Algal cell count data from the Marlborough Sounds:

Marlborough Sounds Quality Programme and Marlborough District Council data

Prepared for New Zealand King Salmon Ltd

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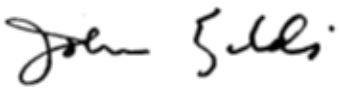
Contents

Figures

Reviewed by



John Zeldis

Approved for release by

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Executive summary

The consent conditions governing the licenses for the New Zealand King Salmon fish-farm sites in the Marlborough Sounds require that existing water-quality data for the Marlborough Sounds be summarized before the farms are occupied. An earlier report (Broekhuizen 2013) summarized a large set of NIWA and Marlborough District Council water-quality data-sets for Pelorus and Queen Charlotte Sounds. This report summarizes two further data-sets. One is the taxon-specific algal cell-count data from the long-running Marlborough Sounds Quality Programme; the other is conceptually similar data gathered by Marlborough District Council since July 2011.

The Marlborough Sounds Quality Programme (MSQP) has been measuring phytoplankton concentrations (as taxon-specific cell counts) at stations throughout the Sounds on a weekly basis since the early 2000s. All sampling sites are close to the shore (they are associated with mussel farms). Whilst the data do not include other water-quality variables, they provide detailed information upon phytoplankton composition and dynamics over a prolonged period. Thus, they can be used to establish the bounds of 'natural phytoplankton variability' within the Marlborough Sounds. This is relevant to NZKS because the NZKS consent conditions require that algal composition data (in addition to other water-quality characteristics) be gathered during the baseline monitoring period. The MSQP data provide a means of determining whether the data that will stem from the NZKS baseline monitoring are representative of longer-term average conditions.

The MSQP data-set comprises two types of count: *full-count* and *routine-count*. In a full-count, all individuals are identified and recorded. In a routine-count, records are kept only for: (i) the most abundant two taxa (at the regional scale), (ii) all toxic phytoplankton. For the most-part, full-counts have been restricted to the West Beatrix Bay, Laverique Bay and Nydia Bay sampling sites. Taxon-specific algal abundance is recorded as cells L^{-1} – and that is the form in which we have analysed the data. It is, however worth noting that cell dimensions (hence cell volumes and biomasses) vary dramatically between different taxa. Thus, species which are dominant by cell-count may not be dominant by biomass.

Whilst the MSQP data includes data from almost 100 distinct locations, some of these are outside the Marlborough Sounds, and many of the remaining stations have been sampled only a few times. There are 15 sites within the Marlborough Sounds that have been sampled on more than 260 occasions (span a period of > five years). We have restricted our analysis to the data from these 15 locations.

Marlborough District Council (MDC) have been gathering data which are conceptually similar to the MSQP full-count data at seven sites within Pelorus Sound since July 2011 and at five sites within Queen Charlotte Sound since July 2012. Unlike the MSQP sampling locations, the MDC ones are far from the shore-line (mid-channel or mid-bay) and sampling is monthly rather than weekly. The MDC data include counts of zooplankton (not reported here). NZKS are required to collect information on the taxonomic composition of the phytoplankton community as a part of their baseline sampling. The samples that NZKS are gathering are analysed by the taxonomist who analyses the MDC samples.

Taxonomies are revised from time-to-time. This can lead to two different names being applied to the same species. There are instances of this in the MSQP data. Similarly, the taxonomic level to which cells have been identified have varied through time in the MSQP and differ in the MSQP and MDC/NZKS data. In our analysis of the NZKS data, we have endeavoured to eliminate species synonyms. In some of our analyses, we also chose to amalgamate individual species to the genus level to further reduce the scope for false species distinctions or to accommodate situations where individuals were identified only to genus level on some sampling occasions, but to species level on others.

We present the raw time-series for the abundance (cells L^{-1}) of the dominant (most-frequently present in the time-series) taxa in the MSQP data and derivatives thereof (total diatoms, dinoflagellates and others; total toxic algae, etc.). We also present box-plots to illustrate the probability-distributions of cellular concentration for each of these dominant taxa within each month-of-the-year. Whilst most of the taxa do show a clear average annual-cycle (as inferred from the median monthly concentrations), the within-month-of-the-year variability usually exceeds the amplitude of the annual cycle of median-monthly concentration. The amplitude of the annual cycle (as inferred from monthly median counts) is usually around 10-fold whereas the within-month-of-year variability in the raw-counts can exceed 100-fold.

In the routine-count time-series, some of the dominant (by frequency of presence) taxa appear to be present only at some times of the year (members of the genera *Leptocylindricus*, *Heterosigma* and *Skeletonema* being good examples). The first, and last of these are non-toxic diatoms. In the full-count data, they are present throughout the year. This implies that their apparent seasonal absence in the routine-count data is an artefact of the recording method – they will have been recorded only on those occasions when they were amongst the regionally dominant taxa (by cell concentration).

The MDC data and the MSQP full-count data are both conceptually similar to the corresponding algal count data that NZKS are required to gather. The MDC and NZKS data could be rendered conceptually similar to the MSQP routine-count data by filtering to remove non-toxic, sub-dominant taxa but it would also be necessary to adopt a common taxonomic classification for all three data-sets.

Whilst care will need to be taken to ensure that like-is-compared with like (by subsampling from the MDC/NZKS algal count data when comparing against MSQP routine-count data and by ensuring consistency between taxonomies in the various data-sets), there is no doubt that the MSQP data provide a means of deriving robust estimates of monthly-taxon-specific cellular abundances against which ongoing algal-count data could be compared in order to provide an indication of whether fish-farming activities might be modifying the phytoplankton component of the pelagic biota.

The algae recorded within the MSQP and MDC sampling programmes include several taxa that are known to be toxic to fish (for example, members of the genera *Pseudochatonella, Prymnesium*, and *Karlodinium*). Similarly, though non-toxic, at sufficiently high concentrations, some members of the genus *Chaetoceros* can be harmful to fish because their hard-spiny skeletal structure causes irritation to the gills.

Detailed comparisons between the dynamics of these harmful algae and records of fish health, condition/quality and growth rate lie outside the scope of this review. However, these comparisons might be helpful to NZKS in determining the causes of past fish-health or loss-of-condition events – and, perhaps, thereby determining how to minimise the future occurrence/severity of such events.

1 Introduction

The consent conditions governing the development of four new salmon farms by New Zealand King Salmon Co. Ltd. (NZKS) in the Marlborough Sounds region require a synthesis and review of all existing historical data related to water quality monitoring in the Marlborough Sounds. The consent conditions also required that 'Baseline Monitoring' (prior to establishment of the new farms) be undertaken. The precise nature of this Baseline Monitoring was to be resolved through development of a Baseline Monitoring Plan (BMP) that was to be submitted to a review panel for approval prior to the onset of said monitoring. The data-review and BMP were to be submitted to the review panel in tandem by June 30 2013 – with an expectation that sampling would begin in late July 2013.

An earlier report (Broekhuizen 2013) summarized the Marlborough District Council water-quality data and NIWA's MSQP-related water-quality data. In this report, we will summarize the MSQP species-specific counts of algae[1] and the much smaller (but conceptually similar) species-specific counts within the MDC data-set.

[1] We were not provided with the bacterial contaminant (coliform bacteria) data.

2 MSQP taxon-count data

The primary purpose of the MSQP sampling scheme has been detection of potentially toxic algae. Sampling began in 2001 and continues to the present day.

The MSQP data-set includes records from 96 unique locations. Stations in Tasman/Golden Bay and Port Underwood are not relevant to the NZKS farms and have been excluded. The remaining sites are located within the Marlborough Sounds (Pelorus Sound, Forsyth Bay, Anakoha Bay, Port Gore, Queen Charlotte Sound and Tory Channel). Figure 2-1 illustrates the locations of the MSQP stations that lie within (or close to) the Marlborough Sounds. Several of the MSQP sites (Cannon Bay, Richmond Bay, Port Gore and Tio Point) are relatively close to the newly consented NZKS farm sites.

The majority of stations have been sampled on only a few occasions, but a core-group have been sampled on a weekly basis for five or more years.

2.1 Sampling details & laboratory analyses

The ensuing descriptions of sampling techniques and laboratory analyses are based upon information provided to me by Jenny Robinson (Cawthron Institute, by email September 3 & 5, 2013).

The members of aforementioned core-group are sampled on Monday, Tuesday or Wednesday of each week. At each station, a hose-pipe is lowered to 12 m depth and sealed. It is then retrieved and the contents are drained into a bucket. Two 100 mL samples are drawn from the bucket. One is preserved with Lugols solution. The second is chilled. Both are returned to the laboratory for analysis. Laboratory analyses take place on the day after the sample was collected. Usually, only the Lugols sample is analysed, but the fresh-sample may be referred to when an individual cannot readily be identified to taxon within the Lugols samples.

Each 100 mL water sample is allowed to settle, and sub-samples of the deposited material are inspected under a microscope. Individual cells are identified to the lowest practicable taxon. The detection limit is 1 cell $(100\ mL)^{-1}$ = 10 cell L^{-1}.

The MSQP data contain records from two distinct types of counting procedure: *full-count* and *routine-count*.

In a *full-count*, all micro-algal taxa are counted and recorded.

In a *routine-count*, a preliminary scan of all the samples (that were collected on the preceding day – up to 8 samples) is made to determine which diatom taxa are most abundant (by cell-count, averaged across all samples). Thereafter, only the two (sometimes three) most abundant diatom taxa (across all samples), together with all dinoflagellates, all toxic and icthyotoxic species are counted in each taxa. Ultimately, only the counts of the two most abundant taxa (whether diatom or dinoflagellate) together with all toxic and ichthyotoxic taxa are recorded and reported to the client (i.e., information on non-dominant, non-toxic diatoms, dinoflagellates etc., is lost).

The spreadsheets that were provided to us do not explicitly record whether a *full-count* or a *routine-count* was performed upon any given sample, but some taxa are never counted in *routine-counts*. These include: *Cryptomonas spp*. *Euglena* spp.*,* small flagellates and ciliates (other than *Mesodinium rubrum* – which is recorded as low, moderate or high within the *full-counts* and in more recent *routine-counts*). Whilst the absence of any of these taxa in the records from a particular sample does not guarantee that it was a *routine-count*, the presence of even one such record guarantees that the sample was a *full-count*.

With very few exceptions, the full-counts have been restricted to Beatrix Bay (two stations: west Beatrix Bay, Laverique bay) and Nydia Bay.

The fact that routine-counts are not guaranteed to have recorded even relatively abundant non-toxic taxa makes analysis of the data for non-toxic species difficult: does the absence of a record for a particular non-toxic taxa imply that it was genuinely absent, or merely that it did not rank amongst the most abundant non-toxic algae at the Sounds-wide scale (even if locally the most abundant)? The net result is that, the time-series for non-toxic taxa may provide deceptive impressions of how frequently each taxa is truly, entirely absent and may not provide robust impressions of which taxa are locally dominant. In particular, it is not possible to calculate robust estimates of the probability distributions of taxon-specific abundance at each site – because the value (actively searched for but not found (thus zero cell concentration) vs not-counted (thus unknown, but possibly non-zero concentration) that should be associated with absent species-counts is unknowable.

The data for toxic species do not introduce the same difficulties – if found, each toxic species was always recorded.

As noted above, the MSQP records algal abundance as cells L^{-1}. The largest algal species are 10-100 times larger (by length and/or width and/or height) than the smallest, and thousands of times larger by cell mass. Whilst the larger species are usually less abundant (by cell concentration) than the small ones, it is important to recognise that the large species will often be substantial (or even dominant) contributors to the total algal biomass. Unfortunately, estimates of the cell-specific mass of individual cells within a given taxa vary by a factor of two or more. To avoid introducing this additional source of uncertainty, we chose to analyse the raw cell-concentration data rather than transform the cell concentrations to biomass and analyse those.

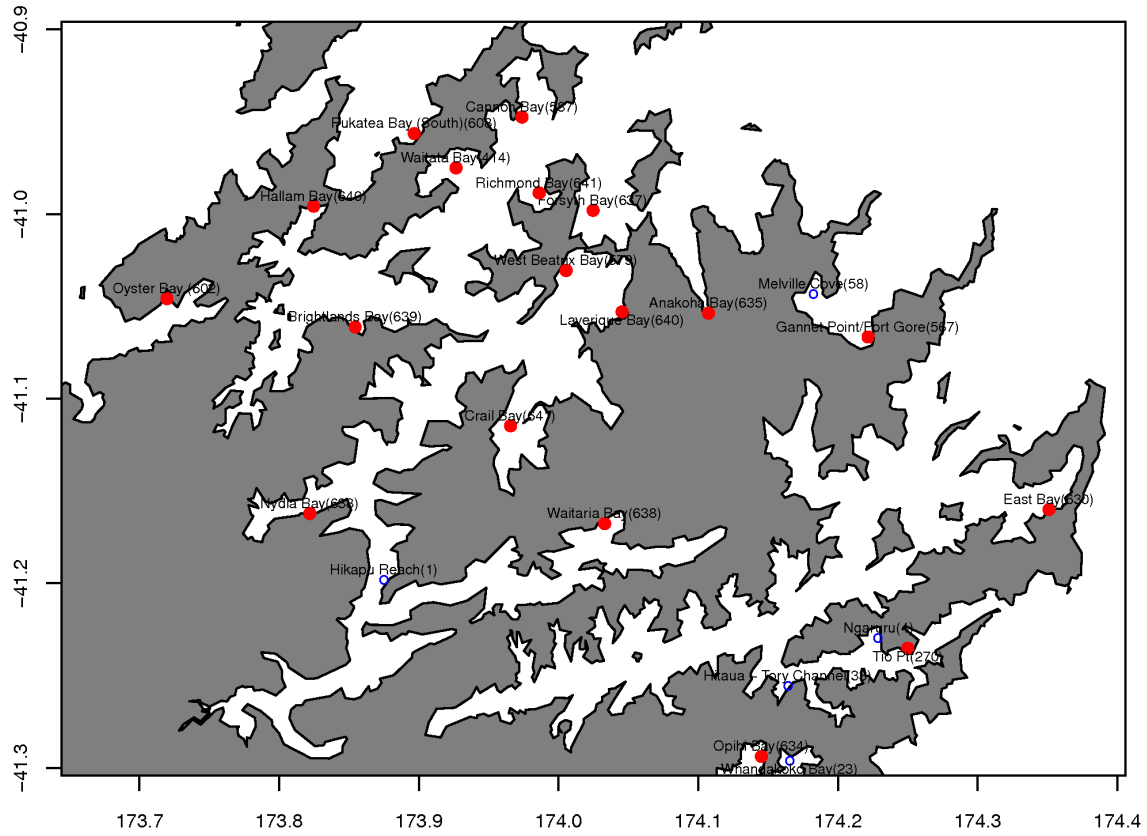

Figure 2-1: MSQP sites within the vicinity of the Marlborough Sounds. The bracketed figures indicate the number of occasions upon which each site was sampled. Sites marked in red were sampled on >260 occasions. Sites outside the Marlborough Sounds were excluded from the analysis (as were those sites in the Sounds with fewer than 260 sampling occasions). The Marlborough Sounds are deemed to include Pelorus Sound, Forsyth Bay, Anakoha Bay, Melville Cove (port Gore), Queen Charlotte Sound and Tory Channel.

2.2 Summary of the MSQP data-set

We were provided with two spreadsheets ("All MSQ Phyto Results from 2001.xlsx" and "MSQP phyto data (Sept 08 to April 13).xlsx"). The former spreadsheet contains data for the period 5 January 2001 – 29 Sept 2008 (inclusive). The latter contains data for the period 29 Sept 2008 – 10 April 2013 (inclusive).

In total, the data-set contains 78824 records, of which 76601 are counts (cells L^{-1}) of a taxon[2,3]. The remaining records are for items such as temperature, salinity, estimated phytoplankton biomass (categorical variable) and sundry other derived characteristics.

[2] Only non-zero counts generate a record. There is no way to ascertain whether any particular taxon that was not recorded was looked for but not found.

[3] Throughout this document, I use the term taxon to refer to the name-field item within the MSQP data-base. In some cases, individuals were recorded to species-level, in other cases, what were probably the same species were recorded only to genus. The reported taxon-totals consider these two names as unique taxa. Taxonomic revisions/operator differences mean that there are some species-synonyms within the data-base. Those which we noticed were combined to a shared species name before any analyses were performed.

There are records from 96 unique locations – but some of these are outside Pelorus Sound (in Tasman/Golden Bay or around the Port Underwood region) and the majority of sites have only been sampled on a few occasions[4]. Of the 96 unique sites, 26 contain more than 260 unique date records (span a period of at least five years) and 18 contain more than 520 unique sampling dates (span a period of at least 10 years). Of the 26 that were sampled on more than 260 occasions, 15 are within the Marlborough Sounds. I restrict my analyses to these 15 sites. For convenience, I will adopt the term core-site(s) to refer to a member(s) of these 15 sites in the remainder of this report. The core-sites are all near-shore (they are associated with mussel farms).

Figure 2-1 illustrates the time-series of number-of-taxa-recorded-per-sampling-occasion at each of the 15 sites. The natures of the *routine*- and *full*- counts imply that the latter should usually always yield a larger total taxon count, but a higher proportionate occurrence of toxic taxa. That expectation proves to be true for those sites where both types of count have been performed (West Beatrix, Laverique and Nydia). An unanticipated finding is that the average number of toxic taxa recorded in *routine* counts appears to be lower than the number recorded in a *full* count (note the abrupt drops in the counts of toxic taxa when full counting ceased in West Beatrix and Laverique, Figure 2-1). This may indicate that greater volumes of water were examined in the *full*-counts (providing a greater probability of detecting rare taxa). Alternatively, it may be that some taxa were identified to a finer resolution (e.g., species rather than genus) in *full* counts than in *routine* counts. If there were several species of the genus present (or if some of the component species were deemed toxic but the genus was not deemed toxic), this would yield an apparent drop in the number of toxic taxa.

[4] We infer that additional sampling sites have been temporarily introduced upon occasion in response to detection of serious toxic algal blooms

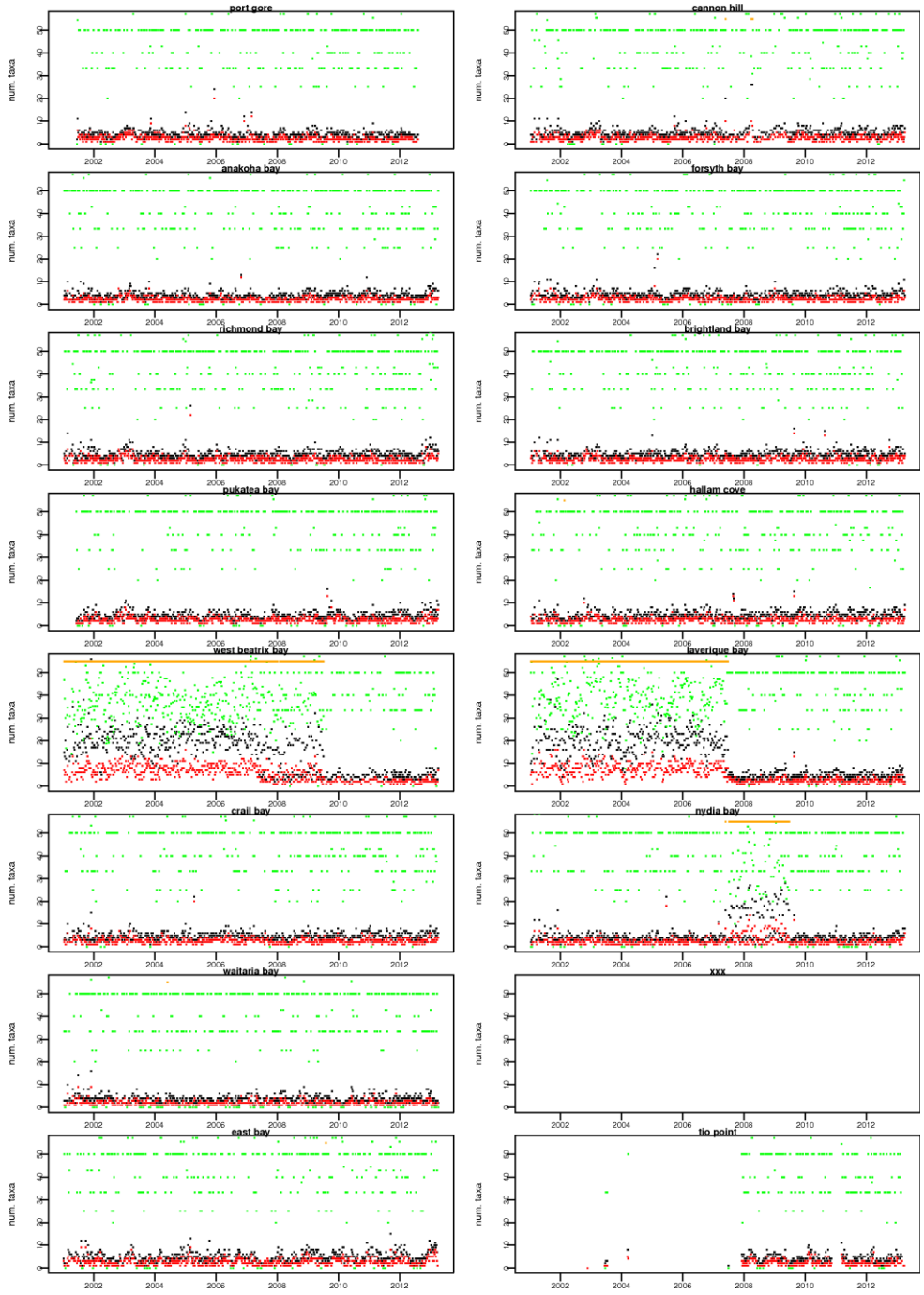

Figure 2-2: Time-series of total number of taxa recorded at each site (black dots). Also shown: number of toxic taxa (red) and percentage of the total taxa which are toxic (green) Orange dots at the top of a panel indicate dates on which the taxa-counts were derived from full-counts (cf routine counts). The natures of the *full-* and *routine-* counts imply that the latter will always yield a lower total taxon count, but a higher proportionate occurrence of toxic taxa.

