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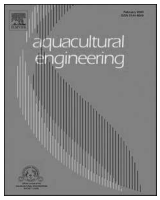

Waste characterization and biofilter sizing criteria for oligotrophic RAS egg incubators

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ABSTRACT

Nitrogenous and carbonaceous waste associated with decaying eggs, eggshell debris, and hatching-related fluids, can overload egg incubation systems. In this work, BOD_5, nitrogen, and protein are measured and compared across decaying eggs of 8 species to determine similarity in loading behavior. A statistical analysis shows that marine and freshwater species display similar egg loading. Results suggest that a unified sizing criteria developed for application across many species would suffice for incubation biofiltration sizing. A conservative loading of 12 mg-$Nd^{-1}g^{-1}$ is estimated for the egg masses of species investigated. Assuming a 50% hatching rate and decay over a five-day incubation period, generic sizing criteria of 0.08 and 0.16 L g^{-1} eggs for floating bed and moving bed filter media volume are calculated. Hatchery specific information on hatching percentage, ammonia sensitivity, and pH would likely reduce filter sizing criteria.

1. Introduction

Recirculating technologies are increasingly being utilized in hatchery operations to support egg incubation and hatching operations. These technologies facilitate precise control of salinity and temperature in a biosecure environment. Historically regulated to a largely flow-through operations, specific sizing criteria for recirculating components are often lacking. This is particularly true for non-commodity or emerging commercial species. In these cases, engineers tend to resort to oversizing the biofilters for this critical production step.

Information on both carbonaceous and nitrogenous loading is required to size biofiltration units. In incubation and hatching systems, the biofiltration sizing is controlled by nitrogenous loading that stems from the decay of eggs which have failed to successfully hatch during the incubation period. Thus, the loading is directly driven by the nitrogen content of the eggs. The level of biodegradable organics is usually of secondary importance in determining the degree of biofilm harvesting required.

The specific objectives of this effort were to develop a biofilter sizing rationale for an exotic, freshwater ornamental species, *Balantiocheilus melanopterus* egg incubation operation, and to identify a more common species whose eggs could be used as surrogates in the testing and evaluation of the incubation designs. In order to focus solely on biofilter performance, acclimation will remain outside the scope of this study. The sizing approach assumes the initial biofilm acclimation is complete. It may be noted that, depending upon the species and cultural methods, some hatcheries for eggs may operate via flow through or upflow systems. For these apparatuses, water quality beyond oxygen levels and biosecurity has little relevance. However, for this study, the scope is limited to the problem of how to size a biofilter (bioclarifier) for a recirculating incubator system.

2. Background

2.1. Egg and fry mortality

Early attempts to breed exotic species such as bala sharks (*Balantiocheilus melanopterus*) in the U.S. in recirculating formats were complicated by high mortalities in the hatching stage. These procedures involved the maturation of pond raised fish (Ng and Tan, 1997), whereby eggs are placed in flow-through tanks, hatched, and left to grow for one week before their introduction to grow-out ponds. High mortalities were attributed to poor water quality conditions in the hatching tanks.

While Southgate (2010) noted that numerous disease-causing agents and bacteria were ubiquitous in the tank itself, Salvesen and Vadstein

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(1995) indicated that intensive incubation exposes eggs and larvae to certain bacteria that they would not encounter in the natural process. Industrial RAS fish reproduction tends to promote fish diseases and bacteria transmission from fish eggs to the live larvae and fish in the hatchery tank. Brown et al. (2010) explained that the high rate of egg mortality is largely due to genetic deformities. However, it can be argued that nutrition as well as tank hygiene are more important.

The presence of disease-causing agents and salinity can also affect mortality rates among eggs and fry. Exposure to nitrogen (unionized or free ammonia) and inappropriate dissolved gas concentrations (oxygen shortage, for instance), due to the presence of dead eggs, seems to be the greatest hazard to egg survival (Finn et al., 1991). In incubation tanks more specifically, waste is produced not only by hatching egg fluids, but also by dead eggs. These by-products of the hatching process must be addressed in any recirculating designs.

