Huon Estuary/D'Entrecasteaux Channel nutrient enrichment assessment

Establishing the potential effects of Huon Aquaculture Company P/L nitrogen inputs

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Non-technical summary

Background

Aquaculture, like most farming practices, has the potential to impact the environment through the introduction of nutrients; nitrogen (N) in particular can have an impact as it generally limits primary productivity of coastal marine systems. N enters the environment as overfeed, faeces and urine. Approximately 85% of the N released is dissolved (urine) and immediately available to primary producers in the water column. The remainder is released as particulate material (faeces and feed), which settles onto the seabed where sedimentation processes break down the particulates, consuming oxygen in the process, and releasing various forms of N back into the water column, providing an additional nutrient source to feed primary productivity. This, along with naturally occurring forms of N, can lead to nutrient enhancement and potentially eutrophication and algal blooms.

Nutrient inputs from the salmonid aquaculture industry in the D'Entrecasteaux Channel and Huon region are regulated through a feed cap, to help ensure levels remain environmentally sustainable. In the Huon River and Port Esperance Marine Farming Development Plan (MFDP) area and the D'Entrecasteaux Channel MFDP area each salmonid company has been allocated a Total Permissible Dissolved Nitrogen Output (TPDNO) limit. This limit and the period over which it applies has been determined in accordance with the provisions of Management Controls contained in each of the MFPDs. Dissolved nitrogen outputs that can be discharged within the MFDP area must not exceed the prescribed limit during any 12-month period. In July 2015, Huon Aquaculture Company (HAC) reported to government that it had exceeded its TPDNO limit in the Huon/Port Esperance MFDP area. Further, it was also clear that they would continue to exceed this limit for some time.

As a result the present study was commissioned to 1) document the nature, timing and location of HACs N exceedance, 2) assess the extent of any adverse ecological effects using available monitoring data, and 3) evaluate the potential risk of adverse effects using modelling. The findings of the resultant report would then be used to help determine what (if any) management response might be needed to reverse any observed negative impacts in the Huon Estuary/Port Esperance MFDP area.

The primary resource available for this assessment is data collected for the Broadscale Environmental Monitoring Program (BEMP). The BEMP is a comprehensive environmental monitoring program that was designed to track broadscale changes in the system; both natural and in response to changes in salmon aquaculture inputs and other sources of nutrients. It is a legislative requirement of finfish aquaculture license holders in the D'Entrecasteaux Channel and Huon/Esperance MFDP areas. As a result, a range of nutrient, water chemistry and algal composition data are gathered monthly throughout the year and fortnightly during summer.

The nature, timing and location of nitrogen inputs

In the period running up to the reported exceedance, HAC slightly exceeded their TPDNO limit in the D'Entrecasteaux Channel MFDP area from January – December 2014, but were under their limit for the 12-month periods investigated. However, in the Huon estuary HAC exceeded their TPDNO limit for the 12-month periods beginning March 2014 – February 2015 and were still above the limit for the last 12 month period investigated (April 2015 –

March 2016). The maximum exceedance, of 292.5 t of N, occurred from December 2014 – November 2015 and was 44% above the limit. This resulted in the total limit for the Huon/Port Esperance TPDNO being exceeded by 17%. Tassal were farming below their TPDNO in both the D'Entrecasteaux Channel and Huon/Esperance MFDP areas for each 12-month period investigated. As a result when considering the combined limit across both companies and MFDP areas, the total TPDNO for the southeast was not exceeded.

To provide broader context for the exceedance it is informative to consider how the distribution and size of farm inputs have changed since the inception of the industry in the region in the 1980s. Initially established in the Huon estuary system, the industry expanded into the D'Entrecasteaux Channel from the mid-1990s to 2000s. During this time, aquaculture operations, and thus N inputs, were relatively stable and evenly dispersed throughout the D'Entrecasteaux Channel and Huon/Esperance MFDP areas. In the late 2000's, increased production saw both companies increasing their N inputs, most notably in the lower D'Entrecasteaux Channel and lower Huon estuary.

The majority of the N exceedance in 2015 primarily occurred at two leases (Flathead Bay and East of Redcliffs) between the Huon Estuary mouth and Port Esperance. These two leases contained the majority of HAC's production biomass for this region. Inputs remained comparatively low at all other HAC leases during this time.

In addition to aquaculture nutrient inputs, nutrients are introduced into the system via river runoff, wastewater treatment plants and industrial sources. The Huon River provides the greatest natural catchment nutrient inputs/ water flows in the D'Entrecasteaux Channel/Huon River catchment, although there can also be significant flows from the Esperance, Mountain and Kermandie rivers. The inputs from Snug and Northwest Bay Rivulets are negligible. Total N, ammonia and nitrite inputs from these rivers are largely dictated by flow, with the Huon River having by far the highest input. However, nitrate inputs are not as closely related to river flow and as such there can be/ has been significant inputs from the Huon, Kermandie and Mountain Rivers. Elevated nitrate levels in the latter two rivers are potentially due to inputs from wastewater treatment plants (Kermandie River) and agriculture (Mountain River).

Wastewater treatment plants are spread throughout the system and, although their inputs may be localised (i.e. at their outfall sites) they tend to be quite low in comparison to river and aquaculture inputs, and as such are unlikely to have a large influence on the system as a whole. Industrial inputs are very low.

During the HAC exceedance nutrient contributions from river flows, wastewater treatment plants and industrial sources were typical, or lower than average. This would tend to suggest that such processes/ inputs were unlikely to be driving any system wide shifts, should such shifts have been observed in the D'Entrecasteaux Channel/Huon Estuary system.

Environmental influence of nitrogen exceedance

The HAC exceedance occurred in a relatively localised region, based on this, the spatial extent of other inputs and bathymetry of the system, it made sense to divide the system into three separate regions for the assessment: 1) the central/northern D'Entrecasteaux Channel; 2) the upper Huon estuary; and 3) the southern region, incorporating the lower Huon estuary and southern D'Entrecasteaux Channel where the majority of N inputs and the exceedance occurred.

There was no clear long term trend in total N, nitrate or total phosphorus evident in the data. However, all these nutrients fluctuated seasonally due to inputs from nutrient rich subantarctic waters and rivers during the cooler months. For dissolved reactive phosphorus there was some indication that concentrations have declined throughout the time series investigated, particularly in surface waters, independent of seasonal variation. There has been a stable, or slightly decreasing, trend in surface and bottom ammonia in the northern D'Entrecasteaux Channel and upper Huon estuary regions during the time series investigated. The same trend was also observed in the southern region up until the HAC N exceedance. Since late 2014 (i.e. during the HAC exceedance) there was a notable increase in bottom water ammonia concentrations in the southern region, with an increased frequency of ammonia concentrations >2 mg/l in the summers of 2014/15 and 2015/16. As a result, a number of the BEMP sites in this region exceeded the level 1 draft performance indicators in 2015 (i.e. summer mean >25% or mean for any one site increased by >50% of baseline), and a number of sites in the lower Huon Estuary and southern D'Entrecasteaux Channel have exceeded the level 3 draft performance indicators (i.e. summer mean >50% or mean for any single site up 200%).

Dissolved oxygen concentration displayed no obvious trend in any of the three regions and notably, dissolved oxygen did not show any declining trend at those sites where bottom ammonia was elevated in the southern region. As a result none of the proposed performance indicators for dissolved oxygen were exceeded during the period which HAC were exceeding their TPDNO.

Response of algal communities to the nitrogen exceedance

Nutrient concentrations and their relative ratios can have a large influence on algal abundance. As such, the regions used for algal analysis were based on observed nutrient concentrations and ratios. As a result, the upper Huon Estuary, the lower Huon Estuary and the D'Entrecasteaux Channel were the areas applied. Analysing the data in this way suggests no significant difference in any of the nutrient ratios between the pre- (2009–2014) and post- (2015–2016) exceedance periods in any region. At all sites, the ratio of N to phosphorus ratios was below 16:1, indicating that the system is N limited. In the D'Entrecasteaux Channel, silicate levels were low, which may limit diatom growth. Nonetheless, diatoms were the most abundant phytoplankton group throughout the system, with dinoflagellates being the second most abundant group (diatoms and dinoflagellates represent 92% of the phytoplankton abundance). However, in terms of the exceedance, there was no significant difference in diatom or dinoflagellate abundance pre- (2009–2014) and post- (2015–2016) exceedance. Consistent with previous studies, higher peaks in abundance of dinoflagellates were typically observed in the Huon Estuary.

Of all of the environmental variables, total N, nitrate, the ammonia:nitrate ratio and temperature appear to be the key drivers of changes in phytoplankton communities in the D’Entrecasteaux Channel/Huon Estuary system. This suggests that seasonal changes in temperature and the influx of nitrate (and concomitant variation in total N and ammonia:nitrate ratio) associated with the influence of sub-Antarctic currents are major drivers of phytoplankton dynamics in the D’Entrecasteaux Channel/Huon Estuary system.

Modelled dispersion and ecological response to nutrient inputs

A biogeochemical model of the D'Entrecasteaux Channel/Huon Estuary system was originally developed as part of the Aquafin Cooperative Research Centre project undertaken to advise managers and industry during the initial expansion of the salmon farming industry. The model is linked to hydrodynamic and sediment models enabling it to predict the movement of nutrients throughout the system in all forms and states. This model provides an estimate of the cycling of particulate and inorganic forms of carbon, N and phosphorus, incorporating phytoplankton, zooplankton, macrophyte and detrital components and incorporates nutrient inputs from rivers, WWTP and industrial sources. As a result, this model can be used to simulate and predict the potential ecological response to changes in both natural and anthropogenic drivers. In this study, biogeochemical modelling was undertaken to assess the ecological response to the N exceedance under two scenarios. In the first scenario the actual farm inputs that occurred during the exceedance were used and in the second scenario the model inputs were identical but the HAC N inputs were adjusted such that they remained within their proscribed TPDNO limit. The difference between these two scenarios was used to identify what, if any, difference the additional nutrient inputs might have had on the D'Entrecasteaux Channel/Huon Estuary system.

The modelled ammonia dispersion resulted in a spatially and temporally variable response in ammonia concentration, particularly in surface waters and at 10 m depth near where the exceedance occurred during winter 2015. The response in bottom waters was less pronounced and more evident in the upper Huon Estuary and central/northern D'Entrecasteaux Channel in late winter and spring 2015. Furthermore, due to a net water flow south past the leases where the exceedance occurred, an increase in ammonia concentrations was predicted in Port Esperance. The model predicted that excess ammonia would be either rapidly assimilated by primary producers or nitrified, with nitrate being more persistent, particularly in bottom waters, where it would be more widespread throughout the system by northward flowing currents.

Despite elevated nitrogenous concentrations at all depths in winter during the exceedance, the modelled chlorophyll-a concentration was similar in both scenarios, probably because the reduced photoperiod was not conducive to primary productivity. However, as the photoperiod began to increase in August the model predicted increased chlorophyll-a concentrations during the exceedance, with levels peaking in September and October, and then returning to similar concentrations from November onward, when HAC N inputs were comparatively low. The model also predicted a small (~3%) decline in bottom dissolved oxygen in the immediate vicinity of the leases in the southern Huon Estuary and southwestern D'Entrecasteaux Channel during autumn and spring 2015. For the rest of the year there was no clearly discernible difference between the two model scenarios.

Conclusion

Other than an increase in bottom ammonia concentration in the southern D'Entrecasteaux Channel and lower Huon Estuary, there was little indication that the N exceedance by HAC has had a large scale impact on the system as a whole. Few changes were apparent in the BEMP results. However, this may also reflect the fact that there are no BEMP sites within the immediate vicinity of where the exceedance occurred, and since the BEMP was designed to detect system wide shifts in ecological function and not the impacts of localised point source nutrient emissions this result is perhaps not surprising. Biogeochemical modelling indicated that there could have been relatively large localised changes to a suite of water quality variables in the immediate vicinity of the exceedance. The model also predicted that there may have been other more subtle changes over larger scales; again such changes are not likely to be detected in the BEMP sampling given the inherent natural variation in the system.

By the time the present study commenced, HAC N inputs had already reduced to comparatively low levels and there was therefore little benefit in conducting additional sampling. This is partly because the TPDNO limit is based on inputs over a rolling twelve-month period, and as a result, it is possible for high nutrient inputs to occur prior to an exceedance of the TPDNO limit. As such, aquaculture companies should be encouraged to report that an exceedance is likely to occur to enable sampling during periods when nutrient inputs are greatest. In the event of a future exceedance, it is recommended that targeted sampling is undertaken as soon as possible around the offending lease(s), and that sampling continue whilst nutrient inputs are elevated. This would greatly increase the capacity to detect and monitor for any impact.

We also recommend a number of other variations to BEMP sampling that would increase the likelihood of detecting any impacts of a future N exceedance should it occur, and increase the effectiveness of the BEMP monitoring program in general. These include: greater consideration of sample collection times with respect to the tidal cycle; increasing the frequency of BEMP sampling, particularly during exceedance events; increased resolution of water column (CTD/Sonde) profiling; addition of phytoplankton performance indicators for important species (e.g. species responsible for harmful algal blooms, *Noctiluca scintillans*).

Finally, it is also important to note that despite the exceedance by HAC, the overall TDPNO limit for the system was not exceeded. This was largely because Tassal were farming well below their limit. This may also help explain why there was limited evidence of any broadscale change observed in the system. As such, this assessment should not be considered indicative of the likely system response should N inputs from farming exceed the existing overall limit.

Acknowledgements

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Abbreviations

ANOVA	Analysis of variance
BEMP	Broadscale Environmental Monitoring Program
CTD/Sonde	Oceanography instrument used to measure conductivity, temperature and depth
DO	Dissolved oxygen
DPIPWE	Department of Primary Industries, Parks, Water and Environment
FCR	Food Conversion Ratio
FRDC	Fisheries Research and Development Corporation
HAB	Harmful algal bloom (phytoplankton)
HAC	Huon Aquaculture Company
MFDP	Marine Farming Development Plan
N	Nitrogen
NOx	Nitrate + nitrite
P	Phosphorus
PCA	Principal component analysis
Si	Silicate
TPDNO	Total permissible dissolved nitrogen output
WWTP	Wastewater treatment plant

1 Introduction

Aquaculture now provides half of all of the fish consumed globally with annual production increasing by 5.8% on average from 2005 – 2014 (FAO, 2016). Salmonid aquaculture has grown rapidly in Tasmania over the last two decades and in the 2013–14 financial year comprised 95% of Tasmania's aquaculture value ($531.3 million, 40,405 tonnes) (ABARES, 2015).

Potential environmental impacts of salmonid aquaculture, on both the water column and sediments, are well documented (Read and Fernandes, 2003; Soto and Norambuena, 2004; Buschmann, 2006; Cubitt *et al.*, 2008). In the water column impacts stem predominantly from the introduction of nitrogen (N) and phosphorus (P) to the system, with only ~30% of the nutrients added through fish feed being removed as fish when harvested (Volkman *et al.*, 2009). N has the potential to limit the primary productivity of coastal marine systems (Howarth and Marino, 2006) and consequently the availability of N has the potential to influence ecosystem dynamics.

N from aquaculture enters the marine environment as overfeed, faeces and urine (Knoph and Thorud, 1996). Estimates of total N inputs range from 20 – 43 kg per tonne of Atlantic salmon produced (reviewed by Cubitt et al. (2008)). Around 80% of the nutrients released into the environment are dissolved and immediately available to primary producers (e.g. phytoplankton, macro-algae and plants) (Volkman *et al.*, 2009). The major nutrient released is ammonia in urine (Brett and Zala, 1975; Bergheim *et al.*, 1991), which is both readily available to phytoplankton and preferentially utilised because less energy is required to transport it through the cell membrane than other naturally abundant forms of N such as nitrate (McCarthy, 1981; Raven *et al.*, 1992)). As such, these ammonia inputs can affect phytoplankton abundance, including toxin-forming species that are associated with harmful algal blooms (HAB) and can lead to eutrophication (see Box 1 for a more detailed description of phytoplankton dynamics and the role of nutrients and other environmental drivers).

The particulate organic N entering the environment as feed and faeces will also contribute to the dissolved pool of N in the water column. If not directly consumed by animals (e.g. scavengers or filter feeding invertebrates) the organic matter is broken down by sedimentation processes, and dissolved N in the form of ammonia and nitrate, is released back into the water column (see Box 2 for a more detailed description of the relationship between finfish aquaculture and the N cycle).

To minimise negative impacts on the environment, and to prevent unrestricted production of the salmonid aquaculture industry in SE Tasmania, N inputs are regulated by the Department of Primary Industries, Parks, Wildlife and Environment (DPIPWE) with each salmonid aquaculture company being allocated a total permissible dissolved N output (TPDNO) in any 12 month period (i.e. a 12 month rolling limit). The TPDNO effectively caps the total amount of feed aquaculture companies can put into the system, thereby limiting the quantity of fish that can be produced. The TPDNO limits were set at a level designed to prevent shifts in the trophic state of the system, and are based on the combined findings of two independent research projects; the Huon Estuary Study (Butler *et al.*, 2000) and an Aquafin Co-operative Research Centre study (Butler *et al.*, 2000; Volkman *et al.*, 2009) but most notably, the outputs of the biogeochemical model developed for the system as part of the Aquafin Cooperative Research Centre study (Wild-Allen, 2008). The TPDNO limits were introduced in 2009, with independent control levels being set for the D'Entrecasteaux Channel Marine Farming Development Plan (MFDP) Area (1, 140.67 t) and the Huon/Port Esperance MFDP Area (1,084.63 t) (DPIPWE, 2011). Each salmonid aquaculture company is issued a TPDNO limit for each MFDP area and is legally obliged to remain within this limit during any 12-month period.

The review of monitoring data for the Huon Estuary and D'Entrecasteaux Channel between 2009 – 2013 found N inputs to be within the TPDNO limits, with inputs in the Huon Estuary/Port Esperance MFDP Area of ~1000 tonnes and inputs in the D'Entrecasteaux Channel MFDP area ranging from 709 – 849 tonnes (Ross and Macleod, 2013). However, in July 2015 Huon Aquaculture Company (HAC) reported to DPIPWE that its N inputs for the Huon/Esperance MFDP area were greater than planned and that they had exceeded their TPDNO limit for the 12-month period from August 2014 to July 2015. Furthermore, due to feed inputs being elevated over several months it was clear that they would continue to exceed their TPDNO limit in the Huon/Esperance MFDP area for some time to come.

As a result of the above, this project was commissioned to 1) characterise the nature (excretion versus waste feed/faeces), timing and location of HACs nutrient inputs using information sourced from company records, 2) assess the extent of any adverse ecological effects using available monitoring data, and 3) evaluate the potential risk of adverse effects using modelling. The findings of this report will then be used to consider what (if any) management response might be needed to reverse any observed negative impacts to the Huon Estuary/Port Esperance MFDP area.

The primary resource available for this assessment is data collected for the Broadscale Environmental Monitoring Program (BEMP). The BEMP is a comprehensive environmental monitoring program undertaken by finfish aquaculture license holders in the D'Entrecasteaux Channel and Huon River/Port Esperance MFDP areas that was designed based on recommendations by Volkman *et al.* (2009) and Thompson *et al.*(2008). The BEMP was initiated in 2009 in accordance with the *Marine Farming Planning Act 1995* and is a regulatory requirement described in Schedule 3 BEMP of marine farming licences.

The first analysis of BEMP data was reported in 2013 (Ross and Macleod, 2013) and indicated that most measures were performing within acceptable bounds and that there had was no evidence of large-scale changes in the measured parameters over the study period (2009 – 2012). However, comparisons with previous studies (Thompson *et al.*, 2008; Volkman *et al.*, 2009) suggested that there had been some conspicuous changes in certain water quality measures (increases in ammonia and a decrease in dissolved oxygen in the Huon River estuary), indicating that whilst the system appeared to be coping with the nutrient and organic inputs it was under pressure.

Box 1: Phytoplankton dynamics - the role of nutrients and other environmental drivers

The release of nutrients in bio-available forms due to aquaculture can increase primary productivity and potentially lead to eutrophication and algal blooms. As eutrophication increases, the bacteria in sediments increase in anaerobic metabolism and this sets up a series of positive feedback mechanisms that increase the release of both N and P to the water column thereby maintaining eutrophication (reviewed by Cloern (2001)). While photosynthesis does produce oxygen, several negative effects can result from large algal blooms: 1) many algal species (dinoflagellates in particular) produce toxins that can be harmful to farmed fish and wild fish; 2) filter feeding shellfish (e.g. mussels and oysters) can accumulate these toxins and become toxic for human consumption; 3) the boom and bust nature of phytoplankton means that large blooms inevitably die off and up to half of the carbon, N, and P contained within the phytoplankton needs to be processed by benthic aerobic and anaerobic mineralisation (Jørgensen, 2013).

While nutrients play a significant role in phytoplankton dynamics a variety of physical forces and interactions between various components (e.g. nutrient ratios) play an important role. The complexity of the interaction among these forces is presented in Figure 1 and takes into account: (1) relative preference for chemically reduced vs chemically oxidized forms of N; (2) relative availability of inorganic N and phosphorus; (3) adaptation to high vs low light or the tendency to be purely autotrophic vs mixotrophic; (4) cell motility; (5) environmental turbulence; (6) relative pigmentation quality, from higher relative proportion of carotenoids (brown) to higher relative proportion of phycobiliproteins and chlorophylls (blue-green or green); (7) temperature; (8) cell size; (9) relative growth rate; (10) relative production of bioactive compounds such as toxins or reactive oxygen species; (11) ecological strategy, varying from short lived, fast growing r strategists (e.g. diatoms) to slow growing, long lived K strategists (e.g. dinoflagellates); and (12) fate of the production in terms of grazing.

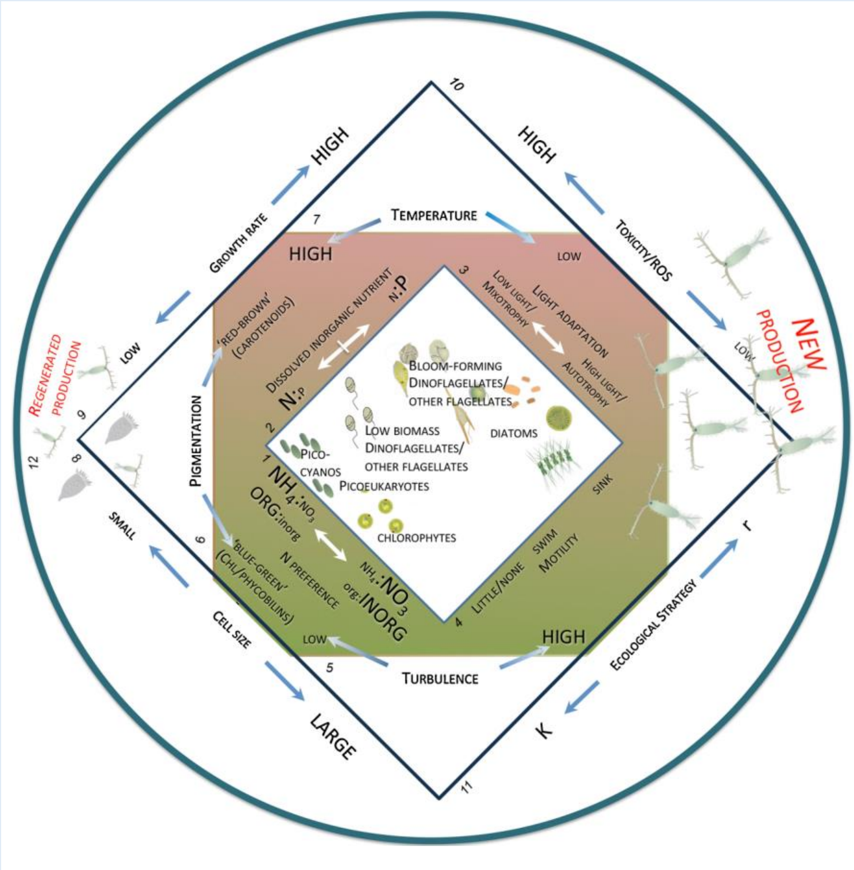

Figure 1: Schematic summarising factors that dictate phytoplankton abundance (adapted from Glibert (2016)). Transition of phytoplankton functional types is depicted along 12 axes.

Box 2: The relationship between finfish aquaculture and N cycling

N from aquaculture enters the marine environment as overfeed, faeces and urine, the last of which is predominantly across the gill membrane (Knoph and Thorud, 1996). Estimates of N inputs range from 20 – 43 kg per tonne of Atlantic salmon produced (reviewed by Cubitt et al. (2008)). Around 80% of the nutrients released into the environment are dissolved and immediately available to primary producers. Particulate organic matter (i.e. faeces, and feed), if not directly consumed by animals (e.g. scavenging vertebrates or filter feeding invertebrates), is broken down by sedimentation processes (see Figure 2). Firstly, in oxygenated sediments (oxic), organic matter is mineralised by aerobic bacteria and some N is released into the water column as ammonia or, following nitrification, nitrate (Ross *et al.*, 2015). Both of these forms are then available for primary production. In the absence of oxygen (anoxic) nitrates are converted to gaseous N via denitrification and released from the system as it is not suitable for primary production. Nitrate can also be converted to ammonia via dissimilatory nitrate reduction, which occurs under anoxic conditions and acts to recycle bio-available N rather than removing it from the system.

The above processes, which occur under both oxic and anoxic conditions, strongly dictate the availability of various forms of N and also influence the availability of oxygen for other marine organisms. Large nutrient loads can result in a drawdown of sediment oxygen concentration, which can lead to inefficient N processing and an inability to remove nitrate from the system via denitrification. The release of various nitrogenous compounds into the system in bio-available forms, along with the ammonia directly released from fish, can increase primary productivity leading to eutrophication and algal blooms (detailed in box 1).

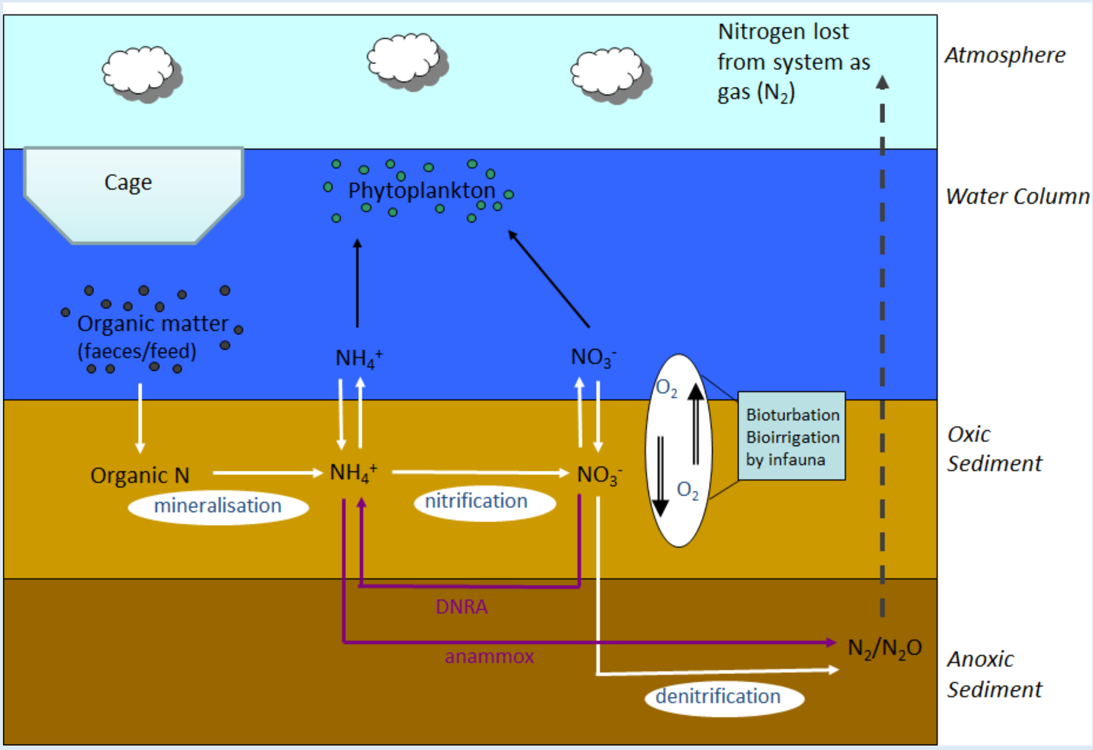

Figure 2: Schematic representation of N cycling and oxygen consumption during marine farming operations Source: Ross *et al.* (2015).

2 Objectives

1. Establish the nature, timing and location of HACs reported dissolved N inputs in the context of historical patterns.

2. Assess the extent to which the HAC N inputs in the Huon River and Port Esperance Marine Farming Development Plan (MFDP) area has influenced local and broadscale environmental conditions – using information from the BEMP, farm inputs (objective 1. above) and other external nutrient sources, and any targeted regional sampling.

3. Using the data obtained from the farm evaluation together with other forcings, model the dispersion and ecological response of the system to nutrient inputs (i.e. where does it go and what does it do).

3 Methods

3.1 Aquaculture inputs

A variety of marine aquaculture operations exist in the D'Entrecasteaux Channel/Huon Estuary system including molluscs (oysters and mussels) and salmonids. Molluscan aquaculture serves to remove nutrients from the system as these species filter plankton that has extracted nutrients from the water column. However, finfish aquaculture does contribute nutrients to the system through the addition of feed and fish excretion. As such, spatial and temporal trends in aquaculture inputs (i.e. N via fish feed) were investigated, along with trends in stocking density during the HAC exceedance.

Each salmonid aquaculture company is legislatively required to provide DPIPWE with a monthly breakdown of the quantity of feed input into each lease. Historically, N inputs were calculated based on an agreed N content of the feed (7.2%), however the exact N content of the feed is now known and this has been used to calculate N inputs in recent years to ensure a more accurate assessment. Other factors used to convert feed quantities into N input are an agreed food conversion ratio (FCR) for the Tasmanian industry of 1.35 (i.e. 1.35 kg of dry feed produces 1 kg of fish), a digestibility coefficient of 90%, and a final estimate of the N content of the fish produced (3%) (Wild-Allen *et al.*, 2005). When all these measures are combined this suggests that ~5% of the feed enters the environment as N (with 15% of this being particulate and the remaining 85% being dissolved). Environmental P inputs from feed was estimated as comprising 0.4% dissolved inorganic P and 0.4% particulate P of feed mass, based on results from Wild-Allen *et al.* (2005).

To provide additional insight into the nature and location of the HAC exceedance, HAC provided a monthly breakdown of fish biomass for the present study. The interaction between fish biomass and feed inputs can provide insight into the form of N inputs (i.e. dissolved or particulate), which has implications for where any negative impacts are likely to occur (i.e. water column VS benthic).

As the number of fish, their weight, and hence biomass, is generally only estimated when individual cages are bathed to treat fish for amoebic gill disease, when they are moved for some other purpose, or when harvesting, there were periods of time for which there was no fish biomass data available. To account for this, in months where fish biomass was not available, or where the same values were duplicated for multiple months, linear growth was assumed during the period of time from the minimum starting biomass through until the maximum end biomass. Further, when fish are moved, it results in multiple biomass records in a given month (i.e. a biomass record for each location). To account for this, the biomass was divided evenly between the locations (i.e. if the same cohort was reported in two leases in a given month, half of its biomass was allocated to each lease). In addition, at harvest the fish cages are moved to Hideaway Bay and harvesting typically occurs over several days, weeks, or sometimes months, often resulting in many biomass entries over this period with the biomass decreasing through time. In this case, we assumed that in the last month half of the initial biomass was present, which is effectively the same as if the entire cage was harvested in the middle of the month. When partial harvesting occurred in other months, biomass was estimated as the mean of the maximum initial biomass and minimum end biomass.

3.2 River inputs

In addition to nutrient inputs from aquaculture, nutrients reach the D'Entrecasteaux Channel/Huon Estuary system from a variety of natural and anthropogenic sources (river inputs, wastewater treatment plants (WWTP), industrial inputs). The following details how these various sources were incorporated into the analyses in order to place the contribution of aquaculture in context, and to provide input data for the biogeochemical modelling.

River flow data for the Huon River (station 635), Esperance River (station 7200), Northwest Bay Rivulet (station 5201) and Snug Rivulet (station 5202) were sourced from the Water Information System Tasmania (WIST) database (http://wrt.tas.gov.au/wist/ui). Following Bobbi (1998), and Ross and Macleod (2013), river flow was estimated for the Kermandie River using a scaled relationship with Riley’s Creek (Kermandie River flow = 7.1 X Riley’s Creek flow) where the flow of Riley’s Creek was estimated using a linear relationship with the Huon River (Riley flow = 0.004 X Huon flow - 2.810) during 1992–1999 when these two stations were monitored concurrently.

Nutrient concentrations for the Huon River, Esperance River and Snug Rivulet are typically measured once monthly. There was no recent nutrient concentration data for the Mountain River, Northwest Bay Rivulet, or Kermandie River. As such, median nutrient concentrations presented in Ross and Macleod (2013) were used.

In the absence of a relationship between river flow and nutrient concentration for all nutrients, other than total N, nutrient loads were calculated following Ross and Macleod (2013), whereby nutrient concentration (mg/l) is multiplied by river flow (l/day). For total N, where a positive relationship exists between flow and nutrient concentration during high flows, loads were calculated as per other nutrients during low flow, but during high flows (>7500 ML/day for Huon River; >150 ML/day for Esperance River) loads were calculated using the power relationships developed by Ross and Macleod (2013). A relationship could not be established for the other rivers; thus TN was calculated using the median concentration as per the other nutrients.

3.3 Wastewater treatment plants

The data required to estimate nutrient loads from WWTP was provided by TasWater. Nutrient loads were calculated by multiplying mean monthly flow rate by nutrient concentration, which is measured once per month. When nutrient concentration data were unavailable, the last available value was carried forward in time until new data were available, although this was rarely necessary.

An important component of WWTP inputs for the biogeochemical modelling is NOx (nitrite + nitrate) concentration, which was not measured. In previous model runs (Wild-Allen *et al.*, 2005; Wild-Allen *et al.*, 2010; Wild-Allen and Andrewartha, 2016), WWTP NOx concentration was ~0.1 mg/l; as such, 0.1 mg/l, along with actual measured quantity of ammonia, was subtracted from total N, and the remainder was assumed to be detrital labile N. For total P, 83% was assumed to be dissolved inorganic P and the rest detrital labile P, as has been assumed previously (Wild-Allen *et al.*, 2005; Wild-Allen *et al.*, 2010; Wild-Allen and Andrewartha, 2016).

3.4 Industrial inputs

There are three fish processing plants that discharge into the D'Entrecasteaux Channel/Huon Estuary system and therefore contribute to its nutritional state: Tassal plants at Dover and Margate, and the Tas Seafoods plant at Margate. Tassal provided an annual estimate of the total N, total P and ammonia concentration of their discharge. No data were available for Tas Seafoods outputs, however in previous analyses (e.g. Ross and Macleod (2013)) the N outputs from this plant were exceedingly low (<1% of that from the Tassal plant at Margate) and therefore were deemed to be effectively irrelevant in terms of nutrient inputs within the D'Entrecasteaux Channel/Huon estuary system.

3.5 BEMP analyses

The BEMP program was designed based on the recommendations of the Aquafin CRC project (Thompson *et al.*, 2008; Volkman *et al.*, 2009), which investigated the cumulative impacts of finfish aquaculture on the D'Entrecasteaux Channel/Huon Estuary system. Sites were selected to cover both MFDP areas spatially and to include areas that are most likely to be impacted by marine farming operations, but the emphasis was on maximising the probability that the design would detect a system wide shift should it occur (see Figure 3 for spatial representation of BEMP sites and proximity to aquaculture leases).

BEMP surveys are undertaken monthly for most of the year and fortnightly during summer when there is an elevated risk of algal blooms. Nutrients measured during BEMP surveys include both surface and bottom concentrations of total N, ammonia (comprising both NH_3/NH_4), nitrate (NO_3), nitrite (NO_2), nitrate + nitrite (NOx), total P, dissolved reactive P (principally PO_4) and silicate (SiO_4). However, both NOx and nitrite have not been measured throughout the entire BEMP monitoring period, thus, only results pertaining to nitrate are presented in this study (noting that nitrate will generally represent the majority of NOx). BEMP nutrient samples are analysed by Analytical Services Tasmania and reported in terms of mg of the main analyte in question (e.g. mg of N as NH_3).

Physiochemical measurements (salinity, temperature and oxygen) are taken at the surface, 5 m depth (termed middle hereafter) and one meter above the bottom (hereafter termed 'bottom'). In addition, an integrated water sample is taken from the surface to 10 m depth and this samples is used to measure chlorophyll-a concentration and to obtain algal counts (to species level where possible).

The D'Entrecasteaux Channel/Huon Estuary system has three distinct regions: the relatively oceanic, deep, southern basin; the estuarine upper Huon Estuary; and the narrow, relatively shallow central and northern D'Entrecasteaux Channel region. To investigate how the HAC N exceedance affected the system at a regional scale, the BEMP sites were grouped into the central and northern D'Entrecasteaux Channel (BEMP sites 1, 2, 3, 4, 5), upper Huon Estuary (11, 13, 14) and southern region (BEMP sites 6, 7, 8, 9, 10, 12) (see Figure 3). Site 15, the reference site in Recherche Bay, was treated individually.

Temporal trends in mean nutrient, dissolved oxygen and chlorophyll-a concentrations were investigated at the abovementioned regional level. When trends were identified in regional level analyses, further analyses were undertaken at the site level and, where appropriate, generalised additive models (GAMs) were fitted to the time series to characterise these

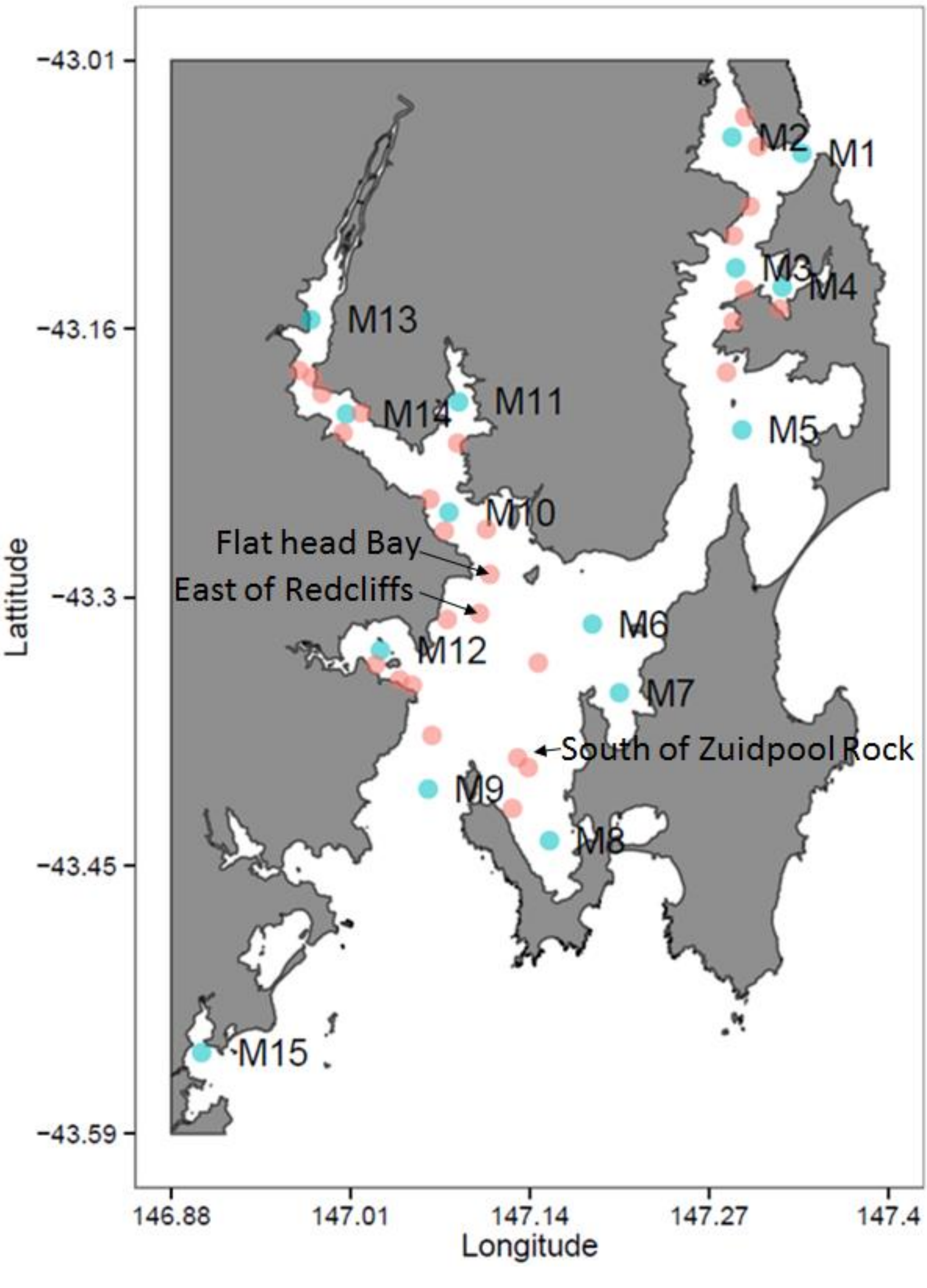

Figure 3: Map of salmonid aquaculture leases (red) and BEMP monitoring sites (blue). Site numbers shown alongside with 'M' referring to monitoring site. Note that farm locations are correct as of when this study was commissioned (January 2016).

trends. This was done using the default parameters of the 'mgcv' package in R software (version 3.2.2; http://www.r-project.org).

As detailed in Box 1, phytoplankton abundance is dictated by complex interactions between nutrient concentrations, their ratios, and a variety of physio-chemical parameters. For example, the Redfield ratio (Redfield, 1934) is a widely accepted as representing the ratio of C:N:P (106:16:1) found in marine phytoplankton and changes in this ratio can be used to make inferences about factors influencing phytoplankton communities. To analyse the relationships between phytoplankton abundance at each BEMP site and the environmental factors most likely to influence phytoplankton dynamics (i.e. nutrient concentrations and ratios), principal component analysis (PCA) was conducted using the 'prcomp' function of the Vegan R package. PCA results were summarised by applying the 'ggbiplot' function (https://github.com/vqv/ggbiplot) to PCA outputs, which incorporate 68% probability distribution to the ellipses of the output.

In order to establish whether environmental factors had a significant effect on phytoplankton abundance pre- (2009–2014) and post- (2015–2016) HAC N exceedance (dependent variables), a generalised linear model (GLM) was fit to the PCA output for the first two principal components both independently and in combination (PC1, PC2 and PC1+PC2). Akaike Information Criteria was used to determine which principal components created the best model.

The monthly response of phytoplankton abundance against environmental factors was subjected to an analysis of variance (ANOVA). Normality and homogeneity of variances were assessed by Kolmogorov–Smirnov and Levene's tests. Post hoc Tukey's tests were used to investigate differences in the interaction terms (i.e. among months and years) where relevant. The critical significance level of 95% ($\alpha = 0.05$) was applied and all statistical tests were undertaken using R.

To assess the temporal and spatial changes in the dominance of diatoms or dinoflagellates in the D'Entrecasteaux Channel/Huon Estuary system, a G Index (Clément and Guzmán, 1989) was calculated based on species richness for pre (2009–2014)- and post (2015) N exceedance (Figure 40). G index values range from -1 (dominance of dinoflagellates) to +1 (dominance of diatoms).

An important element in the design of the BEMP monitoring program was the need to provide data that could be compared with the baselines or trigger values proposed by Thompson *et al.* (2008) as being relevant for the identification of change in ecological function. These recommended baselines and triggers (Thompson *et al.* (2008); Volkman *et al.* (2009); Table 1) were based on a combination of current knowledge, as well as evaluation of both data collected and the outputs of the high resolution biogeochemical modelling developed as part of the Aquafin CRC studies (Volkman *et al.*, 2009). The trigger values were designed to incorporate three levels of management response consistent with the relative risk to ecological function associated with increasing temporal or spatial changes in ecological conditions relative to the baseline, regardless of cause (natural/anthropogenic). In accordance with this objective, the performance of ammonia, dissolved oxygen and chlorophyll-a was assessed relative to the defined baselines and trigger limits for the entire BEMP time series, including the HAC exceedance.

Table 1: Baselines and recommended trigger levels proposed by Volkman *et al.* (2009). Source: Ross and Macleod (2013). Note: ammonia baseline and triggers were adjusted to account for the inter-laboratory variation in ammonia measurement (see below).

Parameter	Standard or Baseline value[1]		Level 1	Level 2	Level 3
	Huon Estuary	D'Entrecasteaux Channel	(low risk)	(moderate risk)	(high risk)
Sediment biota (infauna)	To be determined TBD	To be determined TBD	Significant change over time since start of assessments at one or more sites + other indicators TBD	Significant change in multivariate community structure at 1 site since last assessment + other indicators TBD	Significant change in multivariate community structure at ⩾ 2 or more locations since last assessment + other indicators TBD
Seagrass and other macrophytes	TBD	TBD	Significant change over time or relative to control site.	As level 1 + TBD	As level 1 +TBD
Sediment chemistry	ANZECC guidelines for metals and TBD	ANZECC guidelines for metals and TBD	Significant change over time at one site.TBD	Significant change at 2 sites in ⩾ 2 indicators. Exceeds ANZECC guidelines for low metal concentrations. TBD	Significant change at ⩾ 3 sites in ⩾ 2 indicators. Exceeds ANZECC guidelines for high metal concentrations. TBD
Nutrients	Summer NH_4^+ surface = 0.32 µM. Bottom = 0.42 µM	Summer NH_4^+ surface = 0.12 µM. Bottom = 0.27 µM	summer mean up 25%, or 3 successive annual means > baseline, or mean for any one site +50%	summer mean up 50%, or 8/10 annual means > baseline for any site, or mean for any single site up 200%	Summer mean +100%, or summer mean > 1 µM (~ ANZECC)
chlorophyll *a*	sites 10 to 14 annual = 1.4 µg/L.Summer = 1.7 µg/L	Sites 1 to 9 summer mean = 0.66 µg/L. Annual mean = 0.80 µg/L	Any site[5]: annual mean +100%; or average summer mean +50%	Any site[5]: annual mean +200%; or average summer mean +100%; or average annual mean +50%	Any site[5]: annual mean +400%; or average summer mean +200%; or average annual mean +100%
Phytoplankton blooms	7% obs. > 3x median chla	3.6% obs. > 3x median chla	% obs. > 3x median rise 50%	% obs. > 3x median rise 100%	% obs. > 3x median rise 200%
Harmful Phytoplankton *G. catenatum*[4]	TSQAP data from 7 areas 1997-2007	TSQAP data from 7 areas 1997-2007	# of days 7 areas are closed to shellfish harvest due to HAB >226 days	# of days 7 areas are closed to shellfish harvest due to HAB >336 days	Not defined
Absolute DO[3]	Channel mean > 6 ppm. Bay mean > 5 ppm	Channel mean > 6 ppm. Bay mean > 5 ppm	Any 2 channel observations ⩽ 6 ppm. Any 2 bay observations ⩽ 5 ppm	50% of channel observations ⩽ 6 ppm. 50% of bay observations ⩽ 5 ppm. Any 2 observations < 2 ppm	Channel mean ⩽ 6 ppm. Bay mean ⩽ 5 ppm. Any 2 measurements < 1 ppm
Relative DO (percent saturation)	Set at 20th percentile from 1st year of observations	Set at 20th percentile from 1st year of observations	[2]Number of observations below baseline increases 50%	[2]Number of observations below baseline increases 100%	Mean falls 10% from baseline (~ ANZECC)

3.5.1. Changes in detection limits and laboratories

During the BEMP monitoring period the minimum detectable limit of some analytes changed. For the most part this doesn't affect either the analysis or interpretation, however, when the results are regularly below the detection limit (which is often the case for ammonia) it is necessary to assign an arbitrary low level value to the full dataset so that the concentration is not classified as zero, as i) below detection level does not necessarily mean not present and ii) too many zeros in the dataset can create problems in subsequent analyses. In previous studies using the BEMP data (e.g. Ross and Macleod (2013)), this discrepancy was not an issue, and a value of half of the detectable limit was used (i.e. when <0.002 was reported for ammonia a value of 0.001 was assigned). However, now the detection limit for ammonia has changed (new detection limit is <0.005), using this method to assign concentrations will result in two separate values (i.e. 0.001 and 0.0025) and will therefore influence temporal trends. Further, the new detection limit eliminates any results between 0.002 and 0.005 in more recent data. The only logical approach available was to treat all values of 0.0025 (i.e. undetectable in recent data), and any values <0.0025 from early data, as 0.0025 throughout the entire time series.

