

The Effects of Suspended Solids on *Oreochromis niloticus* Filter Feeding Selection Mechanism and Ingestion of *Microcystis aeruginosa*

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ABSTRACT

Oreochromis niloticus species are filter feeder having gill raker on the bronchial arch modified to selectively sieve suspended solids into the alimentary canal. They have pharyngeal teeth for breaking algae cells and produce gastric acid that lyses blue-green algae in the stomach. Silt particles obtained from the bottom mud collected from Airthrey Loch (Lake) were passed through a series of sieves down to 63 μ m were used and sand particles used were also sieved through a series of sieves down to 68 μ m. *O. niloticus* fed on micro-pelleted diet of mean weight of 7.50 mg reared in re-cycled water system maintained at 27^oC were put into aerated three 5 litre experimental tanks with two replicates and control. Concentrations of 50 and 150mg/l of sand and algae and the same for silt and algae were introduced in the tanks to which 50 and 150mg/l dry weight of algae were added respectively. Experimental fish were left for 8 hours then removed and killed by piercing using a scalpel at the back of the brain at intervals of 15, 30, 45, and 60 minutes after feeding. The gill arches were carefully removed and put into a Petri-dish and particles observed under dissecting microscope. The stomach and gut contents were removed by micro-pipette and put on a microscope slide and examined under microscope. In this study, it was found that silt particles were entangled and bound in the copious mucus of the gill structures but not algae and sand particles. The stomach and gut contents had algae and silt particles which proved difficult to quantify because the remnants of ingested particles invariably included both detritus material. Sand particles were not seen in the entire gill apparatus stomach and gut. Thus it can be concluded that silt and algae particles are ingested but not sand particles.

Key words: algae, silt, sand, ingestion, filter-feeding

INTRODUCTION

In developing countries where industrially manufactured feeds are costly and scarce due to low level of technology, tilapia can be extensively cultured by artisanal fish farmers. In this respect planktonic algae which grow successfully in freshwater ponds in the tropical countries may prove a substitute for expensive fishmeal and other animal by-products. Many species of carp and tilapias readily accept algae as feed. Favourable results have been reported by Ahmed (1966), using micro algae as a protein source in the feeds of warm water fishes.

Oreochromis niloticus are mainly omnivorous and feed on phytoplankton (Moriarty and Moriarty, 1973). They are reported to successfully feed on phytoplankton because they have the ability to digest blue-green algae which are widespread in freshwater in the tropics (Moriarty, 1973, Fries and Iles, 1972). Filter feeding has evolved in a number of fishes. It is a specialized form of feeding which has evolved in many of the cichlids, enabling them to exploit a variety of common food sources (Fryer and Iles, 1972)

The Total Suspended Solids (TSS) influence on fish reported in the literature range from beneficial to detrimental. There has been some benefit from increased suspended sediments in aquatic ecosystems. These include increased protection to prey fish from predators (Bruton, 1985, Doan, 1941) as well as the predators themselves (Gregory and Northcote, 1993), increased production for some species such as channel catfish (Homer, 1956), enhanced fishing success for species including eels (Deelder, 1970). With increase in concentration, Johnson and Wildish (1982) noted a depression in the feeding rate of larval herring. For pacific herring, Boehlert and Morgan (1985) found that the incidence and intensity of maximum feeding occurred at levels 500-1000mg/l of suspended solids which were significantly greater than in controls 0mg/l. They hypothesized that suspended solids may have acted to improve visual contrast thereby increasing feeding efficiency.

Suspended sediments are usually silt and clay particles that are between 2 and 60 micrometers in diameter. In the tropics the effect of suspended solids in fish in water bodies and ponds can be a problem following torrential rains accompanied by surface run-off where streams and rivers are major source of water for some ponds. The actual

effect on fauna would depend on the physical characteristics of the particles. Fish farmers have often used pond color and turbidity as measure of phytoplankton standing crop (Lin, 1986, Boyd, 1990).

Effects of suspended particles on fish include effects on fish behaviour, physiology and fish habitat. While suspended sediments are often the main contributors to turbidity, organic turbidity consisting of algae, phytoplankton and algae-derived detritus, zooplankton and fecal matter also contribute to turbidity.