In total, 155 unique taxa have been recorded[5]. For some taxa, individuals were sometimes identified and recorded to species-level, at other times individuals within this same genus were recorded only to genus-level. There are no occasions where some individuals were recorded to species level whilst others (of the same genus) were recorded only to genus level. The aforementioned figure of 155 unique taxa was calculated by counting a record of <genus>_spp. as an additional unique taxon. Since records of the form <genus>_spp. and <genus>_<species> do not co-occur, it is certain that they are disjoint sets. Thus, one can synthesize 'missing values' in the time-series of <genus>_spp, by summing the cell-counts in any corresponding <genus>_<species> records (if those exist for the sampling date and location in question). Doing so also reduces the likelihood that we have falsely treated records from species-synonyms (that we failed to recognise) as unique taxa.

Figure 2-3 - Figure 2-10 indicate the total number of records (across all sampling dates) for each taxon at each core site. Given the nature of the routine-counts, it is no surprise that a disproportionate fraction of the most frequently-found-to-be-present-taxa are toxic and/or dinoflagellates. Whilst present, many of these are present only in relatively small concentrations (Figure 2-11 - Figure 2-20). At all sites, the most frequently recorded as present taxa are (in alphabetical order): *Chaetoceros* spp. (diatoms), *Dictyocha* spp. (silicoflagellates)*, Leptocylindricus* spp. (diatoms), *Pseudo-nitzschia* spp. (diatoms, some species are toxic) and *Rhizosolenia* spp. (diatom). Recall, however that most sites used the routine-count method, and the decision about which diatoms to count was made on a 'global basis' rather than a site specific basis. *Chaetoceros* spp are [6]non-toxic diatoms, yet they almost always recorded in the routine-counts (Figure 2-11) – suggesting that they are almost always one of the two or three most abundant taxa (by cell concentration) within the Sounds. In contrast, in the routine-count data, the non-toxic, diatoms *Leptocylindricus* and *Skeletonema* are recorded only at particular times of the year – despite being recorded throughout the year in the full-count data. The inference must be their frequent absence from the routine-counts indicates they are sub-dominant for much of the year (conversely, that they are regular, seasonal dominants).

Inevitably, those sites which experienced numerous full-counts (West Beatrix Bay, Laverique Bay and Nydia Bay) appear to have a more diverse plankton community than the 'routine count' sites. This is almost certainly an artefact arising from the two different counting methods rather than a genuine feature of the region.

[5] Within the databases, members of some genera were sometimes recorded only as members of a particular genus. At other times, they were identified and recorded to species-level.

[6] Though they do not produce toxins, *Chaetoceros* have a spiny exo-skeleton which appears to be an irritant to fish gills when the alga is sufficiently abundant. Cawthron have recorded three distinct *Chaetoceros* classes: *C. concavicornis, C. convolutus* and *Chaetoceros spp.* They classify the first two as ichthyotoxic but have not flagged *Chaetoceros spp* as being harmful to fish.

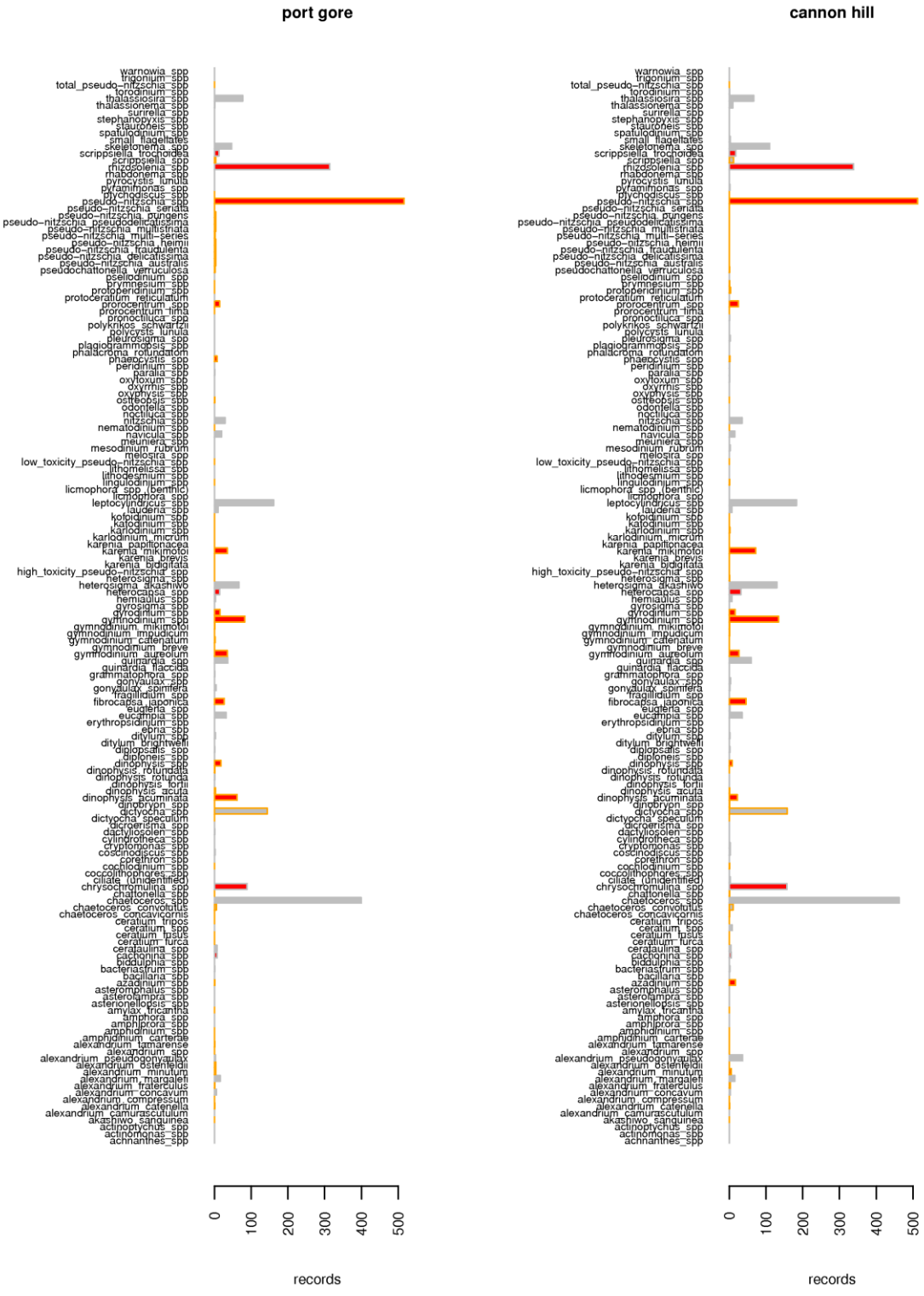

Figure 2-3: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. Port Gore & Cannon Hill. Red bars denote taxa that are harmful to humans (respiratory toxins and shellfish toxins etc.). Grey bars denote taxa that are not known to be harmful to humans. Bars that carry an orange outline denote taxa that are harmful to fish.

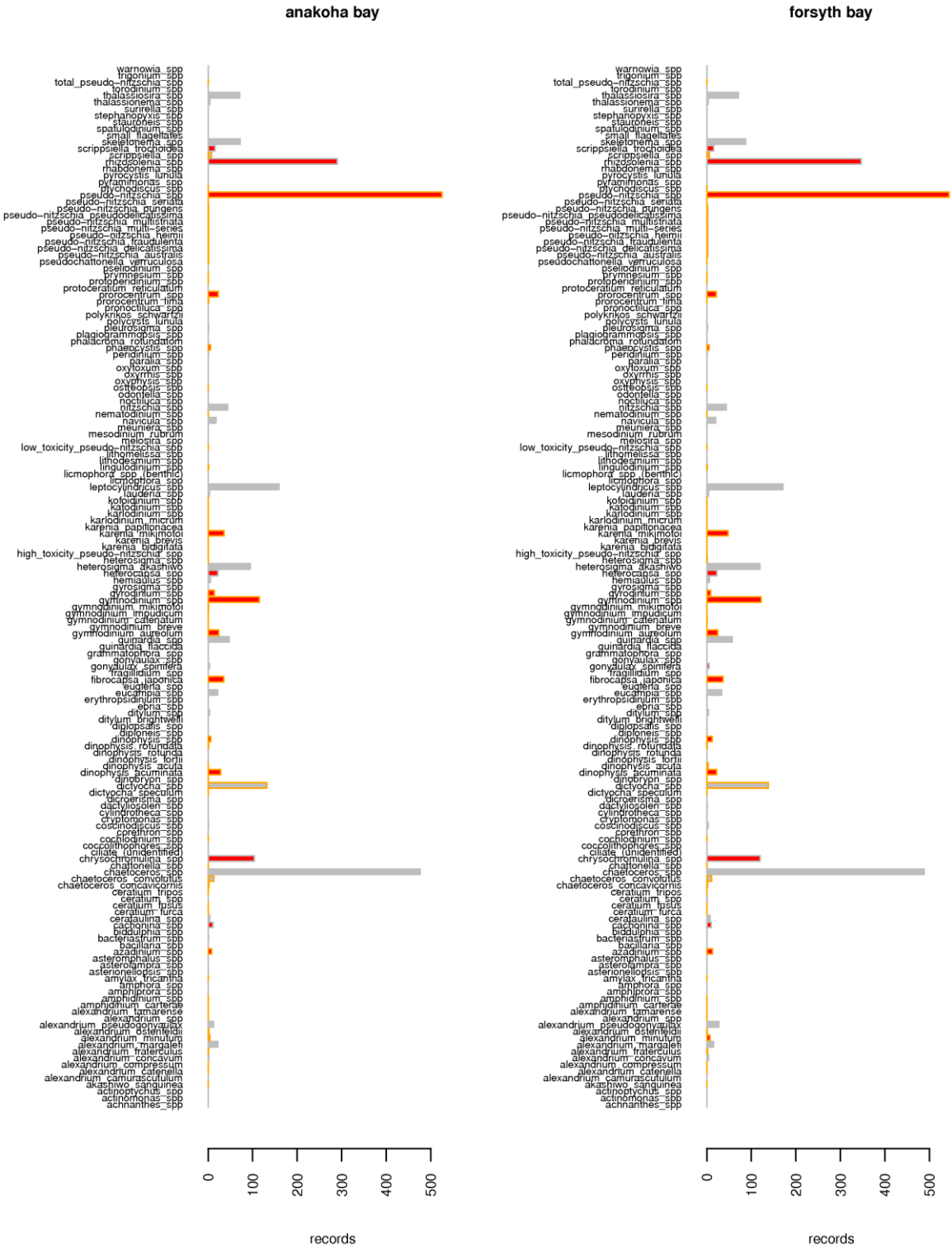

Figure 2-4: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. Anakoha & Forsyth Bays.

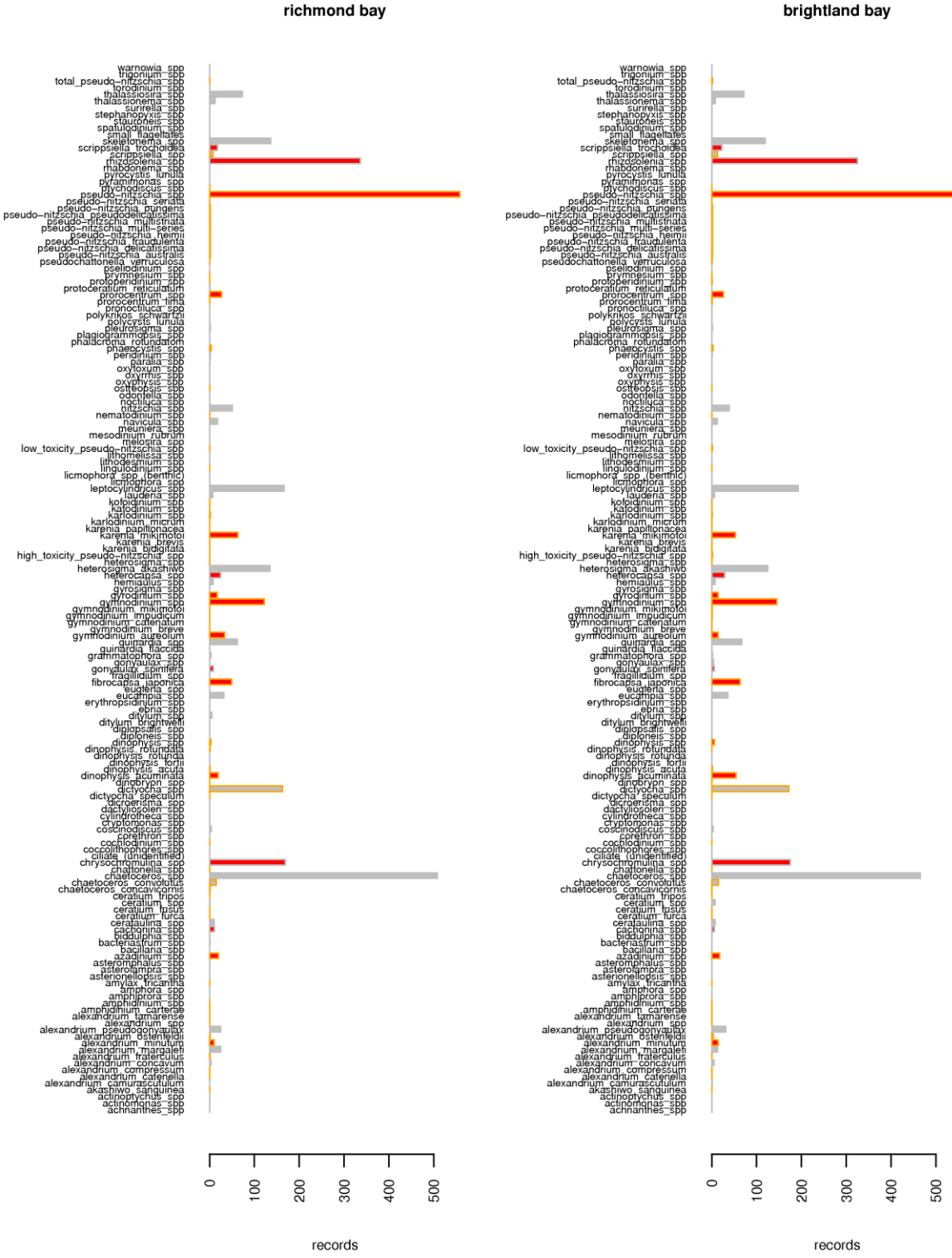

Figure 2-5: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. Richmond & Brightland bays

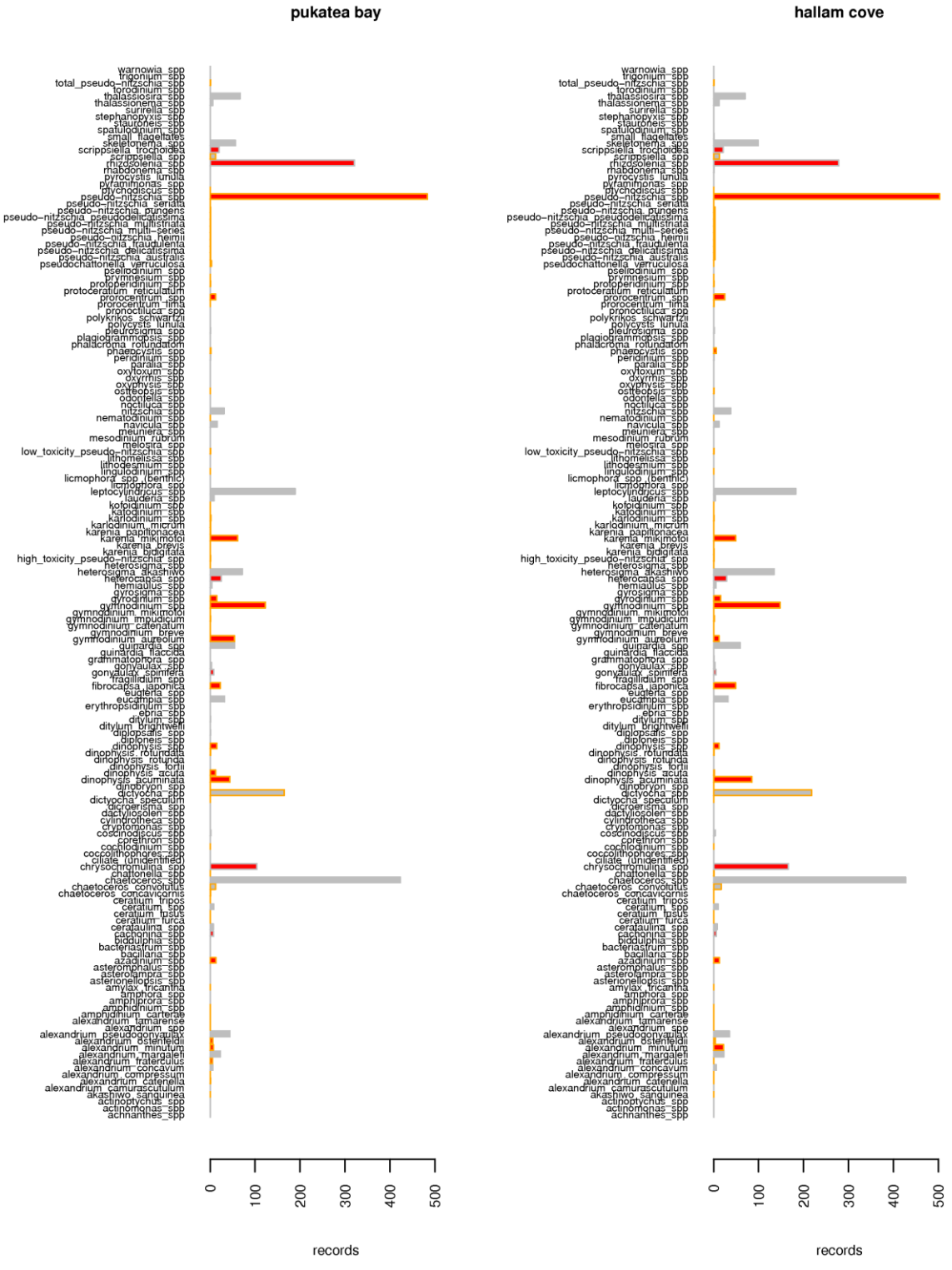

Figure 2-6: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. Pukatea Bay and Hallam Cove

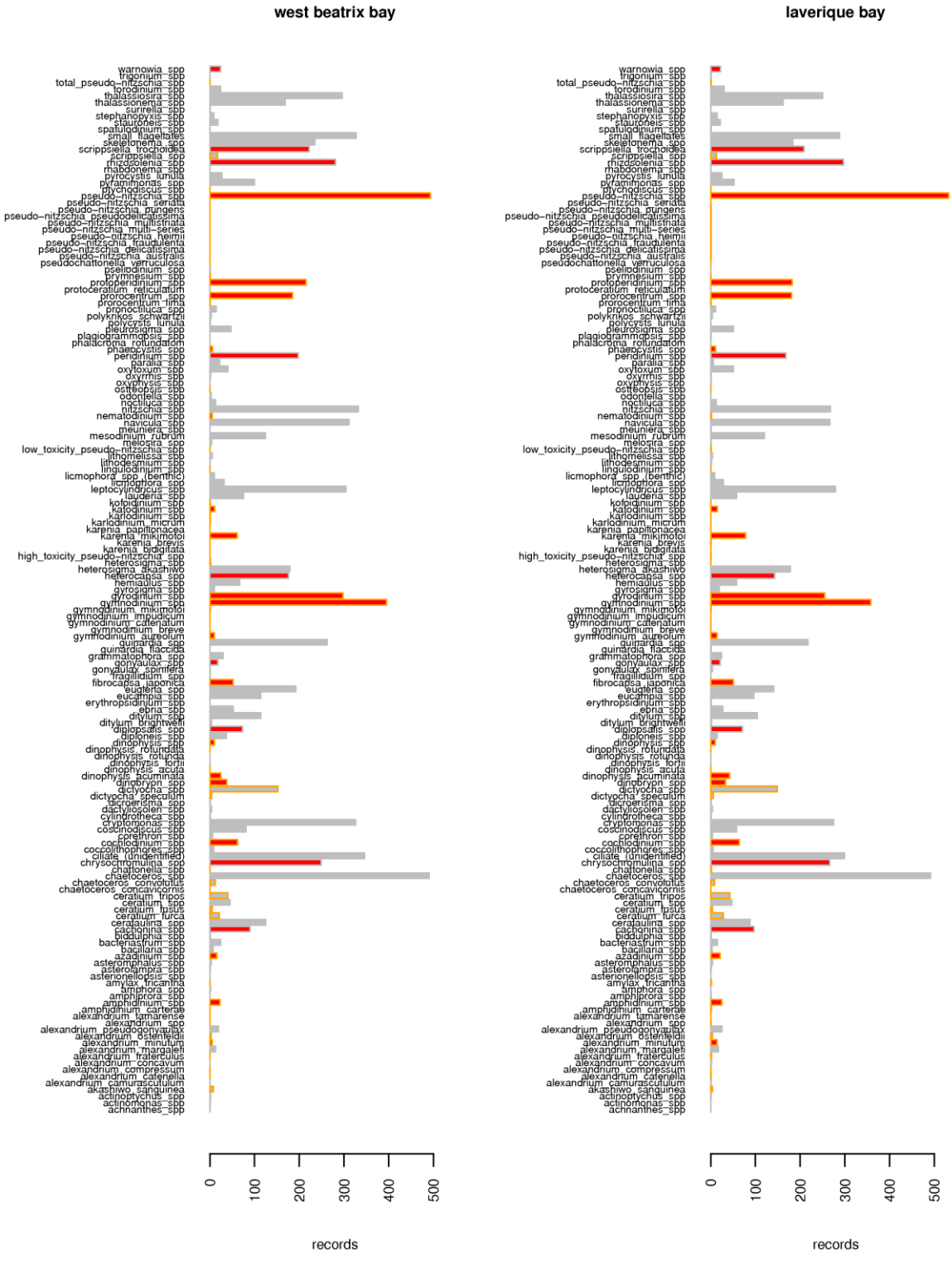

Figure 2-7: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. West Beatrix & Laverique Bays

