# Interannual variability of *Alexandrium fundyense* abundance and shellfish toxicity in the Gulf of Maine

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**Abstract**

Six years of oceanographic surveys of *Alexandrium fundyense* concentrations in the Gulf of Maine are combined with shellfish toxicity records from coastal monitoring stations to assess covariations of these quantities on seasonal to interannual time scales. Annual mean gulf-wide cell abundance varies by less than one order of magnitude during the time interval examined (1993-2002). Fluctuations in gulf-wide annual mean cell abundance and shellfish toxicity are not related in a consistent manner. This suggests that interannual variations in toxicity may be regulated by transport and delivery of offshore cell populations, rather than the absolute abundance of the source populations themselves.

## 1. Introduction

The causative link between blooms of *Alexandrium fundyense*[1] and outbreaks of Paralytic Shellfish Poisoning (PSP) in the Gulf of Maine has been known for many years. Nevertheless, the mechanisms regulating interannual variability in PSP outbreaks have remained obscure. Some years, toxicity is extremely high, reaching levels that require extensive closures of harvesting areas. Other years, toxicity is virtually absent, or is well below quarantine levels (Shumway et al., 1988). One obvious hypothesis is that interannual variations in shellfish toxicity are caused by interannual variations in the abundance of vegetative *A. fundyense* cells. Testing of this hypothesis requires concurrent records of both cell abundance and shellfish toxicity, and it is only relatively recently that such data have become available. Although shellfish monitoring programs in the Gulf of Maine have been in place since the late 1950s (Shumway et al., 1988), systematic surveys of the abundance and distribution of *A. fundyense* were first undertaken in the 1980s (Martin and White, 1988). Herein we compare seasonal to interannual trends in cell abundance with toxicity records from selected coastal monitoring stations for six years between 1993 and 2002. For a detailed description of PSP toxicity during 1997-2001 see Bean et al. (submitted).

## 2. Methods

Abundance of *A. fundyense* was estimated from near-surface shipboard observations (Figure 1, upper panel) and shellfish toxicity records were derived from selected coastal

[1] Both *A. tamarense* and *A. fundyense* occur in the Gulf of Maine (Anderson et al., 1994). We consider these to be varieties of the same species (Anderson et al. 1994; Scholin et al. 1995). Neither antibody nor oligonucleotide probes can distinguish between them, and only detailed analysis of the thecal plates on individual cells can provide this resolution. This is not practical for large numbers of field samples. Accordingly, for the purpose of this study, the name *A. fundyense* is used to refer to both forms.

monitoring stations (Figure 1, lower panel). Toxicity measurements were based on the blue mussel *Mytilus edulis*, using the standard mouse bioassay (Association of Official Analytical Chemists, 1984). These data were kindly provided by the Maine Department of Maine Resources (http://www.state.me.us/dmr/) and the Massachusetts Division of Marine Fisheries (http://www.mass.gov/dfwele/dmf/).

Shipboard surveys of *A. fundyense* cell abundance ranged from single transects to spatial grids that cover large portions of the Gulf of Maine (Figure 2). Detailed descriptions of these surveys are provided in Townsend et al. (2001), Anderson et al. (2005), Keafer et al. (submitted-a), Keafer et al. (submitted-b) and Townsend et al. (submitted-b). Although vertical distributions of *A. fundyense* can exhibit pronounced subsurface maxima tens of meters deep (Townsend et al., submitted-a; Townsend et al., 2001), we focus herein on near-surface cells because of their more direct impact on shellfish in the intertidal zone.

The near-surface depth intervals sampled were not uniform among the different studies, nor were the methods used to enumerate the cells (Table 1). Although there are significant methodological differences in the various data sets (geographic scope, depths sampled, and cell counting methods), these do not preclude assessment of seasonal and interannual variability in the mean abundance of *A. fundyense*. Specifically, the data from each survey is used to estimate the mean *A. fundyense* concentration on a per-cruise basis. Weekly toxicity data from the coastal stations are treated in the same way. In both cases, confidence intervals around the estimated means are computed assuming the data are normally distributed.

## 3. Results

Cell abundance observations reveal a complex mixture of regional, seasonal, and interannual variability (Figure 3). Some data sets resolve seasonal blooming of *A. fundyense* in specific geographic locations. For example, the ECOHAB-GOM study documented both the onset and demise of the blooms in Casco Bay in 1998 (Figure 2c) and 2000 (Figure 2d) (Keafer et al., submitted-a). The 2000 bloom started a few weeks later in the year than the 1998 bloom, but the peak in abundance was almost identical to 1998 in both timing (late May / early June) and magnitude (mean concentration ca. 200-300 cells $l^{-1}$). Peak values persisted longer in 2000 than in 1998, such that termination of the bloom was not resolved by available observations in 2000. Similarly, RMRP studies in 1993 (Figure 2a) and 1994 (Figure 2b) (Anderson et al., 2005) documented seasonal increases in abundance from ca. 10 cells $l^{-1}$ to 100-200 cells $l^{-1}$, with the disappearance of the bloom captured only in 1994. Interannual variability is clearly evident in small-scale surveys in Massachusetts Bay (Figures 2e,f), with peak abundances reaching 100 cells $l^{-1}$ in 2002 and only background levels present in 2001.

In contrast to the relatively plentiful regional studies, far fewer gulf-wide surveys are available, and only in the years 1998, 2000, and 2001 (Figures 2c-e). ECOHAB-GOM large-scale surveys in June, July, and August of 1998 (Townsend et al., 2001) revealed relatively constant mean abundance of 200-350 cells $l^{-1}$, whereas 2000 surveys in April/May and June (Townsend et al., submitted-b) documented a sharp seasonal increase in mean abundance from ca. 20 cells $l^{-1}$ to 300 cells $l^{-1}$. Large-scale surveys in 2001 (Keafer et al., submitted-b; Townsend et al., submitted-b) also showed an early season rise in mean abundance, from 10 cells $l^{-1}$ in May to 40 cells $l^{-1}$ in June. However, abundance continued rising to 700 cells $l^{-1}$ in July, significantly

higher than that observed in July 1998. Note that the relative paucity of large-scale surveys precludes assessment of both bloom initiation and termination in any one year.

Shellfish toxicity records reveal well-defined seasonal to interannual trends with clear regional differences (Figure 4). In the western Gulf of Maine, toxicity typically begins in late April to early May, and generally ends by late June to early July. Both the onset and demise tend to occur later in the eastern Gulf of Maine, with the principal period of toxicity being June through August (Bean et al., submitted). In 1993, 1994, and 1998, significant toxicity events occurred in both eastern and western Gulf of Maine regions, although their timings and magnitudes varied considerably. In 2000 and 2001, eastern and western regions experienced alternating bouts of toxicity: in the former there was strong toxicity in the west and none in the east, and in the latter the opposite occurred. Lastly, in 2002 there was prolonged low-level toxicity in the western subdomain and a modest spike in the eastern subdomain in late summer.

