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Clinical evaluation of the HYDROFLOW water disinfection system

FINAL REPORT

May 2013

GOAL:

Monitoring the clinical effect of the HYDROFLOW water disinfection system on the following parameters:

1. Gram (-) Bacterial pathogens of fish.
2. Total Bacterial counts in water.
3. Morbidity rate.
4. Mortality rate.

PROTOCOL:

Four (4) Lg. Tanks (10 cubic Meters each) were re-circulated for a period of 90 Days in the Aquavet Wet – Lab facility in Israel. (See enclosed pictures)

TANK # II and III (DISEASE RESEVOIRS) were stocked with 300 Hybrid Tilapia (each) characterized by heavy presence of Bacterial, Fungal and Parasitic Infection.

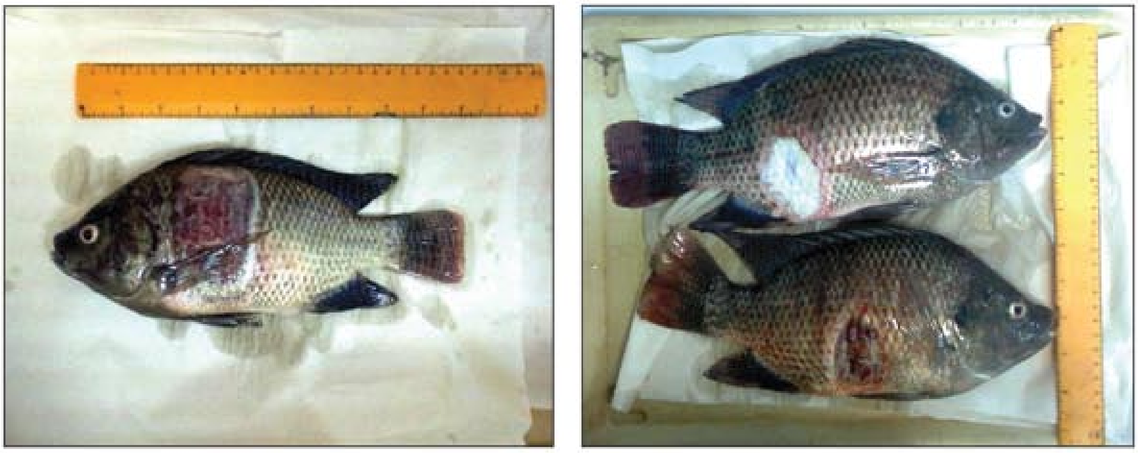
Tilapia from Tank II and III

Note severe gill rot and presence of *Saprolegnia* spp.

Isolation of Bacterial disease causing agents:

Presence of Gram (-) *Aeromonas* spp. in these fish was established following specific bacteriological isolation procedures:

- Bacteriological samples were collected from internal organs of clinically symptomatic fish
- Samples were collected by a sterile inoculation loop, and inoculated on plates with Brain Heart Infusion (BHI) agar. The plates were incubated at 25ºC for 24-48 h.
- Bacteria were identified as *Aeromonas* spp. using the following criteria:
  - Morphological features
  - Gram stain
  - Biocemical reactions (API – 20)



Gram (-) Bacterial infection in Tilapia spp.

TANK # I (POSITIVE CONTROL) and TANK # IV (NEGATIVE CONTROL) were stocked with 300 Hybrid Tilapia (each), free of infectious disease.

TANK # I (POSITIVE CONTROL) and TANK # IV (NEGATIVE CONTROL) were treated with 2 consecutive treatments of Formalin (Formaldag®) and Naled 50 (Bromex®) at the recommended doses in order to eliminate any presence of Parasitic pathogens prior to the beginning of recirculation.

In addition, TANK # I (POSITIVE CONTROL) and TANK # IV (NEGATIVE CONTROL) were treated with medicated feed (O.T.C. at 100 mg per Kg. of body weight x 10 days) in order to eliminate any presence of Bacterial pathogens and/or Bacterial disease symptoms in this population prior to the beginning of recirculation.

Water from TANK # II (DISEASE RESEVOIR) were not treated prior to transfer to TANK # I (POSITIVE CONTROL)

All Incoming water from TANK # III (DISEASE RESEVOIR) to TANK # IV (NEGATIVE CONTROL) were disinfected & treated by the HYDROFLOW disinfection system.

