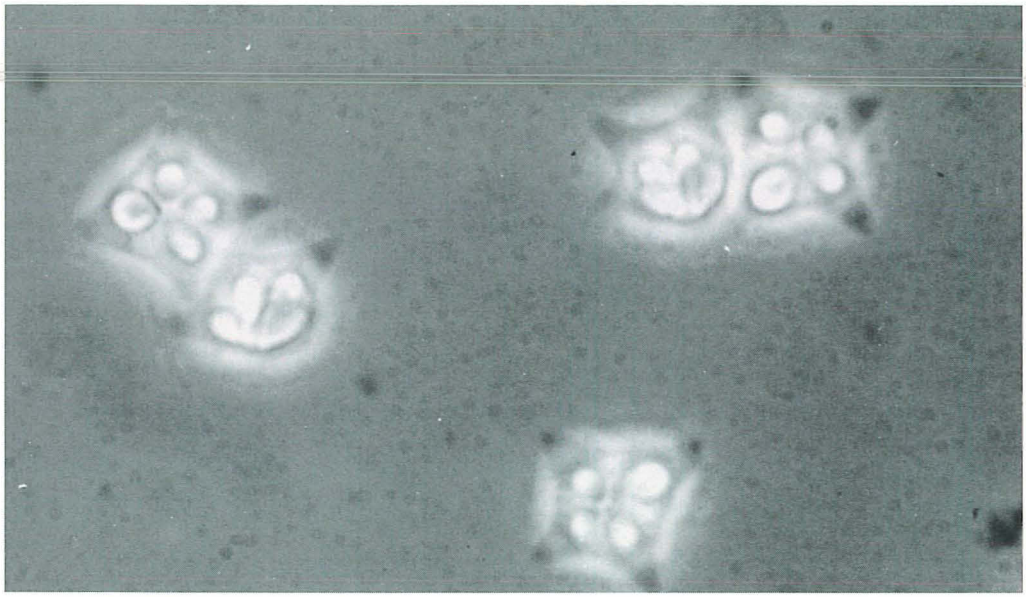


Figure 8-10. Wet mount of *Kudoa thyrsites*. Note stellate spores with four unequal polar capsules.

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily infected fish that are held on ice for 3-6 d may develop extreme softening of the flesh texture (Fig. 8-2f). The soft flesh also will occur following smoking at cool temperatures (below 70°C). In this case, white patches in the muscle are readily seen (Fig. 8-2e).

MICROSCOPY. Wet mount preparations of infected fillets reveal stellate myxosporean spores with four unequal polar capsules converging at one end (Fig. 8-10). The spores are approximately 13-15 µm from tip to tip.

Developing parasites occupy muscle fibers forming large "pseudocysts" filled with developing spores. Heavy infections are associated with extensive inflammation in the surrounding muscle fibers and released spores are found within phagocytes. Infections have also been observed in the cardiac muscle of wild Pacific salmon (Kabata and Whitaker 1989) and Atlantic salmon (Brackett et al. 1991).

DIAGNOSIS. Diagnosis is based on the observation of the characteristic stellate spores of the parasite (Fig. 8-10). The spores are best detected by microscopic examination of fluid collected from the freshly cut surface of a fillet or by crushing a small piece of muscle. The parasite shows up well in Giemsa-stained histological sections. Detection of the parasite in fish in the round presents a problem that is, as yet, unresolved.

St-Hilaire et al. (1997b) reported that wet mount examination of the *m. hyoid* muscle in the underside of the operculum is a relatively sensitive and specific method for detecting the infection without damaging the body musculature. Although this method may miss a few light infections in the body muscle, this is not a great concern because such infections do not cause soft flesh.

Many copies of the rDNA sequence occur within a cell, and thus this sequence is useful for developing very sensitive PCR-based tests. Hervio et al. (1997) developed a sensitive PCR test for *K. thyrsites*. In addition to detecting light infections, we are using the probe to identify the source of infection for salmon (e.g., potential invertebrate alternate hosts).

CONTROL AND TREATMENT. No drugs are commercially available for treating *Kudoa* infections at this time. Fish become infected in sea water, so it would be very difficult to eliminate exposure to infections. Because sexually mature fish and reconditioned grilses are more prone to the infection, removing such fish from the population before harvest (e.g., thorough screening for grilses in the winter) will greatly minimize the problem.

Myxobolus species

Myxobolus aeglefini, a parasite of the cartilage and bone of gadid fishes, was reported from the retrobulbar tissues of pen-reared Atlantic salmon in Norway (Mo et al. 1992). The infection was of particular concern because the spores could be confused with those of *Myxobolus cerebralis*, the causative agent of whirling disease. We have observed *Myxobolus arcticus* in the brain of pen-reared chinook salmon in British Columbia. Infections by this non-pathogenic myxosporean are contracted in fresh water but persist throughout the life of the fish (Kent et al. 1994b).

DIAGNOSIS. *Myxobolus* species are usually identified by observing the spore stage in either wet mount preparations (Fig. 8-11) or histological sections.

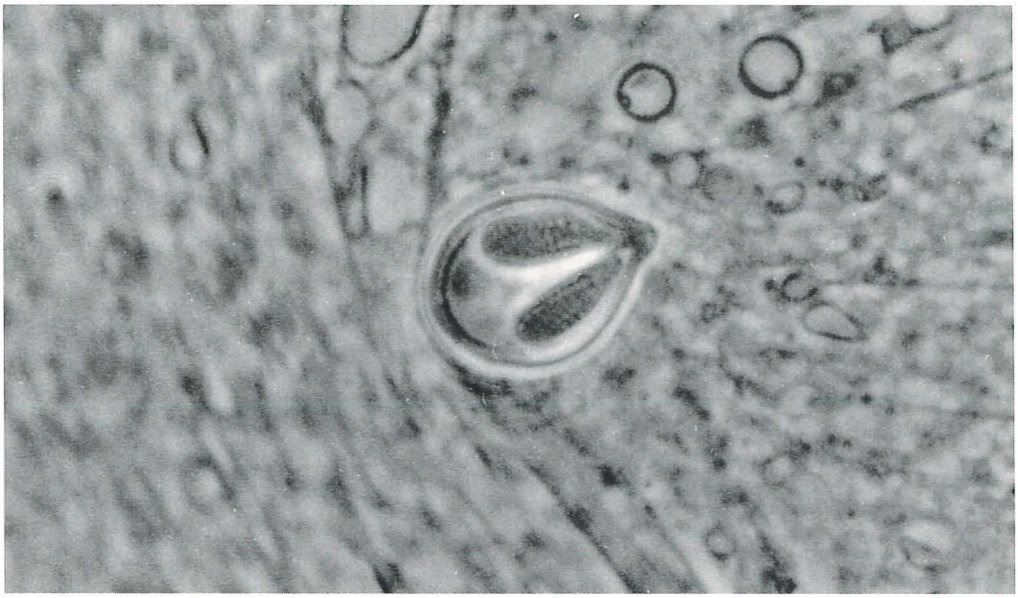


Figure 8-11. *Myxobolus arcticus* from the brain of sockeye salmon.

CONTROL AND TREATMENT. There are no commercially-available drugs for myxosporeans at this time. See discussion on fumagillin (page 57).

Chloromyxum truttae

Chloromyxum truttae, a coelozoic myxosporean that infects the liver, bile ducts and gall bladder, was reported to cause liver damage in coho salmon reared in brackish water netpens in the Baltic (Vismanis et al. 1984). Although salmonids usually contract the infection in fresh water, the authors suspected that they became infected from rainbow trout held in adjacent netpens.

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily infected fish may exhibit a dark or gray liver. The gall bladder is markedly enlarged and filled with red or yellow fluid.

MICROSCOPY. Examination of the bile reveals numerous myxosporean trophozoites and spherical spores with 4 polar capsules and prominent ridges on the surface of the spore (Fig. 8-12).

DIAGNOSIS. The infection is identified by observing spores (round with 4 polar capsules together at the apical end) or trophozoites in the bile (Fig. 8-12).

CONTROL AND TREATMENT. There are no commercially-available drugs for myxosporeans at this time. See discussion on fumagillin (page 57).

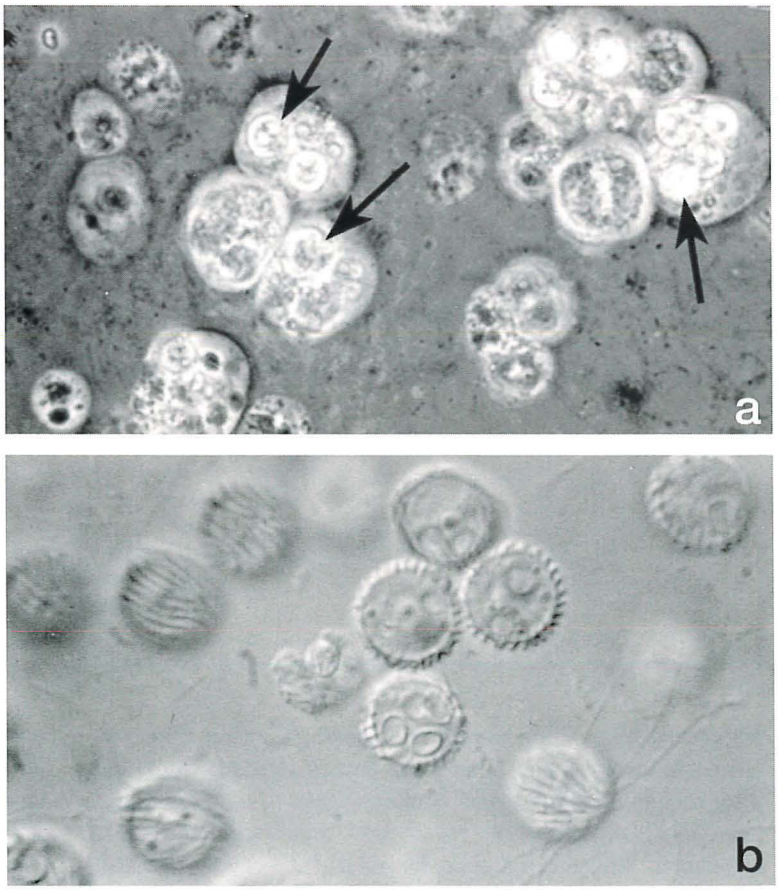


Figure 8-12. a. *Chloromyxum truttae* in the bile. Note spores (arrows) within plasmodia. b. Spores of *Chloromyxum* sp. with prominent ridges on valves.

Nervous Mortality Syndrome

Nervous system infections by trophozoites of an unidentified myxosporean, referred to as nervous mortality syndrome (NMS), has been associated with high mortalities in post smolt Atlantic salmon in netpen sites in Ireland (Rodger et al. 1995; Scullion et al. 1996; Frasca et al. 1998). The disease generally begins about 6 to 8 weeks after transfer to sea water, and mortalities have been as high as 90%. As only trophozoites have been observed, the precise taxonomic status of this myxozoan-like parasite is unknown (Frasca et al. 1998).

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish may be lethargic and accumulate at the surface of the pens. Other fish may exhibit signs of neural disease, such as disorientation, circling or sudden swimming bursts. Fish may also be anorexic. Necropsy reveals no remarkable gross pathological changes.

MICROSCOPY. Histological examination of the brain reveals protozoan-like parasites in aggregates or aligned in string-like patterns (Rodger et al. 1995). The parasites are basophilic and multinucleate. Infections are associated with mild to severe, multifocal inflammation. The parasites appear to be focalized in the mesencephalon, whereas the inflammatory changes are more common in the myelencephalon (Frasca et al. 1998). Electron microscopy reveals the myxozoan characteristics of the parasites. For example, they are multicellular, with primary cells containing daughter cells, and they contain mitochondria (a feature absent from microsporidia).

DIAGNOSIS. The infection is diagnosed by observing parasites as described above in histological sections.

CONTROL. Rodger et al. (1995) reported that unspecified antiprotozoal drugs administered orally were not effective for controlling the disease. However, some infected fish appear to recover from the disease.

MICROSPORIDIANS

Microsporidians (phylum Microsporidia) are common protozoan parasites in the aquatic environment and many cause disease in fishes and invertebrates (Canning and Lom 1986). Like myxosporeans, they undergo complex development that culminates in a spore with a coiled polar tube (Fig. 8-13). In contrast to myxosporeans, microsporidian spores contain no

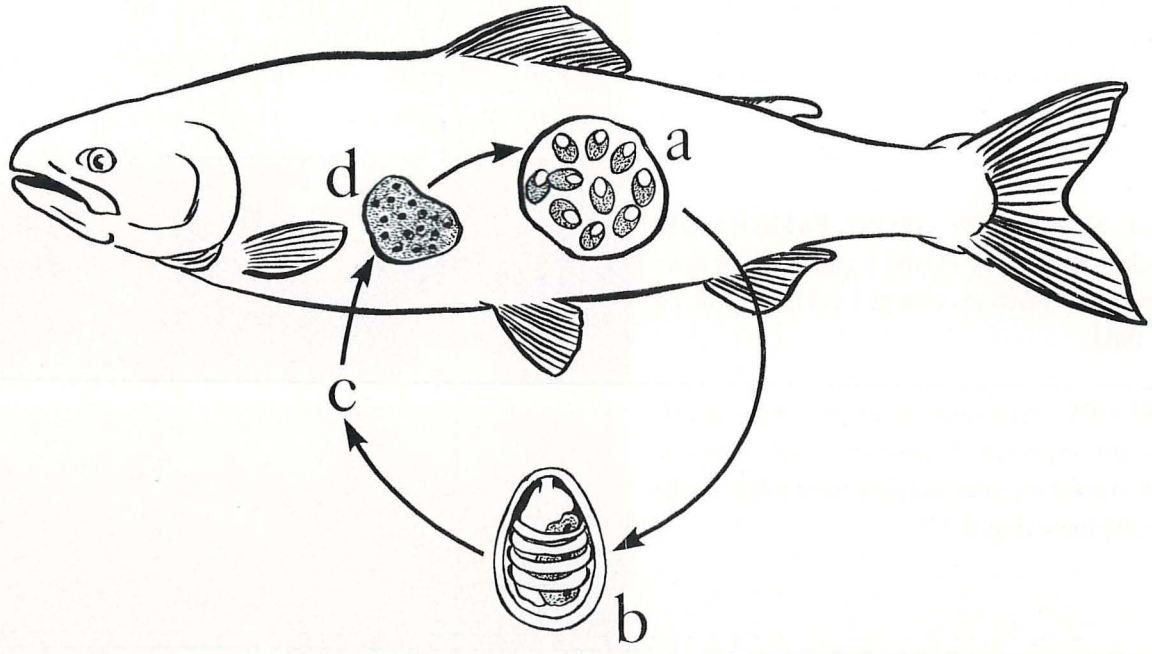


Figure 8-13. Generalized life cycle of microsporidians: a, xenoma with mature spores; b, spores released from host after xenoma ruptures (possibly after death of the host) - typical microsporidian spore with coiled polar tube in the peripheral cytoplasm; c, fish are apparently infected directly by ingesting spores; d, a sporoplasm is released from the spore and infects a host cell - the parasite undergoes intracellular vegetative development (merogony and sporogony), culminating in the formation of spores in xenomas.

polar capsules and the polar tube lies free in the sporoplasm. Additionally, microsporidians contain no mitochondria and all are obligate intracellular parasites. Phylogenetic studies have shown that microsporeans are very primitive eukaryotes, and have thus been placed in a separate kingdom, the Archizoa. Other researchers have recently proposed that Microsporea are more closely related to fungi. Three microsporeans have been reported to cause disease in seawater-reared salmonids; *Loma salmonae* infects the gills and visceral organs of coho and chinook salmon, *Nucleospora (Enterocytozoon) salmonis* infects the nuclei of blood forming cells in chinook and Atlantic salmon, and *Microsporidium cerebralis* was described from the brains of Atlantic salmon.

Loma salmonae

Loma salmonae infects the gills and other vascularized tissues of salmonids reared in fresh water (Putz et al. 1965; Putz and McLaughlin 1970; Hauck 1984; Morrison and Sprague 1981, 1983; Poynton 1986; Markey et al. 1994; Bruno et al. 1995). Severe gill infections have been reported in rainbow trout, steelhead trout and kokanee salmon (Wales and Wolf 1955), and Hauck (1984) observed high mortality in chinook salmon due to systemic infections by a *Loma* sp. (presumably *L. salmonae*). Infections in coho salmon in fresh water are usually mild and cause little disease (Magor 1987). Whereas all *Oncorhynchus* species are apparently susceptible to the infection, Atlantic salmon are resistant (Kent et al. 1995a). The infection is directly transmissible from fish to fish by either ingestion of infected tissue or by free spores (Kent et al. 1995a), and can easily be transmitted by co-habitation with infected fish (Shaw et al. 1998; Speare et al. 1998a). Fish exhibit xenomas about 4-6 wk after exposure.

Infections can persist after fish are transferred to sea water, and the associated lesions in the gills can become severe in the pen-reared salmon (Kent et al. 1989; Speare et al. 1989). Although the gills are the primary site of infection, parasites and associated lesions can occur in the heart, spleen, kidney and pseudobranchs. Although the disease can originate in fresh water, we have seen a high prevalence of *L. salmonae* infection in chinook salmon from netpens that had been reared solely on ground water during the freshwater phase. The spores of other fish microsporidians have been found in the mature ova of the host. This form of transmission has been reported for other fish microsporidia (Vaney and Conte 1901; Summerfelt and Warner 1970), and vertical transmission of *Loma salmonae* from infected females to progeny should be considered as a possibility. Docker et al. (1997a) found a high prevalence of the infection in the ovaries, but not the eggs, of sexually mature chinook salmon. Therefore, the progeny of infected females could become exposed to the parasite through contaminated ovarian fluid. Furthermore, we have found that the spores of *L. salmonae* can survive iodine treatment at 100 ppm for 15 min., a dose typically used for disinfecting salmonid eggs after spawning.

Seawater horizontal transmission of *L. salmonae* to netpen-reared salmon from wild marine fishes is another possible source of the infection because the parasite can easily be transmitted from fish to fish while in sea water (Kent et al. 1995a). Several *Loma* species have been described from marine fishes (Canning and Lom 1986), and we are investigating the relationship of these microsporidians to *L. salmonae*. We were particularly interested in a *Loma* species found in shiner perch because this is one of the most common wild fish species found around netpens. However, using transmission studies and ribosomal DNA sequence comparisons, Shaw et al. (1997) substantiated that the *Loma* from shiner perch was a different species than *L. salmonae*, and was thus given the name *L. embiotocia*. To date, we have examined *Loma* isolates from several marine fishes, and we have yet to identify a marine non-salmonid reservoir for the infection. Moreover, we have found *L. salmonae* in ocean-caught salmon (Kent et al. 1998), and thus a source of the infection for fish in netpens may be wild marine-phase salmonids.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish do not exhibit any distinguishing behavioral signs, but fish with severely damaged gills may exhibit respiratory distress and are lethargic. Close examination of heavily infected fish may reveal small white cysts in the gills (Fig. 8-2d). A hallmark gross pathological change in infected pen-reared salmon are the presence of multiple petechiae in an otherwise pale gill. Infected gills may also appear lumpy (Fig.8-2c). Systemic infections in chinook may cause enlargement of the spleen and kidney.

MICROSCOPY. *Loma salmonae* spores can be easily detected in wet mount preparations of moderately to heavily infected gills. The spores are bean-shaped, about 5.5 X 3 µm, and have a posterior vacuole (Fig. 8-14). Gram-stained smears of heavily infected tissue reveal the Gram-positive spores of *L. salmonae* (Fig. 5-3d). Histological examination reveals the cyst-like xenomas formed by the parasite (Fig. 8-15). Xenomas that develop in the secondary lamellae of the gills cause relatively little damage and inflammation, whereas those

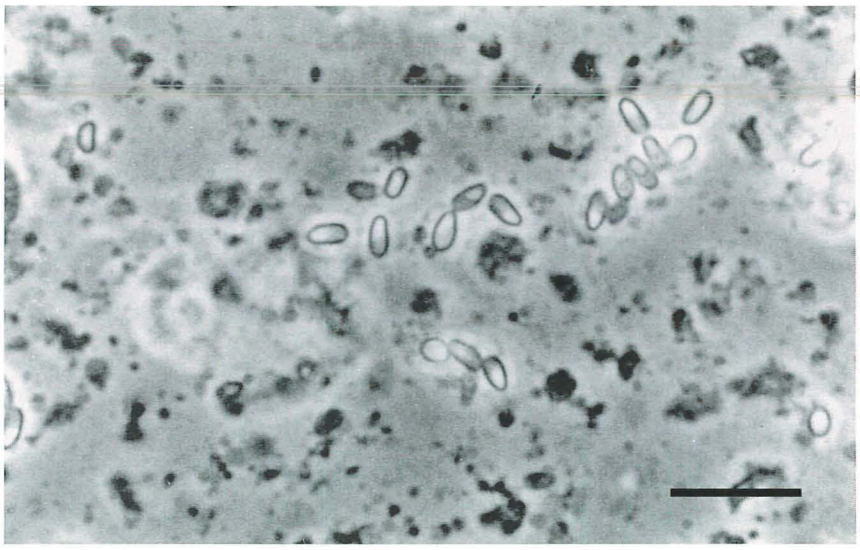
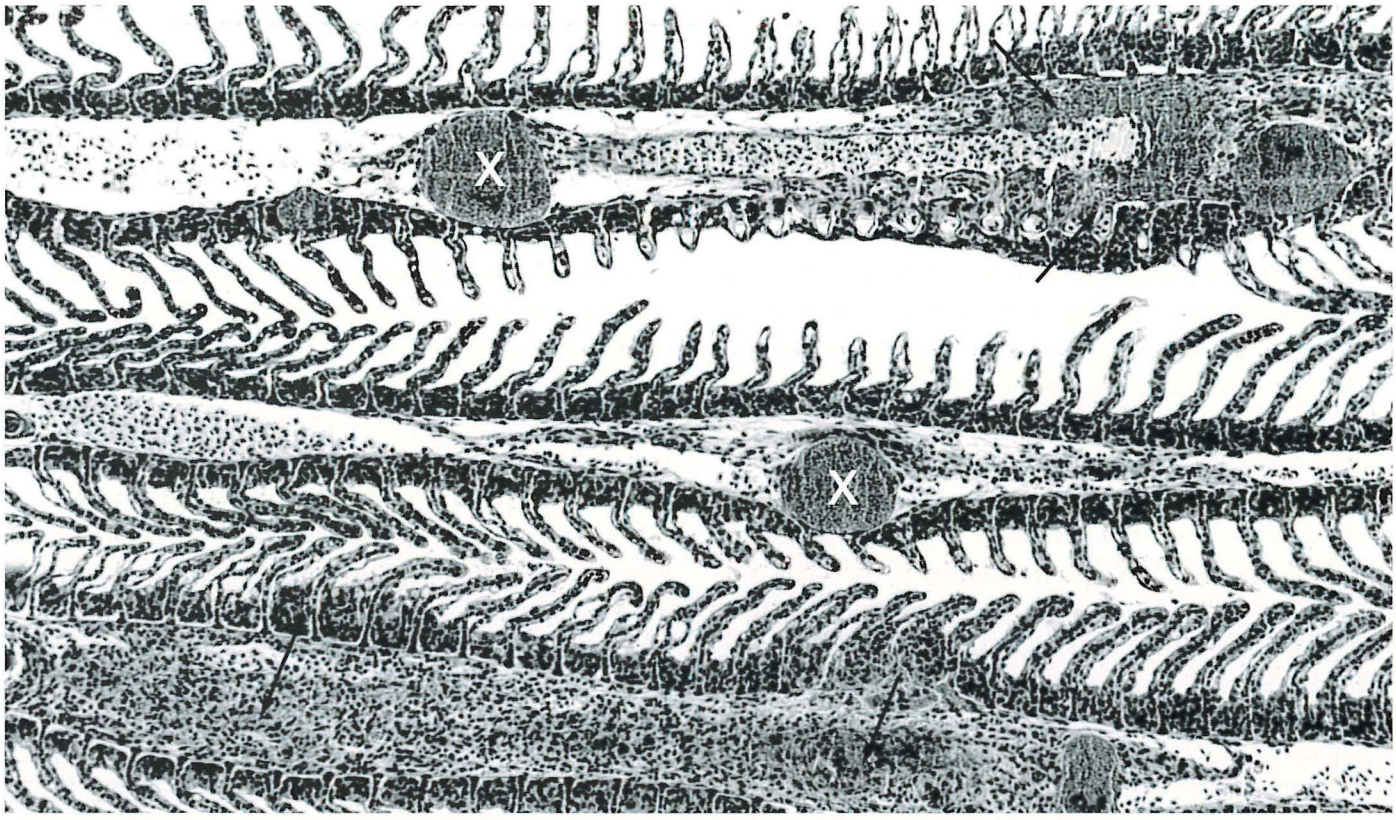


Figure 8-14. Wet mount of *Loma salmonae* spores (arrows). Bar = 15 µm.

Figure 8-15. Tissue section of coho salmon gills with *L. salmonae* infection. H & E. Intact xenomas (X) with little tissue reaction. Chronic inflammation in the primary lamella (arrows) due to *L. salmonae* infection.



that form deep within the blood vessels of primary lamellae can cause severe inflammation and tissue destruction (Fig. 8-15). In such cases, microsporidian spores are dispersed throughout inflammatory lesions and individual spores are found within phagocytic cells. Similar inflammatory lesions caused by ruptured xenomas are found in the spleen and kidney, especially in chinook. Release of spores from xenomas into surrounding tissue is believed to be a factor contributing to the inflammatory response (Hauck 1984; Kent et al. 1989, 1995a).

DIAGNOSIS. *Loma salmonae* infections are diagnosed by detecting the characteristic spores. The spores can be demonstrated in wet mounts or Gram-stained tissue smears of gills when infections are heavy. However, the gills may show severe pathological changes with relatively few spores, and histology should be conducted on suspect tissues because the parasites are difficult to detect in wet mounts when the xenomas have ruptured. Furthermore, tissue sections will reveal the characteristic inflammatory lesions in the gill primary lamellae and visceral organs.

Docker et al. (1997b) developed a sensitive PCR test for the parasite using rDNA sequence. This may be useful for screening fish (i.e., broodstock) for subclinical infections. These specific primers can differentiate *L. salmonae* from other *Loma* species.

CONTROL AND TREATMENT. In laboratory studies, infections in chinook salmon were prevented by feeding fumagillin at 10 mg drug/kg fish/day for 30 days (Kent and Dawe 1994). Our recent experiments demonstrated that the infections can be controlled with lower doses of fumagillin - i.e., 2 or 4 mg drug/kg fish.

We have also found that a synthetic analog of fumagillin, TNP-470 (Takeda Chemical Industries, Ltd.) can also be effective at reducing *L. salmonae* infections. Oral treatment with this compound at 0.1 mg or 1.0 mg drug/kg fish for 4 wk greatly reduced the intensity of infections, with no apparent toxic side effects (Higgins et al. 1998). Rainbow trout show strong protection to reinfection (Speare et al. 1998b), which suggests that this infection is a good candidate for vaccine development.

Recent experiments conducted at the Pacific Biological Station showed that chinook salmon that have recovered from *Loma* infections are also resistant to re-infection. The disease could also be controlled by avoiding infections. At least some infections apparently originate in fresh water and persist in fish after they are transferred to netpens. Ensuring that fish are free of infection during their freshwater development, therefore, may help reduce the infection in netpens. In laboratory transmission studies, Speare et al. (1998b) demonstrated that rainbow trout were susceptible to the infection when held at 14.5 °C, while they were resistant when held at 10 °C.

Nucleospora (Enterocytozoon) salmonis

Nucleospora salmonis is an unusual microsporidium that infects the nuclei of hemoblasts, particularly lymphoblasts or plasmablasts, in salmonid fishes (Chilmonczyk et al. 1991). This microsporidium was first observed in pen-reared chinook in Washington State, where it was associated with anemia (Elston et al. 1987). The parasite has also been reported in freshwater-reared chinook, kokanee, and steelhead trout in Washington (Morrison et al. 1990), California (Hedrick et al. 1990; 1991a, b) and Idaho (MacConnell et al. 1991). Although we have only seen one case of *N. salmonis* in freshwater-reared salmon in British Columbia, it has been observed in chinook at several seawater netpen sites in the province. The infection has also been observed in seawater-reared Atlantic salmon in Chile (Bravo 1996).

This microsporidium was originally described as *Nucleospora salmonis* (c.f. Hedrick et al. 1991b), but was described shortly thereafter as *Enterocytozoon salmonis* by Chilmonczyk et al. (1991). Rules of zoological nomenclature, morphological data, and ribosomal DNA sequence data support the validity of the genus *Nucleospora*, and its placement in the family Enterocytozooidae (Docker et al. 1997b). Intranuclear microsporidia were reported in Atlantic lumpfish (Mullins et al. 1994), and Atlantic halibut (Nilsen et al. 1995), and these organisms were also assigned to the genus *Enterocytozoon*. Based on the data available to date, the intranuclear microsporidia found in these fish should also be transferred to the genus *Nucleospora*.

Nucleospora infections are usually associated with a concurrent neoplastic condition involving massive lymphoproliferation, known as plasmacytoid leukemia (PL) in chinook salmon in British Columbia (Kent et al. 1990a). Laboratory transmission studies indicate that *N. salmonis* may not be the primary cause of all cases of PL (Kent and Dawe 1990; Newbound and Kent 1991a). See pages 106–110 for further details on the relationship of *N. salmonis* with PL.

Nucleospora salmonis is transmitted by cohabitation or feeding infected tissues to fish in fresh water (Baxa-Antonio et al. 1992). We have repeated these findings in our laboratory, but were unable to transmit the infection by cohabitation in sea water. The microsporidium has been maintained in lymphocyte cultures, and soluble fractions of these cultures stimulate uninfected cells (Wongtavatchai et al. 1995a). Infected fish apparently have an impaired humoral and cellular immune response (Wongtavatchai et al. 1995b).

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily infected fish are anemic, with a packed blood cell volume as low as 5%. Typical of severe anemia in fish, affected salmon exhibit prominent pallor of the gills. See page 108 for description when infections are associated with PL. In one instance, the infection in pen-reared Atlantic salmon was associated with multiple focal lesions mainly on the head. Histologically the lesions were characterized by massive fibroplasia in which the nuclei of proliferating fibrocytes were infected by the parasite.

MICROSCOPY. This microsporidium is very small and is identified by careful examination of nuclei of hemoblasts in histological sections (Fig. 5-3e, f) or in Gram-stained imprints (Fig. 5-3g). In tissue sections stained with hematoxylin and eosin, the parasites appear as eosinophilic spherical bodies (2-4 µm) in host cell nuclei, surrounded by a rim of basophilic

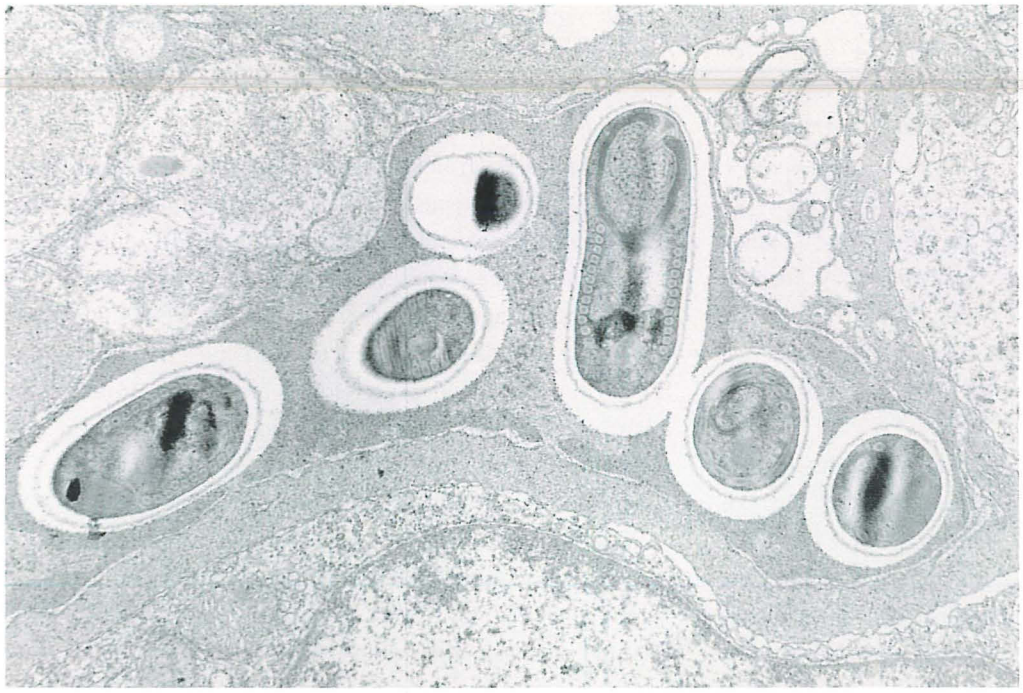


Figure 8-16. Electron micrograph of *Nucleospora salmonis* spores in a host cell nucleus.

host cell chromatin. The Warthin-Starry stain combined with hematoxylin and eosin enhances the detection of the parasite in tissue sections (Kent et al. 1995c). In these preparations, prespore stages stain brown or black, and spores stain dark black (Fig. 5-3f). Some researchers have also used Giemsa-stained impression smears to identify the parasite. In Giemsa imprints, the parasites appear as clear spheres within the host cell nuclei. The spores of microsporidians are Gram positive, and *N. salmonis* spores can be detected in Gram-stained smears of infected tissues (Fig. 5-3g). In these preparations, the spores stain blue, have a characteristic bean shape, measure about 2 X 1 µm. Electron microscopy is required to demonstrate more details of the morphology of spores and presporogonic stages (Fig. 8-16).

