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Futureproofing the green-lipped mussel aquaculture industry against ocean acidification

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Report

Final report for Sustainable Seas National Science Challenge Innovation Fund project
Ocean acidification mitigation strategies for the mussel industry (IF2.2.2.1)

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More information on this project, visit

www.sustainableseaschallenge.co.nz/mitigating-mussel-acidification



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Executive summary

Ocean acidification is a potential threat to the growth, condition and survival of the Green-lipped mussel (*Perna canaliculus*) and may have implications for the New Zealand shellfish aquaculture industry. This study first assessed the current status of the pH and carbonate system in the primary mussel aquaculture areas of the Marlborough Sounds and Firth of Thames, and generated future projections of pH and carbonate availability for the Marlborough Sounds. Pilot studies were then carried out to assess the potential of two intervention techniques to increase pH and dissolved carbonate availability at mussel farm and dropper line scales. This was carried out in consultation with Sanford Limited, with in situ measurements and tests at the Ecofarm in Old Homewood Bay in the Marlborough Sounds. The potential of waste shell to raise dissolved carbonate and pH was assessed by determining the factors influencing the dissolution of waste shell, and also shell dissolution rates in the laboratory and on dropper lines. These results were applied to determine the impact of incorporating waste shell on dropper lines and waste shell deposition on the seafloor. In addition, tests were carried out to determine the effect of waste shell on the short-term growth rates of juvenile mussels. The second intervention technique, using aeration to strip CO₂ and so raise carbonate and pH, was assessed in controlled tests in a seawater pool, with results scaled up to a mussel farm. Aeration was also trialled on a mussel farm to determine the practicality of this approach. Recommendations are presented based on these results and alternative mitigation options briefly considered.

Primary results:

- The inner Firth of Thames currently experiences the lowest seasonal pH of the sites monitored, with a daily minimum of 7.84 (7.79–7.96) in autumn, with short-term (15-minute) pH minima as low as 7.2. Time-series data in the inner and outer Firth of Thames, and also on a mussel farm in the western Firth, show episodic declines in carbonate saturation to the critical carbonate saturation state $\Omega_{AR} = 1.0$ at which solid aragonite (the form of carbonate in mussel shells) will start to dissolve. Consequently, mussels in the Firth of Thames experience episodic corrosive conditions.
- The mean pH in the Marlborough Sounds region is projected to decrease by 0.15–0.4 by 2100 depending on future emission scenario. The corresponding decline of 0.5–1.25 in the saturation state of aragonite (Ω_{AR}), results in the critical threshold of $\Omega_{AR} = 1$ being reached by 2100 under the worst-case scenario. These projections are based only on future CO₂ emission scenarios and do not consider other coastal sources of acidity in coastal waters which may also alter in the future.
- At night pH within the boundary layer on a mussel dropper line may be up to 0.1 lower than the surrounding water due to respiration in the absence of photosynthesis. Consequently, mussels experience a more corrosive environment at night on dropper lines relative to the surrounding water.
- To raise pH from the present-day minimum to present-day mean in a one-hectare mussel farm would require instantaneous addition of 0.6 tonnes of dissolved carbonate. However, dilution by currents is high, and so to maintain this supply of dissolved carbonate would require 3.5–36 tonnes/day, depending on mussel farm location and current regime.
- Screening tests using mild acid to assess the factors influencing dissolution of waste shell identified shell particle size as a primary factor, with powdered shell dissolving faster than whole, fragmented, or crushed shell. Older shells dissolved more readily than fresh shell, and highest dissolution rates were obtained after waste shell had been submerged in coastal water or buried in sediment for one month.
- Laboratory tests using coastal seawater confirmed the threshold for mussel shell dissolution at a $\Omega_{AR} = 1.1$, slightly higher than the accepted Ω_{AR} value of 1.0. This Ω_{AR} value is already experienced episodically on mussel farms in the Firth of Thames.
- In dissolution tests, waste shell maintained on dropper lines showed an average mass loss of 0.012%/day (range 0.002–6%), which is lower than reported dissolution rates at the sediment surface. Maintenance of waste shell on dropper lines also had negligible measurable effect on pH.

Executive summary

- To raise pH from the present-day minima to present-day mean within the boundary layer ($\sim 10\text{m}^3$) of a dropper line would require dissolved carbonate addition of 8.5–85 kg/day at current speeds of 0.01–0.1 m/s. If this were delivered by waste shell the slow dissolution rate would necessitate loading of 0.9–3.6 tonnes waste shell/m dropper line at the lowest current speeds, which is not practical.
- The impact of waste shell addition on growth and survival of spat could not be tested on dropper lines due to low spat availability for seeding. Instead, juvenile mussels were tested in laboratory experiments under present-day pH and future pH projected for the year 2050. The presence of waste shell had no effect on juvenile mussel growth (shell length or width), or their overall physical or physiological condition over a 5-week experimental timescale.
- Although deposition of waste shell on the seafloor may be practical an excessive amount of waste shell (>11,000 tonnes) is required to significantly raise pH and carbonate availability on a one-hectare mussel farm. The waste shell would need to be at least 1.5 km upstream of the farm to ensure vertical mixing and availability of the resulting dissolved carbonate to mussels on the upper dropper lines.
- Aeration tests in a coastal seawater pond determined the efficiency of different tubing and flow rates, with the results extrapolated to a mussel farm. This technique has potential to raise pH and carbonate availability, but its effectiveness is also reduced by dilution. For example, aeration of 50% of the volume of a one-hectare mussel farm would raise dissolved carbonate levels by less than 5%. The associated air delivery requirement would be high and incur significant capital and power generation costs.
- Location of aeration needs to be assessed as a pilot test on a mussel farm identified that near-surface aeration (4 m depth) within the farm resulted in re-suspension of particulate matter and a reduction in pH and dissolved oxygen.

Recommendations

Within the scope of this study the application of waste shell has minimal potential for ameliorating ocean acidification within mussel farms. Alternative applications of waste shell, such as incorporation within the matrix of dropper lines and calcination to produce lime may represent more effective ways of using waste shell. CO_2 removal by aeration has limited potential for raising pH over a mussel farm, although further studies could investigate the potential of microscale aeration within dropper lines, and other potential “bio-buffering” options such as macroalgae beds upstream of mussel farms.



Introduction and rationale

Ocean acidification (OA)

Ocean acidification (OA) is the long-term decrease in seawater pH that is primarily driven by the increase in atmospheric CO₂. OA is not just a decline in seawater pH, but also involves changes in the concentration of different dissolved inorganic carbon species in the marine carbonate system (Figure 1). The overall process of acidification arising from CO₂ transfer from atmosphere into seawater results in (1) an increase in dissolved CO₂ (pCO₂), which reacts with water to form carbonic acid (H₂CO₃) (2). This acid then (3) dissociates to produce bicarbonate ions (HCO₃⁻) and hydrogen ions (H⁺), with the latter reacting with carbonate ions (CO₃²⁻) to form more bicarbonate (4). Consequently, OA results in an increase in dissolved CO₂ and bicarbonate, and decreases in pH (i.e., an increase in H⁺) and dissolved carbonate (Figure 1).

Of relevance to shellfish, and so the mussel aquaculture industry, is the impact this has on dissolved carbonate availability. The decline in carbonate ions causes a corresponding decrease in the carbonate saturation state (Ω) which affects the stability of solid carbonate (e.g. shells and exoskeletons). Generally, when Ω is >1 solid carbonate

is thermodynamically stable, whereas when Ω falls below 1 it will dissolve; consequently, $\Omega = 1$ is regarded as an important threshold for organisms that form carbonate shells, such as shellfish. Solid carbonate exists primarily in two forms, calcite and aragonite, which have different crystalline structure and different solubilities.

As a result of increasing atmospheric CO₂ there has been a 30% increase in the acidity of seawater in the open ocean since the start of the industrial revolution, corresponding to a decrease in pH from 8.2 to 8.1 and an associated 10% decline in carbonate concentration (Orr et al, 2005). Furthermore, the current rate of increase in CO₂ emissions is projected to cause a further decline in pH to 7.8 and a 60% decrease in carbonate concentrations by 2100 (Bopp et al, 2013).

Coastal acidification

Coastal waters experience greater variability in pH and the carbonate system than the open ocean; while the mean pH of the open ocean is 8.1 with an annual variation of <0.1, pH in coastal waters ranges from 7.0 to 8.8, with a daily variation as high as 0.5 in some regions (Cornwall et al. 2013; Law et al 2018a).

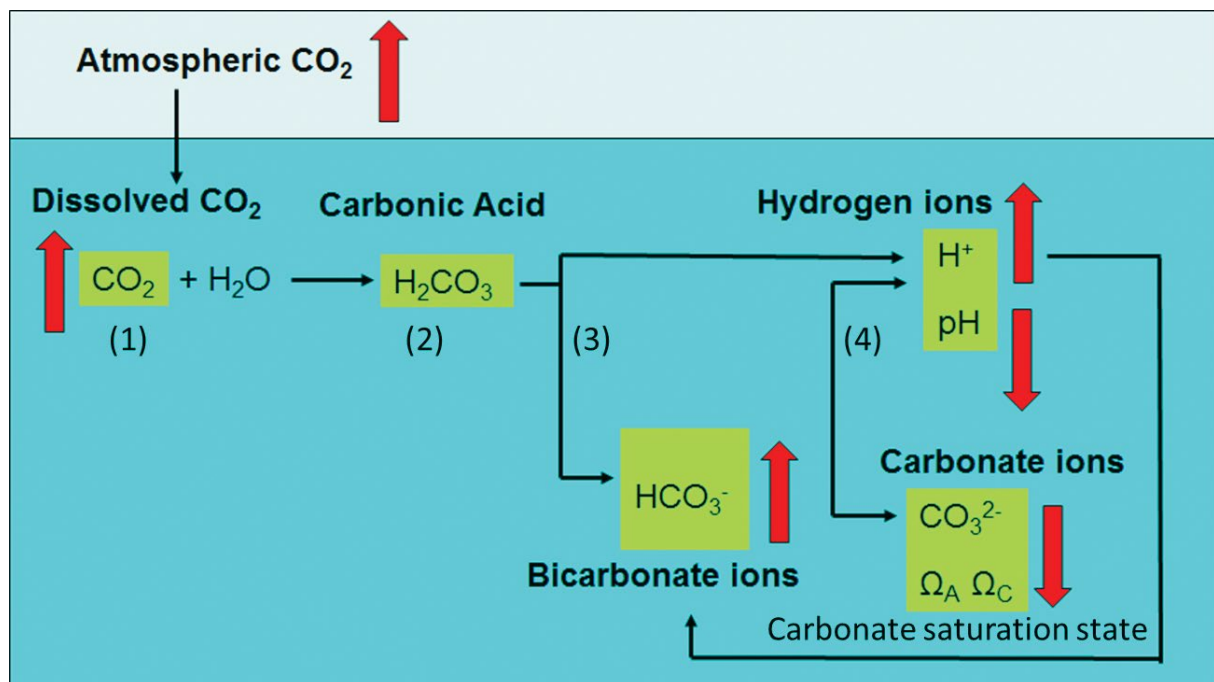


Figure 1. Conceptual diagram of the marine carbonate system (black arrows), with the red arrows indicating the net change resulting from increasing atmospheric CO₂, that characterise ocean acidification (adapted from Law et al, 2018a).

Introduction and rationale

This is due to several factors, including the composition and volume of terrestrial input, tidal cycles and seasonal temperature. In addition, the balance between algal primary production (carbon dioxide (CO₂) removal) and respiration (carbon dioxide production) is a strong determinant of the daily and seasonal oscillation in pH. The increased input of CO₂ from the atmosphere is just one source of acidity in coastal waters; deposition of acids from industrial and vehicular emissions, addition of low alkalinity river water, and the indirect effect of nutrient and organic matter input in terrestrial run-off on primary production and respiration also accelerate the acidification of coastal waters (Kelly et al, 2011; Duarte et al, 2013; Zeldis and Swaney, 2018). Consequently, organisms that live in the coastal waters experience lower and more variable pH than those in the open ocean.

OA impacts on shellfish

It is well established that OA hinders calcification, the process of carbonate formation, in marine organisms (Kroeker et al. 2010, 2013), and a synthesis of studies has identified a reduction in shell growth and length for molluscan species under lower pH in more than a third of all experiments reported (Gazeau et al., 2013). The carbonate saturation state (Ω) is the primary parameter in the carbonate system that affects the shell of molluscs (Waldbusser et al., 2015). Shell dissolution may occur at Ω (Ries et al., 2016), but even at $\Omega > 1$ calcifying organisms may expend more energy building and maintaining their shells. OA also causes changes in the intracellular acid-base balance of molluscs, with the result that energy is diverted from processes such as growth and reproduction in order to maintain basic functioning (Pörtner 2008; Gazeau et al., 2013). There is strong evidence that the early-life-history stages of carbonate-forming organisms are most vulnerable to OA, with general reductions in larval performance (growth, development, survival, and recruitment) reported at lower Ω for several mollusc species (Kurihara, 2008; Kurihara et al. 2008; Kroeker et al. 2010, 2013; Barton et al. 2012, Parker et al. 2013, Waldbusser et al. 2015, Cummings et al. 2019).

Consequently, OA represents a significant threat to coastal ecosystems and particularly to the viability of molluscs. For the reasons outlined above calcifiers inhabiting coastal environments may already experience seawater that is low in pH and carbonate, and so some degree of shell dissolution may already occur (Andersson and Mackenzie, 2012). Whereas coastal species may have some level of adaptation to lower carbonate availability projected increases in OA and warming may further increase the vulnerability of organisms with carbonate shells in coastal waters.

OA impacts on green-lipped mussels

In addition to the potential loss of coastal biodiversity, and the ecosystem functions that molluscs support (Cooley et al, 2009), OA may also influence the sustainability of shellfish aquaculture. The impacts of OA on this sector are already recognised as a global issue, with recent losses of ~80% in oyster hatchery production in the N.W. USA attributed to OA (Barton et al, 2015). Green-lipped mussels (*Perna canaliculus*) underpin New Zealand's aquaculture sector, with an export revenue in excess of USD250 Million p.a. (Jefferies et al., 2018); however, this sector has recently experienced major spat failures and die-offs with socio-economic repercussions that have been tentatively attributed to warming¹ but may also be influenced by OA. Mussel physiology is known to be deleteriously affected at lower pH, with reports of increased mortality, thinner shells and weaker byssal attachment (Gazeau et al, 2010; Fitzer et al, 2015; Pfister et al, 2016). The shells of green-lipped mussels are primarily composed of aragonite which is stronger than calcite but also more soluble as the saturation state of Ω_{AR} is lower than the corresponding Ω_C value. Consequently green-lipped mussels may be impacted by OA earlier than molluscs with calcite shells.

Recent studies within the CARIM (Coastal Acidification: Rates Impacts and Management) project indicate that the average growth and survival are not significantly impacted for most life stages in the Green-lipped mussel under conditions projected for 2100 (1100 μatm pCO₂, pH 7.64) (N. Ragg, pers. comm.). However, the embryonic stages (between egg fertilization and formation of the first larval shell) represent a potential bottleneck, as lower net growth and survival occurred under future low pH conditions relative to present day conditions (N. Ragg, Cawthron Institute, pers. comm.; Law et al, 2019). Ragg et al. (2019) have also reported that less than 1% of green-lipped mussel eggs maintained below the critical Ω_{AR} threshold of 1.0 developed to the veliger stage and, although larvae of greater than two days old showed greater resilience their survival was reduced and final shell length 25% lower at $\Omega_{AR} < 1$. OA also results in shell thinning in 13-day-old green-lipped mussel larvae (prodissoconch II stage), with shell thickness decreasing by 50% from pH 8.3 to 7.3 (Ericson 2010). However, the impacts of OA are not restricted to the larval stage, with the average growth of juvenile green-lipped mussels (4 – 6 months old) also reduced under end-of-century conditions (N. Ragg, pers. comm.; Law et al, 2019).

1. <http://www.stuff.co.nz/business/industries/67674016/sanford-blames-warming-sea-for-christchurch-plant-closure>

Ren et al. (2020) developed a Dynamic Energy Budget model to investigate the potential influence of OA on growth and reproduction of green-lipped mussel. The model used data obtained from laboratory experiments where mussels were grown at projected future lower pH (elevated pCO₂) levels to estimate consequences of OA to mussel performance. The model indicates that decreasing pH may cause reductions in shell length growth, flesh weight and reproductive capacity. Specifically, they identified a 7–13% higher energy requirement for respiration and 10% lower final shell length under lower pH and carbonate projected for the end of this century compared to present day conditions (Ren et al. 2020).