Both the recovery efficiency and settling velocity of particles generally have a direct relationship to the weight and size of particles (the heavier the particle, the faster the deposit; Han *et al.*, 1999). Newcombe (2003) created a model that takes into account of duration of exposure and suspended sediments concentration to project possible effects on fish. The model SEV is a scale ranked 0 to 14 on a 15 step scale, where 0 represents nil effect and 14 100 percent mortality (Newcombe and Jansen 1996). On severity of Ill Effects Scale and Corresponding Effects on Salmonids he showed that at SEV 4 level there was short-term reduction in feeding rate; short-term reduction in feeding success whereas at SEV 8 there was indication of major physiological stress, long-term reduction in feeding rate, long term reduction in feeding success, poor condition regarding severity of effects at sub-lethal level.

The use of algae as fish food has a great potential. They are generally recognized as the natural food for many fish species especially the omnivorous and herbivorous. Le Roux (1956) found the phytoplankton to be the only food for the juvenile *Oreochromis* and Tilapia. Alikunhi (1957) found the proportion of algae consumed in major carps to vary with their size. Algae have been used in tilapia diets to substitute or as an alternative source of limiting essential amino acids. Boyd (1976) found the amino acid composition of blue-green *Anabaena circinalis* to be higher at 0.94% and 0.88% for histidine and methionine respectively compared to fish meal.

*Microcystis auraginos*a used in this work are planktonic and are aided by pseudovacuoles for buoyancy. They live in suspension in water and are thus consumed by plankton feeders by filter feeding mechanism. Due to this *microcystis* has been the subject of considerable research. Algal blooms due to blue-green algae mainly of the genera *microcystis*, *anabaena*, *oscillatoria* and *spirulina* are common in fishponds in Israel during summer months leading to occasional heavy mortalities (Sarig 1974)

Suspended solids in water bodies can originate from either external or internal water sources. The external load of suspended solids depends on natural weathering of rocks that result in the formation of sand and silt, algal bloom and various chemical pollutants. Human activities such as farming, tree felling and engineering works, sewage and paper milling can load rivers with suspended solids (Alabaster, and Lloyd 1982). 50 mg/l of suspended particles have been found in clear water bodies whereas 150mg/l are commonly found in turbid flowing after short rains and muddy pond where yields tend to be much reduced (Alabaster and Lloyd 1982).

Gill rakers are functionally adapted to collecting food particles that come with the respiratory water. They collectively function as a sieve device which entraps suspended particles that would otherwise be carried out through the opercula aperture with exhalent respiratory current. The feeding selectivity of many fish species is linked to gill arch morphology (Lock, 1974, Lagler *et al.*, 1977, Hyatt, 1979). Bardach *et al.*, (1972) proposed that numerous long, thin, closely spaced gill rakers indicate plankton feeding habit. The gill rakers collect smaller particles which may be further sieved by the smaller microbranchiospines into even finer particles which are entangled in the mucus secreted in the gill cells (Fryer, and Iles, 1972).

Breathing and coughing reactions are associated with the rate of entrapment of particles in the gills and subsequent swallowing. Using concentrations of 50mg/l and 150mg/l of silt and algae and sand and algae Olendi (2015) reported that breathing rates and cough rates increased significantly in *O. niloticus*. Increased cough rate signify ingestion of particles (Briggs, 1985, McNally 1988). In a separate experiment algae and silt particles have been found to be ingested by tilapia although sand particles are not (McNally 1988). Drenner *et al.*, (1984) found *Sarotherodon galilaeus* of 80mm standard length selectively grazed on particulate plankton of greater than 20 microns. Various food particles other than the algae have been found to be successfully digested (Fish 1960, Greenwood, 1974). Particles in suspension in freshwater, sea and estuarine water carry a range of negative surface charges as measured by electrophoresis (Loder and Liss 1985). The presence of charge in larger algae ranging in size from 5-50 microns was assumed, although it was not proved experimentally until 1955 as reported by Ives (1959).

Tilapia filter-feeding studies have involved gut content analysis of field captured individuals (Moriarty and Moriarty, 1973; Hofer and Schiemer, 1983; de Moor *et al.*, 1986; Getachew and Fernando, 1989; Abdelghany, 1993). Their studies showed those tilapia filtrations are influenced by environmental conditions.

In this study the ability of tilapias to ingest food and to distinguish between particles in terms of electric charge, size and density at different concentrations were studied in order to elucidate selectivity mechanism during filter feeding process.