The initial analysis of dissolved nutrients for the BEMP was undertaken by CSIRO, and the draft baselines and performance indicators were based on CSIRO measurements. Since June 2012 (BEMP survey 52) AST has been contracted to analyse the dissolved nutrients. It is known from an interlaboratory comparisons (Eriksen, 2009) that there is some discrepancy in the measurement of ammonia concentration between the laboratories. Fortunately, AST were contracted to measure total N and total P for the entire BEMP monitoring period and, when measuring total N, also measured ammonia concentration and provisioned this data for the current study. As such, ammonia data were available from both AST and CSIRO for the first 51 BEMP surveys (June 2010 – May 2012). Using these data, a linear model was developed to convert the baseline ammonia estimates proposed by Volkman *et al.* (2009) (i.e. based on CSIRO measured ammonia) to values that align with AST measured ammonia (see Appendix i).

3.6 Biogeochemical modelling

The biogeochemical model of the D'Entrecasteaux Channel/Huon systems was developed within the Aquafin CRC project (Volkman *et al.*, 2009). Detailed methodology for the model can be found in Volkman *et al.* (2009), with further information on more recent application of the model in local systems in Wild-Allen *et al.* (2010), Wild-Allen *et al.* (2013) and Wild-Allen and Andrewartha (2016).

The biogeochemical model has been developed as a package to work alongside the relevant hydrodynamic and sediment models, which enable it to predict the movement of nutrients throughout the system. It cycles particulate and inorganic forms of carbon, N and P, incorporating phytoplankton, zooplankton, macrophyte and detrital components. It also incorporates nutrient inputs from rivers, WWTP and industrial sources (detailed above). As a result, it can provide an understanding of how anthropogenic inputs, such as N from aquaculture, might affect key components of the ecosystem, such as algal communities and oxygen, which can in turn impact on aquaculture and the broader environment.

Two modelling scenarios were undertaken as part of the present study, both for the time period of October 2014 – March 2016 (i.e. the time period encompassing the HAC N exceedance). In both scenarios the non-farm nutrient loads were as detailed above. The differences between the scenarios related to the farm inputs: scenario 1 used the actual N inputs for the two aquaculture companies whilst in scenario 2 the Tassal inputs and HAC inputs for the D'Entrecasteaux Channel MFDP area remained as reported, but the HAC inputs for the Huon/Esperance MFDP were adjusted so that they did not exceed their TPDNO limit. This was achieved by re-proportioning the monthly N contribution of each lease so that the 12-month period from December 2014 until November 2015 (i.e. the time period of the largest N inputs) equalled the TPDNO limit. Proportion contribution was based on the relative monthly contribution of each lease from 2012 – 2014 (i.e. when operations were assumed to be normal).

Several methods were trialled to adjust HAC N inputs (see Appendix ii) but the above method provided the most realistic scenario. Other methods resulted in either unrealistically low N inputs during the time period, unrealistic temporal trends, or exceedance of the Huon/Esperance TPDNO during some twelve month periods.

Modelling results were displayed as the difference between the two scenarios (i.e. the N inputs during the exceedance minus the adjusted N inputs) to highlight how the HAC N exceedance could have affected ammonia, nitrate, dissolved oxygen and chlorophyll-a concentrations throughout the D'Entrecasteaux Channel/Huon Estuary system. Modelling results were reported for the surface, 10 m and bottom depths.

4 Results & discussion

4.1 The nature, timing and location of N inputs

4.1.1. Aquaculture inputs

HAC exceeded their TPDNO limit in the D'Entrecasteaux Channel from January – December 2014 (Figure 4), were close to the limit from February 2014 – January 2015 and below the limit for the remaining 12-month periods investigated. In the Huon/Esperance MFDP area, HAC exceeded their TPDNO limit for the 12-month periods beginning March 2014 – February 2015 and were still exceeding in the last 12 month period investigated, which was April 2015 – March 2016 (Figure 5). The maximum exceedance, of 292.5 t of N, occurred from December 2014 – November 2015 and was 44% above the limit. This resulted in the total Huon/Esperance TPDNO being exceeded by 17%; despite Tassal operating at around 75% of their permissible TPDNO. Tassal remained below their TPDNO limits in both the D'Entrecasteaux Channel and Huon/Esperance MFDP areas for all of the 12-month periods investigated (January 2014 – December 2014 to April 2015 – March 2016) (Figures 4 and 5). It is important to note that despite the HAC inputs being above permissible limits the total TPDNO for the south-east (i.e. combined D'Entrecasteaux Channel and Huon/Esperance MFDP areas) was not exceeded (Figure 6), because the Tassal inputs were below their limits in both MFDP areas.

To provide some context for the exceedance, it is important to consider how the inputs from farming have changed in both space and time since farming first began in the Huon Estuary in the 1980's. By the mid-2000's, aquaculture operations, and thus N inputs, were relatively consistent and evenly dispersed throughout the D'Entrecasteaux Channel and Huon/Esperance MFDP areas (~50–60% Huon Esperance and ~40–50% D'Entrecasteaux Channel from 2006 to present) (Figure 7)[1]. In the mid-late 2000's, overall production increased significantly (>300% increase in N inputs from 2004–2009), particularly in the lower D'Entrecasteaux Channel and lower Huon estuary. This is potentially because previous studies found that the upper Huon Estuary was showing signs of elevated ammonia and decreased dissolved oxygen in bottom waters (Ross and MacLeod 2013) and the acknowledgment that areas of high water currents are more suitable for farming, as this facilitates the dispersal of waste products (Soto and Norambuena, 2004) thereby improving environmental outcomes. Water generally flows from south to north in the D'Entrecasteaux Channel (Herzfeld et al., 2010), as such the southern regions of the D'Entrecasteaux Channel and Huon/Esperance MFDP areas will get more benefit from oceanic waters, and are therefore thought to be less susceptible to aquaculture inputs (Volkman et al., 2009), hence the increase in production in these areas.

For HAC, the increase in production is most evident by the increase in farming at their Flathead Bay and East of Redcliffs leases (see Figure 1 for lease locations) in the south-western D'Entrecasteaux Channel between the Huon Estuary mouth and Port Esperance, but within the Huon/Port Esperance MFDP area. They have also increased production in the South of Zuidpool Rock lease within the D'Entrecasteaux Channel MFDP area[2]. In 2014, the

[1] Several finfish aquaculture companies have operated in the D'Entrecasteaux Channel/Huon Estuary system throughout the years and we have allocated inputs based on the companies who currently operate the leases.
[2] It must be noted, however, that HAC do not have any leases in the northern D'Entrecasteaux Channel.

South of Zuidpool Rock lease had the greatest N inputs (Figures 7 and 8) and highest fish biomass (Figure 9). Although Hideaway Bay also had a high fish biomass, fish are generally only transferred there briefly for harvesting/bathing and therefore biomass estimates reported for this site (herein) are likely to be an overestimate; this is best reflected in the relative low N inputs at this lease. In 2015, when the South of Zuidpool Rock lease was being divided in two, the Flathead Bay and East of Redcliffs leases had the highest fish biomass of HACs leases (Figure 9), and hence highest N inputs (Figures 7 and 8). The increase in farming on these leases contributed to the exceedance in the Huon/Esperance MFDP area. At these leases, both biomass and N inputs increased in late 2014 and remained consistently high until late 2015 before decreasing markedly in 2016 (Figure 8). In all other leases, monthly N inputs and biomass remained comparatively low during this time period.

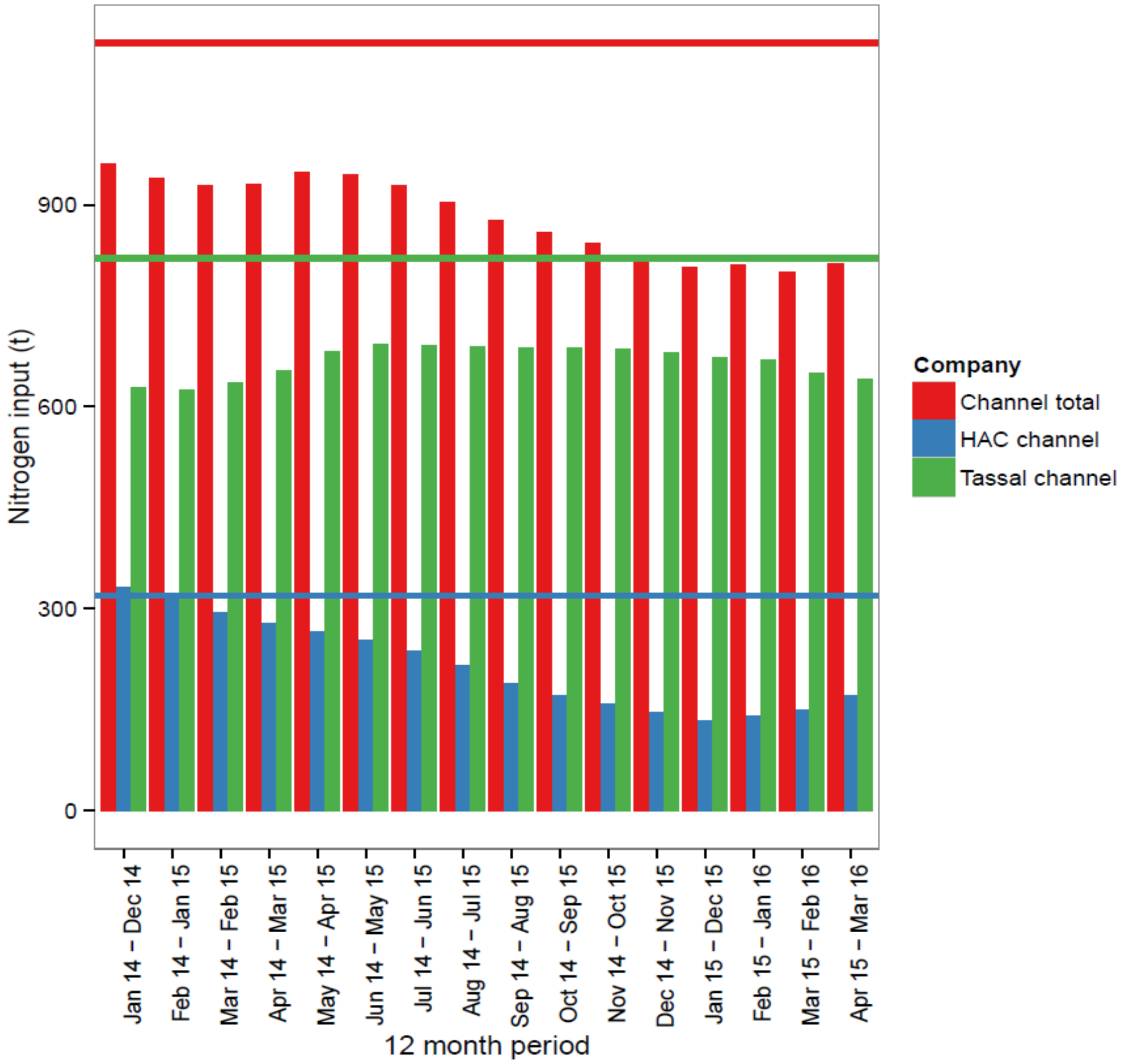

Figure 4: N inputs by Tassal and Huon Aquaculture Company in the D'Entrecasteaux Channel MFDP area. TPDNO represented by horizontal lines.

FCR's are a measure of how efficiently farmed fish (or other animals) convert food into biomass. They can also provide insight into how N enters the environment (i.e. as urine and faeces or overfeed). To calculate FCR's accurately, precise feed and biomass data are essential. The level of detail in the HAC biomass data was insufficient to calculate FCR accurately because there were a number of issues with how the information was captured, which resulted in occasions where there was duplication within months, or months where there was no data recorded. As a result, a large proportion of the data was estimated. Therefore, it was not possible to determine whether an unusually large amount of feed, relative to fish biomass, was used during the exceedance period.

Figure 5: N inputs by Tassal and Huon Aquaculture Company in the Huon/Esperance MFDP area. TPDNO represented by horizontal lines.

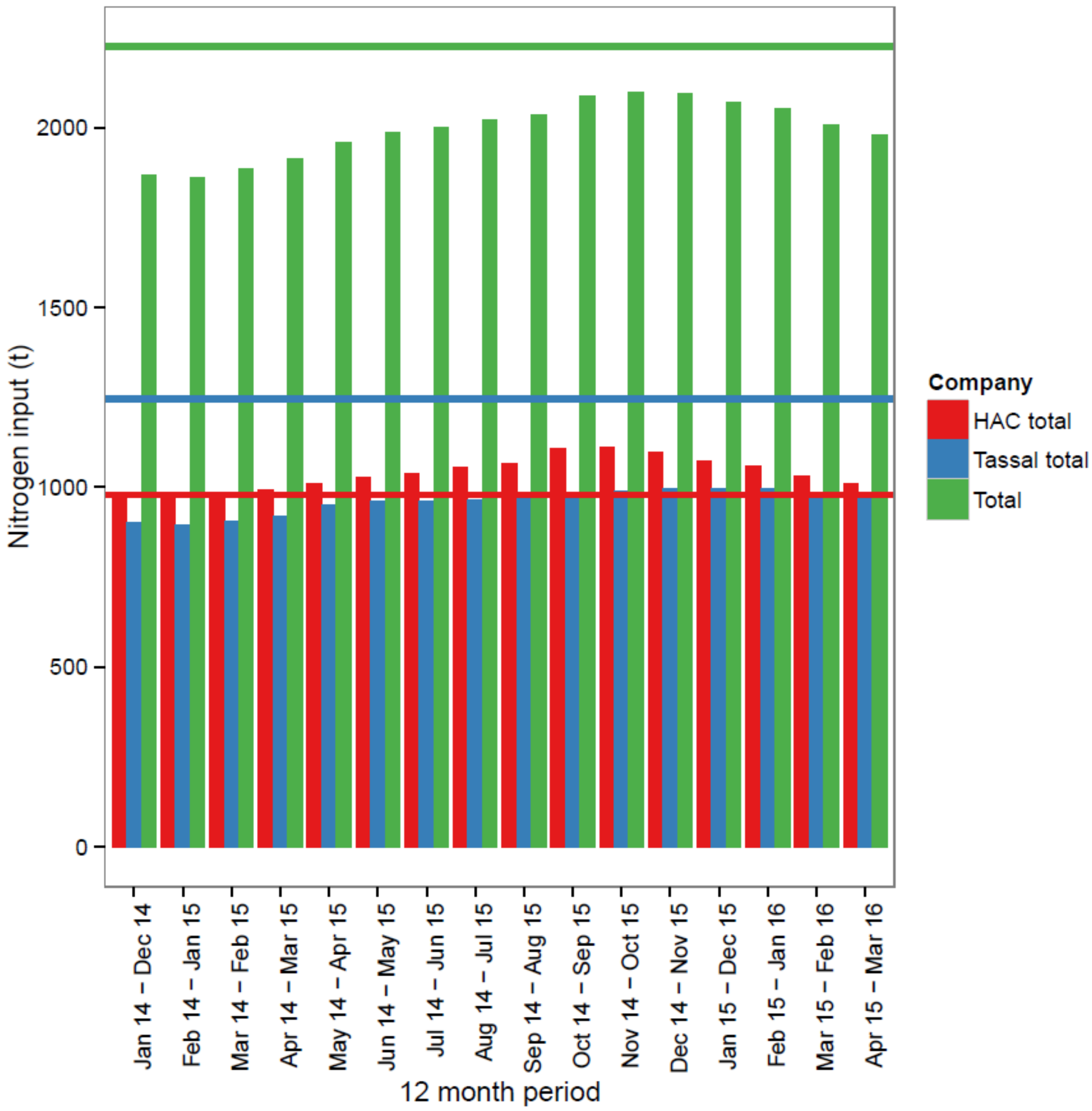

Figure 6: N inputs by Tassal and Huon Aquaculture Company in the combined D'Entrecasteaux Channel and Huon/Esperance MFDP area. TPDNO represented by horizontal lines.

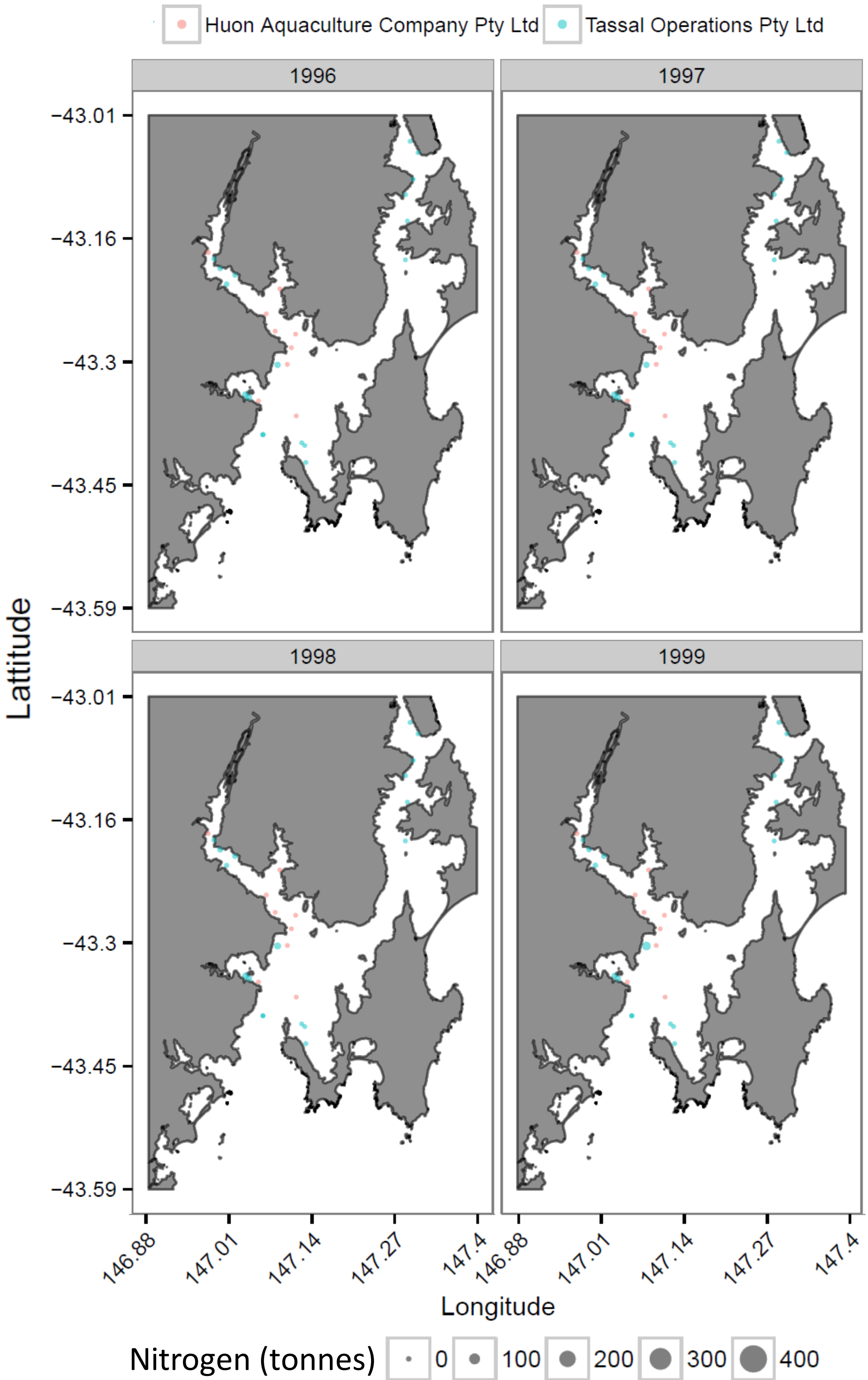

Figure 7: Spatial depiction of annual N inputs by Tassal and Huon Aquaculture Company in the D'Entrecasteaux Channel and Huon/Esperance MFDP area from 1996 – 2015.

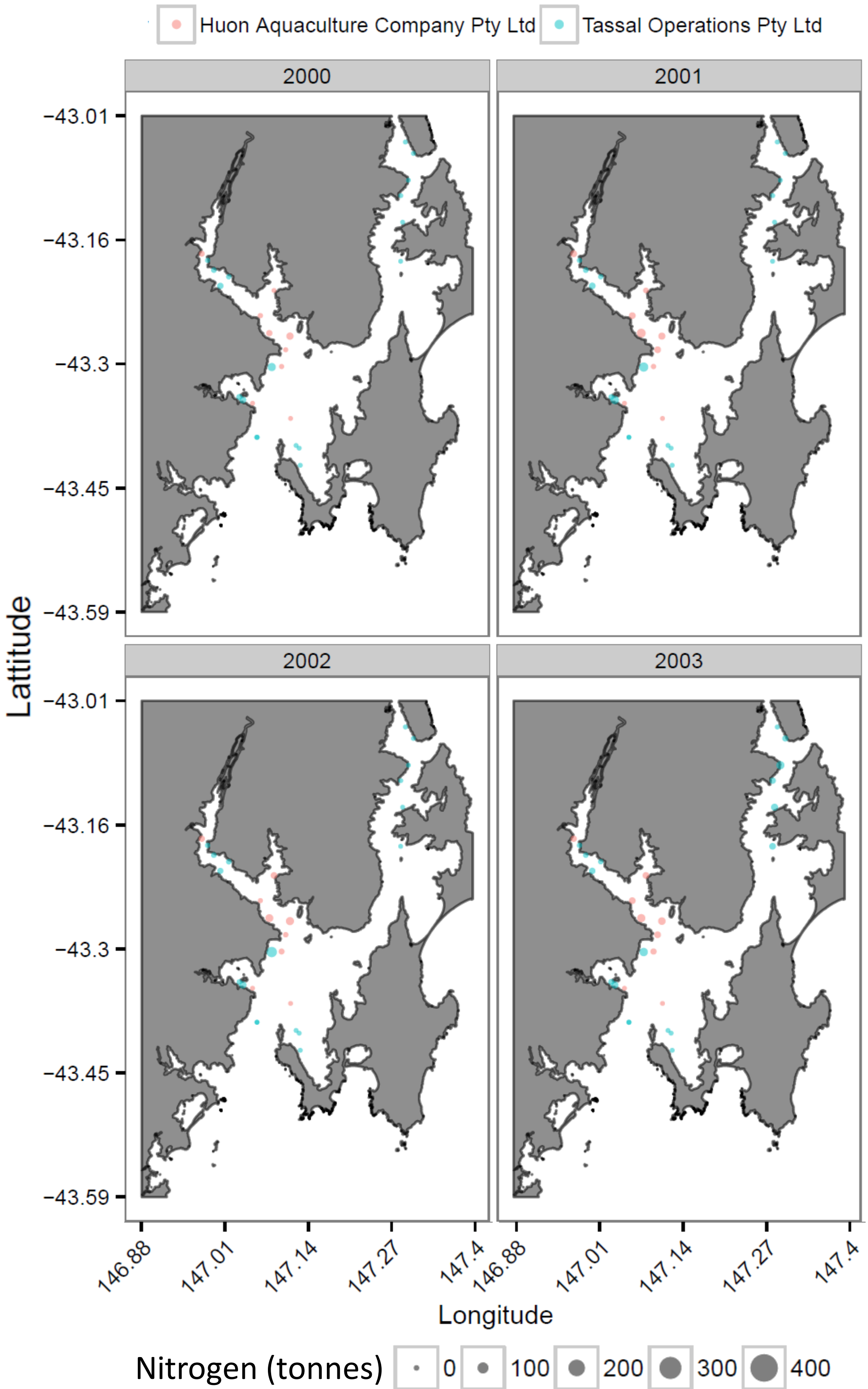

Figure 7 continued: Spatial depiction of annual N inputs by Tassal and Huon Aquaculture Company in the D'Entrecasteaux Channel and Huon/Esperance MFDP area from 1996 – 2015.

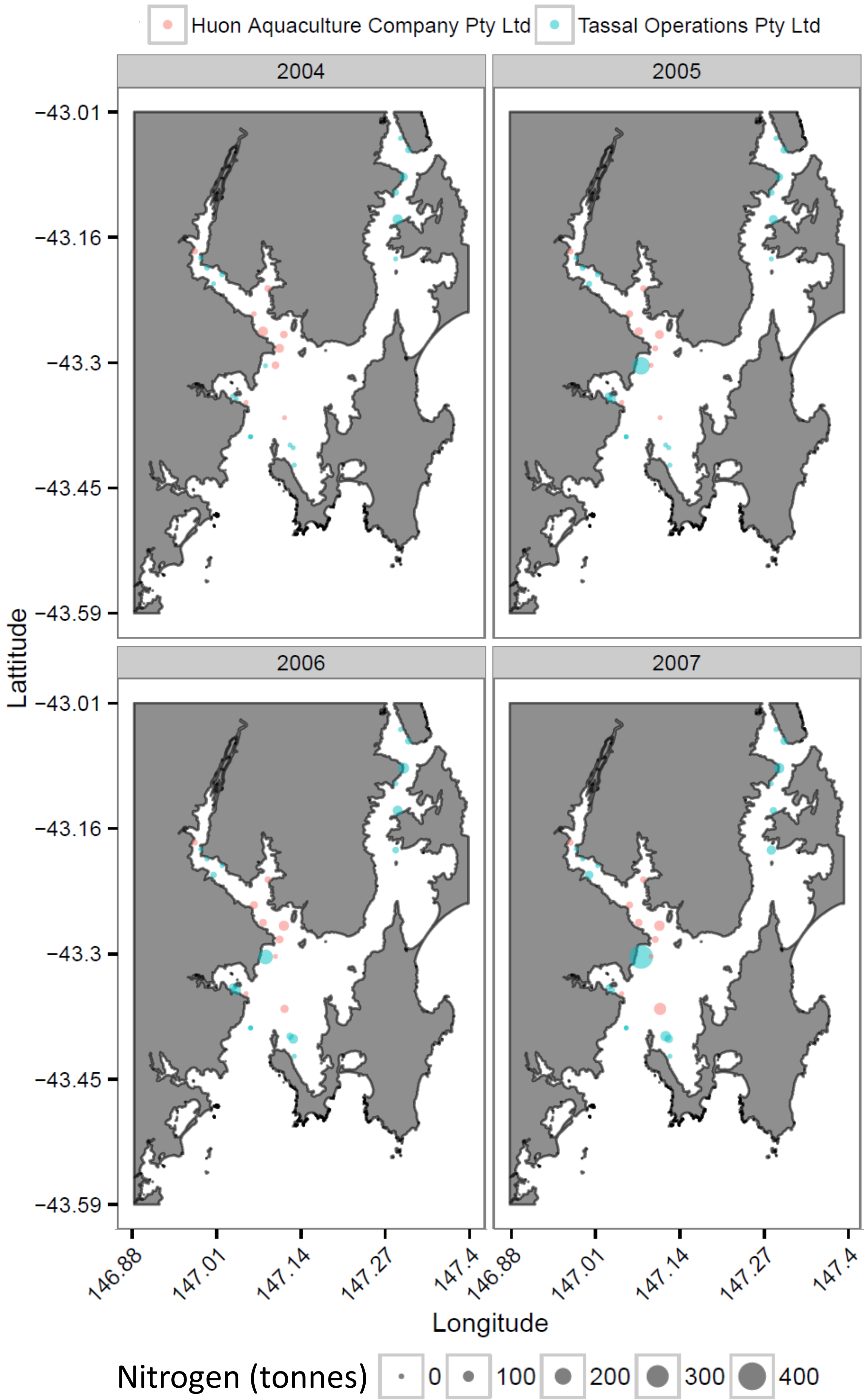

Figure 7 continued: Spatial depiction of annual N inputs by Tassal and Huon Aquaculture Company in the D'Entrecasteaux Channel and Huon/Esperance MFDP area from 1996 – 2015.

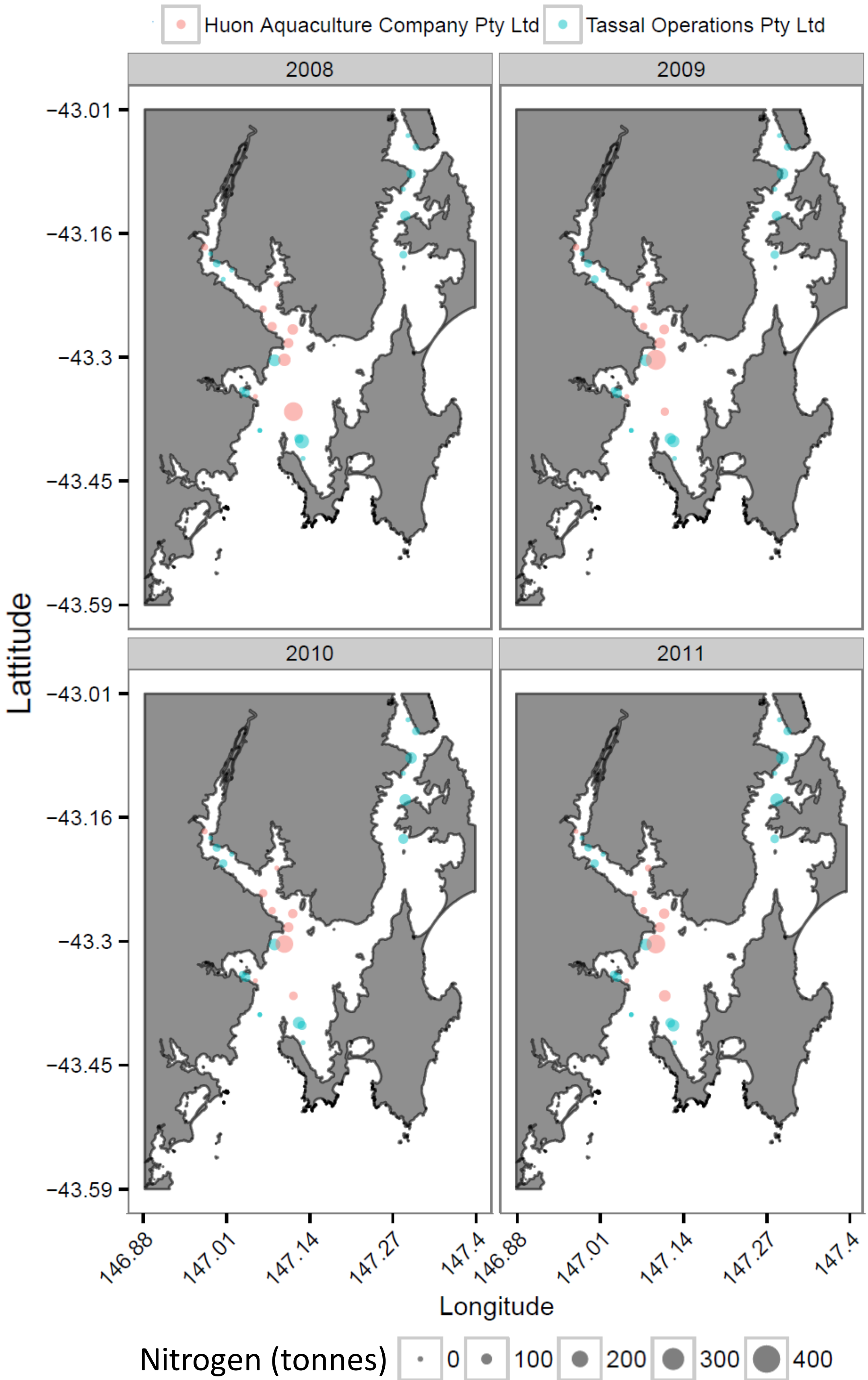

Figure 7 continued: Spatial depiction of annual N inputs by Tassal and Huon Aquaculture Company in the D'Entrecasteaux Channel and Huon/Esperance MFDP area from 1996 – 2015.

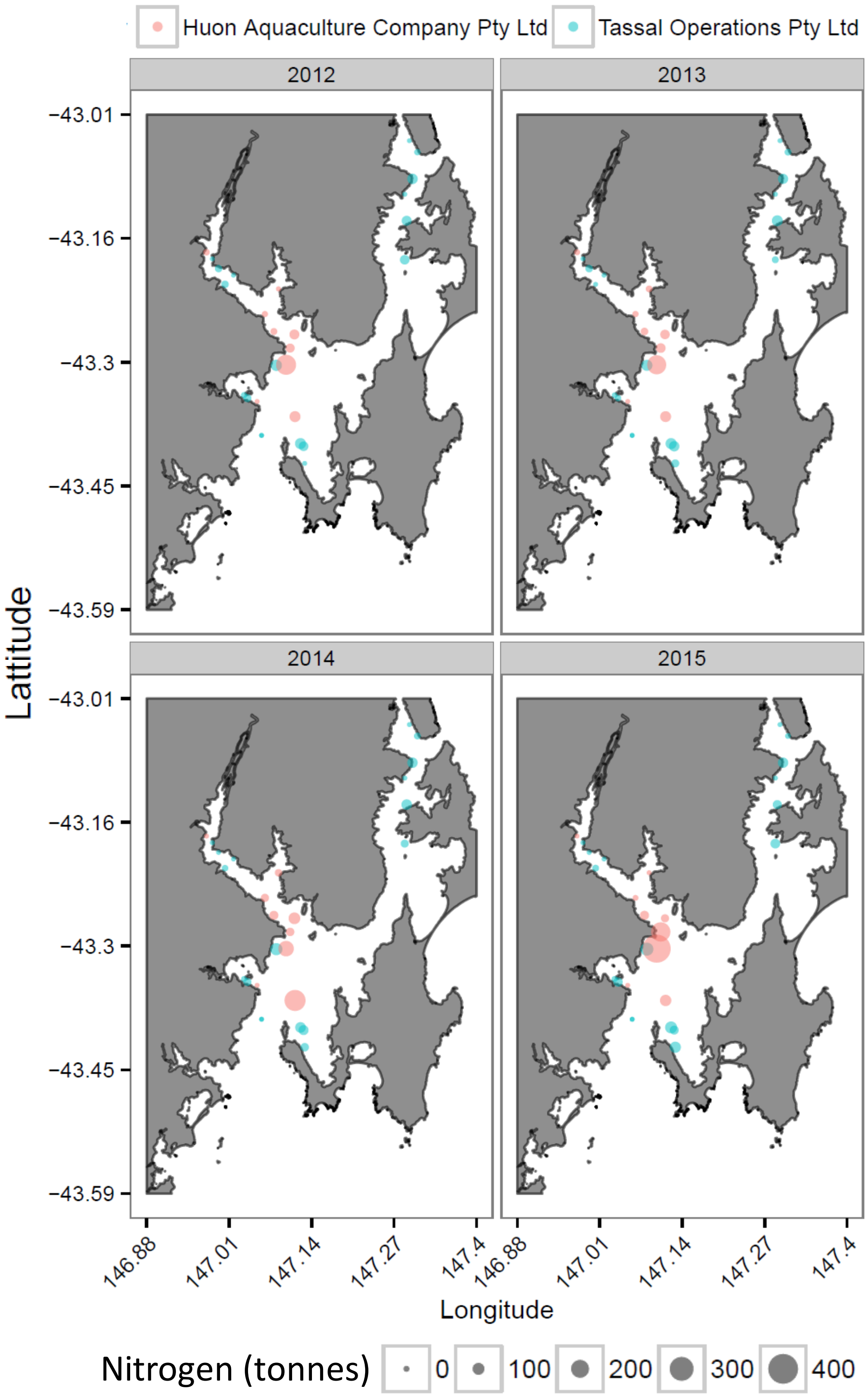

Figure 7 continued: Spatial depiction of annual N inputs by Tassal and Huon Aquaculture Company in the D'Entrecasteaux Channel and Huon/Esperance MFDP area from 1996 – 2015.

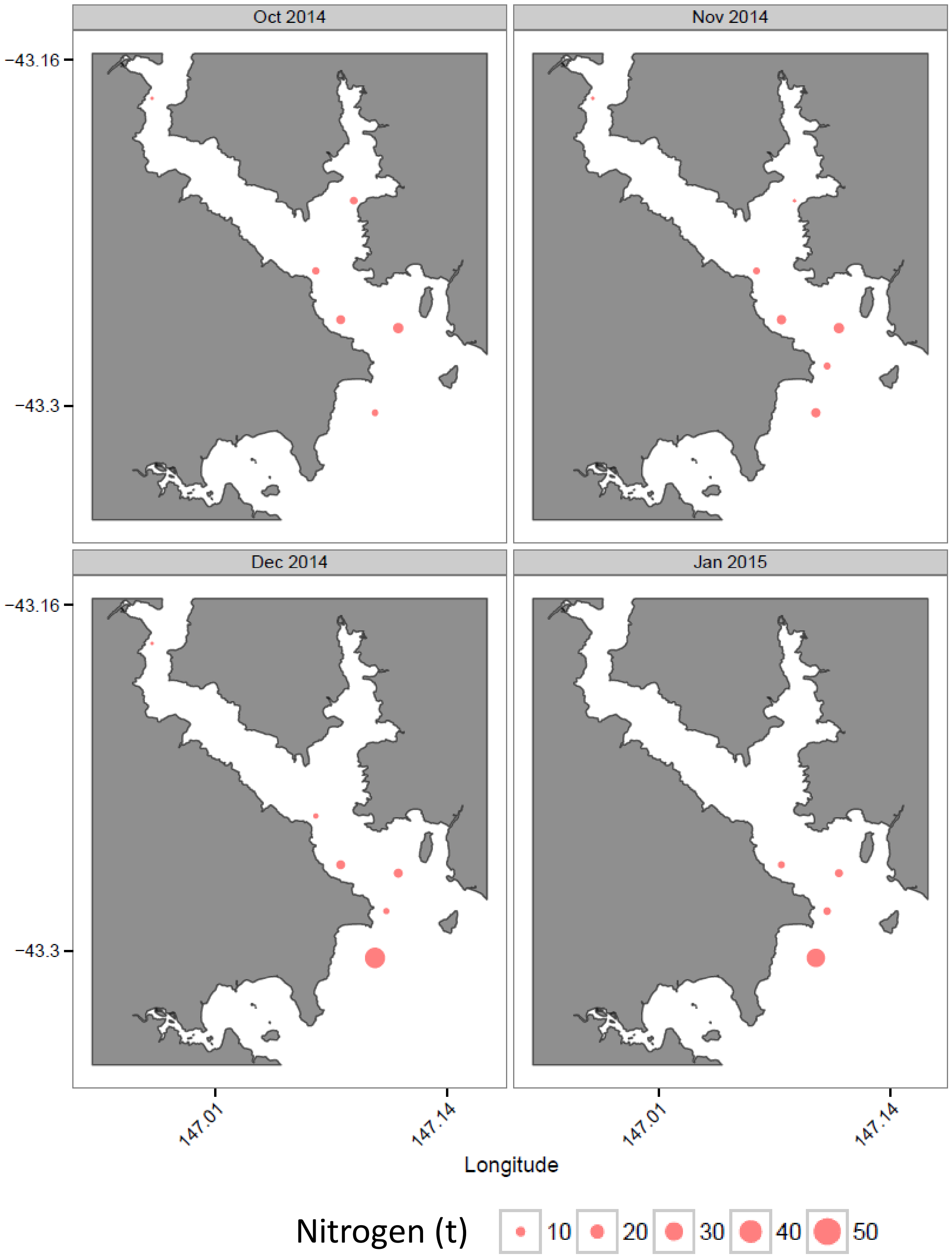

Figure 8: Spatial depiction of monthly N inputs by Huon Aquaculture Company in the Huon/Esperance MFDP area from October 2014 – March 2016.

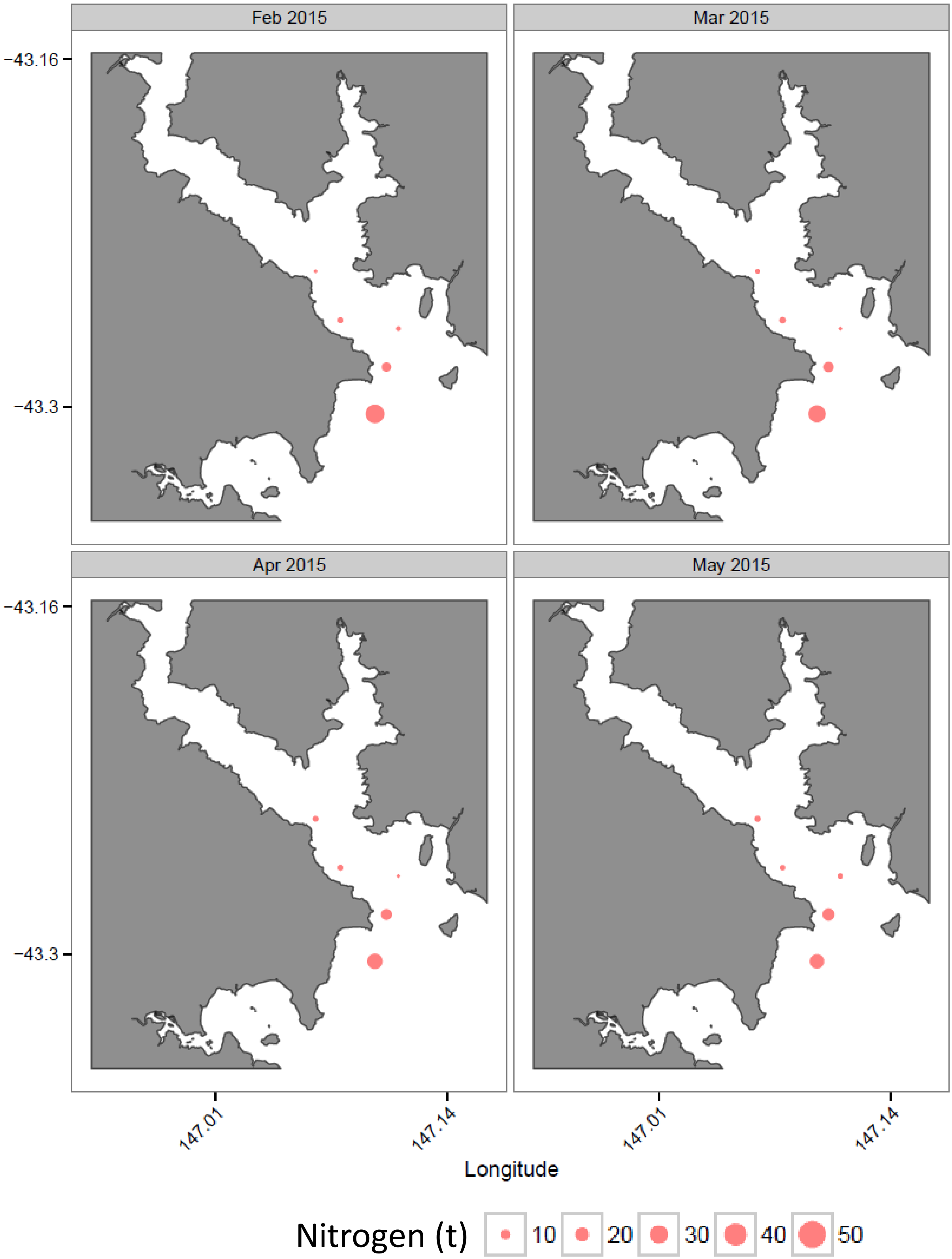

Figure 8 continued: Spatial depiction of monthly N inputs by Huon Aquaculture Company in the Huon/Esperance MFDP area from October 2014 – March 2016.

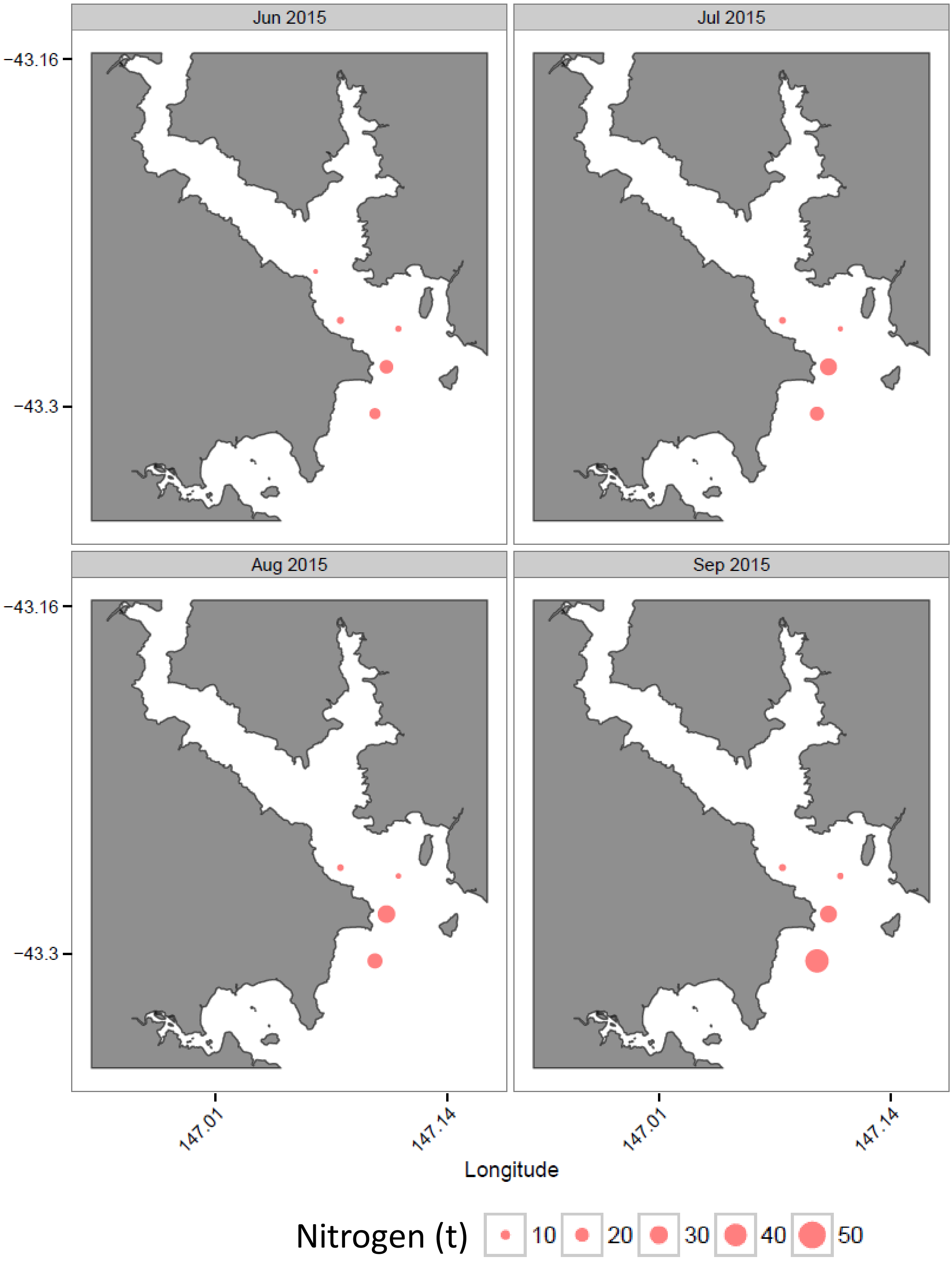

Figure 8 continued: Spatial depiction of monthly N inputs by Huon Aquaculture Company in the Huon/Esperance MFDP area from October 2014 – March 2016.