2.2. Loading and biofouling

Total ammonia nitrogen (TAN) loading measurements are used as part of the physicochemical characterization of water, although experts agree that a high level of free ammonia is a better indicator of water toxicity. Therefore, tests such as total Kjeldahl nitrogen (TKN) that capture both total ammonia nitrogen and organic nitrogen are valuable in determining both actual and potential ammonia loading on an incubation system. High TKN levels indicate not only immediate protein contamination but also forecast ammonia production (Wright and Fyhn, 2001). Nitrogen loading is a source of great contamination for live fish (Bergheim et al., 1984; Handy and Poxton, 1993; Shinn et al., 2013). Elevation of free ammonia levels can decrease hatching success increasing the number of decaying eggs. The egg decay releases both nitrogenous waste and organics that impact not only the remaining eggs, but also the emerging fry.

Although biofouling may not be directly lethal for marine species' eggs, (Steer and Moltschaniwskyj, 2007; Hattori et al., 2004) studied the effect of varying amounts of dissolved oxygen in the rearing tank. Dead, decaying eggs stimulate a build-up of bacterial biomass in the tank, prompting an increase in oxygen consumption, and potentially creating a septic environment for the remaining eggs (Lovegrove, 1979; Cronin et al., 1999). Hatcheries demand oligotrophic or ultra-oligotrophic conditions characterized by low biodegradable organic concentrations typically estimated by Biochemical Oxygen Demand (BOD_5) (Duarte et al., 2019; Almeida et al., 2021). Wickett's (1954) experiments measured respiration of live salmon eggs in the Nile Creek and determined oxygen demand to be between 0.00013 and 0.0003 mg/egg/hour at a temperature of 0.1–8.2 °C. Lowering oxygen content was found to result in higher egg mortality.

2.3. Hatcheries and RAS

Fig. 1 illustrates a compact recirculating system supporting the incubation of eggs and fry capture from a dozen McDonnald jars in a large tilapia production facility. Water pulled from a sump is first passed through a floating bead bioclarifier for solids removal and biofiltration before passing through an ultraviolet light sized to disinfect recirculating waters. The water is then passed to a constant head configuration that passes about half the flow directly back to the sump. The other half is passed through the McDonald jars then to a fry capture tank before cascading back into the sump. Aeration and carbon dioxide stripping is addressed as the water cascades back into the sump. These incubation modules facilitate the isolation and biosecurity if genetic strains in large

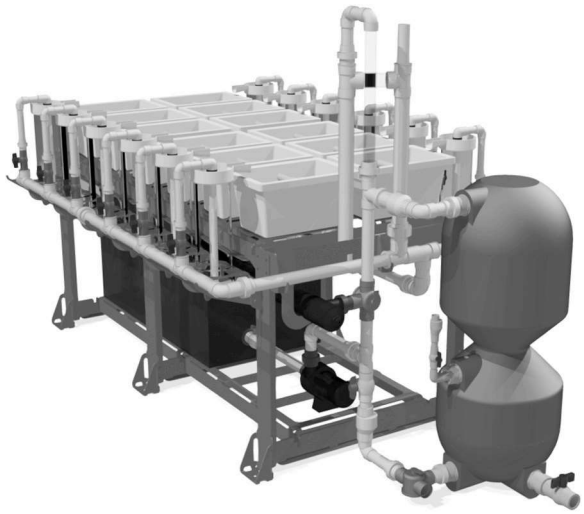

Fig. 1. This compact RAS supports 12 McDonald Jars with a reservoir, recirculation pump, ultraviolet light and a static floating bead filter. Used for tilapia production, this is a freshwater system with a water reuse of about 1 week. (Courtesy of Miryam Aquaculture Products and Aquaculture Systems Technologies).

facilities.[1] Anticipating that shock loading may be an issue, the reservoir functions to buffer rapid changes in water quality behavior.

When evaluating a RAS, transitional phenomena such as acclimation differ in length based on habitat-specific parameters, such as temperature (Saidu, 2009) and salinity (Gutierrez-Wing and Malone, 2006). While Gutierrez-Wing and Malone (2006) calls for the development of new acclimation procedures for marine RAS, Malone and Pfeiffer (2006) advises that studies focused on "the performance of biofilters that are operated in a steady-state mode (on a daily time scale) with respect to their organic and nitrogen loading" should disregard acclimation and other management parameters. If the system's units are to be used continuously, it is common to indicate no seed while using chemical addition to stimulate nitrifier growth (Manthe and Malone, 1987). If as a practical matter disease is not an issue then seeding from broodstock system or another incubator's biofilter is recommended. These bacterial communities have evolved to match the loading pattern and have developed resistance to backwashing abrasion.