DIAGNOSIS. The infection can be diagnosed by detecting the characteristic spores in Gram-stained kidney or eye imprints. However, in some infections very few spores are found, and histology is required to detect the presporogonic forms within the nuclei of hemoblasts. Giemsa-stained imprints have been used by some for detecting these presporogonic forms, but because the parasites only appear as clear vacuoles in these preparations they could be easily confused with artifacts.

Sensitive and specific PCR tests have been developed for the detection of *N. salmonis* based on ribosomal DNA sequence from the small subunit region (Barlough et al. 1995) or ITS region (Docker et al. 1997b).

TREATMENT AND CONTROL. There is no commercially available drug for treating *N. salmonis* infections. However, Hedrick et al. (1991a) controlled the infection in experimentally infected chinook with oral treatment of fumagillin DCH at 1.0 mg drug/kg fish/day for 4 wk. We have found the fumagillin analog, TNP-470 (Takeda Chemical Industries, Ltd.), was very effective at controlling experimental infections. Fish that received an oral treatment at either 0.1 or 1.0 mg drug/kg fish/day for 4 wk showed a dramatic reduction in infection compared to untreated controls (Higgins et al. 1998).

To reduce the effects of the associated anemia, clinically affected fish should not be handled or transported, and feed should be reduced to decrease oxygen demand.

Microsporidium cerebralis

Microsporidium cerebralis infects the central nervous system of pen-reared Atlantic salmon (Brocklebank et al. 1995). The infection has been found at two sites in British Columbia in fish from 1- 2 kg.

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish exhibit abnormal swimming behavior, including spiral swimming, and loss of equilibrium. No other external or internal macroscopic signs are associated with the infection.

MICROSCOPY. Wet mount preparations of the hind brain reveal aggregates of ovoid spores, which are 5.7 X 3.0 µm (Fig. 8-17). Histological sections reveal the infection predominantly in neurons of the myelencephalon, including giant motor neurons (Mauthner's cells). Degenerating and necrotic changes are associated with the infection.

DIAGNOSIS. The infection is diagnosed by observing aggregates of microsporean spores in the hind brain, in either wet mounts or histological sections.

TREATMENT AND CONTROL. Based on research with other fish microsporidia (see *Loma salmonae* and *Nucleospora salmonis*), fumagillin may be useful for treating this infection. Because the source of the infection is unknown (fresh water or sea water), recommendations for avoidance cannot be made. To prevent entrapment of impaired fish in the nets, additional downhauls were placed in the corners and along the sides of the pens (Brocklebank et al. 1995).

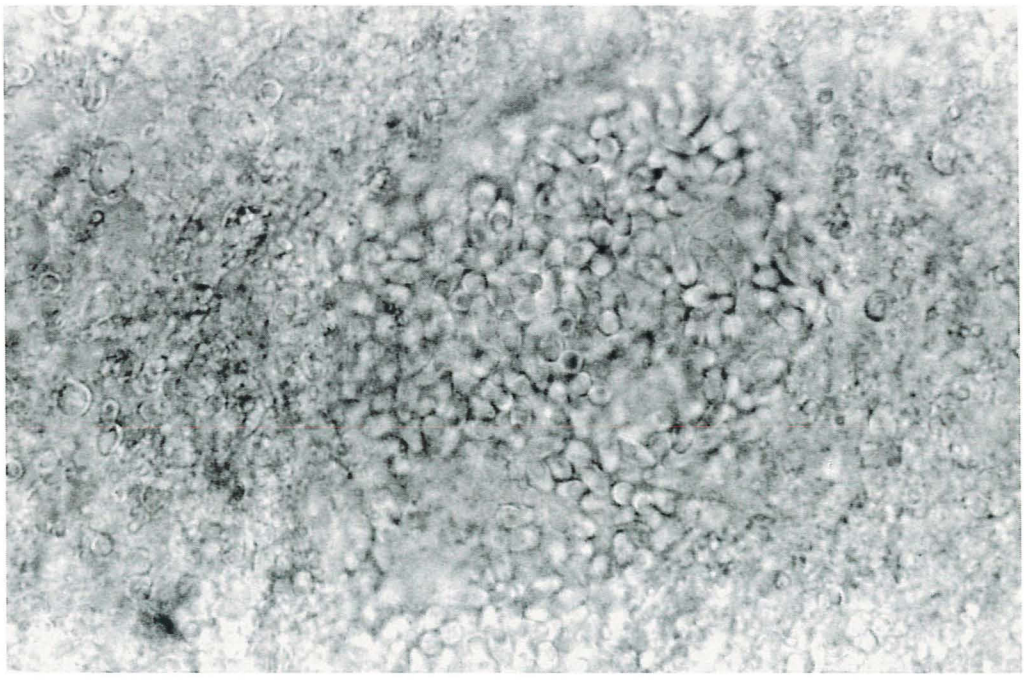


Figure 8-17. *Microsporidium cerebralis* in the brain of Atlantic salmon. Wet mount preparation.

HELMINTH AND MOLLUSCAN PARASITES

M. L. Kent and L. Margolis

Fishes are infected with a wide variety of parasitic worms, collectively referred to as helminth parasites. Although these parasites are very common in wild fishes, and occasionally infect cultured species, most often they do not cause severe disease. However, certain helminths can cause disease when infections are heavy or when they infect a critical organ. In addition, some helminth parasites of fishes can infect humans or cause unsightly lesions. The following are helminth parasites of importance in pen-reared salmon.

Cestodes (Tapeworms)

Cestodes (tapeworms) are one of the main groups of parasitic helminths. Two life stages of cestodes are found in fish: adults infect the digestive tract and metacestodes (juveniles) are found in the internal organs or muscle, or occasionally other sites (Fig. 9-1). The first intermediate hosts of tapeworms that infect fishes are usually crustaceans (e.g., copepods), although in one major taxon, the Caryophyllidae of freshwater fishes, oligochaetes are the intermediate hosts.

Fishes are the second intermediate hosts for tapeworms in which a fish-eating mammal or bird, or another fish, are the definitive hosts. Fish usually acquire metacestode infections by eating infected crustaceans. Metacestodes in fish tissues often cause an inflammatory response to the encapsulated or migrating parasite. The only reported significant metacestode disease of salmon reared in marine netpens is caused by *Gilquinia squali*, which infects the eyes of chinook salmon.

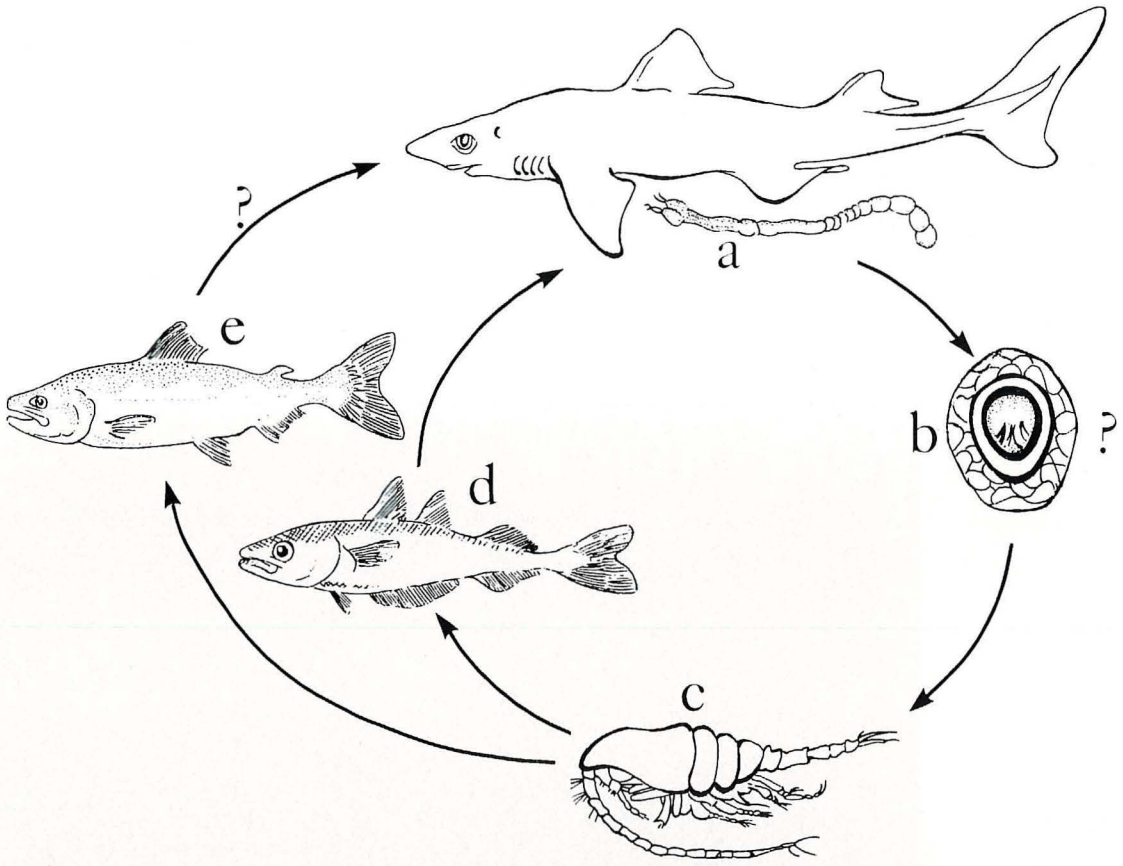


Figure 9-1. Life cycle of the trypanorhynch cestode *Gilquinia squali*: a, dogfish eats infected teleost and worm becomes adult in the spiral valve; b, coracidium develops within egg; c, the invertebrate first intermediate host (presumably a crustacean) is probably infected by eating an embryonated egg and the parasite becomes a procercoid in the coelom of the invertebrate; d, e, teleost fish (e.g., salmon, whiting) ingests invertebrate infected with the procercoid, which migrates to eye and becomes plerocercoid (metacestode).

Adult Cestodes - *Eubothrium spp.*

Eubothrium spp. are common cestode parasites of salmonid fishes both in fresh and salt water. Adults develop in the gut. Infections with one species in pen-reared Atlantic salmon in Norway (Fig. 9-2) have been associated with reduced growth and, occasionally, mortality (Bristow and Berland 1991b; Håstein and Lindstad 1991). Berland and Bristow (1994) have noted that the weight of infected market-size farmed Atlantic salmon in Norway is 10-15% less than uninfected salmon. The species of *Eubothrium* in marine Atlantic salmon has usually been considered to be *E. crassum*, which was originally described from fish in fresh water (Kennedy 1978). However, Berland and Bristow (1994) regard the marine form as distinct from *E. crassum*. We have seen infections with a similar cestode in broodstock of pen-reared chinook salmon in British Columbia.

The fish acquire infections of *Eubothrium* species by ingesting first intermediate hosts (presumably copepods) infected with the procercoid stage, or possibly transport hosts infected with plerocercoids. The life cycle of this tapeworm has not been elucidated, but its freshwater counterpart, *E. salvelini*, in sockeye salmon in British Columbia uses copepods (*Cyclops* spp.) as its intermediate host. Procercoids that develop in *Cyclops* are directly infective for juvenile sockeye salmon (Boyce 1974). *Eubothrium salvelini* is known to affect survival, growth, and stamina, and to have other debilitating effects on juvenile sockeye salmon (Boyce 1979; Boyce and Behrens-Yamada 1977; Boyce and Clarke 1983).

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily infected fish are often smaller than average. Dissection of the gut will reveal numerous, white, flat, "tape-like" worms in the intestine and pyloric caeca (Fig. 9-2). Heavy infections may induce anemia, and when extremely severe may cause death due to blockage of the intestinal tract.

MICROSCOPY. Histological examination of infected pyloric caeca reveals minimal pathological changes.

DIAGNOSIS. Adult cestodes are usually long, flat, whitish, and segmented; some, such as the Caryophyllidae, are smaller unsegmented worms. Identification of cestodes is based largely on whether the body is segmented or not, on the morphology of the scolex (anterior end), and on the structure and arrangement of the components of the reproductive system within the segments (Schmidt 1986; Khalil et al. 1994). *Eubothrium* lacks hooks on the scolex, which is elongate with two shallow grooves - one dorsal and the other ventral.

CONTROL AND TREATMENT. Oral treatment for adult tapeworms with antihelminthic drugs, such as praziquantel, may be effective (Mitchell 1993). Avoiding marine worm infections is difficult because infected intermediate hosts (i.e., crustaceans) move freely throughout netpens.

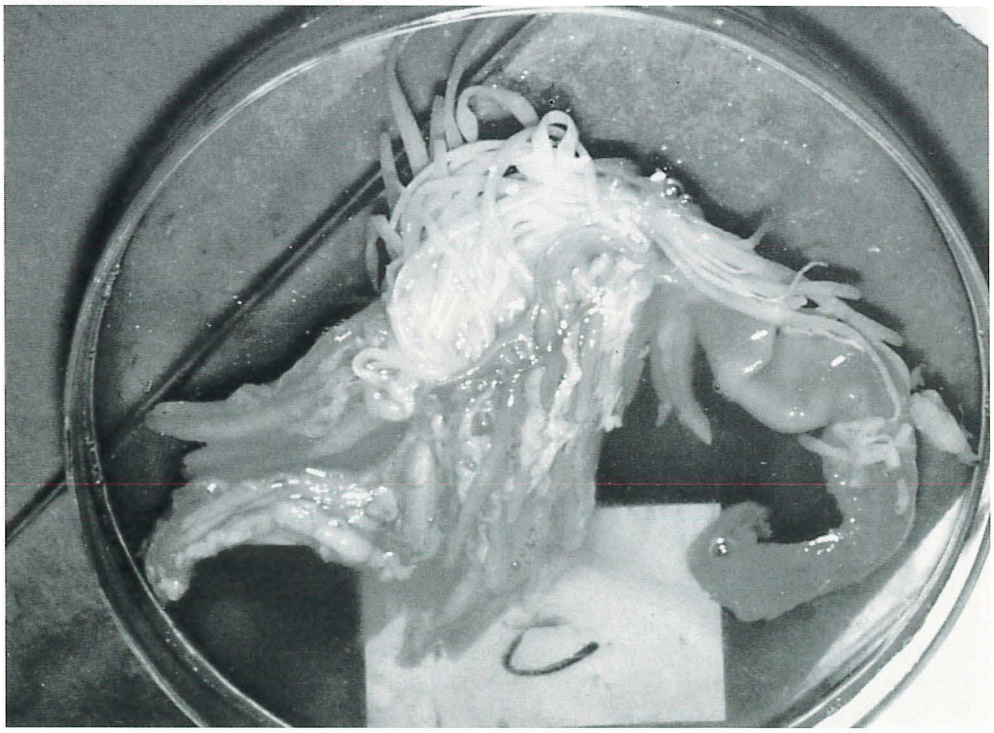


Figure 9-2. *Eubothrium* sp. adults in the digestive tract of an Atlantic salmon. (Courtesy of B. Berland)

Gilquinia squali metacestodes in chinook salmon

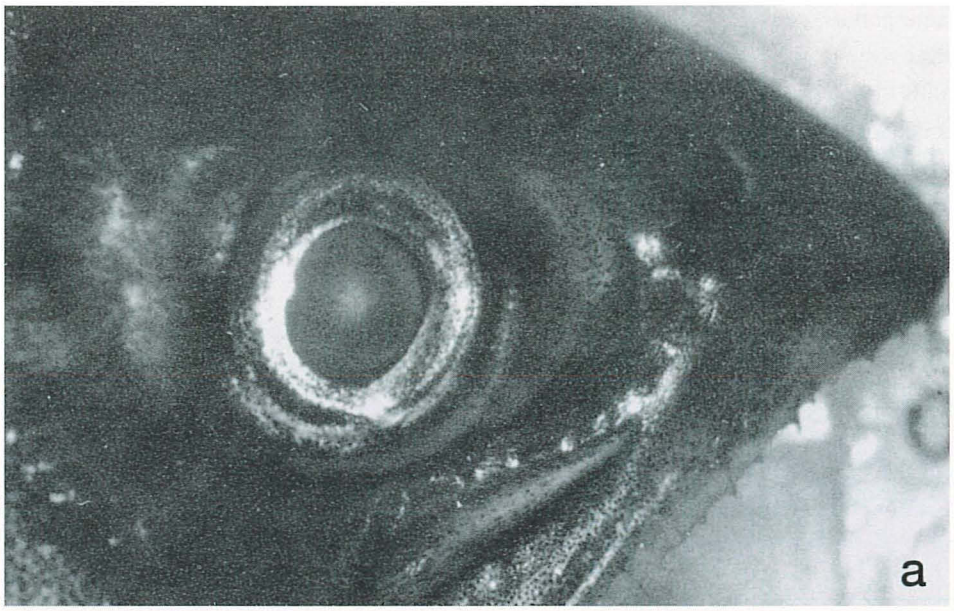
Eye infections by metacestodes of *Gilquinia squali* (order Trypanorhyncha) have been associated with mortality of young chinook salmon at netpen sites in British Columbia (Kent et al. 1991b). The definitive hosts for trypanorhynch tapeworms are sharks and rays, and the definitive host for *G. squali* is the spiny dogfish (Fig 9-1). The metacestode is common in the eyes of North Sea whiting (MacKenzie 1965, 1975). Kuitunen-Ekbaum (1933) reported this metacestode from the muscle of speckled sanddab from British Columbia coastal waters. Observations of the infection in pen-reared chinook salmon is the first reported occurrence of *G. squali* metacestodes in a salmonid fish, which suggests that wild salmon are probably not a normal intermediate host for the worm.

The adult worms are prevalent in dogfish throughout marine waters in British Columbia, and gravid adult worms are commonly found in dogfish in the spring. Dogfish are frequently found in or around netpens at this time, thus providing infective coracidia for the first intermediate host near the pens. Based on the few trypanorhynch cestode life cycles that have been investigated (Mundry and Dailey 1971; Overstreet 1978; Mattis 1986; Sakanari and Moser 1989), it is likely that a crustacean, which has yet to be identified, is the first intermediate host for *G. squali*. Chinook salmon in netpens presumably acquire the infection by eating this crustacean.

CLINICAL SIGNS AND GROSS PATHOLOGY. Moribund fish are lethargic and remain near the bottom of the pen. The fish are easily captured by hand by divers, suggesting that they are blind. However, fish are not usually emaciated and contain food in their stomachs, which indicates that they continue to feed. The actual mechanism that causes death is unknown.

The lens often appears normal, whereas the iris and vitreous chamber may be white and opaque and occasionally exhibit hemorrhages. The lens in more severely affected eyes is also opaque, suggesting cataractous changes (Fig. 9-3a). In extreme cases, the globe is ruptured and the lens is extruded. However, many fish die with the globe of the eye still intact. The eye lesions are often bilateral.

MICROSCOPY. Dissection of affected eyes that are still intact reveals one or two metacestodes of *G. squali* in the vitreous chamber (Fig. 9-3b). The worms are white and are about the size and shape of rice



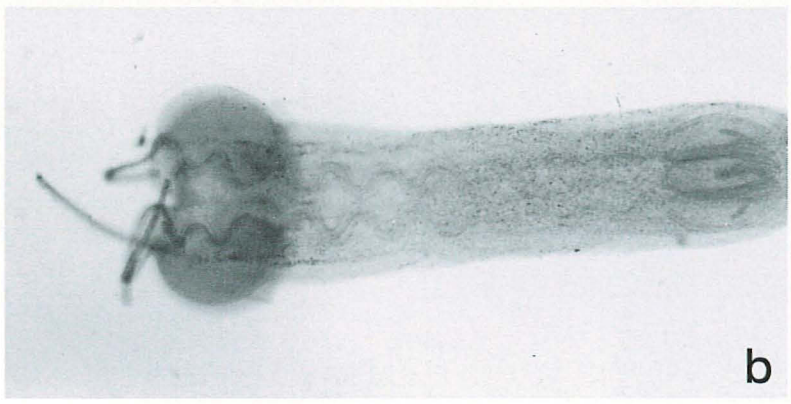


Figure 9-3. *Gilquinia squali* in chinook salmon. a. Chinook salmon with opaque lens associated with *G. squali* infection. b. Whole mount of *G. squali* metacestode from the eye of chinook salmon. Note the four eversible, spiny tentacles that emerge from the apex of the scolex, which are characteristic for trypanorhynch tapeworms. Semichon's acetocarmine.

grains when examined by the naked eye. Kent et al. (1991b) described the histopathological changes of *G. squali* infections in the eyes of chinook salmon. Histological examination of infected eyes often reveals an inflammatory infiltrate in the vitreous humor surrounding the worm (Fig. 9-4).. Separation of the sensory retina from the pigment epithelium causes moderate to severe folding of the retina, resulting in apparent retinal duplication. The lens of infected eyes shows a spectrum of cataractous changes. Some severely affected eyes exhibit hemorrhages in the globe, congestion of the choroid, and extrusion of the lens. Panophthalmitis is prominent in eyes with a ruptured cornea. A tapeworm metacestode resembling *G. squali* was detected in the optic nerve of a chinook salmon (R. Armstrong, Aquatic Animal Health Consulting, Forestville, California, pers. comm.), which suggests that this may be the route of entry of the worm into the globe of the eye.

DIAGNOSIS. The infection is identified by detecting trypanorhynch metacestodes in the vitreous humor. Trypanorhynch cestodes are identified by the presence of four eversible, spiny tentacles that emerge from the apex of the scolex (Fig 9-3b).

CONTROL AND TREATMENT. At present there is no known treatment for *Gilquinia* infections in fish. The disease, therefore, can only be controlled by preventing infections. Fish that are feeding well on commercial diets and thus feed less on natural biota appear less susceptible to the infection. Therefore, assuring that fish are feeding well on pellets should reduce the prevalence of infection. The complete life cycle of the parasite is unknown, so precise recommendations for avoiding the infection are not available. Furthermore, preventing transmission of the parasite from dogfish to salmon via the arthropod first intermediate hosts would be very difficult because of uncontrolled water movement into netpens and unrestricted movement of dogfish around netpens.

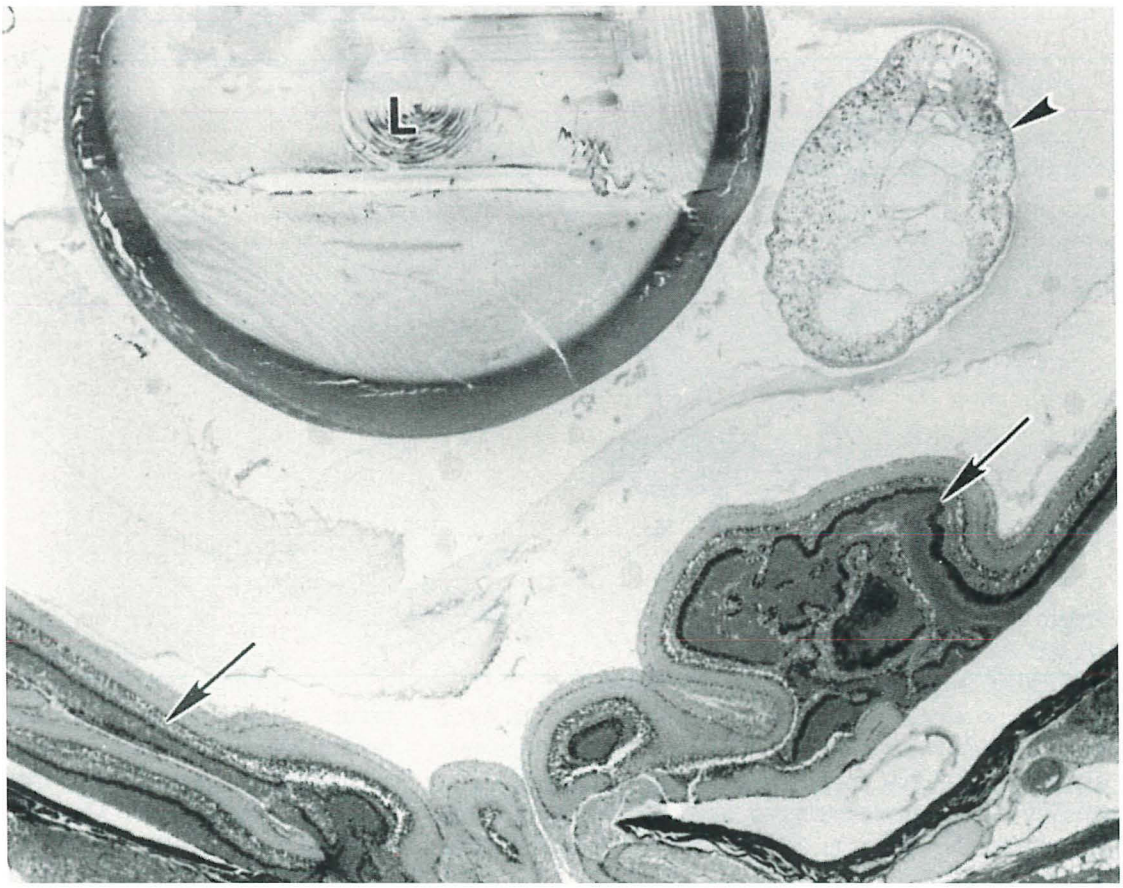


Figure 9-4. *Gilquinia squali* (arrowhead) the vitreous humor of the eye of a chinook salmon. Arrows indicate retinal folding and apparent duplication. H & E. (Courtesy of J. Aquat. Animal Health).

Digenetic trematodes (flukes)

As with cestodes, fish can be intermediate hosts or definitive hosts for digenetic trematodes (Fig. 9-5). Almost all flukes have either a 2-host or 3-host life cycle, but there is a wide variety of life cycle patterns (Williams and Jones 1994). With a few exceptions among the marine fish blood flukes of the family Sanguinicolidae, molluscs (either snails or bivalves) are the first intermediate hosts of digeneans. Second intermediate hosts, where such occur in the life cycle, may be an invertebrate or a fish. Except in occasional circumstances where the life cycle has been foreshortened, the definitive hosts are to be found among the vertebrates. The species of concern in marine farming of salmon use either birds or fish as definitive hosts, with the fish serving as the second intermediate host. Cercariae of these species emerge from molluscs and infect the salmonid host by direct penetration of the skin or gills, subsequently developing into a resting stage known as a metacercaria, which may be encysted or unencysted depending upon the final site of infection in the fish.

Heavy infections by metacercariae are of concern because they can cause morbidity. In addition, metacercarial infections of the skin or muscle can be important because they may reduce the aesthetic quality of the fish. Except for blood flukes and a group of tissue parasites of the family Didymozoidae found mainly in scombroid fishes, most adult flukes of fish infect the alimentary tract and seldom cause significant tissue damage.

The metacercariae of four digenean trematodes have caused problems in seawater pen-reared salmonid fishes: "neascus"-type, *Diplostomum* sp., *Cryptocotyle lingua* and *Stephanostomum tenue*.

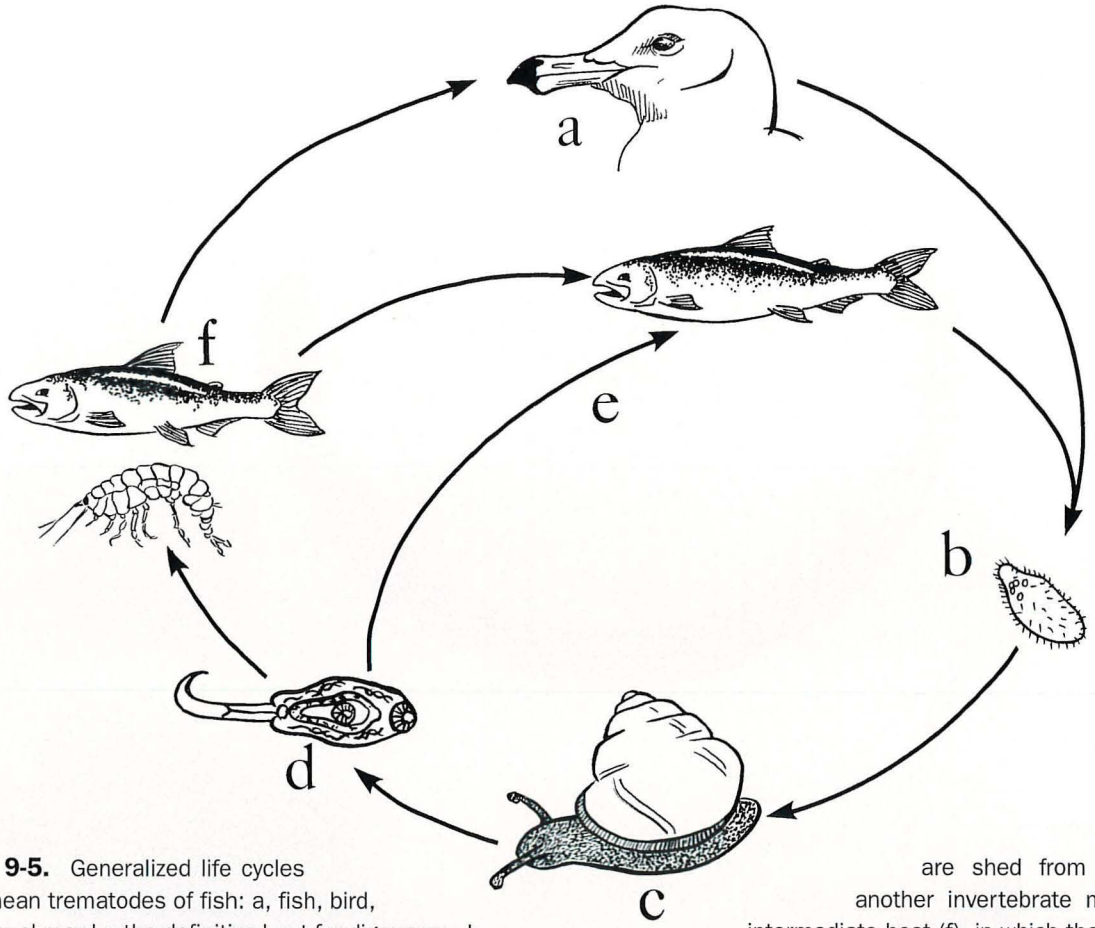


Figure 9-5. Generalized life cycles of digenean trematodes of fish: a, fish, bird, or mammal may be the definitive host for digeneans; b, adult worms release eggs and miracidia hatch from eggs in the external environment or within the next host; c, invertebrates, especially snails and bivalves, are first intermediate hosts in which the miracidium undergoes several developmental and proliferative stages culminating in numerous cercariae (d) that are shed from the host. Fish or another invertebrate may be the second intermediate host (f), in which the cercariae encyst in tissues and becomes a metacercariae. Definitive host (a) eats second intermediate host and the parasite develops into an adult, usually in the gut. Certain digeneans (e.g., blood flukes) do not have a metacercarial stage and the cercariae infect the definitive host directly (e).

Black Grub in Coho Salmon

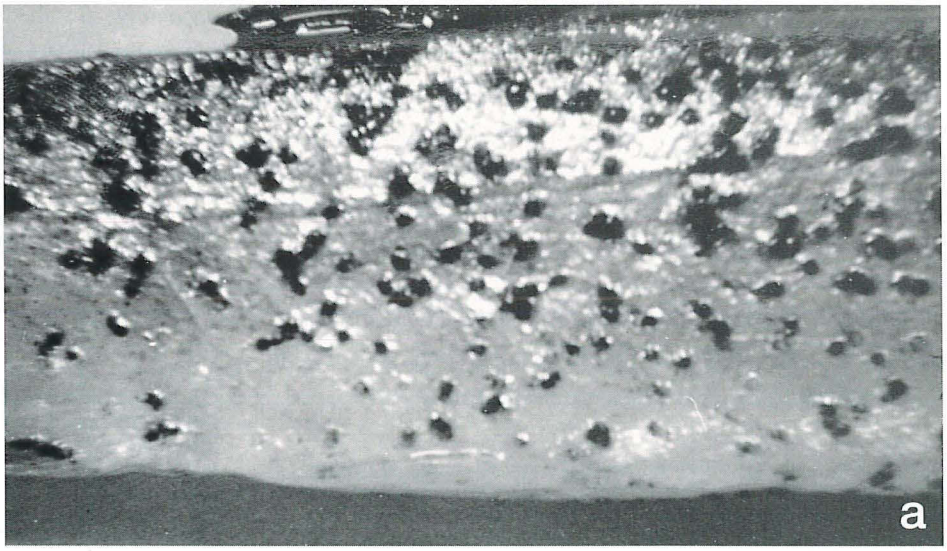
Black grub (larval type neascus) is the metacercarial stage of certain species of the family Diplostomatidae, which includes several genera (see Gibson 1996). Black grub infect a variety of fishes in fresh water, including salmonids. Snails are the first intermediate hosts, and cercariae released from infected snails penetrate beneath the scales in the dermis of the fish host. Fish-eating birds serve as the definitive hosts. Mortality and morbidity have not been reported in infected salmon, but Lemly and Esch (1984) demonstrated that heavy infections of the "neascus"-type metacercariae of *Uvulifer ambloplitis* could kill bluegill sunfish when water temperatures decline.