## 4. Discussion

There is no coherent relationship between interannual variations in time series of gulf-wide mean *A. fundyense* abundance and shellfish toxicity (Figure 5, top panel). Annual mean cell abundance fluctuations were confined to within a factor of 5 (40 to 200 cells $l^{-1}$), whereas annual mean toxicity scores ranged from 6 to 50 μg toxin per 100g shellfish tissue. Annual mean cell abundance in the eastern region was strikingly stable during the three years in which cells were counted in that region (Figure 5, middle panel). That same time interval saw two years of low toxicity (1998 and 2000) followed by a large rise (2001). In the western region, *A. fundyense* abundance and toxicity appear to show some degree of correspondence (Figure 5, bottom panel). In particular, the cell abundance maximum in 2000 and minimum in 2001 are

mirrored by local extremes in toxicity. However, toxicity in 1993 was the highest of all years examined, during which the abundance of cells was not significantly different from either 1994 or 1998.

It is notable that the successive changes in *A. fundyense* abundance and shellfish toxicity in the western Gulf of Maine have in all cases the same sign. Goodman and Grunfeld (1961) described a simple test for independence between time series based on these so-called co-movements. For these data, the approximate significance level for a one-sided test for positive co-movement is 0.08. Although this is not significant by conventional standards ($P<0.05$, or 95% confidence), given the shortness of the time series and the consequent low power of the test, it is suggestive of a relationship in the western subdomain.

Aggregate statistics reveal that annual mean cell abundance and shellfish toxicity are not correlated in a consistent manner (Figure 6). Intercept and slope parameters of linear least squares fits to the observations vary widely between the full data set and eastern and western subdomains. The highest numerical value of the correlation coefficient (0.80) is found in the eastern region, but with only three points available to determine that relationship there is a 41% chance such correlation could have arisen purely by random processes. The correlation is also positive in the western region, albeit weaker (0.45). Again the observed correlation can easily be explained by random chance ($P=0.37$). Interestingly, the full data set exhibits slightly negative correlation, although that value is not statistically different from zero either ($P=0.75$). Clearly these data do not, either in whole or in part, meet the traditional statistical metric of $P<0.05$ required to reject the null hypothesis of no linear correlation between the variables. Thus it appears that something other than the annual mean *A. fundyense* concentration controls the annual mean shellfish toxicity.

There are several caveats to our interpretation. One consideration is that different counting methods were used for the different surveys. This resulted in part from an evolution in our understanding of the extent to which *Alexandrium ostenfeldii* co-occurred with *A. fundyense* and the manner in which some counting methods (e.g., an antibody-based technique) could not distinguish between these two species (Anderson et al., submitted). The approach taken here assumes that all *Alexandrium* cells counted were capable of PSP toxin production, so samples containing significant numbers of *A. ostenfeldii* cells would be overestimates. *A. ostenfeldii*, which is now known to co-occur with *A. fundyense* in the Gulf of Maine (Gribble et al., submitted), is a producer of the recently identified spirolide class of algal toxins, but is not yet associated with PSP toxin synthesis in North American waters (Cembella et al., 2000; Gribble et al., submitted). It is difficult to assess retrospectively the extent of *A. ostenfeldii* influence on estimates of *A. fundyense* abundance. Anderson et al. compared abundance data generated by an oligonucleotide probe (demonstrated to label *A. fundyense* and not *A. ostenfeldii*) with results from antibody (M8751-1) staining for samples collected in the Casco Bay region during the spring, 2000 (see Anderson et al. submitted, Figure 6). A regression analysis of oligonucleotide- vs. antibody-based counts showed a slope of 0.9 and an $r^2$ value of 0.75. Thus the antibody tended to overestimate *A. fundyense* abundance due to its cross-reactivity with *A. ostenfeldii*, but the influence of the latter species was not large. This view is supported by the general agreement between the timing of peak antibody-based *A. fundyense* estimates and the peak in 1998 shellfish toxicity at moored mussel sites near Casco Bay coincident with this study (Keafer et al., submitted-a; Luerssen et al., submitted). This uncertainty is unfortunate, but unavoidable in this retrospective study. Our view is that this does not preclude the interannual analyses we are presenting since the counting errors are likely to be similar among surveys, given their common

timing and geographic coverage. Future studies, however, should be careful to eliminate the potential complication of the co-occurrence of the two *Alexandrium* species.

Another potentially confounding factor is the presence of subsurface populations of *A. fundyense* known to be present in the Gulf of Maine (Townsend et al., 2001), yet not included in this analysis of near-surface observations. Thin (<5m) layers of *A. fundyense* tend to be located tens of meters deep in association with the pycnocline and nutricline, and are most prevalent in offshore waters on the frontal boundaries of the Maine Coastal Current (Townsend et al., submitted-a). Given their spatial separation from the intertidal zone (both vertically and in terms of cross-shore distance), a transport pathway to shellfish exposure is not clear. Nevertheless, we cannot discount the possibility that these subsurface populations might contribute to interannual variability in PSP.

## 5. Conclusions

Gulf-wide mean cell concentrations exhibit little interannual variability, with all six of the yearly mean values bracketed by less than one order of magnitude. This is particularly surprising given the tremendous patchiness present in synoptic distributions of the organism (e.g. Townsend et al. 2001; Anderson et al. 2005). Perhaps the organism's life history strategy, including a resting cyst stage, lends intrinsic stability to the population on interannual to interdecadal time periods (Wyatt and Jenkinson, 1997).

This analysis does not support the hypothesis that interannual variations in shellfish toxicity are caused by interannual variations in vegetative cell abundance, insofar as yearly mean values are appropriate metrics for both quantities. Other statistical treatments may be more revealing, but we have yet to identify one. Nevertheless, these findings are suggestive of an

alternative hypothesis of *persistent abundance and fluctuating transport*: under this scenario, gulf-wide cell concentrations vary little from year-to-year; interannual variability in regional outbreaks of PSP would then be controlled by physical transport of offshore vegetative cell populations to the shellfish beds. Indeed, episodic transport of *A. fundyense* populations has been shown to influence specific toxicity events in the western Gulf of Maine (Keafer et al., submitted-a; Luerssen et al., submitted). Whether or not hydrodynamic transport holds the key to interannual variations in shellfish toxicity cannot be resolved by existing data. Regular observations of *A. fundyense* populations and coastal currents, in concert with ongoing shellfish monitoring programs, will be needed to test this hypothesis.

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## References


Anderson, D.M., Keafer, B.A., Geyer, W.R., Signell, R.P., Loder, T.C., 2005. Toxic *Alexandrium* blooms in the western Gulf of Maine: the plume advection hypothesis revisited. Limnol. Oceanogr., 50(1): 328-345.

Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. Marine Biology, 120: 467-478.

Anderson, D.M., Kulis, D.M., Keafer, B.A., Gribble, K.E., Marin, R., Scholin, C.A., submitted. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep Sea Research II.

Association of Official Analytical Chemists, 1984. Association of Official Analytical Chemists. In: S. Williams (Editor), Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Arlington, Virginia, pp. 59-60.

Bean, L.L., McGowan, J.D., Hurst, J.W., submitted. Annual variations of paralytic shellfish poisoning in Maine, USA 1997-2001. Deep Sea Research II.

Cembella, A.D., Lewis, N.I., Quilliam, M.A., 2000. The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolide shellfish toxins. PHYCOLOGIA, 39: 67-74.