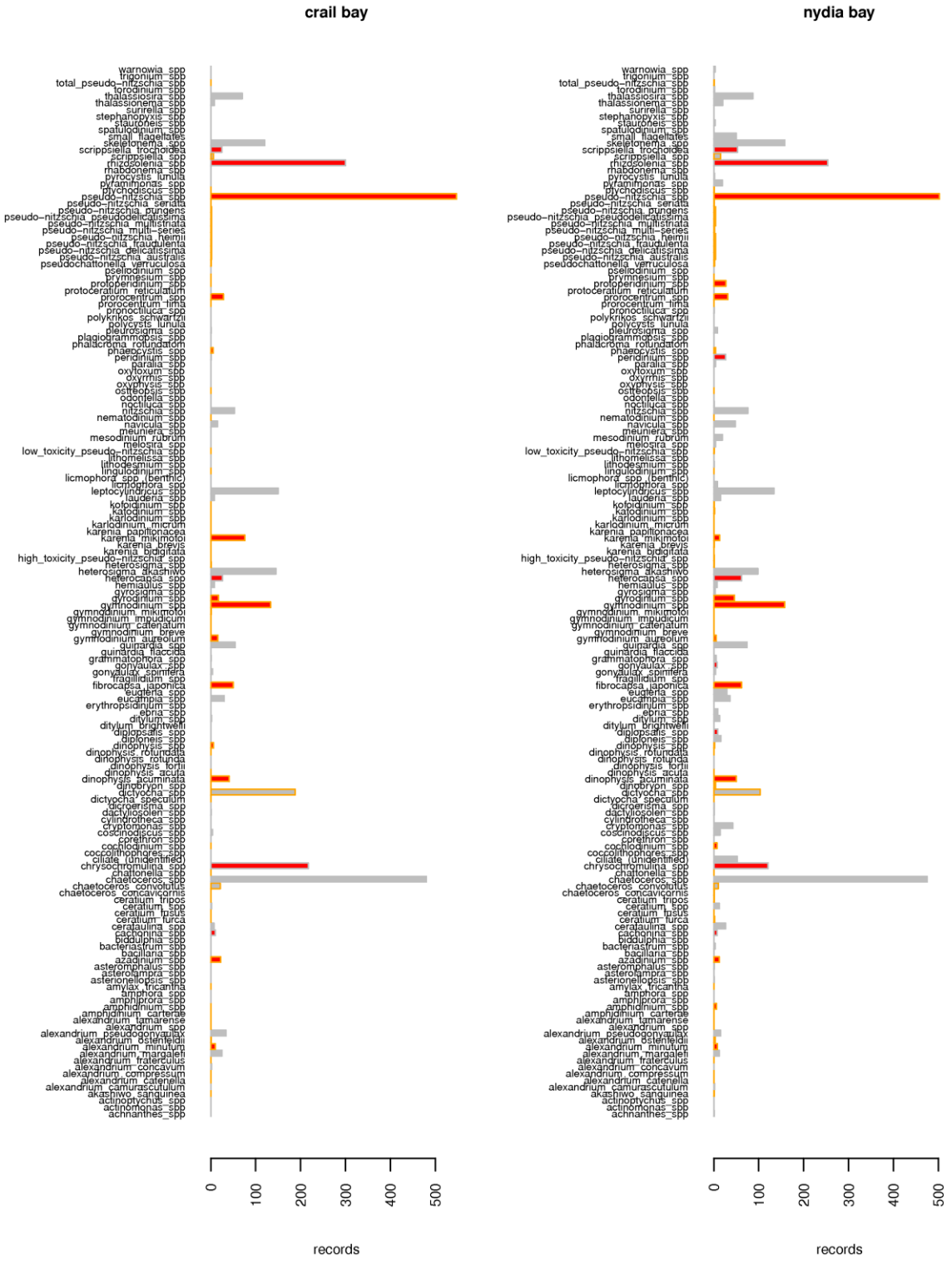

Figure 2-8: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. Crail & Nydia bays

Figure 2-9: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. Waitaria bay.

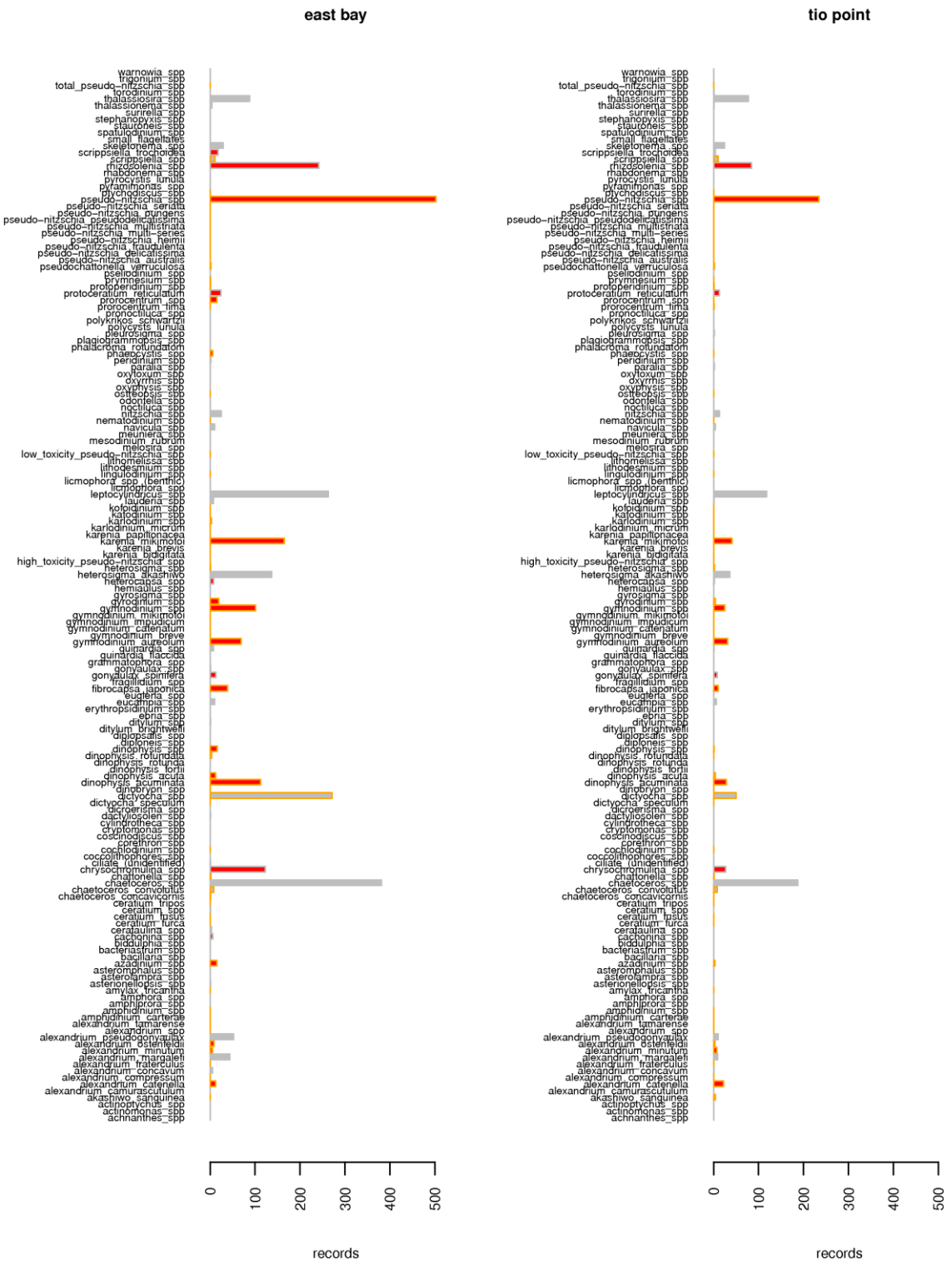

Figure 2-10: Bar plots illustrating the total number of occasions that each taxon has been recorded as present within a station's samples. East bay & Tio point.

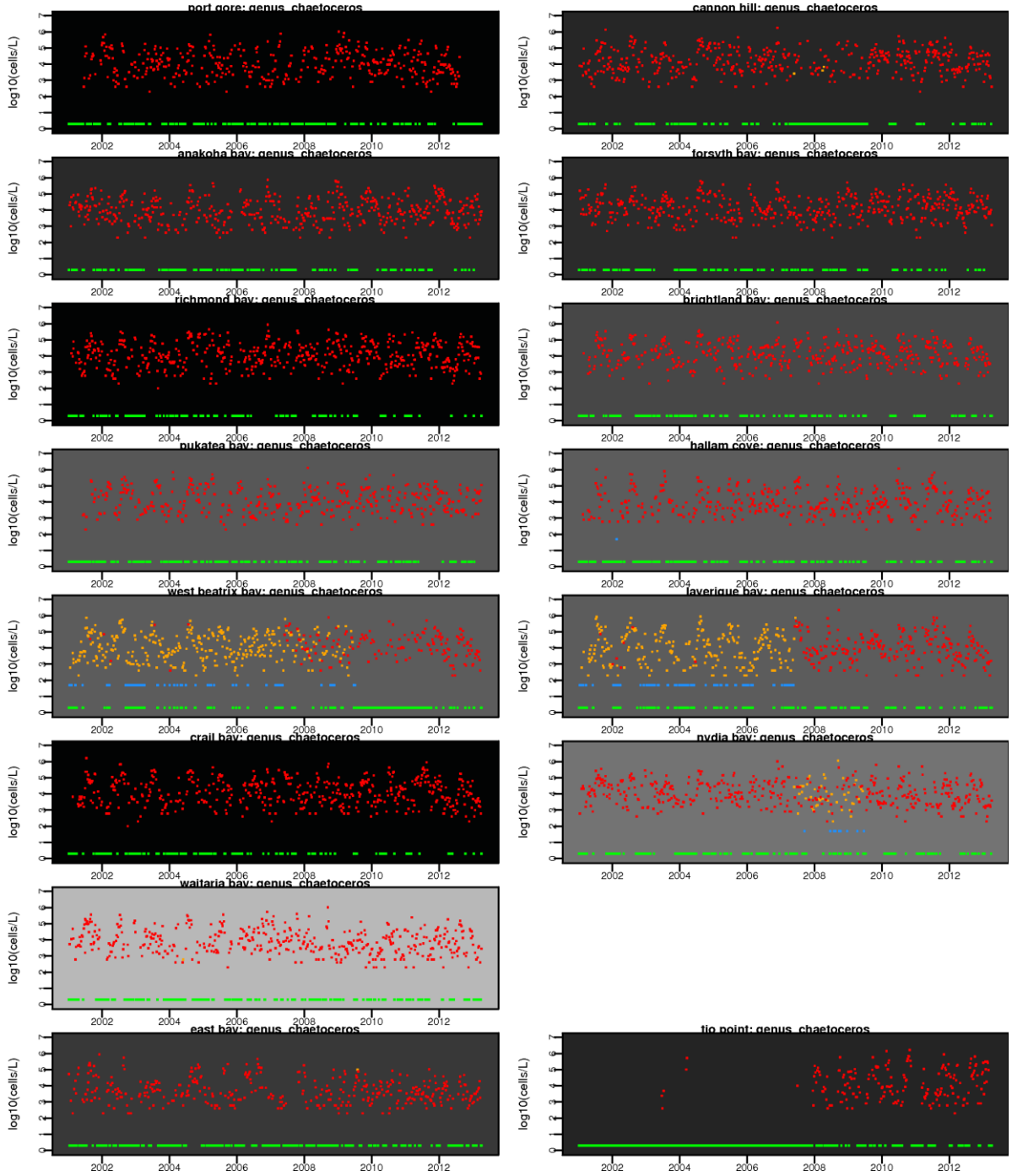

Figure 2-11: Time-series of recorded abundance of genus *Chaetoceros* (red & orange dots). Orange dots indicate dates upon which the recorded abundance is derived from a full-count. Blue dots correspond to full-count sampling dates on which no members of the genus were found at the station. For such dates, the measured cell concentration can safely be inferred to be zero (plotted as $\log_{10}$(50 cells/L) – being half of the detection-limit cell concentration). Green dots indicate dates on which there are missing-values in routine-count time-series. Within full-counts and for toxic taxa, a missing value is indicative of zero abundance. For non-toxic taxa within routine-counts, it merely indicates that the taxon was not amongst the most abundant at the Sounds-wide-scale. The background grey-scale is indicative of the through-the-Sounds-distance between the open Cook Strait and the sampling location (paler grey backgrounds indicate larger distances).

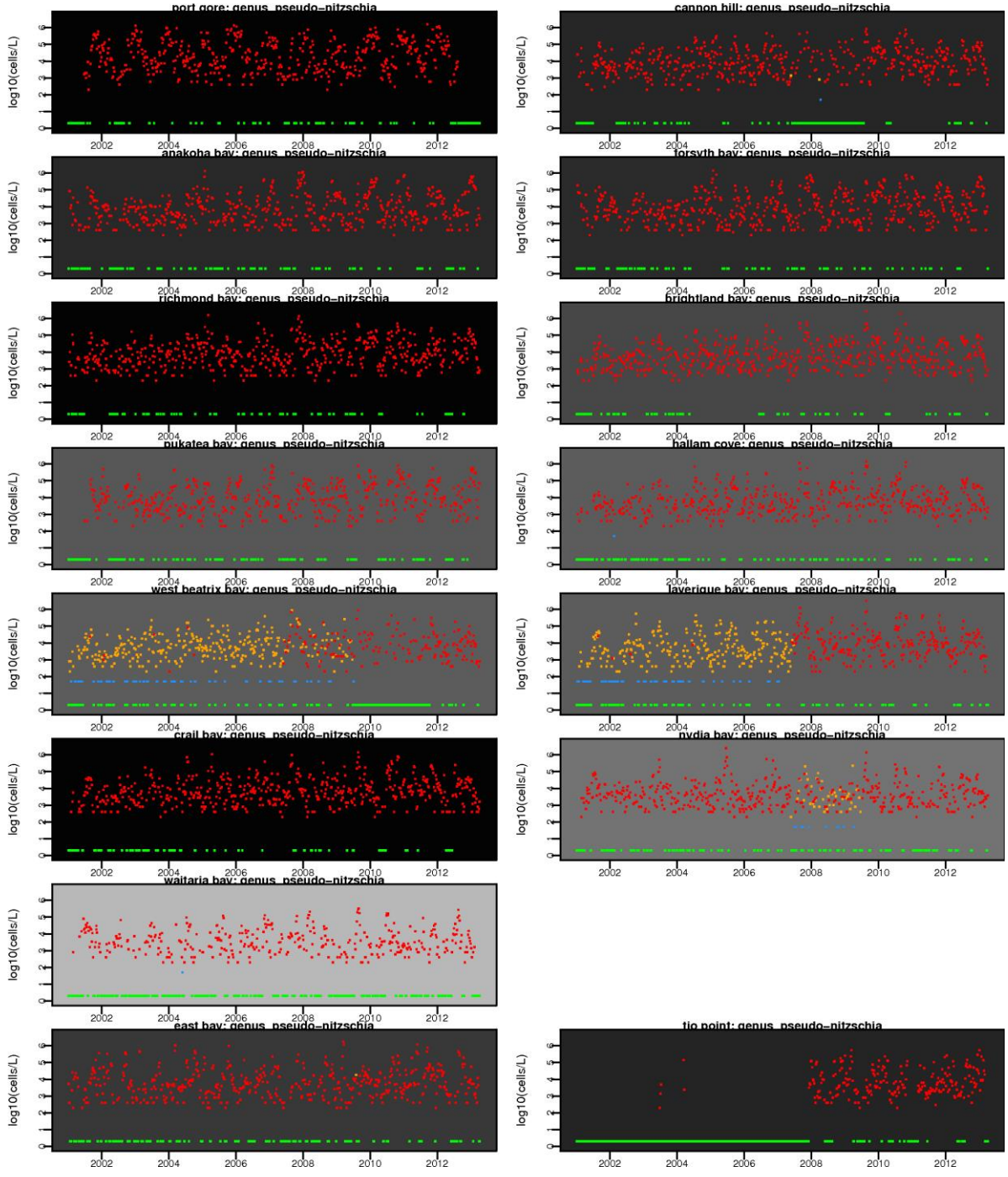

Figure 2-12: Time-series of recorded abundance of members of the genus *Pseudo-nitzschia*. See the legend of Figure 2-11 for a description of the colour-scheme.

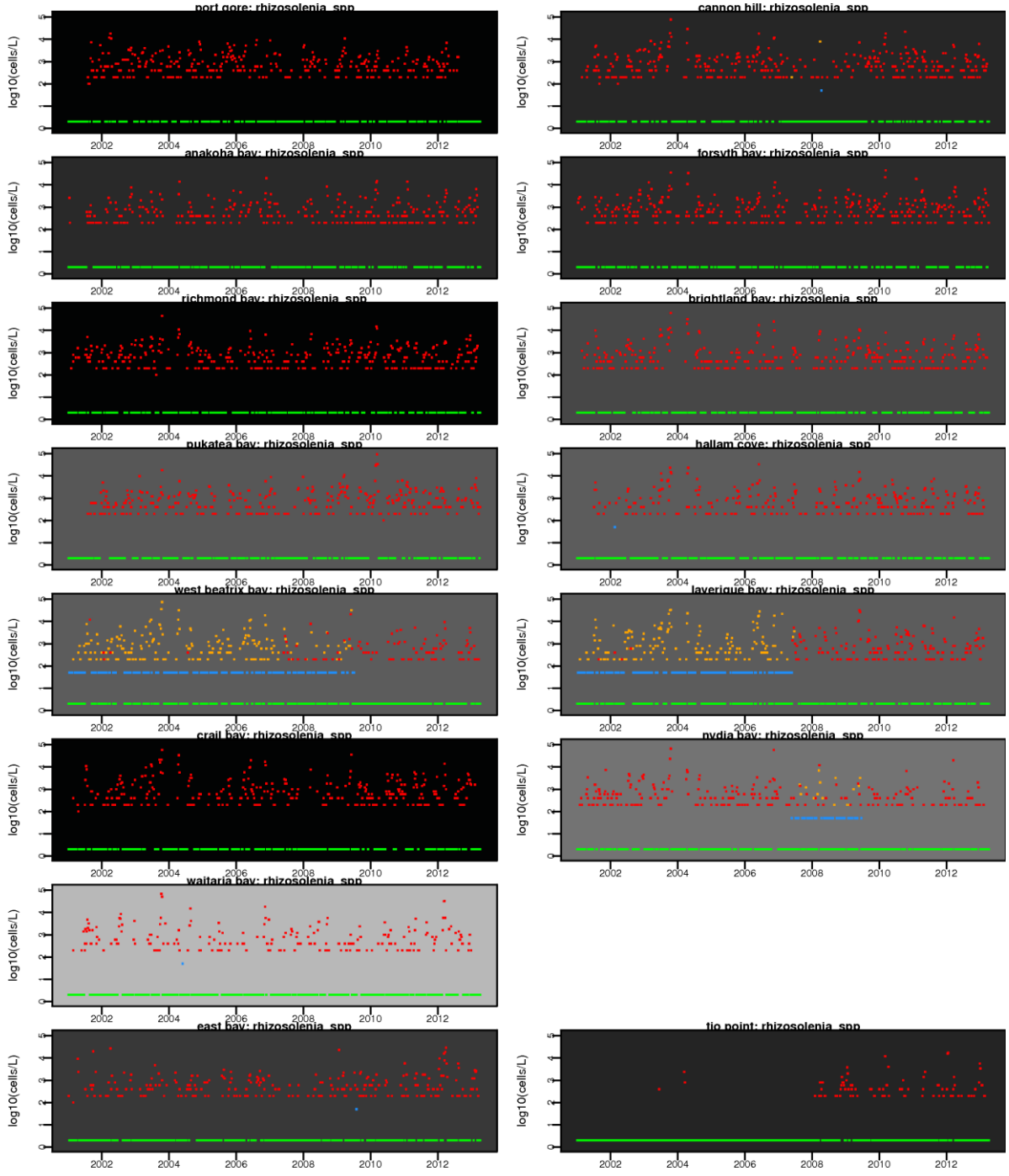

Figure 2-13: Time-series of recorded abundance of *Rhizosolenia* spp. See the legend of Figure 2-11 for a description of the colour-scheme.

