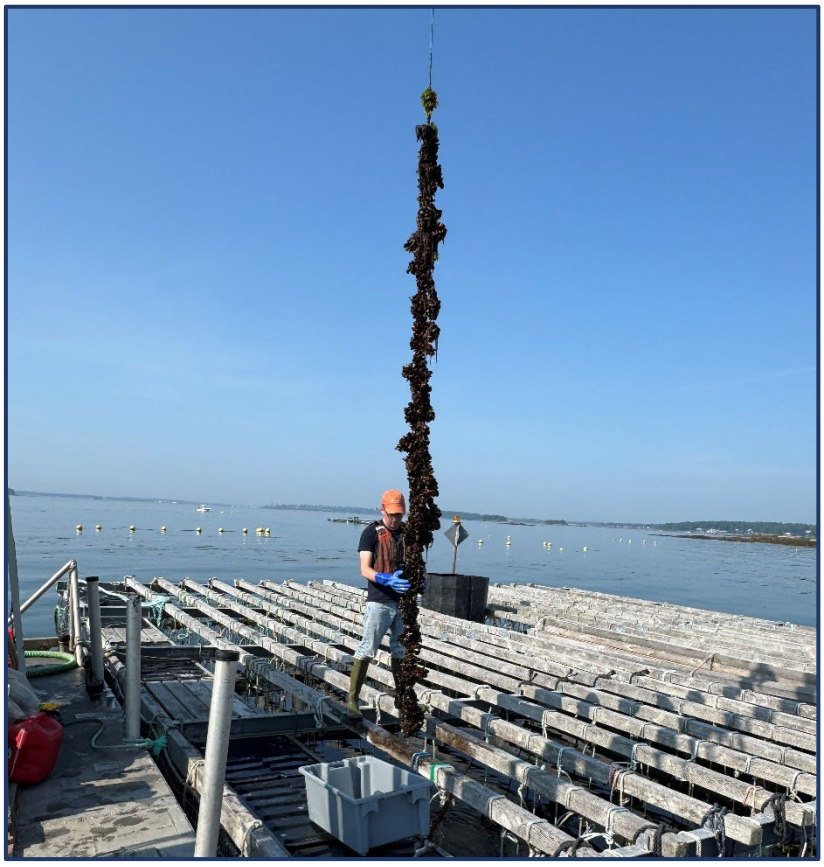
December 28th, 2025

Technical Report for Industry Stakeholders

Live Microalgae Diet Optimization and Seawater Buffering for Improving Blue Mussel Resilience to Ocean Acidification



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I. Background

As wild blue mussel (*Mytilus edulis*) seed declines in the Gulf of Maine, mussel growers are relying increasingly on hatchery production to stock their farms. While mussel populations are currently under pressure from predation by the invasive European green crab, ocean acidification (OA) is expected to be another stressor – especially among earliest life stage mussels – as climate change progresses. Traditionally, shellfish hatcheries have implemented seawater buffering to mitigate low pH in their incoming seawater. Here, live microalgae diet optimization is proposed as an alternative strategy for rendering blue mussel larvae inherently more resilient to OA in a hatchery scenario.

This Technical Report for Industry Stakeholders describes the methodology and preliminary results from a series of experiments conducted in the OA Lab at Downeast Institute to test microalgae diet optimization and seawater buffering as methods for improving blue mussel survival/settlement success, growth, and condition when the natural seawater pumped into a facility to fill culture tanks is acidified. The project included a preliminary microalgae nutrition trial in which nine microalgae species produced at Downeast Institute were cultured in triplicate and tested for caloric and lipid content, and the results of this trial are presented in the first section of this report to aid shellfish hatcheries in selecting species to optimize various nutritional parameters. The project also included a preliminary seawater buffering trial in which eight different buffering additives (or ratios of additives) were tested for their effectiveness and practicality in buffering seawater for bivalve culture, and the results of this trial are presented in the second section of this report to aid hatcheries in selecting additives for the treatment of natural seawater. Finally, the third section of this report includes preliminary results from two lab experiments and field deployments that tested blue mussel responses to various live microalgae diets with and without seawater buffering at both ambient and acidified seawater pH. Full results from these experiments, including analyses of additional morphometrics and a third experiment testing the responses of commercial-size mussel cultures, will be reported in a forthcoming manuscript to be published in a peer-reviewed scientific journal.

Figure II.1: Microalgae culture flasks in the mezzanine at Downeast Institute.

II. Microalgae Nutrition

The project described in this technical report sought to improve the resilience of blue mussels (*Mytilus edulis*) to ocean acidification using microalgae diet optimization and/or seawater buffering. To that end, three lab experiments were designed to test mussel responses to ambient and acidified seawater pH, and each featured multiple microalgae diet treatments. In order to aid in selecting the microalgae species to be used in each experiment, a preliminary microalgae nutrition trial was completed to determine the fatty acid and caloric content in various species cultured for shellfish feeds at Downeast Institute. The microalgae species analyzed in this trial are listed in Table II.1 below.

Species	Shorthand	Classification	Suitable for
Chaetoceros calcitrans	Chaet B	Diatom	Larvae
Chaetoceros muelleri	Chgra	Diatom	Larvae
Pavlova lutheri	Mono	Flagellate	Larvae
Thalassiosira pseudonana	T. pseudo	Diatom	Larvae
Tisochrysis lutea	T. iso	Flagellate	Larvae
Rhodomonas lens	Rhodo	Flagellate	Veliger
Tetraselmis chuii	Chuii	Flagellate	Veliger
Tetraselmis maculata	MC:2	Flagellate	Veliger
Thalassiosira weissflogii	Weiss	Diatom	Veliger

Table II.1: Summary of microalgae species cultured at Downeast Institute and analyzed for fatty acid and calorie content, including full species names, shorthand names used elsewhere in this report, classification (flagellate/diatom), and earliest bivalve life stage each is consumable by (larvae/veliger).

Microalgae Culture: For each of the microalgae species listed in Table II.1, three independent cultures were set up in a temperature-controlled environmental chamber and harvested after 1 week.

Lipid Analysis: Cell density was quantified in each replicate microalgae culture before 10 ml was filtered through a pre-combusted disk, placed in a vial of preservative, and stored in a -20°C freezer. Samples were transported to the Bigelow Laboratory for Ocean Sciences for lipid analysis. The fatty acid profile and total lipid content in each sample was determined by extracting weighed samples and derivatizing to FAMES (fatty acid methyl esters) for analysis by gas chromatography-mass spectrometry (Shimadzu QP2010 Ultra GC/MS) using the approach of Breuer et al. 2013. Measurements were validated using a Supelco FAME37 mix standard.

Caloric Analysis: Along with the filtered samples for lipid analysis, 1 L samples of each of the same microalgae cultures were stored in screw top bottles, frozen, and transported to Bigelow for caloric analysis. 10 ml of each sample was centrifuged to form a wet pellet, weighed, lyophilized, and weighed again to quantify pellet dry weight. The nitrogen content in each sample was measured using an elemental analyzer to determine protein content, which was converted to calories using the Atwater calculation. The carbon content in each sample was also measured using an elemental analyzer and used to determine carbohydrate content, which was converted to calories using the same calculation. The caloric content of the protein, carbohydrate, and lipid components was summed to calculate total caloric content in each sample.

Dry Weight Determination: For each replicate microalgae culture, two 25mm Whatman GF/C glass microfiber filters were rinsed with 10 ml of ultrapure DI water using a vacuum pump filtration assembly. Each filter was then placed in an aluminum weigh boat and combusted at 450°C in a muffle furnace for 2 hours. Once cool, filters were weighed using a microbalance. For each replicate microalgae culture, duplicate 10 ml samples were passed through filters using the

vacuum pump filtration assembly, followed by 20 ml of 0.5 M ammonium bicarbonate solution. Filtered samples were then dried in a drying oven at 100°C for 4 hours. Once cool, filters were weighed again and sample dry weight was calculated by subtracting the initial weight from the final weight.

II.i Caloric Content

The results of the caloric content analysis are presented below. Caloric content was determined in part to test a hypothesis that the energy content of food, which limits the energy gained per unit feeding effort, will be a more important factor than food availability for herbivorous marine calcifiers growing in the future ocean. Further, the caloric content was analyzed by component in part to aid in selecting a high-protein diet treatment, as protein is a vital macronutrient for growth. A summary of total caloric content, as well as protein, carbohydrate, and lipid caloric content by cell of each microalgae species is listed in Table II.2 and visualized in Figs. II.2-II.5 below.

Species	Dry Weight (pg/cell)	Total (ncal/cell)	Protein (ncal/cell)	Carb. (ncal/cell)	Lipid (ncal/cell)
Mono	22.35 (3.84)	141.17 (26.2)	37.07 (8.39)	86.80 (18.83)	17.30 (0.78)
Chgra	25.37 (6.39)	85.70 (16.87)	15.10 (3.41)	46.43 (10.58)	24.17 (5.58)
T. iso	25.58 (4.80)	106.08 (12.33)	21.09 (2.65)	68.42 (10.33)	16.56 (4.89)
Chaet B	29.85 (6.77)	151.01 (21.56)	23.22 (1.91)	30.61 (2.37)	97.19 (20.78)
T. pseudo	48.57 (6.53)	160.21 (28.54)	21.55 (5.18)	17.85 (9.60)	120.81 (15.84)
Rhodo	108.02 (13.08)	609.10 (14.19)	182.04 (10.33)	255.08 (11.53)	171.98 (23.35)
Weiss	208.27 (24.40)	1198.08 (127.77)	219.80 (7.85)	352.35 (153.24)	625.92 (17.62)
Chuii	487.23 (47.22)	1966.37 (93.23)	549.96 (77.52)	1229.21 (250.80)	187.20 (80.06)
MC:2	556.75 (40.73)	3199.81 (4.12)	851.87 (3.24)	2206.96 (8.06)	140.98 (15.41)

Table II.2: Summary of microalgae dry weight and caloric content per cell, including total caloric content and protein, carbohydrate, and lipid components. Species shorthand names are listed in Table II.1. Rows are presented in order of increasing dry weight from top to bottom. Values in parentheses represent standard deviations of replicate cultures (n = 3).