Goodman, L.A., Grunfield, Y., 1961. Some nonparametric tests for comovements between time series. Journal of the American Statistical Association, 56: 11-26.

Gribble, K.E., Keafer, B.A., Quilliam, M., Cembella, A.D., Kulis, D.M., Anderson, D.M., submitted. Distribution and toxicity of *Alexandrium ostenfeldii* (dinoflagellata) in the Gulf of Maine, USA. Deep Sea Research II.

Keafer, B.A., Churchill, J.H., Anderson, D.M., submitted-a. Blooms of the toxic dinoflagellate *Alexandrium fundyense* in the Casco Bay region of the western Gulf of Maine: advection from offshore source populations and interactions with the Kennebec River plume. Deep Sea Research II.

Keafer, B.A., Churchill, J.H., McGillicuddy, D.J., Anderson, D.M., submitted-b. Bloom development and transport of toxic *Alexandrium fundyense* population within a nearshore coastal plume in the Gulf of Maine. Deep Sea Research II.

Luerssen, R., Thomas, A.C., Hurst, J.W., submitted. Relationships between satellite-measured thermal features and *Alexandrium*-imposed toxicity in the Gulf of Maine.

Martin, J.L., White, A.A., 1988. Distribution and abundance of the toxic dinoflagellate *Gonyaulax excavata* in the Bay of Fundy. Can. J. Fish. Aquat. Sci., 45(11): 1968-1975.

Scholin, C.A., Hallegraeff, G.M., Anderson, D.M., 1995. Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae): Dispersal in the North American and West Pacific regions. Phycologia, 34(6): 472-485.

Shumway, S.E., Sherman-Caswell, S., Hurst, J.W., 1988. Paralytic shellfish poisoning in Maine: Monitoring a monster. Journal of Shellfish Research, 7(4): 643-652.

Townsend, D.W., Bennett, S.L., Thomas, M.A., submitted-a. Diel and vertical distribution of *Alexandrium* spp. in the Gulf of Maine. Deep Sea Research II.

Townsend, D.W., Pettigrew, N.R., Thomas, A.C., 2001. Offshore blooms of the red tide dinoflagellate *Alexandrium* sp., in the Gulf of Maine. Continental Shelf Research, 21: 347-369.

Townsend, D.W., Pettigrew, N.R., Thomas, A.C., submitted-b. On the nature of offshore *Alexandrium fundyense* blooms in the Gulf of Maine. Deep Sea Research II.
Wyatt, T., Jenkinson, I.R., 1997. Notes on *Alexandrium* population dynamics. Journal of Plankton Research, 19(5): 551-575.

Table 1. Sampling depths and *A. fundyense* assays used in the various data sets.

| Year | Data Set | Depth Sampled | Cell detection method | Reference |
|---|---|---|---|---|
| 1993 | RMRP | surface | light microscopy | Anderson et al. (2004) |
| 1994 | RMRP | surface | light microscopy | Anderson et al. (2004) |
| 1998 | ECOHAB-GOM: Casco Bay | pooled (1, 3.5, 7m) | immunofluorescence assay | Keafer et al. (submitted) |
| 1998 | ECOHAB-GOM: large scale | 2m | immunofluorescence assay | Townsend et al. (2001) |
| 2000 | ECOHAB-GOM: Casco Bay | 2 ± 2m | immunofluorescence assay | Keafer et al. (submitted) |
| 2000 | ECOHAB-GOM: large scale | 2 ± 2m | immunofluorescence assay | Townsend et al. (submitted) |
| 2001 | ECOHAB-GOM: large scale | 2 ± 2m | oligonucleotide probe | Keafer et al. (submitted) Townsend et al. (submitted) |
| 2001 | MWRA Mass Bay | 2 ± 2m | oligonucleotide probe | Unpublished data |
| 2002 | MWRA Mass Bay | 2 ± 2m | oligonucleotide probe | Unpublished data |

## Figure Captions

Figure 1. Sampling locations for *A. fundyense* (top) and shellfish toxicity (bottom). Eastern and western subdomains are indicated.

Figure 2. Station locations for *A. fundyense* samples by year: (a) 1993, (b) 1994, (c) 1998, (d) 2000, (e) 2001, (f) 2002. See Table 1 for depths sampled in each survey.

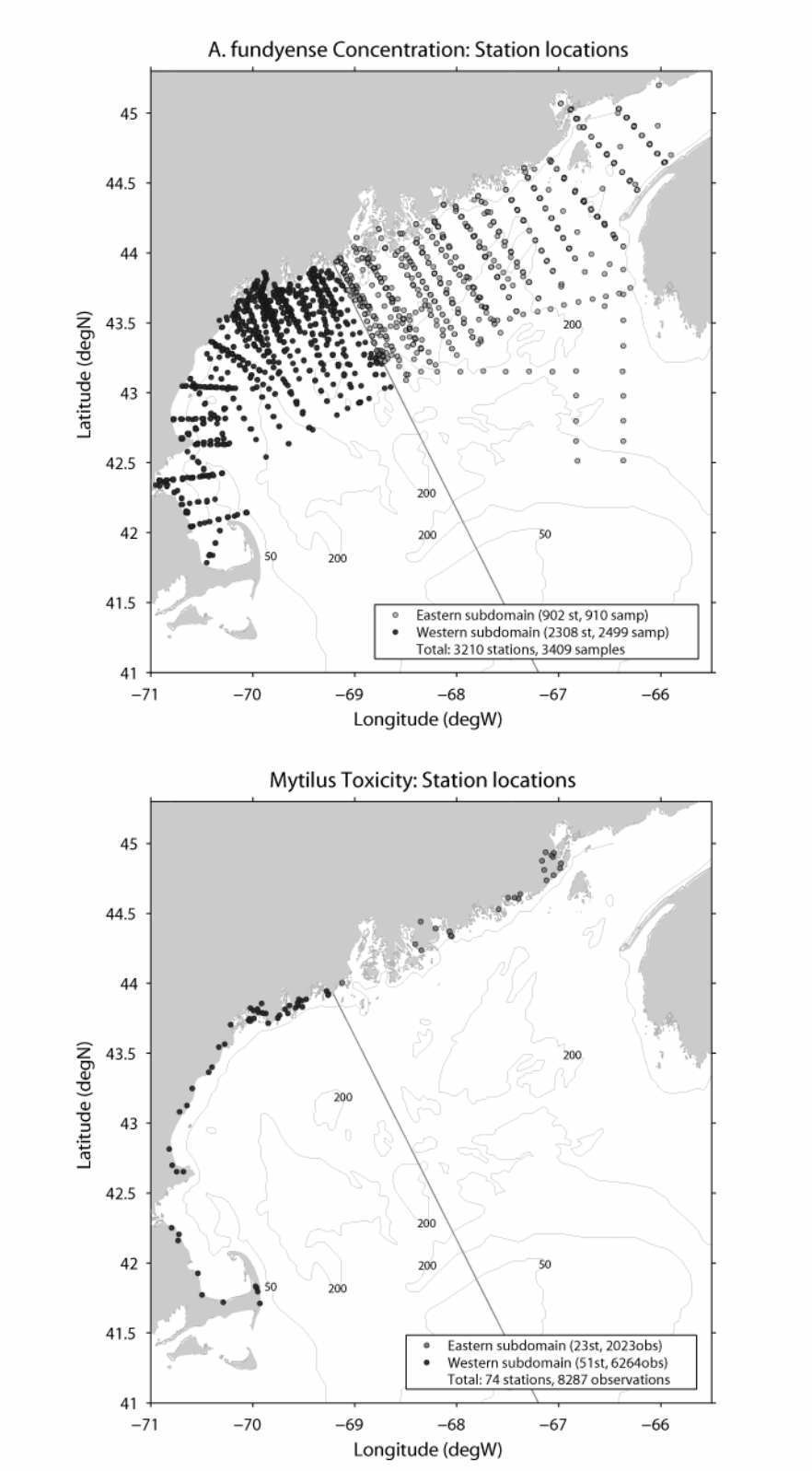
Figure 3. Mean *A. fundyense* concentrations computed from each cruise in shown in Figure 2. Error bars indicate 95% confidence intervals for the means, assuming the data are normally distributed. Crosses indicate mean abundances less than 1 cell $l^{-1}$.