Furthermore, we made sure that all water flow from TANK III (DISEASE RESEVOIR) to TANK # IV (NEGATIVE CONTROL) was stopped in case of a malfunction of the HYDROFLOW system.

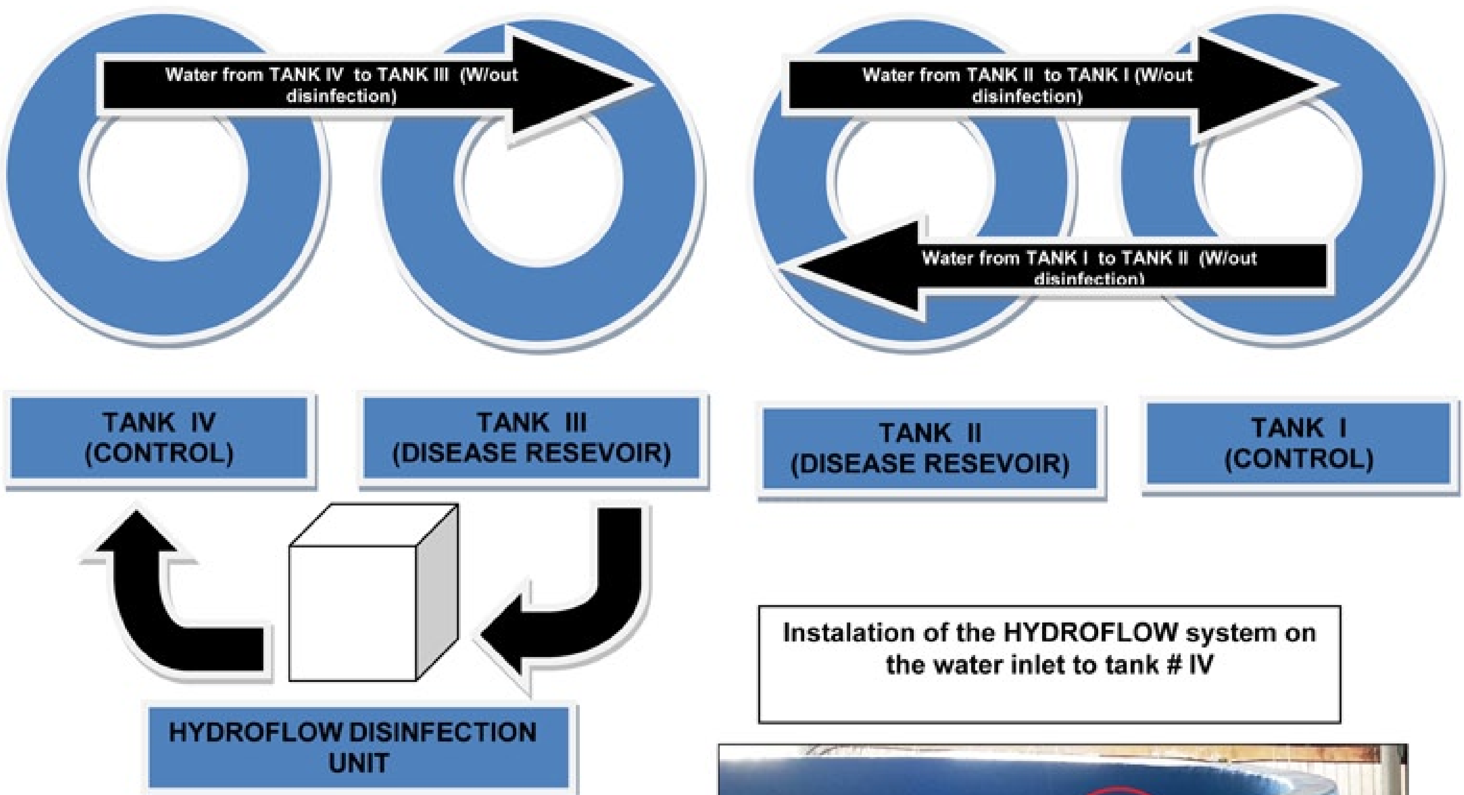
These controls were critical in preventing the flow of "un-treated" water from TANK III (DISEASE RESEVOIR) to TANK # IV (NEGATIVE CONTROL)

Fish in all 4 tanks were monitored for a period of 90 days.

Presence of morbidity and mortality in all three tanks was monitored on daily basis.

