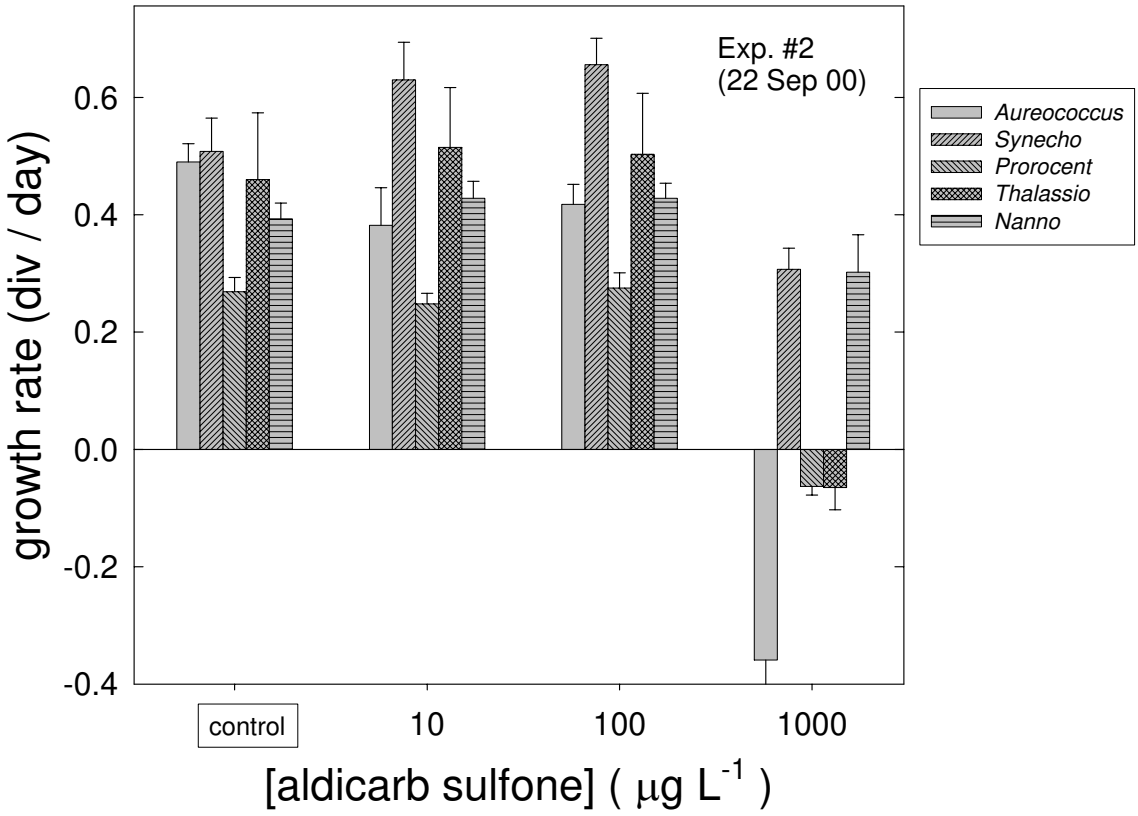

Fig. 24. Growth responses of all microalgal species to variations in exposures to the pesticide metabolite, Aldicarb sulfone, Trial #2.