Figure 4. Seasonal variations in shellfish toxicity (score expressed as μg toxin per 100g shellfish tissue) at the coastal stations shown in the lower panel of Figure 1. Error bars indicate 95% confidence intervals for the means, assuming data are normally distributed. Crosses indicate mean toxicity score of less than 1.

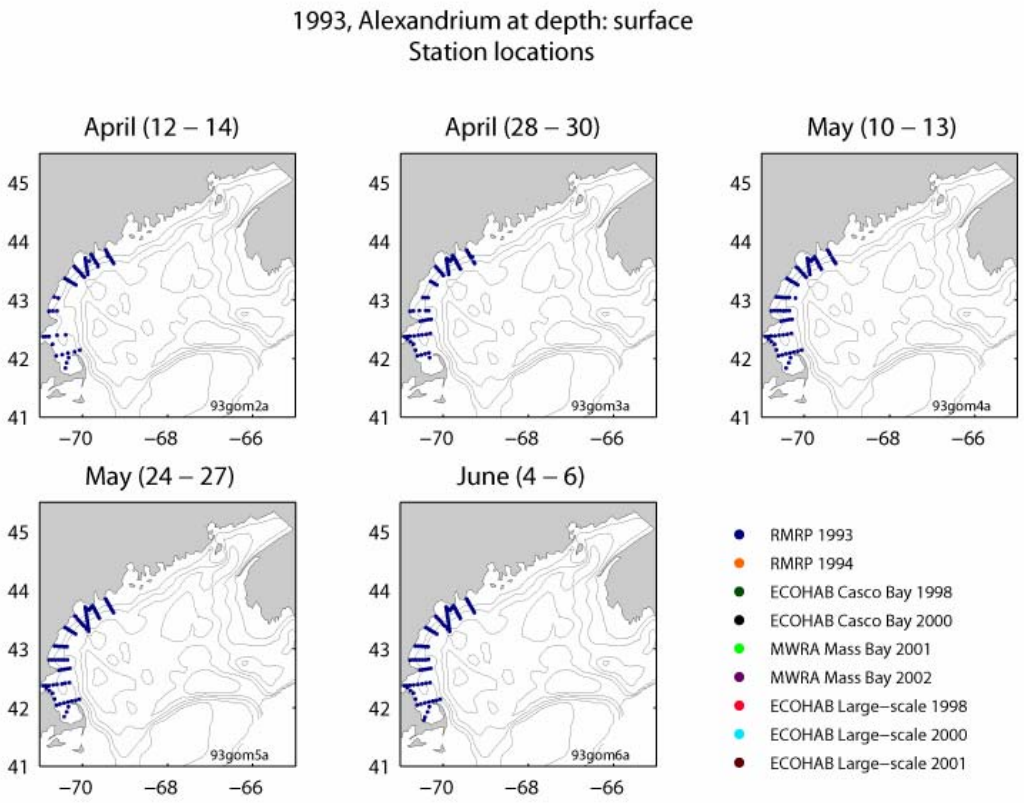
Figure 5. Time series of yearly mean *A. fundyense* concentration and shellfish toxicity: all observations (top), eastern subdomain (middle) and western subdomain (bottom). Eastern and western subdomains are indicated in Figure 1. Error bars indicate 95% confidence intervals for the means, assuming the data are normally distributed.

Figure 6. Correlations of yearly mean *A. fundyense* concentration and shellfish toxicity: all observations (top), eastern subdomain (middle) and western subdomain (bottom). Eastern and western subdomains are indicated in Figure 1. Statistical parameters are provided in the upper right of each panel: slope and intercept of the linear least squares fit, correlation coefficient (r), and associated P value.

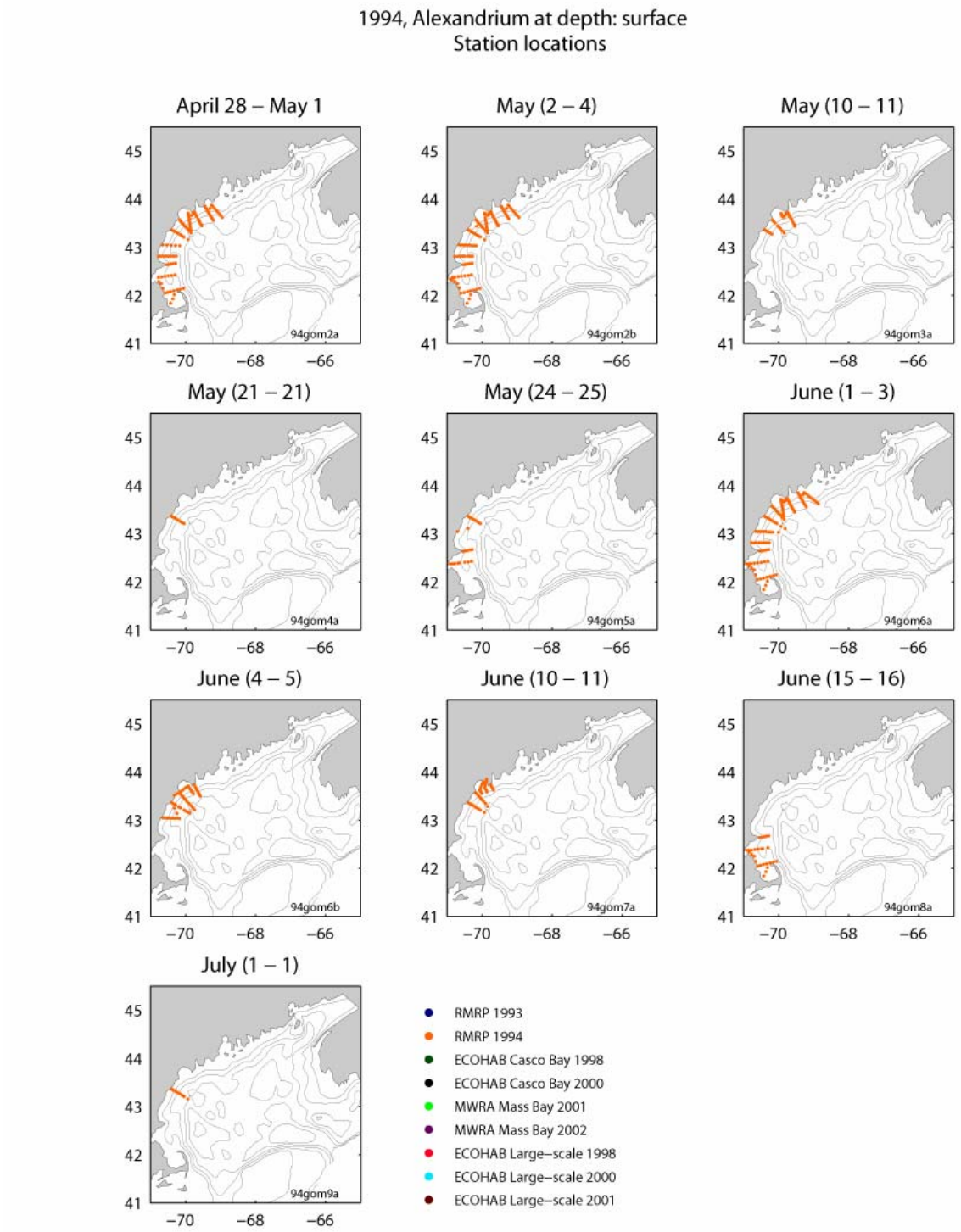
McGillicuddy et al., Figure 1.