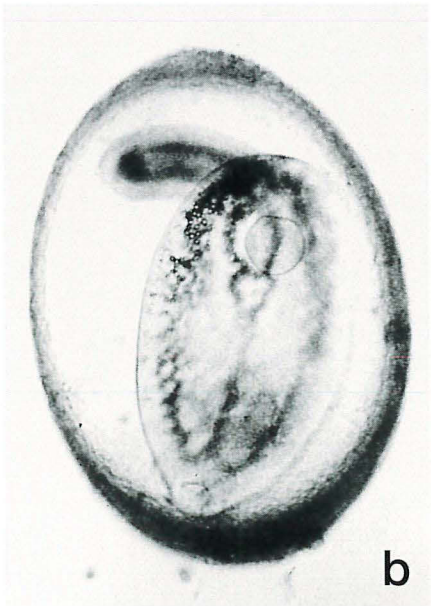
CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish exhibit numerous small, black, raised, nodules in the skin, which are about 1-2 mm in diameter (Fig 9-6a).

MICROSCOPY. Careful examination of wet mount preparations of the black cysts reveals a metacercaria identified by the presence of two suckers (Fig. 9-6b). Tissue sections of infected skin reveal encysted metacercariae surrounded by a thick fibrinous capsule (Fig 9-6c). The periphery of the capsule contains numerous melanocytes.

DIAGNOSIS. Presumptive diagnosis can be obtained by the observation of small, multifocal, slightly raised black spots in the skin. Confirmation is obtained by observing metacercariae in the cysts in wet mount preparations or histological sections.

CONTROL AND TREATMENT. Fish become infected in fresh water by exposure to surface water containing infected snails. Disinfection of influent water or using ground water should eliminate or greatly reduce the infection. Based on reports from one fish farm, removing fish from freshwater hatcheries and introducing them to netpens before June or July may reduce the intensity and prevalence of the infection.





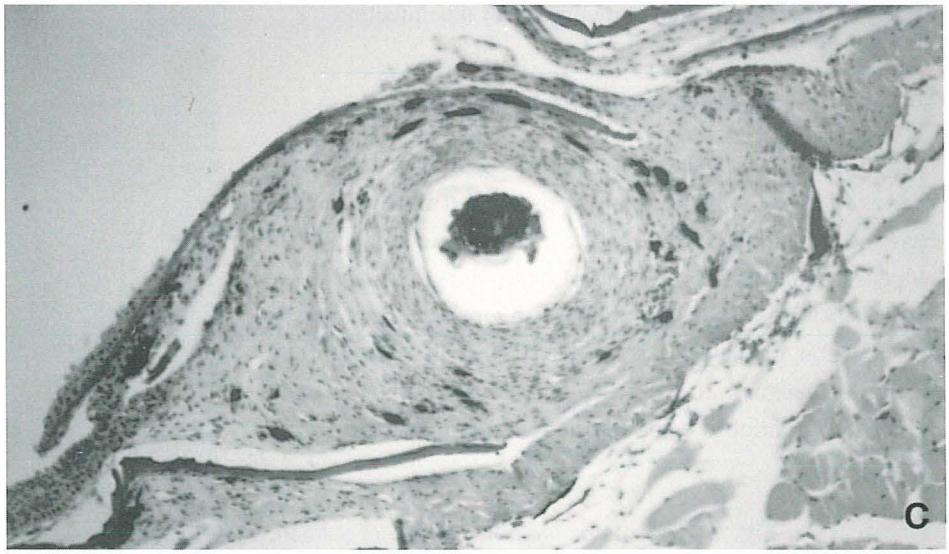


Figure 9-6. Black grub metacercariae. a. black grubs in the skin of a coho salmon smolts. b. Parasite excised from a cyst. Note two suckers, which are characteristic of digeneans. (Courtesy of G.L. Hoffman). c. Black grub (arrow) encysted in the dermis of coho.

Black Spot Disease in Atlantic salmon

A condition similar to black grub in coho salmon is "black spot disease" caused by metacercariae of the digenean *Cryptocotyle lingua* in Atlantic salmon. In contrast to the former condition, this parasite has a marine life cycle, which involves a definitive (adult) stage in fish-eating birds and a cercarial stage in snails.

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish exhibit few to numerous slightly raised black spots up to 1 mm in diameter in the skin, fins, cornea, and gills. Occasionally, encysted metacercariae may be found in internal organs. Fish seldom become clinically affected unless they are heavily infected.

MICROSCOPY. Both wet mounts and histological sections of the skin show similar findings as with black grub of coho salmon.

DIAGNOSIS. Diagnosis of metacercarial infections in general is relatively easy using wet mounts or histological sections. However, more precise identifications to the genus or species level usually requires careful preparations of the metacercariae in stained whole mounts and examination of the internal anatomy.

Information on the first occurrence of the infection (i.e., marine vs freshwater) is useful for differentiating neascus from *Cryptocotyle*.

CONTROL AND TREATMENT. As the cercarial stage is common in the periwinkle (*Littorina littorea*), cages located in shallow water and close to the shore are more prone to the infection. There is no known treatment for this infection.

Diplostomum - Eye Fluke

In low salinity waters of the Baltic Sea, metacercariae of a species of *Diplostomum*, usually a parasite of freshwater fish, have been reported to infect the eyes of rainbow trout in netpens located in shallow water, close to shore (Vismanis et al. 1984). Buchmann and Uldal (1994) also reported *Diplostomum* sp. infection in the eyes of rainbow trout in a mariculture facility in Denmark. In this case, the fish had become infected in fresh water prior to being transferred to sea water.

CLINICAL SIGNS AND GROSS PATHOLOGY. In one year (1977) blindness occurred in more than 17% of the trout reared in the Baltic Sea due to infection with *Diplostomum* metacercariae (Vismanis et al. 1984). In pens further from shore, where water depths were 9-12 m, intensities of infection were much lower than in the inshore pens (maximum 30 metacercariae per fish compared to more than 120 per fish in the inshore pens).

In the Danish case of trout diplostomosis, there was a significant negative correlation between the number of parasites in the least infected eye and fish weight, but not between the total number of parasites in both eyes and fish weight. Buchmann and Uldal (1994) suggested that the feeding ability of the trout was determined by the vision of the least infected eye.

MICROSCOPY. Examination of infected eyes reveals metacercariae in the lens or other tissues (e.g., the retina) depending on the particular *Diplostomum* species (Gibson 1996). Lens infections are associated with cataractous changes, and may result in lens dislocation, capsular rupture and detachment of the retina (Shariff et al. 1980; Wilcock and Dukes 1989).

DIAGNOSIS. In eye infections with *Diplostomum* (diplostomosis), cataracts may develop and a positive diagnosis is made by finding the characteristic unencysted metacercariae (Fig. 9-7) in the lens, vitreous humor, or retina.

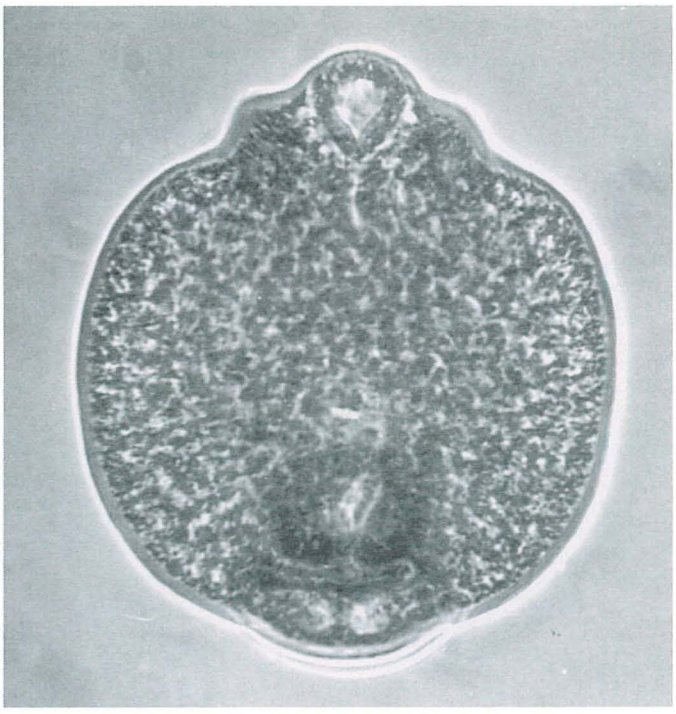


Fig. 9-7. *Diplostomum* from the eye of salmon.

CONTROL AND TREATMENT. As with black grub, the infection can be avoided by preventing exposure of fish to infectious fresh water. Diplostomosis in rainbow trout in the Baltic Sea netpens was prevented by locating netpens in deeper water where the snail first intermediate hosts, *Limnaea* spp., are absent (Vismanis et al. 1984).

Although Heckman (1984) reported the efficacy of praziquantel and mebendazole against the eye-fluke *Diplostomum spathaceum* in the freshwater sculpin *Cottus bairdi* (administered by injection), there is no information available on the practical application of these drugs in mass treatment of salmonids.

Stephanostomum Heart Infections

Heart (pericardial cavity) infections by metacercariae of *Stephanostomum tenue* caused high mortalities in pen-reared rainbow trout in Atlantic Canada (McGladdery et al. 1990). Rainbow trout are accidental hosts for this marine fluke, which normally infects mummichog or silversides as second intermediate hosts, and American eels as definitive hosts in the vicinity of the affected sea-pen sites. The first intermediate host was the mud dog whelk (*Nassarius obsoletus*), which was common around the affected netpens.

CLINICAL SIGNS AND GROSS PATHOLOGY. The infection was associated with high mortalities in the summer months when water temperatures increase, presumably due to cardiac dysfunction (McGladdery et al. 1990).

MICROSCOPY. Histological sections reveal encysted metacercariae and a thick fibrogranulomatous layer on the surface of the heart and extending anteriorly on the bulbus arteriosus.

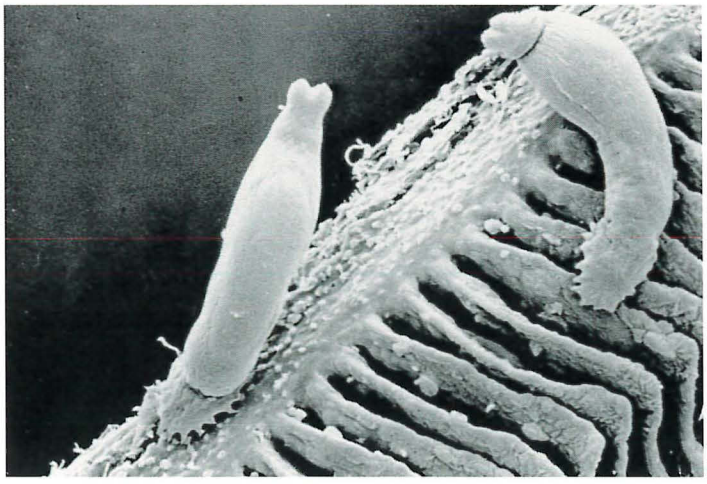


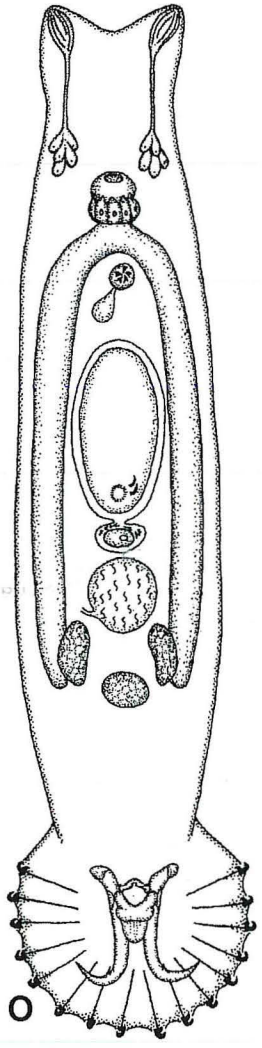
Figure 9-9. Scanning electron micrograph of *Gyrodactyloides bychowskii* on the gills of Atlantic salmon (Courtesy of T. Atle Mo).

CONTROL AND TREATMENT. Maintaining netpens in water with over 7 m clearance from the bottom may reduce the intensity of infection (McGladdery et al. 1990).

Monogenean Flatworms

Monogenean flatworms (Figs. 9-8, 9-9), often incorrectly called monogenetic trematodes, are common parasites of the skin, fins and gills of fishes. Several species cause disease in captive fishes. Unlike digenetic trematodes, monogeneans have a direct life cycle, and some are viviparous. Therefore, they can rapidly proliferate and spread in the confined environment of aquaculture systems. The only monogenean that we have detected in seawater-reared salmon on the Pacific coast of North America is *Laminiscus strelkowi*. Typical of its family, it is a small worm, approximately 0.5 mm in length. This parasite is frequently found on the gills of salmonids reared in netpens, and we have seen heavy infections in Atlantic and coho salmon.

Figure 9-8. Monogenean trematode *Laminiscus strelkowi*. O = opisthaptor, used to attach to host tissue.



Very heavy infections have also been observed on the gills of pen-reared cutthroat trout in Puget Sound, Washington, USA (L.W. Harrell, National Marine Fisheries Service, Manchester, Washington, pers. comm.). The precise role of the parasite in disease in pen-reared salmon is unclear. In Norway, Mo and MacKenzie (1991) and Berland (1994) reported the occurrence of a similar monogenean, *Gyrodactyloides bychowskii*, on netpen-farmed Atlantic salmon.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with heavy monogenean infections of the gills are often lethargic, exhibit labored respiration, and accumulate near the surface.

MICROSCOPY. Wet mount preparations of the gills of heavily infected fish will reveal numerous, actively moving monogeneans. Histological examination reveals the worms attached to the gill surface by the posterior attachment organ, the opisthaptor. Examination of coho salmon with moderate *L. strelkowi* infections revealed no significant histological changes in the gills. *Gyrodactyloides bychowskii* causes hypertrophy and hyperplasia of the gill epithelium (Mo and MacKenzie 1991).

DIAGNOSIS. Monogenean infections are diagnosed by observing the parasites in wet mount preparations of the gill or skin.

CONTROL AND TREATMENT. Monogenean infections are controlled with external therapeutic agents, such as formalin and organophosphates (Schmahl et al. 1989; Schmahl 1991, 1993). However, organophosphates are not legal for treating food fishes in Canada and the United States, although proprietary formulations of formalin are available.

Nematodes

Nematodes (round worms) are common parasites of fishes, and occasionally infect pen-reared salmon. As with cestodes and digenetic trematodes, fish can be either definitive or intermediate hosts for nematodes. Crustaceans and, less frequently, other invertebrates are the first intermediate hosts for nematodes that infect fish. One nematode, *Philonema agubernaculum* causes severe visceral infections in salmon reared in netpens in freshwater lakes. It is possible that these infections could persist in fish if they were transferred to netpens.

Members of the family Anisakidae are the only important (and reported) nematodes of pen-reared salmon. The only nematode that has been reported to be associated with disease in salmon netpens is *Hysterothylacium aduncum*, which in its adult stage infects the digestive tract of fishes. However, we include a discussion of another anisakine nematode, *Anisakis* sp., which can infect humans (Figs.9-10, 9-11). Larvae of this

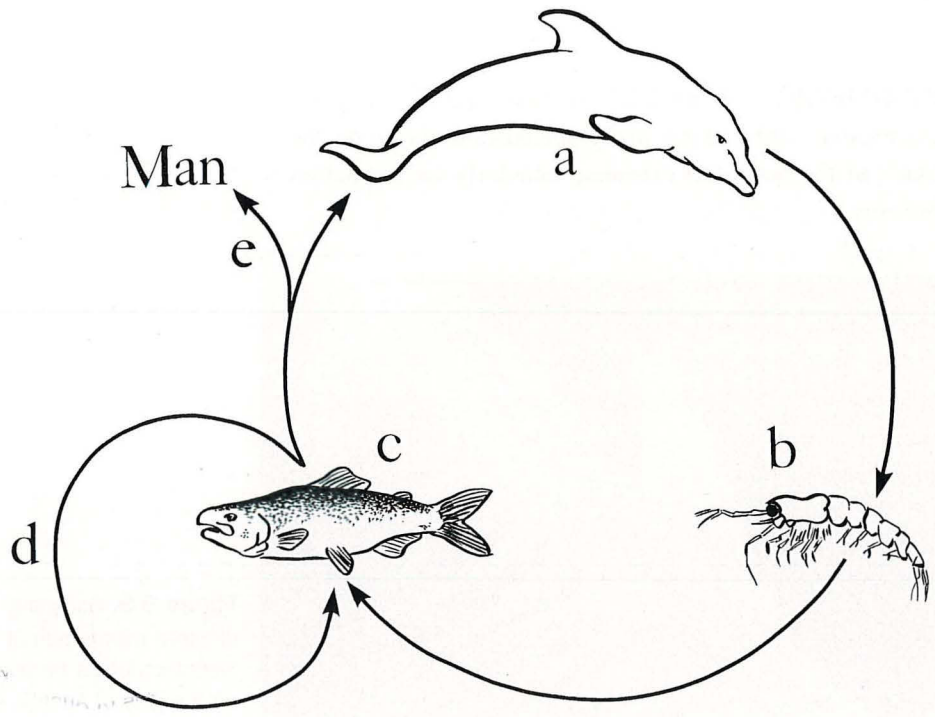


Figure 9-10. Life cycle of anisakine nematodes: a, marine mammals eat fish infected with third stage larvae (L3) - larvae develop into fourth stage larvae, then adults, in the gut of the mammal - eggs shed from mammals, hatch and second stage larvae (L2) eaten by crustaceans (intermediate host); b, L2 develops into L3 in crustacean; c; fish or invertebrate (e.g., squid) are paratenic (transport) hosts; d; L3 can be transmitted from fish to fish, squid to fish, or vice versa; e, man becomes infected after eating undercooked or raw fish - man is a dead end host in which the worms remain as L3 larvae or molt into L4 larvae.

worm commonly occur in wild-caught salmon, and have the potential to infect pen-reared salmon.

Hysterothylacium

Large numbers of adult worms of the species *Hysterothylacium* (=*Thynnascaris*) *aduncum* were found blocking the anterior intestine of seapen-reared rainbow trout in Norway some months after feeding the trout fresh wild sprats (*Sprattus sprattus*) that contained juvenile *H. aduncum* in their viscera (Berland 1987). Intestinal infections of seapen-reared coho salmon and rainbow trout with a species of *Hysterothylacium* have also been observed in Chile (González and Carvajal 1998). Calanoid copepods are the first intermediate host of *H. aduncum*, whereas fish and various invertebrates, such as polychaetes, barnacles and amphipods act as second intermediate hosts (Svendsen 1990; González 1994).

In rainbow trout farms in the Baltic Sea, infections of the liver with juvenile nematodes identified as *Contracaecum* (= *Hysterothylacium*) *aduncum* have been reported by Vismanis et al. (1984). The parasite was considered to be identical with the juvenile nematode pathogen of Baltic cod that reduces liver size and decreases body condition factor in heavily infected fish. Fagerholm (1978, 1988) determined that the nematode in Atlantic cod livers is actually *Contracaecum osculatum*, and it is possible that the trout liver parasite also belongs to this species. Seals and other pinnipeds are the definitive hosts of *C. osculatum*.

Infection intensity in the Baltic farmed trout has been low and an impact comparable to that seen in heavily infected cod has not been observed in the rainbow trout. However, infection of the trout had been increasing through the late 1970s, raising the possibility that abundances could reach damaging levels.

CLINICAL SIGNS AND GROSS PATHOLOGY. Carvajal and González (1990b) suggested that heavily infected fish exhibit poor growth, and Berland and Egidius (1980) have attributed mortalities among pen-reared rainbow trout in Norway to heavy intestinal infections with *H. aduncum*.

DIAGNOSIS. Diagnosis is based on the identification of the worm. Presumptive diagnosis is based on the observation of nematodes in gut lumen. The worms are whitish and cylindrical, and adults are about 40 to 80 mm in length (Gonzáles and Carvajal 1994). Confirmation of the worm's identity requires microscopical examination of cleared or dissected worms for certain pathognomonic anatomical features of the worm's digestive tract (Möller and Anders 1986; Berland 1989). *Hysterothylacium* and *Anisakis* spp. can be differentiated from *Pseudoterranova* and *Contracaecum* in that they have straight digestive tracts, without a ventricular appendix or intestinal caecum. *Hysterothylacium* can be separated from *Anisakis* in that the former has the excretory pore at the level of the nerve ring, whereas it occurs near the anterior tip with *Anisakis*.

CONTROL AND TREATMENT. To prevent infections with *Hysterothylacium* or *Contracaecum* species, or keep infection abundances low, the use of fresh wild marine fish or fish offal as feed for farmed fish should be avoided (Berland and Egidius 1980; Vismanis et al. 1984). Such fish serve as intermediate or paratenic (transport) hosts for the nematodes. González and Carvajal (1994) also referred to use of anthelmintics to reduce infection levels but did not specify which anthelmintics they employed.

Anisakis

Larvae of *Anisakis* spp., and the closely related genus *Pseudoterranova*, are commonly found in wild marine fishes. These worms are of concern because they can infect humans who eat raw or undercooked fish (Margolis 1977; Bier et al. 1987; Deardorff and Overstreet 1990). The general life cycle of

Figure 9-11. Larval *Anisakis* nematodes in the viscera of a Pacific herring.

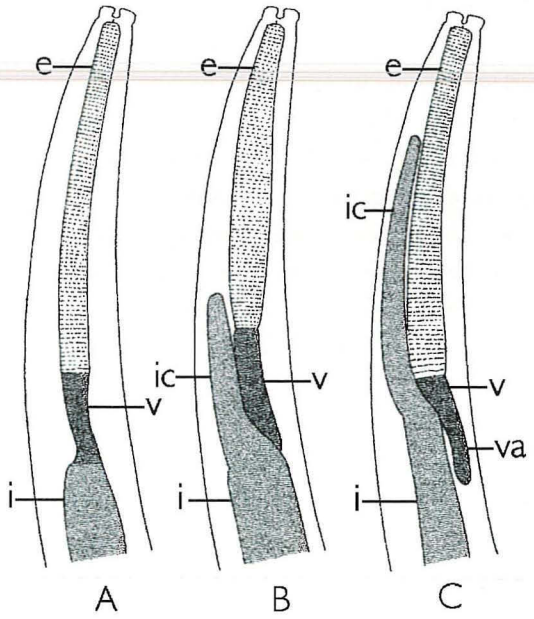


Figure 9-12. Distinguishing characteristics of anisakine larval nematodes. A. *Anisakis*-type. B. *Pseudoterranova*-type. C. *Contracaecum*-type. e = muscular portion of esophagus, i = intestine, ic =intestinal caecum, v = ventriculus, va = ventricular appendix. (Courtesy of A.C. Olson and Am. Soc. Clin. Sci.)

these worms (Fig. 9-10) involves crustaceans as the first intermediate hosts, fish as a second intermediate or transport hosts, and marine mammals as definitive hosts (Nagasawa 1990). Wild caught Pacific salmon are often infected with *Anisakis* larvae (Margolis 1982; Deardorff and Throm 1988), whereas the parasite has not been detected in salmon reared in netpens (Deardorff and Kent 1989; Bristow and Berland 1991a). However, farmed fish could become infected by feeding on infected fish (e.g., Pacific herring) or invertebrates that frequent netpens.

CLINICAL SIGNS AND GROSS PATHOLOGY. *Anisakis* larvae are relatively nonpathogenic to salmon and morbidity has not been associated with the infection. Dissection of infected fish reveals encysted worms in the liver, mesenteries, and flesh (Fig. 9-11).

DIAGNOSIS. Diagnosis is based on the identification of the worm. Presumptive diagnosis is based on the observation of encapsulated, whitish nematodes in the viscera or body musculature. The worms are cylindrical, and usually range in length from 17 to 34 mm. Confirmation of the worm's identity requires microscopical examination of cleared or dissected worms for certain pathognomonic anatomical features (Olson et al. 1983; Berland 1989) (Fig. 9-12). See diagnosis for *Hysterothylacium*.

CONTROL AND TREATMENT. The apparent absence of *Anisakis* larvae in farmed salmon is probably because most of the diet of salmon consists of manufactured feeds. Some fish farmers have used fresh Pacific herring to supplement the diet of their fish. This practice should be avoided because this fish is often heavily infected with the worm, which can be transmitted from prey to predator fish. However, frozen herring or other fish could be used because freezing fish for at least 24 hr at -20ºC will kill the worm.

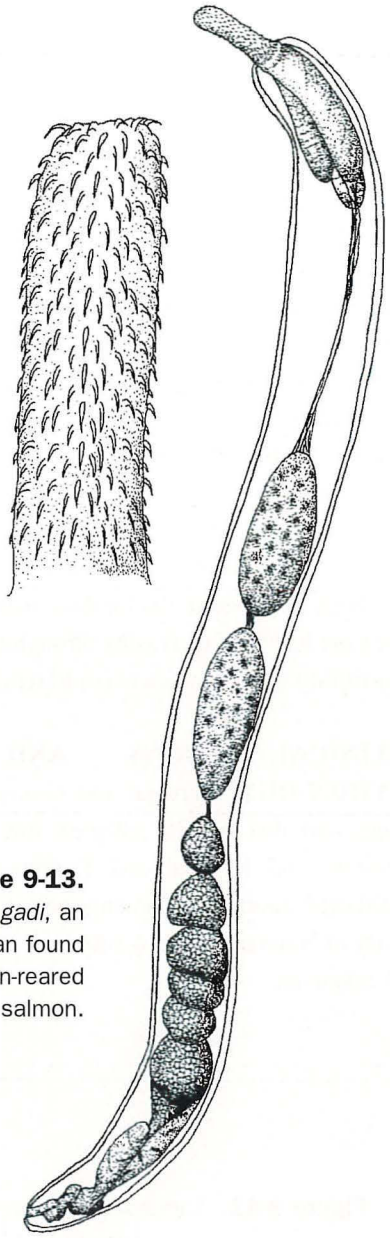


Figure 9-13. *Echinorhynchus gadi*, an acanthocephalan found occasionally in pen-reared salmon.

Acanthocephala

Acanthocephala (spiny-headed worms), as adults, are parasitic in the intestine of vertebrates. Many species occur in fishes. The life cycle involves one intermediate host, an arthropod, and for some species a paratenic or transport host also plays an important role in transmission to the definitive host but is not biologically essential for completion of the life cycle. For species using fish as definitive hosts, the intermediate hosts are small crustaceans.

No species of acanthocephalan has been reported to cause significant disease in seapen-reared salmonids. Two species, *Echinorhynchus gadi* (Fig. 9-13) and *Pomphorhynchus laevis*, have been reported to occur in low numbers in rainbow trout farms off the Baltic coast (Vismanis et al. 1984), and *E. gadi* has been observed in seapen-reared Atlantic salmon on the east coast of Canada (Burt 1994).

MUSSEL LARVAE

Larvae of certain freshwater mussels (glochidia) frequently infect the gills of fishes. Gill infections by larvae of the mussel *Mytilus edulis* have been reported in seawater pen-reared Atlantic salmon in Scotland (Bruno 1987, 1989) and Norway (Flesjaa 1992). In both cases, heavy infections were associated with severe gill damage and mortality.

CLINICAL SIGNS AND GROSS PATHOLOGY. The infection was associated with reduced growth and mortalities as high as 45% (Bruno 1987). Infected fish shake their heads and gasp, whereas others are listless before death. Infected gills exhibit increased mucus production and small white bodies in the secondary filaments.

MICROSCOPY. Wet mounts will reveal the bivalved larvae (Fig. 9-14). Histologically, heavily infected gills exhibit extensive epithelial hyperplasia and fusion of the secondary lamellae associated with the veliger larvae.

DIAGNOSIS. Numerous small, raised, whitish nodules throughout the filaments is suggestive of the infection. Positive diagnosis is achieved by observing post-veliger mussel larvae (about 0.4 mm in diameter with typical bivalve shells) by microscopy.

CONTROL AND TREATMENT. There is no available treatment for this infection. As with other marine parasites, avoiding the infection in netpens would be very difficult. Fortunately, this infection appears to be rare, with only two reported occurrences.

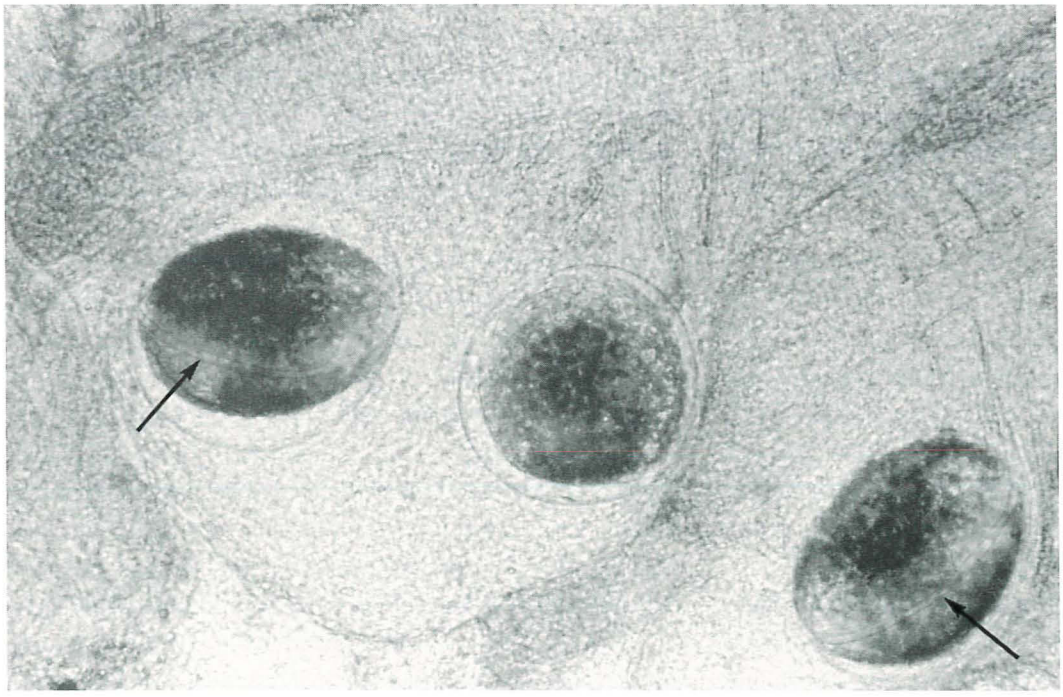


Figure 9-14. Mussel larvae in gills. Arrows = valve opening.

CRUSTACEAN PARASITES

Stewart C. Johnson

Parasitic crustaceans are often important pathogens in aquaculture and severe disease has been associated with caligid copepods (sea lice) in netpen farms. A large section of this chapter is, therefore, devoted to sea lice, with an extensive review on their biology and treatment.

ECTOPARASITIC COPEPODS

Members of three families of parasitic copepods have been reported to cause disease in sea-farmed salmonids. These families are the Pennellidae, the Ergasilidae and the Caligidae. Of these three families, members of the Caligidae (also referred to as sea lice) are the most important with respect to the marine-rearing of salmon.

Family Caligidae (Sea Lice)

The term sea lice is commonly used to refer to several species of marine ectoparasitic copepods of the family Caligidae that infect salmonids. Reported species of sea lice from marine-reared salmon include *Caligus clemensi*, *Caligus curtus*, *Caligus elongatus*, *Caligus orientalis*, *Caligus teres*, *Caligus* sp., *Lepeophtheirus cuneifer*, and *Lepeophtheirus salmonis*. Other species in the family Caligidae are economically important parasites of a wide variety of both wild and marine-reared fishes (reviewed in Costello 1993). Of these species, *Lepeophtheirus salmonis* (also referred to as the salmon louse) and *Caligus elongatus* are responsible for the majority of serious disease outbreaks and high economic losses to salmon farmers throughout the Northern Hemisphere. Other *Caligus* species are responsible for disease outbreaks in stocks of salmon in the Southern Hemisphere.

Lepeophtheirus salmonis is limited in its host range to salmonids, except for very rare cases (Kabata 1979). In comparison, the other species of sea lice have broad host ranges that include both non-salmonid teleost and elasmobranch hosts (Parker et al., 1968; Margolis et al. 1975; Kabata 1974b, 1979, 1988; Bruno and Stone 1990; Urawa and Kato 1991). Many of these non-salmonid hosts are common in the vicinity of netpen sites and serve as a source of parasites for salmon (Bruno and Stone 1990; Shaw and Opitz 1993).