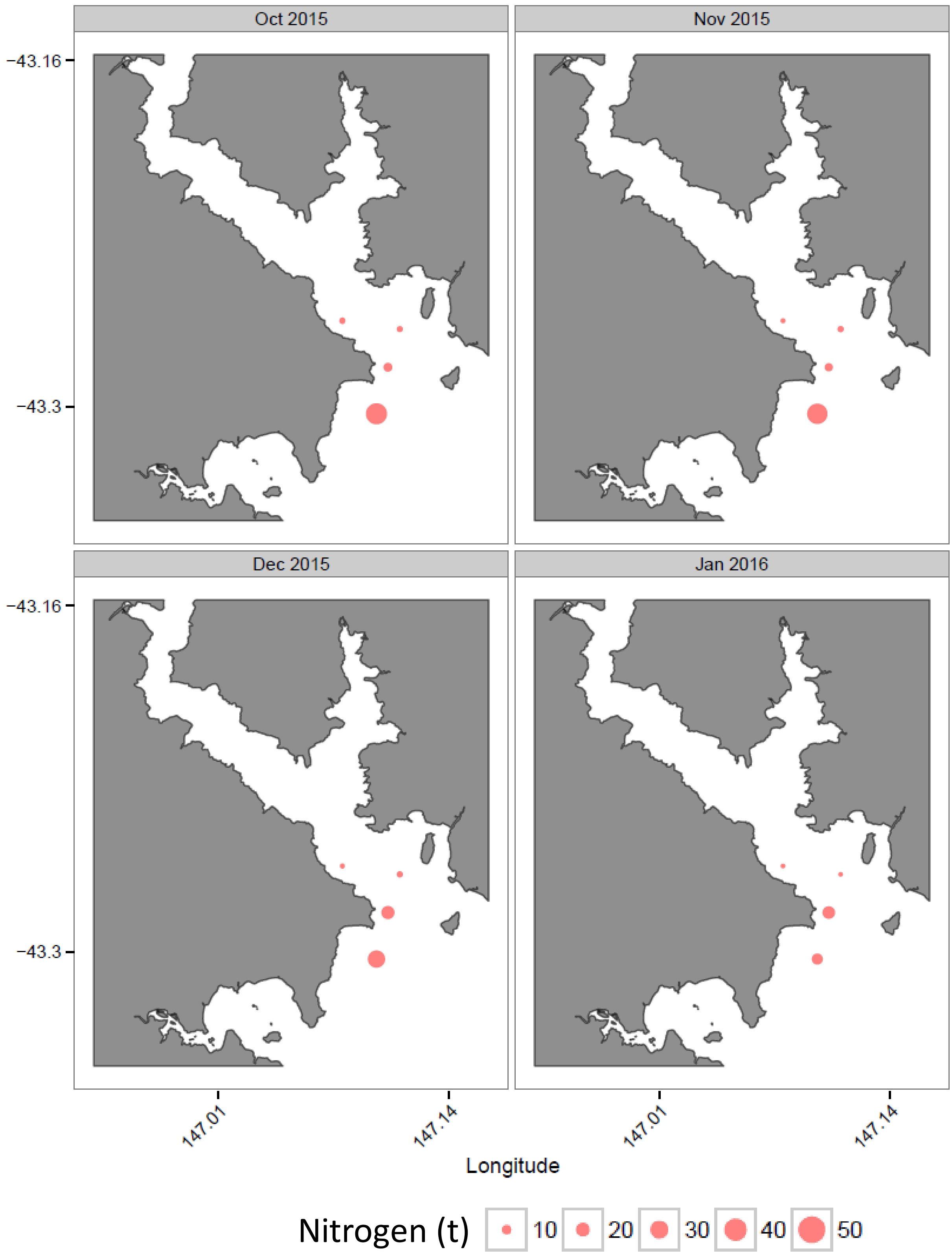

Figure 8 continued: Spatial depiction of monthly N inputs by Huon Aquaculture Company in the Huon/Esperance MFDP area from October 2014 – March 2016.

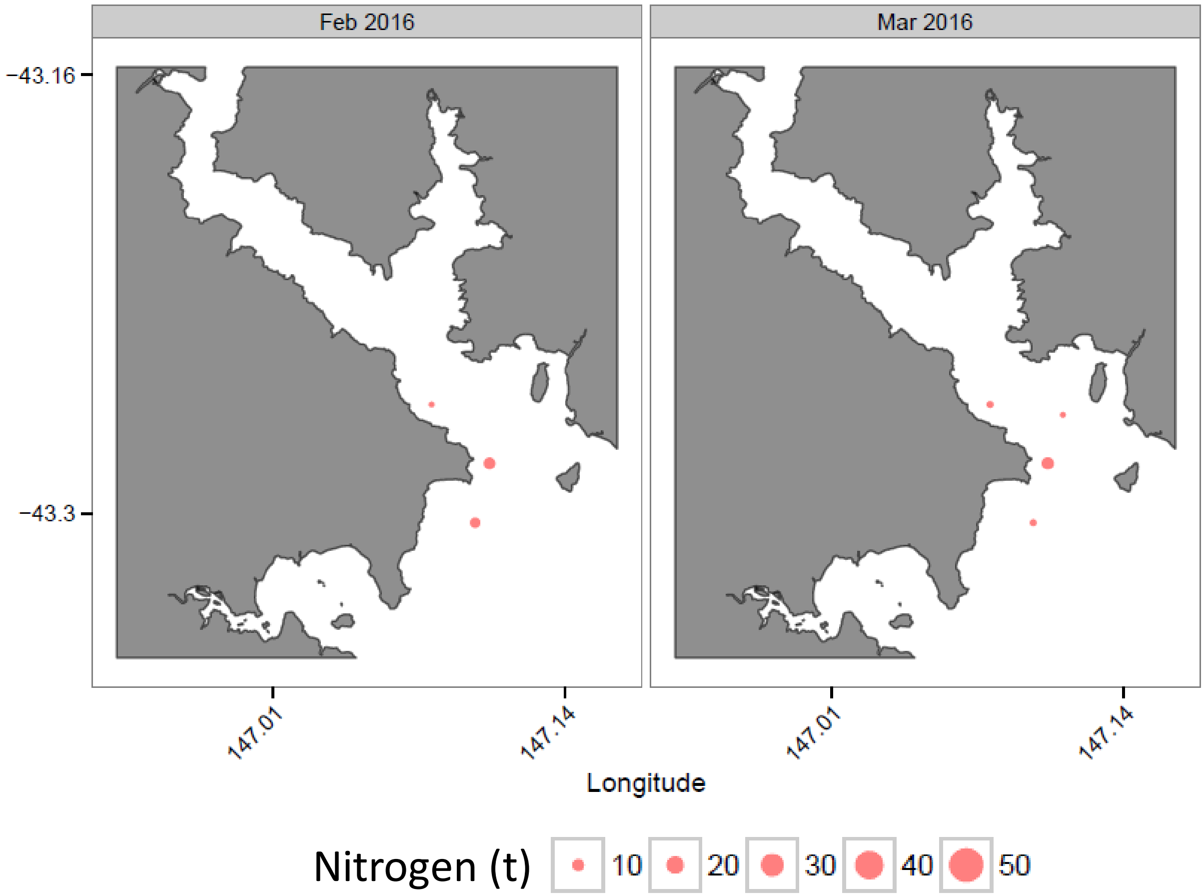

Figure 8 continued: Spatial depiction of monthly N inputs by Huon Aquaculture Company in the Huon/Esperance MFDP area from October 2014 – March 2016.

4.1.2. Riverine inputs

The Huon River, is by far the greatest source of freshwater and riverine nutrients in the D'Entrecasteaux Channel/Huon Estuary catchment (Figure 10). At times there are also significant flows from the Esperance, Mountain and Kermandie Rivers, with only relatively low inputs from the Snug and Northwest Bay Rivulets (Figure 10). All of the rivers have periods of relatively low flow (or no-flow in the case of Snug and Northwest Rivulets) with high flow spikes being more frequent in winter and spring (Figure 11).

Flow would appear to be the primary determinant of total N, ammonia and nitrite inputs from the rivers, and the Huon River by far had the highest input of these nutrients (Figure 12). Nitrate inputs, were not as closely related to river flow, with significant inputs from the Huon, Kermandie and Mountain Rivers. The majority of nitrate in the Huon River is likely to be from natural sources within its largely pristine catchment. In addition to natural sources of nitrate, the Kermandie River also receives inputs from the wastewater treatment plant that discharges near its mouth (see section on wastewater treatment below), whilst the nitrate in the Mountain River is potentially also associated with agricultural sources. There is no evidence that riverine nutrient inputs were any greater during the HAC N exceedance than at other times, if anything they may have been slightly lower due to relatively reduced flows throughout much of 2015 (Note: information on earlier years can be found in the BEMP report (Ross and Macleod, 2013) and the State of the D'Entrecasteaux Channel report (Parsons, 2012).

Wild-Allen and Andrewartha (2016), suggest that inputs from the ocean are likely reduced during periods of low river flow due to a concomitant reduction in return flows transporting oceanic nutrients into the system. However, this combination of low river flow and reduced ocean inputs will increase residence times within the system (Wild-Allen and Andrewartha, 2016), which could have a counteractive effect on the lower nutrient inputs from the Huon River and ocean.

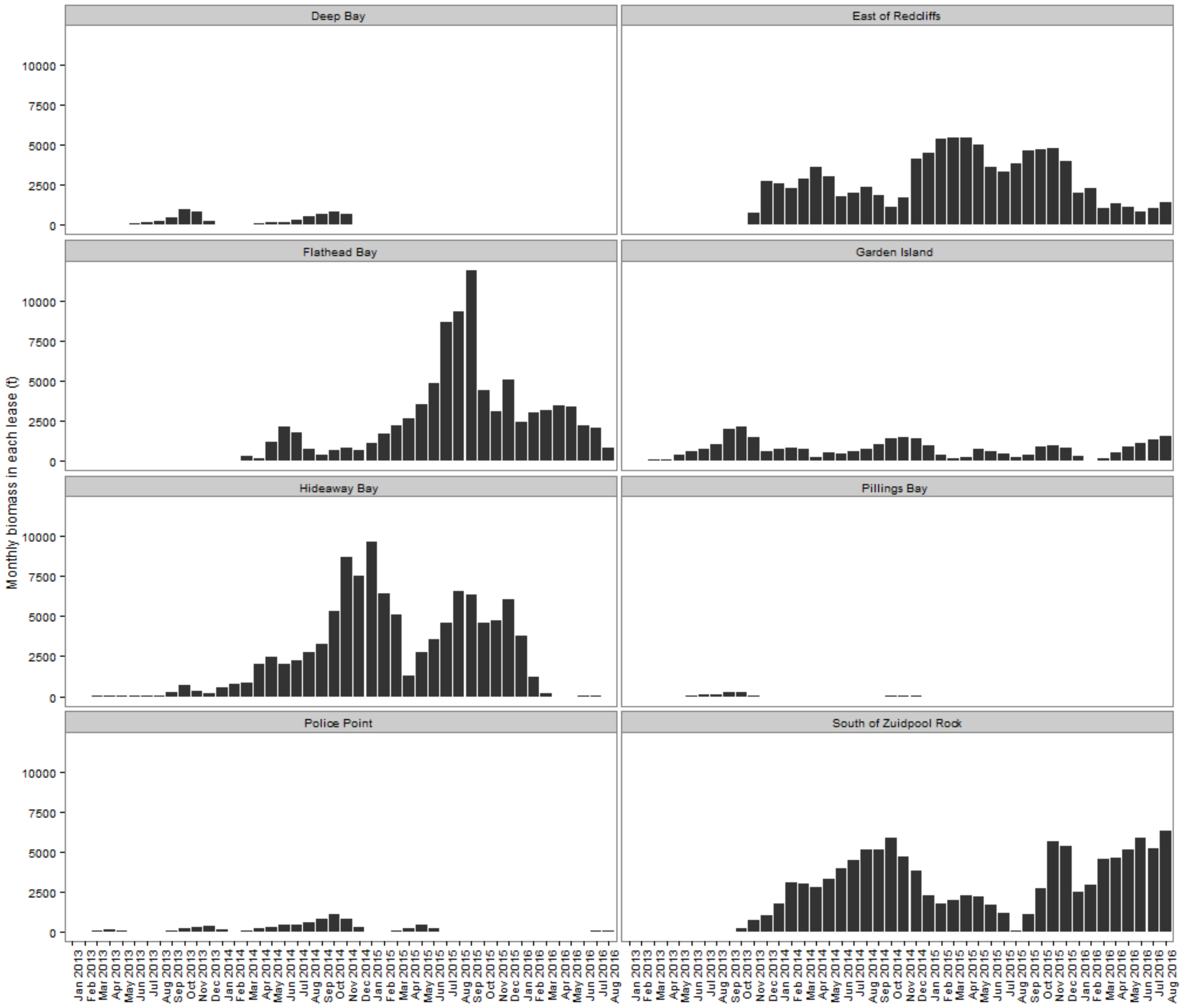

Figure 9: Monthly Atlantic salmon biomass of Huon Aquaculture Company in all of their active leases in both MFDP areas. Figure only includes cohorts that were cultured post-2013.

4.1.3. Wastewater treatment plant inputs

Eight WWTP discharge into the D'Entrecasteaux Channel/Huon estuary (four into each MFDP area). Over 20 t of N was input into the system by these WWTPs annually between 2013 and 2015, >10 t of this input was ammonia (Figure 13). Around 3 t of P are input annually. Margate and Electrona treatment plants contribute the greatest quantity of nutrients, both of these plants have outfalls in Northwest Bay, in the northern D'Entrecasteaux Channel. As such, these are unlikely to contribute to any effects that occur within the vicinity of the HAC N exceedance.

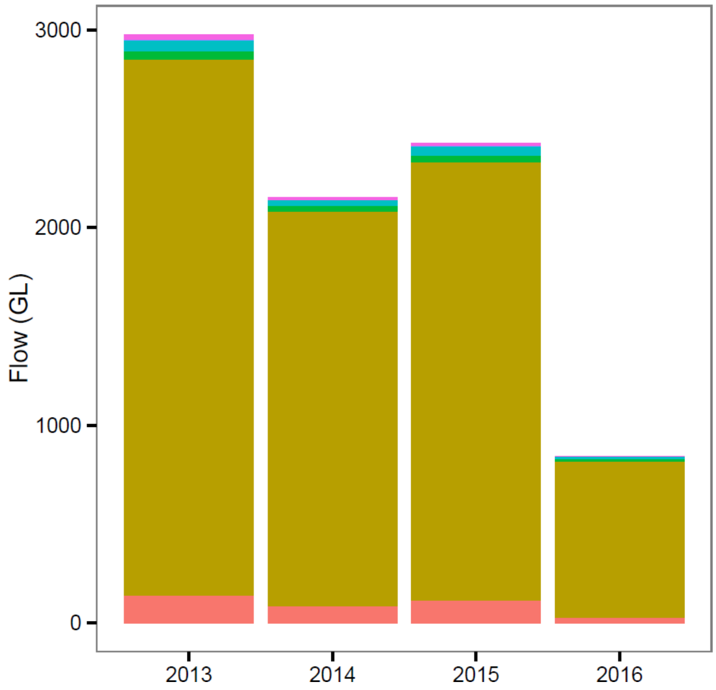

Figure 10: Annual river flow from the major tributaries feeding the D'Entrecasteaux Channel/Huon Estuary system.

4.1.4. Industrial inputs

Between 2012 and 2015 there was a marked reduction in total N, ammonia and total P inputs from the Tassal Dover processing plant (Table 2) as a result of upgrades. In contrast, nutrient outputs have increased through time at the Margate plant; although it should be noted that the total outputs for all three nutrients from this plant are very low. Industrial inputs from the third processing plant in the system, Margate Tasmanian Seafoods, were <100 kg for each of the nutrients measured when last reported (Ross and Macleod, 2013) and therefore, along with the Tassal processing plants, are negligible in a system-wide assessment.

Table 2: Annual total N, ammonia and total P inputs (tonnes) from the Tassal Dover and Margate processing plants. Source: Tassal.

Processing plant	Year	Total nitrogen	Ammonia	Total phosphorous
Tassal Dover	2013	8.37	6.04	0.69
	2014	6.09	4.58	0.29
	2015	1.44	1.05	0.11
Tassal Margate	2013	0.46	0.23	0.09
	2014	0.51	0.40	0.13
	2015	0.57	0.66	0.16

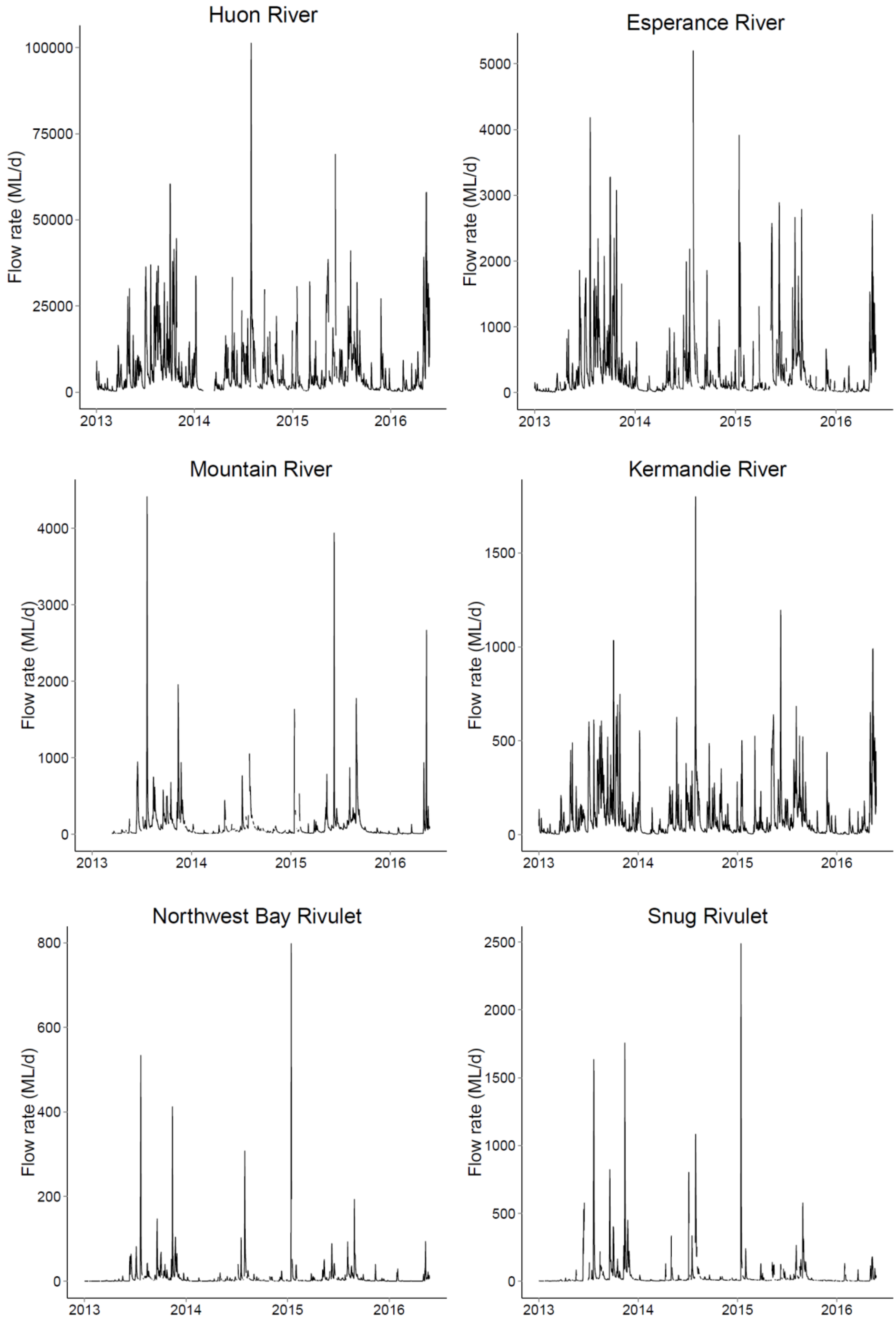

Figure 11: River flow from the major tributaries feeding the D'Entrecasteaux Channel/Huon Estuary system.

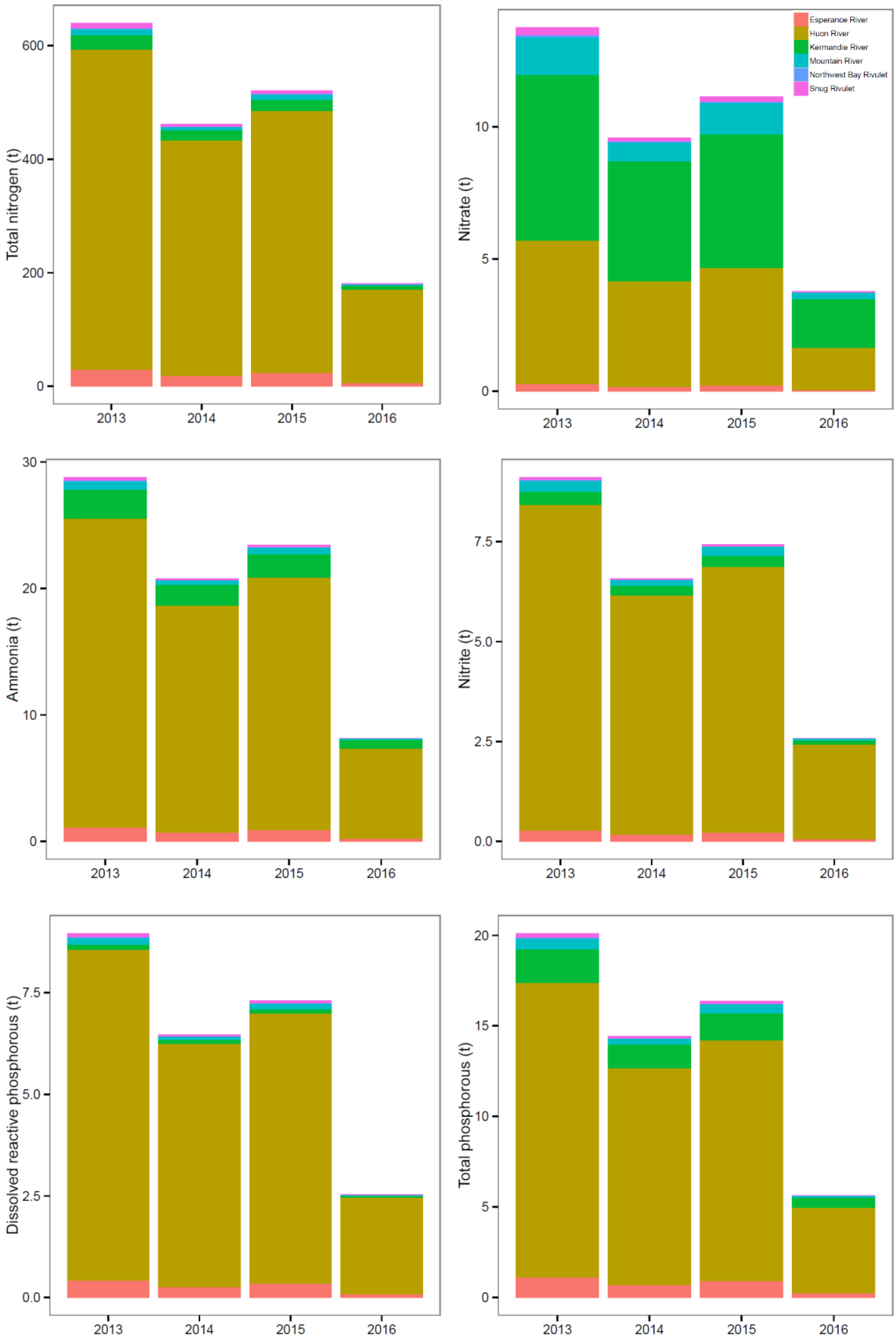

Figure 12: Nutrient inputs from each of the major tributaries feeding the D'Entrecasteaux Channel/Huon estuary system.

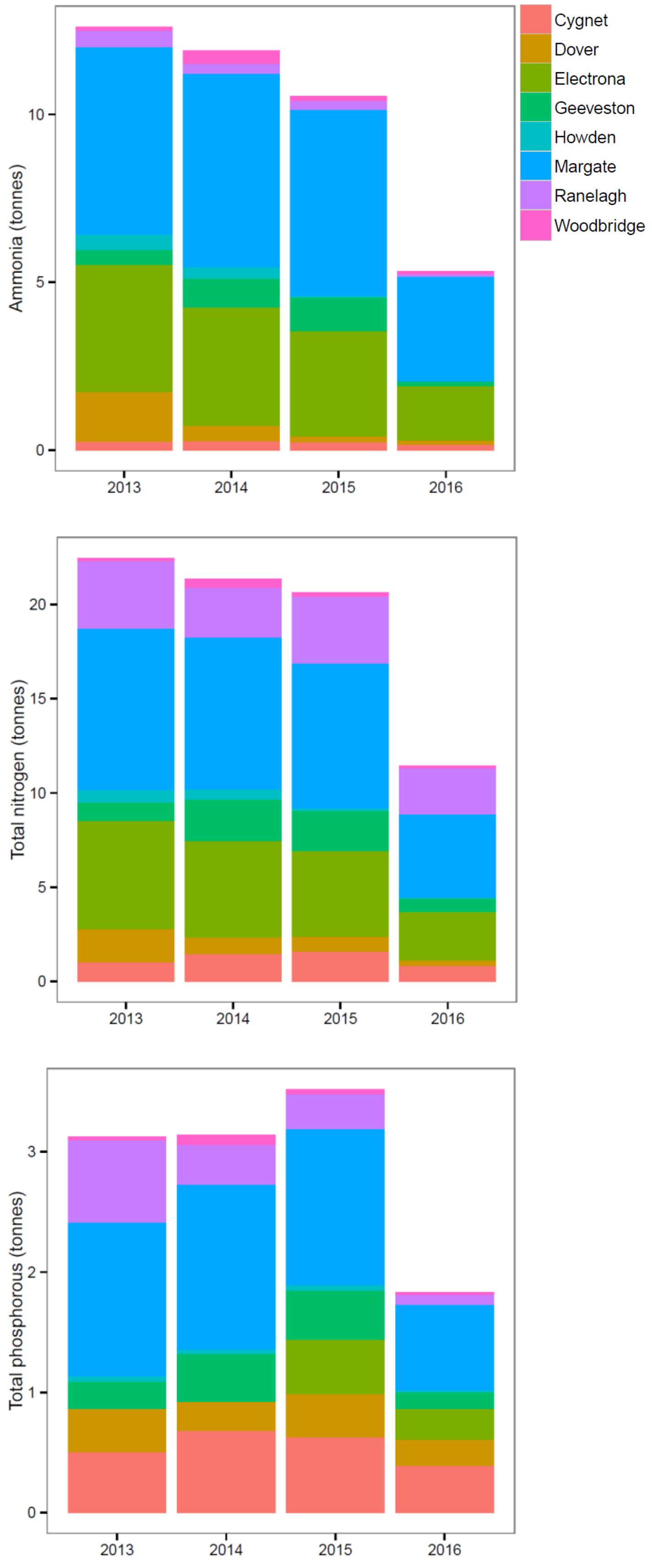

Figure 13: Annual WWTP inputs. Data for 2016 only extend until June. Note: nutrient inputs are calculated using a single value each month for both nutrient concentration and mean monthly flow and should therefore be interpreted as an estimate only.

4.2 Environmental influence of the HAC exceedance

4.2.1. Nitrogen

Both surface and bottom water total N concentration were highly variable in all regions with no discernible temporal trend (Figures 14 and 15). Nitrate concentrations (surface and bottom) are highly seasonal in all four regions (Figures 16 and 17), being lowest in summer and highest in winter. In Recherche Bay, the northern D'Entrecasteaux Channel and the southern region, these seasonal patterns are likely to be largely driven by cool, nutrient rich, subantarctic water penetrating northwards during winter (Crawford *et al.*, 2011). In the upper Huon estuary, the seasonal distinction was less obvious in surface waters but winter elevation was still observed in bottom waters, albeit at a lower level than in the Channel waters. Winter nutrient inputs in this case are likely a combination of subantarctic water incursion and increased nutrient loads from the Huon and Mountain Rivers during high flow periods.

In the northern D'Entrecasteaux Channel, both surface and bottom ammonia concentrations were highly variable, and there was no obvious seasonal or temporal trend (Figures 18 and 19). Similarly, there was no obvious seasonal or temporal trend in ammonia concentration in surface of bottom waters at the Recherche Bay reference site with nutrient concentrations, in general, being considerably lower at this site than those observed in the other three regions. Bottom water ammonia concentration was notably higher in the south and upper Huon regions (Figure 19). Both regions displayed a similar pattern, with typically lower levels in autumn and winter before increasing throughout spring to reach a maximum in late spring and summer. This would appear to be linked to increases in aquaculture inputs but may also be influenced by riverine inputs. Mean bottom water ammonia concentrations were higher in the southern region over the summer of 2014/15 and spring of 2015, with a particularly high proportion of values >0.03 mg/l recorded over this period, particularly at site 10 (see Appendix iv for a site level breakdown of ammonia concentration and Figure 1 for site locations). To further investigate which BEMP sites in the southern region were responsible for driving this trend, GAMs were fitted to the time series. These indicate that sites 8, 9, 10 and 12 have all displayed an increasing trend in later years (Figure 20) with site 10, the closest site to the exceedance, displaying the greatest increase and having the highest ammonia concentrations of any BEMP site in the D'Entrecasteaux Channel/Huon Estuary system. There was no observable increase at the other sites in the southern region (sites 6, 7 and 12). As there was no notable increase in riverine nutrient inputs during this time period, and the sites that displayed the greatest increase were those near to where the majority of inputs occurred during the HAC N exceedance it could reasonably be assumed that these changes may be a result of the increased biomass, and N inputs, from the HAC farms during this time period.

As ammonia is the principle nitrogenous compound produced by fish, and is also created due to mineralisation of faeces and overfeed, it is useful to compare the data against the draft performance indicators for ammonia proposed in the BEMP. The elevated ammonia concentration in 2015 exceeded the level 1 performance indicators (i.e. summer mean >25% or mean for any one site increased by +50% of baseline) in the Huon/Esperance MFDP area and four performance indicators exceeded the level 2 specifications (i.e. summer mean >50% or mean for any single site up 200%) (Figure 21). BEMP site 10 (the closest site to the main source of the HAC exceedance) and site 12 in Port Esperance were above the specified trigger values for a level 3 breach but individual sites cannot trigger this level (only summer mean can result in a level 3 breach). Similarly, sites 6, 8 and 9, in the southern D'Entrecasteaux Channel, were above the level three trigger in the D'Entrecasteaux Channel MFDP area (single sites do not represent a breach of level three triggers) with the summer and annual means, and the remaining sites, all being below the level two trigger (Figure 22).

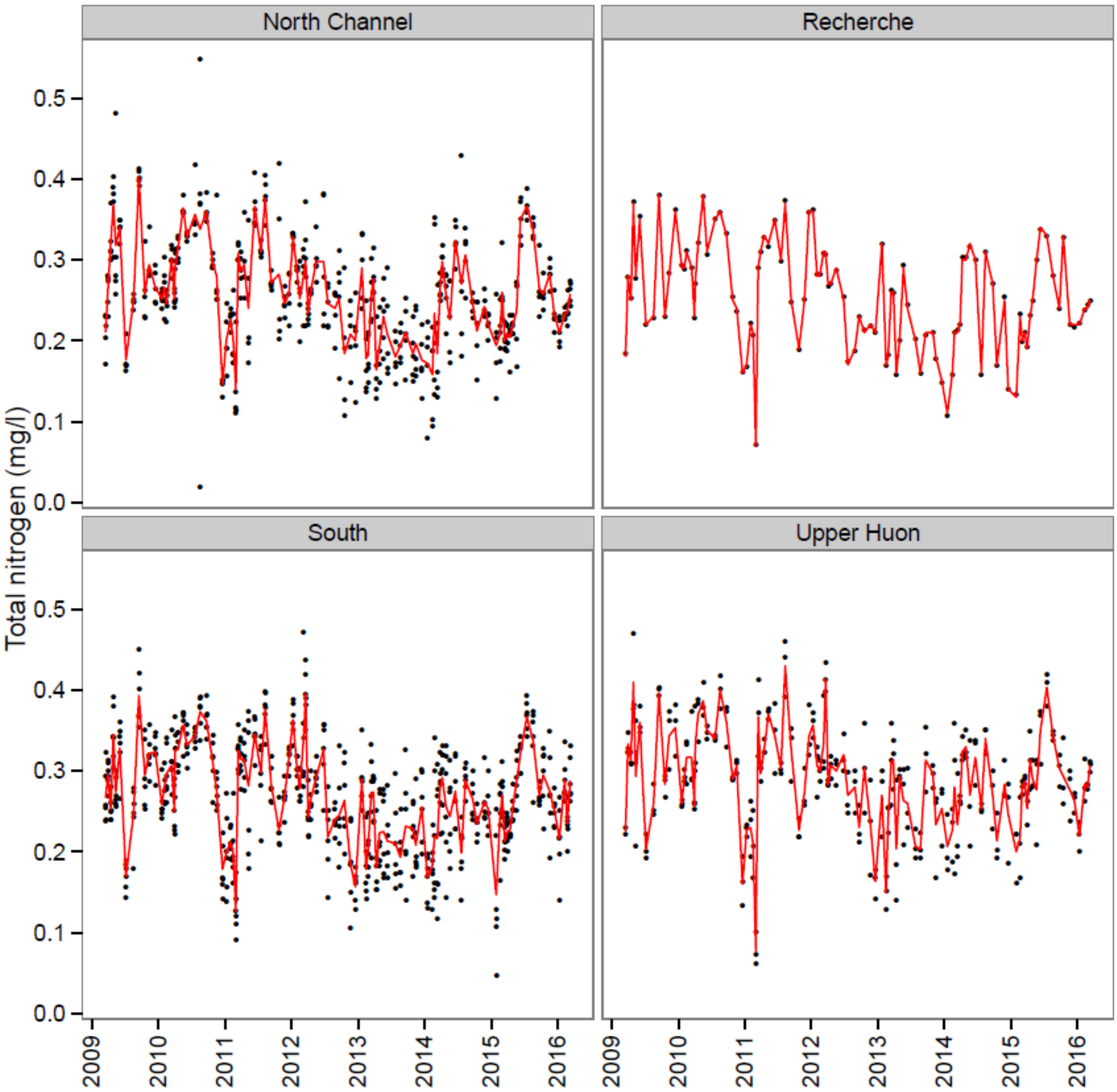

Figure 14: Bottom mean total N concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

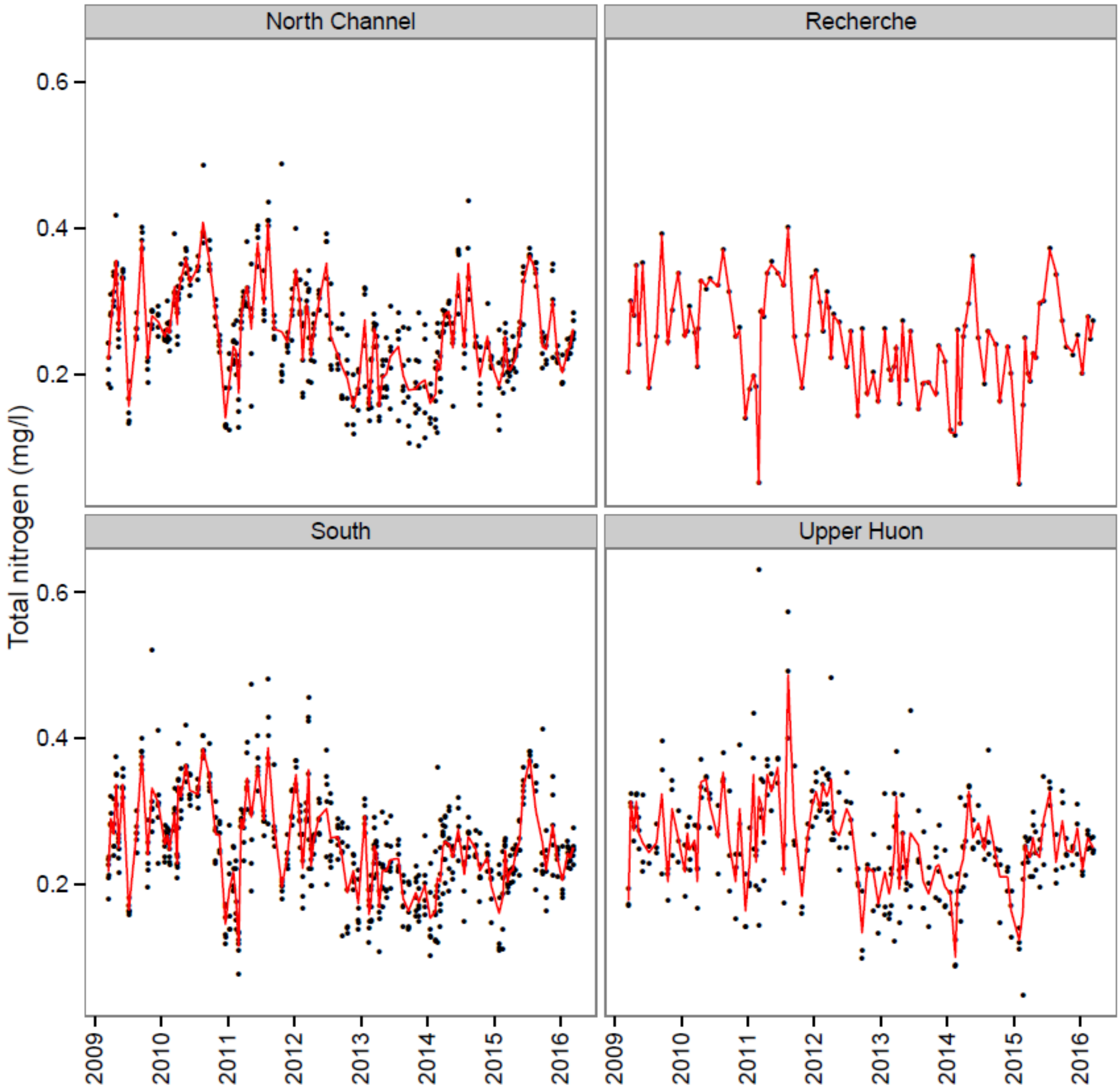

Figure 15: Surface mean total N concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

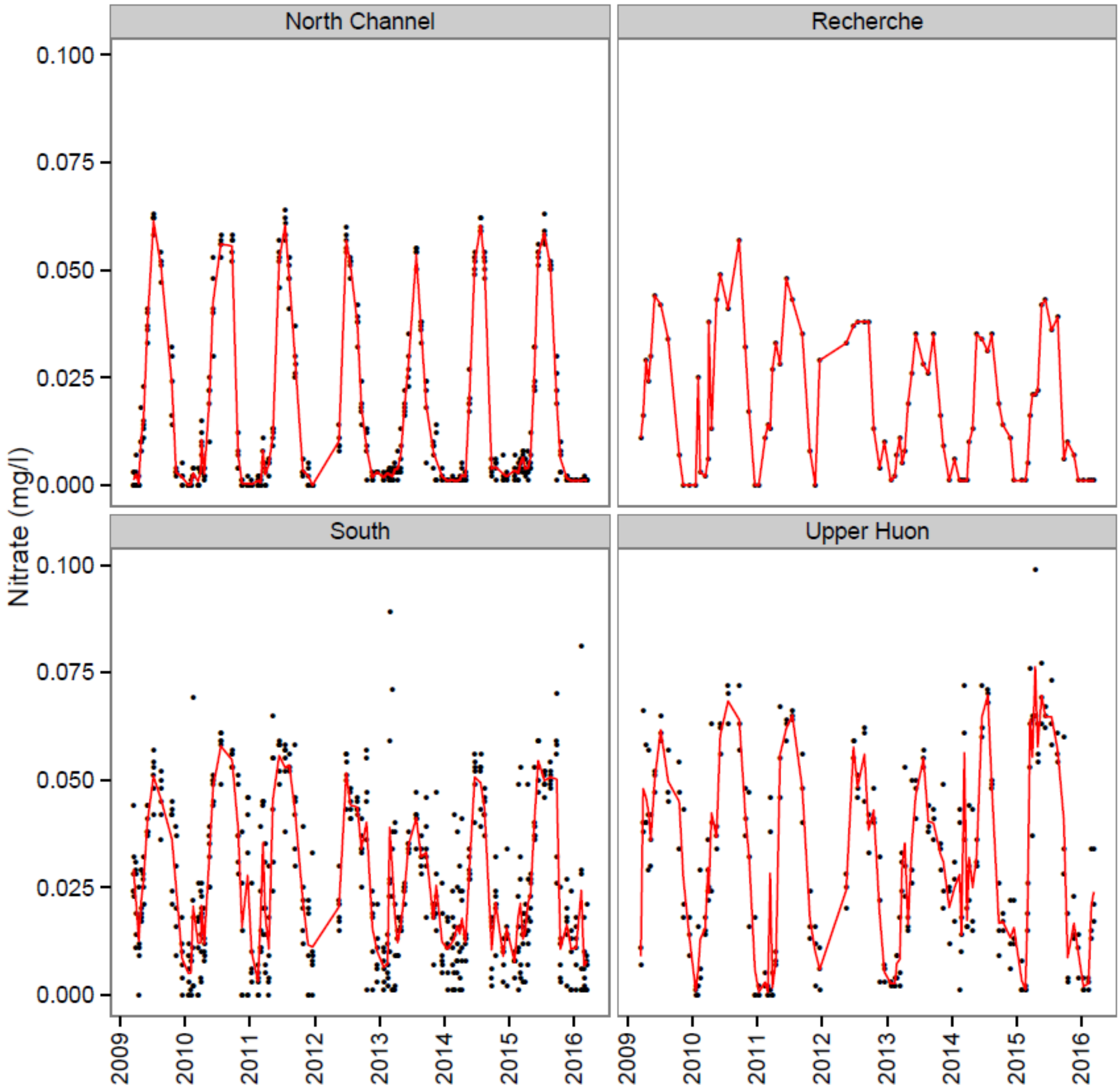

Figure 16: Bottom mean nitrate concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

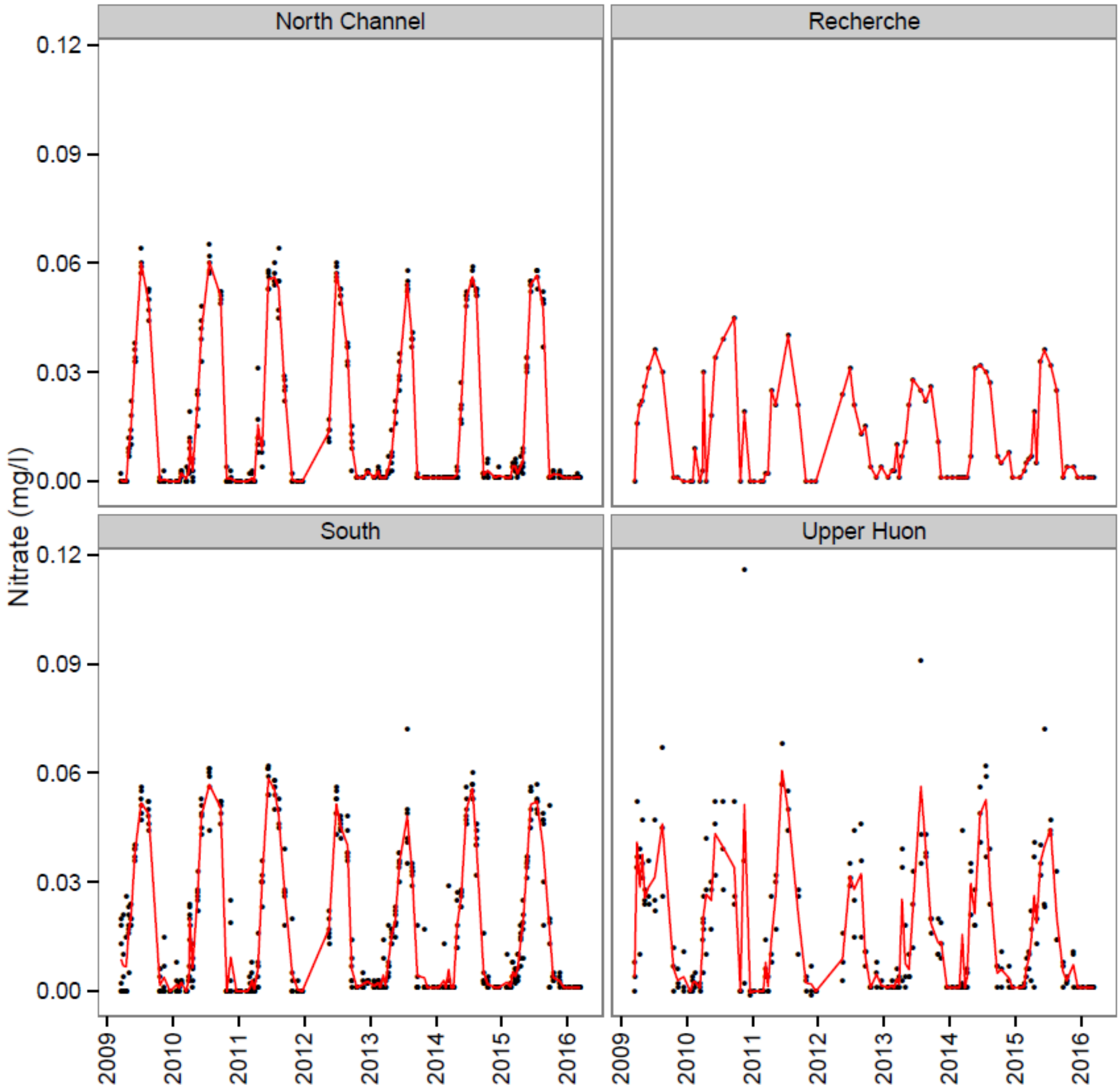

Figure 17: Surface mean nitrate concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

