Assessment of Measured vs. Modeled Ammonia-Nitrogen and Zone of Mixing-Dilution Analysis at Blue Ocean Mariculture, Island of Hawai'i

Final Report Prepared for

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Cover Photos. Left: SeaStation 3000 cage at Blue Ocean Mariculture on surface. Right: *Seriola rivoliana*. Both courtesy of Blue Ocean Mariculture.

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EXECUTIVE SUMMARY

- The purpose of this study was to measure and computer model the assimilative capacity for water quality parameters that are measured within the existing current zone of mixing (ZOM). The study collected water quality and water flow data to investigate dilution parameters and assess the effectiveness and design of the current ZOM boundaries. The results indicate that the existing ZOM configuration is sufficiently large enough to allow for mixing and dilution without any changes.
- Field measurement and computer-modeled estimates of dissolved inorganic nitrogen wastes were made from a single, large submersible net pen offshore of the leeward coast of the Big Island of Hawai'i that rears a high-quality amberjack known as Hawaiian Kanpachi (*Seriola rivoliana*). Most marine fish excrete waste nitrogen, principally in the form of ammonia nitrogen, from normal metabolism of protein in their diet.
- Sampling upstream and downstream of a submersible net pen at the Blue Ocean Mariculture fish farm was conducted by Dr. John Burns in July 2018. Protocols that I previously developed for fish aquaculture in other regions were used and coordinated with the author of this report. Current velocity was modest and near unidirectional while the fish cage had near-maximum density and biomass of fish during the tests.
- The results were as expected and similar to prior studies of properly-sited net pens in other regions that I previously conducted. A low background concentration of ammonia nitrogen, just above the laboratory detection limit, was found using analytical services of a research-grade oceanographic laboratory in Hawai'i.
- In the net pen there was a modest increase of about a factor of five above the background that declined to statistically insignificant compared to background concentrations between 10- and 25-meter distance downstream at both the surface and 6m depth. Further downstream at 50m distance nominal but statistically insignificant differences compared to upstream were documented.
- Using published literature conversion rates, the Hawai'i cages had measured concentration of the toxic form of ammonia nitrogen (un-ionized NH_3) that was 1,700 to 8,600 times less than the LC_{50} (lethal concentration of 50% of tested fish over 96 hours exposure) of studied marine fish or rainbow trout in seawater. Thus, there is no remote possibility of acute toxicity in the cages or downstream. Sensitivity testing of AquaModel at relatively slow water velocities showed only nominal increases at some sampling stations.
- There have been few studies about the chronic, long-term exposure of larger marine fish to low levels of un-ionized ammonia nitrogen, particularly in the ocean. The Canadian Governments has recently and purposely not recommended standards or guidelines for chronic exposure limits. The U.S. EPA standard of 35 µg/L is 88 times higher than the highest values observed outside and adjacent to the subject net pens. Ammonia toxicity in marine waters is extremely rare in

aquaculture but occasionally occurs in freshwater systems. There is no apparent risk of ammonia toxicity to the cultured fish or pelagic sea life downstream of the Blue Ocean Mariculture cages.

- In Southern Chile a recirculating seawater aquaculture system rearing a very similar fish species to *Seriola rivoliana* reared in Hawaii (i.e., *Seriola lalandi*, California Yellowtail) for over 400 days had an average concentration of total ammonia that was thirty times higher than that measured in the Blue Ocean Mariculture cage. Yet the fish grew normally and without problems. Recirculating seawater systems are generally not economical or reliable at present for large-sized fish that the market demands.
- In my experience and the literature, ammonia toxicity from aquaculture is restricted to freshwater habitats due to differing salinity and pH conditions. In my 45 years of limnology and oceanography experience around the world I have never investigated or read about a marine net pen aquaculture source of ammonia that resulted in acute or other forms of toxicity of the cultured fish or other species in downstream waters. However, large municipal or industrial wastewater treatment plants that can in some circumstances be a significant source of toxic ammonia when they malfunction.
- One reasonable way to think about this is that the cultured fish in a net pen are most at risk to ammonia toxicity as the highest values of un-ionized ammonia occur there. Often the high-quality fish that are cultured are among the most sensitive to un-ionized ammonia or other toxicants. A few tens of meters downstream there is a great dilution in all forms of ammonia and moreover, it is rapidly converted to nitrate by ambient bacteria in any oxygenated aquatic habitat such as the Pacific Ocean near Hawai'i.
- The computer model AquaModel™ was setup with the same conditions that occurred during field sampling and produced a reasonably good estimate of the distribution of ammonia nitrogen with a correlation coefficient of 0.76 out of a possible maximum of 1.0. In this case, the model slightly overstated measured nitrogen production for reasons explained herein that do not apply to normal model use. Generally, the total amount of ammonia produced by fish cages is more likely to be accurately modeled than measured because of the difficulty of making rapid, accurate and highly replicated sample collections over the cross section of the cages downstream.
- Slight increases of the concentration of ammonia nitrogen were noted when AquaModel was run again with the same settings, but with a much slower current velocity of 5 cm sec^{-1}. The estimated ammonia concentrations remained far less than chronic or acute concentrations as reported in the literature or by U.S. EPA.
- Terrestrial, anthropogenic ammonia nitrogen from domestic sewage facilities has recently been documented in shallows nearshore and fringing coral reef water on the Big Island of Hawai'i A recent study from the Kohala coast of Hawai'i documents the growth of nuisance algae and possible adverse effects on the reef. Stable isotope tracking methods traced the source of the problem to sewage discharge from residential housing. Open ocean net pens as presently located do not seem to affect such areas.

- To date, open ocean fish aquaculture near the island of Hawai'i has maintained a high standard of avoiding adverse water column or benthic effects. A routine targeted chemical and biological monitoring system that has evolved over the years has helped manage and document this conclusion.
- Based on a prior study of the northern leeward shore of the Big Island of Hawai'i for NOAA in 2011, my colleagues and I demonstrated that future expansion of open ocean aquaculture in the region is technically possible without adversely affecting sensitive nearshore habitat. All marine habitats have a carrying capacity for aquaculture wastes and AquaModel provides first-order or better estimates of what they may be and guidance for optimum cage or fish farm locations to ensure that nitrogen discharge does not impact sensitive marine habitats such as nearshore reefs.
- Field studies using drifters (drogues) that are both graphic and easily understood can be used to validated the AquaModel results when it is powered by far-field circulation model input that provide a representation of varying currents and direction of flows over large areas, rather than a single-point current meter as was appropriately used in the present study.

INTRODUCTION

Environmental monitoring of fish net pens usually focuses on the sea bottom effects as they are typically the most measurable results of such operations. The sea bottom acts to integrate the amount of oxygen-demanding wastes deposited that is then assimilated and oxidized by bacteria and benthic organisms and often flows into the entire food web including pelagic organisms and even seabirds (Rensel and Forster 2007). It has been possible to design and operate fish farms so as to minimize the amount of wastes to limit or entirely avoid adverse biological effects but it requires diligence, advanced forecasting tools and/or monitoring for operational adjustments.

Measurement of water column effects of net pens including dissolved oxygen reduction from fish respiration and dissolved nitrogen enhancement from the metabolic excretion of the fish has been conducted and reported from several geographic locations with different species of cultured fish. Water column effects are more transient and variable due to changes in velocity and direction of flow and variable background water quality conditions. These effects are only detectable near individual cages due in part to dilution but waste nitrogen is a biologically dynamic substance and is assimilated by the aquatic food web. Just like benthic effects, a very small amount of wastes constitute enrichment, but as a threshold of flux increases, the effects can become adverse. In shallow nearshore environments with extensive aquaculture development and weak currents such discharges can be very problematic as has been reported from some countries in Southeast Asia. In the U.S., Canada, Australia, Norway and Scotland managers and regulators use monitoring and increasingly computer modeling to predict and track environmental effects, often before permits are granted.

In Hawai'i, open ocean shelf waters (i.e., offshore of shallow coral reefs in ~50m or more depth) are oligotrophic (nutrient poor) and generally not adversely affected by human development as are shallow inshore coastal waters in urbanized areas. Open ocean aquaculture seeks to site and operate in deep waters that are distant from shorelines for the health of the cultured fish and protection of the sensitive nearshore environments.

The concept of carrying capacity for fish net pen operations is becoming well established in nations with aggressive environmental conscience and regulations. Carrying capacity may be defined as the amount of fish production that results in no measurable or probable adverse change to ecosystem components of the specific habitat where fish farm operations occur. Carrying capacity is usually focused on dissolved nutrient waste management, not benthic effects as the latter are restricted to sediments nearby a fish farm. Dissolved wastes are a factor to be considered when an area has many fish farms that cumulatively can be a significant contributor of nitrogen locally. I am a member of a computer modeling team that previously illustrated the use of such a far-field AquaModel to assess the potential effects of many farms along the north, leeward coast of the Island of Hawai'i (O'Brien et al. 2011). Individual site location is a key factor in the distribution of wastes, some locations are much better than others based on the absence (better) or presence (less suitable) of eddy (gyre) circulation that forces water parallel, offshore or toward nearshore, respectively.

This study reports the results of dissolved nitrogen monitoring upstream and downstream of individual and distantly-spaced submersible net pens at one of five cages operated by Blue Ocean Mariculture offshore of the leeward shore of the Island of Hawai'i. The conditions during sampling were such that the results are considered conservative including maximum fish biomass in the tested cage and flow velocity only about 50% of the long term (two month) current meter average velocity previously measured.

WATER QUALITY MONITORING & MODELING OF NET PENS

Water quality effects of fish net pens are often not measured for individual pens or farms for several reasons compared to sea bottom (benthic) effects. Benthic effects are typically more apparent and measurable and have accordingly been the focus of required monitoring associated with permitting, although open ocean fish farming obviously occurs in much deeper water than inshore or nearshore fish farming, and thus adverse benthic effects are easier to avoid.

I previously conducted numerous field studies of the amount of dissolved nitrogen produced by net pens as part of NPDES permit required monitoring in Puget Sound (Washington State) for relatively large commercial salmon net pens. I also contributed to computer modeling and testing designed to predict the quantity, distribution and biological effects of waste dissolved nitrogen discharge for AquaModel fish farm simulation software.

