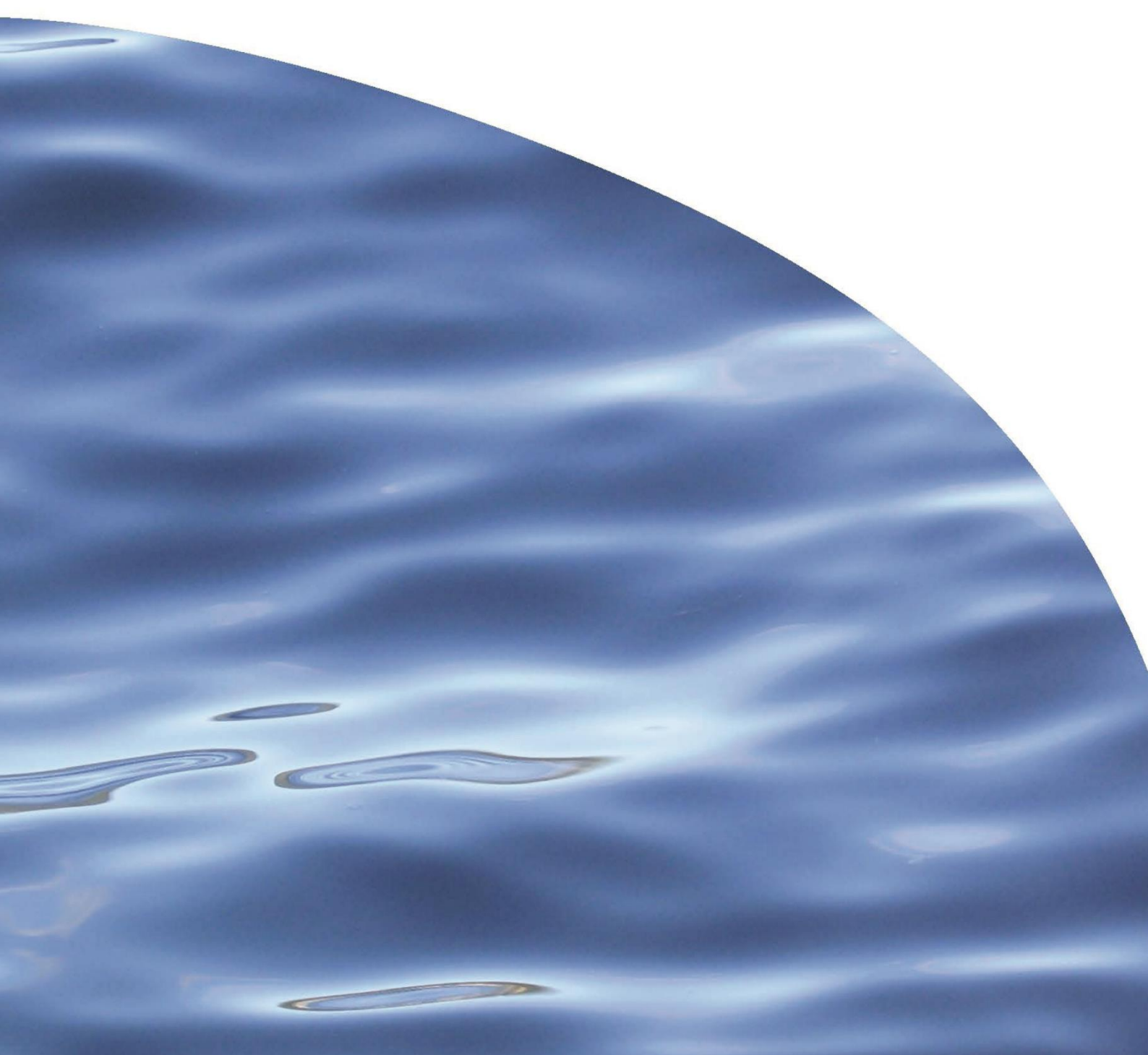




REPORT NO. 3550

MEASURING MUSSEL FARMING EFFECTS ON PLANKTON IN THE MARLBOROUGH SOUNDS



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EMMA NEWCOMBE¹, NIALL BROEKHUIZEN²

¹ Cawthron Institute

² National Institute of Water and Atmospheric Research

Both authors contributed to all sections of this report; NB was lead author for phytoplankton material and EN was lead author for zooplankton material

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CAWTHRON INSTITUTE
98 Halifax Street East, Nelson 7010 | Private Bag 2, Nelson 7042 | New Zealand
Ph. +64 3 548 2319 | Fax. +64 3 546 9464
www.cawthron.org.nz

REVIEWED BY:
Lincoln MacKenzie



APPROVED FOR RELEASE BY:
Chris Cornelisen



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EXECUTIVE SUMMARY

The potential effects of shellfish aquaculture on the marine environment include depletion of plankton (both phytoplankton and zooplankton) in the immediate area of farms, depletion that persists further afield, and, in contrast, the stimulation of plankton growth. The Marlborough District Council (MDC) has recognised that there are concerns about the extent to which mussel farming is affecting plankton in the Marlborough Sounds. As a result, MDC has commissioned the Cawthron Institute and the National Institute of Water & Atmospheric Research Ltd (NIWA) to consider:

- What we know about how the cumulative effects of mussel grazing affect plankton populations, and
- What would be needed to build a better understanding of whether there has been any community change and attribute the reasons for this change.

Much more information is available on phytoplankton populations (largely using chlorophyll-*a* concentration as a proxy for phytoplankton biomass) than for zooplankton. Chlorophyll-*a* (henceforth 'chlorophyll') concentration data are available from Pelorus Sound since the early 1980s from a range of studies. Analysis of these data does indicate that phytoplankton concentrations (as measured by chlorophyll) in the Sound have declined at some sites since the 1980s to the present. This decline is not a localised phenomenon: satellite data reveal that chlorophyll concentrations have declined around much of New Zealand's coastline over the past 20–30 years. The reasons for this decline remain unclear. In a provisional analysis, we have applied Generalized Additive Models (GAMS) to try to seek correlations between anomalies of bay-scale chlorophyll concentration¹ within Pelorus Sound and a variety of candidate explanatory variables. Using the available historical data, we found no evidence of a correlation between chlorophyll anomalies and expansion of the marine farming industry (as determined by area of approved marine farms measured at bay scale). In contrast, there is evidence that inter-annual fluctuations in river flow are correlated with the anomalies and it is possible that rising sea temperatures may also play a role.

There are too few data on zooplankton in the Marlborough Sounds to test whether zooplankton has been affected by mussel farming. Those data which do exist were collected in very small samples, and as a result are a very unreliable indicator of the densities of species of particular interest, such as fish eggs and copepods.

Unfortunately, zooplankton are very difficult to sample sufficiently well to establish the information needed to assess cumulative change in an area such as the Marlborough Sounds. Ways in which better datasets may be gathered include collection of background data as part of State of the Environment monitoring, or research projects that measure farm-scale effects of mussel farms on zooplankton communities. However, these approaches would not provide an assessment of the cumulative effects of multiple farms.

¹ i.e. differences between instantaneous chlorophyll concentrations and the appropriate long-term month-of-year median concentration

Much of the simulation modelling that has been undertaken for the Marlborough Sounds has been targeted explicitly towards understanding cumulative effects. That said, most of these simulations have focussed upon cumulative effects of nutrient emissions from fish farms rather than cumulative influences of mussel farms. All models are simplistic representations of reality and each has limitations which restrict the accuracy with which they can reproduce past states or predict future outcomes. This report includes a discussion of the key limitations (with respect to describing the cumulative effects of mussel farming) that are associated with the two best known models of the Marlborough Sounds. It also suggests investigations aimed at alleviating some of the limitations and better understanding the consequences of the limitations and argues (with some supporting reasons and evidence) that these models have over-estimated the impacts that mussel farms have upon plankton.

It is our opinion that, at the scale of large bays/reaches, mussel farms are not the dominant influence upon the plankton of the Marlborough Sounds—but we concede that, at least for zooplankton, there are scant data to support that opinion. We provide options for field and modelling work aimed at better understanding the influences that mussel farms are having upon plankton of the Marlborough Sounds. We believe that this body of work would enable us to reduce the uncertainties concerning mussel farm influences upon plankton in the Sounds but we are unable to confidently determine how much uncertainty will be removed or how much residual uncertainty will remain even after completion of a large (costly), integrated field and modelling programme.

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1. INTRODUCTION AND BACKGROUND

The potential effects of shellfish aquaculture on the marine environment include depletion of plankton (both phytoplankton and zooplankton) in the immediate area of farms (MPI 2013). Further afield, several outcomes are possible: the depleting effects of different farms may accumulate ('cumulative effects'), or mixing of water bodies and natural processes may diminish any depletion effects, or natural processes may lead to counter-intuitive change, such as increased growth of plankton.

Mussels consume particulate organic matter including both living plankton and fine detrital material. While some of the ingested material is retained by mussels as they grow, a large fraction is released back into the environment as faeces, pseudofaeces and inorganic nutrients. Mussels therefore cycle detrital material back into inorganic forms (dissolved nutrients, which can promote phytoplankton growth). While mussels certainly consume phytoplankton and zooplankton, this nutrient regeneration will tend to promote more rapid growth of the surviving phytoplankton if nutrient availability is limiting phytoplankton growth. It is, however, widely recognised that plankton populations can be depleted immediately downstream of mussel farms. Assessment of 36 mussel farms found some evidence of localised depletion in and near mussel farms in the Marlborough Sounds, including Port Underwood (Keeley et al. 2009; Appendix 1). However, further downstream, phytoplankton populations may be enhanced in the presence of mussel farms.

The prediction and measurement of effects of shellfish aquaculture on plankton communities in the environment is difficult, in large part because of high background variability caused by multiple natural and human-mediated processes that influence both plankton and shellfish (and many other organisms). Marlborough District Council (MDC) has commissioned the Cawthron Institute (Cawthron) and the National Institute of Water and Atmospheric Research Ltd (NIWA) to: (a) review existing data from the Marlborough Sounds to determine whether there is any evidence that mussel farms have influenced plankton abundance or biomass and (b) to consider the requirements to build a better understanding of mussel farming effects upon plankton communities in the coastal waters of the Marlborough region.

1.1. Plankton

Plankton are organisms that live in the water column (i.e., not associated with the seabed or other structures) but have little or no control over their large-scale movements. Some plankton are sufficiently motile to be able to influence/regulate their depth within the water column, but none are able to materially influence their horizontal movements.

Phytoplankton are usually single celled and are primary producers, i.e., like higher plants, they obtain their energy through photosynthesis². Single-celled phytoplankton range in size from $< 1 \mu\text{m}$ ($1000 \mu\text{m} = 1 \text{mm}$) to tens of μm (a few taxa have cell sizes of a few hundred μm). Some taxa have a colonial growth form. Colonies range from 10s–100s of μm in size. Cell concentrations can reach hundreds of thousands of individuals per litre, dependent upon the taxon³. The phytoplankton community of temperate coastal waters is diverse. A small sample of water (e.g. 100 mL) will usually contain individuals from a few tens of different taxa (albeit that the sample will be dominated by cells (or mass) from just a few of those taxa⁴).

Zooplankton are the animal component of the plankton community. The zooplankton community consists of both single-celled forms (protozoa) and multicellular organisms (e.g. copepods). The former span much the same size range as phytoplankton and may occur in densities of thousands of individuals per litre. The latter range from 10s of μm to several centimetres, or even larger (e.g. in the case of large jellyfish or salps). Some zooplankton (e.g. protozoans and most copepods) spend their entire life as plankton (and are termed holoplankton), others (such as urchin or fish eggs and larvae) spend only a part of their life cycle as plankton (termed meroplankton). For the purposes of this report, we restrict consideration of zooplankton to those organisms small enough to conceivably be consumed by a large mussel. Filter-feeding green-lipped mussels can extract particles larger than approximately $2 \mu\text{m}$ (Safi & Hayden 2010; James et al. 2001) and at least as large as $430 \mu\text{m}$ (Zeldis et al. 2004).

1.2. Marine farming in the Marlborough Sounds

Shellfish farming is rare in Queen Charlotte Sound (including Tory Channel), and widespread in Pelorus Sound, Port Underwood, and Squally Cove (eastern Croisilles Harbour, where oyster farming is common). Mussel farms in Pelorus Sound and other areas are often adjacent to other farms, forming strips of farms that run parallel to the coast (Figure 1). Many farms are sited in waters between 10 and 30 m deep, although both shallower and deeper areas are farmed. The density of cultured mussels within farms can be high in Marlborough Sounds farms, compared to more recently developed mussel farming areas elsewhere. Culture lines suspended from double backbones can be $< 10 \text{m}$ apart in the Marlborough Sounds, while distances of over 50 m between lines occur in some of the larger, offshore developments (e.g. in eastern and western Tasman Bay).

² Some phytoplankton are mixotrophic. These taxa can meet at least some of their energetic and nutrient needs by consuming organic matter rather than through photosynthesis and uptake of inorganic compounds.

³ A taxon (plural taxa) is a classification of an organism, i.e., it may be a species, or a higher grouping such as genus or family.

⁴ Cells from different taxa can be of vastly different size. Thus, taxa which are numerically dominant by cell-count may not be the dominant taxa by mass



Figure 1. Orange shading indicates the location of mussel farms in some of the relatively high-density farming areas in Pelorus Sound. From 'Cruise Guide to the Marlborough Sounds' (<http://www.cruiseguide.co.nz/maps/>).

Fish farming is established in 5 sites in Queen Charlotte Sound and 6 in Pelorus Sound, although some of the smaller farms in Pelorus are currently fallowed. Effects of fish farming are not considered in this report.

1.3. Report scope

The focus of this report is the effects of mussel farming in the far-field within the Marlborough Sounds; however, we include brief consideration of near-field effects (scales of 10s–100s of metres around individual farms) as needed. In considering the cumulative effects of multiple farms this report seeks to answer:

- What do we know about how the cumulative effects of mussel grazing affect plankton populations?
- What would be needed to build a better understanding of whether there has been any community change and attribute the reasons for this change?

We first consider the methods that are available for possible data collection and modelling approaches to assess whether shellfish farming affects plankton communities (Section 2). We then review the existing information on phytoplankton and zooplankton in the Marlborough Sounds, and assess the extent to which we can test whether the cumulative effects of mussel grazing are affecting plankton populations (Section 3). Finally, in Section 4 we consider options for better understanding whether shellfish farming has caused change in phytoplankton and zooplankton communities.

2. MONITORING METHODS

2.1. Phytoplankton

Phytoplankton-sized organic particles are generally considered to be the primary food source for cultured mussels, although other food sources may also contribute (e.g. Hulot et al. 2020, and references therein). Data from the Marlborough Sounds indicate that living phytoplankton usually account for < 30% of the particulate organic carbon suspended within the water column (Broekhuizen & Plew 2018) and mussel condition tends to be better correlated with concentrations of particulate nitrogen than with chlorophyll in Pelorus Sound (Zeldis et al. 2008). Measurements of phytoplankton to assess depletion have been the focus of effects assessments of mussel farming on the water column (e.g. Newcombe 2018).

2.1.1. Laboratory analyses of water samples

Up until around 20 years ago, the only way to monitor phytoplankton was to collect water samples for analysis. Samples might be collected using bottle-like devices which can be opened and closed while underwater, or by lowering an open hose through the water, then sealing it before recovery. The former method allows sampling around moderately accurately prescribed depths. The latter allows for easy 'depth integrated' sampling across the upper 10–15 m of the water-column.

Sometimes, the samples may be inspected under the microscope. Doing so allows cells to be counted, identified and sized—but the microscope work is slow and labour-intensive. A simpler (and much more common) approach relies upon the fact that phytoplankton contain photosynthetic pigments⁵ that are not found in chemotrophic and heterotrophic organisms (though they are found in other photosynthetic organisms such as vascular plants and seaweeds). These chemicals can be made to fluoresce. Each photosynthetic pigment fluoresces at characteristic wavelengths. The fluorescence is comparatively easy to measure with methods that have been available for many years. If water samples are passed through a filter⁶, the filters (and associated retained particulates) can then be ground and chemically digested to extract the pigments into solution. The fluorescence (across relevant wave lengths) of the solution is then measured to provide an indication of the abundance of photosynthetic pigments. Whilst most phytoplankton taxa contain several pigments (and some of those pigments are unique to specific taxonomic groupings), fluorescence is usually measured across the wavelengths at which chlorophyll-a

⁵ i.e. pigments involved in photosynthesis of sugars from water and CO₂ driven by sunlight energy

⁶ The cell-specific chlorophyll content can vary through time in response to fluctuations in temperature, light, nutrients etc. Filtration should take place as soon after the water-sample has been gathered as practical. Thereafter, filters should be frozen to slow chlorophyll degradation. Water samples are usually chilled and kept dark until they are sampled. Chilling slows all biological processes that will influence chlorophyll concentration (intra-cellular physiological change, grazing activities of zooplankton etc). Keeping samples in the dark ensures all samples experience similar light histories in the time leading up to filtration. That helps to standardise/stabilise the cell-specific chlorophyll content of different samples.

(being the dominant pigment in most phytoplankton) emits most strongly. Fluorescence intensity is converted back to chlorophyll-a concentration by means of empirically determined calibration curves.