With exception of *C. elongatus*, all species of sea lice found on salmon that have been studied have ten developmental stages (see Piasecki 1996). These stages include two free-living nauplius stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two preadult stages, and one adult stage (Johnson and Albright 1991b; Schram 1993) (Fig. 10-1). Sea lice molt between each of these developmental stages.

In laboratory studies the occasional transfer of preadult and adult *L. salmonis* between salmon has been observed, but transfer between hosts was considered a insignificant route of infection (Bruno and Stone 1990). However, a recent study by Ritchie (1997) suggests that mobile stages of *L. salmonis* do transfer among fish in netpens. Both the preadult and adult stages of *Caligus* species have been reported free swimming in the plankton, and infection of sea-farmed salmonids with preadult and adult stages of *C. elongatus* and *C. clemensi* is known to occur (Wootten et al. 1982; Hogans and Trudeau 1989b; unpublished data for British Columbia). Infection by *Caligus* preadults and adults most commonly occurs when large numbers of infected non-salmonid hosts become resident in the vicinity of net pens.

Sea lice carry their eggs as elongate cylindrical strings that are attached to the genital segment (Fig. 8-2g). The number of eggs produced by sea lice is highly variable depending on the species of sea lice, as well as environmental (seasonal) and host (Hogans and Trudeau 1989b; Tully 1989; Costello 1993; Pike et al. 1993; Ritchie et al. 1993; Tully and Whelan 1993; Hogans 1995). Species of host, host age (maturation state), general condition of the host, and other host factors can also effect the number of eggs carried by sea lice. Sea lice living on older (sexually mature), highly stressed or diseased hosts generally carry more eggs than those on younger and/or non-stressed or healthy hosts. Atlantic salmon immunized with extracts derived from adult *L. salmonis* were shown to have a lower proportion of ovigerous copepods than control fish. Furthermore, those from immunized fish showed a significant reduction (26%) in the average number of eggs carried (Grayson et al. 1995).

Egg production in sea lice is a continuous process, and newly formed egg strings are visible within the genital segment. For both *L. salmonis* and *C. elongatus*, new egg sacs can be extruded within 1 day of hatching. As egg strings near hatching they develop a darker coloration which is due to the development of pigmentation in the nauplii. Low salinities (< 15 ppt.) and low water temperatures (< 3 °C) markedly reduces egg hatching success in *L. salmonis* (Johnson and

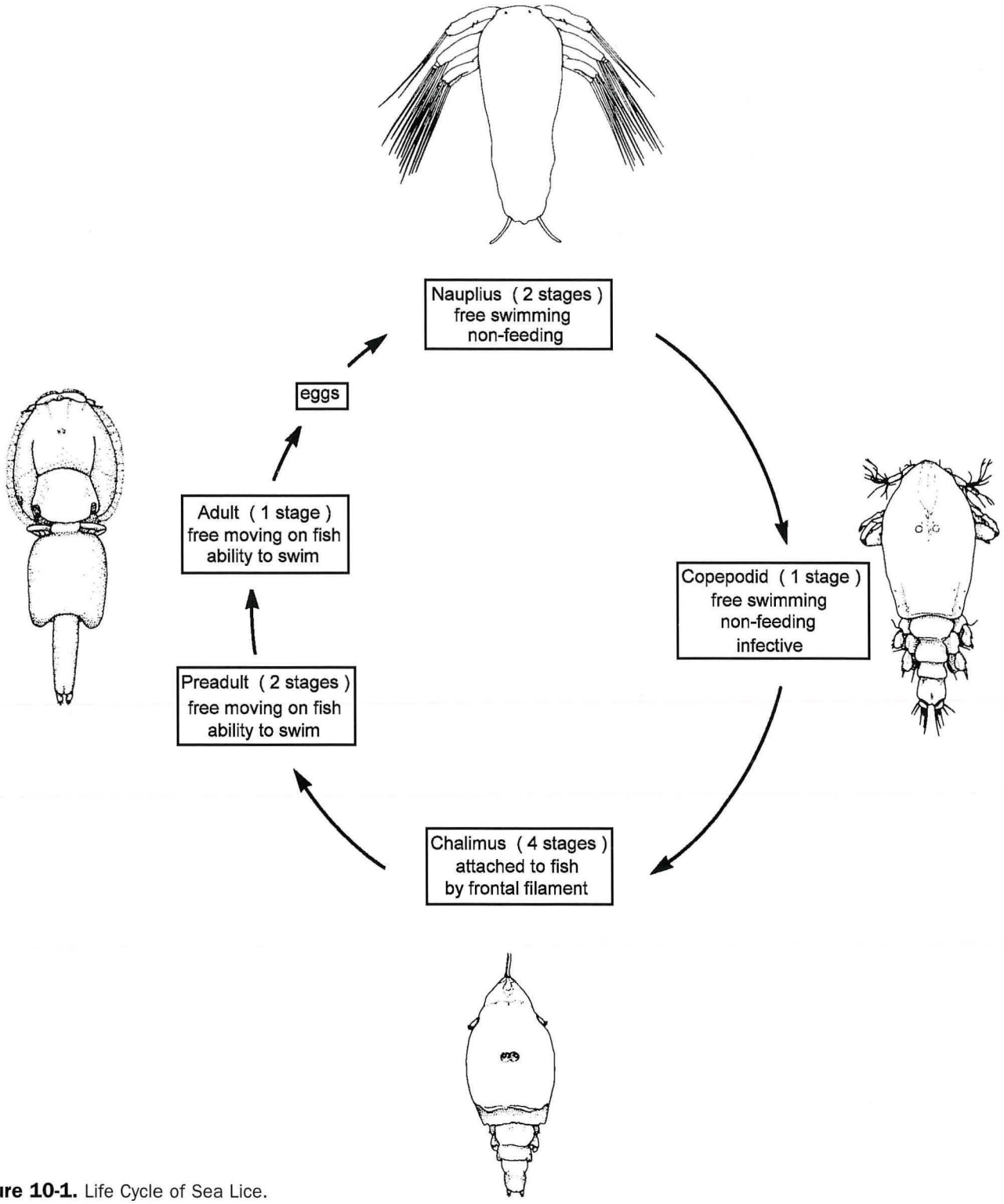


Figure 10-1. Life Cycle of Sea Lice.

Albright 1991c; Hogans 1995). Development of the egg to the first naupliar stage is primarily controlled by water temperature, although other factors such as salinity may have an effect. Egg development times for *L. salmonis* were 17.5, 8.6, and 5.5 days at 5, 10 and 15 ^{o}C , respectively (Johnson and Albright 1991c).

The two naupliar stages are free-swimming in the plankton and serve as dispersal stages. The naupliar stages are non-feeding and not infectious. The duration of this planktonic phase (nauplius I to copepodid) is primarily controlled by temperature, although other environmental parameters such as salinity may also have an effect. In laboratory studies survival

of the planktonic (naupliar and copepodid) stages of sea lice is strongly effected by the culture conditions under which they are maintained (Johnson and Albright 1991c; Pike et al. 1993).

The free-swimming infectious copepodid stages of sea lice are non-feeding up until they attach to a suitable host. Prior to attachment, copepodids rely upon maternally derived lipid droplets for energy and for this reason their survival as free-swimming organisms is limited. At salinities of 15 to 30 ppt and temperatures of 5 to 15 °C average survival times for *L. salmonis* copepodids range between 2 and 8 days (Wootten et al. 1982; Johnson and Albright 1991c).

The infectious copepodid stage of sea lice attaches to the host by means of well developed second antennae. Copepodids are capable of changing position on the host, and can become dislodged from the host (Bron et al. 1991, 1993a; Kabata 1979). After a period of feeding the copepodid stage molts to the first chalimus stage. All chalimus stages are physically attached to the host by means of frontal filaments that serve to anchor them to hard structures such as scales, cartilage and fin rays (Bron et al. 1991; Pike et al. 1993). For this reason chalimus larvae are not removed by most chemical treatments. In naturally and experimentally infected fish, copepodids and chalimus larvae can be found on all the body surfaces but are generally more abundant on the fins (Bron et al. 1991; Johnson and Albright 1992a,b; Tully et al. 1993; Johnson et al. 1996).

With the molt to the first preadult stage the copepods move off the fins on to the other body surfaces. Except during molting, both preadult and adult sea lice are mobile and remain in contact with the host by using the anterior portion of their body as a suction cup. When present in low densities, preadult and adult sea lice are most commonly found in the perianal region and on the dorsal surface behind the dorsal fin.

Development rates for *L. salmonis* on both Atlantic and chinook salmon have been determined from both laboratory studies and field observations. Based on laboratory studies development from copepodid to adult male takes from 234 to 280 degree days on naive Atlantic salmon and 380 degree days on naive chinook salmon (Johnson 1993). Development from copepodid to adult female takes 281 to 400 degree days on Atlantic salmon and about 444 degree days on chinook salmon (Johnson 1993). Based on laboratory data, *C. elongatus* developed from copepodid to adult males and adult females in 247 degree days (Piasecki and MacKinnon 1995). Generation times for *C. elongatus* and *L. salmonis* have been estimated from field collected data (Tully 1989). The generation time of *C. elongatus* was approximately 81, 66 and 50 days when the average seawater temperature was 8.0, 13.0 and 16.0 °C, respectively. The generation time of *L. salmonis* was approximately 93, 76, 56 and 48 days when the average seawater temperature was 7.0, 8.0, 12.0 and 15.5 °C, respectively.

Species of salmon are known to differ in the susceptibility to infection with *L. salmonis*. Experimental infections with *L. salmonis* of Atlantic, chinook and coho salmon showed that Atlantic salmon were the most susceptible to infections, while coho salmon were the most resistant species (Johnson and Albright 1992a). In Canada, Atlantic salmon and rainbow trout are generally more heavily infected with sea lice than chinook or coho salmon when raised at the same site (unpublished data). Based on laboratory studies, differences in susceptibility between species is related to the magnitude of their innate host responses to *L. salmonis*. In coho salmon these responses include well developed epithelial hyperplasia and inflammatory responses, while in Atlantic salmon only minor tissue responses occur (Jones et al. 1990; Johnson and Albright 1992a). Examination of the attachment and feeding sites of *C. elongatus* chalimus larvae on the body of Atlantic salmon revealed only minor tissue responses (MacKinnon 1993).

Administration of the stress hormone cortisol increases the susceptibility of coho and chinook salmon to infection with *L. salmonis* by reducing the magnitude of their tissue responses (Johnson and Albright 1992b; unpublished data). Depressed immune function due to other acute or chronic disease states or the process of sexual maturation can result in increased susceptibility to sea lice. In British Columbia, higher numbers of sea lice with higher numbers of eggs per female were found on sexually mature verses immature coho salmon that were raised at the same site (unpublished data).

Aspects of the Behavior of Sea Lice

Important to our understanding of the dynamics of sea lice epizootics is a knowledge of their distribution and behavior in the field. This is especially true for the naupliar and infectious copepodid stages. At present our knowledge is limited to studies of the distribution and behavior of *L. salmonis*. Cues such as light, chemical, pressure, and water flow have been suggested to be important factors controlling the distribution and behavior of these stages, including host location by copepodids (reviewed in Bron et al. 1993a). In the laboratory, both the naupliar and copepodid stages of sea lice have been demonstrated to have a strong positive phototaxis to direct light sources suggesting that they may be found in surface water during the day (Johannessen 1978; Wootten et al. 1982; Bron et al. 1993a). However, caution should be exercised when using this information to infer distribution in the field, as it is well

recognized that for many zooplankton species that positive phototaxis to direct light sources in the laboratory is artifactual (see Forward 1988).

The naupliar stages showed only small differences in depth distribution between the night and day, whereas copepodids were seen to display a distinct diurnal vertical migration, gathering near the surface during the day, and spreading out into deeper layers at night (Heuch et al. 1995). With respect to pen-reared salmon, these authors suggest that salmon become infected during daytime when they come to the surface to feed. Huse and Holm (1993) reported that infestation of Atlantic salmon by *L. salmonis* was significantly higher with fish maintained in a 6 m deep netpen when compared to a 20 m deep netpen, suggesting higher rates of infection occur in surface waters.

Any factor that decreases water movement through sea cages may result in the retention of high numbers of nauplii and copepodids, both which are relatively weak swimmers within the sea cage. Costelloe et al. (1996) investigated the dispersion of *L. salmonis* larvae (nauplii and copepodids) from sea cages. The highest levels of larvae were consistently found within the sea cages, and relatively few larvae were recovered from waters surrounding the sea cages. Reduced water movement through the sea cages, due to net fouling, was responsible for the high retention of larvae within the cages.

Sea lice may also function as vectors of viral and bacterial diseases of fish (Nylund et al. 1991, 1993). It has been demonstrated that the sea lice species *L. salmonis* can function as a vector for the agent of infectious salmon anaemia (ISA), especially during the epidemic and endemic phases (Nylund et al. 1993, 1994). Although not implicated in the transfer of *Aeromonas salmonicida*, the causative agent of furunculosis, this bacterium has been isolated from the surface of *L. salmonis* (Nese and Enger 1993).

CLINICAL SIGNS AND GROSS PATHOLOGY. The attached copepodids, chalimus, preadult and adult stages of sea lice feed on mucus, skin, and blood (Kabata 1970; Brandal et al. 1976; Bron et al. 1993b). Disease is caused by the feeding activities of the sea lice. Due to their small size, damage by copepodid and chalimus larvae is generally limited to a small area around their point of attachment, where they erode the epidermis and sub-epidermis (Bron et al. 1991; Johnson and Albright 1992a,b). However, heavy infections of *C. clemensi* on wild pink salmon and *L. salmonis* on wild sea trout have been reported to cause serious fin damage – e.g., complete removal of the fins (Parker and Margolis 1964; Tully et al. 1993).

Because the preadult and adult parasites are larger and capable of moving on the surface of the fish, damage by these stages is more severe and widespread, with heavily infected salmon commonly show gray patches (extensive areas of skin erosion and hemorrhaging) on the head and back, and a distinct area of erosion, dark coloration, and sub-epidermal hemorrhages in the perianal region (Wootten et al. 1982). In seriously diseased salmonids, open lesions, in which the epidermis is breached and the underlying tissues exposed, commonly occur on the head and/or behind the dorsal fin

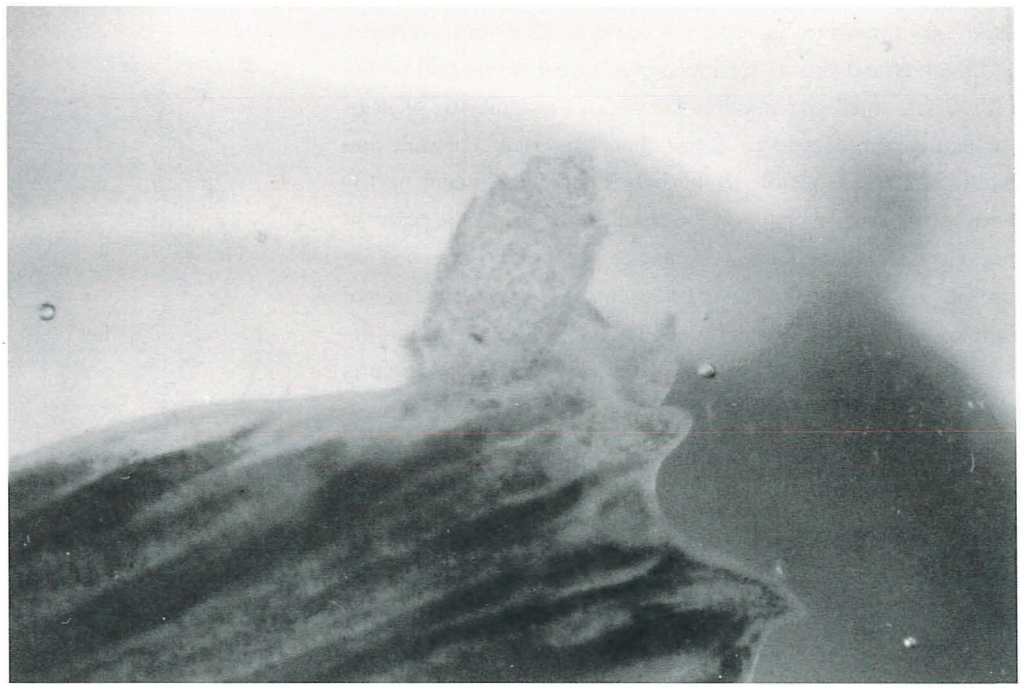


Figure 10-2. II and IV stage larvae of *Lepeophtheirus salmonis* attached to a fin.

(Jónsdóttir et al. 1992; Johnson et al. 1996). Heavy infections reduce the market value of the fish and ultimately result in death. Death may occur due to the development of secondary diseases (e.g., vibriosis, furunculosis) exacerbated by the high levels of accompanying stress, or in severe cases where the epidermis is breached, death may be due to a loss of physiological homeostasis including osmotic stress, anemia, and hypoprotenemia (Wootten et al. 1982; Tully et al. 1993; Nylund et al. 1993; Grimnes and Jakobsen 1996). The relationship of the number of sea lice to severity of the disease is dependent on 1) size and age of the fish, 2) the general state of health of the fish, and 3) the species and developmental stages of the sea lice present.

MICROSCOPY. Examination of the fins, gills and skin under a dissecting microscope will demonstrate the developmental stages of the parasite (Fig. 10-2). Histological examination of tissue sites of copepod feeding reveals that the epidermis of infected Atlantic salmon is eroded, and the dermis exhibits hemorrhagic areas (Wootten et al. 1982; Bron et al. 1991; MacKinnon 1993). Fins of Atlantic and chinook salmon infected with chalimus larvae of *L. salmonis* become eroded. In contrast, the skin and fins of coho salmon infected with the chalimus larvae of *L. salmonis* show pronounced epithelial hyperplasia and an inflammatory response (Johnson and Albright 1992a,b).

DIAGNOSIS. Diagnosis is confirmed by observing the copepods and determining their specific identity through microscopic examination. Copepodids and chalimus larvae are small (< 4 mm in length) and can occur on all exterior surfaces of the body and fins as well as in the buccal cavity and on the gills. Their small size requires the use of a magnifying glass or dissecting microscope to detect their presence. Preadult and adult sea lice are visible to the naked eye. They occur on the body surfaces, especially on the head, back, and in the perianal region. Preadult and adult stages of *Caligus* species can be distinguished from *Lepeophtheirus* species by the presence of lunules on their anterior margin (Fig. 10-3). A key to aid in the identification of adult sea lice of the Northern Hemisphere is given in Johnson and Margolis (1994).

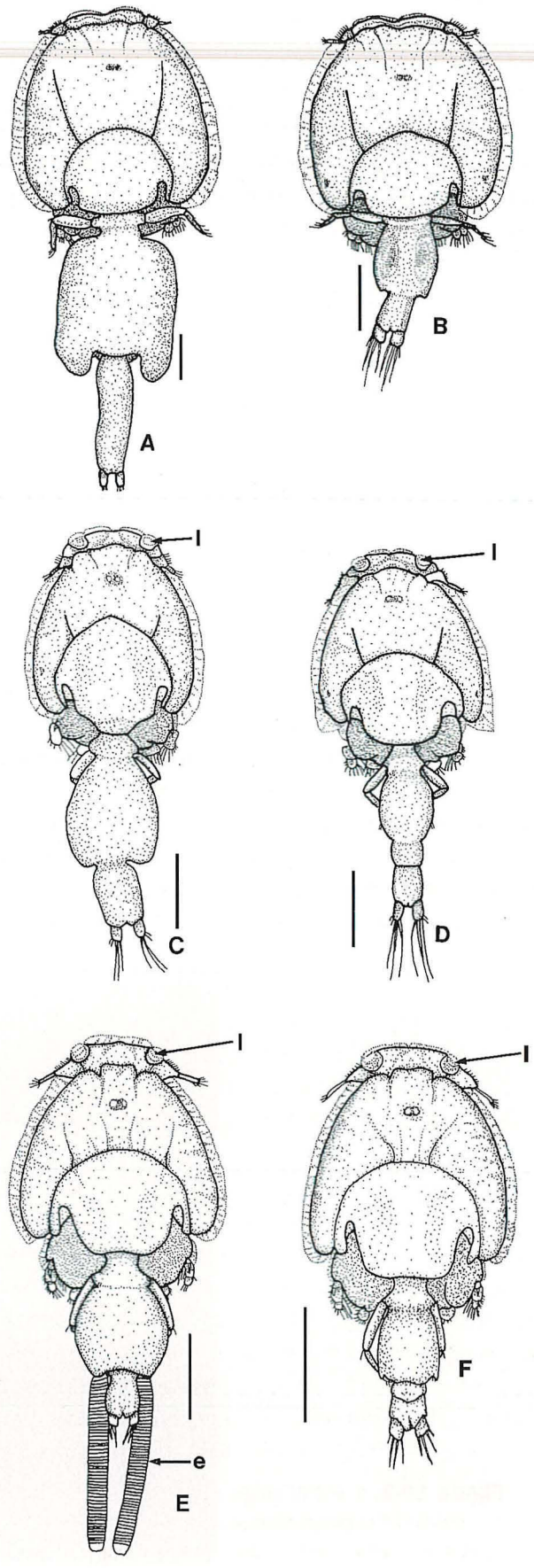


Figure 10-3. Adult stages of sea lice. *Lepeophtheirus salmonis*, female (A), male (B). *Caligus elongatus*, female (C), male (D). *Caligus teres*, female (E), male (F) (A, B, C, D, redrawn from Kabata 1979; E, F, redrawn from Wilson 1905). e = egg sac; l = lunule.

It is possible to identify the copepodid and chalimus stages but their identification is difficult and requires a detailed knowledge of copepod structure. The developmental stages of *C. clemensi*, *C. elongatus* and *L. salmonis* can be identified by reference to Kabata (1972), Piasecki (1996) and Johnson and Albright (1991b), respectively. Supplementary descriptions of the developmental stages of *L. salmonis* are given in Schram (1993). The copepodid and chalimus stages of *C. clemensi* and *L. salmonis* can be identified by reference to Kabata (1972), Johnson and Albright (1991b) and Schram (1993). The earlier developmental stages of the other sea lice species have not been described.

CONTROL AND TREATMENT. Sea lice control is best carried out by the implementation of an integrated pest management scheme. Such approaches utilize information on the biology and behavior of sea lice and the efficacy of various treatments to develop management plans that reduce the frequency of treatments and amount of drugs needed to control the infestations.

Proper site selection and husbandry practices can reduce the incidence of sea lice disease. For example, farm sites should be situated in areas where water current patterns are such that the infectious copepodid stages are not retained on site. Areas with abundant wild hosts of sea lice should also be avoided. Fish reared in sites with poor water quality may be placed under stress resulting in higher incidences of disease, including sea lice disease. The use of single year class sites, and the fallowing of farm sites between restocking can also significantly reduce the need for treatments for *L. salmonis* (Bron et al. 1993c; Grant and Treasurer 1993). This approach is not as effective against *C. elongatus* due to its broad host range and ability of the preadult and adult stages to move from fish to fish (Bron et al. 1993c). In cases where farms belonging to different companies are in close proximity, cooperative agreements between companies with respect to single year class stocking, periods of fallowing, and timing of sea lice treatments have proven useful in reducing the severity of sea lice outbreaks (Grant and Treasurer 1993).

A great deal of effort has gone into the development of treatments for sea lice. Bath or dip treatments with dichlorvos, trichlorfon, azamethiphos, cypermethrin, carbaryl, pyrethroids and hydrogen peroxide have been used to control sea lice infections on farmed salmonids (Brandal and Egidius 1979: Costello 1993; Johnson et al. 1993; Roth et al. 1993a,b; Thomassen 1993a,b). Excellent reviews on the chemotherapeutic control of sea lice are given in Roth et al. (1993a) and Costello (1993). It is important to remember that there can be marked differences between salmon species in their ability to tolerate sea lice treatments (see Johnson and Margolis 1993; Johnson et al. 1993)

The organophosphorus insecticides, dichlorvos, marketed as 'Nuvan 500EC' or 'Aquaguard SLT', or in its related trichlorphon form as 'Neguvon', were the first chemicals widely used to control sea lice (Brandal and Egidius 1977; Grave et al. 1991a,b). Brandal and Egidius (1977) reported the first use of trichlorphon for the treatment of salmonids infected with sea lice. In their study, trichlorphon, which was administered orally, resulted in a decline in the number of sea lice present and a high level of mortality in the treated fish. Dichlorvos and trichlorphon have been used since the 1960's as a bath treatment for parasites in pond fish culture (reviewed in Schmahl et al. 1989). These compounds were administered to salmonids as a bath treatment following the methods described by Brandal and Egidius (1979). These treatments effectively remove both the preadult and adult stages of sea lice but not the chalimus larvae. Therefore, successive treatments usually at two to four week intervals are required to control infections (Wootten et al. 1982). It was thought that by early 1990 resistance of *L. salmonis* to dichlorvos was being seen at some farm sites (Jones et al. 1992).

The organophosphate insecticide azamethiphos (marketed as Salmosan) is presently used in Europe and Canada for sea lice control. This chemical is administered to salmon as a bath treatment, and like the other organophosphates shows little efficacy against the attached chalimus stages (Roth et al. 1996) This chemical is more efficacious against *L. salmonis*, has a wider therapeutic margin and appears to be more tolerated by Atlantic salmon than the other organophosphates (Hodneland et al. 1993; Roth et al. 1996).

Although organophosphates are recognized as toxic to a wide variety of marine organisms, they are released into the surrounding waters after treatment is completed, where it is argued that their impact on non-target species is minimal due to the rate of dilution and their rapid breakdown (Egidius and Moster 1987; Cusack and Johnson 1990; Dobson and Tack 1991). This practice has resulted in conflict between environmental groups and salmon farmers over the use of these chemicals, and has led Scotland and Ireland to consider banning their use.

Pyrethrin and pyrethroid compounds are currently being used for sea lice control (Boxaspen and Holm 1991 a,b; Roth et al. 1993). The synthetic pyrethroid cypermethrin is believed to be more efficacious than azamethiphos for the control of *L. salmonis* on Atlantic salmon. Clinical field trials are ongoing in Northeastern United States using cypermethrin, which is widely used in Norway.

Bath treatments with hydrogen peroxide have been used to control sea lice in Norway, Faeroe Islands, United Kingdom and North America (Bruno 1992; Thomassen 1993a,b; Bruno and Raynard 1994). Thomassen (1993 a,b) reported that bath treatments of hydrogen peroxide at a concentration 1.5 g/l for 20 minutes effectively removes from 85 to 100% of the preadult and adult stages of sea lice without toxicity to Atlantic salmon, but had no significant effect on the intensity of infection with the attached chalimus stages. In addition, high proportion of the preadult and adult stages removed from the fish recovered after treatment (Johnson et al. 1993; Bruno and Raynard 1994). With respect to *L. salmonis*, these stages are unlikely to reinfect the treated hosts. Preadult and adults of species of *Caligus* are generally more active swimmers and reinfection is possible if they recover. The use of hydrogen peroxide is rather impractical and its use has been essentially abandoned in Norway.

The toxicity of hydrogen peroxide to fish increases with increasing water temperatures, concentrations, and exposure times (Johnson et al. 1993; Roth et al. 1993a; Bruno and Raynard 1994). Atlantic salmon are less sensitive to hydrogen peroxide than chinook salmon (Johnson et al. 1993). Histological sections of gills from fish that have experienced acute toxicity to hydrogen peroxide show extensive epithelial lifting and necrosis (Johnson et al. 1993; Thomassen 1993a,b).

Bath treatments cause high levels of stress that can result in the development of secondary diseases (e.g., vibriosis and furunculosis) following treatment. In addition, production levels of treated fish are compromised due to lower growth and feed conversion rates. To avoid physical damage during treatments, associated high levels of stress, and the high costs of bath treatments, a great deal of effort has gone into developing oral treatments for sea lice.

Palmer et al. (1987) reported the results of preliminary studies on the efficacy of oral doses of ivermectin for the control of sea lice on Atlantic salmon. Although this drug was found to be effective in reducing populations of sea lice, the drug had a narrow margin of safety. Ivermectin has been demonstrated to be effective in controlling all developmental stages of sea lice (Smith et al. 1993; Johnson and Margolis 1993). Our studies showed that ivermectin can be very toxic to Atlantic salmon and that the level of toxicity varied between salmon species. Atlantic salmon fed 0.05 mg/kg on alternate days became anorexic after 20 days, and ivermectin was lethal to fish fed at higher doses (Johnson et al. 1993). Fish suffering from ivermectin toxicity are listless, show ataxia, and then die in a few days. Due to long tissue withdrawal times and concerns about the impact of ivermectin residues in the sediments beneath the netpens, this drug is unlikely ever to be licensed or registered for use in aquaculture (Burridge and Haya 1993; Costello 1993). At present, however, ivermectin can be prescribed in some jurisdictions for treatment of sea lice, especially on smolts.

The oral administration of insect growth regulators to control sea lice was first described by Hoy and Hornberg (1991 cited in Roth et al. 1993a). In their study oral administration of diflubenzuron, a chemical that inhibits chitin synthesis, resulted in significant reductions in both adult and larval stages of sea lice. Oral treatments with the insect growth regulators Lepsidon (containing diflubenzuron) and Ektobann (containing teflubenzuron) are currently under development in Norway. Treatments using these chemicals are being conducted on a limited scale in Norway and the Faeroe Islands under special permit. These compounds when administered orally are highly efficacious against the copepodid, chalimus and preadult stages. However, they have no efficacy against the adult stages which no longer molt. A portion of these compounds when fed may be deposited to the sediments in the feces, with a maximum uptake by the fish of 30%. Associated with this deposition is the potential for a negative impact on benthic crustaceans, including commercially important species. This problem will have to be addressed before these compounds can undergo licensing and registration for use. Licensing of these compounds in some areas, such as the United States, may be very difficult due to laws which limit the use of diflubenzuron within 5 km of the coast (Roth et al. 1993a).

The effectiveness of alternative treatments such as hanging bags of sliced onions in sea pens and the addition of garlic to salmon feeds is at best questionable (see Costello 1993).

Biological control of sea lice using cleaner-fishes (wrasses) is widely used in Norway, Shetland, Scotland and Ireland (reviewed in Costello 1993; Treasurer 1993; Kvenseth 1993; Tully et al. 1996). The use of wrasses to control *L. salmonis* on Atlantic salmon was first proposed by Bjordal (1988, 1990). In both laboratory and field studies wrasses removed sea lice from salmonids, but not always in a predictable manner. A survey of fish farmers in Scotland that have tried wrasses to control sea lice showed that the majority felt that this sea lice control measure was beneficial, particularly when used in conjunction with Dichlorvos treatments (Anonymous 1991). However, problems encountered using wrasses to control sea lice included the requirement of smaller mesh size in nets to prevent their escape, some wrasses were intimidated by larger salmon and tended not to clean them, while other wrasses became aggressive and inflicted scale and eye damage to the salmon, and some farmers feared that the wrasses ate salmon

feed and would abandon their cleaning behavior (Anonymous 1991). Other problems with the use of wrasse is their high over-winter mortalities, limited availability, and high purchase costs. The efficacy is limited to early in the season (when lice are active). Nevertheless, wrasses are used in over half of the fish farms in Norway. A bonus is that they also reduce the fouling of the cages.

There are no vaccines available for the control of sea lice. Ongoing research has demonstrated that Atlantic salmon can produce antibodies when injected with crude extracts of *L. salmonis* (Grayson et al. 1991). However, salmon naturally infected with *L. salmonis* and *C. elongatus* fail to produce an antibody response (Grayson et al. 1991; MacKinnon 1991). There was no difference in the number of copepods carried on control and Atlantic salmon that had been immunized with extracts derived from adult *L. salmonis* when challenged under laboratory conditions (Grayson et al. 1995). However, there were fewer copepods with eggs and a significant reduction in the average number of eggs carried by those copepods on the immunized fish. These results support the view that development of a vaccine for sea lice is possible.

Family Ergasilidae

In the family Ergasilidae only one species, *Ergasilus labracis,* has been reported to cause disease in marine-reared salmon (Fig. 10-4). High levels of mortality in Atlantic salmon parr reared in brackish water have been attributed to this species (O'Halloran et al. 1992). Other species in the family Ergasilidae have been reported to be economically important parasites of wild and marine-reared non-salmonid fishes (see Paperna 1975).

DIAGNOSIS. In this family only the adult female copepods are parasitic. Adult males and juvenile copepods of both sexes are free-living. Females attach to the host using modified second antennae that terminates in a strong claw. Diagnosis is confirmed by detecting the presence of copepods, and determining their specific identity through microscopic examination. As adult females are small (approximately 1 mm in length), the use of a hand lens or dissecting microscope may be required to confirm their presence. *Ergasilus labracis* can be easily distinguished from the other parasitic crustaceans of salmonids by its body shape and structure of its second antenna. Keys to aid in the identification of *Ergasilus* species are given in Kabata (1979; 1988).