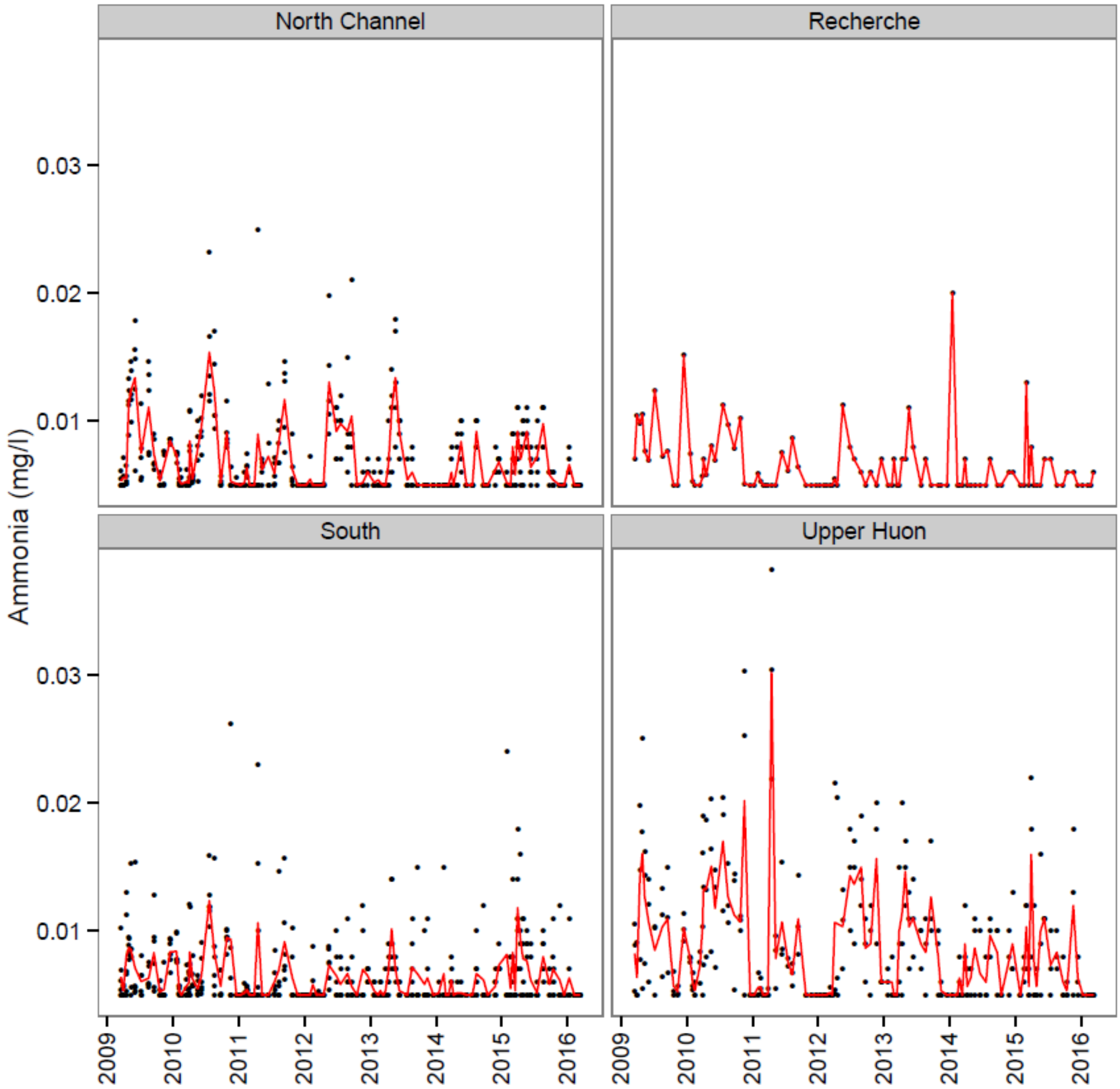

Figure 18: Surface mean ammonia concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

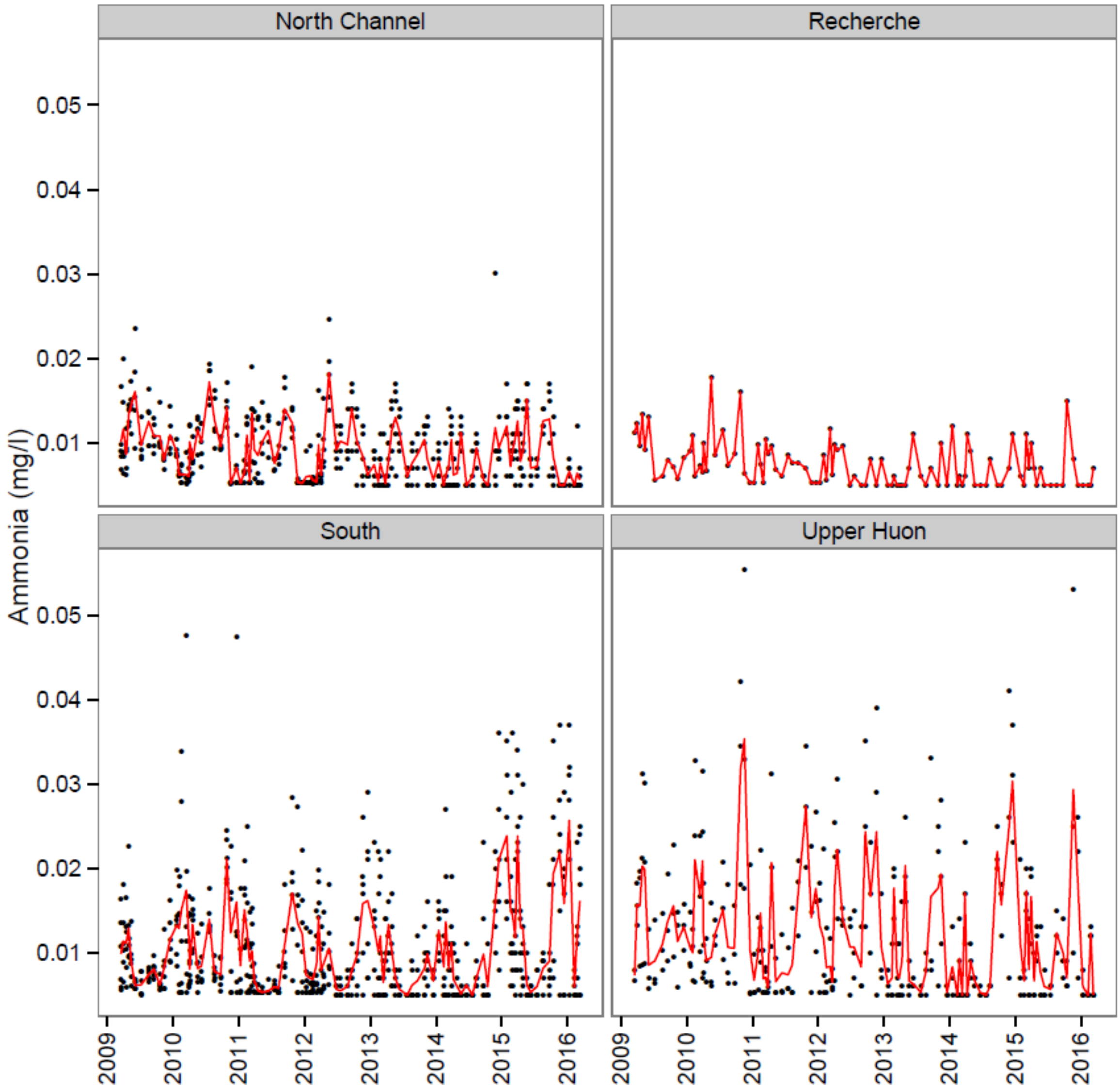

Figure 19: Bottom mean ammonia concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

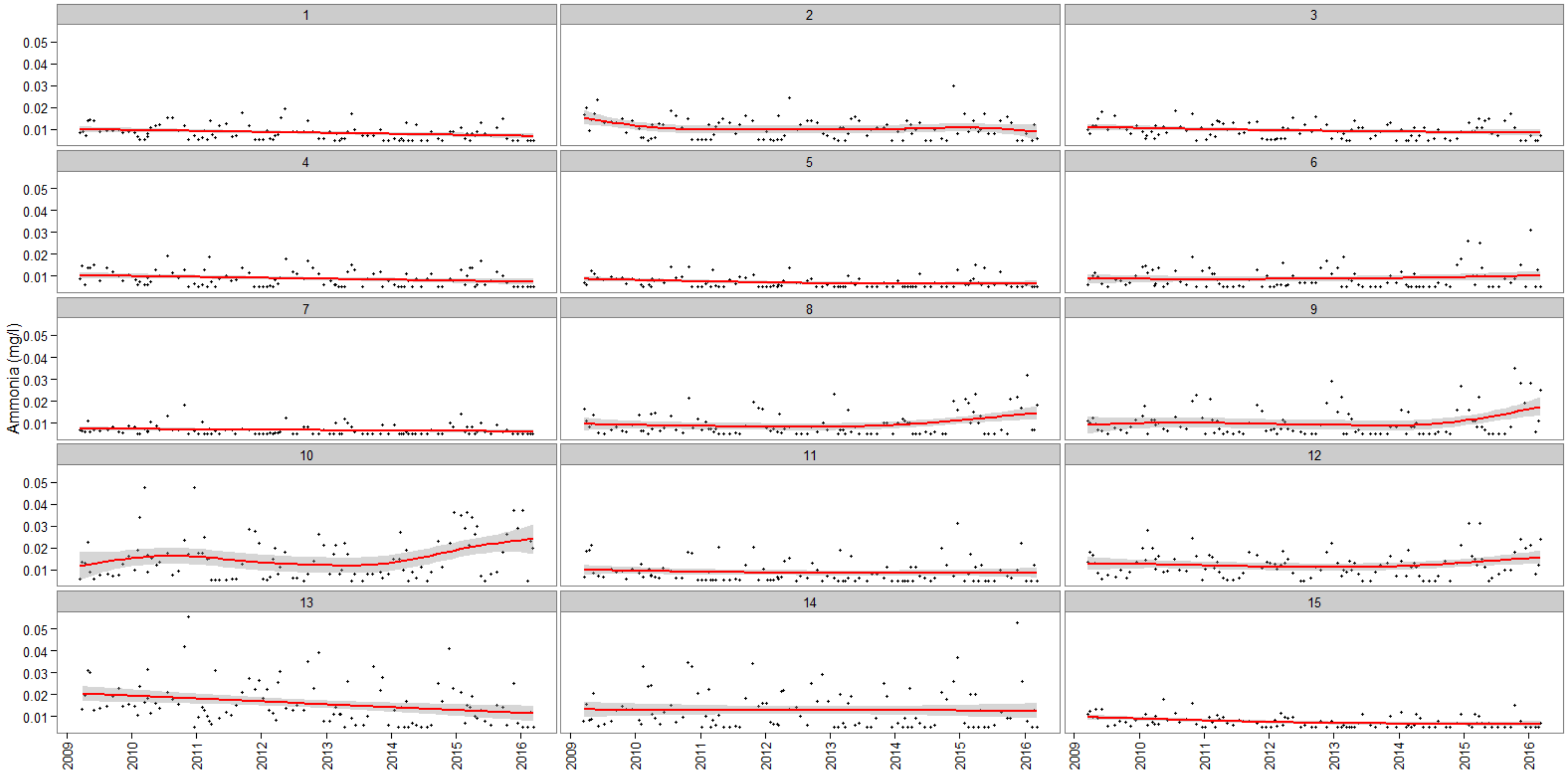

Figure 20: Bottom ammonia concentration at each BEMP site from March 2009 – March 2016. Red line is a GAM and shaded area represent 95% confidence intervals of the model.

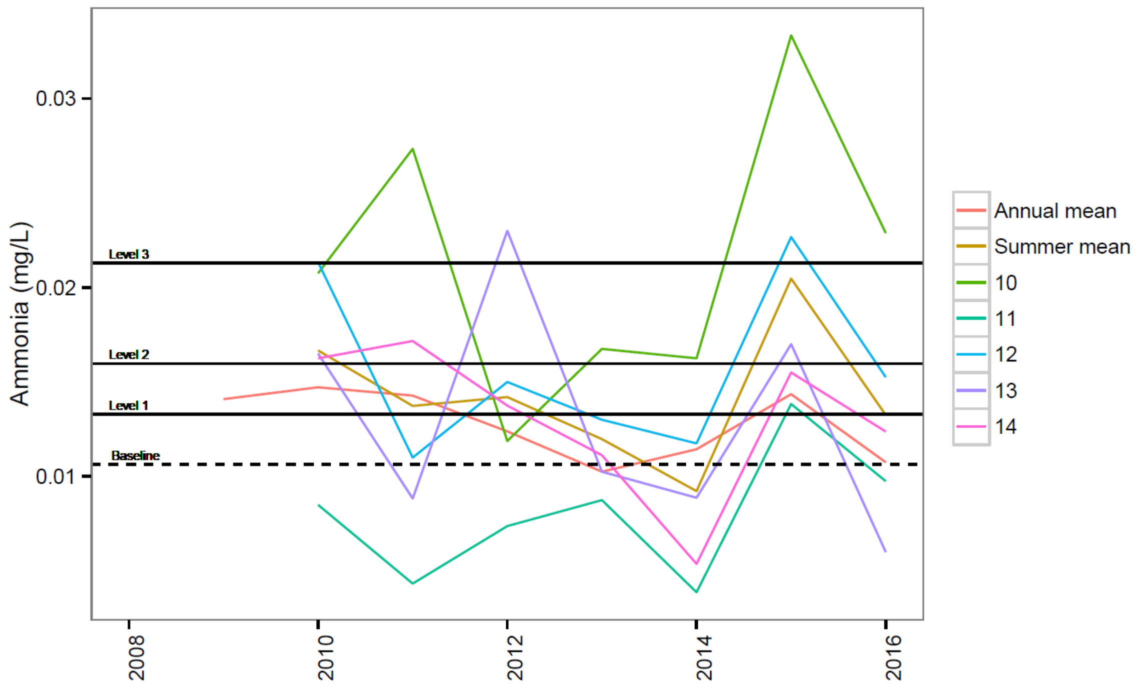

Figure 21: Performance against recommended bottom ammonia trigger levels in the Huon/Esperance MFDP area. The recommended baseline and triggers were proposed by Volkman *et al.* (2009) and are summarised in the methods.

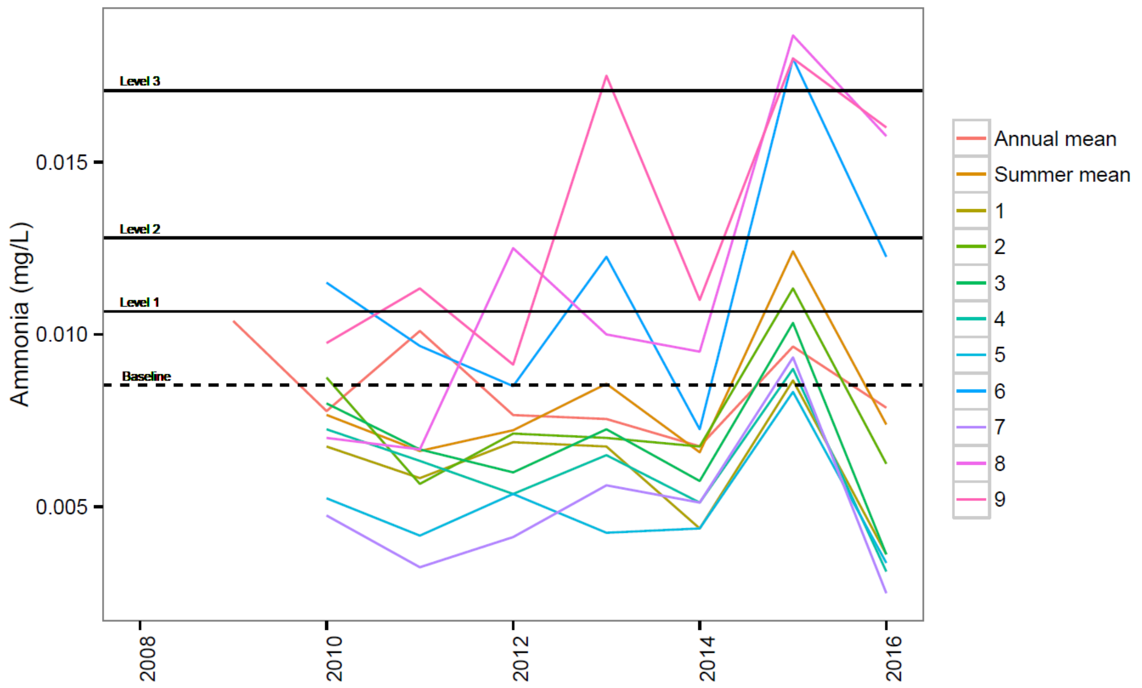

Figure 22: Performance against recommended bottom ammonia trigger levels in the D'Entrecasteaux Channel MFDP area. The recommended baseline and triggers were proposed by Volkman *et al.* (2009) and are summarised in the methods.

4.2.2. Phosphorus

Total P concentration was highly variable in all four regions with no discernible trend through time (Figures 23 and 24). However, the ability to discern fine-scale changes in total P concentration is confounded by the fact that the analytical sensitivity at which total P is reported has changed over the study timeframe (i.e. there has been a reduction in reporting level from 0.001 mg/l to 0.01 mg/l). As such, it is difficult to interpret whether there are any trends in recent years, with the coarser measurement potentially influencing interpretation of recent data. That said, there was a clear seasonal pattern in dissolved reactive P (i.e. phosphate) concentration, which was particularly evident in surface waters (Figures 25 and 26). It also appears that dissolved reactive P concentrations have declined through time in both bottom and surface waters throughout the system, independent of any seasonal variation.

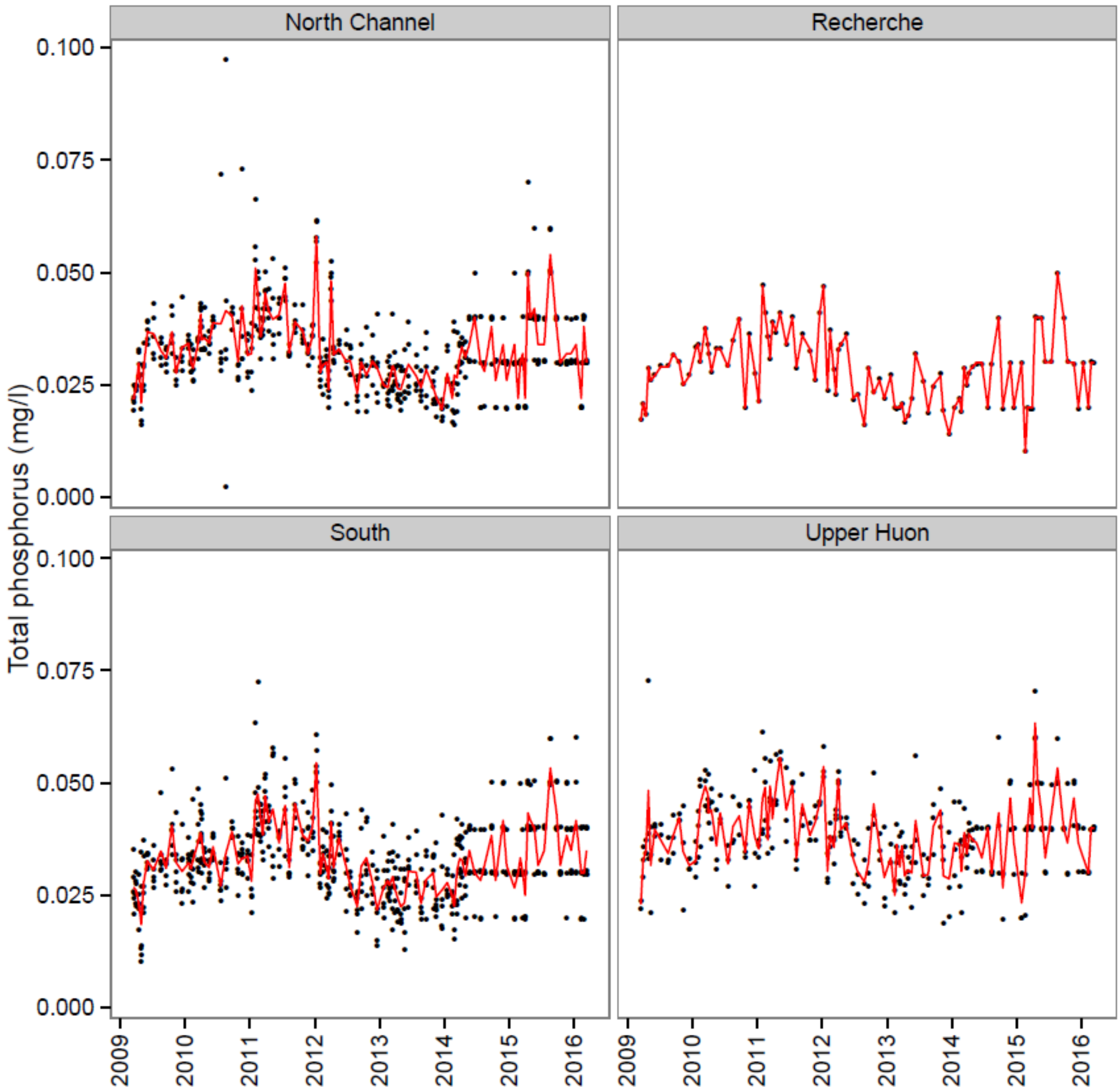

Figure 23: Bottom total P concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

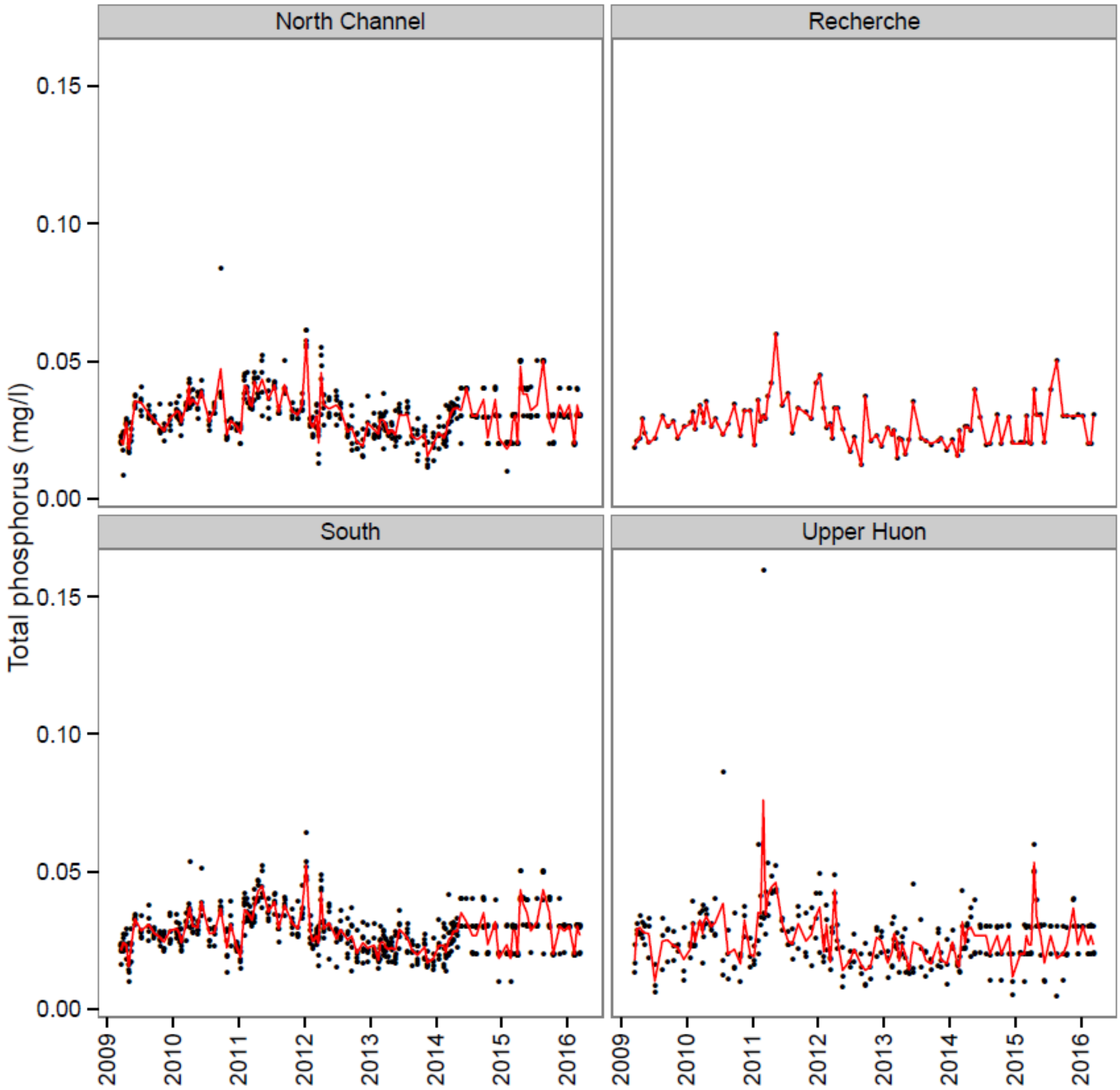

Figure 24: Surface total P concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

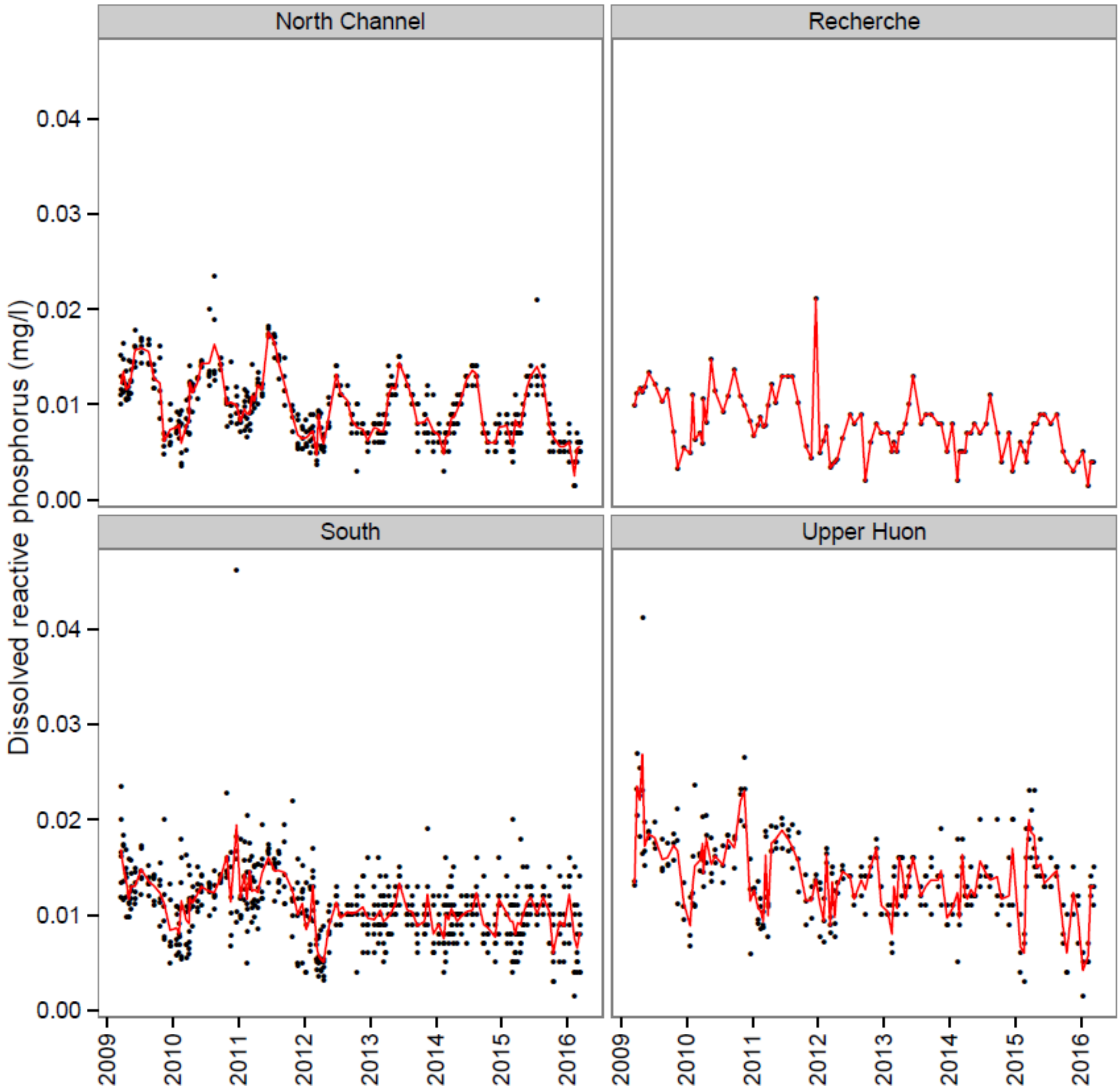

Figure 25: Bottom dissolved reactive P concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

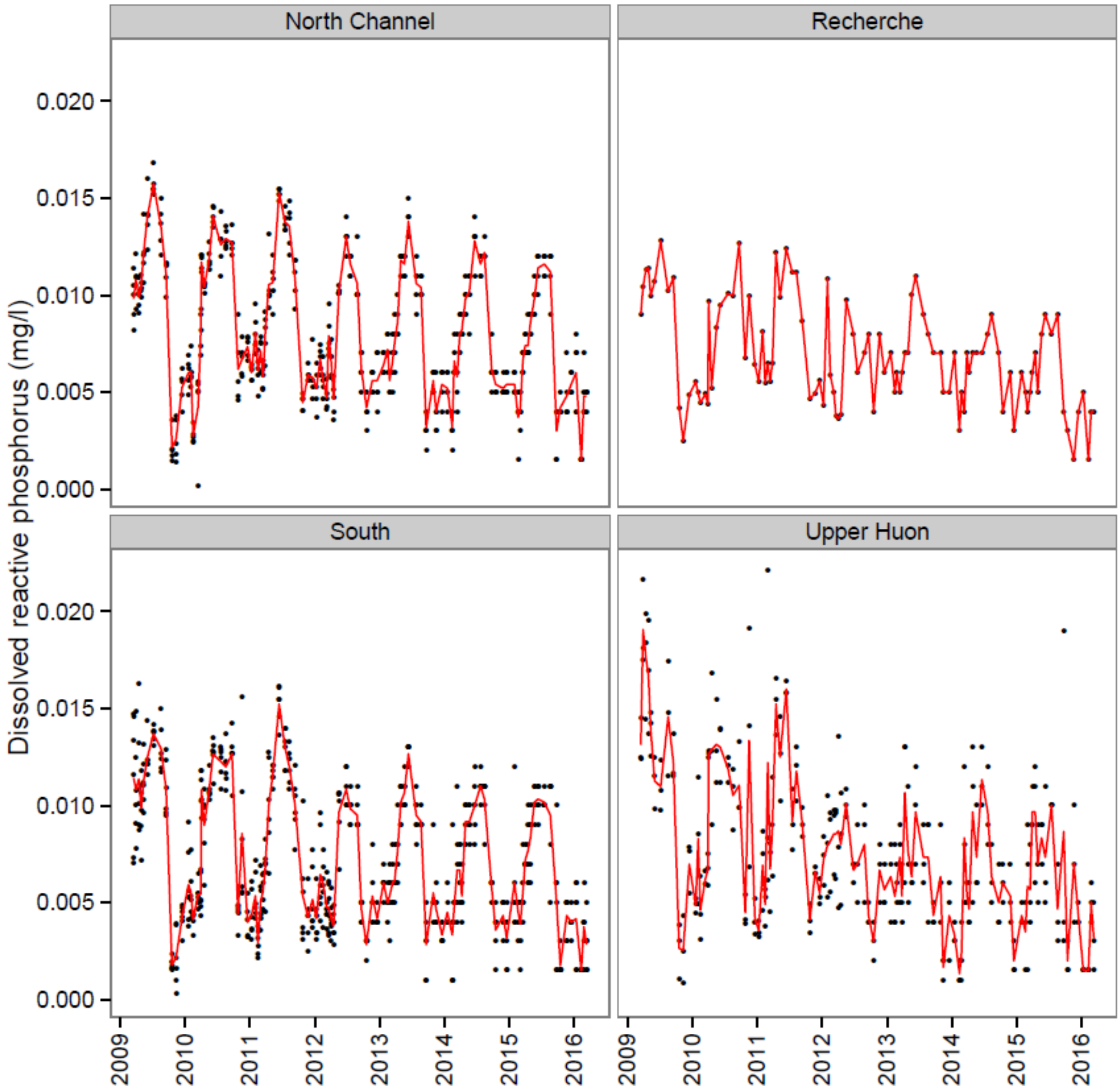

Figure 26: Surface dissolved reactive P concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

4.2.3. Dissolved oxygen

There was no obvious long term trend in surface or bottom water dissolved oxygen concentration in any of the regions over the time period investigated (Figures 27 and 28). Nor was there any obvious change in bottom water oxygen concentrations at those sites where elevated bottom water ammonia concentrations were observed (i.e. sites 6, 8, 9, 10 and 12) during 2015 (see Appendix v for a site level breakdown of bottom dissolved oxygen). There was, however, a seasonal pattern in both surface and bottom dissolved oxygen concentration with oxygen levels generally increasing over winter and decreasing over summer. This is not surprising and probably reflects the influence of natural seasonal biotic and abiotic processes (e.g. variability in oxygen saturation with temperature, river induced stratification and reduced mixing, breakdown of algal matter, nitrification). It is also possible that anthropogenic factors have influenced this seasonal response. There are strong temporal differences in the aquaculture production cycle, with feed inputs and fish biomass peaking in late spring and summer before harvesting (see feed and biomass section). There are several biogeochemical interactions associated with fish production that could exacerbate localised oxygen depletion. Fish not only draw down oxygen directly via respiration, the breakdown of particulate waste (feed and faeces) in the sediments consumes oxygen during mineralisation, and the ammonia excreted during fish respiration can also lead to oxygen consumption via nitrification, which coverts ammonia to nitrate, consuming oxygen in the process.

Dissolved oxygen concentrations were generally lower in the bottom waters of the upper Huon estuary (often <5 mg/l), as a result of stratification (i.e. a lack of mixing between the dense marine bottom layer and the less dense freshwater from river flow). Interestingly, between 2009 and 2012 all three of the sites in the upper Huon estuary (sites 11, 13 and 14) experienced particularly low dissolved oxygen (<4 mg/l) levels in the bottom waters. It is not clear what caused this but such low levels have not been observed since 2012 suggesting the shift southward in the Huon Estuary, and into the D'Entrecasteaux Channel, may have improved conditions in the upper Huon Estuary.

Draft performance indicators have been outlined in the BEMP for both dissolved oxygen (%) and absolute oxygen (mg/l). For dissolved oxygen, baselines levels have been established for each MFDP area based on the lower 20th percentiles from the first year of BEMP sampling (March 2009 – February 2010), with only the samples from the open channel sites being included, as it was felt that the bays would be naturally prone to lower DO. For absolute oxygen, baseline levels of 5 mg/l and 6 mg/l were proposed for bays and mid-channel sites respectively (Volkman *et al.*, 2009). During the exceedance period in 2015 none of the trigger limits for dissolved oxygen (percent or absolute) were breached for any depth, MFDP area, or bay/channel combination (Tables 3 and 4), nor was there any indication that the proportion of values falling below the baselines was increasing through time.

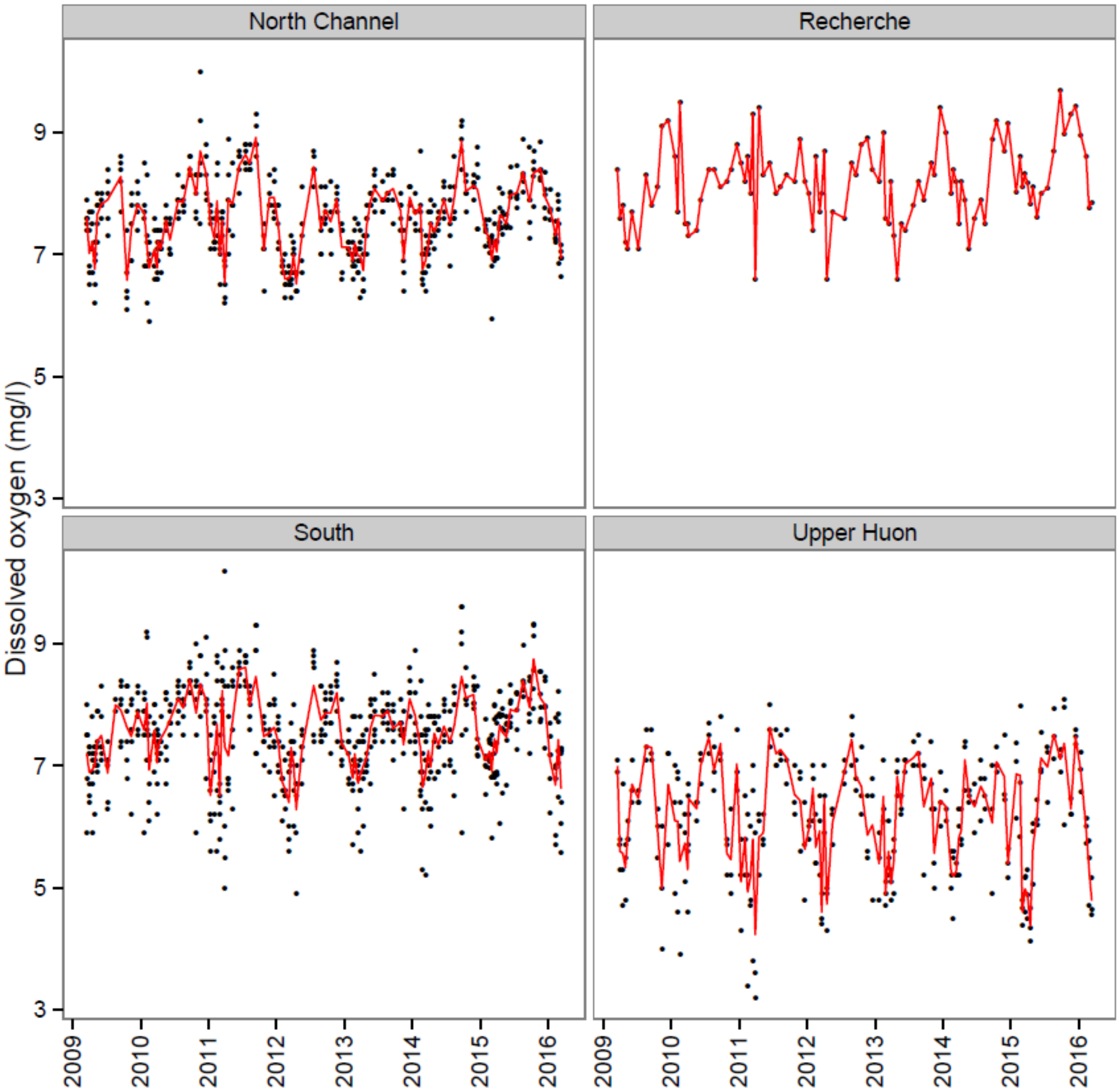

Figure 27: Bottom mean dissolved oxygen concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

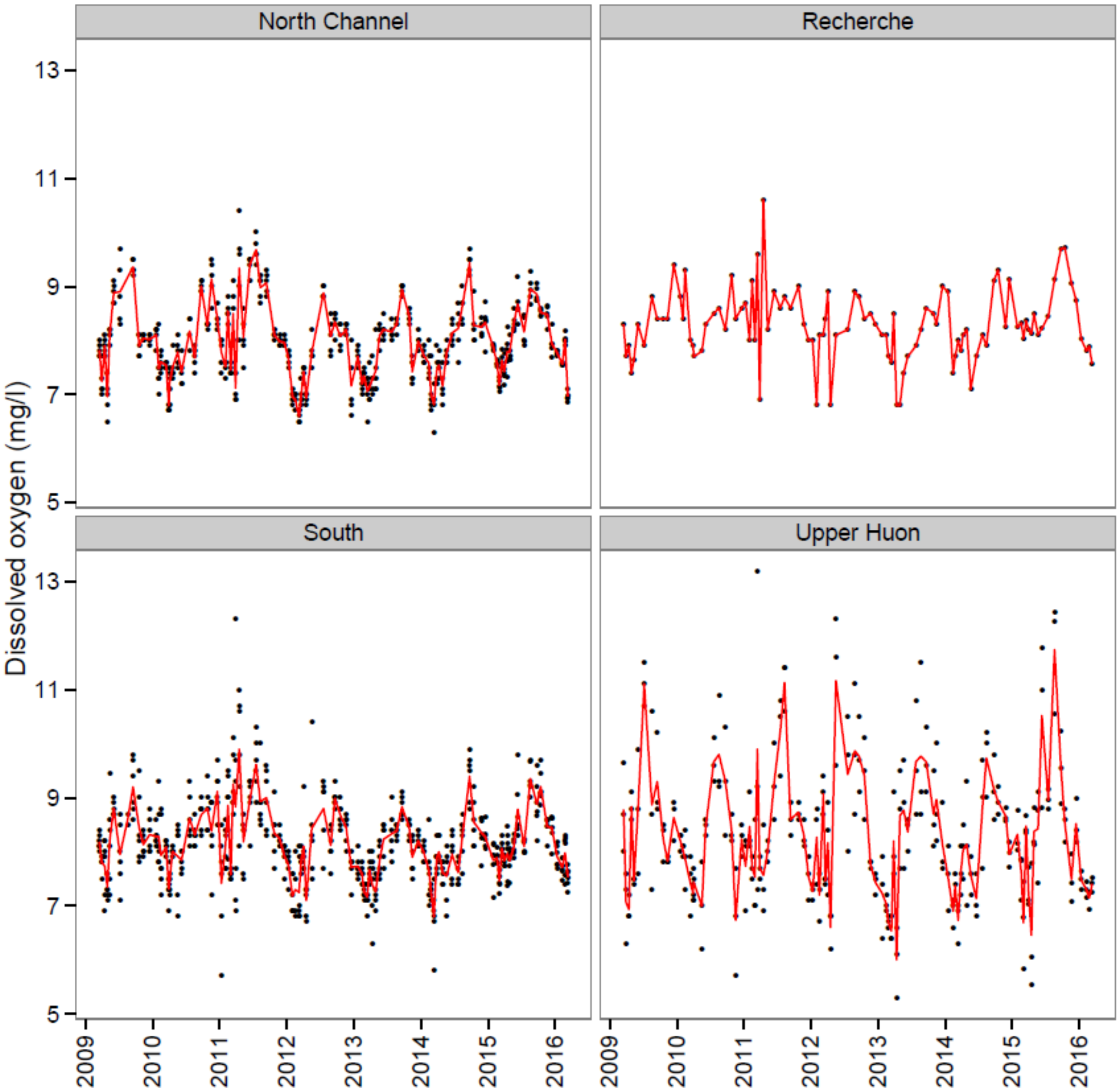

Figure 28: Surface mean dissolved oxygen concentration at the regional scale (northern D'Entrecasteaux Channel, upper Huon estuary, southern region and Recherche Bay) from March 2009 – March 2016. The trend line is the mean during each BEMP sampling event in each region.

Table 3: Performance against recommended absolute oxygen (mg/l) trigger levels for bay and mid-channel sites within each MFDP area. The recommended baseline and triggers were proposed by (Volkman *et al.*, 2009) and are summarised in the methods.

	D'Entrecasteaux Channel MFDP area				Huon Estuary MFDP area			
	Bay		Channel		Bay		Channel	
Year	n	Percent below baseline	n	Percent below baseline	n	Percent below baseline	n	Percent below baseline
2009	144	0.00	180	1.11	72	1.39	108	14.81
2010	180	0.00	225	0.00	90	2.22	135	11.85
2011	180	0.00	225	1.78	90	5.56	135	14.07
2012	180	0.00	225	0.44	90	3.33	135	14.07
2013	180	0.00	225	0.00	90	2.22	135	14.81
2014	180	0.00	225	0.00	90	3.33	135	13.33
2015	180	0.00	225	0.00	90	4.44	135	15.56
2016	48	0.00	60	0.00	24	4.17	36	22.22

Table 4: Performance against recommended dissolved oxygen (% saturation) trigger levels for each depth, within bays and open channel sites in each MFDP area. The recommended baseline and triggers were proposed by (Volkman *et al.*, 2009) and are summarised in the methods.

		Bays						Channel					
		Bottom		Middle		Surface		Bottom		Middle		Surface	
MFDP area	Year	n	Percent below baseline	n	Percent below baseline	n	Percent below baseline	n	Percent below baseline	n	Percent below baseline	n	Percent below baseline
D'Entrecasteaux Channel	2009	48	20.83	48	25.00	48	25.00	60	21.67	60	21.67	60	23.33
	2010	60	5.00	60	18.33	60	20.00	75	4.00	75	25.33	75	34.67
	2011	60	5.00	60	10.00	60	8.33	75	10.67	75	9.33	75	10.67
	2012	60	13.33	60	36.67	60	45.00	75	20.00	75	38.67	75	45.33
	2013	60	10.00	60	11.67	60	31.67	75	9.33	75	22.67	75	37.33
	2014	60	5.00	60	16.67	60	33.33	75	12.00	75	28.00	75	40.00
	2015	60	3.33	60	11.67	60	28.33	75	1.33	75	9.33	75	28.00
	2016	16	12.50	16	0.00	16	12.50	20	0.00	20	0.00	20	10.00
Huon/Esperance	2009	24	16.67	24	25.00	24	25.00	36	19.44	36	22.22	36	25.00
	2010	30	16.67	30	6.67	30	13.33	45	15.56	45	15.56	45	17.78
	2011	30	33.33	30	13.33	30	13.33	45	15.56	45	11.11	45	13.33
	2012	30	16.67	30	13.33	30	13.33	45	17.78	45	17.78	45	11.11
	2013	30	16.67	30	13.33	30	16.67	45	15.56	45	17.78	45	20.00
	2014	30	20.00	30	16.67	30	30.00	45	20.00	45	13.33	45	26.67
	2015	30	13.33	30	13.33	30	6.67	45	22.22	45	17.78	45	17.78
	2016	8	12.50	8	12.50	8	0.00	12	25.00	12	16.67	12	8.33

4.2.4. Algal abundance and chlorophyll-a

4.2.4.1 Site characterization

The main environmental variables that can affect the spatial distribution, abundance and composition of phytoplankton (temperature, N:P ratio, ammonia, nitrate, ammonia:nitrate ratio, salinity or silicate (Si)) are spatially variable, with greater fluctuations of most variables in the Huon Estuary (Figure 29). Principal component analysis (PCA) clearly shows how sites cluster based on temperature, salinity, N:P ratio, ammonia:nitrate ratio, and Si, ammonia and nitrate concentrations (Figure 30). The first two-principal components account for 60.1% of the overall variation in the site distributions. Ammonia, N:P ratio and Si strongly correlate along PC1 and respond negatively to changes in salinity. This highlights the influence of the Huon River discharge on the nutritional state of the region. In contrast, total N input and nitrate strongly correlate along PC2 and respond to changes in temperature and ammonia:nitrate ratio. These results would tend to suggest that a key driver of phytoplankton composition is the input of nutrient rich-sub-Antarctic waters (nitrate) that reach southern Tasmania via the Antarctic Circumpolar Current in winter.

The BEMP sites clustered into three sub-groups (each site cluster includes 68% of probability distribution) based on PCA of the spatial and temporal patterns of key physiochemical variables in the water column from 2009–2014 (see Figure 30). As such, for the purposes of further analyses pertaining to algal communities during the HAC exceedance, sites were grouped into the D'Entrecasteaux channel (sites 1–9), lower Huon Estuary (sites 10–12), and upper Huon Estuary (sites 13 and 14). The Recherche Bay reference site (site 15) clustered with D'Entrecasteaux Channel sites but was treated individually as it is unlikely the exceedance had any influence on this site.