Malone and Pfeiffer (2006) categorize filters and filter performance to match RAS applications based on total ammonia nitrogen (TAN) loading and provide biofilter classifications based on trophic levels from ultra-oligotrophic for larval production to hypereutrophic and acidic hypereutrophic. Oligotrophic production systems are characterized by a severely limited amount of nutrients. These systems are ideal for hatchery tanks because the low concentration of nitrogen and organic waste minimizes oxygen consumption to the eggs' benefit (Ip et al., 2001; Smart, 1976; Burrows, 1964; Wright and Fyhn, 2001; Lovegrove, 1979; Cronin et al., 1999). The oligotrophic standard for biosecure fry production is under 0.3 g N/m^3 (Malone and Pfeiffer, 2006; Weaver, 2006). Controlled maturation studies indicate that some species' eggs require an even lower (ultra-oligotrophic) TAN level < 0.1 g N/m^3 (Watanabe et al., 2002; Malone et al., 2006).

Larval fish are extremely sensitive to trophic water quality changes (Brown et al., 2010). In RAS filters under oligotrophic conditions, biofilms are considerably thin and heterogeneous (Malone and Pfeiffer, 2006). Diffusion to nitrifiers is relatively fast and nitrification rates are

[1] For the scope of this study, the sizing criteria are not dependent on the system configuration. The biofilter is sized to accommodate the eggs that fail to hatch assuming 100% of the TKN in an egg is converted to TAN. Whether the system is operated in parallel or series does not impact the sizing.

moderate despite low TAN levels. Bacterial counts are relatively low as well (Michaud et al., 2006). However, the oligotrophic water conditions are altered in the presence of decaying eggs, which inevitably impact the rest of the RAS. As the eggs decay, there is an increase in both carbon and nitrogen levels. The tank's biofilms thicken, and diffusion into the biofilms slows. In terms of biofiltering capacity, increased nitrogen loading pushes these oligotrophic RAS systems (designed for low loading) toward an unacceptable mesotrophic or even eutrophic condition, thereby making the filter sizing inadequate for the filtering needs of the eggs and fry as the bacterial count rises (Malone and Pfeiffer, 2006). A conservative design approach is thus imperative.

3. Methods

3.1. Overview

Eggs of seven different species were selected for comparison. BOD_5, nitrogen, and protein content were determined for each. These selected species, apart from the bala shark, are relatively abundant and readily available (Greensword, 2015). Table 1 lists the species selected.

3.2. Egg Collection

Eggs of potential surrogate species (blackfin tuna (*thunnus atlanticus*), cobia (*rachycentron canadum*), snapper (*lutjanus campechanus*), speckled trout (*cynoscion nebulosus*), and yellowfin tuna (*thunnus albacares*)) were collected from CoCo Marina in Cocodrie, Louisiana, with the help of Dr. Ed Chesney, from Louisiana Universities Marine Consortium (LUMCON). Dr. John Hargreaves from Aquaculture Assessments, LLC., provided eggs of catfish (*ictalurus punctatus*) and tilapia (*oreochromis niloticus*).

3.3. Solidifying the samples

All eggs were freeze-dried in a Labconco LYPH-Lock 18 freeze-dryer, and placed in a Whirlpool® upright freezer to preserve the organic composition while removing the handling limitations related to moisture. Wet eggs yielded a 30 ± 3.6% dry mass across species. Freeze-dried samples were powdered via a grinder. Powdering dry eggs increases the surface area of the particles and facilitates the manipulation and measurement of each sample. The increased surface area facilitates the rapid degradation in the BOD_5 test, thereby providing a worst-case scenario for O_2 consumption. Powdering the dry eggs also improved accuracy in weight measurements.

3.4. Loading measurements

The O_2 probe (Accumet XL40 Benchtop Dissolved Oxygen Meter) was calibrated using the Winkler method (McCormick, 1972) as protocol to determine dissolved oxygen. The Winkler method was selected for its accuracy (Carpenter, 1965; Peck and Uglow, 1990; Helm et al., 2009). A calibration curve of the BOD_5 probe was created with 5 calibration data points. A fixed weight of freeze-dried eggs was measured (1.5 mg) and added to the BOD bottles.