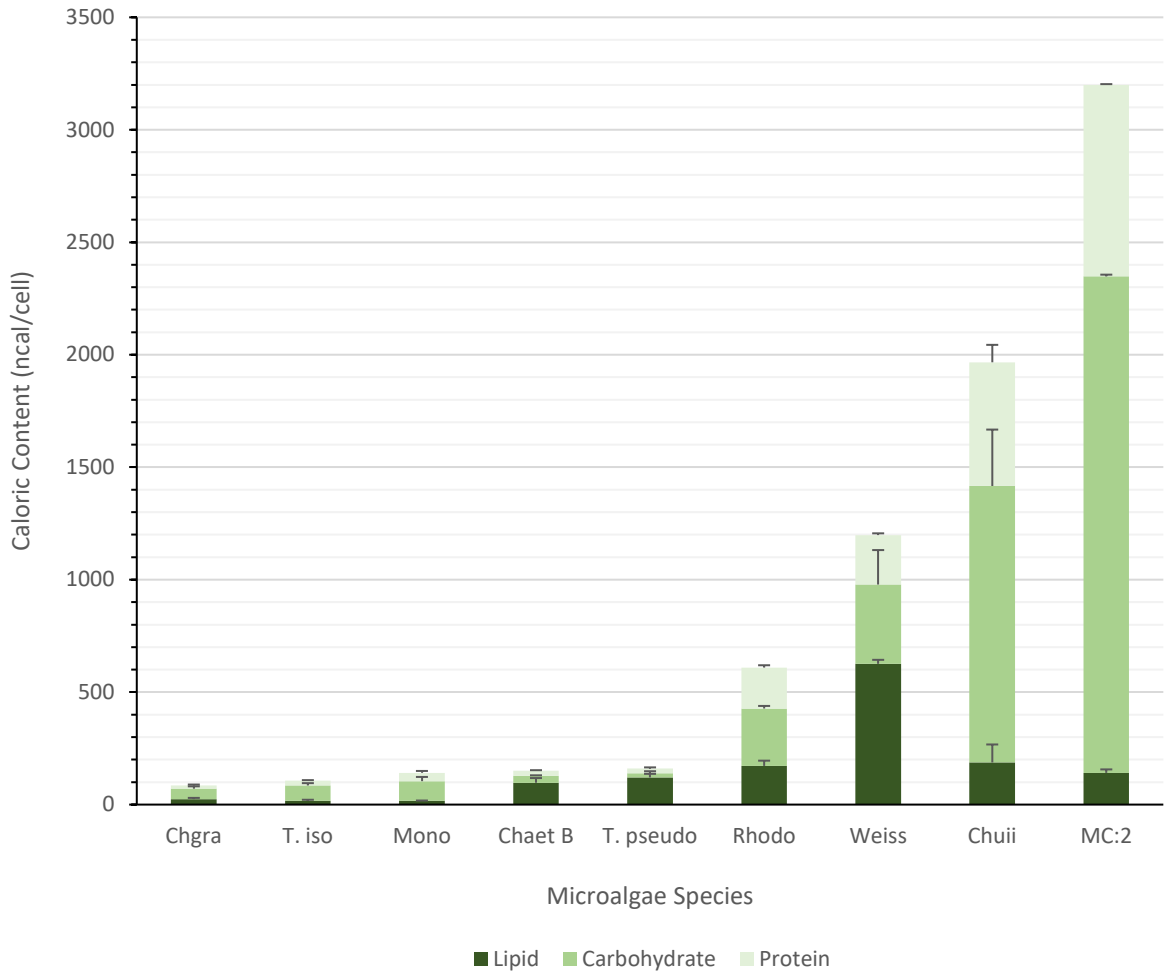

Figure II.2: Total caloric content (nanocalories) per cell by microalgae species, subdivided by lipid, carbohydrate, and protein components (legend). Species shorthand names are listed in Table II.1. Columns are presented in order of increasing total caloric content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

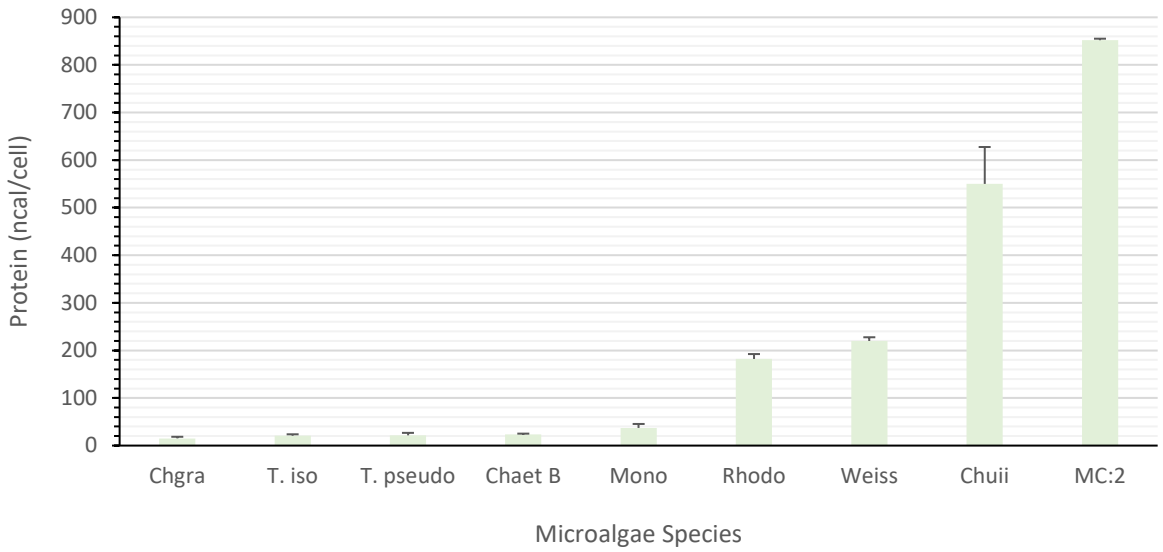

Figure II.3: Protein content (nanocalories) per cell by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing protein content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

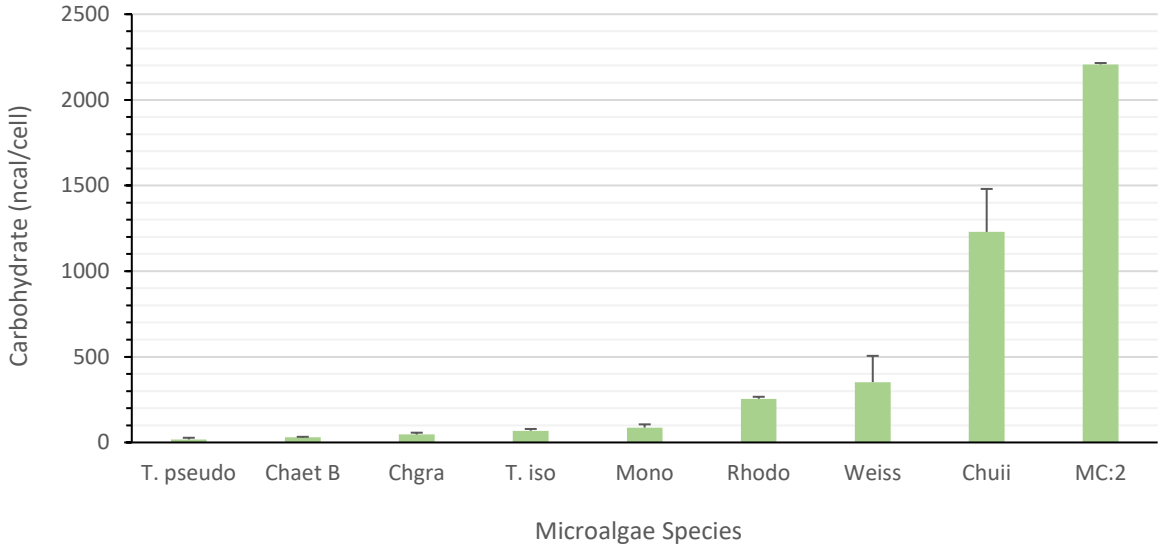

Figure II.4: Carbohydrate content (nanocalories) per cell by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing carbohydrate content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

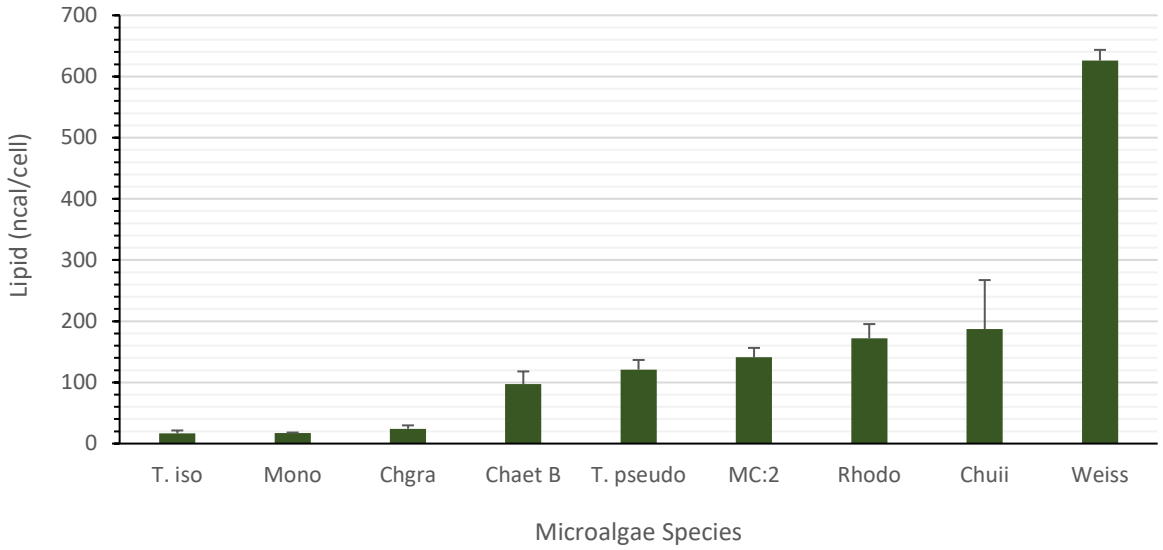

Figure II.5: Lipid content per cell (nanocalories) by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing lipid content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

II.ii Fatty Acid Content by Cell

Lipid/fatty acid content of microalgae is a limiting factor for shellfish growth and condition. In particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both omega-3 fatty acids, are identified as vital for bivalve nutrition due to a limited ability to be synthesized from less highly unsaturated precursors (FAO 2004). However, EPA and DHA do not always co-occur in optimal quantities within the same microalgae species, underscoring the importance of providing a balanced diet. Fatty acid content was analyzed in order to aid in selecting high-EPA and high-DHA diet treatments. A summary of total fatty acid content (TFAME), as well as EPA and DHA content for each microalgae species by cell, is listed in Table II.3 and visualized in Figs. II.6-II.8 below. Fatty acid content is here standardized to cell in order to be most useful to shellfish hatchery managers, since microalgae cell density is the most commonly-monitored estimate of biomass in shellfish feeds.

Species	Dry Weight (pg/cell)	TFAME (fg/cell)	EPA (fg/cell)	DHA (fg/cell)
Mono	22.35 (3.84)	1.92 (0.09)	585.46 (30.36)	296.8 (11.52)
Chgra	25.37 (6.39)	2.69 (0.62)	587.96 (127.51)	72.2 (53.93)
T. iso	25.58 (4.80)	1.84 (0.54)	69.16 (19.02)	293.81 (92)
Chaet B	29.85 (6.77)	10.8 (2.31)	1586.96 (204.45)	75.53 (9.7)
T. pseudo	48.57 (6.53)	13.42 (1.76)	1289.82 (88.23)	85.82 (4.46)
Rhodo	108.02 (13.08)	19.11 (2.59)	3247.78 (505.36)	1442.67 (183.36)
Weiss	208.27 (24.40)	69.55 (1.96)	7580.46 (2441.88)	907.09 (387.08)
Chuii	487.23 (47.22)	20.8 (8.9)	1625.55 (561.4)	0
MC:2	556.75 (40.73)	15.66 (1.71)	2975.25 (314.47)	0

Table II.3: Summary of microalgae dry weight and fatty acid content per cell, including total fatty acid methyl ester (TFAME), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) content (femtograms). Species shorthand names are listed in Table II.1. Rows are presented in order of increasing dry weight from top to bottom. Zero values were below detection limits. Values in parentheses represent standard deviations of replicate cultures (n = 3).