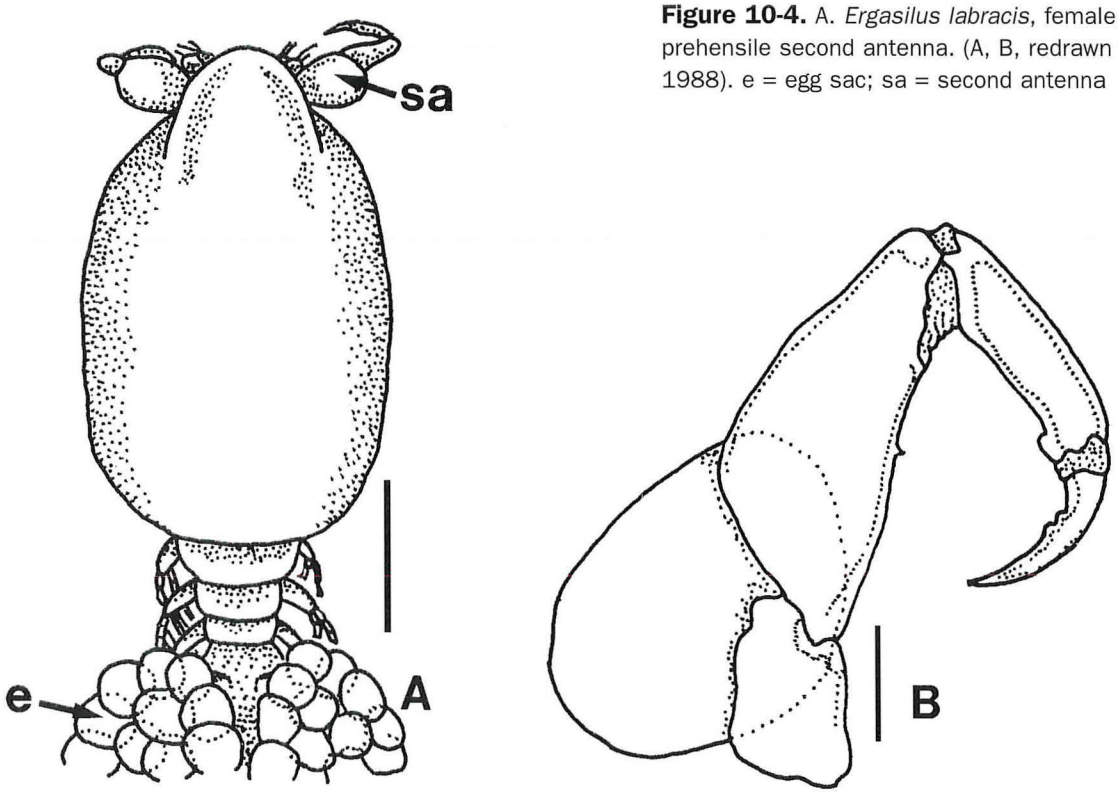


Figure 10-4. A. *Ergasilus labracis*, female, dorsal; B. Same, prehensile second antenna. (A, B, redrawn from Kabata 1988). e = egg sac; sa = second antenna

CONTROL AND TREATMENT. O'Halloran et al. (1992) reported that a single dose of ivermectin fed at 0.2 mg/kg fish resulted in a 98-99% reduction in the intensity of *E. labracis* on Atlantic salmon parr, but also caused some mortality in the fish. Feeding at a dose rate of 0.05 mg/ kg fish twice weekly gave a similar result without an increase in the mortality rate. Although not yet tested, some of the other compounds used to control sea lice may work on this species.

Family Pennellidae

One member of the family Pennellidae, *Haemobaphes disphaerocephalus*, has been reported in pen-reared Atlantic salmon (Kent et al. 1997b). This parasitic copepod normally infects eulachon (family Osmeridae), and this was the first report of a *Haemobaphes* species infecting salmon. The parasite penetrates the branchial vasculature, and causes anemia. Fortunately, the infection has been observed in only a few Atlantic salmon reared in British Columbia.

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish are anemic and may be lethargic. Examination of the opercular cavity reveals the coiled egg sacs and blood engorged body of the parasite (Fig. 10-5). The long neck and anterior hold fast are internal within the gill arch (Fig. 10-5).

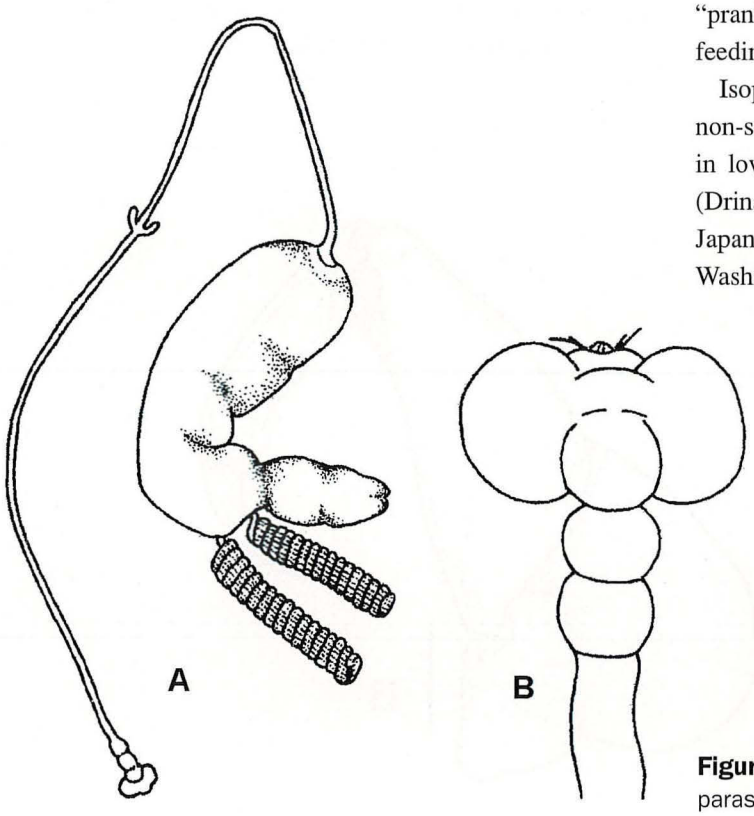


Figure. 10-5. *Haemobaphes disphaerocephalus*. a. entire parasite. b. close-up of anterior holdfast.

DIAGNOSIS. *Haemobaphes* is characterized by attachment at the gill arch and coiled egg sacs. Specific identification requires examination of the anterior holdfast (see Kabata 1988), which must be very carefully dissected from tissues.

CONTROL AND TREATMENT. There is no suitable drug available for treating this infection. As the infective larvae are free-swimming, it would be difficult to prevent the infection in netpens.

ECTOPARASITIC ISOPODS

Four species of isopods, *Ceratothoa gaudichaudii*, *Rocinela maculata*, *R. belliceps pugettensis*, and *Gnathia* sp. have been reported from seawater-reared salmonids (Novotny and Mahnken 1971; Awakura 1980 1983; Drinan and Rodger 1990; Inostroza et al.1993). These species belong to the two suborders Flabellifera and Gnathiformes. Members of the suborder Flabellifera, which includes the species *C. gaudichaudii* and *R. maculata* might be best thought of as micropredators or scavengers. All members of this suborder have a typical isopod shape (Fig. 10-7) and follow a typical isopod life cycle. Members of the family Gnathiformes (e.g., *Gnathia* sp.) have a modified body shape with the larval, adult female and adult males differing considerably in their morphology (Fig. 10-6). Only the larval stages (referred to as "praniza") are parasitic, and feed on host blood. Adults are non-feeding and live in tubes in the sediments.

Isopods have been reported to cause serious disease in both non-salmonid and salmonid fishes. Isopods have been reported in low numbers from pen-reared Atlantic salmon in Ireland (Drinan and Rodger 1990), and pen reared coho salmon in Japan (Awakura 1980, 1983), Chile (Inostroza et al. 1993), and Washington State, USA (Novotny and Mahnken 1971). In Chile, *C. gaudichaudii* has been reported from a wide variety of native hosts. This low host specificity has allowed this parasite to successfully infect the coho and Atlantic salmon, causing disease at certain netpen sites (Inostroza et al. 1993). Novotny and Mahnken (1971) report that feeding experiments using natural plankton as a food source for pink salmon (*Oncorhynchus gorbuscha*) resulted in their exposure to *Rocinela belliceps pugettensis*. Isopods were seen to quickly attach to the fish and cause death within a few minutes of settling.

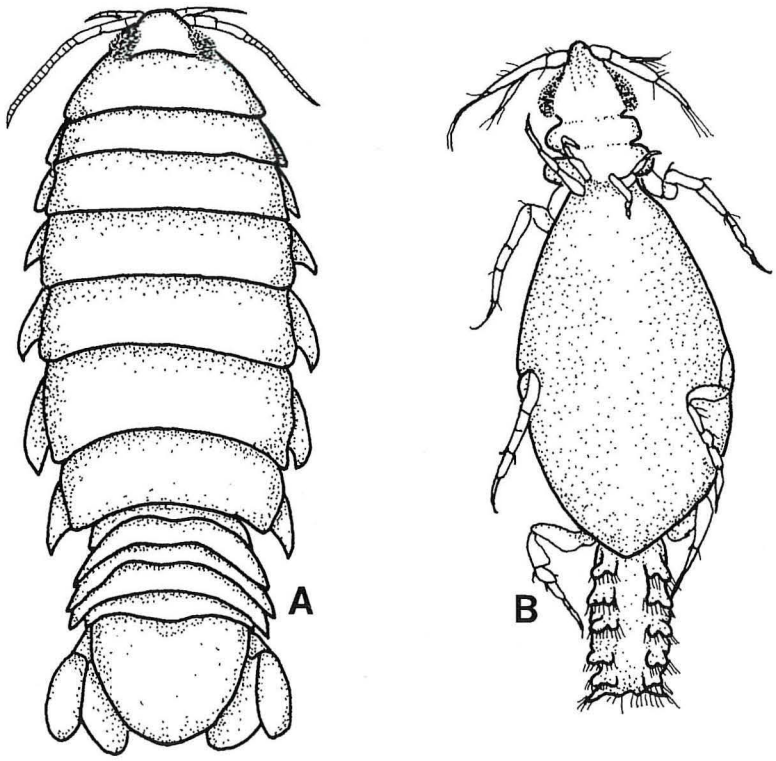


Figure 10-6. General structure of Flabellifera and Gnathiform isopods. A. *Rocinela* sp., dorsal; B. *Gnathia* sp., ventral. (A, redrawn from Kabata 1988; B, redrawn from Kabata 1970).

CLINICAL SIGNS AND GROSS PATHOLOGY. *Ceratothoa gaudichaudii* feeds on host blood, attaching to the inner mouth surfaces and less frequently to the gills (Sievers et al. 1995). Disease is caused by their attachment and feeding activities. Damage to the host includes severe erosion of gill lamellae and ulcers on the gill arch and inside the mouth.

DIAGNOSIS. Adult Flabellifera of the species (e.g., *Ceratothoa*) which have been reported from marine-reared salmonids are large (1 - 4 cm) and are easy to see with the naked eye. They have been reported from the mouth, the gills, and the body of salmonids (Fig. 10-7). The small size of praniza larvae (up to 4 mm) requires the use of a hand lens or dissecting microscope to detect their presence (Fig. 10-6).

CONTROL AND TREATMENT. Sievers et al. (1995) evaluated the efficacy of eight commercial insecticides against *C. gaudichaudii* on Atlantic salmon. Sixty minute bath treatments with the organophosphates, trichlorfon (Neguvon) and dichlorvos (Nuvan 1000) at concentrations of 300 and 3 ppm, respectively were found to be 100 percent effective against this parasite without toxicity to the fish.

Atlantic salmon infected with three to six praniza per fish were found to have no isopods after a lice treatment with an organophosphate (Drinan and Rodger 1990). Praniza infection of eels in tanks was successfully controlled by switching the tanks from salt to freshwater (Mugridge and Stallybrass 1983).

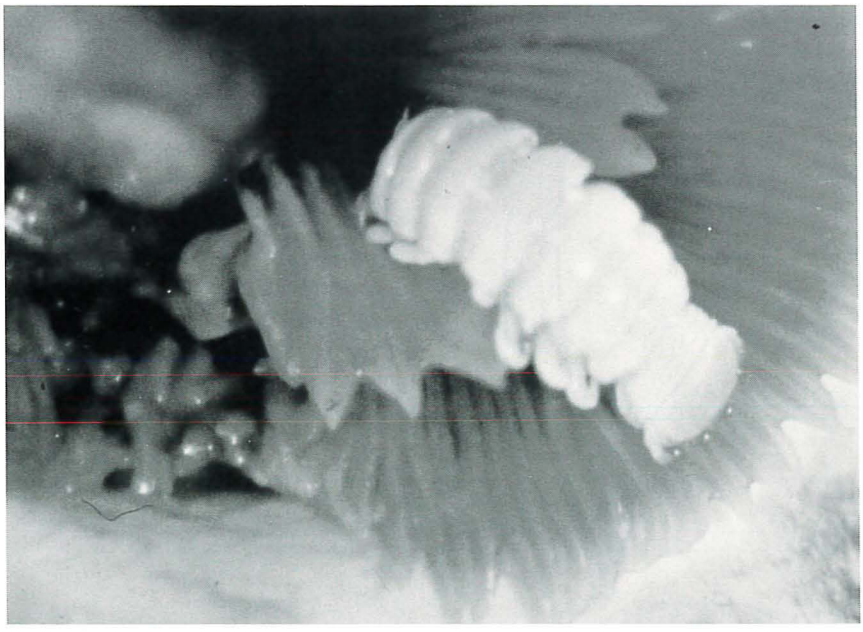


Figure 10-7. *Ceratothoa gaudichaudii* isopod on the gills of Atlantic salmon (Courtesy of R. Inostroza).

BRANCHIURANS

The parasitic crustaceans of the order Branchiura (commonly called fish lice) are economically important parasites of salmonids and other fish species cultured in freshwater (Fig. 10-8). Although present in the brackish and marine waters, their occurrence on salmonids has only rarely been reported. Several specimens of an unidentified species of *Argulus* have been collected from marine reared chinook salmon in Washington State, U.S.A. (Novotny and Mahnken 1971). In British Columbia we occasionally find *Argulus pugettensis* on pen-reared coho salmon but have not seen any disease associated with their presence (unpublished observation). Stuart (1990) reported the presence of *Argulus* sp. on marine reared salmonids in Nova Scotia, Canada, but does not report any disease.

Branchiurans, although parasitic, can spend considerable time off their hosts and are strong swimmers easily capable of transferring between hosts. Female branchiurans mate while swimming and deposit their fertilized eggs on underwater structures. They develop through several planktonic and/or benthic larval stages before infecting hosts. Branchiurans feed on host body fluids and tissues. Digestive secretions from the mouth and a specialized feeding apparatus called the "preoral stylet" which pierces the skin, prepare host tissues for maceration and ingestion.

Morphological features characteristic of branchiurans include: an oval shaped dorsal shield that covers most of the appendages, an abdomen with a Y-shaped posterior margin, the preoral stylet, and cup-like suckers (modified first maxillae) which are used to maintain position on the host (Fig. 10-8).

DIAGNOSIS. Adult branchiurans reported from marine-reared salmonids range in size up to 1.8 cm and are generally easy to see with the naked eye or a hand lens. They are generally found on the external body surfaces of salmonids.

CONTROL AND TREATMENT. There are no reported treatments for branchiurans on marine reared salmonids. Branchiuran infections of freshwater fishes have been treated with a wide variety of chemicals, including organophosphates (Schmahl et al. 1989).

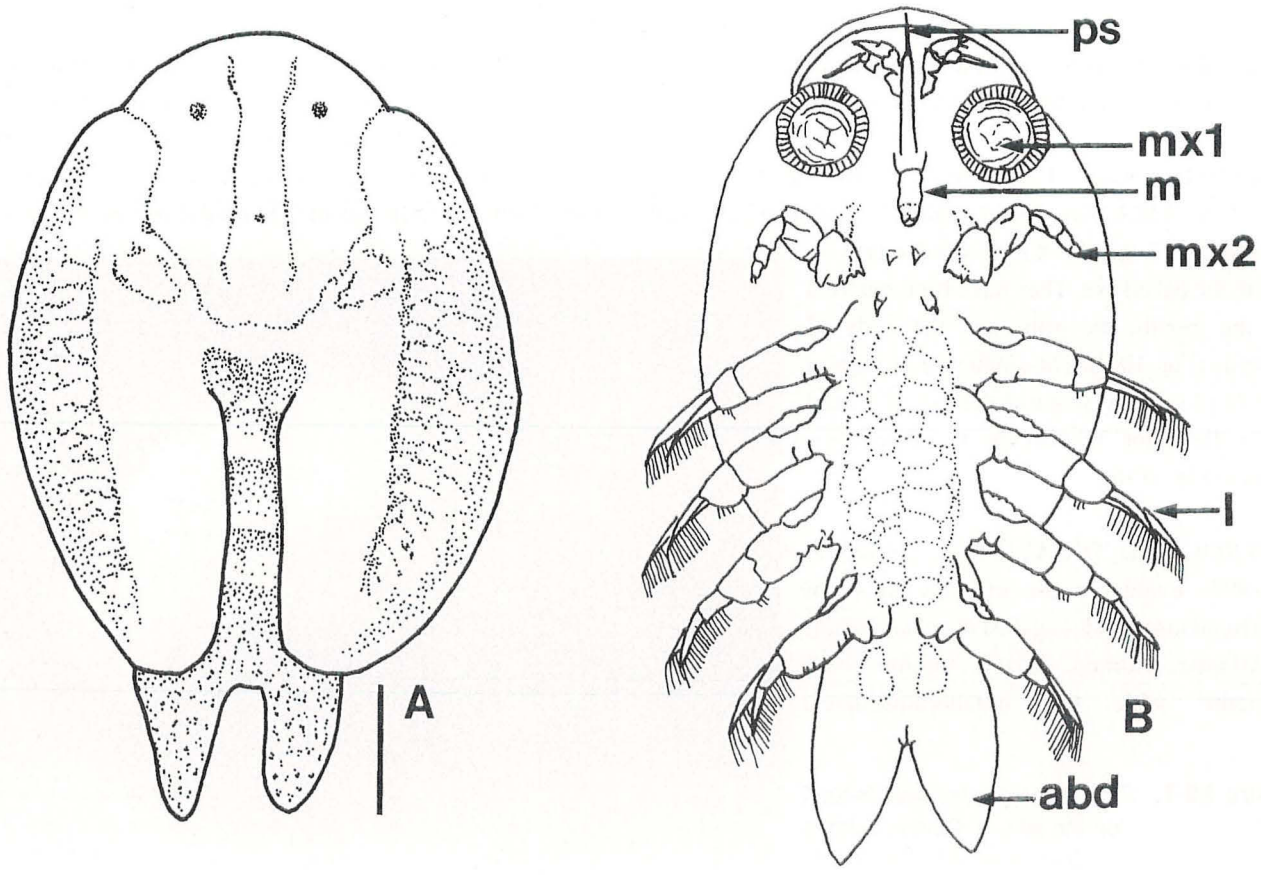


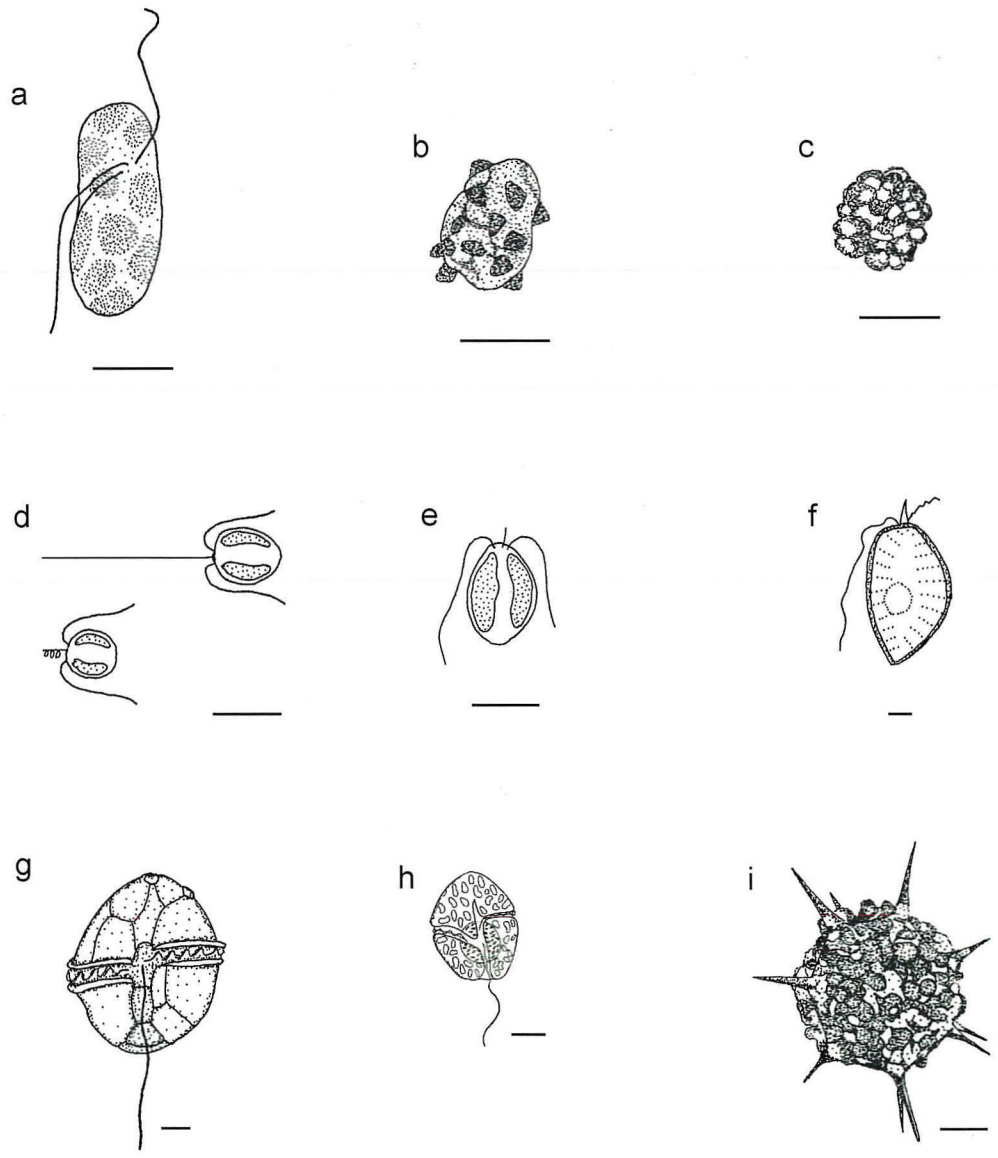
Figure 10-8. General structure of *Argulus* spp. A. *Argulus pugettensis*, female, dorsal; B. *Argulus alosae*, ventral. (A, redrawn from Kabata 1988; B, redrawn from Cressey 1978).

HARMFUL ALGAL BLOOMS

M.L. Kent and J.N.C. Whyte

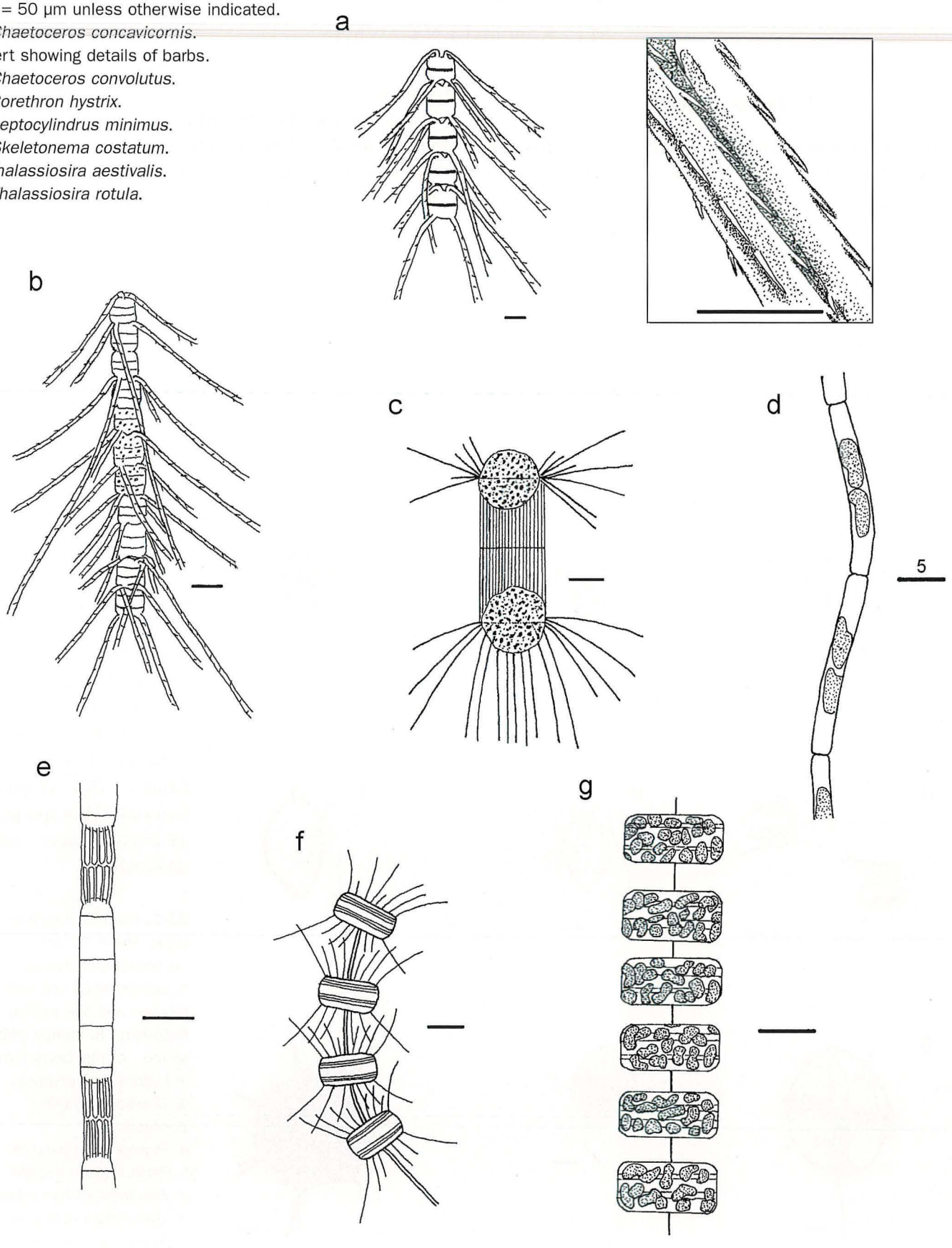
Introduction

The term harmful algal blooms (HABs) is now referred to 'red tides' or 'brown tides' commonly used for describing dense visible patches of pigmented microalgae, that have grown fast or 'bloomed' to color the surface water. Not all harmful phytoplankton species are highly colored, yet produce potent phycotoxins or can inflict physical damage to fish (Fig. 11-1, 11-2). The occurrence, geographic distribution, and duration of these natural phenomena have increased throughout the world and have become a major economic threat to the commercial fisheries and aquaculture industries (White 1988; Hallegraeff 1993; Taylor and Horner 1994; Bruslé 1995). Classes of microalgae containing genera with known fish lethality include chloromonads (*Chattonella*, *Fibrocapsa*, *Heterosigma*); diatoms (*Chaetoceros*, *Skeletonema*, *Thalassiosira*, *Nitzschia*, *Amphora*; *Leptocylindrus*); dinoflagellates (*Alexandrium*, *Gambierdiscus*, *Gymnodinium*, *Gyrodinium*, *Pyrodinium*, *Amphidinium*, *Pfiesteria*); silicoflagellates (*Dictyocha*); cyanobacteria (*Microcystis*, *Anabaena*, *Aphanizomenon*) and prymnesiomonads (*Chrysochromulina*, *Prymnesium*). In general, blooms of these genera of algae can be lethal to fish from anoxia caused by the process of bloom decay, asphyxiation caused by excess mucus formation due to mechanical damage or irritation of gill tissue, gas-bubble trauma due to extreme oxygen saturation from algal photosynthesis or from the production of ichthyotoxins producing gill toxicity, hepatotoxicity, neurotoxicity or hemolysis. Netpen reared fish have less opportunity to avoid HABs than wild fish, resulting in considerable economic hardship to the farmers. This chapter is focused on algal species that adversely affect netpen salmonids.



11-1. Harmful flagellate algae. Bar = 10 µm.
Heterosigma carterae.
a. bean-shaped cell with one forward and one trailing flagellum. b. bumpy, potatoe shape. c."raspberry form" in Lugol's preservative.
d. *Chrysochromulina polylepis.*
e. *Prymnesium parvum.*
f. *Prorocentrum micans.*
g. *Alexandrium tamarense.*
h. *Gyrodinium aureolum.*
i. *Dictyocha speculum.*

11-2. Harmful diatoms.
Bar = 50 µm unless otherwise indicated.
a. *Chaetoceros concavicornis.*
Insert showing details of barbs.
b. *Chaetoceros convolutus.*
c. *Corethron hystrix.*
d. *Leptocylindrus minimus.*
e. *Skeletonema costatum.*
f. *Thalassiosira aestivalis.*
g. *Thalassiosira rotula.*



Heterosigma carterae (formerly *H. akashiwo*, often misidentified as *Olisthodiscus luteus*), *Chaetoceros concavicornis*, *C. convolutus*, *Corethron* spp., are the major causes of pen-reared salmonid mortalities in North America (Brett et al. 1978; Harrison et al. 1983; Gaines and Taylor 1986; Rensel et al. 1989; Speare et al. 1989; Horner et al. 1990; Black et al. 1991; Albright et al. 1992, 1993; Rensel 1992; Taylor, 1993; Taylor and Haigh 1993; Taylor et al. 1994). Mortalities of farmed salmon in New Zealand have been caused by *Prymnesium calathiferum* and *Heterosigma carterae* (Taylor et al. 1985; Chang et al. 1990, 1993; MacKenzie 1991). In Europe, major kills to farmed salmonids have been caused by *Gyrodinium aureolum* (considered identical or closely related to *Gymnodinium mikimotoi*), *Chrysochromulina polylepis*, *C. leadbeateri*, *Prymnesium parvum*, *Chaetoceros wighamii*, *Dictyocha speculum* (= *Distephanus speculum*) and the flagellate X (most likely *Heterosigma*) (Tangen 1977, 1979; Ayres et al. 1982; Dahl et al. 1982; Gowen et al. 1982; Jones et al. 1982; Parker et al. 1982; Johnson 1988; Bruno et al. 1989; Erard-le-Denn and Ryckaert 1989; Aune et al. 1992; Dahl and Tangen 1993; Graneli et al. 1993; Heidal and Mohus 1995). Farmed salmonids have been killed by *Alexandrium tamarense* (formerly *Protogonyaulax tamarensis* = *Gonyaulax tamarensis* = *G. excavatum*) in the Faeroe Islands (Mortensen 1985; Simonsen et al. 1995). *Chaetoceros convolutus* and *Leptocylindrius minimus* have caused mortalities in farms in Chile (Clément and Lembeye 1993).

Mitigation Techniques

As HABs are natural phenomena, the process of ameliorating the adverse effects on farmed fish must rely on improved husbandry, technologies, and procedures that include monitoring of algae at various depths on farmed sites. Site selection for fish farms is fundamentally important because HAB events are annual occurrences in certain coastal areas. Bloom avoidance can be managed by moving pens away from an encroaching bloom, however this can be equally stressful and lethal to caged fish. Stationary pens can be made deeper to allow the fish to swim below the surface bloom, or can be constructed in a manner that allows for lowering of the pens during a surface bloom. Non-permeable perimeter skirting of pens with polyester aprons allows for upwelling of deeper colder water either by simple deep aeration within the pen or the pumping of deep water by air-lift or hydraulic pumps. Using these techniques prevents advection of surface blooming algae into the pens, reduces any anoxic conditions caused by the algae, inhibits growth of many alga by lowering water temperature, and de-stratifies the water column by vertical convection to inhibit growth of flagellates, such as *H. carterae,* that require calm stratified water for growth. Care should be exercised, however, in using these techniques if the blooms are of harmful diatoms because maximum cell densities of these species can be at depths of about 20 m, hence the need for accurate monitoring of the water column. In addition, care must be exercised with use of air-lift pumps that can cause gas supersaturation with resultant gas bubble trauma to the fish. Newly developed self-contained bag-culture systems for fish with pumped water from controllable depths into PVC-coated woven polyester bags, promises to negate many of the problems associated with HABs. Intake water level into these bags can be governed by the depth of the bloom adjacent to the pen.