Some of the field data were reported in WDF (1990) and subsequently the requirement to monitor was eliminated by the Washington Department of Ecology as the results indicated no measurable change more than about 30m downstream. For several species of fish, it is simpler and more accurate to use laboratory or physiology models with fish biomass data to estimate nitrogen production compared to the complexities of sampling around these large structures, particularly when there are changes in flow direction that relates to complex bathymetry.

My experience is that even very large net pens that are suitably-located have little measurable effect on ambient levels of dissolved oxygen or concentrations of ammonia outside the pens. This is due to the tremendous volumes of water that flow through net pens, and the fact that ammonia is rapidly oxidized to nitrate by bacteria on and around fish farm structures as the water never becomes hypoxic.

In the 1970s and early 1980s there were large fish farms in some locations in the U.S. and elsewhere that had significant adverse effects on the sea bottom including the previously largest fish farm in the world in Clam Bay Washington. The fish farm was located in relatively shallow water and had 160 contiguous net pens and food conversion ratios and feed loss rates resulted in the sea bottom becoming anaerobic (Weston 1986, 1990). But the net pen system was totally revamped many years ago, reconfigured, moved to deeper water with better currents. Feed loss decreased and food conversion to fish flesh ratios improved. Routine monitoring for decades has shown that it has since then never exceeded sediment effects standards at the 30m distance (Washington Department of Ecology Routine NPDES Sampling Reports). I conducted most of the routine monitoring in this case and the data reports are available from the Department of Ecology. NOAA scientists summarized some of these types of advances for net pens in the U.S. in a journal publication (Rust et a. 2014).

For inshore aquaculture sites, the tidal currents vary in direction throughout the tides and it is difficult to find a suitably-long period of time to measure downstream at different distances without a change in flow direction that results in measurement accuracies due to the lack of a straight through pipeline effect. In the open ocean and in tropical areas tidal effects are usually less important and prevailing currents and winds are primary factors controlling the water circulation. At the Blue Ocean Mariculture site a comparison of one-month with a second consecutive month's current meter record indicated that the current direction and velocity are somewhat repetitive, as discussed further below.

Water quality effects such as nutrient or oxygen monitoring of most commercial-sized net pens farms are:

- **difficult to measure** due to sampling constraints such as the need for replicated but separate samples from individual stations for adequate statistical analysis. The complex hydrodynamics and turbulence downstream of net pens requires that replicate samples be sampled in order to ascertain the variability of the resulting effects.
- **minimal** due to the large amount of initial dilution and volume of flow-through water compared to on land, pumped water facilities that funnel all their discharge through a single or set of pipes.
- **transient** as there are temporal changes in fish waste production related to time of first daily feeding and in tidally-influenced environments dilution greatly increases with peak tidal flows. Dissolved oxygen effects of very large fish farms, and presently this usually means salmon farms, are virtually non-existent as all major salmon farming companies use mechanical aeration to maintain reduce the possibility of fish stress that can slow growth or predispose the fish to fish health issues. As a result, these cages that used to have a measurable reduction of dissolved oxygen downstream a few tens of meters are now a source of elevated dissolved oxygen. Large but isolated single cage farms such as Blue Ocean Mariculture do not have the concentrated biomass of fish that many salmon farms have with many contiguous surface cages.
- **possibly misleading** unless accurate collection and analyses of dissolved nutrient discharge is performed. This demands access to an accredited, professional or academic laboratory that uses autoanalyzer technology and experience. Although reasonably priced, there are mandatory field filtering and sample processing and storage requirements and if not done correctly, some measures like ammonia nitrogen may result in major inaccuracies. Inexpensive test kits and even test equipment that costs thousands of dollars but is designed for wastewater discharge assessment are without exception useless for net pen water quality monitoring.
- **sometimes difficult to interpret** as nutrients such as nitrogen are not conservative properties but change rapidly over time and space through the nitrogen cycle. Oceanographers rely on and

generally report their results in terms of molar units for many reasons and flux calculations (i.e., flow rate times concentration) rather than concentrations. The reaction of micro and macroalgae to nutrients is particularly complex, as the physiological requirements vary among species and there can be beneficial as well as adverse environment effects of adding nutrient to coastal or open ocean waters at varying rates.

This report focuses on one form of nutrient effects, i.e., the excretion of ammonia by the fish and the resulting concentrations that occur immediately downstream. In countries and regions where fish aquaculture is massively large compared to the U.S. there are cumulative impact issues that are often not addressed where terrestrial sources of waste nutrients have flooded coastal waters with massive loads resulting in harmful algal blooms (i.e., the so-called "red, green, blue-green and other colored tides"). Hawai'i has issue with nearshore aquatic eutrophication in some locations, but is surrounded by the open Pacific Ocean where the background concentrations of nitrogen and phosphorus are extremely low and transport rates (circulation) is relatively strong.

Aquatic eutrophication is a cumulative process, often of multiple sources, so that all sources of anthropogenic nutrients should be controlled where waters are sensitive to perturbation. The east coast of Florida is a good example with persistent harmful benthic macrophyte blooms clearly linked both to sewage injection into groundwater and possibly the persistent fish kill "red tides" that may be exacerbated by human development. Evidence of eutrophication in shallow, nearshore waters of Hawai'i from human waste disposal systems, golf courses and other nearshore sources do exist. My prior work for the native Hawai'ian community on the leeward shore of O'ahu allowed me to examine the extent of this in published government data records and with field sampling that had to be adjudicated by federal circuit court in favor of the local people over the federal government.

It is instructive to briefly examine what other states have done with regard to nutrients and commercial fish aquaculture. In Washington State, routine monitoring of net pens required by the Department of Ecology once included annual measurement of the effects of fish net pens on dissolved oxygen and dissolved nitrogen (mostly ammonia) production under worst-possible-case conditions. Most teleost fishes produce ammonia nitrogen that is excreted from their gills as a result of protein deamination. I conducted most of this monitoring in addition to sea bottom monitoring and developed methodologies to accurately measure the effects that were reported in annual permit reports and partly summarized in a State of Washington Programmatic Impact report (WDF 1991 and appendices). I have also conducted several laboratory experiments to determine the amount of ammonia produced by various fishes including moi (*Polydactylus sexfilis*, Pacific threadfish, a culturally-important fish in Hawai'i), that also produced some measurable urea (an organic form of dissolved nitrogen) in addition to ammonia nitrogen. These laboratory experiments confirmed estimates of nitrogen excretion estimable from mass balance computer modeling of fish physiology that my colleagues and I use for operation of AquaModel, as described below.

After about a decade of routine annual monitoring of dissolved oxygen and nitrogen flux in Washington State, I petitioned the government that it was clear that the effects were not measurable more than about 30 meters downstream and that the necessity to repeat monitoring annually was unclear. Moreover, all the commercial net pens in Washington State are purposely located in non-nutrient sensitive waters and do not affect harmful bloom initiation (Rensel et al. 2010, Lewitus et al. 2012). For dissolved nitrogen flux results, we found that the levels of dissolved inorganic nitrogen (i.e., total ammonia, nitrate and nitrite) were very slightly above upstream conditions during worst case conditions of peak annual biomass and low flow velocity and not statistically different than upstream beyond about 30m downstream and often less. In the case of oxygen flux, only very minor reductions of dissolved oxygen were found within a few tens of meters downstream.

Open ocean fish aquaculture has developed on a relatively small scale in two locations in the Hawaiian Islands over the past few decades and presently only occurs at the Blue Ocean Mariculture. Prior routine sampling and reporting of the benthic effects of the submersible net pens near Kona on the Big Island of Hawai'i in the deep waters on the coastal shelf commenced in 2007 involving the measurement of sediment total organic carbon and other measures including biological observations of benthic algal species assemblages.

Working with a team of NOAA and other scientists, Rensel et al. (2015) measured benthic conditions at the Blue Ocean Mariculture fish farm northeast of Kona on the Big Island of Hawai'i using field data collected by Dr. John Burns who also provided data for this present report. The data was used to test and validate the benthic component of a numerical computer model known as AquaModel, developed by myself and System Science Applications, Inc. Strong current flow mostly north and south mostly parallel to shore at the farm site and benthic conditions both measured and modeled indicated a tiny (0.01%) increase of total organic carbon in sediments near the cages versus reference areas a few kilometers distant.

This report presents the results of field sampling and AquaModel computer program simulation of waste nitrogen production and distribution downstream of one of the cages. Unlike many inshore farms with multiple cages placed contiguously, these SeaStation™ cages are configured to have a significant amount of distance among them allowing for excellent dispersion of wastes. It is critical to understand that dispersion of wastes is only a part of the open ocean waste consideration. More important is assimilation of the wastes by the marine food web over large areas in the strong currents prevents adverse effects of eutrophication of sea bottom and water column if planned properly. On shore non-point sources of pollution seep into the sensitive, shallow nearshore area and are poorly dispersed and as discussed herein, may lead to adverse changes in nearshore flora and fauna. The goal of open ocean mariculture is to avoid contact with the shallow, nearshore zone and utilize nutrient dispersion into the often oligotrophic (nutrient poor) waters of the mid Pacific Ocean. Nitrogen is the principal nutrient limiting algae and microalgae in the region, and thus the focus of the study.

The primary goal of this study was to assess the distance of measurable transport of ammonia nitrogen in comparison to cage spacing.

Secondarily, I compared the result of the present field work to the output of a computer software package I helped develop to assess water column and benthic effects of net pens. The program known as AquaModel. It was previously calibrated and validated for benthic effects monitoring at the Blue Ocean Mariculture site by NOAA, company technicians and the model developers as reported by Rensel et al. (2015).

The report begins with a brief description of AquaModel, then describes the field methods, software settings and results with an interpretation of the findings and comparison to known and published conditions elsewhere along the leeward coast of the Big Island of Hawai'i.