If the original water sample is passed through sequential filters (of declining pore size), it is possible to get some information about the size-structure of the phytoplankton community by measuring the fluorescence associated with each filter—but this provides scant information about the detailed taxonomic composition of the algal community.

Environmental DNA (eDNA) techniques are another emerging approach for monitoring algal (and other) communities. These techniques seek to determine what organisms are present within a sample by identifying known DNA sequences. The method cannot distinguish between DNA arising from live and dead organisms (or fragments of dead organisms). Thus, while the method is very useful as a means of determining what types of organisms may be associated within a general region, it cannot be used to determine what organisms were unequivocally alive within the collected sample. The method cannot yield quantitative estimates of abundance—it is best regarded as a presence/absence method that operates on a spatial scale that may be much larger than that of individual samples.

2.1.2. In situ phytoplankton monitoring

Traditionally, chlorophyll concentration has been measured in the laboratory as described above. Unfortunately, it has remained difficult to manage large numbers of samples because of the time and effort demanded to gather and process these large volumes of water.

More recently, sensors have been developed to measure fluorescence in intact water samples. A suitable light source is used to briefly illuminate the water sample and a sensor measures any subsequent fluorescence. The sensors can be preprogrammed to measure fluorescence at high frequency (e.g. every few seconds). The fluorescence sensor is usually mounted alongside other sensors (e.g. temperature, salinity, turbidity, dissolved oxygen) to allow simultaneous monitoring of several important water properties.

This instrument (commonly known as a 'sonde') opens the possibility of more frequent/extensive sampling. For example, it can be hung from a buoy to permit quasi-continuous monitoring at one location (Figure 2a), slowly lowered through the water column to make measurements of the vertical structure in the water column (Figure 2b) or towed to cover larger areas (Figure 2c).

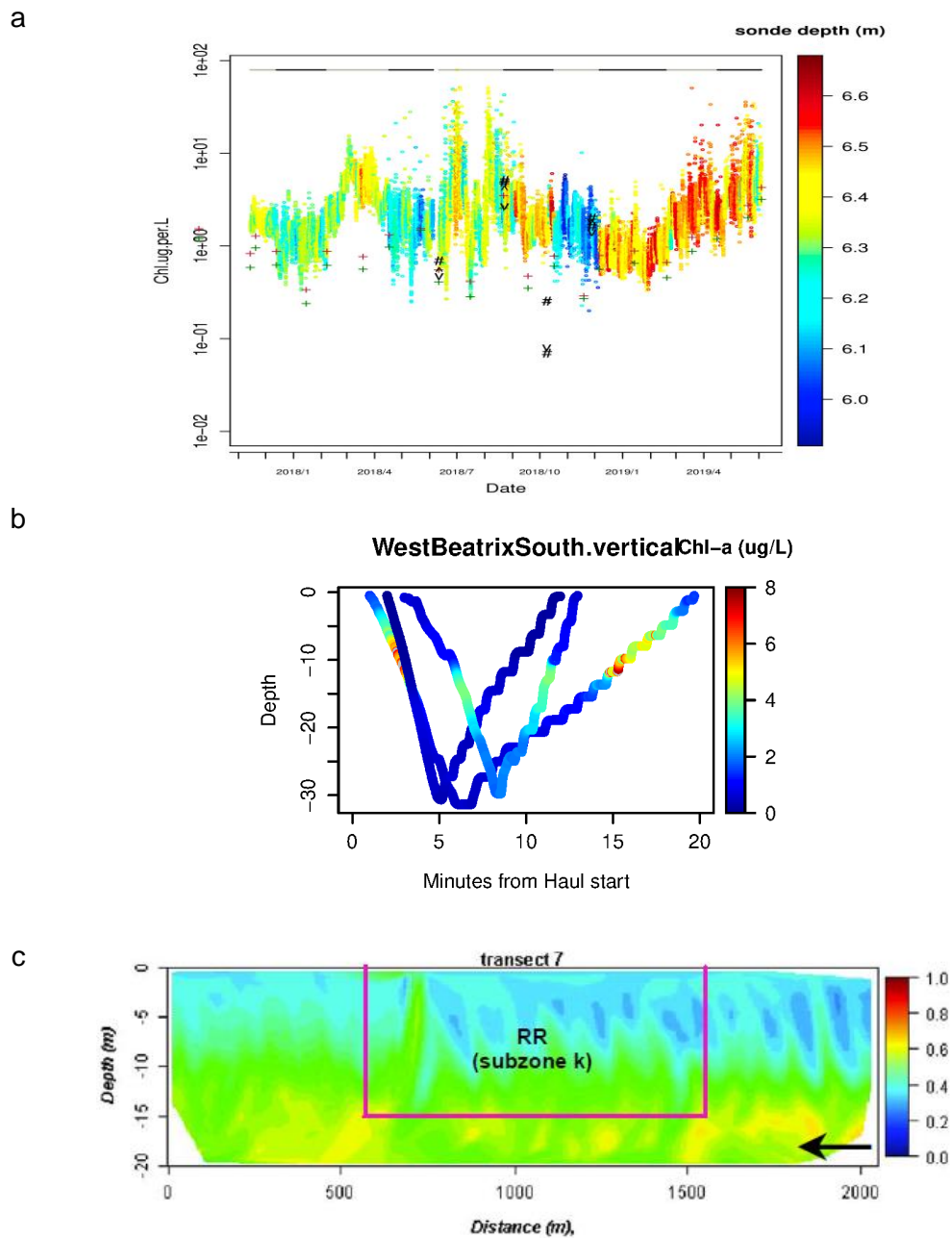


Figure 2. Examples of fluorimeter data : (a) High frequency, long-term measurements of chlorophyll measured at 15 minute intervals over ten sequential deployments during 2017–2019 in Laverique Bay (sonde hanging below a mussel backbone at approx. 6 m depth). Coloured circles are indicative of the instantaneous sonde depth. The black symbols are estimates of chlorophyll averaged across the upper 6 m (#) or 15 m (^, v) using the sonde in depth-profile mode. The green and red coloured crosses are depth-averaged concentrations of chlorophyll and phaeophytin measured in water samples that were returned to the laboratory (to 15 m) from vertical profiles with the sonde at instrument turnaround time. (b) Vertical distribution of chlorophyll measured on several occasions at several sites within Pelorus Sounds using a fluorimeter in vertical profile mode. Haul start-times were offset from one another to make it easier to distinguish the different profiles gathered at each site. (c) Chlorophyll concentration measured in and around a mussel farm (pink box) in Tasman Bay with a towed fluorometer (from Clark et al, 2012). The black arrow shows the direction of the current flow.

While not yet widely used and still very expensive, coupled microscope-camera systems are now being developed that can be hung from buoys or operated from vessels. The images that they take have a pixel resolution of a few μm . This is fine enough to allow medium and large phytoplankton cells to be identified to moderate taxonomic resolution but insufficient to allow smaller phytoplankton cells (e.g. many flagellates) to be further categorised. The camera systems can also distinguish protozoa, fine sediments and fine particulate detrital matter. After sufficient images have been characterised by experienced taxonomists, they can be used to train image analysis software. Once trained, that software can quickly analyse further images.

In situ imaging has been tested in the Marlborough Sounds with an Imaging Flow Cytobot (IFCB), an autonomous, submersible microscope made by McLane Lab USA (MacKenzie et al. 2019). The research project using this microscope was focussed on the detection of phytoplankton species with the potential to cause harmful algal blooms. The IFCB was found to enable near real-time phytoplankton sampling and analysis and greater spatial coverage with reduced labour. While it has some clear benefits for sampling for the purpose for which it was tested, application to the measurement of cumulative effects of aquaculture was not considered, and the researchers note that work is still required to customise the image analysis cell classifier to New Zealand species.

2.1.3. Remote-sensed chlorophyll fluorescence

Absorption of light by algal pigments (and re-emission of some of this light at other wavelengths through fluorescence) induces subtle changes in both the overall intensity of light emitted from water and the apparent colour of water. Hyper-spectral cameras suited to measuring ocean colour have been carried on satellites since the 1990s and smaller, lighter versions (which can be carried by aerial drone) are now coming to the market.

In oceanic waters, the colour spectrum of light emitted from the water surface can provide a moderately reliable indicator of chlorophyll concentrations in the upper few metres of the water column. In coastal waters, it can be much more difficult to extract a reliable chlorophyll signal because concentrations of materials derived from the catchment and seabed (notably suspended sediments and tannins, etc. from decaying terrestrial vegetation) are sufficiently high that their influence upon colour and intensity of the water often outweighs that of the phytoplankton.

There are several important differences between the natures of the data which can be gathered using *in situ* sampling (collection of water samples, or in-water fluorimetry) and those gathered by satellite observations. The former allows for measurements that are precisely controllable—the user can determine when and where (horizontal and vertical location) to sample with high precision (especially since GPS navigation became commonplace). Furthermore, ancillary data (water samples for nutrient

analyses, etc.) are easily gathered whenever instruments are deployed or retrieved. In contrast, satellite sensors are restricted to making measurements of only near-surface water properties⁷. The horizontal resolution of satellite 'samples' is dictated by the pixel density of the camera system and the elevation of the observation point. Satellite data for the New Zealand region have a resolution of approximately 1 km. The timing of individual samples is dictated by the satellite orbit. Furthermore, for measurements that are dependent upon visible light, usable images are restricted to daytime and to pixels which are not obscured by cloud, mist, foam (from breaking waves) etc. For this reason, analysis of satellite data usually starts by grouping several sequential images together to create a composite image (e.g. an image in which the value within each pixel is an average all the available, useable values at this location as extracted from the individual images in the sequence). *In situ* measurements are essentially 'point measurements' while satellite ones are clearly 'spatial average' measurements.

2.1.4. Limitations upon using chlorophyll as a measure of phytoplankton abundance

Regardless of how chlorophyll concentrations are estimated, it is important to recognise that they provide only a very approximate indication of the total abundance of the phytoplankton community biomass. The chlorophyll content (whether expressed as chl cell⁻¹, chl (unit cell biomass)⁻¹ or chl (unit biovolume)⁻¹) can be very variable. Indeed, carbon:chl ratios can vary from around 35:1 mg carbon mg⁻¹ chl to more than 200:1 mg carbon mg⁻¹ chl (e.g. Chan 1980; Bowie et al. 1985). Diatoms tend to contain more chlorophyll per unit cell carbon than other taxa and dinoflagellates tend to contain less than other taxa—but there is a lot of overlap amongst taxa. Indeed, the absolute and relative chlorophyll content varies through time at the level of an individual phytoplankter—being influenced by the ambient light intensities, nutrient concentrations and temperatures that the plankter has experienced over preceding hours/days (e.g. Geider et al. 1998)⁸.

It is also important to recognise that different means of estimating chlorophyll abundance sometimes yield results which are only poorly correlated. For example, chlorophyll measurements stemming from laboratory determinations of chlorophyll captured on filters are not always closely correlated with chlorophyll inferred from fluorescence in the antecedent 'whole seawater'. There are several reasons for this:

⁷ The efficiency with which even pure water absorbs electromagnetic radiation depends upon the wavelength of the radiation. Water absorbs infra-red (heat) radiation more efficiently than it absorbs visible light. Thus, satellite measurements of water-temperature typically amount to measurements of temperature in the upper few tens of cm of the water-column. In contrast, measurements made in the visible spectrum (e.g. measurements of chlorophyll) typically amount to measurements across the upper few metres. Since the concentrations of coloured solutes and particulates (which also influence absorption) can fluctuate day-to-day at a location, the depth across which measurements extend can vary day-to-day at any one location.

⁸ As a result of these dependencies, it can be the case that the *in situ* fluorescence associated with a given biomass of phytoplankton living at depth will differ from that of the same biomass concentration living near the surface. This is a lesser problem for laboratory-determined chlorophyll—because the whole water sample will usually have been stored under standard conditions (cold and dark) for some time after collection before particulates are extracted onto filters for analysis.

1. *In situ* instruments are prone to becoming biofouled. They must be cleaned frequently to minimise artefacts arising from biofouling.
2. Even in the absence of fouling, some of both the light used to stimulate fluorescence and the resultant fluorescence signal will be absorbed by other (coloured) solutes and particulates (dead phytoplankton, suspended sediments) in the intact seawater before it reaches the sensor element in any *in situ* sensor. Fluctuations in the concentrations of these potential absorbants serve to disrupt the correlation between (apparent) fluorescence intensity and true concentration of chlorophyll in living cells. This is a lesser problem in laboratory determinations of chlorophyll because filtration can be used to remove solutes and particles of unwanted size classes.
3. Conversely, unless very fine-pored filters are used in laboratory determinations, some of the chlorophyll which would be measured by *in situ* methods may be lost during laboratory filtration. Unfortunately, fine filters are prone to becoming clogged. This often makes it difficult to use them in waters that carry substantial quantities of suspended sediments (as coastal waters sometimes do)⁹.
4. Some intracellular change is almost inevitable between gathering a water sample and subsequent laboratory determination of chlorophyll—despite efforts to reduce and standardise the nature of these changes. Similarly, some phytoplankton (and accompanying chlorophyll) may be consumed by zooplankton before the samples can be filtered and frozen.
5. At any instant, an *in situ* instrument measures fluorescence in only a few mL of water. A laboratory determination is usually based upon filtration of several hundred mL. Thus, the *in situ* sensor may detect fine space-scale fluctuations that are 'invisible' to methods based upon filtering seawater (or to satellite imagery). Time-averaging results from *in situ* determinations serves to increase the sample volume—thereby masking any fine-space-scale fluctuations.

By way of analogy, using chlorophyll as an index of algal concentration is somewhat akin to estimating the density of trees in a forest from the quantity of green in an aerial photograph of a misty deciduous forest canopy without good knowledge of whether the trees are in full leaf, whether they are water stressed and/or nutrient deficient or how much caterpillar damage the leaves have suffered. For the reasons outlined above, we believe that traditional laboratory chlorophyll analyses and *in situ* methods should be seen as complementary approaches rather than alternative/substitute approaches. *In situ* camera systems show promise as valuable tools for the future (and may supplant) chlorophyll methods in some circumstances—but they remain expensive at present.

⁹ The smallest phytoplankton taxa are too small to be efficiently captured by mussels. Thus, in the context of assessing phytoplankton availability to mussels, and perhaps, also in the context of assessing mussel impacts upon phytoplankton, estimates of chlorophyll captured on GF-C-like filters (1.2 µm) may be more relevant than measures based upon *in situ* fluorescence of whole water (esp. in situations where the smallest phytoplankton contribute substantially to fluctuating fractions of the chlorophyll biomass).

2.1.5. Summary of phytoplankton sampling methods

Table 1. Phytoplankton measurement options for field studies, and the resultant data, availability, and cost of the technology.

	Physical samples		<i>In situ</i> fluorimetry	<i>In situ</i> camera imagery	Satellite & drone imagery
	Morphological analysis ¹	Genetic analysis			
Sample size	Up to 100s of mL	Up to a few L (subsampled)	Few mL per reading	Few ul per image	Satellite: Large, national + (several ha per pixel) ² Drone: variable ³ , 100s of m ² +. Resolution–10s of m feasible.
# sites feasibly sampled	Many per day	Many per day	Many sites or 10s of km of transect per day	1 or very few per campaign	Satellite: High areal coverage ⁴ . Drone: limited by flight times, speeds, regulations
Sample frequency	Limited by field time	Limited by field time	High ⁵	High ⁶	Satellite: approx. daily ⁷ . Drone: limited by field time
Parameter measured	Chlorophyll, Cell Counts/sizes (hence, biomass). PC, PN.	Presence/absence or sequence abundance ⁸	Fluorescence–wavelengths relevant to chlorophyll-a ⁹	Density and size	Inferred chlorophyll concentration
Species ID	Reliable	Presumably reliable for species of main interest.	No. ¹⁰	Partial	No
Technology availability	Readily	Readily	Readily	Limited	Satellite: readily Drone: limited
Cost	\$10,000s for small studies	\$10,000s for small studies	Several \$10,000s	Several/many \$100,000s	Satellite: data varies widely Drone imagery: moderate-high

¹. Taxonomist-processed or automated

². Restricted to upper part of water column

³. Dependent upon camera system and flight elevation

⁴. Data are more frequent for outer Sounds than for central/inner Sounds

⁵. Finest resolution is around record per 5–10 sec. Requires frequent (e.g. monthly) servicing of instruments

⁶. Finest resolution is around record per 5–10 sec. Requires frequent (e.g. monthly) servicing of instruments

⁷. Composite/time-averaged data usually used

⁸. Uncertain/poor correlation with living biomass e.g. Harvey et al. (2017)

⁹. Can be converted to an inferred chlorophyll concentration if the instrument has been properly calibrated. It is recommended that water samples be collected and analysed for chlorophyll to assist the calibration

¹⁰. but it is sometimes possible to augment the generic chlorophyll sensor with sensors that detect fluorescence by pigments that are specific to e.g. blue-green algae

2.2. Zooplankton

Substantially more research has been undertaken regarding phytoplankton depletion by mussel farms than for depletion of zooplankton. Some research findings are important in considering how effects of mussel farming on zooplankton would best be researched or monitored. It has been established from field observations that mussels in a natural or farmed setting can indeed consume zooplankton (e.g. Alfaro 2006; Davenport et al. 2000). Additionally, other organisms that have colonised mussels or farm structures may also graze zooplankton (Woods et al. 2012). However, little research exists identifying zooplankton depletion resulting from consumption by mussels (Hulot et al. 2020; but see Maar et al. 2008; Cranford et al. 2014). A second means by which mussel farming may affect the abundance and structure of zooplankton communities is by affecting their food supply. Mussels may out-compete zooplankton for food (by consuming phytoplankton, or even smaller zooplankton e.g. Gibbs 2004), or stimulate regrowth of phytoplankton by releasing dissolved nutrients into the water (Ogilvie et al. 2003; Trottet et al. 2008; Ren et al. 2010), providing a localised increase in productivity to the food web. Both of these effect types may lead to cumulative effects of multiple farms, and this adds substantial complexity to any assessment of effects.