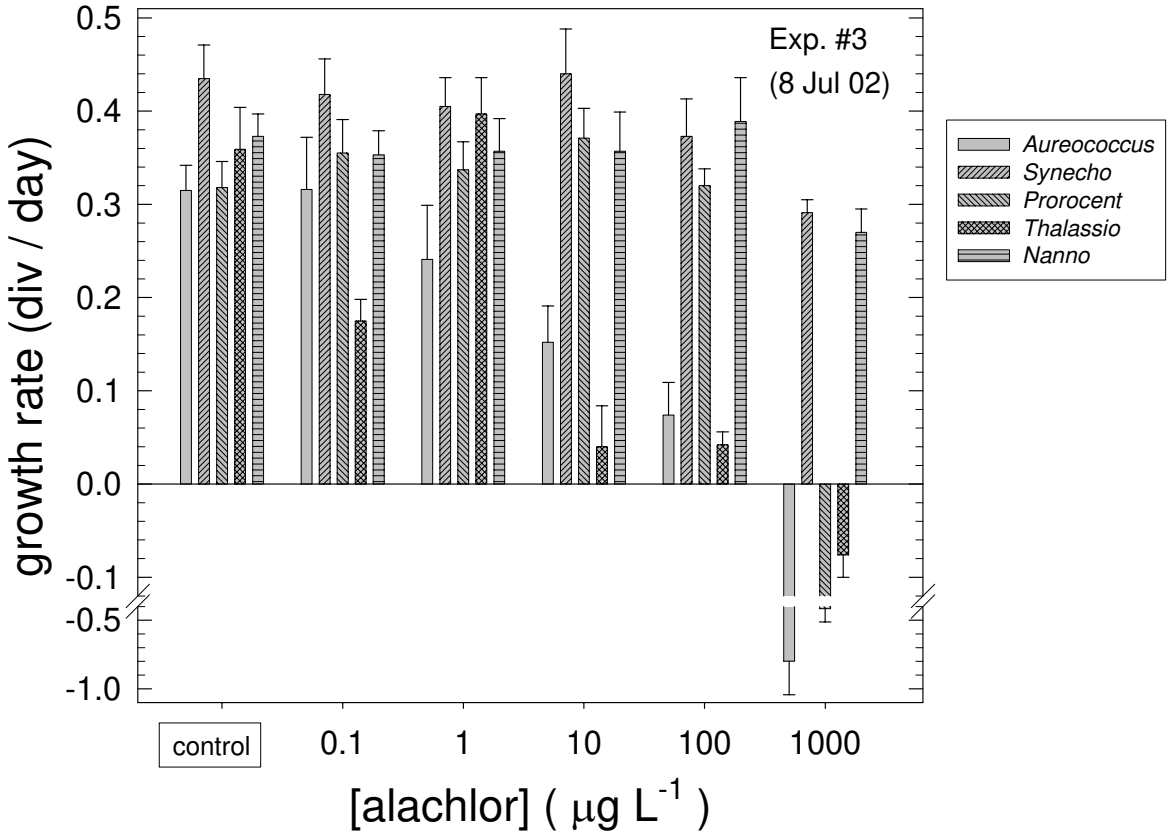

Fig. 25. Growth responses of all microalgal species to variations in exposures to the pesticide, Alachlor Trial #3.

