PART I SUMMARY

PROJECT TITLE: Adapting Aquaculture to Changing Water Chemistry in the Pacific Northwest

REPORT GIVEN IN YEAR: 2020

PROJECT WORK PERIOD: 6/30/2016 to 8/31/2020

AUTHOR: George Waldbusser

PARTICIPANTS: G. Waldbusser* (OSU), B. Haley (OSU), B. Hales (OSU-no longer on project as of August 2019), A. Barton (Whiskey Creek), B. Warren (Global Ocean Health), S. Cudd (Whiskey Creek), C. Langdon (OSU), J. Sanders (Global Ocean Health), Benoit Eudeline (Taylor's Shellfish), Marnie Jo Zirbel* (OSU)

REASON for TERMINATION: Completion of funded work, funds expended.

PROJECT OBJECTIVES:

Objective 1. Evaluate previous and ongoing nutrient and carbon chemistry data at Whiskey Creek Hatchery (WCH) and Taylor Hatchery (TH).

Objective 2. Establish monitoring in both hatcheries, including protocols for trace metals, reduced sulfur species, and dissolved organic matter for incoming water and within later stage larval culture tanks.

Objective 3. Measure oxygen demand of the larger culture tanks used for the later stage larvae with and without larvae and algae present.

Objective 4. Determine condition and fitness of later stage larvae in larger culture tanks.

Objective 5. Integrate outcomes of research and with extension/outreach to inform shellfish hatcheries and setting locations from Alaska to California.

The above objectives were designed around the supposition, and anecdotal evidence, that the water quality issues facing the hatchery project partners were related to the products or indirect effects of metabolism. There are two major directives that are supported by the above objectives, **first** is that metabolic effect derived from environmental effects in estuarine waters or from practices within the hatcheries (or some combination of both), and **second** is what property (or properties) of these metabolically active waters are likely causing the problems for the hatchery. This point is critical if the research is to lead to adaptive strategies for mitigating the water quality issues. Notably, as well, we included Objective 4 to parameterize more fully the condition of larvae and seed as a possible covariate, given many of the issues seem to arise at times not only of high metabolic demand, but also, at times, on the fringes of the prime spawning time for Pacific oysters.

PRINCIPAL ACCOMPLISHMENTS:

Principal accomplishments for Objective 1 (existing data and monitoring).

Carbonate chemistry data QA/QC'd at the Whiskey Creek Shellfish Hatchery per Fairchild thesis from 2015 to 2019. Carbonate chemistry data from Taylor's Hatchery has yet to be QA/QC'd due to internal logistics among the entities responsible for maintaining the instrument.

A predictive model of carbonate chemistry in Netarts Bay was developed based on non-carbonate chemistry parameters, much like a weather forecast model. While not originally part of the proposal, this was work a former graduate student was completing as we awaited funds to begin field work and was to be part of his dissertation. The model had a generally high level of coherence in the 3-4 day forecast window (based on tides, wind, cloud cover) when the model was hindcast and compared to existing measurements. The model was intended as a mechanism to help demonstrate to hatchery operators an approach that would limit the need for permanent, high-precision and high-frequency carbonate chemistry monitoring. Unfortunately, the graduate student who had developed the model is currently on a leave of absence and I don't currently anticipate him completing the degree program.

The two hatcheries over the project period and prior, have different record keeping practices. I have done a cursory review of Taylor's hatchery records in relation to their existing monitoring data and found no clear and apparent relationships with nutrient data and production data. The issue with any current tank sampling is that discrete one-time samples do not speak to changes in water quality within tanks. We have been on hold for the QA/QC Taylors high precision carbonate chemistry data, due to external to our project data management issues.

The WCSH books are kept in hard-copy form, and thus need to be transcribed. Thus, over the past year, in addition to the 2017 records that were previously worked up, the 2018 through summer of 2019 records were digitized and utilized to further examine possible links between oxygen demand/metabolic events and production failures. Analyses below focus on the 2019 data, given the ZAPS instrument was offline for a considerable part of the 2018 year.

Principle accomplishments with regards to Objective 2. (new monitoring in hatcheries)
Over the course of the project period we collected nearly 1750 samples for analyses of metals, nutrients, sulfide, carbonate chemistry, and oxygen demand/oxygen concentration. Of those 1150 have been analyzed with the remainder either being de-prioritized or on hold due to facilities related issues of analytical instruments. A brief summary of conclusions we can make from those discrete samples follows.

Sampling campaigns for sulfide at both hatcheries in 2018 found sulfide levels almost always below detection limits, with the exception of lime slurries added to culture tanks (but that did not seem to translate to significant sulfide in tanks).

Unfortunately, of the over 400 trace clean metals samples collected, we have only been able to analyze just over a 100 of them. About 70 or so are redundant samples from the WCSH 2017 campaign, but many of the remainder are still a priority. A significant fire adjacent to the clean metals lab in Dec of 2018 shut all operations down, closed the entire building due to safety concerns, and required purchasing new mass spectrometers due to smoke damage. We were able to run some samples in late spring of 2020, after a temporary lab space was established, following Covid shutdowns. In addition, unfunded Co-PI Haley left OSU in late summer of 2019, and has continued to assist, but in a diminished capacity. The temporary facility was closed in late June 2020 to move back into the renovated building. Despite the setback, the metals data demonstrated two interesting findings, varying between the facilities. First, in Taylors we found elevated metals in culture tanks that appeared to have some link to the lime slurry. Measurements of incoming water were lower than slurry measurements, and levels measured in tanks. Second it appears that during low tide events we see elevated trace metals, likely due to mobile metals in naturally anoxic sediments that drain during lower low tides.

