analysis and best applicable technology be referenced instead of the existing Paragraph 7 and blue highlighted line below it.

Responses to Maine DEP's Questions included in email on March 19, 2021

From the email sent by the Cindy Dionne on Friday, March 19, 2021, Kingfish is responding to the following questions, which are also referenced by number in the comments above.

> "In particular, the Department is seeking clarification/communication from Kingfish on the following items:
>
> 1. Specific information as to alternatives that Kingfish scoped (this includes treatment/technology and resulting concentrations from each identified alternative);
> 2. Technical information that supports Kingfish's assertion that they cannot reduce nitrogen concentrations any further;
> 3. Clarification of culture/process wastewater flow (application states 6.5MGD and 6.4MGD); and
> 4. Collaboration with the Department's Compliance unit to determine a potential sampling point for BOD/TSS and discussion of potential saltwater interference with TSS/TRC sampling."

1. *"Specific information as to alternatives that Kingfish scoped (this includes treatment/technology and resulting concentrations from each identified alternative"*

And

2. *"Technical information that supports Kingfish's assertion that they cannot reduce nitrogen concentrations any further;"*

Response: Kingfish would like to address these two questions together as many of the points would be repetitive otherwise.

Kingfish Maine (Kingfish) is proposing an aquaculture facility, which will be used to grow Yellowtail Kingfish using a Recirculating Aquaculture System (RAS) approach. Yellowtail Kingfish is a marine species requiring full salinity seawater (i.e., 30ppt and above), thus why Kingfish has selected a site with direct seawater access on Chandler Bay in Jonesport, Maine. The benefits of RAS technology include reduced water consumption, improved control and greater stability of culture process, isolation from the external environmental factors, and efficient footprint.

Fish are reared in contained systems which incorporate multi-step filtration processes that allow for the majority of water to be recycled or reused in the systems multiple times prior to exchanging with an external water supply. Within the culture systems, the components of Kingfish's filtration process include standard steps (Timmons and Ebeling, 2010): mechanical filtration to remove solids, CO_2 extraction, biological filtration or bio-reactors to process nutrient loads through nitrification, protein skimming, sterilization, oxygenation, and chemical addition for pH adjustment (see Kingfish Application Attachment 4, page 7, "Conceptual Flow Diagram of RAS System"). A fraction of the total system volume is replaced on a continuous basis with new water, commonly termed "makeup water". An equivalent volume of the existing system water must leave the system to make room for the makeup water, and this is where Kingfish's wastewater treatment system takes over.

Processing plant water also goes through a primary treatment process before combining with culture water to be sent to the wastewater treatment plant (see Kingfish Application Attachment 4, page 8, "Conceptual Flow Diagram of Discharge Water Filtration"). The steps are, again, standard within the seafood processing sector and include primary flocculation followed by particle removal via mechanical filtration (typically belt filters), and a secondary flocculation and clarification. As stated, this flow then combines with the culture water leaving the culture systems to be directed to the wastewater treatment building.

Water leaving the culture systems and processing area is gathered in a collective pipe from the culture buildings (hatchery and growout) to the wastewater treatment building. Kingfish's wastewater treatment system incorporates standard steps within the aquaculture sector but also those more familiar to conventional wastewater treatment applications, which are still considered early phase development within the aquaculture sector (see Kingfish Application Attachment 4, page 8, "Conceptual Flow Diagram of Discharge Water Filtration"). Culture water goes through another round of mechanical filtration (drum or disk filters) to remove solids, followed by an anoxic chamber or upflow sludge bioreactor (USB) for denitrification. This reactor utilizes recycled fish waste from sludge collection and influent flow as an internal carbon source; internal recycle flow leaving the nitrifying bioreactor is circulated back to this anoxic chamber to break down nitrate into nitrogen gas; this is commonly referred to as a Modified Ludzak-Ettinger process or MLE. Combined flow leaves the anoxic chamber and goes through an aerobic bioreactor for nitrification and finally a sterilization step (0.4mg/L ozone or 30mJ/cm^2 UV). An additional clarification step may be incorporated after the nitrifying bioreactor if deemed necessary. All solids or sludge waste captured are collected, concentrated, and trucked offsite for disposal. Post-sterilization, water will combine with Heat Recovery Flow in a discharge reservoir prior to discharging to Chandler Bay via Outfall A or B as described in Kingfish's permit application.

Within the draft permit presented and the questions above, nitrogen removal is the characteristic of Kingfish's effluent treatment system for which the Department is seeking further clarification.

