Dr. Ra'anan Ariav
AquaVet Technologies Ltd.
POB 1725, N. Habaron
Zichron Yakov 30900
Israel
Tel. +972-4-6291-833
Fax. +972-4-6390-957
E-Mail: aquavet@netvision.net.il
Website: www.aqua-vet.co.il

17/1/14

Clinical evaluation of the HYDROFLOW water disinfection system

FINAL REPORT

I - GOAL:

This clinical evaluation has monitored the effect of the HYDROFLOW water disinfection system on *Gyrodactylus* spp.

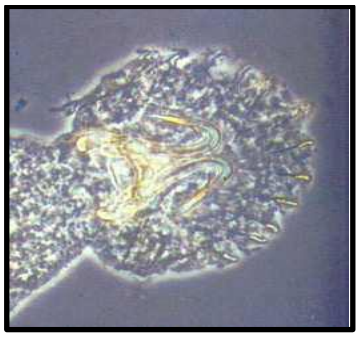
As such, we evaluated the following parameters:

1. Presence of parasites in gill tissue and skin.
2. Morbidity rate.
3. Mortality rate.

II - BACKGROUND INFORMATION:

Gyrodactylus spp. are part of the monogenean group of parasites. The monogeneans are parasites that have annex foliating action on the skin and gills of fish. They feed on the mucus and epithelium of the body surface, producing external lesions that erode and expose the dermis to infection. Monogeneans are considered extremely aggressive organisms.

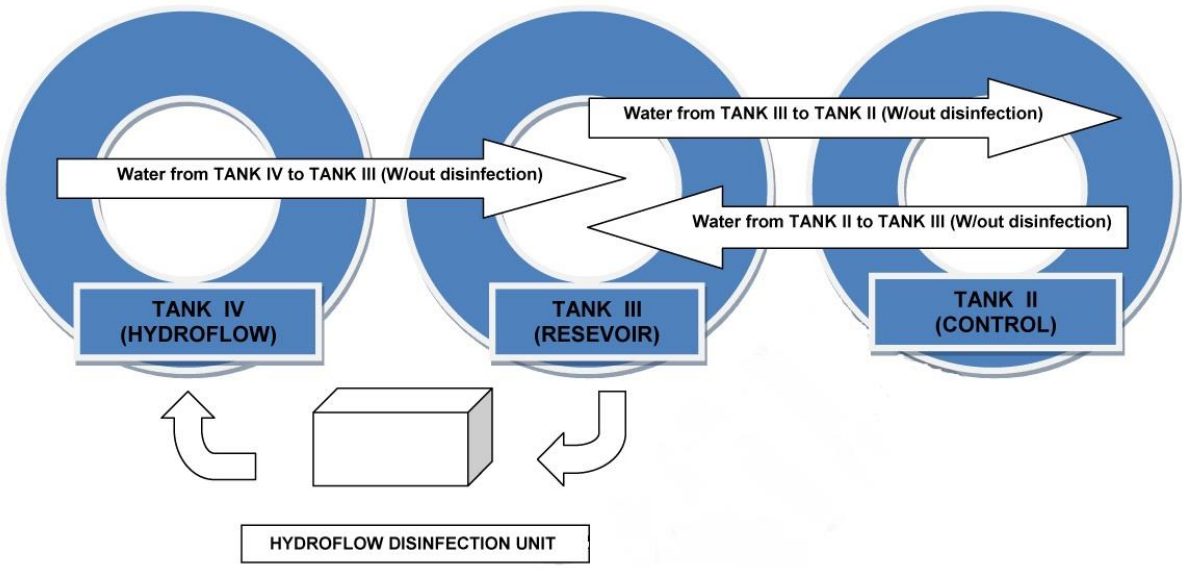
As such, these parasites (e.g. *Gyrodactylus* spp.) cause heavy mortalities within a short period of time. They can be treated by baths in formalin, potassium permanganate or organo-phosphorus compounds.