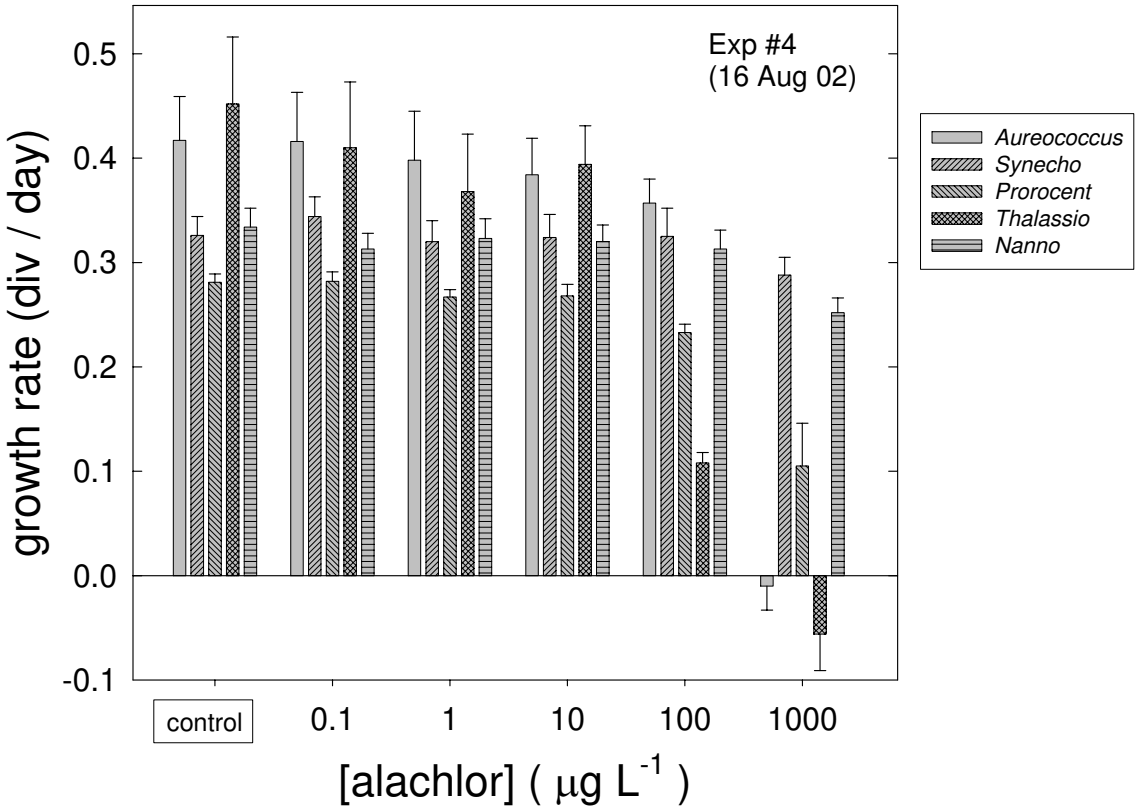

Fig. 26. Growth responses of all microalgal species to variations in exposures to the pesticide, Alachlor Trial #4.