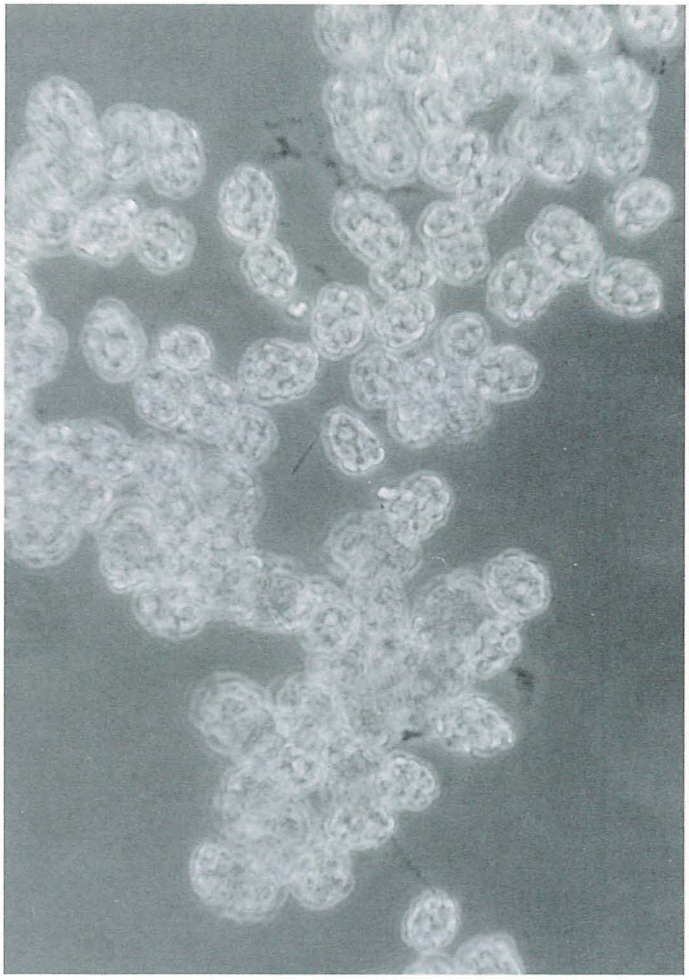
If contact with the bloom cannot be avoided, then losses can be reduced by lowering the oxygen demand and general stress on the fish. This can be achieved by cessation of feeding just prior to and during the bloom, and minimizing personnel traffic on the walkways, both strategies that discourage the fish from moving up into a surface bloom. It is particularly important to lower oxygen demands on exposed fish because most harmful algae damage the gills. Yang and Albright (1994a) prevented suffocation of coho salmon on exposure to *Chaetoceros concavicornis* cells by suppressing gill mucus production with the mucolytic agent L-cysteine ethyl ester in the diet. Mucolytic agents should be used with caution because mucus is an important barrier to pathogens. This therapy has yet to be demonstrated in field trials. Yang et al. (1995) prevented death of rainbow trout when exposed to *H. carterae* by adding the enzymes superoxide dismutase and catalase to the toxic culture of the alga. Both *Chattonella antiqua* and *H. carterae* produce superoxide, hydroxyl radicals, and hydrogen peroxide, which are destroyed by these enzymes (Tanaka et al. 1992, 1994). Oxygen radicals can strip the mucus from fish gills leading to osmoregulatory failure and fish death.

Heterosigma carterae

Several blooms of *Heterosigma carterae* (formerly *H. akashiwo* = *Olisthodiscus luteus*) have been associated with high mortality in pen-reared salmon in the Pacific Northwest since 1986 (Taylor and Haigh 1993). Fish kills associated with blooms of *H. carterae* have also been reported in Europe (Johnson 1988), Japan (Honjo 1994), and New Zealand (MacKenzie 1991). Bioassays with juvenile chinook salmon in blooms of *H. carterae* indicated that morbidity in fish was due to a labile ichthyotoxic agent (Black et al. 1991). Blooms

generally occur during the summer, and massive mortalities have been observed in all species of pen-reared salmon throughout British Columbia (Egan 1990; Black 1991) and Washington (Rensel et al. 1989). High mortalities were observed in pen-reared chinook during two separate blooms when the concentration of *H. carterae* in water was 200,000 and 800,000 cells/ml (Black et al. 1991). Hershberger et al. (1997) reported that the alga was attracted to low salinity waters, which may account for blooms occurring during warm weather following heavy rains. Disturbance of the water column by wave action and wind disperses most *Heterosigma* blooms.

CLINICAL SIGNS AND GROSS PATHOLOGY. Rapid, high mortality (often reaching near 100%) occurs shortly after water containing a toxic bloom enters the netpens. Not all blooms of this alga are toxic as formation of toxicity depends on the physiological stage of the alga, which is reflective of environmental conditions. In general, toxicity is triggered in the alga by nutrient deprivation when in the stationary phase of growth (Black, E. British Columbia Ministry of Fisheries, pers. comm.). Affected fish accumulate at the surface in the net corners, are lethargic, often appear anesthetized and exhibit labored respiration. Fish do not exhibit any distinctive external or internal pathological changes. However, some affected fish may exhibit excessive mucus on the gills.



11-3. *Heterosigma carterae* preserved in Lugol's.

MICROSCOPY. In wet mount preparations, the live alga can appears in a number of morphological shapes from bean-shaped (nutrient rich) to flattened warty bean-shaped (lacking nutrient) to bumpy potato-shaped (usually nutrient deplete stationary phase) motile cells (Fig. 11-1, a, b, c). The following is a description of *H. carterae* by Gaines and Taylor (1986):

> *"The organism occurs as microscopic, bean shaped single cells, usually 12-22 µm long, propelled by two very fine, whip-like flagella which arise from the side of the cell near the front, one pointing forwards and one trailing behind. The cells turn slowly as they swim forward. The shape varies from being smoothly oval (flattened in the same plane as the flagella) to being bumpy and potato-like in outline. Each cell contains usually 20 or fewer (but may have up to 50) greenish-brown bodies termed chloroplasts.... Small shining dots on its surface are mucus-producing bodies, termed mucocysts".*

As the alga has no cell wall, preservation in Lugol's solution (see Appendix III) causes the cell membrane to collapse to the appearance of a cluster of grapes (Fig. 11-1, c).

The histopathological changes in fish dying from exposure to *H. carterae* have not been adequately described. We have observed acute, diffuse necrosis of the gill epithelium in dying fish collected from the field during a *Heterosigma* bloom. These fish were suffering the affects of the bloom for several hours before collection, and this change was not observed in fish that were killed by more acute exposure (Black et al. 1991).

DIAGNOSIS. Because moribund fish do not exhibit distinctive pathological changes, the diagnosis of disease caused by *H. carterae* is based on the observation of a high concentration of the alga in the water associated with rapid mortality in the exposed fish. Methods for collecting and enumerating planktonic algae are described by Gaines and Taylor (1986).

Chaetoceros and *Corethron* spp.

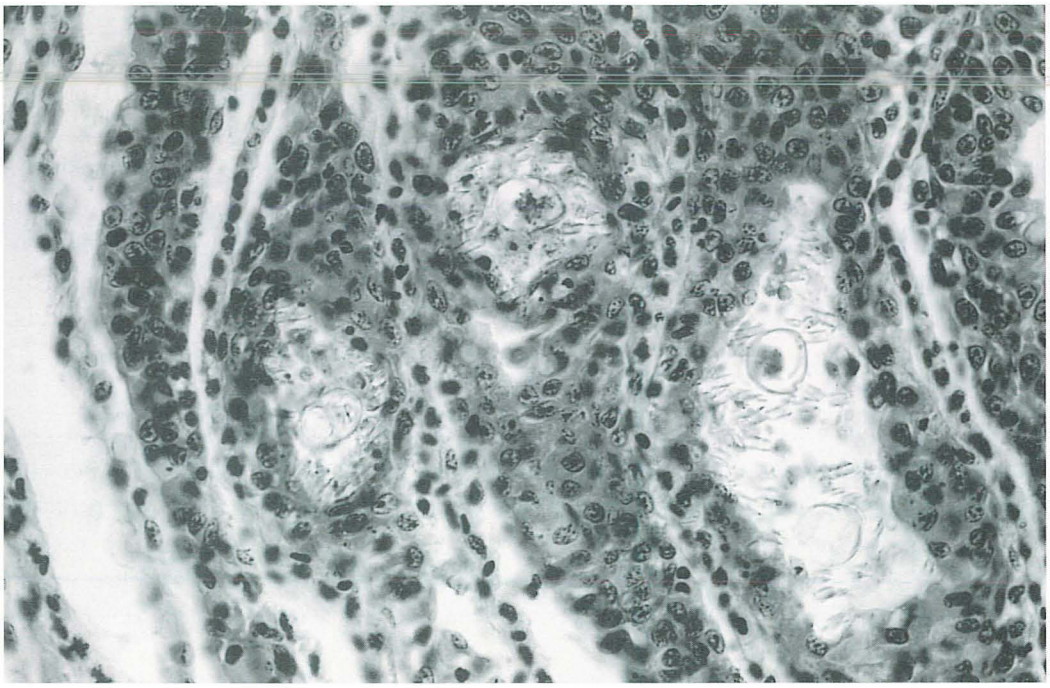
Chaetoceros concavicornis (Fig. 11-2a), *C. convolutus* (Fig. 11-2b) and a *Corethron* sp. (Fig. 11-2c) have been associated with mortality at several locations where salmon are reared in seawater netpens. Mortalities due to *Chaetoceros* spp. have been reported in pen-reared salmon at many sites in British Columbia and Washington State (Gaines and Taylor 1986; Rensel et al. 1989; Horner et al. 1990; Albright et al. 1992), in Alaska (Martin et al. 1981; Farrington 1988), and Chile (Clément and Lembeye 1993). Mortality caused by *Chaetoceros* is usually very rapid, and high mortality can occur within a few days during heavy blooms. All salmon species are susceptible. Atlantic salmon and sockeye salmon appear to be the most susceptible species, and larger fish are more susceptible than smaller fish (Brett et al. 1978; Marsh 1988). Speare et al. (1989) reported high mortality in pen-reared coho associated with a bloom of *Corethron* sp., a diatom similar to *Chaetoceros*. Both genera of diatoms cause disease in fish through physical damage to the gill (Bell 1961; Bell et al. 1974; Farrington 1988; Speare et al. 1989). The diatom initially causes massive increase in gill mucus production, despite no signs of penetration by the spines of the diatom, followed by degenerative changes of the gill epithelium. Hypoxia due to respiratory dysfunction is the ultimate cause of death (Rensel 1993; Yang and Albright 1992).

In netpens, mortality in sockeye salmon associated with *Chaetoceros* blooms occurred at about 1 cell/ml (Kennedy et al. 1976). High mortality occurred in sockeye, coho and chinook during a bloom of *Chaetoceros* at 8-32 cells/ml (Brett et al. 1978), and 8 cells/ml killed Atlantic salmon in netpens (Marsh 1988). In laboratory experiments 3,000-18,000 cells/ml were required to achieve an LC_{50} in chinook and chum salmon (Farrington 1988). The high concentration of *Chaetoceros* that is required to kill salmon in the laboratory has been attributed to a loss of barbs on the setae when the alga is grown in culture. Sublethal cell concentrations, 0.4 to 5.0 cells/ml, may increase fish's susceptibility to infectious diseases, such as vibriosis, due to suppression of the immune system (Albright et al. 1993; Yang and Albright 1994b).

CLINICAL SIGNS AND GROSS PATHOLOGY. Typical of gill diseases, affected fish accumulate near the surface, are lethargic, and exhibit labored respiration and flaring of the opercula. Fish may also exhibit a coughing response (Rensel 1992). Examination of gills reveals excessive mucus and may reveal white patches of hyperplastic tissue at the base of the primary filaments, particularly at the bend in the gill arch (Fig. 12-3b). The internal organs of affected fish appear normal.

MICROSCOPY. Wet mount preparations of the gills may reveal masses of diatoms entrapped in excessive mucus. Histological examination of the gills reveals severe hyperplasia and necrosis of the epithelium (Fig 11-5). Hyperplasia of the epithelium results in fusion of the secondary lamellae, and in severe cases, the primary lamellae may be fused. Affected gills

11-4. *Chaetoceros convolutus*. Wet mount.



11-5. *Chaetoceros* sp. entrapped in coho salmon gills associated with epithelial hyperplasia

often exhibit prominent inflammation. Speare et al. (1989) observed a marked neutrophil response in the extravascular spaces and in the lymphatic-like central venous sinusoidal spaces of the gills. Diatoms can be found on the surface of the gill epithelium or embedded in the hyperplastic tissue, particularly at the base of the primary lamellae (Fig. 11-5). The embedded portions of the diatoms may be entrapped by multinucleate giant cells. Mild fibroplasia may be observed associated with diatom remnants in recovering fish (Speare et al. 1989).

DIAGNOSIS. Diagnosis of *Chaetoceros* or *Corethron* disease can be based on the occurrence of large numbers of diatom chains with barbed spines or setae in the water associated with respiratory distress in the salmon. Histological examination, which reveals the presence of entrapped diatoms and associated gill damage, is also useful.

Miscellaneous algae

***Leptocylindrus* sp.**

Blooms of the diatom *L. minimus* (Fig. 11-2d) have occurred at several locations in Chile since 1989 (Clément and Lembeye 1993; Clément 1994). Although not confirmed, it appears that the diatom causes mechanical damage to the gills. Atlantic salmon accumulate in the corners or bottom of the pen, while exposed rainbow trout accumulate at the surface, often with their dorsal fin exposed (Clément 1994). The gills of affected fish exhibit pallor, increased mucus secretion, and epithelial hyperplasia. Death occurs when concentrations of the diatom exceed 10,000 cells/ml.

***Chrysochromulina* spp.**

A massive and widespread bloom of the prymnesiomonad flagellate *C. polylepis* (Fig. 11-1d) occurred in May and June 1988 at the Kattegat and Skagerrak coasts of Norway, Denmark, and Sweden, and affected more than 120 fish farms (Lindahl and Dahl 1990; Graneli et al. 1993). In addition to killing numerous pen-reared salmon in Norway, this alga also killed many naturally occurring marine organisms (Saunders 1988; Nielsen et al. 1990). In 1991, a bloom of a similar alga, *C. leadbeateri*, killed about 500 tonnes of pen-reared Atlantic salmon in northern Norway (Johannessen et al. 1991; Aune et al. 1992; Heidal and Mohus 1995). The mechanism by which *Chrysochromulina* spp. kills fish has not been completely elucidated, but Edvardsen et al. (1990) demonstrated that the alga *C. polylepis* produces a hemolysin. The toxin also damages the gills, causing increased gill permeability. Salmon quickly died following exposure to 4 to 8 million cells/L in full strength sea water, while fish were unharmed when exposed to the same concentration at 16 ppt, which is much closer to isotonic conditions (Underdal et al. 1989). Toxic extracts collected from the *C. leadbeateri* bloom were similar to those of *C. polylepis* (Aune et al. 1992). Blooms of *C. polylepis* are often harmless, and thus Nielsen et al. (1990) suggested that the alga is only toxic under certain environmental conditions.

***Skeletonema* sp. and *Thalassiosira* sp.**

Mortality and gill lesions in Atlantic salmon reared in a seawater netpen in British Columbia were associated with a dense bloom of diatoms containing predominantly *Skeletonema costatum* (Fig. 11-2e), *Thalassiosira aestivalis* (Fig. 11-2f) and *T. rotula* (Fig. 11-2g) (Kent et al. 1995b). Gills of moribund and dead fish exhibited excessive mucus production. Histological examination revealed necrosis of the gill epithelium and edema at the base of secondary lamellae. The edematous spaces contained an inflammatory infiltrate. The mechanism of gill damage was not determined, but was likely due to physical irritation by the algae. None of these diatoms had been previously reported to cause disease in fish, which illustrates that many diatoms have the potential to be harmful if the occur in high enough density..

***Gyrodinium* species**

The dinoflagellate *G. aureolum* (Fig. 11-1h) is the most harmful alga to aquacultured fish in Europe. Blooms in the late summer or autumn have caused deaths of between 2000-3000 tons of pen-reared Atlantic salmon and rainbow trout in Norway, Scotland and Ireland (Tangen 1977, 1983; Dahl and Tangen 1993). Mortalities usually occur when concentrations exceed 10,000 cells/ml, whereas lower concentrations (e.g., 1,000 cells/ml) are associated with inappetence. The alga produces the hemolytic and ichthyotoxic agents, 1-acyl -3-digalactosylglycerol and octadecapentaenoic acid, also found in *C. polylepis* (Yasumoto et al. 1989), which causes necrosis of the gill epithelium (Roberts et al. 1983). Furthermore, blooms have been associated with liver necrosis (Dahl and Tangen 1993). Blooms of unidentified *Gyrodinium* species have also caused inappetence in pen-reared salmon, but not abnormal mortalities in Chile (Clément and Lembeye 1993).

Prymnesium parvum

This eurohaline prymnesiophyte flagellate *P. parvum* (Fig. 11-1e) has caused losses of 750 tonnes of pen-reared salmon in the Ryfylke fjord system of Norway (Johnsen and Lein 1989). The alga produces a toxin, which is cytolytic, hemolytic and neuroactive.

Prorocentrum micans

A bloom of the dinoflagellate *P. micans* (Fig. 11-1f) was associated with mortality of pen-reared coho salmon in Chile in April of 1983 (Lembeye and Campodonica 1984). Affected fish were excited and the organism apparently caused obstruction of the gills.

Alexandrium tamarense

Exposure of pen-reared salmon and trout in the Faeroe Islands to an intense bloom of the dinoflagellate *A. tamarense* (Fig. 11-1g) caused 77% mortality. Examination of gills revealed necrosis and sloughing of the epithelial of secondary lamellae, and hemorrhaging. No other organ revealed any histopathological features (Mortensen 1985). The high hemolytic activity in cell extracts of *Alexandrium* (Underdal et al. 1989) is not caused by the associated PSP toxins (Simonsen et al. 1995).

***Dictyocha* spp.**

Mortalities of netpen rainbow trout in France and Atlantic salmon in the Shetland Isles were caused by the silicoflagellate *D. speculum* (Fig.11-1i). Gills of the affected fish showed extensive necrosis and sloughing with separation of the secondary gill lamellae and moderate hyperplasia at the base of the filaments (Bruno et al. 1989; Erard-Le-Denn and Ryckaert 1989). Mortalities of pen-reared salmon on the west coast of Vancouver Island also have been attributed to this alga.

IDIOPATHIC AND NON-INFECTIOUS DISEASES

M.L. Kent and T.T.Poppe

Several diseases of unknown or non-infectious causes have afflicted pen-reared salmonid fishes. These include cataracts (caused by various etiologies); netpen liver disease, caused by microcystin from an unknown natural source; heart diseases such as cardiomyopathy syndrome and coronary arteriosclerosis; and "water belly", which may be caused by a nutritional or osmoregulatory problem. Furthermore, chronic peritonitis has been associated with oil-adjuvant vaccines administered by intraperitoneal injection.

Heart Diseases

Various idiopathic lesions of the heart and pericardial cavity are occasionally observed both in wild and farmed fish. Coronary arteriosclerosis has been known for a long time in feral Pacific salmonid stocks, but is also described from Atlantic salmon. Different types of malformations seem to be an increasing problem in farmed Atlantic salmon.

Cardiomyopathy syndrome (CMS)

This chronic, progressive disease has been observed since 1984 in farmed Atlantic salmon in Norway and a few cases have been diagnosed in the Faeroe Islands (Bruno and Poppe 1996). The cause(s) have not been determined, but recently Grotmol et al. (1997) reported the presence of a nodavirus-like agent in affected heart tissue. Although transmission experiments have been negative, viral particles have been observed by electron microscopy and the lesions and epidemiology may be consistent with a viral etiology. The most serious losses typically occur in the autumn 12-18 months after transfer to sea water.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish in the terminal stages of the disease are often in good body condition, showing no or few clinical signs before they die. They may however, go off the feed and swim sluggishly around for a few days before they die. Such fish frequently develop skin hemorrhage and edema, exophthalmia and ascites. At necropsy, fibrous peritonitis, ascitic fluid and blood or a blood clot surrounding the heart are typical findings (Fig. 12-1c). The atrium and sinus venosus are usually dilated and may contain blood clots. Sometimes, clotted blood may also be found on the dorsocranial surface of the liver.

MICROSCOPY. Characteristic lesions are found in the spongious myocardium of the atrium and ventricle (Amin and Trasti 1988; Ferguson et al. 1990). These lesions are comprised of muscular degeneration, proliferation of the endocardial cells, and infiltration with macrophages and lymphocytes subendocardially and in the degenerated muscle. Blood clots will frequently be found in atrium. Focal necrosis in the hepatic parenchyma may also occur.

DIAGNOSIS. The diagnosis is based on the characteristic gross and pathognomonic histopathological lesions. Diseased fish may also be diagnosed while still alive by means of ultrasound imaging (Sande and Poppe 1995). The disease has little resemblance to other diseases, but hemopericardium may be observed in fish dying from other causes.

CONTROL AND TREATMENT. There are great differences in susceptibility to CMS between different families, and selective breeding may be a good way to control the disease in the future. Also, many farmers have experienced that fallowing of sites for a year or two before new fish are introduced has reduced the problem considerably.

Coronary Arteriosclerosis

This condition is so common in many salmonids that it has been discussed whether this is a "normal abnormality" or genuine pathological changes.

CLINICAL SIGNS AND GROSS PATHOLOGY . There are no specific clinical signs of the disease. Fish with coronary arteriosclerosis may die suddenly for no other apparent reason.

MICROSCOPY. The condition is characterized by hyperplasia of the endothelium and smooth muscle in the walls of the coronary vessels, resulting in narrowing of the lumen and thereby reduction of the blood-flow. The significance of moderate lesions is unknown and there is usually no clinical signs or grossly visible lesions. Occasionally, a complete occlusion of the vessel may occur, thereby leading to degeneration with subsequent calcification of those parts of the outer compact myocardium supplied with arterial blood from the coronary vessels.

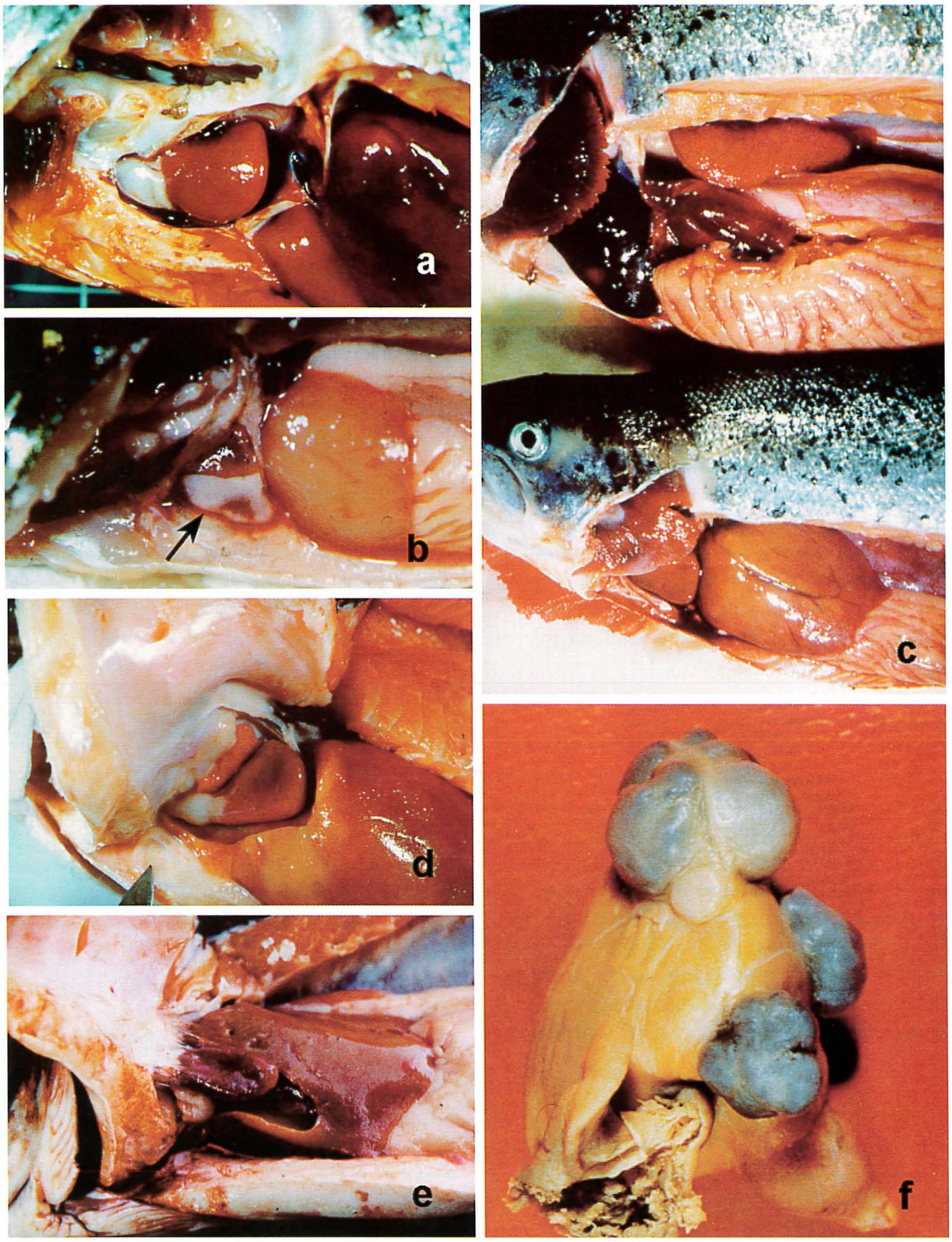


Figure 12-1. Heart diseases in Atlantic salmon. a. Situs inversus (up-side-down heart). b. Hypoplasia of the ventricular compact myocardium. Note fatty tissue (arrow) around heart. c. Upper fish with cardiomyopathy syndrome. Note blood clot surrounding the heart. Lower fish is normal. d, e. Hypoplastic septum transversum. Note indentation in the liver caused by the posteriorly enlarged, sac-shaped ventricle. f. hemangiomas on the ventricle wall.

DIAGNOSIS. The diagnosis is based upon histological examination of the coronary vessels.

CONTROL AND TREATMENT. The lesions are related to the absolute size of the heart and accelerate in parallel to factors responsible for rapid growth and can be modified by sex hormones and feed composition (Davie and Thorarensen 1996; Farrell et al. 1986; House et al. 1979; Saunders et al. 1992; Schmidt and House 1979). Excessively rapid growth has been associated with coronary arteriosclerosis, and thus reducing the feeding rate may ameliorate the problem.

Malformations of the heart

Several types of malformations of the heart have been observed in farmed Atlantic salmon in recent years. The most common types seem to be aplasia of the septum transversum and situs inversus of the ventricle. Hypoplasia of the outer compact layer of the ventricular myocardium has also been observed recently. The end result of the three malformations described is reduced cardiac output and more strain on the heart. This will lead to cardiac failure with secondary lesions as a result of poor blood supply, altered pressure and reduced circulation.

Hypoplastic septum transversum

In fish with this malformation, the natural restraints for myocardial growth are absent, and the ventricle may grow to a considerable size and become dislodged into a depression in the liver surface (Fig. 12-1d,e). The shape of the ventricle is often like a tube or sac in contrast to the normal triangular or pyramidal shape. The normal function of the heart is depending upon the subambient pressure inside the pericardium created when the ventricle contracts and the characteristic shape which is important for optimum force during contraction (Farrell and Jones 1992). As the heart itself is dislocated, vessels may become compressed and the end result will be reduced cardiac output.

Situs inversus

In some cases the heart seems to be turned more or less upside down within a normal pericardial cavity (Fig. 12-1a), thereby taking an irregular shape and compression of the vessels may result. This condition is being seen more frequently in Atlantic salmon in Norway, both in the freshwater and in the seawater phase. At necropsy, the ventral part of the heart (with the apex) is rotated counterclockwise on its transverse axis, resulting in the atrium being located under the ventricle. The shape of the ventricle is altered and the bulbus arteriosus is stretched. Fish with this malformation are usually smaller than normal and seem to be less tolerant to stressors, such as handling and grading. In some populations, this condition has been observed in 5 to 10% of the fish.

Hypoplasia (or aplasia) of the ventricular compact myocardium

Fish with this lesion are typically smaller than their siblings and have ascites and exophthalmia. At necropsy, the heart is partly surrounded by fatty tissue and the ventricle is considerably smaller than normal. Histology reveals a total aplasia or severe hypoplasia of the outer, compact myocardium of the ventricle. Muscle cells and nuclei in the inner, spongious myocardium are hyperplastic. The epicardium is typically heavily infiltrated by fatty tissue.

Cardiac Hemangiomas

Hemangiomas (blood vessel neoplasms) originating from the wall of the ventricle has recently been observed at harvest in farmed Atlantic salmon in Iceland and Norway. To date, less than 1% of fish have been affected. The condition is characterized by cyst-like extrusions from the muscular ventricle wall (Fig. 12-1f). The cysts may be multiple and are up to 10 mm in diameter. The wall may also be thickened. Histological examination reveals that the lumen of the cysts communicate with the ventricular lumen.

DIAGNOSIS. The diagnosis of heart malformations are based upon gross and histological findings.

CONTROL AND TREATMENT. No treatments are known for these conditions. The causes are not known, but hereditary factors and high temperature during incubation seem to be the most likely explanations.

Skeletal Deformations

Numerous different malformations, in particular skeletal malformations, have been described in several freshwater and marine fish species, both wild and farmed, over the years. Such lesions are usually easily observed even for the untrained eye and the descriptions of such lesions date back several hundred years. In farmed salmonids, the skeletal malformations most frequently seen are lordosis (vertical deviation) and scoliosis (horizontal deviation). Fusion of vertebrae may also result in local shortening of the spine leading to humpback formation or shortening of the tail. Malformations of cranial bones

(e.g., jaws, operculum) are also frequently seen. Such abnormalities are quite common in fry and usually leads to impaired survival in wild fish. Farmed fish, on the other hand, may survive longer in their sheltered environment and may therefore develop lesions seldom seen in wild fish.

Although several factors are known to cause skeletal malformations in farmed fish, the vast majority of such lesions have an obscure and probably multifactorial etiology. Inadequate levels of different nutrients like phosphorous, amino acids and vitamins (e.g., vitamins C and D) are known to cause such lesions. Infections due to *Myxobolus cerebralis* and *Flavobacterium psychrophilium* that originate during the freshwater phase of development should also be considered if the presmolts were reared on surface water. Hypoxia, high temperatures and exposure to acidic water, heavy metals and organophosphates during incubation and early life-stages may also cause skeletal deviations (Ferguson 1989). Inbreeding is also believed to be a possible cause of this condition (Aulestad and Kittelsen 1971; Poynton 1987). Healing of fractures after electric shocks or after medication with chelating drugs such as oxytetracycline should also be considered (From et al. 1985).

CLINICAL SIGNS AND GROSS PATHOLOGY. Malformations may be confined to one particular part of the skeleton, or include most of the length of the vertebral column. Commonly occurring head deformities include shortening of the opercula, shortening of jaws like in "pug-heads" or ventral deviation of the mandible (Fig. 12-2). In the vertebral column, the malformations may be in the form of shortening of the anterior ("humpback") or posterior ("short-tail") part of the vertebral column, or deviations and curvatures like in "saddlebacks". Most malformations are easily seen, but sometimes they are not discovered until the fish are filleted. Depending on the extent and location of the malformation, affected fish may show variable clinical signs that may include aberrant swimming behavior or impaired food intake.

MICROSCOPY. Depending on the etiology, microscopy of affected parts of the vertebral column may reveal reduced length and increased diameter of individual vertebrae. This may frequently be associated with mineralization and fusion. If *Myxobolus cerebralis* is suspected, particular attention should be paid to the possible destruction of cartilage and bone in affected areas.

DIAGNOSIS. The diagnosis is based on characteristic gross lesions and microscopic examination of affected areas. It is important to exclude the possibility of myxosporean infection (*Myxobolus cerebralis*) or healed fracture.