Best Available Technology in Aquaculture

One of the greatest challenges to the RAS sector, according to surveys from industry leaders, consultants, commercial producers, and researchers, is system complexity and lack of trained personnel to operate them (Badiola et al, 2012). Kingfish addresses both of these concerns in the use of a system tried and tested within our own facility in the Netherlands, designed by Billund Aquaculture, and with the building of a diverse and skilled team with backgrounds stretching across the aquaculture, and specifically RAS, sector globally. As previously stated, typical waste treatment processes in RAS effluent include coarse and/or fine particle filtration, biofilters for the conversion of dissolved excretion products in particles and gases, chemical conditioning of the water, and sterilization (Martins et al. 2010; Stavrakidis-Zachou et al. 2019; Timmons and Ebeling 2010). Kingfish utilizes each of these in its wastewater treatment design, both in primary processing within the RAS systems themselves as well as the wastewater treatment plant process. TSS, BOD, and phosphorus are adequately reduced within the proposed regulatory limits using this treatment method; some of the constituents of total nitrogen are reduced as much as possible as well. However, a significant characteristic of the effluent from conventional RAS treatment sequence is nitrate, which is the end product of the nitrification process. Kingfish Maine and its parent, The Kingfish Company, incorporate an additional step to reduce this parameter, denitrification via upflow sludge bioreactors (USBs), which is far less studied or applied in the aquaculture sector, let alone in marine applications (Almeida et al. 2020; He et al. 2018; Lindholm-Lehto et al. 2020; Martins et al. 2010; Stavrakidis-Zachou et al. 2019). Incorporation of this step in Kingfish's wastewater treatment application is a step in advancement of large-scale marine RAS. Lindholm-Lehto et al. 2020 states that trials to date have been limited and that this treatment process is still understudied with only few exceptions demonstrated beyond small scale trials. Kingfish chooses incorporation of this additional treatment as it sees (1) benefit in its existing application in reducing total nitrogen in the effluent, but also (2) the future potential for this technology in marine RAS and because (3) commercial application at scale is critical to technology advancement. With what Kingfish sees in its own facility in the Netherlands and those results demonstrated in the research sector, Kingfish's proposed effluent concentration of 6.6mg/L is verifiable and achievable for Kingfish's proposed system.

US EPA Guidance suggests MLE systems as a preferred method for nitrogen removal in wastewater treatment applications (US EPA Nutrient Control Design Manual, State of Technology Review Report 2009; US EPA Wastewater Treatment Fact Sheet: External Carbon Sources for Nitrogen Removal 2013). An MLE process "enables contact between nitrates formed at the back end of the wastewater treatment process and soluble COD generally found in the influent wastewater streams by recycling nitrate laden process flows to the head of the treatment stream" (US EPA Onsite Wasewater Treatment SystemsTechnology Fact Sheet 9: Nitrogen Removal). This process can still require an external carbon source; however, when denitrification technology has been applied in RAS, it has most typically involved an MLE or similar application utilizing fish waste as an internal carbon source just as Kingfish is proposing in its application. Utilizing fish waste in denitrification as an internal carbon source offers significant environmental and economic benefits (He et al, 2018); this will be discussed in further detail below. According to US EPA Guidance, typical N removal ranges for managed systems are anywhere from 40-80%, with the MLE process in a typical configuration demonstrating efficiency as high as 80%. This system has the highest removal efficiency listed in US EPA guidance. However, given the specific characteristics of Kingfish's effluent, achievable efficiency rates in any configuration are significantly less than EPA Guidance suggests. This is largely due to the interference of saline conditions. In addition, these processes, particularly the anaerobic upflow filters, need greater hydraulic retention times to achieve EPA's removal rates. The necessary footprint to achieve this in the case of Kingfish's development is impractical as it creates a larger land impact in addition to the management costs for a system this size; again, this characteristic will be discussed further below.

Despite its limitations in this circumstance, the system configuration Kingfish is proposing, inclusive of conventional RAS treatment processes as well as denitrification set up in an MLE configuration, is the best available technology within this industry and for this application that has been demonstrated at scale for removal of total nitrogen. Kingfish sees incorporation of denitrification at the end of the standard RAS effluent treatment train as an important part of advancing wastewater treatment technology in the industry. "The use of anaerobic denitrification to remove nitrate is not yet widely applied to commercial RAS due to its level of efficiency, its complexity, and cost" (Almeida et al, 2020). While it has been addressed in many types in municipal wastewater application, within the context of RAS, it is not widely understood or applied. Research into RAS and its wastewater treatment systems tends to focus on specific situations or setups; comparisons are difficult as these systems are not identical and thus creating a good performance standard is challenging (Badiola et al, 2012). Commercial application of new technology is critical to the industry. At the basis of Kingfish's wastewater treatment process is a tested and trusted industry standard for treatment; in addition, the company is advancing the process through incorporation of additional technology in adding denitrification for optimization of nitrogen removal.