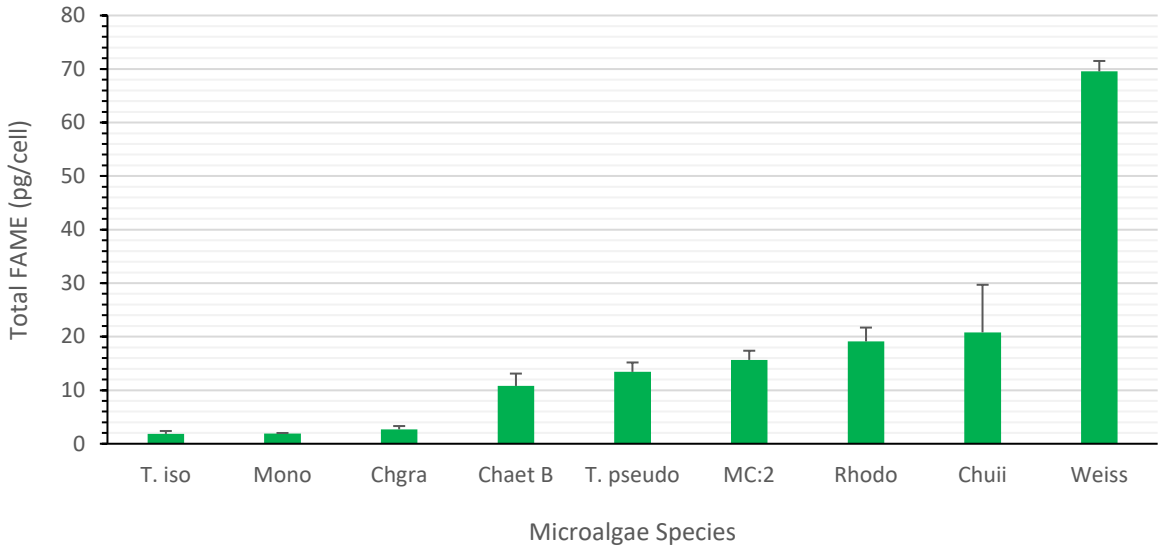

Figure II.6: Total fatty acid methyl ester (TFAME) content (picograms) per cell by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing TFAME content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

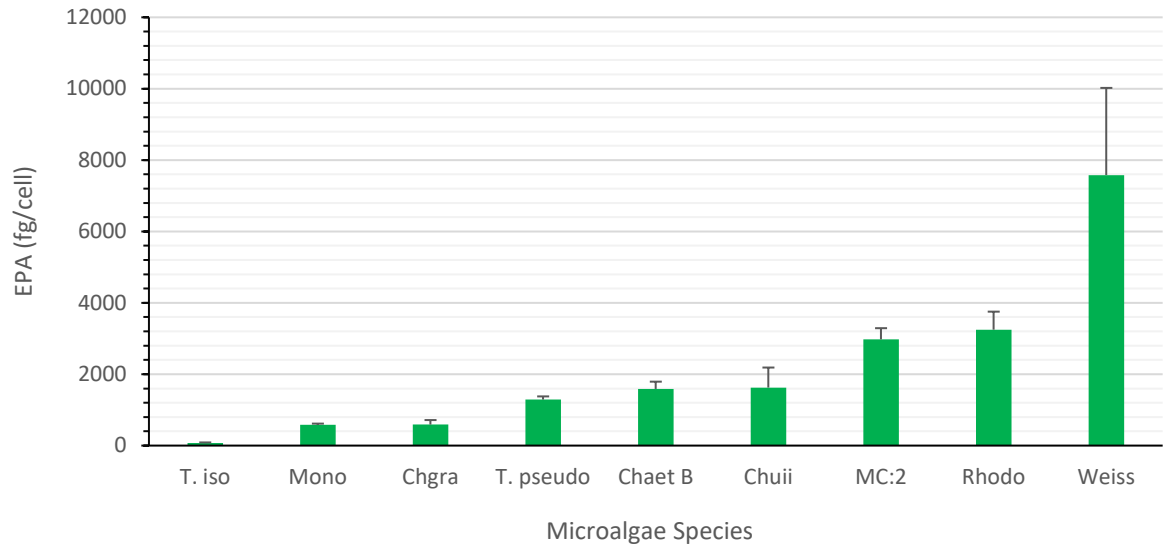

Figure II.7: Eicosapentaenoic acid (EPA) content (femtograms) per cell by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing EPA content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

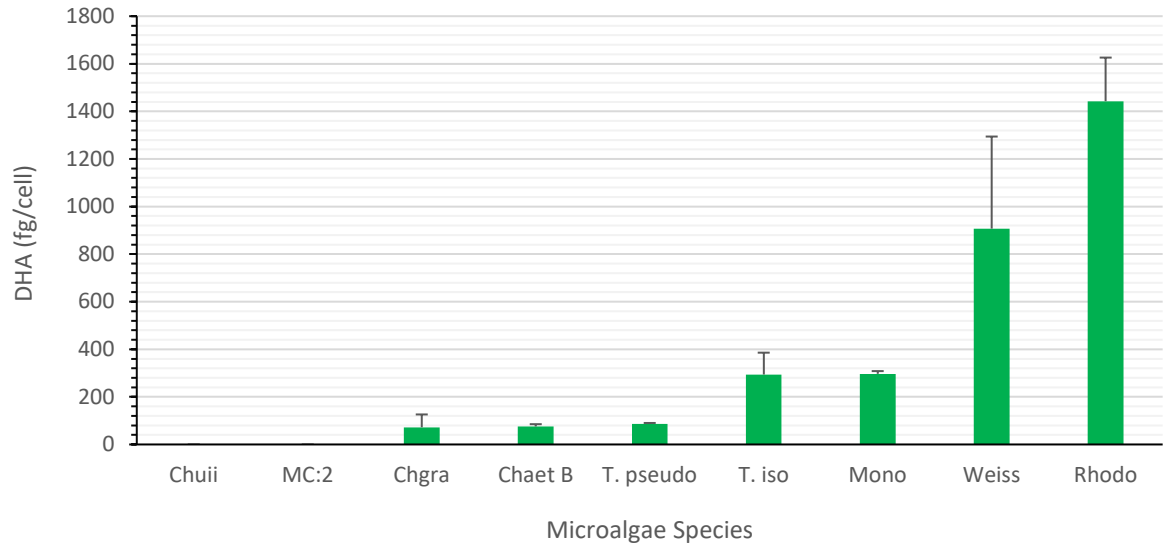

Figure II.8: Docosahexaenoic acid (DHA) content (femtograms) per cell by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing DHA content from left to right. Zero values were below detection limits. Error bars represent standard deviations of replicate cultures (n = 3).

II.iii Fatty Acid Content by Calorie

Although microalgae cell density is more easily quantified in the day-to-day operations of a shellfish hatchery, the lab experiments comprising this project used isocaloric study designs (i.e., feeds were standardized to calorie instead of cell count) in order to eliminate the confounding influence of cell size on nutritional content. A summary of total fatty acid content (TFAME), as well as EPA and DHA content for each microalgae species by calorie, is listed in Table II.4 and visualized in Figs. II.9-II.11 below.

Species	Calories (ncal/cell)	TFAME (µg/cal)	EPA (µg/cal)	DHA (µg/cal)
Chgra	85.70 (16.87)	31.43 (4.25)	6.93 (1.17)	0.96 (0.92)
T. iso	106.08 (12.33)	17.43 (4.88)	0.65 (0.16)	2.79 (0.84)
Mono	141.17 (26.2)	13.94 (2.71)	4.25 (0.88)	2.15 (0.43)
Chaet B	151.01 (21.56)	70.98 (5.69)	10.54 (0.73)	0.5 (0.05)
T. pseudo	160.21 (28.54)	84.36 (5.79)	8.2 (1.41)	0.55 (0.12)
Rhodo	609.10 (14.19)	31.35 (3.93)	5.33 (0.79)	2.37 (0.26)
Weiss	1198.08 (127.77)	58.47 (7.87)	6.47 (2.73)	0.74 (0.24)
Chuii	1966.37 (93.23)	10.7 (5.03)	0.83 (0.33)	0
MC:2	3199.81 (4.12)	4.9 (0.53)	0.93 (0.1)	0

Table II.4: Summary of microalgae caloric content per cell and fatty acid content per calorie, including total fatty acid methyl ester (TFAME), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) content (micrograms). Species shorthand names are listed in Table II.1. Rows are presented in order of increasing calories from top to bottom. Zero values were below detection limits. Values in parentheses represent standard deviations of replicate cultures (n = 3).

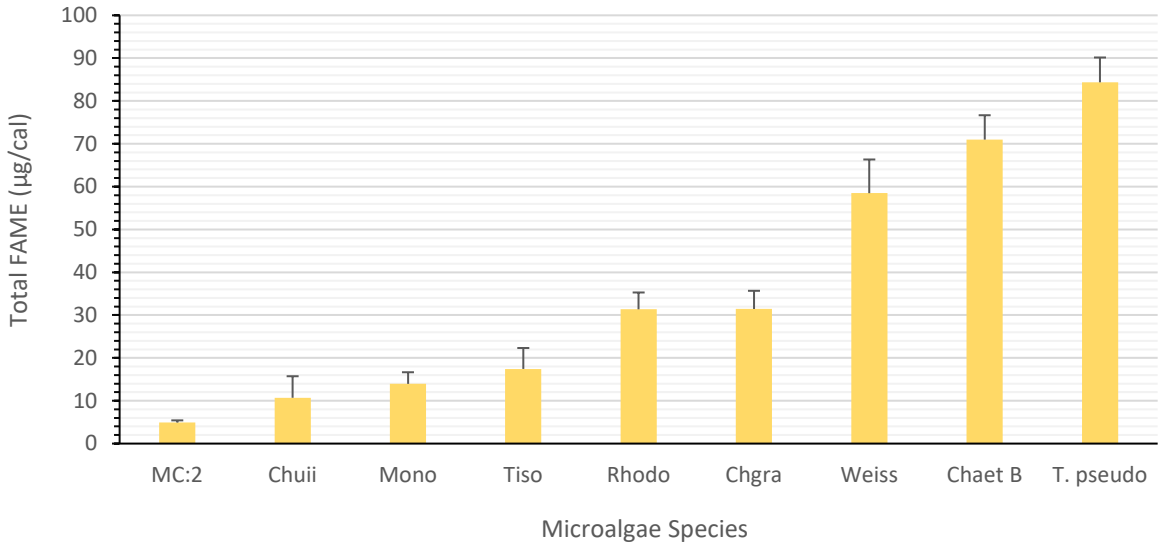

Figure II.9: Total fatty acid methyl ester (TFAME) content (micrograms) per calorie by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing TFAME content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

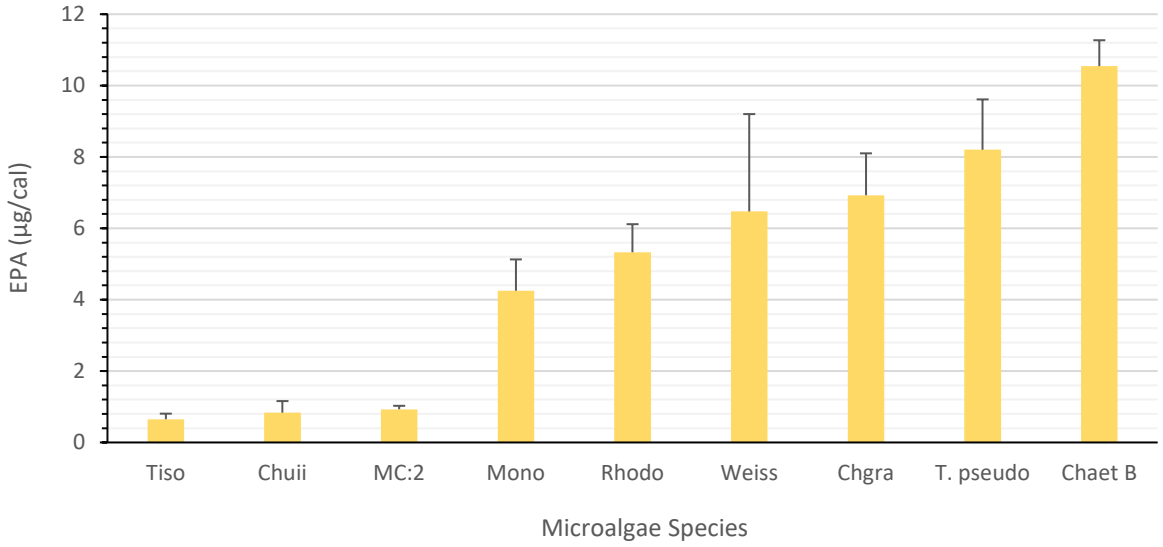

Figure II.10: Eicosapentaenoic acid (EPA) content (micrograms) per calorie by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing EPA content from left to right. Error bars represent standard deviations of replicate cultures (n = 3).