To prepare the egg solution, distilled water was aerated at room temperature. The volume for each sample of stock solution was set at 300 mL dilution water. The initial and final (5 day) O_2 were measured with the meter in mg L^{-1}, enabling the calculation of how much O_2 was consumed. All measurements were triplicated. The BOD_5 in gram per gram of sample was then calculated.

For TKN measurements, 1.025 g of dry weight power was measured in triplicate for each species' egg sample into a foil paper. Samples were inserted into a nitrogen analyzer (LECO FP 528) that analyzes organic samples and gives a reading of % nitrogen. Samples of the same weight (1.025 g) were analyzed for % protein in a LECO FP 528 as well.

3.5. Statistical analysis

The data acquired from the measurements were input into SAS and an ANOVA analysis was conducted. A Tukey Studentized test identified similarities by grouping, based on the mean value.

4. Results

Table 1 displays averaged BOD_5 results as well as nitrogen and protein content for each species tested. BOD_5 results range from 0.622 g/g to 0.750 g/g. Nitrogen levels range between 9.24% and 11.70%, and protein levels between 61.41% and 73.17%. Results were consistent across the board for all trials of the triplicated tests.

The BOD_5 results for the Tukey's Studentized Range Test also in Table 1 showed the mean in g/g of the triplicate results. Identical Tukey grouping letters indicated the mean BOD_5 results were not significantly different. Likewise, the box plot shows the distribution the BOD_5 and the grid location of each species. Tilapia eggs had the highest mean out of all species with a BOD_5 of 0.75 ± 0.01 g/g and the Tukey grouping letter 'A.' Catfish, cobia and bala shark eggs showed no significant difference with means at 0.663 ± 0.01 g/g, 0.644 ± 0.01 g/g, and 0.64 ± 0.004 g/g, respectfully. They were all characterized by the Tukey grouping letter 'C.' Bala shark also showed no significant difference with blackfin and yellowfin tuna which BOD_5 (g/g) mean was 0.622 ± 0.01 g/g and 0.630 ± 0.01 g/g, respectfully. They shared the Tukey grouping letter 'D.' Snapper and speckled trout eggs had the second and third highest BOD (g/g) of 0.715 ± 0.02 and 0.714 ± 0.01, respectively. They shared the Tukey grouping letter 'B.'.

The next variable tested in triplicate and analyzed was nitrogen content. Table 1 shows the mean nitrogen in g g^{-1}. Identical Tukey grouping letters indicate the mean nitrogen of each species' eggs that are not significantly different. The results indicate that blackfin tuna, tilapia, and yellowfin tuna have the highest means, with nitrogen content of 0.117 ± 0.11, 0.117 ± 0.31, and 0.117 ± 0.001 g/g, respectfully, and a Tukey grouping letter of 'A.' Bala shark, catfish, and cobia showed no significant difference with mean nitrogen content of 0.110 ± 0.06, 0.109 ± 0.09, and 0.109 ± 0.06 g/g, respectfully, and a Tukey grouping letter of 'B.' Speckled trout and snapper eggs show the lowest mean of nitrogen of 0.102 ± 0.29 and 0.093 ± 0.15 g/g, respectively, and a Tukey grouping letters 'C′ and 'D′.

The final variable tested and analyzed was mean percent protein for all 8 species of fish egg in triplicates. Table 1 shows the mean percent

Table 1
Levels from BOD_5, nitrogen, and protein and Tukey's Studentized Range (HSD) test for the eggs of all 8 species tested.

Scientific Name	Water Type	BOD_5 (g/g)	BOD Grouping	Nitrogen %	Nitrogen Grouping	Protein %	Protein Grouping
Balantiocheilus melanopterus	Freshwater	0.64 ± 0.00	C, D	10.97 ± 0.06	B	68.55 ± 0.36	B
Thunnus atlanticus	Marine	0.62 ± 0.01	D	11.67 ± 0.12	A	72.84 ± 0.60	A
Ictalurus punctatus	Freshwater	0.66 ± 0.01	C	10.92 ± 0.09	B	68.30 ± 0.28	B
Rachycentron canadum	Marine	0.64 ± 0.01	C, D	10.83 ± 0.06	B	67.74 ± 0.22	B
Lutjanus campechanus	Freshwater	0.71 ± 0.02	B	9.24 ± 0.15	D	61.41 ± 0.92	C
Cynoscion nebulosus	Marine	0.71 ± 0.01	B	10.13 ± 0.29	C	68.40 ± 0.55	B
Oreochromis niloticus	Freshwater	0.75 ± 0.01	A	11.67 ± 0.31	A	73.10 ± 0.42	A
Thunnus albacares	Marine	0.63 ± 0.01	D	11.70 ± 0.00	A	73.17 ± 0.04	A