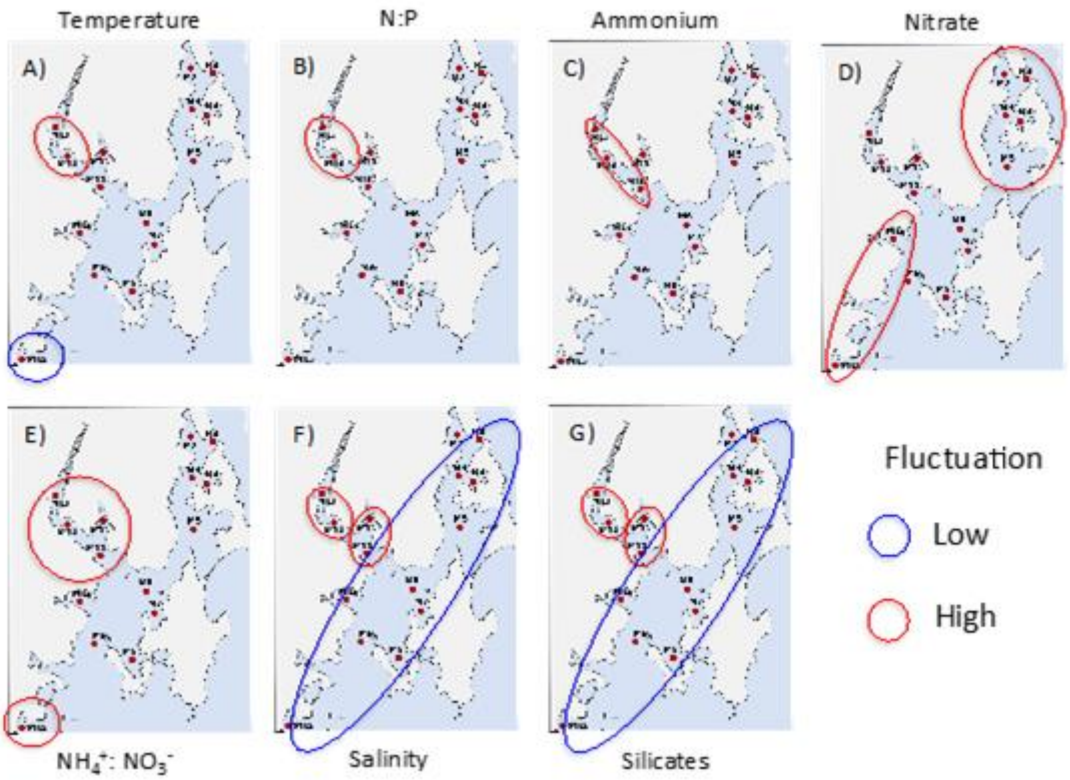

Figure 29: Schematic representation of patterns in nutrient concentration, nutrient ratio and physiochemical variation at BEMP sites for the assessment of nutrient exceedance in 2015. PCA analyses (see Figure 30) indicate that sites grouped into the D'Entrecasteaux channel (sites 1 to 9), lower Huon (sites 10 to 12), upper Huon (sites 13 and 14) and control (site 15).

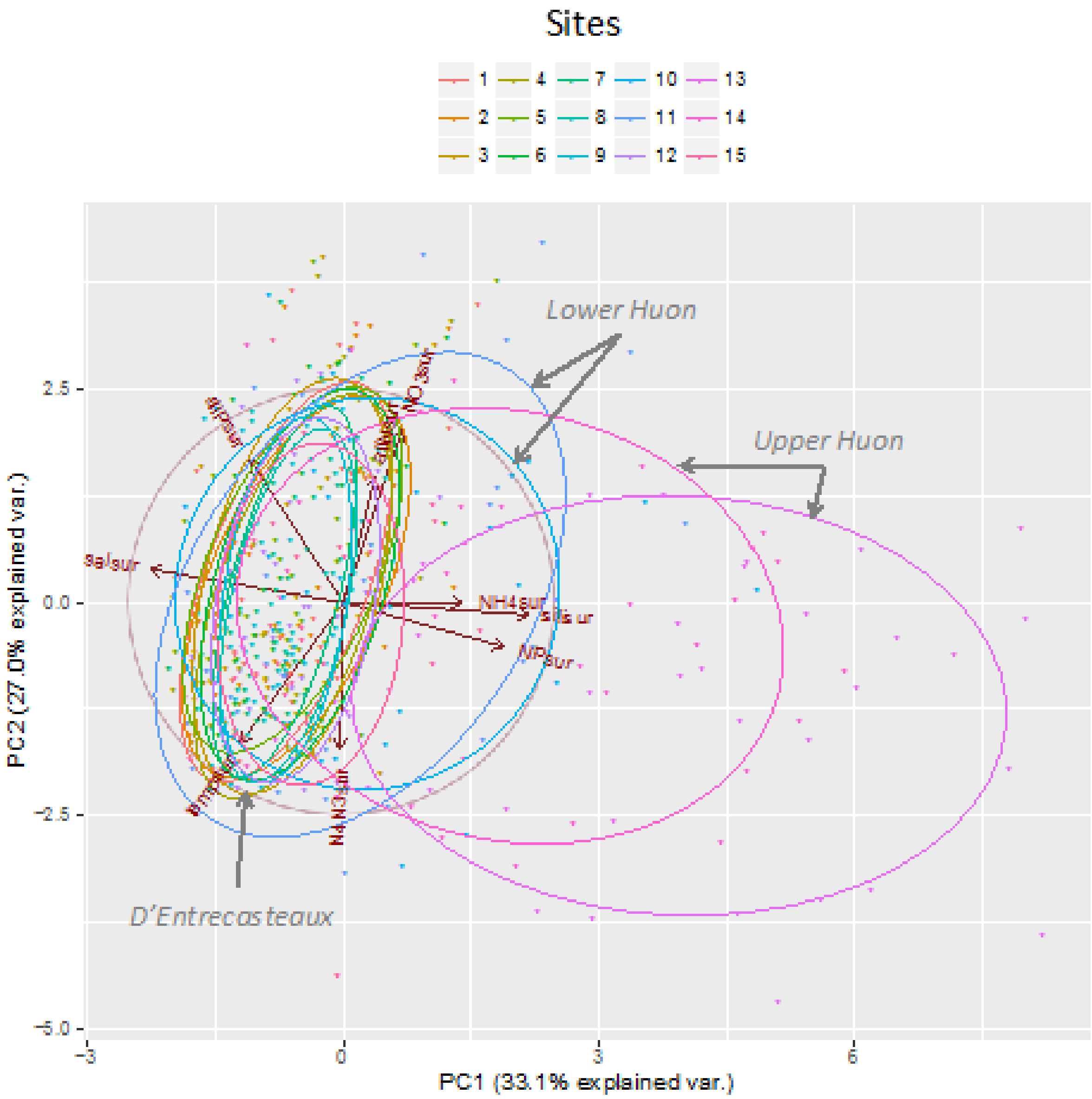

Figure 30: Principal component analysis ordination showing the relationship between the 15 BEMP study sites based on superficial environmental variables measured from 2009-2014 (pre-nutrient exceedance period). Clusters were created using the default parameters of the 'ggbiplot' function in R and include a 68% probability of inclusion.

4.2.4.2 Pre- and post- HAC N exceedance assessment

The PCA of combined environmental data (temperature, N:P ratio, ammonia, nitrate, ammonia:nitrate ratio, salinity or silicate) during, and after, the HAC exceedance (2015–2016; hereafter termed post-exceedance period) is shown in Figure 31. The first two-principle components account for 66.4% of the overall variation in the distribution of sites. In both the pre-exceedance period (2009–2014) (Figure 30), and post-exceedance period (2015–2016) (Figure 31) there is strong correlation between total N, nitrate, ammonia, N:P ratio and Si along the two main component axes, suggesting there was no change in the principal environmental variables that lead to site clustering.

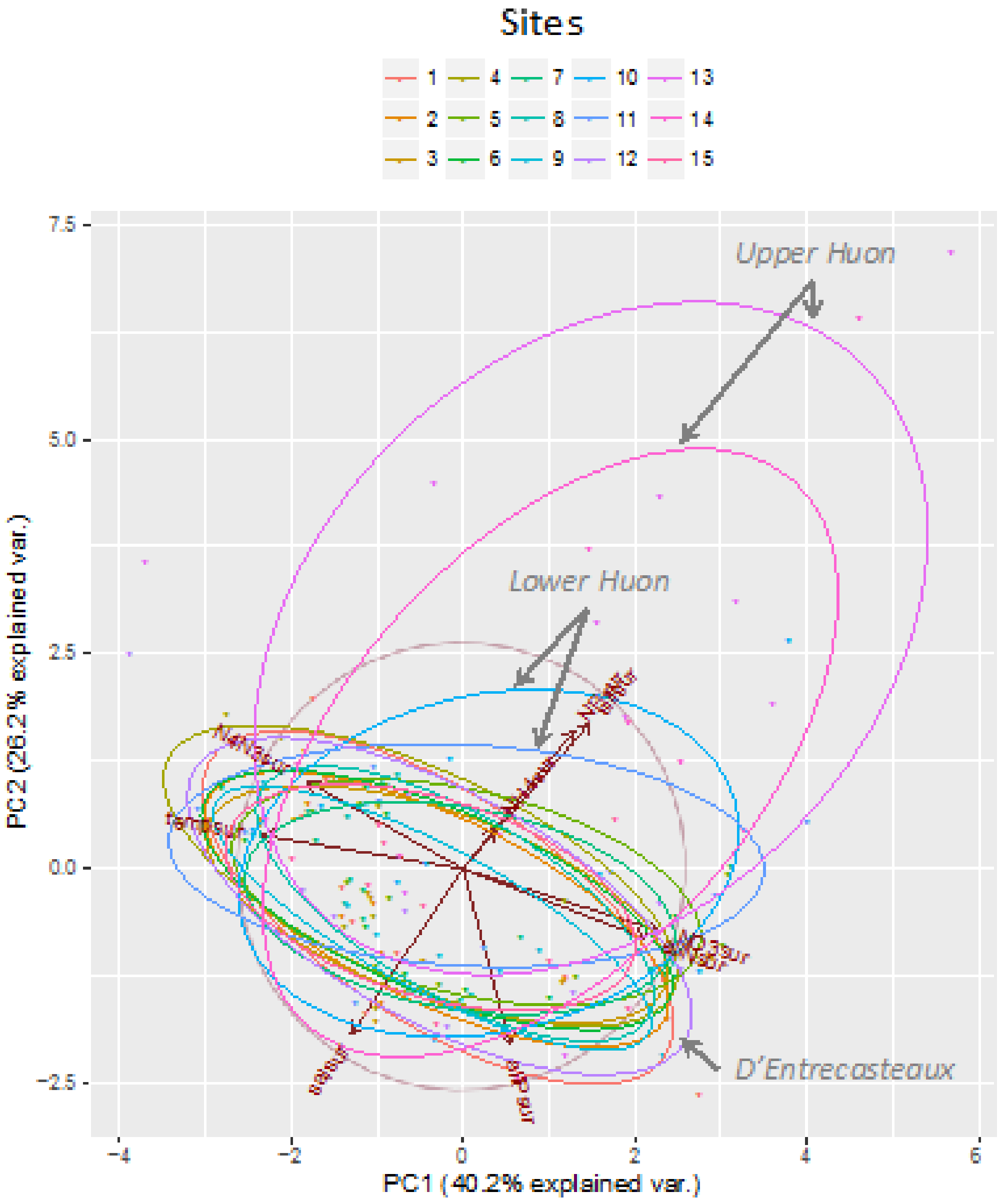

Figure 31: Principal component analysis ordination showing the relationship between the 15 BEMP study sites based on environmental variables measured between 2015–2016 (post-nutrient exceedance period).

4.2.4.3 Nutritional state

Based on measures of N, P and Si in surface waters (i.e. the key drivers of phytoplankton composition and abundance), the nutritional condition of the entire D'Entrecasteaux/Huon system is shown in Figure 32. In the context of the system as a whole, N:P, Si:N and Si:P ratios pre (2009–2014) - and post (2015–2016) N exceedance were not significantly different (ANOVA, p = >0.05). At all sites, during both time periods, there was a high level of variability in the Si:N ratio, and the N:P ratios were consistently lower than the Redfield ratio of 16:1 (Redfield, 1934), suggesting N limitation.

In contrast, when the system is divided into the regions used in the subsequent algal analysis (i.e. D'Entrecasteaux, upper Huon and lower Huon) there were significant differences between the N:P, Si:N and Si:P ratios in the different regions (ANOVA, $p = <0.05$). Between 2009 and 2014, Si levels in the upper Huon were high and there were sporadic peaks of high N:P ratios > the Redfield ratio (16:1), and similar patterns were observed during 2015–2016 (Figure 33). The sites in the D'Entrecasteaux channel were highly Si limited, especially before the N exceedance (Figure 33). The lower Huon would appear to be a transition area that is characterized by highly fluctuating Si:P and Si:N ratios. Given where the majority of N inputs occurred during the HAC N exceedance, we might expect to see the greatest response in the lower Huon Estuary (i.e. sites 10, 11 and 12), however, there were no significant differences in nutrient ratios detected between pre- and post- HAC exceedance at these sites (ANOVA, $p = >0.05$).

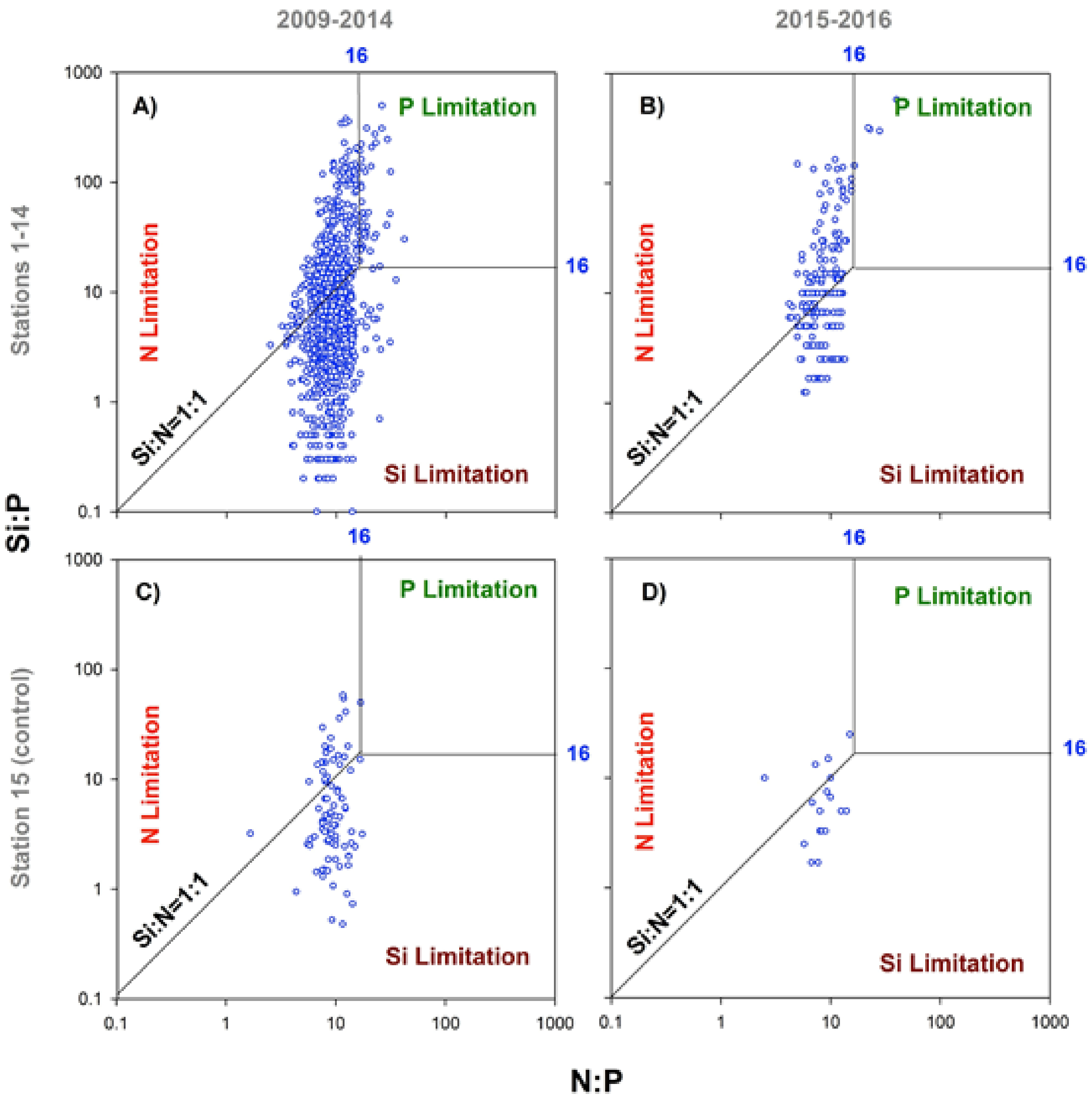

Figure 32: Si:P versus N:P molar ratios for pre- (2009–2014) and post (2015–2016) nutrient exceedance in the impacted D'Entrecasteaux-Huon system and control site. The number of data points in each quadrant indicates the tendency for potential limitation by a particular nutrient.

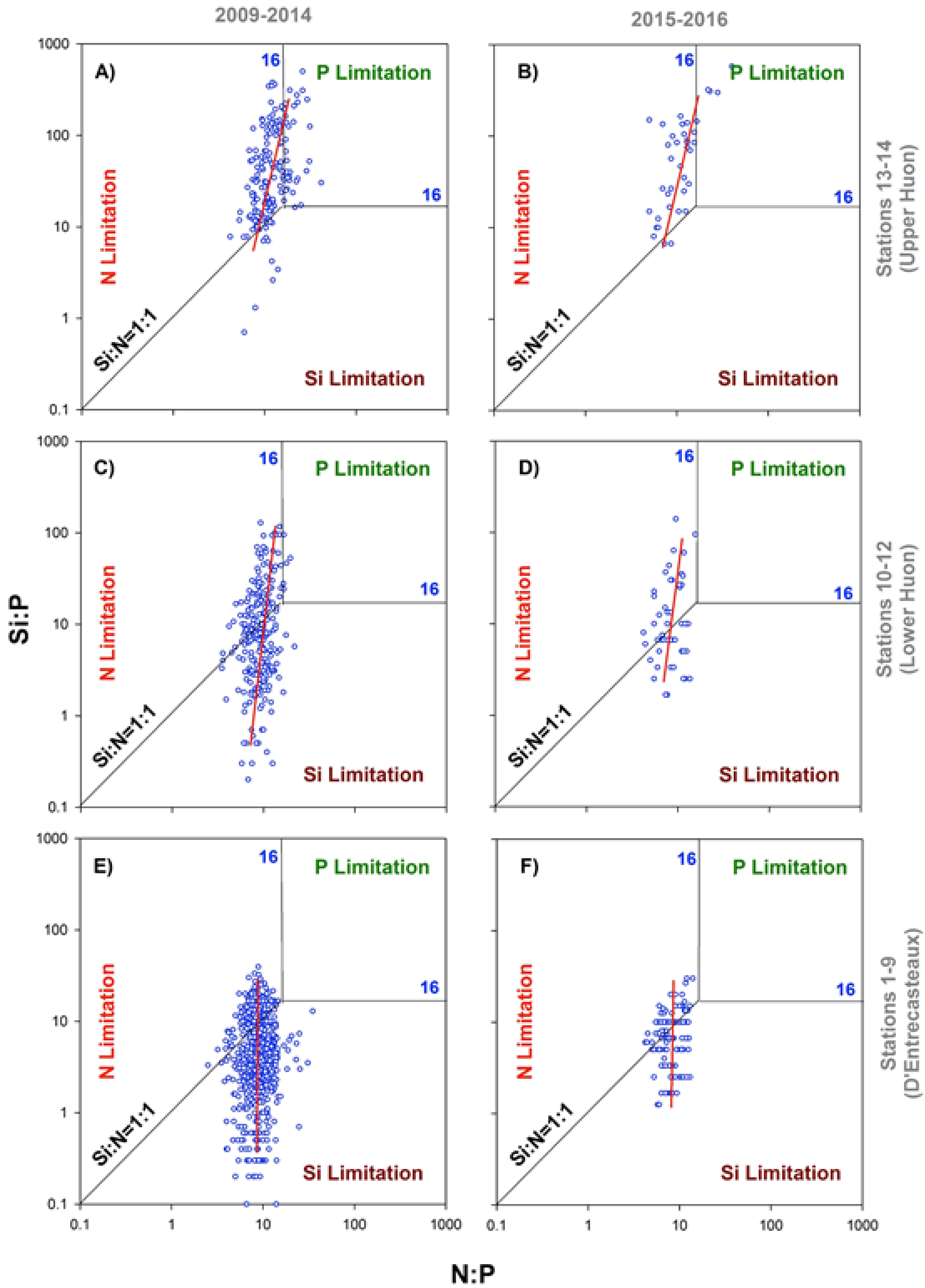

Figure 33: Si:P versus N:P molar ratios for pre- (2009-2014) and post (2015-2016) nutrient exceedance in site clusters. The number of data points in each quadrant indicates the tendency for potential limitation by a particular nutrient.

4.2.4.4 Phytoplankton response

Among the 10 classes of phytoplankton discriminated at the 15 monitoring sites between 2009 and 2016 (Cryptophyceae [non-toxic group and important only between 2009–2012], Cyanobacteria, Dinophyceae, Euglenophyceae, Bacillariophyceae, Raphidophyceae, Chrysophyceae, Prymnesiophyceae, Prasinophyceae and Chlorophyceae) the Bacillariophyceae (diatoms) were the most abundant group throughout the entire system (Figures 34 and 35).

Diatoms and dinoflagellates represented >92% of the phytoplankton in the system (all BEMP surveys and all sites). Higher peaks in abundance of dinoflagellates were often observed in the Huon Estuary compared to the D'Entrecasteaux channel (Figure 36). In contrast, diatom abundance was relatively homogeneous at almost all monitoring sites (Figure 36), with slightly higher abundances (e.g. in 2014) in the northern D'Entrecasteaux Channel (sites 3 and 4). An exception was the reference site, which consistently had low abundance of diatoms throughout the monitoring period.

In order to assess how phytoplankton abundance responded pre- and post-HAC N exceedance, generalized linear models (GLMs) were applied using the principal components PC1, PC2 and the additive PC1+PC2 as explanatory variables (i.e. reflecting the combined environmental variable responses) from the PCA analyses in Figure 30 and Figure 31. In both periods, pre- and post-exceedance, the additive models (~sPC1 + PC2) had the best fit based on Akaike Information Criteria (Table 5). This suggests a complex interaction exists among all variables when predicting phytoplankton abundance. GLMs also showed that of the principal components, total N, nitrate, ammonia:nitrate ratio and temperature play key roles in phytoplankton abundance (Table 5 and Figure 30). This suggests that the seasonal dynamics of decreasing temperature in the water column coupled with an inversely correlated increase of nitrate (and concomitant variation in total N and ammonia:nitrate ratio – see PCA) due to the sub-Antarctic currents, had the greatest effect on phytoplankton community structure in the D'Entrecasteaux Channel/Huon Estuary system.

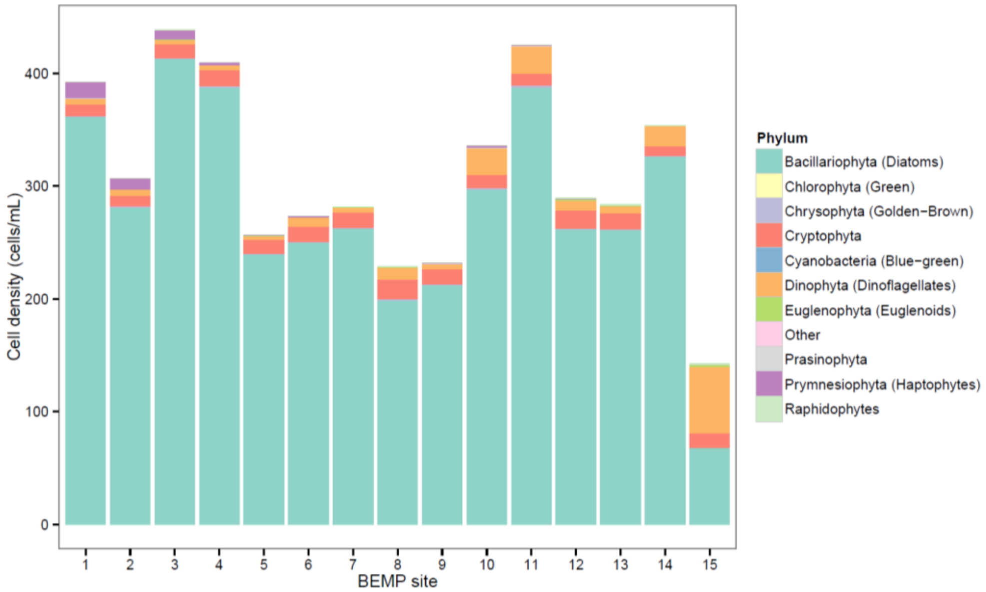

Figure 34: Mean algal abundance at each BEMP site for between 2009–2016.

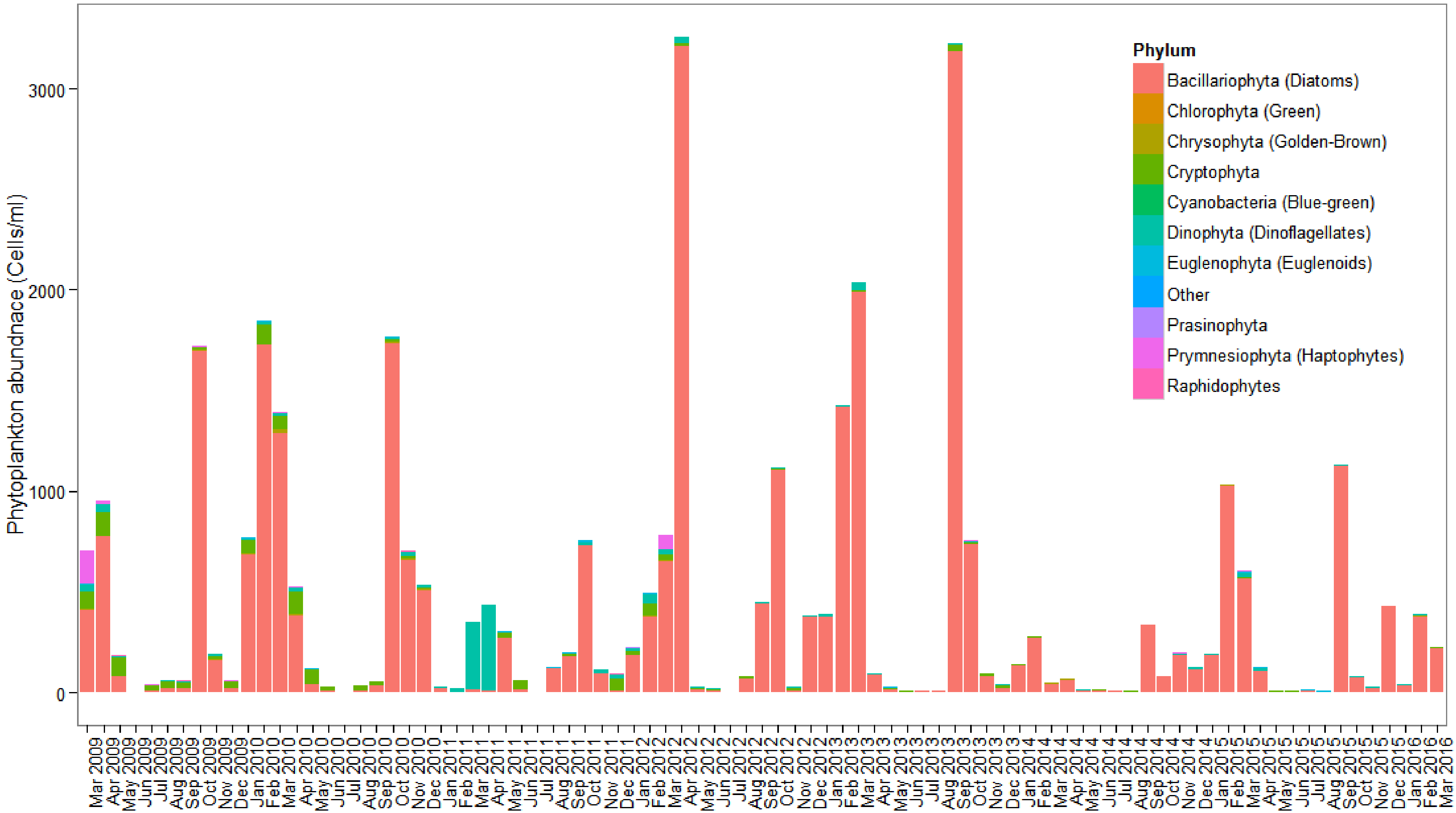

Figure 35: Temporal variation of the phytoplankton community (mean number of cells/ml per BEMP site) in the D'Entrecasteaux Channel/Huon Estuary system from March 2009– March 2016.

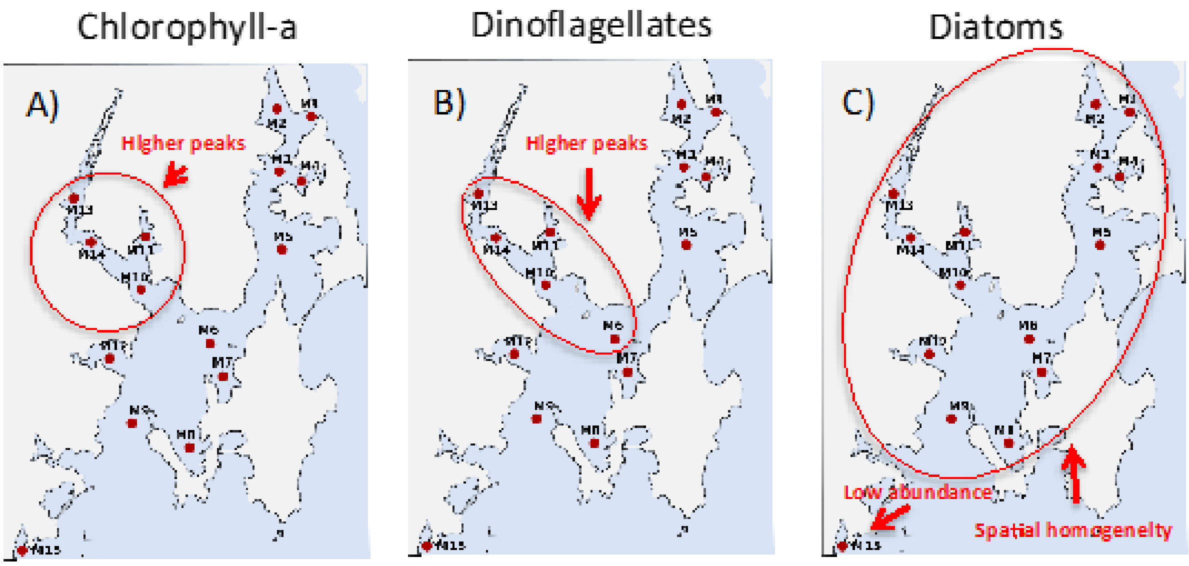

Figure 36: Overall spatial patterns of superficial chlorophyll-a, dinoflagellates and diatoms between 2009 and 2016 in response to nutritional and physical environmental drivers.

Table 5: Output of GLMs based on PCA pre- and post-HAC N exceedance. AIC is Akaike Information Criteria.

		~*s*(PC1)	**~*s*(PC2)**	**~*s*(PC1 + PC2)**
Pre (2009–2014)	*p* value	<0.001	<0.001	<0.001
	AIC	2272.7	2180.5	2162.8
Post (2015–2016)	*p* value	<0.001	<0.010	<0.010
	AIC	584.78	634.89	575.76

4.2.4.5 Nitrogen to phosphorus ratio

There is no evidence to indicate that changes in the N:P ratio are triggering blooms of phytoplankton in the D'Entrecasteaux Channel/Huon Estuary system. Between 2009 and 2016, >85% of the N:P ratios were <16:1 (i.e. below the Redfield ratio), which suggests a N limited system for phytoplankton growth. When the N:P ratio exceeded 16:1 there was no corresponding increase in phytoplankton abundance (Figure 37). The peaks in total phytoplankton concentration observed after the HAC N exceedance were not as high as those observed from 2009–2014 when N:P ratios were lower (i.e. between 8–12:1). This may suggest that the phytoplankton species that bloomed in those periods had a high P requirement relative to N and/or that other physical factors were more important in dictating growth.

4.2.4.6 Ammonia to nitrate ratio

There was no significant difference in ammonia:nitrate ratios between 2015/16 and 2009–14 (ANOVA, p = >0.05). Phytoplankton abundance peaked overall at ammonia:nitrate ratios of 4 – 7 (Figure 37). Even though ammonia is important in promoting species specific algal blooms, such as the diatom *Skeletonema* spp. (Suksomjit *et al.*, 2009; Tada *et al.*, 2010), there was no evidence in this dataset to suggest that higher ammonia:nitrate ratios stimulate phytoplankton growth in the D'Entrecasteaux Channel/Huon Estuary system. In this case the highest ammonia:nitrate ratios were in fact strongly correlated with low phytoplankton concentrations (Figure 37).

4.2.4.7 Diatom and dinoflagellate abundance

This section presents a temporal and spatial analysis of the main microalgal groups (diatoms and dinoflagellates) in the D'Entrecasteaux Channel/Huon Estuary system. Note, to eliminate any seasonal biases, these analyses compare 2009 – 2014 with 2015 and do not include early 2016.

Monthly comparison of diatom and dinoflagellate abundance pre- and post-HAC N exceedance in the D'Entrecasteaux Channel/Huon Estuary system is shown in Figure 38. There was no significant temporal variation (ANOVA, p= >0.05) in the abundance of diatoms or dinoflagellates between 2015 (exceedance period) and 2009–2014. Only two peaks in abundance of the diatoms *Skeletonema* sp. and *Chaetoceros socialis* occurred in 2015 in the Huon Estuary in February and September, respectively. Both species have a high surface/volume ratio (small cells; r-strategists) and rapid growth rates (compared to big cells e.g. *Rhisozolenia*; K-strategist) under nutrient depleted conditions.

Figure 39 shows a comparison between the monthly historical (2009–2014) patterns in species richness (diatoms and dinoflagellates) and the monthly species richness in 2015 (i.e. during the HAC N exceedance). In general, the number of diatom species in 2015 was similar to historical levels with slightly higher species diversity in September in the entire D'Entrecasteaux Channel/Huon Estuary system. Dinoflagellate species richness during 2015 was also similar to historical levels (anomalies ~<2 species). An exception was at Recherche Bay (Fig. 12H) which had four dinoflagellate species present when ~2 was more typical.

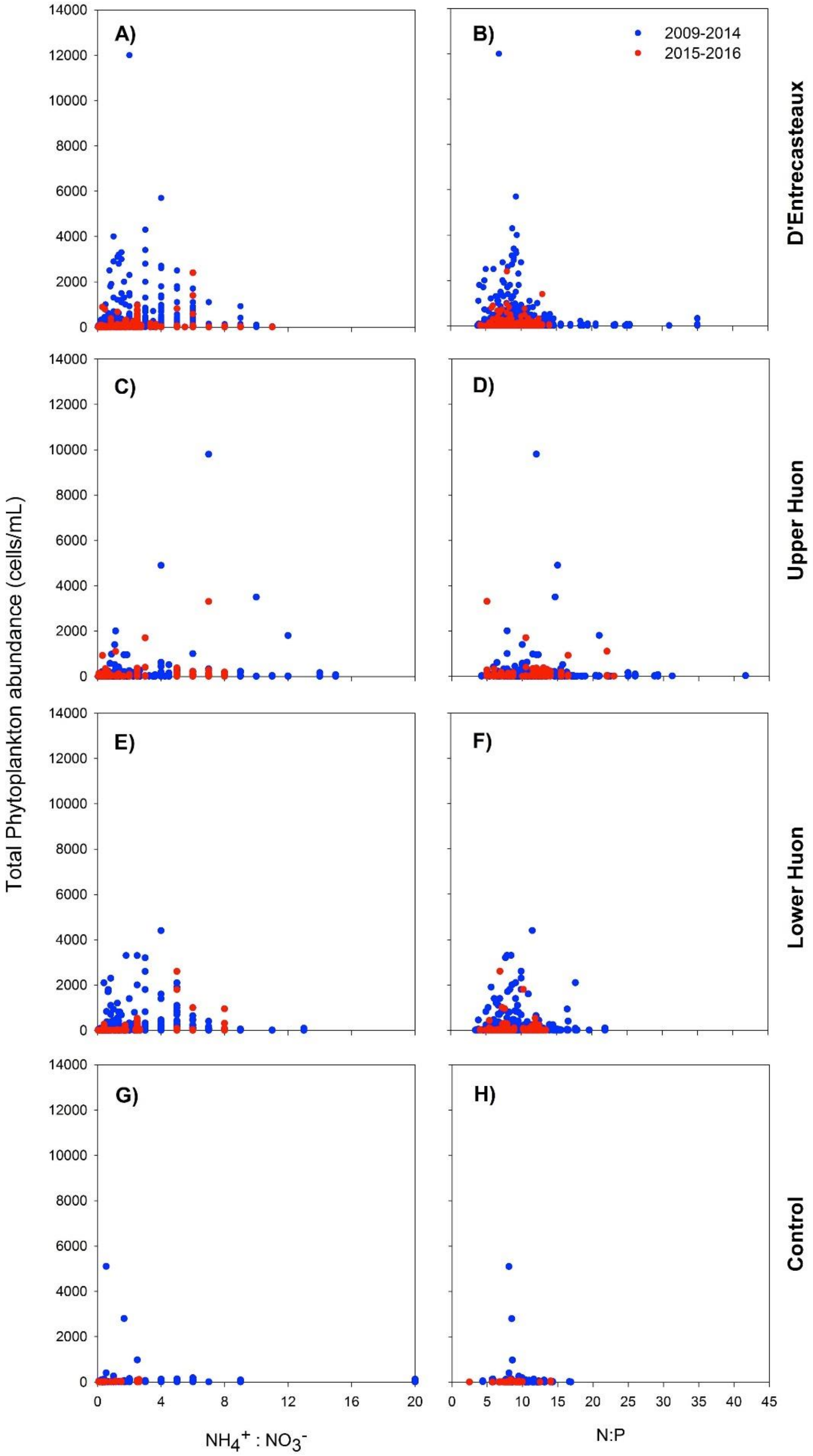

Figure 37: Relationship of total phytoplankton abundance with ammonia:nitrate and nitrate:phosphorus ratios at four sub-regions of the D'Entrecasteaux-Huon system. Blue dots represent data obtained between 2009 and 2014 (pre-exceedance) and red dots data obtained between 2015 and 2016 (post-exceedance).

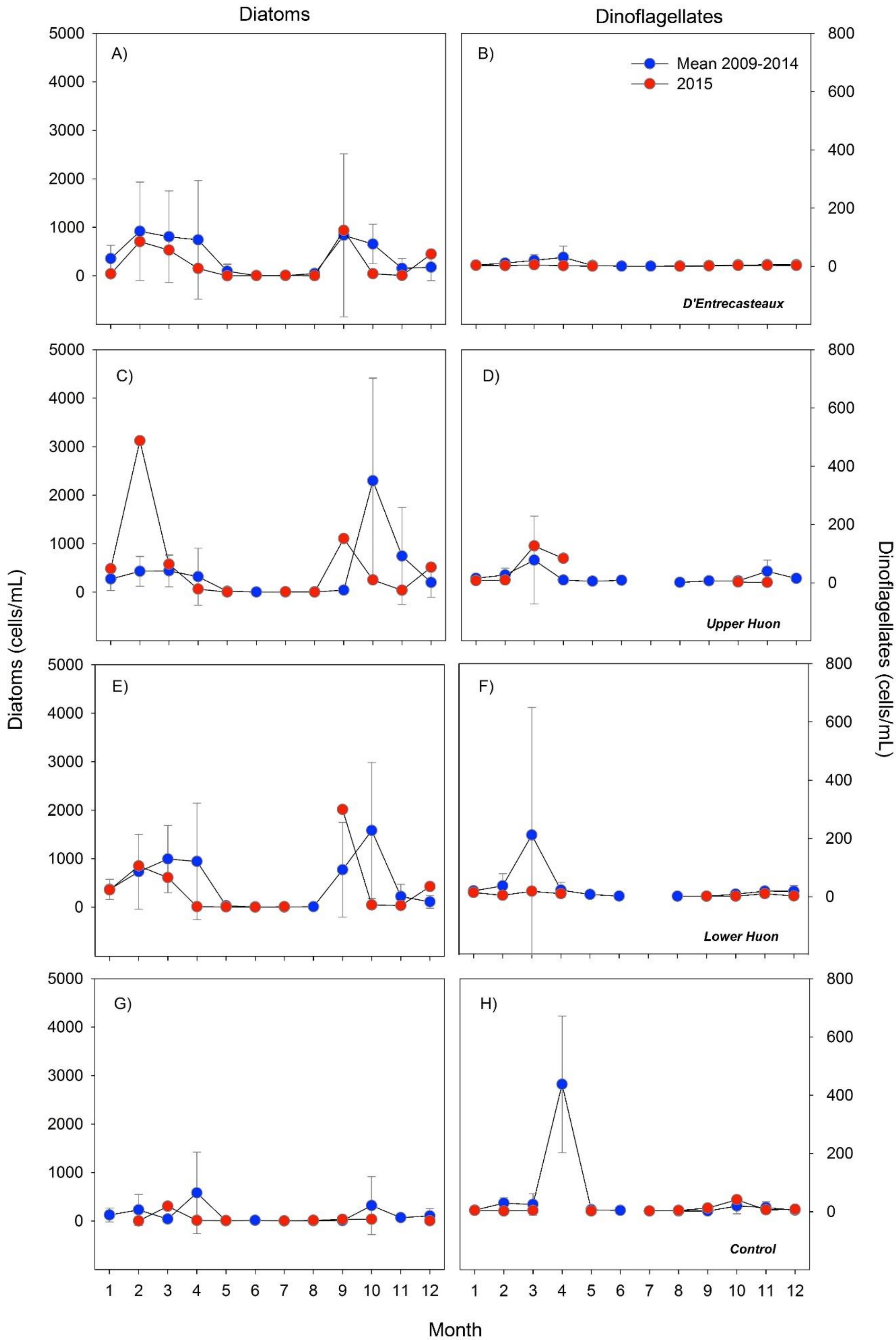

Figure 38: Comparison of historical monthly variation in the abundance of diatoms and dinoflagellates between 2009-2014 (mean±SD) and 2015 at the D'Entrecasteaux-Huon system.

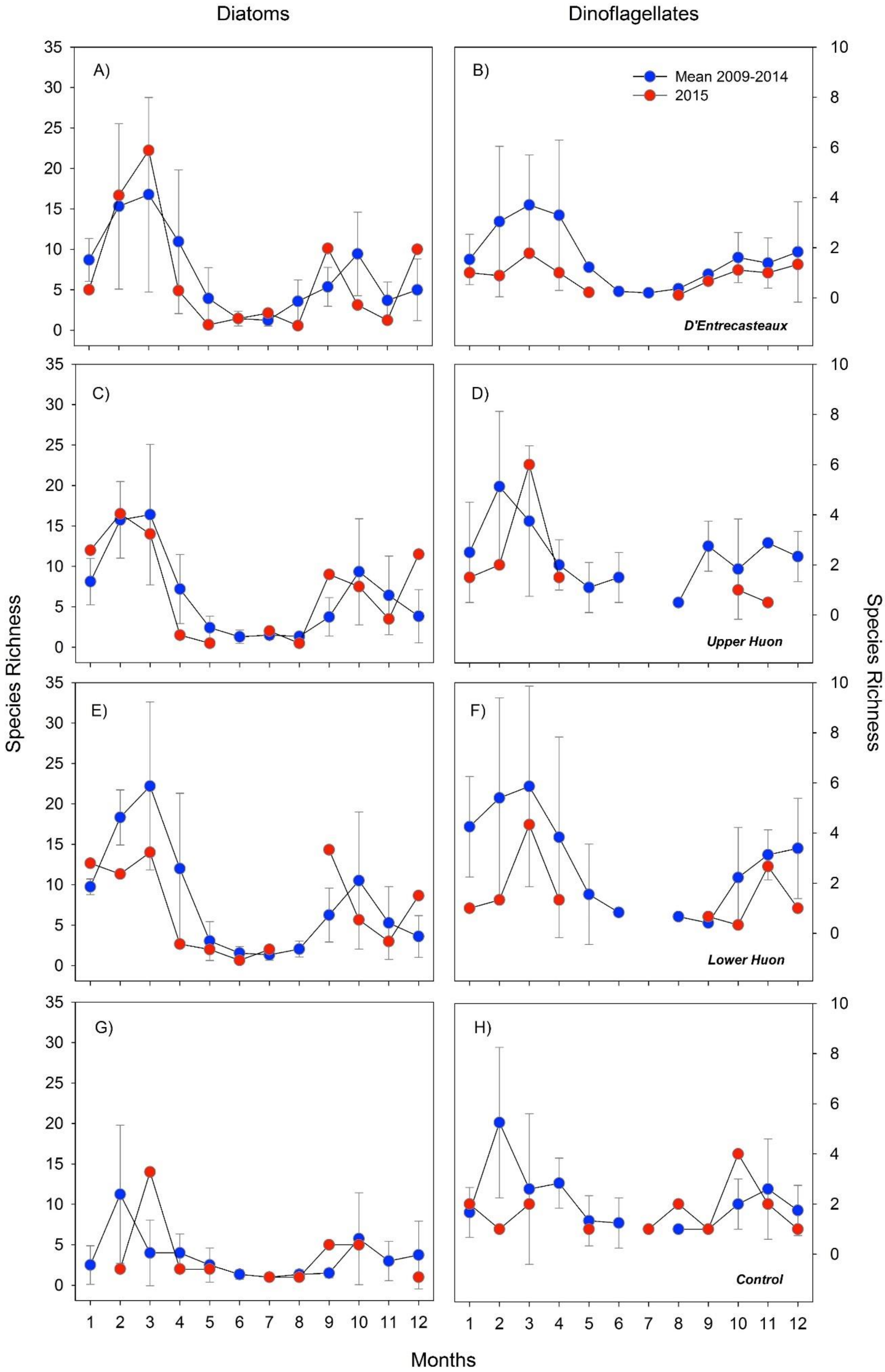

Figure 39: Comparison of historical monthly variation in species richness of diatoms and dinoflagellates between 2009-2014 (mean±SD) and 2015 at the D'Entrecasteaux-Huon system.

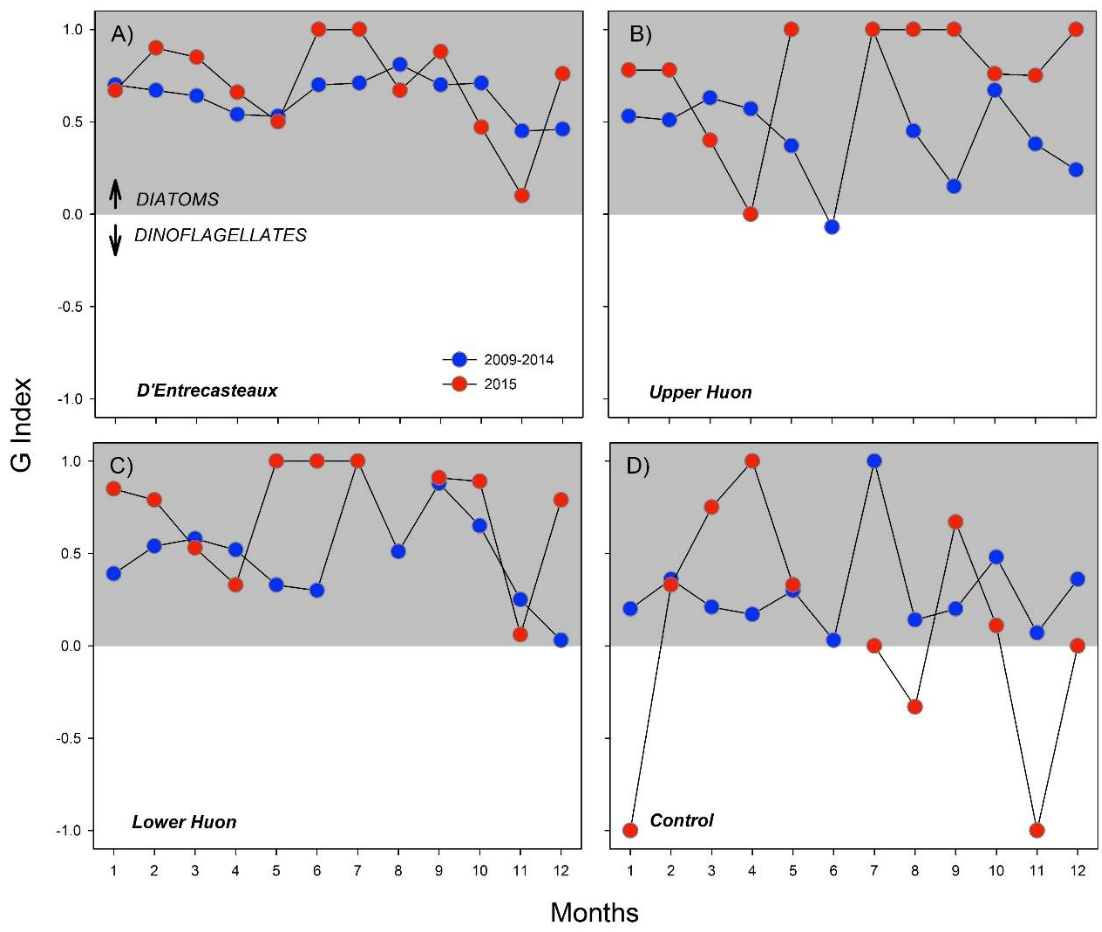

Figure 40: Comparison between the monthly historical and 2015 G Index in the D'Entrecasteaux Channel/Huon Estuary system.