McGillicuddy et al., Figure 2a.



McGillicuddy et al., Figure 2b.



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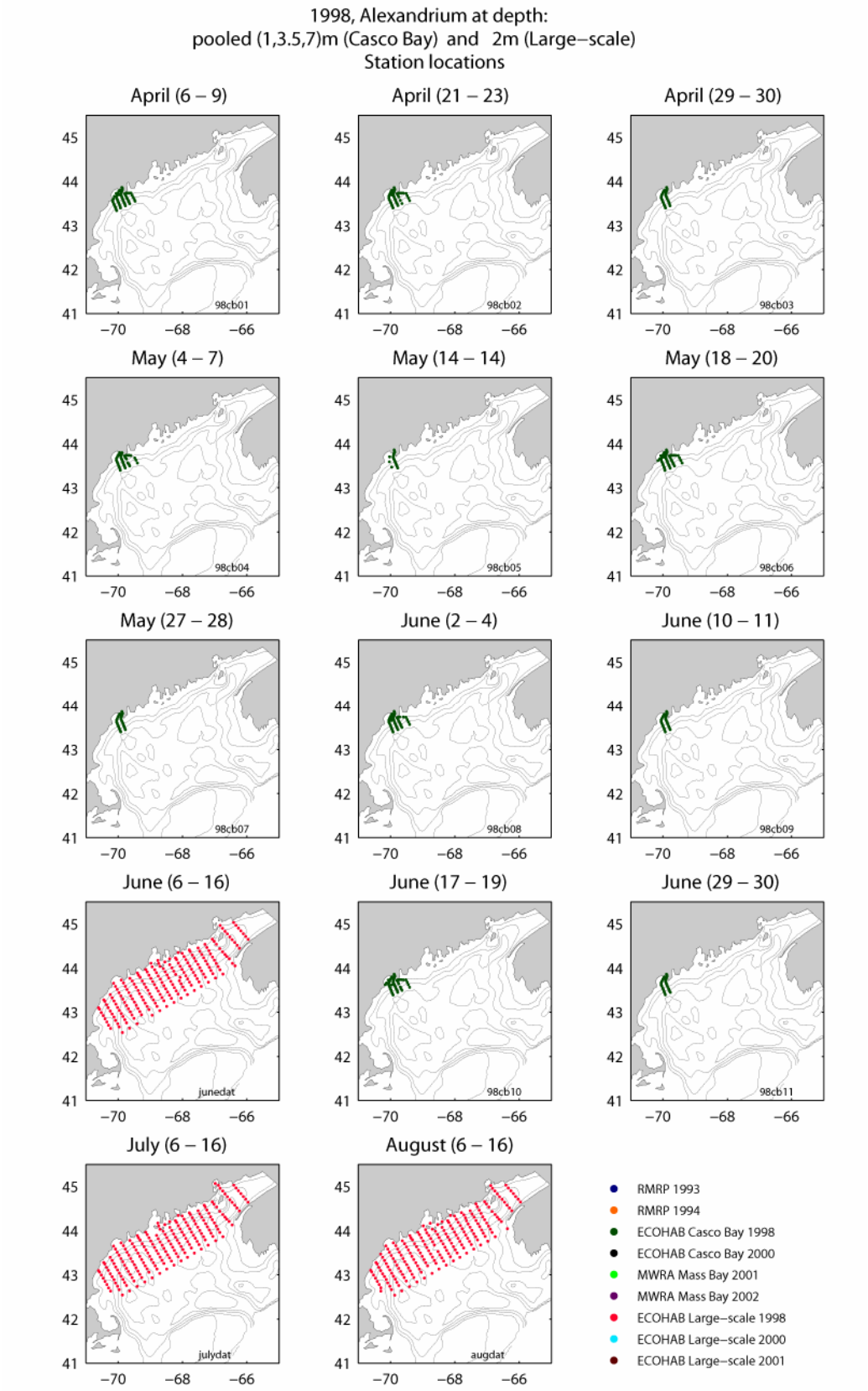