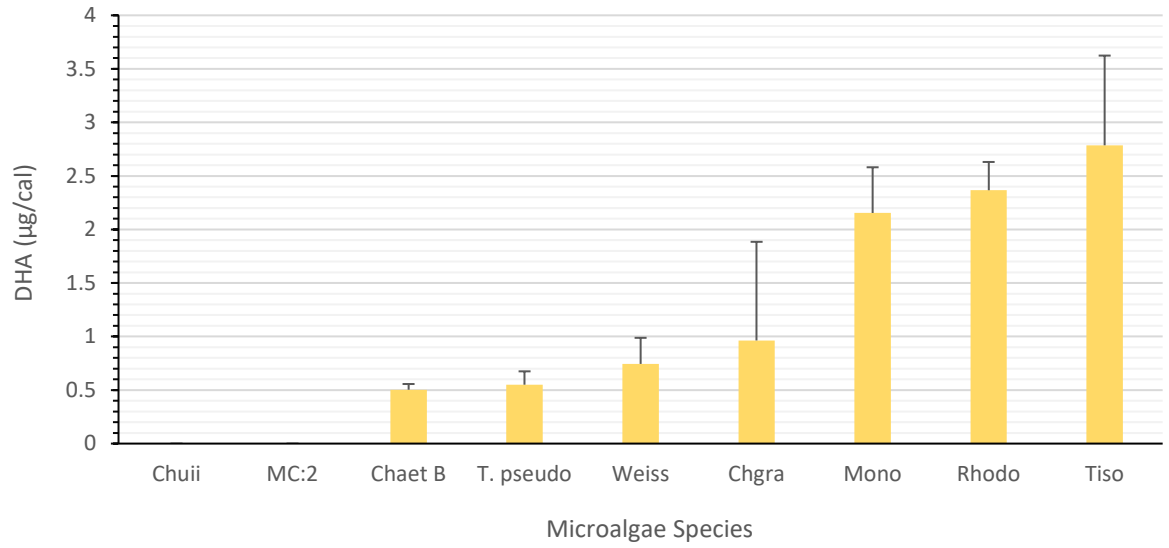

Figure II.11: Docosahexaenoic acid (DHA) content (micrograms) per calorie by microalgae species. Species shorthand names are listed in Table II.1. Columns are presented in order of increasing EPA content from left to right. Zero values were below detection limits. Error bars represent standard deviations of replicate cultures (n = 3).

II.iv Conclusion

In summary, and as expected, the microalgae species with the largest cell sizes (Rhodo, Weiss, Chuii, MC:2) also exhibited the highest total calorie content per cell, while those with smaller cell sizes (Chgra, T. iso, Mono, Chaet B, T. pseudo) exhibited lower total calorie content (though not necessarily in order of cell size). The species with the largest cell size, MC:2, also exhibited the highest total calorie content, while the species with the second smallest cell size, Chgra, exhibited the lowest total calorie content. Among the larger species, MC:2 exhibited the highest protein and carbohydrate content per cell, while Rhodo had the least, and Weiss exhibited the highest lipid content, while MC:2 had the least. Among the smaller species, Mono exhibited the highest protein and carbohydrate content per cell, while Chgra had the lowest protein and T. pseudo had the lowest carbohydrate, while T. pseudo exhibited the highest lipid content, and T. iso had the lowest.

In terms of fatty acid content, the microalgae species with the largest cell sizes also exhibited the highest EPA content, with Weiss exhibiting the most and Chuii the least. Among the smaller species, Chaet B exhibited the highest EPA content and T. iso had the least. Although Rhodo and Weiss also exhibited the highest DHA content per cell, the other large species (Chuii and MC:2) exhibited none within detection limits. Among the smaller species, Mono exhibited the highest DHA while Chgra had the least. When standardized to calorie instead of cell, the confounding influence of cell size was removed. T. pseudo exhibited the highest TFAME per calorie, followed by Chaet B and Weiss, while MC:2 had the least, followed by Chuii and Mono. Chaet B exhibited

the highest EPA per calorie, followed by T. pseudo and Chgra, while T. iso had the least, followed by Chuii and MC:2. Finally, T. iso exhibited the highest DHA per calorie, followed by Rhodo and Mono, while Chuii and MC:2 had none, and Chaet B otherwise had the least, followed by T. pseudo and Weiss.

In conclusion, the microalgae species cultured at Downeast Institute exhibited varied caloric and lipid/fatty acid content. While it is not always known how different bivalve species will respond to different feeds, providing them with a diverse and balanced diet will help to ensure that their particular nutritional needs are met. This analysis supports the idea of swapping smaller-celled microalgae species with larger-celled alternatives once shellfish are large enough to consume them, a common practice in hatcheries, as shellfish will receive more nutritional "bang for their buck" per unit feeding effort. The data presented here can be used as a reference by shellfish hatcheries to help optimize caloric content and fatty acid ratios in their feeds, or to help choose new microalgae species where they are not already cultured.

Figure III.1: Various solutions prepared for testing in the seawater buffering trial.

III. Seawater Buffers

Buffering additives are increasingly used by shellfish hatcheries to raise the pH and saturation state of aragonite (Ω_{Ar}) – likely the most important carbonate chemistry parameters for bivalve survival and condition – in the seawater used to fill culture tanks. While several alternative buffers can be used to achieve the same end, they differ in terms of cost, instantaneity of feedback, and stability of the effect over time. In order to determine which buffer might be most practical for use in shellfish aquaculture in general, as well as aid in selecting a buffer to be used in two out of the three experiments for this project, a simple preliminary experiment was conducted to compare the effectiveness of different additives.

Cost: Buffering additives are available from a number of different vendors in a variety of grades and form factors, including bulk pails marketed for commercial aquaculture, swimming pool maintenance, or other domestic uses, and smaller containers marketed for aquarium hobbyists. The cost of additives varies greatly, with bulk and non-aquarium grade options tending to be cheaper.

Instantaneity of Feedback: In shellfish hatcheries, buffer solutions are often added to seawater reservoirs or header tanks automatically using a peristaltic dosing pump interfaced with an aquarium controller and pH probe, which monitors the pH of the seawater continuously and adds buffer until the desired setpoint is reached. In a system that operates in this sort of feedback loop,

and especially in a flow-through system in which seawater is exchanged continuously, it is important to achieve snappy feedback for everything to run smoothly without overshooting the setpoint. While some additives mix into seawater and increase its pH near-instantaneously, others are slower to have an effect, and sodium bicarbonate (baking soda) will initially decrease the pH until equilibration of the carbonate system is achieved.

Stability Over Time: All else being equal, the longevity of the buffering effect in seawater may be different depending on which additive is used. If buffers are added to seawater manually, shorter-lived buffers will need to be replenished more frequently. This is worth considering in a scenario in which shellfish larvae are reared in static culture tanks and seawater is exchanged once every 48 hours, since buffers cannot be added directly to cultures without harming larvae. It is less of a concern in a different type of scenario in which buffer addition is automated with a dosing pump, as the system will correct any pH drops as needed.

Mixing Sodium Carbonate and Sodium Bicarbonate: It is common practice in aquaculture and the aquarium hobby to prepare a buffer solution consisting of both sodium carbonate (soda ash) and sodium bicarbonate (baking soda), typically in a ratio of 9:1 parts sodium carbonate to sodium bicarbonate. This stems from the notion that sodium carbonate is more effective at boosting seawater pH, while sodium bicarbonate is more effective at boosting seawater alkalinity, which buffers future pH drops. It is commonly believed that sodium carbonate will not sufficiently boost alkalinity on its own, making it necessary to supplement it with sodium bicarbonate.

Experiment: 500 ml each of eight buffer solutions were prepared according to the manufacturers' suggested concentrations: sodium carbonate (soda ash), sodium bicarbonate (baking soda), sodium hydroxide (lye), calcium hydroxide (lime), and four mixtures of sodium carbonate to sodium bicarbonate (1:9, 1:1, 9:1, 7:3). The mixtures of sodium carbonate and sodium bicarbonate were mixed to the same total concentration as each of those components in isolation. Nine 10 L experimental aquaria were filled with natural, filtered seawater sourced from just outside Downeast Institute. The initial pH and total alkalinity (TA) of the ambient (unbuffered) seawater was measured: pH was measured on the total hydrogen ion scale using an electrode calibrated to TRIS and 2-aminopyridine synthetic seawater buffers, and TA was measured using a spectrophotometric titration method (Liu et al. 2015) and verified using certified reference material from the Dickson Lab. Each buffer solution was used to buffer the seawater in one experimental aquarium to a pH of 8.25 at 25°C (the temperature in each tank was held constant using a custom heat jacket wrapped around the outside of the tank), while one additional tank was left unbuffered. Each bottle of buffer was weighed prior to dosing, buffer solutions were added manually with a pipette, seawater pH was monitored using the electrode, and bottles were weighed again after the desired pH was achieved to calculate the quantity of solution used. 50 ml of seawater from each tank was then sampled to measure initial (post-buffering) TA. The saturation state of aragonite in each tank was calculated by inputting pH and TA into CO2sys, with output conditions set to 16°C. Finally, the tanks were left undisturbed overnight before pH was measured again the following morning.

Buffer Solution	Molarity	Initial pH_T	TA (µmol/kgSW)	Ω_{Ar}	Final pH_T
Ambient Seawater		7.88	2,152.29		1.74
Sodium Carbonate/Soda Ash	1.19	8.26	2,660.61	5.40	8.22
Sodium Bicarbonate/Baking Soda	1.19				
Sodium Hydroxide/Lye	1.87	8.33	2,377.11	5.37	8.34
Calcium Hydroxide/Lime	0.01	8.25	2,304.97	4.48	8.18
1:9 Sodium Carbonate : Sodium Bicarbonate	1.19				
1:1 Sodium Carbonate : Sodium Bicarbonate	1.19	8.24	3,535.73	7.19	8.15
9:1 Sodium Carbonate : Sodium Bicarbonate	1.19	8.25	3,108.66	6.21	8.22
7:3 Sodium Carbonate : Sodium Bicarbonate	1.19	8.26	3,262.34	6.64	8.19

Table III.1: Results of the seawater buffer experiment listing eight buffer solution preparations, their concentration, the initial (post-buffer) seawater pH, alkalinity, and saturation state of aragonite, and the final seawater pH after equilibrating overnight. The first row of the table provides the ambient, untreated seawater pH, alkalinity, and saturation state for comparison.

Results: The results of the experiment are given in Table III.1 above. The ambient (unbuffered) seawater started with a pH of 7.88, an alkalinity of 2,152.29 µmol/kgSW, and a saturation state of 1.74. After being buffered to 8.26 with the sodium carbonate (soda ash) buffer, the alkalinity rose approximately 500 units, and the saturation state rose by nearly 4 units. The seawater pH changed near-instantaneously as buffer was added, and it decreased by only 0.04 units overnight. Upon adding the sodium bicarbonate (baking soda) buffer, seawater pH decreased to 7.85, so this buffer was discontinued. After equilibrating overnight, the pH rose above ambient to 8.03. Adding the sodium hydroxide (lye) buffer raised seawater pH to 8.33 (overshooting the target a bit), the alkalinity by just over 200 units, and the saturation state to near the level of the sodium carbonate buffer. Seawater pH changed near-instantaneously as buffer was added, and it remained stable overnight, increasing by 0.01 unit. After being buffered to 8.25 with the calcium hydroxide (lime) buffer, the alkalinity rose by about 150 units, and the saturation state rose by nearly 3 units. The pH decreased by 0.07 units overnight. The 1:9 sodium carbonate : sodium bicarbonate buffer was not practical, with the seawater pH rising to only 8.19 after adding the entire bottle of buffer, before decreasing to 7.57 overnight with a portion of the buffer precipitating out of solution. The resulting alkalinity was too high to measure using conventional methodology. The other sodium carbonate : sodium bicarbonate mixtures resulted in the highest seawater alkalinity and saturation states, with the 9:1 sodium carbonate : sodium bicarbonate buffer increasing the alkalinity by nearly 1,000 units and the saturation state by 4.5 units, the 1:1 sodium carbonate : sodium bicarbonate buffer increasing the alkalinity by nearly 1,400 units and the saturation state by 5.5 units, and the 7:3 sodium carbonate : sodium bicarbonate buffer falling in between. The higher the proportion of sodium bicarbonate, the less stable the pH overnight. A summary of the cost of each buffering additive is given in Table III.2 below.