Potential solutions

Whereas reductions in CO₂ emissions would be the most direct route to reduce OA this presents major economic, social and technical challenges. Deliberate intervention techniques such as large-scale geoengineering have been considered but not assessed practically. For example, the addition of dissolved carbonate to the ocean, known as alkalinisation, may reduce OA, but presents a formidable challenge to implement (Feng et al, 2017). However, at smaller scales such as the coastal ocean, this buffering technique may be more tractable. For example, the addition of waste shell to sediments has been shown to raise the pH of sediment porewater (Green et al. 2009) and shell return to sediment has shown positive responses in terms of survival and recruitment in other bivalve species (Green et al, 2013) that may be related to carbonate regeneration (Waldbusser et al, 2013). Adaptation measures including carbonate addition have proven successful in addressing the elevated oyster mortality mediated by OA on the north-western US seaboard, although these oyster hatcheries have the advantage of being land-based controlled systems (Barton et al, 2015). Although mitigating acidification in coastal waters presents many challenges, in part due to the different sources of acidity and large variability in pH and the carbonate system in coastal waters, these regions have advantages of accessibility, particularly to infrastructure and resources. In the Firth of Thames, where present day seasonal (autumn) pH reductions are already rivalling those expected by 2100, coastal acidification could be potentially managed by control of runoff (Zeldis and Swaney 2018).

Structure of report

In light of the increasing awareness of the potential impacts of OA on NZ shellfish aquaculture, particularly following the decimation of the oyster industry on the Pacific NW US coast over the last decade (Barton et al. 2012), this project was initiated to examine the potential to mitigate OA impacts on green-lipped mussels in New Zealand coastal waters. The aim was to assess techniques that could be deployed at the scale of a mussel farm to alleviate the effects of OA by providing buffering capacity to low pH and carbonate. This report assesses proof-of-concept pilot studies of two potential remediation techniques – return of waste shell and strategic aeration – with a regional focus on the two main shellfish aquaculture regions in New Zealand – the Firth of Thames and Marlborough Sounds. The Marlborough Sounds supports ca. 68% of the NZ\$204 million per annum national production of New Zealand green-lipped mussels (75000 t fresh weight), which are grown on hundreds of individual farms throughout its main channel, side-arms and embayments (Zeldis et al. 2013). The Firth of Thames supports the largest spawning and nursery grounds for NZ's most valuable inshore fin-fishery and ~20% of the national mussel aquaculture industry, but also experiences seasonally low pH (Law et al, 2018a; Zeldis and Swaney, 2018).

The field research in this report was carried out in collaboration with Sanford Limited who provided access to their Ecofarm in Old Homewood Bay in the Marlborough Sounds for measurements and testing (see Appendix Figure 1). This collaboration enabled discussion regarding the feasibility of deployment of these OA mitigation methods. The report provides information on the current status and future threat of OA in the Firth of Thames and Marlborough Sounds regions and then uses model estimates, in situ observations and laboratory experimentation to assess the efficacy of the two potential remediation strategies at farm and dropper line scales. In addition, the impacts of waste shell on juvenile green-lipped mussels were assessed. The report considers practical deployment issues and costs and provides recommendations for the NZ shellfish aquaculture industry.

Current status and future projections of pH and carbonate system

Regional variation in pH and the carbonate system

pH and carbonate system parameters are routinely monitored by the New Zealand Ocean Acidification Observing Network (NZOAN; <https://marinedata.niwa.co.nz/nzoa-on-map/>) in the Pohuenui Growing Area 1508 Site P043/G31 in West Beatrix Bay (41.03S 174.02E) in the Marlborough Sounds (see Appendix Figure 1), with sample collection at fortnightly intervals by the Marlborough Shellfish Quality Programme and sample analysis in the Department of Chemistry, University of Otago. Figure 2 shows the Temperature, Dissolved Inorganic Carbon ($\text{DIC} = \text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^-$), pH_T (pH measured on the total scale) and Ω_{AR} of surface water (upper 4m, sampled during day) over 3.5 years from mid-2015, with seasonal minima for pH and Ω_{AR} identified in Table 1. On a seasonal basis maximum pH generally occurred in late winter and early spring (Figure 2) with the lowest pH (7.948 (7.85–8.01); mean and quartile range), in summer and autumn (see Table 1). Conversely, Ω_{AR} was highest in late summer with a minimum (1.9) in winter reflecting the lower temperature. An extended period of low pH and carbonate saturation was apparent from autumn to spring 2016, with the minimum observed pH of 7.8 in spring 2016 equivalent to the pH projected for the open ocean around New Zealand by the end of this century (Law et al, 2018b). This highlights that coastal waters are ahead of their oceanic counterparts in terms of acidification. The lowest observed Ω_{AR} of 1.45 in spring 2016 exceeded the critical Ω_{AR} value of 1.0.

pH was also monitored in the Firth of Thames as part of a long-term monitoring programme by NIWA (Zeldis and Swaney, 2018; Law et al, 2019), using in situ SeaFET sensors mounted on two moorings in the Inner and Outer Sounds (see Appendix Figure 1), with the inner site being closer to the Wilson A and B and Waimangu Point mussel farm locations. Figure 3 shows the daily median values for Temperature, DIC, pH_T and Ω_{AR} of surface water over 5 years from mid-2014 at both sites, with seasonal minima for pH and Ω_{AR} also identified in Table 1. The high resolution (15-minute) sensor pH data are also summarised in the violin plots in Figure 4. The Inner Firth site was characterised by lower pH than the Marlborough Sounds site and Outer Firth site for all seasons, with the lowest seasonal pH minimum of 7.84 (7.79–7.9) in Autumn. Seasonal pH trends were similar between sites, with highest pH in early spring, and pH minima in summer to autumn reflecting elevated CO_2 production from organic matter respiration (Law et al, 2019). However, seasonal trends in Ω_{AR} differed between sites, with a minimum of 1.83 in the Inner Sounds in Autumn, whereas the lowest seasonal minimum in the Marlborough Sounds was 1.9 in winter (see Table 1). The high-resolution (15-minute) data show short-term Ω_{AR} minima falling below 1.0 at both Firth of Thames sites (Figure 4), indicating that calcifying organisms such as mussels already experience conditions that are corrosive to their carbonate shells.

Current status and future projections of pH and carbonate system

	MS minimum pH	MS minimum Ω_{AR}	Outer FoT minimum pH	Outer FoT minimum Ω_{AR}	Inner FoT minimum pH	Inner FoT minimum Ω_{AR}
Summer	7.949 (7.93–7.95)	2.07 (1.94–2.18)	7.92 (7.89–7.99)	2.32 (2.17–2.65)	7.867 (7.79–7.96)	2.08 (1.75–2.40)
Autumn	7.948 (7.85–8.01)	1.95 (1.55–2.22)	7.978 (7.94–8.05)	2.58 (2.34–2.88)	7.84 (7.79–7.9)	1.83 (1.65–2.99)
Winter	8.002 (8.01–8.05)	1.9 (1.98–2.01)	8.044 (8.03–8.10)	2.35 (2.32–2.55)	7.949 (7.92–8.0)	1.85 (1.76–1.99)
Spring	7.975 (7.86–8.05)	1.99 (1.59–2.22)	7.997 (7.99–8.09)	2.27 (2.14–2.60)	7.963 (7.86–7.99)	2.04 (1.76–2.33)

Table 1. Mean and range (quartiles) for minimum pH and Ω_{AR} for each season derived from monthly data for Marlborough Sounds (MS, Puhenui Growing Area 1508 Site P043/G31 in West Beatrix Bay, 41.03oS 174.02°E) for mid-2015 to the end of 2018, and derived from daily data for the Outer Firth of Thames site (Outer FoT, 36.763°S 175.301°E) and Inner Firth of Thames site (Inner FoT, 37.025°S 175.383°E) for mid-2014 to mid-2019.

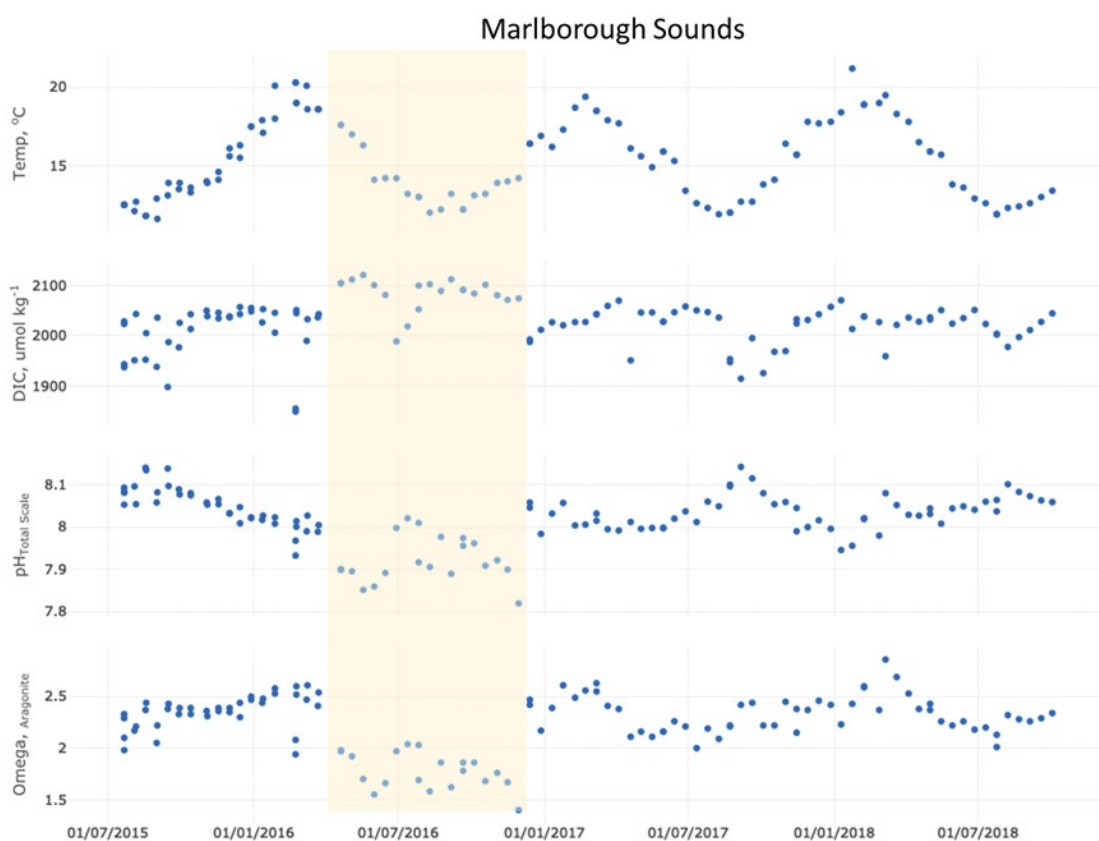


Figure 2. Temperature, Dissolved Inorganic Carbon (DIC, $CO_2 + H_2CO_3 + HCO_3^-$), pH_T (Total scale) and Ω_{AR} of surface water from mid-2015 to mid-2018 at the Puhenui Growing Area 1508 Site P043/G31 in West Beatrix Bay (41.03S 174.02E) in the upper 4m surface water in the Marlborough Sounds, as determined by the NZOAN (<https://marinedata.niwa.co.nz/nzoa-on-map/>). The shaded region indicates the extended period of low pH and Ω_{AR} in 2016.

Current status and future projections of pH and carbonate system

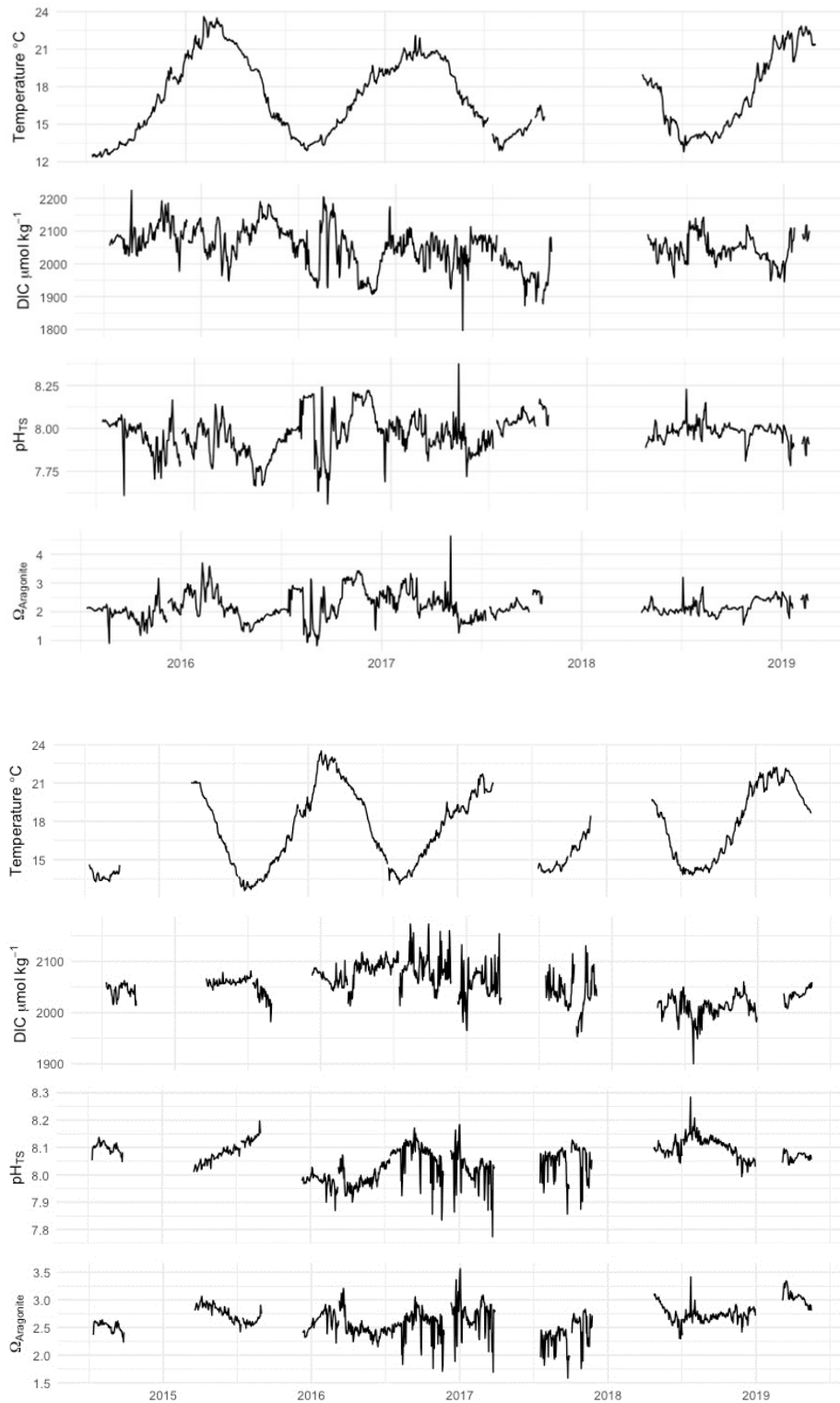


Figure 3. Daily median values for Temperature, Dissolved Inorganic Carbon (DIC, $\text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^-$), pH_T (Total scale) and Ω_{AR} of surface water from mid-2014 to early-2019 at the Inner Mooring site (upper figure) and outer Mooring site (lower figure) in the Firth of Thames (Zeldis and Swaney, 2018; Law et al, 2019). pH was recorded using a SeaFet sensor, and carbonate system parameters were determined by water sample collection and analysis in the Department of Chemistry, University of Otago. DIC and Ω_{AR} were calculated from pH and Total Alkalinity (TA), with TA estimated from the linear relationship with salinity. Data were omitted during certain periods due to excess biofouling on the pH sensor.