AquaModel Primer

AquaModel is a computational tool for planning and evaluating proposed aquaculture sites, assisting in permit evaluations, and assessing investment risks and opportunities. It operates on a standard PC and provides a simple interface to enter environmental and operational information. Graphical outputs map the distribution over time of key parameters including oxygen, particulate organic and dissolved nutrient wastes, algal and plankton effects and dozens or other environmental and fish cultural/management parameters.

The model is being used in by governments in the U.S. (offshore San Diego and Hawai'i), Canada, South Korea, Chile, Hong Kong, and the Arabian Sea at this time. Model validation studies have been completed in some of these areas and are ongoing in other areas including for the governments of the U.S. and Chile. It is also used for carrying capacity estimates to determine potential interactions among separate fish farms and broad-scale eutrophication from nutrients. AquaModel is the model of choice for the U.S. National Oceanic and Atmospheric Administration modeling group in Beaufort, North Carolina.

AquaModel is presently being adapted for seaweed (sugar kelp) aquaculture to provide ocean acidification refugia for species sensitive to ocean acidification with funding from the Paul Allen Foundation's Ocean Challenge Competition and the U.S. Navy.

AquaModel is a simulation program that provides for assessment of both farm operations and their environmental effects on coastal or offshore waters. The model describes the nutrient transformations by fish farms of both dissolved and particulate materials in the water column and sea bottom.

A system of equations describing fish growth and physiology that integrates with flow field data to transport waste from farms, assimilate dissolved nutrients by plankton, and simulate the sinking, deposition, resuspension and mineralization of fish feces and uneaten feed. A mathematical description of fish growth and metabolism consists of a nutrient budget for carbon, oxygen, and nitrogen as determined by the size of the fish, water temperature, oxygen concentration, swimming speed, feed rate and composition. Optimal feeding rates for varying environmental conditions are provided as outputs in addition to all other simulation output data in tabular form.

Figure 1 shows an aerial view of dissolved oxygen concentration simulation at an existing 12-pen farm in British Columbia. Plots surround the main image including oxygen transect (left center, along a user specified red line), a dissolved oxygen vertical profile through one of the pens (center, top), a time series of surface and bottom current speed and sediment organic carbon concentration transect (top right). Many other plots and forms of numerical output are available (including dissolved nitrogen and phytoplankton response) and all are updated at each user specified time steps varying from minutes to hours to days or months. Benthic outputs include sediment total organic carbon, oxygen and sulfide concentrations and virtual aerobic and anaerobic bacteria population abundance that respond to changes in temperature, organic carbon loading and effects of particle resuspension and transport.

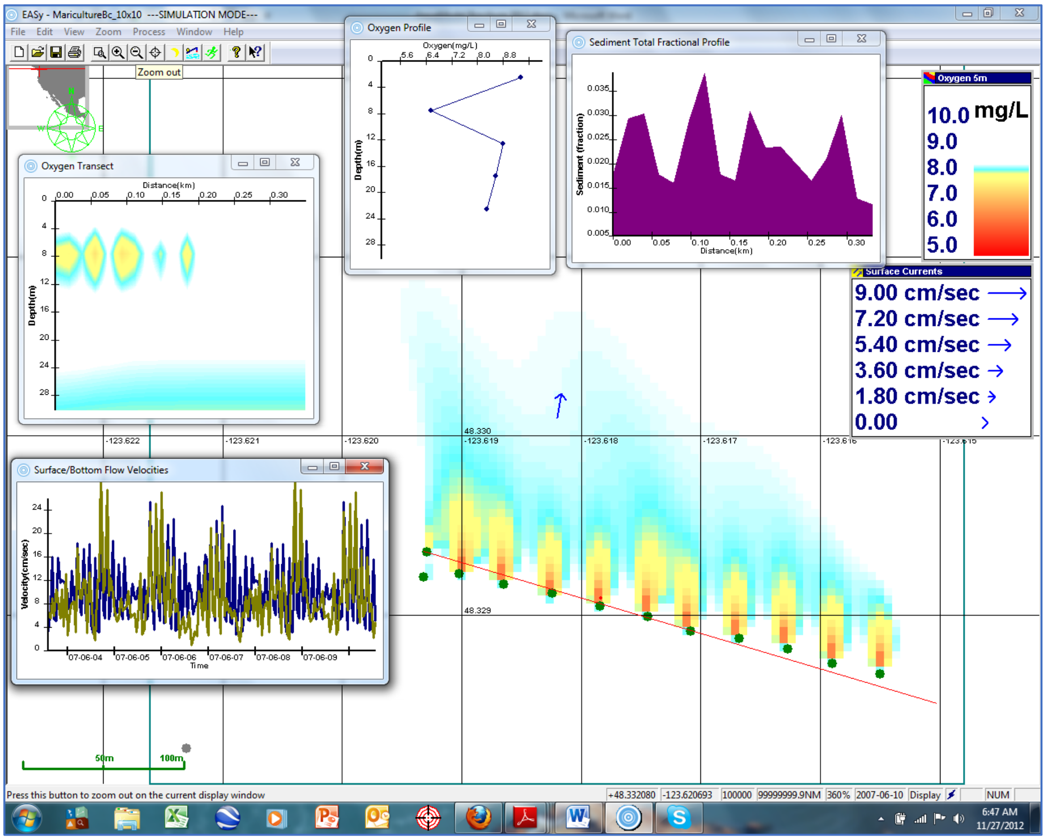

Figure 1. Example of eleven fish cages in British Columbia showing oxygen reduction and recovery downstream along with current velocity record being used and a plot of sediment organic carbon effects[1/]

AquaModel has been used to simulate single (near field) and multiple (far field) fish farms over broad areas for Atlantic salmon (*Salmo salar, three variations for west coast North America, East Coast N.A. and Chile*), rainbow trout (*Oncorhynchus mykiss*), cobia (*Rachycentron canadum*), striped bass (*Morone saxatilis*), longfin yellowtail (*Seriola riviolani* aka Hawaiian Kanpachi), moi *Polydactylis sexfilis*), sea bream (*Sparus aurata*), hybrid grouper (two species), yellowtail (*Seriola lalandi*, aka Kingfish) and California yellowtail (*S. dorsalis*).

AquaModel provides a dynamic 4-dimensional display (3D plus time) of aquaculture and environmental processes and resides within our EASy Geographic Information System. This GIS was specifically designed for marine applications and provides interfaces to import diverse types of environmental data including satellite imagery, current meter data, modeled 3-D current data, bathymetry, and coastlines allowing site or regional-specific information to be incorporated into the simulations

[1] Note that nowadays, almost all salmon farms use aeration systems so rather than oxygen reduction in the cages they produce a plume of increase dissolved oxygen downstream!

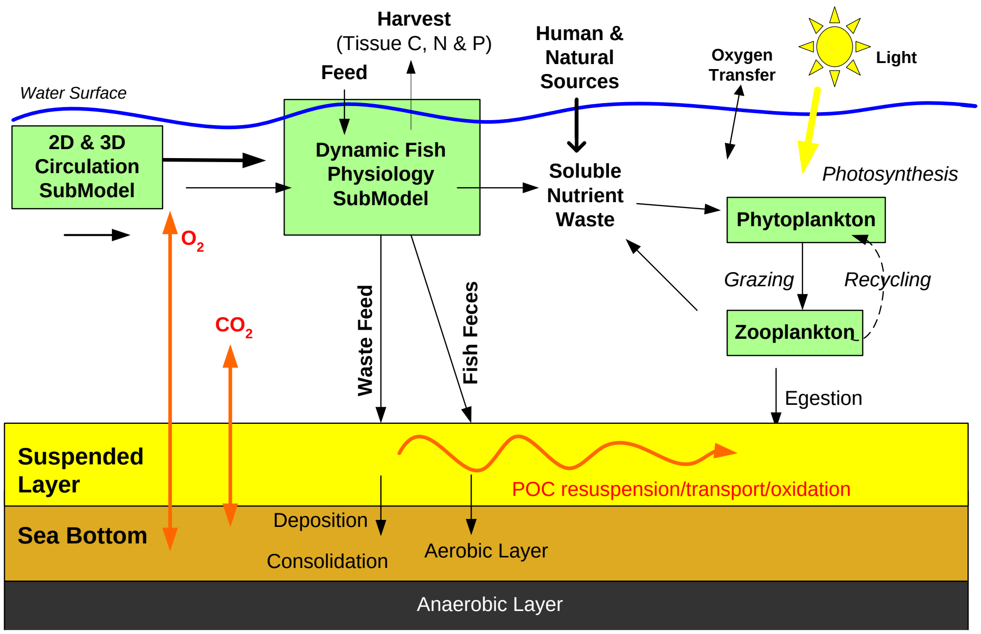

Figure 2. Conceptual water column and benthic interactions within AquaModel software.

AquaModel has been validated by comparison to detailed measurements of the growth and physiology of target fish species using extensive laboratory and by use of fish farm field data from several locations around the world. It is now used by several governments worldwide including NOAA National Ocean Survey in the U.S. It is the only software available that is capable of assessing both individual and multiple fish farms over large regions. See www.AquaModel.org for more information.

FISH FARM LOCATION & CURRENT FLOWS

The subject fish farm is located north-northeast of Kona on the Island of Hawai'i and offshore of the Ellison Onizuka Kona International Airport at Keāhole as shown in Figure 3. There are currently five SeaStation submersible cages being used by Blue Ocean Mariculture, one SS 3000 and four SS 8000. Field sampling was conducted around one cage with a high amount of fish biomass. Current flow velocity data were collected 49 times during this survey, while collecting the water samples and averaged 15.3 cm sec-1 (SD = 2.2). This is an ideal flow velocity for net pen aquaculture, relatively strong to provide exercise for the fish (that actually grow better and are healthier when they swim continuously) and also provide good flushing of the cages to remove wastes and supply dissolved oxygen. This is a typical flow rate for the Blue Ocean Mariculture net pen site, based on our prior measurements.

Currents at the center of the fish farm lease area were previously measured over a two-month period using an acoustic doppler current meter positioned in a gimbaled mount on the seafloor and looking upward toward the surface (Rensel et al. 2015). Figure 4 is a polar vector current flow diagram from that study. The term polar refers to a 360-degree circular plot matching a compass rose. The term vector indicates the plot shows combined velocity and direction frequency.