Discrete nutrient sampling was used to trace metabolic processes through hatchery production and estimate metabolism in both static and flow-through culture tanks. Our data demonstrated significant amounts of metabolism in the Taylors flow-through and discrete culture tanks in 2018 (discussed more fully under Objective 3). We collected an additionally 80 samples from both hatcheries in 2019 and into 2020. However our nutrient analyzer malfunctioned in late 2019, necessitating a repair that may run thousands of dollars. Thus, remaining samples have not yet been analyzed, but when they are analyzed, we will be able to more fully describe the extent of in tank metabolism.

Under an agreement with ZAPS Technologies, we were able to extend the original 6-month monitoring agreement to multiple years. Like so many other aspects of this project, this also became problematic. An ownership dispute between the founder and investor left the instrument in limbo (and without support) for a significant portion of 2018. We were able to bring it back online in late 2018, and collected data through the production season of 2019. The oxygen demand data alleviated the need for more tedious dissolved organic carbon measurements, as originally proposed (as these are generally tightly coupled). The primary findings from these data were that oxygen demand events (defined as significant, transient conditions above baseline conditions) increased the likelihood of production failures. Additionally, we have since linked these oxygen demand events to lower low tides or higher tidal ranges.

The links among lower tides, oxygen demand events, increased metals, and production failures, while not providing a silver bullet mechanism for failures, does provide real and tangible actions for hatcheries, in avoiding water around times of extreme tides, particularly in locations associated with extensive marshes and tide-flats.

Principle accomplishments for Objective 3. (oxygen demand)
We made both direct measures of oxygen demand within culture tanks, and inferred metabolism from inorganic nitrogen accumulation through widely used stoichiometric relationships between oxygen consumption and nitrogen excretion through respiration. Our findings demonstrated large metabolic signals in culture tanks, with possible concerns stemming from nitrogen accumulation and possibly metals speciation. Generally, we did not find low oxygen concentrations in tanks, as hatcheries have since the inception of this project incorporated oxygen concentrators to deliver air with oxygen saturations of 200-300% to tanks. However, oxygen demand (which is analogous to metabolism, but not exactly), may lead to nitrogen increases if due to biological

activity, and can alter metals speciation, making metals more bioavailable. As noted above, there is also an environmental signal of oxygen demand we found at the WCSH that would presumably exacerbate any within-tank oxygen demand associated with metabolism.

Principle accomplishments for Objective 4. (larval condition)
We were unfortunately unable to make many advancements in terms of direct measurements of larval condition. The primary intent was to determine the degree with which initial larval condition may covary with production failures. This stemmed from changes in total FTE associated with personnel changes in the project and prioritization of monitoring and identification of effects on hatchery production. Analyses of hatchery records have been more enlightening at the WCSH where we found seasonal trends in larval survivorship, and significant effects of low/extreme tide series (and correlated water quality variables) on production.

Principle accomplishments for Objective 5. (outreach and engagement)
A concise Best Management Practices document has been drafted and is being finalized before being submitting first to industry partners, then to the advisory panel, and then to WRAC for approval before release. The guide includes measures and strategies that hatchery operators or growers can take and includes references to primary literature as appropriate. Global Ocean Health assembled an advisory panel consisting of scientists, industry personnel, and other stakeholders. We have presented some of our findings to this group. We will include a more complete summary in the BMPs guide including the degree of certainty and areas where additional research is needed.

IMPACTS:

Relevance: The oyster seed crisis in the mid to late 2000's struck multiple commercial shellfish hatcheries on the US Pacific Coast, creating a shortage of seed for growers. The primary cause of the crisis was identified to be ocean acidification, and strategies adopted by hatcheries to mitigate these effects were generally successful, including monitoring carbonate chemistry, buffering hatchery waters, and changing the timing of spawning oysters, both in day and seasonal time scales. Despite these efforts, hatchery operators still contended with unexplained failures, and demand for seed was growing within the industry.

Response: Discussions among the project scientists and industry partners resulted in a plan to conduct exploratory research into possible causes of these unexplained failures that occurred despite the acidification mitigation strategies. A work plan was developed around a series of most likely hypotheses examining the both possible external and internal causes, primarily around metabolism byproducts, heavy metals, and other water quality issues. The team was to develop a Best Management Practices guide for water quality in shellfish hatcheries and for growers, incorporating the knowledge previously developed by the team about acidification mitigation, and the results from the current study.

Results: The results from measurements of water quality outside and inside the hatcheries, discussions with hatchery operators, and synthesis of existing and new knowledge has narrowed the potential scope to a few key issues (that vary between the hatcheries). First, tidal effects in some estuaries and bays increase the probability of hatchery failures. Second, metabolic effects in tanks are significant, with large increases in nitrogen and possible interactions with metals and buffering slurry suggest care must be taken in managing these other possible effects. Third, it is reasonable to assume that the interaction of these external (and somewhat predictable events) co-occurring with internal tank effects can amplify production challenges.

Impact: Within the first years of the project, study hatcheries have already included oxygen concentrators to help alleviate possible oxygen consumption issues. Identification of low tide issues at WCSH or other deep-water issues at TH provide cost-effective manageable mitigation strategies. The production of a BMPs for hatchery and grower water quality management will help transfer knowledge from this funded work and industry partners to other industry stakeholders, as more growers appear to be setting up their own smaller hatcheries.

Collaborators: An advisory panel of 12 members from the US Pacific and Atlantic coasts, of industry, stakeholder, and academic partners have been interfacing through the Outreach PI/Program of Global Ocean Health.

RECOMMENDED FOLLOW-UP ACTIVITIES:
Specific adaptation and mitigation strategies for different hatcheries and locations will vary by site, size, resources, etc, and thus we can identify some general approaches that may provide additional clarity on causes and mechanisms surrounding external and internal water quality issues. First, to the degree possible, careful record keeping and identifying quantitative measures of larval success should help identify clear more finely failures. Hatchery operators have tremendous intuition and an eye to see problems, but, for example, the oxygen demand events being on the order of an hour or less duration, requires fine scale record keeping. Second, we were unable, and did not propose to experimentally test mechanisms for failures. This would be a useful area of future work to better understand root causes and design treatment systems to target key parameters. Third, we were able to provide some predictable conditions to avoid. For example, developing predictive models for 'bad times' based on a subset of more easily measurer parameters will let hatchery operators to better avoid or treat culture waters. Finally, as related to the first point, targeted environmental measurements with high time frequency and within hatcheries may help to utilize resources more effectively than daily samples taken on an ad hoc basis (assuming capacity to collect and process samples).