All fish in all 4 tanks were continuesly exposed to low temperatures and hypothermia, thus maintaining an effective disease reservoir of Bacterial, Parasitic and Fungal agents through the trial period.

HYDROFLOW - SCHEMATIC VIEW OF EXPERIMENTAL DESIGN



Instalation of the HYDROFLOW system on the water inlet to tank # IV

Water quality parameters in all 4 tanks was monitored on daily basis.

These parameters included:

- Ammonia:
- Nitrite:
- pH
- Temperature:
- Oxygen:

BACTERIOLOGY:

BACCKGROUND INFORMATION: [Gram (-) Bacteria of Tilapia]

Tilapia affected by gram negative bacteria show signs of darkening, exophthalmia, loss of appetite, hemorrhagic and ulcerated areas at the bases of the pectoral and ventral fins and in the eye region.

Internally, it is common to find pale livers and the presence of hemorrhagic foci. Necrosis of the liver, heart, spleen, and skeletal muscle is often detected, in addition to necrosis of the renal hematopoietic tissue.

External lesions of Gram (-) infection in Red TILAPIA SPP.

Gram (-) Bacterial infections in Tilapia can lead to losses of 5-100% in freshwater and brackish waters.

We monitored TOTAL COUNTS and SPECIFIC BACTERIAL COUNTS in TANK I, II, III and IV

Total Bacterial Contamination of Incoming and out flowing water was evaluated by utilization of the Hy-Giene monitoring system. The Hy-Giene monitor line of dip slides provides the solution for reliable monitoring kits of bacteria.

In addition, we monitored specific levels of both Gram (-) *Aeromonas* spp. in the fish.

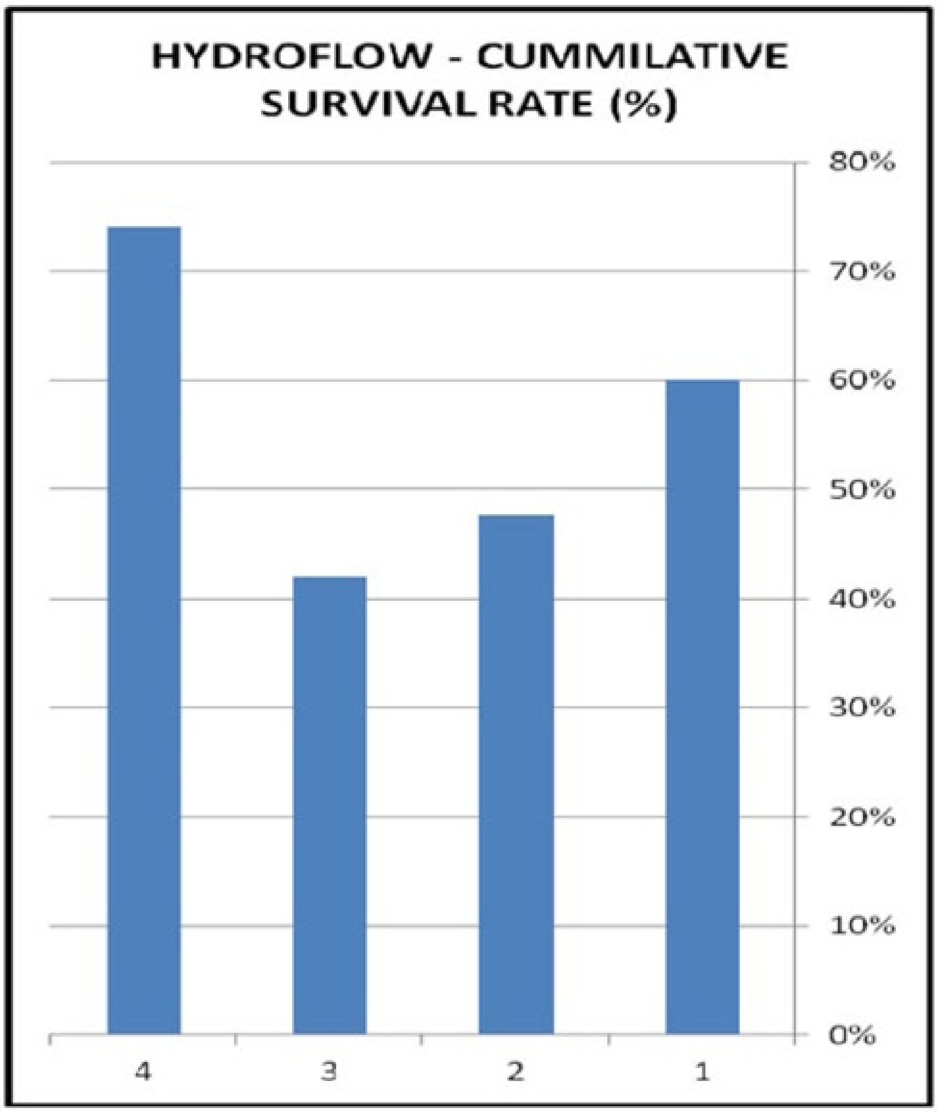
Clinical evaluation of the fish in the HYDROFLOW trial