Flow velocity often decreases with depth in the ocean both inshore and offshore. In the 2015 study the deeper level flow velocity averaged from 9.3 to 13.0 cm sec^{-1}, or slightly less than that measured with a hand-held meter in this 2018 study. I tentatively conclude that flow velocity during the 2018 survey reported herein was probably similar to the two-month average previously recorded, but must caution that it is not possible to know exactly due to the above factors.

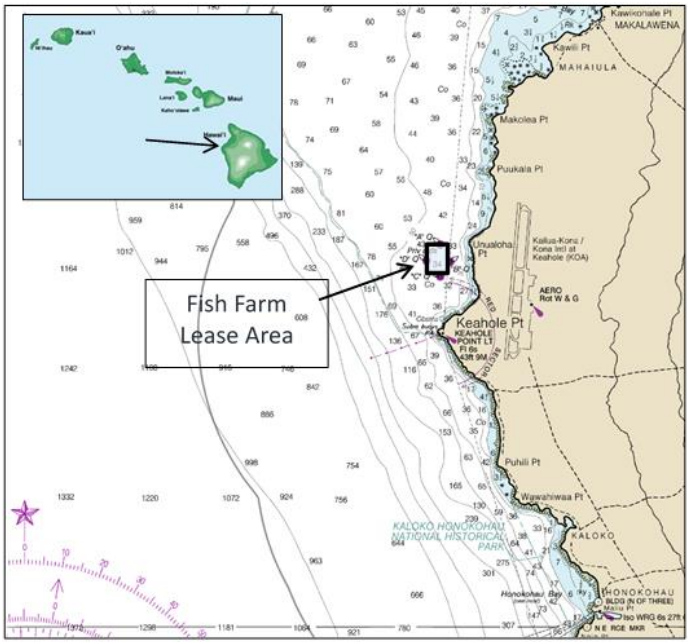

Figure 3. Vicinity map (inset) and area map of fish farm lease near Keahole Point, Hawai'i. Chart based on Blue Ocean Mariculture (2014).

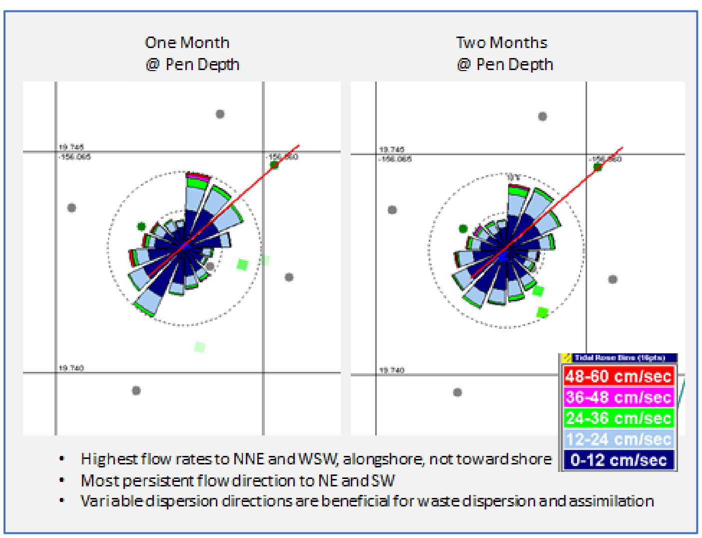

Figure 4. Polar current flow vector diagram for the Blue Ocean Mariculture lease area comparing one- and two-month duration plots from Rensel et al. (2015).

For both one- and two-month time periods the 2015 flow direction results indicate strong north and south distribution of flows with some variation to the east or west that probably associated with the effects of Keahole Point causing non-linear flow. Flow direction was uniformly to the north during this 2018 survey, as manually observed on the sampling boat's compass.

Figure 5 indicates the Blue Ocean Mariculture site and surrounding zone of mixing.

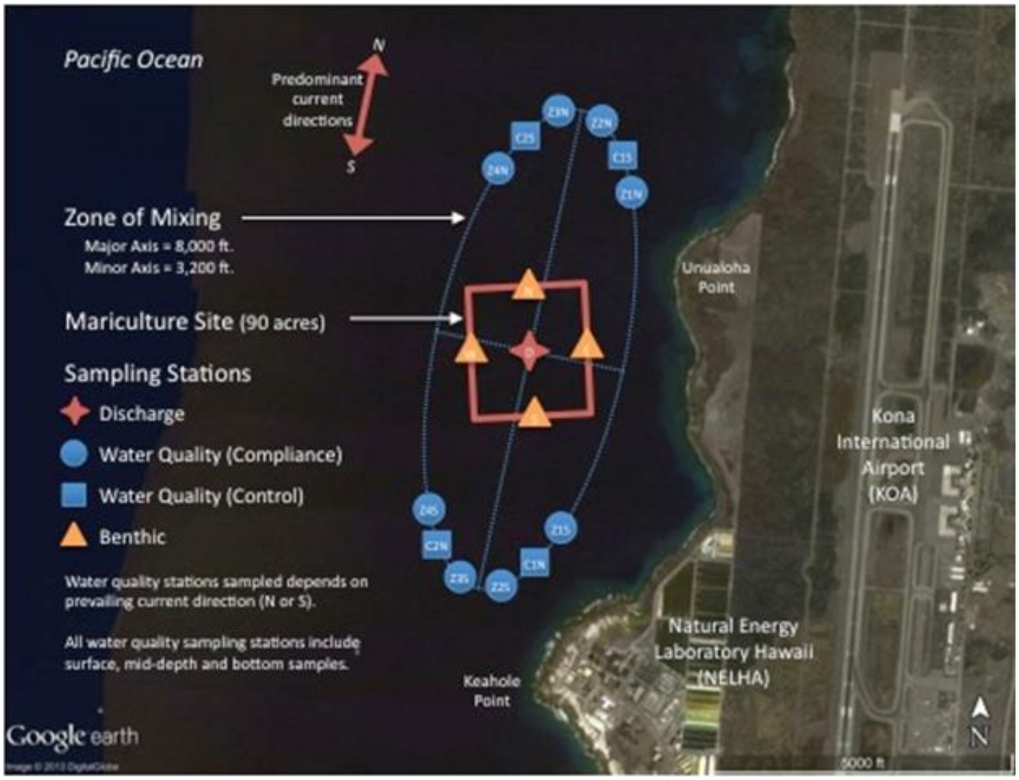

Figure 5. Map of Blue Ocean Mariculture lease area and zone of mixing specified in Permit No. HI 0021825 for the time period October 1, 2015 – August 23rd, 2020.

METHODS

FIELD STUDY METHODS

Sampling was conducted on 19 July 2018 using an acid cleaned and distilled water rinsed water bottle sampler to collect triplicate but separate samples at each sampling location from upstream to downstream. Concurrently, a portable current meter was utilized from the boat while held in a fixed location to record current velocity and direction.

Water samples collected from the monitoring sites were immediately and, in the field, filtered through pre-combusted (500° C, 6h) GF/F (Whatman) filters. These water samples were transported to the University of Hawai'i NELHA laboratory on ice and frozen at -20°C until analysis. Samples were analyzed for nitrate + nitrite (NO3- + NO2-), ammonium (NH4+), phosphate (PO43-), total dissolved phosphorus (TDP), and total dissolved nitrogen (TDN) using standard autoanalyzer methods. Values recorded below the minimum detection limit (MDL) were noted as '<MDL'. The MDL is calculated with analysis of seven of the same samples (Gravimetric Standard C4, 10, 100, 10, 10 ug/L for NH3, Si, PO4, NO3, and NO2, respectively). These samples are analyzed in order to determine the standard deviation, which is multiplied by the degree of freedom in order to calculate the precise MDL. MDL was for ammonia nitrogen for the analyses associated with this study was 4.0 µg/L (or 0.3 µM units). The MDL for dissolved inorganic nitrogen (total ammonia + nitrate + nitrite) was 8.0 µg/L (or 0.6 µM units). These

are high quality, oceanographic research-grade results that are far more sensitive and accurate than test kits and various instrumentation used in wastewater treatment analyses.

The gravimetric standards are analyzed throughout the runs to determine calibration drift. Only total ammonia data were used from the field data as the total (filtered) nitrogen data were judged to be inaccurate, a condition of that was previously found in another recent study. Un-ionized ammonia was calculated by applying coefficient to total ammonia results derived from the tables of La Ruyet et al. (1995).

AQUAMODEL METHODS

AquaModel nitrogen results include all forms of dissolved total nitrogen that are based on known food conversion and growth rates that were previously calculated for Seriola rivoliana (Rensel et al. 2015). However, there is no definite method to partition those results into ammonia (inorganic nitrogen) and urea, amino acids and other forms of organic nitrogen production for this species. I could find no published and peer-reviewed literature regarding (e.g., including urea that many marine fish produce but in lesser quantities than ammonia) and accordingly were reduced by a factor of 0.3 to estimate total ammonia concentrations.

Standard settings previously utilized for use in Hawai'i (Rensel et al. 2015, O'Brien et al. 2011) were employed for the modeling task. These included the following:

Current velocity: 15 cm s^{-1} (to match that measured in the field) for trial one, 5 cm sec^{-1} for trial two.

Current direction: True North

Horizontal dispersion: 0.1 m^2 sec^{-1} (See Kiefer et al. 2011 for origin of this factor and Rensel's field work validation)

Vertical dispersion: 0.001 m^2 sec^{-1}

Dissolved inorganic nitrogen background: 0.4 µM

Water temperature: 27˚C

Wind speed: 4.5 m sec^{-1}

Dissolved oxygen background: 7.4 mg L^{-1}

Fish physiology submodel: *Seriola rivoliana*

Horizontal array grid size: 5 x 5 meters

Fish biomass: 280 MT

Feed loss rate: 4%

Data was acquired from nitrogen plots using the hover over with cursor method that highlights specific depths and distances from a cage.