MATERIALS AND METHODS

Study Area

The study was conducted at the Institute of Aquaculture, University of Stirling, Scotland. *Microcystis aeruginosa* was cultured in BG II Algal growth medium as adopted by Stanier *et al.*, 1971. Algae in suspension were introduced into flasks containing autoclaved BG II growth media. The initial culture was sub-cultured in a five liter autoclaved demijohn. The culture vessels on shelves were maintained at 28°C and illuminated for 24 hours. The vessels were aerated and placed on Stuart SM4 Magnetic Stirrer. Interpret combined Heater/Thermostat was submerged into each 5 liter aquaria glass tank and maintained at 25⁰ - 27⁰C.

The algae stock used originated from Cambridge collection of Algae and protozoa unit via department of Biology, University of Dundee.

Microcystis used was obtained by filtering algal-rich suspension through a weighed filter paper(name) and the retained algae was air dried together with filter paper on a tray. Dry algae weight was then calculated. The procedure was repeated until adequate quantity of dry algae was achieved.

Silt particles were extracted from the mud of Airthrey Lock (Lake) (Fig 1) by sieving through a series of sieves down to 63µm. The resulting suspension was poured into a 1 liter measuring in cylinder and left to settle for about 5 minutes and bner clay particles were then decanted. 100 ml of collected decanted material was resuspended and poured into a tray placed in a drier set at 60°C for 8-12 hours. The process was repeated until enough silt was obtained.

Study area

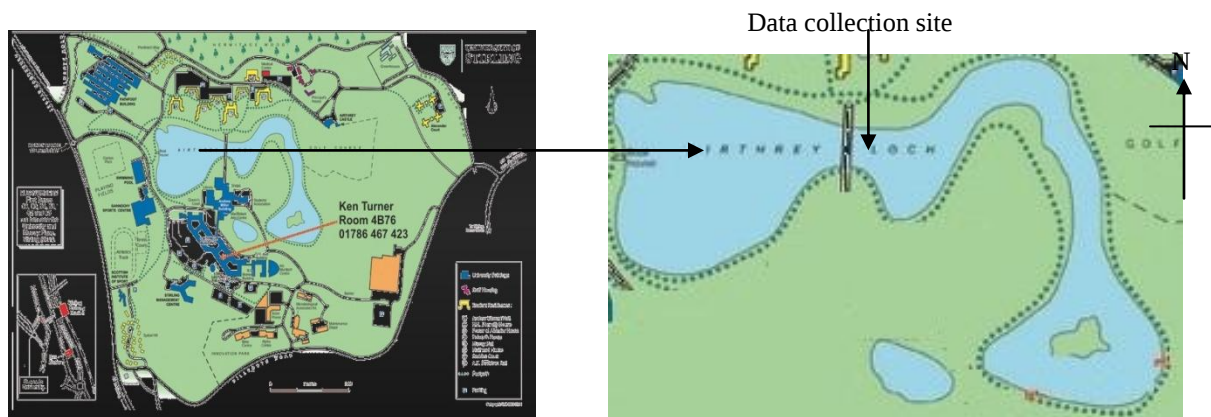


Fig 1: Source: Google map of University of Stirling, Scotland showing Lock Airthrey

The acid washed sand particles used were obtained from Fisons Chemical Company. The sand was passed through a series of sieves down to 68µm and collected in a tray. The same procedure was repeated until an adequate concentration was obtained.

Juvenile Bangkok strain of *O. niloticus* of mean weight of 7.50mg fed on a micro-pelleted diet were reared in a re-cycle water system at 27⁰-28⁰C were selected for this work.

Fish were transferred from rearing tank to holding tank and left for 24 hours without feeding to allow evacuation of gut contents. From holding tanks fish were taken and weighed after drying on absorbent tissue paper. Five fish of mean weight of 7.50gm were then transferred into three static 5 liter aquaria with two replicates and control and left for 3 hours to acclimatize before experiment.

Concentrations of 50mg/l and 150mg/l of sand and algae and the same for silt and algae were introduced in the tanks. To each of these corresponding of 50 and 150mg dry weight of algae were added. Control aquaria had water only. Each tank was vigorously aerated by a six-inch airstone connected to airpump by hose. Interpet combined Heater/Thermostat was submerged into each tank to maintain temperature at 25-27c.