RESULTS & CONCLUSIONS:

I – Morbidity and MORTALITY:

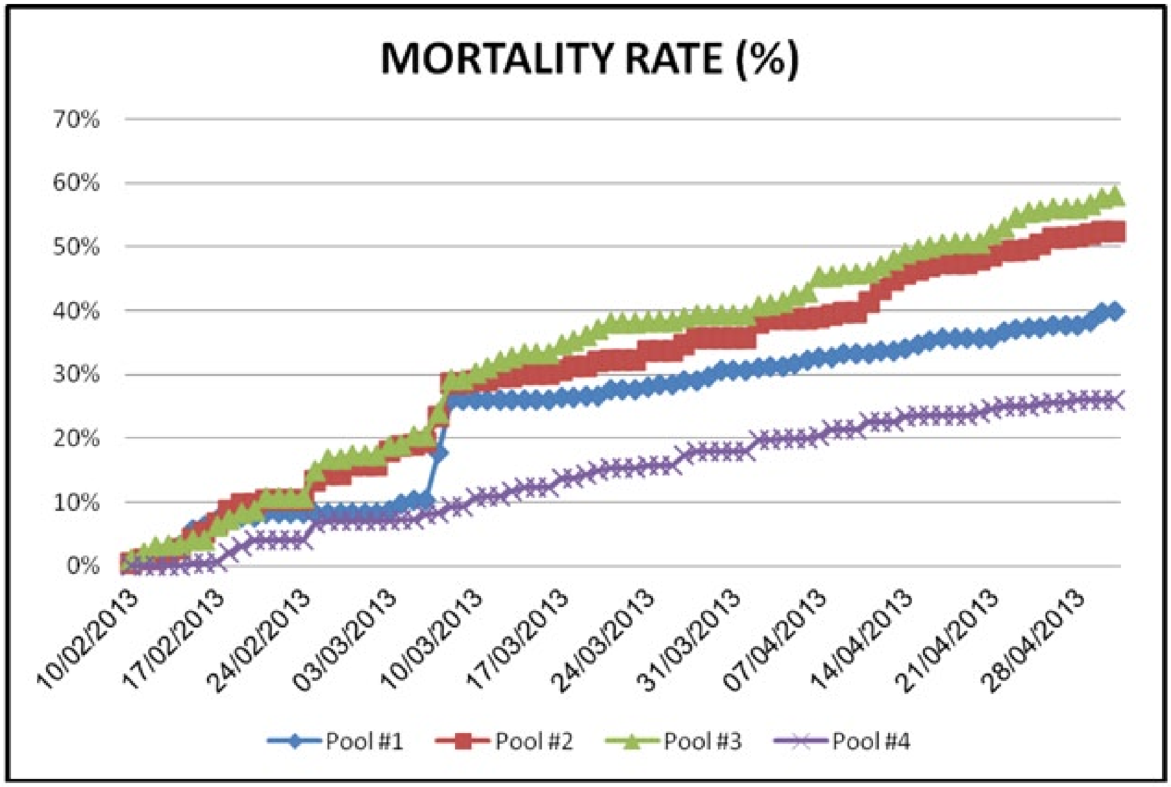
Following 90 days of detailed monitoring we may conclude the following:

- Overall survival rate in TANK # IV (HYDROFLOW Treated incoming water) is considerably higher (74%) when compared to survival rate in TANKS # I, II and III. (60%, 48% and 42% Respectfully)
- The Tilapia population in TANK # IV (HYDROFLOW Treated incoming water) exhibited increased survival rate and improved health as expressed by lower level of bacterial, parasitic and mycotic infection during 90 days of observation.



