Southern Regional Aquaculture Center



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Protozoan Parasites

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Most parasitic infections in farmraised fish are caused by protozoan parasites. The protozoa causing the most significant problems in aquaculture are discussed below. (Ich, *Ichthyophthirius multifiliis*, is covered in SRAC 476, "Ich—White Spot Disease.")

Trichodina

Trichodina species are in the family Trichodinidae that includes the genera *Trichodina*, *Paratrichodina*, *Trichodonella*, *Tripartiella* and *Vauchomia*. Many *Trichodina* species are pathogenic and the disease caused by them is called trichodinosis.

When viewed from the top, Trichodina is circular; side views of the organism reveal a saucer or dome shape (Fig. 1). It has three rings of cilia (small, hair-like projections) encircling its body and oral cavity, which are used for locomotion and feeding. Its body is supported by a rigid ring of interconnected discs called a chitinoid or denticular ring (Fig. 2). Each disc has a thorn-like inner ray projecting into the center of the ring. Trichodina glides rapidly over the gill and skin surfaces. It is usually found on the gills but also can

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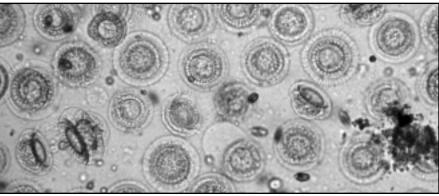


Figure 1. *Trichodina* is shaped like a flying saucer or an upside down soup bowl (Photo by Glenn Hoffman). (View video clip of *Trichodina*)

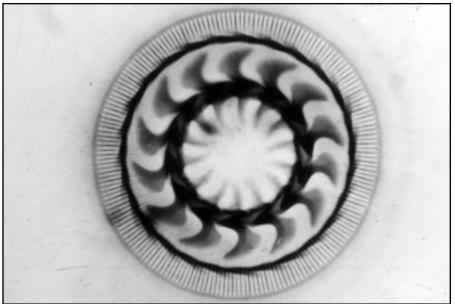


Figure 2. *Trichodina* is supported by a rigid ring of interconnected discs called a chitinoid or denticular ring (Photo by H. S. Davis).

Video clips mentioned in this publication can be viewed in the online version of this publication at *www.msstate.edu/dept/srac*. Click on Publications and then Fact Sheets.

be found on the rest of the body, especially when the fish has become weakened. *Trichodina* can infect almost all fish species and directly or indirectly cause a fish's death.

Trichodina infections cause no distinctive lesions, so diagnosis is made almost exclusively by microscopic examination. Gill swelling often can be seen. Infected fish often display lethargic behavior, weight loss and flashing (abrupt movements where the silvery underside of the fish flashes during the fish's attempt to get rid of the parasite). Protozoan parasites such as *Trichodina* can be present in low numbers and not cause disease; the experience of the diagnostician in weighing the overall parasite load in combination with other pathogenic and environmental factors is important in determining whether or not a particular protozoan is causing a disease condition.

Trichodina infestations are typically caused by high stocking densities and generous feeding rates, both done to maximize production and profits. Such aggressive management can be profitable up to a point, but when the culture system's limits are exceeded, adverse conditions such as poor water quality can lead to lower production levels, higher mortalities from disease, off-flavor problems and, ultimately, lower profits. High feeding rates can lead to high ammonia concentrations, creating an ideal environment for the reproduction of *Trichodina* and a greater chance of a full-blown infection. If the fish are also crowded (as in cage-raised fish) the infestation can spread very rapidly among the fish.

Treatments for *Trichodina* infestations (at the time of this publication) include formalin (Formalin-F[®], Paracide-F[®] and Parasite-S[®]); copper sulfate (CuSO₄), which is on deferred status with FDA; potassium permanganate (KMnO₄), also on deferred status;* and acetic acid. *Trichodina* are typically killed with a single treatment and the fish can recover after treatment. A qualified fish health professional should be consulted for treatment rates and the current legal status of specific chemicals for treating food fish. Help in calculating treatment rates can be found in SRAC publication 103, "Calculating Area and Volume of Ponds and Tanks," and SRAC 410, "Calculating Treatments for Ponds and Tanks."