Assessment of Denitrification in Marine Aquaculture & Kingfish's Specific Application

As stated, Kingfish's approach to the wastewater treatment process surpasses aquaculture industry standards. Kingfish's facility in the Netherlands is a demonstration of this in its certifications: the first land-based farm globally to be Best Aquaculture Practices certified and the first Aquaculture Stewardship Council certified source of yellowtail kingfish. Kingfish Company also operates in a Natura 2000 nature reserve and is thus closely monitored for its practices. Incorporation of a denitrification process, particularly in a marine farm application, is ahead of industry standard at present; it presents challenges in terms of demonstrable efficiency (He et al. 2018; Stavrakidis-Zachou et al. 2019). For this reason and detailed further below, Kingfish is conservative in our application to the Department for nitrogen removal levels; Kingfish only proposes a removal level that it has achieved in its existing farm with the same species. A higher removal rate may be achievable, but it is not yet proven specifically in a commercial scale, marine aquaculture application. While Kingfish and its parent company continue to actively engage in ongoing research both in house at commercial scale and with its university partners in order to improve operational efficiency, Kingfish feels inclusion of removal rates above what is confirmed at commercial scale in existing facilities to be presumptuous and overly optimistic. There are many limitations to denitrification's application within the operating parameters of marine RAS and specifically, Kingfish's proposed facility, namely high salinity water, limitations to retention time, and efficacy of available carbon sources.

In addition to the removal rates Kingfish sees demonstrated in its Netherlands facility, several studies have validated that increasing salinity reduces the efficiency of denitrification (Dincer and Kargi, 1999; He et al, 2018; von Ahnen et al. 2019). Kingfish's proposed facility operates with ambient salinity in Chandler Bay, which is 32.5ppt on average. At this level of salinity, salt inhibition to denitrification is significant; von Ahnen et al. 2019 found removal rates decreased between 54-69% in identical denitrifying reactors from 0 ppt (freshwater) to 35 ppt; reductions like this have been demonstrated across multiple carbon sources in saline conditions to date. In addition, less alkalinity increase was achieved in the denitrification process in the higher salinities, one of the major benefits of applying these systems in RAS; this is presumably due to a shift in microbiome in the denitrification reactor. Microbial ecologies of these systems, particularly in saline applications, are not completely understood; this will drive the success of commercial implementation as one of the research priorities (Martins et al, 2010). With what Kingfish sees in its own facility and those results demonstrated in the research sector, Kingfish's proposed removal rate included in the application to the Department is in range of what has been shown in seawater settings. Therefore, the effluent concentration of 6.6mg/L is verifiable and achievable for Kingfish's proposed system.

Although there are several water quality parameters that affect nitrogen removal rate and efficiency, hydraulic retention time (HRT) also plays a significant role (Torno et al. 2018). While retention times demonstrated within the aquaculture industry range from 0.75 – 7 hours, there is not a general recommendation for optimal HRT due to variability of denitrification performance in the various aquaculture applications. Those principles which would impact HRT include the microbiome, which, as discussed above, shifts in seawater applications, reactor type, temperature, and carbon source, among others (Hamlin et al, 2008; Lindholm-Letho et al, 2020; Torno et al, 2018); thus, suggested HRT "should be evaluated for every denitrification type separately" (Torno et al, 2018). In Kingfish's application, due to the significant flow rates, longer HRTs result in the requirement of enormous anoxic reactors, which have implications in Kingfish's land resource utilization as well as increased operational costs of energy, oxygenation in the downstream nitrification process, and additional filtration and controls required in subsequent treatment steps to accommodate changes in water chemistry resulting from longer HRT (Almeida et al, 2020; Hamlin et al, 2008; Martins et al, 2010; Stavrakidis-Zachou et al, 2019; von Ahnen et al, 2019). Kingfish's denitrification design works to optimize the balance between denitrification efficiency (percent removed) and denitrification rate (removal per unit time), which oppose one another in application of longer or shorter HRTs (Torno, et al, 2018). The system maximizes the efficiency in seawater denitrification without requiring a reactor so large as to make the process unmanageable from the perspective of denitrification stability and operational costs.

