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Inland Marine Fish Culture in Low Salinity Recirculating Aquaculture Systems

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Abstract：Expansion of marine aquaculture is challenged by the high cost and limited availability of coastal land and water resources, effluent concerns, high production costs, restricted growing seasons, lack of quality fingerlings, and inadequate regulatory and permitting processes. Many of these constraints can be addressed with inland marine fish culture in low salinity recirculating systems as production models. We describe recent and ongoing development of technologies in four principal areas: 1) engineering and system design; 2) year-round fingerling production; 3) diet development; and 4) physiological adaptation of marine fish to low salinity environments using genomic approaches. It is anticipated these technologies could find application for rearing euryhaline marine fish throughout approximately 2/3 of the U.S. where low salinity groundwater is available. This approach will reduce the need to be located near the coast, reduce the volume of saltwater effluent, and reduce the carbon footprint of marine finfish production.

Keywords: low salinity, marine fish, recirculating systems

Introduction

A growing and increasingly health-conscious population, coupled with declining capture fisheries, is driving increased global demand for farm-raised seafood that can only be met through expansion of aquaculture (Delgado *et al.*, 2003). In 2007, aquaculture represented 33% of total global seafood production and is projected to increase to as much as 71% by 2030. The U.S. aquaculture industry represents a US$ 1 billion/year industry; however, the U.S. still imports 84% of its edible seafood resulting in a US$ 9 billion trade deficit (NOAA, 2007). The U.S. aquaculture industry is principally based on production of freshwater finfish, with salmon representing only 5% of the industry being the only notable exception. This suggests tremendous potential for growth in a developing U.S. marine aquaculture industry. However, development and expansion of marine aquaculture is challenged by the high cost and limited availability of coastal land and water resources, effluent concerns, high production costs, restricted growing seasons, lack of quality fingerlings, and inadequate regulatory and permitting processes (FAO, 2009). Many of these constraints can be addressed with inland marine fish culture in low salinity recirculating aquaculture systems (RAS) as the production model. We describe recent and ongoing development of technologies for this production model in four principal areas: 1) engineering and system design; 2) year-round production of fingerlings; 3) diet development;

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and 4) physiological adaptation of marine fish to low salinity environments. It is anticipated these technologies could find application for rearing euryhaline marine fish throughout approximately 2/3 of the contiguous United States (Fig.1) where lightly saline groundwater is available (Alley, 2007). We have worked with several species, including southern flounder (*Paralichthys lethostigma*), summer flounder (*Paralichthys dentatus*), hybrid striped bass (*Morone chrysops* × *M. saxatilis*), black sea bass (*Centropristis striata*), red drum (*Sciaenops ocellatus*), and cobia (*Rachycentron canadum*), but this paper is focused principally on our work with Florida pompano (*Trachinotus carolinus*), the project's model species.

Engineering and System Design

Although RAS performance for freshwater production has been well characterized, less information is available for saltwater RAS production. Saltwater RAS present several challenges, including: 1) saline water holds less dissolved oxygen (DO) than freshwater; 2) lower oxygen transfer and nitrification rates; 3) solids are more buoyant and difficult to remove; and 4) carbon dioxide (CO_2) is more difficult to measure using standard methods (Pfeiffer *et al.*, 2010).

Traditional RAS utilize high pressure centrifugal pumps to move water for filtration, aeration, degassing, and delivery to culture tanks. However, centrifugal pumps can account for more than 50% of the system's energy consumption as their operating efficiency is typically about 80% (Pfeiffer and Wills, 2009). An alternative is to utilize axial flow propeller pumps and airlift technology to move large volumes of water under low pressure. Those technologies have been found to reduce energy use by approximately 33%.