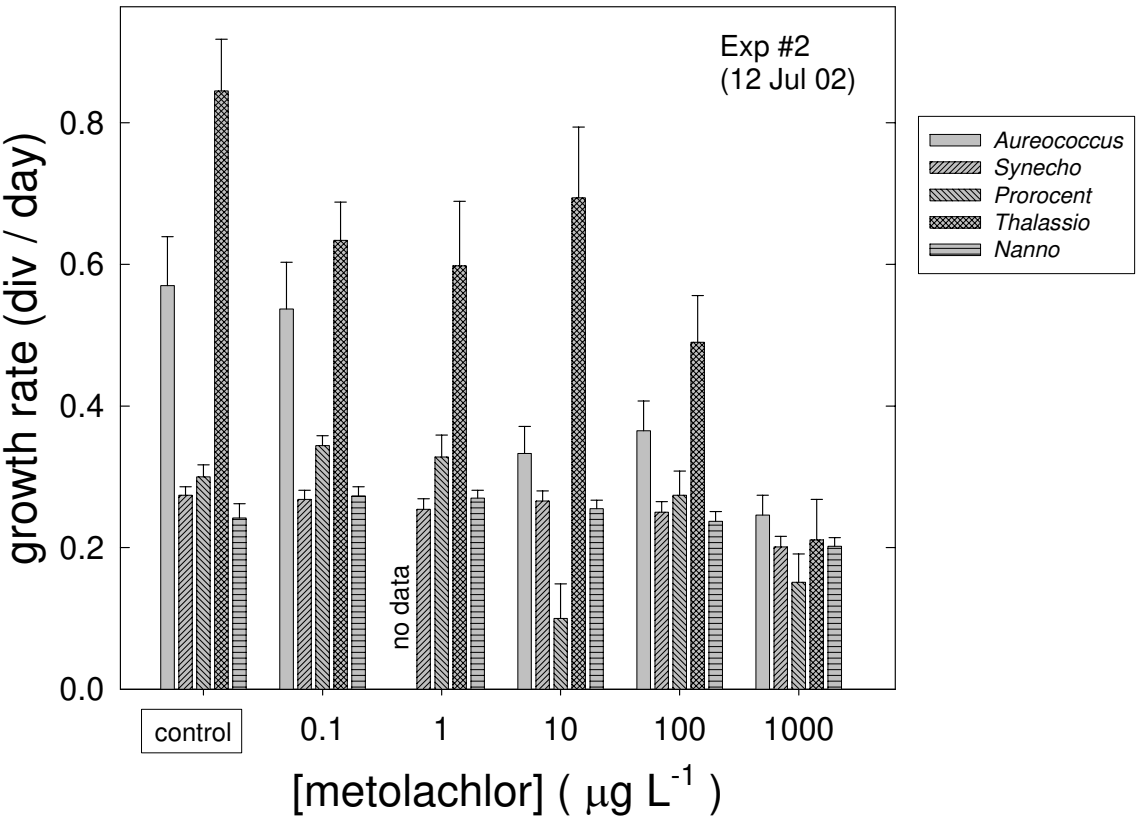

Fig. 27. Growth responses of all microalgal species to variations in exposures to the pesticide, Metolachlor Trial #2.

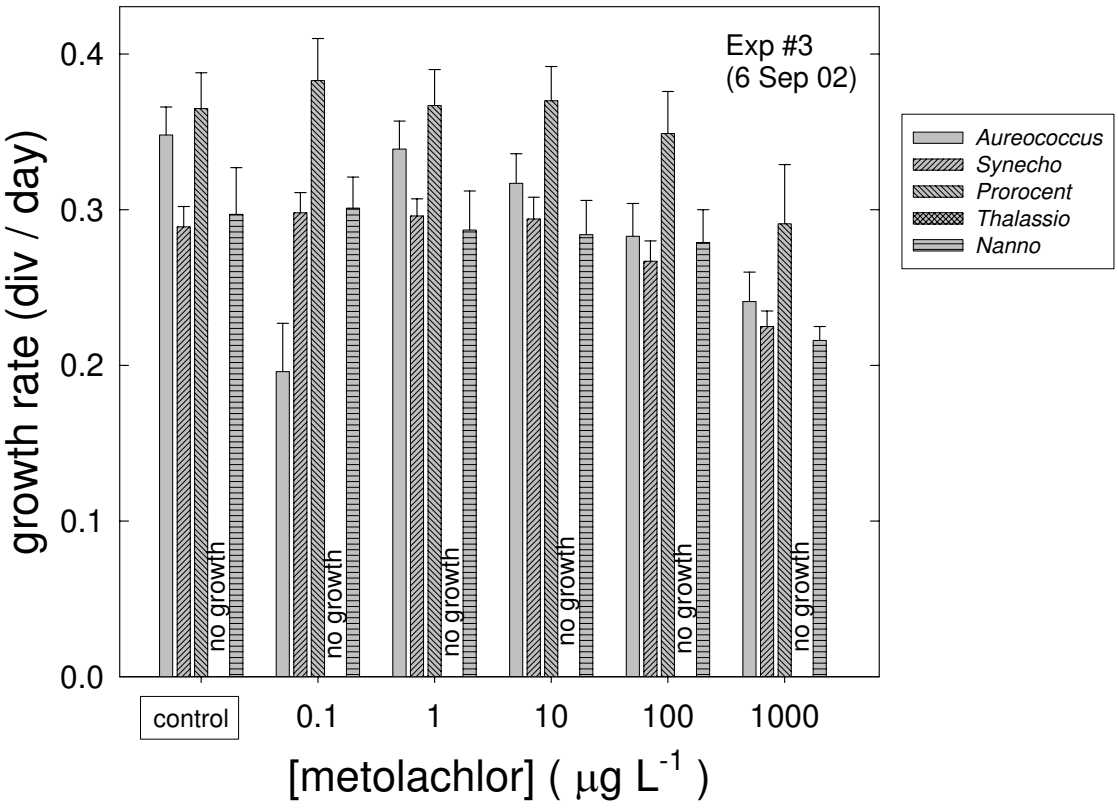

Fig. 28. Growth responses of all microalgal species to variations in exposures to the pesticide, Metolachlor Trial #3.