Display settings were set to actual data output rather than contouring mode.

Data was acquired in molar units (µM = microgram atoms per Liter) and converted to weight basis (µg/L = micrograms per liter also known as parts per billion) by multiplying by molecular weight of nitrogen (14). Capture runs were made for one day at 10-minute external time step intervals based on internal ~second intervals of computation. Bathymetry was from Rensel et al. (2015) but not an important factor. Modeled data were collected from 0.5 m and 7 m depths to compare to measured data.

The plume emerging from the cage is subject to horizontal diffusivity coefficients that I have personally confirmed by simultaneous multiple drogue release and high-resolution GPS tracking around net pens. In O'Brien et al. (2011) we reviewed the diffusivity literature and noted that great variation occurs in some case but both my measurements and that a colleague scientist working in the Mediterranean Sea (Dr. Chris Cromey, formerly the principal architect of another single fish farm model that only estimates benthic effects) both validated in field work downstream of net pens. In the ocean, horizontal diffusivity far surpasses vertical diffusivity, a fact that first emerged from the pioneering hydrographic work of Professor Akira Okubo at Stoney Brook University.

One cage was measured and modeled. The other four cages were the same volume and dimensions but were spaced over relatively large distances from each other.

AMMONIA TOXICITY BACKGROUND

Ammonia toxicity to marine life is a function primarily of pH as well as temperature and salinity that controls the relative amount of toxic unionized ammonia (NH_3) versus the ionized form (NH_4^+) that together comprise total ammonia nitrogen (TAN). Compared to freshwater, the toxic version in seawater is a much smaller percentage and as a result, ammonia toxicity associated with marine aquaculture is rare except in malfunctioning recirculated seawater systems.

There are two categories of consideration in this topic and most all other pollutants: acute (short term, higher concentration) exposures that may result in fish mortality and chronic (long term, low concentration). Acute toxicity is of un-ionized ammonia is relatively well known for some species of fish including juveniles or in some cases adult fish as well as for invertebrates. Chronic exposure concentrations and effects are much more poorly described because of the difficulty in maintaining constant conditions for long periods of time with live fish that are eating and excreting actively.

There are few simple standards for ammonia discharge worldwide, either initial or outside a dilution zone due to the complexity of the literature with regard to species-specific acute and chronic exposure levels. This is particularly true for saltwater as it is difficult to adjust and maintain pH in bioassays, except for short term acute exposures.

Environment Canada (2010) concluded that "*It should be noted that due to the paucity of ammonia toxicity data on marine organisms; currently, there is insufficient information to adequately derive a full or interim guideline for the protection of marine life. As a result, no marine guideline is recommended*".

U.S. EPA (1999) makes the same conclusion in a very dated and less-well-documented manner and since then has focused on freshwater habitats for ammonia standards exclusively. Ten years previous, U.S. EPA (1989) proposed a chronic effects-based limit of 0.035 m L^{-1} based on sensitive marine species studies (equivalent to 35 µg L^{-1}, the units principally used in this report).

In the present study, the ratio of ionized ammonia to un-ionized ammonia was calculated to be about 20.2 or in percentage terms, 4.95% of measured TAN values. This was estimated using published tables (reference) at pH 8.0, water temperature 27˚C and salinity of 35 psu (Spotte and Adams 1983). The pH was conservatively set slightly below that seen in recent years for University of Hawai'i data from ocean station ALOHA to be conservative.

For example, LeRuyet et al. (1994) performed controlled laboratory assays of ammonia toxicity (LC50 for 96-hour exposure) for several marine fish finding a range of 1.7 to 2.6 **mg/L** for 96-hour period based on the LC50 (lethal concentration for 50% of the test fish)**.** I highlight the units as they are a factor of 1000x more than **µg/L** and are therefore equivalent to 1,700 to 2,600 µg/L. In another study, the 96-hour LC50 with un-ionized ammonia exposure to rainbow trout (*O. mykiss*) in seawater was tested in various concentrations of dissolved oxygen ranged from 2.6 to 8.6 **mg/L** (see review by Environment Canada 2001, much better than existing U.S. EPA reviews). There are very few reported results of long term, low level exposures of large fish to un-ionized ammonia as such tests are extremely difficult to control.

The range of TAN measured in the present study was from 3.0 to 26.6 µg/L (again, same as parts per billion). Accordingly, the toxic un-ionized ammonia fraction would have been approximately 0.2 to 1.2 µg/L. As discussed below, the literature indicates that these values are far below acute toxic levels for several marine fish.

For long term exposure in the Environment Canadian approach and for each endpoint, ‘‘*a conservative Estimated Exposure Value (EEV) is selected and an Estimated No-Effects Value (ENEV) is determined by dividing a Critical Toxicity Value (CTV) by an application factor. A conservative (or hyperconservative) quotient (EEV/ENEV) is calculated for each of the assessment endpoints in order to determine whether there is potential ecological risk*".

This approach results in maximum allowable "guideline" values of about 0.75 mg/L total ammonia for pH, salinity and temperature conditions similar to those in Hawai'i (Ministry of Environment, Province of British Columbia, 2009). This is equivalent to 750 µg L^{-1}, the unit principally used in this report and the same as 53 µM and about 30 times greater than the maximum value seen in this report. I am not stating that this is or should be a standard for net pens in Hawai'i or elsewhere, but only offer this for perspective to illustrate one benchmark that is clearly several orders of magnitude higher than found in the Blue Ocean Mariculture cages in Hawai'i.

It is beyond the scope of this document to interpret the findings of this study with regard to existing legal standards or regulations in Hawai'i or the U.S. in general. In many forms of fish culture, the cultured fish themselves are as sensitive or much more sensitive than receiving water flora and fauna. This is why rainbow trout exposure bioassays are often used by EPA and other environmental agencies to measure effluent toxicity for routine discharge quality assessment. Fish such as *Seriola rivoliana* are no exception to this generality as they are highly energetic and require quality conditions to thrive in culture.

RESULTS AND DISCUSSION

Measured Total Ammonia

Results of the upstream to downstream ammonia measurements are presented in Table 1 and Figure 6 in terms of average, standard deviation, net change from upstream to downstream and the estimated concentration of potentially toxic un-ionized ammonia. The last two column present average and net change in the units of micromoles (µM = mg atoms/m^3, the units preferred by oceanographers).

As explained below, these data indicate low concentrations of total ammonia and extremely low concentrations of un-ionized ammonia at all locations including within the cages although the concentrations there were greater than outside the cages as is to be expected. It is important to note that ammonia is rapidly oxidized by the presence of aerobic bacteria to nitrate-nitrogen that is not toxic.

Table 1. Measured total ammonia nitrogen (TAN) and un-ionized ammonia nitrogen (both as nitrogen) from up and downstream of the Blue Ocean Mariculture facility with about 280 metric tons of fish biomass, unidirectional flow and current velocity of ~15 cm sec^{-1}.

Location and Depth	Mean TAN in µg/L	TAN Standard Deviation µg/L	TAN in µM	Net Mean Change µg/L	Mean Un-ionized Ammonia	Mean TAN in µM	Net Change µM
50m Upstream Surface	4.2	1.2	0.3	NA	0.2	0.3	0
50m Upstream 6m Depth	7.7	2.1	0.5	NA	0.4	0.5	0
Cage Surface	11.4	0.4	0.8	7.2	0.6	0.8	0.5
Cage 6m Depth	25.5	1.9	1.8	17.8	1.2	1.8	1.3
Cage 12m Depth	18.1	1.5	1.3	ND	0.9	1.3	ND
1m Dowstream Surface	8.3	4.1	0.6	4.2	0.4	0.6	0.3
1m Downstream 6m Depth	9.1	1.2	0.6	1.4	0.4	0.6	0.1
5m Downstream Surface	9.6	1.9	0.7	5.4	0.5	0.7	0.4
5m Downstream 6m Depth	10.5	1.4	0.7	2.8	0.5	0.7	0.2
10m Dowstream Surface	13.5	0.6	1.0	9.4	0.7	1.0	0.7
10m Downstream 6m Depth	8.0	0.6	0.6	0.4	0.4	0.6	0.0
25m Dowstream Surface	8.1	1.5	0.6	3.9	0.4	0.6	0.3
25m Downstream 6m Depth	9.7	0.3	0.7	2.0	0.5	0.7	0.1
50m Dowstream Surface	5.6	1.0	0.4	1.5	0.2	0.4	0.1
50m Downstream 6m Depth	7.8	0.7	0.6	0.1	0.4	0.6	0.0

Recall from the method section that the detection limit for total ammonia for this survey was 4 µg/L and the surface background concentration upstream of the cage just slightly higher than that value. By 50 meters downstream the surface concentration was 5.6 µg/L and nearly returned to ambient conditions. The six-meter depth water had returned to background condition on a statistical basis (i.e., 7.7 µg/L upstream and 7.8 µg/L at 50m downstream).

The plot of surface and six-meter depth total ammonia shows the maximum values occurred at 10m downstream which is somewhat unusual. In other such surveys the maximum concentration I have found was within a few meters of the cage and declined progressively. The large standard deviation bar around the 1m downstream distance surface results suggests that water flow turbulence may have been high and thus a large difference in the replicate results occurred there. Figure 7 shows the net change of total ammonia concentration between 50m upstream and the other measurement points in the cages and downstream.