Trichophrya (also seen in the scientific literature as *Capriniana*)

There are several species of Trichophrya. This ciliated protozoan parasite is not motile in the adult stage. It reproduces by budding and the newly formed teletroch (the free-swimming juvenile stage) resembles Trichodina but does not have a denticular ring (see Trichophrya video clip). It has feeding tubes or tentacles that protrude from a spherical cell, resembling pins stuck in a pin cushion (Fig. 3). These tentacles, however, are often absent. There are characteristic orangish-brown granules in the *Trichophrya* cell.

Although some scientists consider *Trichophrya* to be a commensal (i.e., not parasitizing the fish but simply living on the fish and feeding on debris in the environment), others view it as a parasite that can stress fish and cause mortality

when present in large numbers. The author has observed that large numbers of *Trichophrya* can cause mortalities in fish, but not always. Perhaps there are differences in virulence among different strains of the parasite and/or differences in resistance among fish species. *Trichophrya* is specifically a gill parasite that may cause death simply by blocking the flow of oxygen. An organically rich, eutrophic environment (e.g., fish culture ponds) allows this parasite to multiply.

Copper sulfate (CuSO₄) is the chemical of choice for treating *Trichophrya*.

Ambiphrya and Apiosoma

Ambiphrya and Apiosoma are ciliated protozoa that are non-motile in the adult stage and attach themselves to the gills and skin of fish. *Ambiphrya* (formerly *Scyphidia*) is barrel-shaped and attaches to the fish's surface layer of cells with a broad, flattened scopula or holdfast organ on the posterior end (Fig. 4). There is a ring of cilia around the mouth and one around the middle called a ciliary girdle. *Ambiphrya* usually has a ribbon-shaped nucleus.

Apiosoma (formerly Glossatella) has an elongated vase shape with a smaller base of attachment (Fig. 5). It has no ciliary girdle, only an oral ciliary ring, and a more compact conical or triangular nucleus.

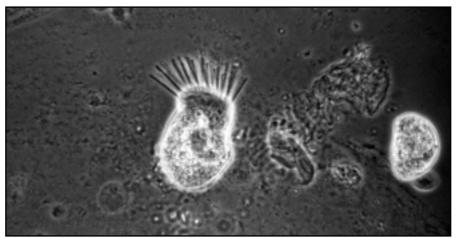


Figure 3. *Trichophrya* has feeding tubes or tentacles that protrude from a spherical cell and resemble pins in a pin cushion (Photo by Glenn Hoffman). (View video clip of *Trichophrya* budding)

^{*}Deferred status connotes that the treatment is allowed to be used on food fish but is not officially approved; FDA has deferred that decision until a later time.



Figure 4. *Ambiphrya* is barrel-shaped and is attached to the fish's epithelium by a broad, flattened scopula on the posterior end (Photo by Glenn Hoffman). (View video clip of *Ambiphrya*)

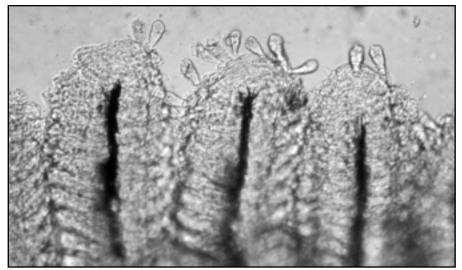


Figure 5. *Apiosoma* has an elongated vase shape with a base of attachment smaller than that of *Ambiphrya* (Photo by Fred Meyer). (View video clip of *Apiosoma*)

Ambiphrya and Apiosoma usually are treated with one application of formalin, $CuSO_4$ or $KMnO_4$. Unless environmental conditions are improved, the infection may recur.