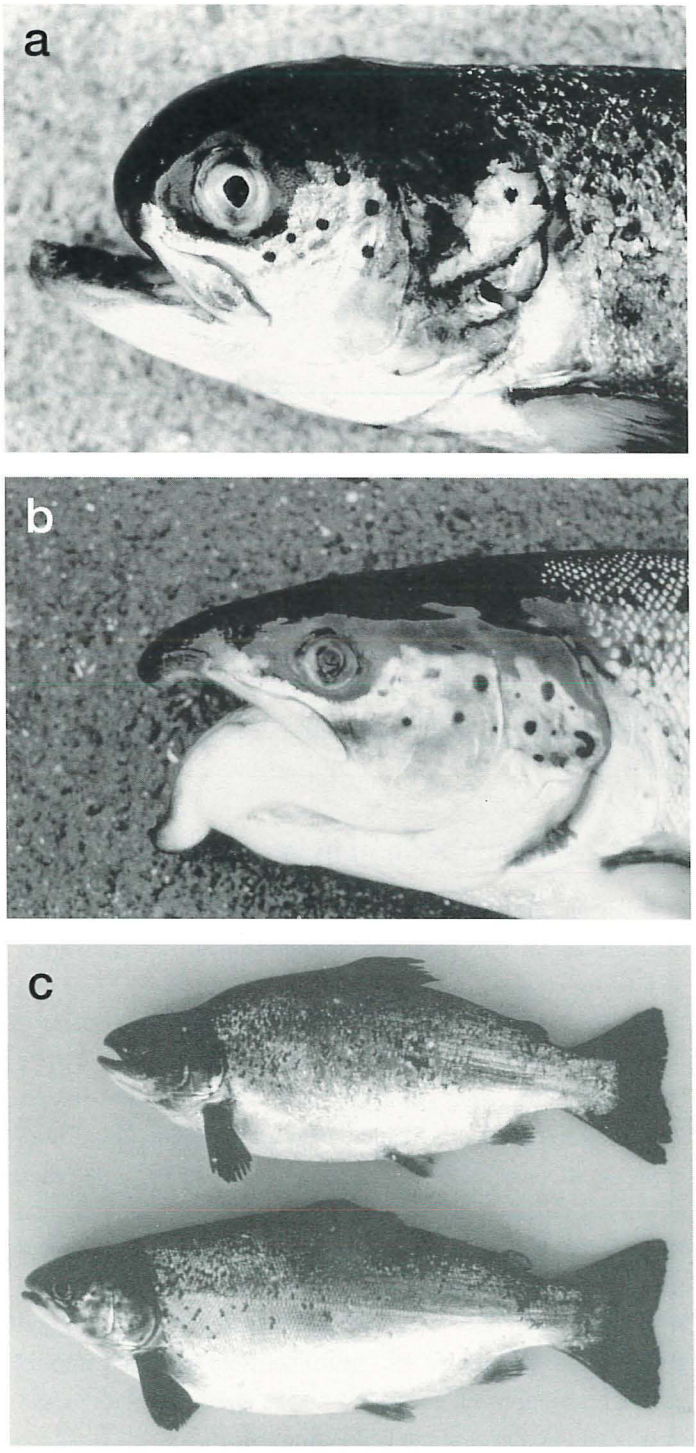


Figure 12-2. Skeletal deformities. Malformation of the head (a) and jaws (b). c. Fusion of vertebrae causing humpback appearance.

TREATMENT AND CONTROL. No treatment is known for the malformations described above. As long as the exact etiology of most of the spinal deformations seen in modern aquaculture is unknown, it is difficult to give any advice for the control of the malformations other than optimizing the environmental factors including feeds.

Cataracts

Cataracts resulting in opaqueness of the lens of the eyes are frequently observed in pen-reared salmon. They can be caused by infectious agents (e.g., larval helminths), by nutritional imbalances, sudden drop in temperature, by intoxication, or by genetic factors (Hargis 1991). Furthermore, they may be induced in smolts poorly adapted to sea water.

CLINICAL SIGNS AND GROSS PATHOLOGY. Opacity of the lens is characteristic of cataracts (Fig 9-3).

MICROSCOPY. Cataracts are comprised of various histological lesions including fiber lysis and regeneration, and other dysplastic changes. See Wilcock and Dukes (1987) and Hargis (1991) for reviews of ocular pathology of fishes.

DIAGNOSIS. Cataracts are usually diagnosed by observing opacity of the lens.

CONTROL AND TREATMENT. Cataracts are difficult to control because the precise etiology is seldom verified in field situations.

Post-Vaccination Peritonitus

The aquaculture industry has benefited considerably from the oil-adjuvanted vaccines for intraperitoneal immunization against furunculosis and other septicaemic diseases (Erdal and Reitan 1992; Midtlyng 1996). One of the few negative effects is the development of adhesions in the abdominal cavity (Lillehaug et al. 1992; Midtlyng et al. 1996). In most cases, these lesions are of acceptable extent and severity and of little or no significance for the fish or the end product. In a few cases, however, the abdominal reaction to the vaccine components can be extremely severe and lead to very serious lesions (Poppe and Breck 1997). There can be little doubt that the most extensive lesions may interfere to a considerable degree with normal motility and function of the gastrointestinal tract and normal gonadal development. Furthermore, the animal welfare aspect of the condition should not be overlooked.

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily affected fish are typically smaller and darker than unaffected fish in the same cages. They are usually inactive and congregate near the surface. A distention of the anterior part of the abdomen is a frequent finding. Adhesions are typically found near the injection site, i.e. cranially to the pelvic fins and usually involves the spleen and pyloric caecae with adhesions attached to the visceral wall by fibrous strands. More severe lesions are often found dorsally and cranially to the liver which is often adhered to the anterior part of the swimbladder and kidney, the gonads, the oesophagus and the septum transversum that separates the pericardial cavity from the peritoneal cavity. Adhesions may also be found in the posterior portion of the abdominal cavity, where they may interfere with bladder and hindgut function. In most cases the lesions are white or light grey, but adhesions stained black due to accumulation of melanomacrophages have also been seen (Fig. 12-3a). The lesions may develop into fairly thick aggregates, up to 10-15 mm in thickness.

MICROSCOPY. The tissue between the visceral organs and the body wall is dominated by fibrous tissue often arranged in sworls and granulomas. Round or oval spaces with a sharp periphery represent oil droplets from the vaccine are engulfed by the fibrous tissue (Fig. 11-8). Lymphocytes and macrophages are frequently found, and in some cases eosinophilic granular cells (EGC) dominate completely. Numerous melanomacrophages are found when the lesions are macroscopically dark. Granulomas of variable extent may also be encountered in internal organs like the kidney and liver. Pancreatic tissue is often completely surrounded by the fibrotic lesions.

DIAGNOSIS. The diagnosis is based upon the characteristic gross and histopathological lesions with numerous EGC's plus anamnestic information. The lesions may have some resemblance to post-spawning peritonitis, encapsulated tapeworm larvae (*Diphyllobothrium* spp.) and chronic BKD, but the adhesions associated with these diseases are usually not as firm as in post-vaccination peritonitis.

CONTROL AND TREATMENT. It is not known what causes the extensive adhesions in a few cases and it is therefore difficult to give any advise other than the vaccination should be performed on healthy fish that are subjected to a minimum of stress, before and after the vaccination. Furthermore, the vaccination should be done in accordance with the information given by the producer.

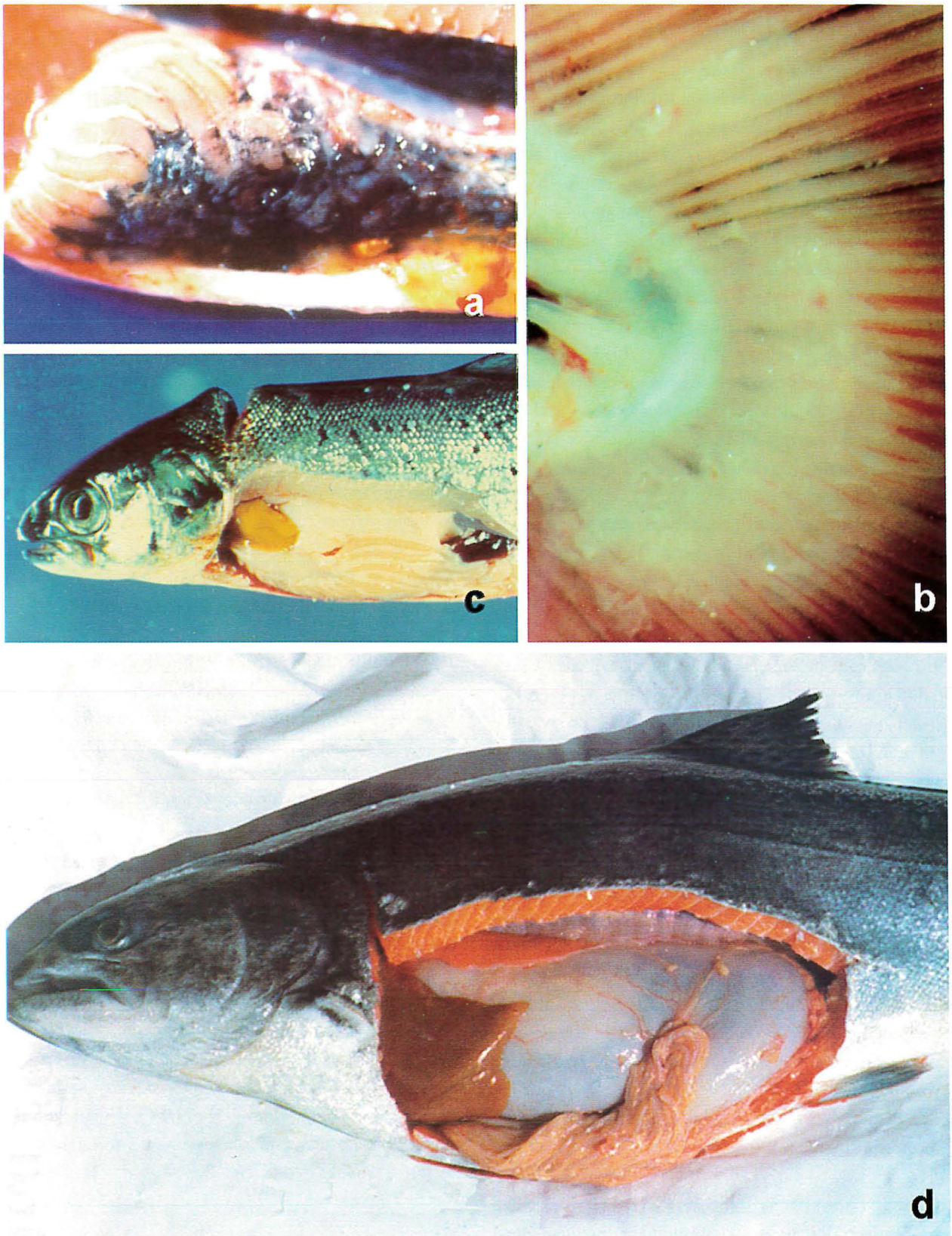


Figure 12-3. a. Post-vaccine peritonitis. Note extensive melanization of granulation tissue in adhesions around pyloric caeca. b. White patches in gills due to epithelial hyperplasia associated with entrapped *Chaetoceros* diatoms. c. Netpen liver disease. Note atrophied, yellow liver. d. Water belly in a chinook salmon. Note extremely enlarged stomach with very thin wall.

Netpen Liver Disease

A toxicopathic liver disease, referred to as netpen liver disease (NLD), has repeatedly been observed in Atlantic salmon (*Salmo salar*) during the summer at several netpen sites in Washington State, USA and British Columbia, Canada (Kent et al. 1988b; Kent 1990). The disease is characterized by diffuse hepatic megalocytosis, and diffuse necrosis and inflammation of the liver. Although NLD affects primarily Atlantic salmon during their first year in sea water, the disease has been also observed in pen-reared chinook salmon and rainbow trout. We have also observed essentially identical liver lesions in wild-caught chinook salmon from the Strait of Georgia, British Columbia (Stephen et al. 1993). Netpen liver disease begins in the summer, usually is chronic with steady, persistent mortality, and may continue through the early autumn. Fish that survive until the winter recover. Mortality associated with the disease is extremely variable; fish from some sites may exhibit only mild to moderate liver damage and no significant increase in mortality, whereas in other sites there has been severe liver damage and almost 90% cumulative mortality by the end of the summer.

Kent (1990) proposed that NLD is most likely caused by a naturally occurring toxin, possibly an algal toxin. Andersen et al. (1993) presented biological and chemical evidence which suggested that a toxin indistinguishable from microcystin LR, a potent hepatotoxin produced by blue-green algae, was the cause of NLD. Liquid chromatography-linked protein phosphatase analysis (LCPP) of liver tissue from salmon with NLD demonstrated the presence of a phosphatase inhibitor identical to microcystin LR, whereas control fish showed complete absence of the compound. In addition, salmon injected with purified microcystin LR exhibited liver lesions similar to those seen in NLD, including hepatic megalocytosis. Microcystin LR has also been detected in the biota growing on netpens (Andersen et al. 1993; Williams et al. 1998).

Atlantic salmon post-smolts often feed on the natural biota around netpens, and thus they probably contract the disease by eating zooplankton or other biota containing the toxin (Kent et al. 1996). Although microcystin occurs in zooplankton, the actual alga that produces the toxin in the marine environment has not been identified.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish may be dark, lethargic and accumulate in the netpen corners. The liver of affected fish may be small, yellow, opaque, and friable (Fig. 12-3c). However, fish can have severe liver damage without any obvious macroscopic changes to the liver. Other organs appear normal.

MICROSCOPY. The hallmark histological change of NLD is prominent hepatic megalocytosis (Fig 12-4). Affected livers also exhibit a variety of other pathological changes. Early in the disease the liver shows vacuolation of hepatocytes, individual cell necrosis of hepatocytes scattered throughout the parenchyma (hepatocyte dropout), nuclear pleomorphism of hepatocytes, and bile preductual cell proliferation. Moderate and severe lesions are characterized by prominent nuclear pleomorphism leading to hepatic megalocytosis, diffuse necrosis and hydropic degeneration of hepatocytes (Fig. 12-4), loss of the liver parenchyma architecture, perivascular and peritubular cuffing by inflammatory cells, and ceroid deposition in macrophages. Islands of regenerating hepatocytes are frequently observed, demonstrating concurrent degeneration and regeneration.

After affected fish are moved to clean water they exhibit almost complete regeneration of the liver in 3 mo. (Kent 1990). However, individual hepatocytes with megalocytosis can be found in regenerated livers at least 9 mo. after fish are moved to clean water. Therefore, the presence of these megalocytic hepatocytes in fish collected during the winter can be used as an indication that the fish previously had NLD.

DIAGNOSIS. Diagnosis of NLD is based on the presence of the histological changes described above in pen-reared salmon. However, this change can be induced by a variety of hepatotoxins and may be found in fish with other liver diseases.

CONTROL AND TREATMENT. Reports to us from veterinarians and fish farmers have suggested that post-smolts exhibiting inappetence toward commercial pellets often have a higher prevalence of NLD. These observations suggest that fish that are well-imprinted on commercial pellets will feed less on the natural biota, and thus may be less prone to NLD. In controlled field experiments we confirmed this hypothesis (Kent et al. 1996). Therefore, assuring that fish feed well (i.e., are imprinted well on pellets) shortly after seawater entry will greatly reduce the severity of the disease. In addition, introduction of fish in the winter or early spring, which is before the seasonal occurrence of NLD, will allow time for the post-smolts to adapt to sea water and re-establish a strong feeding response to artificial diets.

Water Belly (Bloat)

Distention of the abdomen due to massive fluid accumulation in the stomach has been observed in pen-reared Atlantic salmon, chinook and coho throughout the Pacific Northwest. The syndrome has also been observed in pen-reared rainbow trout in Europe (Staurnes et al. 1990). Apparently the fluid in the stomach is primarily sea water. The cause of water belly is unknown, but some have suggested that it is exacerbated by rapid changes in pellet size or overfeeding. Staurnes et al. (1990) proposed that water belly may be related to disturbances in fat and carbohydrate metabolism.

CLINICAL SIGNS AND GROSS PATHOLOGY. Although water belly has been associated with morbidity and mortality, affected fish often appear otherwise normal and can survive for weeks with the condition. Fish with water belly exhibit severe distention of the abdominal wall. Dissection reveals a massively enlarged stomach with a very thin wall (Fig. 12-3d). The stomach is filled with watery, clear fluid. Atrophy of the liver has also been observed in affected fish (Hicks 1989).

MICROSCOPY. Fish do not exhibit any significant histological changes, other than thinning of the stomach wall.

DIAGNOSIS. The disease is diagnosed by observation of massive accumulation of watery, clear fluid in the stomach.

CONTROL AND TREATMENT. Some farmers have reported that decreasing the feeding rate may alleviate the problem (Hicks 1989).

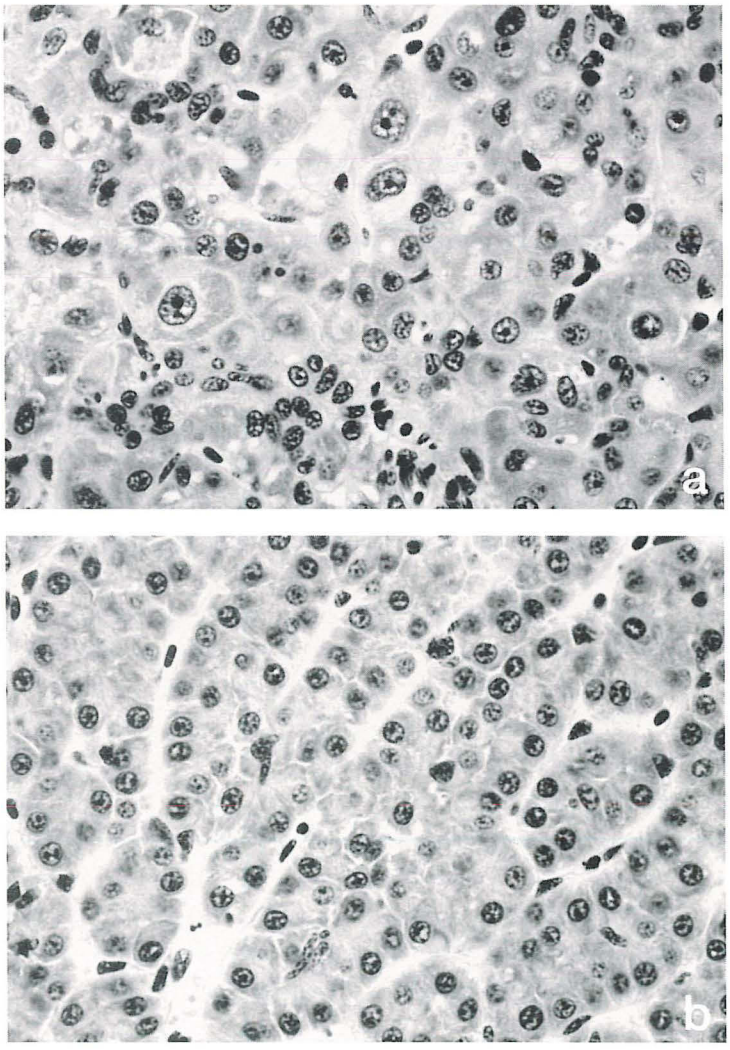


Figure 12-4. Atlantic salmon liver with netpen liver disease (A). Note loss of parenchyma architecture and nuclear pleomorphism of hepatocytes leading hepatic megalocytosis. B = normal liver. Note cord pattern of parenchyma and homogeneity of hepatocyte nuclei. H & E.

NEOPLASTIC DISEASES AND RELATED DISORDERS

M.L. Kent

As with other forms of agriculture, neoplasms are occasionally observed in individual fish (often at harvest). However, most of these do not pose a significant economic problem to fish farmers because only a few fish are usually affected. However, some of these diseases (e.g., plasmacytoid leukemia, swimbladder sarcoma) affect enough fish in a given population to be of concern. The following is a description of some common neoplasms found in pen-reared salmon. Cardiac hemangiomas are covered in the previous chapter (see page 100).

Plasmacytoid Leukemia

A plasmacytoid leukemia (PL) of salmon, referred to as marine anemia by fish farmers, caused high mortality at several chinook farms in British Columbia in the late 1980's (Kent et al. 1990a; Newbound and Kent 1991b). Now the disease is considered endemic, rather than epidemic, and has been reported at essentially all chinook farms in British Columbia (Stephen and Ribble 1995b; Stephen et al. 1996). Plasmacytoid leukemia has also been observed in pen-reared coho and Atlantic salmon in Chile. Diseases histologically identical to PL have also been observed in freshwater-reared chinook in California (Hedrick et al. 1990) and Washington (Harshbarger 1984; Morrison et al. 1990), and we have seen PL in freshwater-reared chinook in British Columbia.

Plasmacytoid leukemia is characterized by infiltration and proliferation of immature plasma cells (plasmablasts) in the visceral organs and retrobulbar tissue. The disease is usually detected in chinook after they have been in sea water for approximately one year, and outbreaks are often recognized following BKD epizootics. Mortalities associated with PL have varied considerably; whereas mortalities are usually chronic and moderate, at least one farm has lost approximately 50% of the production stock in the fish's second year in sea water.

The etiology of PL has not been determined, but field observations and laboratory transmission studies clearly indicate that the disease is caused by an infectious agent (Kent and Dawe 1990; Newbound and Kent 1991a). Plasmacytoid leukemia of chinook is readily transmitted by injection of homogenates of affected kidney and spleen tissue. In addition, we have experimentally infected sockeye salmon and Atlantic salmon by injection of affected chinook tissues (Newbound and Kent 1991a). Two agents have been suggested to be the cause of PL; a microsporidian parasite, *Nucleospora salmonis* (pages 65 and 66), and an oncogenic virus (e.g., retrovirus). The microsporidium has been observed in the nuclei of the plasmablasts from many fish with PL in British Columbia and in similar diseases in United States and Chile. However, experimental studies have produced conflicting results.

Our earlier transmission studies suggested that the microsporidium is not the primary cause of PL, and that an oncogenic retrovirus is the cause of the disease (Eaton and Kent 1992; Kent et al. 1991a). Essentially all infectious leukemias and related disorders are caused by oncogenic viruses (particularly retroviruses) and these have been associated with some blood neoplasms of fishes (Papas et al. 1976; Gross 1983). Virus-like particles similar to retroviruses have been detected in affected fish (Kent et al. 1991a), and Eaton and Kent (1992) consistently found increased reverse transcriptase activity, a retroviral enzyme, in fish with PL.

It is possible that PL actually represents two separate diseases; one caused by the virus and one caused by the microsporidium. Studies with fumagillin and TNP-470 (Hedrick et al 1991a; Higgins et al. 1998) support the microsporidian hypothesis. Morever, in essentially all cases that we have investigated in recent years, and in reports from other countries, *N. salmonis* is consistently observed in the proliferating plasmablasts.

Interestingly, the only other member of the family Enterocytozooidae, *Enterocytozoon bieneusi*, is associated with a retroviral disease, AIDS in humans (Desportes et al. 1985; Bryan et al. 1990). *Renibacterium salmoninarum*, the cause of BKD, may also be a cofactor in PL. Laboratory transmission studies indicate that the bacterium is not the primary cause of the disease. However, given the high prevalence of BKD that proceeds most outbreaks of PL, it is possible that the bacterium may exacerbate the proliferation of plasmablasts seen in PL. Conversely, PL-affected fish may be predisposed to BKD.

Transmission studies suggest that PL is not easily transmissible in sea water. However, the disease can be transmitted by *per os* exposure (Baxa-Antonio et al. 1992), and we have transmitted the disease by cohabitation in fresh water. Anecdotal field observations from Chile and British Columbia suggest that vertical transmission of PL is a possibility (Kent et al. 1993), but we have not been able to achieve vertical transmission of the disease in laboratory field studies.

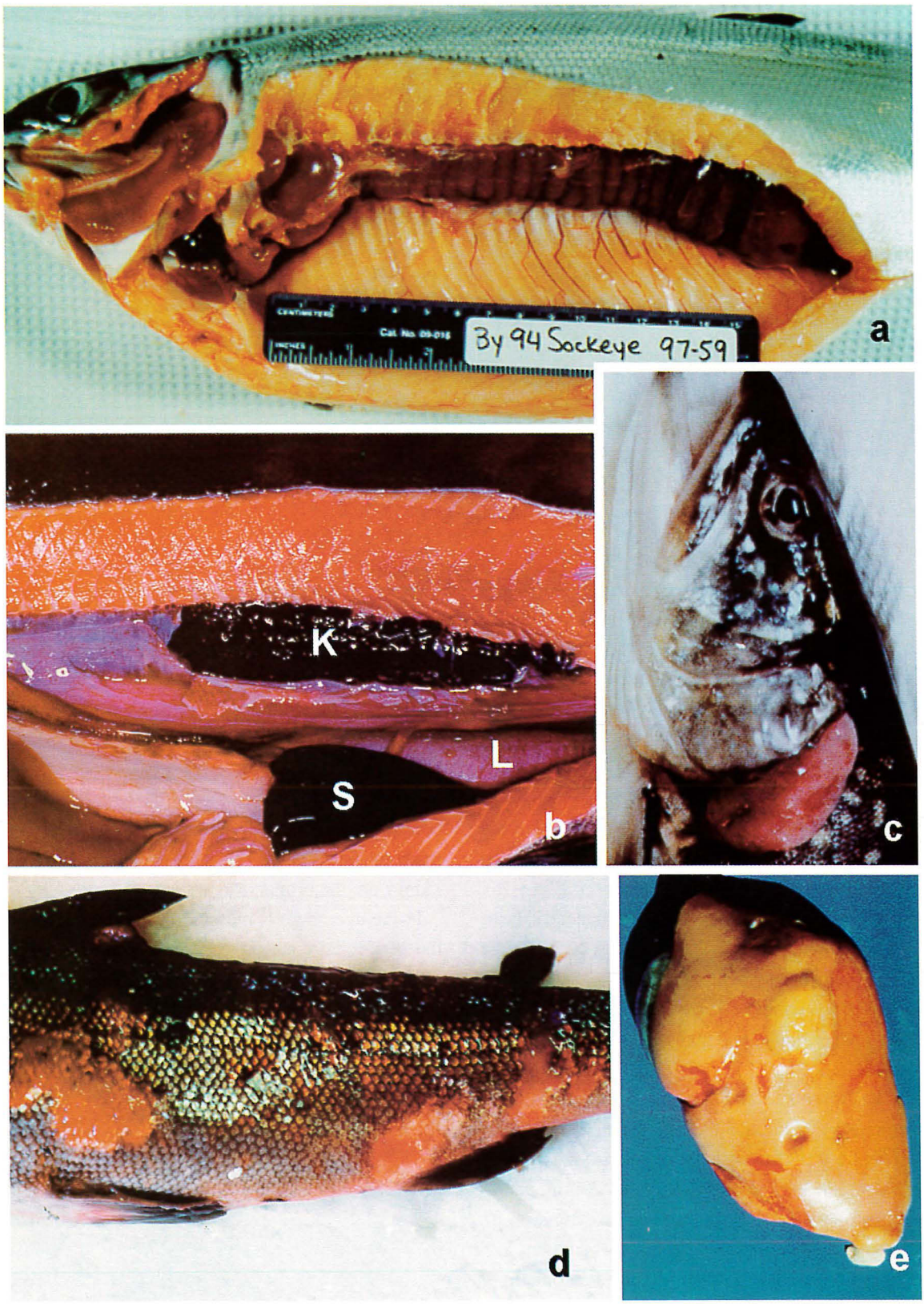


Figure 13-1. a. Lymphosarcoma in sockeye salmon. Note extremely enlarged kidney (Courtesy of K. Johnson). b. Plasmacytoid leukemia in chinook. Note enlargement of the spleen (S), lower intestine (L) and kidney (K). c. Thymic lymphoma in lake trout. Note pink tumor extending out of opercular cavity (Courtesy of C. Smith). d. epidermal papillomata in Atlantic salmon. Note raised, hyperemic skin lesions. e. Hepatocellular carcinoma in rainbow trout. Note raised yellow nodules in liver (Courtesy of C. Smith).

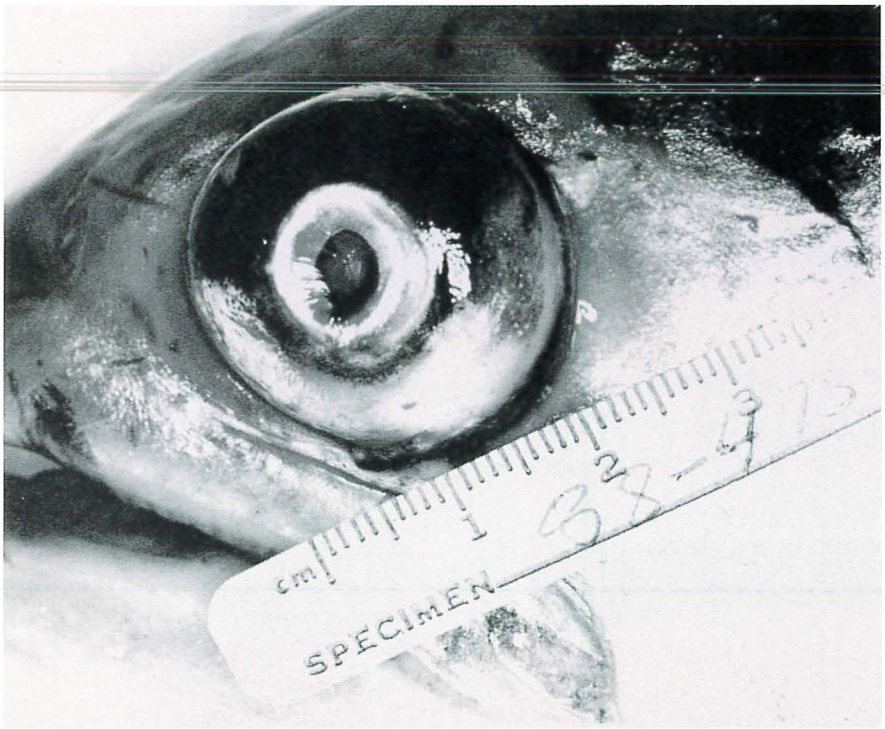


Figure 13-2. Severe exophthalmus in chinook salmon with plasmacytoid leukemia.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are dark, lethargic, and often swim near the surface. Pallor of the gills, indicating anemia, is a common finding. Many of the of fish with PL exhibit severe bilateral exophthalmus (Fig. 13-2). Some of these fish may exhibit severe eye involvement with relatively minimal visceral changes and anemia. Dissection of affected eyes reveals that the exophthalmus is due to massive accumulation of white or hyperemic tissue in the orbit of the eye. When visceral involvement occurs, internal examination reveals enlargement of the spleen and kidney (Fig. 13-1b). Petechiae may occur in the liver, mesenteric fat, pancreas, heart and skeletal muscle. The lower intestinal wall may be markedly thickened. Some fish have ascites consisting of a clear or serosanguinous fluid. Hematocrit values of blood from affected fish are quite variable, and may reach as low as 2%.

MICROSCOPY. Imprints stained with Giemsa or Diff-Quik from the kidney, liver, and the eye reveal numerous plasmablasts (Fig. 13-3). In these preparations, the cells have a smooth contour, vary in size from 10 to 20 µm, and contain sparse to moderate amounts of basophilic cytoplasm. Nuclei are large, occasionally indented, and contain a smoothly homogeneous to moderately reticulated chromatin pattern. A juxtanuclear hof is visible in some cells.

Histological examination of affected fish reveals a proliferation of plasmablasts essentially in every organ (Figs. 13-4), including kidney, spleen, liver, intestine, pancreas and associated mesenteric fat, meninges, heart, skeletal muscle, skin, and the eye (Kent et al. 1990a). In histological sections, the plasmablasts contain large, often deeply clefted or lobated nuclei, and prominent nucleoli. They have a moderate amount of finely granular, eosinophilic or amphophilic cytoplasm, and many of these cells are mitotically active. The eye, spleen, and kidney are the primary organs affected.