2.2.1. Laboratory experiments

Because so little has been published regarding the effects of mussel farming on zooplankton, here we consider the role of experimental work in assessing likely effects of mussel farms. This also ensures that material that has been raised with the authors by interested parties is considered.

Laboratory experiments (or, in many cases, experimental setups in containers or flow-through tanks at or near field sites) have been undertaken and depletion rates calculated accordingly (e.g. Zeldis et al. 2004). These are informative to an extent, for example, by identifying size classes of zooplankton that may be consumed by mussels. However, scaling the results up to calculate a measure of real-world depletion is fraught with difficulties (Cranford et al. 2014). For example, experimental plankton densities are often quite different from real-world densities, current conditions are generally not comparable to real-world situations, and re-filtration of water as it passes through the farm cannot be considered. Conversely, experimental systems are usually of small volume—such that zooplankton have limited ability to avoid the feeding currents of filter-feeders such as mussels. Similarly, extrapolation from laboratory or field observations of the presence of zooplankton in mussel guts to a measure of depletion from a water body passing through a farm is no simple task. This calculation would require knowledge of the density of zooplankton in the water, the rate of movement through the farm, and the amount of water filtered (or re-filtered) by each mussel. Accordingly, experiments or measurements from individual or few mussels, in either artificial or natural settings, are likely to be only a useful

complement to a suite of measures that may be able to approximate real-world in-farm depletion.

2.2.2. Scales of field assessments

To most effectively assess, or monitor, real-world zooplankton depletion, field measurements would therefore be more appropriate than laboratory-type measures, both in terms of avoiding experimental artefacts, but also to ensure that the role of biofouling filter feeders are considered (as per Woods et al. 2012). Direct consumption of zooplankton within a single farm can conceivably be assessed on a small scale, for which collections or measurements *in situ* are likely to be most appropriate. Comparison of zooplankton communities in water bodies entering a farm with those in water exiting the farm would indicate the degree of grazing of zooplankton that has occurred within the farm area. Depletion of zooplankton has been demonstrated with this type of experiment around high-density culture rafts in Spain (e.g. Maar et al. 2008). While such experiments and measurements above can be informative, it is important to recognise their limitations with respect to assessment of ongoing mussel farming. For example: measured grazing rates differ under different conditions of food availability, hydrodynamics and other factors.

A larger spatial scale must be considered to address whether mussels affect zooplankton communities by competing for food resources (or, by direct grazing and competition for food resources), and to consider cumulative effects of either depletion or competition. This is because effects are not expected to manifest at an individual farm site, but would occur further afield, once waterbodies passing through multiple farms have mixed (in the case of cumulative effects) or once zooplankton communities have responded to food competition (e.g. a reduction or change in structure of the food source).

A number of approaches can be taken to field measurements, and three key options are outlined below; laboratory analysis of physical zooplankton samples, *in situ* monitoring (with moored imaging or genetic instrumentation), and acoustic technologies.

2.2.3. Physical samples

The volume of water sampled is an important consideration when sampling zooplankton, due to their inherent patchiness and low densities (in comparison to phytoplankton). Sampling with a large plankton net allows for collection of zooplankton from a large volume of water (e.g. > 10 m³), although substantial variability is still apparent (e.g. Morrissey & Newcombe 2018). Sampling of smaller volumes of water means that samples reflect even more environmental variability. Sampling of small volumes is also more subject to bias if some taxa can escape the sampling device (e.g. Harvey et al. 2012).

Laboratory analysis can be undertaken by morphological analysis by a taxonomist, or with the use of imaging and automated identification of taxa. In both cases individuals can be counted, and size information can be collected in addition to species identification. Genetic techniques can also be used for identification of the taxa present in physical samples. This is an extremely useful technology for the precise identification of large numbers of different taxa, and undoubtedly could be applied to numerous research questions. The main limitation in the context of mussel farm monitoring is that genetic techniques measure the frequency of genes, rather than counts of individuals (as also discussed in Section 2.1.2). These environmental DNA (eDNA) methods can provide qualitative (perhaps, semiquantitative) information about changes to the relative abundances of different taxa, but they do not provide a means of accurately assessing the absolute concentrations of individuals. Moreover, genetic techniques cannot distinguish between life stages (e.g. eggs vs. adults, or small vs. large individuals). To understand the effects of mussel farming on zooplankton populations, these are important parameters to measure. Some limitations do exist in both imaging and genetic techniques, in that rarer taxa may not be recognised by existing computer algorithms, nor represented in existing molecular databases. However, in the context of monitoring of zooplankton around shellfish farms, these limitations are probably not of great concern, as the most common taxa are likely to be of primary interest, at least in the early stages of investigation. The greater limitation of assessment of physical samples is the sampling effort required to overcome the high patchiness of zooplankton over space and time, particularly where effects are not expected to be strong.

Physical samples can also be collected by deployed samplers, and either preserved or processed *in situ* for genetic analysis, however this technology generally samples very small organisms, very small volumes, and/or is limited to a small number of samples (McQuillan & Robidart 2017). Furthermore, automated samplers can be unreliable when left untended for more than a few days in coastal waters.

2.2.4. *In situ* imaging

In situ monitoring of plankton populations with high-tech sensors can reduce issues of high variability in zooplankton communities over time by sampling at a high frequency over long periods. Sensors are available that can be deployed for weeks at a time but programmed to capture an image of a small volume of water at high frequency (e.g. every few minutes). However, a number of issues do arise. Firstly, the sample volumes tested are very small (a few millilitres); this has two disadvantages, firstly, that many samples are required before the volume of a single net haul is met. Secondly, highly mobile zooplankton are likely able to escape the intake currents of sampling mechanisms, causing a sampling bias. An additional limitation is that a given unit is able to sample a limited size range, and as a result, it is our understanding that a single imaging unit would not be capable of sampling the size range of zooplankton

potentially subject to mussel grazing. A third limitation is that the instruments are very costly to purchase at present.

In situ imaging has been effectively undertaken in the Marlborough Sounds (MacKenzie et al. 2019). However, this research was focussed on harmful algal blooms, and considered organisms below the size range of those zooplankton (e.g. copepods, fish eggs) that have been identified as of concern to stakeholders in the top of the South Island.

2.2.5. Acoustic sensing / particle analysis

Acoustic sampling can measure the occurrence of particles of particular sizes, and has been used to estimate abundance of relatively large zooplankton (e.g. several mm or larger) for decades (e.g. Greenlaw 1979; Stanton 2012). Thirty or more of size classes can be measured with modern instruments (e.g. Cranford et al. 2014). Acoustic monitoring technology can be vessel mounted, which has the potential to survey large areas, or it can be incorporated into much smaller moored or mobile units.

In the context of mussel farming effects assessment, it is important to be able to identify the species of zooplankton, as farms may deplete some zooplankton taxa (those that mussels and other resident filter feeders consume) but act as a source of others (e.g. eggs and larvae of mussels and biofouling organisms growing in the farm). Without an ability to distinguish between these zooplankton groups, correct interpretation of data could be impossible. Identification of particular taxa from acoustic signals can require large amounts of validation data and seems to be much less well developed for smaller zooplankton such as copepods than larger taxa such as euphausiids (e.g. Stanton 2012). The suitability of this technology for assessment of mussel farming effects is therefore likely to be very limited at this stage.

It is beyond the scope of this report to undertake a thorough assessment of the international status of acoustic sensing technology development. However, it is our interpretation that the application of this technology within a suitable effects assessment would be a substantial undertaking in terms of both required technology, and calibration of instruments to ensure the collection of appropriate data. However, it could conceivably form part of a research project to address the question of mussel farming effects on zooplankton.

2.2.6. Summary, zooplankton sampling methods

In summary, there is no single solution that is capable of robustly addressing the question of the effect of mussel farming on zooplankton communities and all methods are costly. Perhaps for those reasons, very little sampling/observational effort has been devoted to determining whether or not mussel farms are having a material influence upon zooplankton dynamics at bay or regional scales. The key points of the

main available field survey options are summarised in Table 2. It is likely that a substantial amount of research would be required before a feasible monitoring strategy could be developed for assessment of the effects of both depletion and competition on zooplankton communities. Even in the case where substantial field data were available, it is likely that modelling would need to form a key part of any programme to understand the effects of mussel farming.

2.3. Simulation models

By design, models are simplified caricatures of the real-world system that they seek to approximate. Before constructing a model, it must be designed. This requires that interested parties:

- Identify what the purpose of the model is (for example, what questions are to be addressed using the model)
- Identify what features/quantities the model must contain in order to best address those questions (this might also include a description of what degree of adequacy is required)
- Identify all the processes/factors/events which may influence the features/quantities that are of interest and then rank those processes by anticipated relative influence
- Decide which of the ranked processes/factors will be explicitly or implicitly included in the model and which ones will be entirely disregarded/excluded
- Assemble data to assist with parameterising, calibrating and validating the model

Ultimately, a simulation model consists of:

1. State variables (quantities that characterise the state of the system: e.g. water temperature, salinity, water velocities, nutrient and phytoplankton concentrations etc.)
2. Process descriptions (equations describing the rates of state change as functions of the present state of the system [incl. both internal state and state at the boundaries]). Some process descriptions (such as those describing the evolution of water temperature and water motion) derive from well understood fundamental relationships. Others (notably those relating to many of the biological processes) are better described as 'theoretically plausible but merely empirically determined relationships'.
3. Scalar numerical coefficients that appear within the process description equations.
4. Scalars or time-series vectors representing boundary conditions

Table 2. Zooplankton measurement options for field studies, and the resultant data, availability, and cost of the technology. * Would require frequent (e.g. monthly) servicing of instruments. ** Variable correlation with biomass e.g. Harvey et al. (2017).

	Physical samples			<i>In situ</i> imagery	Acoustic sensing / particle analysis
	Morphological analysis (taxonomist-processed or automated)	Genetic analysis			
		lab processing	<i>in situ</i> ¹		
Sample size	Up to 10s of mL for protozoa, up to 10s of m ³ for larger e.g. copepods ² .	Up to 10s of m ³ (subsampled)	Few ml.	Few ml.	Large (up to hectares for vessel mounted systems?)
# sites feasibly sampled	Many per day	Many per day	1 or very few	1 or very few	High areal coverage (vessel mounted) 1 or very few (moored)
Sample frequency	Limited by field time	Limited by field time	Limited by unit capability (10s?)	High*	Limited by field time (vessel mounted) High* (moored)
Parameter measured	Counts/sizes, can convert to biomass	Presence/absence or sequence abundance**		Density and size	Counts/sizes, can convert to biomass
Species ID	Reliable	Presumably reliable for species of main interest. Likely to require research to confirm.		Size classes only (potentially calibrated for species ID)	Presumably reliable for species of main interest. Likely to require research to confirm.
Life stage discrimination	Yes	No (e.g. Harvey et al. 2017)		No (size classes possible)	Yes (limited size range)
Availability of technology	Readily	Readily	Extremely limited	Limited	Unknown
Cost	\$10,000s for small studies	\$10,000s for small studies	Many \$100,000s	Several/many \$100,000s	Unknown

1. Automated samplers

The simulation models such as those that have been applied in the Marlborough Sounds (Hadfield et al. 2014; Broekhuizen et al. 2015) are designed to address questions related to:

- a. how river flows, winds and oceanic conditions influence water-flows in the coastal zone
- b. how plankton dynamics within the coastal zone are influenced by water circulation patterns, nutrient (nitrogen) delivery into the region.

The aforementioned Marlborough Sounds models were based upon standard codes which were modified in order to allow them to address questions related to marine farming. Specifically, the codes were modified to include (some of) the influences that marine farms may have upon plankton dynamics:

1. nutrient input from fish-farms (as ammonium, faeces and (small quantities of) uneaten fish feed)
2. consumption of particulate organic material (living phytoplankton and zooplankton and plankton-sized particulate organic detritus) by mussels
3. release of solutes (specifically, ammonium) and particulate organic matter (faeces and pseudofaeces) by mussels¹⁰.

These models encompass only a small fraction of the factors and processes present in the real world. Conditions along the 'outside perimeter' of the model's spatial domain world (i.e. at the river mouths, across the open-ocean boundaries, at the air/sea interface etc) are represented by so-called boundary conditions. Since many of the characteristics of the models' marine farms (locations, stocking characteristics etc) are prescribed *a priori* at model setup time we choose to think of these characteristics as also being boundary conditions (albeit internal boundaries).

Results from a model do not constitute 'monitoring data'. Nonetheless, results from a suitably designed model (which has been calibrated and validated), can legitimately be used to help interpret field data and to provide qualitative (sometimes quantitative) forecasts of the manners in which the system will change in response to changes in one or more of the boundary conditions (or to changes in one or more scalar parameters of the model). In particular, model features (2) and (3) above imply that the model offers some ability to elucidate the net outcome of near-field seston depletion and net conversion of (some of) the ingested particulates back into nutrients that may stimulate subsequent phytoplankton (re-)growth.

Appendix 1 lists seven assumptions (and associated implications) made by Hadfield et al. (2014) and Broekhuizen et al. (2015) that are believed to be particularly relevant

¹⁰ Note that, whenever mussels are exhibiting net positive growth, the implication is that consumption of particulate organic matter exceeds the sum of production of particulate organic matter and of inorganic nutrient by mussels.

to assessing the models' utilities for assessing influences of mussel and fish farms upon plankton dynamics. In a majority of cases, we believe that a key consequence of the assumptions is that the models are likely to overestimate the magnitude of any far-field (scales in excess of several hundred metres) plankton depletion induced by the mussel crop. Indeed, they may also overestimate depletion at scales in excess of a few tens of metres and perhaps even scales of tens of centimetres. Furthermore, the over-estimation is likely to be greater for larger (more motile) mesozooplankton and fish eggs/larvae than for phytoplankton and small zooplankton (refer to Appendix 1 for the reasoning behind this belief). In support of those beliefs we note that the same model code has been applied in the Firth of Thames/Hauraki Gulf region. It predicts that the Wilson Bay Area A marine farming zone (1200 ha arranged as a rectangle several km offshore in the NE part of the Firth of Thames) will be inducing time-averaged plankton depletion of up to about 5% during the summer months and up to about 20% in the winter ones. In contrast, detailed *in situ* plankton sampling and an analysis of satellite imagery both indicate that annual-average depletion extends only out to an area about 50% greater than the marine farming zone itself and averages around 1–2% across that area (with a maximum of around 6% within some parts of the farming zone) (Pinkerton et al. 2018a). Currently, NIWA is working to reformulate some details of the model to enable it to better reproduce observed levels of depletion in the Firth. NIWA anticipates that the reformulated model will be equally applicable to the Sounds. In the meantime, we believe that the models can provide reliable qualitative indicators of the relative susceptibility of different bays to mussel-farm induced plankton change, but that the absolute quanta of change at any location are likely to be over-estimated (particularly in shallower areas of the Sounds).

The strengths of simulation models over empirical data alone include that:

- models can be used to make predictions on larger scales than it is feasible to measure with *in situ* sampling and at higher temporal resolution than is practical with satellite/drone data
- models may be able to make predictions about future change.

Limitations of models include that:

- models are simplifications of the real world
- some model coefficients and boundary conditions are known only imprecisely
- validation with real-world data is desirable to enable assessment of the confidence with which we can view simulation results.

In a monitoring context, models are perhaps most usefully used to provide context to real-world data.

3. PLANKTON COMMUNITIES IN THE MARLBOROUGH SOUNDS

3.1. Phytoplankton data sources

3.1.1. *Near-field snapshot studies*

There have been numerous studies of phytoplankton distributions in the immediate vicinities of mussel farms. Examples from overseas and from New Zealand are summarized in Hulot et al. 2020. Ogilvie et al. (2000) measured vertical profiles of chlorophyll fluorescence within and outside three or four farms (dep. upon sampling occasion) within Beatrix Bay in seven different months. In three farms that were visited in May, August 1997 and February 1998 (statistically) significantly lesser chlorophyll was measured inside the farms than outside (across the 1–8 m depth interval). In November 1998, four farms were visited and all exhibited greater fluorescence inside the farms (across the 1–8 m depth interval). In other months (September 1997, April 1998 and May 1998), some farms exhibited greater fluorescence inside the farms and others exhibited greater fluorescence outside the farms (across the 1–8 m depth interval). In most cases, the difference between inside- and outside fluorescence amounted to < 20% of the outside fluorescence, but in August 1997, it amounted to more than 50% of the outside fluorescence at two of the three farms. Waite (1989) reports results of monthly surveys of chlorophyll around a mussel farm in Crail Bay across the period August 1983–September 1984. The following are quotes from his thesis: (i) ‘chlorophyll concentrations 10–100 m down-current from a mussel farm were often reduced 5-20% compared with those up-current from the farm but, as water flowed beyond this distance from the farm plankton chlorophyll increased’ and (ii) ‘Up to 60% of available food was consumed as water moved through the farm ...’.