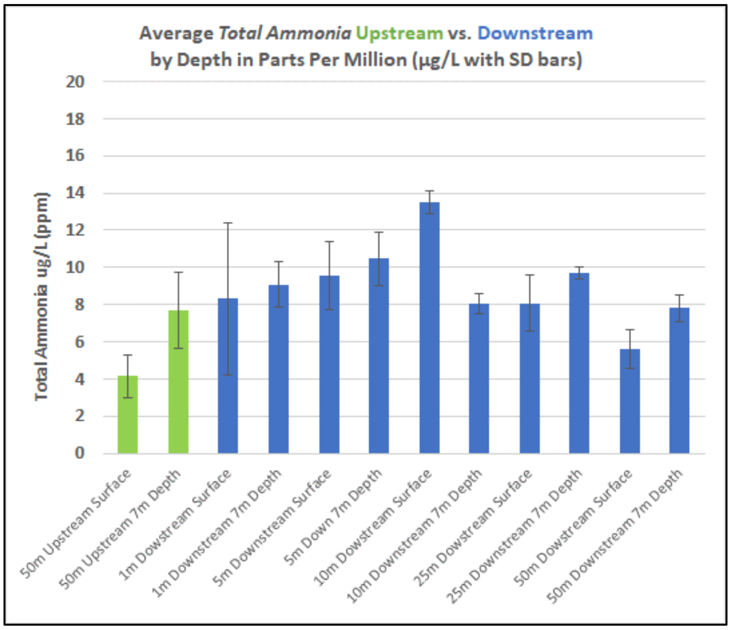

Figure 6. Average total ammonia at sampling location with one standard deviation error bars.

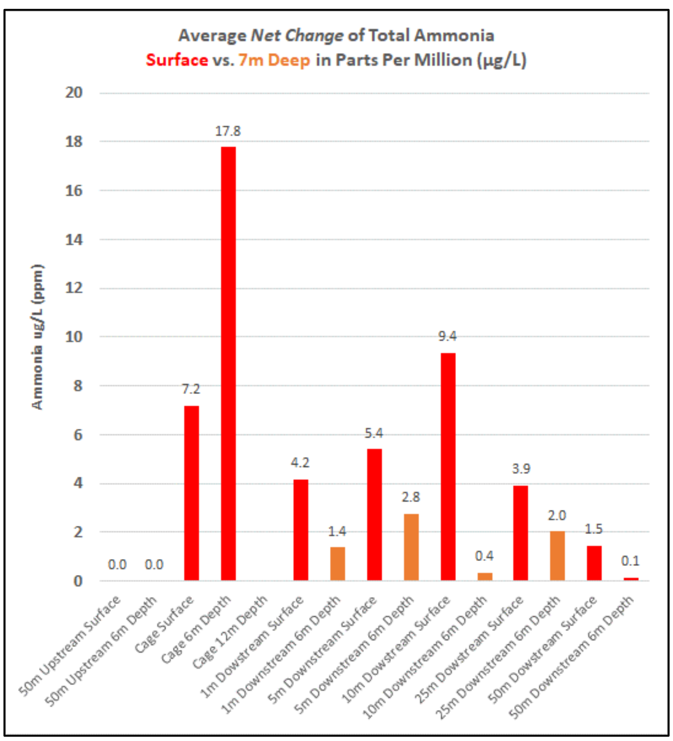

Figure 7. Net change of total ammonia from upstream to within cage and downstream.

The largest difference was in the 6m depth inside the cage of 17.8 µg/L and the largest difference at any of the downstream stations was 9.4 µg/L at the 10m downstream surface station.

Measured Un-ionized Ammonia Results

Figure 8 indicates the results converted to un-ionized ammonia units only to illustrate that very low percent toxic amount of ammonia nitrogen. I retained the Y axis the same as the prior two plots for perspective and note that all results were much less than 1.0 µg/L and therefore not detectable by the advanced laboratory analysis and reflects the estimated value derived from published tables that use pH, salinity and temperature to convert total ammonia measurements into estimated un-ionized ammonia.

All these values are far below any short- or long-term exposure sensitivities of tested marine organisms. Compared to U.S. EPA's 1989 chronic exposure criterion of 35 µg L-1, discussed above, the highest concentrations outside the cages were about 88 times less than the government's standards, developed to protect the most sensitive species.

In my work with fish culture around the world I have never found a situation where marine aquaculture produces a result that would be injurious to the cultured fish or other aquatic organisms. But in freshwater that occurs occasionally, particularly with very large hatcheries or net pens that have biomass far in excess of the 280 MT present during this study.

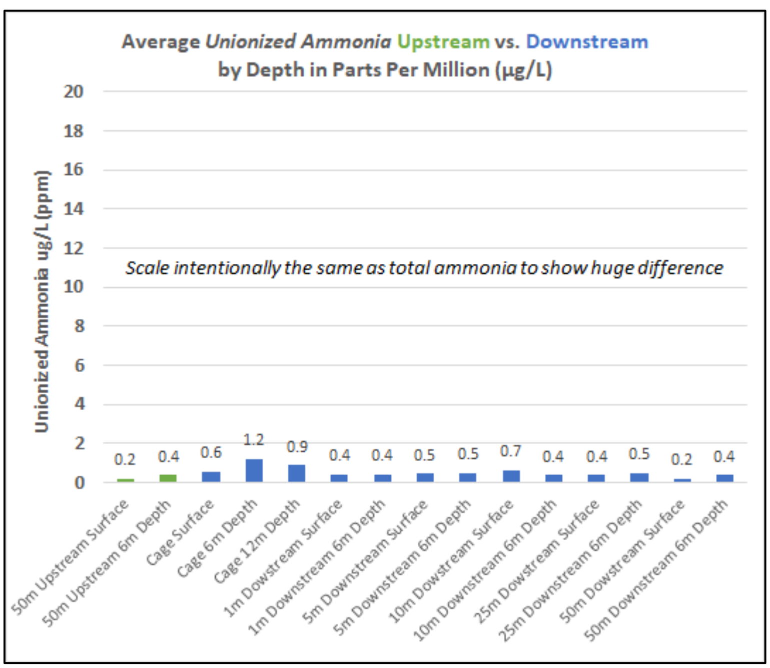

Figure 8. Un-ionized (potentially toxic) ammonia concentration estimates from field work measurements.

An example of seawater aquaculture in recirculated tanks from Chile published by Orellana et al. (2014) is useful for perspective at this point. The authors cultivated a similar fish species (*Seriola lalandi*, California Yellowtail, aka kingfish in Australia) for 448 days an average concentration of total ammonia of 420 µg L^{-1} that was thirty times higher than that measured in the Blue Ocean Mariculture cage. The authors found that the fish grew normally and without problems but recirculating seawater that was slightly cooler than Hawaiian waters. Some may opine that fish should be raised in recirculating seawater systems as they are technically possible, but repeated studies in North America and elsewhere have demonstrated they are not economical or reliable for culture of large size fish such as yellowtail or salmon but rather have a place in some cases for juvenile fish production. It is also illogical to extract fish fecal wastes and then dump them back in the sea as the salt content does not allow them to be used for terrestrial agriculture fertilization.

One important factor in marine fish culture is that flow direction in marine cages will vary at least slightly and more often more so on hourly or faster time scales. Thus, for contiguous cages or individual cages that are closely spaced, the variation in flow direction as well as velocity results in better-than-expected dilution. In rivers, this is often not the case and that is where I have measured the largest concentrations of un-ionized ammonia from large net pen facilities due to the lack of flow direction variability and the much higher percentage of toxic ammonia resulting from higher pH water.

STATISTICAL ANALYSES OF FIELD DATA

I conducted paired (two tailed) t-tests of upstream versus downstream stations for both measured depths using Statistix Version 9 Statistical software. Table 2 indicates the results of the triplicate analyses with 2 degrees of freedom in every case.

Table 2. Paired t-test results for measured data downstream or in cage vs. upstream 50m.

Station	Surface	Six meters depth
Center of Cage	Significant Difference P = 0.0008*	Significant Difference P = 0.002*
10 m Downstream	Significant Difference P = 0.0012*	No Significant Difference P = 0.008*
25 m Downstream	No Significant Difference P = 0.121	No Significant Difference P = 0.188
50 m Downstream	No Significant Difference P = 0.180	No Significant Difference P = 0.156

* = significant difference with alpha of 0.05

In addition to the above, there was no significant difference in measure total ammonia for shallow versus six meters depth with P = 0.061 although that result is borderline and with more replicates could have been significant.

The analyses indicate that significant greater ammonia occurred at both depths within the cage and at 10 meters downstream in surface waters both in surface and 6m depths but by 25m downstream no significant differences were detected. Compared to prior analyses performed at salmon net pens that were slightly larger, this is what was expected, that for this size of fish biomass detectable and significant differences downstream rarely exceed beyond 30m.

The paired t-test is a parametric and robust test that depends on the assumption of normally distributed data. With a sample size of three it is difficult to judge exactly, but no obvious outliers were apparent in the raw data. Because the samples were true, separate and independent sample grabs, it would have been difficult to take 4 or more samples at each station as the risk that the homogeneity of the water mass entering the cages may have changed with the delay that more samples would have involved.

I could have elected to use “one-tailed” tests instead and that would have minimized the statistical differences observed as that addresses only the possibility that downstream would be larger than upstream, but not less. Either way, the results are clear.

MODELED VS. MEASURED AMMONIA RESULTS

An example screen print of AquaModel output is shown below in Figure 9. The color image in the center of the screen prints is the plan (aerial) view of the net pen shown by the red circle with the north pointing current vector arrow showing the 7.5m depth only as a plan view is two dimensional only in length and width. The plume stretches out to the north and the green and yellow colors represent the estimated concentration of dissolved nitrogen that forms three parallel lines each the width of the 5m modeling array resolution.

On the left is a 2-dimension length x depth profile of the dissolved nitrogen plume with crossed lines (i.e., cross hairs) indicating the concentration found at one particular depth by hovering over with the computer mouse cursor. The value at 1m depth there was 1.35 µM associated with the yellow color. By just less than 50 meters downstream that indicator showed less than one-half of that concentration and that is how I measured the model output for various depths and distances from the net pen. One can model to higher resolution than can be measured in the field but more importantly, recall that the model output is in molar units and includes all forms of inorganic and organic dissolved nitrogen, not simply the ammonia measured in the field. This is why the values are not the same as the measured data that were in µg/L units and for total ammonia only.