Current status and future projections of pH and carbonate system

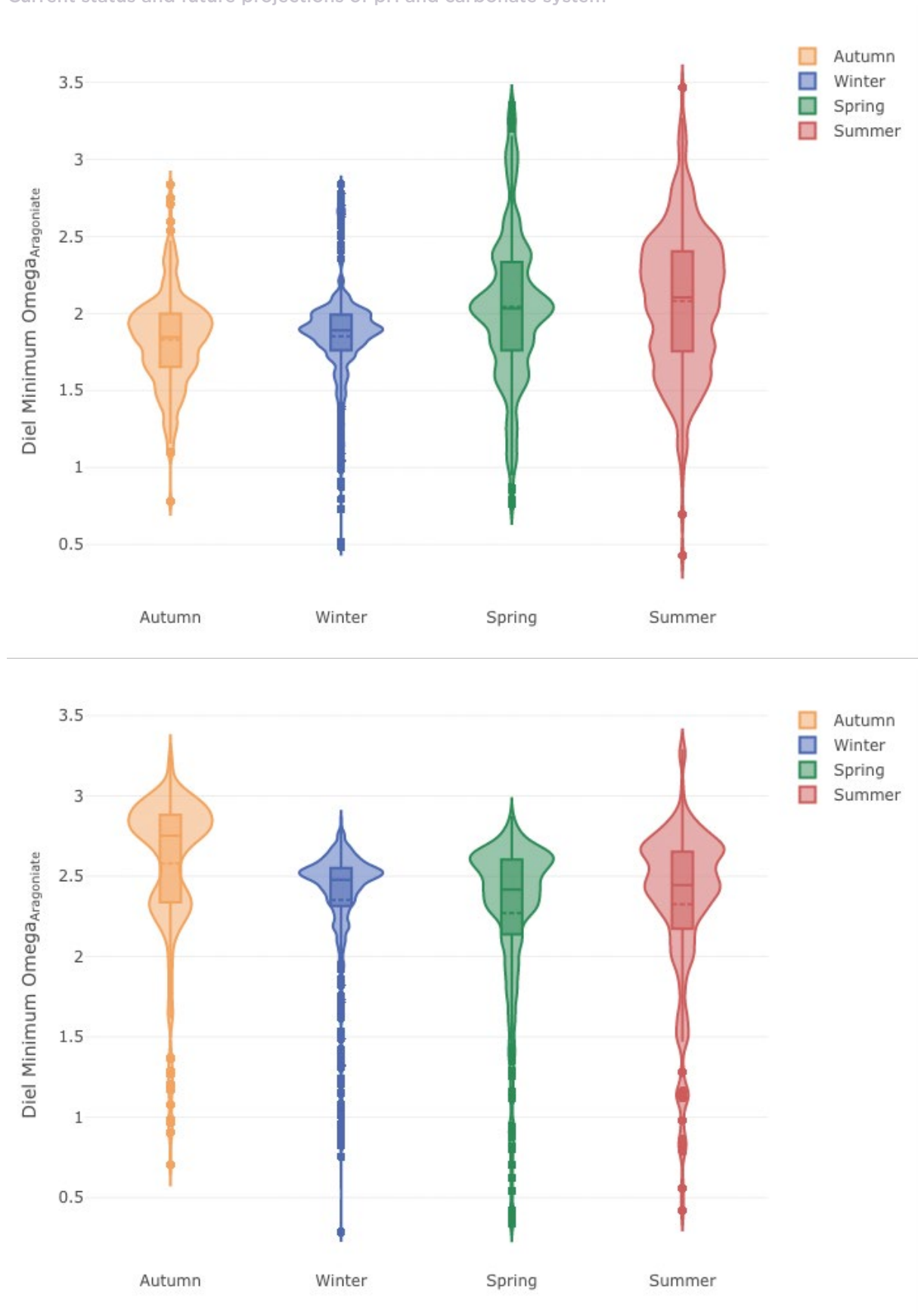


Figure 4. Violin plot summary of the high-resolution (15-minute resolution) values for minimum Ω_{AR} at the Inner site (upper figure) and Outer Site (lower figure) in the Firth of Thames. The solid horizontal line is the median and the dashed line is the mean. The box spans the inter-quartile range with the bulges indicating the probability density for given values.

pH on mussel dropper lines

To establish the pH variability experienced by mussels, pH sensors were maintained at depths of 2–4m on dropper lines at the Sanford Limited Ecofarm for periods of 2–3 weeks in summer and winter 2019 (see Figure 5). On both occasions the pH sensor head was placed within the mussels and associated biofouling community on the dropper line, with a second sensor deployed in the surrounding water to measure background pH in summer. Boundary layer pH on the dropper line was higher during winter, with a mean of 8.02, relative to the average pH of 7.97 in summer. A diel trend was apparent in both pH datasets, reflecting the domination of algal photosynthesis (CO_2 removal) during the day and respiration (CO_2 production) at night. The increase in diel pH variability from midway through the summertime-series coincided with an increase in water temperature ($\sim 1^\circ\text{C}$) and dissolved oxygen saturation, and a decrease in turbidity, but no change in salinity or chlorophyll (data not shown). During summer pH in the dropper line boundary layer was generally lower than in the surrounding water, regardless of the diel trend, with a pH minimum of 7.87 during night-time on occasions in February 2019. As dissolved oxygen in the dropper line boundary layer decreased by 10% relative to the surrounding water (data not shown), the

lower pH on the dropper line during summer and at night likely reflected CO_2 production during respiration by the mussels and associated biofouling. The lower pH at night demonstrates that mussels experience more corrosive conditions on the dropper line relative to the water flowing through the farm. The seasonal difference in temperature had a greater effect on Ω_{AR} with a marginally lower minimum Ω_{AR} in winter (1.88) than in summer (1.95) in the dropper line boundary layer.

Although pH measurements were not obtained within the boundary layer on a mussel dropper line in the Firth of Thames region a pH time-series record was obtained on a mussel farm (Tuwhitu Mussel Farms, 36.88°S 175.249°E) on the western side of the Firth of Thames (see Appendix Figure 1) during June 2016–April 2017 as of the CARIM project (Frost, 2019; Figure 6). Although this dataset was collected at a different location to the NIWA Moorings, the pH and Ω_{AR} results show consistent trends particularly with the Inner site (Figure 3), with a sharp pH minimum in August 2016 and subsequent minima in January and April 2017 evident in both time series. Ω_{AR} values at the Tuwhitu Mussel Farm site also decreased to 1.0 in early spring and late autumn (Figure 6), confirming that the Firth of Thames already experiences conditions that are corrosive to solid carbonate.

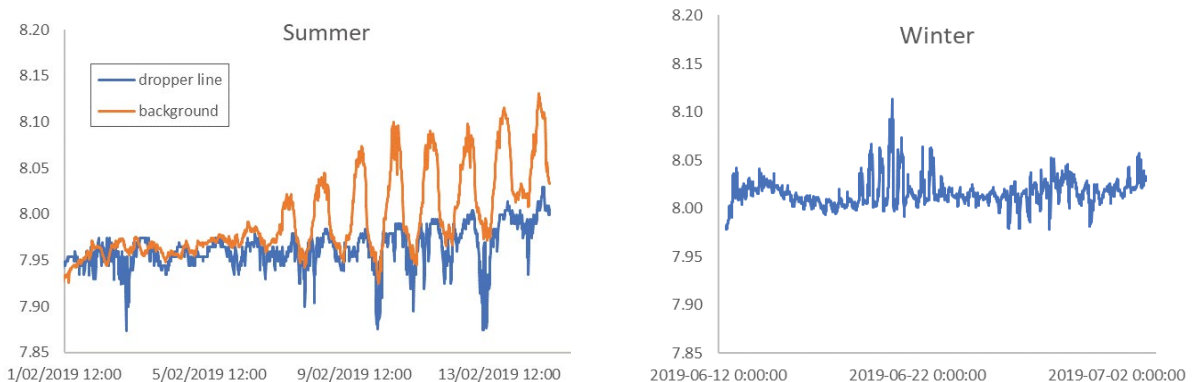


Figure 5 Left figure, pH in the boundary layer of a dropper line (blue line), and in background water 12 cm from the surface of mussels on a dropper line (orange line), over a 2-week period in February 2019, and in the right figure pH over a 3-week period on a dropper line during June–July 2019 at the EcoFarm in Marlborough Sounds.

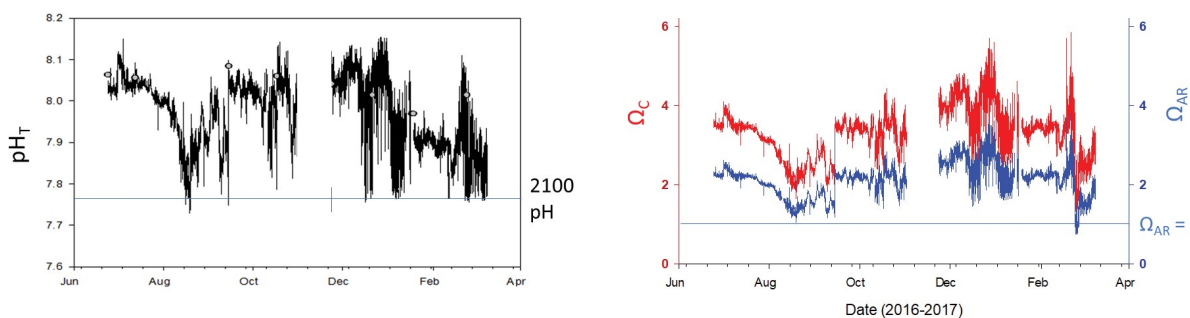


Figure 6. pH_T (Total scale, black line), and Ω_{Ca} (Calcite saturation state, red) and Ω_{AR} (blue) in surface water at 3–4m on Tuwhitu Mussel Farm (western Firth of Thames $36^\circ 59' 48''\text{S}$, $175^\circ 17' 41''\text{E}$) from June 2016 to April 2017. The horizontal blue line in the pH plot indicates the projected pH for 2100 using the RCP8.5 scenario for NZ open ocean waters (Law et al, 2018b), and $\Omega_{\text{AR}} = 1$ in the Ω_{AR} plot (reproduced from Frost, 2019).

Future regional projections

Future projections of pH and Ω_{AR} were derived for the Marlborough Sounds region using an Earth System Model (GFDL-ESM2; Law et al, 2018b; Rickard et al, 2016) from CMIP6, and two different future CO₂ emission scenarios. The SSP5-8.5 scenario represents the “Fossil-Fuel Development” scenario, whereas the SSP2-4.5 is a “middle of the road” scenario in which trends do not diverge significantly from historical pathways. The model projections for the nearest geographical grid point were adjusted for the recorded pH trends by using the time series data from mid-2015 to mid-2018 for the Marlborough Sounds (Figure 2).

The projected pH decline is ~0.4 with the SSP5-8.5 projection (Figure 7), with mean values of 7.89 and 7.62 at mid-century (2050) and end-century (2100) for the Marlborough Sounds. The projected change in pH under the SSP2-4.5 scenario is lower and does not diverge from the SSP5-8.5 projections until after mid-century, with mean values of 7.93 and 7.85 at mid-century (2050) and end-century (2100). Comparison of the current time-series data (2015–2018) in Figure 2 with projected trends indicates that the projected mean pH values fall below the current pH range by 2065.

The projections for Ω_{AR} in the Marlborough Sounds (Figure 8) show an overall decline of 1.25–1.47 by 2100 with the SSP5-8.5 projection, to values of 1.83

and 1.00 by 2050 and 2100, respectively. As with pH the Ω_{AR} changes projected under the SSP2-4.5 scenario are also lower, and do not diverge from the SSP5-8.5 projection until after mid-century, with mean values of 1.96 and 1.68 at mid-century (2050) and end-century (2100). The higher Ω_{AR} projected by the SSP2-4.5 scenario may be more conducive to development of larval stages, based upon the observations of Ericson (2010) and Ragg et al (2019), than the SSP5-8.5 scenario.

These projections are based upon ocean models that only consider the influx of CO₂ from the atmosphere and do not account for other processes (described above) that influence pH and the carbonate system in coastal waters. Although pH and carbonate availability in coastal waters are influenced by respiration, which is sensitive to temperature and may be expected to increase CO₂ production at future warmer temperatures, the net effect of future climate and environmental change on processes influencing coastal acidification remains unclear (Duarte et al, 2013). However, the data presented here shows that pH on a mussel dropper line falls below that of the surrounding water (see Figure 5 and 6), and furthermore that short-term pH decreases occur that are significantly lower than mean values, as in the Firth of Thames (see Figure 4). Consequently, lower pH and the Ω_{AR} threshold of 1.0 may be reached on mussel dropper lines earlier than indicated in these projections.

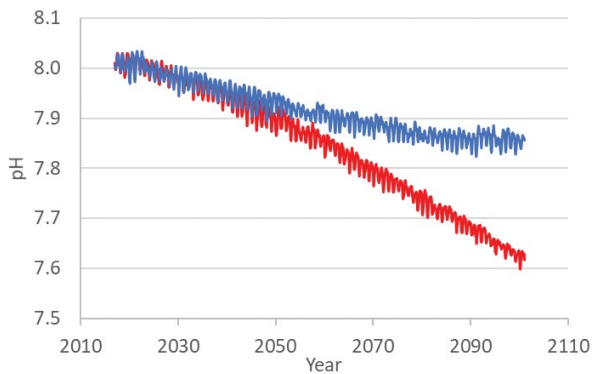


Figure 7. Projected pH trend from 2020 to 2100 for the Marlborough Sounds for two CO₂ emission scenarios (SSP2-4.5, blue and SSP5-8.5, red).

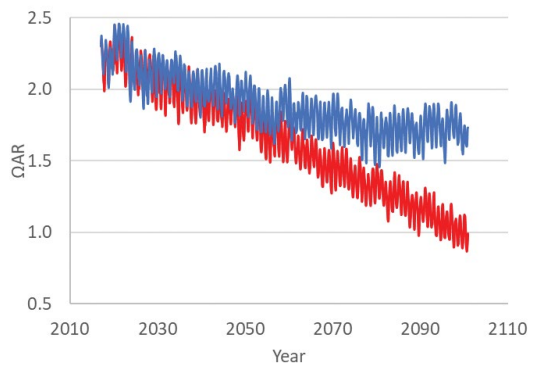


Figure 8. Projected Ω_{AR} trend from 2020 to 2100 for the Marlborough Sounds for two CO₂ emission scenarios (SSP2-4.5 (blue), and SSP5-8.5 (red)).

Potential OA mitigation solutions

The following section considers two approaches to elevating pH and dissolved carbonate by the application of waste shell and aeration. The efficacy of the two mitigation techniques are assessed at the farm scale and at the dropper line scale for the waste shell technique, using the information in Table 2.

How much dissolved carbonate is required to raise pH at the farm scale?

To raise pH from the observed minimum (7.82) to the mean value (8.02) in the Marlborough Sounds region (Figure 2) instantaneously would require injection of ~0.6 tonnes carbonate across the volume of the farm (see Table 2). Similarly, to raise the pH from the mean projected for the Year 2100 (pH 7.68)

would require instantaneous injection of 0.88 tonnes carbonate across the volume of the farm. However, this is a purely volumetric estimate, and dilution by currents would greatly increase the carbonate supply rate required. Current flow through a mussel farm is critical for supplying food and oxygen to the mussels and removing carbon dioxide and waste products; however, dilution by currents effectively reduces gradients within the farm. When dilution is factored in the amount of dissolved carbonate required to raise pH by 0.2 is 3.6 tonnes/day in a low current regime such as the Inner Sounds, increasing to ~36 tonnes/day in the higher current regime of the Outer Sounds. Consequently, dilution is a major barrier to any remedial solution to OA.

Statistics	Farm	Dropper Line
Depth (m)	20	20
Surface area (m ²)	10000 (one hectare)	51.3
Volume (m ³)	200000	10
Replacement time (min) at background tidal speeds:		
• Outer Sounds (0.1 m/s)	23.6	0.2
• Inner Sounds (0.01 m/s)	226	2.0
Dilution rate (/d) at background tidal speeds:		
• Outer Sounds (0.1 m/s)	61.1	6900
• Inner Sounds (0.01 m/s)	6.1	690

Table 2. Scales and dimensions used for a mussel farm and a dropper line (including boundary layer) in the current study. The dilution rates are calculated on the assumption of a 50% reduction in current velocity within a mussel farm (see Appendix for references for this and other parameters).

Potential of waste shell to mitigate OA

Mussel shell is the primary waste product of the NZ aquaculture industry with considerable volumes generated of >1x 10⁵ tonnes shell per annum, that is currently used for stockpiling, landfill burial, track fill & horticulture (Barnaby, 2004). Removal of >20,000 tonnes of GSM in the Nelson/Marlborough region (1960–1984) represents a significant carbonate loss (Handley and Brown, 2012) of potential buffer capacity against ocean acidification. Restoration of natural shellfish reefs via waste shell deposition on the seafloor is currently being investigated in NZ as a strategy to enhance natural mussel populations

(Handley and Brown, 2012,²). Although there is interest in returning waste shell material to the seafloor around mussel farms, this is currently not permitted.