Collectively, these studies indicate: (i) chlorophyll (and indeed, particulate organic matter in general) is usually present in lesser quantities within/immediately downstream of mussel farms than upstream of them (but sometimes, the reverse has been observed), (ii) the difference appears to fall back towards zero within tens to a few hundred metres of the downstream edge of the farm, (iii) the magnitude of difference can be very variable across time at any one farm and across farms. When depletion has been observed, it has tended to be greater at times of the year when phytoplankton are expected to be growing only slowly (higher summer—when inorganic nutrients are scarce, and mid-winter when temperature and light-levels are low).

3.1.2. *In situ data – time series*

Observations of chlorophyll concentration for the Pelorus Sound complex stretch back to the early 1980s (albeit that we know of no sampling between the mid-1980s and the mid-1990s). The earlier observations stem from one-off (but sometimes, long-term)

research studies, but Marlborough District Council initiated a formal monitoring program of their own in July 2012. We know of no early chlorophyll observations for the Queen Charlotte Sound/Tory Channel region, but Marlborough District Council commenced a program of regular monitoring in that region in July 2011. In addition, the Cawthron Institute have undertaken intermittent studies in the Onapua Bay region of Tory Channel in relation to blooms of toxic algae. New Zealand King Salmon Ltd are required to monitor chlorophyll concentrations in the vicinities of their more recently established farms. Table 3 provides a summary of the studies that we are aware of.

Table 3. Summary of *in situ* sampling studies in which chlorophyll has been measured. In most cases, chlorophyll concentrations were measured using a GF-C filter (nominal pore-size = 1.2 μm). The Safi and Gibbs (2003) and NIWA2015 datasets contains some measurements of chlorophyll in size fractions smaller than this.

Study	Time-span	Locations	References
DSIR 1981	July 1981 & May 1982	Various throughout Pelorus Sound	Bradford et al. (1987)
Cawthron 1982	October 1982 & January 1983	Schnapper Point	Kaspar et al. (1985), MacKenzie et al. (1986)
DSIR1984	1984-1985, bi-monthly, 7 sampling occasions	Mills Bay, Schnapper Point, Four Fathom Bay, Crail Bay, Hallam Cove, Richmond Bay + numerous stations along a transect through Kenepuru and Pelorus Sound main-stem	Gibbs et al. (1991), Hickman et al. (1991), Gibbs et al. (1992) Vincent et al. (1989)
Cawthron1986	June 1986-Nov 1987 Approx. 3-6 monthly intervals	Numerous throughout Pelorus, Kenepuru Sound system	MacKenzie (2018)
Waite1989	August 1983-Sept 1984, monthly	Kenepuru Sound (two locations), Four Fathom Bay, Crail Bay, Richmond Bay	Waite (1989)
NIWA1997	Location-dependent, fortnightly. Overall data span 1997-2010 ¹¹ .	Schnapper Point, Nydia Bay, South East Bay, Tawero Point, West Beatrix Bay, Laverique Bay, Cannon Hill, central Outer Pelorus	Figure. Some data appear in Zeldis et al. (2008), Ogilvie et al. (2000), (James et al. 2001; Safi & Gibbs 2003). Data are also summarised in Broekhuizen (2013)
MDC2012	July 2012 onwards, monthly	Seven sites within Pelorus Sound. 5 sites within Queen Charlotte Sound/Tory Channel	The most recent summary of these Marlborough District Council data are summarised in Broekhuizen and Plew (2018)
Fox1997	November 1997-October 1998, fortnightly (approx.)	Anakoha Bay, Beatrix Bay, Hallam Cove, Kenepuru Sound, Nydia Bay	Figure A2.2. Fox (2003), Ren et al. (2019)
NIWA2015	July 2015 – June 2019. Monthly	West Beatrix Bay, Laverique Bay	Unpublished
New Zealand King Salmon Ltd	July 2013 onwards, monthly	Various in vicinities of Kopaua (Richmond Bay), Waitata Bay and Ngamahau salmon farms	e.g. McGrath et al. 2020a, 2020b, 2020c.
Cawthron 2000	Nov 1996 - Jan 2000. Approx 20 samples taken sporadically in summers	Wedge Point algal bloom	Unpublished
Cawthron Seafood Safety programme 2019	May to June 2018 and Feb to July 2019. \leq 2-weekly intervals	up to 12 sites in Nydia Bay and adjacent sites in Pelorus Sound	Unpublished

¹¹ West Beatrix, Laverique and Schnapper Point were sampled through much of that period. Other locations only for much shorter periods

3.1.3. Satellite-sensed chlorophyll

Satellite data are available for the Sounds region from 2002 onwards—albeit that image pixels from the nearshore regions have usually been deemed to contain ‘bad values’ (due to mist cover, breaking waves etc) – such that data are abundant only in the central parts of the main-stems and large bays of the mid- and outer Sounds.

Two different satellite systems have carried sensors from which near-surface marine chlorophyll concentrations can be inferred. The SeaWiFs system was in operation from 1997–2010, while the MODIS-Aqua system has operated since 2002. In the New Zealand region, these have spatial resolutions of approximately 4 km and 1 km, respectively. Pinkerton et al. (2018b) have recently published an analysis of the data for the New Zealand coastal zone. The data which we will present in this report stem from that same analysis—but we focus only upon the MODIS-Aqua data (which have sufficient spatial resolution to resolve at least the outer parts of the Sounds). Readers should consult Pinkerton et al. (2018b) for a description of the methods employed to process the satellite data. An important point to note is that, chlorophyll concentrations were inferred from the raw satellite data using generic (rather than site-specific) coastal water algorithms. Thus, while the chlorophyll concentrations inferred from the satellite data may not be as reliable as those which might have been inferred using a region-specific algorithm, they are entirely independent of the *in situ* data described in the preceding section.

3.1.4. Analysis of historical *in situ* chlorophyll measurements

Schnapper Point, Beatrix Bay and Outer Pelorus

In various combinations, the Cawthron1982, Cawthron1986, DSIR1984, NIWA1997, MDC2012 and NIWA2015 studies held some sampling stations in common. By concatenating the data from the various studies, it is possible to create composite time-series that span several decades. In particular, it is possible to create composite time-series using data gathered in the vicinities of Schnapper Point, West Beatrix Bay, Laverique Bay (eastern side of Beatrix Bay) and outer Pelorus (mid-channel around Cannon Hill) by concatenating data from different studies. The composite time-series are presented in Figure 3.

All four composite time-series stretch back to the mid (or even, early) 1980s—albeit there are no data from the mid-1980s to the mid-1990s. Within all four time-series, the maximum chlorophyll concentrations have been more than ten times greater than the minimum ones (Figure 3).

While the composite time-series are not true time series in a strict statistical sense¹², we have nonetheless endeavoured to determine whether they show any evidence of

¹² The sampling interval was not constant across all component studies, there were gaps between sequential studies, and in some cases, the sampling locations were not coincident across sequential studies (i.e. were more than a few 100 m apart).

long-term trend by adopting the methods described in McBride (2019). More specifically, the data were deseasoned by subtracting the long-term monthly median values from corresponding (by month) raw values, subsampling the resultant residuals to monthly resolution¹³ and calculating the Sen's slope¹⁴ in these subsampled residuals. In addition, we determined the probability that the sign of the slope is likely to have been correctly determined¹⁵. If the probability that the sign of the slope has been correctly determined exceeds 90%, we argue that the direction of trend has been confidently determined. This is equivalent to a statement that the 95% confidence bounds on the estimated value of the slope do not span zero.

When the data are subsampled to monthly resolution (as described above), the direction of trend cannot confidently be determined at Schnapper Point. At the other three locations, the trend direction has been determined to be downward with probability > 90% (Figure 4). When the data are subsampled down to two-monthly resolution, the directions of trend at Schnapper Point and Outer Pelorus cannot confidently be determined, but those at West Beatrix and Laverique remain negative (downward).

We note that Helsel and Hirsch (2002) recommend that for analyses such as that reported here, no one third of the calendar span should contain fewer than 20% of the data points in the subsampled composite time-series. Even after subsampling to monthly (or two-monthly) sampling, this condition is not met in some of our time series—because there are so few data from the 1980s/early 1990s. This is therefore a potential limitation of this analysis. However, if the (scarce) data from the 1980s are excluded, the remaining data still indicate that chlorophyll concentrations reported in the NIWA1997 campaign tended to be higher than those that have been recorded in the MDC sampling (and also higher than those recorded in the various shorter-term studies from the 1980s).

¹³ This helps to minimise the bias (towards any trend evident during the time spanned by the NIWA1997 data) that would otherwise arise from the fact that the NIWA1997 would contribute a disproportionate number of slopes since sampling was fortnightly in that series, but monthly or several monthly in other series.

¹⁴ The Sen's slope is the median of the slopes calculated between all pair-wise combinations of data-points. In comparison with other slope estimators (e.g. least-squares regression), it is insensitive to missing values and outlying values.

¹⁵ Traditional statistical approaches test a null-hypothesis that states 'the slope of the sample time series is (exactly) zero'. McBride argues that this is an inappropriate test because the null hypothesis is unlikely to be true in any real-world situation. He argues that it is more appropriate to accept that a non-zero sign will arise (perhaps, by chance alone) and focus upon determining the probability that the sign of the slope has been correctly determined. We have followed McBride's recommendation.

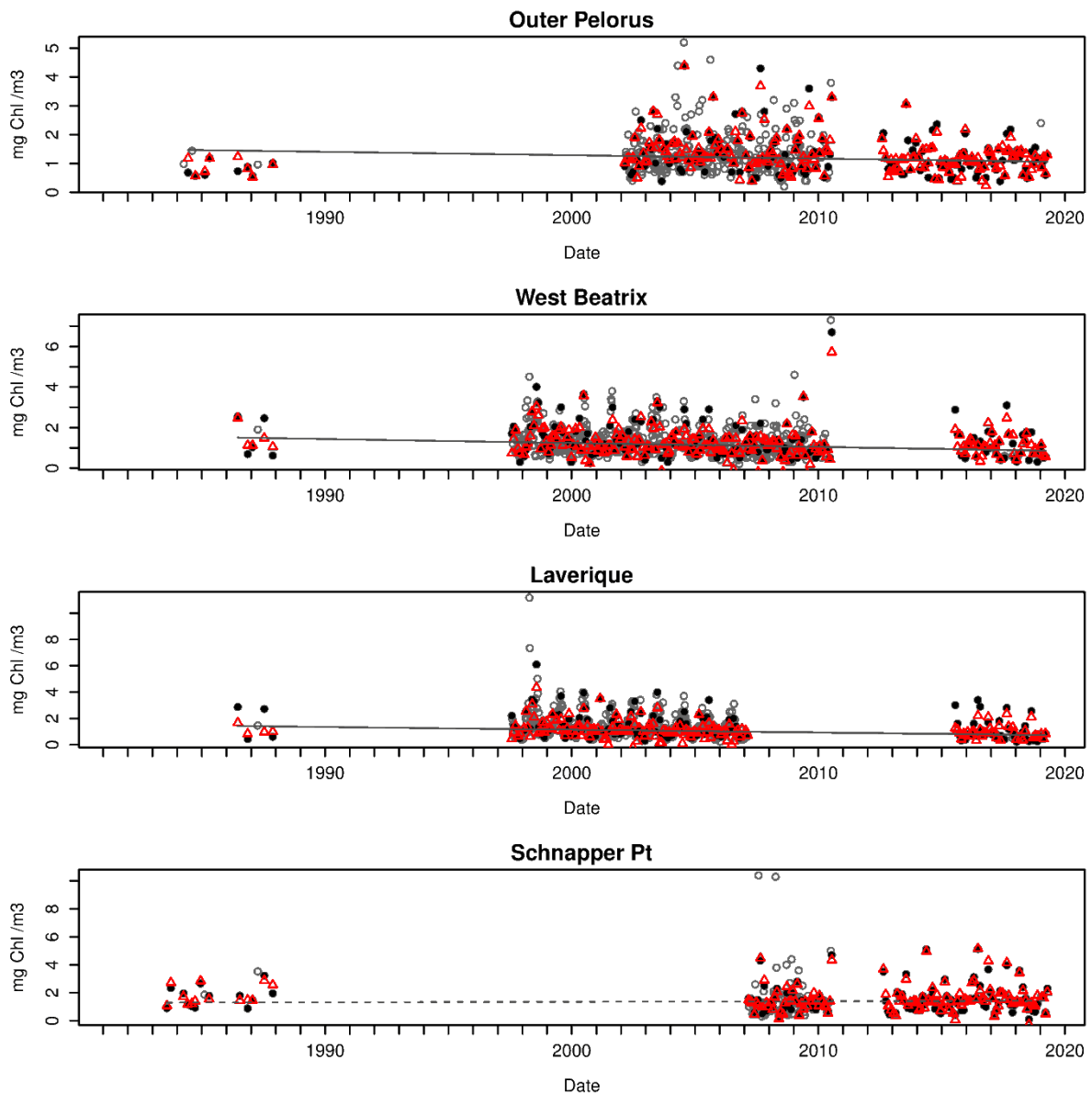


Figure 3. Chlorophyll concentrations measured across several sequential studies in the vicinities of four locations in Pelorus Sound (black and grey symbols). Water was collected using a hose-sampler extending to 12–15 m below the surface and samples were filtered onto a GF-C filter (nominal pore size 1.2 μm). The lines illustrate the best fit (Sen's slope) linear trend line fitted through the deseasoned data (red symbols). Robust estimates of trend require that the sampling frequency in the data be similar throughout the time series. In reality, it has varied from approximately three monthly down to approximately weekly across studies. To mitigate that against bias arising from this variation, the high-frequency data were subsampled to monthly resolution (solid symbols) before Sen's slopes were estimated. Where the Sen's slope line is solid, the distribution and quantities of data are sufficient to allow the sign of the trend-slope to be determined with confidence of 95% or greater (McBride 2019). Note that, whilst we have relied upon MDC data for the Schnapper Point and Outer Pelorus site in the post-2010 period, we have used NIWA data collected at the locations of the original (pre-2010) West Beatrix and Laverique Bay locations.

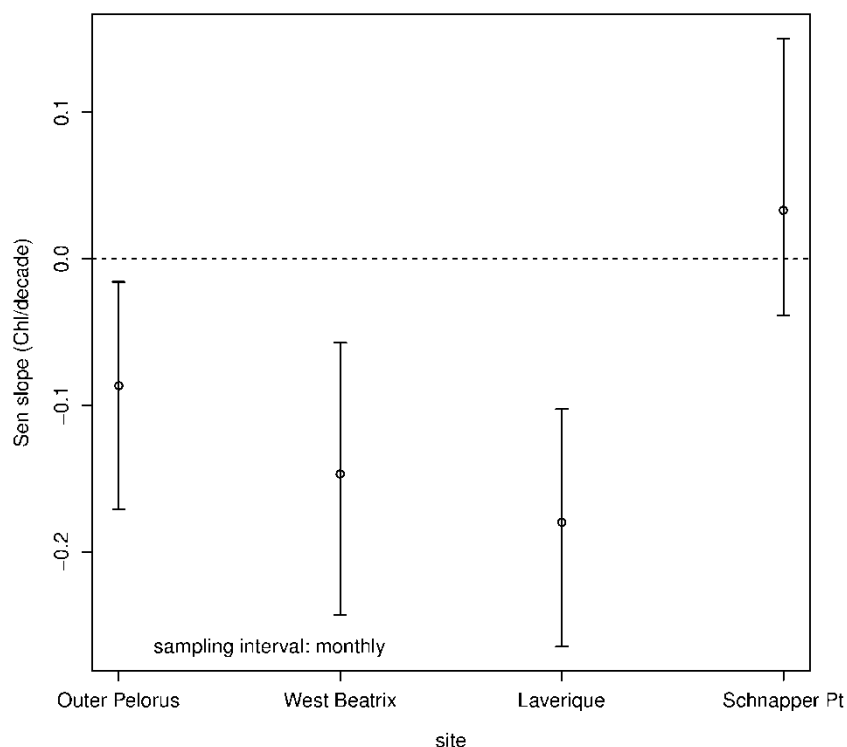


Figure 4. Sen slope estimates for the deseasoned and subsampled chlorophyll records from four locations in Pelorus Sound (see also Figure 3). Vertical bars denote the 95% confidence intervals associated with the estimated Sen slope values. Note that the time series that yielded these slopes were generated by subsampling the raw data onto monthly resolution. If one instead subsampled onto two-monthly resolution, the Sen slope for the Outer Pelorus station becomes positive (with confidence bounds that span zero), whilst the slope at Schnapper Point becomes negative (with confidence bounds that span zero). The slopes at West Beatrix and Laverique remain similar and their confidence bounds do not extend up to zero.

Sites elsewhere in Pelorus Sound

Data are also available for several other bays within / close to Pelorus Sound (Table 3) Results from two of the largest data-sets are summarised in Appendix 2.