SUPPORT: No additional support to report.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:
See attached, per WRAC Termination Report Guidance

SUBMITTED BY:

9/16/2020

Title: PI ,George Waldbusser — Date

APPROVED:

9/25/20

Project Monitor: Ron Hardy — Date

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

<u>Publications and Manuscripts</u>

Best Management Practices for addressing Ocean Acidification and other water quality concerns. (in prep) Sanders, J. G.G. Waldbusser et al.

C.P. Allan, G.G. Waldbusser, and B. Hales (in prep) Empirical Prediction of Estuarine Carbonate Weather. To be submitted to Limnology and Oceanography: Methods

Waldbusser, G.G. (in development) The links among tidal forcing and water chemistry in tidally dominated embayments.

Fairchild, W. (2020) High-resolution carbonate dynamics of Netarts Bay, OR from 2014-2019. OSU MS Thesis, B. Hales advisor, 106 pages.

Related Public Outreach Events

Speaker/Panelist, Rep. Bonamici's Virtual Town Hall (2020)
Panelist/Speaker Healthy Oceans OSU Event, Portland, (2020)
Panelist/Speaker Shuck Portland, Portland, (2019)
Panelist, Rep. Bonamici's Ocean Health Forum, Astoria (2019)
Panelist, Oregon's Coastal Caucus Economic Summit, Lincoln City (2018)

<u>Papers Presented</u>

Waldbusser, GG, SR Smith, B Hales (2019) Effects of Seagrass and Tides on Carbonate Chemistry Exposure in Juvenile Oysters. ASLO Aquatic Sciences Meeting, Puerto Rico. (data presented included WRAC funded data)

Warren, B. and R. Feely (2018) Accelerating ocean change in the PNW: New research, and new policy tools to protect healthy waters. Pacific Coast Shellfish Growers Association Meeting, Blaine, WA.

Allan, C.P. G.G. Waldbusser, and B. Hales (2018) Omega Oracle: Forecasting estuarine carbonate weather. Salish Sea Meeting, Seattle, WA.

PART II DETAIL

PROJECT TITLE: Adapting Aquaculture to Changing Water Chemistry in the Pacific Northwest

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AUTHOR: George Waldbusser

PARTICIPANTS: G. Waldbusser* (OSU), B. Haley (OSU), B. Hales (OSU-no longer on project as of August 2019), A. Barton (Whiskey Creek), B. Warren (Global Ocean Health), S. Cudd (Whiskey Creek), C. Langdon (OSU), J. Sanders (Global Ocean Health), Benoit Eudeline (Taylor's Shellfish), Marnie Jo Zirbel* (OSU)

PROJECT OBJECTIVES:

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The above objectives were designed around the supposition, and anecdotal evidence, that the water quality issues facing the hatchery project partners were related to the products or indirect effects of metabolism. There are two major directives that are supported by the above objectives, **first** is that metabolic effect derived from environmental effects in estuarine waters or from practices within the hatcheries (or some combination of both), and **second** is what property (or properties) of these metabolically active waters are likely causing the problems for the hatchery. This point is critical if the research is to lead to adaptive strategies for mitigating the water quality issues. Notably, as well, we included Objective 4 to parameterize more fully the condition of larvae and seed as a possible covariate.

TECHNICAL SUMMARY AND ANALYSIS:

Overall Summary

The data and evidence are convergent on a few key issues that the study hatcheries (and by extension others) should be increasingly aware of with regards to possible water quality issues associated with production losses that are not due to acidification impacts. While these issues are not consistent across both hatcheries, they likely play varying roles in each, and will require other hatcheries to assess possible risk factors for these key issue areas.

The first issue revolves around the role that extreme tides and low tides in generating water quality concerns that are likely due to water draining from tide flats and fringing marshes. In Netarts Bay we found that the probability of increased transient water quality issues (and associated production losses) increase significantly with increasing tidal range/lower tides. Analyses of the WCSH production records have also shown a significant increase in the probability of production losses when these oxygen demand events occur (which appear to also correlate with increases in trace metals). We lack inferential power to determine exactly the mode of action on larvae from the conditions in bay during these tides, but with confidence can state that avoiding large outgoing and extreme low tides would decrease the likelihood of production losses in systems that have large fringing marshes and tide flats.

The second issue is overall tank metabolism and a possible interaction with general water quality conditions. In some culture tanks we measured large and significant metabolic rates (via both oxygen consumption and nitrogen generation), which is not unexpected due to culture practices. The potential result of these metabolic effects can exacerbate environmental water quality issues, and to some degree appear to be changing the availability of metals in the culture tanks. One of the key issues here, is that high rates of metabolism appear to be mobilizing metals that are adsorbed to particles coming into the tanks and may further become bioavailable if pH shifts in culture tanks.

The interaction of waters having high oxygen demand, that are derived from organically rich sediments within embayments (or deep stratified water in deeper systems), with high metabolic demand cultures would also cause concern.

We found that the carbonate-based slurries used to buffer against high CO2/low pH (acidified) waters to have elevated metals and sulfide, although sulfide was never detected in culture tanks. The slurries could be a small source of metals to tanks, however several years of buffering hatchery waters has demonstrated the overall benefit of this approach. It is important to ensure metabolic conditions in tanks are managed well, to prevent possible metal loading. Anecdotally more frequent water changes in one hatchery helped alleviate production failures one season.