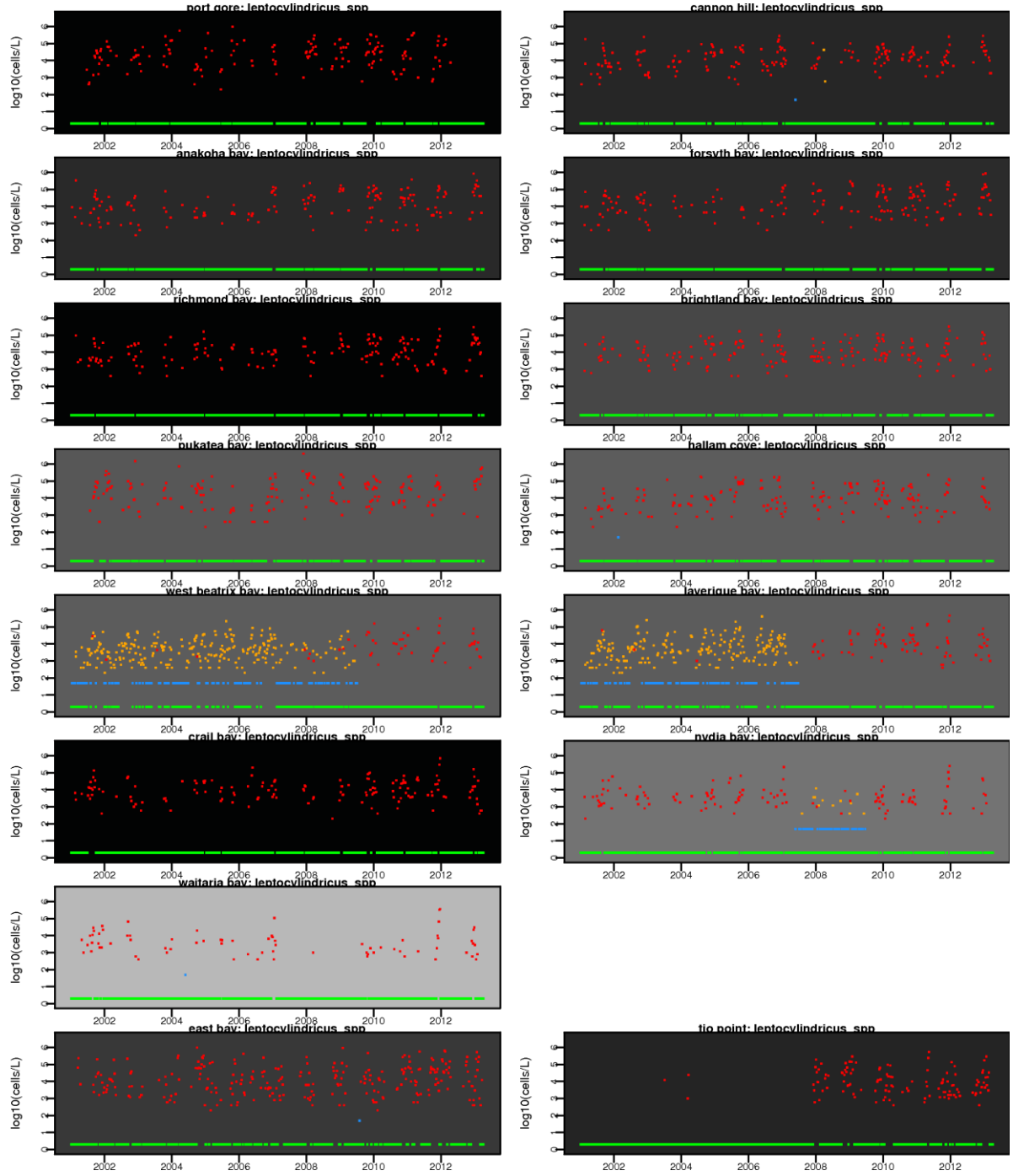

Figure 2-14: Time-series of recorded abundance of *Leptocylindricus* spp. See the legend of Figure 2-11 for a description of the colour-scheme.

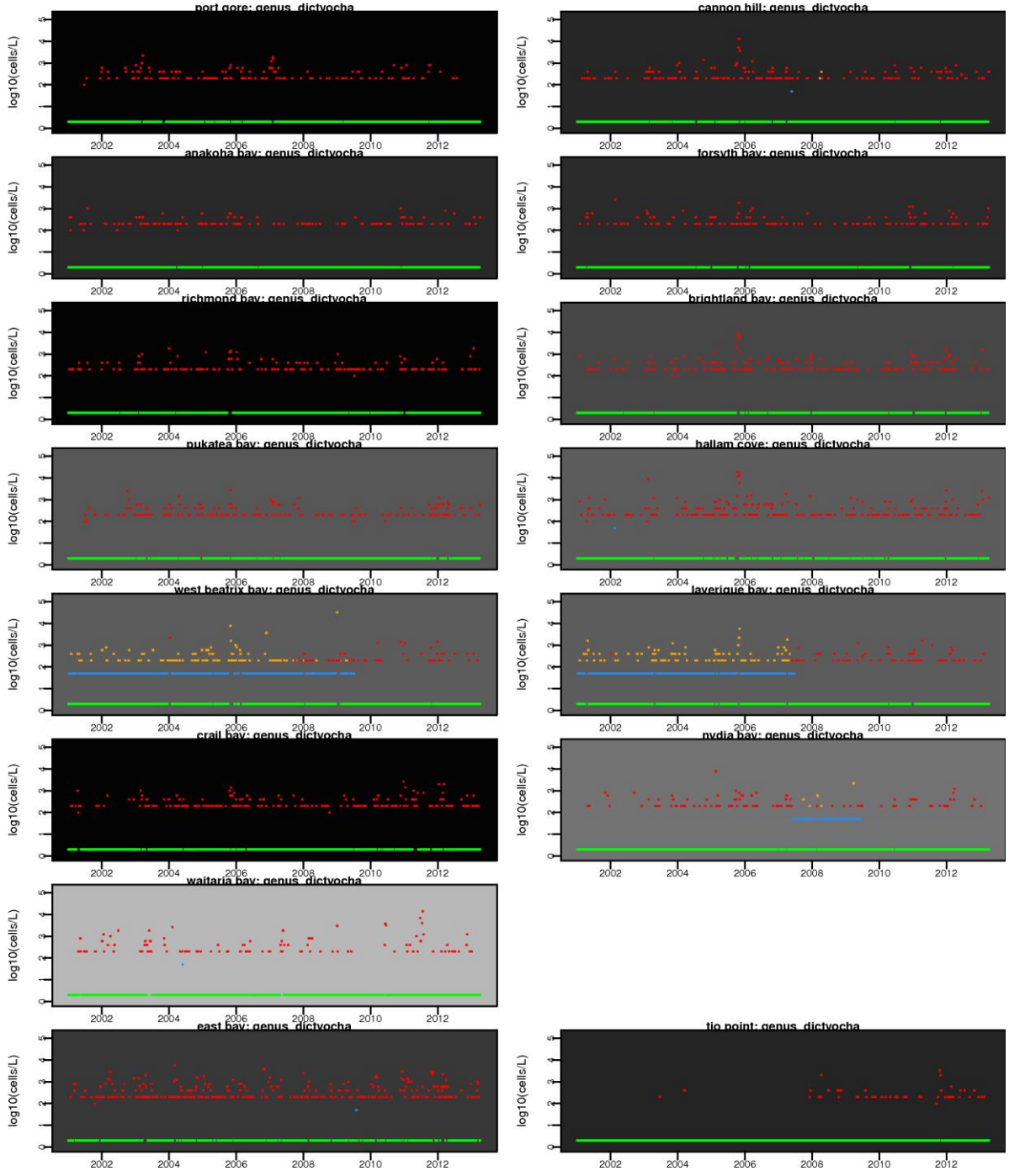

Figure 2-15: Time-series of recorded abundance of members of the *Dictyocha* genus. See the legend of Figure 2-11 for a description of the colour-scheme.

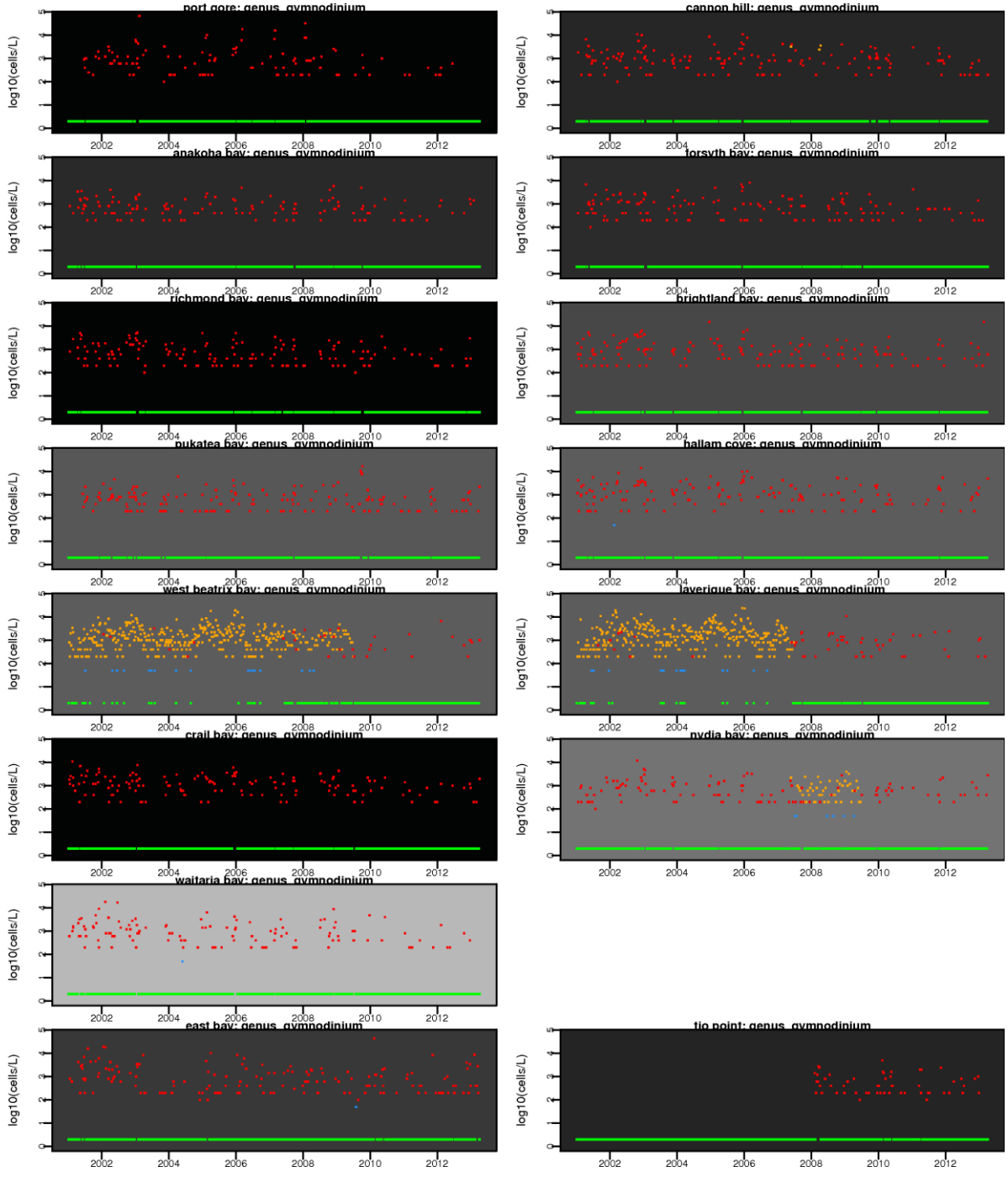

Figure 2-16: Time-series of recorded abundance of members of the *Gymnodinium* genus. See the legend of Figure 2-11 for a description of the colour-scheme.

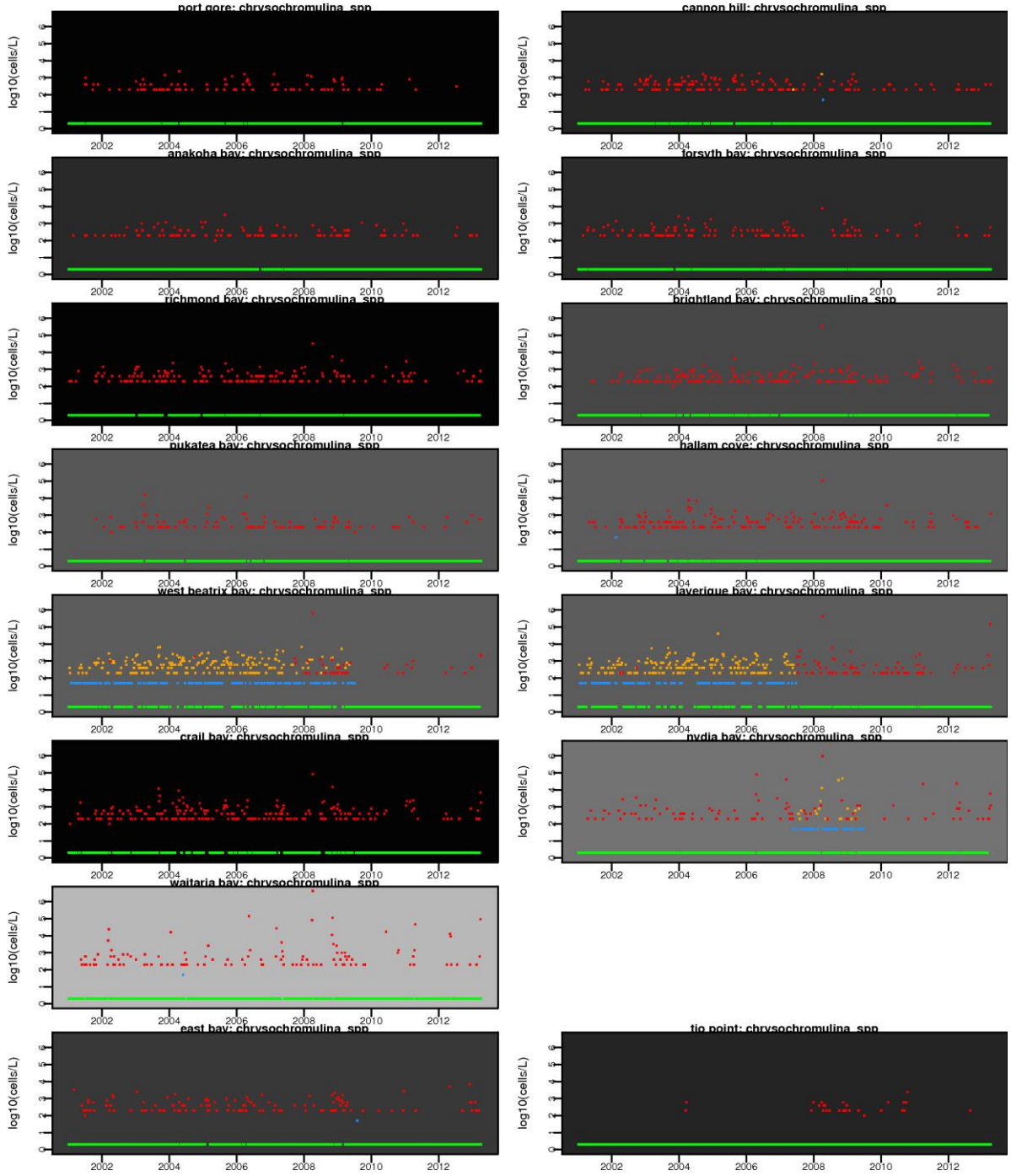

Figure 2-17: Time-series of recorded abundance of *Chrysochromulina* spp. See the legend of Figure 2-11 for a description of the colour-scheme.

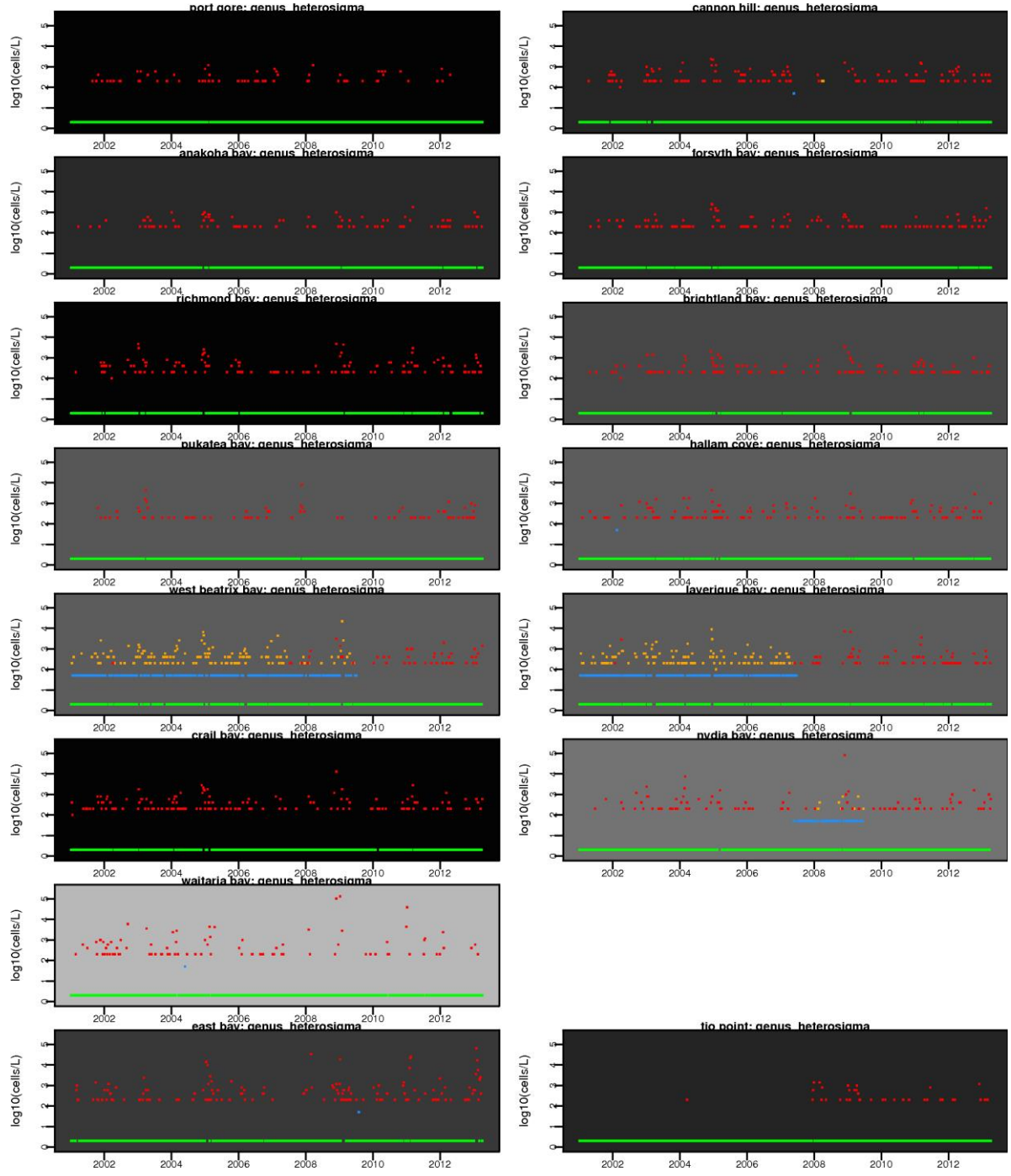

Figure 2-18: Time-series of recorded abundance of *Heterosigma akashiwo*. See the legend of Figure 2-11 for a description of the colour-scheme.

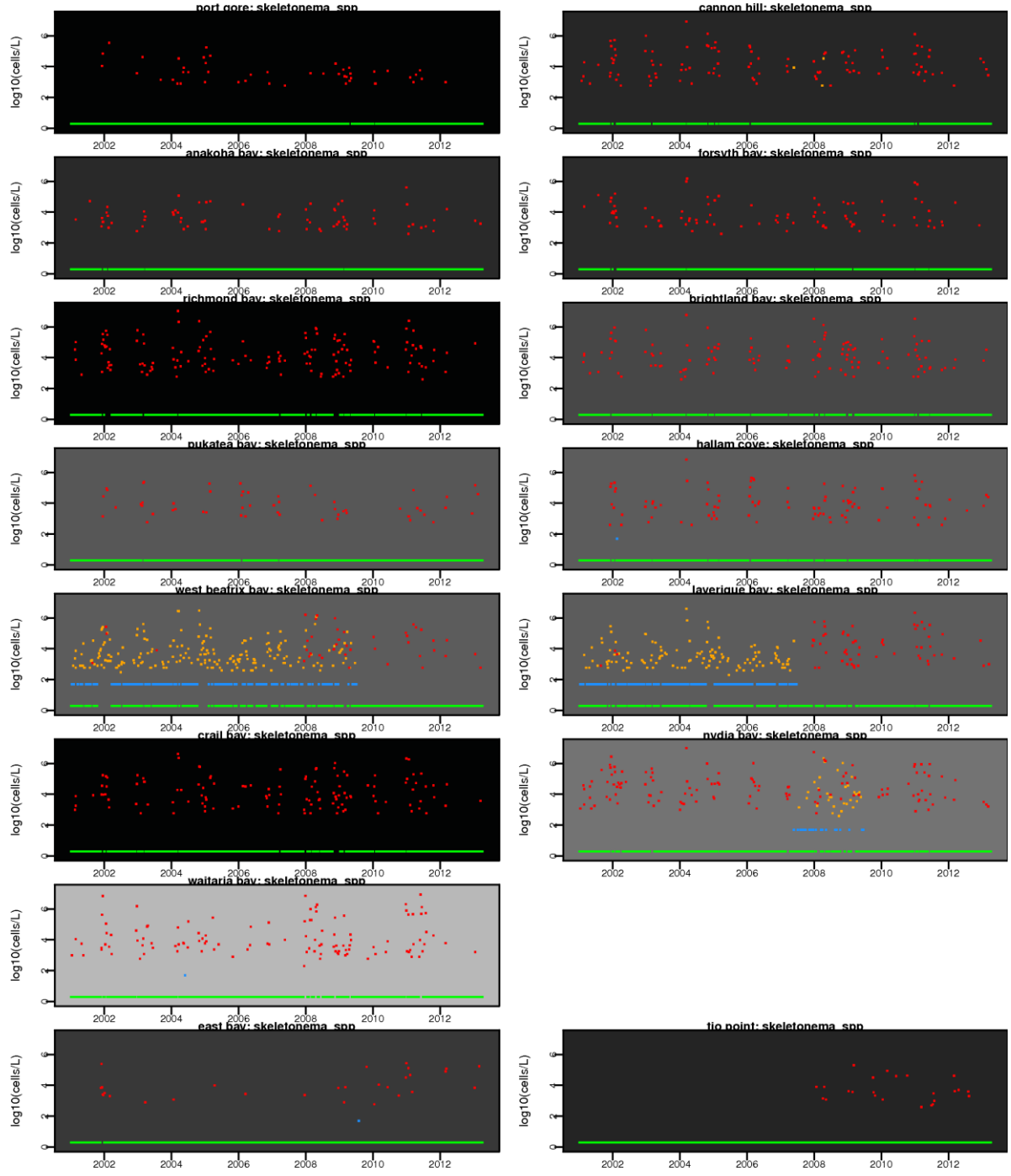

Figure 2-19: Time-series of recorded abundance of *Skeletonema* spp. See the legend of Figure 2-11 for a description of the colour-scheme.