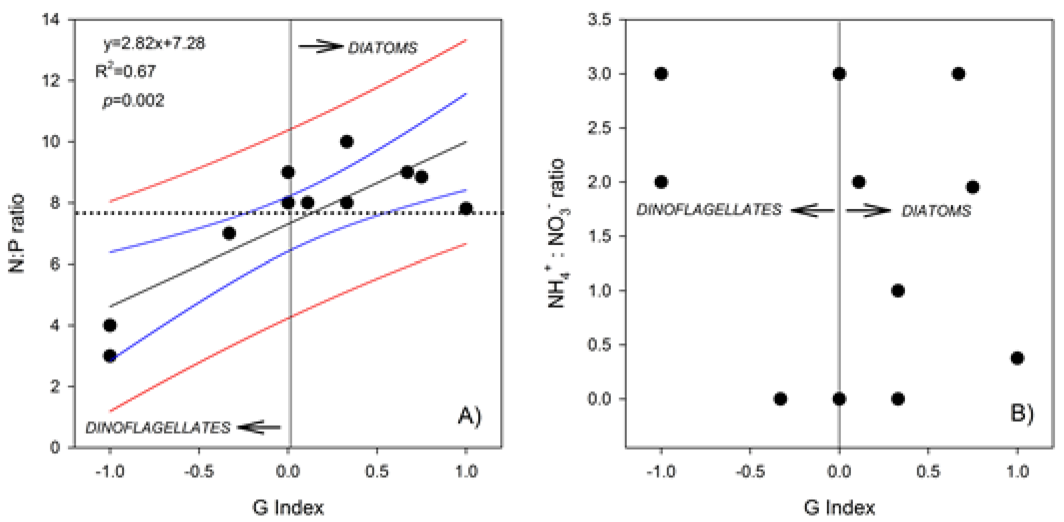

Figure 41: Relationship between estimated G Index and A) nitrate:phosphorus ratio and B) ammonia:nitrate ratio.

4.2.4.8 Harmful algal bloom species

This section details the temporal frequency of HAB occurrence between 2009 and 2015. Figure 42 displays the annual frequency of HAB species that may present a risk for salmon farming activities. There is no evidence indicating that the N exceedance in 2015 caused a HAB, or increased the number of HAB forming species compared with previous years (ANOVA p = >0.05), with HAB frequency being below average during 2015 (red horizontal lines in Figure 42).

Annual frequencies of HAB species that may present a risk for shellfish farming, and consequently for human health due to toxin production, are shown in Figure 43. Shellfish HAB species were not significantly more abundant in 2015 compared to historical levels (ANOVA p = >0.05). Most of the HAB observations for the D'Entrecasteaux Channel corresponded to the genus *Pseudo-nitzschia*, which is widely distributed in the area. High-density *Pseudo-nitzschia* blooms (>100,000 cells/L) have also reportedly been associated with stressed Atlantic salmon in North West Bay (Gustaaf Hallegraeff, pers. com).

Six *Pseudo-nitzschia* spp. have been identified in the BEMP monitoring, with two dominating in terms of frequency of occurrence, and abundance; *P. seriata* and *P. delicatissima*, with only the former being responsible for HAB.

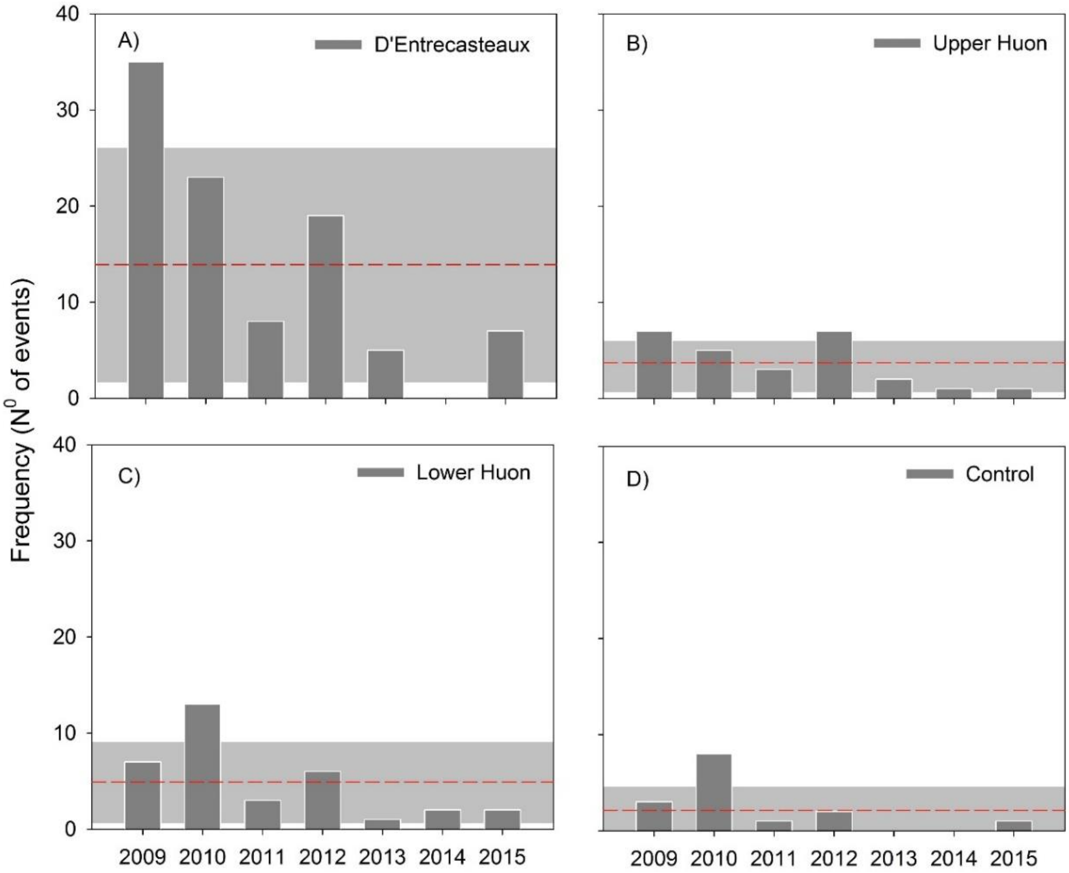

Figure 42: Annual frequency of harmful algal species that may represent a potential risk for salmon farming activities (*Chaetoceros convolutus*, *Chaetoceros criophilis*, *Chattonella* spp., *Dictyocha speculum*, *Heterosigma akashiwo*, *Noctiluca scintillans*, *Phaeocystis* spp., and *Rhizosolenia* group), detected between 2009 and 2015. Dashed red line indicates the inter-annual mean of the observations and the grey area the standard deviation.

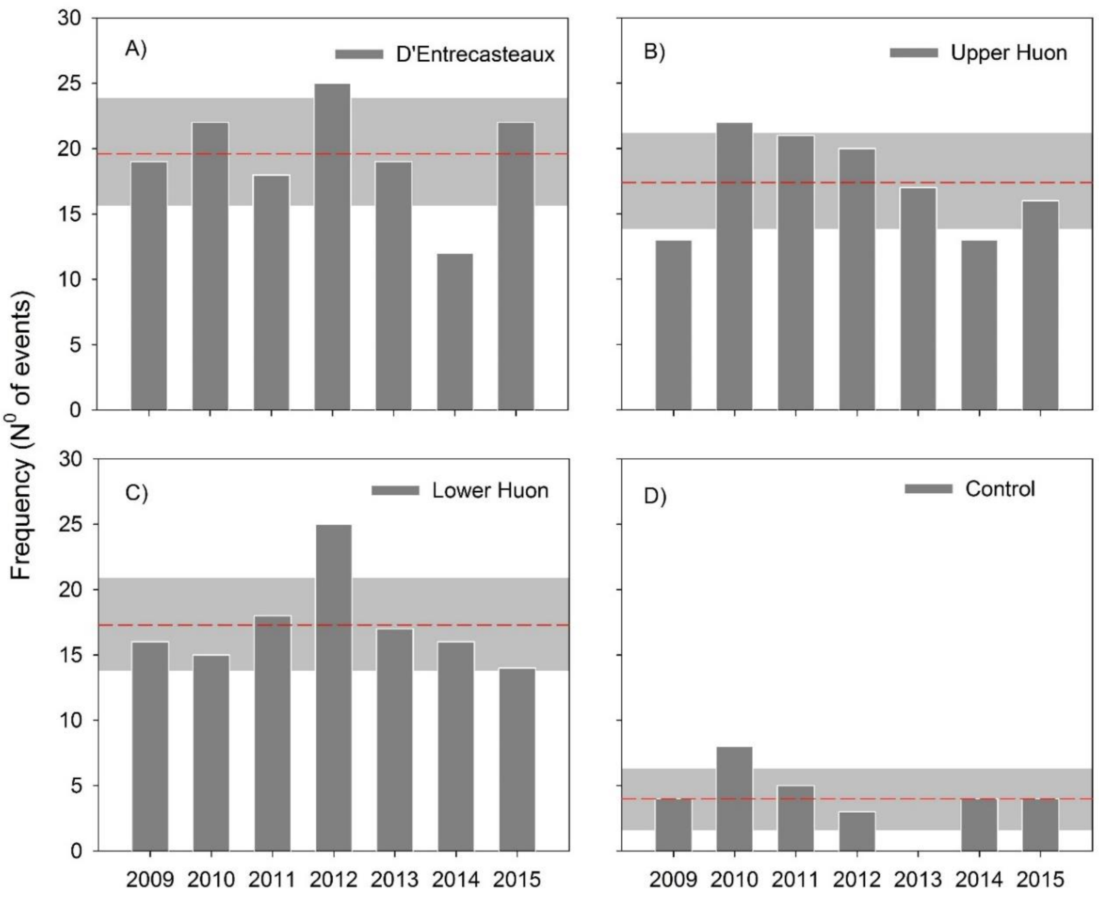

Figure 43: Annual frequency of harmful algal species that may represent a potential risk for shellfish farming activities due to phycotoxins production (*Alexandrium minutum, A. ostenfeldii, A. tamarense, Dinophysis acuminata, D. fortii, Gymnodinium catenatum, Phalacroma rotundatum, Pseudo-nitzschia* spp.), detected between 2009 and 2015. Dashed red line indicates the inter-annual mean of the observations and the grey area the standard deviation.

4.2.4.9 Performance against chlorophyll-a trigger limits

The level 1 and 2 chlorophyll-a trigger limits were frequently exceeded in the D'Entrecasteaux Channel MFDP area between 2011 and 2013 with level 3 triggers also being exceeded on several occasions (Figure 44). Level 1, and some level 2, triggers were also exceeded in the Huon/Esperance MFDP area in 2011 (Figure 45).

The annual mean for 2015, the summer means for 2014/15, and the summer mean for six sites were above the level one trigger in the D'Entrecasteaux Channel MFDP area (Figure 44). One site (site 12) exceeded the level 2 trigger for the summer mean. However, the observed chlorophyll-a concentrations were well below historic highs. Only two sites were above the level 1 trigger the following summer (2015/16).

Only one site was above the level 1 trigger in the Huon/Esperance MFDP area (site 13); this site is in the far north of the Huon estuary and not close to where the exceedance occurred (Figure 45). No triggers were breached in the summer of 2015/16, nor did the annual mean reach the level 1 trigger limit.

Despite several triggers being reached during the exceedance time period, in 2011 in the Huon/Esperance MFDP area, and from 2011–2013 in the D'Entrecasteaux Channel MFDP area, level 1 and 2 triggers were frequently breached and occasionally level 3 triggers so the N exceedance by HAC does not appear to have caused a large scale increase in algal abundance.

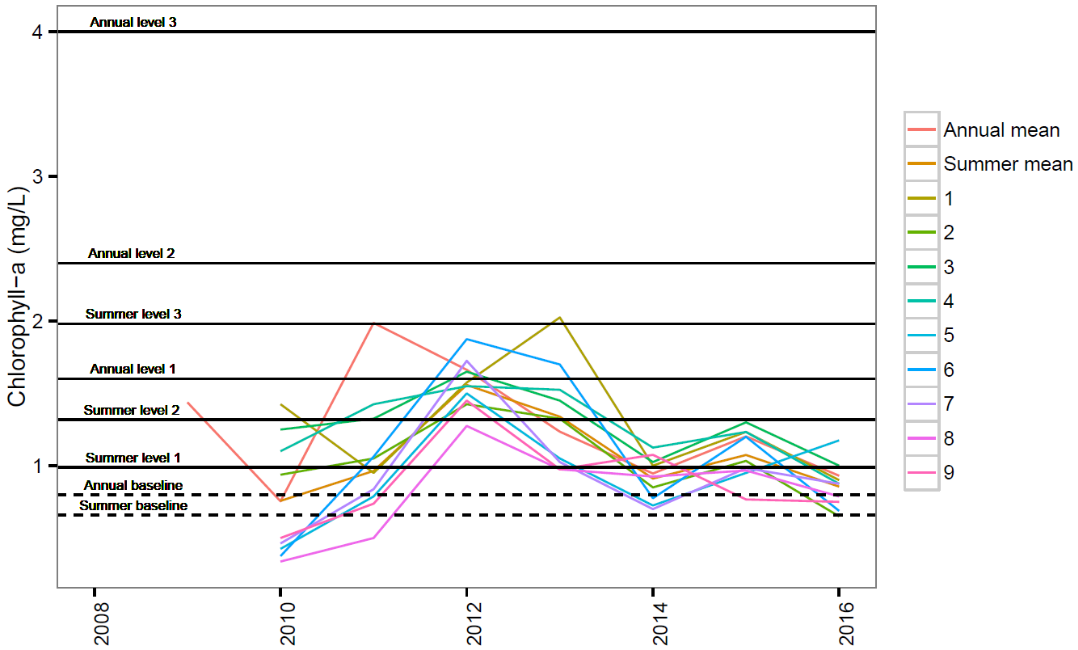

Figure 44: Performance against recommended chlorophyll-a trigger levels in the D'Entrecasteaux Channel MFDP area. The recommended baseline and triggers were proposed by (Volkman *et al.*, 2009) and are summarised in the methods.

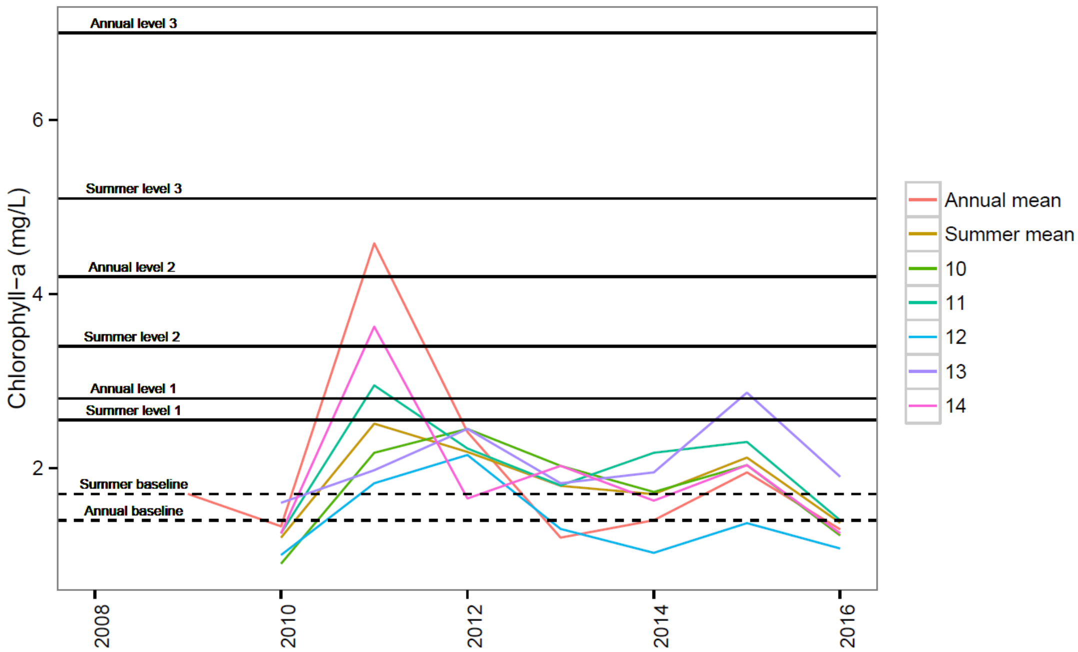

Figure 45: Performance against recommended chlorophyll-a trigger levels in the Huon/Esperance MFDP area. The recommended baseline and triggers were proposed by (Volkman *et al.*, 2009) and are summarised in the methods.

4.3 Summary of nutrient and algal results

Riverine and industrial nitrogenous inputs into the D'Entrecasteaux Channel/Huon Estuary are relatively low compared to oceanic nitrate, and aquaculture ammonia inputs, particularly with respect to dissolved forms. Nitrate concentrations display a clear seasonal pattern with a maximum in winter (May – September) when nutrient rich sub-Antarctic currents push northwards (Crawford *et al.*, 2011; Ross and Macleod, 2013). In contrast, ammonia concentrations tend to reflect aquaculture production, with maximums typically occurring in spring and early summer when fish biomass is highest pre-harvest. In 2015, this pattern was slightly different, with elevated bottom water ammonia concentrations extending into autumn in the southern region. This was most likely because HAC feed inputs remained high throughout summer of 2014/15 and into autumn, particularly at the Flathead Bay and East of Redcliffs leases. This resulted in the level three draft ammonia trigger levels being exceeded at a number of the BEMP monitoring sites near where the exceedance occurred.

Ammonia is the major nitrogenous waste product produced by fish (Knoph and Thorud, 1996). Adult Atlantic salmon have an ammonia excretion rate of 10 – 40 g N/kg feed with the variation due to feeding rate, activity, season and potentially other factors (Bergheim *et al.*, 1991). Using these figures, 326 – 1304 t of ammonia would have entered the Huon/Esperance MFDP area in 2015 from fish excretion alone. In the context of the TPDNO for the Huon/Esperance MFDP area, the HAC exceedance equated to an additional 46 – 185 t of ammonia above that which is permitted at the maximum TPDNO.

In the environment ammonia is preferentially utilised by phytoplankton over other nitrogenous compounds as ammonia requires less energy to assimilate – it is more easily transferred across the cell wall than other forms such as nitrite and nitrate (Glibert, 2016). High ammonia concentrations (c.a. 1 μmol/l; 0.017 mg/l) can actually repress the assimilation of nitrate (Syrett, 1981). Ammonia concentrations at this level are occasionally observed in the D'Entrecasteaux Channel/Huon Estuary system, particularly in the Upper Huon Estuary, and were relatively common in the bottom waters of the southern region during the HAC N exceedance.

Ammonia concentrations exceeding 5 μmol/l (some reaching >45 μmol/l) are often associated with large changes in phytoplankton composition (Glibert *et al.*, 2016). The highest ammonia concentrations in this study were in the bottom waters of the southern region during the exceedance, but these did not exceed 2 μmol/l. Thus it may be that ammonia concentrations in the system (at the observed levels) are not a key driver of phytoplankton dynamics. Further, the abovementioned increases in ammonia were observed in bottom waters, and as such would largely be below the photic zone, which would in turn suggest that much of this additional ammonia was unavailable to primary producers and was either nitrified, or diluted during dispersal and flushed from the system.

The results of this study have highlighted the importance of key water quality parameters including nitrate, temperature, ammonia:nitrate ratio and total N in affecting the spatial and temporal patterns in determining phytoplankton composition and abundance (see BOX 3 for a synopsis of phytoplankton dynamics and environmental drivers). Most notably, reinforcing that seasonal changes in temperature and the influx of nitrate (and concomitant variation in total N and ammonia:nitrate ratio) associated the influence of sub-Antarctic currents are major drivers of phytoplankton dynamics in the D'Entrecasteaux Channel/Huon Estuary system. There was no significant difference in any of these key water quality parameters between the pre- and post- HAC N exceedance periods. As such, it is not surprising that there was also no difference observed in phytoplankton abundance and composition, including the prevalence of HAB species, between these two time periods.

Diatoms were shown to be the major component of the phytoplankton community and biomass in the D'Entrecasteaux Channel/Huon Estuary system at all sites during most of the BEMP time series (2009–2016), with dinoflagellates being the second most abundant group. Higher peaks in abundance of dinoflagellates were typically observed in the Huon Estuary, which is consistent with previous observations (Volkman *et al.*, 2009). At all sites, the ratio of N to P was below 16:1 (the Redfield ratio) indicating that the system was N limited. Interestingly, in the D'Entrecasteaux Channel silicate levels were also low which may limit

Box 3: Synopsis phytoplankton dynamics and environmental drivers

Following the spatial and temporal analyses of nutrient concentrations, nutrient ratios, and algal abundance, the nutrient and algal interactions in the D'Entrecasteaux Channel/Huon Estuary over this study period can be summarised as follows (see Figure 46 for schematic representation): the Huon Estuary has high organic matter, silicate, N and P inputs, which leads to high silicate:P and ammonia:nitrate ratios. Tide and flow regimes produce fluctuations in the physio-chemical properties of the water column and vertical mixing, which influences vertical phytoplankton distribution and results in thin layer formation (high phytoplankton concentration at the halocline). The D'Entrecasteaux channel is characterized by a low silicate:P ratio and spatial homogeneity in diatom distribution with dominance of diatoms of the *Pseudo-nitzschia* genus. Given their dominance, the relative abundance of this group would likely make them a good indicator genus. N and P inputs from finfish aquaculture contribute to dissolved organic N and P, dissolved inorganic N and P in the water column and particulate organic N and P in the bottom waters. Low ammonia:nitrate ratio in surface waters of the D'Entrecasteaux Channel, influenced by nitrate input from deep ocean waters, results in a vertical gradient whereby ammonia and nitrate increase with depth.

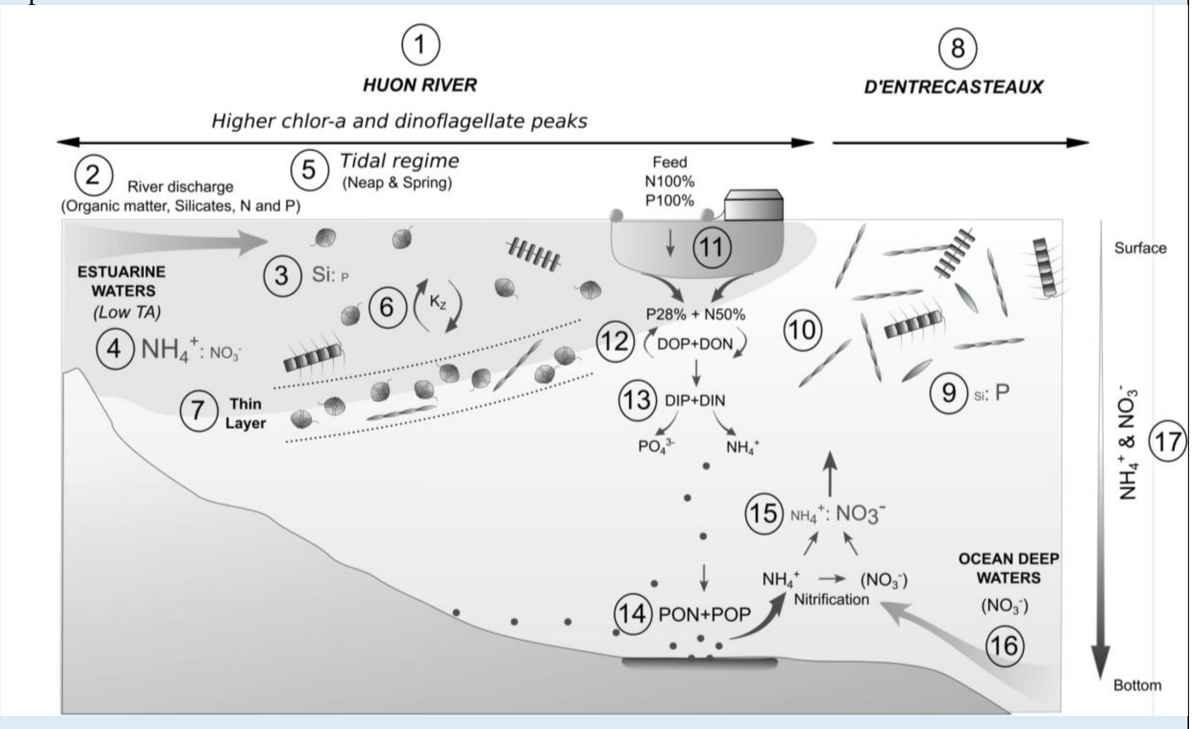

Figure 46: Schematic representation of the phytoplankton dynamic in the D'Entrecasteaux Channel/Huon Estuary system associated with environmental drivers.

diatom growth. However, as discussed diatoms were the most abundant group throughout the system. Diatoms from the *Pseudo-nitzschia* genus dominated the entire D'Entrecasteaux Channel. *Pseudo-nitzschia* diatoms are lightly silicified r-strategists that prosper in low Si conditions and are able to respond rapidly to nutrient inputs (Fehling *et al.*, 2006), as such they are particularly well adapted to the Si-depleted conditions of the D'Entrecasteaux Channel.

An important species to consider when investigating the potential consequences of the N exceedance was the heterotrophic dinoflagellate *Noctiluca scintillans* (included in the analyses of Dinoflagellates and HAB species). During the initial expansion of marine farming in southeastern Tasmania, *N. scintillans* established in the D'Entrecasteaux Channel/Huon Estuary system. This species feeds on phytoplankton, small zooplankton and faecal pellets and has been shown to accumulate, and excrete, large amounts of ammonia (Volkman *et al.*, 2009). *Noctiluca* species have also been implicated as the cause of fish kills in both the wild and aquaculture due to the high quantities of ammonia that they excrete (Okaichi and Nishio, 1976). As such, *N. scintillans* has the capacity to influence the abundance of other algal species, reduce the sedimentation of faecal pellets and influence ammonia concentrations (Volkman *et al.*, 2009). There was no evidence that *N. scintillans* increased in abundance as a result of the HAC exceedance so were not responsible for controlling the abundance of other species or influencing ammonia concentration within the system.

4.4 **Considerations for future monitoring**

4.4.1. Location and timing of sampling

Despite the localised nature of the excess N inputs by HAC, the BEMP was able to detect a measureable increase in ammonia at several of the sites closest to where the exceedance occurred, and in the southern region as a whole. However, there were no BEMP sites within the immediate vicinity of the exceedance and as such a detailed analysis of localised impacts was not possible. The BEMP was designed to provide an early warning of sustained system wide impacts and was predominantly based on modelling outcomes that identified the best locations to place sites to detect a system wide changes. Enclosed water bodies and embayments tend to have low water movement and are therefore likely to be more susceptible to the accumulation of nutrients and concomitant ecological impacts such as HABs. As a result, a large number of the BEMP sites are located in bays and not necessarily near aquaculture leases; again because the BEMP was designed around detecting broadscale system change. As such, the capacity of the BEMP program to detect near field effects or a gradient of impact associated with changes in inputs from one or more specific leases in a particular region is limited. If the exceedance did however lead to a system wide response, the BEMP is well designed to detect it, although given the inherent natural variability in the system, and the power issues associated with that, it would be more suited to detecting moderate to large responses.

By the time data were provisioned in the present study (March 2016), it was apparent that fish biomass was low and ammonia concentrations had already declined. As such, in the event of a TPDNO exceedance in future, it is recommended that additional sampling takes place in the vicinity of the inputs as soon as the exceedance is reported. This will enable a more detailed and comprehensive impact assessment of localised nutrient concentrations and algal communities to be investigated more thoroughly. Alternatively, the BEMP program is reviewed such that it has the capacity to detect both near field and system wide change (i.e. sites are positioned nearer to where the majority of N inputs occur under modern aquaculture practices).

Benthic sediments are known to be very sensitive to nutrient enrichment (reviewed by Cloern (2001)), and the benthic response to organic enrichment from finfish aquaculture is well established in southeast Tasmania (Crawford *et al.*, 2001; Macleod *et al.*, 2004). As such, it would have been useful to sample sediments in the area of the HAC N exceedance while the exceedance was taking place, or preferably when the majority of nutrients were being input into the system as the two did not align in the present study. The presence of species known to flourish in nutrient enriched conditions (e.g. *Beggiatoa* bacteria and the abundance of opportunistic fauna such as the polychaete *Capitella* spp.) would provide insight into the magnitude and extent of benthic impacts associated with the exceedance.

Regulatory compliance monitoring was undertaken on the 12th May 2015 at the original Flathead Bay lease and no *Beggiatoa* bacteria was found; however, this lease was moved in January 2015 so the fish biomass and N inputs over the previous several months had occurred at the new lease location. Compliance monitoring was undertaken at the East of Redcliffs lease on the 5th December 2014 and 21st January 2016. Some *Beggiatoa* was found at one compliance site during the first survey but none was present during the second survey. Unfortunately, these surveys occurred either side of the majority of N inputs (December 2014 to November 2015) from the exceedance and may have missed any impacts should they have occurred.

By the time this study was commissioned, HAC had already reduced biomass and feed inputs in the Huon/Esperance MFDP and inputs had been at normal levels for several months. In some ways, this highlights a potential downside of the TPDNO rolling average management system – there can be a lag between when the majority of N is added to a region and when a TPDNO limit is exceeded during any 12-month rolling period. This was the case in early 2015 with HAC only beginning to exceed in the period March 2014 – February 2015, despite N inputs being very high from December 2014 onward. Further, there can be a lag in when the highest nutrients are put in the system and when the peak TPDNO exceedance occurs within any given 12 month period. For example, in the present case, peak exceedance was not reached until the 12-month period from December 2014 – November 2015 but N inputs were actually relatively low in late 2015.

As shown in Figure 47, tidal regime (neap and spring tides) can affect the vertical distribution of phytoplankton cells, but likely also influences the vertical diffusion of nutrients and other physio-chemical water chemistry properties as many of these vary with depth. Estuarine phytoplankton biomass can fluctuate at a time scale of days to weeks, and much of this variability is associated with fluctuations in tidal energy (Cloern, 1991). During neap tides, vertical mixing (parameterized as an eddy diffusivity K_z) decreases and phytoplankton aggregate, forming patches in the surface and/or in the pycnocline zone (thin layer formation). In contrast, during spring tides vertical mixing increases, distributing phytoplankton cells through the water column. Thus, phytoplankton sampling at different tidal regimes can potentially lead to under- or over-estimation of cell density at spring or neap tide, respectively. Figure 48 shows the tidal regime of BEMP sampling between 2009 and 2016 and sampling dates were widely distributed between both tide regimes. Sampling

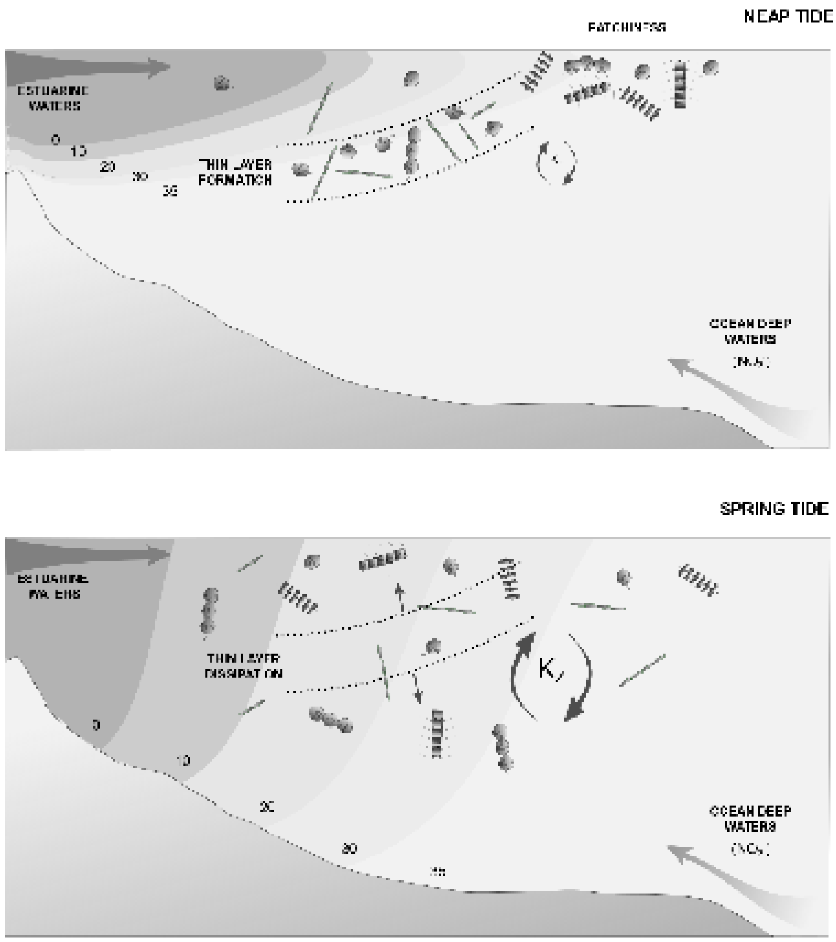

Figure 47: Tidal regimes and phytoplankton dynamics in an estuarine system.

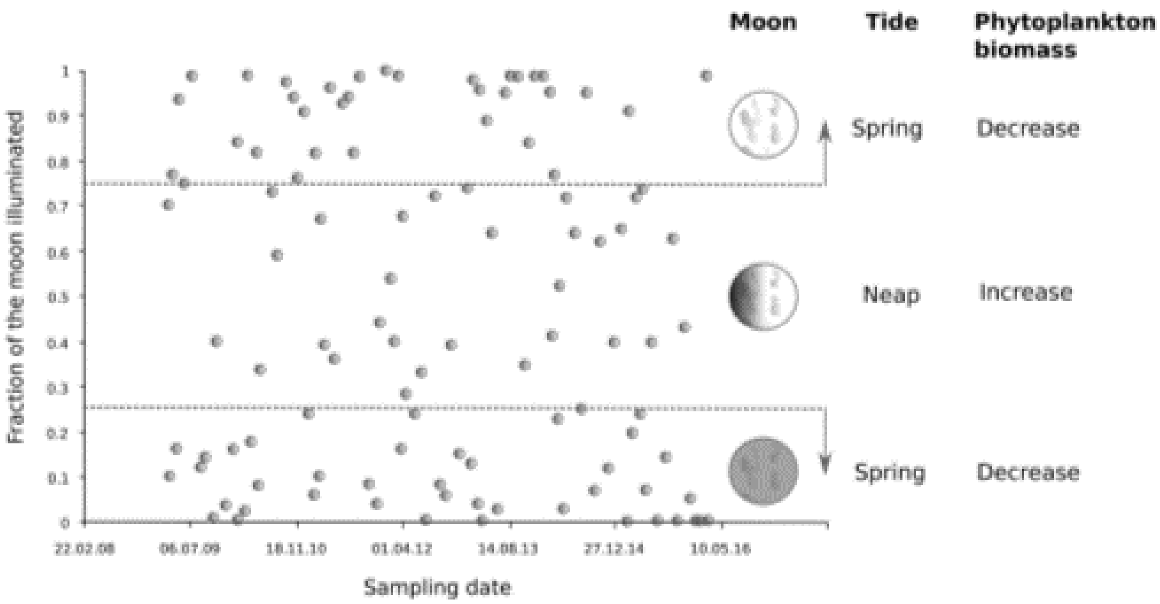

Figure 48. Estimation of the tide regime based on the fraction of the moon illuminated (Data from Astronomical applications department {http://www.aa.usno.navy.mil }) when BEMP-phytoplankton sampling was undertaken between 2009 and 2016.

phytoplankton at a specific tide phase (neap tide recommended) might significantly reduce the likelihood of under- or over-estimation of phytoplankton abundance and diversity through time.

Increasing the frequency of sampling, particularly during exceedance events, could enhance the ability to detect any relationship between phytoplankton biomass and nutrient concentration. The monthly sampling regime may have missed major peaks, or blurred relationships between phytoplankton abundance and environmental variables. For example, it is rare for high nutrient concentrations to occur concurrently with high phytoplankton abundance; more commonly, when high nutrient concentrations occur it means there is a lack of phytoplankton present to utilise them, and, by the time phytoplankton increase in abundance, they have stripped the water of nutrients. It is also recommended that CTD/Sonde sampling is undertaken at greater resolution (currently only done at surface, 5 m and bottom). This would enable the depth of the pycnocline/halocline to be identified, which is a key determinant of phytoplankton aggregations (e.g. *Pseudo-nitzschia*). This is particularly important in the Huon Estuary.

4.4.2. Algal performance indicators

We suggest a review of phytoplankton bloom performance indicators (i.e. triggers) proposed by Thompson *et al.* (2008) with the addition of performance indicators that are HAB species-specific; currently the only phytoplankton performance indicators relate to chlorophyll-a concentrations. Several toxic dinoflagellate species, such as the genus *Dinophysis* (diarrhetic toxins producers), can cause gastrointestinal illness (diarrhetic shellfish poisoning) even at relatively low cell densities (<103 cells/l) (Reguera *et al.*, 2014). Thus, HAB species cannot be adequately managed based on chlorophyll performance indicators alone and HAB species should have specific performance indicators.

For example, *N. scintillans* is an important species in the D'Entrecasteaux Channel/Huon Estuary system (Volkman *et al.*, 2009). It is a heterotrophic dinoflagellate and therefore does not photosynthesize itself (i.e. does not possess its own chlorophyll-a) but may contain chlorophyll-a if it has consumed phytoplankton for either food or endosymbiosis (Goes and Gomes, 2016). Given the relative importance of this species in the system, and its ability to influence phytoplankton abundance, faecal deposition and ammonia concentration, and potentially act as a HAB species, it may be worth considering specific performance indicators for this species.

Additionally, in the present study, the *Pseudo-nitzschia* group were shown to be the most abundant phytoplankton throughout the system. Given their high abundance, and the ability of some species to create HABs, their abundance would be a good indicator of shifts within the system, although it must be noted that chlorophyll-a is a better indicator of diatom abundance than it is of dinoflagellates.

4.5 Modelled dispersion and ecological response to nutrient inputs

Modelling was undertaken for two scenarios: i) the first reflects the actual inputs (i.e. including the exceedance) and ii) the second represents a ‘capped’ scenario (where the HAC

N inputs did not exceed their TPDNO limit for the Huon/Esperance MFDP area). In all other respects the model parameterisation for the two scenarios was identical.

4.5.1. Ammonia

The modelled results suggest that ammonia concentrations were spatially and temporally different between the two scenarios. Not surprisingly, at surface, 10 m and bottom depths, the greatest differences occurred near the East of Redcliffs and Flathead Bay leases (Figures 49–51) where the majority of the excess fish biomass and feed inputs were reported. Notably, during the exceedance scenario, higher ammonia concentrations were predicted in surface waters and at 10 m depth during winter (Figure 50-51). The difference between the two scenarios was less pronounced in bottom waters near the East of Redcliffs and Flathead Bay leases (Figure 49) but was more evident in the upper Huon Estuary and central/northern D'Entrecasteaux Channel in late winter and spring 2015 than it was in surface waters. The modelling suggests that, due to a net water flow south past the leases where the exceedance occurred and into Port Esperance (Figure 52), ammonia concentrations would have increased in Port Esperance. However, the model predicted that during the exceedance excess ammonia would be either rapidly assimilated by primary producers or rapidly nitrified in the Port.

HAC do not farm within Port Esperance so all of the model parameters (WWTP, river and aquaculture inputs) were identical within the Port during the two model runs indicating it was externally sourced ammonia likely to be driving the differences within.

4.5.2. Nitrate

Nitrate is typically higher in concentration than ammonia at all depths (surface, 10m and bottom) throughout the D'Entrecasteaux Channel/Huon Estuary system (Figures 53–55). The modelling suggests that temporal nitrate distribution was similar at all three depths, but that levels increased with increasing depth, being greatest near the bottom (Figure 53). As a result, nitrate was found to be widespread throughout the system as deep currents pushed it northward up both the Huon Estuary and the D'Entrecasteaux Channel. This predominantly occurred during winter when nutrient inputs were high (both natural and anthropogenic) and photosynthesis was low due to the reduced photoperiod. In spring, modelled nitrate levels decreased markedly, probably because HAC decreased production but also because the excess nitrate inputs during winter were either assimilated by primary producers as photoperiod increased, or were flushed from the system.

4.5.3. Chlorophyll-a

Despite the suggestion that N species concentrations would be elevated at all depths in winter during the exceedance, modelled chlorophyll-a concentration was similar at all depths (surface, 10m and bottom) in both scenarios (Figure 56–58); this is probably because the reduced photoperiod in winter would not be conducive to primary productivity. However, as the photoperiod began to increase in August, the model predicted an increase in chlorophyll-a concentration under the exceedance scenario, with this peaking in September and October, before returning to similar concentrations in both scenarios from November onward. Interestingly, in the lower D'Entrecasteaux Channel, the model predicted a larger increase in chlorophyll-a under the exceedance scenario at 10 m depth (Figure 57) than it did at the surface (Figure 58), presumably because these slightly deeper waters had higher nutrient concentrations, and the light penetration in the largely oceanic southern region would be sufficient at this depth to enable photosynthesis. In the central D'Entrecasteaux Channel, the modelling suggested a greater increase in chlorophyll-a in bottom waters, presumably because this region is shallower than the southern D'Entrecasteaux Channel where much of the bottom waters are >20 m depth.

The greatest difference in chlorophyll-a concentration between the two modelling scenarios occurred in Port Esperance. This is likely to be because during the exceedance additional ammonia and nitrate was flushed into this bay where flushing rates are lower (see Figure 52) and depths are relatively shallow, notably in the west of the Port, thereby enabling light to penetrate through much of the water column.

Apart from the predicted increase in chlorophyll-a outlined for Port Esperance above, there was very little difference between the two modelled scenarios for most of the system: chlorophyll-a concentrations varied by <0.5 mg/m^3 (<0.005 mg/l) between the two scenarios, which is less than the sensitivity of chlorophyll-a measurement in the BEMP.

4.5.4. Oxygen

The biogeochemical model estimated that during the exceedance there would have been a decline in bottom water dissolved oxygen of up to ~3% in the immediate vicinity of the leases in the southern Huon Estuary and southwestern D'Entrecasteaux Channel during autumn and spring 2015 (Figure 59). The modelling suggested minimal difference between the two scenarios throughout the rest of the year in most of the D'Entrecasteaux Channel/Huon Estuary system with a single exception: at the beginning of 2015 dissolved oxygen concentration in western Port Esperance was estimated to be around 5% lower during the exceedance scenario. Given all model drivers were the same within Port Esperance at this time, this is potentially driven by the relatively high N inputs associated with the HAC exceedance entering Port Esperance in December 2014. The modelling suggests that the decreased oxygen levels in Port Esperance remained throughout the year until after spring.

4.5.5. Comparison of observed and modelled results

There was generally good agreement between the observed physio-chemical conditions (i.e. BEMP analyses) and the modelling results, particularly when you take into account the magnitude of change predicted by the model and the inherent variation in the BEMP data. The model predicted elevated ammonia levels near the East of Redcliffs and Roaring Beach leases (Figures 49–51), and this was observed at both the site level (sites 8, 9, 10 and 12) (Figure 20) and for the southern region as a whole (Figure 19). Although the model predicted elevated nitrate concentrations throughout much of the system (Figures 53–55) this was not overly apparent in the observational data (other than at site 10); this may reflect nitrification of the excess ammonia. This is also perhaps not surprising as the large, but highly seasonal sub-Antarctic inputs, can potentially mask local signals. As the model only predicted relatively small changes to most nutrients and physio-chemical conditions it would be difficult to detect these subtle changes in the observational data, particularly given the inherent variation in the BEMP data and the relatively high detection limits in the analytical samples.

The major difference between the model and BEMP analyses occurred in Port Esperance. In Port Esperance, the model predicted large changes to all of the variables. The observational data (BEMP results) indicated that there was an increase in ammonia during 2015, but did not detect any subsequent increase in nitrate, chlorophyll-a, or any decrease in oxygen. It may be that the BEMP lacks the sensitivity required to detect the modelled changes within Port Esperance because of the temporal and spatial frequency of the sampling – there is only monthly (fortnightly in summer) sampling of a single centrally located site – whereas the greatest changes predicted by modelling occurred in the west of the Port. Alternatively, it may be that the biogeochemical model was not particularly accurate at small spatial scales within Port Esperance in the present case. The model cycles carbon and natural/anthropogenic nutrients, while incorporating phytoplankton, zooplankton and detrital components throughout the entire D'Entrecasteaux Channel/Huon Estuary system. As such, localised effects, for example in western Port Esperance, may not be completely representative and should be interpreted with this in mind. Nevertheless, the fact that a relatively small additional contribution from HAC was predicted to have an impact on both nutrient and dissolved oxygen concentrations within Port Esperance, particularly in the shallower areas, highlights the potential susceptibility of this location.