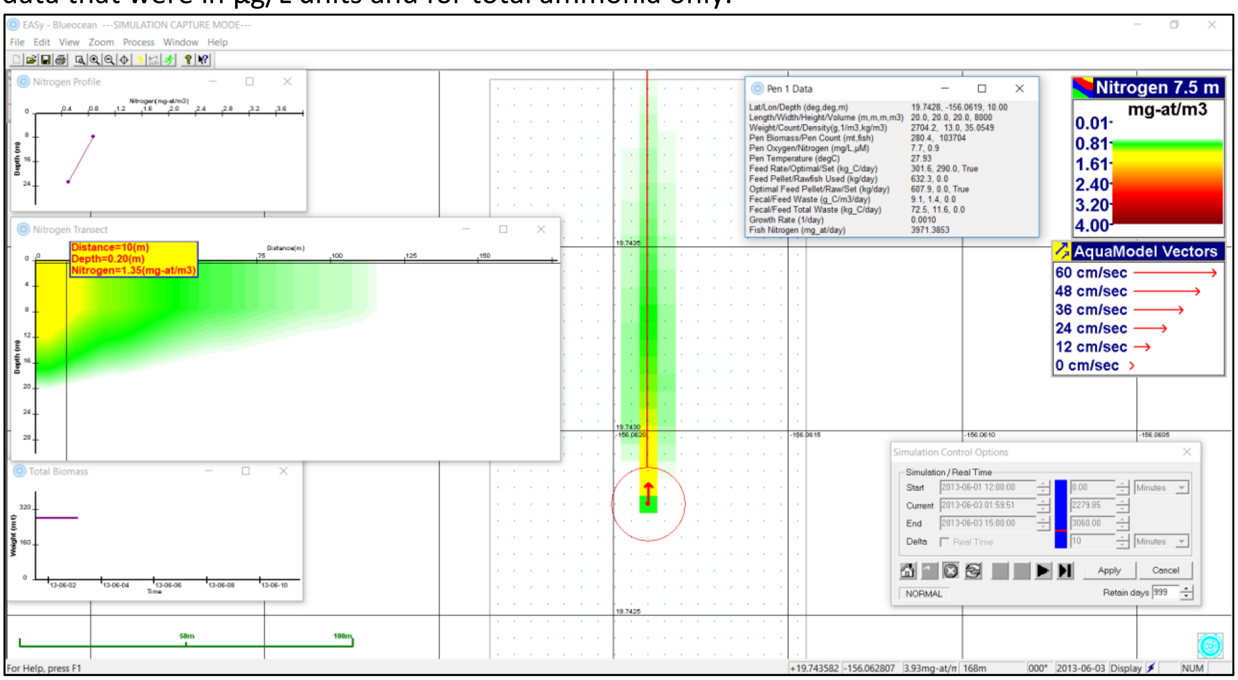

Figure 9. AquaModel screen print showing plan (center) and side profile plot (left side) of the simulation in process using a steady current velocity of 15 cm sec^{-1}.

As previously stated, the measured amount of dissolved nitrogen from nitrate and nitrite did not vary from upstream to downstream. The important factor is that the AquaModel plume overstates the amount of ammonia by virtue of being an estimate amount of total dissolved nitrogen and thus I normalized the model output in the spreadsheet analysis to include the total ammonia content only by applying a coefficient of 0.7 to the model output that represents an average found in other marine fish that have been tested. Figure 10 shows the results of the comparison.

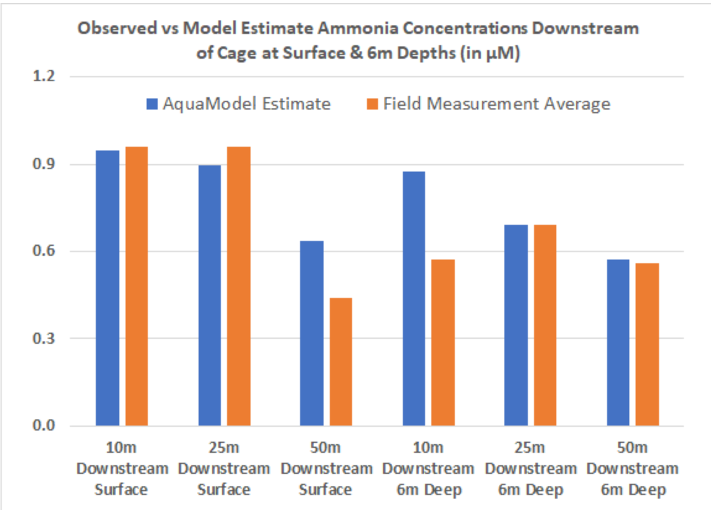

Figure 10. Observed versus AquaModel estimated water column nitrogen distribution from the studied net pen.

Given the above considerations, the final outcome shown in Figure 10 represents a relatively good correlation between measured and modeled results. The correlation coefficient was 0.76 (of a possible 1.0) for the relationship and was very tight for four of the six stations and the other two stations (50m downstream surface and 10m downstream at 6m depth) were less accurate.

The relatively small discrepancy could have been done to a few uncontrolled factors. Because the model was set to run at a constant flow rate of 15 cm sec^{-1} and the fish growth rate only changes very slightly over a day, the output remained relatively constant during the model runs so a single figure is an adequate but not exact representation of what occurred. In reality, the current velocity changed slightly during the field measurements and direction was only estimated and could result in variation not available to input to the model. The location of the bulk of the fish in the cage have changed during the measurements that took several hours. The AquaModel representation of the data are affected by the number and depth of vertical cells in the modeling array and it may be possible to disperse the fish more realistically, if that could be measured in the field. This is not, however, a top priority in model development at this time as it is more important to get the total amount of dissolved nitrogen produced correct and know its general distribution downstream and in the case of multiple farms, use these data to estimate cumulative effects.

A primary reason that water column nitrogen is included in AquaModel is to assess cumulative effects of multiple pens or fish farms over broad areas for planning and avoiding fish-waste caused water column or benthic algal eutrophication. When 3-dimensional circulation data are available, the model can also be used to estimate the frequency that a dissolved nitrogen plume from a coastal net pen farm might impinge on shallow nearshore reefs or other special habitats. 3D circulation modeling data are available from the University of Hawai'i, but in the past the available far-field model spatial resolution was relatively poor (O'Brien et al. 2011) and it would take some work to modify such models to be useful in the 50m or less resolution range although this has been achieved recently near O'ahu (Azevedo Correia de Souza and Powell, 2016) and elsewhere in the world.

SENSITIVITY ANALYSIS

Dilution of discharged dissolved substances from net pen decreases with less water current. Thus, I used AquaModel exactly as above except that the current velocity was reduced to a steady 5 cm sec^{-1} to examine any differences in the plume size and ammonia concentrations at the same distances as previously evaluated. The velocity was selected as it was the 25th percentile flow rate, i.e., 75% of the time flows were greater than that value in the two-month long ADCP current meter record for the submerged cages (Rensel et al. 2015).

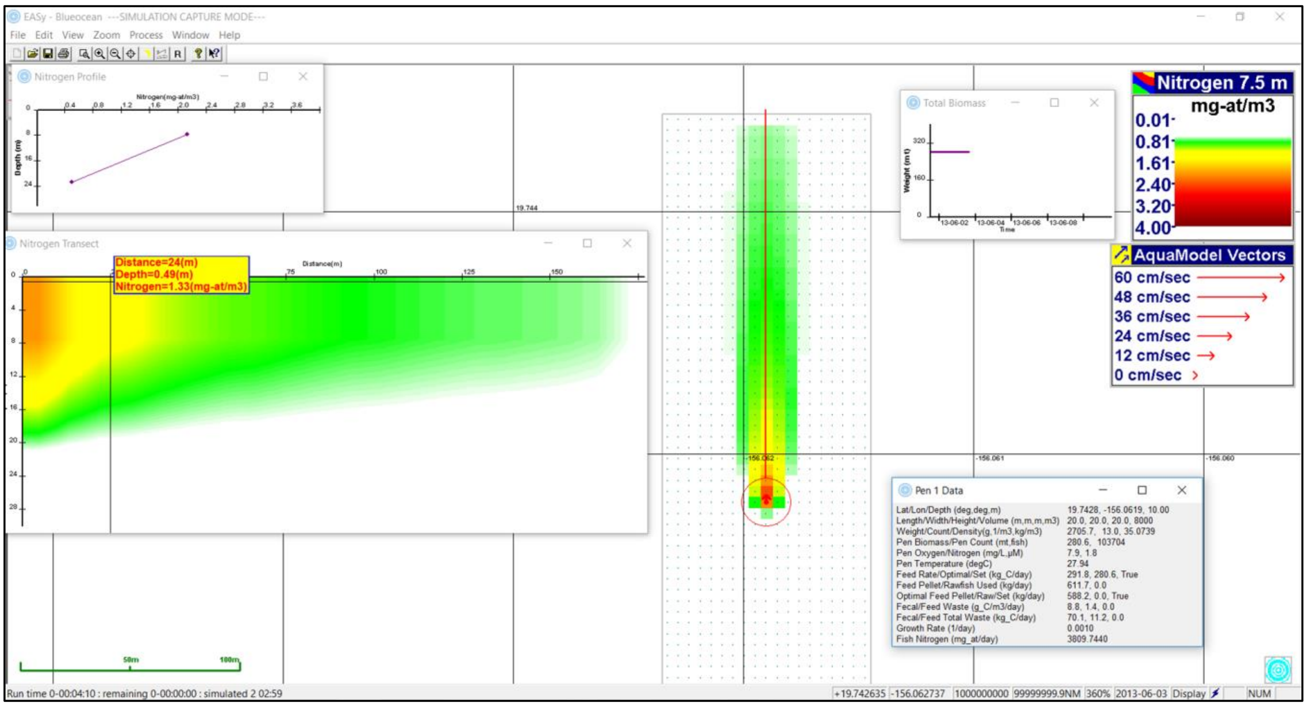

Figure 11. AquaModel screen print showing plan (center) and side profile plot (left side) of the simulation in process using a steady current velocity of 2 cm sec^{-1} displaying total dissolved nitrogen in µM units.