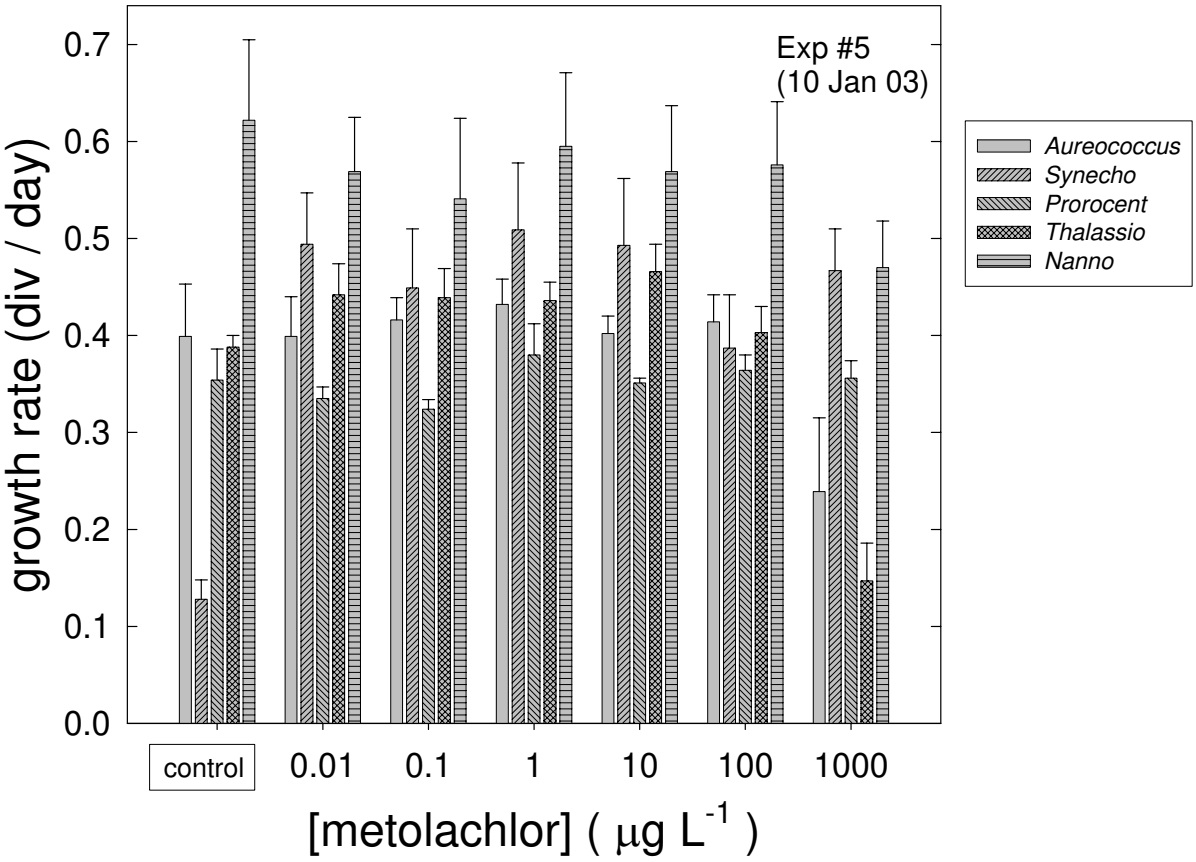

Fig. 29. Growth responses of all microalgal species to variations in exposures to the pesticide, Metolachlor Trial #5.