Conventional gas transfer technologies require a large amount of space, capital investment, and contribute to energy demand. In addition, diffused aeration in a circular tank can interfere with the hydrodynamics of water rotation and efficiency of solids removal. We evaluated low-head systems utilizing airlift technology for water movement,

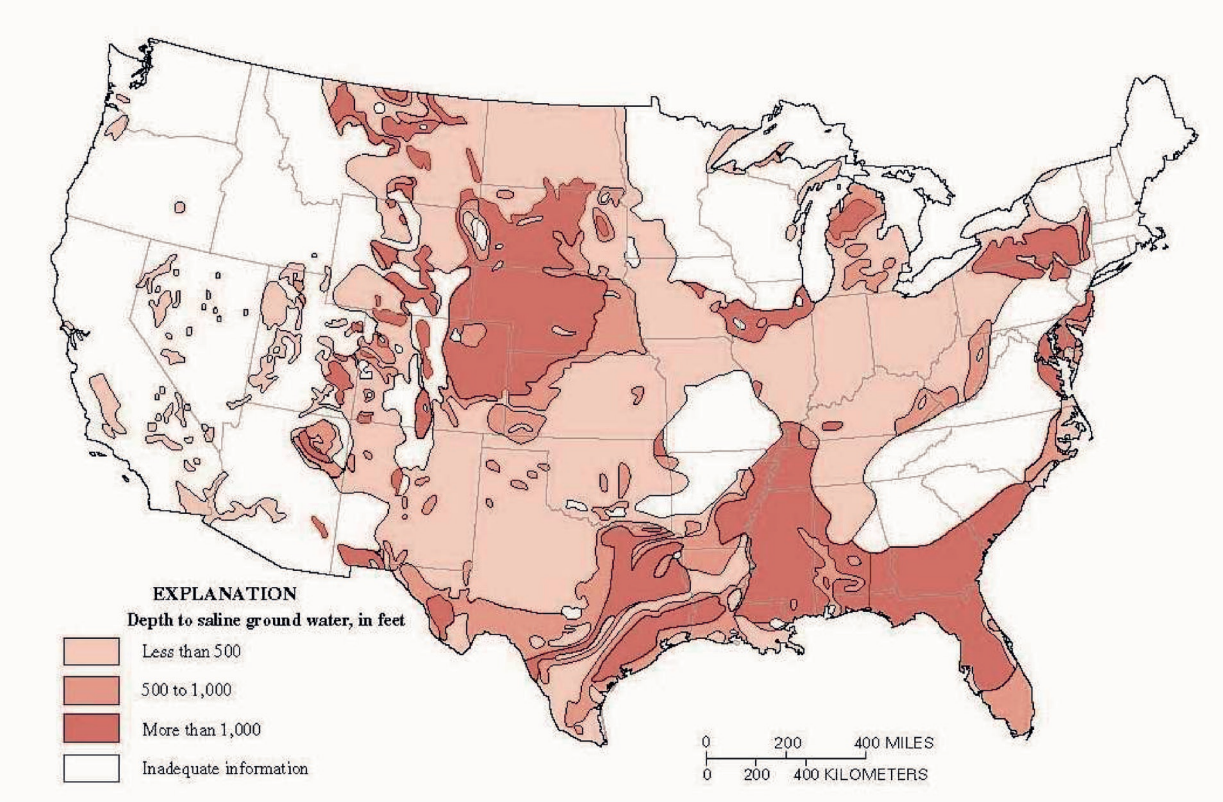

Fig. 1. Preliminary perspective on the location and depth to reach saline groundwater in the contiguous United States (adapted from Alley (2007) as generalized from Feth and others).

aeration, and degassing. Gas:liquid ratios, lift:submergence ratios, and flow rates in the airlift systems were also evaluated for their effect on gas transfer rates. Oxygen mass transfer rates ranged from 0.01 to 0.09 kg O_2 hr^{-1} based on airlift pipe diameter, water flow rate, air-flow, and dynamic head. A modification of the airlift design to give higher water flow at low dynamic head improved oxygen addition and CO_2 removal, providing 0.54 mg l^{-1} of O_2 to the water and removing 1.6 mg l^{-1} of CO_2 per pass.

In moving and static fixed film biofilters the ammonia removal rate varies with feed loading, salinity, and hydraulic flow rates. Ammonia removal rates ranged from 50 to greater than 1,200 g TAN m^{-3} media day^{-1} in static submerged fixed film biofilters using standard floating plastic media and removal rates correlated with influent TAN concentrations. With increasing salinity the nitrification rates decreased approximately 30%. When plastic media offering greater protective surface area was compared with the standard plastic media, significant differences in TAN removal rates were observed once feed loading exceeded 0.4 kg feed m^{-3} media. The range of TAN removal in a 0.71 m^{-3} moving bead biofilter ranged from 20 to 250 g TAN m^{-3} media day^{-1}. In airlift moving bed torrus filters with kaldness media (0.37 m^3) TAN removal rates were under 300 g TAN m^{-3} media day^{-1}. Increased TAN removal efficiency from 36 g to 180 g TAN removed per dollar spent was realized. Increases in nitrification rates at higher feed loads allow biofilters to support a greater biomass of fish and improve production costs.