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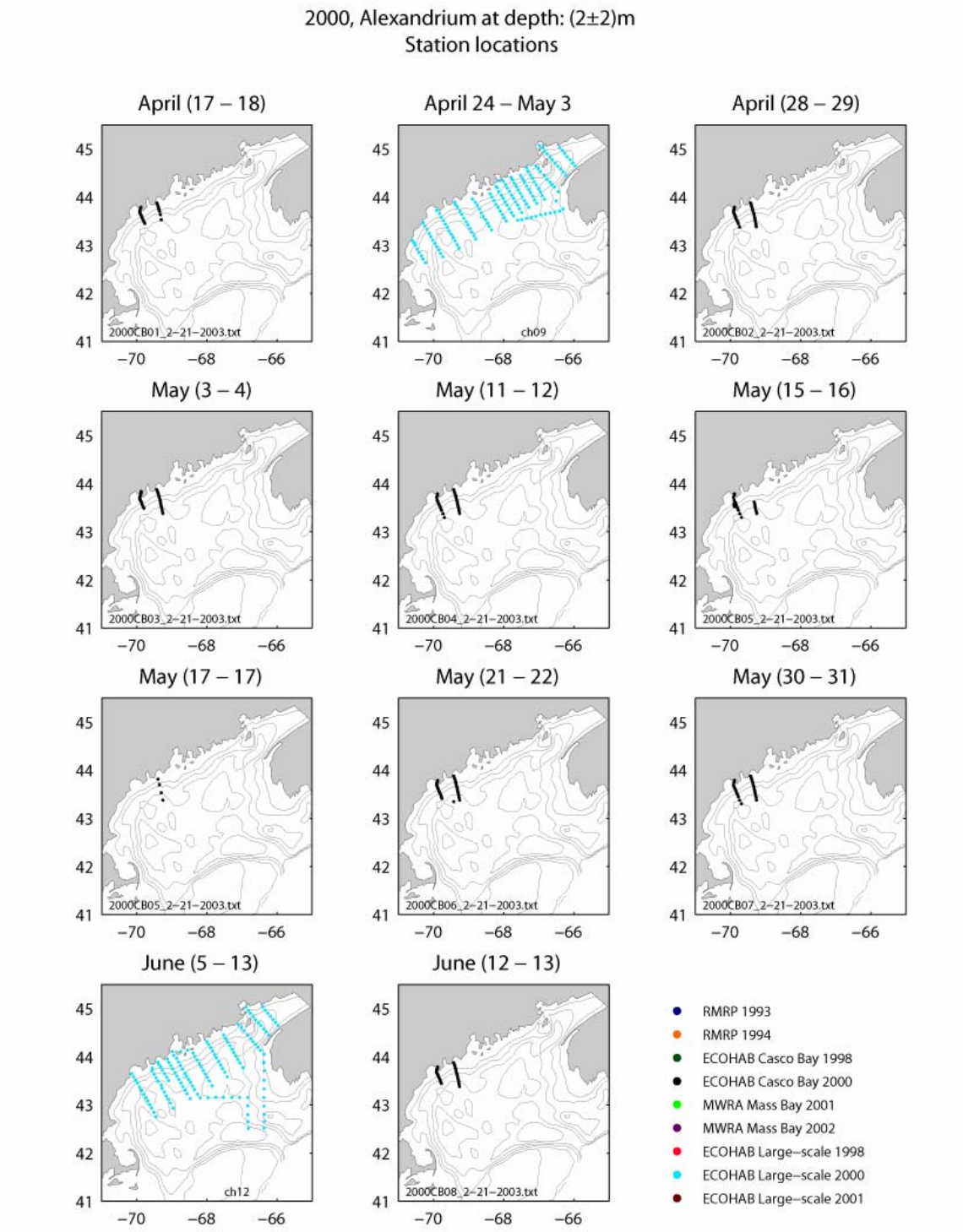

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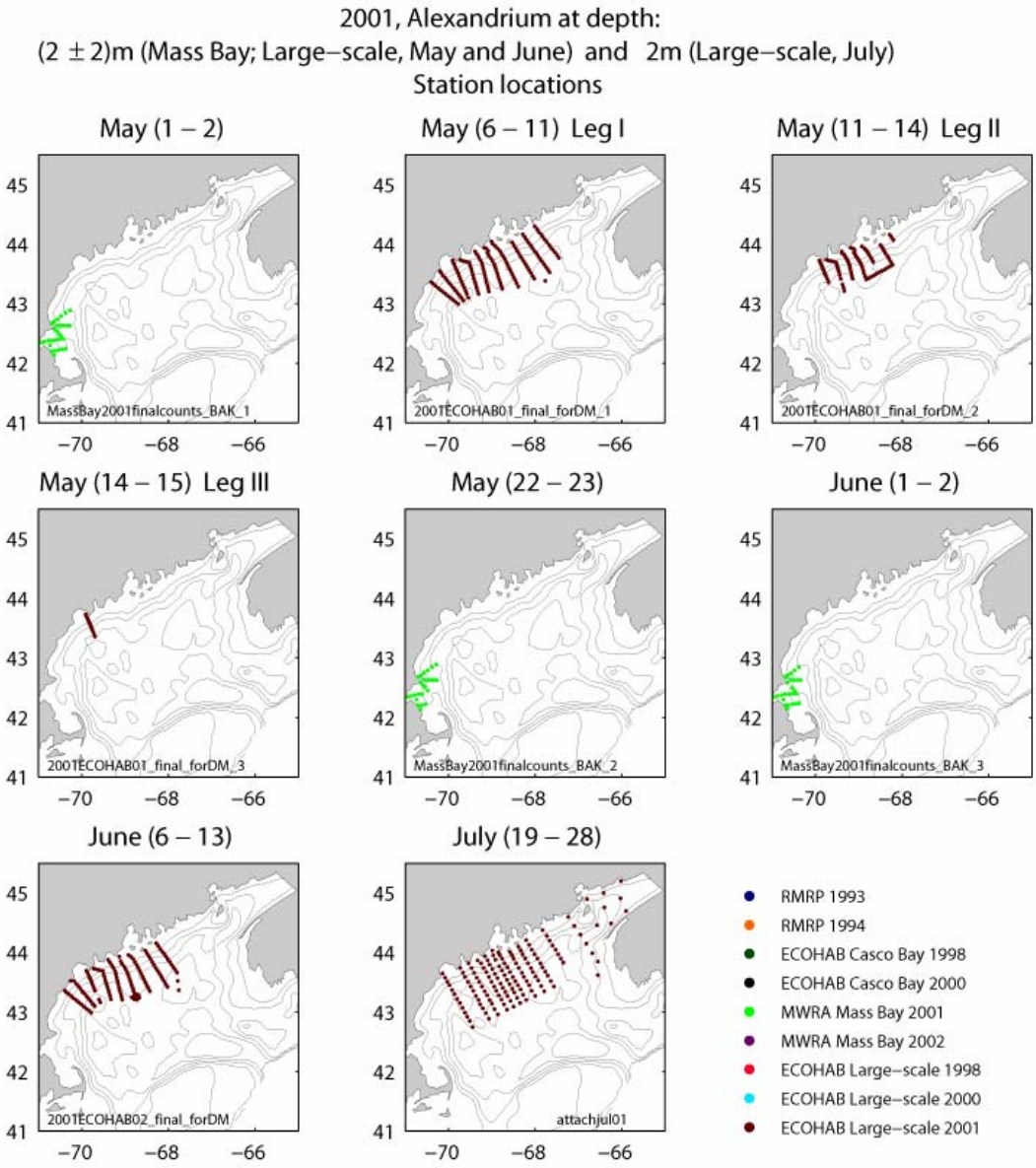
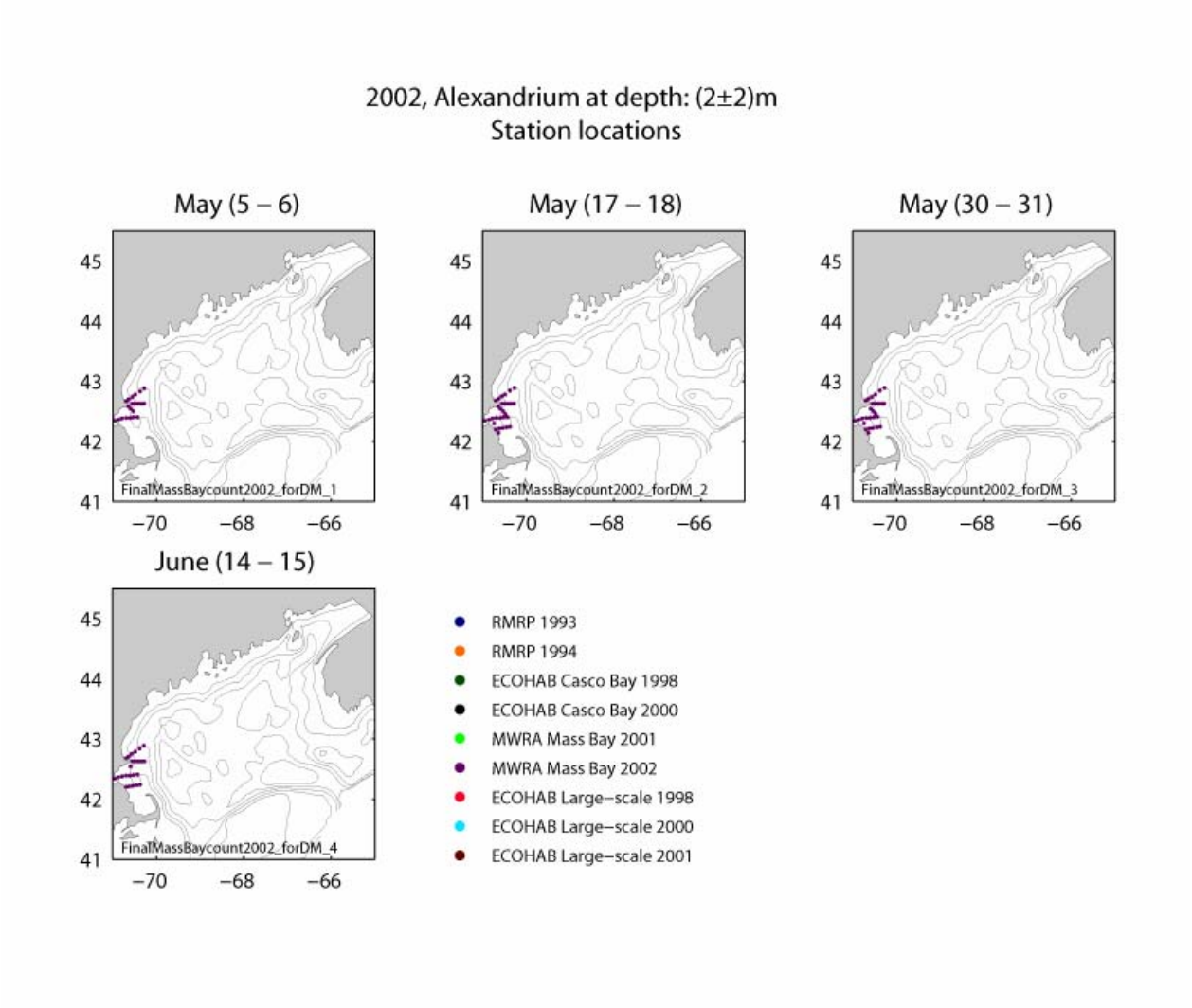