Buffer Solution	Brand	$ / g Solute	$ / 500 ml Soln	$ / Soln Used
Sodium Carbonate/Soda Ash	Bulk Reef Supply	0.013	0.818	0.004
Sodium Bicarbonate/Baking Soda	Bulk Reef Supply	0.013	0.648	
Sodium Hydroxide/Lye	Red Crown	0.005	0.187	0.001
Calcium Hydroxide/Lime	Bulk Reef Supply	0.016	0.006	0.002

Table III.2: Cost summary of each buffering additive used in the experiment, including the brand of additive purchased, the cost per gram, the cost per 500 ml buffer solution, and the cost per volume of solution used to buffer seawater from pH 7.88 to ~8.25. All costs are given in USD.

Conclusion: In summary, the buffer solutions that resulted in the highest seawater alkalinity and saturation state of aragonite at an equivalent pH were the various mixtures of sodium carbonate (soda ash) and sodium bicarbonate (baking soda), with higher values resulting from higher proportions of sodium bicarbonate. Among the buffer solutions composed of a single additive, sodium carbonate produced the highest alkalinity and saturation state, followed by sodium hydroxide (lye) and calcium hydroxide (lime). Sodium bicarbonate in isolation, as well as the 1:9 sodium carbonate : sodium bicarbonate mixture, failed to achieve the target pH and were not considered further. In terms of instantaneity of feedback, the sodium carbonate and sodium hydroxide buffers provided near-instantaneous results, while the sodium carbonate : sodium bicarbonate mixtures were slower, and the mixtures with the higher proportions of sodium bicarbonate were slowest. The calcium hydroxide buffer was also slower than the sodium carbonate and sodium hydroxide buffers, likely because its concentration was lower; however, higher concentrations result in the solute precipitating out of solution. In terms of pH stability overnight, the sodium hydroxide buffer performed best, followed by the sodium carbonate buffer. The calcium hydroxide buffer and sodium carbonate : sodium bicarbonate mixtures performed relatively poorly. In terms of cost of solution used, the sodium hydroxide was the cheapest additive, followed by calcium hydroxide and sodium carbonate. The sodium carbonate : sodium bicarbonate mixtures become cheaper as more sodium bicarbonate is added, because although these additives cost the same per gram from Bulk Reef Supply, sodium bicarbonate has a lower molecular weight making it cheaper per mole. However, the cost varies greatly depending on the vendor and volume purchased. In conclusion, while the sodium carbonate : sodium bicarbonate mixtures produced the highest seawater alkalinity and saturation state of aragonite, these values probably far exceed those of anywhere in the natural ocean, so the necessity of adding sodium bicarbonate at all is called into question. For most aquaculture applications, the more-than-adequate buffering effectiveness of sodium carbonate and sodium hydroxide is probably sufficient, and the superior feedback and stability of these additives – not to mention the relative simplicity of mixing them into solution – makes them more favorable. Likewise, sodium carbonate is recommended in most situations, while sodium hydroxide is recommended as a cheaper (though slightly less effective) option, and the 9:1 sodium carbonate : sodium bicarbonate mixture is recommended when extreme high alkalinity and saturation state are desired.

Figure IV.1: The experimental aquaria and controllers in the OA Lab at Downeast Institute.

IV. Blue Mussel Experiments

Shellfish hatcheries typically produce a variety of live microalgae species to use as feeds, and an increasing number of hatcheries have adopted seawater buffering as a means of addressing suboptimal natural water quality, especially as ocean pH and saturation state of aragonite have declined due to ocean acidification. However, few hatcheries have the ability or opportunity to conduct formal scientific experiments testing different combinations of microalgae diets and seawater buffering levels to enhance standard operating procedures and improve seed condition and yield. This section describes the methodology and preliminary results from a series of experiments conducted at Downeast Institute that tested various microalgae diets with and without seawater buffering, and at both ambient and acidified seawater pH levels, with the aim of identifying optimal conditions for making hatchery-reared blue mussels (*Mytilus edulis*) more resilient to ocean acidification stress in the future.

Ocean Acidification Experimental System: The OA Lab at Downeast Institute contains a pH-stat CO_2-dosing apparatus built around the Neptune Apex aquarium controller platform, consisting of 30 11-L conical experimental aquaria configurable to any combination of seawater pH and temperature treatments (each precise up to 0.01 units). Seawater pH control operates in a feedback loop: temperature-compensated pH is monitored in real time in each aquarium using pH and temperature probes; the aquarium controller activates a solenoid valve when pH rises above the programmed setpoint, dosing CO_2 gas through a diffuser (reducing pH); the aquarium controller deactivates the solenoid valve when the setpoint is reached (stabilizing pH). This ensures that any deviations from setpoints (e.g. from passive CO_2 outgassing, microalgae photosynthesis, experimental animal respiration) are quickly corrected, resulting in stable experimental treatment conditions. Seawater temperature is adjusted using custom-designed heat jackets that wrap around each aquarium.

Treatment	pH	Diet	Shorthand
1	7.30	*T. iso* + *T. pseudo*	7.30, IP
2	7.80	*T. iso* + *T. pseudo*	7.80, IP
3	7.30	*T. iso* + *Chgra*	7.30, IC
4	7.80	*T. iso* + *Chgra*	7.80, IC
5	7.30	*Mono* + *Chgra*	7.30, MC
6	7.80	*Mono* + *Chgra*	7.80, MC
7	7.30	*T. iso* + *T. pseudo/Rhodo* + *Weiss*	7.30, RW
8	7.80	*T. iso* + *T. pseudo/Rhodo* + *Weiss*	7.80, RW

Table IV.1: Summary of treatments used in Experiment #1, including seawater pH, microalgae diet, and the shorthand denoting each treatment in the figures. Full names for microalgae species are given in Table II.1.

Experiment #1 Lab Methods: Experiment #1 tested blue mussel survival and growth in response to four microalgae diet treatments and two seawater pH treatments in a full factorial design (Table IV.1). The diet treatments were selected to optimize different nutritional parameters according to the results of the preliminary microalgae nutrition trial. The diet treatments included: *Tisochrysis lutea* + *Thalassiosira pseudonana* ("industry standard"), *Tisochrysis lutea* + *Thalassiosira pseudonana* fed to mussel larvae and *Rhodomonas Lens* + *Thalassiosira weissflogii* fed to mussel veligers (high calorie), *Tisochrysis lutea* + *Chaetoceros muelleri* (high DHA), and *Pavlova lutheri* + *Chaetoceros muelleri* (high EPA, protein). The seawater pH treatments included pH 7.80, chosen to represent present-day coastal Maine, and pH 7.30, chosen to represent a year 2100 business-as-usual carbon emissions scenario. Blue mussels were spawned in the hatchery and stocked in 24 experimental aquaria assigned a factorial combination of pH x diet treatment levels (and replicated 3 times). Live microalgae were fed in an isocaloric study design, i.e. feeds were standardized to caloric content rather than cell counts to eliminate bias related to cell size. Tanks were drained, cleaned, and refilled every 48 hrs. Mussels were reared under experimental conditions for two weeks until the majority achieved settlement competency, upon which 5 sections of rope (1/3 meter) were placed in each tank (in the event that there weren't enough swimming larvae in a tank to cover 5 ropes, the number of ropes placed in the tank was scaled to the number of larvae). Mussels were then reared under experimental conditions for a further 3 weeks until they were large enough to move outside.

Treatment	pH	Diet	Buffer	Shorthand
1	7.30	*T. iso* + *T. pseudo*	No	7.30, IP
2	7.30	*T. iso* + *T. pseudo*	Yes	7.30, IP, B
3	7.75	*T. iso* + *T. pseudo*	No	7.75, IP
4	7.75	*T. iso* + *T. pseudo*	Yes	7.75, IP, B
5	7.30	*T. iso* + *T. pseudo/Rhodo* + *Weiss*	No	7.30, RW
6	7.30	*T. iso* + *T. pseudo/Rhodo* + *Weiss*	Yes	7.30, RW, B
7	7.75	*T. iso* + *T. pseudo/Rhodo* + *Weiss*	No	7.75, RW
8	7.75	*T. iso* + *T. pseudo/Rhodo* + *Weiss*	Yes	7.75, RW, B

Table IV.2: Summary of treatments used in Experiment #2, including seawater pH, microalgae diet, seawater buffering, and shorthand denoting each treatment in the figures. Full names for microalgae species are given in Table II.1.

Experiment #2 Lab Methods: Experiment #2 tested blue mussel survival and growth in response to two microalgae diet treatments (*Tisochrysis lutea* + *Thalassiosira pseudonana*, and the same as larvae and *Rhodomonas Lens* + *Thalassiosira weissflogii* as veligers), two seawater pH treatments (pH 7.75, pH 7.30), and two seawater buffering treatments (buffered with a sodium carbonate solution, unbuffered) in a full factorial design (Table IV.2). The seawater used to fill tanks in the buffered treatments was treated in buckets on an individual basis before being added to tanks: pH 7.75 seawater was buffered before being acidified back down to pH 7.75 or pH 7.30. Blue mussels were spawned in the hatchery and stocked in 24 experimental aquaria assigned a factorial combination of pH x diet x buffering treatment levels (and replicated 3 times). Otherwise, the experimental trial was conducted in the same way as in Experiment #1.

Figure IV.2: Closeup of an experimental aquarium during the Experiment #2 lab trial, featuring the ropes placed once mussels achieved settlement competency (pH probe mounted in the center of the rope rack).

Experiment #1 and #2 Field Deployment Methods: Upon completion of each experimental trial, one settlement rope from each tank was preserved in the freezer, while the remaining ropes were divided between each of the mussel farm field sites (Bangs Island Mussels in Portland, Maine, and Blue Hill Bay Mussels in Mount Desert, Maine). At each field site, experimental mussel ropes were attached at intervals to longer, 4m ropes and hung from floating rafts. Experimental mussel ropes were left undisturbed for 8-10 months before being retrieved and processed.

Data Collection and Measurements: Experimental mussel ropes frozen following each experimental trial were thawed in warm water, and mussels were sieved, transferred into petri dishes, and allowed to dry. All mussels from each rope were counted to quantify survival at the end of each trial, while 200 mussels per rope were subsampled to quantify shell surface area. Shell surface area was quantified by photographing mussels at 2x magnification on a dissection microscope and analyzing with ImageJ software. Experimental mussels retrieved from the mussel farm field sites were removed from each rope and counted to quantify post-deployment survival.

Subsamples of 15 mussels from each short rope were removed by pulling with a handheld force gauge to quantify byssal strength. The same 15 mussels from each rope were weighed to quantify whole wet weight, then the tissue was removed and weighed to quantify tissue wet weight. Meat yield was calculated as the ratio of tissue weight to whole weight.