protein. Again, identical Tukey grouping letters indicate the mean percent protein of each species' eggs that are not significantly different. The results indicate that tilapia, yellowfin, and blackfin tuna eggs have the highest mean percent protein of all species with 73.10 ± 0.42, 73.17 ± 0.04, and 72.84 ± 0.60, respectfully, and a Tukey grouping letter of 'A.' Bala shark, speckled trout, catfish, and cobia eggs show no significant difference with mean percent protein of 68.549 ± 0.36, 68.40 ± 0.55, 68.30 ± 0.28, and 67.74 ± 0.22, respectively, and a Tukey grouping letter of 'B.' Snapper eggs show the lowest mean percent protein of 61.41 ± 0.92 with a Tukey grouping letter of 'C.'.

5. Application examples

Warmwater oligotrophic water quality conditions (Malone and Pfeiffer, 2006) are presumed appropriate for a wide variety of egg incubation and hatching operations. It is presumed that ammonia removal would limit the sizing of the biofilter, and that the principal nitrogen loading to the system is decaying eggs rather than egg excretion. For this generic analysis, the nitrogen loading is derived from the yellowfin tuna content (rounded up to 12% or 120 mg-N g^{-1}). Finally, assuming that the egg loss occurs evenly across a 5 -day incubation, the load for a 50% egg loss can be estimated as:

$$load = \frac{120mg - N}{g - eggs} * .5 * \frac{1}{5days} = \frac{12mg - N}{g - d} \quad (1)$$

Definition of the *load* in this manner makes the overall sizing process very conservative. The assumption of 50% egg loss incurs a safety factor of 1–2 as high losses are unlikely in a well-managed system. Thus, the design load has an inherent safety factor of the order of 2–4, rendering the load difference amongst species moot.

The nitrogen production information generated facilitates the sizing of biofilters for egg hatching completely mixed RAS under the assumption that the biofilter is acclimated. The following mass balance equation is thus presented in terms of TAN:

$$\frac{d \quad TAN * V}{dt} = W_{eggs} * load + Q_f * TAN_b - Q_f * TAN_c - Conv \quad (2)$$

Where:

W_{eggs}=the dry weight of eggs incubated (g).

load=total ammonia generation rate of a gram of dry weight eggs (mg-N $d^{-1}g^{-1}$).

TAN_b=total ammonia nitrogen concentration in the supply water (mg-N L^{-1}).

TAN_c=critical total ammonia nitrogen concentration in incubator RAS (mg-N L^{-1}).

V=volume of the system (L).

Q_f=flushing flow (L d^{-1}).

Conv=nitrification capacity of the biofilter (mg-N d^{-1}).

Under a normal assumption of steady state conditions, while conservatively neglecting the flushing flow the equation simplifies to:

$$Conv = W_{eggs} * load \quad (3)$$

As an example for a static bed floating bead filter (Malone and Beecher, 2000), the conversion capacity is estimated (Malone et al., 2006) as:

$$Conv = \tau * TAN_c * V_b \quad (4)$$

Where:

τ=the concentration normalized conversion rate (d^{-1}).

V_b=the volume of the packed bead bed (L).

Assuming a typical conservative design value for τ of 750 d^{-1} and a target oligotrophic TAN_c level of 0.2 mg-N L^{-1}:

$$\tau * TAN_c * V_b = W_{eggs} * load \quad (5)$$

Normalizing the filter bed volume to the weight of eggs allows the filter sizing constant to be defined:

$$\tau * TAN_c * \frac{V_b}{W_{eggs}} = load \quad (6)$$

$$\tau * TAN_c * v_b = load \quad (7)$$

Where:

v_b = the weight normalized biofilter sizing constant (L g^{-1}).

Rearranging and substituting in known values while solving for v_b:

$$v_b = \frac{12}{0.2 * 750} \quad (8)$$

$$v_b = \frac{0.08liters \quad of \quad beads}{gram \quad eggs} \quad (9)$$

The weight normalized sizing criteria that can be used to size a bead filter for any batch size of eggs:

$$V_b = W_{eggs} * v_b \quad (10)$$

Eq. 10 can then be used to determine the bead bed sizing for various incubator processes.