Mollusc shells generally consist of a mineral phase (95–99% calcium carbonate) and an organic matrix (1–5%) (Gazeau et al, 2013), with the outer periostracum consisting of an organic layer composed primarily of polysaccharide chitin that may reduce dissolution. The shell of *P. canaliculus* is primarily aragonite (92%) with a small proportion of calcite (8%, Checca & Navarro, 2001).

2. <https://www.stuff.co.nz/national/politics/local-democracy-reporting/300095562/mussels-to-be-poured-on-mussel-shell-reef-to-revive-devastated-population>

Potential of waste shell to mitigate OA

Mollusc shells are a temporary sink for alkalinity in coastal waters and following mortality their dissolution rebalances the carbonate system (Waldbusser et al, 2013). However, shell dissolution under current conditions in coastal waters is very slow, as evidenced by the presence of aged shell material on a typical beach. This is because the Ω value is generally >1.0 in coastal waters (see Figures 2–4 and 6), and so solid carbonate is stable and will not dissolve. Although waste shell represents a potential source of carbonate that could theoretically be used to raise pH and carbonate saturation state (Ω) the challenge is to determine if there are locations and processes that could accelerate the conversion of solid carbonate in waste shell to dissolved carbonate.

Screening shell for optimal dissolution rate

Waste shell was screened for 3–18 hours in a dilute acid solution under different conditions to determine the factors that influence dissolution. Approximately 10 g sample/mussel was placed in 600 ml 4% acetic acid at room temperature (22°C) on a shaker. Different factors were assessed including shell size (whole, fragmented, crushed and powdered), source location (Marlborough Sounds or Firth of Thames), and age (fresh versus old shell/air dried). In addition, the effect of pre-treatment was determined by

exposure of fresh waste shell to coastal water, and coastal sediment, for four months prior to screening.

There was no apparent effect of source location (Figure 9), although in comparisons of fresh and old shells, the latter tended to show higher dissolution rate, which was surprising given that exposure of oyster shell material is reported to decrease dissolution (Waldbusser et al, 2011). As expected, a decrease in size of the waste shell particles and corresponding increase in surface area : volume ratio enhanced dissolution, with the powdered shell showing the highest rates of dissolution, consistent with reported observations (Cubillas et al, 2005). Pre-exposure of crushed and powdered shell in coastal water and sediment for one month resulted in the highest dissolution rates. Several studies have investigated the dissolution kinetics of carbonate shells and skeletons (see summary in Ries et al, 2016), some of which have shown that dissolution rates increase with microstructural surface area (Pickett and Andersson, 2015). Pre-exposure of the mussel shell may have increased shell surface area due to boring organisms, which could accelerate dissolution, but epiphytic settlement and organic coating may alternatively reduce dissolution (Waldbusser et al, 2011). The dissolution screening tests confirm that simple relatively low-cost treatments could be implemented to enhance shell dissolution.

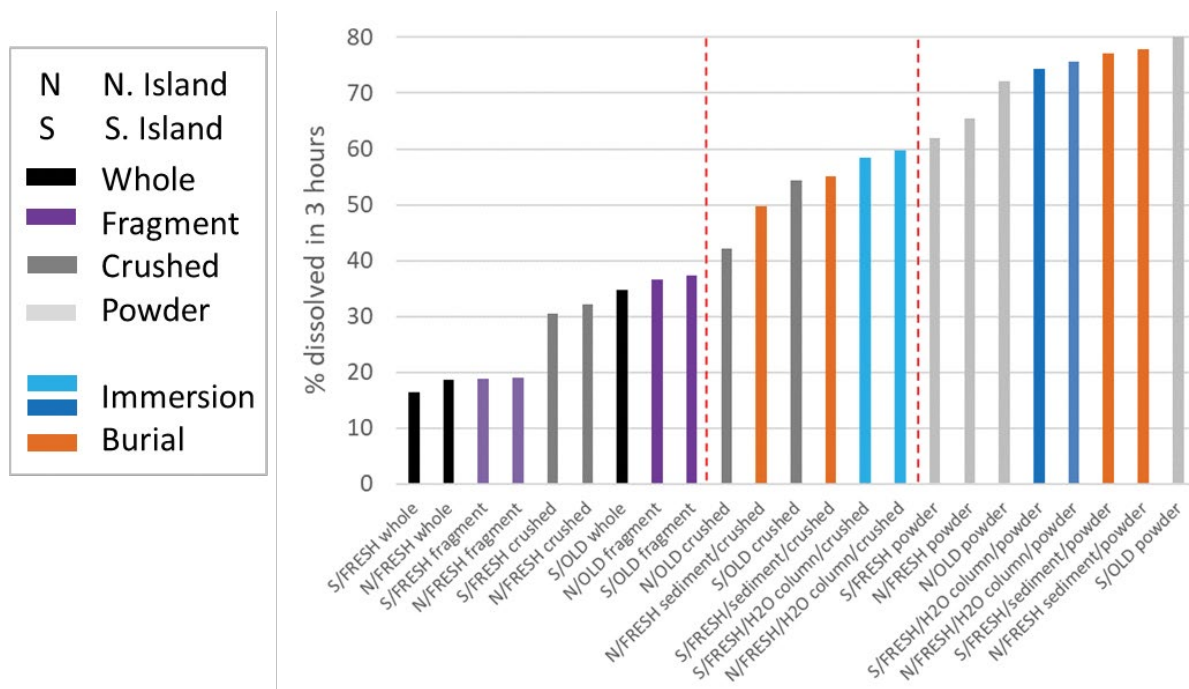


Figure 9. Comparison of mass loss (expressed as % shell mass decrease over three hours in a 4% acetic acid solution) of green-lipped mussel shell of different size, age, and source and following pre-screening treatment, as identified in the legend following immersion in 4% acetic acid for 3 hours. Key: S – South Island (Marlborough Sounds); N – North Island (Firth of Thames); Fresh – recently obtained from organisms on dropper lines; Old – weathered shells from piles at extraction sites; whole – complete shells, fragment – shell fragments of ~20 mm; crushed – shell particles of ~4–5.5 mm size; and powdered (<2.4 mm) shell; H2O column – shells maintained in a mesh bag in Otago coastal waters for 4 months; sediment – shells maintained mesh bag in Otago coastal sediments for 4 months.

Sensitivity of waste shell dissolution to Ω_{AR}

The main barrier to shell dissolution is the carbonate saturation state (Ω_{AR}). The sensitivity of waste shell to Ω_{AR} was tested in short-term incubations in the laboratory. Mussel shells from the Marlborough Sounds region were crushed and sieved, and the 3.4–5.5 mm size fraction retained, washed and dried. A mesh bag containing 40 g of crushed mussel shell was then placed in each of series of bottles containing 2-Litres of coastal water in which the carbonate chemistry was adjusted by addition of equimolar sodium bicarbonate and hydrochloric acid, to give a range of Ω_{AR} values. Two control treatments without mussel shells were included, and each treatment carried out in duplicate. All bottles and cubitainers were placed on a shaker table to maintain gentle agitation and kept at 18.8°C in the dark for 14 days. Water samples from each bottle were analysed for pH, DIC and alkalinity.

Assuming that the mussel shells are 100% carbonate, the shell mass loss under the differing Ω_{AR} values was calculated from the change in measured dissolved carbonate concentration (Figure 10). The results confirm the stability of solid carbonate above a Ω_{AR} value of 1.0, although there was some loss of mass at a slightly higher saturation state of $\Omega_{AR} = 1.1$. This has been observed in other dissolution studies, including for carbonate shells of live organisms of 10 different species (Ries et al, 2016). The dissolution at $\Omega_{AR} > 1.0$

in the latter study was attributed to the presence of soluble phases such as amorphous calcium carbonate. Only 0.014% of the waste shell dissolved at the lowest experimental Ω_{AR} (0.85, $pH_T = 7.48$; Temperature = 18.8°C) in the current study. However, the experiment was designed primarily to identify the critical Ω_{AR} threshold at which waste mussel shell dissolution is initiated, and not to determine actual rates of mass loss which will be additionally influenced by in situ processes in coastal waters such as dilution and respiration.

Waste shell dissolution on dropper lines

Waste shell mass loss was measured in nine permeable bags, each containing 36–54 g waste shell, following deployment at 2–4 m depth on dropper lines for a period of 3 weeks on the Sanford Ecofarm. Before deployment and after recovery the waste shell was washed, dried and weighed to estimate mass loss. The average shell mass loss was 0.005 (0.003–0.1) g/day, or 0.012% per day (range 0.002–6% /day), although this may represent an overestimate if loss of unweighed ultrafine particles or shell organic content were significant (Ries et al, 2016). This observed rate is one-quarter of the mass loss rate recorded for shells of the mussel species *M. galloprovincillus* under corrosive conditions ($\Omega_{AR} = 0.59–0.91$) at the sediment surface, in which shell mass decreased by 0.05%/day (Abdulghani, 2014).

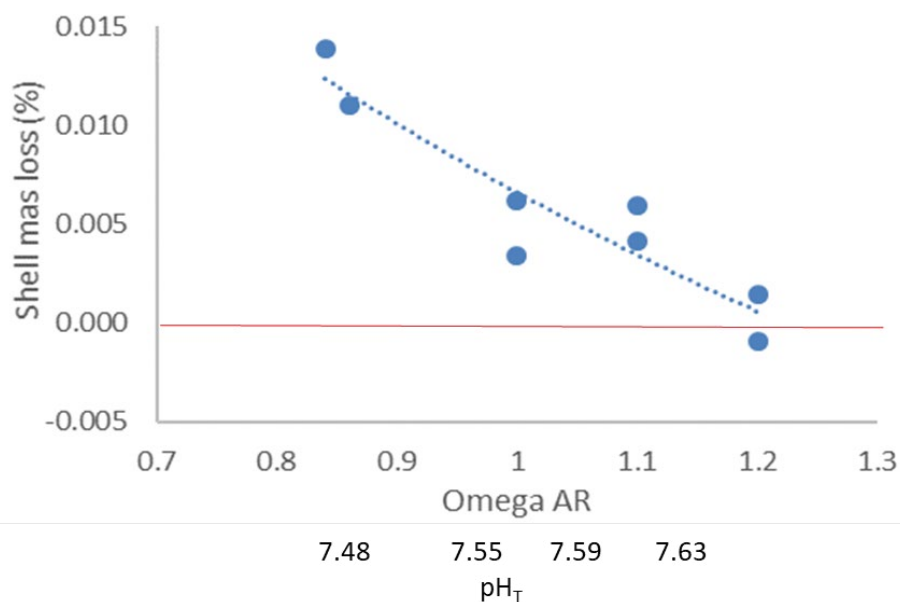


Figure 10. Mass loss (%) of waste shell as a function of Ω_{AR} in 14-day laboratory experiments using 40g shell, with the red horizontal line indicating zero shell mass loss. Relative pH_T values at temperature 18.8°C and Total Alkalinity 2275 $\mu\text{mol/kg}$ are also shown for reference.

Potential of waste shell to mitigate OA

A further experiment was carried out to examine the effect of waste shell on boundary layer pH on a dropper line. Three pH sensors were placed on a dropper line with one inserted in a permeable bag containing waste shell, a second in a permeable bag containing glass beads which provided a control, and the third suspended in the surrounding water. After two weeks deployment the pH records from the three sensors showed no significant differences (see Figure 11b) – in fact, the waste shell bag exhibited some of the lower pH_T values – and so the presence of waste shell did not elevate pH under these conditions.

How much dissolved carbonate is required to raise pH on the dropper line?

Calcium carbonate cycling in surficial marine sediments is strongly coupled to respiration of organic matter (Waldbusser and Salisbury, 2014), which lowers carbonate saturation state and promotes dissolution of aragonite. Respiration by mussels and associated biofouling may create an analogous zone of elevated DIC in the boundary layer of a dropper line, particularly at night when CO₂ is not removed by photosynthesis. This is inferred from the lower pH on the dropper line relative to the surrounding water at night on the Ecofarm (Figure 5). The resulting lower Ω_{AR} would create a more challenging environment for mussels, but conversely may offer a potential buffer if waste

shell incorporated on the dropper line dissolves more rapidly relative to the surrounding water. This could be beneficial as any dissolved carbonate release from waste shell dissolution would be in the immediate vicinity of the mussels. If Ω_{AR} was low enough to stimulate shell dissolution, then the approach of adding waste shell to a dropper line may be more tractable than raising the dissolved carbonate across the entire volume of a mussel farm.

To raise pH in the boundary layer of a dropper line instantaneously by 0.1 (present-day minimum to mean pH recorded on dropper lines in Marlborough Sounds; Figure 2) would require the addition of 30 g dissolved carbonate to the 10m³ volume of the dropper boundary layer. However, when dilution losses and shell dissolution rates are considered the carbonate delivery requirement increases substantially. For example, to raise dropper line pH by 0.1 at a background current speed of 0.01 m/s would require dissolved carbonate addition of 8.5 kg/d to the 10m³ dropper line volume. Applying the observed and reported mussel shell dissolution rates of 0.012 (this report) and 0.05% (Abdulghani, 2014), indicates this would require addition of 0.9–3.6 tonnes waste shell per metre of dropper line. It is not practical to incorporate this volume of waste shell. This estimate does not account for the reduction in dilution around a dropper line which, though not well-constrained, has been inferred from modelling (Smith et al, 2006) and small-scale simulations (Plew, 2005).

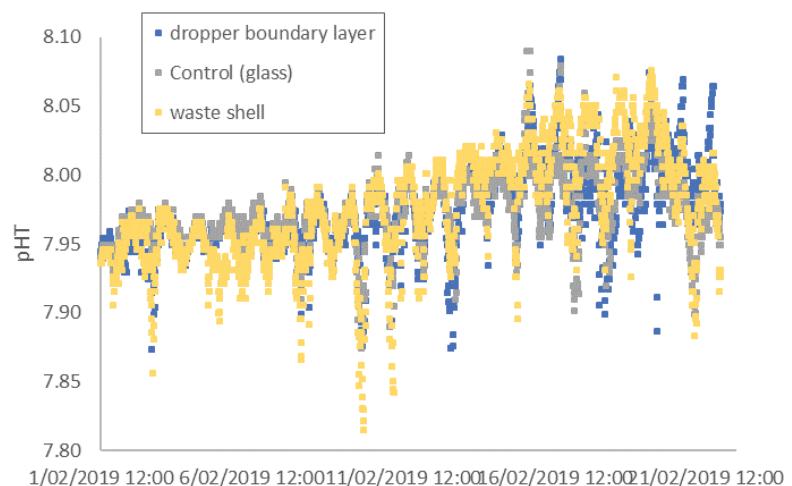


Figure 11. a) Location of pH electrodes on dropper lines at the Ecofarm (Sanford Limited), with upper electrode in control bag containing glass beads, the middle electrode in a bag containing waste shell, and the lower electrode in the outer boundary layer. b) pH_T record from the three electrodes over a two-week period, showing no significant differences in pH_T.

Is incorporation of waste shell on dropper lines practical?

Although preliminary discussions with Sanford scientists indicated that inclusion of small volumes of waste shell could be achieved using the standard industry practise of spat and seed enclosure within a spat sock (see Figure 12), the large shell volume requirement calculated above could not be physically supported on a dropper line and would likely impair the supply of food & space for mussel growth. Even if a lower volume of waste shell was used other logistical considerations would include coordination of spat sock mesh size and shell fragment size to prevent shell loss, and waste shell would require smoothing to reduce sharp edges that could damage and split the spat sock. In addition, the waste shell would only be retained on the dropper line until the spat sock decayed.

Influence of waste shell on mussel growth

Unfortunately, tests of waste shell addition on a dropper line during spat deployment within this project were prevented by the failure of spat collection in 2018 and 2019, and so there were no *in situ* deployment tests of waste shell. In the absence of *in situ* tests on a dropper line, a laboratory experiment was carried out instead. Juvenile mussels (<30 mm shell length; from Mahanga Bay, Wellington Harbour), were grown for 39 days in aquaria containing either waste mussel shell, or glass beads (as an inert 'control' substrate), while also exposed to seawater pH representative of current and future projected levels. The experiment took place in the Marine Experimental Manipulation Facility at NIWA Wellington.