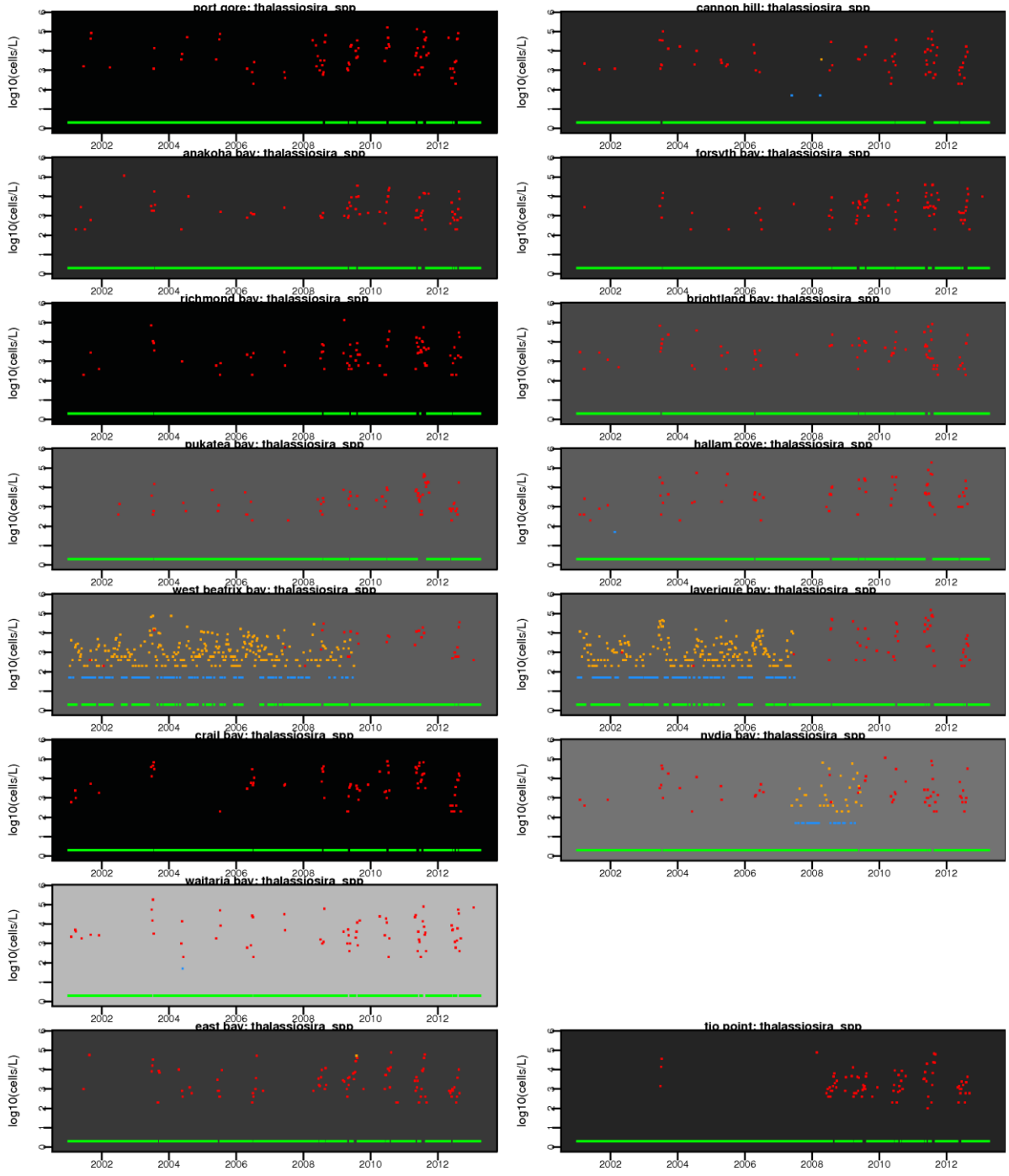

Figure 2-20: Time-series of recorded abundance of *Thalassiosira* spp. See the legend of Figure 2-11 for a description of the colour-scheme.

Figure 2-21 shows the time-series for total abundances of diatoms, dinoflagellates and other algal taxa at each site. Diatoms are almost invariably the dominant taxa (by cell concentration) – particularly in the routine-count records. The diatoms of the sites closest to Cook Strait (Port Gore, Anakoha Bay, Forsyth Bay, Pukatea bay) exhibit much more regular annual cycles than those evident at other sites. That said, East Bay (Cook Strait entrance of Queen Charlotte Sound) does not exhibit such regular dynamics whilst Tio Point (Tory Channel) does. Given that flow is believed to be clockwise within the Tory Channel/outer Queen Charlotte system), these results are consistent with a view that diatom dynamics are more regular in Cook Strait than in the central or inner parts of the Marlborough Sounds.

The total diatom population (measured as cell concentration) differs little between full and routine counts. This implies that non-toxic diatoms (usually members of the genus *Chaetoceros*) are the numerical dominants in the plankton system).Conversely, the concentrations of dinoflagellates and of other phytoplankton are markedly lower in the routine counts – because the non-toxic members of these taxa are rarely (if ever) amongst the numerical dominants by cell concentration (so rarely counted in routine counts).

Total phytoplankton abundance (as cell concentration) fluctuates through one-two orders of magnitude over the course of a year (Figure 2-21) – which is consistent with the magnitude of seasonal fluctuations evident in the most frequently present taxa (Figure 2-22 - **Figure 2-30**[7]).

[7] These box-plots for monthly abundance in the MSQP data (and later ones for monthly abundance in the MDC data) are based exclusively upon the records of species that were found on any given data. In effect, non-detections (zero-counts) have been treated as missing values. For rarer/infrequently recorded species, the box-plots will be biased (tend to over-estimate true cell concentration) as a result. Since the MSQP cell counts are derived from scans of smaller volumes of water than those of the MSQP counts, the over-estimation will tend to be greater in the MSQP data.

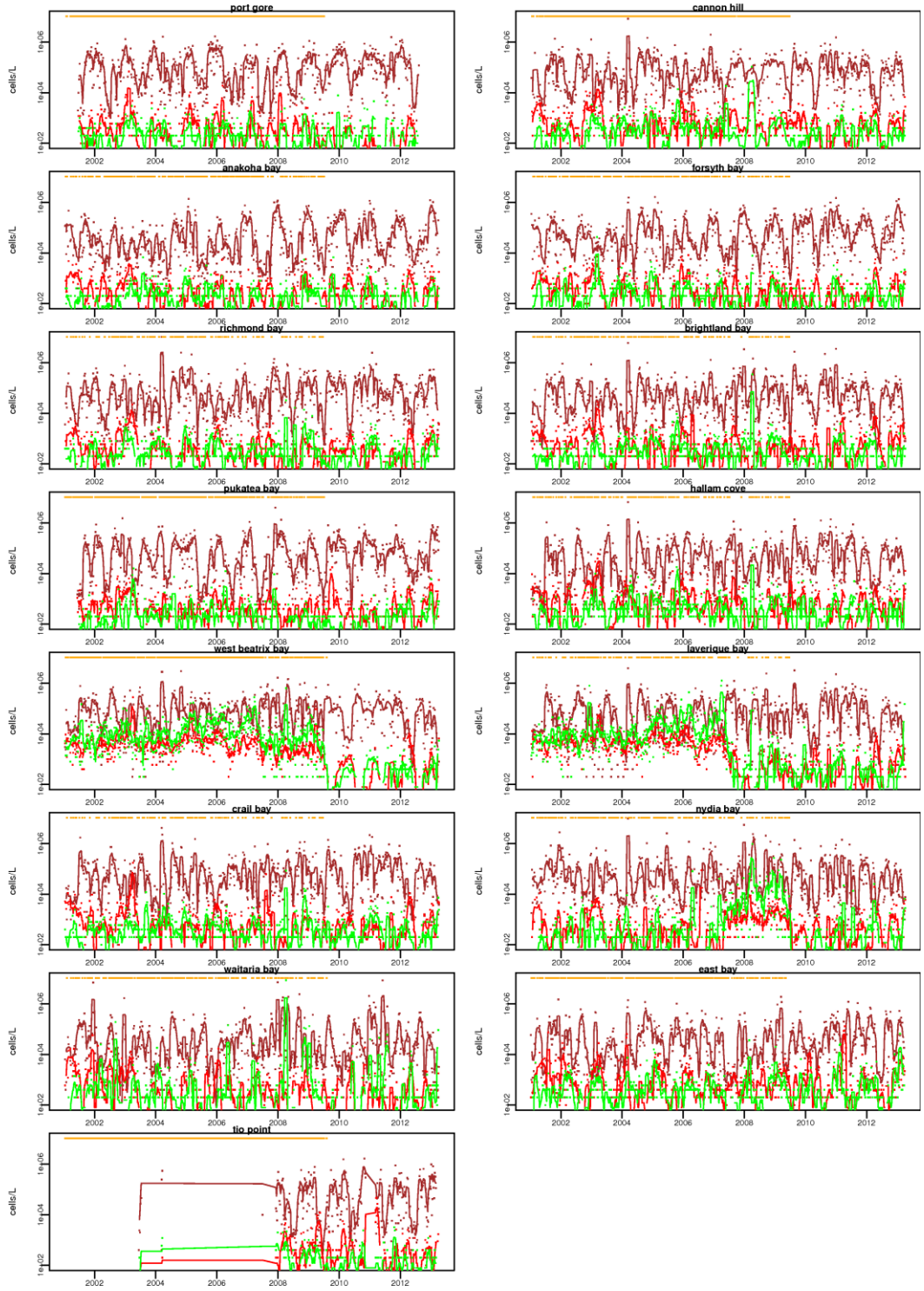

Figure 2-21: Time-series of total cell concentrations for diatoms (brown), dinoflagellates (red), and other plankton (green). Dots are the raw data; lines are 5-point time-centred moving averages.

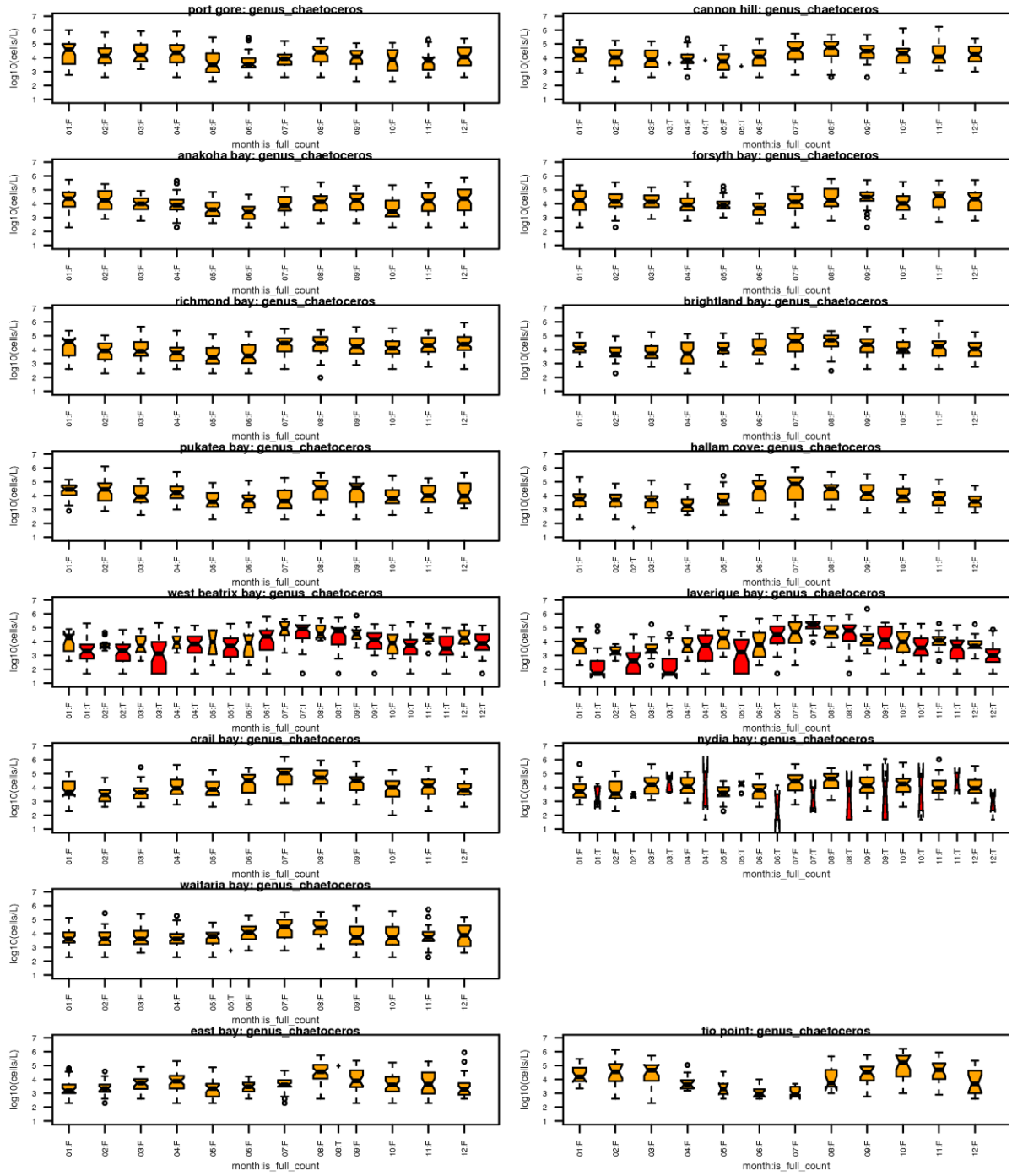

Figure 2-22: Boxplots revealing the seasonal-scale dynamics of members of the *Chaetoceros* genus. Red polygons are based upon full-counts. Orange polygons are based upon routine-counts. Months are numbered 1-12 (January-February) in the legend below each box-plot. The 'waist' of each box marks the median. The 'notches' denote the confidence bounds on the median – if notches do not overlap, it is 'strong evidence' that the two medians are differ significantly different at the 95% level. The whiskers extend to 1.5 x the inter-quartile range of the data.

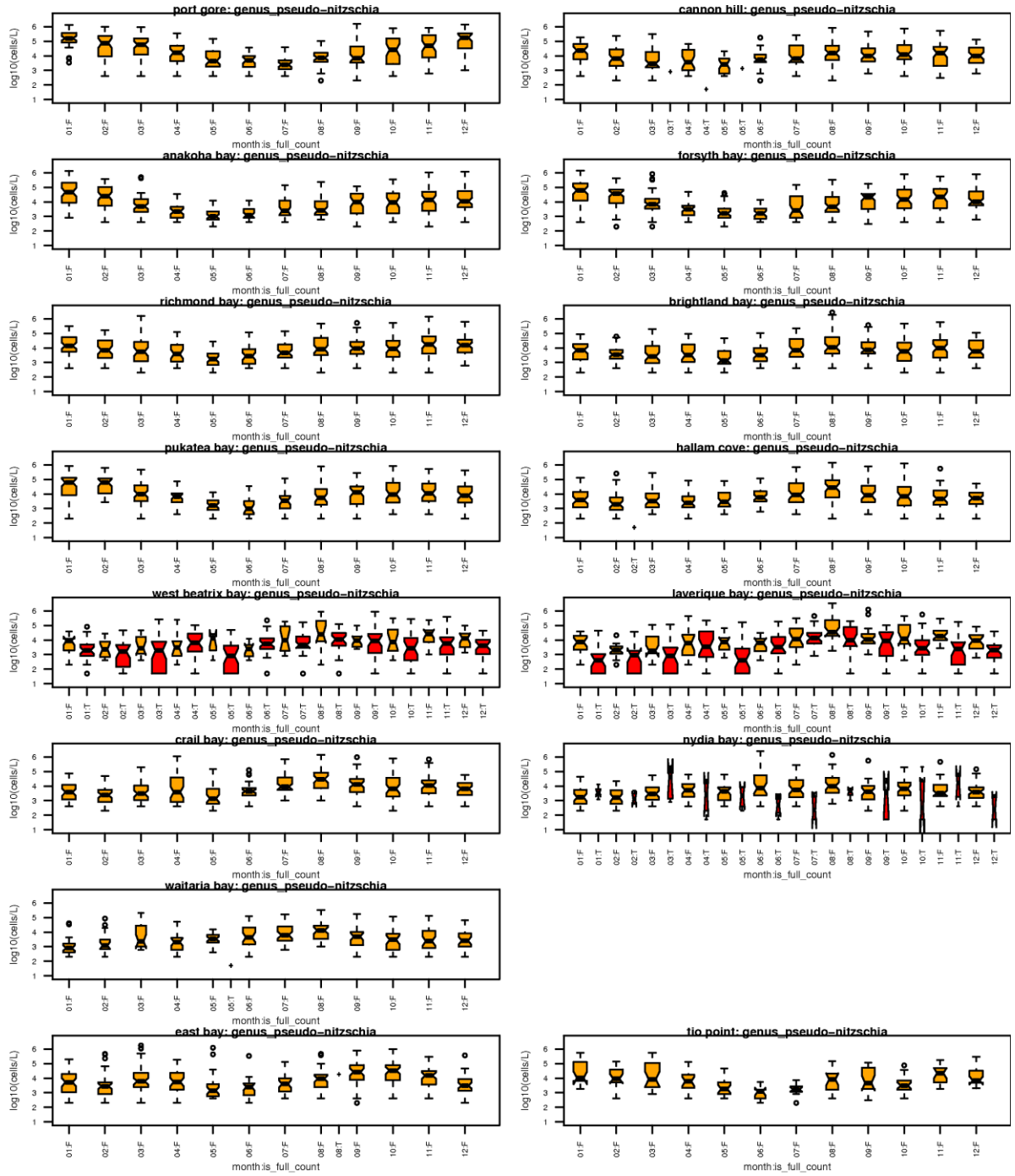

Figure 2-23: Boxplots revealing the seasonal-scale dynamics of members of the *Pseudo-nitzschia* genus.

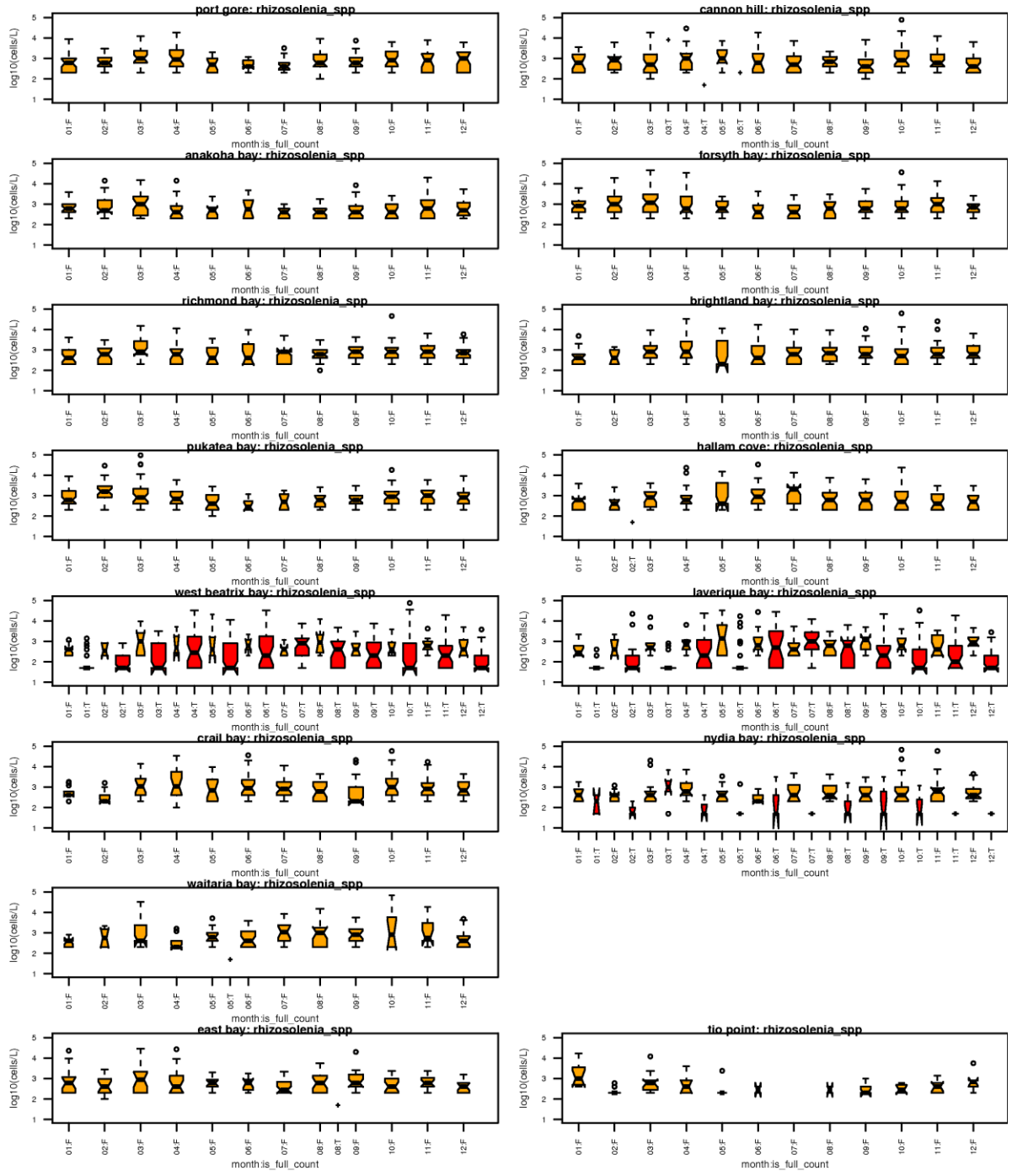

Figure 2-24: Boxplots revealing the seasonal-scale dynamics of members of the *Rhizosolenia* genus.