Ichthyobodo necator (formerly Costia necatrix)

Ichthyobodo is a very small protozoan about the size of a red blood cell. It is a single-celled parasite shaped like a comma or tear drop. It uses flagella for motility and to attach to the host fish (Fig. 6). Flagella are long, whip-like structures used to propel an organism or cell through a water environment. Flagellated protozoa usually have one to four flagella, while ciliated protozoa often have hundreds of cilia. When unattached from the fish, *Ichthyobodo* swims erratically in a corkscrew pattern. When attached, a flagellum remains fixed to the *fish's* surface and the flagellar movement of the cell body sometimes looks like a flickering candle flame. Ichthyobodo also can be seen lined up motionless along the edge of the gill, giving the gills a serrated appearance (Fig. 7). Irritation from the parasites can cause gill swelling.

Because of its small size, *Ichthyobodo* can easily be missed under 100x magnification, especially with an average or below average quality microscope. It can be seen clearly under 200x magnification with most scopes.

These parasites do not cause distinctive lesions on the fish but do block the flow of oxygen when heavily loaded on the gills. As with most protozoa, environmental degradation and crowded conditions cause them to become more damaging. However, prevention measures such as reducing stocking densities and lowering feeding rates may make fish production unprofitable. But stocking and feeding rates should be kept reasonable. Contact a qualified aquaculture or fisheries scientist for advice on proper stocking densities for the fish species you are raising.

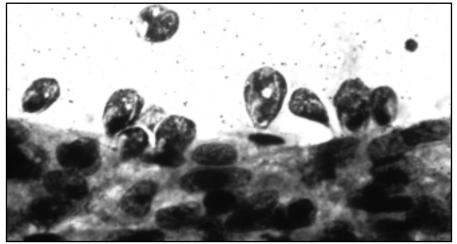


Figure 6. *Ichthyobodo* is shaped like a comma or tear drop. This single-celled parasite uses flagella for motility and to attach to the host fish. (View video clip of unattached *Ichthyobodo*)

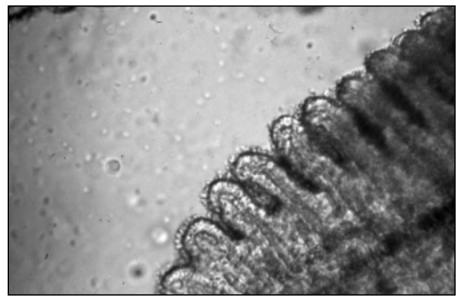


Figure 7. *Ichthyobodo* are often seen lined up motionless along the edge of the gill, giving the gills a serrated appearance (Photo by Andrew Mitchell). (View video clip of attached *Ichthyobodo*)

Ichthyobodo occurs on the skin and gills. The organism is notorious for affecting fish in crowded environments such as cages and can cause significant mortalities when infestations are heavy. It can cause infections at temperatures of 36 °F to 86 °F.

Treatments include formalin, $CuSO_4$ and $KMnO_4$; a single dose at the appropriate concentration usually is effective.

Chilodonella

Several species of this genus are described in scientific literature; two of them are pathogenic to fish. *Chilodonella* is an oval, flat protozoan with parallel rows of cilia and a notched anterior end (Fig. 8). It swims erratically like *Ichthyobodo*, but is much larger. *Chilodonella* glides over the fish's gill and skin surfaces. Heavy concentrations of this parasite can cause mortalities. Swelling of the gills, as noted under a microscopic wet mount, is often seen with *Chilodonella* infections.

Formalin, $CuSO_4$ and $KMnO_4$ have been used successfully to treat *Chilodonella*.

Epistylis and Heteropolaria

The protozoan parasites *Epistylis* and *Heteropolaria* are very similar.