Table 3. Data for comparison of AquaModel estimated ammonia concentrations downstream of cage at 15 versus 5 cm sec^{-1}

Steady Current Velocity →	**5 cm sec^{-1}**	**15 cm sec^{-1}**
Distance Downstream and Depth	**Estimated Model Ammonia Results µM**	**Estimated Model Ammonia Results µM**
10m Downstream Surface	1.23	0.96
25m Downstream Surface	0.93	0.96
50m Downstream Surface	0.76	0.44
10m Downstream 6m Deep	0.88	0.57
25m Downstream 6m Deep	0.69	0.69
50m Downstream 6m Deep	0.57	0.56

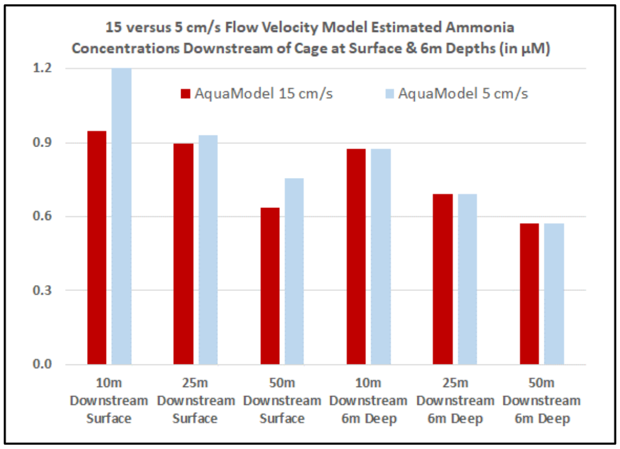

Figure 12. Graphic comparison of AquaModel estimated ammonia concentrations downstream of cage at 15 versus 5 cm sec^{-1}.

There were nominal increases in the ammonia concentration at the slower current velocity at 10m downstream surface and 50m downstream surface (Table 3, Figure 12). But no apparent differences for the other stations. However, the width of the plume increased (compare Figure 11 versus Figure 9, the two different current velocity screen prints). This indicates there was an increased amount of ammonia nearer to the cage with the slower velocity, as dilution rate had decreased. But overall the total ammonia values for either speed remained minimal for a fully stocked cage of fish. The model has the ability to measure concentrations of about 50 different parameters including ammonia at 100 different locations in the modeling domain. This utility was not used as the visual output screen print effectively communicates the outcome. I must also emphasize that these modeling estimates overstate the ammonia concentrations as there is always some small amount of current direction variation even over a short time period but the simulations run here used a fixed direction.

NEARSHORE HABITAT EFFECTS AND PROTECTION

Nearshore and shallow waters of Hawai'i can be pristine and unaffected by human influence. But in several cases, they are presently adversely affected by terrestrial development. I have worked on the issue in O'ahu where there are non-point fecal coliform and nutrient issues in some locations. On the Big Island near Puakō, a coastal community with a fringing coral reef ecosystem located in the South Kohala region, a recent study measured nutrient concentrations in shallow nearshore waters, on the reef adjacent and on the outside of the reef toward the open ocean (Abaya et al. 2018, see also associated PowerPoint presentation). Table 4 below was drawn from that study and is of interest to this present study as follows.

Note the furthest offshore waters from the reef slope surface were similar in total ammonia concentration to those measured in our study upstream of the Blue Ocean Mariculture cages. Further inshore at the surface over the reef bench, the concentration increased slightly but, in the shallows of the nearshore the concentration was about four times greater than offshore. Using stable isotope tracing techniques, the authors traced the nearshore increase to human sewage leakage and found that nuisance macrophyte algae (Ulva) contained the tracer and coral cover was inversely correlated with the apparent effects of the discharge. The authors concluded that "pollution scores revealed that sewage was largely concentrated along the shoreline, results showed some reached the reef and may be contributing to its declining condition".

Table 4. Total ammonia nitrogen (averages as N in different units) near South Kohala Coast from Abaya et al. (2018).

South Kohala Coast Study Habitat	µg/L units	SD	µM units	SD
Shoreline (nearshore)	21.28	2.24	1.52	0.16
Reef Bench Surface (at 6m deep)	7.98	1.96	0.57	0.14
Reef Slope Surface (15m deep, furthest Offshore)	5.32	1.54	0.38	0.11

Although the South Kohala nearshore results are similar to the maximum observed values of total ammonia measured in the cages in this study there are profound differences between the findings. The Blue Ocean Mariculture site is ten times deeper than the reef bench at South Kohala and the spatial extent of the fish farm plume was a few tens of meters long and a few meters wide, not over several kilometers in the Kohala Coast as shown by Abaya et al. (2018).

There was no apparent increase of nitrate and nitrite downstream (not analyzed here for brevity) of the Blue Ocean Mariculture cage but a massive increase of nitrate plus nitrite in Hawai'i coastline study of Abaya et al. (2018) for the shoreline samples of about 54 times greater than background areas offshore. This is undoubtedly due to ammonia that was oxidized by bacteria to nitrate and further shows the sensitivity of the shallow nearshore. Many algal forms can use either ammonia or nitrate as a primary nitrogen source and nitrogen is often the most important (i.e., limiting) nutrient in the sea for algal growth. The affected area received pulses of freshwater input from waste sources, a common occurrence in Hawai'i due to the coarse nature of the ground that allows rapid subsurface flows.

The above publication by University of Hawai'i scientists from Hilo demonstrate the extreme sensitivity of shallow, nearshore waters to nutrients from human sources. The housing development in the adjacent area appears not to be particularly large, but the effects were pronounced. The implication for fish mariculture in the region is to be sure that proposed sites are researched sufficiently to ensure that dissolved nitrogen from their operations rarely impinges upon shoreline areas within short time periods that could limit dilution of dissolved nitrogen. Fortunately, use ocean drifters (aka drogues) with GPS tracking is a simple but accurate means to determine circulation patterns. Whenever I use the word "dilution" I must strongly emphasize that dilution is NOT the solution to nutrient waste discharge. Rather it is only the first step and then food web assimilation over large areas without perturbation of existing aquatic fauna or floral communities should be the goal and endpoint achieved.

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ABOUT THE AUTHOR

Dr. Jack Rensel operates a small consulting company (Rensel Associates Aquatic Sciences) and is a partner in a software development firm (System Science Applications, Inc.) that specializes in in aquaculture siting, operations and environmental effects simulation modeling. Born and raised in Olympia Washington, he spent his formative years working, playing and living on South Puget Sound waters and SCUBA diving everywhere possible and as a commercial fishing boat owner and skipper fishing in the Bering Sea along the northern Aleutian Peninsula of Alaska.

At the University of Washington, he completed M.Sc. graduate research on shellfish and fish aquaculture in South and Central Puget Sound inlets with a strong emphasis on aquaculture effects studies and effects of harmful algae on cultured species. Subsequently he was a fisheries biologist and Natural Resource manager for the Squaxin Island Tribe near Shelton Washington building hatcheries, marine and freshwater habitat improvement projects and managing the treaty tribe fisheries in South Puget Sound. He returned to the UW for more training and a Ph.D. dissertation addressing harmful algal blooms (HABs) in Puget Sound at a time when little was known about it. He investigated HAB physiological and pathology effects on key aquaculture species and has since been a leader in HAB mitigation for aquaculture since then. This work led him into contacts and projects throughout the nation and worldwide that continue to this day that has resulted in 45 peer-reviewed journals articles or book chapters.

Rensel Associates is purposely named that way and not Rensel and Associates to emphasize the importance of collaborators and subcontractors in Jack's work. These relationships have resulted in several long-term research and consulting projects in six of seven continents with colleagues in leading academic institutions including Woods Hole – University of Southern California, Dalhousie University, University of Virginia, University of Hawai'i Manoa and others.

Rensel Associates is known for work with leading international shellfish and fish farming corporations, domestic and foreign governments as well as NGOs involved in native peoples' environmental rights protection. Contracts have been performed by Rensel Associates for most of the treaty Indian Tribes in Western Washington on fisheries and aquaculture topics. For example, he conducted an extensive study of Dungeness Bay Washington physical characterization as they relate to shellfish farming issues including coliform sources for EPA, Ecology and the Jamestown S'Klallam Tribe. He also has worked for several years Earthjustice in Hawai'i on behalf of native Hawai'ians and their interest in protecting the Makua valley and nearshore marine environment that has been damaged by military training operation pollutants including high concentrations of toxic arsenic in limu (edible seaweed) and explosive compounds that flow off the land into the leeward coast of O'ahu. The work including defending his positions in Federal Circuit Court in Honolulu working with Earthjustice attorneys.

Prior projects in Western Washington include numerous net-pen siting and optimization studies, development of aquaculture performance standards and regulations for or with governments and assistance to industry in special field studies including those for Taylor Shellfish and Coast Shellfish Co. Dr. Rensel has conducted stable isotope tracing studies in the Columbia River designed to assess fish farm carrying capacity and help fine tune production facilities and maintain sediment and water quality.

Rensel Associates for decades has provided special research studies and consulting for regulatory governments, fish farmers, native peoples' organizations as well as other consultants and industry in Puget Sound, Southern California, Maine, Puerto Rico, Dominican Republic, North Carolina, New Brunswick, Nova Scotia, Chile, Peru, South Korea, Hong Kong, Egypt, Italy and the United Arab Emirates.

Rensel Associates is a partner in AquaModel software development with System Science Applications Corporation, Inc. (SSA) and together they produce various types of GIS-based, ocean simulation software including Spatial Analysis (high seas stock assessment aided by satellite imagery tools) and AquaModel. Development and application of the Spatial Analysis software is principally with NASA and the Joint Propulsion Laboratory as well as the Inter-American Tropical Tuna Commission.

AquaModel is now the software of choice used by the U.S. NOAA National Ocean Survey for federal water development and is being used in South Korea, Hong Kong, UAE, Oman and Eastern Canada by governments, consultants or both. The AquaModel team has developed and are now testing a kelp aquaculture model known as Seaweed AquaModel with funds from the Paul Allen Foundation and the US Navy via the Puget Sound Restoration Fund. This model not only predicts growth of kelp, but nitrogen and carbon sequestration and seawater sweetening (increasing aragonite concentration) and related factors as a means to potentially offset ocean acidification and create local refugia for OA sensitive species.