Fish were left for eight hours then removed and killed by piercing by a scalpel at the back of to the main at internal of 15, 30, 45 and 60 minutes after feeding. The gill arches were carefully removed into a Petri-dish and the particles examined under microscope. The same procedure was repeated for the examination of the stomach and intestinal contents.

The surface electric charge was determined using Zeta-Potential Meter (Repap). The dispersion (algae, silt and sand) under investigation was put in a glass cell in a thermostatic bath filled with water at 20⁰c and electrode voltage set at 90V at both terminals. The rate of movement of a given particle was determined in seconds along a graticule (1mm²) observed under a microscope. The rates of movement of several particles were recorded and mean values and standard deviation calculated.

The diameter of the silt and sand particles were measured using an eye piece graticule calibrated on dissecting microscope with a magnification of x10. Silt and sand was put on different Petri-dishes and fitted on the graticule scale to coincide with a single particle whose reading was recorded. Ten readings were taken and mean diameter calculated. .

The specific gravity of planktons algae was calculated as follows:- It was assumed that the algae consisted of 90% cytoplasm and 10% cellulose. According to Smayda (1970), cellulose has a specific gravity of 1.5 and cell cytoplasm of between 1.03 and 1.10. Hence *Microcystis* could be expected to have a density of 1.07 to 1.14. This is more dense than water (1.0)

RESULTS

Algae and silt particles were ingested by *O. niloticus*, however there was no evidence to suggest sand particles were ingested. The ingestion of algae and *silt* were supported by their presence in the stomach and entanglement the gill apparatus

The examination of the gill structure showed that silt particles were entangled in the copious mucus on the gill surface but not algae and sand particles. Fig. 2.

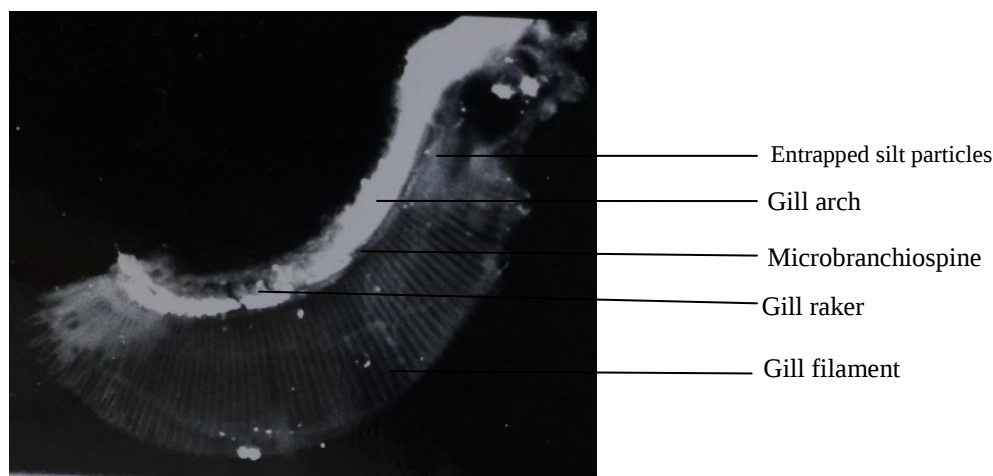


Fig 2: Filter feeding Gill apparatus (x40) of *O. niloticus*

The examination of stomach and intestines showed algae and silt particles were ingested but not sand particles. This showed that algae and silt were raked through gill filaments. However, it proved difficult to quantify the particles which invariably included both detritus materials and remnants of ingested food prior to the experiment.

Table 1 shows $\bar{X}$, Z-potential of $\bar{X}$, =84.31, S=14.4 and $\bar{X}$, =14.95, S=2.06 for algae and silt respectively. It shows that algae has higher surface charge value than silt particles at 90V.

Table 1: Z-Potential of particles (Millivolts)

Algae		Silt	
Time (s)	Z- Potential	Time (s)	Z- Potential
1.14	79.9961	6.36	14.2868
1.07	85.1869	5.11	17.8375
1.05	86.8095	7.17	12.7126
0.98	93.0102	5.73	15.9075
0.96	94.9479	7.04	12.9474
0.86	105.9479	7.27	12.5378
1.48	61.5879	5.36	17.0055
1.36	67.0220	6.74	13.5237
	$\bar{X} = 84.31$ S = 14.4		$\bar{X} = 14.95$ S=2.06

Silt particles mean size diameter of 0.076 mm and sand particles mean size diameter was 0.28 mm were recorded in table 2 below. Algae particles were too small to be determined, however their size were expected to be smaller than silt and sand particles. Larger algae particles sizes have been found to range from 5-50 microns as was reported by Ives (1959).