They are single-celled, but live in stalked colonies (Fig. 9). The branching stalks are rigid and do not contract; the cells at the ends of the stalks are called zooids. They contain cilia around the oral opening and contract when feeding. *Epistylis* or *Heteropolaria* colonies on fish resemble white tufts of fungus (Fig. 10), but can be differentiated from fungus by microscopic examination. These parasites are usually found on the

skin and fins. The base of the stalk attaches to a hard, calcified surface such as scales and fin rays or spines.

Epistylis and *Heteropolaria* reproduce by budding and form a teletroch or motile juvenile stage. The teletroch produces a stalk and uses it to attach to an existing colony. *Epistylis* is often an ectocommensal in that it simply attaches to the fish and feeds on environmental debris such as bacteria. Poor quality water encourages the growth of *Epistylis* on fish.

These parasites can weaken and kill fish. Ulcers caused by *Epistylis* infections may make fish more vulnerable to bacterial infections. For example, red sore disease involves the combination of *Aeromonas* bacteria and *Epistylis*.

The classic treatment for *Epistylis* and *Heteropolaria* infections is uniodized salt (sodium chloride).

Henneguya sp.

Henneguya sp. is a sporozoan parasite that typically has very little effect on fish health. However, the disease of farm-raised channel catfish called proliferative gill disease, which has caused significant mortalities over the past two decades in the fall and especially

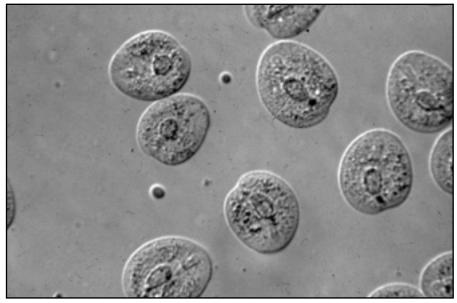


Figure 8. *Chilodonella* is an oval, flat protozoan with parallel rows of cilia and a notched anterior end (Photo by Glenn Hoffman, provided by Drew Mitchell). (View video clip of *Chilodonella*)

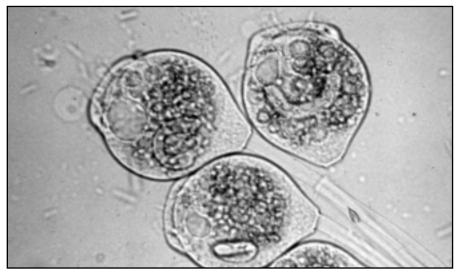


Figure 9. Epistylis is single-celled, but lives in stalked colonies.

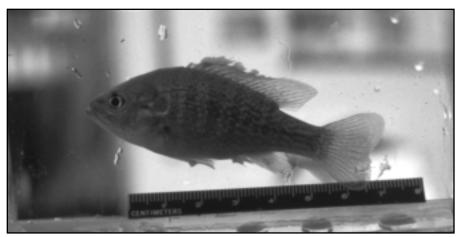


Figure 10. Colonies of *Epistylis* or *Heteropolaria* infecting fish resemble white tufts of fungus, as shown here on the dorsal fin (Photo by B.L. Moore, provided by Andrew Mitchell).

spring, has recently been blamed on a life stage of *Henneguya* sp. (see SRAC publication number 475). Many references still refer to the causative organism as *Aurantiactinomyxon icatluri*.

Proliferative kidney disease (*Tetracapsula bryosalmonae*; formerly called PKX)

Tetracapsula bryosalmonae is a myxosporean protozoan that causes proliferative kidney disease (PKD) in several species of trout and salmon in western North America and Europe. PKD typically occurs in the spring, and mortalities are highest when water temperatures are in the mid 50s (°F).