Summary By Objective

Objective 1 (previous and ongoing hatchery data)

Examination of the nutrient data from Taylors and other discrete samples from WCSH have largely been abandoned, due to the fact that the sampling frequency and lack of targeted sampling around specific events or times provides little inferential power. Our focus has been on the higher resolution carbonate chemistry data and hatchery records (to couple to project specific sampling/monitoring efforts). A simple binning of the carbonate data to examine net community metabolism of Netarts Bay water (Figure 1) demonstrates seasonal phasing of tides and daylight. Earlier in the year it appears metabolism is the bay (as seen as the difference between outgoing night and day values), does not cause significant shifts in median values, with some slightly higher counts of increased CO_2. However, summertime shows an approximate shift of 150-200 ppm of CO_2 between outgoing tides. Predicted tides also show an increase of roughly 20% in the frequency of nighttime low tides from spring through fall.

This all points to a key observation, that the bay water is altered significantly by within bay metabolism and primary production, and the seasonal changes in tide coupled with biomass accumulation and temperatures will create a decreasing frequency of good chemistry conditions as the season progresses.

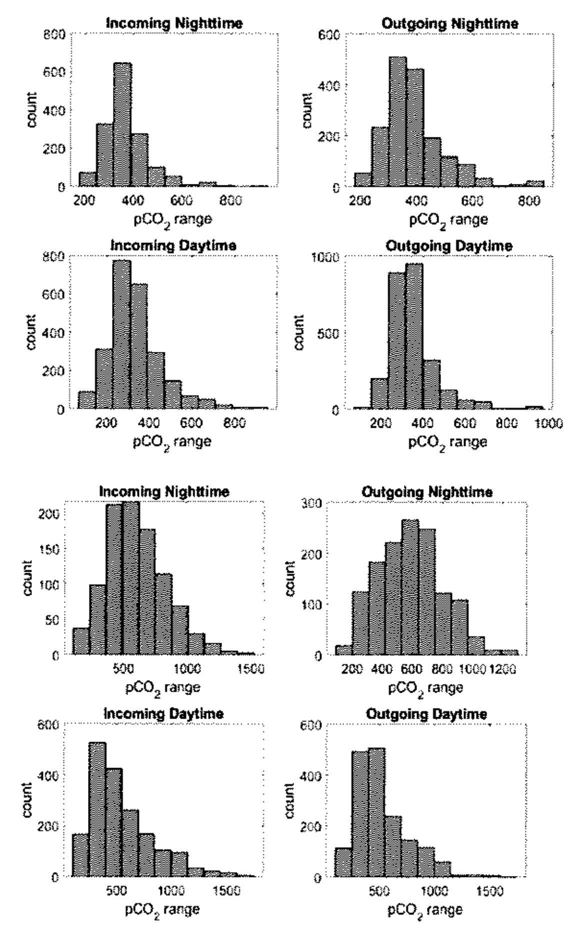


Figure 1. Frequency distribution of season, day/night, and tidal stage for P_{CO2} at the WCSH. The four panels on the left are Apr-June and on the right July-Sept.

Analyses of production records from WCSH alongside the continuous monitoring data has demonstrated that water quality events result in production losses. Data analyses from 2017 found than an exposure to an oxygen demand event more than doubled the likelihood of a production failure. An extended downtime of the ZAPS instrument prevented any similar comparison in 2018, however data from 2019 provide additional support for a role of these events. To more quantitatively examine production losses, rather than noted events in log books, the number of combined cohorts was used to document when numbers were diminishing and groups are combined (Figure 2). Two primary windows of time stand out, Feb into Mar, with a larger signal in the May through July window, focused around mid-May to early June. The oxygen demand data for these periods will be highlighted in more detail under the respective objective (Obj. 2).

The other primary and significant outcome under this objective was the development of a chemistry forecasting model. While not originally part of the proposed work, this was developed by the GRA as a component of their anticipated dissertation. Utilizing other readily available environmental data, such as wind, tides, and cloud cover, the model was able to forecast carbonate chemistry conditions to a operational scale that would be generally acceptable to hatchery operators (defined from previous workshops to be 0.2 units of saturation state). Unfortunately, the student is on leave of absence and I and colleagues have tried to get a manuscript from this work to no avail. Disappointingly most of the work is done, and draft was completed, but it still needs additional work before it would be ready for publication, and we lack the computer code to complete this currently.

Objective 2 (establish monitoring of key variables)

Over the course of the project period we collected nearly 1750 samples for analyses of metals, nutrients, sulfide, carbonate chemistry, and oxygen demand/oxygen concentration (Table 1). Of those 1150 have been analyzed with the remainder either being de-prioritized or on hold due to facilities related issues of analytical instruments. Of those samples, the metals, sulfide and

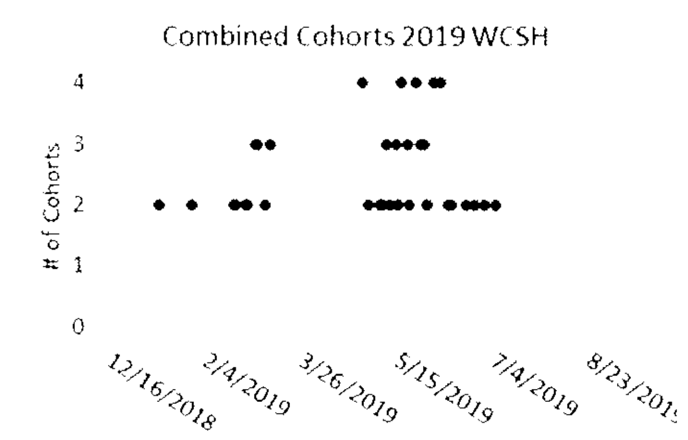


Figure 2. The number of cohorts combined during production cycles in the WCSH, 2019 records through July. Higher numbers indicate fewer numbers of larvae from each cohort that is being combined.

oxygen demand data will be discussed more fully below. In addition, the operation of the ZAPS instrument from late 2016 through 2019 (with some gaps in data coverage), provided some unprecedented insights into water quality conditions in Netarts Bay. Despite an initial plan to move the instrument to Taylors for a deployment, Co-PI Eudeline, the R&D principal for Taylors determined due to space and value at the other hatchery he would prefer to keep it at WCSH. Key overall finds are highlighted.