Queen Charlotte Sound

Marlborough District Council have run a programme of monthly sampling in Queen Charlotte Sound and Tory Channel since July 2011. A recent summary of those data can be found in Broekhuizen and Plew (2018).

3.1.5. Satellite data

Figure 5a illustrates the 1997–2018 average of satellite-sensed near-surface chlorophyll in the coastal strip around New Zealand. The averages sit around 0.3-1.5 mg chl m⁻³ around most of New Zealand. Figure 5b illustrates the corresponding average satellite-sensed near-surface temperatures—which range from around 11 °C up to around 18 °C.

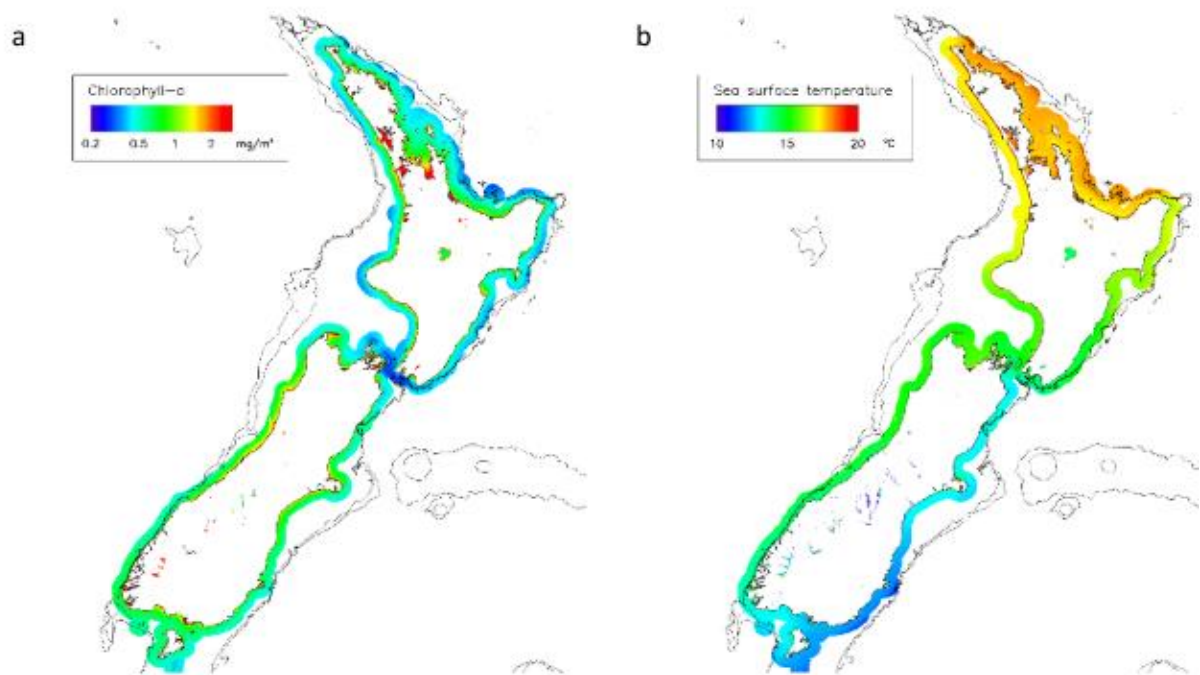


Figure 5. Plots illustrating long-term (a) near-surface satellite-sensed chlorophyll concentration and (b) near-surface satellite-sensed temperatures around New Zealand (reproduced from figures 3-10 of Pinkerton et al. (2018b)). The time-average periods are 1997–2018 for chlorophyll and 1981–present for temperature.

Figure 6 illustrates the estimated 1997–2018 time-trends for (a) satellite-sensed temperature and (b) satellite-sensed chlorophyll concentrations around the New Zealand coastline. Sea-surface temperatures have tended to rise around all parts of the coastline. Warming has been most rapid along the west coast of the South Island and least rapid around the SE flank of the North Island and the open, east coast of Marlborough. Conversely, the chlorophyll concentration has been downward around much (but not all) of New Zealand’s coastline including the western and central region of Cook Strait.

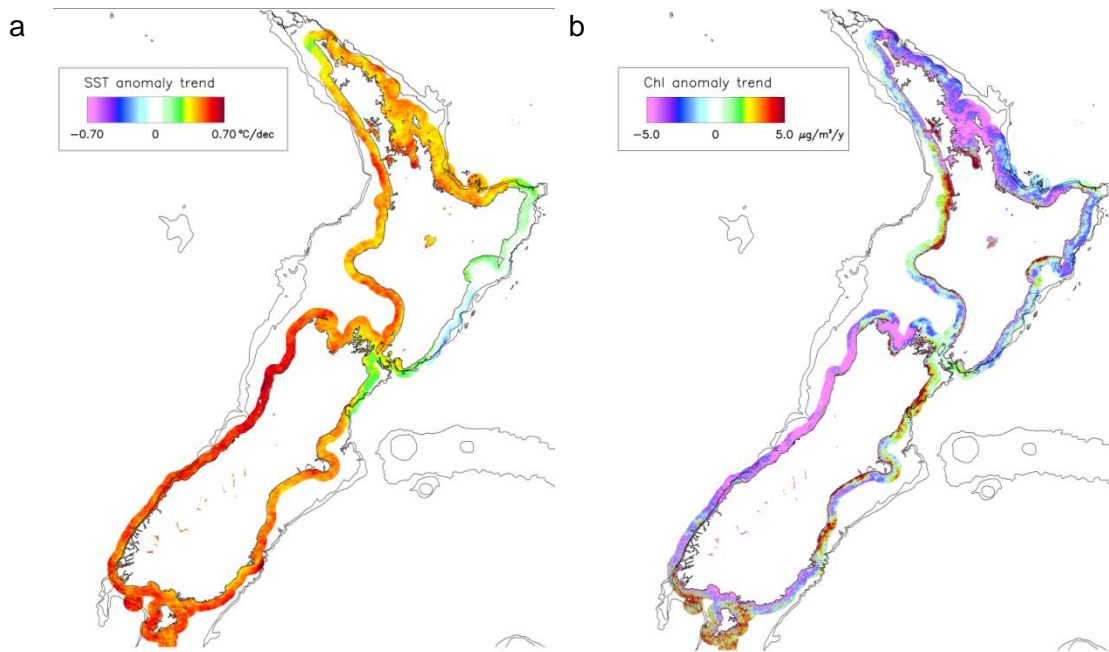


Figure 6. Plots illustrating the long-term trend (Sen slope) for deseasoned monthly satellite-sensed temperature and satellite-sensed chlorophyll concentrations (adapted from figures 3-14 of Pinkerton et al. (2018b)). Trends are calculated for the 2002–2018 period.

Figure 7 presents the same trend-data, focussing upon the Marlborough/Cook Strait region. At this scale, it becomes apparent that warming has been more rapid on the western and northern side of Cook Strait and may also have been more rapid in Queen Charlotte Sound/Tory Channel than in Pelorus Sound (Figure 7a). Chlorophyll concentrations have tended to decline in the western and northern region of Cook Strait and in the Marlborough Sounds, but risen in the waters of the southern Cook Strait to the east of Pelorus Sound (Figure 7b). Inside the Sounds, the trend in concentration of satellite-sensed chlorophyll concentration has amounted to approximately -3 to $-5 \mu\text{g chl m}^{-3} \text{ y}^{-1}$ (-0.03 to $-0.05 \text{ mg chl m}^{-3} \text{ decade}^{-1}$). The direction of trend is the same as that inferred from *in situ* measurements but the magnitude of the trend is smaller than that inferred from the *in situ* data (around 20–35% of the rate inferred from *in situ* data).

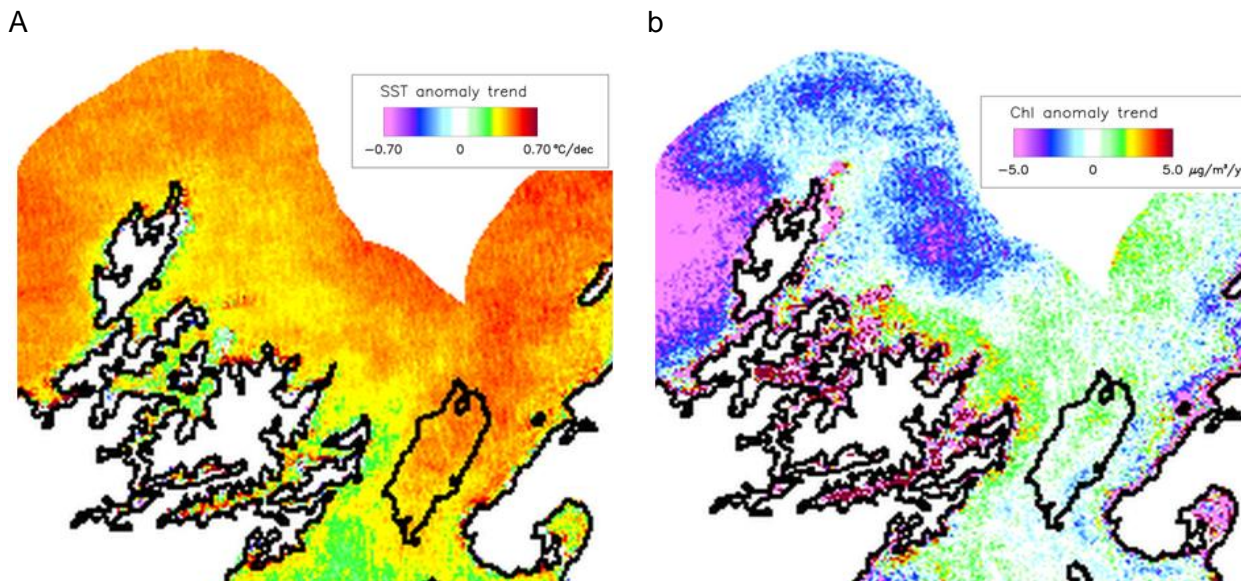


Figure 7. Long-term trends (Sen's slopes) for (a) near-surface temperature and (b) near-surface chlorophyll in the Cook Strait region using satellite sensed data.

3.1.6. Phytoplankton cell count data

The Marlborough District Council monthly monitoring programme includes making counts of phytoplankton cells in the near-surface water samples. The contents of approximately 100 mL of water are settled onto microscope slides. The cells within a subsample of the grid-squares on the slide are identified to lowest practical taxonomic resolution (often species level) and counted. Crude estimates of taxon-specific cell biovolume (hence, cell biomass) are also made. Any one water sample will usually contain representatives from around 20 taxa but most will be relatively scarce. It is not practical to present data for every taxon, but they can be aggregated to higher taxonomic levels to provide a broad impression of the patterns (three sites from Pelorus Sound are illustrated in Figure 8.). Three features are evident in this data: (a) overall biomass is usually greatest in central Pelorus (PLS-4 and PLS-5, respectively, Beatrix Bay and Tawhitinui Reach), (b) at all sites, colonial diatoms are usually the dominant group by biomass concentration. Small unicellular algae form a significant component of the biomass at the inner sites (PLS-1 to PLS-3) while dinoflagellates are relatively more important at the outer sites (PLS-6 and PLS-7), (c) biomass concentration varies seasonally and across years—having been higher since mid-2016).

A subset of corresponding data from Queen Charlotte Sound/Tory Channel are presented in Figure 9. Colonial diatoms are relatively less abundant in those samples. Indeed, dinoflagellates are often the biomass dominant—particularly at the two sites within Grove Arm (QCS-1 and QCS-2). Once again, seasonal variations and inter-annual variability are plentiful.

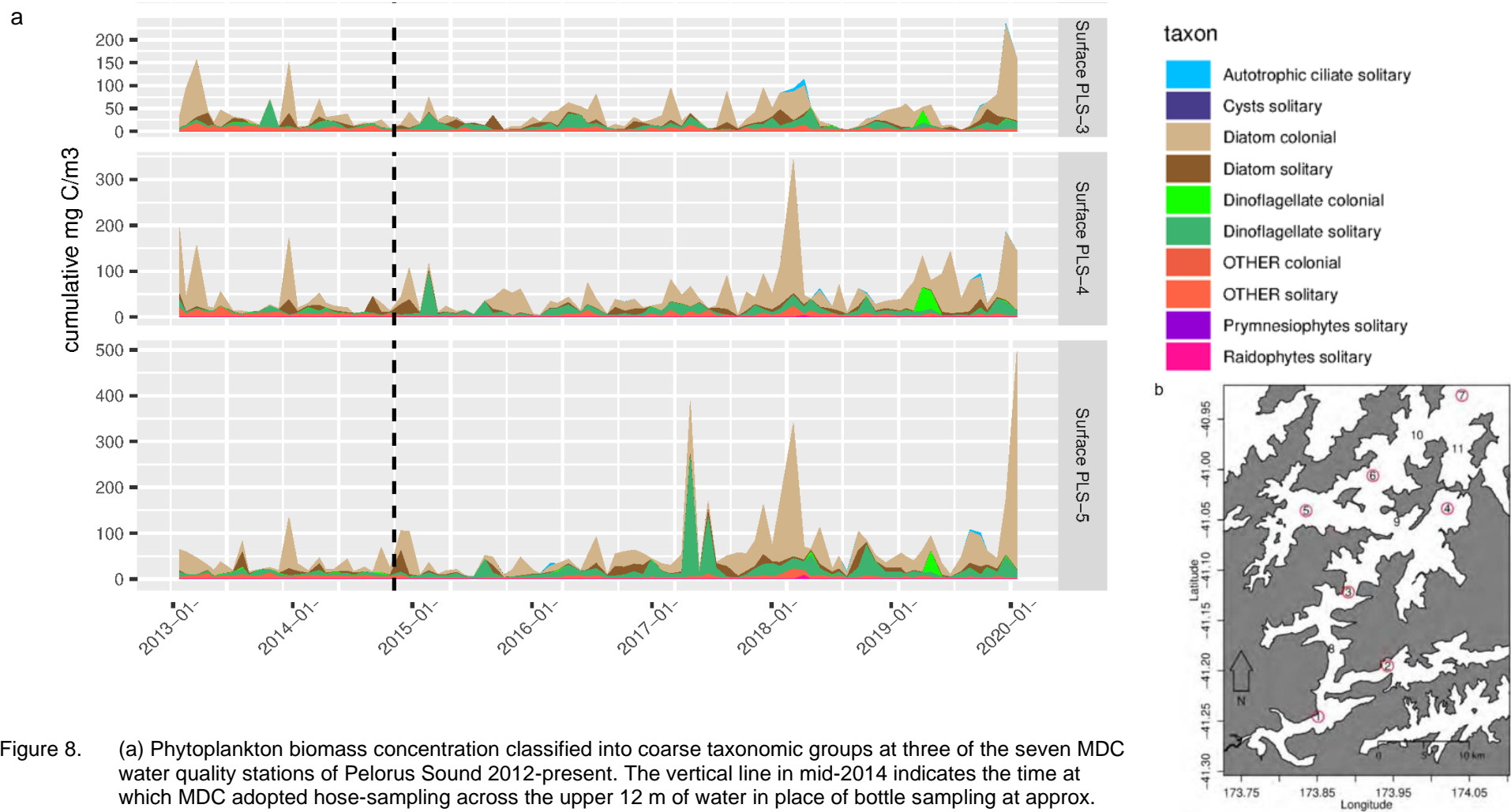


Figure 8. (a) Phytoplankton biomass concentration classified into coarse taxonomic groups at three of the seven MDC water quality stations of Pelorus Sound 2012-present. The vertical line in mid-2014 indicates the time at which MDC adopted hose-sampling across the upper 12 m of water in place of bottle sampling at approx. 4 m. (b) Site-location map.