Other recent accomplishments include the evaluation of individual system water treatment components and development of design criteria. Ammonia removal efficiency of two types of filters, efficacy of TAN removal with different substrates, and solids removal efficiency of three additional system components were determined. Ammonia removal rates and performance curves for a fixed film biofilter were established over a wide range of salinities, providing models to design filtration devices for specific salinity needs.

A swirl separator, static bed filter, and moving bed torrus filter were evaluated for suspended solids removal during low salinity culture of red drum. Total suspended solids analyses were conducted to determine the particle size distribution and percent removal efficiency by each filter type. The static bed clarifier removed 23% of particles ≦ 55 μm. Overall, the static bed exhibited the best removal efficiency of particles of all size ranges and was the most efficient clarifier.

To minimize labor costs associated with filter maintenance, foam fractionation technology was also evaluated for its ability to remove suspended solids under low salinity conditions (11-13 ppt). Efficiency of a foam fractionator was analyzed using different methods and design criteria including microbubble generation, ozonation, and hydrodynamic head. Results indicated greater particle removal with the use of ozone and at a higher operating water height.

These advances and component optimizations have been implemented in a research scale production facility with four replicated 45 m^3 high-head and four replicated 43 m^3 low-head RAS, each with four 3.05 m diameter tanks, rotary microscreen drum filters, biofiltration, supplemental oxygenation, ultraviolet light sterilization, degassing, and water and energy monitoring capabilities. The tanks have automatic feeders and oxygen sensors outfitted with programmable logic controllers (PLC) for feed delivery and controlled oxygen input.

In intensive RAS the use of supplemental oxygen increases carrying capacity of the system. Liquid oxygen can reduce unit production costs through production of greater biomass per unit volume. However, since saline water holds less DO at saturation than freshwater, the margin between DO saturation and deficiency is much narrower, which can result in wasteful overuse of oxygen, reduced production, or mortalities. Utilization of PLC maintains the percent saturation levels in the tanks between designated set points. Implementation of PLC technology for individual tank oxygen control has reduced daily oxygen to one-fifth of that in uncontrolled systems.

The production facility has been used for experimental trials with hybrid striped bass (Rawles *et al.,* 2006), Florida pompano (Weirich *et al.,* 2009; Pfeiffer and Riche, in press) and cobia (Weirich *et al.,* 2010) reared to market size. This was the first

demonstration that pompano and cobia could attain market size utilizing RAS technologies. Furthermore, it was demonstrated that pompano could reach market size under low salinity conditions (5 g l^{-1}). Future research activities include: 1) developing nitrification and aeration curves for a moving bed biofilter with different volumes of media evaluated at various feed loading rates in a low-head RAS under low salinity conditions; 2) determining the solids removal efficiency and relative cost to benefit of implementing foam fractionation technology to improve removal of fine and suspended solids; and 3) determining solids removal efficiency of various microscreen mesh sizes on a rotary drum filter in a low-head RAS. We also propose to design a sustainable effluent treatment system to recover effluent wastewater for reuse as rinse water on microscreen drum filters, and convert the sludge to a salable secondary by-product. In addition, we are exploring alternative means for addressing off-flavor problems that can arise during final production in RAS.

Year-round Production of Fingerlings

Lack of techniques for sustained production of fingerlings is a principal bottleneck for marine fish production. Therefore, a domestication program for Florida pompano was developed. Protocols for the capture, quarantine, feed training, conditioning, and spawning induction were established and described elsewhere (Weirich and Riley, 2007). During conditioning and spawning, Florida pompano were fed a modified commercial gelatin-based broodstock diet formulated for pompano (Gelly Belly, Florida Aqua Farms, Inc., Dade City, FL). Volitional spawning was induced via administration of a gonadotropin releasing hormone analogue (GnRH-a). In 2004 and 2005 a total of nine spawning trials were conducted with mixed but generally increasing success (Table 1). Since then, methods for rearing pompano larvae using RAS have been established, with post-larval transformation occurring at 17-days after hatch (DAH) and survival rates as high as 50% from egg to metamorphosed juvenile (Weirich *et al.,* 2005; Weirich, 2009). Recently we were able to attain year-round spawning, with one of six populations of females spawning as many as 10 consecutive months, and three others at least 7 months with no diminishment of egg quality.