Clinical signs include swelling of the kidney and spleen, pale gills, darkened body, swollen abdomen containing fluid (ascites), and pop-eye (exophthalmia). The enlargement of the kidney is often noticeable externally even before the internal examination is performed. The swollen kidney can have a nodular or "ropy" appearance (Fig. 11). This extreme reaction of the kidney and the inability of the parasite to produce mature spores in the infected fish indicate that trout and salmon may be unnatural or aberrant hosts for *Tetracapsula bryosalmonae*. Microscopically, stained tissue smears show the primary cell of the parasite with secondary or daughter cells inside or near the primary cell.

To prevent PKD, fingerling trout should not be stocked until summer when the main threat has passed. Once the disease has occurred, mortalities have been decreased somewhat by increasing salinities to 8 to 12 parts per thousand (about one-third the strength of seawater) in trout-rearing facilities.

Whirling disease (*Myxobolus cerebralis*; formerly *Myxosoma cerebralis*)

Whirling disease, caused by the myxosporean Myxobolus cerebralis, occurs worldwide and in all trout and salmon species. Rainbow trout are particularly vulnerable and it is most severe in trout less than 6 months old. The disease attacks cartilage (younger fish have more cartilage). Myxobolus cerebralis infections in the spine can cause the fish's tail to turn black (Fig. 12) and the spine to curve (Fig. 13). Infections in the head cartilage create head and jaw deformities, while infections in the auditory capsule cause young trout to become disoriented and chase their tails in a



Figure 11. The swollen kidney in fish with Proliferative Kidney Disease can have a nodular or "ropy" appearance.



Figure 12. *Myxobolus cerebralis* infections in the spine can cause the fish's tail to turn black. These infected trout are whirling (Photo by Glenn Hoffman, provided by Drew Mitchell).

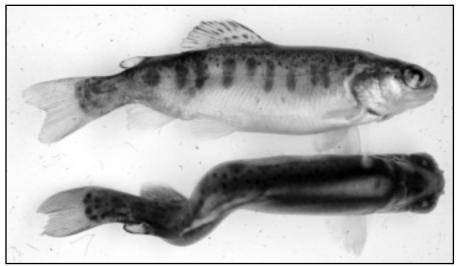


Figure 13. *Myxobolus cerebralis* infections in the spine can cause spinal curvature (Photo by Glenn Hoffman).

whirling motion. Heavy infections can kill fish before clinical signs have a chance to develop.

Myxobolus cerebralis spores are oval and have two distinct polar capsules hat can be seen with a microscope.

This protozoan has a complex life cycle. Spores can be shed from infected live fish as well as from dead and decomposing fish. The spores also can be spread via bird feces. Spores are ingested by an annelid worm intermediate host, *Tubifex tubifex*, which lives in the bottom mud of ponds, streams and earthen raceways. The spores develop into actinosporeans that penetrate fish (or the fish ingest the actinosporeans when they eat tubifex worms). Plasmodia develop in the fish's cartilage and eventually produce the characteristic spores.

Whirling disease can be prevented by not stocking trout fry in infested waters until they are older than 6 months. Raising fish only in concrete tanks or raceways also can eliminate the disease. If earthen raceways are used, they can be disinfected between production cycles with 380 grams (0.84 pounds) of unslaked lime (calcium oxide, also called burnt lime or quick lime) per square meter of pond bottom.

Chemicals Used to Treat Protozoa

Un-iodized salt (sodium chloride). Salt is used to treat *Epistylis* and some other external protozoa at 1,000 to 2,000 ppm as an indefinite treatment or in hauling tanks. This is equal to 1 to 2 parts per thousand or 0.1 to 0.2 percent (3.8 to 7.6 g per gallon or 28.3 to 56.6 g per cubic foot). For short-term treatments (usually lasting less than an hour or until fish show signs of stress), 10 to 30 ppt treatments have been used.

Potassium permanganate.