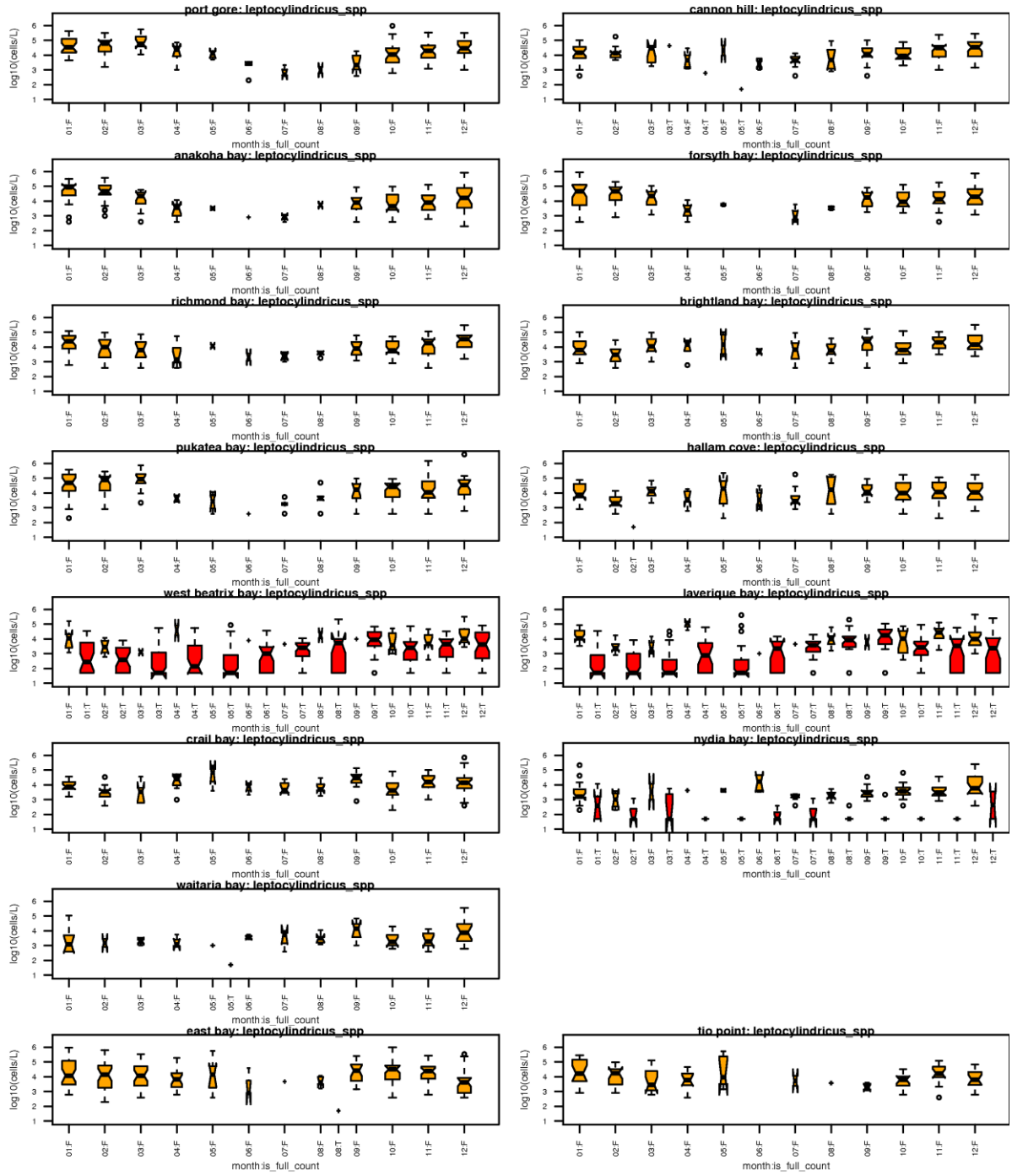

Figure 2-25: Boxplots revealing the seasonal-scale dynamics of members of the *Leptocylindricus* genus.

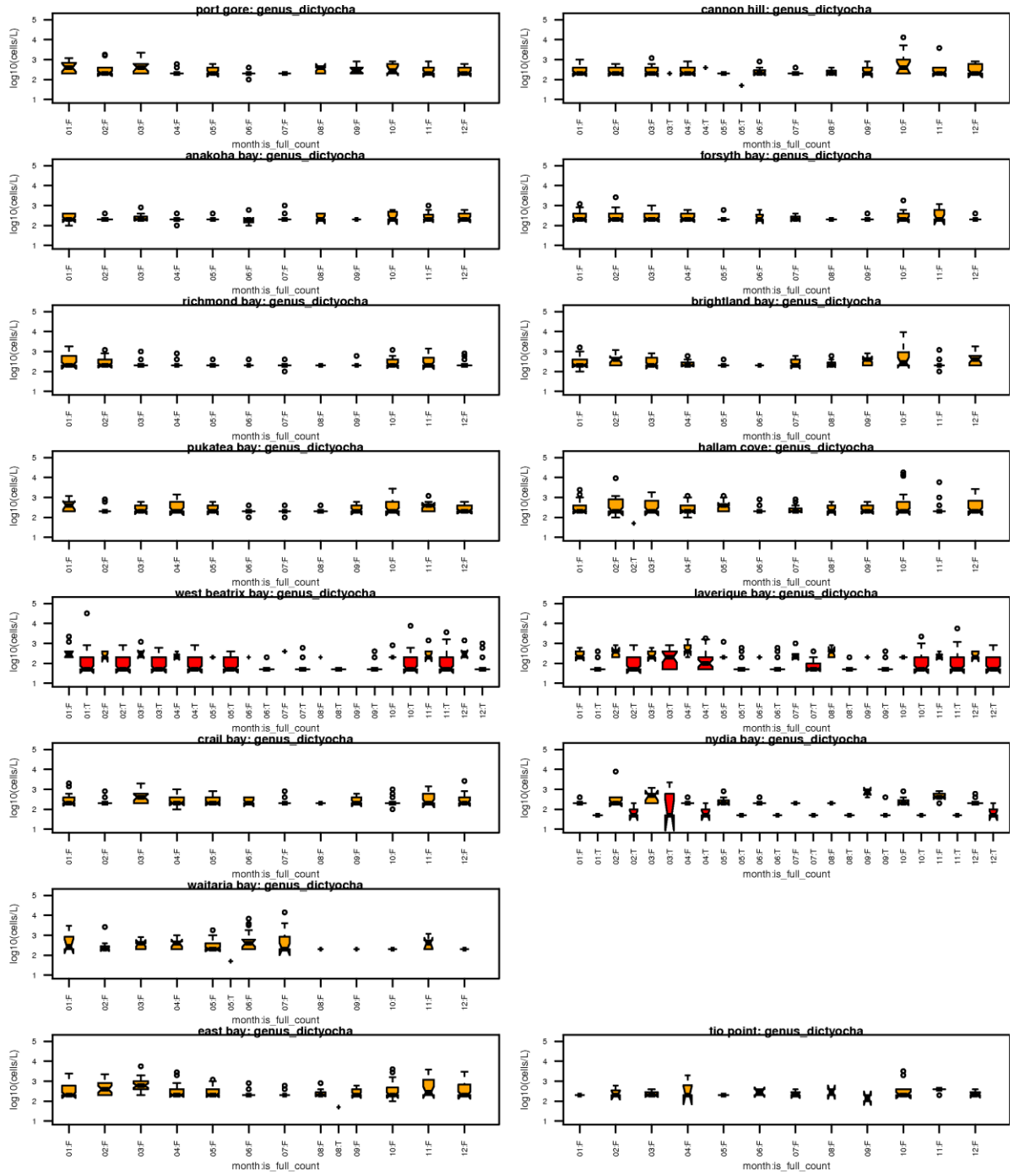

Figure 2-26: Boxplots revealing the seasonal-scale dynamics of members of the *Dictyocha* genus.

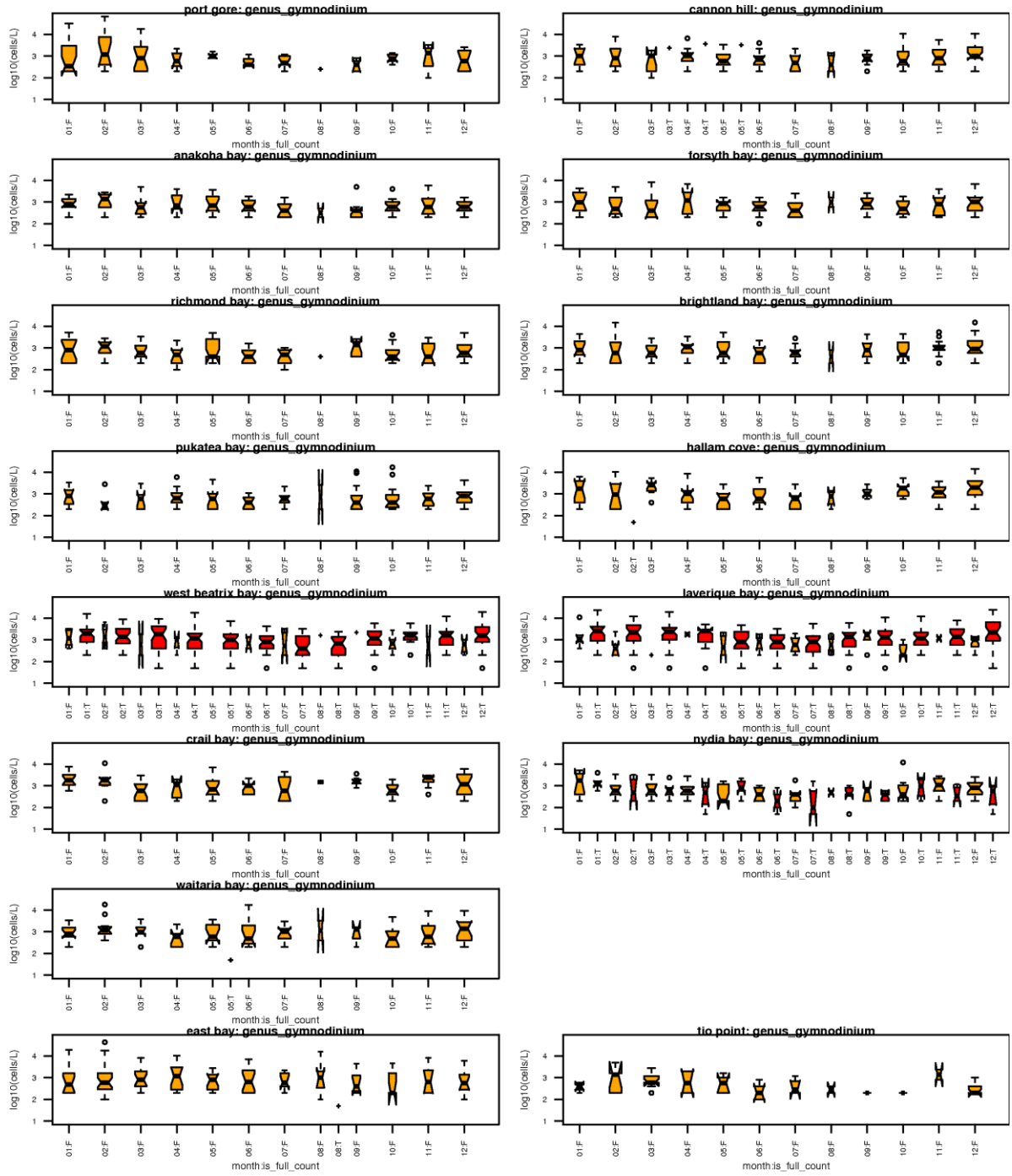

Figure 2-27: Boxplots revealing the seasonal-scale dynamics of members of the *Gymnodinium* genus.

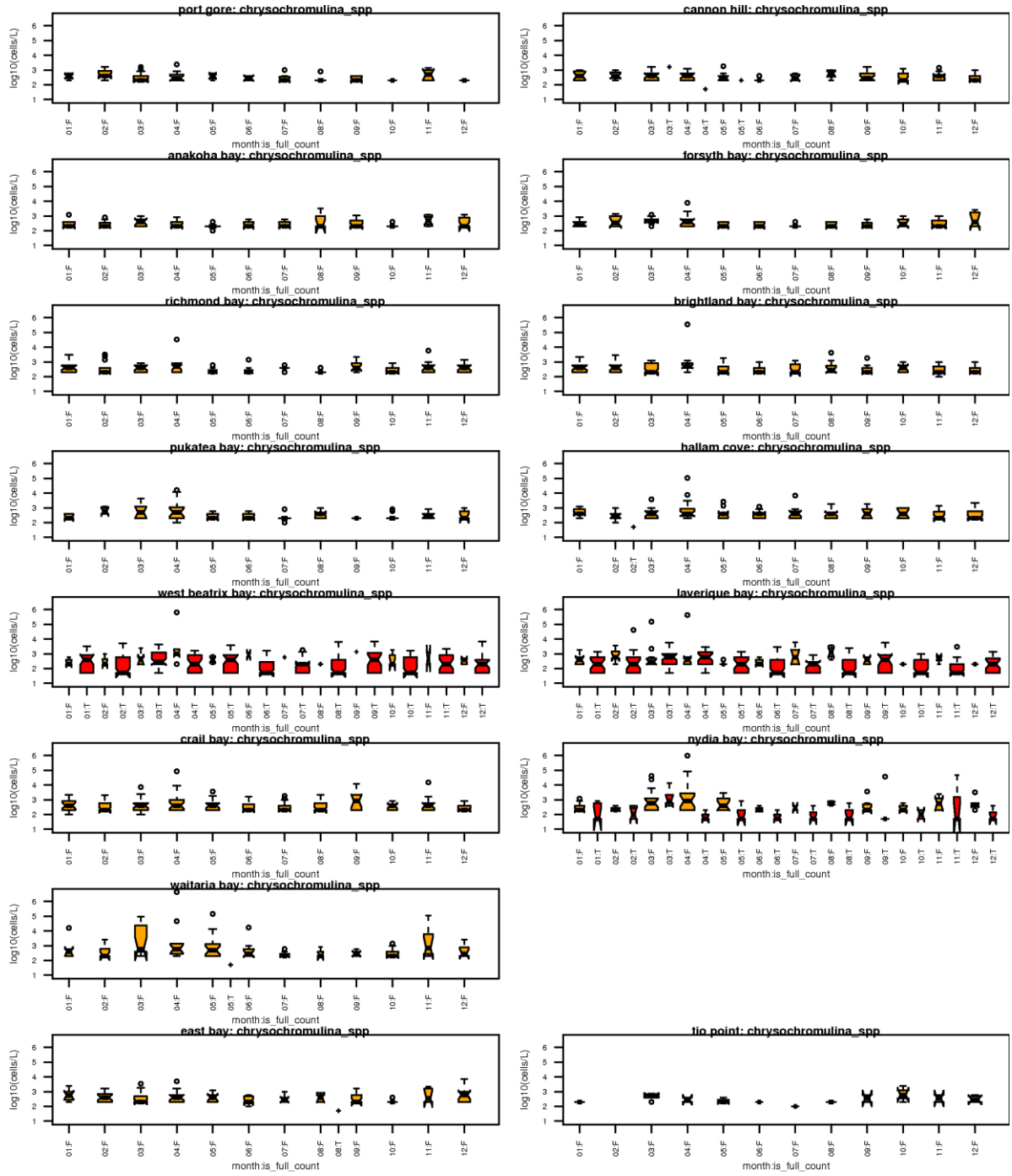

Figure 2-28: Boxplots revealing the seasonal-scale dynamics of members of the *Chrysocromulina* genus.

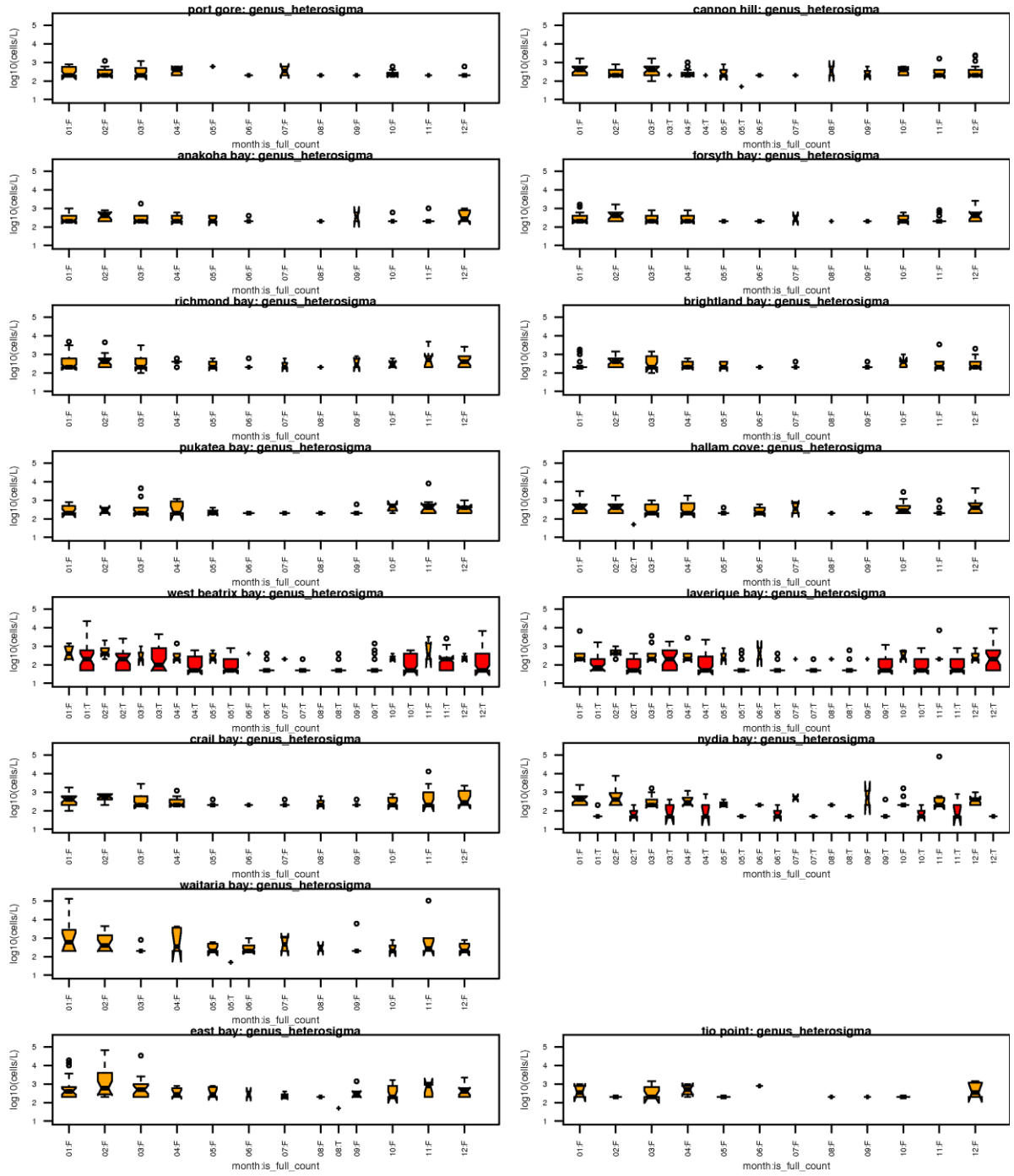

Figure 2-29: Boxplots revealing the seasonal-scale dynamics of members of the *Heterosigma* genus.

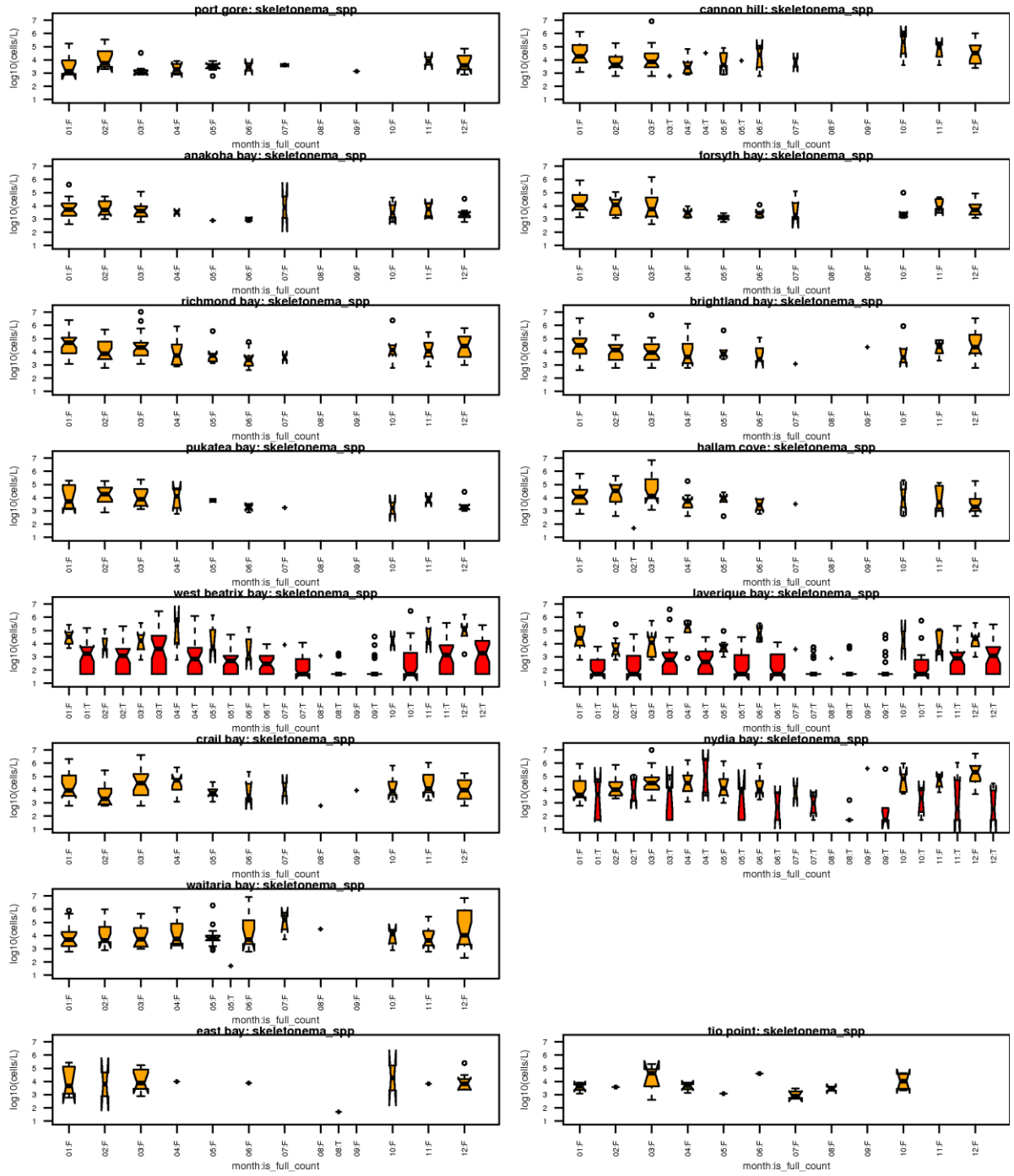

Figure 2-30: Boxplots revealing the seasonal-scale dynamics of members of the *Skeletonema* genus.