Table 2: The diameter of silt and sand in millimeters (mm)

Silt particle (mm)	Sand particle (mm)
0.08	0.20
0.10	0.31
0.05	0.30
0.09	0.34
0.10	0.40
0.06	0.40
0.11	0.15
0.04	0.35
0.08	0.24
0.05	0.20
$\bar{X} = 0.076$	$\bar{X} = 0.28$

The specific gravity of *microcystis* is expected to range from 1.07 to 1.14 a little more dense than water (1.0). The specific gravity of sand range between 1.9 and 2.5 while silt was 1.57. Perry and Chitton, (1973).

DISCUSSION

Examination of particles on the gill structures showed that algae and sand were not trapped in the gill rakers, gill filaments and microbranchiospines. However, silt particles were entangled in the copious mucus of the gill structures.

Whitehead (1959), concluded that although algae were ingested by tilapia they could not be seen entangled on the gill surface. Briggs (1985) on the other hand, reported small quantities of algae trapped on the mucus on the

microbranchiospines and gill filaments. For these reasons it is hard to explain the relative importance of the individual gill structures in trapping and subsequent raking of the particles ingested.

Newcombe and Jensen (1996) summarized the acute and chronic effects of channel suspended sediment on a variety of fish species, and used these data in developing a method for synthesizing and quantitatively assessing the resulting degree of risk and impact to fish. In determining the severity of ill effect to fish, the authors stressed the importance of considering both the duration of exposure and the concentration of suspended sediment (the sediment dose). Newcombe and Jensen (1996) described four categories of severity of ill effects associated with excess suspended sediment, beginning with no effects and progressively worsening through behavioral effects, sub lethal effects, and paraethal and lethal effects. Behavioral effects include alarm reaction, abandonment of cover, and avoidance response, while sub lethal effects include short-term to long-term reduction in feeding rate and success, increased rate of coughing and respiration, impaired homing, poor condition, and moderate habitat degradation. Paraethal and lethal effects include reduced fish growth rate and density, delayed hatching, increased predation, moderate to severe habitat degradation, and stepwise incremental rates of mortality increasing from 0 to 20 percent to 80 to 100 percent.

The stomach and the gut contents had algae and silt particles which proved difficult to quantify because the ingested particles were digested and invariably included both detritus material, algae and remnants of food eaten earlier which were hard to differentiate. Further according to (Moriarty, 1973) the acid in the stomach decomposes the chlorophyll and lyses blue-green algal cell walls which makes subsequent intestinal digestion possible by allowing enzymes access to the algal cell contents. Thus there is evidence that particles in the gut and stomach may not be distinguished. Juvenile and adult Nile tilapia; *O. niloticus*, blue, *O. aureus* and, *O. mossambicus*, are reported to filter phytoplankton (McDonald, 1985a,b; de Moor *et al.*, 1986; Northcott *et al.*, 1991). Abdelghany (1993) found green algae (*Ankistrodemon*, *Pediastrum* and *Closterium*) and cyanobacteria (i.e *Anabaena*, *Oscillatoria* and *Mycrosistis*) in Nile tilapia stomach from River Nile, Egypt. The actual mechanism involved in ingestion had been a major debate. Many workers, Whitehead (1959), Fryers and Iles (1972), found that blue-green algae and green algae were ingested or consumed by tilapias including *O. niloticus* (Chokaa 1984). Bowen (1982) and Beveridge *et al.*, (1987) showed evidence for the involvement of gill rakers, microbranchiospines and the mucus in the process.

Microbranchiospines described as micro-gill rakers by Fryer and Iles (1972) have been found to be present on the outer side of the second, third and fourth gill arches. The first gill arch has none of these fine structures as have been found in several species of Tilapia. The role of the microbranchiospines is believed to be two-fold; to facilitate collection of food particles during filter feeding and protection of the delicate gill tissue from damage by fine particles (Fryer and Iles, 1972). The particles are further refined by the microbranchiospines which thus complete the selection before the food is finally backwashed and swallowed (Fryer and Iles, 1972).