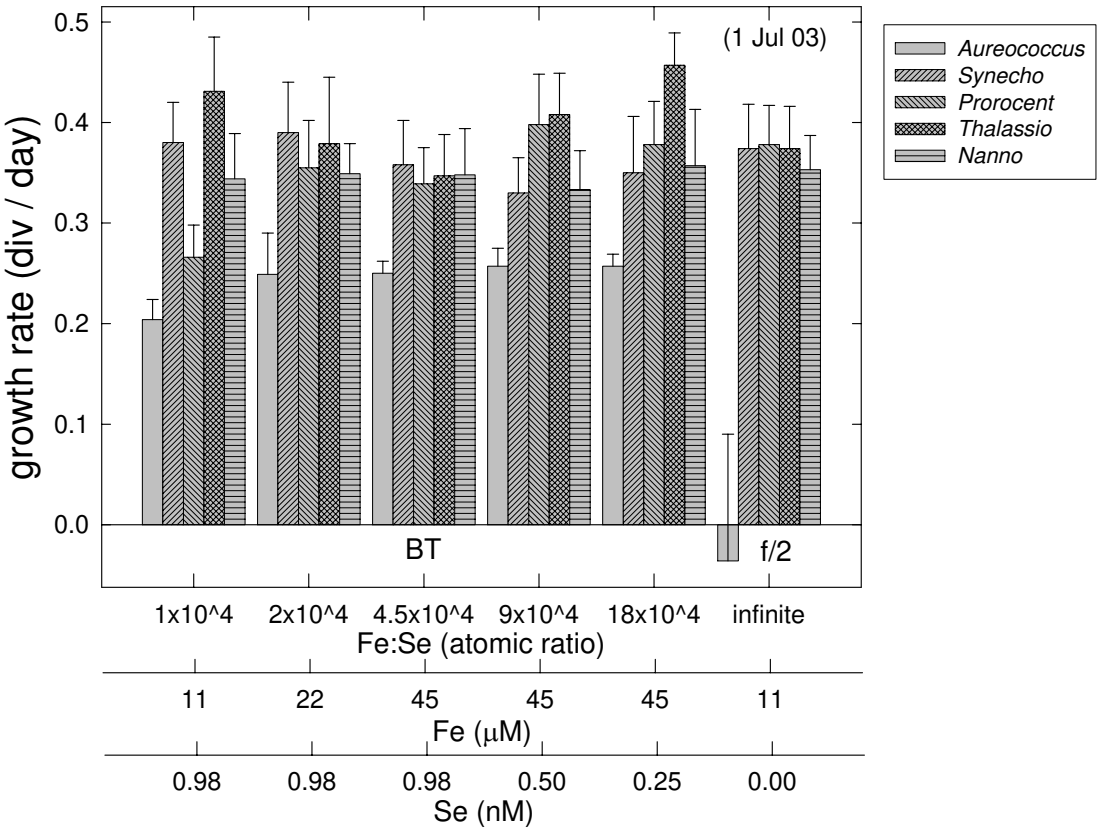

Fig. 30. Growth responses of all microalgal species to variations in exposures to Fe and Se. Experiment designed to evaluate responses to f/2 and BT media and media with intermediate Fe and Se compositions.

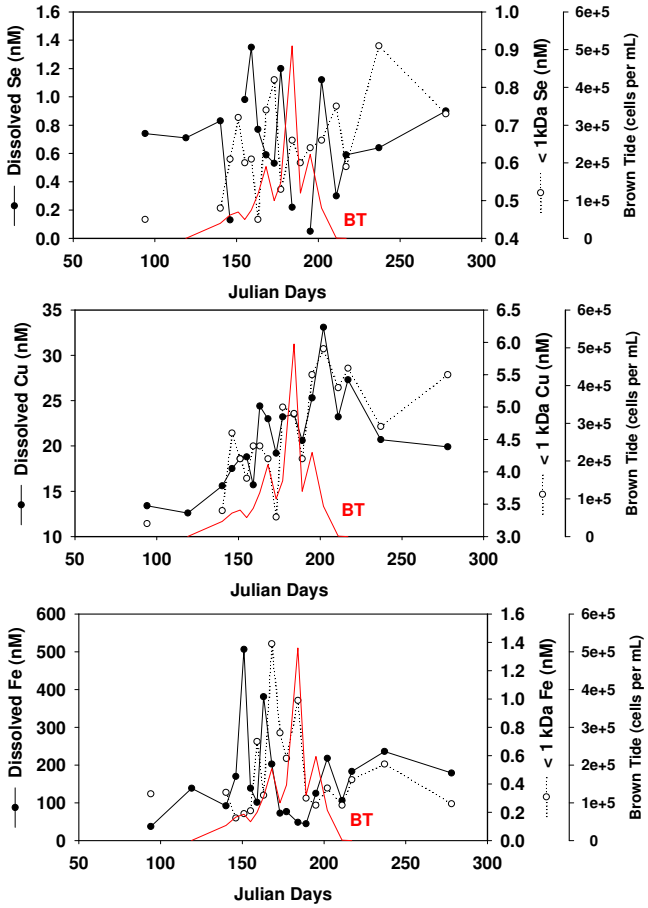

Fig. 31. Temporal distribution of dissolved and low molecular weight (< 1 kDa) selenium, iron and copper measured in surface waters of West Neck Bay in 1998. This figure also shows the distribution of Brown Tide cells.