Larval and juvenile development have been characterized for pompano, black sea bass, and southern flounder via imaging analysis to document ontogenetic development of early life stages. Events such as yolk absorption rate, first feeding, and flexion as well as monitoring of growth, mouth development, and metamorphosis in Florida pompano have been reported elsewhere (Riley *et al.,* 2009). Mouth gape and other rates of development are utilized to appropriately size live feeds and microparticulate diets. Additionally, the tolerance of pompano eggs and larvae to ammonia and nitrite, as well as their sensitivity to salinity and copper have been determined. As RAS are prone to buildup of the metabolites ammonia and nitrite, and toxicity is dependent on salinity and other water chemistry parameters we have determined ammonia and nitrite toxicity for post-metamorphic juveniles at various salinities (Weirich and Riche, 2006).

Optimal feeding protocols for pompano larvae have been established (Riley *et al.,* 2009). Efforts to improve live feed production and nutritional value have been addressed. Rotifer and *Artemia* enrichment protocols were established, and nutritional value of enrichment products evaluated via growth, survival, and fatty acid analyses (Cavalin and Weirich, 2009). Due to the small mouth gape of pompano at first feeding, evaluation of the copepod *Pseudodiaptomus pelagicus* was also undertaken (Cassiano *et al.,* 2011). Co-feeding and weaning strategies were developed and results indicate that pompano can be weaned to dry diets without compromising growth and survival beginning as early as 14 DAH.

Future research activities include evaluating alternative strategies to reduce time to weaning with the goal of eliminating *Artemia* from the larval production cycle. Additionally, we propose to evaluate commercial larval diets and develop novel microparticulate diets to meet the nutritional requirements of pompano larvae.

Table 1. Initial attempts at volitional spawning of Florida pompano following GnRH-a administration (data adapted from Weirich and Riley 2007)

Spawning Event	Number Females	Eggs Collected	Number Floating	Number Sinking	% Fertilization (total eggs)	% Fertilization (floating eggs)	% Hatch (fertilized eggs)	Eggs per Female
Year 2004								
1	8	2,786,000	459,000	2,327,000	14.3	86.8	73.1	348,000
2	4	921,000	368,000	553,000	28.5	71.3	76.5	230,000
3	6	736,000	152,000	584,000	18.0	87.3	79.7	123,000
Year 2005								
1	3	1,156,000	428,000	728,000	36.7	99.0	88.2	385,000
2	3	454,000	21,000	433,000	4.5	96.9	79.4	151,000
3	3	452,000	322,000	130,000	63.7	89.4	83.8	151,000
4	4	753,000	278,000	475,000	36.7	99.3	80.7	188,000
5	4	662,000	179,000	483,000	26.7	98.8	83.0	166,000
6	3	2,316,000	1,243,000	1,073,000	52.5	97.8	95.4	772,000

Diet Development

Nutrient requirements for Florida pompano are poorly understood. Early investigations used diets formulated for other species, typically trout diets with 40% crude protein (CP), which resulted in good growth and survival, but poor feed efficiency (FE). Pompano are highly active, suggesting those early diets had insufficient digestible energy (DE) to support the high metabolic demand. Recently, it was determined that the optimal digestible protein (DP) and DP:DE ratio for juvenile pompano in saltwater (SW) are 360 g kg^{-1} DP and between 23.8 and 25.1 mg DP kJ^{-1} (Riche, 2009).

Apparent digestibility coefficients of feed ingredients exist for only a few fish species and are limited for Florida pompano. Compounding the problem is evidence that salinity affects nutrient digestibility (Dabrowski *et al.*, 1986). Recent evidence suggests transcripts of amino acid solute carriers are differentially expressed in the gastrointestinal tract in pompano held in saltwater and low salinity (see next section), which may result in differences in amino acid (AA) availability in the two environments. Nutrient availability and established nutrient requirements are needed to implement least-cost feed formulation of balanced diets.