III - PROTOCOL:

Three (3) large tanks (10 cubic meters each) were re-circulated for a period of 45 days in the AquaVet Wet - Lab facility.

HYDROFLOW - SCHEMATIC VIEW OF EXPERIMENTAL DESIGN



The HYDROFLOW 60i disinfection system within the AquaVet Wet - Lab facility

TANK # III (DISEASE RESEVOIR) was stocked with 200 Hybrid Tilapia characterized by heavy presence of parasites.

TANK # II and IV were stocked with 200 Hybrid Tilapia (each) free from infectious disease. Water was recirculated between TANK # III and TANK # II continuously, without any treatment. Water was recirculated between TANK # III and TANK # IV continuously, with treatment by the HYDROFLOW water disinfection system. All incoming water to TANK # IV was disinfected, continuously.

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

Water quality parameters in all three tanks were monitored on a daily basis. These parameters included: Ammonia, Nitrite, pH, Temperature and Oxygen.

Parasitic loads on the fish in TANKS II, III and IV (Gill and Skin) were evaluated microscopically every 10 days.

Fish were graded as follows: (1 – 5 Scale)

1. Very low parasitic load.
2. Moderate parasitic load.
3. High parasitic load.
4. Very high parasitic load
5. Parasitimia: Extremely high parasitic load

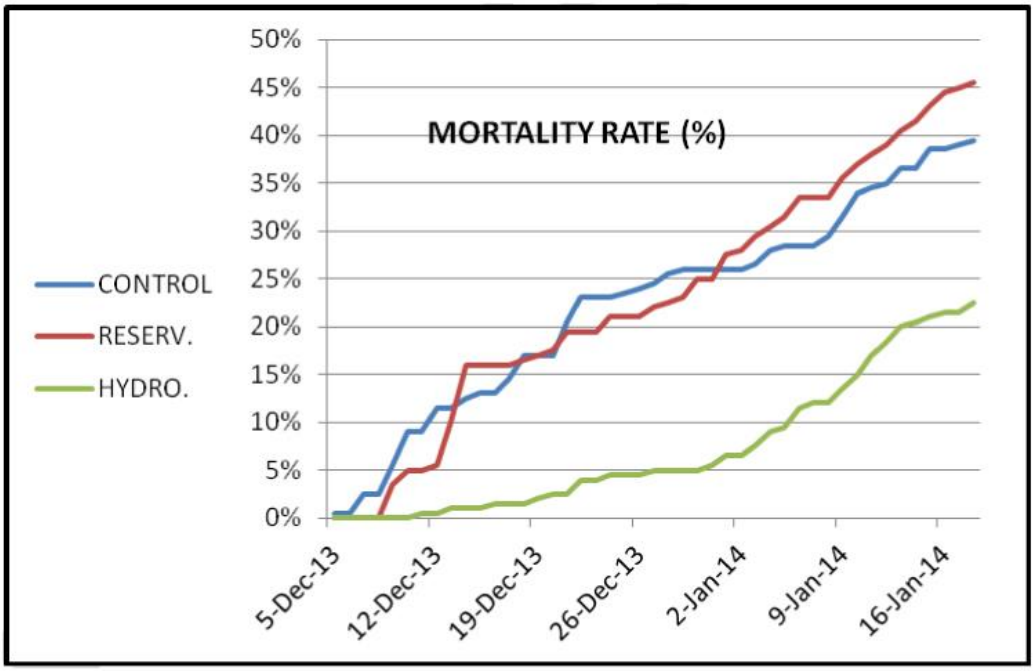
IV - RESULTS:

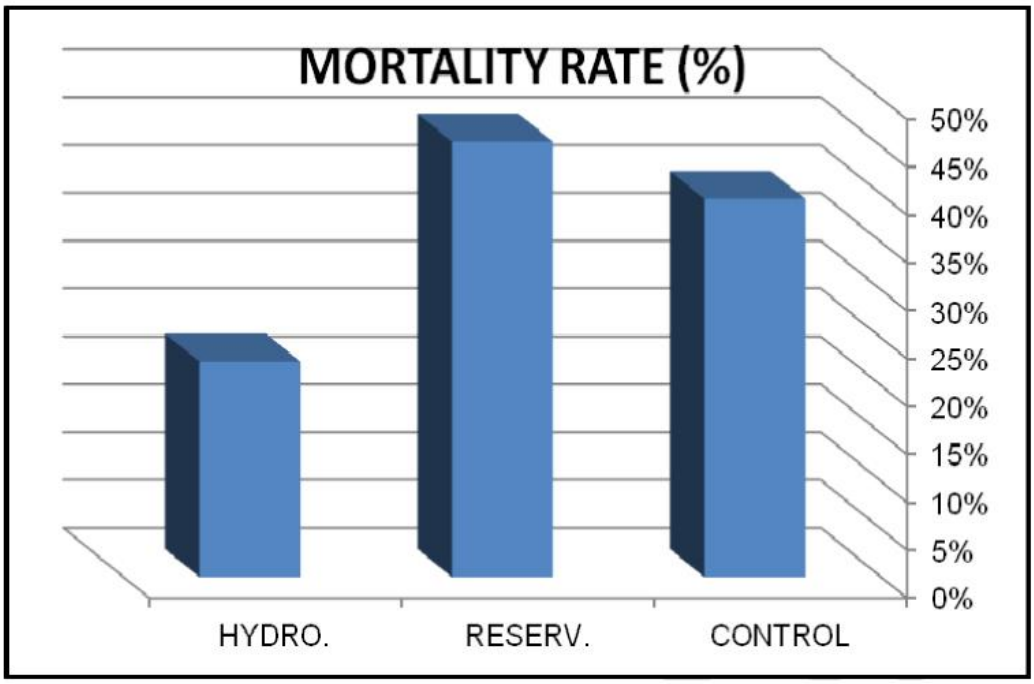
DAILY MORTALITY CHART:

Days	Date	II	III	IV
		CONTROL	RESERV.	HYDRO.
1	5-Dec-13	-1	0	0
2	6-Dec-13	0	0	0
3	7-Dec-13	-4	0	0
4	8-Dec-13	0	0	0
5	9-Dec-13	-6	-7	0
6	10-Dec-13	-7	-3	0
7	11-Dec-13	0	0	-1
8	12-Dec-13	-5	-1	0
9	13-Dec-13	0	-9	-1
10	14-Dec-13	-2	-12	0
11	15-Dec-13	-1	0	0
12	16-Dec-13	0	0	-1
13	17-Dec-13	-3	0	0
14	18-Dec-13	-5	-1	0
15	19-Dec-13	0	-1	-1
16	20-Dec-13	0	-1	-1
17	21-Dec-13	-7	-4	0
18	22-Dec-13	-5	0	-3
19	23-Dec-13	0	0	0
20	24-Dec-13	0	-3	-1
21	25-Dec-13	-1	0	0
22	26-Dec-13	-1	0	0
23	27-Dec-13	-1	-2	-1
24	28-Dec-13	-2	-1	0
25	29-Dec-13	-1	-1	0
26	30-Dec-13	0	-4	0
27	31-Dec-13	0	0	-1
28	1-Jan-14	0	-5	-2
29	2-Jan-14	0	-1	0
30	3-Jan-14	-1	-3	-2
31	4-Jan-14	-3	-2	-3
32	5-Jan-14	-1	-2	-1
33	6-Jan-14	0	-4	-4
34	7-Jan-14	0	0	-1
35	8-Jan-14	-2	0	0
36	9-Jan-14	-4	-4	-3
37	10-Jan-14	-5	-3	-3
38	11-Jan-14	-1	-2	-4
39	12-Jan-14	-1	-2	-3
40	13-Jan-14	-3	-3	-3
41	14-Jan-14	0	-2	-1
42	15-Jan-14	-4	-3	-1
43	16-Jan-14	0	-3	-1
44	17-Jan-14	-1	-1	0
45	18-Jan-14	-1	-1	-2