While we found 98.8 mmol of sulfide in samples of buffering slurry in TH (which was well above the calibration curve, and may have actually been higher), all other samples collected were below detection limits, indicating sulfide contamination, as one initial hypothesis was not an issue (in samples from both hatcheries).

The primary outcomes from the metals monitoring at both hatcheries (and from field locations), were that trace metals were generally not elevated in environmental samples (except at low tides), but were elevated in buffering slurries, and within culture tanks. The amount of metals added from slurries do not appear to a primary source but may be contributing to some of the elevated metals in tanks. It is important to note that we measured metals in solution, not particulates, and thus adsorption or other phase changes could be a consideration, as I will briefly outline. It appears that WCSH has slightly elevated metals relative to TH, and their water looks more aligned with the 30 m intake at TH, and less like the 3 m intake. Based on a series of measurements at WCSH, it appears one mechanism is manganese cycling and precipitation may be serving to adsorb and desorb trace metals. Incoming waters loose Mn through the WCSH, via precipitation of Mn-oxides, which adsorbs trace metals, which appear to be desorbed in tanks, likely due to changes in tank chemistry associated with metabolism and redox state. A similar pattern is seen in TH with lower Mn in the early treatment steps, and increasing later, with correlated increases in nickel and cobalt, although copper, cadmium, and lead do not follow this trend. These findings demonstrate a potential for treatment options, but as previously noted, the concentrations on their own are likely a primary culprit in production losses (but possible interactions with changes of within tank water is an important potential issue).

Table 1. Inventory of discrete samples collected and analyzed from each hatchery by year.

	Whiskey Creek 2020		Taylor's 2019		Whiskey Creek 2019		Taylor's 2018		Whiskey Creek 2018		Whiskey Creek 2017	
	Collected	Analyzed	Collected	Analyzed	Collected	Analyzed	Collected	Analyzed	Collected	Analyzed	Collected	Analyzed
Metals (hatchery collected)					30	20	146	10	0	0	0	0
Metals (sampling campaign)	22		26		10	10	25	25	21	0	161	48
Nutrients	22		23	0	40	0	225	201	21	21	161	161
Sulfide							45	45	21	21	0	0
Discrete PCO2/DIC	22		6	0	6	0	15	15	21	21	114	0
pH							200	200	21	21	0	0
BOD or O2	36	36	48	48	25	25	9	9	14	14	200	200

The most important finding from the monitoring work is the connection among oxygen demand, tides, and larval production issues at the WCSH. As noted above, oxygen demand events increase the likelihood of poorly performing cohorts (doubling the probability of observed failures in 2017 and being associated with increased numbers of combined cohorts in 2019). We installed a pressure sensor at the WCSH intakes in late 2018 to measure directly tide state, as anecdotal observations found occasional co-occurrences of low tide and oxygen demand events (Figure 3). The 2019 data was used to compute a logistic regression of the probability of an oxygen demand event (defined as 2 standard deviations above the mean) (Figure 4). At tidal ranges from 2.5 to 3.0 meters, the probability of an oxygen demand event occurring (as defined at 2 standard deviations from the overall average) increases from 16% to 35% (Wald Chi-sq = 8.2170, P = 0.0042).

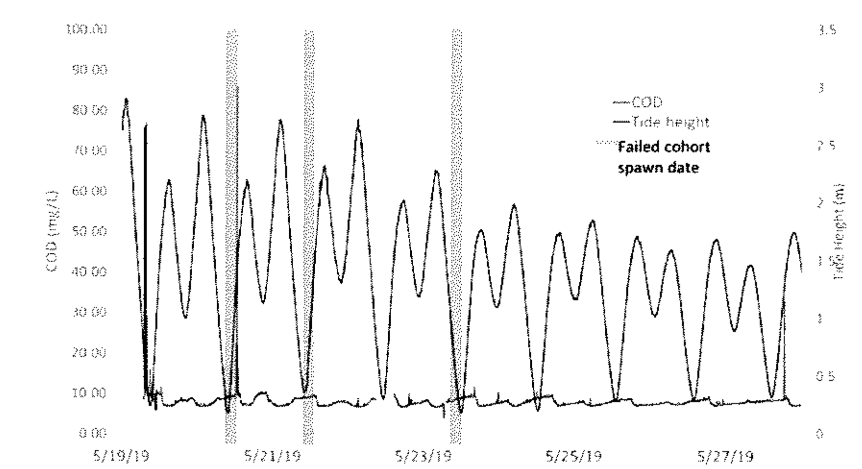


Figure 3. Several day sequence of tide, observed production losses, and oxygen demand (with the event resulting in a transient 8 fold increase). This tide sequence was one of the largest of the 2019 season.