One of the advantages of using a floating bead filter in this application is the ability to manipulate the biofilm. During normal operation (few dead eggs) the backwash frequency can be dramatically reduced preserving bacterial numbers even as endogenous respiration proceeds. When eggs die these starving bacteria rapidly assimilate (C and N) and backflush frequency can be increased to disproportionally harvest the heterotrophs shifting the filters operation back more fully to a nitrifying performance. Some fluidized beds operate with fixed abrasion as well as a balancing abrasion and harvesting across a narrow range. Bead filters' ability to harvest bacteria (abrasion) across a wide range of loading (dead eggs) allows for an approach that is applicable across a wide range of species and system designs.

Alternatively, Rusten et al. (2006) presents design and operational guidelines for moving bed biofilm reactors (MBBR) that use Kaldnes media. The sizing and influence of concentration on conversion parallels the bead bed's conversion rate, except that the moving bed reactor's performance is traditionally reported in areal and not volumetric terms. This areal value must then be adjusted by the media's effective specific surface area (SSA) (Rusten et al., 2006). If we presume at TAN_c = 0.2 mg/L a reasonable areal specific conversion (Conv'= 0.15 g m^{-2} d^{-1}) and noting that Kaldnes filter media K1 has a SSA of 500 m^2/m^3 (Rusten et al., 2006) the Conv is defined as:

$$Conv = Conv^{'} * SSA * V_b \quad (11)$$

And again, normalizing to a gram of eggs then we can similarly derive a sizing constant for the moving bed reactors of:

$$v_b = \frac{0.16liter \quad of \quad beads}{gram \quad eggs} \quad (12)$$

Eq. 10 can then be applied to determine the moving bed reactor sizing for a RAS designed to support egg hatching of a known weight of eggs.

A variety of different conversion values could be used in the development of Eqs. 9 and 12. However, regardless of the biofilter used or values selected, it is known that the nitrification performance rates are sensitive to temperature, pH, C/N ratios, and turbulence (Chen et al., 2006). Performance must be assured by relatively large safety factor. Here, the safety factor inherent in the loading estimates, our neglect of in-situ nitrification, and the availability of flushing flows render this simplified analysis adequate for broad application. These calculations can be used with confidence provided the filter is fully acclimated and a reasonable biofiltration (bioclarification) management addressing pH, alkalinity and flow is in place. The value of the fry justifies the expense of over sizing of the filtration system.

If the scale of the hatching operation justifies it, the impact of flushing flows and in situ nitrification can be taken into account, but more importantly, specific information about the hatching percentage, the critical un-ionized ammonia concentration for eggs and fry, and the incubator's pH can dramatically alter (typically reduce) the filter sizing constant below the generic values calculated here.

6. Discussion and conclusion

Results show a difference between the nitrogen/protein content trend and that of BOD_5, as seen in Table 1. Bala shark, cobia, and catfish emerge as the species with no statistical difference in all test categories (BOD_5, nitrogen, and protein). The ANOVA test shows that all three species display similar dead egg loading. A synthesis of the ANOVA results is displayed in Table 2.

Eggs from the species listed in bold can be selected as surrogates for the bala shark eggs, if biofilter sizing verification is desired. Using eggs of more abundant species and finding no statistically significant dissimilarity with bala shark eggs will greatly facilitate experimental research and trials.

The loading factors developed for organics (BOD_5) and nitrogen (TAN) were similar across all the eggs tested. This suggests that a unified sizing criteria could be developed for application across many species. Robust safety factors (2−3) should be employed to overcome biofilter performance differences driven more by factors such as temperature, pH and ammonia sensitivity rather than intraspecies differences in loading. Further refinement in sizing equations is largely dependent on development of species-specific information on hatching percentages, definition of critical free ammonia concentrations, and pH ranges associated with commercial incubator operations.

All in all, the water quality required for egg hatching and maintaining the larva until first feeding is very high-quality water. The specified the generic loading baseline for oligotrophic applications is thus based on Malone and Pfeiffer (2006) with a TAN limit< 0.3 mg/L. Eqs. 7 and 9 can be adjusted to comply with any TAN target that the culturist chooses to target. On the other hand, tilapia, are relatively insensitive to free ammonia concentrations and do quite well with TAN levels of 1–2 ppm with a pH in the range of 7–8.