Digestibility and AA availability values for soybean meal (SBM), soy protein isolate (SPI), and corn gluten meal (Riche and Williams, 2010), as well as poultry by-product meal, meat and bone meal, and distillers dried grains (Williams, 2008) exist for pompano adapted to both SW and low salinity. Digestibility of CP and AA availability were high for the soy products but slightly lower for corn gluten meal. Digestible energy was inversely correlated to dietary carbohydrate, as is generally observed with carnivorous fish. The digestibility of CP (54-72%) and energy (64-76%) were lower and more variable for the by-product meals. In general, digestibility of the plant proteins appeared to be lower in SW than in low salinity water (Riche and Williams, 2010), but not for the by-product meals (Williams, 2008). To determine if verifiable differences in the

availability of nutrients exist between salinities we have designed a trial to evaluate seven additional ingredients in pompano held at 28 g l^{-1} and 3 g l^{-1}. Sample analysis is ongoing. This will result in expanding the database of digestibility values to 17 ingredients for pompano in both saltwater and in water of low salinity.

Riche and Williams (2011) evaluated SBM and SPI as fishmeal (FM) replacements in diets fed to pompano reared at 3 g l^{-1} salinity. Soybean meal could replace up to 80% and SPI 20% of FM protein without loss of growth or FE. Additional trials were conducted to evaluate nitrogen (N) utilization (digestibility, ammonia and urea excretion, and N accretion) and oxygen consumption in pompano fed five different poultry processing co-products as partial FM substitutes. No differences in growth, FE, or CP digestibility were detected relative to the FM control, and minor but significant differences were observed in ammonia and urea excretion. This suggests that poultry processing co-products are well utilized by pompano, even without indispensable the amino acid supplementation required by other species (Rawles *et al.,* 2006). However, establishing AA requirements for pompano will increase FE and decrease waste production.

Following evaluation of test diets utilizing three AA profiles (fish meal, pompano egg, and pompano whole body) and two intact protein sources (corn gluten meal and casein-gelatin) we developed a suitable test diet for determining AA requirements utilizing casein-gelatin supplemented with purified AA to match the pompano whole body profile (Riche and Williams, 2007). Recently, we determined the lysine (Lys) requirement of pompano reared at 3 g l^{-1} salinity using a traditional dose-response experiment. A broken saturated kinetics model was applied to the weight gain response and the Lys requirement was established as 22.5 g kg^{-1} diet (20.0 g available Lys). The Lys requirement and the ratio of Lys to total indispensable AA in pompano whole body (Kaushik, 1998) were used to estimate the requirements for the remaining AA (Table 2). The estimated AA requirements can be used to formulate more efficient diets for pompano until the true requirements can be established.

In the future we will: 1) continue to develop nutrient digestibility values for traditional and novel feed ingredients for pompano; 2) establish the total sulfur AA and arginine requirements for pompano in low salinity water and seawater; 3) reduce reliance on FM and fish oil during pompano production; and 4) evaluate dietary strategies to improve growth and performance of marine fish reared in low salinity environments.

Table 2. Florida pompano whole body IAA[1] and estimated IAA requirements

Amino Acid	Whole Body Profile (%)	Estimated Requirement (g kg^{-1} diet)
Arginine	3.40	20.6
Histidine	1.15	7.0
Isoleucine	2.05	12.4
Leucine	3.52	21.3
Lysine	3.73	22.5
Methionine	1.69	10.2
Phenylalanine	1.90	11.5
Threonine	2.29	13.9
Tryptophan	0.22	1.3
Valine	2.39	14.5

[1] Mean based on analysis of 3 fish.

Physiological Adaptation of Marine Fish to Low Salinity

Rearing marine species in low salinity water has been established by the U.S. aquaculture industry as a priority research area for the development of sustainable commercial production of marine fish. Although we have successfully reared Florida pompano to market size at 5 g l^{-1} salinity, we have observed that some populations of pompano exhibit low-grade chronic mortality when reared in low salinity environments. The cause of that mortality remains unknown. Interactions between salinity and fish health are complex and difficult to describe using conventional techniques. Genomic approaches using fish promise increased investigative power and have already provided insights into the mechanisms that underlie short-term and long-term environmental adaptations. Environmental genomics explores how the genome interacts with and integrates cues from the environment to produce both the effects of environmental stress and adaptive responses to stress. This approach can provide a description of genes involved in metabolic and osmoregulatory functions important for adaptation of pompano and other euryhaline marine species to low salinity conditions.