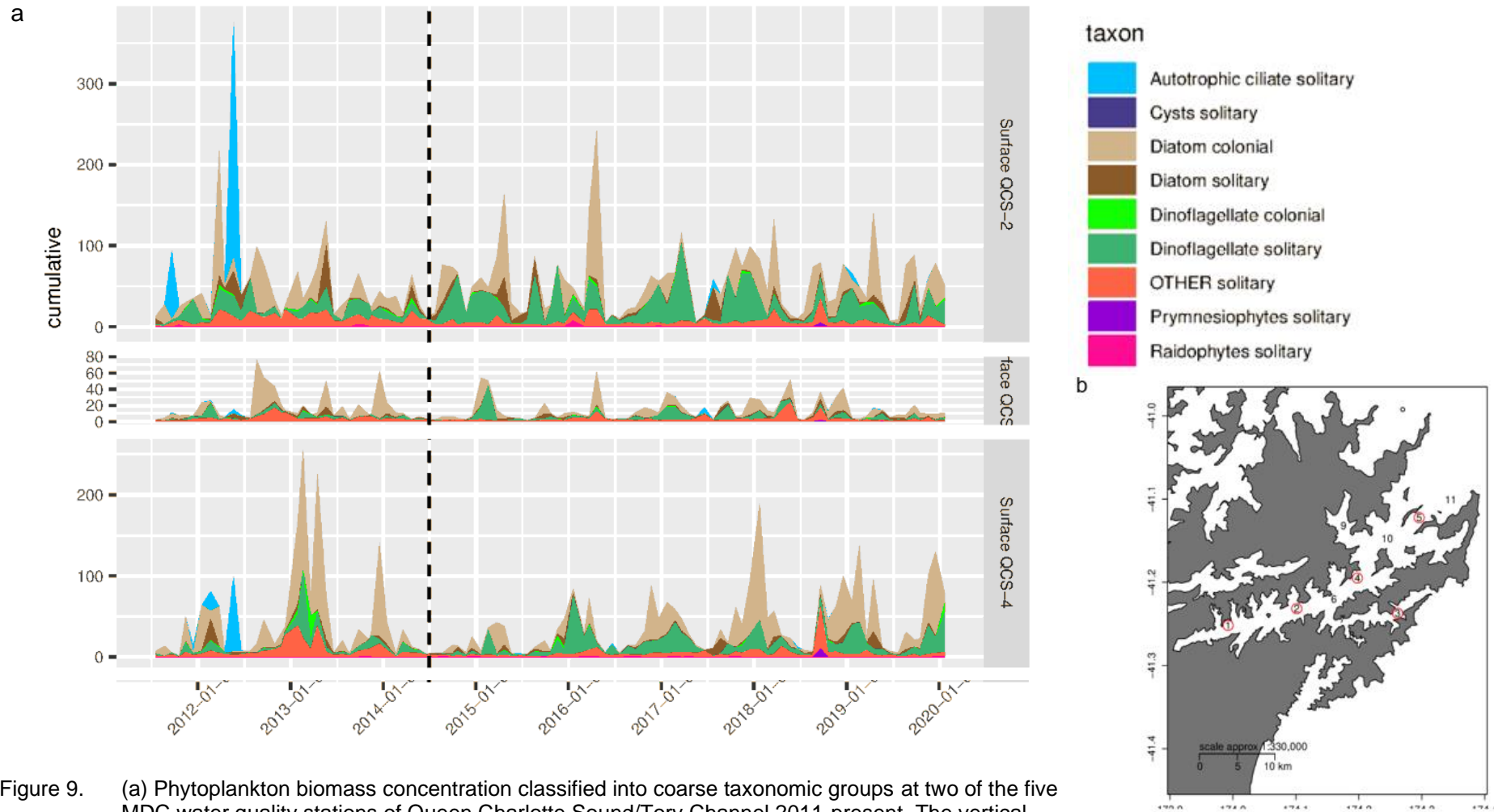


Figure 9. (a) Phytoplankton biomass concentration classified into coarse taxonomic groups at two of the five MDC water quality stations of Queen Charlotte Sound/Tory Channel 2011-present. The vertical line in mid-2014 indicates the time at which MDC adopted hose-sampling across the upper 12 m of water in place of bottle sampling at approx. 4 m. (b) Site map.

3.2. Evidence for phytoplankton community change in the Marlborough Sounds

The *in situ* data and the satellite data are independent of one another and each has unique limitations that imply that one must be somewhat wary of the inferences one draws. The *in situ* measurements of chlorophyll within the composite time-series are composed from data from sequential studies. It is possible that some of the (seeming) temporal trend in the composite time-series is an artefact arising from a combination of the facts that sequential research studies have not always been exactly co-located, and the probability that there are persistent spatial gradients of chlorophyll¹⁶. Inevitably, laboratory instruments have changed from time to time and this may have introduced (small) step-changes into chlorophyll estimates. The satellite time-series extend over a shorter time-span. Furthermore, the confounding influences of suspended sediments and dissolved coloured solutes imply that the inferred chlorophyll concentrations may be less accurate and/or less precise than those that can be made in the laboratory. For these reasons, we are not overly concerned that the magnitudes of chlorophyll trend in the two datasets are not more similar. Despite the limitations of each dataset, both indicate yield the same qualitative inference: chlorophyll concentrations within the Marlborough Sounds have declined in recent decades.

While there is evidence that chlorophyll concentrations in the Sounds have declined in recent years, we have already noted (Section 2.1) that chlorophyll provides only an imprecise indicator of phytoplankton abundance. A decline in chlorophyll concentration indicates that something about the phytoplankton community has changed—but there are several possible changes that might account for the chlorophyll change. For example, (a) taxa with high chlorophyll:carbon (or chlorophyll:biovolume) ratios (e.g. diatoms) may have been displaced by chlorophyll poor ones (e.g. dinoflagellates) with little/no change in properties such as total phytoplankton carbon, nitrogen or particle count, (b) the composition and abundance (as measured by cells, carbon, nitrogen etc) may have remained nearly constant but the average chlorophyll content of individual cells may have changed, (c) the abundance (as measured by cells, carbon, nitrogen etc) of one or more phytoplankton taxa may have changed.

Marlborough District Council have monitored the composition of the phytoplankton community since 2011 (Queen Charlotte, Figure 9) or 2012 (Pelorus Sound, Figure 8), but the time series are too short to merit tests for long-term community structure trends.

¹⁶ We note that the outer Pelorus station (PLS-7) that MDC has sampled since 2012 is several km seaward of the earlier outer Pelorus station and has persistently higher salinities (i.e. is less influenced by the freshwaters that flow into Pelorus Sound).

3.3. Assessment of aquaculture effects on phytoplankton

If we assume that apparent chlorophyll trends are genuine (rather than an artefact arising from differences between studies), it becomes relevant to try to determine what may have driven the chlorophyll fluctuations and long-term decline. NIWA is applying Generalized Additive Models (GAMs) to explore the correlations between deseasoned chlorophyll concentration (i.e. differences between instantaneous chlorophyll concentration and the long-term average for the time of year) and candidate explanatory variables (including anomalies of time-averaged river flow, water temperature, ENSO-state, wind stress, and bay-scale area of approved mussel farms). Preliminary indications are that the approved area of mussel farms is not retained as a useful explanatory variable (of chlorophyll fluctuations and long-term decline) in the optimal GAMs¹⁷. In contrast, river flow¹⁸ is invariably retained and date or study ID are sometimes retained as useful explanatory variables in the optimal GAMs.

River flow has previously been identified as a key driver of interannual variations in phytoplankton standing stock in Pelorus Sound (Zeldis 2008) and this analysis supports that earlier conclusion (the two analyses are not entirely independent as the NIWA1997 data were used in both analyses). Anomalously high time-averaged river flows tend to be associated with anomalously high chlorophyll concentrations. Rivers deliver nutrient into the Sound and play a big part in determining how strongly it stratifies. Phytoplankton productivity tends to be increased when nutrients are plentiful and, at least during the winter, stratification also promotes greater phytoplankton accrual in the surface waters (because those phytoplankton are less likely to be mixed into poorly illuminated near-bed waters when the water column is stratified).

While river flow does appear to be an important driver of interannual fluctuations in season-average phytoplankton fluctuations, it is not clear whether or not subtle changes in river flows have contributed to the decadal-scale declines that are evident in chlorophyll concentration in Pelorus Sound. Nonetheless (but subject to some caveats¹⁹), the outcomes from this provisional analysis provides some evidence that the expansion of mussel farming in Pelorus Sound has not had a major influence upon chlorophyll concentrations there²⁰.

¹⁷ Strictly, those which minimise the information loss (infidelity of prediction ability) that inevitably accrues as candidate explanatory variables are sacrificed, or (conversely) those which can explain the largest fraction of the variability in the data without requiring an excessive number of explanatory variables.

¹⁸ Strictly, the 30 day average (over the period leading up to each chlorophyll sampling occasion) of the difference between instantaneous river flow and long-term average month-of-year median river flow

¹⁹ Our measure of mussel farming intensity (area of approved mussel farms within the bay/reach in where the phytoplankton were monitored) is crude. It takes no account of: (i) delays between approval and farm establishment, (ii) inter-annual variability in stocking intensities (which is influenced by factors such as availability of mussel spat (market forces and no account of seasonal-scale fluctuations in crop characteristics) and (iii) seasonal-scale cycles in crop characteristics.

²⁰ The crude nature of our measure of farming intensity might reduce the level of confidence that an individual chooses to attach to inferences that rely upon this measure of farming intensity but we believe that the

In the absence of controlled, experimental manipulations, the GAM analysis (or any alternative one) can only exploit the realised environmental time-series for chlorophyll, temperature, total area of approved marine farms, river flow, study ID, etc. Thus, our GAM analysis is analogous to a correlation analysis and it is worth repeating the aphorism 'correlation need not imply causation'. The mere fact that river flow anomalies (and, in some instances) other independent variables are retained in the optimal models is not incontrovertible evidence that those characteristics are the ones which have driven the seeming decline of chlorophyll. The relationships may be entirely spurious, or they may be an indication that both river flow and chlorophyll are responding to some other characteristic that has been evolving through the decades spanned by these data. On the other hand, the fact that cumulative area of approved marine farms is not retained in the optimal model provides at least a little evidence to indicate that the expansion of mussel farming has not had a measurable influence upon chlorophyll concentrations at these four 'sites' (Schnapper Point, West Beatrix Bay, Laverique Bay, and outer Pelorus) within Pelorus Sound.

Of course, we acknowledge that our measure of marine-farming intensity (cumulative bay-scale area of approved mussel farms) is only a crude proxy. For example, in any one farm, the stocking density (whether measured as mussels/m dropper or mussel mass.m⁻¹.dropper⁻¹ or filtration capacity m⁻¹ dropper) varies throughout the cropping cycle and between crop-cycles. Stocking characteristics will also vary amongst farms. Furthermore, the regions across which we calculated the bay-scale area of approved marine farms were defined primarily by crude geographical considerations (e.g. Tawhitinui Reach, Beatrix Bay, Kenepuru Sound) without reference to characteristics such as the magnitude of the tidal-ellipse or flushing times, etc. A measure of harvest by area may be better indicator of real-world mussel farming pressure.

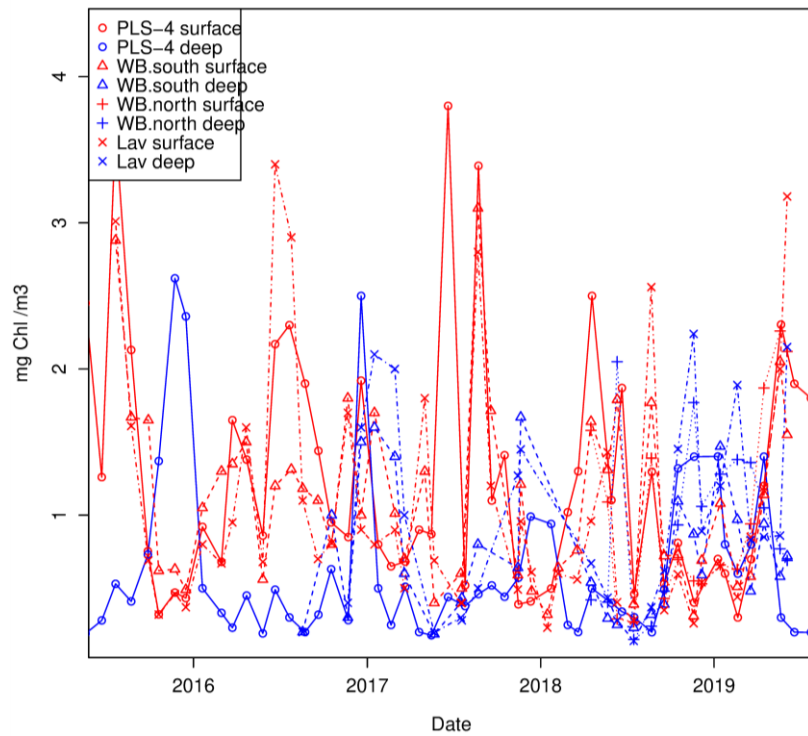
We also acknowledge that, with the exception of the outer Pelorus site, the sampling sites for *in situ* sampling that were chosen in the 1980s and 1990s were selected to be adjacent to existing marine farms. Thus, chlorophyll concentrations at those 'precise' locations may already have been somewhat reduced by the activities of the already-present local crop. Nonetheless, our inability to detect evidence that subsequent farms induced additional decline at those sites suggests that any cumulative effects arising from those additional marine farms have been very small relative to the scales of fluctuation that are evident in the chlorophyll time-series. Given that we have been unable to find any evidence to indicate that the substantial expansion of mussel farming since the 1980s has induced measurable chlorophyll change, we consider it unlikely that the initial development of marine farming in the preceding decades would have had a meaningful influence upon chlorophyll concentration at these locations.

inference (that expanded farming activities have not contributed meaningfully to chlorophyll declines) is more likely than not to be correct.

Whilst certainly not conclusive evidence, the fact that satellite data indicate that chlorophyll declines have not been restricted to the Marlborough Sounds region (or even to regions which harbour shellfish farms) lends some further support to our suggestion that an expansion of mussel farming have not been the main driver of chlorophyll concentration declines in Pelorus Sound. The reasons for the widespread decline in chlorophyll remain unclear but both *in situ* and satellite data indicate that waters around New Zealand are warming and numerical models of the oceanic waters around New Zealand suggest that warming will be associated phytoplankton declines (Law et al. 2018). Thus, it may be plausible that the warming that has already taken place drove the observed chlorophyll declines.

The NIWA2015 study (Table 3) monitored near-surface and near-bed chlorophyll beside three mussel farms in Beatrix Bay at approximately monthly intervals. Marlborough District Council have monitored chlorophyll in the centre of the bay at approximately monthly intervals since July 2012. Whilst the sampling dates have not always been identical, it may be revealing to compare the measured chlorophyll concentrations in each time-series (Figure 10). At the south-western and central Beatrix stations, median near-surface chlorophyll concentrations have tended to be greater than or similar to near-bed ones. Conversely, in north-western Beatrix and Laverique, near-bed chlorophyll concentrations have tended to exceed near-surface ones. Near-surface median chlorophyll concentrations at the south-western station tend to exceed those at the other stations, but near bed median chlorophyll concentrations tend to be lower at the southwestern and central station than at the other two stations. During this 2015–2019 period, median chlorophyll concentrations inside Beatrix Bay have proven to be approx. 0.2–0.3 mg chl.m⁻³ lower than those at the two nearest stations in the main-stem of Pelorus Sound. If one could safely assume that chlorophyll concentrations would be horizontally homogenous in the absence of mussel farms, most (but not all) of the spatial gradients that were evident across these sites during the 2015–2019 period are consistent with an assumption that mussel farms induce some degree of phytoplankton suppression. Unfortunately, the hydrodynamic regimes in the vicinities of each are likely to be somewhat different—such that it is unsafe to assume that chlorophyll concentrations would be spatially homogenous in the absence of marine farms. Consequently, these data do not allow us to confidently determine whether there is any evidence that the mussel farms are (or are not) inducing depletion within Beatrix Bay, or inducing spatial variations across Beatrix Bay.

a



B

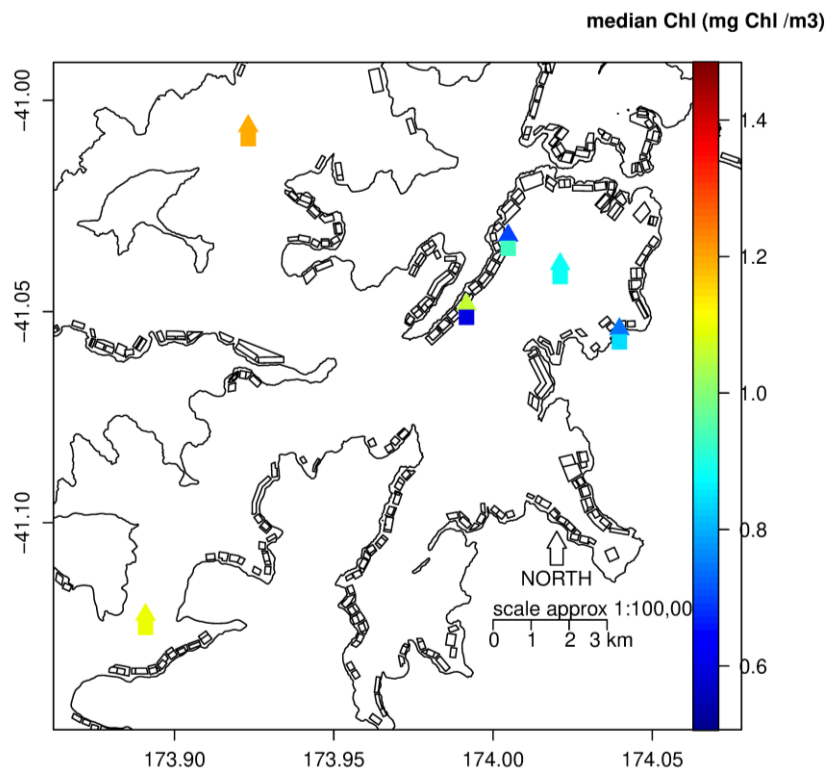


Figure 10. (a) Time-series of chlorophyll measured in water samples gathered from four locations in Beatrix Bay during 2015–2019 (GF-C filter). (b) Map illustrating the median chlorophyll (GF-C filter) measured at each of four locations in Beatrix Bay 2015–2019. Triangles: hose-samples to approx 15 m depth; squares: Van Dorn samples collected near the seabed. The triangles (rather than the squares) indicate the locations that were sampled.