IV.i Results: Experiment #1 Lab Trial

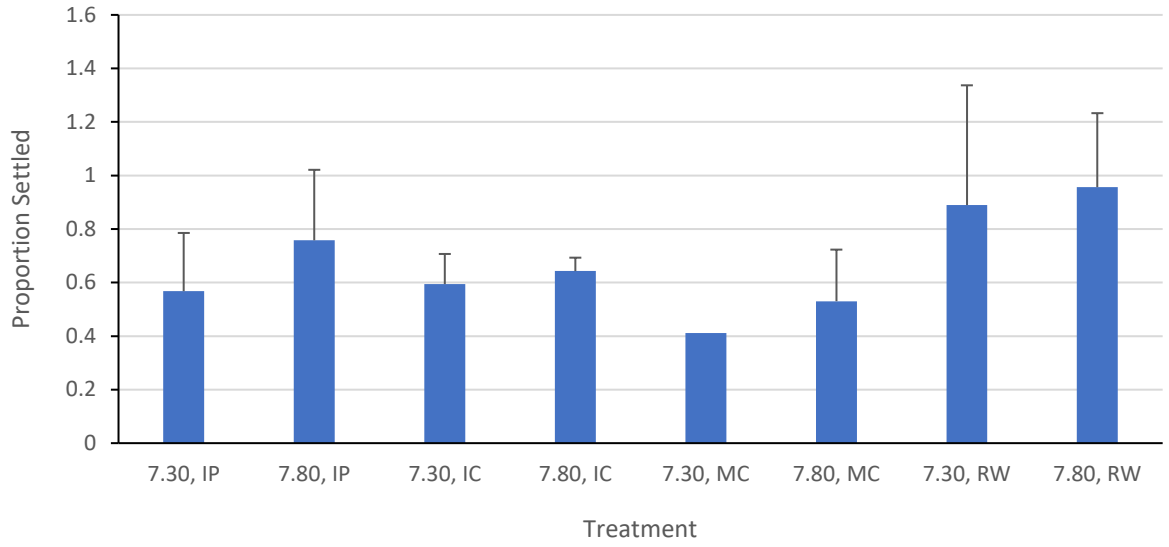

Figure IV.3: Proportion of juvenile mussels that successfully settled on one rope placed in each tank for settlement, quantified as the count of larvae settled on the rope divided by the count of swimming larvae prior to placing ropes divided by the number of ropes placed. Full treatments are listed in Table IV.1. Data is averaged by seawater pH x microalgae diet treatment. Error bars represent standard deviation (n = 3).

Within every microalgae diet treatment, mussels exhibited higher settlement success in the control versus acidified seawater pH treatments (Fig. 1V.3). Mussels fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers exhibited the highest settlement success overall, with even those in the acidified pH treatment outperforming every other pH x diet treatment combination. Mussels fed only *T. iso* + *T. pseudo* exhibited the second-highest settlement success, followed by those fed *T. iso* + *Chgra*, and those fed *Mono* + *Chgra* had the lowest. In a quasibinomial logistic regression testing the effects of pH and diet on the proportion of mussels settled on the preserved rope versus the number of pre-settlement swimmers, those fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers exhibited statistically significantly higher settlement success than those fed *Mono* + *Chgra* ($p = 0.024$). However, the variance within groups was high, and neither pH, diet, nor the interaction of pH and diet had an effect on mussel settlement success in the overall model. In another quasibinomial logistic regression testing the effects of pH and diet on the proportion of

pre-settlement mussel survival versus mortality counts, neither pH, diet, nor the interaction of pH and diet had an effect on pre-settlement survival.

Figure IV.4: Experimental blue mussels preserved immediately following the Experiment #1 lab trial, photographed at 2x magnification. The length of the scale bar represents 2 mm.

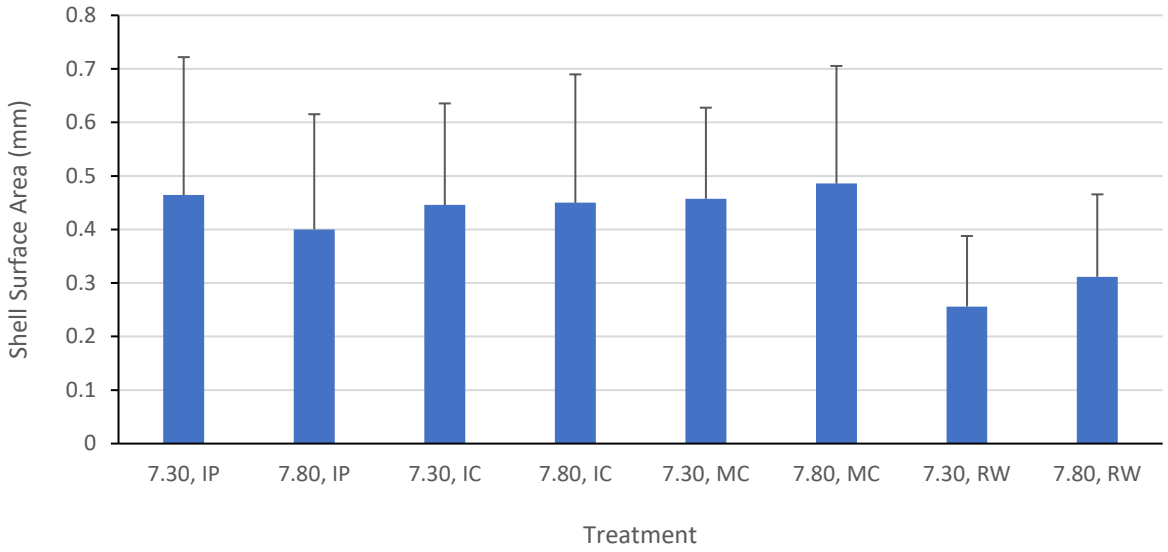

Figure IV.5: Juvenile mussel shell surface area 3 weeks post-settlement averaged by seawater pH x microalgae diet treatment. Full treatments are listed in Table IV.1. Error bars represent standard deviation (n = 3).

Within every microalgae diet treatment except *T. iso* + *T. pseudo*, mussels exhibited higher shell surface area – a measure of growth – in the control versus acidified seawater pH treatments (Fig. IV.5). Mussels fed *Mono* + *Chgra* exhibited the highest shell surface area overall, followed by those fed *T. iso* + *Chgra* and only *T. iso* + *T. pseudo*, while mussels fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers had the lowest. In an analysis of covariance (ANCOVA) testing the effects of seawater pH and microalgae diet on mussel shell surface area while controlling for mussel count in each tank, diet but not pH had an effect on area ($F = 4.247$, $p = 0.027$). In a test of post hoc Tukey multiple comparisons, mussels fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers exhibited statistically significantly lower shell surface area than those fed any of the other diets, while there were no differences between any of the other diet contrasts.

IV.ii Results: Experiment #1 Field Deployment

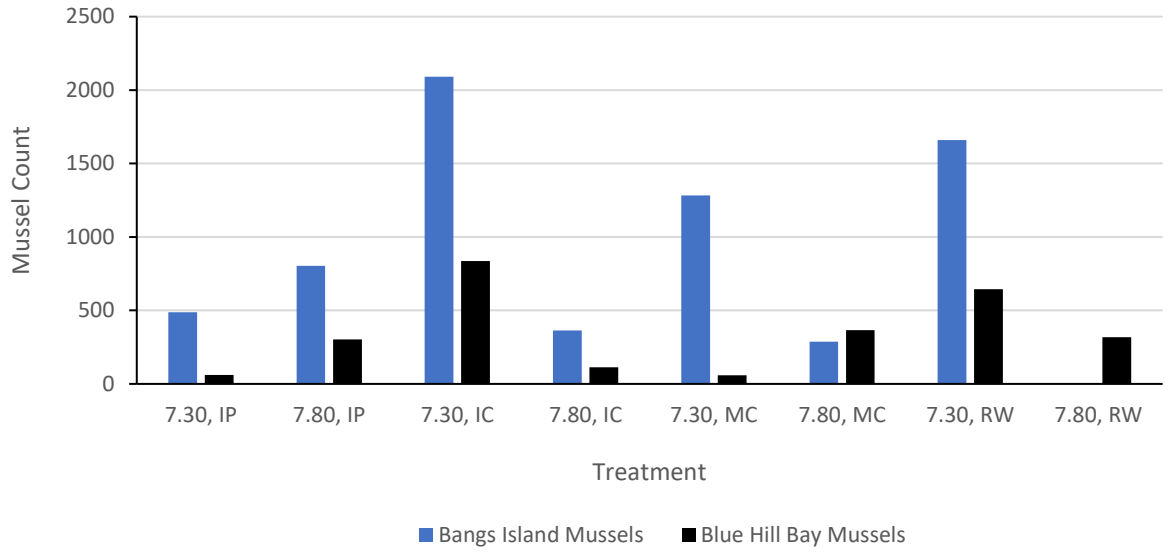

Figure IV.6: Count of adult mussels retrieved from ropes deployed at two mussel farm field sites, grouped by seawater pH x microalgae diet treatments larval/juvenile mussels were exposed to in the lab. Full treatments are listed in Table IV.1.

The count of post-deployment adult mussels retrieved from the mussel farm field sites varied with seawater pH and microalgae (lab) treatments and site (Fig. IV.6). Among mussels retrieved from the Bangs Island Mussels field site, those originating from the pH 7.30, *T. iso* + *Chgra* treatment yielded the greatest numbers, followed by pH 7.30, *T. iso* + *T. pseudo*/*Rhodo* + *Weiss* and pH 7.30, *Mono* + *Chgra*. Those from the pH 7.80, *Mono* + *Chgra* treatment yielded the lowest numbers. Among mussels retrieved from the Blue Hill Bay Mussels field site, those originating from the pH 7.30, *T. iso* + *Chgra* treatment yielded the greatest numbers, followed by pH 7.30, *T. iso* + *T. pseudo*/*Rhodo* + *Weiss* and pH 7.80, *Mono* + *Chgra*. Those from the pH 7.30, *Mono* + *Chgra* treatment yielded the lowest numbers.

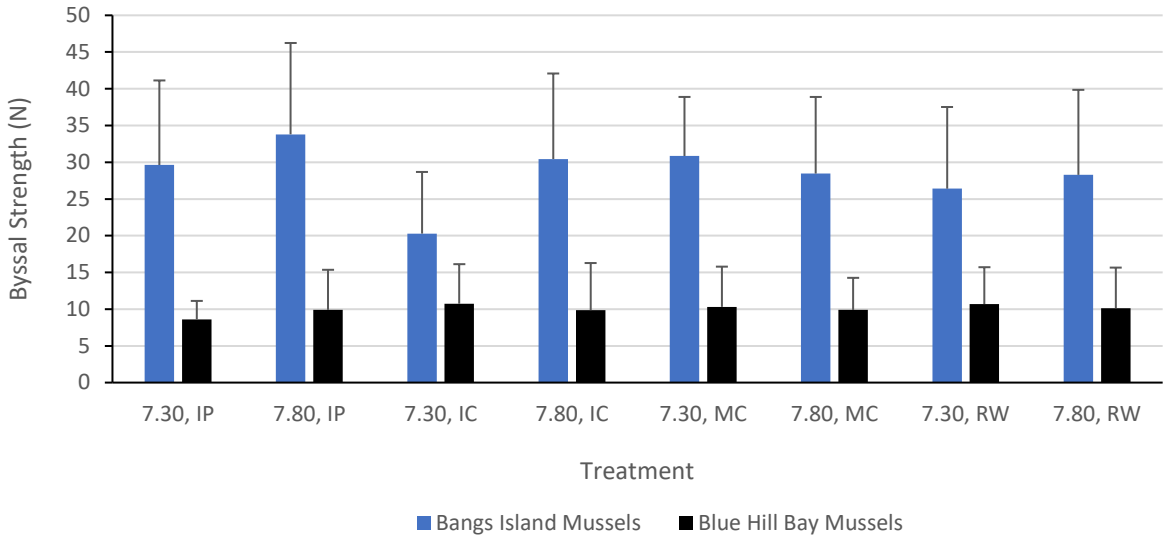

Figure IV.7: Byssal strength (newtons) of adult mussels retrieved from ropes deployed at two mussel farm field sites, grouped by seawater pH x microalgae diet treatments larval/juvenile mussels were exposed to in the lab. Full treatments are listed in Table IV.1.

Post-deployment adult mussels retrieved from Bangs Island Mussels exhibited significantly greater byssal strength across the board compared to those retrieved from Blue Hill Bay Mussels (ANOVA: $F = 658.620$, $p < 0.001$) (Fig. IV.7). Among those retrieved from Bangs Island Mussels, both seawater pH and microalgae diet treatments had effects on byssal strength: mussels reared in the control seawater pH treatment exhibited greater byssal strength than those in the acidified treatment (Tukey's HSD: $p = 0.047$), and those fed only *T. iso* + *T. pseudo* had greater byssal strength than those fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers (Tukey's HSD: $p = 0.032$). Among the mussels retrieved from Blue Hill Bay Mussels, neither seawater pH nor microalgae diet had an effect on byssal strength.