Mortality rate (%) at day 90

DAILY CUMILLATIVE MORTALITY RATE (%) at day 90



DAILY CUMILLATIVE MORTALITY CURVE (%) at day 90

II – BACTERIOLOGY: (Total counts)

Date	TANK WATER	Inlet	TANK WATER	TANK WATER	Inlet	TANK WATER
	TANK # III	TANK # IV	TANK # IV	TANK # II	TANK # I	TANK # I
01/02/2013	1.00E+07	1.00E+07	1.00E+07	1.00E+07	1.00E+07	1.00E+07
07/02/2013	1.00E+07	1.00E+04	1.00E+04	1.00E+06	1.00E+07	1.00E+07
14/02/2013	1.00E+06	1.00E+04	1.00E+06	1.00E+07	1.00E+06	1.00E+07
21/02/2013	1.00E+07	1.00E+05	1.00E+07	1.00E+07	1.00E+07	1.00E+07
28/02/2013	1.00E+07	1.00E+05	1.00E+05	1.00E+07	1.00E+07	1.00E+07
07/03/2013	1.00E+07	1.00E+07	1.00E+06	1.00E+07	1.00E+07	1.00E+06
14/03/2013	1.00E+07	1.00E+06	1.00E+07	1.00E+07	1.00E+06	1.00E+07
21/03/2013	1.00E+07	1.00E+06	1.00E+06	1.00E+06	1.00E+07	1.00E+05
28/03/2013	1.00E+07	1.00E+06	1.00E+05	1.00E+07	1.00E+07	1.00E+07
04/04/2013	1.00E+07	1.00E+05	1.00E+04	1.00E+07	1.00E+07	1.00E+06
10/04/2013	1.00E+07	1.00E+05	1.00E+05	1.00E+07	1.00E+06	1.00E+06
17/04/2013	1.00E+07	1.00E+05	1.00E+06	1.00E+07	1.00E+07	1.00E+07
24/04/2013	1.00E+07	1.00E+05	1.00E+06	1.00E+07	1.00E+07	1.00E+07
01/05/2013	1.00E+07	1.00E+06	1.00E+06	1.00E+07	1.00E+07	1.00E+07

Sampling of total Bacterial counts

Morbid Tilapia from TANKS # II and III (DISEASE RESERVOIRS)

Note: Heavy presence of Fungal infection (*Saprolegnia* spp.) fin rot, hemorrhages and superficial ulceration

Limited water resources and an increased emphasis on effluent pollution reduction have created a more difficult regulatory, economic, and social environment for aquaculture production facilities.

Certain production practices and key production inputs, as utilized currently, are no longer assuring future profitability or sustainability for the aquaculture industry.

Developing *new intensive fish growing technologies* that improve the production efficiency of aquaculture species has become of utmost importance to addressing the problems of 1) physical scale, design and economic viability, 2) challenges of sustainability and environmental compatibility, and 3) overall management practices that improve stock health and enhance production efficiency. A maturing aquaculture sector now requires more cost-effective, reliable, biosecure, and environmentally compatible production systems and management practices.

Infectious diseases of aquatic organisms have become the most important limiting factor in the world-wide expansion of the aquaculture industry.

The F.A.O. estimates that aquatic diseases are responsible for severe economic losses in every part of the industry. These losses are estimated at well over 7 Billion U.S. Dollars per year.

As such, there is great importance in introducing new measures which will decrease the levels of mortality and morbidity among cultured fish populations.

Current measures for control of infectious disease include eradication of infected populations, aggressive drug therapy (antibiotics, antihemlminthics, antiprasitic medications and such) and wide spread utilization of chemicals, (Formalin, Cupper sulfate, Malachite Green, etc.)

The intensification process of this industry and the concurrent increase in disease outbreaks has led to heavy dependency on aggressive drugs and medications which in turn contributed to development of new and even more aggressive disease agents.

Many of these medications (Malachite Green, Formalin, Chloramphenicol) have been proven to be a severe health hazard for human populations. As example, Malachite Green, one of the most common anti-parasitic and anti-mycotic treatments has been proven to be both carcinogenic and teratogenic in its action. Regardless, it is widely used in every part of the industry.

Others, (Naled, Diflurobenzuran) have severe side effects through their effect on the environment and non-target species.

Most of the chemicals and medications which are currently used by this industry are not registered by regulatory offices and as such represent a serious danger to both the users of these products and the consumers of the fish.