3.4. Zooplankton

Few studies have described zooplankton communities in the Marlborough Sounds, or nearby waters. MDC initially collected zooplankton data as part of the State of the Environment water quality monitoring programme; however, these samples were taken in low-volume samplers, and accordingly provide only 'very imprecise estimates of zooplankton abundance' (Broekhuizen et al. 2015). The only other published measures we are aware of are associated with the development of mussel farming in eastern Tasman Bay (Table 4). These data provide an indication of the dominant species in the area, and illustrate the variability in abundance of zooplankton over time. Note that the plot of these data (Figure 11) employs log axes, substantially reducing the apparent variability.

Table 4. Summary of *in situ* sampling studies in which zooplankton has been measured.

Study	Time span	Sample type	Locations	References
MDC SoE data	July 2012–June 2014	Van Dorn / hose	Pelorus and Queen Charlotte Sounds	Broekhuizen et al. 2015
FRIA	October and December 2003, January 2004	Net hauls	West Durville (Tasman Bay)	Hopkins et al. 2004
Wakatu Inc. consent monitoring	March 2017 – October 2018. Approximately fortnightly, 26 sampling occasions	Net hauls	West Durville (Tasman Bay)	Morrissey & Newcombe 2018

Additionally, a series of net haul samples have been collected from Kenepuru Sound by NIWA, but have not been processed. Another dataset from Queen Charlotte Sound collected in or about early last decade may exist (see Leader 2014), but the location and status of these samples/data are unknown to us. These samples were collected in investigations related to the blue cod fishery, and genetic analysis was apparently planned²¹.

²¹ <http://www.stuff.co.nz/marlborough-express/editors-picks/8795822/Lobbyist-thrown-out-of-forum>

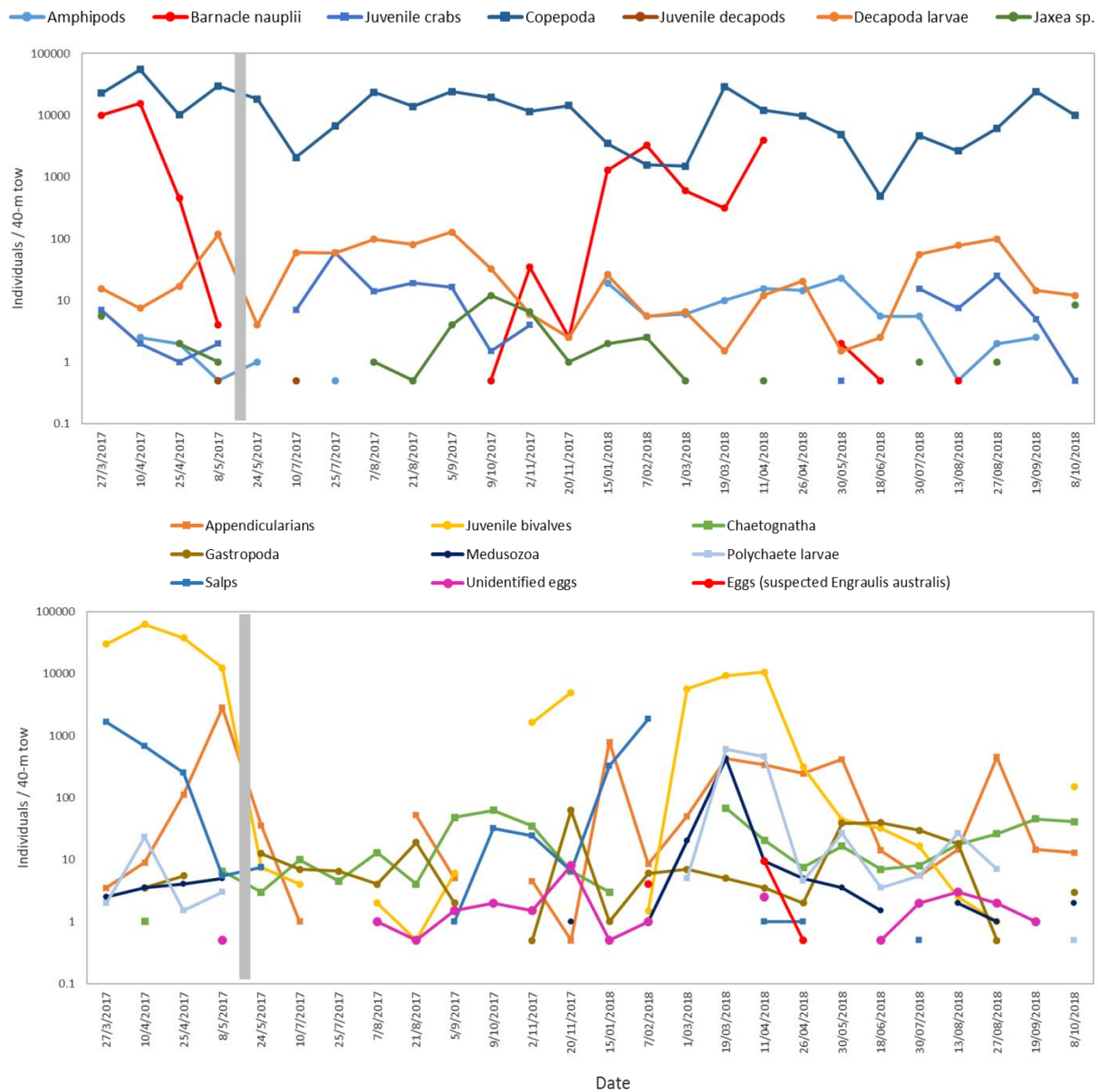


Figure 11. Zooplankton from a site in eastern Tasman Bay. Abundances of different categories of crustacean (top) and non-crustacean (bottom) taxa (number of individuals per 40-m tow averaged across two sampling stations). Note that the y-axis is on a log₁₀ scale. Note also that on the first four sampling dates the volume of water sampled was approximately 40% greater than on subsequent dates, the grey bar indicates the change in net diameter. From Morrisey & Newcombe (2018).

Insufficient data exist to even tentatively assess whether any effect of mussel farming is apparent in the Marlborough Sounds. The best available data is from a series of small sample volumes collected in the early days of the Marlborough District Council SoE monitoring programme (note limitations of small sample volumes above). The sample sites from Pelorus Sound (where most mussel farming takes place) are mapped in Figure 8b, and the data (presented as derived biomass) in Figure 12. The authors noted that while no statistical analysis was undertaken, ‘zooplankton biomass

concentrations are not markedly lower in two regions which have high densities of mussel farms (Kenepuru [Site PLS 2] and Beatrix Bay [Site PLS 4]) than in other regions.'

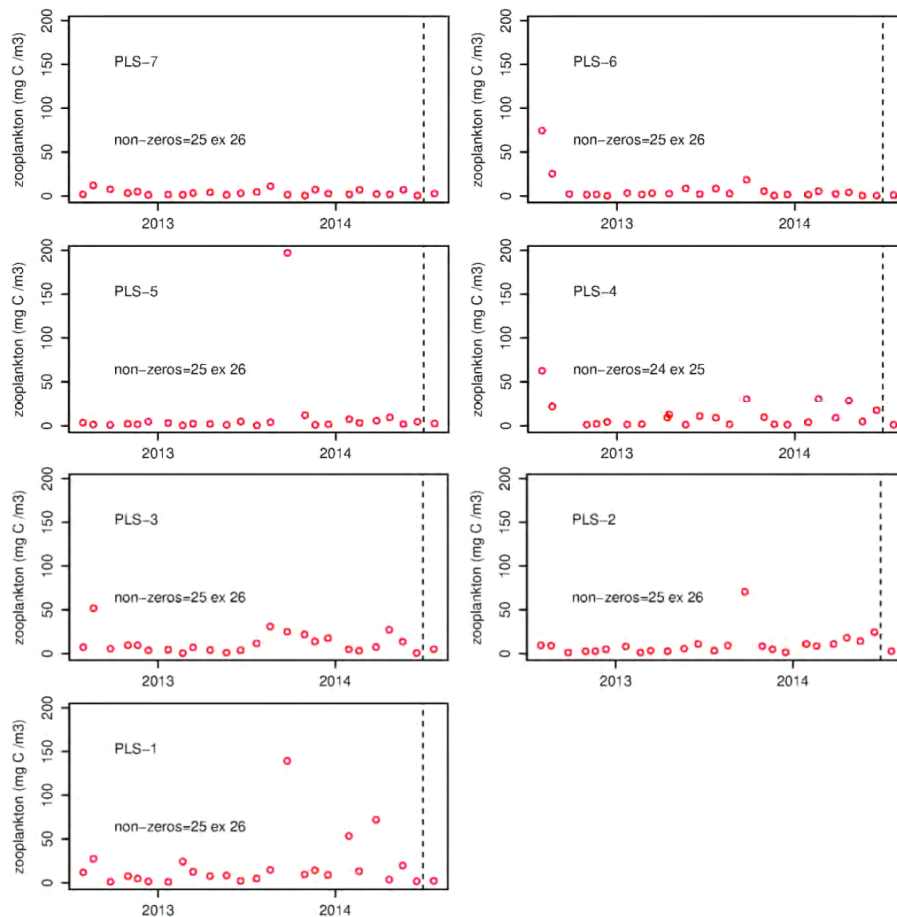


Figure 12. Zooplankton biomass inferred from counts and dimensions at the seven Marlborough District Council Pelorus sites (near surface water samples). The dashed vertical line (1 July 2014) separates data collected with a Van Dorn bottle at 1 m depth (to left of line) from data collected from the upper 15 m of the water column using a hose-sampler. From Broekhuizen & Plew (2018).

Possible experiments and measurements for assessing the effects of mussels on zooplankton communities are presented in Section 4.1.2 below.

3.5. Summary

Our knowledge about the cumulative effects of mussel grazing on plankton populations is therefore better for phytoplankton than for zooplankton. Depletion of both phytoplankton and zooplankton is known to occur in some cases when water passes through mussel farms. Measurements from water samples indicate some

minor reduction in chlorophyll in the Marlborough Sounds over recent decades, however the trend is weak and not evident at all monitoring locations. Satellite data suggest that near-surface chlorophyll concentrations have fallen around much of the country in recent decades. That may indicate that the chlorophyll reductions evident within Pelorus Sound have been driven by processes that were operating at scales larger than bay-, reach- or even Sound-scale (e.g. top-of-south scale or national-scale). Certainly, the analysis of the relationship between (far-field) chlorophyll anomalies suggests that the declines are not strongly correlated with expansion of marine farming within the bays. Insufficient data exists on which to make an assessment of change in zooplankton communities.

4. OPTIONS FOR IMPROVED EFFECTS ASSESSMENTS

4.1.1. *Phytoplankton*

Data concerning the abundance and nature of the phytoplankton community are moderately plentiful for the Marlborough Sounds—but satellite data do not extend into the inner Sounds or even small side-bays of the outer Sounds. Similarly, a majority of the *in situ* data stem from large, moderately open waterbodies (even if samples were collected near the coastline of those bodies).

There are few data from within more constricted/poorly flushed side-bays—though MDC do make CTD and fluorimeter casts within Opuia/Onapua bays on the flank of Tory Channel and Cawthron have also collected some water-samples from that region. The plankton populations within poorly flushed side-bays are likely to be more susceptible to any disturbance that might arise from local activities (whether from catchment inputs, marine farming or other factors). For example, while there are extensive data from Beatrix Bay (which is moderately intensively farmed), there are none from nearby (but more intensively farmed) Clova Bay and distal parts of Tawhitinui Reach (Tennyson Inlet, Hallam Cove). It will never be possible to find bays which are exactly equivalent in terms of depth, flushing characteristics, nutrient inputs etc., but different in terms of farming intensity, but if there is particular concern about some side arms, then monitoring in those side arms might be warranted. Note, however, that if the site already contains farms and has never been monitored before the farms were introduced, it will not be possible to use the newly-acquired simple monitoring data alone to determine whether the existing farms are having a meaningful effect.

The Marlborough aquaculture industry has made regular counts of algal cells on a regular basis since 2001 under their Marlborough Sounds Quality Programme (MSQP). On most occasions, they aim to count all toxic taxa in each sample and also the abundance of a few (two or thereabouts) of the most abundant taxa. Since they (usually) have not counted all taxa, most of the data are unsuitable for examining community structure trends. Nonetheless, examination of those data (if they were to be made available) would reveal whether or not there is evidence for trends among toxic taxa.

The next section (4.1.2) is focused upon zooplankton and lists some possible experimental studies. Conceptually similar studies could be undertaken for phytoplankton. Indeed, even if the study is primarily focused upon zooplankton, it would be wise to include measurements of the phytoplankton community to assist with interpretation of the zooplankton data.

4.1.2. Zooplankton

Given the very scarce data on zooplankton communities, it is not possible to determine a baseline from which to assess whether there has been a change in zooplankton communities over time. Nor do datasets exist on which to build a monitoring programme for the effects of mussel farming on zooplankton.

There are a number of options for field sampling of zooplankton with respect to mussel farm impacts. Both physical sampling and imaging techniques could be a useful component of these surveys depending on the details of the sampling design (and availability of technology). Remote sensing would be unlikely to form a useful component of these surveys due to the substantial need for validation, as discussed in Section 2.2.5. Some options for field sampling, and associated limitations, are briefly outlined below.

Field monitoring of ambient zooplankton populations could be re-established as part of the MDC State of the Environment water quality monitoring programme. However, to gather data on larger zooplankton, such as copepods and fish eggs, net-haul sampling would be most appropriate. This would be a substantial addition to the fieldwork effort required and is likely to involve large cost-increases to that programme (in addition to sample processing costs). SoE monitoring would not give any direct measure of the effect of mussel farming on zooplankton populations, but would provide improved data on which to base future modelling, and, if sufficient data were collected (replicated sampling at multiple sites over many years), may provide a baseline for future broad-scale change.

Field monitoring of zooplankton depletion at individual farms is likely the most feasible approach to measuring an effect of mussel farming on zooplankton populations. Sufficiently well-replicated up-stream, within-farm and down-stream measurements of zooplankton and currents could measure the within-farm depletion. Sampling with increasing distance from the farm may give an indication of the size of the depletion footprint, particularly where the focal farm is not surrounded by other stocked mussel farms. However, as discussed above, measurements at a farm scale would not incorporate consideration of mussels competing for, or otherwise affecting, zooplankton food sources. Nor would this provide a measure of cumulative effects (although it would likely provide useful data for model calibration). Any survey design and interpretation of field measurements would need to consider the ways in which the effect of mussels on zooplankton may change over time. For example, the zooplankton community changes in structure (e.g. eggs present at some times more than others, see Figure 11), mussel grazing changes dependent on growth stage of both the mussel and the zooplankton along with other factors such as current speed (James et al. 2001; Hulot et al. 2020). Measurements would therefore need to be taken under different seasonal and stocking conditions, and at multiple sites, before a

clear picture of the nature and extent of any depletion was able to be reliably estimated.

Larger-scale field manipulations would be informative in a case where mussel farms were able to be removed and re-installed within a relatively heavily-farmed area, ideally with relatively long water residence times, to allow for effects to manifest. Focal-site measurements would need to be compared with control sites, in an attempt to capture inter-annual variability. The necessary sampling effort would be substantial, and the challenges with regard to industry participation required would be considerable (essentially, forgoing the use of farm sites for at least a year on at least two occasions). This could provide data on a bay-scale, but generalisation to the whole of the Sounds would require modelling.

4.1.3. Modelling to assist with assessment of effects

Improved modelling of the effects of mussel farming on zooplankton communities is likely to be the most feasible way of estimating the cumulative effects of mussel farms on zooplankton populations—although models will always have limitations, as discussed in Section 2.3 and Appendix 1. Better field data would be one way in which the potential for modelling to provide useful predictions of zooplankton dynamics could be improved. There are a number of other ways in which an existing model of the Sounds (Broekhuizen et al. 2015) could be improved to better assess the effects of mussel farming on both zooplankton and phytoplankton, and these are summarised in Table 5.

Table 5. Options for improving modelled estimates of mussel farm effects on zooplankton (and phytoplankton) communities in the Marlborough Sounds.

Investigation	Justification	Qualitative cost	Comments
Sensitivity of simulation model results to increasing the horizontal resolution of the simulation models	Will reduce the extent to which simulated mussel crop has false (or at least overly-rapid) access to plankton beyond the immediate dropper environs	Moderate-large	Will require regriding of the model domain and reconstruction of boundary conditions, farm-maps etc. The runtime for annual-scale simulations will be prohibitive. It will not be possible to make simulations with a model that unquestionably has sufficient spatial resolution (tens of cm)
Sensitivity of simulation model results to improved representation of spatio-temporal variation of mussel crop size and density structure in simulation models	Simulations to date likely over-estimate mussel crop density in shallow areas—especially during winter	Low-Moderate	Requires help/information from mussel farmers in order to generate more realistic maps and time-series of farming intensity
Inclusion of farm-induced drag effects into simulations of plankton dynamics	Farm-induced drag may tend to isolate water within the farms from water around it (but promote greater mixing downstream). This may imply greater plankton depletion within the farm and lesser depletion in the far-field.	Moderate-High	Earlier, crude model investigations that focused upon Port Ligar and Waihinau Bay (Plew 2011) indicated that inclusion of realistic farm drag led to greater depletion inside and close to the Port Ligar farms but lesser depletion throughout much of the remainder of the bay. We know of no similar work for other parts of the Sounds, or for plankton with specific recovery rates slower than 0.1 d^{-1} (doubling time of approx. 7 d).
Sensitivity of (zoo)plankton depletion results to assumptions regarding maximum specific growth-rates of zooplankton and vulnerabilities of zooplankton to consumption by mussels	Simulations made to date have assumed that zooplankton are no less vulnerable than phytoplankton. In reality, there is evidence to suggest that larger/more motile zooplankton are less vulnerable.	Low-moderate	

Another potential modelling approach (which would also apply equally to phytoplankton and zooplankton) would be to calculate the proportion of water in the Sounds that passes through mussel farms. This would give a crude measure of the maximum impact that mussel farms could have on the overall water bodies.