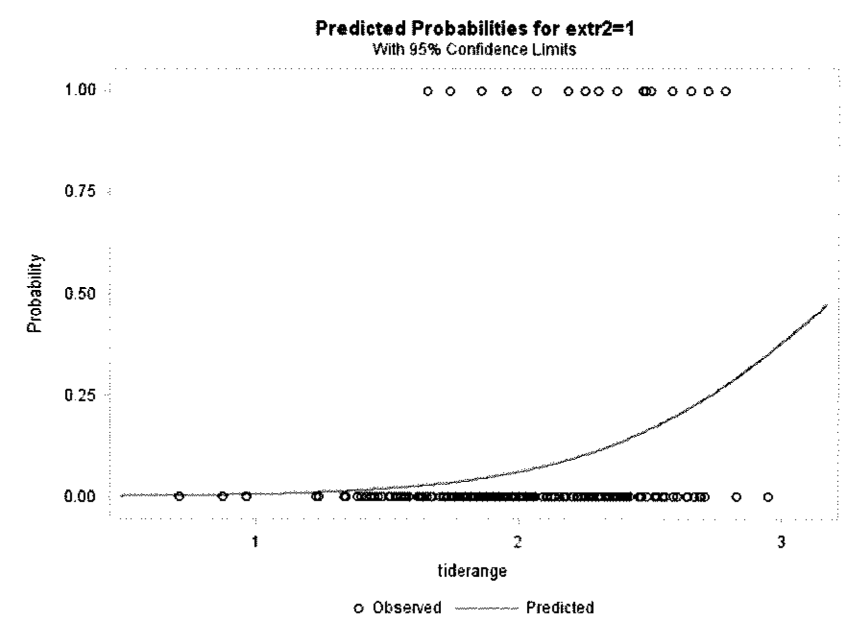


Figure 4. Logistic regression of probability of an oxygen demand event occurring as a function of tidal range.

These findings, coupled to the production data **provides a relatively high level of confidence that some component of the low-tide water, likely derived from porewaters in contact with anoxic tide-flat and salt-mash sediment, significantly increases the risk of production failures.**

Water samples collected in May 19th, 2019 also indicate low-tide waters likely have a signature of anoxic (reduced) biogeochemistry, as we found a two-fold increases in cadmium and iron, but no increases in nickel and lead.

One final notable observation suggesting a role for these waters that are being metabolically altered through contact with inter-tidal sediments was from BOD measurements made in Feb and June of 2020. Samples were collected up bay from WCSH at Jacobson's Salt company and at the intake pipes, and we found a 20-30% increase up bay in BOD between the sites on both dates, but a factor of 4-8 difference between Feb and Jun BOD (data shown below in relation to tank BOD under Objective 3).

Objective 3 (oxygen demand/metabolism within tanks)

At the time of submission of the proposal for this work, the industry partners had noted production problems in their larger culture tanks and had been previously advised to not over bubble tanks due to possible negative effects on larvae. During the early days of this project, hatcheries experimented with increasing bubbling, with success, but also had installed oxygen concentrators to deliver air that is super-saturated with oxygen upwards of 200%. These approaches seemed to help resolve some of the immediate issues, so we prioritized other components of this work (also in agreement with industry partners). Thus, most of the oxygen demand measurements came in the final couple years of the project, and we used these direct measurements to validate the ZAPS continuous data values and confirm some of the patterns observed. In addition, we utilized nutrient sampling as a proxy for the oxygen demand direct measures, given the well-established relationship between oxygen uptake and nitrogen and phosphorous release (though respiration). Our approach was to directly measure BOD with a limited set of whole tank incubations (given the impacts on production associated with not keeping tanks in culture).

Within the tanks at both hatcheries we find high rates of metabolism, which is not surprising at all. We do see some significant variance among tanks, and very high rates of metabolism in conical flow-through tanks utilized at TH.

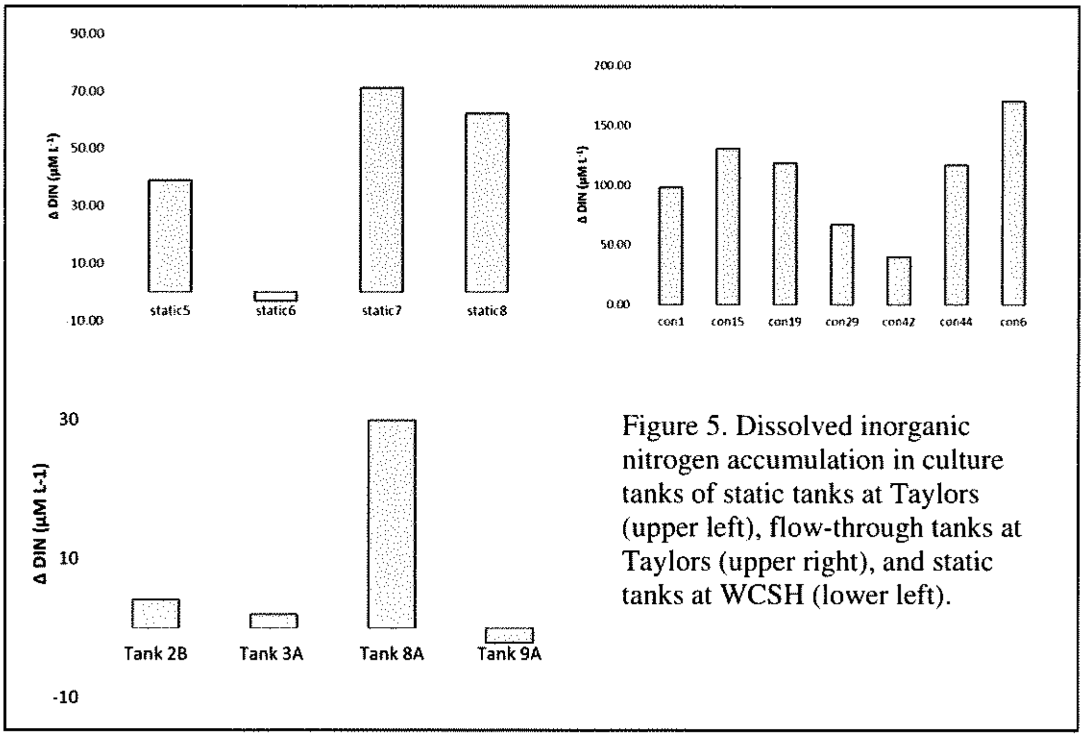
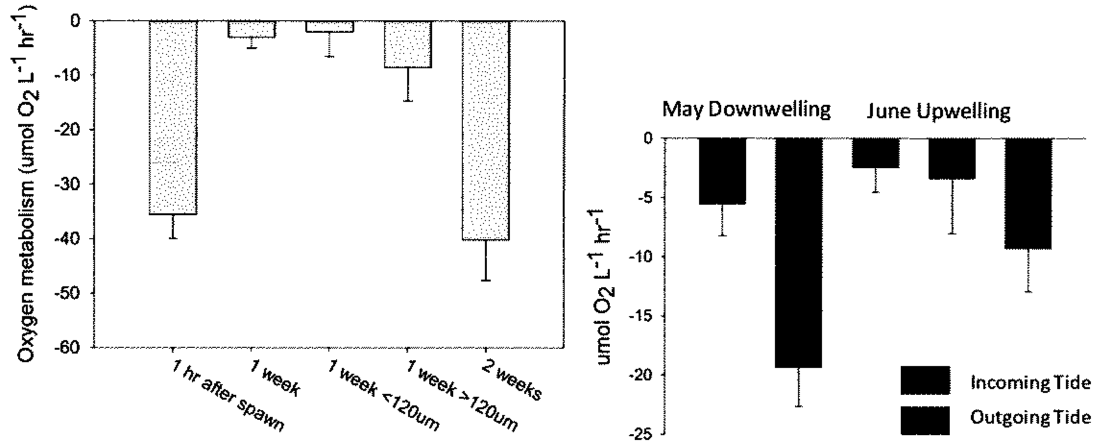