Recirculating aquaculture systems, also known as water reuse systems, have become more and more popular. Recirculating systems are commonly found in aquaculture facilities, wholesale and retail tropical fish facilities, and public aquaria. However, in order to successfully and most efficiently operate one of these systems, a good understanding of fish health management considerations is critical.

Water quality can be more unstable in recirculating systems than in large ponds or flow-through systems. Water quality fluctuations, such as temporary increases in ammonia or nitrite, can, by themselves, result in disease or significant losses. These environmental fluctuations often lead to suppressed immune systems and greater susceptibility to pathogens (i.e., disease-causing organisms, such as bacteria, parasites, fungi, and viruses) and disease outbreaks.

Re-circulating systems favor the growth of many disease-causing organisms and spread of disease. There are a number of reasons for this tendency, including higher densities of fish when compared to other culture systems; build up of sediment and subsequently pathogens in tanks, sumps, or filtration components (especially mechanical and biological filters); and slower turn over of water.

Over time, pathogens can become concentrated (i.e., present in high numbers). Most pathogens are considered opportunistic, causing disease only in fish with suppressed immune systems. However, if pathogens become sufficiently numerous they can also cause disease in healthy fish. In addition, the continuous flow of water throughout a system can spread pathogens rapidly, especially in a system lacking adequate disinfection protocols or components.

Adequate control of pathogens in a system, and consequently reduction of disease in these systems, requires an understanding of where pathogens may be found, how they can be transmitted to fish, and how their numbers may be reduced. In addition, understanding the proper use of chemicals to reduce or eliminate pathogens is an essential part of good management.

As described previously, water may spread pathogens and also be a potential reservoir for them. Water from a tank containing sick fish often carries numerous disease-causing microorganisms. When this same water enters another tank of fish, those fish are then exposed to the microorganisms and they will have an increased risk of developing disease, which greatly helps reduce the spread of some pathogens.

As such, the development and implementation of new technologies for water disinfection in recirculated culture systems is a priority in the aquaculture industry which will result in numerous benefits.

The HYDROFLOW water disinfection system has been evaluated for a period of 90 days in the AquaVet testing facility in Israel.

During this period we evaluated the clinical effect of the HYDROFLOW water disinfection system on the following parameters:

1. **Gram (-) Bacterial pathogens of fish.**
2. **Total Bacterial counts in water.**
3. **Morbidity rate.**
4. **Mortality rate.**

During 90 days of observation, overall survival rate in TANK # IV (HYDROFLOW Treated incoming water) is considerably higher (74%) when compared to survival rate in TANKS # I, II and III. (60%, 48% and 42% Respectfully)

In addition, the Tilapia population in TANK # IV (HYDROFLOW Treated incoming water) exhibited improved health status as expressed by active feeding behavior and lower level of parasitic, bacterial and mycotic infection during 90 days of observation.

During the majority of the observation period, we recognized a clear effect on prevention of disease transmission (Parasitic, Mycotic and Bacterial pathogens of fish) through the water.

Furthermore, The HYDROFLOW system was effectively able to prevent the transmission of these infectious agents during the majority of the observation period as expressed in these parameters.

- Overall Bacterial counts in TANK # IV (HYDROFLOW Treated incoming water) were lower when compared to TANKS # I, II and III during most of the observation period.
- Overall mortality in TANK # IV (HYDROFLOW Treated incoming water) were lower when compared to TANKS # I, II and III during most of the observation period.
- Overall morbidity in TANK # IV (HYDROFLOW Treated incoming water) was considerably lower when compared to TANKS # I, II and III during most of the observation period.

As such, we may conclude that HYDROFLOW water disinfection system was very effective in preventing transmission of Bacerial, Parasitic and Mycotic infection in a recirculated experimental system during 90 days of observation.

The implementation of this innovative technology for disinfection of water in re-circulated systems may result in the following benefits:

- Higher yields due to decreased losses from morbidity and mortality
- Higher yields due to increased feed conversion efficiency
- Reduced utilization of medications & chemicals
- Higher prices due to enhanced public perception of quality (both specific markets and aquaculture in general)
- Value-added products for penetration of growing niche markets (e.g., "organically" grown)
- Reduced trade barriers and enhanced ability to export products
- Reduced regulatory pressure due to lessened environmental impacts and concerns.

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