The options given above would all have different strengths and limitations which should be recognised in any consideration of future work. We do not consider that a large-scale project assessing the cumulative effects of mussel farming on zooplankton communities should be a priority for marine monitoring in the Marlborough Sounds, although it could be an interesting research question. The costs involved in the assessment of cumulative effects in particular can be very high, and even with a substantial field and modelling effort, it may still not be possible to identify change, or causes of change, with a high degree of certainty (though the existing levels of uncertainty are likely to be reduced). A small study, for example collecting and analysing data from a limited area (perhaps one bay or reach) on only a few occasions would cost several tens of thousands of dollars (as per Table 3, Table 4).

Based on our interpretation of the available data and modelling results, we consider it unlikely that mussel farming has induced unacceptable change within the plankton community at reach or Sounds scales. We acknowledge that there are more data for phytoplankton than for zooplankton on which to base our assessment. To further assess the cumulative effects of mussel farming would require substantial field effort and complementary modelling, and would easily cost several hundreds of thousands of dollars. The costs and limitations of large-scale assessments should also be balanced against the likelihood that mussel farming is causing unacceptably large effects. The extent and degree of likely effects from mussel farming should also be considered in the context of the numerous other sources of environmental degradation that are present in the environment.

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6. APPENDICES

Appendix 1. Modelling assumptions which are believed to have a particular relevance to the utility of biophysical models for assessing the influences of mussel and fish farms upon plankton dynamics in the Marlborough Sounds.

Point	Modelling choice/assumption	Reason	Implication
1	Horizontal resolution of 400 m	Run-time constraints. At this resolution an individual simulation took several days to complete on NIWA's super-computer. It was not practical to make simulations at finer resolution	Individual fish-pens, individual mussel farms and individual mussel droppers are not resolved. Processes that operate on scales of cm (e.g. mussel feeding currents) or even tens of metres (nutrient and detritus release from droppers and fish-pens) cannot be accurately represented. For example, in reality mussels have access only to the food which passes within a few cm of them. Should they remove all the food from that water, they must starve until the water is replaced. In the model, mussels have access to all of the particles within the 400 x 400 x layer-depth control-volume that they inhabit. Even once their real-world equivalents have exhausted the food-supply that would be immediately available to them in the real-world, the virtual mussels will continue to perceive & ingest food. In areas with low current speeds, the grid-cell average farm-induced changes (i.e. the quantity predicted by the model) that are predicted in grid-cells that contain droppers are probably under-estimates the change that arises at immediate distances (tens of cm) of mussel droppers. Conversely, they probably over-estimate the change that occurs at radial scales in excess of tens of metres from the dropper. There is evidence that indicates grid-cell average changes predicted by these types of model are larger than the true levels of change that would be measured at those scales (Pinkerton et al. 2018a).
2	Farm structures have no influence upon patterns of flow	Simplifying assumption	In reality, the drag associated with fish-pens and mussel farms tends to cause water currents to diverge as they approach the farms. The water that flows around the outside of the farms tends to flow a bit faster than it would otherwise do and the water inside the farm tends to move a bit more slowly. Vertical and horizontal mixing in the downstream wake may be increased. These effects extend out to length-scales similar to those of the farm-structures. For mussel farms, one implication arising from the absence of farm influences upon flow is that simulated plankton depletion within the mussel farm perimeters is probably lower than it would be were farm-induced drag included. Conversely, simulated depletion beyond the farm perimeter is probably greater than would be the case if drag effects were better represented within the models.
3	All mussel farms were assumed to carry 3 km of dropper/110 m of backbone	Simplifying assumption made at a time when the primary focus of the modelling effort was assessment of the possible effects of fish-farms upon the trophic status of the system	In reality, it is probable that farms in shallower parts of the Sounds (e.g. Mahau/Kenepuru sounds, Tennyson Inlet and inner Clova Bay) probably carry less dropper than this. Consequently, the concentration of crop mussels (mussels m ⁻³ of water-column) is likely to have been over-estimated in shallower parts of the Sounds

Point	Modelling choice/assumption	Reason	Implication
4	The size (age) structure and population density of the mussel crop was assumed to be identical across all farms.	Simplifying assumption made at a time when the primary focus of the modelling effort was assessment of the possible effects of fish-farms upon the trophic status of the system	In reality, operators with access to sufficient sites tend to favour placing spat on farms in the outer Sounds. When the crop is thinned and reseeded, they move the mussels onto farms in the inner Sounds. On average, therefore, farms in the inner Sounds probably house larger mussels. Whilst these are stocked at lower densities /m of dropper than smaller/younger mussels are, they have greater per-capita filtration capacity and greater filtration capacity /m dropper. These crops of larger mussels probably generate greater filtration /m of dropper than the smaller/younger ones do.
5	The size structure and numerical density of the crop was assumed identical throughout the year	Simplifying assumption made at a time when the primary focus of the modelling effort was assessment of the possible effects of fish-farms upon the trophic status of the system	In reality, spat tend to be seeded on to droppers during the late-winter: mid-autumn period whilst harvesting takes place during the spring-early winter period. Thus, during mid-late winter, the model may be over-estimating crop-mussel filtration rates (because, in reality, the standing mussel crop is dominated by younger/smaller mussels and the bay-scale density of crop mussel biomass is probably lower than at other times of year). Conversely, it may be under-estimating filtration rates in summer.
6	The model has only one class of phytoplankton and one class of zooplankton	Simplifying assumption. Alternative models which resolve the plankton into additional classes do exist – but they would be computationally more expensive to run, more difficult to parameterise and demand additional data for parameterisation, calibration and validation.	Whilst it possible that all phytoplankton are equally vulnerable to being consumed by mussels, it is certain that some types of zooplankton are less vulnerable to being consumed than others. For example, protozoa and copepod eggs appear no less vulnerable than phytoplankton but copepod nauplii are less vulnerable than the eggs, copepods are less vulnerable than their nauplii predecessors and adult copepods are less vulnerable than their copepodite predecessors (Zeldis et al. 2004).
7	The mussel pumping rate (m^3 mussel d^{-1}) is assumed to increase with mussel size, but the relative vulnerability of different plankton size classes/taxonomic types is assumed independent of mussel size	Simplifying assumption made at a time when the primary focus of the modelling effort was assessment of the possible effects of fish-farms upon the trophic status of the system	In reality, there is some evidence to suggest that mesozooplankton only become a meaningful component of the mussel diet once the mussels exceed about 60 mm (Alfaro 2001).

Point	Modelling choice/assumption	Reason	Implication
8	The mussel pumping rate is assumed to be constant throughout each 24 hour period	Implicit assumption made when the model was constructed.	In reality, mussels are open (perhaps, feeding) continuously at night but open only for 50-90% of day-light hours (Lurman et al. 2013). The model parameterisation of feeding is based upon results from short-term, laboratory feeding studies. The lighting conditions used in the studies that have informed parameterisation of mussel feeding have varied. It is therefore unclear whether the simulated pumping rates are over- or under-estimates of those achievable in the field.
9	Filtration activities of the biofouling community are ignored	Simplifying assumption	Some members of the biofouling community are filter-feeders. The fouling community gradually accrues after each (re-seeding) event – and also exhibits seasonal cycles of abundance and activity. In overseas studies, the filtration activity of the (well-developed) fouling community has been estimated to amount to around 30-47% of that of the mussel crop (see references within Hulot et al. 2020)

Appendix 2. Summary data from two of the largest data sets from within or close to Pelorus Sound (Table 3). Figure A2.1 (NIWA1997 study) and Figure A2.2 (Fox 1997 study).

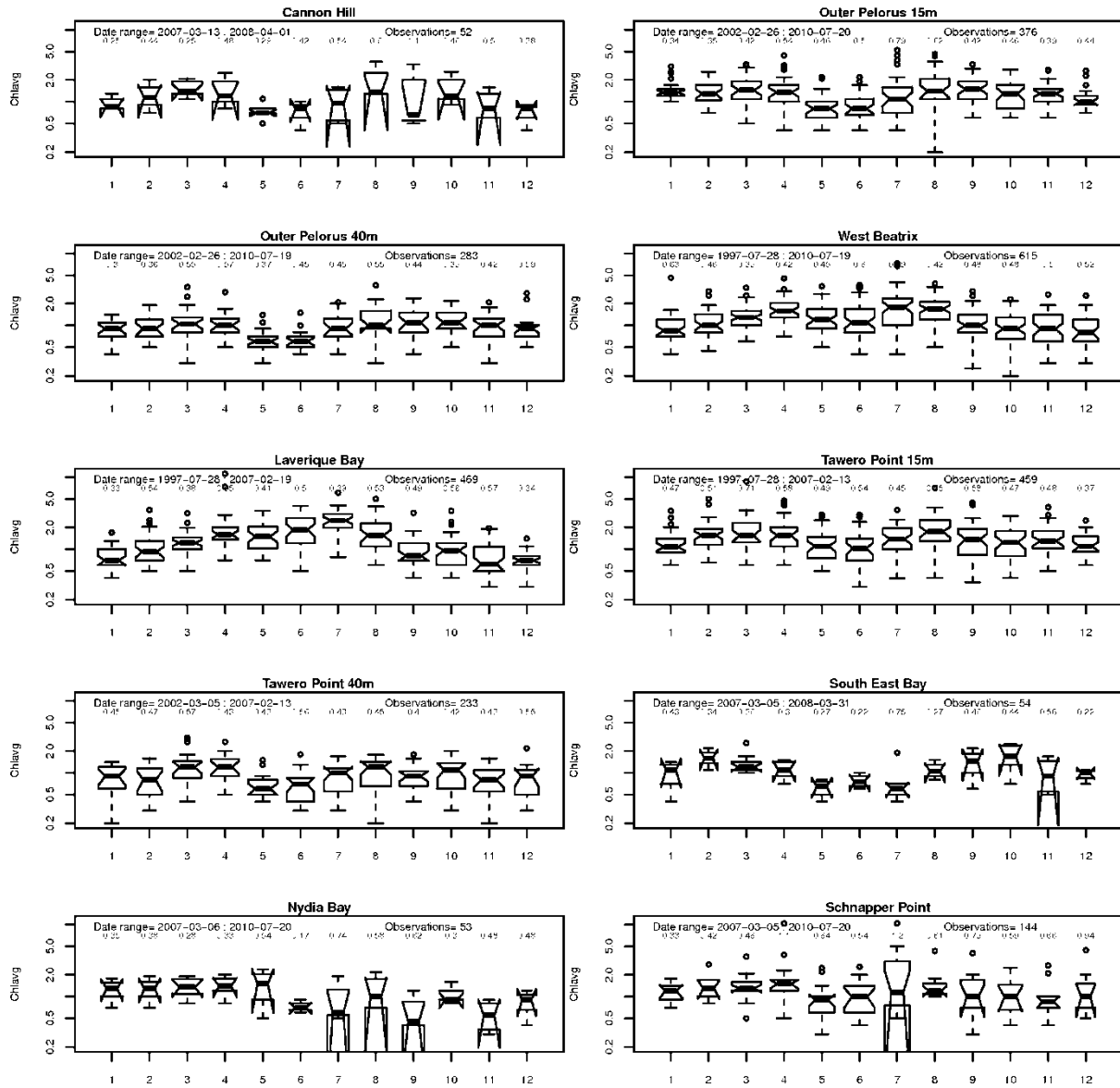


Figure A2.1 Box-plots illustrating distribution of chlorophyll concentrations measured by NIWA at various marine-farm locations within Pelorus Sound (NIWA1997 data-set). NIWA acknowledges the help offered by the Marlborough Sound Quality Program – which gathered the water samples on our behalf. Note that data from Outer Pelorus, The West Beatrix, Schnapper Point and Laverique Bay data also contribute to the time-series discussed in Section 3.1.4.

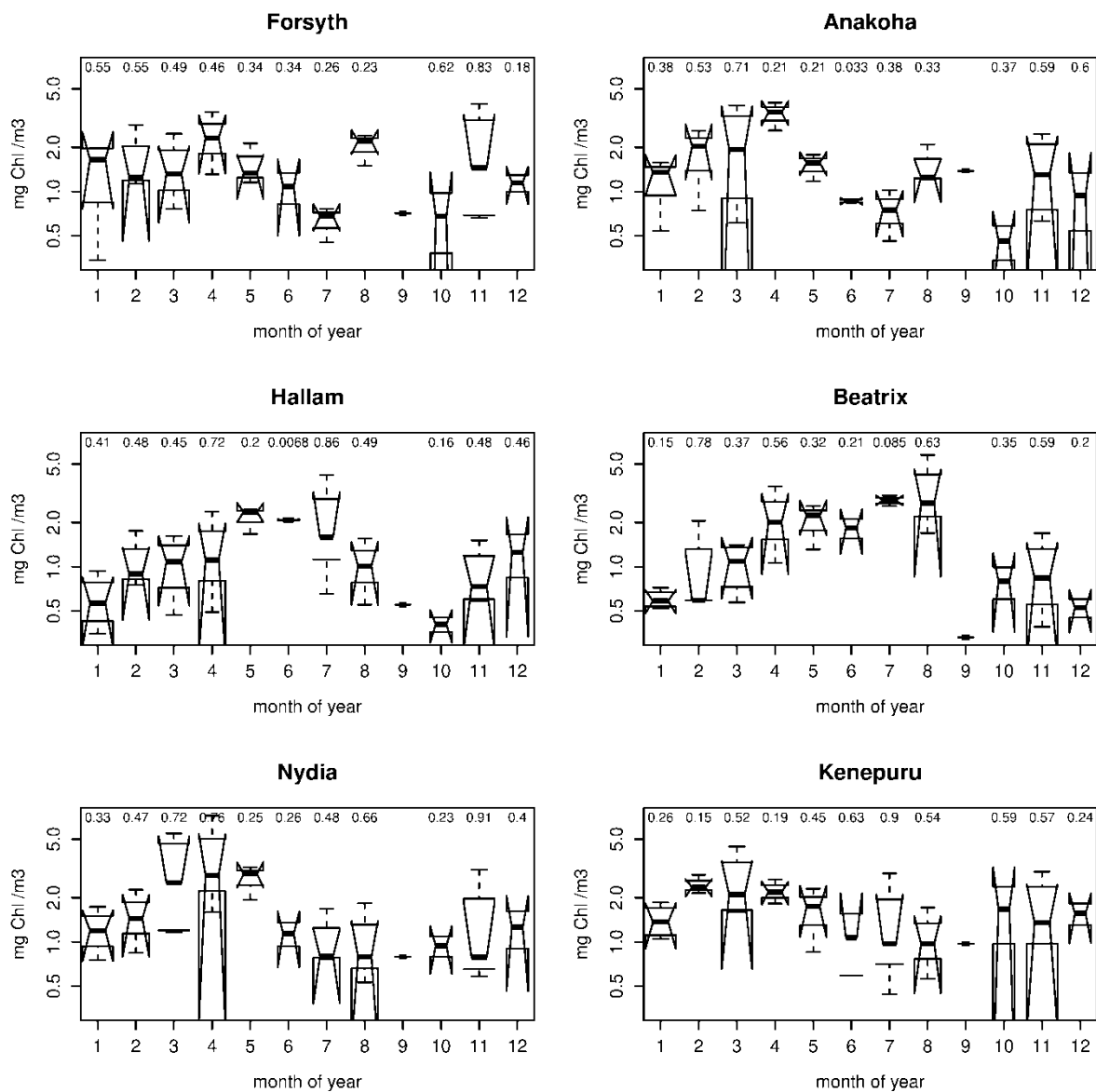


Figure A2.2 Box plots illustrating the distributions of chlorophyll concentrations measured at six locations around Pelorus Sound (and nearby bays) during 1997/98 (data from Fox (2003)). The values above each boxplot are the corresponding coefficients of variation for the data.

Note: The boxplot usually has a bowtie-like shape. Whiskers usually extend beyond the bowtie. The centre of the bowtie denotes the median. The outer limits of the notch in the bow-tie extend to $\pm 1.58 \text{ IQR}/\sqrt{n}$ of the median (where n is the sample-size and IQR is the interquartile range (the difference between the 75th and 25th quartile values)). The outer limits of the bowtie indicate the approximate locations of the first and third quartiles of the data. The whiskers extend to the most extreme data-point which is no more than 1.5 times the interquartile range from the relevant outer limit of the bowtie. If the data have a very asymmetric distribution (and/or if there are

few data-points), the notches may extend beyond the first- and third-quartiles. In such instances, the box-plot appears to have 'fangs'. Similarly, the whiskers may not extend beyond the 25th or 75th quartiles.