The juvenile mussels were exposed to either present-day ambient pH (mean 7.99) and "future" pH (Year 2050, mean pH 7.75), with pH varied on a daily cycle in both treatments to simulate natural *in situ* variation of ~0.2 pH units. This diel pH cycle resulted in a minimum pH of 7.80 and 7.62 in the Year 2020 and Year 2050 pH treatments, respectively (see Figure 13). Coincidentally, the Year 2050 pH is similar to the current minimum pH measured on the mussel farm (see Figure 5), and so this treatment assessed response to both present day extremes and future mean pH values.

Twelve 60-litre header tanks supplied 72 "raceways" (each 240 mm long x 38 mm wide x 38 mm deep) containing the juvenile mussels (see Figure 13). Seawater pH was adjusted and maintained in each header tank (six for each pH/Year treatment) using pH controllers (Sensorex S150C pH probes (Garden Grove, CA, USA), and validated several times per day using a spectrophotometer. Water flow rate through the raceways was 50 ml/min, resulting in a residence time of ~7 minutes, which exceeds the estimated residence time of water in the boundary layer on a dropper line (0.2–2.0 minutes at background current speeds of 0.01–0.1 m/s). Two juvenile mussels of different sizes were added to each raceway: "large juveniles" of 28.45 + 0.14 mm to 15.11 + 0.08 mm shell length (initial sizes average + standard error), and "small juveniles" of 17.00 + 0.14 mm shell length to 9.57 + 0.07 mm shell length. The experiment ran for 37 days, during which the mussels were fed daily with algae (Shellfish Diet 1800; Reed Mariculture, US) added directly to the header tanks.

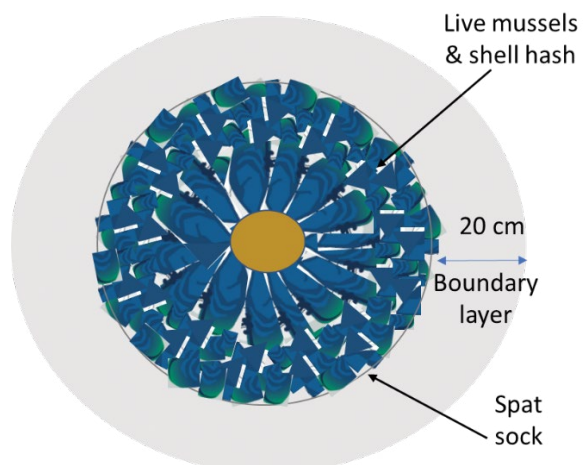


Figure 12. Filling spat sock with mussels onboard a seeding vessel b) cross-section of dropper line showing central rope surrounded by live mussels and waste shell hash enclosed within spat sock, with boundary layer shown.

Potential of waste shell to mitigate OA

The growth and condition of all mussels were assessed at the end of the experimental period. Shell length (SL, mm) and width (SW, mm) were measured using electronic callipers, before the juveniles were dissected to determine dry flesh weight (g DFW; after drying at 60°C for 48 h) and dry shell weight (g DSW; air dried to constant weight). These data were used to calculate mussel growth as a percentage of initial size (% change in length, % change in width), and Condition Indices (CI, calculated following Lucas & Benninger, 1985). We determined physical condition (ratio of dry shell weight to shell length; $CI_{DSW:SL}$) and two biological condition indices incorporating ratios of flesh weight to shell weight and length (i.e., $CI_{DFW:DSW}$; $CI_{DFW:SL}$). These indices reflect the overall metabolic state of the organism (Mann, 1978; Roper et al., 1991), although they are

generally used to examine changes over longer time periods than the duration of this experiment.

Responses of the large and small juvenile mussels were analyzed separately, as mussel growth rates are size-dependent. Differences in each of these five response-types depending on the substrate (waste shell, glass beads) or pH (Year 2020, Year 2050) was investigated using a two-way ANOVA with interactions (pH/Year, substrate, pH/Year*substrate), after first satisfying assumptions for normality and homogeneity of variance. Information from all 72 raceways was used in this analysis, however where the same header tank supplied water to multiple raceways, data from these raceways were averaged before being analysis to avoid pseudo-replication. This resulted in $N = 3$ for each of the four experimental treatment combinations.

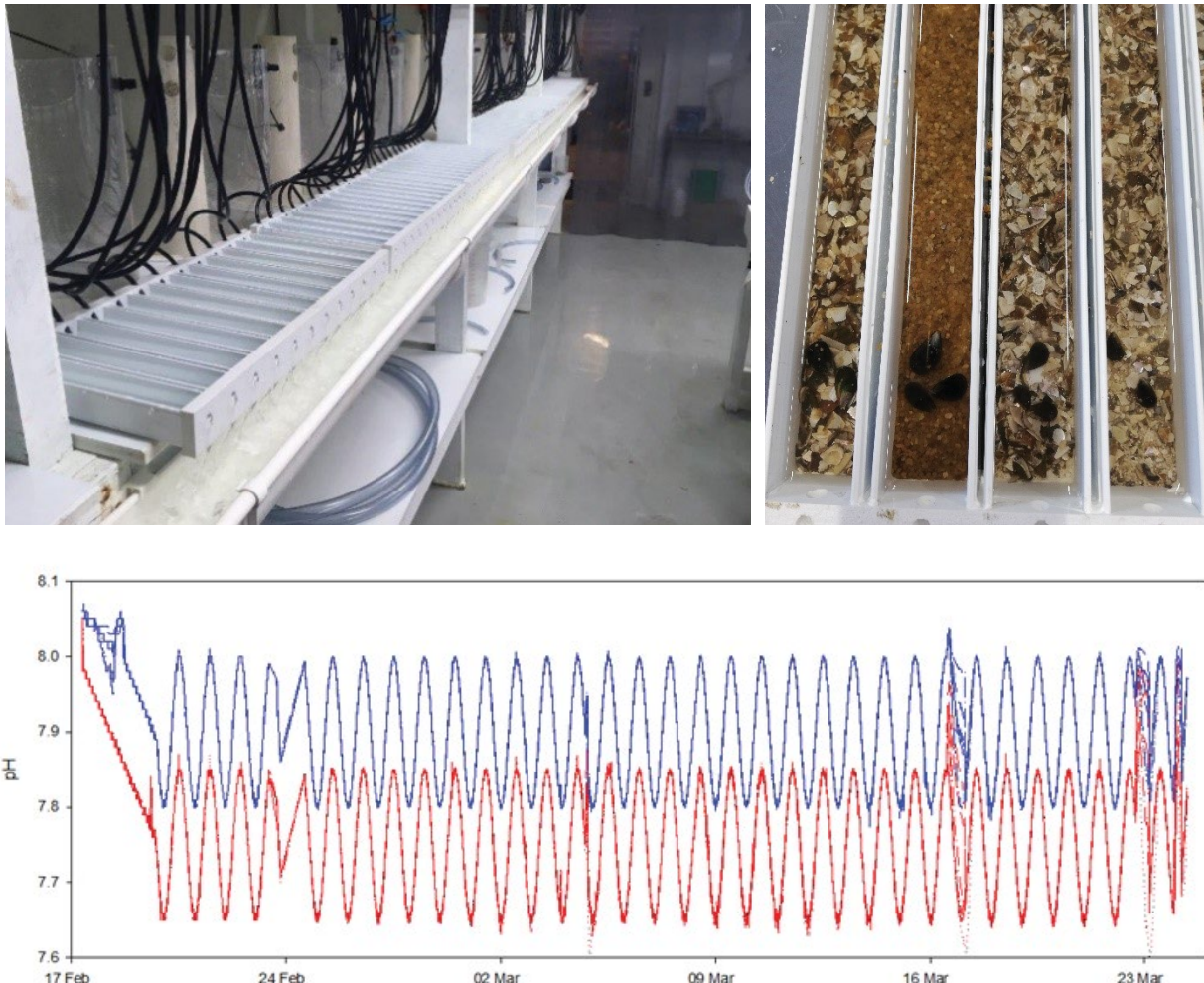


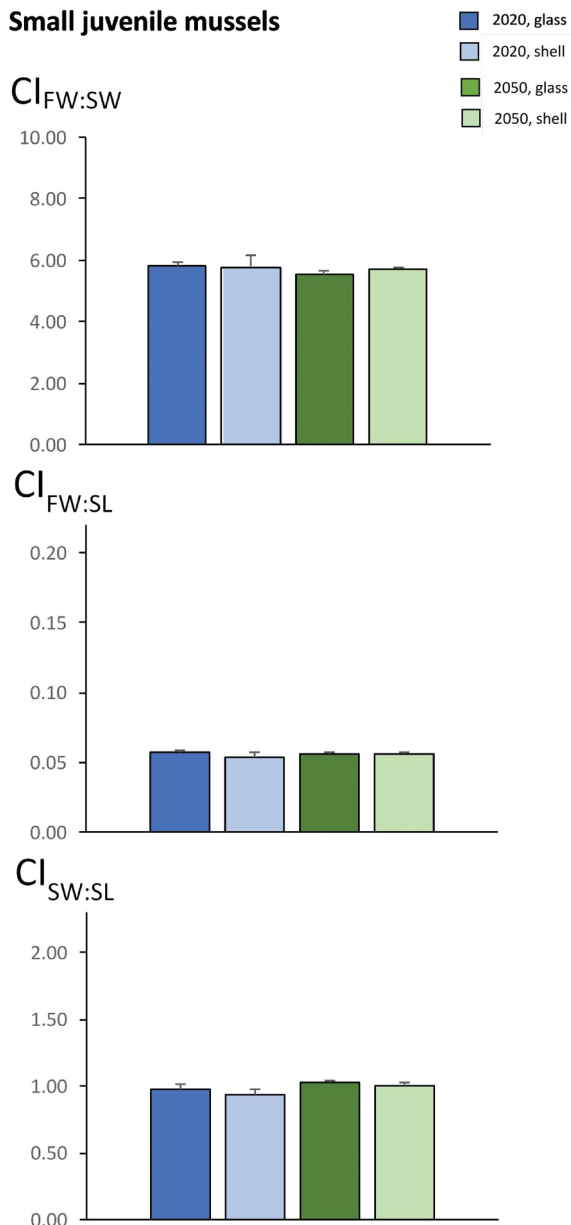
Figure 13. Experimental set up for testing the effect of waste shell on juvenile mussel growth showing multiple raceways in the Marine Experimental Manipulation Facility (upper left figure), and waste shell in four adjacent runways with juvenile mussels visible at the end of each raceway (upper right figure). The lower figure shows pH throughout the 37-day experiment with the control treatment in blue and the 2050 treatment in red. The sinusoidal pH trend reflects a simulated diel variation of 0.2 pH units (as shown by field pH measurements).

Potential of waste shell to mitigate OA

Growth of the juvenile mussels was low over the relatively short (37 day) experimental period. In absolute terms, small mussels grew an average (+ standard error) of 0.22 mm + 0.03 in length and 0.20 + 0.02 mm in width. This equates to an increase of around 0.9 to 1.9% in length, and 1.4 to 2.4% width since the beginning of the experiment (Figure 14). Large mussels grew slightly more in absolute terms (0.31 + 0.03 mm in length, 0.30 + 0.02 in width), but percentage growth was similar (increased by 0.8 to

1.2% in length, 1.4 to 2.0% width; Figure 14). Neither the presence of waste shell, or the seawater pH, had any influence on juvenile mussel growth or condition, irrespective of size class (Figure 14, Appendix Tables A and B). Re-assessment of these results to investigate responses of the mussels to pH/Year alone (i.e., ignoring the substrate type) did not show any effect of pH level (Appendix Tables C and D for small and large mussels, respectively).

Small juvenile mussels



Large juvenile mussels

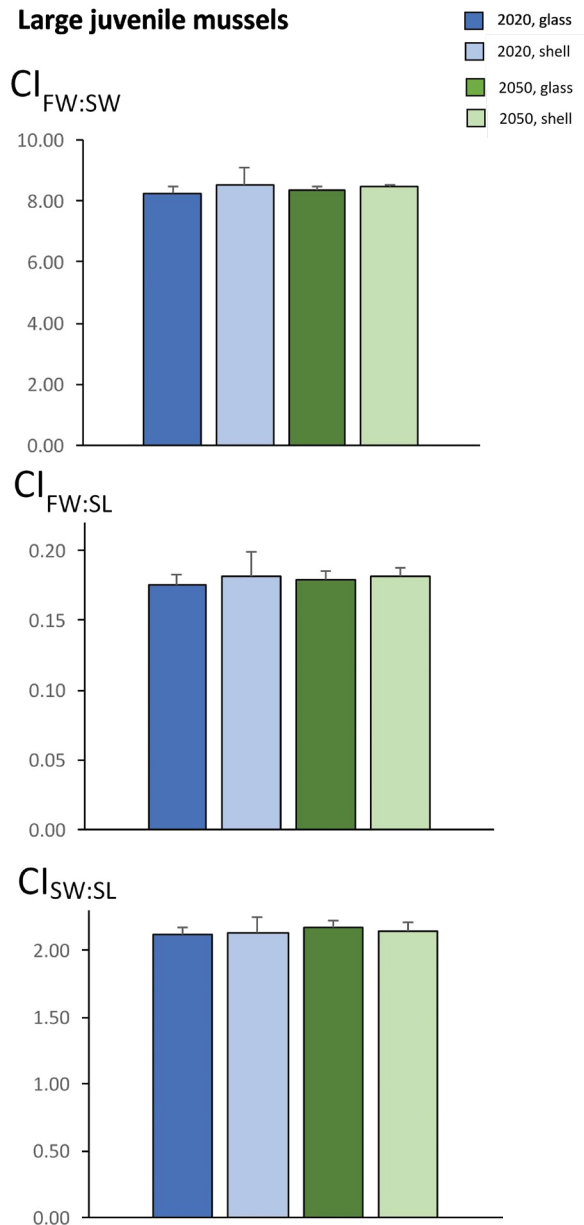


Figure 14. Percentage growth of small (left) and large (right) mussels in the different experimental treatments after 37 days ($N = 3$), showing two biological condition indices involving ratios of flesh weight to shell weight ($CI_{FW:SW}$, upper graphs) and length ($CI_{FW:SL}$, middle graphs), and one physical condition index (ratio of dry shell weight to shell length; $CI_{SW:SL}$, upper graphs).

Is seafloor deposition of waste shell a viable strategy for raising pH on a mussel farm?

Various studies have identified potential ecological benefits of returning waste shell to the seafloor, for example in providing settlement habitat for species that may facilitate shellfish larval settlement (Handley and Brown, 2012), or recruitment of other organisms that contribute to water quality improvement and biodiversity. The Washington State Blue Ribbon Panel on Ocean Acidification (2012) recommends waste shell return to sediment as one method for combatting OA. Addition of clam shell material to sediment resulted in an increase in porewater Ω_{AR} that was reflected in an increase of live clams (Green et al., 2009; Green et al., 2013). The Ω_{AR} at the sediment-water interface may be lower than in the overlying water column, so increasing the potential for shell dissolution, although, Abdulghani (2014) reported mass loss of only 2.6% in *M. galloprovincialis* shells (83% aragonite) on the sediment surface over eight weeks in waters that were undersaturated with aragonite (Ω_{AR} 0.59–0.91).

Deposition of shell on the sediment surface is more practical than addition to a dropper line and offers a potential low-cost option for the mussel aquaculture industry. However, transfer of dissolved carbonate released by shell dissolution on the sediment surface beneath a mussel farm may be influenced by the altered current flow and mixing generated by the farm (Plew et al, 2005, Stevens, 2010; Stevens et al, 2011; see Figure 15). The presence of the dropper lines acts as a partial barrier by deflecting flow below the droppers generating an undercurrent, and shear and associated turbulence, at the base of the dropper lines. The net effect of this is unclear, but it is unlikely that the dissolved carbonate originating from

waste shell deposited directly under the farm would be transported vertically up to the dropper lines.

Delivery of dissolved carbonate from waste shell at the sediment surface would instead require placement of waste shell upstream of the farm to ensure complete vertical mixing in the water column (see Figure 15), and so further dilution. Application of a 1-D vertical model of flow (Rutherford, 1981) indicates that a plume originating at the sediment surface would reach the bottom of the dropper lines if placed ~500 m upstream and would require another ~250 m to be fully mixed over the lower half of the farm. To ensure vertical mixing to the surface before the plume reaches a farm, the waste shell would need to be deposited ~1.5 km upstream of the mussel farm. This estimate is based upon a current speed of 0.1 m/s and water column depth of 25 m and assumes no water stratification. As Pelorus Sound is generally stratified during summer (Stevens, 2003) vertical mixing to the surface would be suppressed restricting supply to mussels on the upper dropper line.