Advances in sequencing technologies have created the next generation of transcriptomics (Mardis, 2008). Similar to microarray, 454 sequencing can provide relative abundance of gene transcripts, but the method also provides species-specific sequences (Schirmer *et al.,* 2010). The method can provide insight into the molecular and physiological responses within cells and tissues to discover the complex physiological interactions between salinity and metabolism, growth, stress, and immune function.

Gill, kidney, intestine, and liver were individually collected from eight Florida pompano within three distinct groups; eight reared at 28 g l^{-1} salinity (high salinity, HS), eight performing well (healthy) and eight performing poorly (moribund) following approximately eight months at 3 g l^{-1} salinity (low salinity, LS). RNA from each tissue sample was individually extracted using TRIsure™ (Bioline USA Inc, Randolph, MA). Total RNA was spectrophotometrically quantified and equal amounts of RNA from each of the eight fish within a group were pooled for each tissue. The resulting pooled samples were used to construct 12 native libraries: HS-gill, HS-liver, HS-kidney, HS-intestine, LS1-gill, LS1-liver, LS1-kidney, LS1-intestine, LS2-gill, LS2-liver, LS2-kidney, and LS2-intestine[1]. Libraries were sequenced on a Roche GS-FLX (454) sequencer using Titanium chemistry (454 Life Sciences, a Roche Company, Branford, CT) by Purdue Genomics Core (Purdue University, West Lafayette, IN). Contigs (gene transcripts) were aligned from individual reads using Newbler 2.5 mapping and assembly software (Kumar and Blaxter, 2010). Contigs, isotigs and singletons were then annotated using BlastX and assigned gene ontogeny terms (GO-terms) using Blast2Go.

Gene transcripts, and their relative abundances, were compared between the HS healthy fish and LS healthy fish to identify potential effects of rearing pompano at LS (Table 3). Relative abundances of gene transcripts were also compared between the healthy pompano reared at LS with moribund pompano reared at the same salinity (Table 4) to discover potentially significant mechanisms that enable marine fish to successfully adapt to low salinities.

Gene ontogeny searches have to date focused on the abundance of solute carriers in the tissues known to play significant roles in osmoregulation: gill, kidney, and intestines. It appears that more solute carriers were up-regulated in kidneys of fish reared at 3 g l^{-1} salinity while more were down-regulated in the intestines (Table 3). Carriers that transport the cationic amino acids Lys, arginine and ornithine were more abundant in the kidneys of healthy fish reared at LS, but less abundant in the moribund fish. Carriers that transport those same substrates appear to be down-regulated in the intestines of healthy fish reared at LS relative to both healthy fish reared at HS and moribund fish. Transcript abundance is currently being validated using qPCR. Future research will focus

[1] HS, high salinity (28 g l^{-1}); LS-1, low salinity healthy fish (3 g l^{-1}); LS-2, low salinity moribund fish (3 g l^{-1}).