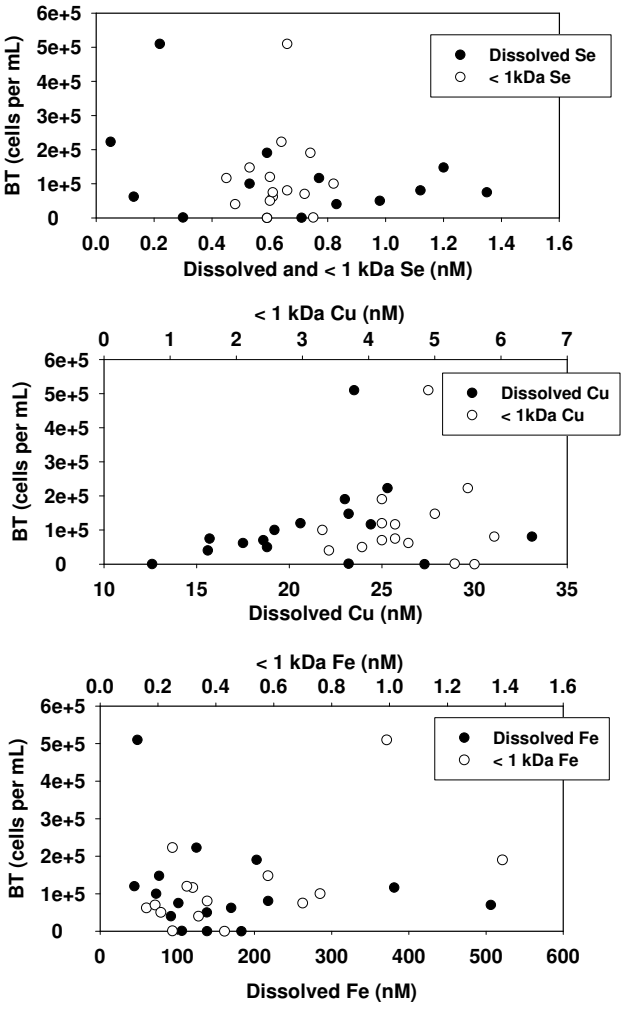

Fig. 32. Brown Tide cells versus dissolved and low molecular weight selenium, copper and iron.