McGillicuddy et al., Figure 2f.



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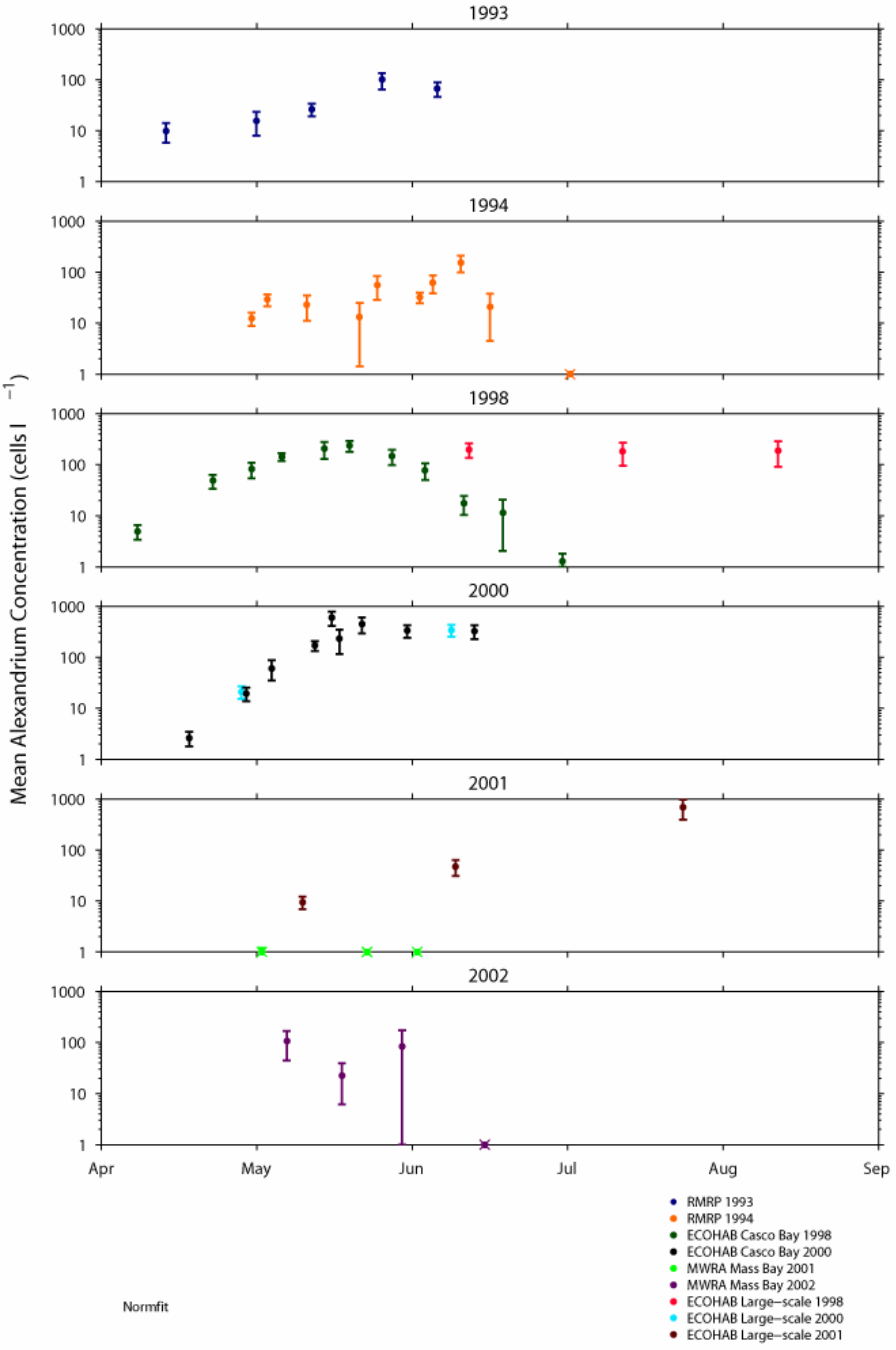