To raise pH by 0.1 would require delivery of ~75 kg/h dissolved carbonate at a background current speed of 0.01 m/s. Based upon the measured waste shell dissolution rates of 0.012%/d on the dropper lines, this delivery rate would require deposition of 44400 tonnes of waste shell. Even assuming a higher dissolution rate at the sediment surface, using the 0.05% rate reported by Abdulghani (2014), this would require deposition of 10560 tonnes of waste shell, equivalent to 10% of national annual waste shell generation (Barnaby, 2004). Although there may be other benefits of waste shell deposition on the sediment surface, these estimates indicate that significant shell (and associated resource use) would have minor to negligible benefit in terms of OA remediation.

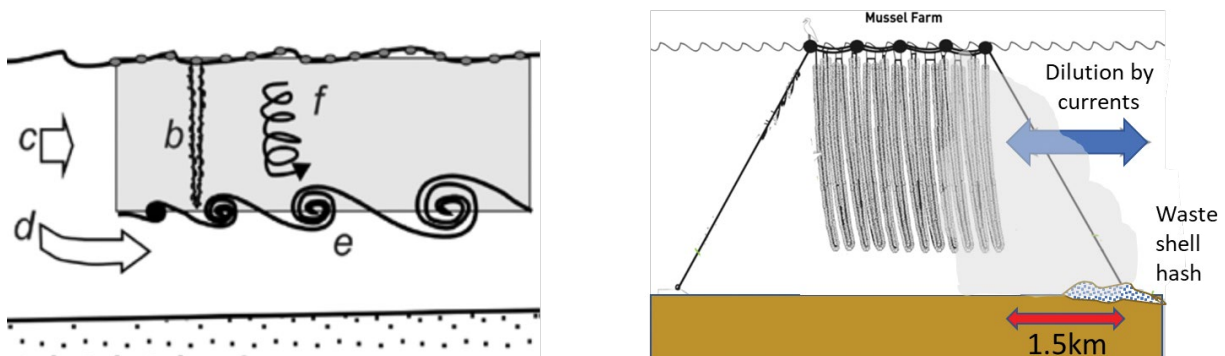


Figure 15. Conceptual figure (left) of horizontal currents around a mussel farm (from Plew et al, 2005) indicating (b) vertical dropper lines, (c) main flow (d) accelerating undercurrent. The shear between the undercurrent and the fluid within the farm-proper generates (e) a mixing layer that augments (f) the turbulence due to the droppers. Conceptual figure (right) of shell deposition on seafloor upstream of mussel farm illustrating the vertical dispersion of the carbonate plume required to reach mussels on the upper dropper line.

Other options for using waste shell to combat OA

Calcination

Pre-treatment of waste shell via calcination results in the production of quicklime (calcium oxide). Lime is highly soluble and would rapidly convert to dissolved carbonate on addition to water on a farm. However, the thermal decomposition of calcium carbonate by calcination requires high temperatures (>600°C, Rashidi et al, 2011), and so significant energy input, resources and expense. Furthermore, because quicklime reacts with air, it needs to be stored in the absence of air to prevent it converting back to calcium carbonate.

Incorporation of shell into the matrix of dropper lines

Although it would not be feasible to accommodate large volumes of waste shell onto a dropper line incorporation of waste shell into the structural matrix of a dropper line may benefit mussel growth and

condition by slow sustained delivery of carbonate in the immediate vicinity of the mussel. This would need to be confirmed by testing and it would need to be established how dissolution of the incorporated shell would affect the lifetime of the dropper line.

Recommendations

As a long-term strategy to ameliorate OA, waste shell does not appear to be a viable approach as benefits appear to be minor to negligible. However, further studies could assess:

- the impact of waste shell on larvae and spat, as these are the most sensitive life stages, over longer duration experiments
- the dissolution rate of mussel shell at the sediment surface in New Zealand coastal waters
- the viability of incorporating waste shell into the structural matrix of dropper lines
- incorporation of pH and carbonate measurements in studies of waste shell and live mussel deposition on the seafloor.

Potential of aeration to mitigate OA

The magnitude of diel pH change varies regionally in NZ coastal waters, with significant daily pH variation and Ω_{AR} range in the Firth of Thames (see Figure 3). The diel pH quartile range in the outer Firth of Thames is generally 0.1, with a maximum daily range approaching 0.8 (see Figure 16). This is reflected in a quartile range of <0.6 for Ω_{AR} , that is generally higher in summer and autumn. The diel cycle in both pH and Ω_{AR} may be exacerbated on a mussel farm by the additional CO₂ production at night during respiration by mussels and associated biofouling communities in the absence of photosynthesis. In addition, extreme pH minima may occur in response to heatwaves and elevated terrestrial run-off.

Consequently, there may be benefit of short-term approaches to alleviating OA, such as the removal of excess dissolved CO₂ by strategic aeration

(Koweek et al, 2016). This approach has potential co-benefits including increasing dissolved oxygen during periods of hypoxia which often co-vary with periods of low pH. In addition, the mixing associated with aeration may increase food supply to mussels and reduce stratification and associated CO₂ accumulation and hypoxia in subsurface waters. Indeed, reducing coastal acidification and hypoxia concurrently via aeration may have a “double-whammy” effect of reducing both the individual and interactive effects of these stressors (Melzner et al, 2013). However, CO₂ does not behave as other dissolved gases such as oxygen; instead of a simple exchange between dissolved and gaseous phase, CO₂ is converted to bicarbonate and carbonate (see Figure 1), which means that CO₂ stripping by aeration requires a longer duration to influence the carbonate system and pH than would be required to alter the oxic status.

Potential of aeration to mitigate OA

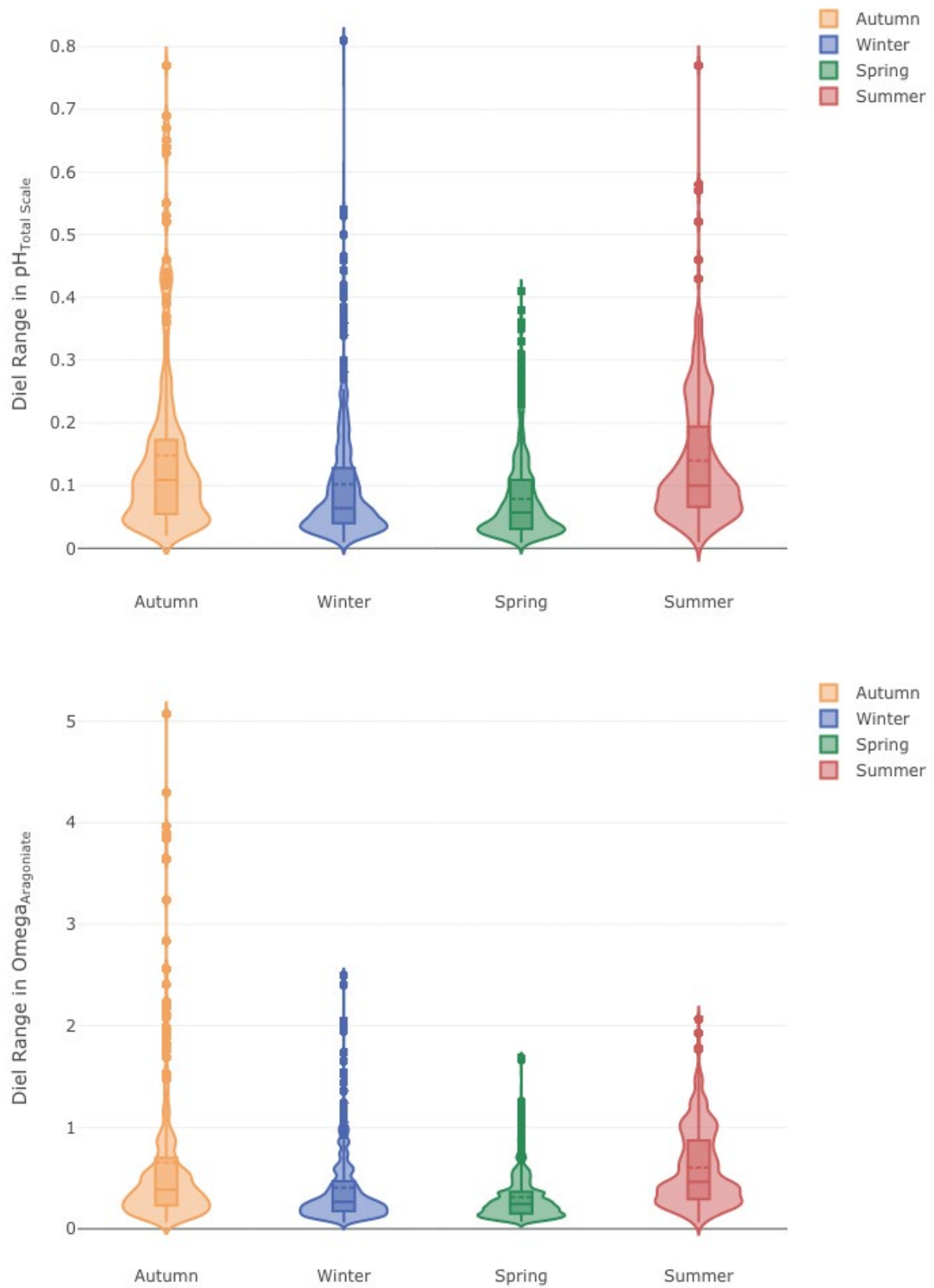


Figure 16. Daily ranges for a) pH and b) Ω_{AR} at the Outer Site in the Firth of Thames. The solid horizontal line is the median and the dashed line is the mean. The rectangular boxes span the 25%–75% quartiles of observations, and the bulges represent the probability density for given values.

Pond aeration experiments

To determine the effect of aeration rate on pH a series of tests were carried out using different tubing, flow rates and depths, with a single in situ test also carried out on the Ecofarm in Marlborough Sounds. Three different types of aeration tubing were assessed, including a flexible permeable hose constructed of a woven polyester fibre (Bubblemac, <https://bubblemacairdiffusers.com/products/bio-weave-air-diffuser-hoses>) and two pore size (1/2' and 3/4') variants of a more rigid PVC-based Bubble tubing® (Canadian pond, <https://canadianpond.ca/bubble-tubing-fine-bubble-linear-diffuser/>). Following initial tests, the Bubble tubing® was subsequently used as it had advantages for deployment on a mussel farm of robustness, anti-fouling properties and lower cost. The Canadian pond tubing is also specifically designed to generate bubble sizes of <1mm diameter to elevate oxygen transfer, and so improve the oxic status of receiving waters (<https://canadianpond.ca/bubble-tubing-fine-bubble-linear-diffuser/#test>). The production of sub-millimetre bubbles may be beneficial for the removal of dissolved CO₂ (Koweek et al, 2016).

Twenty experiments were carried out in a 128 m³ pond at NIWA Wellington containing local coastal seawater. The tubing was deployed as 8 m, 16 m and 24 m lengths at depths of 2 m and 3.75 m and



Figure 17. Aeration tests showing bubble production by the Canadian pond aeration tubing (upper) and surface view of the resulting bubble curtain (lower).

connected to an air compressor (Ash Air) which delivered air at two different flowrates (200 and 400 L/min) for the tests (see Figure 17). The impact of aeration was determined by the change in pH over time, as measured by pH sensors suspended at 0.5 m depth across the pond, and samples were also collected for Total Alkalinity and Dissolved Inorganic Carbon. Initial pH was adjusted by a flow of pure CO₂ gas through permeable tubing immersed in the pond, which was switched off and removed when the target pH was achieved. A bag of 4.2 m³ volume containing pond water that was open to the atmosphere provided a non-aerated Control. Experiments were run for 8–12 hours overnight to exclude the influence of plankton photosynthesis. Experimental pH levels were initially set at two different levels: pH 7.9, to simulate the minimum pH recorded in the Marlborough Sounds (see Figure 2), and pH 7.7, to simulate the minimum pH measured on the mussel farm in the Firth of Thames (see Figure 6).

The change in pH overnight over the course of the experiments was generally linear (see Figure 18). The Control bag without aeration exhibited a decrease in pH in all experiments, with a range of -0.0005 to -0.01 /h, reflecting differing biological states and ambient temperature variation. The net pH change (Δ pH) due to aeration was obtained by subtracting the Control rate, scaled to the pond, from the pH change rate recorded in the pond. The effect of aeration on pH was relatively consistent with tubing length with the pH increase ranging from 0.0008 to 0.009 /hr. In addition, the experiments showed only minor variation in CO₂ stripping capacity in relation to the depth and length of the aeration tubing. The relatively shallow depth of the pond (3.75 m) meant that vertical mixing by the bubble plume resulted in rapid mixing which was ideal for testing but limited the capacity to determine the effect of tubing depth.

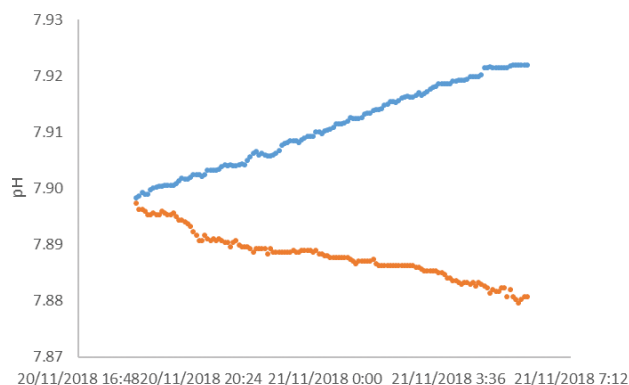


Figure 18. pH during overnight aeration using 3/4" tubing in the pond (blue) and Control bag in the absence of aeration (orange).

Potential of aeration to mitigate OA

As expected, air supply rate and initial pH were the primary determinants influencing pH change and so CO₂ degassing. With an initial pH anomaly of -0.1 (pH 7.9) ΔpH was 0.004/h (+/-0.0008) with an air supply rate of 0.08 L/m²/s, which equates to a decrease in pCO₂ of 5.4 μatm/h and an increase in dissolved carbonate concentration of 1.1 μmol/L/h. With a larger initial pH anomaly of -0.3 (pH 7.77) and higher flow rate of 0.2 L/m²/s, ΔpH was 0.011/h (+/- 0.0005), which equates to a decrease in pCO₂ of 26.5 μatm/h and a dissolved carbonate concentration increase of ~2.0 μmol/L/h. A laboratory simulation and modelling study of aeration obtained a larger decrease in pCO₂ of ~60 μatm/h with aeration at 0.2 L/m²/s (Koweek et al, 2016), although this was modelled using a larger aeration plume radius (50 m), for only the first hour of aeration during which the pCO₂ gradient would be highest, as opposed to the estimate based upon mean pCO₂ loss over 8-12 hours in the current study.

Table 3 considers the impact of scaling up the pond aeration results to different volumes of a mussel farm, using the two initial pH levels and pH adjustment rates in the pond tests. The results show that although aeration is relatively effective at the pond scale the benefits are significantly ameliorated by dilution with surrounding water at the farm scale. For example, aeration of 20% of the volume of a mussel farm would result in a change

of <1% in dissolved carbonate concentration, and it is only when aeration is scaled up to ~50% of farm volume under low current speeds that the increase in dissolved carbonate approaches 5%.

To achieve a 2-5% increase in carbonate availability over 24 hours would require air delivery of ~10,000 L/s (see Table 3). This could be delivered using a large, multistage blower or smaller single stage high-volume low-pressure blowers in series (Koweek et al, 2016). Using four single stage blowers with discharge pressure of 0.5-1.0 Bar would require capital outlay of ~NZD1-1.4M (based upon quotes from Nu-Way Energy NZ Ltd and Ash Air Ltd). Power consumption would be ~680-1000 kW, respectively which, at standard commercial 3-phase rate of ~NZD20 per kw/h, would entail running costs of NZD1440-2000/day. Additional hardware costs including aeration tubing (~2 km) and connectors would be relatively inexpensive at ~NZD10K. Aeration technology is readily available and used in freshwater waterways, lakes and by the finfish aquaculture industry. Degassing systems, such as CO₂ strippers and aeration towers are also used in aquaculture hatcheries (Summerfelt et al, 2000), but will be less effective and costly to deploy in coastal waters around mussel farms. However, bubble curtains, which are used for large-scale applications such as oil spills containment and sediment control in aquatic systems may be more viable.