Table 3. Solute carriers (SLC) altered in Florida pompano reared at low salinity

Solute Carriers	Tissue	Log (ΔTPM)[1]	Substrates
Up-Regulated			
SLC 12 member 6	Gill	3.95	potassium, chloride
SLC 12 member 9	Gill	3.95	potassium, chloride
SLC 7 member 6	Gill	3.95	cationic amino acids
SLC 7 member 7	Gill	3.95	cationic amino acids
SLC 7 member 3	Gill	4.26	cationic amino acids
SLC 12 member 5	Kidney	4.07	potassium, chloride
SLC 24 member 3	Kidney	4.07	sodium, potassium, calcium
SLC 7 member 8 isoform 1	Kidney	4.07	cationic amino acids
SLC 7 member 7	Kidney	4.07	cationic amino acids
SLC 16 member 10	Kidney	4.07	aromatic amino acids
SLC 6 member 10	Kidney	4.37	neutral amino acids
SLC 24 member 2	Kidney	4.37	sodium, potassium, calcium
SLC 12 member 9	Intestine	4.68	potassium, chloride
SLC 6 member 19	Intestine	4.68	neutral amino acid transporter
Down Regulated			
SLC 24 member 3	Gill	4.32	sodium, potassium, calcium
SLC 7 member 9	Gill	4.32	cationic amino acids
SLC 7 member 2 isoform 1	Gill	4.02	cationic amino acids
SLC 12 member 2	Kidney	4.08	potassium, chloride
SLC 12 member 7	Kidney	4.08	potassium, chloride
SLC 24 member 6	Intestine	4.41	sodium, potassium, calcium
SLC 7 member 1	Intestine	4.41	cationic amino acids
SLC 7 member 2	Intestine	4.41	cationic amino acids
SLC 7 member 6	Intestine	4.41	cationic amino acids
SLC 12 member 6	Intestine	4.11	potassium, chloride
SLC 12 member 7	Intestine	4.11	potassium, chloride
SLC 3 member 1	Intestine	4.11	activator of dibasic and neutral amino acid transport
SLC 3 member 2 isoform 1	Intestine	4.11	activator of dibasic and neutral amino acid transport
SLC 7 member 4	Intestine	4.11	cationic amino acids
SLC 7 member 2 isoform 1	Intestine	4.11	cationic amino acids

[1]Log_{10} ((transcripts per million, from healthy fish reared at high salinity) - (transcripts per million, from healthy fish reared at low salinity)).

Table 4. Solute carriers (SLC) altered in moribund Florida pompano reared at low salinity relative to healthy fish reared at low salinity

Solute Carriers	Tissue	Log (ΔTPM)[1]	Substrates
Up-Regulated			
SLC 12 member 7	Gill	4.12	potassium, chloride
SLC 24 member 3	Gill	4.12	sodium, potassium, calcium
SLC 7 member 4	Gill	4.12	cationic amino acids
SLC 7 member 8	Gill	4.12	cationic amino acids
SLC 7 member 2 isoform 1	Gill	4.12	cationic amino acids
SLC 6 member 19	Gill	4.43	neutral amino acids
SLC 6 member 14	Kidney	4.14	amino acids
SLC 7 member 1	Kidney	4.14	cationic amino acids
SLC 36 member 1	Kidney	4.44	proton, amino acids
SLC 7 member 2	Kidney	4.62	cationic amino acids
SLC 12 member 6 isoform 2	Intestine	4.28	potassium, chloride
SLC 12 member 7	Intestine	4.28	potassium, chloride
SLC 24 member 6	Intestine	4.28	sodium, potassium, calcium
SLC 7 member 14	Intestine	4.28	cationic amino acids
SLC 7 member 3	Intestine	4.28	cationic amino acids
SLC 7 member 5	Intestine	4.28	cationic amino acids
SLC 7 member 6	Intestine	4.28	cationic amino acids
SLC 7 member 8	Intestine	4.28	cationic amino acids
Down-Regulated			
SLC 7 member 14	Gill	3.95	cationic amino acids
SLC 7 member 6	Gill	3.95	cationic amino acids
SLC 7 member 7	Gill	3.95	cationic amino acids
SLC 6 member 10	Kidney	4.37	neutral amino acids
SLC 24 member 2	Kidney	4.37	sodium, potassium, calcium
SLC 12 member 5	Kidney	4.07	potassium, chloride
SLC 12 member 9	Kidney	4.07	potassium, chloride
SLC 12 member 5	Kidney	4.07	potassium, chloride
SLC 24 member 3	Kidney	4.07	sodium, potassium, calcium
SLC 7 member 5	Kidney	4.07	cationic amino acids
SLC 7 member 8 isoform 1	Kidney	4.07	cationic amino acids
SLC 7 member 7	Kidney	4.07	cationic amino acids
SLC 12 member 5	Intestine	4.68	potassium, chloride
SLC 12 member 9	Intestine	4.68	potassium, chloride
SLC 12 member 5	Intestine	4.68	potassium, chloride
SLC 6 member 19	Intestine	4.68	neutral amino acids

[1]Log_{10} ((transcripts per million, from healthy fish reared at high salinity) - (transcripts per million, from healthy fish reared at low salinity)).

on addressing the problem of low-grade chronic mortality at low salinity by utilizing nutritional, physiological, and environmental approaches.

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