KMnO₄ is used to treat most protozoa at approximately 2 ppm indefinitely, or at higher concentrations if the organic content of the culture water is higher. A 15minute KMnO₄ demand test should be performed on the culture water to determine the amount of KMnO₄ needed. Four beakers of culture water are set up at 1, 2, 3 and 4 ppm KMnO₄ and observed for 15 minutes. The number half-way between the KMnO₄ concentration that turns clear and the one that remains pink is multiplied by a factor of 2.5 to calculate the concentration (in ppm) needed for the KMnO₄ treatment. For example, if the beaker containing 1 ppm KMnO₄ turns clear after 15 minutes while the 2 ppm beaker remains pink, then the number in between the two (1.5 ppm) is multiplied by the factor 2.5 to get 3.75 ppm KMnO₄ needed for treatment. If the culture water is high in organic content, concentrations higher than 4 ppm may need to be used in the demand test. Please contact a qualified fish health specialist for further details on this demand test or to have the test run for you. For a shorter term tank treatment, as much as 10 ppm KMnO₄ can be applied for up to 20 minutes; however, the fish must be observed throughout the treatment, and if they show signs of stress, water should be flushed through the tank to dilute the treatment. At the time of this writing, $KMnO_4$ is on deferred status by the FDA.

Copper sulfate. CuSO₄ treats most protozoan parasites on nonsalmonid fish at a rate calculated by dividing the culture water's total alkalinity by 100 and using that concentration in ppm for the CuSO₄ treatment. For example, culture water with a total alkalinity of 80 ppm would need 0.8 ppm CuSO₄. Great caution, however, must be used when treating trout with $CuSO_4$. Protozoa on trout can be treated with approximately 0.050 parts per million CuSO₄ (which equals 50 parts per billion) when total alkalinity in the culture water is about 10 ppm (typical of trout culture water in western North Carolina). Consult a qualified fishery biologist before using CuSO₄ on trout or other salmonids. (At the time of this writing, $CuSO_4$ is on deferred status by the FDA.)

Formalin-F[®], **Paracide-F[®]** and **Parasite-S[®]** (37% formaldehyde gas). Formalin is applied at 15 to

25 ppm as an indefinite treatment on non-salmonid fish. A shortterm formalin treatment of 125 to 250 ppm can be used for approximately an hour. The treatment should not be more than 167 ppm at temperatures of 70 °F and higher. The treatment should be diluted quickly if fish show signs of stress. Trout are treated with 167 ppm formalin for an hour at temperatures between 50 and 65 °F, and up to 250 ppm at temperatures below 50 °F

Before treating for protozoa, trout growers are advised to pre-treat with salt to help slough-off excess mucus. The mucus acts as a protective barrier for the parasites, so reducing the amount of mucus helps to increase the amount of contact between the therapeutant and parasite. Approximately 1 pound of salt is dissolved in the trout raceway for every 2 gallons per minute of water flow. The therapeutant is applied immediately after the salt dissolves.

Before using any of these treatments consult a qualified fish health specialist for the current legal status of the therapeutant. Other SRAC publications on fish diseases are:

- SRAC 472, "Submitting a Sample for Fish Kill Investigation"
- SRAC 473, "Medicated Feed for Food Fish"
- SRAC 474, "The Role of Stress in Fish Diseases"
- SRAC 475, "Proliferative Gill Disease (Hamburger Gill Disease)"
- SRAC 476, "Ich (White Spot Disease)"
- SRAC 477, "ESC—Enteric Septicemia of Catfish"
- SRAC 478, "Aeromonas Bacterial Infections—Motile Aeromonad Septicemia"
- SRAC 479B, "Columnaris Disease —A Bacterial Infection Caused by Flavobacterium columnare"
- SRAC 4700, "Saprolegniasis (winter fungus) and Branchiomycosis of Commercially Cultured Channel Catfish"

These can be found at *www.msstate.edu/dept/srac*.

SRAC fact sheets are reviewed annually by the Publications, Videos and Computer Software Steering Committee. Fact sheets are revised as new knowledge becomes available. Fact sheets that have not been revised are considered to reflect the current state of knowledge.



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