MORTALITY RATE: (%)

Date	II	III	IV
	CONTROL	RESERV.	HYDRO.
5-Dec-13	1%	0%	0%
6-Dec-13	1%	0%	0%
7-Dec-13	3%	0%	0%
8-Dec-13	3%	0%	0%
9-Dec-13	6%	4%	0%
10-Dec-13	9%	5%	0%
11-Dec-13	9%	5%	1%
12-Dec-13	12%	6%	1%
13-Dec-13	12%	10%	1%
14-Dec-13	13%	16%	1%
15-Dec-13	13%	16%	1%
16-Dec-13	13%	16%	2%
17-Dec-13	15%	16%	2%
18-Dec-13	17%	17%	2%
19-Dec-13	17%	17%	2%
20-Dec-13	17%	18%	3%
21-Dec-13	21%	20%	3%
22-Dec-13	23%	20%	4%
23-Dec-13	23%	20%	4%
24-Dec-13	23%	21%	5%
25-Dec-13	24%	21%	5%
26-Dec-13	24%	21%	5%
27-Dec-13	25%	22%	5%
28-Dec-13	26%	23%	5%
29-Dec-13	26%	23%	5%
30-Dec-13	26%	25%	5%
31-Dec-13	26%	25%	6%
1-Jan-14	26%	28%	7%
2-Jan-14	26%	28%	7%
3-Jan-14	27%	30%	8%
4-Jan-14	28%	31%	9%
5-Jan-14	29%	32%	10%
6-Jan-14	29%	34%	12%
7-Jan-14	29%	34%	12%
8-Jan-14	30%	34%	12%
9-Jan-14	32%	36%	14%
10-Jan-14	34%	37%	15%
11-Jan-14	35%	38%	17%
12-Jan-14	35%	39%	19%
13-Jan-14	37%	41%	20%
14-Jan-14	37%	42%	21%
15-Jan-14	39%	43%	21%
16-Jan-14	39%	45%	22%
17-Jan-14	39%	45%	22%
18-Jan-14	40%	46%	23%


MORTALITY RATE (%)
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
CONTROL
RESERV.
HYDRO.
5-Dec-13
12-Dec-13
19-Dec-13
26-Dec-13
2-Jan-14
9-Jan-14
16-Jan-14


MORTALITY RATE (%)
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
HYDRO.
RESERV.
CONTROL

INFECTION RATE: (1 – 5 SCALE)

Date	TANK II	TANK III	TANK IV
5-Dec-13	(CONTROL)	(RESERV.)	(HYDRO.)
1	1	5	0
2	0	4	1
3	0	5	0
4	1	4	0
5	0	5	0
MEAN VALUE	0.4	4.6	0.2

Date	TANK II	TANK III	TANK IV
14-Dec-13	(CONTROL)	(RESERV.)	(HYDRO.)
1	1	5	0
2	0	5	0
3	1	5	1
4	1	4	1
5	0	5	1
MEAN VALUE	0.6	4.8	0.6