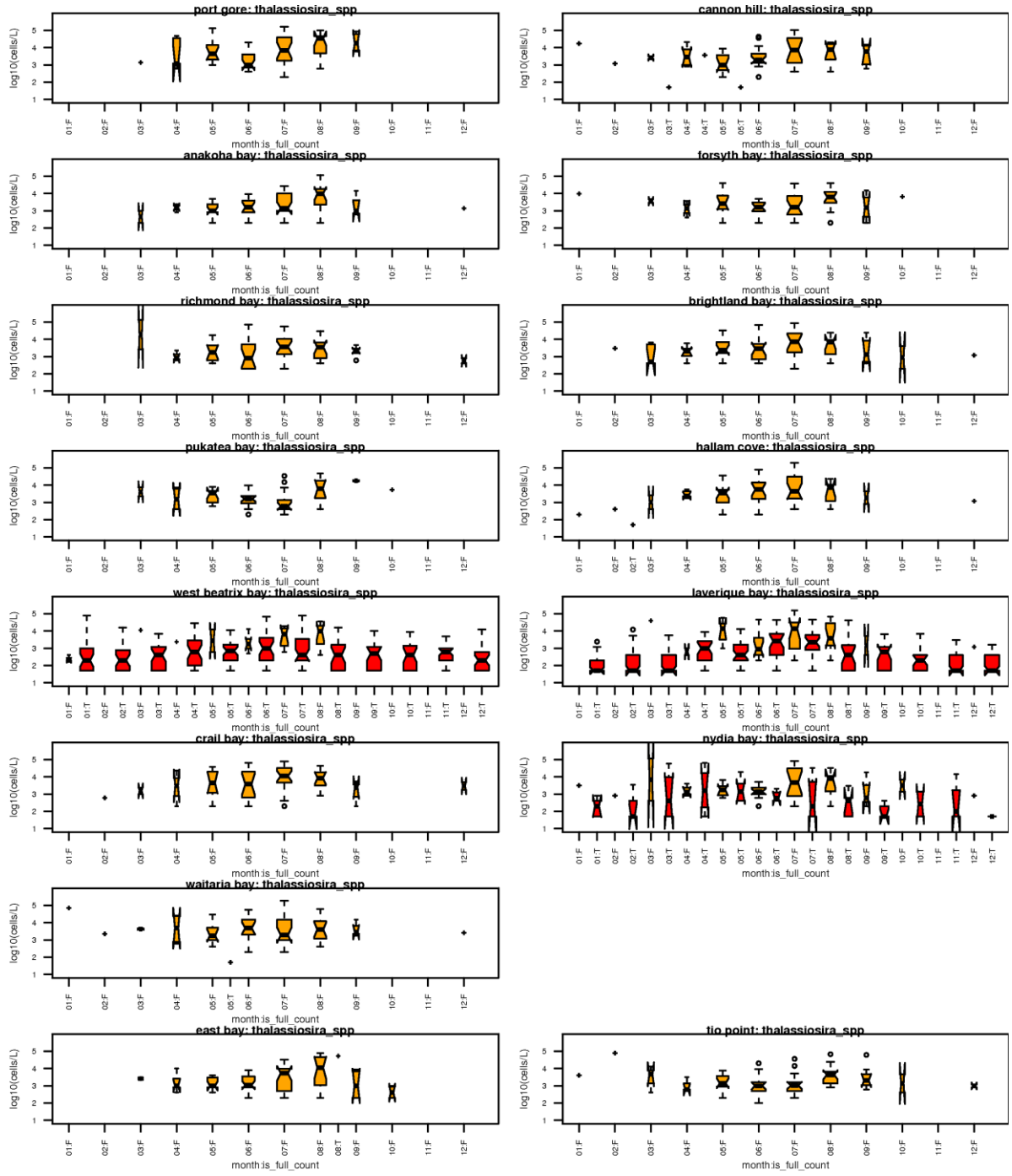

Figure 2-31: Boxplots revealing the seasonal-scale dynamics of members of the *Thalassiosira* genus.

3 MDC taxon-count data

The Marlborough District Council data span a much shorter time-frame than the MSQP data. Monthly water samples have been gathered at five sites in Queen Charlotte/Tory Channel and seven sites in Pelorus Sound (see Figures 4.1 and 4.27 of Broekhuizen (2013) for the locations of the sampling sites). The data span approximately two years for Queen Charlotte/Tory Channel and approximately one year for Pelorus Sound. Sampling has been monthly rather than weekly and comprised a bottle sample taken at 4 m below the water surface rather than a hose-sample. There are too few data-points to derive robust descriptors of the probability distributions of abundance – whether at the monthly or whole-of-time-series time-scale.

Comparisons between MDC and MSQP data must be interpreted with caution because: (a) the sampling sites are not co-located in horizontal space and span differing (but overlapping) depth ranges, (b) the sampling occasions differ, (c) the MDC data are derived from 200 mL water samples rather than 100 ML samples (as in the MSQP) – implying that the MDC data have a lower detection limit. Nonetheless (and, as one would hope), the MDC data appear to be consistent with the MSQP data when compared on a like-for like basis. *Chaetoceros* spp. are usually one of the dominant taxa (by cell counts) and the estimated concentrations are consistent with those measured in the MSQP data (albeit, towards the lower end of those measured in the MSQP – compare Figure 2-11 and Figure 3-1). Data for the other major taxa are also consistent with (but towards the lower end of the range within) the corresponding MSQP data (Figure 3-1 - Figure 3-8)[8]. It is, perhaps, worth noting that two taxa (*Heterosigma* and *Chrysocromulina*) that are frequently recorded (albeit at low concentrations) in the MSQP data have not been recorded in the MDC data. Lugols-preserved *Heterosigma* and *Chrysocromulina* are difficult to identify and have been recorded only as a ‘small flagellate’ in the MDC data.

For the time-being, we are inclined to attribute the differences between MDC and MSQP data primarily to a combination of: (i) the differing detection limits (non-detections were treated as missing data rather than as zeros when drawing the box-plots), (ii) that non-toxic taxa are counted only when they are relatively abundant in the MSQP and (iii) a year-effect.

[8] For the Pelorus Sound sites, there are, at most, two data-points per month. For Queen Charlotte, there are a maximum of three data-points per month. With so few data-points, the distributional characteristics (median and percentiles etc.,) represented by the box-plots are very poorly characterised. Nonetheless, we have chosen to present box-plots to facilitate ready comparison of the MSQP and MDC data.

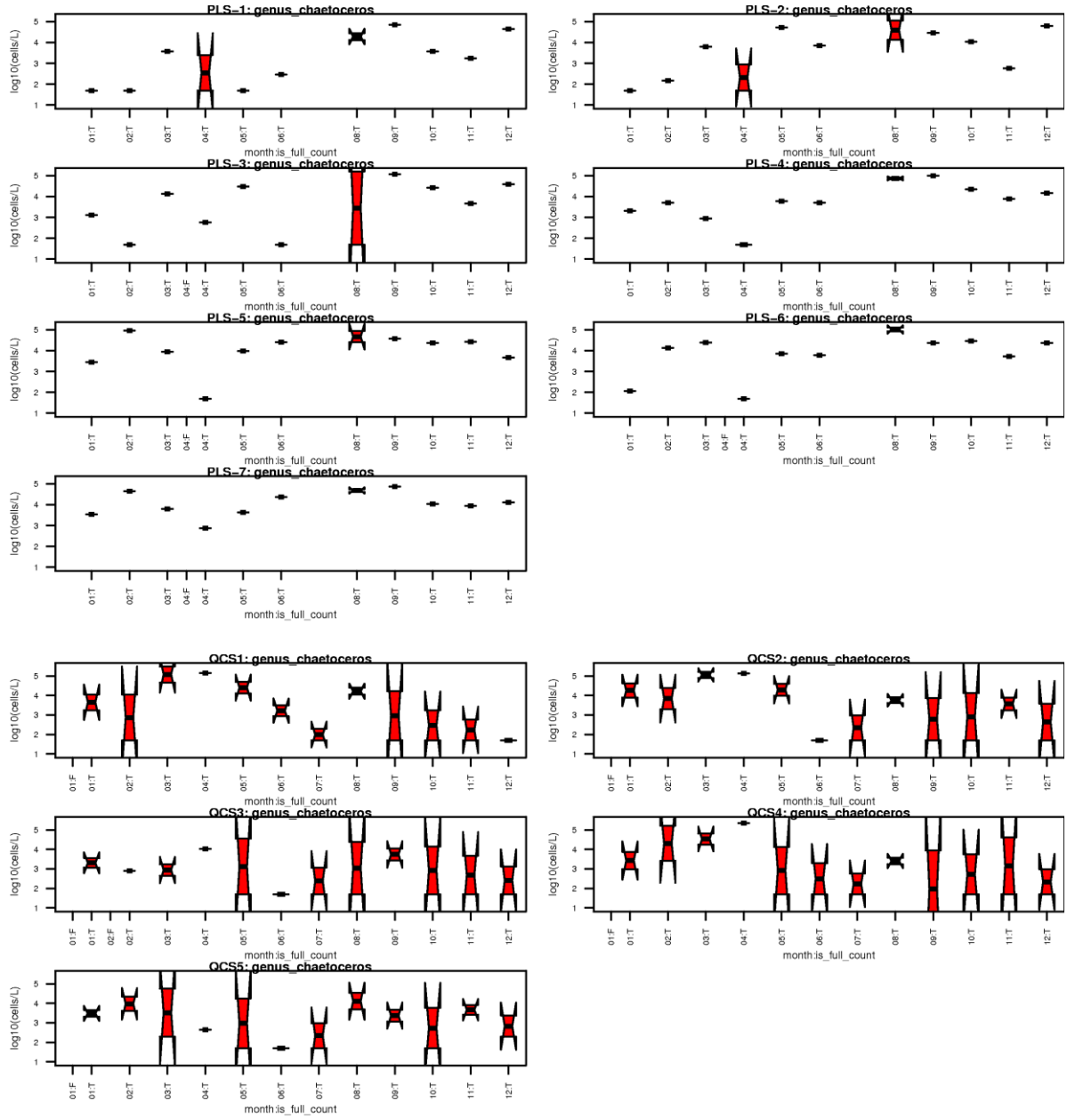

Figure 3-1: Time-series of the measured concentrations (cells/L) of members of the genus *Chaetoceros* measured in the MDC sampling programme. For Pelorus Sound (sites PLS1-PLS7), sampling has been monthly since July 2012. For Queen Charlotte (sites QCS1-6), sampling has been monthly since July 2011. Red dots indicate sampling dates on which no Chaetoceros were recorded.

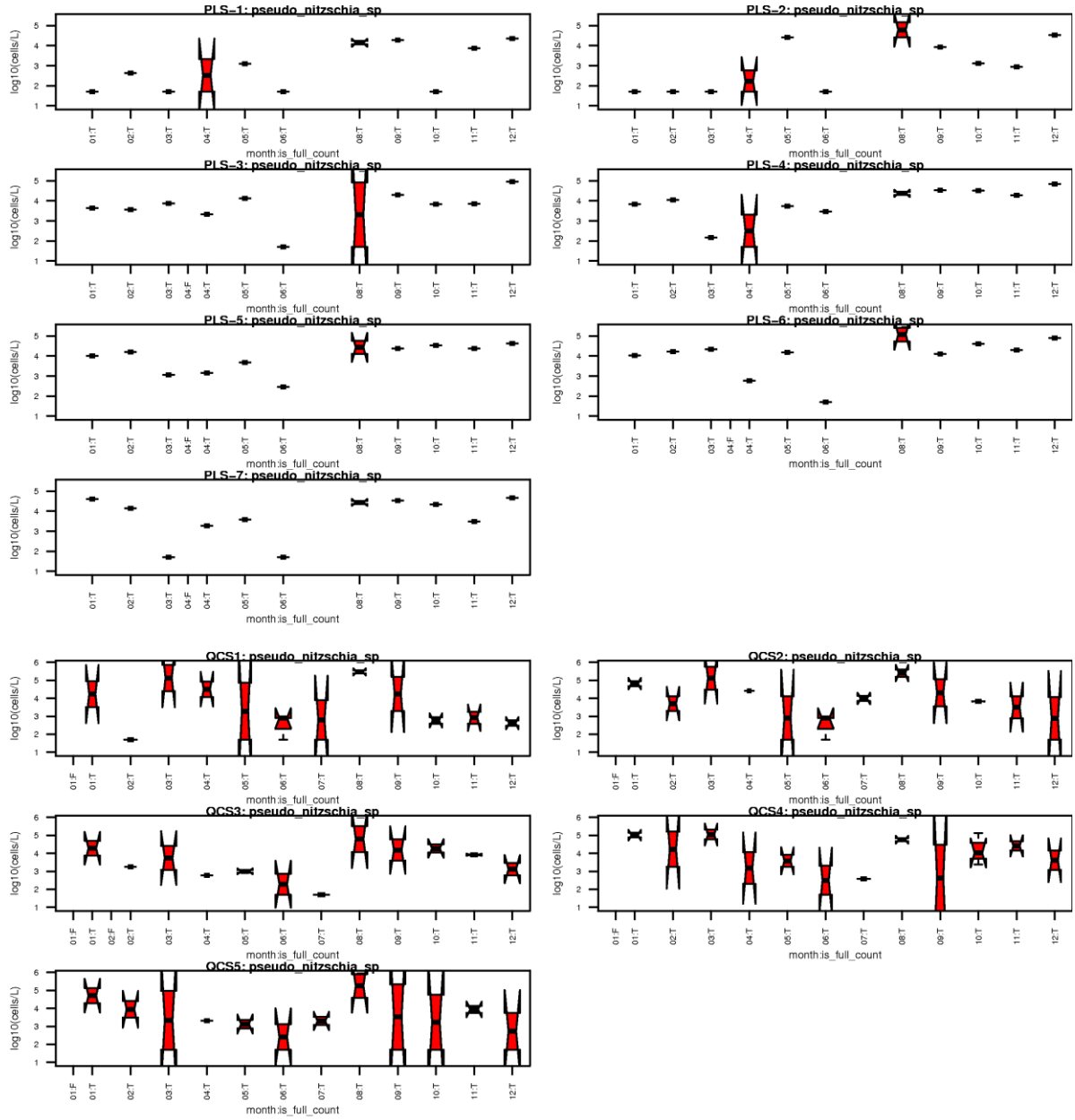

Figure 3-2: Time-series of the measured concentrations (cells/L) of members of the genus *Pseudo-nitzchia* measured in the MDC sampling programme.

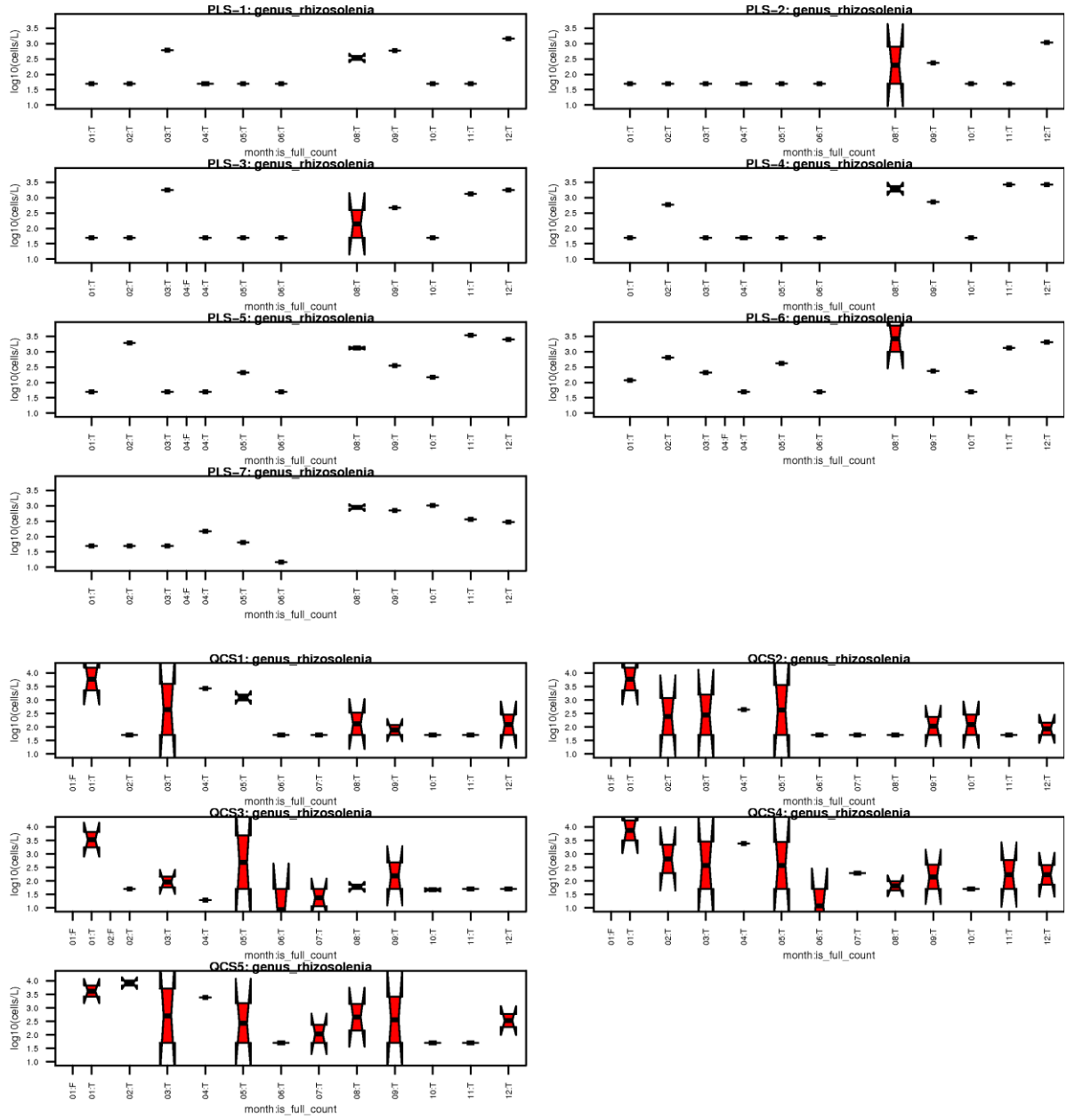

Figure 3-3: Time-series of the measured concentrations (cells/L) of members of the genus *Rhizosolenia* measured in the MDC sampling programme.

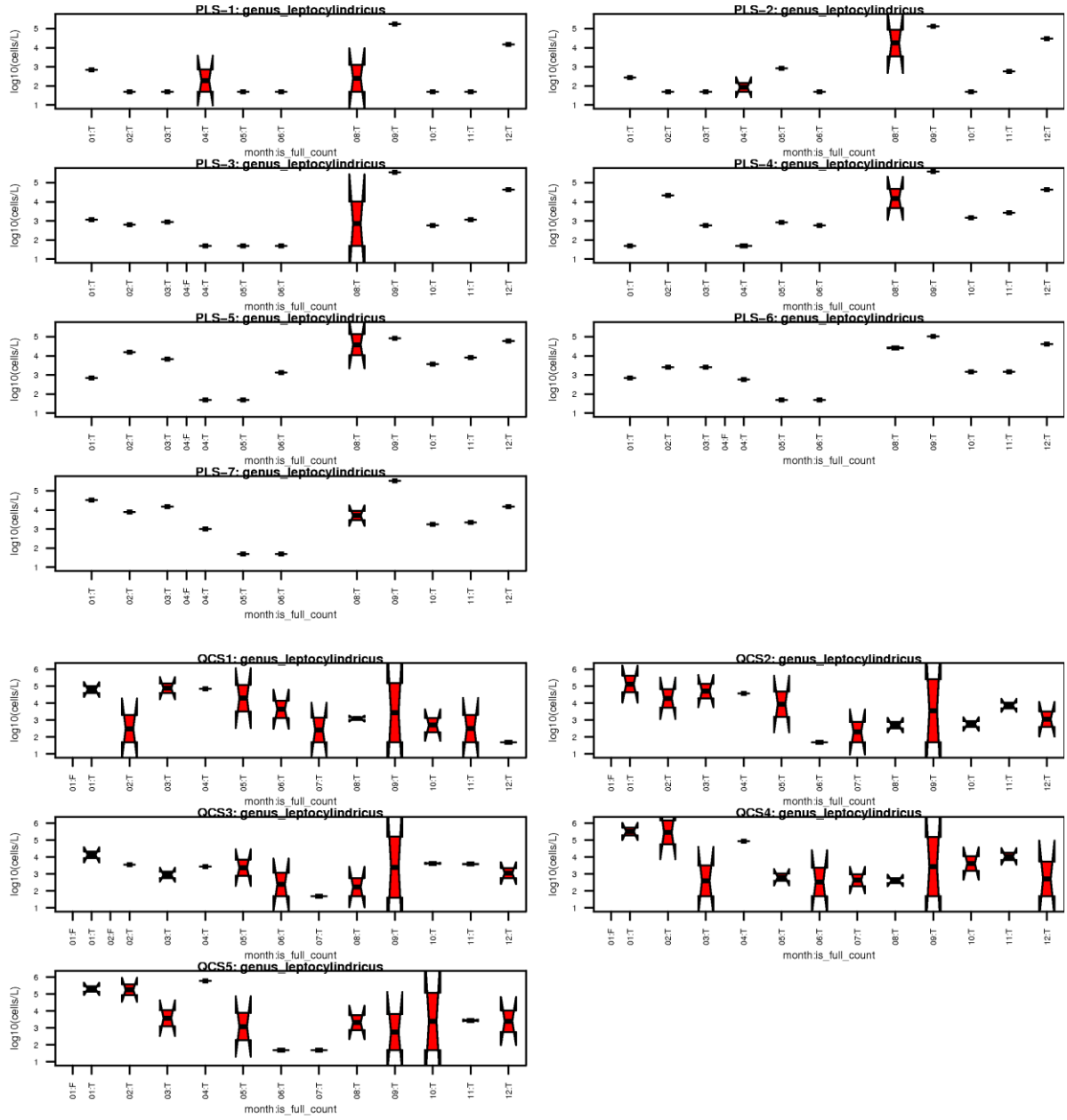

Figure 3-4: Time-series of the measured concentrations (cells/L) of members of the genus *Leptocylindricus* measured in the MDC sampling programme.