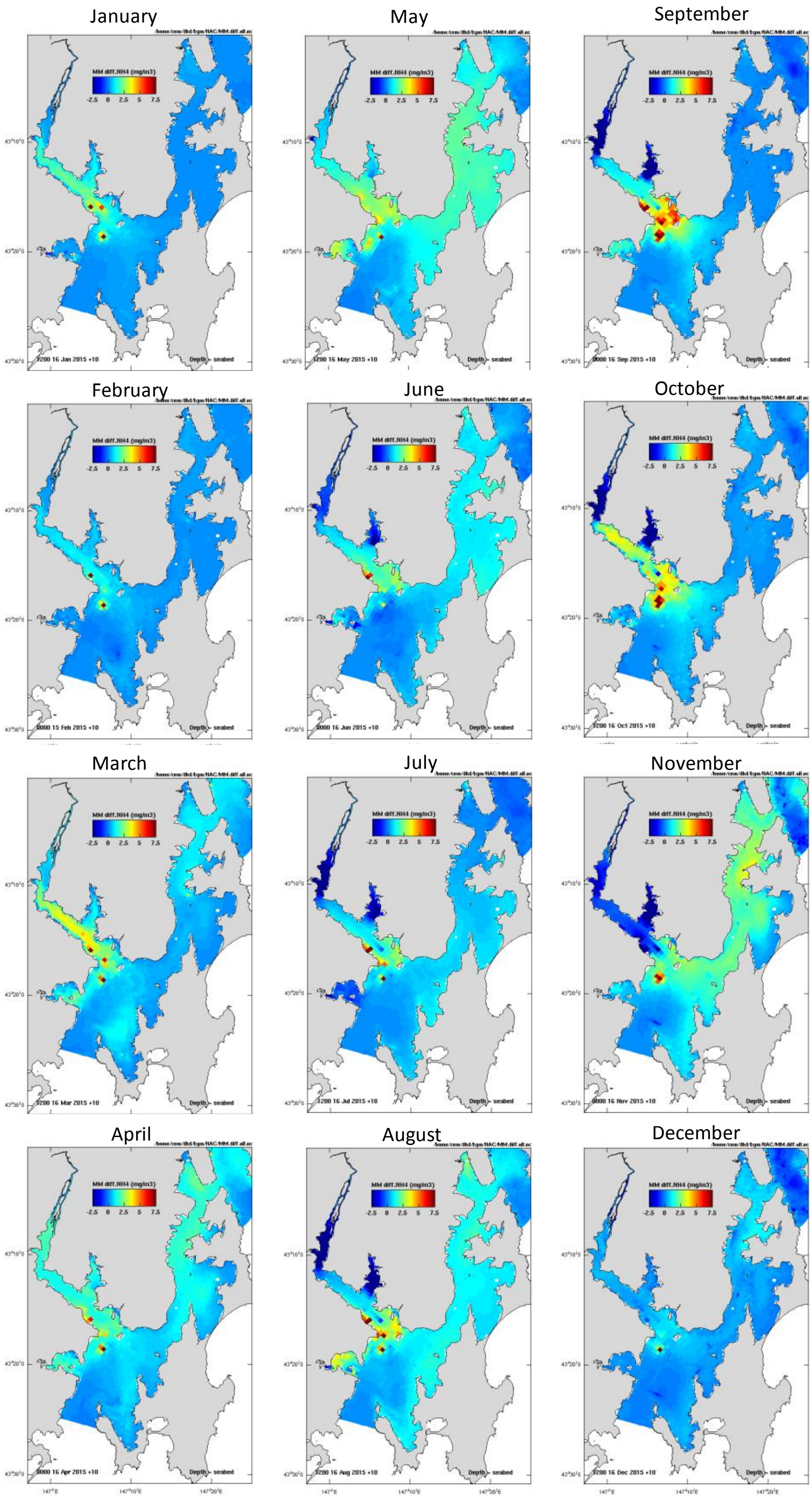

Figure 49: Biogeochemical model output of the difference in bottom water ammonia concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

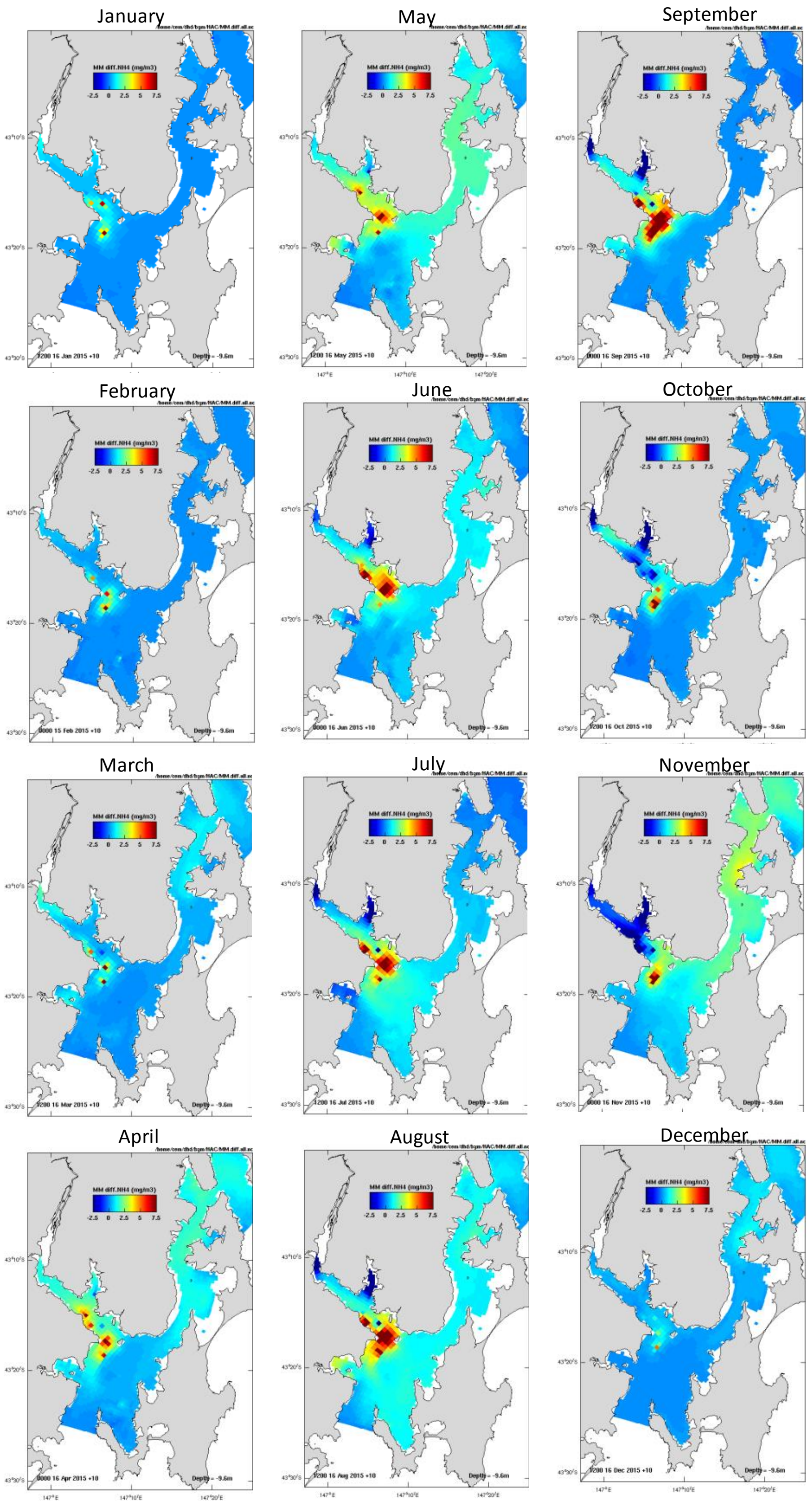

Figure 50: Biogeochemical model output of the difference in 10 m ammonia concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

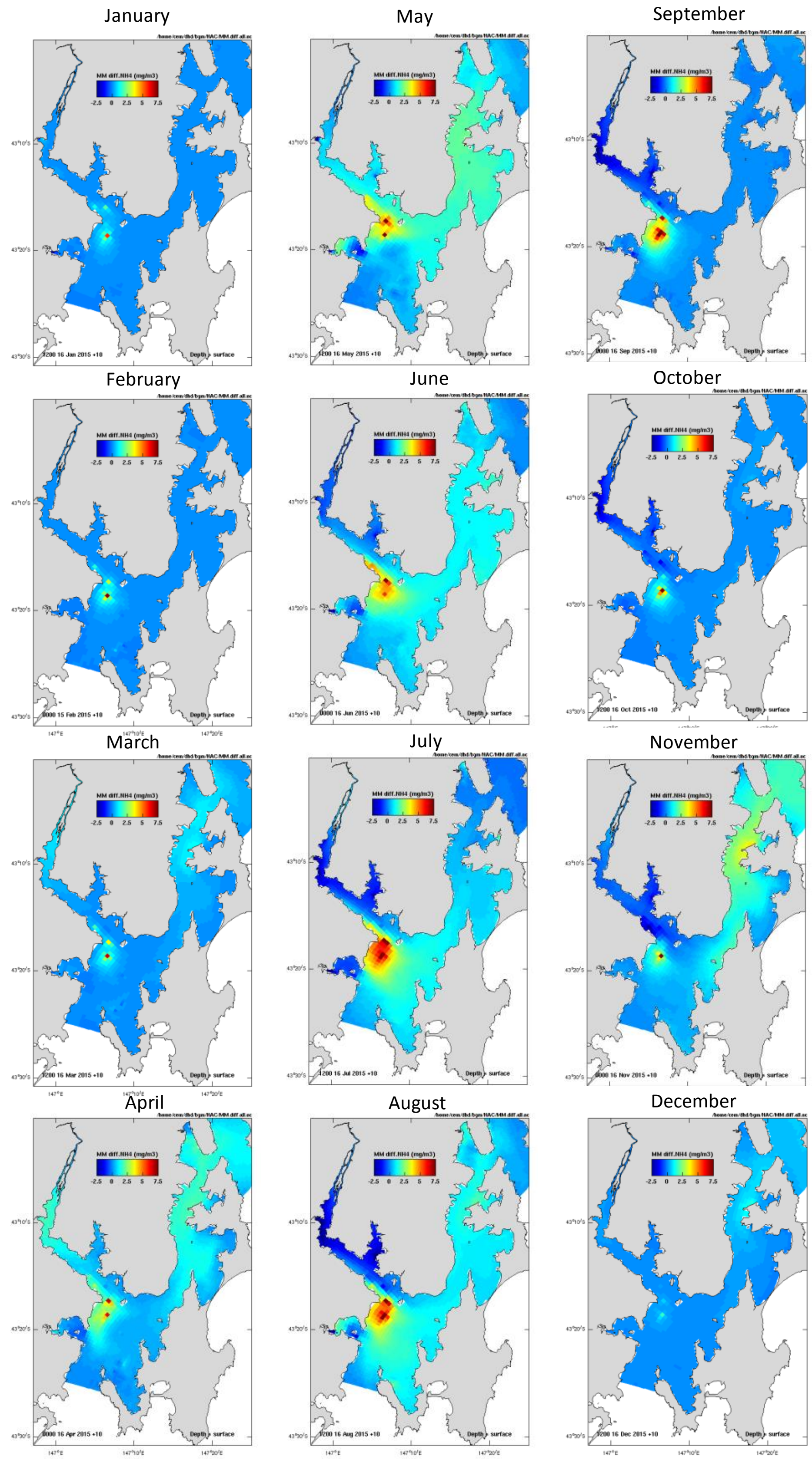

Figure 51: Biogeochemical model output of the difference in surface ammonia concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

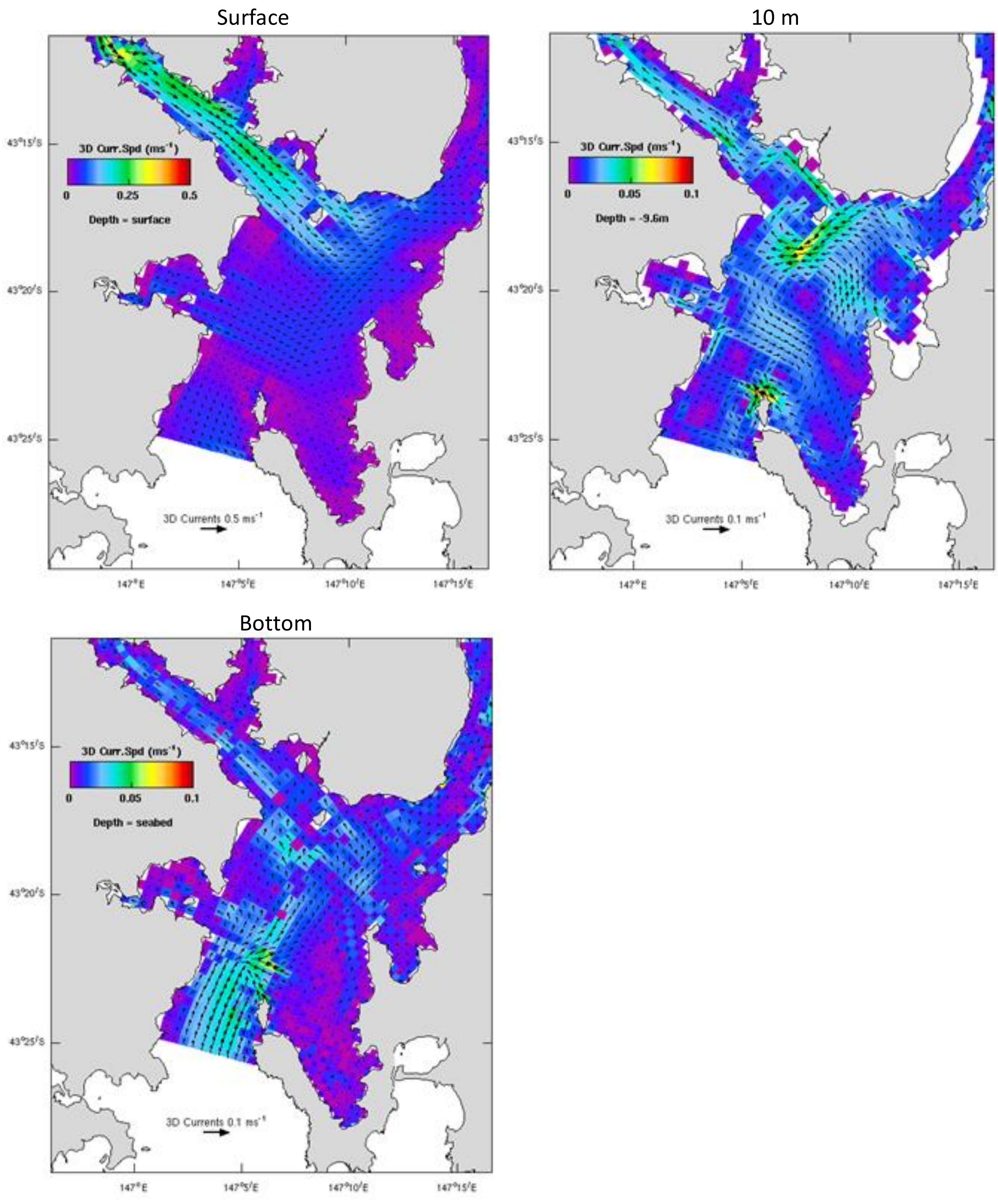

Figure 52: Mean annual current in the southern region of the D'Entrecasteaux Channel/Huon Estuary system estimated by the hydrodynamic model component of the biogeochemical model.

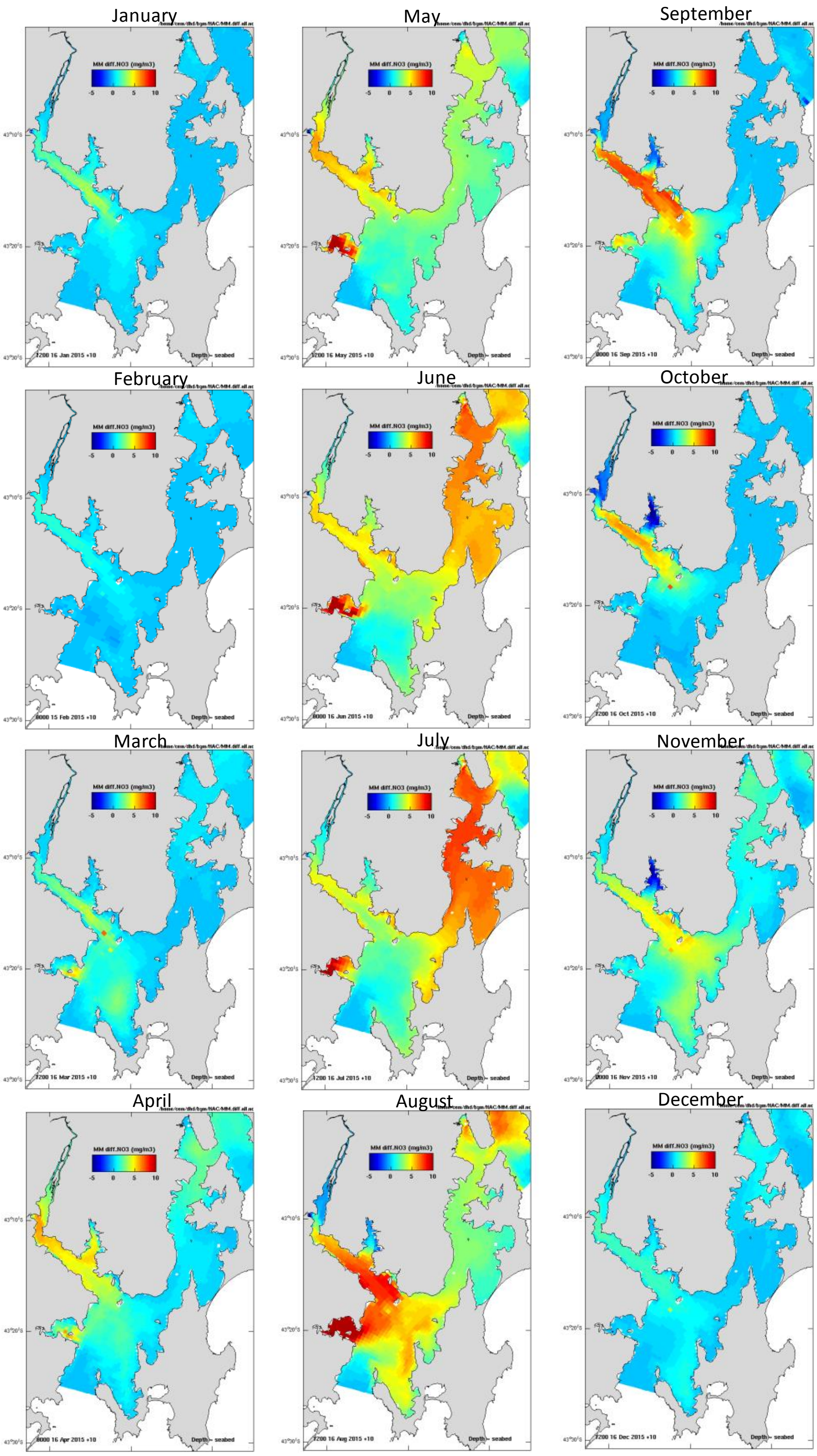

Figure 53: Biogeochemical model outputs showing the difference in the bottom water nitrate concentrations between the two modelled scenarios (i.e. the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

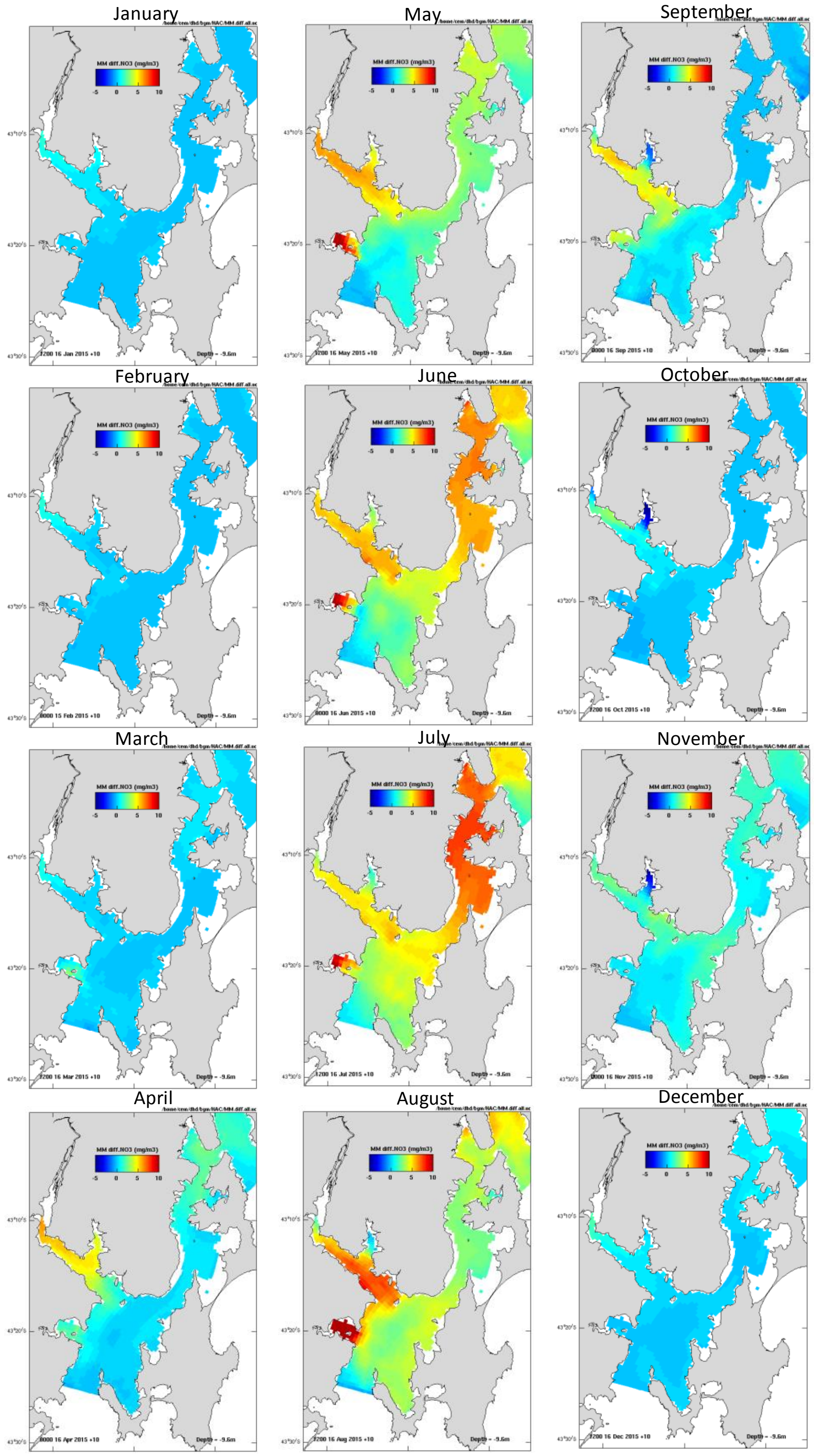

Figure 54: Biogeochemical model output of the difference in 10 m nitrate concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

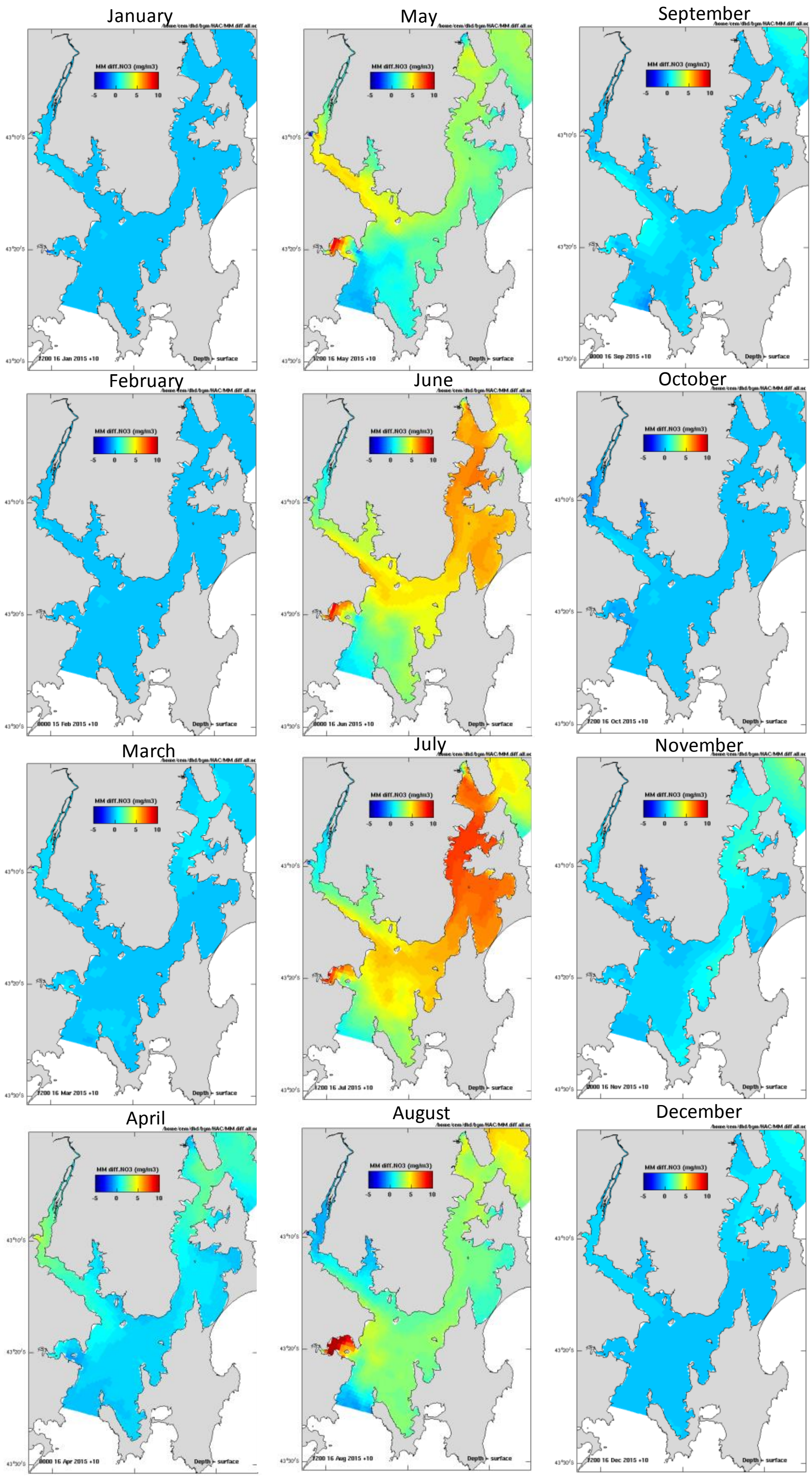

Figure 55: Biogeochemical model output of the difference in surface nitrate concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

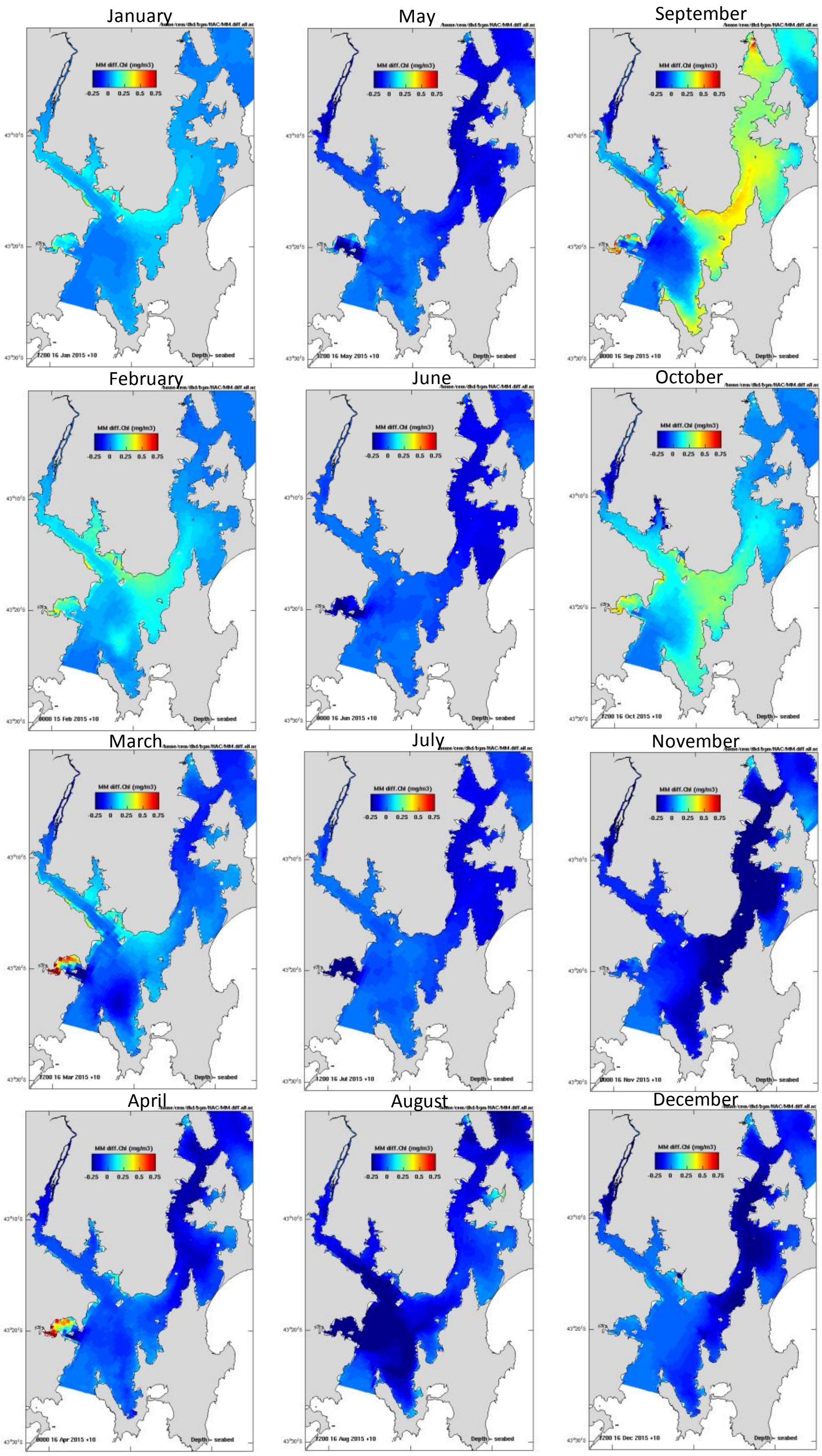

Figure 56: Biogeochemical model output of the difference in bottom chlorophyll-a concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

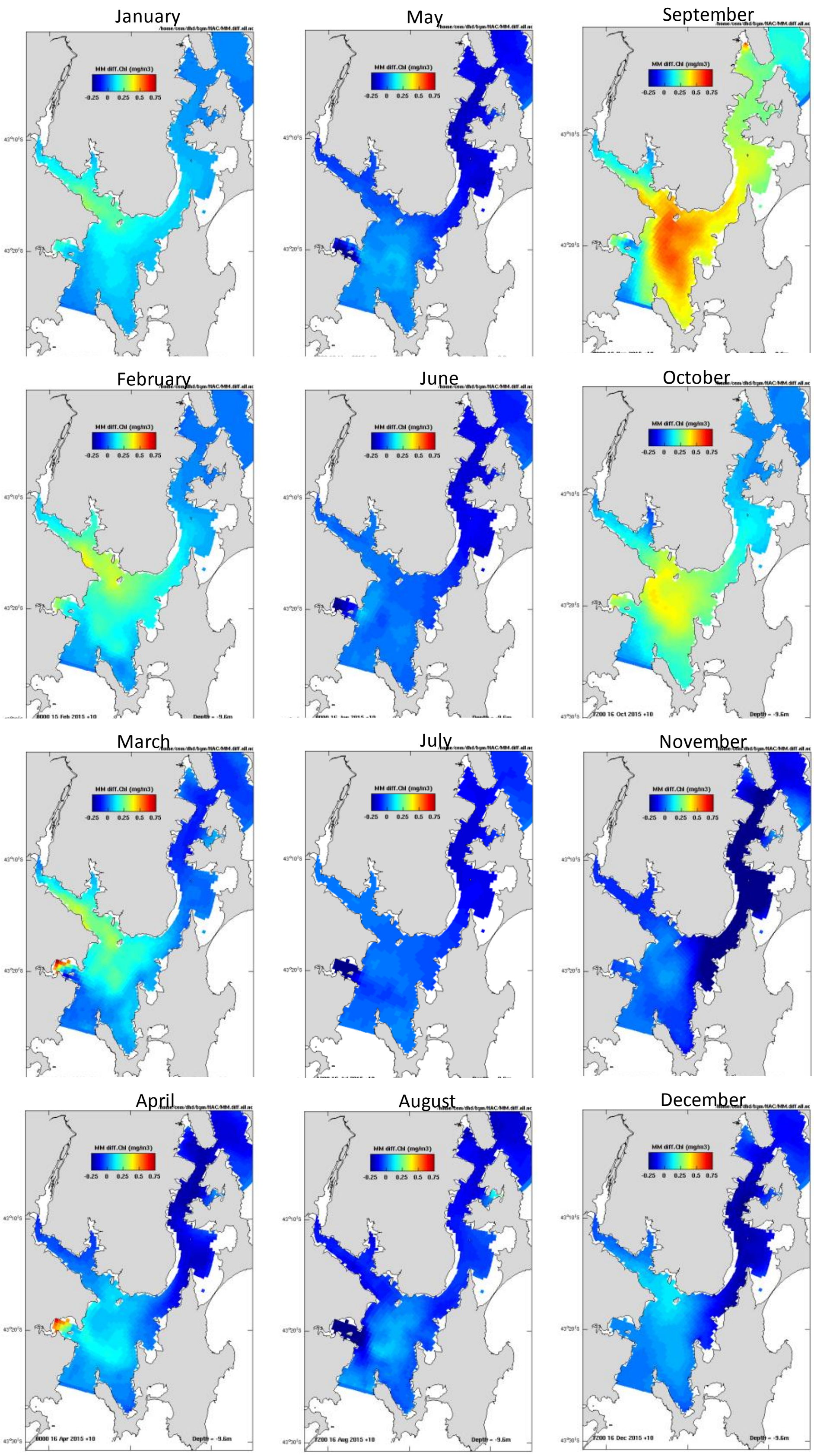

Figure 57: Biogeochemical model output of the difference in 10 m chlorophyll-a concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

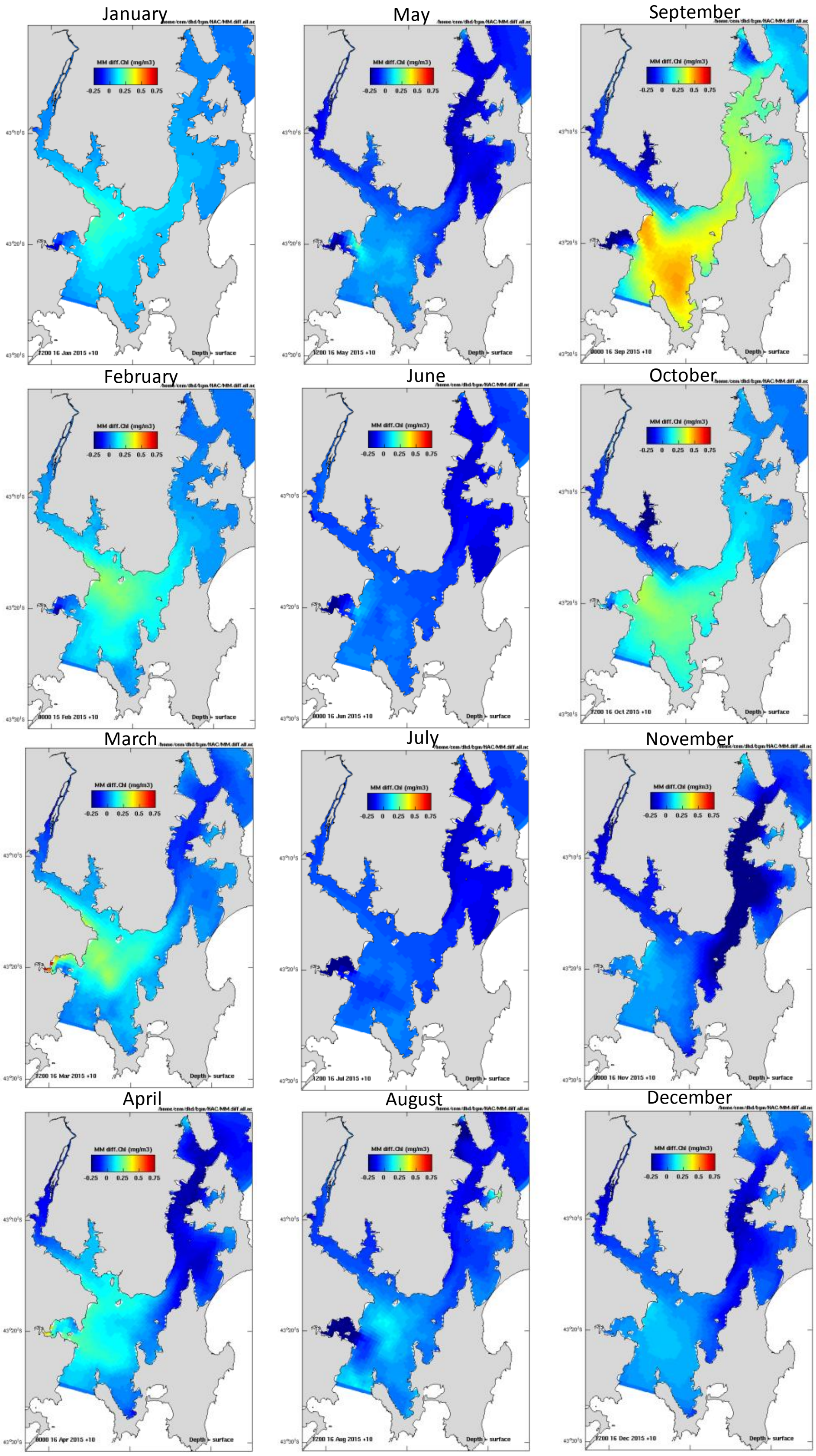

Figure 58: Biogeochemical model output of the difference in surface chlorophyll-a concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

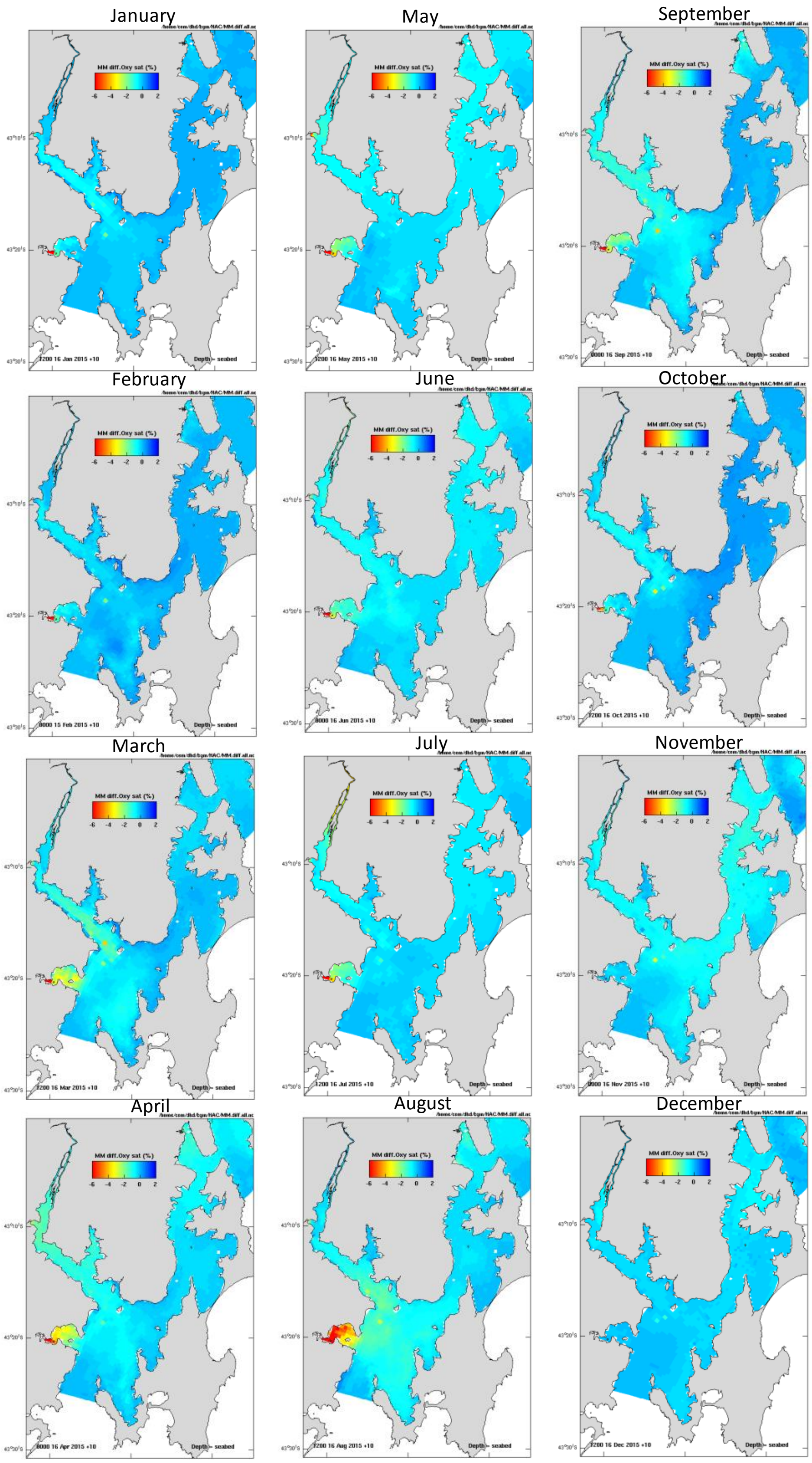

Figure 59: Biogeochemical model output of the difference in bottom dissolved oxygen concentration between the actual nutrient inputs that went into the D'Entrecasteaux Channel/Huon Estuary system during the HAC exceedance minus the capped nutrient input scenario (i.e. the difference between the two scenarios).

4.6 Summary of biogeochemical modelling results

Biogeochemical modelling of the D'Entrecasteaux Channel/Huon Estuary system was conducted for two separate production scenarios, i) the actual inputs that occurred during the exceedance period, and ii) a theoretical scenario in which HAC N inputs were reduced to comply with TPDNO limits. By comparing the modelled outputs for the key response variables and subtracting the results of theoretical capped scenario from those of the actual exceedance scenario, it was possible to identify the spatial and temporal differences that might be expected as a result of the exceedance.

Unsurprisingly, the greatest predicted localised impact of the exceedance was an increase in ammonia concentration in surface and middle waters near the East of Redcliffs and Flathead Bay leases. This ammonia is the result of fish excretion, and is dispersed relatively quickly due to the strong surface currents leaving the Huon Estuary that travel both north and south when entering the D'Entrecasteaux Channel. Some of this ammonia appears to have entered the deep waters where additional ammonia is also produced during the breakdown of particulate N (i.e. faeces) from the farms. The ammonia in bottom waters is dispersed up both the Huon Estuary and D'Entrecasteaux Channel by bottom currents, but the model suggests that ammonia persistence is low and that it is relatively quickly assimilated by phytoplankton or nitrified.

The model predicted a slight increase in chlorophyll-a during the spring and autumn blooms of 2015. However, the predicted increase was relatively small; which might explain why the BEMP monitoring data did not detect any change in either chlorophyll-a or algal composition. Much of the exceedance and additional N input occurred during winter when the photoperiod is short and not conducive to primary productivity. As a result, a large proportion of the ammonia was nitrified and the greatest predicted change to the system between the two modelling scenarios was an increase in nitrate from May–August. Despite this increase in nitrate concentration, there was no major spring algal bloom. This may reflect flushing and dilution of this nitrate prior to photoperiod increasing in spring or removal by denitrification. The model also predicted a relatively small decrease in bottom water dissolved oxygen in the vicinity of the exceedance, again this was not detected by the BEMP.

Other than the increase in ammonia in the immediate vicinity of the East of Redcliffs and Flathead Bay leases, most differences between the two scenarios were relatively small and often not particularly localised. For example, the maximum predicted difference in chlorophyll-a concentration was around 4 mg/m^3 (0.004 mg/l) and a change of this magnitude is below the accuracy of the equipment used to measure chlorophyll-a for the BEMP. Similarly, the maximum observed difference between the scenarios for ammonia and nitrate were in the range of 5–10 mg/m^3 (0.005–0.01 mg/l). To put this in context, ammonia fluctuations in the BEMP data generally range from undetectable to ~0.06 mg/l and nitrate from undetectable to ~0.1 mg/l. There was, however, historically high nitrate and ammonia concentrations at the BEMP site nearest to the exceedance (site 10), and there was an increases in ammonia across the southern region, including Port Esperance, which are both consistent with the biogeochemical models predictions.

The notable exception to the above was in Port Esperance. Despite there being no difference in the parameterisation of the model outside of the change in HAC inputs (e.g. the parametrisation of Port Esperance aquaculture, river and WWTP inputs were identical in each model implementation), the model predicted that the increased ammonia inputs would affect nutrient concentrations, dissolved oxygen and chlorophyll-a within Port Esperance. This is because the hydrodynamic model predicts a net influx of water into the Port by currents that have previously travelled in a southerly direction past the leases where the majority of the HAC N exceedance occurred. Due to the shallow, enclosed nature of Port Esperance, this ammonia was predicted to concentrate within and, once nitrified, resulted in increased nitrate concentrations. The elevated nitrate was then predicted to fuel phytoplankton production (increased chlorophyll-a) with a concomitant decline in dissolved oxygen during autumn and spring 2015 when the phytoplankton senesces and is broken down. Nonetheless, these differences are still relatively small (~0.01 mg/l increase for nitrate; ~0.004 mg/l increase in ammonia; ~0.0075 mg/l increase for chlorophyll-a; ~6% decrease in dissolved oxygen).

Modelling has shown that external drives such as river flow can have a major influence on system dynamics (Wild-Allen and Andrewartha, 2016) and thereby responses to perturbations such as the HAC exceedance. In wet years, river flows from the Huon lead to increased intrusion of marine waters, and hence nutrients, across the southern boundary, whereas in dry years this affect is reduced (Wild-Allen and Andrewartha, 2016). As such, in wet years with strong river flows and increased nutrient inputs from both rivers and the ocean, primary production is likely to be enhanced. During the time of the HAC exceedance river flows were relatively low, and as such, the system response observed may not be representative of the likely response in other background conditions/years.

5 Conclusions & recommendations

The 3 main objectives of this study were:

1. Establish the nature, timing and location of HACs reported dissolved N inputs in the context of historical patterns.

2. Assess the extent to which the HAC N inputs in the Huon River and Port Esperance Marine Farming Development Plan (MFDP) area has influenced local and broadscale environmental conditions – using information from the BEMP, farm inputs (objective 1. above) and other external nutrient sources, and any targeted regional sampling.

3. Using the data obtained from the farm evaluation together with other forcings, model the dispersion and ecological response of the system to nutrient inputs (i.e. where does it go and what does it do).

5.1 The nature, timing and location of N inputs

The feed inputs that were responsible for the exceedance of the Huon/Esperance MFDP TPDNO limit occurred largely at two leases (East of Redcliff's and Flathead Bay) in the south-western D'Entrecasteaux Channel, at the bottom end of the Huon/Esperance MFDP area. N inputs increased in December 2014 and remained high until November 2015, before returning to lower levels between December 2015 and March 2016. Although the N inputs had increased in late 2014, the actual exceedance of the TPDNO limit didn't commence until February 2015 because the TPDNO limit is based on a 12 month rolling calculation. This makes it difficult to pinpoint the timing of the exceedance and link it to the actual point in time where the greatest environmental response would have been observed.

It is important to emphasize that while the TPDNO was exceeded in the Huon/Esperance MFDP area, the major input of excess nutrients was actually in the D'Entrecasteaux Channel. The biogeochemical modelling predicted that these inputs could have influenced conditions within Port Esperance and may have resulted in elevated bottom ammonia concentrations both within Port Esperance and at sites located in the D'Entrecasteaux Channel MFDP area, a situation supported by the BEMP analyses. These findings would suggest that the two MFDP areas are not mutually exclusive, and highlights the complexity of the regional TPDNO management system. This may be particularly relevant given the southward shift of aquaculture practices in both MFDP areas.

At the time of the HAC exceedance in the Huon/Esperance MFDP area, Tassal were operating below their permitted TPDNO in both MFDP areas and HAC were operating below their permitted TPDNO limit in the D'Entrecasteaux Channel MFDP area. As a result, although HAC exceeded their TPDNO allocation of 659.63 t by 44% in the Huon/Esperance MFDP area, and the cap within this MFDP area (1084.63 t) was exceeded by 17%, the combined TPDNO for the south-east was not exceeded.

We were unable to determine whether the exceedance might have altered the way in which nutrients (N) entered the environment because of the challenges in matching biomass and feed inputs due to the complex nature of stock movements and how this is captured in farm records. If the FCR increased due to a period of excess feeding then we would expect more of the N to report to the system as particulate waste reaching the bottom, which would have quite a different effect than would a greater number of fish being fed efficiently. However, as noted we were unable to determine these differences and have therefore relied on observational data to infer the response mechanism.