Tyther and Calon (1985) suggested that filter feeding was a random and continuous process whereby very small food particles in a stream of water were ingested with no evidence of satiation in some filter feeders even after twenty four hours of continuous feeding. The evidence of the ingestion of particles in this study can be linked to the opercula movement and coughing activities. Coughing is believed to precede swallowing process (Briggs, 1985).

Appler (1982), found that *O. niloticus* consumed *Chlorella vulgaris* at all concentrations while *Tilapia zilli* only ingested appreciable amount of algae at the highest and not at the lowest concentrations. Chokaa (1984) using different concentration of three different species of algae found a tremendous decrease in concentration of algae with time when *O. niloticus* was left in the trial tanks. She concluded that *O. niloticus* grazed and ingested *Synechococcus nostoc canina* and *Anabaena cylindrica* depending on density. McNally (1988), found *S. galilaeus* to ingest silt particles two and a half times more than sand particles. It is apparent that *O. niloticus* selectively ingests particles but how the selection is achieved is not clear.

In practice, particles suspended in water have been found to have a net negative surface charge (Loder and Liss 1985). The negative charge on the surface is mainly due to the absorption of the organic matter to the particles. Substances with more organic matter would be expected to be more negatively charged. Algae have been found to possess a negative surface charge which has been used as the basis for their flocculation in water treatment tanks with positively charged flocculants or coagulants (Eves 1959, Lavore and Noue 1983). Silt similarly would be expected to exhibit a net negative surface charge since the organic matter was not removed by ashing prior to experiment.

Nile tilapia feeding rate of green algae and cyanobacteria followed Ivlev's (1961 filter-feeding model; a curvilinear relationship with increasing particulate organic matter concentration. From this work and published work, it appears that fine mucus-bound particles are entrapped by the entire gill arch and that silt and algae are probably adsorbed thus get entrapped together

CONCLUSIONS

It can be deduced from this work that negatively charged particles were ingested more than positively or less negatively charged particles. Hence if surface charges played any role in the selection and ingestion of particles then it can reasonably be concluded that negatively charged particles are preferentially ingested by *O. niloticus*. Subsequently algae and silt particles would be ingested more than the sand particles. Furthermore algae have higher surface charge value (more electronegative) than silt particle with respect to water is ingested preferentially to silt particles.

The diameter of the particles was also postulated to have an influence on the selection process of ingestible particles. Algae measured 5 microns (0.005mm), silt 76 microns (0.076mm) and sand 280 micron (0.28mm). Algae have smaller diameter than silt and sand. It might be suggest and proposed that *O. niloticus* selected smaller particles and rejected larger ones. Algae and silt were perhaps trapped because of their smallness by the delicate gill rakers, microbranchiospines and mucus on the surface of the gills while sand particles might be removed through the mouth following sorting procedure when water flow was reversed with the mouth wide open.

The specific gravity of particles was also thought to have an influence on the selectivity of the particles for ingestion. Whether particles sink, float or remain in suspension depends upon their specific gravity. Specific gravity of water is generally taken to be 1.0. The specific gravity of *microcystis* was estimated to be approximately 1.14. The specific gravity of silt extracted from the mud of Airthrey Loch would be expected to be between the specific gravity of river mud (1.44) and flowing mud (1.7) which is 1.57 while sand has a range between 1.9 to 2.5 (Perry and Chitton, 1973). Based on size algae and silt with small diameter were ingested as opposed to sand particles. The ingestion of algae and silt were supported by entanglement on the gill apparatus and their presence in the stomach. This shows that the less dense particles were ingested more than denser particles.

In this study it was shown that the rate of ingestion of particles decreased with the increased specific gravity. Algae and silt with lesser specific gravity and negatively charged with respect to water were ingested more than the denser sand particles. Logically, filter feeders would take the particles in suspension with the inhalant water in the natural water bodies. Suspended particles, algae and silt, were ingested by *O. niloticus*, however there was no evidence to suggest sand particles were ingested.

RECOMMENDATIONS

Fish farmers should maintain good water quality in pond culture to minimize the effects of suspended solids on the physiology of fish. Water should be free from silt and clay particles that may excavate the occurrence of fish diseases and gill proliferation. This can be achieved by reducing the surface run off and screening the inlets to exclude muddy soil into the ponds. Feeding regimes should be managed in appropriate portions to minimize the effect of unconsumed food becoming suspended in the water column.

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