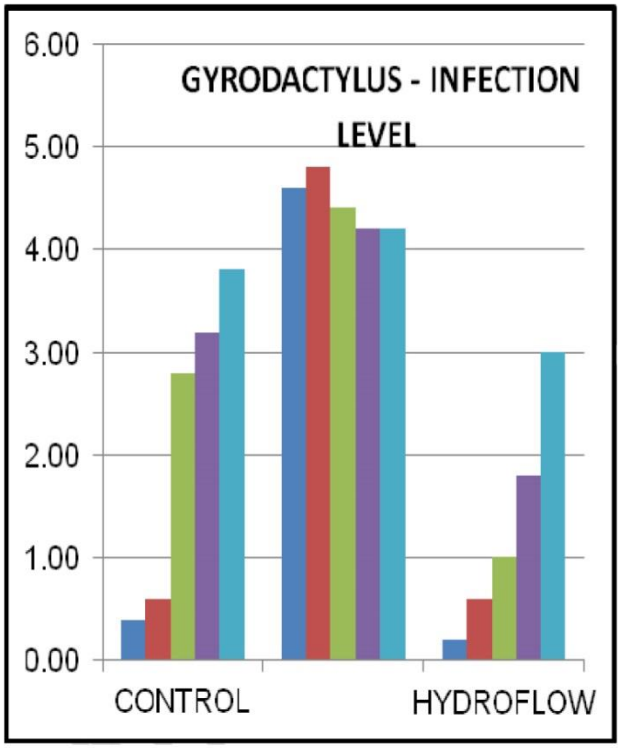
Date	TANK II	TANK III	TANK IV
24-Dec-13	(CONTROL)	(RESERV.)	(HYDRO.)
1	3	4	1
2	3	4	1
3	2	5	1
4	3	5	0
5	3	4	2
MEAN VALUE	2.80	4.40	1.00

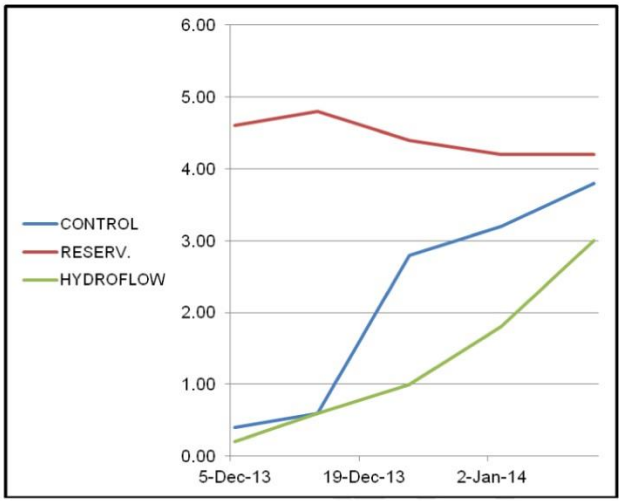
Date	TANK II	TANK III	TANK IV
3-Jan-14	(CONTROL)	(RESERV.)	(HYDRO.)
1	3	4	2
2	4	3	3
3	3	5	1
4	2	4	1
5	4	5	2
MEAN VALUE	3.20	4.20	1.80

Date	TANK II	TANK III	TANK IV
13-Jan-14	(CONTROL)	(RESERV.)	(HYDRO.)
1	4	4	3
2	4	5	4
3	3	4	4
4	4	3	4
5	4	5	4
MEAN VALUE	3.80	4.20	3.00

INFECTION RATE: (MEAN VALUES)

Date	TANK II	TANK III	TANK IV
	CONTROL	RESERV.	HYDROFLOW
5-Dec-13	0.40	4.60	0.20
14-Dec-13	0.60	4.80	0.60
24-Dec-13	2.80	4.40	1.00
3-Jan-14	3.20	4.20	1.80
13-Jan-14	3.80	4.20	3.00





STATISTICAL ANALYSIS:

INFECTION RATE AT DAY # 1

TANK II	TANK III	TANK IV
CONTROL	RESERV.	HYDROFLOW
1	5	0
0	4	1
0	5	0
1	4	0
0	5	0

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	2	0.4	0.3
Column 2	5	23	4.6	0.3
Column 3	5	1	0.2	0.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	61.73333333	2	30.86666667	115.75	**1.4325E-08**	3.885293835
Within Groups	3.2	12	0.266666667			
Total	64.93333333	14				

CONCLUSION - DIFFRENCES BETWEEN GROUPS HIGHLY SIGNIFICANT (P VALUE < 0.05)