Furthermore, this analysis opted for a conservative fertilization rate of 50% in its generic calculation. In addition, there are several "hidden" safety factors involved in the computation. For example the TAN conversion rate set for both the filters illustrated is set at two thirds that easily obtained peak values are 3–4 times higher. It is assumed that 100% of the TKN would be expressed when an egg died. In practice, about 50% of the TKN will be removed by the RAS solids capture devise. Moreover, the impact of water discharge was not considered, because it does not critically affect outcomes. Finally, design was based on TAN, not free ammonia. In the pH range typically encountered, there is another factor of safety of 10: this free ammonia level will be 10% or less than the target TAN in range of pH is between 7 and 8. Thus the net "hidden" safety factor here is of the order of 20–30 times, where the filter is inherently sized 20–30 times larger than is required. Under reasonably good maturation practice the incubator system will operate as predicted, and will be reasonably cost-effective for the industry,

CRediT authorship contribution statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in *Aquacultural Engineering*.

Table 2
Catfish and cobia displayed no significant differences from bala shark for each test.

Test	Species with no significant differences from bala shark
BOD_5	thunnus atlanticus, ***rachycentron canadum, ictalurus punctatus***, *thunnus albacares*
Nitrogen	***rachycentron canadum, ictalurus punctatus***
Protein	*cynoscion nebulosus*, ***rachycentron canadum, ictalurus punctatus***

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interest; Ronald Malone reports financial support was provided by University of Florida. Marlon Greensword reports equipment, drugs, or supplies was provided by CoCo Marina. Marlon Greensword reports equipment, drugs, or supplies was provided by Aquaculture Assessments, LLC. Ronald Malone reports a relationship with Aquaculture Systems Technologies that includes: employment, equity or stocks, and travel reimbursement. Corresponding author currently serving on the Aquacultural Engineering Society's Board of Directors MG.

Data availability

Data will be made available on request.

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References

Almeida, D.B., Magalhães, C., Sousa, Z., Borges, M.T., Silva, E., Blanquet, I., Mucha, A.P., 2021. Microbial community dynamics in a hatchery recirculating aquaculture system (RAS) of sole (Solea senegalensis). Aquaculture 539, 736592.
Bergheim, A., Hustveit, H., Kittelsen, A., Selmer-Olsen, A.R., 1984. Estimated pollution loadings from norwegian fish farms. II. investigations 1980–1981. Aquaculture 36 (1–2), 157–168.
Brown, C.L., Power, D.M., Núñez, J.M., 2010. 5 disorders of development in fish. Fish. Dis. Disord. 2, 166.
Burrows, R.E., 1964. Effects of accumulated excretory products on hatchery-reared salmonids. Bureau of Sport Fisheries and Wildlife,.
Carpenter, J.H., 1965. The accuracy of the Winkler method for dissolved oxygen. Limnol. Oceanogr. 10, 135–140.
Chen, S., Ling, J., Blancheton, J.P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquac. Eng. 34 (3), 179–197.
Cronin, E.R., Cheshire, A.C., Clarke, S.M., Melville, A.J., 1999. An investigation into the composition, biomass and oxygen budget of the fouling community on a tuna aquaculture farm. Biofouling 13 (4), 279–299.
Duarte, L.N., Coelho, F.J., Oliveira, V., Cleary, D.F., Martins, P., Gomes, N.C., 2019. Characterization of bacterioplankton communities from a hatchery recirculating aquaculture system (RAS) for juvenile sole (Solea senegalensis) production. PloS One 14 (1), e0211209.
Finn, R.N., Fyhn, H.J., Evjen, M.S., 1991. Respiration and nitrogen metabolism of Atlantic halibut eggs (Hippoglossus hippoglossus). Mar. Biol. 108 (1), 11–19.
Greensword, M.A., 2015. Life Cycle Analysis of an Airlifted Recirculation Aquaculture Facility. Master's thesis. Louisiana State University,.
Gutierrez-Wing, M.T., Malone, R.F., 2006. Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquac. Eng. 34 (3), 163–171.
Handy, R.D., Poxton, M.G., 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Rev. Fish. Biol. Fish. 3 (3), 205–241.
Hattori, M., Sawada, Y., Kurata, M., Yamamoto, S., Kato, K., Kumai, H., 2004. Oxygen deficiency during somitogenesis causes centrum defects in red sea bream, Pagrus major (Temminck et Schlegel). Aquac. Res. 35 (9), 850–858.
Helm, I., Jalukse, L., Vilbaste, M., Leito, I., 2009. Micro-Winkler titration method for dissolved oxygen concentration measurement. Anal. Chim. Acta 648 (2), 167–173.