Much like HRT, determining the optimal carbon source for the denitrification process in a specific application is also a significant factor in nitrogen removal efficiency. As stated above, Kingfish proposes the utilization of concentrated fish waste from the facility's systems as an internal carbon source. Please note that Kingfish has also included a variety of potential additional external carbon sources as potential chemicals for use in future as research and commercial scale testing demonstrates an improved process (see Kingfish Application, Attachment 9, "Chemical List"). Within Kingfish's ongoing research in the Netherlands as well as that occurring within the marine RAS research community, rates of denitrification with fish waste as an internal carbon source present the best option for a balance between effective nitrogen reduction, system complexity, and cost within proven demonstrations.

Fish waste as an internal carbon source in marine RAS has shown efficient denitrification rates within various demonstrations (He et al, 2018); Kingfish's parent facility has demonstrated work showing 33% TN removal using fish waste. The limitation is the available COD within the fish waste itself; Kingfish has shown 1.2-1.8 kg COD/ kg N, which is approximately 1/3 of that needed for the anaerobic denitrification process. He et al, 2018 results show slightly higher efficiency but with a lesser salinity (15ppt) than that of Kingfish's operations. There are additional benefits of fish waste as a carbon source in comparison to other external sources; with limits to availability of carbon, the system experiences less volatility and a minimal risk of over-application as can be experienced with external inputs, resulting in downstream water quality issues.

While external sources, such as methanol, ethanol, acetic acid, and even wood chips can offer the same or greater efficiency, they also carry significant additional cost from greater physical footprint demand, increased energy and oxygen requirements in downstream processes, increased carbon footprint, and can result in other downstream water quality issues due to dosing system variability (He et al, 2018; Torno et al, 2018; US EPA Nutrient Control Design Manual, State of Technology Review Report 2009; US EPA Wastewater Treatment Fact Sheet: External Carbon Sources for Nitrogen Removal 2013). Addressing each of these points in assessment of external carbon sources as an alternative:

- Most widely used external carbon sources in RAS are methanol and acetic acid (Stavrakidis-Zachou et al, 2019). While the raw product cost alone is not prohibitive, there is extreme volatility in the price (US EPA Nutrient Control Design Manual, State of Technology Review Report 2009) as well as challenges in availability. In addition, there are indirect costs in terms of transport and increased safety measures required for safe storage and handling due to flammability and potential for combustion, particularly in the large quantities that would be required. For example, simply to address the Department's request for further reduction, Kingfish would need an additional 750kg daily or 5,250kg weekly of an external carbon source; again, this is operating at most efficient removal rates. Due to variability common in denitrification systems, there is potential to require significantly greater quantities (Almeida et al, 2020; Stavrakidis-Zachou et al, 2019). Due to supply or availability concerns, substantial onsite storage would be required, thus posing safety concerns with those more volatile sources.

- The denitrification rates of external carbon sources demonstrated in the various experimental scale references included ranges from 250-680 g per m^3 per day. Given the Department's request for an additional 207kg TN daily reduction from Kingfish's requested limit, Kingfish would need an additional $340m^3$, the equivalent of 5 standard shipping containers, at the most efficient demonstration as well as a significantly larger, separate bioreactor and physical footprint to contain it (Hamlin et al, 2008).

- A bioreactor of such significant size, particularly utilizing an external carbon source which can vary in quality and purity, requires meticulous management in order to avoid downstream water quality issues (*see bullet point below*). The system proposed by Kingfish is one that has been tested and managed by its team and shows reliable results. The same cannot be said for the configuration in the bullet point above.

- Increased energy consumption from the need for separate bioreactors to accommodate external sources, additional clarifying filters to handle inevitable downstream water quality variations, and increased oxygen generation for downstream nitrification are all results of the use of external carbon sources. With the control offered by a limited internal carbon source, such as fish waste Kingfish proposes, these are mitigated.

- US EPA Guidance states that increased carbon footprint is a result of incorporation of denitrification and the use of external carbon sources. This is only amplified with larger systems. In the previous bullet point and above in the first bullet, further reduction to the level requested by the Department would result in a significant carbon footprint increase from transport of external sources to the site, increased energy and oxygen use, as well as additional trucking from increased sludge production.