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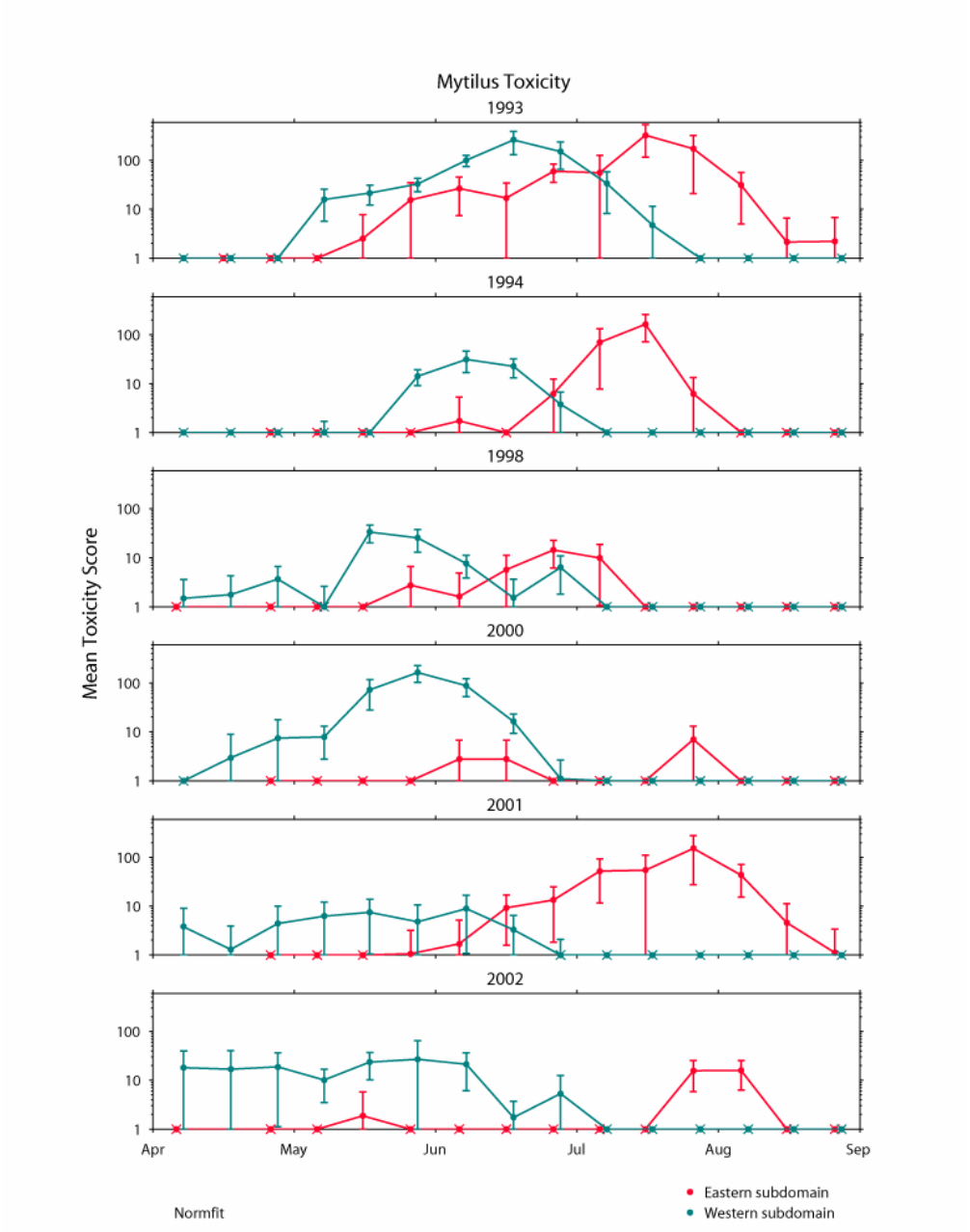

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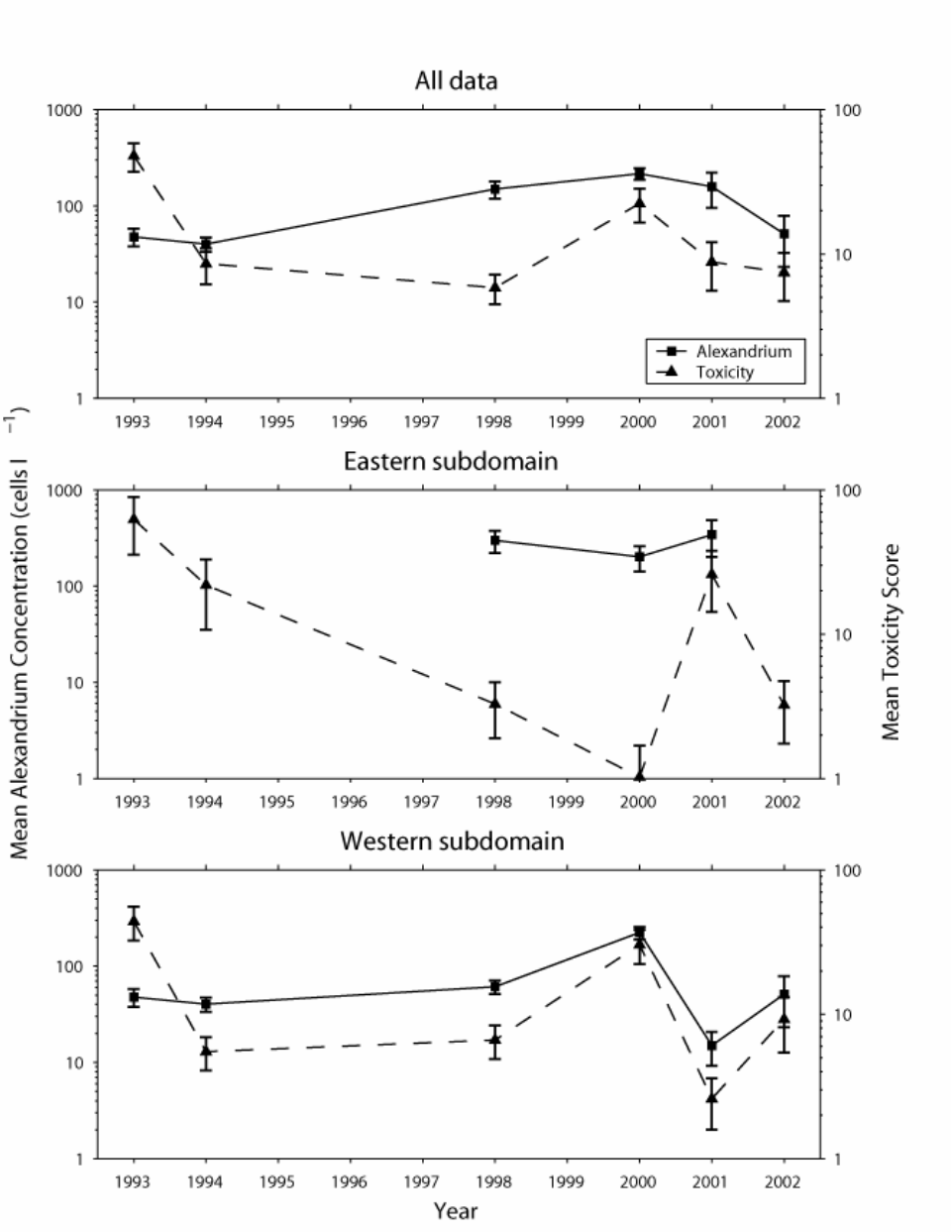

McGillicuddy et al., Figure 6.