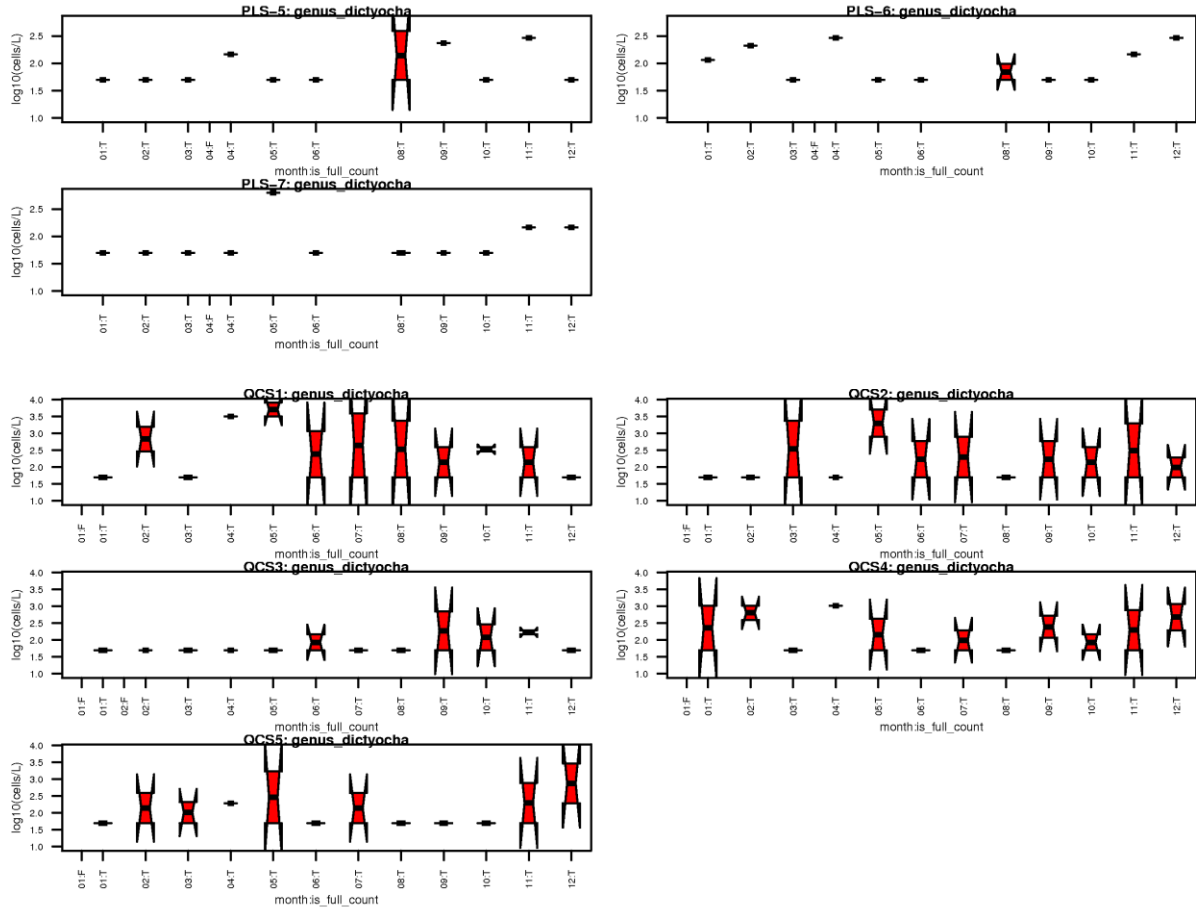

Figure 3-5: Time-series of the measured concentrations (cells/L) of members of the genus *Dictyocha* measured in the MDC sampling programme.

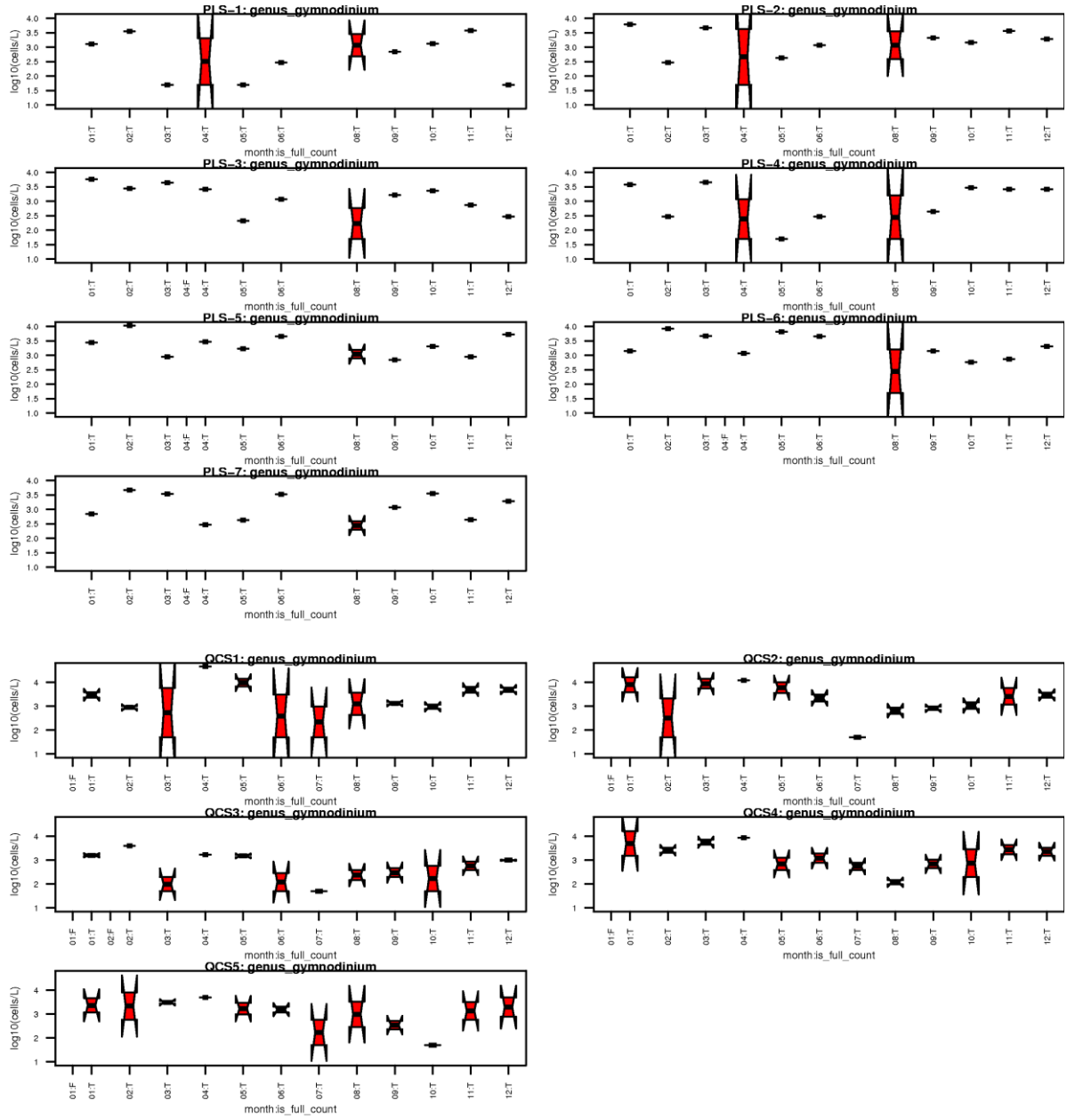

Figure 3-6: Time-series of the measured concentrations (cells/L) of members of the genus *Gymnodinium* measured in the MDC sampling programme.

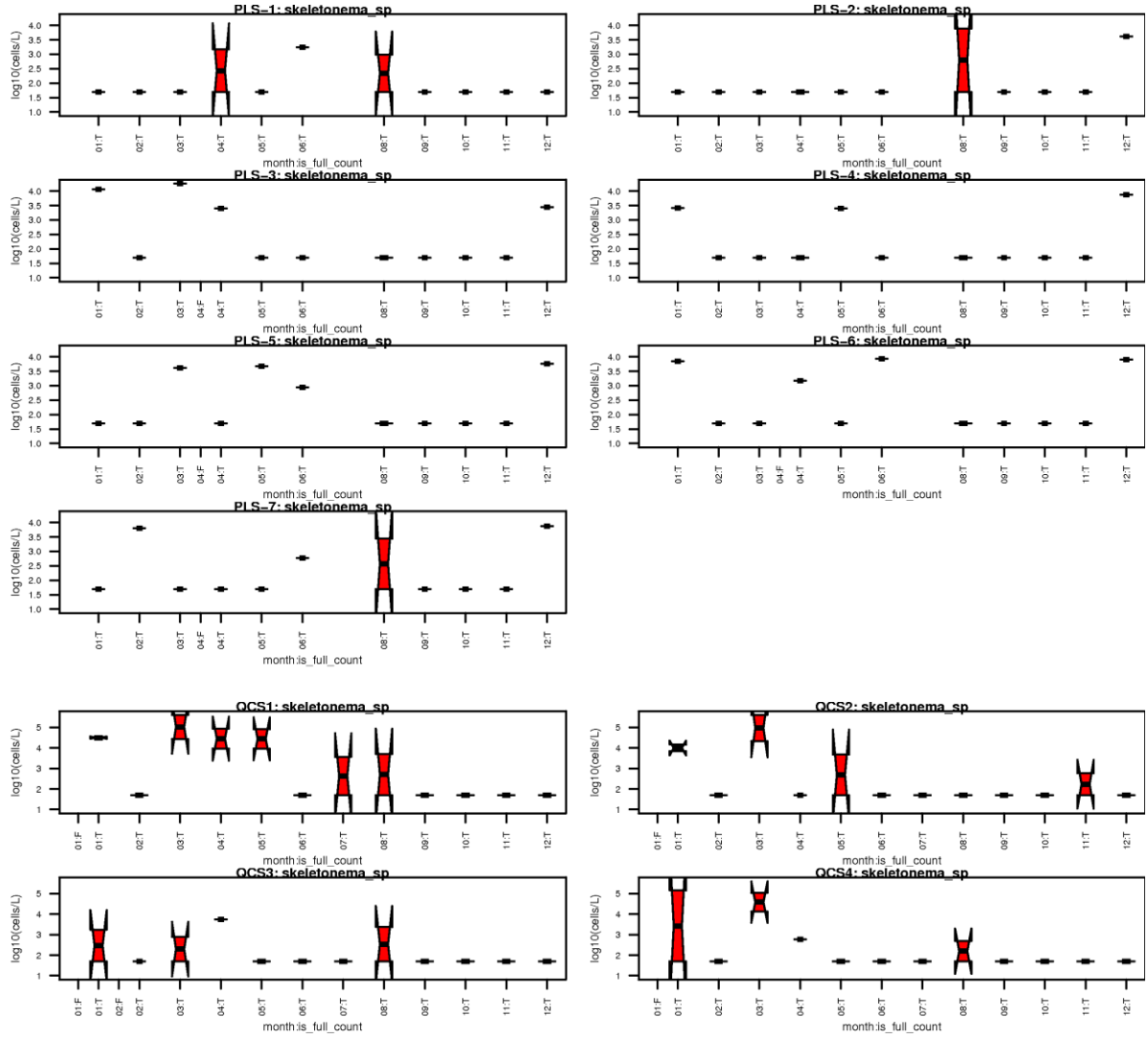

Figure 3-7: Time-series of the measured concentrations (cells/L) of members of the genus *Skeletonema* measured in the MDC sampling programme.

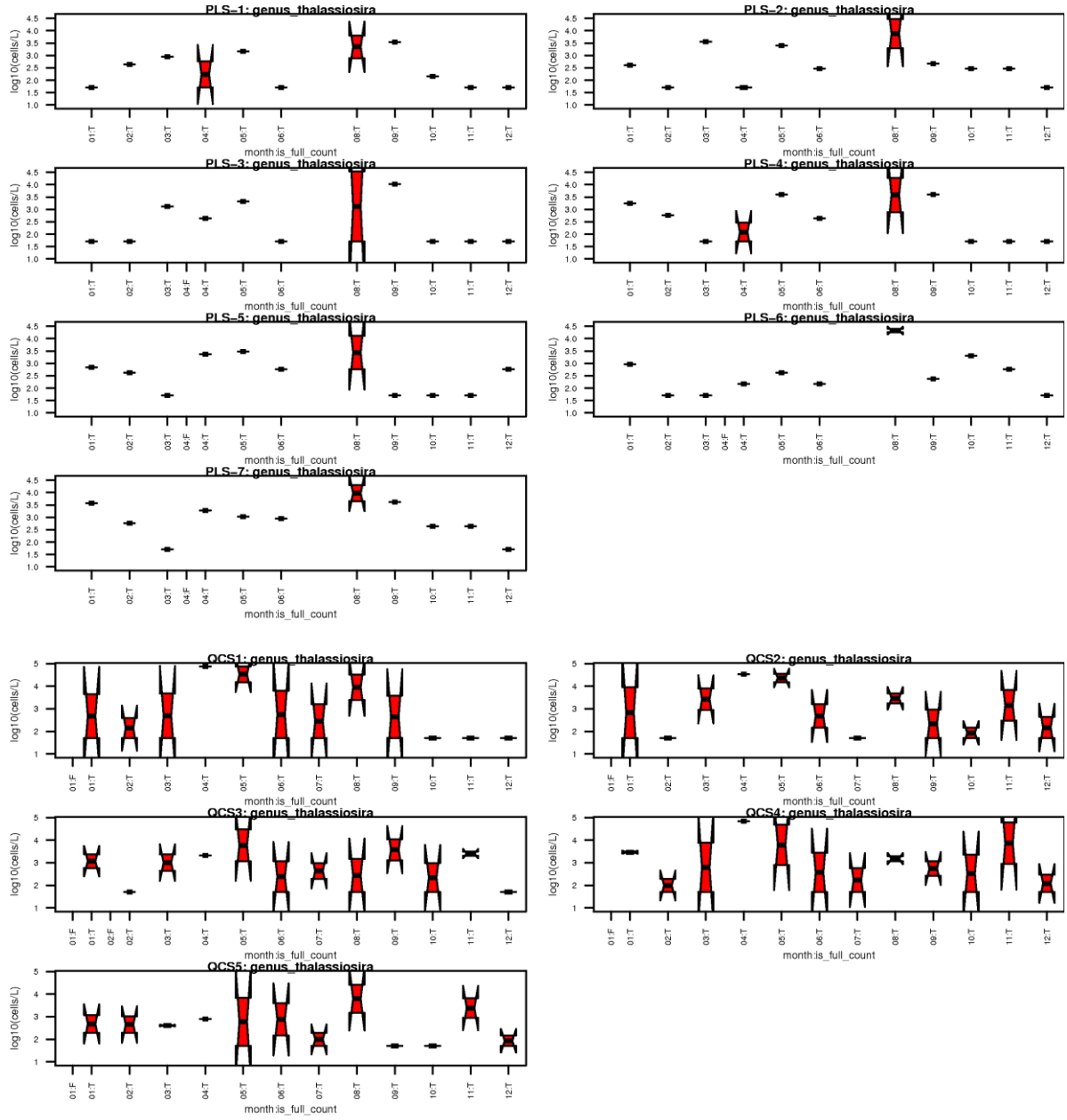

Figure 3-8: Time-series of the measured concentrations (cells/L) of members of the genus *Thalassiosira* measured in the MDC sampling programme.

4 Toxic Algae and Fish Health

The toxic algae recorded within the MSQP and MDC sampling programmes include several taxa that are known to be toxic to fish (for example, members of the genera *Pseudochatonella, Prymnesium*, and *Karlodinium*). Similarly, though non-toxic, at sufficiently high concentrations, some members of the genus *Chaetoceros* can be harmful to fish because their hard-spiny skeletal structure causes irritation to the gills.

Detailed comparisons between the dynamics of these harmful algae and records of fish health, condition/quality and growth rate lie outside the scope of this review. However, these comparisons might be helpful to NZKS in determining the causes of past fish-health or loss-of-condition events – and, perhaps, thereby determining how to minimise the future occurrence/severity of such events.[9].

[9] We do not know whether this would fall within the terms and conditions which currently govern NZKS's usage of the MSQP data.

5 Conclusions and implications

Clearly, the MSQP data-set is spatially and temporally extensive, but the fact that most stations have only *routine*-count data (i.e., count only a non-random subset of the total plankton population) means that comparison between MSQP data and NZKS species-composition data will need to be made with care. Nonetheless, on the basis of the limited MDC data that are available, it does appear that the historical MSQP data are consistent with the MDC data. This suggests that they should also prove similar to the forthcoming NZKS data.

Since the MSQP sites that are closest to the forthcoming NZKS farms are routine-count sites, the NZKS monitoring data will need to be 'filtered'/'resampled' such that it better mimics the nature of an MSQP routine count. When comparisons are made, the 'filtered' data-set should include only:

1. All toxic phytoplankton.
2. The two or three most abundant taxa (by cell concentration and assessed on a regional basis rather than on a site-by-site basis). The MSQP data suggest that these will almost invariably include members of the genus *Chaetoceros*, whilst *Leptocylindricus* and *Skeletonema* can be expected to be near-dominant members at particular times of the year.

It will also be necessary to ensure that taxonomic revisions are properly accounted for such that like can be compared with like. Furthermore, the MSQP data have weekly resolution, whereas the NZKS data have monthly resolution. Thought needs to be given as to how one should deal with this difference. Should one: (a) build probability-density distributions of monthly cell-abundance using all the MSQP data (as we have in this report), or (b) by randomly selecting one of the four/five weekly MSQP samples when building probability-density distributions of monthly abundance, or (c) selecting only those from the closest week-of-year (assuming that the NZKS sampling remains relatively regular, so that (for example) it tends to occur in the third week of every month).

With the possible exception of the Tio Point data-set (which spans only about five years), the time-series from the core MSQP sites are sufficiently long to permit robust characterisations of the probability distributions of cell-concentration for the most-frequently recorded taxa for each month of the year. Our analysis had made no attempt to remove inter-annual trends that might be driven by natural climate cycles etc. Thus, the within-month-of-year variability evident in the scatter-plots (Figure 2-22 - **Figure 2-30**) is a combination of; (a) biologically genuine fine-time-scale (week-to-week), (b) biologically genuine long-time-scale (year-to-year trend) and biologically false sampling error. It is clear that this sum of genuine fine-temporal scale variability and sampling error is of similar or greater magnitude to the seasonal-scale fluctuations. If there are any fish-farm induced changes, they will have to be very, very large to be discernable simply by comparing spot-measurements of water-quality characteristics with the box-plots presented in this report. More formal time-series analysis techniques could be used identify the fine-time-scale (week-to-week), medium-time-scale (seasonal) and inter-annual-time-scale variabilities in the MSQP data (and in NIWA's associated water-quality data). These more sophisticated techniques (detrending, consideration of the (partial) autocorrelation structure in the time-series, etc.,) may render it easier to detect 'statistically-significant' fish-farm effects but would not, on their own, be

sufficient to determine whether such change is 'ecologically significant'. Even if we assume that all of the within-month variability is biologically irrelevant 'sampling error' (rather than genuine fine-temporal-scale variability), the seasonal-scale variability is sufficiently large that one might argue that a farm-induced change would have to be very, very large (or very prolonged) to be judged ecologically significant.

The only way to determine the relative magnitudes of the genuine fine-temporal scale variability and the sampling error would be to take replicate samples at the same location and the same instant in time (such that all between replicate variability can be attributed to sampling error). That has not been done and is beyond the scope of this project.

An earlier report (Broekhuizen 2013) summarized water-quality data, nutrients, chlorophyll, turbidity etc., but not plankton counts) from NIWA data-sets which were gathered in parallel with some of the MSQP data summarized within this report. Like NIWA's chlorophyll data, these MSQP cell-count data also suggest that the plankton dynamics in the central Pelorus region are different from those of the outer-Pelorus (where the new NZKS farms will be). In the outer Pelorus (and in Queen Charlotte) phytoplankton tend to be most abundant in the summer period; in the central Sounds (notably Beatrix & Crail Bays), they tend to be most abundant in mid-winter. In general, one can expect that chlorophyll will be better correlated with algal biomass than with algal cell numbers. Thus, the fact that the MSQP routine-sample cell-count data and the NIWA chlorophyll data indicate similar seasonal dynamics suggests that the routine-count data provide an adequate (albeit crude) indication of the dynamics of the algal community's biomass – despite the uncertainties associated with converting between cell numbers and biomass and despite the fact that the routine-counts do not include record non-toxic, sub-dominant algal taxa.

During the NZKS hearings, Cawthron argued that nitrogen emissions from fish-farms might be expected to induce the greatest chlorophyll concentrations during winter [because, they argued, that is when chlorophyll is most abundant in the Sounds].Other experts argued that the biggest chlorophyll changes might be expected to happen in the summer (because that is when nutrients tend to be most limiting to algal growth in the Sounds). The MSQP and NIWA's own data both suggest that, in the immediate vicinity of the forthcoming fish-farms (as opposed to in Beatrix/Crail Bay), algal abundance tends to be greatest in summer. In combination with the summertime nutrient-limitation, this tends to support the contention that, if fish-farming does induce a change in algal abundance, it will be most likely to induce unacceptably high algal concentrations in the outer Sounds during the summer months.

6 Acknowledgements

We would like to thank Helen Smale (of the MSQP), Catherine Moisan (Cawthron Institute) and, most especially, Jennifer Robinson (Cawthron Institute) for the prompt and helpful answers that they have provided to our questions regarding details of the MSQP sampling programme and the data stemming from the programme.

7 References

Broekhuizen, N. (2013) Review of historical water-quality data from Pelorus Sound and Queen Charlotte Sound: long-term NIWA time-series and Marlborough District Council time-series. *NIWA Client Report (for New Zealand King Salmon Ltd)*, HAM2013-070 (project NZKS13401): 110.