Figure 5. Dissolved inorganic nitrogen accumulation in culture tanks of static tanks at Taylors (upper left), flow-through tanks at Taylors (upper right), and static tanks at WCSH (lower left).

Measurements of nutrient release in the TH tanks in 2018 and WCSH tanks in 2017 find generally lower rates of metabolism in WCSH static tanks relative to TH. However, flow-through culture tanks in TH had nitrogen accumulation rates twice to three times higher than the static tanks, despite the continuous water replacement (Figure 5). The amount of oxygen needed to keep those waters oxygenated would be equal to approximately 3 times the total oxygen concentration (and direct measures of oxygen in tanks put O2 saturations between 70-90%).

Direct measurements of oxygen demand or uptake with tanks or source waters found generally similar values as would be estimated from the DIN measurements, and our direct measurements, when converted to standard BOD5 values, align well with the ZAPS instrument values. While the tank and source water measurements highlighted a few interesting trends (Figure 6). First, we found higher oxygen demand in static hatching tanks, and in the largest size class flow-through tanks at Taylors (approximately 10-20% uptake of total oxygen per hour). The smaller sized flow-through tanks however had a fraction of the oxygen uptake compared to the larger larvae. This may indicate its worth ensuring additional aeration in hatching tanks, and in the largest sized flow-through tanks.



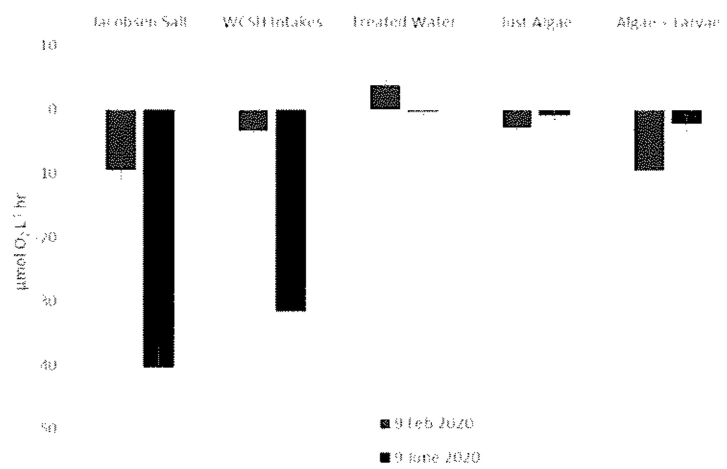


Figure 6. Oxygen demand or consumption in TH static and flow-through tanks (upper left), BOD of Netarts Bay waters during downwelling and upwelling across tides Upper right), and BOD of waters from the upper reaches of Netarts Bay into the hatchery and in tanks with different treatments in Feb and June of 2020 (lower left).

Measurements of oxygen demand during seasonal periods and with tides demonstrate a larger BOD during downwelling (rainy) and outgoing tides, further supporting a within bay source of elevated oxygen demand (Figure 6, upper right). BOD from the upper bay, to the intakes and within the hatchery shows a stronger signal in June 2020 than Feb 2020 (and nearly twice the BOD in 2020 than 2019. Additionally, it appears that the treatment in the WCSH removes much of the BOD (which would be attributed to dissolved organic matter, through their treatment systems), and the addition of larvae and algae increase this again, to levels similar to Feb upper bay waters, but far from June values (figure 6, lower left).

This may initially seem counter to indications that oxygen demand is responsible for the larval failures, but it only indicates that it is not likely that oxygen demand, and by extension dissolved organic matter, are not directly affecting larvae. Rather there is likely something else in the source waters at WCSH that is responsible for the stressors on the larvae. It may be mobilized metals within those waters, as noted with the elevated cadmium found at low tide notes above, with Cd concentrations doubling returning to baseline within 2 hours (as noted above), but at the concentrations measured 30-70 ppb, this is still an order of magnitude below published ED50 concentrations.

Objective 4 (condition and fitness of larvae)

We were unfortunately unable to make many advancements here in terms of direct measurements of larval condition. The primary intent was to determine the degree with which initial larval condition may covary with production failures. Analyses of hatchery records have been more enlightening at the WCSH where we found seasonal trends in larval survivorship, and significant effects of low/extreme tide series (and correlated water quality variables) on production. It may still be possible that seasonal effects, or transient water quality issues affecting broodstock may contribute to losses, but we are unable at this time to make any inferences on whether this may be a factor in each hatchery specifically or associated with specific failures.