In the eye, there is massive infiltration of plasmablasts into the periorbital connective tissue and ocular muscles. Kidneys

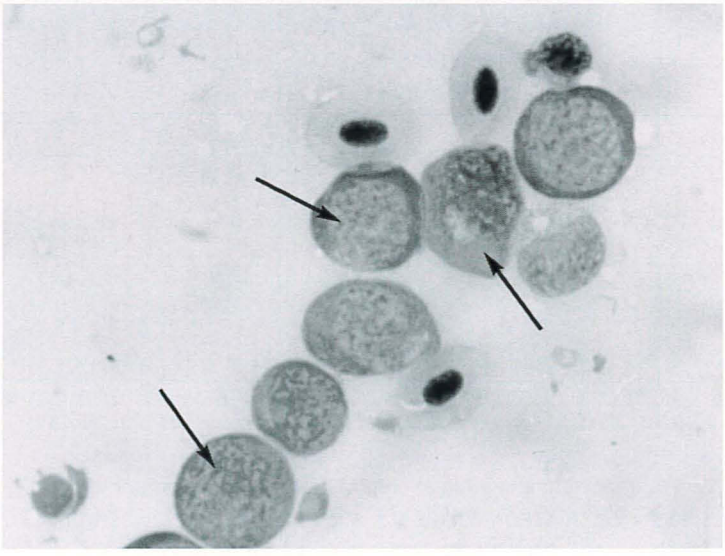
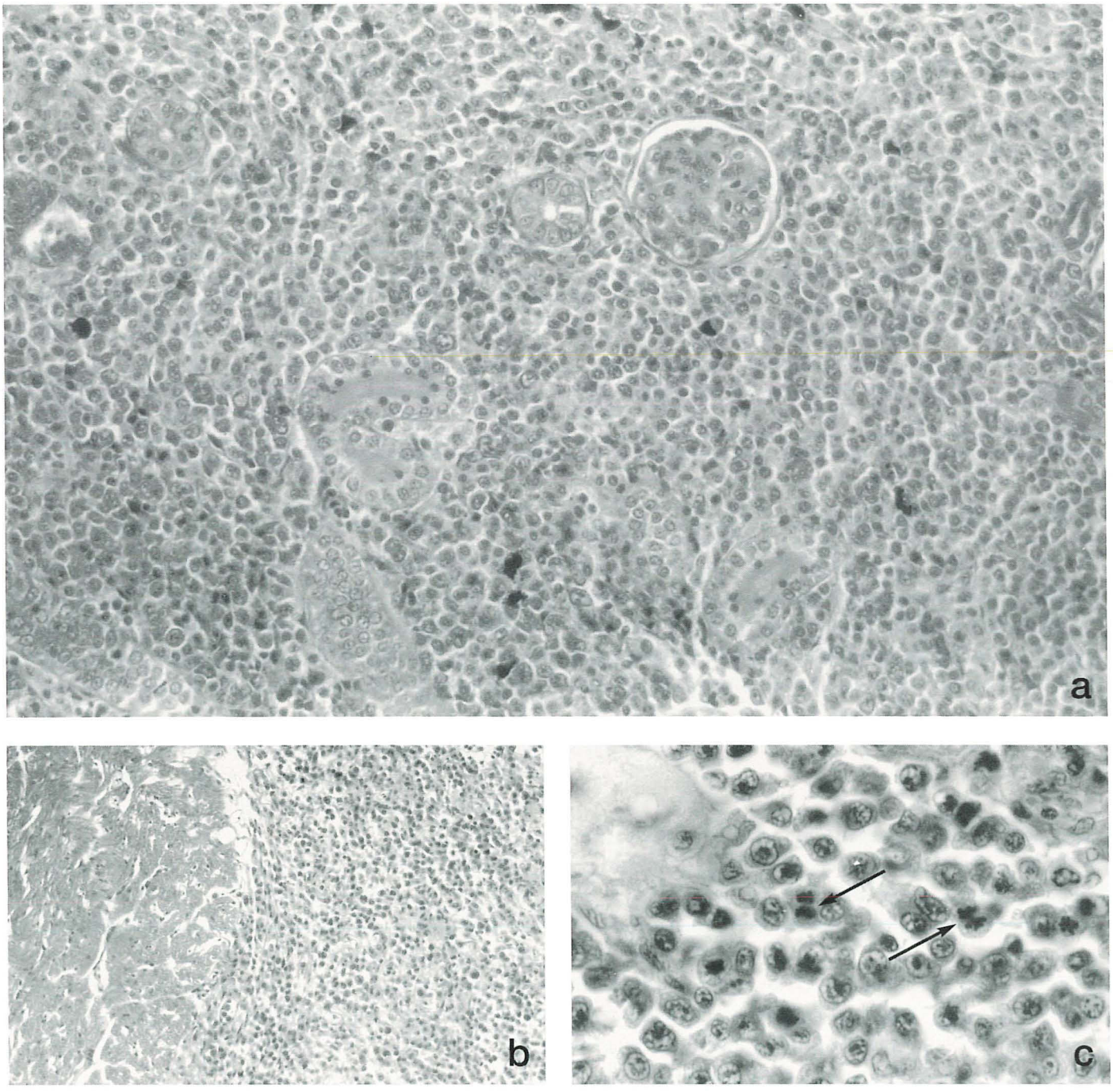


Figure 13-3. Plasmacytoid leukemia. Liver imprint stained with Giemsa. Arrow = plasmablasts.

exhibit prominent hyperplasia of the interstitium due to proliferation of the plasmablasts (Fig. 13-4). Thickened basement membranes in the capillaries of the glomerulus are often observed. In severely affected fish, there is a perivascular infiltration of the plasmablasts within the liver, and the cells often proliferate within the sinusoids.

The pericardium of the ventricle, atrium, and bulbus arteriosus of the heart may be infiltrated by the plasmablasts, which forms a thick cellular capsule that surrounds the heart. The cells may also infiltrate the endocardium, particularly in the bulbus arteriosus, and plasmablasts occur throughout the vascular sinuses of the heart. When the lower intestine is affected, there is massive proliferation of the plasmablasts in the lamina propria and in the submucosa, resulting in expansion of the intestinal villi.

Figure 13-4. Histology of plasmacytoid leukemia (Courtesy of Dis. Aquat. Org.). a. Hyperplasia of the kidney interstitium caused by proliferation of plasmablasts. b. Proliferation of plasmablasts in pericardium. c. Plasmablast proliferation in pancreas. Note numerous mitotic figures (arrows).



DIAGNOSIS. Confirmatory diagnosis of PL based on gross pathological changes is impossible because these changes are very similar to those of fish with BKD. Nevertheless, Stephen and Ribble (1996) developed an alogorithm for field investigations of PL employing gross pathological signs that is reasonably accurate. Presumptive diagnosis and distinction from BKD can be achieved by the examination of Giemsa-stained and Gram-stained imprints of the liver and kidney, respectively. Examination of an organ that is normally not a site of hemopoiesis, such as the liver, is recommended because differentiation of abnormal populations of plasmablasts from normal hemoblasts in organs such as the spleen and kidney may be difficult. The occurrence of large numbers of immature blood cells (presumably plasmablasts) in the liver (Fig. 13-3), and the absence of numerous *Renibacterium* organisms in the kidney and liver allows for a presumptive positive diagnosis for PL. However, a false negative diagnosis can occur using these criteria alone because PL-affected fish may exhibit concurrent BKD, and fish with PL do not always exhibit numerous plasmablasts in the liver.

Therefore, confirmatory diagnosis should be based on histological examination. Diagnosis by histology is based on the occurrence of renal interstitial hyperplasia of cells resembling the plasmablasts, and proliferation of these cells in at least one non-hemopoietic organ. Stephen and Ribble (1996) expanded the case definition of PL based on histology, which included the following characteristics: 1) hyperplasia of the interstitial cells of the posterior kidney; 2) increased proportion (>15%) of large mononuclear cells, blast cells and mitotic figures in the renal interstitium; 3) the presence of mononuclear cell infiltrates, composed primarily of large mononuclear cells and blasts, in at least one organ other than the spleen or kidney; 4) no important signs of granuloma formation or necrosis in the organs examined. Concurrent BKD can mask the histological diagnosis – i.e., the severe, chronic inflammatory response that occurs throughout the viscera and occasionally the eye in fish with severe BKD can mask the detection of the plasmablast infiltrates.

CONTROL AND TREATMENT. The mode of transmission of PL and the source of the infection in the field is still unclear. Therefore, precise recommendations for the control of the disease at affected sites are not available. Given the infectious nature of PL and its ability to cause high mortality, fish should not be transferred from sites with PL to sites where the disease has not been found. Until the risk of vertical transmission has been further evaluated it would be prudent not to use eggs or smolts that originated from brood stock with a history of PL. If this is not possible, the individual spawners should be examined, and eggs and sperm from PL-positive fish should be discarded.

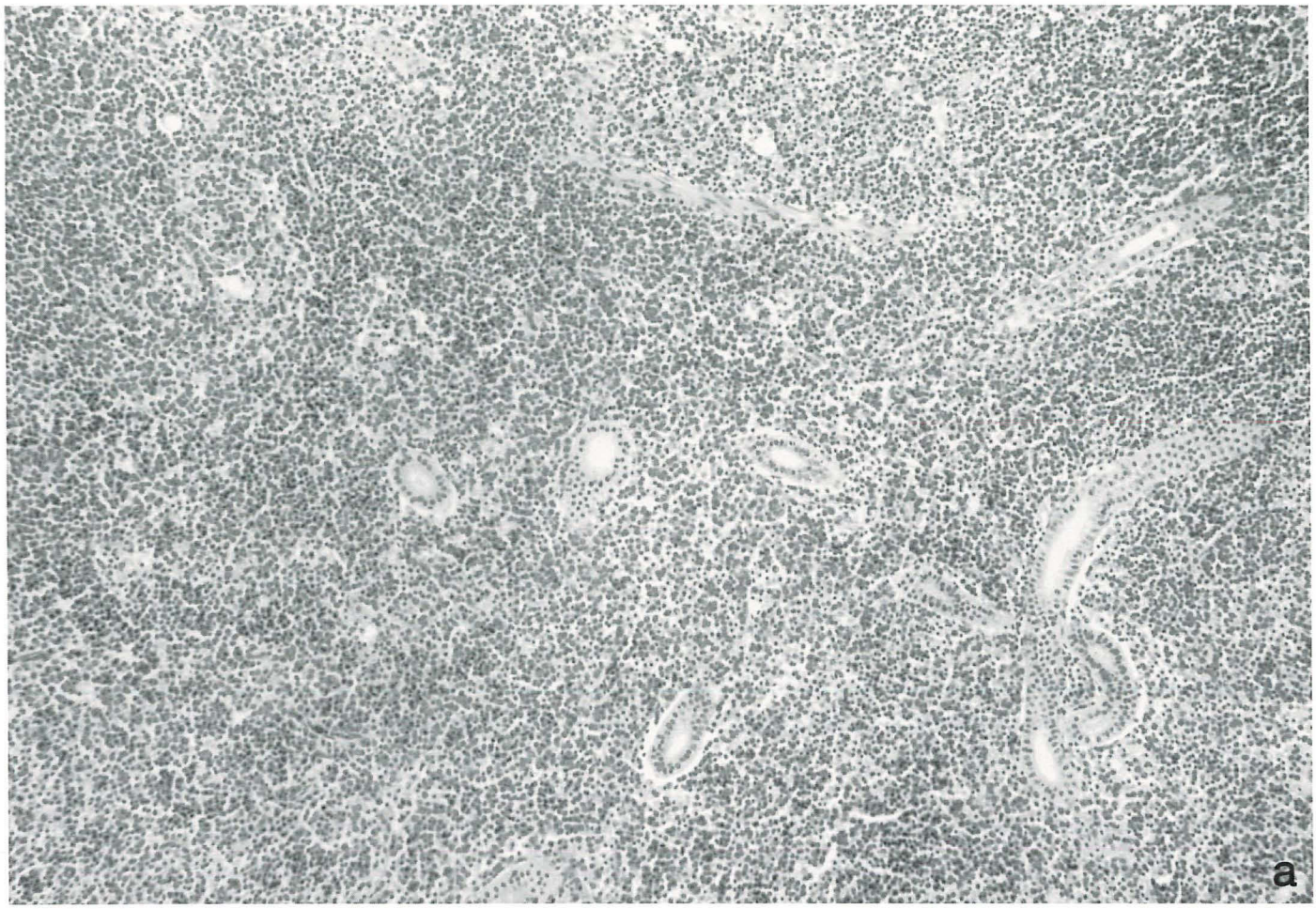
There are no commercially available anti-leukemia drugs for fish. However, Hedrick et al. (1991b) reported that treatment of a disease similar to PL in California with fumagillin, an anti-microsporidial drug, reduced infections by *N. salmonis* and the concurrent leukemia-like condition. We have achieved similar results with a fumagillin analog, TNP-470 (see page 66). This indicates that, although *N. salmonis* is not believed to be the primary cause of the PL, elimination of potential co-factors, such as *N. salmonis* infections and BKD, may be helpful for the control of PL. However, this drug is not useful for treating PL in cases were *N. salmonis* is not associated with the condition (Kent and Dawe 1993).

Lymphosarcoma and Lymphoma

Lymphosarcomas and lymphomas are occasionally observed in wild and cultured salmonids, and there are a few reports of these neoplasms from pen-reared salmonids. One case in Chile, many fish with visceral lymphosarcoma were observed at harvest (Inostroza, R., pers. comm.). The kidney or thymus are usually the primary organs involved, but these neoplasms may occur on the skin or scattered through the trunk musculature (Roald and Håstein 1979). Oncogenic viruses are often the cause of lymphoid tumors, including those of fishes (Papas et al. 1976; Bowser and Casey 1993), but to date none have been isolated from lymphosarcomas of salmonids.

CLINICAL SIGNS AND GROSS PATHOLOGY. As with plasmacytoid leukemia, fish with disseminated lymphosarcomas may be anemic. However, it is remarkable how severely affected a fish may be without showing outward morbidity. Discrete lymphomas are usually solid, whitish lesions. For example, thymic lymphomas appear as white masses in the opercular cavity (Fig. 13-1c). With disseminated (malignant) lymphosarcoma, the kidney is often extremely enlarged along the entire length (Fig. 13-1a).

MICROSCOPY. Lymphoid tumors are usually identified by histology. The infiltrated organ or tumor mass is deeply basophilic. In contrast to PL, the proliferating lymphoblasts of these neoplasms have minimal cytoplasm and are monomorphic (Fig. 13-5). Characteristic of neoplasms, there is often an abundance of mitotic figures.



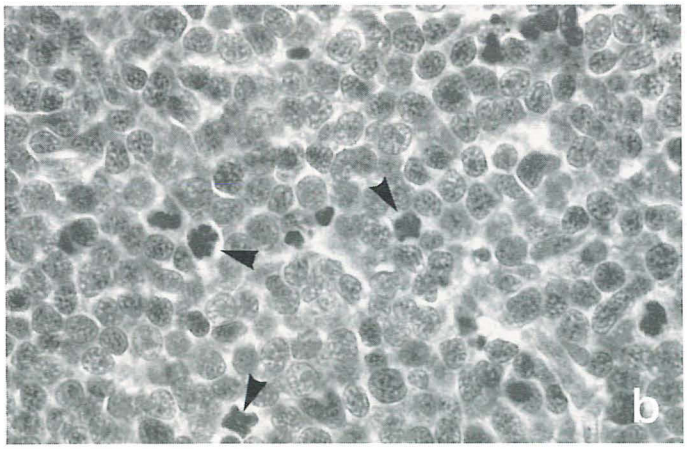


Figure 13-5. Lymphosarcoma in kidney of sockeye salmon. a. Massive infiltration of proliferating lymphoblasts in kidney interestitum. b. high magnification showing enlarged nucleus, prominent nucleolus, and minimal cytoplasm. Note numerous mitotic figures (arrowheads).

DIAGNOSIS. Diagnosis is confirmed by histological examination of affected tissues.

CONTROL AND TREATMENT. Fortunately this neoplasm is usually only an incidental finding in netpen farms. These neoplasms are often caused by oncogenic viruses (e.g., retroviruses), some of which may be vertically transmitted. Therefore, it would be prudent not to use fish with lymphoid tumors for brood stock.

Swimbladder Sarcoma

Leiomyosarcomas of the swimbladder have been reported from pen-reared Atlantic salmon (McKnight 1978). Duncan (1978) observed retrovirus-like particles in the tumors, but the etiology of this neoplastic disease has not been confirmed.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are generally in poor condition and lethargic. Internal examination reveals multiple, large masses in the swimbladder. Typical of sarcomas, the tumors are whitish, hard, and in severely affected fish they may occupy the entire length of the swimbladder (Fig. 13-6).

MICROSCOPY. Histological examination reveals that they arise from the junction of the inner smooth muscle layer and the areolar tissue zone. The tumors are well-differentiated, and consist of bundles of spindle cells (McKnight 1978).

DIAGNOSIS. Presumptive diagnosis is observation of multiple, firm nodules in the swimbladder, and identification of the tumor is based on histological analysis.

CONTROL AND TREATMENT. Duncan (1978) presented evidence that the disease is caused by an oncogenic virus, but the source of the infection has not been elucidated. As with other viral diseases, avoidance of infected stocks is probably the best method for controlling the disease.

Hepatocellular Carcinoma

Neoplasms of the liver are often caused by chemical carcinogens, and the most recognized cause of this lesion in salmonids in aquaculture is caused by aflatoxin. The toxin is produced by *Aspergillus* spp., which commonly grows in rancid feeds containing cotton seed meal. Rainbow trout are particularly sensitive to the toxin (Halver 1976; Wales 1967; Hendricks et al. 1984), and in the early 1960's many epizootics occurred in freshwater trout farms before the cause was recognized. Fish exposed to high doses may develop tumors as short as 4 months post exposure (Hendricks et al. 1984). We know of one report of an outbreak of hepatocellular carcinoma in seawater pen-reared rainbow trout, which occurred in Denmark (Rasmussen et al. 1986).

CLINICAL SIGNS AND GROSS PATHOLOGY. The disease is characterized by the appearance of an enlarged liver with muliple nodules (Fig. 13-1e).

MICROSCOPY. Hendricks et al. (1984) describes the histological progression of aflatoxin-induced liver tumors in rainbow trout. Early in development, preneoplastic nodules are often observed in the liver parenchyma. Aggressive carcinomas are then found. Aflatoxin exposure may also induce other liver lesions, such as ceroidosis, adenofibrosis, hepatic megalocytosis, and pancreatic cell metaplasia (Wales 1967; Hendricks et al. 1984).

Figure 13-6. Swimbladder fibrosarcoma in Atlantic salmon.

DIAGNOSIS. Diagnosis of hepatocellular carcinoma is based on histology. Because other agents may induce this neoplasm, confirmatory diagnosis is obtained by detecting high levels of aflatoxin in the feed.

CONTROL AND TREATMENT. Avoidance of cottonseed meal and proper handling and storage of feeds will eliminate the risk of aflatoxin-induced liver tumors in fish.

Epidermal Papillomatosis

Epidermal papillomas are one of the most common neoplasms of fishes, and have been observed on numerous fish species. Some are associated with exposure to chemical carcinogens (Harshbarger et al. 1993), whereas as others are apparently caused by oncogenic viruses (Wolf 1988a; Bowser and Casey 1993). Epidermal papillomas (warts) have been reported from both wild and farmed Atlantic salmon (Bylund et al. 1980; Rand 1985; Smail 1989), and has been observed in marine-cultured rainbow trout (Roberts and Bullock 1979) and Atlantic salmon (Carlisle 1977; Carlisle and Roberts 1977). The condition is particularly common in fish undergoing smoltification, but may persist or first appear after transfer to sea water. Virus particles have been visualized in the lesions (Carlisle 1977; Shchelkunov et al. 1992), and a virus is probably the underlying cause of the disease. However, stressors, such as environmental and hormonal changes, are probably important co-factors (Smail 1989).

CLINICAL SIGNS AND GROSS PATHOLOGY. The warts appear as multifocal, raised, light-colored or hyperemic plaques on the skin (Fig. 13-1d). The lesions appear to grow in size and coalesce. Advanced lesions may then regress and be sloughed, sometimes leaving an ulcer. The lesions seldom cause high mortality in sea water, but have been a significant problem in a few freshwater outbreaks. Affected fish may be lethargic (Karaseva and Beskrovny 1991).

MICROSCOPY. Typical of epidermal papillomata, histological examination of the lesions reveals massive, focal hyperplasia of the epidermis, which often form convoluted folds.

CONTROL AND TREATMENT. In sea water, Smail (1989) recommended reduced handling to minimize damage to the skin. Bylund et al. (1980) found that baths with formalin or NaCl reduced the severity of the condition in salmon held in freshwater. Karaseva and Beskrovny (1991) reported that BIOMOS (a high polymer melanin-like metal complex) in combination with tetracycline added to the feed reduced the incidence of affected fish. They concluded that this positive effect was due to overall improvement of the health status of the fish. As with other viral diseases, probably the best method for dealing with epidermal papillomatosis is avoidance of affected fish and improvement of culture conditions.

APPENDIX I - **GLOSSARY**

Amphophilic. Staining readily with both acid or basic dyes. Amphophilic material usually is purple in hematoxylin and eosin-stained tissue sections.

Anemia. Reduction below normal levels of red blood cell mass in the circulatory system. Anemia is caused by hemorrhage (hemorrhagic anemia), increased red blood cell destruction (hemolytic anemia), nutritional deficiencies (e.g., iron, folic acid or vitamin B_{12} deficiencies), and suppression of hemopoiesis (aplastic anemia).

Ascites. Accumulation of serous fluid in the peritoneal cavity. A form of edema.

Ataxia. A loss of muscular coordination with impaired balance.

Atrophy. Reduction in mass of a tissue or organ, due to a reduction in cell number or cell size.

Autolysis. The initial degenerative changes in dead cells as a result of the action of endogenous enzymes. Postmortem autolysis occurs after the animal has died.

Basophilic. Having an affinity to basophilic dyes. In hematoxylin and eosin-stained tissue sections basophilic material stains blue.

Caseation. Caseous necrosis. Formation of necrotic tissue which, due to its focal nature, is soft, pasty or cheese-like.

Cataract. A loss of transparency in the lens of the eye.

Cataractous Changes. Denoting pathological changes associated with the formation of cataracts.

Ceroid. Wax-like, golden or yellow-brown intracellular material. A type of lipofuscin. Ceroid is usually formed from remaining indigestible breakdown products in phagolysosomes.

Coelozooic. Living within the lumina (tissue spaces) of organs, such as the lumina of renal tubules or the gall bladder.

Coracidia. Plural for coracidium, a larva with a ciliated epithelium that hatches from the egg of certain tapeworms.

Definitive host. Host in which a parasite achieves sexual maturity.

Digitiform. Finger-like.

Ecchymotic. Denoting hemorrhages larger than petechiae, often up to 2-3 cm.

Edema. Accumulation of abnormal amounts of fluid in the intercellular tissue spaces. Edema can be caused by decreased plasma osmotic pressure, increased blood pressure, lymphatic obstruction, or increased capillary permeability.

Encephalitis. Inflammation of the brain.

Eosinophilic. Having an affinity to acidic dyes. In hematoxylin and eosin-stained tissue sections eosinophilic material stains orange or red.

Epizootic. Denoting a disease attacking many animals in population simultaneously.

Erythema. Redness of the skin.

Etiology. Causation; the study of the cause of disease.

Exophthalmus. Protrusion of the eye; "pop-eye".

Fibroplasia. Abnormal, non-neoplastic increase in fibrous tissue.

Friable. Having a gritty texture. Falls apart when handled and is easily reduced to powder.

Glomerulus. A plexus of capillaries at the beginning of each uriniferous tubule in the kidney.

Golgi body. A complex of membranes in the cytoplasm of a cell, which is distributed in a parallel orientation adjacent to the nucleus. This organelle probably functions as a site of production and packaging of cellular secretions.

Granuloma. Focal accumulation of inflammatory cells comprised primarily of macrophages. Formation of granulomas is associated with chronic inflammation.

Hemoblast. Immature blood cell.

Hemopericardium. Blood accumulation around the heart.

Hemopoiesis. The process of development and formation of blood cells.

Hemorrhage. The escape of blood from the cardiovascular system.

Hemosiderin. A storage form of excess iron. Hemosiderin often accumulates in the liver, spleen, or kidney interstitium, and is associated with the release of iron from lysed red blood cells.

Hematoma. A localized mass of extravascular blood that is confined within an organ, tissue or space. The blood is usually clotted.

Hepatic megalocytosis. Megalocytic hepatosis. The formation of hypertrophied hepatocytes (liver cells) with greatly enlarged, often dark-staining, nuclei. This condition is apparently due to a failure of cell division in mitosis, resulting in polyploid cells. Hepatic megalocytosis is an indication of cellular toxicity, and can be induced by exposure to anthropogenic (man-made) contaminants or natural toxins.

Hepatotoxicant. A toxicant that effects the liver.

Helminth. Denoting a worm. Helminth parasites include digenean trematodes, cestodes, monogeneans, nematodes, and acanthocephalans.

Histozoic. Living within tissues outside of the cell.

Hydropic degeneration. A degenerative process at the cellular level that results in the formation of many vacuoles of variable size in the cytoplasm. Commonly the vacuoles represent distended organelles, especially the endoplasmic reticulum.

Hyperemic. Showing increased quantity of blood.

Hyperplasia. An increase in the number of normal cells. Hyperplasia will not progress, and usually resolves itself, in the absence of the causative stimulus (this is an important distinction from neoplasia).

Hypertrophy. Enlargement of individual cells or an organ.

Idiopathic. Denoting a disease of unknown cause.

Intermediate host. Host in which a parasite develops to some extent but not to sexual maturity.

Juxtanuclear hof. A clear-staining area adjacent to the nucleus representing the Golgi body.

Lamina propria. The stratum of connective tissue and associated cells underlying the epithelium of various organs, including the gut.

Leucopenia. Any situation when the total number of circulating white blood cells are less than normal.

Leukemia. Progressive proliferation of abnormal blood cells found in the hemopoietic tissues, other organs, and usually resulting in increased numbers of these cells in the blood. Leukemias are considered to be neoplasms of the blood.

Lumina. Plural for lumen, the space in the interior of a tubular structure such as a kidney tubule or artery.

Lunules. Disc-like cups on the ventral surface of the anterior margin of the body in *Caligus* spp. (parasitic copepods).

Lymphocyte. A white blood cell important in the immune response of fish. B lymphocytes are produce antibody, whereas as T lymphocytes are important in the cellular arm of the immune response. Approximately 95% of salmonid fish white blood cells are lymphocytes. The cell contains a large nucleus and a narrow rim of basophilic cytoplasm.

Lymphoblast. Immature lymphocyte.

Macrophage. A large, mononuclear phagocytic white blood cell. Macrophages engulf foreign material, including pathogens, and are associated with chronic inflammation. Fixed macrophages (= reticuloendothelial system) are abundant in the kidney interstitium, atrium of the heart, and spleen.

Macroscopic. Visible with the naked eye.

Meningitis. Inflammation of the meninges (the covering of the brain).

Melanomacrophage. A macrophage containing melanin. As with normal macrophages, these cells are often associated with chronic inflammation.

Melanin. Black or brown pigment thought to play a role in the detoxification of free radicals and cations.

Metacercaria. Stage between the cercaria and adult in the life cycle of most digenetic trematodes. Usually encysted and quiescent. This stage is absent in blood flukes.

Metacestode. Juvenile developmental stage of a cestode. In fish, this stage is usually equivalent to the plerocercoid.

Metaplasia. Transformation from one cell type to another.

Megalocytosis. See hepatic megalocytosis.

Merogony. Developmental phase of certain protozoans (e.g., coccidians, microsporidians) involving asexual, vegetative proliferation.

Miliary. Denoting multiple white, cysts throughout the body or in an organ.

Miracidium. The first larval stage of digenean trematodes. Miracidia infect the first intermediate hosts of digenean trematodes, which are usually molluscs. Miracidia are small, ciliated and swim actively.

Multinucleate giant cell. A syncytia of macrophages. This cell is often found in granulomas of higher animals and occasionally in fish.

Myocardium. Heart muscle.

Necrosis. Cell death within a living organism.

Neoplasia. The pathologic process resulting in the growth of a neoplasm.

Neoplasm. An abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissues, and persists after the causative stimulus is removed.

Nephritis. Inflammation of the kidney.

Neutrophil. A white blood cell similar in morphology to neutrophils of higher animals. The cytoplasm is filled with fine granules and the nucleus is lobate and segmented. Although this cell is important in acute inflammation in mammals, the cell is relatively rare in fish and its function in fish is not entirely clear.

Oncogenic. Denoting an agent that causes neoplasia.

Panophthalmitis. Inflammation throughout the eyeball.

Pathognomonic. Characteristic or indicative of a disease.

Petechiae. Minute, focal hemorrhages up to 1 to 2 mm.

Plasma cell. Mature B lymphocyte that has a well organized rough endoplasmic reticulum and produces a relatively large amount of antibody.

Plasmablast. Immature plasma cell.

Plerocercoid. Cestode larva that develops from a procercoid. This stage is often found in the tissues of fishes.

Procercoid. Cestode larva that usually infects the first intermediate hosts. In aquatic cestodes, this host is usually a crustacean.

Pyriform. Pear-shaped.

Retrobulbar. Behind the eyeball.

Septicemia. Systemic infection of the cardiovascular system.

Serosanguineous. Denoting a discharge or exudate containing serum and blood.

Sporogeny. A developmental phase in certain protozoans (e.g., microsporidians) that results in the formation of spores.

Stomatitis. Inflammation of the mouth.

Suppurative. Refers to the formation of pus, which is large comprised of necrotic host cells and debris. Suppurative lesions are often caused by bacteria.

Thrombi. Plural for thrombus, a solid mass formed within a blood vessel.

Vasculitis. Inflammation of blood vessels.

Vitreous chamber. Posterior chamber of the eye, between the lens and retina.

Xenoma. A host cell packed with microsporidian parasites and that is greatly hypertrophied. The host cell nucleus is also greatly hypertrophied, which may be branched or fragmented.

Zoonoses. Diseases transmitted from animals to man.

APPENDIX II - **SCIENTIFIC NAMES OF FISHES**

amago salmon - *Oncorhynchus rhodurus*

American eel- *Anguilla rostrata*

Arctic char - *Salvelinus alpinus*

Arctic grayling - *Thymallus arcticus*

Atlantic cod - *Gadus morhua*

Atlantic halibut - *Hippoglossus hippoglossus*

Atlantic herring - *Clupea harengus*

Atlantic lumpfish - *Cyclopterus lumpus*

Atlantic salmon - *Salmo salar*

big skate - *Raja colliei*

bluegill sunfish - *Lepomis macrochirus*

brook trout - *Salvelinus fontinalis*

brown trout - *Salmo trutta*

chinook salmon- *Oncorhynchus tshawytscha*

chum salmon - *Oncorhynchus keta*

coho salmon - *Oncorhynchus kisutch*

copper rockfish - *Sebastes caurinus*

cutthroat trout - *Oncorhynchus clarki*

grayling - *Thymallus thymallus*

greenling - *Hexagrammos* sp.

eulachon – *Thaleichthys pacificus*

haddock - *Melanogrammus aeglefinus*

Japanese flounder - *Paralichthys olivaceus*

Japanese yellowtail - *Seriola quinqueradiata*

kokanee salmon - *Oncorhynchus nerka*

lumpfish - *Cyclopterus lumpus*

masou salmon - *Oncorhynchus masou*

mummichog - *Fundulus heteroclitus*

North Sea whiting - *Merlangius merlangus*

Pacific cod - *Gadus macrocephalus*

Pacific herring - *Clupea harengus pallasi*

Pacific hake - *Merluccius productus*

pink salmon - *Oncorhynchus gorbuscha*

plaice - *Pleuronectes platessa*

rainbow trout - *Oncorhynchus mykiss*

sea trout - *Salmo trutta*

shiner perch- *Cymatogaster aggregata*

Siamese fighting fish - *Betta splendens*

silversides - *Menidia menidia*

snoek – *Thyrsites atun*

sockeye salmon - *Oncorhynchus nerka*

speckled sanddab - *Citharichthys stigmaeus*

spiny dogfish - *Squalus acanthias*

spotted ratfish - *Hydrolagus colliei*

sprat - *Sprattus sprattus*

steelhead trout - *Oncorhynchus mykiss*

tubesnout - *Aulorhynchus flavidus*

threespine stickleback - *Gasterosteus aculeatus*

walleye pollock - *Theragra chalcogramma*

yamame salmon - *Oncorhynchus masou*

APPENDIX III - **PRESERVATIVES AND CULTURE MEDIA**

Lugol's Preservative

Dissolve 100 g potassium iodide in 1 L distilled water. Then dissolve 50 g crystalline iodine into the solution. Finally, add 100 ml glacial acetic acid. Any precipitate is removed by filtration or decanting. Lugol's is added to the sample until the sample has a weak tea color (about 1 drop/ml).

Davidson's solution

95% ethanol 300 ml
formalin 200 ml
glacial acetic acid 100 ml
distilled water 300 ml

Millonig's buffer for electron microscopy

Solution A 2.62 % NaH_2PO_4
Solution B 2.52 % NaOH

Working solution:

Mix 83 ml Solution A with 17 ml Solution B.
Adjust pH to 7.4 with 10 N NaOH or concentrated HCl.

For transmission electron microscopy of fish tissues, we generally prepare 4% glutaraldehyde in Millonig's buffer. Fix at small pieces of tissue at room temperature for 2 h, then hold overnight at 4 °C. Transfer to Millonig's buffer without glutaraldehyde.

50% Seawater-Cytophaga Medium

tryptone 0.5 g
yeast extract 0.5 g
sodium acetate 0.2 g
beef extract 0.2 g
agar 11.0 g

add above to:

filtered sea water 500 ml
distilled water 500 ml

autoclave and pour into sterile petri dishes.

LEISHMAN'S GIEMSA

This stain is useful for examining protozoa, bacteria and cell morphology in tissue imprints.

Leishman's. Add 1 g Leishman's stain to 500 ml absolute methanol and filter.

Giemsa Stock Solution. Add 1 g powder to 66 ml of glycerol and heat at 60 °C for one hour. Then add 66 ml of absolute methanol and filter. Store at 40 °C.

Phosphate Buffer Solutions.

Solution A - 31.20 g NaH_2PO_4 , 2 H_20 in 1 L distilled water
Solution B - 53.65 g Na_2HPO_4 , 7 H_20 in 1 L distilled water

Giemsa Working Solution.

To prepare phospate buffer, mix 73.5 ml solution A with 26.5 ml solution B. Then add 100 ml distilled water.

Add 3.5 ml stock solution to 50 ml phosphate buffer (pH 6.4). Make fresh for each use.

Staining Procedure:

1. Prepare smear or imprint and allow to dry for about 1/2 hour.
2. Fix in methanol for 2-5 min.
3. Stain in Leishman's for 2-3 min.
4. Stain in Giemsa for 10-12 min.
5. Rinse in distilled water for about 1 min.
6. Air dry.

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