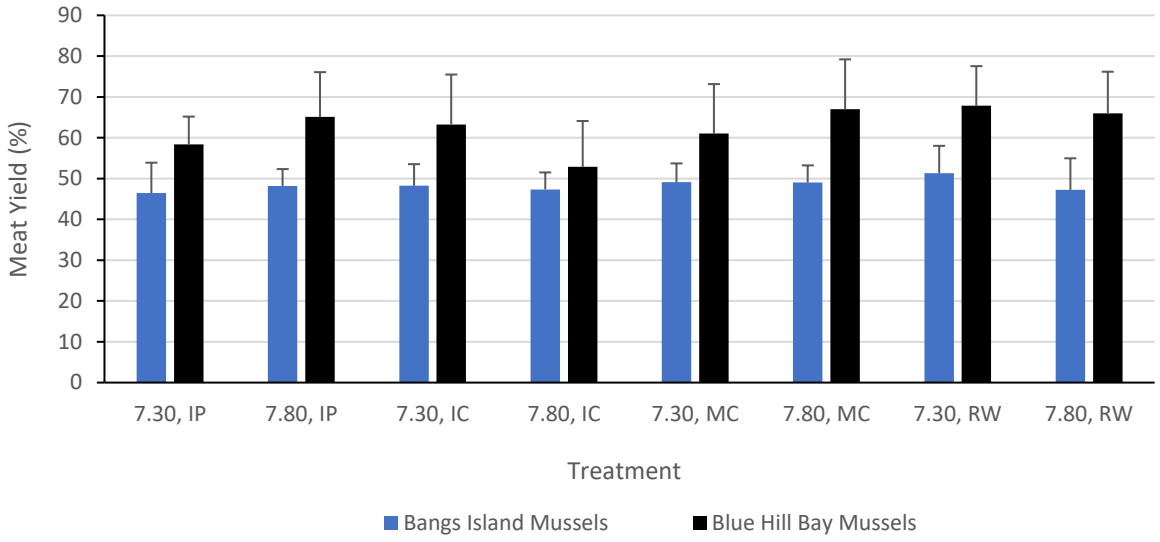

Figure IV.8: Meat yield of adult mussels retrieved from ropes deployed at two mussel farm field sites, grouped by seawater pH x microalgae diet treatments larval/juvenile mussels were exposed to in the lab. Full treatments are listed in Table IV.1. Meat yield is quantified as the proportion of tissue weight wet to whole weight.

Post-deployment mussels retrieved from Blue Hill Bay Mussels exhibited significantly greater meat yield across the board than those retrieved from Bangs Island Mussels (ANOVA: $F = 445.69$, $p < 0.001$) (Fig. IV.8). Among those retrieved from Bangs Island Mussels, neither seawater pH nor microalgae diet had an effect on meat yield. Among the mussels retrieved from Blue Hill Bay Mussels, microalgae diet but not seawater pH had an effect on meat yield: mussels fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers exhibited greater meat yield than those fed *T. iso* + *Chgra* (Tukey's HSD: $p < 0.001$).

Figure IV.9: Experimental mussels retrieved from Bangs Island Mussels following the Experiment #1 field deployment during December 2023.

IV.iii Results: Experiment #2 Lab Trial

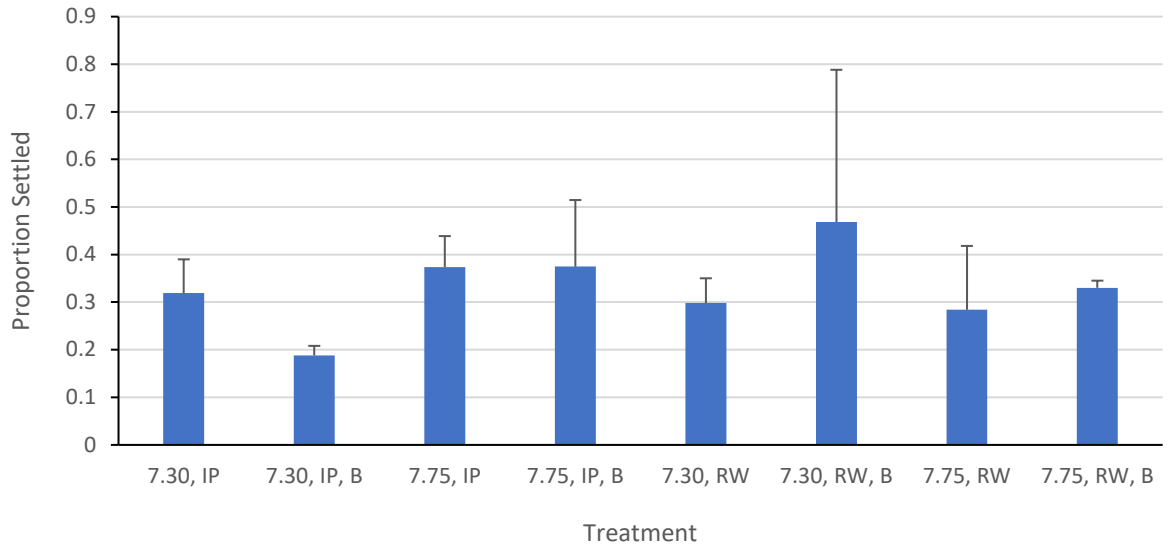

Figure IV.10: Proportion of juvenile mussels that successfully settled on one rope placed in each tank for settlement, quantified as the count of larvae settled on the rope divided by the count of swimming larvae prior to placing ropes divided by the number of ropes placed. Full treatments are listed in Table IV.2. Data is averaged by seawater pH x microalgae diet x seawater buffering treatment. Error bars represent standard deviation (n = 3).

Within each seawater pH x microalgae diet treatment, mussels exhibited higher settlement success in the buffered versus non-buffered treatments, with the exception of the pH 7.30, *T. iso* + *T. pseudo* treatment (Fig. IV.10). Mussels in the pH 7.30, *Rhodo* + *Weiss*, buffered treatment exhibited the highest settlement success overall, although the variance was high, and the corresponding non-buffered treatment had lower settlement success than all non-buffered *T. iso* + *T. pseudo* treatments. The pH 7.75, *T. iso* + *T. pseudo* treatments exhibited the second-highest settlement success, followed by the pH 7.75, *Rhodo* + *Weiss* treatments, and the pH 7.30, *T. iso* + *T. pseudo*, buffered treatment had the lowest (though the corresponding non-buffered treatment had higher settlement success than the pH 7.75, *Rhodo* + *Weiss*, non-buffered treatment). In a quasibinomial logistic regression testing the effects of pH, diet, and buffering on the proportion of mussels settled on the preserved rope versus the number of pre-settlement swimmers, neither pH, diet, nor buffering had an effect on mussel settlement success in the overall model, though the interactions of pH x diet ($F = 6.829$, $p = 0.020$) and diet x buffering ($F = 5.300$, $p = 0.036$) did. Specifically, mussels in the pH 7.30, *Rhodo* + *Weiss* treatments exhibited higher settlement success than those in the pH 7.30, *T. iso* + *T. pseudo* treatments ($p = 0.037$). In another quasibinomial logistic regression testing the effects of pH, diet, and buffering on the proportion of

pre-settlement mussel survival versus mortality counts, neither pH, diet, buffering, nor their interactions had an effect on pre-settlement survival.

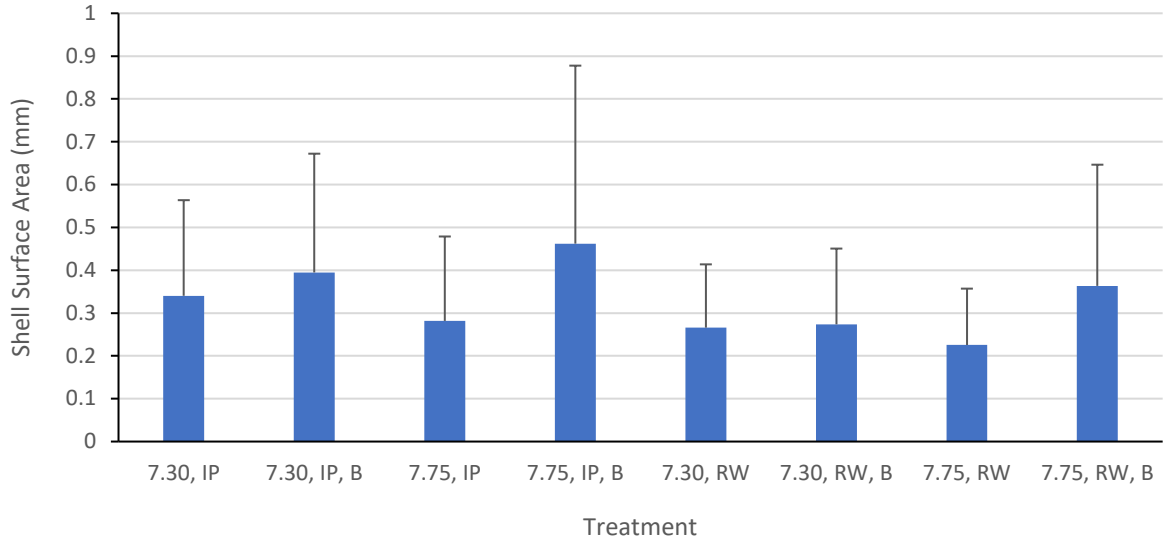

Figure IV.11: Juvenile mussel shell surface area 3 weeks post-settlement averaged by seawater pH x microalgae diet x seawater buffering treatment. Full treatments are listed in Table IV.2. Error bars represent standard deviation (n = 3).

Within every seawater pH x microalgae diet treatment, mussels exhibited higher shell surface area in the buffered versus unbuffered treatments (Fig. IV.11). For the most part, mussels in the pH 7.75 treatments tended to also exhibit higher shell surface area than those in the pH 7.30 treatments. Among the buffered treatments, mussels in the 7.75, *T. iso* + *T. pseudo* treatment exhibited the highest shell surface area overall, followed by pH 7.30, *T. iso.* + *T. pseudo* and pH 7.75 *Rhodo* + *Weiss*, while pH 7.30 *Rhodo* + *Weiss* had the lowest. Among the non-buffered treatments, mussels in the pH 7.30, *T. iso* + *T. pseudo* treatment exhibited the highest shell surface area (higher than the worst-performing buffered treatment), followed by pH 7.75, *T. iso* + *T. pseudo* and pH 7.30, *Rhodo* + *Weiss*, while pH 7.75, *Rhodo* + *Weiss* had the lowest. However, in an analysis of covariance (ANCOVA) testing the effects of seawater pH, microalgae diet, and seawater buffering on mussel shell surface area while controlling for mussel count in each tank, none had a statistically significant effect on shell surface area.