- Water quality issues that can arise from improper dosing of these external carbon sources include carryover of organic substrates, or greatly increased TSS (greater sludge production), bioreactor clogging, and phosphorus leaching, to name a few (Almeida et al, 2020; Torno et al, 2018; von Ahnen et al, 2019). Over application of external carbon sources, which can commonly occur (Almeida et al, 2020; Boley et al, 2000; Lee et al, 2000; Stavrakidis-Zachou et al, 2019), can actually interfere with the downstream nitrification process, which poses a significant threat to efficient operation of Kingfish's overall wastewater treatment system (US EPA Nutrient Control Design Manual, State of Technology Review Report 2009). Use of fish waste has shown the potential to effect TAN; however, through the use of MLE as Kingfish proposes, this can be easily controlled in the downstream nitrification reactor. The limitation of the available carbon in fish waste actually aids in control of the process.

In addition to the above mentioned factors, the technology of denitrification using external carbon sources in marine aquaculture is simply not trialed in significant quantity nor at applicable scale that the investment and the risk to the rest of the effluent treatment process in implementation is far too great (Almeida et al, 2020; Boley et al, 2000; Martins et al, 2010; He et al, 2018; Torno et al, 2018).

With all of these considerations, as Kingfish has stated, there is a balance point between implementation of tested and efficient nitrogen control solutions and application of those systems that potentially offer greater removal efficiency. As stated by Torno et al, 2018, "The optimum carbon dosage should be determined under the guiding principle: as much as necessary, as little as possible." In Kingfish's proven track record investing in sustainable choices, ongoing research, and advancement in the RAS sector, the company's goal is to exceed nitrogen removal expectations and continue to advance the industry as a whole in addition to what goes on in house. Given the information supplied above, Kingfish's approach of implementation of denitrification utilizing fish waste as an internal carbon source achieves these goals without risking unproven methodologies; the company has included in its application materials the request for those external sources so that as technology advances, Kingfish can quickly and efficiently implement greater control measures in a safe and reliable way.

Summary

Through incorporation of standard wastewater treatment practices within the aquaculture sector as well as additional steps more familiar in other wastewater treatment sectors, Kingfish Maine is proposing the best available technology for its facility. The use of the fish waste as an internal carbon source allows for both economic and environmental benefit that is not yet matched by the use of external sources in marine RAS applications. While alternative strategies exist, further development work is needed in marine RAS applications in order for Kingfish to implement them in a verifiable and reliable way; Kingfish's application to the Department has incorporated language that allows for that, and the company continues to push research and development in this area. Within the application materials presented to the Department and this supplement, which includes technical information regarding Kingfish's systems, alternatives, and maximum removal efficiency achievable, Kingfish demonstrates the capability to achieve the levels of treatment requested within the application; however, further reduction as requested by the Department is not proven feasible at a commercial scale in marine applications with reliable and proven technology.

In review of the information presented within Kingfish's application and supplemented here, Kingfish requests that the Department implement the limit on Total Nitrogen of 6.6mg/L or 1,580 lbs per day, which is protective of remaining assimilative capacity for dissolved oxygen but requires justification for the use of greater than 20% of the remaining assimilative capacity for eelgrass as an environmental response indicator. Justification for that use has been provided within Kingfish's application materials.

3. *"Clarification of culture/process wastewater flow (application states 6.5MGD and 6.4MGD)".*

Response: Kingfish requests to clarify this to 6.5 MGD. There is a typographical error in Attachment 6 of our submission materials, which states that heat exchange water is "22.3 MGD" and culture and process water is "6.4 MGD". This should state that Heat exchange water 22.2 MGD and culture and process water is 6.5 MGD. Kingfish did not identify any other areas where this error occurred; however, if the Department identifies any, Kingfish requests that the above correction be utilized.

4. *"Collaboration with the Department's Compliance unit to determine a potential sampling point for BOD/TSS and discussion of potential saltwater interference with TSS/TRC sampling."*

Response: Kingfish will make sure a water sample can be collected from the combined Culture and Process water post effluent treatment system but prior to it entering the discharge reservoir where it mixes with the Heat Recovery Water. Kingfish is also happy to discuss appropriate parameter testing methods for use in saltwater applications with the Department.

This concludes Kingfish's comments to the draft permit as well as the responses to questions posed by the Department in the email dated March 19, 2021. Should any further information or clarification be required, please feel free to email Megan Sorby or Tom Sorby at the email addresses previously provided to the Department. Kingfish would request the opportunity for additional review of the draft permit after incorporation of any amendments prior to finalization of the draft.

Thank you,

Megan Sorby

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