Objective 5 (outreach and extension)

As a project, one of the best possible outcomes of this work would be a set of recommendations that could prevent production failures and provide possible mitigation strategies. While a smoking gun is still elusive, we have found times of day and some water quality parameters that can provide an operational approach to managing non-acidification, water quality issues. The rest of the world has looked to the Pacific Northwest for guidance on mitigating acidification in oyster hatcheries, and this work will help increase the power of the "headlights" in a colloquial sense. The primary outreach and engagement product of this research is a set of BMPs that can help guide current and future operations in regional hatcheries and elsewhere.

A draft of a concise Best Management Practices has been developed, and is being expanded with data, descriptions, a bibliography, and expert opinion on degree of efficacy and certainty surrounding approaches to mitigate water quality issues (including ocean acidification). Before producing and distributing the guide, it will first go to industry partners, then to the advisory panel, and then to WRAC for approval before release. Global Ocean Health assembled an advisory panel consisting of other scientists, industry personnel, and other stakeholders who have been communicated with regarding some of our findings. The BMPs will include approaches for both hatcheries (primary focus) and growers. Global Ocean Health will rely on their network (including a high readership newsletter), the network of other project PIs, and lead PI Waldbusser's position as part of the working group in the NOAA supported Ocean Acidification Information Exchange network to ensure a wide distribution.

Synthesis

While the primary and most relevant findings are included above, this section will clearly state findings in bulleted format.

1) A link has been established between a higher probability of production failures and transient (hour scale) measurements of significantly elevated oxygen demand. These oxygen demand events are far more likely to occur during significant tide sequences. It is important to state that it is likely not oxygen demand that is responsible for production failures, it is most likely a signal associated with waters that have had significant contact with organically rich, chemically reducing environments associated with tidal flat and salt marsh sediments. The lack of an identifiable mode of action, limits the opportunities for direct water treatments, but the link to tides (and seemingly rainfall in the early part of the production season), does allow for selective avoidance of water at the lowest of tides.
2) Buffering slurries used to improve carbonate chemistry in culture tanks, and that have been incredibly effective at stemming failures with upwelling and bay metabolic CO2 effects, contain elevated sulfide and trace metals. The concentrations within the systems studied do not appear elevated enough to have direct effects in culture tanks, but do cause some elevations in these concentrations in the tanks.
3) Additional analyses of metals and culturing indicates a possible case of metals increases (again below toxic values in tanks for aqueous forms), related to adsorption-desorption dynamics with manganese oxides. The fingerprint of this process is evident in the data, where a loss of Mn is seen from source waters to hatchery culture waters, and a strong correlation between Mn and some metals.
4) Culture tanks have a high metabolic demand, by design, however increases in dissolved inorganic nitrogen, and expected changes in redox chemistry may exacerbate the failures related to low tide waters. In many cases oxygen concertation is sufficient, but build up of metabolic waste products may be making conditions worse. Anecdotal evidence of more frequent tank changes or differing sizes of larvae in flow-through systems provides additional support for this posit. This is an additional direct action that can be utilized to help reduce the risk of failures.

Statement on Data Availability:
Following the interest in accessing the primary data that was stated following last year's annual report. I wanted to provide a statement here that I am willing to share any data collected through this project with interested parties, with the following contingencies.

- It will take several months to clean up, fully organize and develop metadata to be associated with the data. These initial steps must be completed before data would be available to others outside the project.
- I will clear all requests for data with the industry partners. Some data collected and presented includes proprietary information.
- It will take a year total to finish working on the data sets and draft 1-3 publications for peer review, prior to making data fully available.
- Any reasonable requests prior to the complete organization or publication submission/one-year timeframe will be happily considered.

IMPACTS:

Relevance: The oyster seed crisis in the mid to late 2000's struck multiple commercial shellfish hatcheries on the US Pacific Coast, creating a shortage of seed for growers. The primary cause of this was identified to be ocean acidification, and strategies adopted by hatcheries to mitigate these effects were generally successful, including monitoring carbonate chemistry, buffering hatchery waters, and changing the timing of spawning oysters, both in day and seasonal time scales. Despite these efforts however, hatchery operators still contended with unexplained failures, and demand for seed was growing within the industry.

Response: Discussions among the project scientists and industry partners resulted in a plan to conduct exploratory research into the possible cause of these unexplained failures that happened despite the acidification mitigation strategies. The work plan was developed around a series of most likely hypotheses examining the both possible external and internal causes, primarily around metabolism byproducts, heavy metals, and other water quality issues. The team was to develop a Best Management Practices guide for water quality in shellfish hatcheries and for growers, incorporating the knowledge previously developed by the team about acidification mitigation, and the results from the current study.

Results: The results from measurements of water quality outside and inside the hatcheries, discussions with hatchery operators, and synthesis of existing and new knowledge has narrowed the potential scope to a few key issues (that vary between the hatcheries). First, tidal effects in some estuaries and bays increase the probability of hatchery failures. Second, metabolic effects in tanks are significant, with large increases in nitrogen and possible interactions with metals and buffering slurry suggest care must be taken in managing these other possible effects. And third, it is reasonable to assume that the interaction of these external (and somewhat predictable events) co-occurring with internal tank effects can amplify production challenges.

Impact: Within the first couple years of the project, study hatcheries have already included oxygen concentrators to help alleviate possible oxygen consumption issues. Identification of low tide issues (at WCSH) or other deep-water issues (at TH) provide cost-effective manageable mitigation strategies. The production of a BMPs for hatchery and grower water quality management will help transfer knowledge from this funded work, and industry partners, to other industry stakeholders, as more growers appear to be setting up their own smaller hatcheries.

Collaborators: An advisory panel of 12 members from the US Pacific and Atlantic coasts, of industry, stakeholder, and academic partners have been interfacing through the Outreach PI/Program of Global Ocean Health.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PUBLISHED:
Attached per WRAC guidance

SUBMITTED BY: ____________________ **9/16/2020**________

Title: PI ,George Waldbusser   Date

APPROVED:

Project Monitor: Ron Hardy　　　　　　　　　　9/25/20
Date