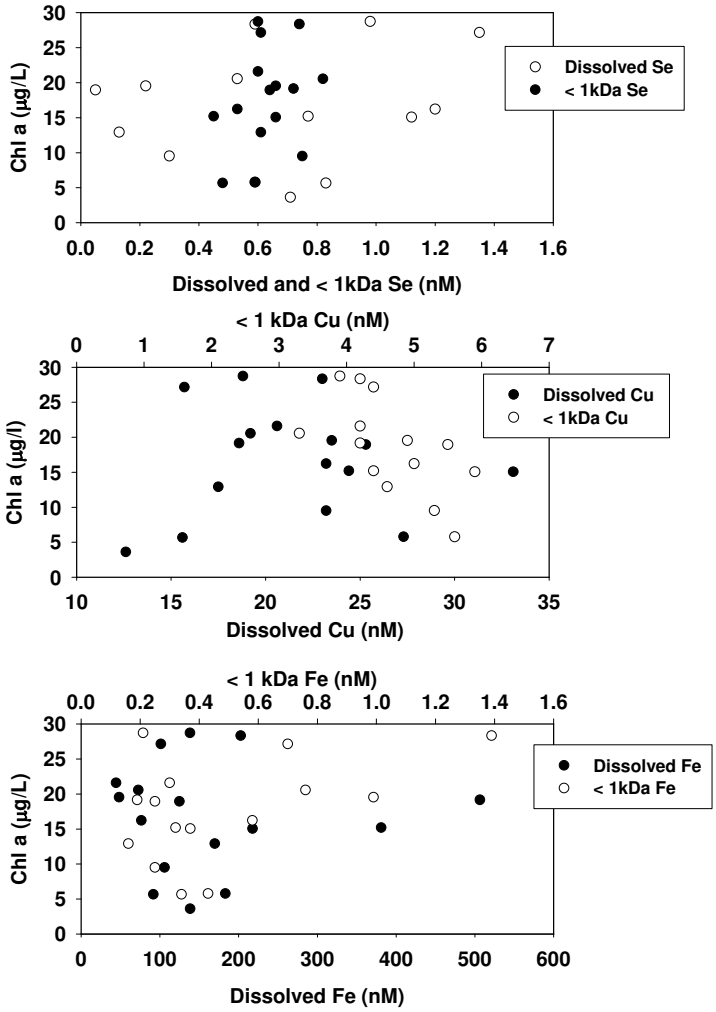

Fig. 33. Total chlorophyll a versus dissolved and low molecular weight selenium, copper and iron.

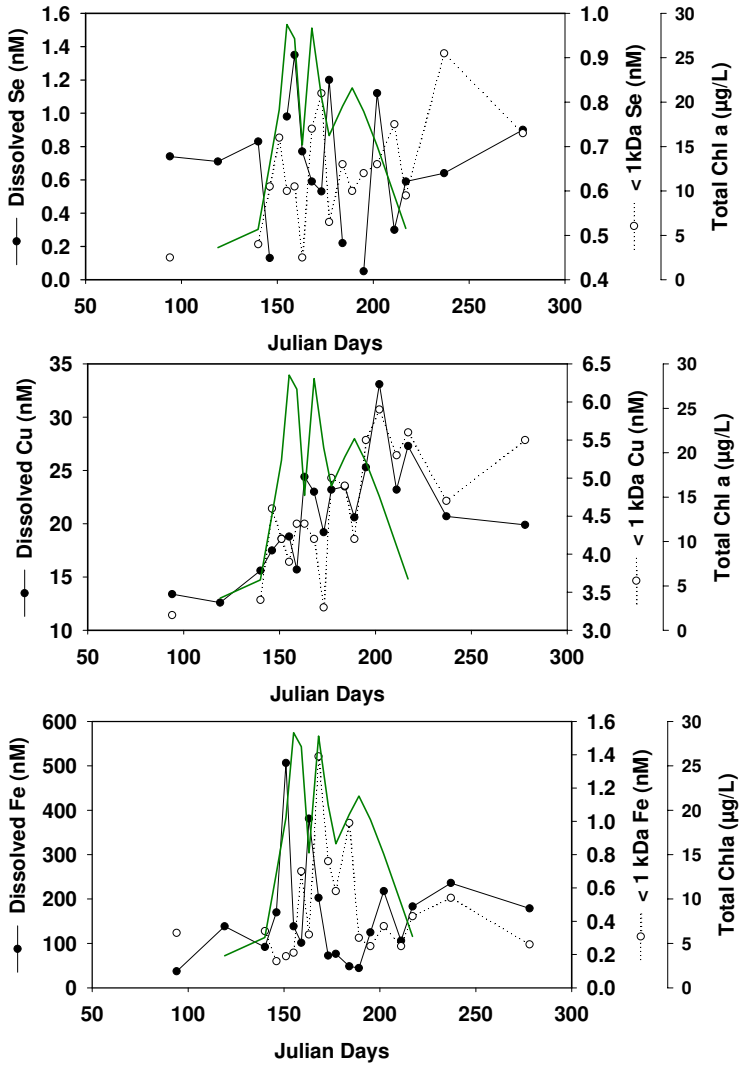

Fig. 34. Temporal distribution of dissolved and low molecular weight (< 1 kDa) selenium, iron and copper measured in surface waters of West Neck Bay in 1998. This figure also shows levels of total biomass represented as chlorophyll a.