		Aeration			Current speed 0.01 m/s		Current speed 0.1 m/s	
Start pH	ΔpH (/h)	Proport farm aerated	Aeration volume (m ³)	Air supply (L/s)	Carbonate increase after dilution (mol/d)	Increase as % initial carbonate	Carbonate increase after dilution (mol/d)	Increase as % initial carbonate
7.9	0.004	0.1	40000	1562.5	168.1	0.30	16.8	0.03
		0.2	80000	3125.0	336.3	0.60	33.6	0.06
		0.5	200000	7812.5	840.7	1.51	84.1	0.15
7.77	0.011	0.1	40000	4166.7	303.3	0.89	30.3	0.09
		0.2	80000	8333.3	606.5	1.78	60.7	0.18
		0.5	200000	20833.3	1516.4	4.46	151.6	0.45

Table 3. Effect of aeration at two different pH levels, based upon the pond tests, scaled to different volumes of a mussel farm using two different current regimes (see Table 2 and Appendix). Total aeration rate required is identified and the resulting increase in dissolved carbonate indicated as both total and percentage concentration change at the farm scale.

In situ testing of aeration on a mussel farm

A test deployment of aeration was carried out following overnight monitoring on the Ecofarm in the Marlborough Sounds in May 2018. Three Exosonde sensor packages were suspended at 2.5 m from the backbone line to provide continuous data for a range of parameters over a tidal cycle (see Figure 19), and a current meter suspended at a depth of 2 m from a parallel backbone line (see Appendix Figure 2). A 24 m length of Canadian pond tubing was deployed at a depth of 3–4 m perpendicular to the backbone lines and between mussel buoys with the aeration provided by an air compressor (Ash Air) located onboard the Sanford vessel *HawkEye*. Aeration took place for approximately 1 hour at 0730–0830, approximately two hours after Low Tide (see Figure 20).

The Exosonde sensor data revealed that the start of aeration coincided with an abrupt change in some parameters, including an increase in temperature of 0.4°C and an increase in turbidity (Figure 20). This coincided with decreases of ~4% in dissolved oxygen saturation and a decrease in pH of 0.4. Aeration also caused a temporary spike in currents against a background of generally intermediate currents (see Appendix Figure 3). These changes were attributed to aeration, as comparable changes in current flow and other parameters at other times during the tidal cycle did not elicit a similar response. These responses were maintained throughout the aeration period, with highest turbidity and lowest pH and dissolved oxygen recorded as aeration was terminated, after which the parameters readjusted towards pre-aeration conditions (Figure 20).

The change in surface properties suggests that aeration degraded the near-surface stratification at 5–10 m depth, resulting in vertical transfer of water from below the farm that was warmer, but had lower oxygen and pH, as indicated in the water column profiles immediately prior to aeration (see Figure 21). The increase in turbidity and declines in pH and dissolved oxygen may also have arisen from disturbance and suspension of particulate material associated with the mussels and other biomass on the dropper lines. Comparison of temperature and salinity profiles across the tidal cycle on the farm suggests that aeration at other points in the tidal cycle when stratification was deeper could have resulted in greater mixing and aeration of near-surface water with less vertical transfer of deep water (data not shown); however, this would be of limited benefit to mussels on the lower portion of the dropper line.

Consequently, the trial showed that aeration exacerbated the conditions it was meant to address, which suggests that aeration within the boundaries of the farm may not be a viable option particularly for addressing short-term extremes in low pH. If aeration was outside the farm boundary the vertical transfer of deeper water may be lower in the absence of dropper lines, although this would introduce further dilution. Calculations suggest that with aeration tubing at a depth of 20 m, a bubble rise time of 50–250 seconds and a cross flow speed of 0.1 m/s, the bubble plume would not interact with the farm if the aeration tubing was 5–25 m upstream. However, aeration outside the farm boundary would require testing to ensure that the entrainment of deeper water was negligible.

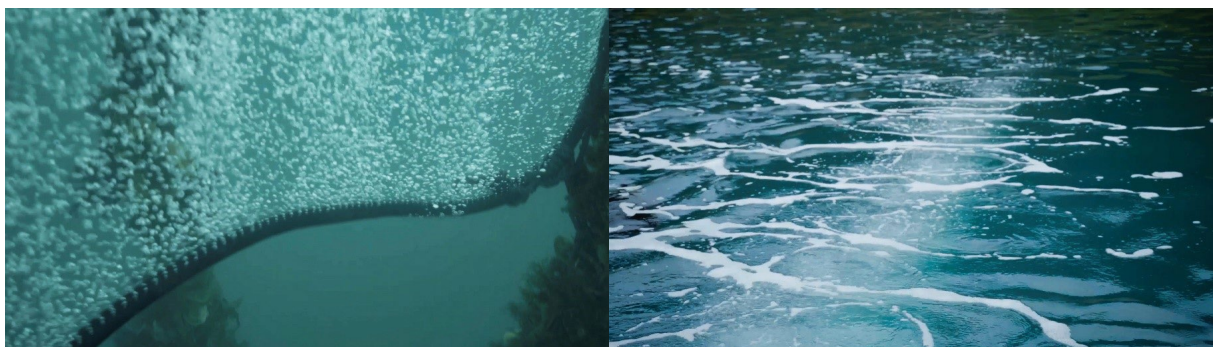


Figure 19. Aeration tests on the Ecofarm (Sanford Limited) using the Canadian pond tubing showing a) aeration at 4m depth between backbone lines and b) the resulting bubble plume at the surface.

Potential of aeration to mitigate OA

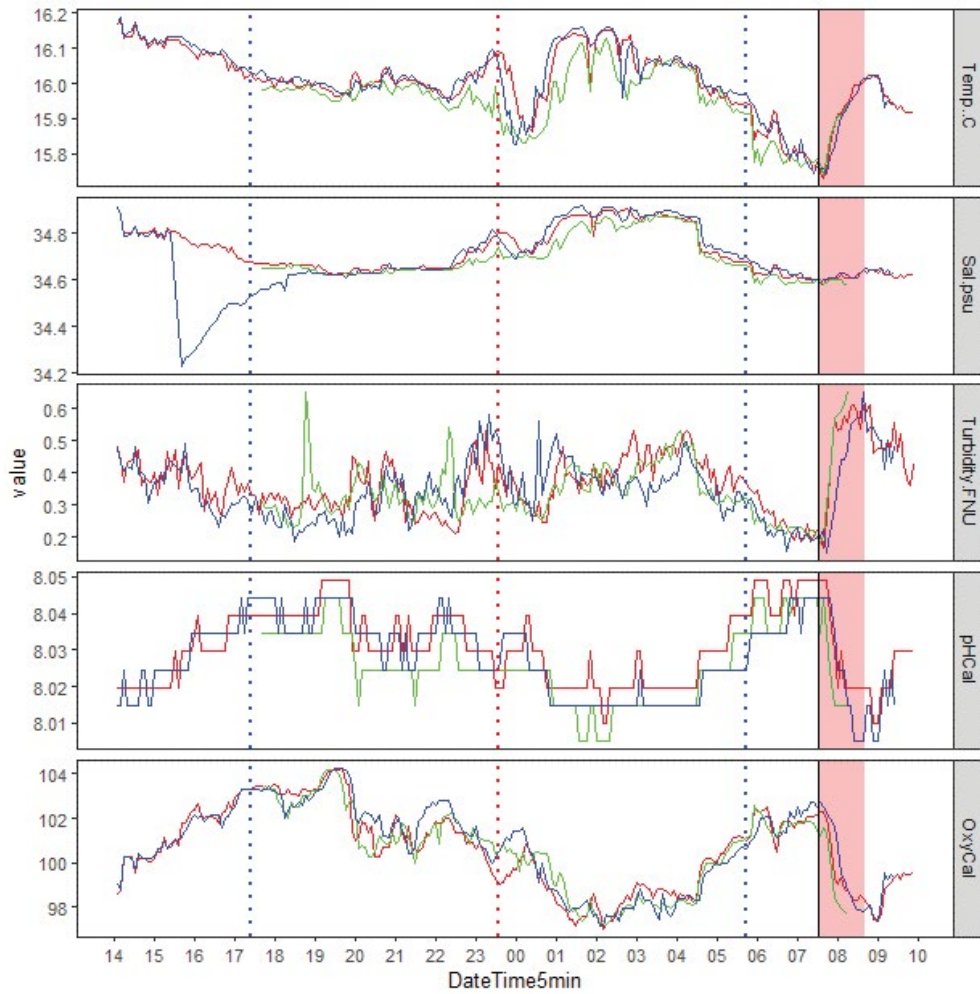


Figure 20. Water column properties at 2.5 m depth in a mussel farm over a tidal cycle, with High Tide (HT, red dashed vertical line) and Low Tide (LT, blue dashed vertical line) indicated. The different colour lines indicate measurements of the Exosonde sensor packages at three different locations on the farm (see Appendix Figure 2). The measured parameters are (from the top) temperature (°C), salinity (psu), turbidity, pH (note the Exosonde pH sensor precision of 0.01 limits resolution), and Dissolved Oxygen saturation (%). The pink shaded region indicates the 1-hour period (0730-0830) during which aeration was supplied by tubing at 4m depth.

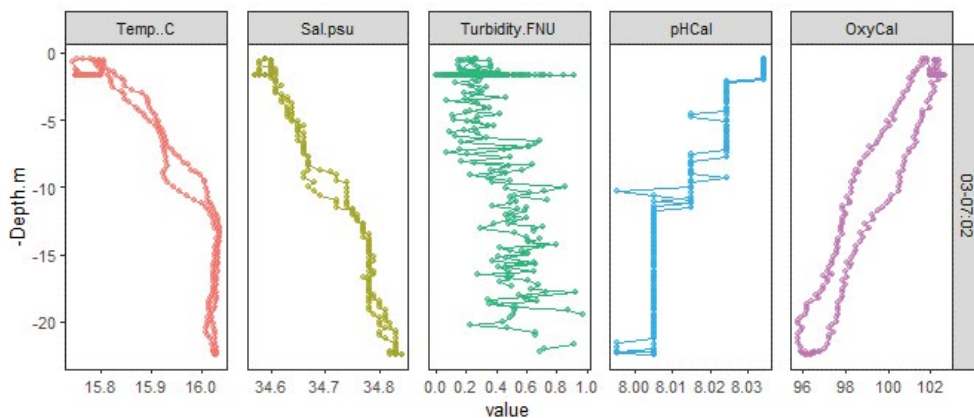


Figure 21. Depth profiles of water column properties on the mussel farm prior to aeration (0700), showing (from the left) temperature (Temp, °C), salinity (Sal, psu), turbidity (Turbidity FNU), pH (pHCal, note pH sensor precision of 0.01 limits vertical resolution), and dissolved oxygen saturation (OxyCal, %). Note the warmer water of higher turbidity, lower pH and lower dissolved oxygen in the deeper water column

Added benefits of aeration

Coastal waters may experience hypoxia as a result of elevated organic matter degradation, which may lead to reduction in biodiversity and in extreme cases mortality. Although NZ coastal waters are generally not characterised by hypoxia, dissolved oxygen saturations as low as 60% have been recorded in the Firth of Thames (J. Zeldis, pers. comm., Law et al, 2019). However, the potential for hypoxia may increase in future coastal waters as elevated temperatures increase the metabolic rate of marine organisms and decrease the solubility of dissolved oxygen. Hypoxia represents an additional stress on marine organisms that can magnify the deleterious impacts of acidification and could be partially ameliorated by aeration.

Aeration alternatives

Dropper aeration

There may be potential benefits of air supply and associated savings in air delivery by aeration in the immediate vicinity of the mussels on the dropper line. The observed drawbacks of vertical transfer of deeper low-oxygen water during bubbling could potentially be reduced by integrating aeration tubing within the dropper line matrix.

Phytoremediation

Maintenance of an upstream algal source may provide mussel farms with some degree of protection

from both acidification and hypoxia. This approach is being considered for protection of coastal ecosystems by using seagrass and macroalgae (seaweed) beds (Washington State Blue Ribbon Panel on Ocean Acidification, 2012; Nielsen et al, 2018, Koweek et al, 2018). This application could have broader economic benefits if incorporated within Integrated Multi-Trophic Assemblage (IMTA) aquaculture (Clements and Chopin, 2017; Han et al, 2017; Fernandez et al, 2019).

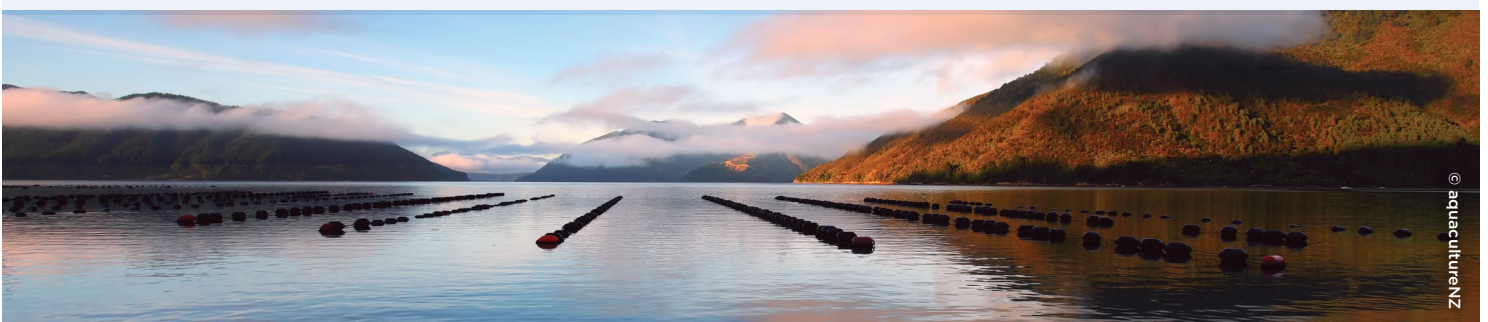
Recommendations

Aeration offers a more responsive intervention option to counter low pH and carbonate during extreme events such as heatwaves, high run-off and disturbance events. However, the resources, energy and cost to achieve this are significant relative to the minor improvements in carbonate availability and pH achievable. Further tests could include:

- aeration tests outside the boundary of the mussel farm to determine if resuspension and stratification issues can be overcome
- assessment of aeration within the dropper line matrix, via permeable or perforated tubing, which may more directly improve carbonate availability and oxidic status in the immediate vicinity of mussels
- Integrated Multi-Trophic Aquaculture (IMTA) using macroalgae to “bio-buffer” against low pH/high CO₂.

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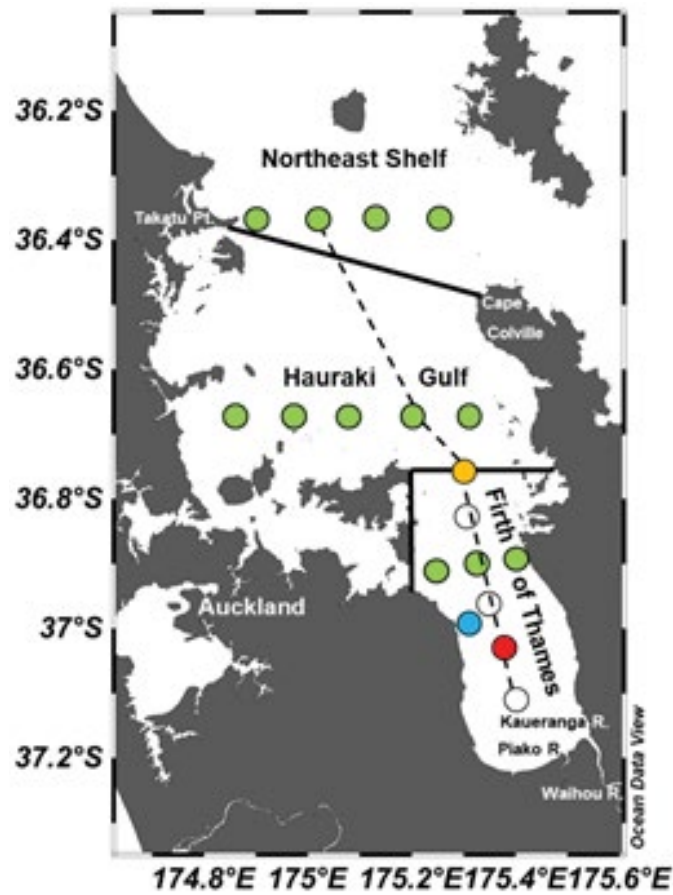
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Appendix



Appendix Figure 1. Location of Sanford Ecofarm (Sanford Limited), Old Homewood Bay, Marlborough Sounds (upper figure). In the lower figure the orange circle marks the location of the long-term (1998–present) NIWA monitoring site in the Outer Firth of Thames, the red circle the more recent NIWA monitoring site in the Inner Firth of Thames (2015–present) and the blue circle the University of Auckland monitoring site at Tuwhitu Mussel Farm (western Firth of Thames). Green circles denote stations used in nutrient mass-balance analysis (Zeldis and Swaney 2018).