Ip, Y.K., Chew, S.F., Randall, D.J., 2001. Ammonia Toxicity Tolerance and Excretion. In: Wright, P.A., Anderson, P.M. (Eds.), Nitrogen Excretion. Academic Press, pp. 109–140.
Lovegrove, T., 1979. Control of fouling in farm cages. Fish. Farming Int. 6 (1), 33–37.
Malone, R.F., Beecher, L.E., 2000. Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. Aquac. Eng. 22 (1–2), 57–73.
Malone, R.F., Pfeiffer, T.J., 2006. Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. Aquac. Eng. 34 (3), 389–402.
Malone, R.F., Bergeron, J., Cristina, C.M., 2006. Linear versus Monod representation of ammonia oxidation rates in oligotrophic recirculating aquaculture systems. Aquac. Eng. 34 (3), 214–223.
Manthe, D.P., Malone, R.F., 1987. Chemical addition for accelerated biological filter acclimation in closed blue crab shedding systems. Aquac. Eng. 6 (3), 227–236.
McCormick, P.G., 1972. The determination of dissolved oxygen by the Winkler method. a student laboratory experiment. J. Chem. Educ. 49 (12), 839–841.
Michaud, L., Blancheton, J.P., Bruni, V., Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquac. Eng. 34 (3), 224–233.
Ng, P.K., Tan, H.H., 1997. Freshwater fishes of Southeast Asia: potential for the aquarium fish trade and conservation issues. Aquar. Sci. Conserv. 1 (2), 79–90.
Peck, L.S., Uglow, R.F., 1990. Two methods for the assessment of the oxygen content of small volumes of seawater. J. Exp. Mar. Biol. Ecol. 141, 53–62.
Rusten, B., Eikebrokk, B., Ulgenes, Y., Lygren, E., 2006. Design and operations of the Kaldnes moving bed biofilm reactors. Aquac. Eng. 34 (3), 322–331.
Saidu, M.M.G., 2009. Temperature impact on nitrification and bacterial growth kinetics in acclimating recirculating aquaculture systems biofilters. Doctoral dissertation. Louisiana State University,.
Salvesen, I., Vadstein, O., 1995. Surface disinfection of eggs from marine fish: evaluation of four chemicals. Aquac. Int. 3 (3), 155–171.
Shinn, C., Marco, A., Serrano, L., 2013. Influence of low levels of water salinity on toxicity of nitrite to anuran larvae. Chemosphere 92 (9), 1154–1160.
Smart, G., 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish. Biol. 8 (6), 471–475.
Southgate, P., 2010. 13 welfare and farmed fish. Fish. Dis. Disord. 2, 357.
Steer, M.A., Moltschaniwskyj, N.A., 2007. The effects of egg position, egg mass size, substrate and biofouling on embryo mortality in the squid Sepioteuthis australis. Rev. Fish. Biol. Fish. 17 (2–3), 173–182.
Watanabe, W.O., Losordo, T.M., Fitzsimmons, K., Hanley, F., 2002. Tilapia production systems in the Americas: technological advances, trends, and challenges. Rev. Fish. Sci. 10 (3–4), 465–498.
Weaver, D.E., 2006. Design and operations of fine media fluidized bed biofilters for meeting oligotrophic water requirements. Aquac. Eng. 34 (3), 303–310.
Wickett, W.P., 1954. The oxygen supply to salmon eggs in spawning beds. J. Fish. Board Can. 11 (6), 933–953.
Wimberly, D.M., 1990. Development and evaluation of a low-density media biofiltration unit for use in recirculating finfish culture systems. M.S. Thesis. Louisiana State University,, Baton Rouge, Louisiana.
Wright, P.A., Fyhn, H.J., 2001. Ontogeny of nitrogen metabolism and excretion. Fish. Physiol. 20, 149–200.