IV.iv Results: Experiment #2 Field Deployment

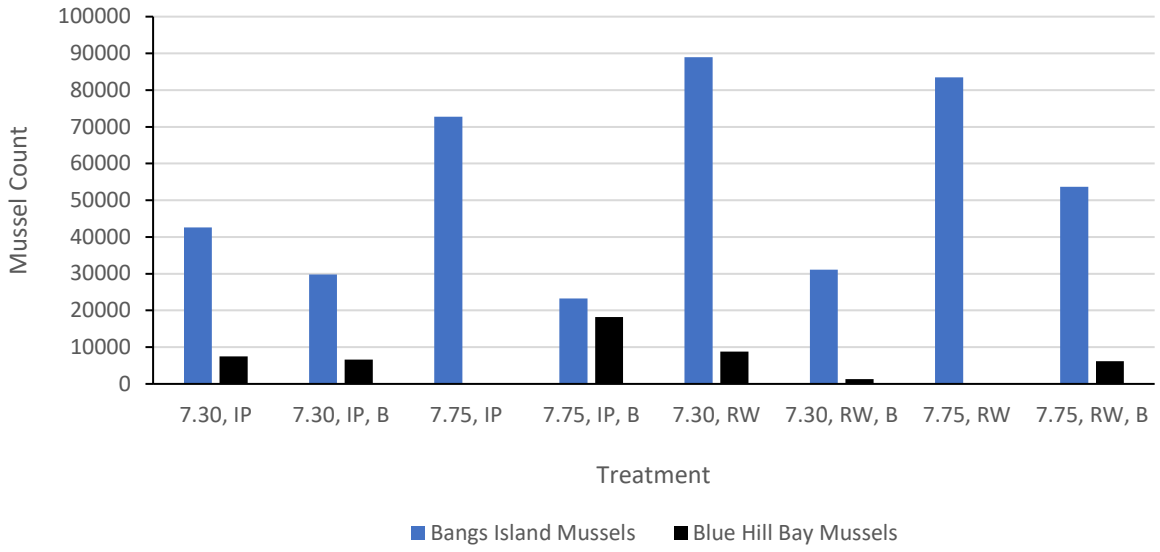

Figure IV.12: Count of adult mussels retrieved from ropes deployed at two mussel farm field sites, grouped by seawater pH x microalgae diet x seawater buffering treatments larval/juvenile mussels were exposed to in the lab. Full treatments are listed in Table IV.2.

The count of post-deployment adult mussels retrieved from the mussel farm field sites varied with seawater pH, microalgae diet, and seawater buffering (lab) treatments and site (Fig. IV.12). Among mussels retrieved from the Bangs Island Mussels field site, those originating from the non-buffered treatments yielded greater numbers than those in the corresponding buffered treatments. Those from the pH 7.30, *Rhodo* + *Weiss*, non-buffered treatment yielded the greatest numbers, while those in the pH 7.75, *T. iso* + *T. pseudo*, buffered treatment yielded the lowest. Only the pH 7.75, *Rhodo* + *Weiss*, buffered treatment yielded higher numbers than the pH 7.30, *T. iso* + *T. pseudo*, non-buffered treatment. Among mussels retrieved from the Blue Hill Bay Mussels field site, there was no clear pattern between the buffered versus non-buffered treatments. Those from the pH 7.75, *T. iso* + *T. pseudo*, buffered treatment yielded the highest numbers, while those from the pH 7.30, *Rhodo* + *Weiss*, buffered treatment yielded the lowest.

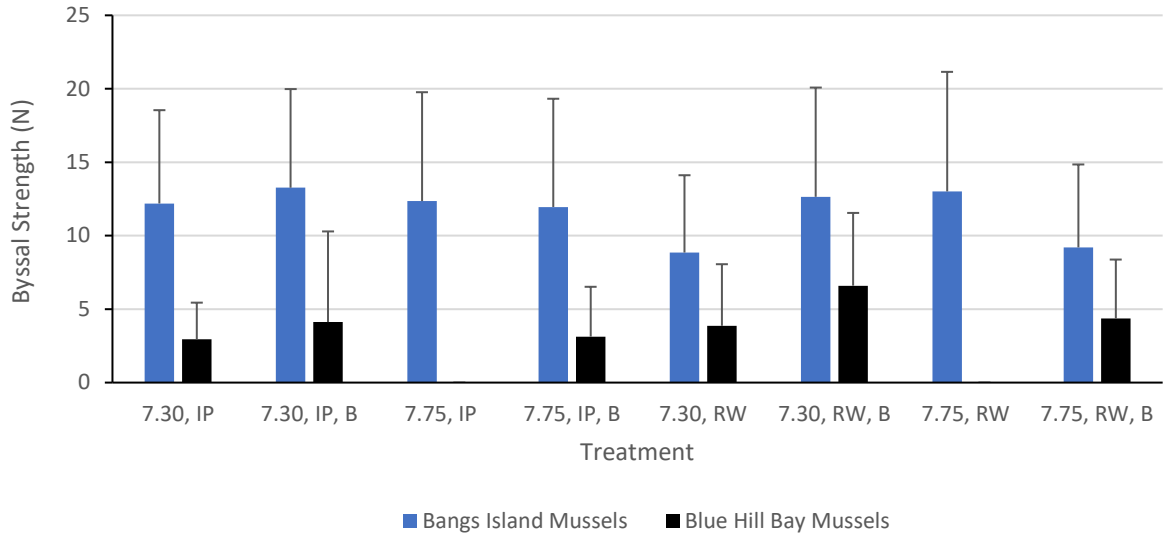

Figure IV.13: Byssal strength (newtons) of adult mussels retrieved from ropes deployed at two mussel farm field sites, grouped by seawater pH x microalgae diet x seawater buffering treatments larval/juvenile mussels were exposed to in the lab. Full treatments are listed in Table IV.2.

Post-deployment adult mussels retrieved from Bangs Island Mussels exhibited significantly greater byssal strength across the board compared to those retrieved from Blue Hill Bay Mussels (ANOVA: $F = 289.620$, $p < 0.001$) (Fig. IV.13). Among those retrieved from Bangs Island Mussels, microalgae diet treatment had an effect on byssal strength: mussels reared in the *T. iso* + *T. pseudo* treatments exhibited greater byssal strength than those in the *Rhodo* + *Weiss* treatments (Tukey's HSD: $p = 0.011$). Among the mussels retrieved from Blue Hill Bay Mussels, both microalgae diet and seawater buffer treatments had effects on byssal strength: mussels fed *Rhodo* + *Weiss* exhibited greater byssal strength than those fed *T. iso* + *T. pseudo* (Tukey's HSD: $p = 0.013$), and those reared in the buffered treatments exhibited greater byssal strength than those reared in the non-buffered treatments (Tukey's HSD: $p = 0.016$).

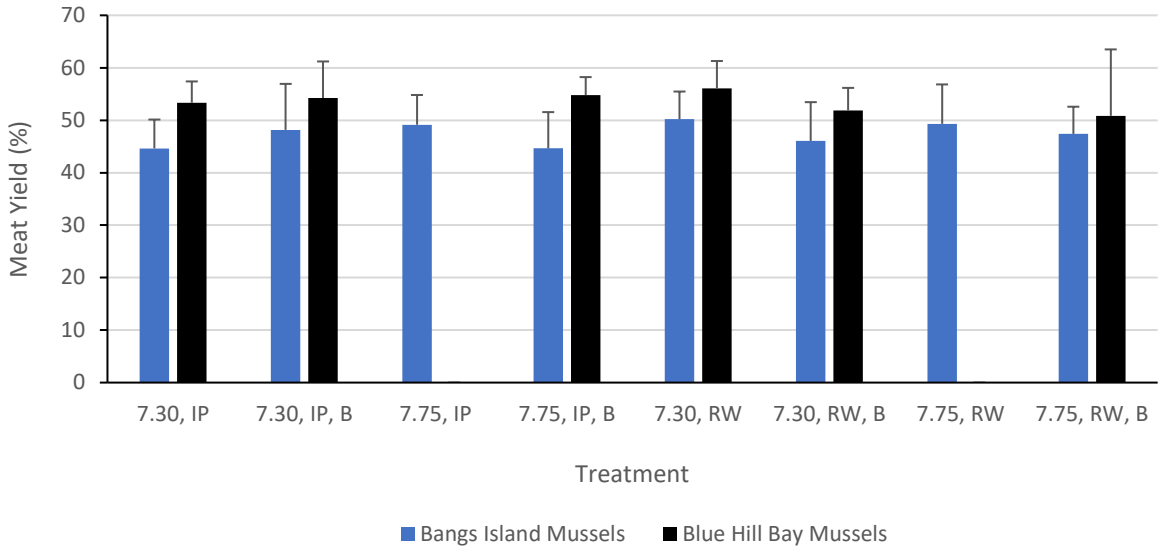

Figure IV.14: Meat yield of adult mussels retrieved from ropes deployed at two mussel farm field sites, grouped by seawater pH x microalgae diet x seawater buffering treatments larval/juvenile mussels were exposed to in the lab. Full treatments are listed in Table IV.2. Meat yield is quantified as the proportion of tissue weight wet to whole weight.

Post-deployment mussels retrieved from Blue Hill Bay Mussels exhibited significantly greater meat yield across the board than those retrieved from Bangs Island Mussels (ANOVA: $F = 445.69$, $p < 0.001$) (Fig. IV.14). Among those retrieved from Bangs Island Mussels, microalgae diet had an effect on meat yield: those fed *Rhodo* + *Weiss* exhibited greater meat yield than those fed *T. iso* + *T. pseudo* (Tukey's HSD: $p = 0.006$). Among the mussels retrieved from Blue Hill Bay Mussels, neither seawater pH, microalgae diet, nor seawater buffering treatments had an effect on meat yield.

IV.v Conclusion

Experimental blue mussels exhibited varied survival and growth with the seawater pH, microalgae diet, and seawater buffering conditions they experienced in their earliest life stages, as well as the field site at which they were deployed post-settlement. Some of these differences were statistically significant, while others were merely trends.

Although seawater pH did not have a statistically significant effect on mussel settlement success, mussels reared in the pH 7.80 treatments did tend to exhibit greater settlement success than those in the pH 7.30 treatments, indicating that low seawater pH can be a stressor for early life stage mussels and should be addressed in shellfish hatchery culture. Mussels fed *T. iso* + *T. pseudo* as larvae and *Rhodo* + *Weiss* as veligers tended to exhibit the highest settlement success in both

experimental trials, suggesting that a high-calorie diet may benefit mussel settlement. Those fed only *T. iso* + *T. pseudo* tended to exhibit the second-highest settlement success, suggesting that the "industry standard" diet combination as used by Downeast Institute is also effective. Mussels also tended to exhibit higher settlement success in buffered treatments, indicating that seawater buffering in combination with diet optimization may be the best practice for mussel culture.

Mussels tended to exhibit higher shell surface area when reared in control versus acidified seawater pH treatments, as well as buffered versus non-buffered treatments, further underscoring the need to address low seawater pH in hatchery culture. However, the microalgae diets that resulted in the highest growth were different than those that resulted in the highest settlement success, as mussels fed *Mono* + *Chgra* (high EPA, protein) and *T. iso* + *Chgra* (high DHA) in Experiment #1 grew the largest. In Experiment #2, in which those diets were not tested, mussels fed *T. iso* + *T. pseudo* tended to grow the largest.

In both Experiment #1 and #2, mussels deployed at Bangs Island Mussels exhibited greater byssal strength across the board than those deployed at Blue Hill Bay Mussels, while mussels deployed at the latter exhibited greater meat yield than those deployed at the former. Mussels originating from control versus acidified pH treatments, as well as those from buffered versus non-buffered treatments, tended to exhibit greater byssal strength, indicating the superior condition of these mussels. Mussels fed *Rhodo* + *Weiss* tended to have higher meat yield in both experiments, suggesting that a high-calorie diet is beneficial for mussel condition at harvest.

In conclusion, it is recommended that shellfish hatcheries take steps to address suboptimal natural seawater quality, especially as ocean pH and saturation state of aragonite continue to decline into the future due to ocean acidification. Both seawater buffering and careful consideration of live microalgae feeds are recommended to prevent the negative impacts of acidified seawater on mussel settlement success and growth. In particular, a high calorie diet such as *Rhodo* + *Weiss* appears to be effective for improving settlement success and promoting adult tissue growth (quantified as meat yield). Other diets, such as *Mono* + *Chgra*, may be more effective for post-settlement growth. Although only simple diet combinations were tested here and held constant throughout each lab trial for experimental purposes (with the exception of *Rhodo* + *Weiss*), shellfish hatcheries typically have a variety of microalgae species at their disposal, and it is recommended that a diversity of species are fed to reap the benefits of each.

V. References

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