Additional information for Table 2

Scales and dimensions relevant to a mussel farm and a dropper line

Tidal speeds of 0.01 and 0.1 m/s for the Inner and Outer Sounds respectively:

Broekhuizen, N., Hadfield, M., Plew, D. (2015) A biophysical model for the Marlborough Sounds. Part 2: Pelorus Sound. *Prepared for Marlborough District Council, NIWA Client Report CHC2014-130*: 175.

Reduction of horizontal currents within a mussel farm on the order of 50%, as in:

Lin, J., Li, C.Y., Zhang, S.Y. (2016) Hydrodynamic effect of a large offshore mussel suspended aquaculture farm. *Aquaculture*, 451: 147-155. 10.1016/j.aquaculture.2015.08.039 (45-90% reduction)

O'Donncha, F., Hartnett, M., Nash, S. (2013) Physical and numerical investigation of the hydrodynamic implications of aquaculture farms. *Aquacultural Engineering*, 52: 14-26. (25-30% reduction)

Plew, D.R. (2011) Shellfish farm-induced changes to tidal circulation in an embayment, and implications for seston depletion. *Aquaculture Environment Interactions*, 1: 201-214. 10.3354/aei00020 (up to 35% reduction - but this averaged across several farms)

Aure, J., Strohmeier, T., Strand, O. (2007) Modelling current speed and carrying capacity in long-line blue mussel (*Mytilus edulis*) farms. *Aquaculture Research*, 38(3): 304-312.

Plew, D.R., Stevens, C.L., Spigel, R.H., Hartstein, N.D. (2005) Hydrodynamic implications of large offshore mussel farms. *IEEE Journal of Oceanic Engineering*, 30(1): 95-108. (36-63%)

Stevens, C.L. and Petersen, J.K., 2011. Turbulent, stratified flow through a suspended shellfish canopy: implications for mussel farm design. *Aquaculture Environment Interactions*, 2(1), pp.87-104.

Farm scale

A typical mussel has dimensions of 100 m x 100 m, with 20 m dropper lines. In these calculations we assume the farm is diagonal to the flow rather than end on.

Volume replacement time(s) $T = \text{volume}/\text{inflow} = 100 \times 100 \times 20 / (\text{sqrt}(100^2+100^2) \times 20 \times U)$

For a farm volume of 200000 m³

$T = 23.6$ minutes at 0.05 m/s (background flow of 0.1 m/s with 50% reduction within the farm)

$T = 236$ minutes at 0.005 m/s (background flow of 0.01 m/s with 50% reduction within the farm)

Dilution rate calculated from steady state mass balance $V \cdot dC/dt = mV - U \cdot A \cdot C$ where:

C = concentration in the farm/dropper (g/m³)

V = volume of the farm/dropper (m³)

A = projected area of the farm/dropper (m²)

U = velocity (m/s)

m = rate that the tracer is added per unit volume (g/m³/s)

At steady state dilution rate $D = UA/V$ (s⁻¹)

For farm of $V = 200,000$ m³

$D = 6.1$ d⁻¹ at 0.005 m/s

$D = 61.1$ d⁻¹ at 0.05 m/s

Dropper line scale

Diameter of 0.8 m (2x the diameter of a harvest size dropper to include boundary layer)

Replacement time = volume/inflow = $10 \text{ m}^3 / (0.8 \times 20 \times U)$

= 12.5 seconds at 0.1 m/s background (0.05 m/s inside the farm)

= 125 seconds at 0.01 m/s background (0.005 m/s inside the farm)

Dilution rate = $UA/V = 0.8 \times 20 \times U/V = 1.6U$ (s⁻¹) = 138240 U

= 6900 @ 0.1 m/s background

= 690 @ 0.01 m/s background

Appendix

	DF	SS	MS	F Value	Pr > F
A. % growth (shell length)					
Model	3	0.508	0.169	0.60	0.6334
Error	8	2.261	0.283		
Corrected total	11	2.769			
pH	1	0.370	0.370	1.31	0.2859
Substrate	1	0.048	0.048	0.17	0.6909
pH*substrate	1	0.090	0.090	0.32	0.5873
B. % growth (shell width)					
Model	3	3.774	1.258	1.29	0.3422
Error	8	7.795	0.974		
pH	1	1.748	1.748	1.79	0.2173
Substrate	1	0.830	0.830	0.85	0.3831
pH*substrate	1	1.196	1.196	1.23	0.3001
C. CI_{FWSW}					
Model	3	0.933	0.311	2.90	0.1013
Error	8	0.857	0.107		
Corrected total	11	1.790			
pH	1	0.191	0.191	1.78	0.2189
substrate	1	0.192	0.192	1.79	0.2175
pH*substrate	1	0.550	0.550	5.14	0.0532
D. CI_{FWSL}					
Model	3	0.000	0.000	1.97	0.1977
Error	8	0.000	0.000		
Corrected total	11	0.000			
pH	1	0.000	0.000	0.00	0.9654
Substrate	1	0.000	0.000	0.08	0.7794
pH*substrate	1	0.000	0.000	5.81	0.0424
E. CI_{SWSL}					
Model	3	0.022	0.007	1.59	0.2660
Error	8	0.037	0.005		
Corrected total	11	0.058			
pH	1	0.005	0.005	1.14	0.3178
Substrate	1	0.007	0.007	1.53	0.2506
pH*substrate	1	0.010	0.010	2.11	0.1848

Appendix Table A. Small juvenile mussels. Results of statistical tests investigating the influence of pH (Year 2020 and 2050) and substrate (shell hash, glass beads) on the growth and condition of small juvenile mussels.

Appendix

	DF	SS	MS	F Value	Pr > F
A. % growth (shell length)					
Model	3	0.292	0.097	0.98	0.4488
Error	8	0.793	0.099		
Corrected total	11	1.085			
pH	1	0.080	0.080	0.80	0.3960
Substrate	1	0.088	0.088	0.88	0.3745
pH*substrate	1	0.124	0.124	1.25	0.2955
B. % growth (shell width)					
Model	3	0.632	0.211	1.04	0.4260
Error	8	1.621	0.203		
pH	11	2.253			
Substrate	1	0.566	0.566	2.79	0.1332
pH*substrate	1	0.018	0.018	0.09	0.7741
C. CI_{FWSW}					
Model	3	0.174	0.058	0.38	0.7720
Error	8	1.232	0.154		
Corrected total	11	1.406			
pH	1	0.001	0.001	0.01	0.9388
substrate	1	0.151	0.151	0.98	0.3517
pH*substrate	1	0.023	0.023	0.15	0.7106
D. CI_{FWSL}					
Model	3	0.000	0.000	0.17	0.9146
Error	8	0.001	0.000		
Corrected total	11	0.002			
pH	1	0.000	0.000	0.07	0.7920
Substrate	1	0.000	0.000	0.35	0.5691
pH*substrate	1	0.000	0.000	0.08	0.7860
E. CI_{SWSL}					
Model	3	0.003	0.001	0.12	0.9470
Error	8	0.073	0.009		
Corrected total	11	0.076			
pH	1	0.002	0.002	0.26	0.6212
Substrate	1	0.000	0.000	0.00	0.9670
pH*substrate	1	0.001	0.001	0.09	0.7745

Appendix Table B. Large juvenile mussels. Results of statistical tests investigating the influence of pH (Year 2020 and 2050) and substrate (shell hash, glass beads) on the growth and condition of large juvenile mussels. DF = degrees of freedom, SS = sum of squares, MS = mean square.

Appendix

	DF	SS	MS	F Value	Pr > F
A. % growth (shell length)					
Model	1	0.370	0.370	1.54	0.2429
Error	10	2.400	0.240		
Corrected total	11	2.770			
B. % growth (shell width)					
Model	1	0.013	0.013	1.75	0.2152
Error	10	0.076	0.008		
Corrected total	11	0.089			
C. CI_{FWSW}					
Model	1	0.191	0.191	1.19	0.3005
Error	10	1.600	0.160		
Corrected total	11	1.790			
D. CI_{FWSL}					
Model	1	0.000	0.000	0.00	0.9705
Error	10	0.000	0.000		
Corrected total	11	0.000			
E. CI_{SWSL}					
Model	1	0.005	0.005	0.98	0.3467
Error	10	0.053	0.005		
Corrected total	11	0.058			

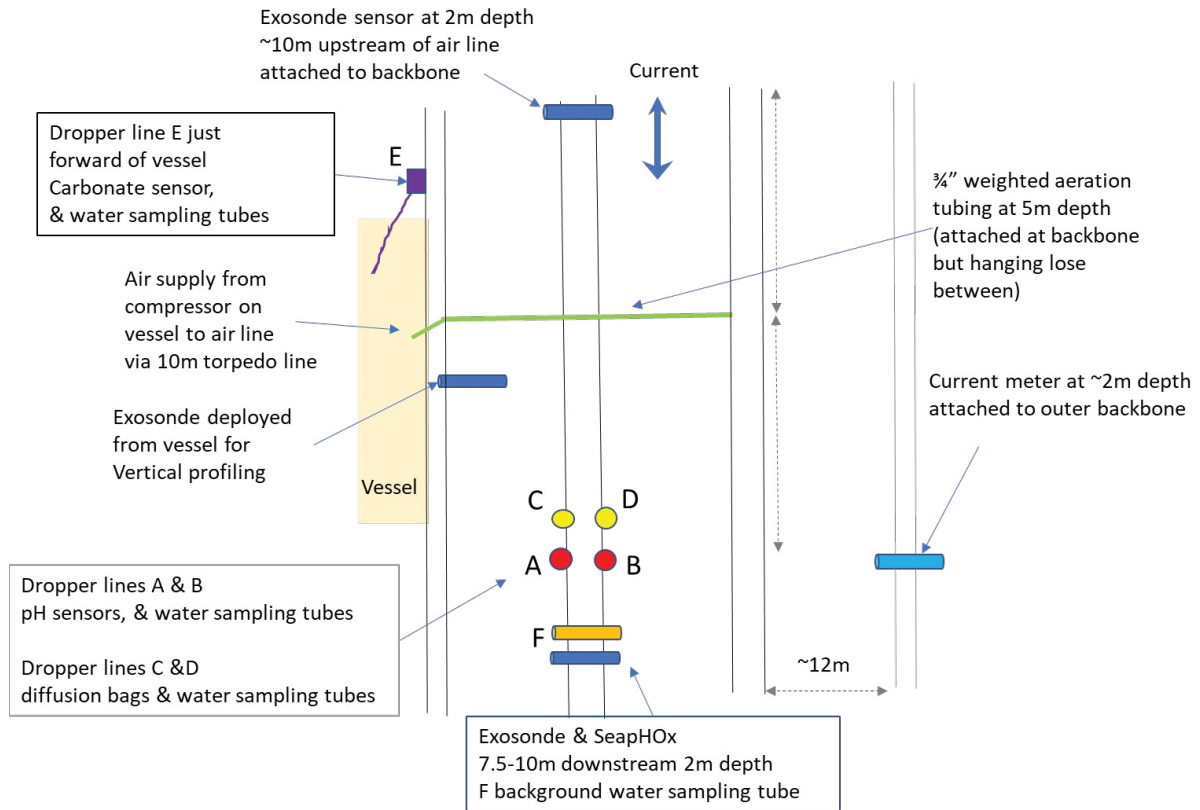
Appendix Table C. Small juvenile mussels. Results of statistical tests investigating the influence of pH (Year 2020, Year 2050) on the growth and condition of small juvenile mussels. N = 6.

Appendix

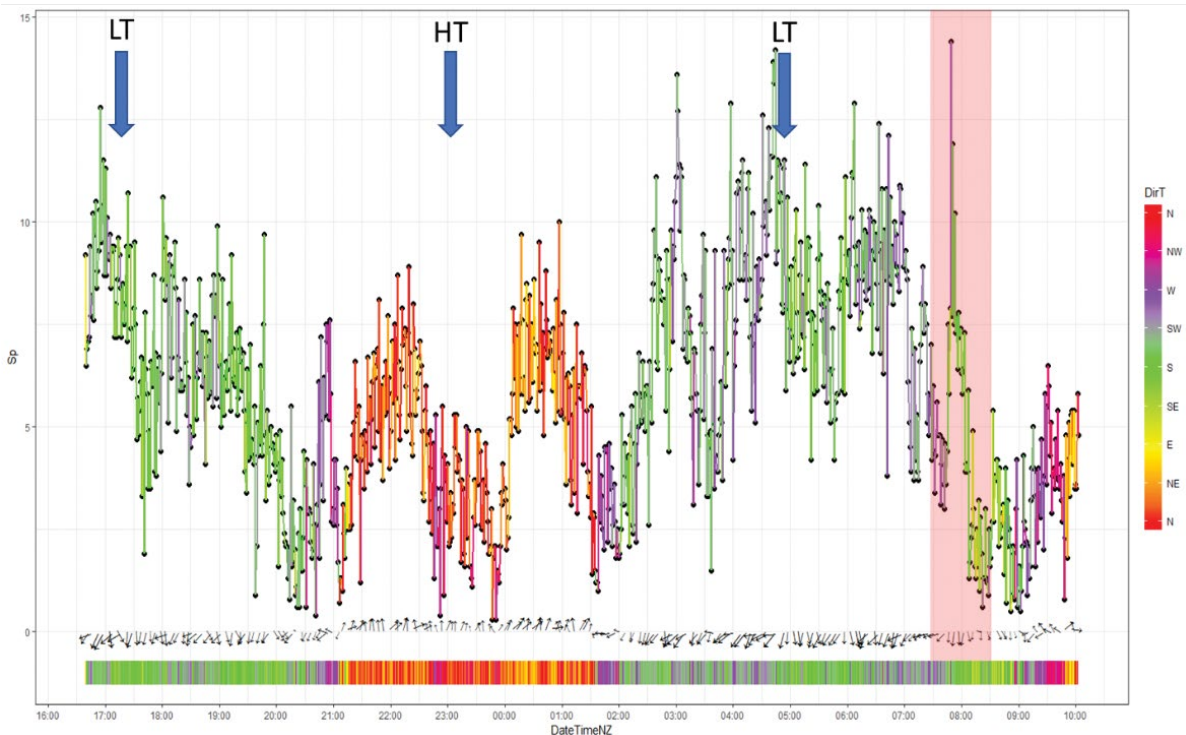
	DF	SS	MS	F Value	Pr > F
A. % growth (shell length)					
Model	1	0.013	0.013	1.75	0.2152
Error	10	0.076	0.008		
Corrected total	11	0.089			
B. % growth (shell width)					
Model	1	1.748	1.748	1.78	0.2118
Error	10	9.821	0.982		
Corrected total	11	11.569			
C. CI_{FWSW}					
Model	1	0.191	0.191	1.19	0.3005
Error	10	1.599	0.160		
Corrected total	11	1.790			
D. CI_{FWSL}					
Model	1	0.000	0.000	0	0.9705
Error	10	0.000	0.000		
Corrected total	11	0.000			
E. CI_{SWSL}					
Model	1	0.005	0.005	0.98	0.3467
Error	10	0.053	0.005		
Corrected total	11	0.058			

Appendix Table D. Large juvenile mussels. Results of statistical tests investigating the influence of pH (Year 2020, Year 2050) on the growth and condition of large juvenile mussels. N = 6.

Appendix



Appendix Figure 2. Arrangement of sampling equipment at the Ecofarm (Sanford Limited) during the May 2018 measurement campaign.



Appendix Figure 3. Current flow and direction (colour bar) recorded by a current meter at 2m depth during the May 2018 measurement campaign on the Ecofarm (Sanford Limited) in the Marlborough Sounds. The pink shaded region indicates the 1-hour period (0730-0830) when aeration was supplied at 3-4m depth.