5.2 Influence on local and broadscale environmental conditions

Although we were unable to determine the actual mechanism for the exceedance, in terms of production strategy and farm management, there was no evidence in the monitoring data to suggest a fundamental change in the way N entered the system. Nutrient dynamics in this system are complex with strong spatial and temporal influences. The highest levels of ammonia observed were associated with the exceedance event, but were in bottom waters and as such are likely to be beyond the photic zone or in areas where they would be nitrified or readily flushed from the system, and therefore unavailable to primary producers. As a result, there was no difference in the phytoplankton dynamics that could be directly related to the exceedance event. This was supported by the biogeochemical model which predicted only relatively minor changes in phytoplankton biomass (modelled as chlorophyll-a) as a result of the exceedance. The results suggest that ammonia concentrations in the system may not be a key driver of phytoplankton dynamics, and other environmental factors, such as temperature, salinity, light limitation and the seasonal influence of nitrate from the open ocean may have a greater effect on the spatial and temporal patterns of phytoplankton composition and abundance.

5.3 Modelled dispersion and ecological response to nutrient inputs

The biogeochemical model predicted relatively localised impact of the exceedance with increased ammonia concentrations in waters (surface, middle and bottom) near the East of Redcliffs and Flathead Bay leases. This ammonia dispersed relatively quickly and as a result only relatively minor changes in phytoplankton biomass (modelled as chlorophyll-a) were observed during the spring and autumn blooms of 2015. There was also some indication of a localised decrease in bottom water oxygen close to the leases where the majority of N inputs occurred. The predicted nutrient and chlorophyll-a increases, and oxygen decreases, were small (in many cases below the level of detection of the analytical equipment available through the BEMP) and therefore it is not surprising that it was not reflected in the BEMP monitoring data.

The model also identified a potential far field effect of the exceedance, with a notable response in Port Esperance. As there was no difference in the model parameterisation within Port Esperance this could reasonably be assumed to be a result of the HAC N exceedance. The modelling predicted that nutrient concentrations, dissolved oxygen and chlorophyll-a within Port Esperance would all be affected by the nutrient exceedance – albeit at relatively low levels. Unfortunately the greatest predicted changes were some distance from the nearest BEMP monitoring site and so there is no observational data to confirm the predictions.

5.4 Other important findings and recommendations

The frequency (monthly or bimonthly in summer) of sampling and distribution of sites in the monitoring program may have had an effect on our ability to detect changes in both nutrient levels and phytoplankton abundance and composition, particularly in the immediate vicinity of where the majority of the exceedance occurred. As noted earlier the BEMP was designed to provide an early warning of sustained system wide impacts. The capacity of the BEMP to detect near field effects or a gradient of impact associated with changes in inputs from one or more specific leases in a particular region is limited. However, if the exceedance did lead to a system wide response, the BEMP is designed to detect it and the results herein suggest that it would likely have done so – given that the data did show the bottom water changes consistent with the modelled scenario outputs.

We have provided some recommendations below to increase the sensitivity of the sampling program and improve the ability to detect ecological responses to short term, or localised, N exceedances such as that investigated in this study should they occur in the future.

- The BEMP is designed to identify major broadscale changes but if causal responses to localised exceedances/impacts are required, targeted, rapid-response programs are necessary.
- Phytoplankton sampling may be inadequate at present to identify short term, or localised, blooms. This is exacerbated by the fact that current CTD/Sonde sampling is inadequate to identify the depth of the halocline, which can affect the depth at which phytoplankton aggregate.
- There is currently inadequate knowledge about the links between local (i.e. lease level compliance monitoring) and broadscale (i.e. BEMP) sampling and how these might better inform management and interactions in the ecosystem as a whole.
- There may not have been large-scale impacts because Tassal were farming below their TPDNO limit during the HAC exceedance meaning the combined TPDNO for the D'Entrecasteaux Channel/Huon Estuary was not exceeded.
- Under the 12-month rolling TPDNO management system, it is possible for a very large quantity of N to be introduced to the system before the TPDNO limit is exceeded. This can influence the ability of studies such as this to identify any negative impacts as it is not necessarily during the period of TPDNO exceedance that the greatest N inputs, and hence impacts, occur. As such, aquaculture companies should be encouraged to report the likelihood of a future exceedance so that additional sampling can be undertaken at appropriate times.
- Although HAC exceeded their TPDNO limit in the Huon/Esperance MFDP area, the majority of their inputs were actually in the D'Entrecasteaux Channel. Further, as aquaculture production has increased there has been a shift toward the southern D'Entrecasteaux Channel and lower Huon Estuary. There is a need to carefully consider the MFDP boundaries and the implications for system management.
- Biogeochemical modelling results have highlighted the susceptibility of Port Esperance with the potential for N inputs outside of the Port to influence the conditions within.
- Increasing the frequency of sampling, particularly during exceedance events, could enhance the ability to detect any relationship between phytoplankton biomass and nutrient concentration.

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7 Appendix i: Correction for inter-laboratory variation in ammonia measurement

As AST were able to provide ammonia concentrations for the first 51 BEMP campaigns, when technically CSIRO were contracted to do so, it was not necessary to correct for inter-laboratory variation in ammonia concentration reported by Eriksen (2009). It was, however, necessary to correct for this difference to allow ammonia to be compared against the baselines and triggers that were proposed by CSIRO using ammonia measurements undertaken in their laboratories (Thompson *et al.*, 2008).

Firstly, units were converted from µM-N to mg/l-N (hereafter abbreviated to mg/l) using its molecular weight (14.0067 µ). To confirm the mean difference of 0.21 µM-N between CSIRO and AST determined by Eriksen (2009), data from surveys 1–42 when the laboratories were measuring ammonia/ammonium concurrently, were compared. A linear relationship between the data sets was identified and modelled (Figure 60; Table 6). The model provided a relatively good fit (R^2 = 0.73). This indicates that the difference between the two laboratories is dependent on concentration, and that using the modelled relationship would be more accurate method for conversion than the offset of 0.21 µM-N determined by Eriksen (2009); this likely reflects the much larger data set now available for the inter-laboratory comparison

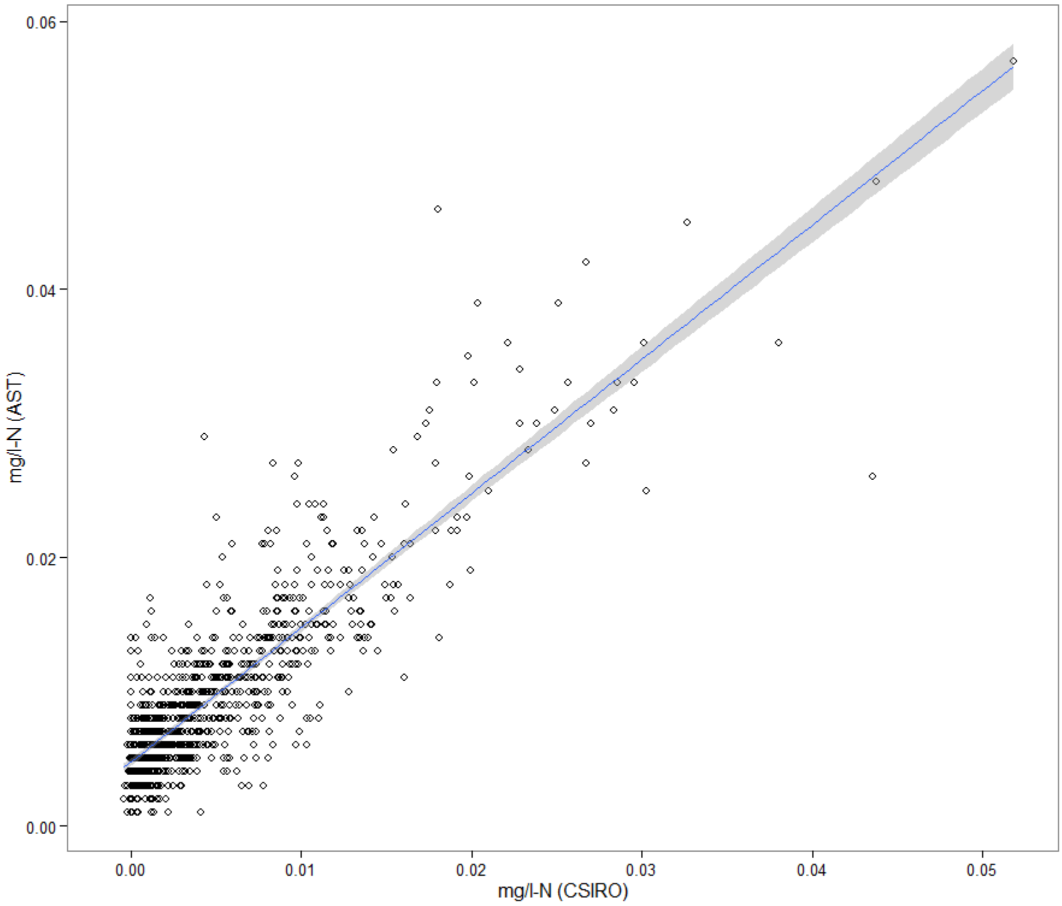

Figure 60: Linear relationship between CSIRO and AST ammonia/ammonium N concentration. Blue line indicates the linear relationship; shaded area represents 95% confidence intervals of the model.

Table 6: Linear model of CSIRO and AST ammonia/ammonium concentration to facilitate the conversion of CSIRO data to align with the latter AST data.

Summary statistics are as follows: residual standard error = 0.003428 on 1086 degrees of freedom, multiple R-squared = 0.7343, adjusted R-squared = 0.7373, F-statistic = 3004 on 1 and 1086 DF, p-value = <0.001.

Coefficient	Estimate	Std. error	t-value	*p*
(Intercept)	0.0047481	0.0001298	36.57	<0.001
AST ammonia	1.0017627	0.0182764	54.81	<0.001

8 Appendix ii: Methods used to adjust HAC N inputs for theoretical modelling scenario

The following methods were trialled to adjust HAC N inputs so that they did not exceed their TPDNO limit:

- Contribution method: the monthly N contribution of each lease from the Huon/Esperance MFDP area were re-proportioned to equal the TPDNO limit during the 12-month period beginning December 2014 and ending November 2015 (i.e. the time period when N inputs were the greatest). The data was re-proportioned using the monthly N contribution from each lease from 2012 to 2014 (i.e. during a period where operations were normal). This method resulted in consistently high N inputs in this MFDP area without exceedance during any 12-month period (Figure 61).
- Replacement method: this method replaces the 2015 N contributions from Flathead Bay and Roaring Beach leases with the contributions from the same two leases in 2014. This resulted in low N inputs for much of the time period as these two leases were not operating at high levels during 2014 (Figure 61)
- Percentage method: in this method, the N inputs of each lease in the Huon/Esperance MFDP area are multiplied by the percentage 69.24% (i.e. multiplied by 0.6924) required to decrease the TPDNO so that the limit is reached during a single 12-month period, but never exceeded. This method mirrors the trend of the actual data accurately but results in unrealistically low values for much of the time period in question (Figure 61).

Following the above, the contribution method was deemed to be most suitable as it maintained high N inputs throughout the time period in question, without exceeding the TPDNO limit.

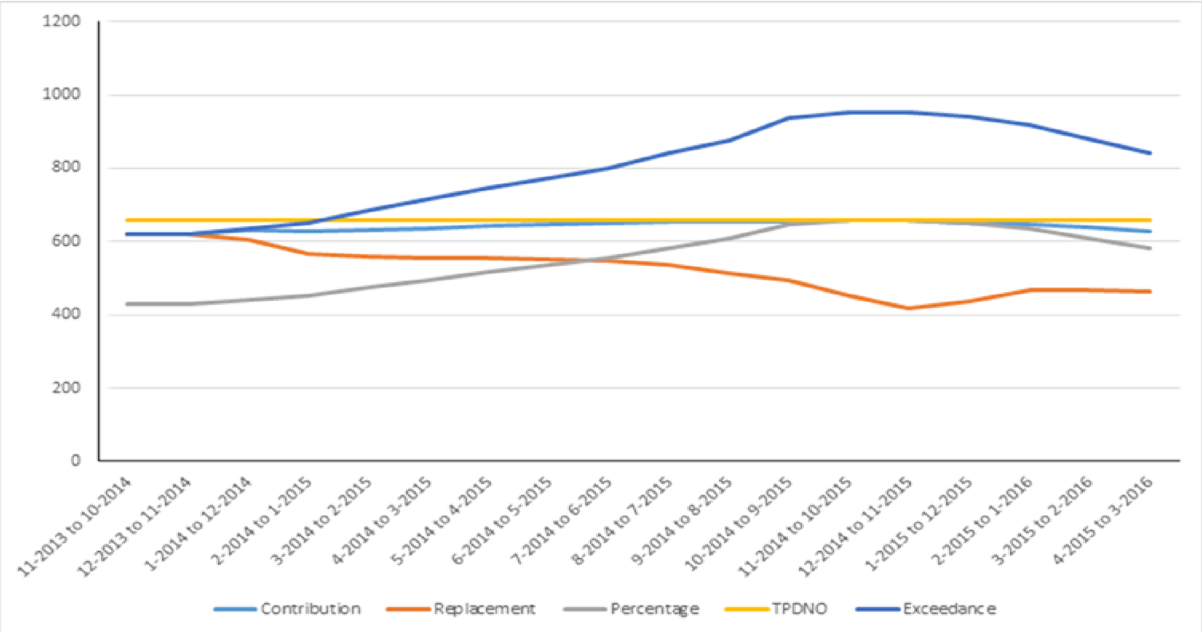

Figure 61: The HAC TPDNO limit for the Huon Esperance MFDP area (yellow line), actual N inputs (dark blue line), adjusted inputs using the previous contribution method (light blue line), adjusted inputs using the percentage contribution method (grey line) and adjusted inputs using the replacement of Flathead Bay and Roaring Beach method (orange line).

9 Appendix iii: Bottom nitrate concentration at each BEMP site

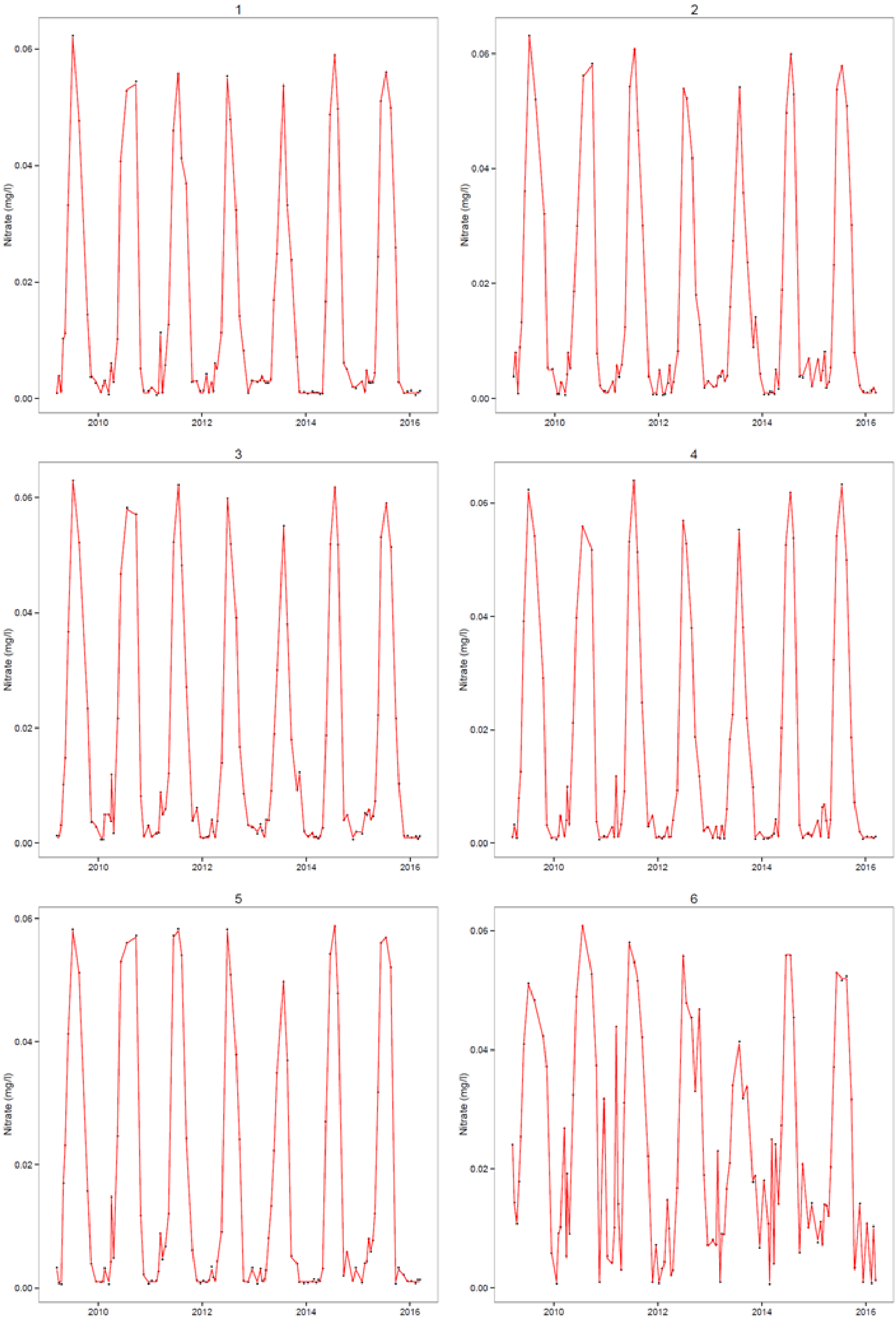

Figure 62: Bottom nitrate concentration at each BEMP site.

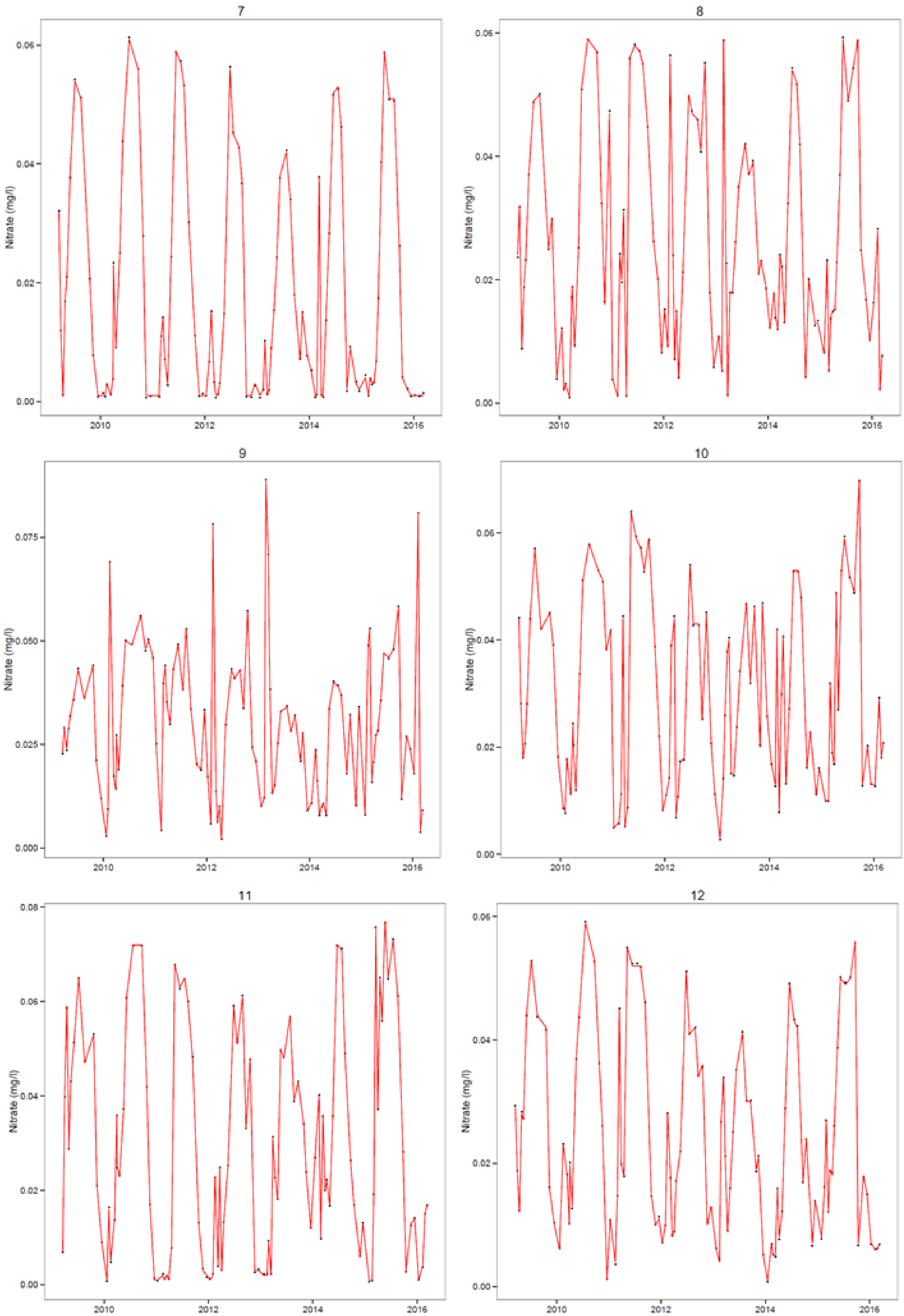

Figure 62 continued: Bottom nitrate concentration at each BEMP site.

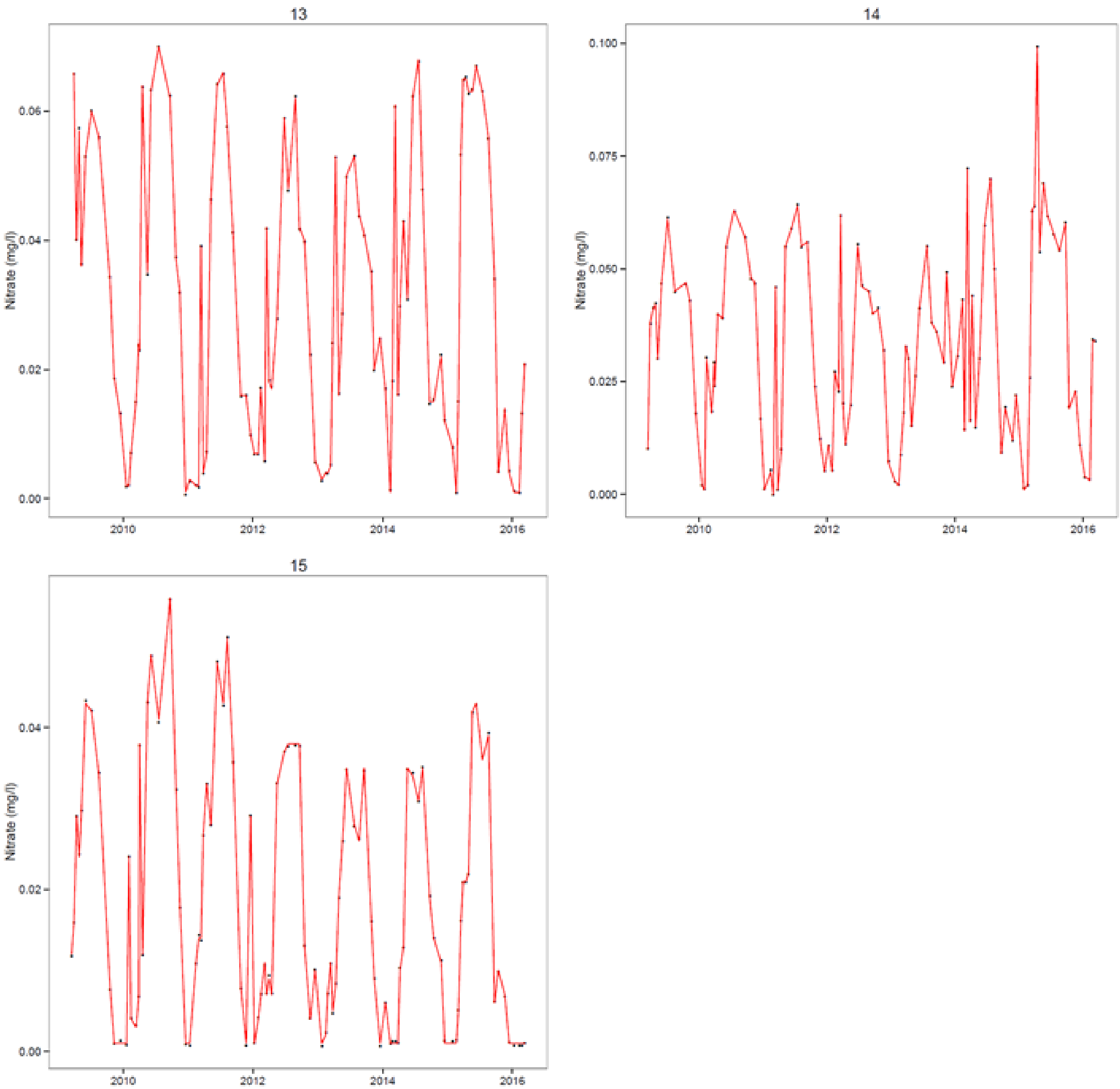

Figure 62 continued: Bottom nitrate concentration at each BEMP site.

10 Appendix iv: Bottom ammonia concentration at each BEMP site

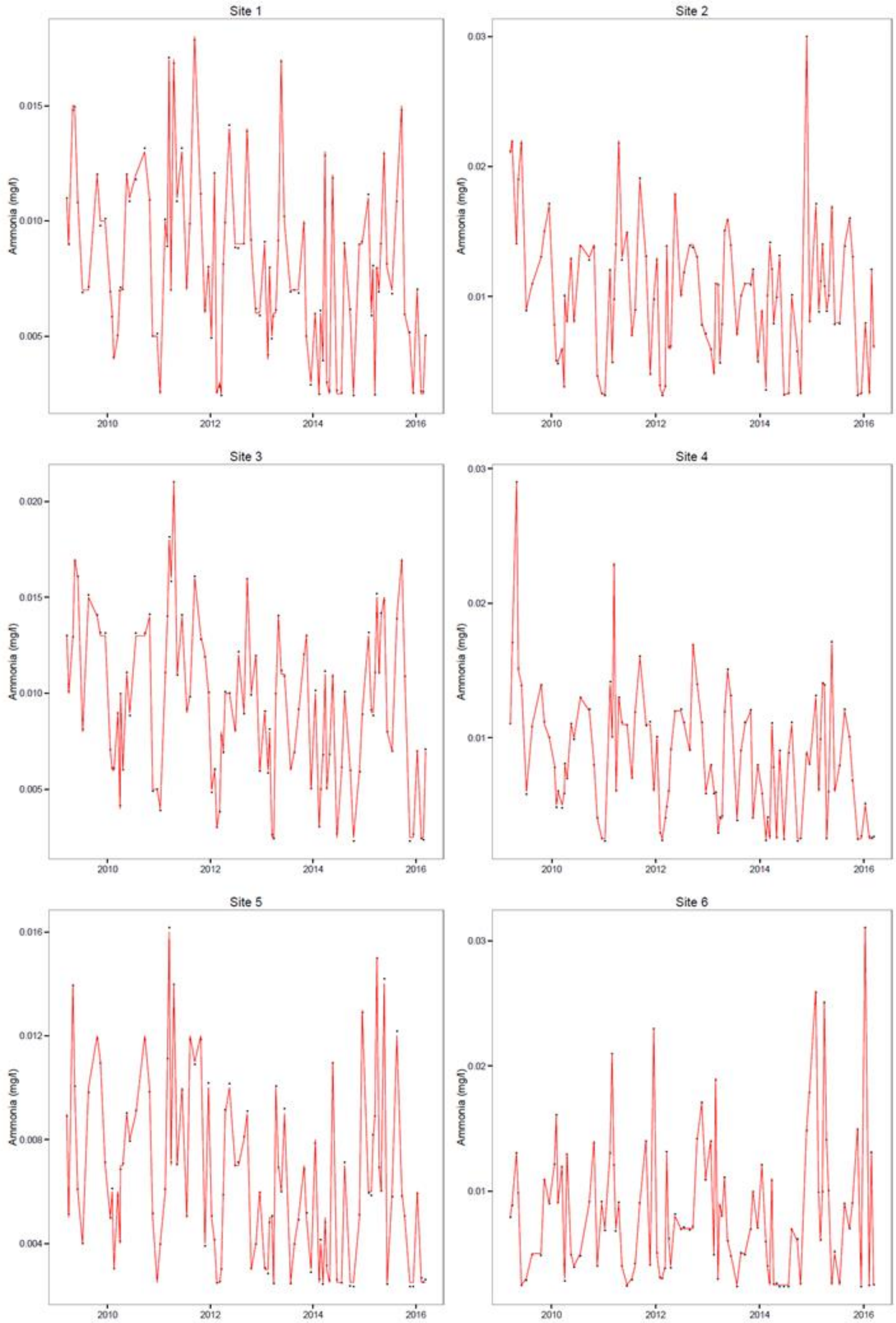

Figure 63: Bottom ammonia concentration at each BEMP site.

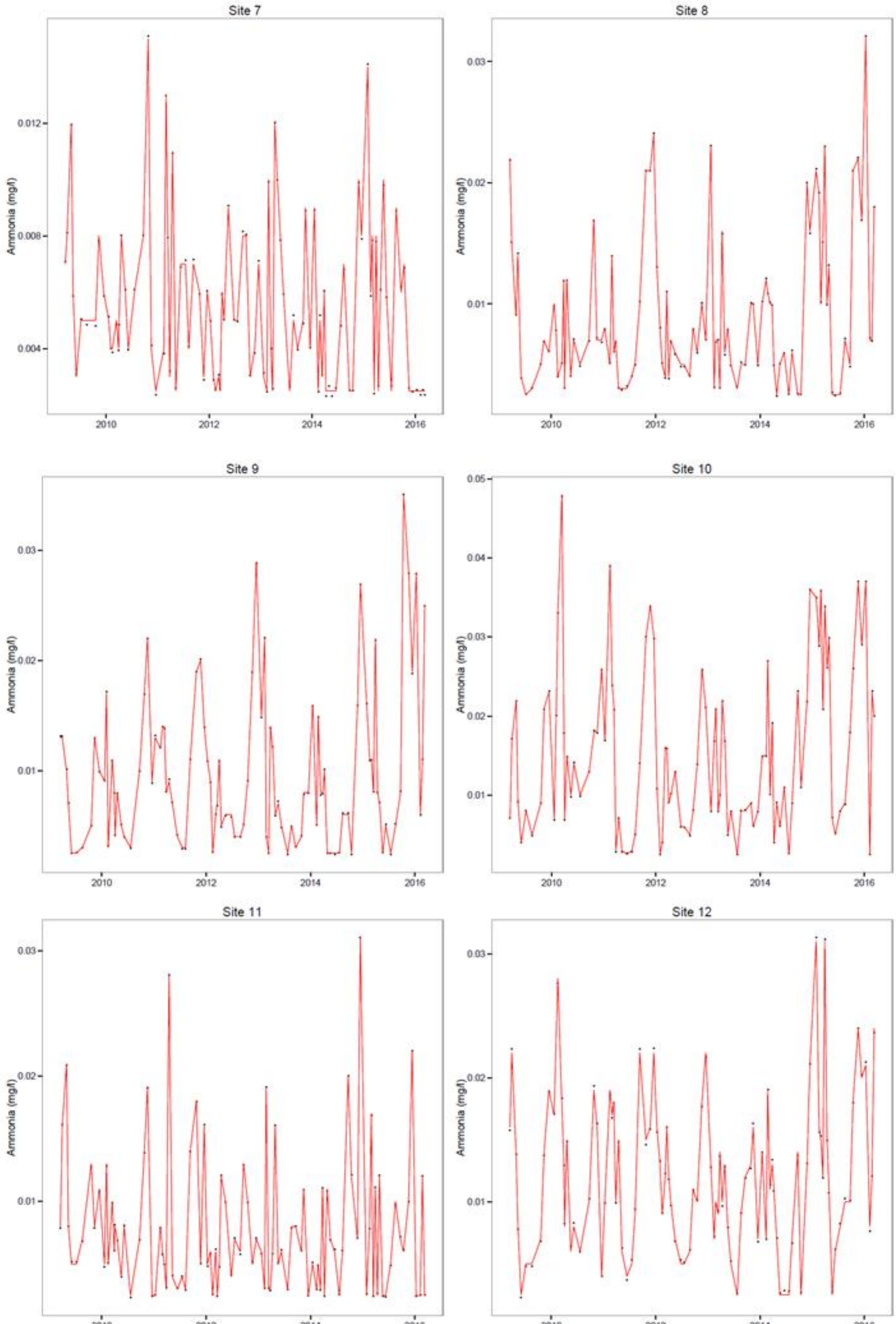

Figure 63 continued: Bottom ammonia concentration at each BEMP site.

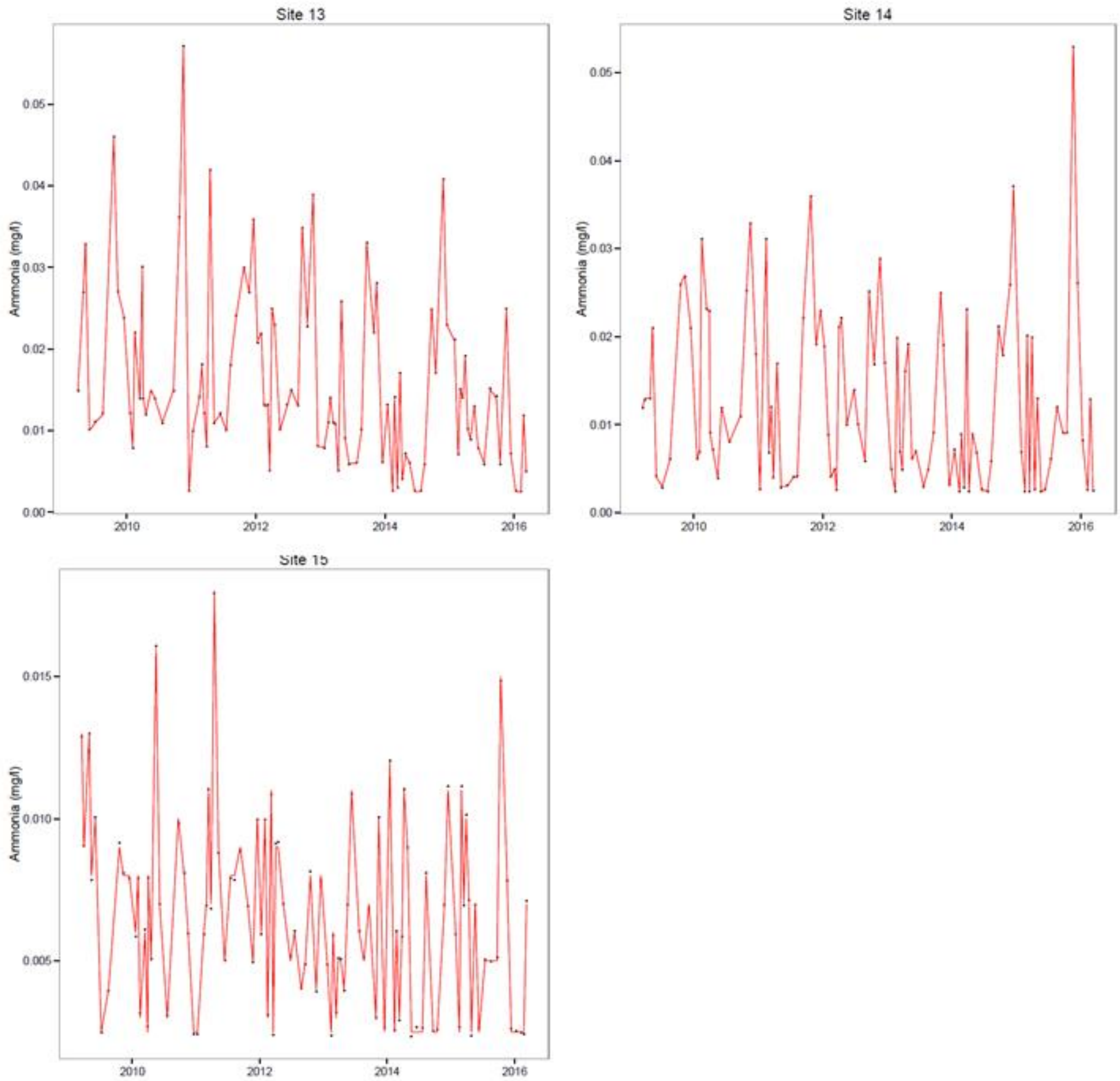

Figure 63 continued: Bottom ammonia concentration at each BEMP site.

11 Appendix v: Bottom dissolved oxygen concentration at each BEMP site

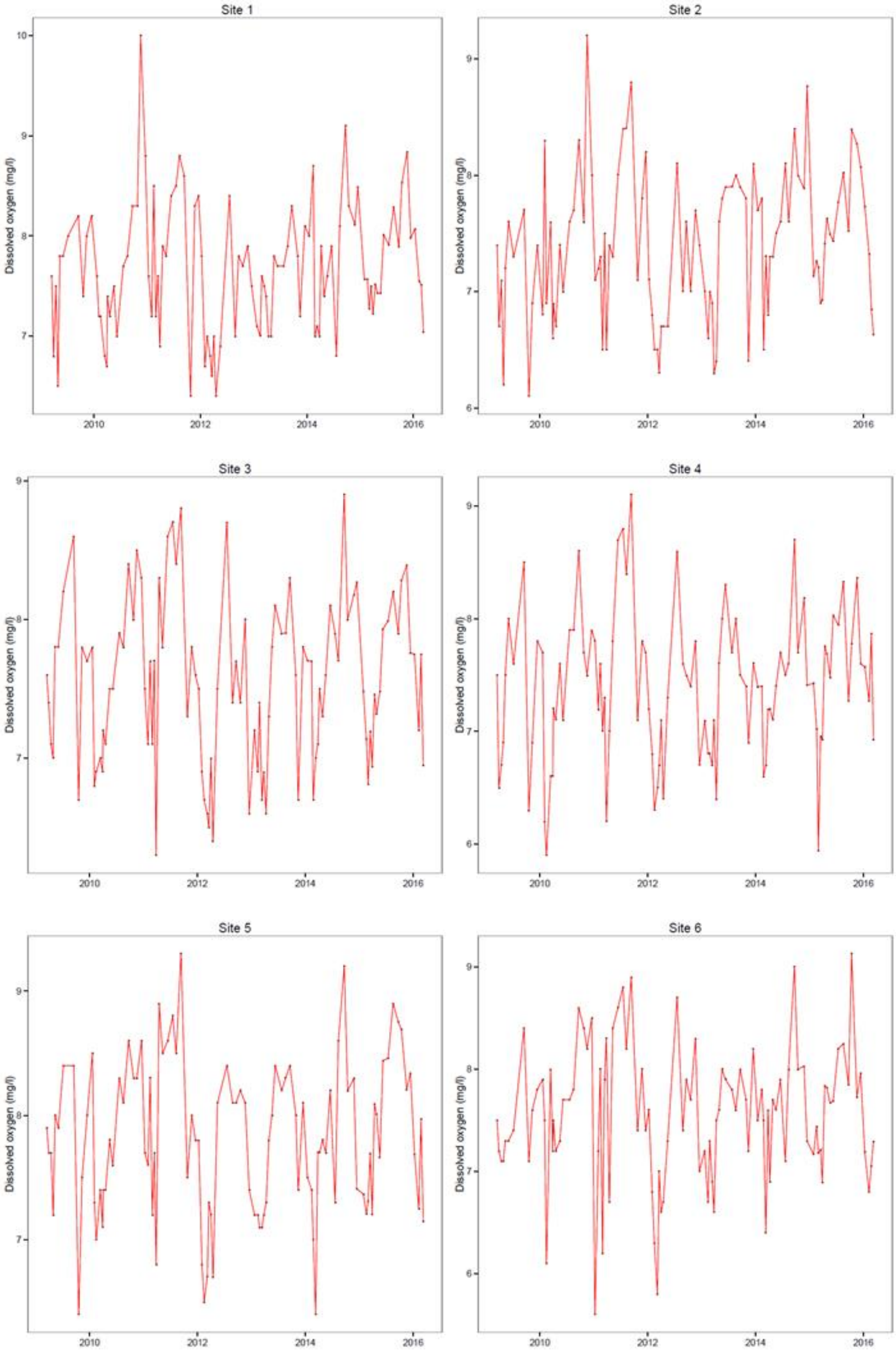

Figure 64: Bottom dissolved oxygen concentration at each BEMP site.

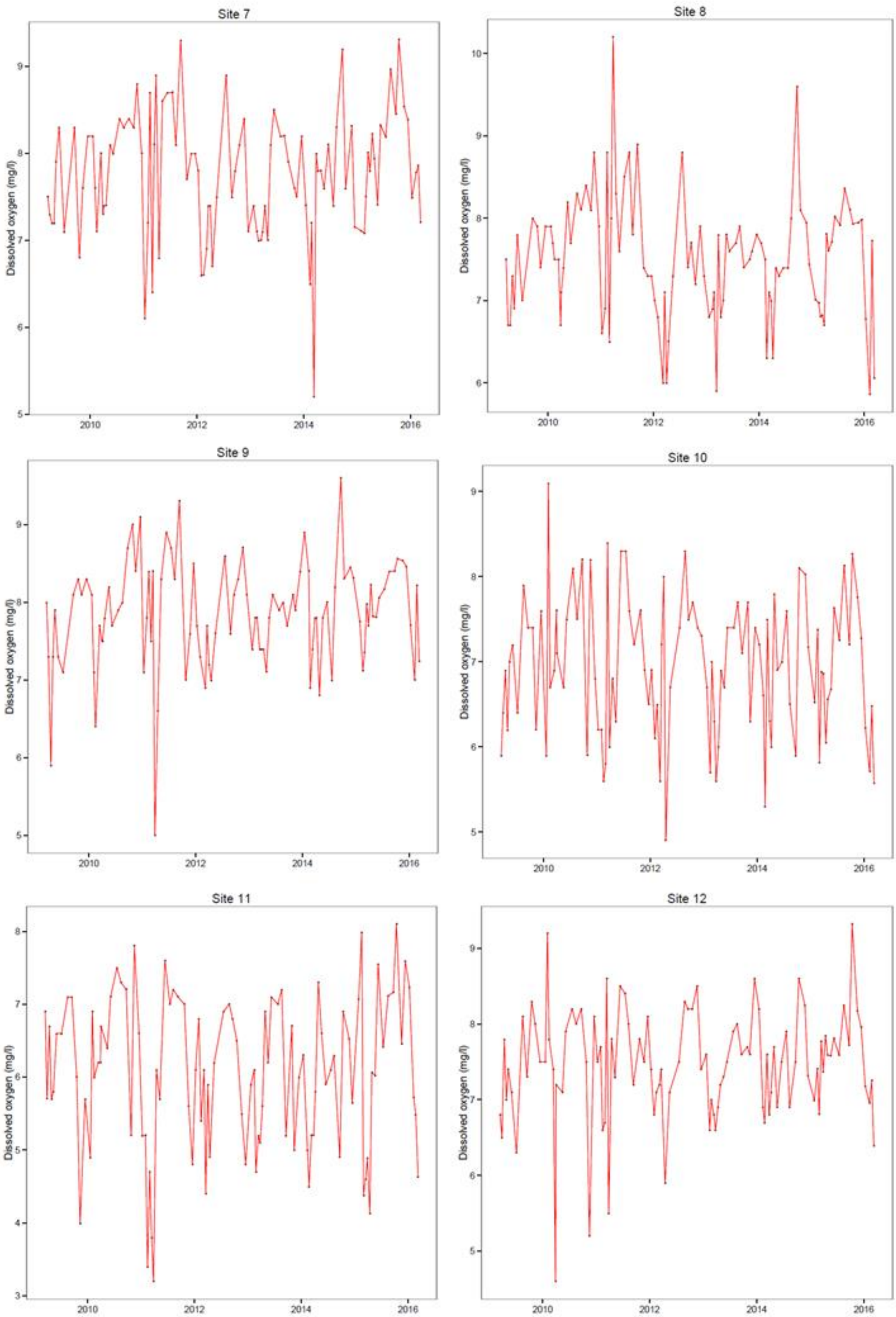

Figure 64 continued: Bottom dissolved oxygen concentration at each BEMP site.

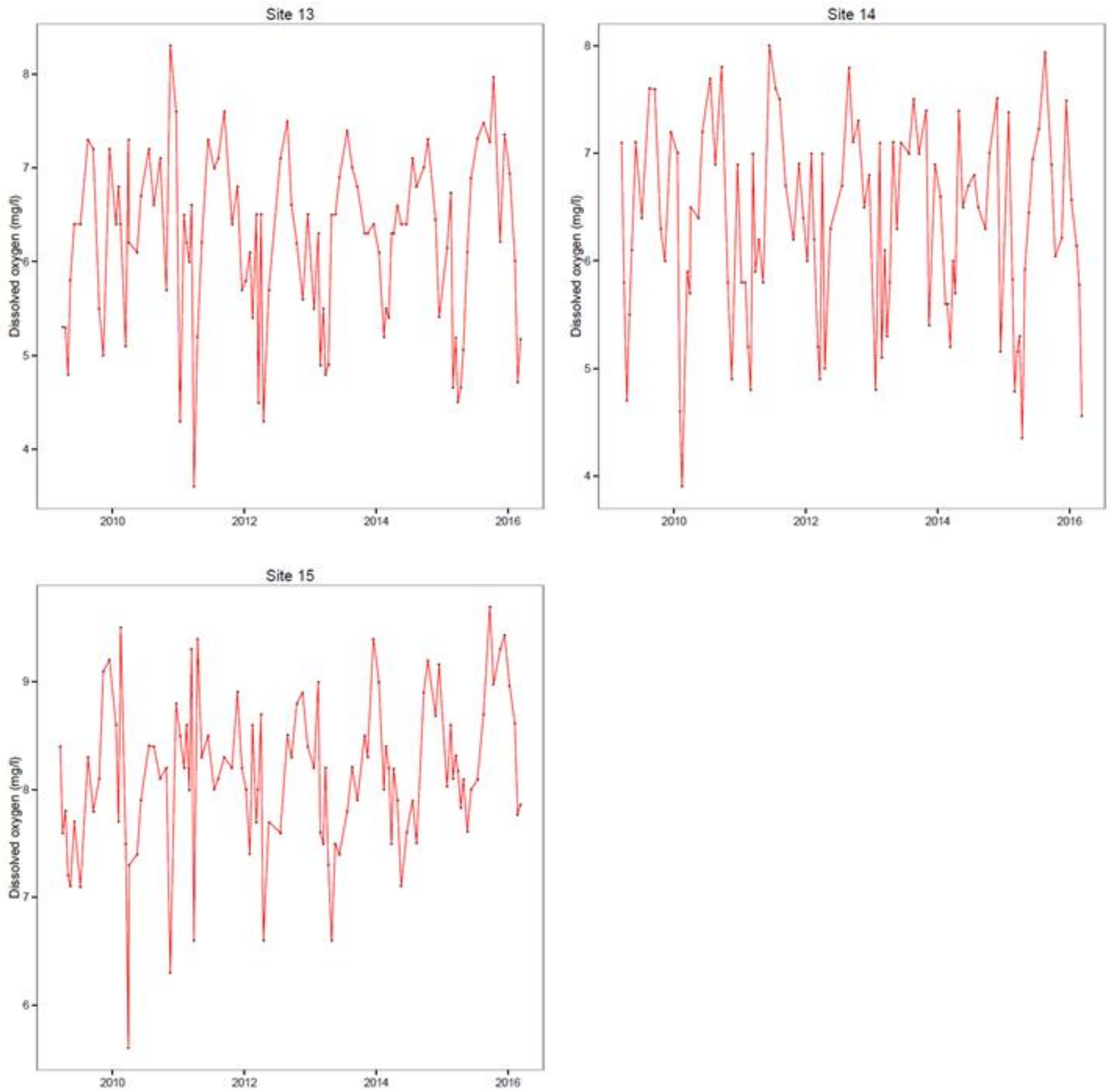

Figure 64 continued: Bottom dissolved oxygen concentration at each BEMP site.