INFECTION RATE AT DAY # 10

TANK II	TANK III	TANK IV
CONTROL	RESERV.	HYDROFLOW
1	5	0
0	5	0
1	5	1
1	4	1
0	5	1

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	3	0.6	0.3
Column 2	5	24	4.8	0.2
Column 3	5	3	0.6	0.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	58.8	2	29.4	110.25	**1.89038E-08**	3.885293835
Within Groups	3.2	12	0.266666667			
Total	62	14				

CONCLUSION - DIFFRENCES BETWEEN GROUPS HIGHLY SIGNIFICANT (P VALUE < 0.05)

INFECTION RATE AT DAY # 20

TANK II	TANK III	TANK IV
CONTROL	RESERV.	HYDROFLOW
3	4	1
3	4	1
2	5	1
3	5	0
3	4	2

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	14	2.8	0.2
Column 2	5	22	4.4	0.3
Column 3	5	5	1	0.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	28.93333333	2	14.46666667	43.4	**3.2103E-06**	3.885293835
Within Groups	4	12	0.333333333			
Total	32.93333333	14				

CONCLUSION - DIFFRENCES BETWEEN GROUPS HIGHLY SIGNIFICANT (P VALUE < 0.05)

INFECTION RATE AT DAY # 30

TANK II	TANK III	TANK IV
CONTROL	RESERV.	HYDROFLOW
3	4	2
4	3	3
3	5	1
2	4	1
4	5	2

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	16	3.2	0.7
Column 2	5	21	4.2	0.7
Column 3	5	9	1.8	0.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.53333333	2	7.266666667	10.38095238	**0.002**	3.885293835
Within Groups	8.4	12	0.7			
Total	22.93333333	14				

CONCLUSION - DIFFRENCES BETWEEN GROUPS ARE SIGNIFICANT (P VALUE < 0.05)

INFECTION RATE AT DAY # 40

TANK II (CONTROL)	TANK III (RESERV.)	TANK IV (HYDRO.)
4	4	3
4	5	4
3	4	4
4	3	4
4	5	4

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	19	3.8	0.2
Column 2	5	21	4.2	0.7
Column 3	5	19	3.8	0.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.533333333	2	0.266666667	0.727272727	**0.503**	3.885293835
Within Groups	4.4	12	0.366666667			
Total	4.933333333	14				

CONCLUSION - DIFFRENCES BETWEEN GROUPS ARE INSIGNIFICANT (P VALUE > 0.05)

V - CONCLUSIONS:

The HYDROFLOW water disinfection system has been evaluated for a period of 45 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. Gyrodactylus spp.
2. Morbidity rate.
3. Mortality rate.

During 45 days of observation, overall survival rate in TANK # IV (HYDROFLOW Treated incoming water) was considerably higher when compared to the survival rate in TANKS # II and III.

In addition, the Tilapia population in TANK # IV (HYDROFLOW Treated incoming water) exhibited improved health status by expressing a lower level of Gyrodactylus infection during the first 30 - 40 days of the clinical evaluation.

During the majority of the observation period, we recognized a clear effect on prevention of parasitic disease transmission through the water.

Variances between the tanks were greatly reduced by day 40. This could be because the clinical study was performed in a closed recirculating system, without a filter. Eventually, the parasitic load became too abundant for the HYDROFLOW system to be effective.

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 mortality in TANK # IV (HYDROFLOW treated incoming water) was lower when compared to TANKS # 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 # II and III during most of the observation period.

- Presence of Gyrodactylus spp. infection in TANK # IV (HYDROFLOW treated incoming water) was lower when compared to their presence in TANKS # II and III during the first 30 - 40 days of the observation period.

As such, we may conclude that that HYDROFLOW water disinfection system was very effective in preventing transmission of Parasitic infection in a recirculated experimental system during the first 40 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

This trial was conducted without any filtration system. Gyrodactylus is less than half a millimeter in size and barely visible to the naked eye. However, an effective filtration system (100 Micron) may show significant benefit when used after the disinfection system due to its ability to flocculate suspended solids.

Dr. Ra'anan Ariav
AquaVet TECHNOLOGIES, Israel