Journal of Fish Diseases 2003, **26,** 627–646

Review

Cryptobia (*Trypanoplasma*) *salmositica* and salmonid cryptobiosis

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Abstract

Salmonid cryptobiosis is caused by *Cryptobia (Trypanoplasma) salmositica*. The haemoflagellate has been reported from all species of Pacific *Oncorhynchus* spp. on the west coast of North America. It is normally transmitted by the freshwater leech, *Piscicola salmositica*, in streams and rivers, and sculpins, *Cottus* spp., are considered important reservoir hosts. The pathogen can also survive on the body surface of fish because it has a contractile vacuole to osmoregulate when the fish is in fresh water. This allows for direct transmission between fish, especially in aquaculture facilities. The parasite divides rapidly by binary fission in the blood to cause disease, the severity of which is directly related to parasitaemia. *Cryptobia salmositica* has a mitochondrium and it normally undergoes aerobic respiration; however, if its mitochondrium is damaged it will switch to glycolysis. Its glycolytic enzymes and catalase are contained in glycosomes. Cysteine protease is a metabolic enzyme, and its neutralization inhibits oxygen consumption and multiplication of the parasite. An important virulent factor in cryptobiosis is a secretory metalloprotease. The protective mechanism involves production of complement fixing antibodies, phagocytosis by macrophages, and cell-mediated cytotoxicity. Recovered fish are protected, probably for life as the immunity is non-sterile. Clinical signs of the disease include anaemia, anorexia, splenomegaly, general oedema and abdominal distension with ascites. The metabolism and swimming performance of infected fish are significantly reduced and the bioenergetic cost of the disease is very considerable. Fish are susceptible to hypoxia and their immune system is depressed during acute cryptobiosis. Severity of the disease and mortality rates vary significantly between species and stocks of salmon. Protective strategies include selective breeding of *Cryptobia*-resistant fish. This is innate resistance to infection and it is controlled by a dominant Mendelian locus. In these fish the parasite is lysed via the alternative pathway of complement activation. In *Cryptobia*-tolerant fish (infected with the pathogen but which do not suffer from disease) the metalloprotease secreted by the parasite is neutralized by α_2 macroglobulin. Hence, the production of a transgenic *Cryptobia*-tolerant salmon is an option. This strategy has the advantage in that human intervention (e.g. vaccination, chemotherapy) is not required once the transgenic fish is produced. Acquired immunity is another option; a single dose of the attenuated live vaccine protects fish for at least 2 years. The protective mechanism in vaccinated fish is similar to that in recovered fish. The trypanocidal drug, isometamidium chloride, is an effective therapeutic and prophylactic agent. It accumulates in the mitochondrium of the parasite and significantly disrupts aerobic respiration by causing lesions in the organelle. Efficacy of the drug is significantly increased after its conjugation to antibodies. This immuno-chemotherapeutic strategy has

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the advantage in that it will lower the drug dosage and hence side-effects of chemotherapy. It will probably reduce the accumulation of the drug in fish, an important consideration in food fish.

Keywords: biology, control, *Cryptobia salmositica*, cryptobiosis, host–parasite relationships, salmonids, systematics.

Introduction

This review is divided into three sections. The first section is on the systematics and the biology of the haemoflagellate, *Cryptobia (Trypanoplasma) salmositica* (Fig. 1) while the second section deals with host–parasite relationships, including the disease mechanism. The final section is on the development of control strategies against the pathogen and disease.

Systematics and biology of *Cryptobia (Trypanoplasma) salmositica*

Systematics

The genus *Cryptobia* was proposed to describe a biflagellated protozoan isolated from the reproductive system of snails (Leidy 1846). It now includes morphologically similar parasites infecting other invertebrates and vertebrates, and there are at least 52 nominal species (five from the body surface and gills, seven from the digestive tract and 40 from the blood) described from fish (Woo 1994). Laveran & Mesnil (1901) proposed the genus *Trypanoplasma* for a biflagellated protozoan in the blood of a freshwater fish. Crawley (1909) synonymized *Trypanoplasma* with *Cryptobia* because he was unable to find morphological differences between the two organisms.

The synonymy has not been accepted by all workers. In general, European workers (e.g. Lom 1979; Steinhagen, Kruse & Korting 1990) retained the genus *Trypanoplasma* for haematozoic species while workers in North America (e.g. Bower & Woo 1977; Newman 1978; Khan, Lobos, Garcias, Munoz, Valdebenito & George-Nascimento 2001; Woo 2001) agreed with Crawley and used the genus *Cryptobia* for haematozoic and non-haematozoic species. Woo (1987, 1994, 1998) has always supported Crawley because there are also biological similarities between the two groups of organisms. For example, *Cryptobia salmositica* (Fig. 1) is a haematozoic species that infects *Oncorhynchus* spp. along the Pacific coast in North America. It is normally transmitted indirectly by the leech, *Piscicola salmositica* in freshwater streams (Becker & Katz 1965a,b). However, this pathogen also has an ectoparasitic phase (Fig. 2) on the body surface of infected rainbow trout, *Oncorhynchus mykiss* (Walbaum), and like the non-haematozoic species it can be transmitted directly between fish in the absence of blood-sucking leeches (Woo & Wehnert 1983). In addition, like the non-haematozoic *Cryptobia*, the blood form of *C. salmositica* has a pulsatile (contractile) vacuole so that it can osmoregulate when it is on the body surface of fish (Paterson & Woo 1983).

Woo & Wehnert (1983) suggested that haematozoic species were derived from free-living *Procryptobia* (Vickerman 1978) via ectoparasitic species that lived on the body surface of fish, while Nohynkova (1984) proposed the haematozoic *Cryptobia* were linked to flagellates that lived in the digestive tract of fish. Both hypotheses agree that the haematozoic and non-haematozoic species are very closely related. Consequently, Woo (1994) proposed to divide the genus *Cryptobia* into two subgenera; this would take into account the similarities and differences between the two groups. Briefly, the haematozoic *Cryptobia* (normally transmitted indirectly by leeches) were placed in the subgenus *Trypanoplasma*, while the non-haematozoic species (parasitic on the body surface or in the digestive tract with direct transmission) were placed in the subgenus *Cryptobia*. This approach indicates a very close phylogenetic relationship between the two groups and yet indicates some distinctive biological differences (e.g. their transmission patterns). Wright, Li, Feng, Martin & Lynn (1999) studied the small-subunit ribosomal RNA gene sequences of kinetoplastids including four species of haematozoic *Cryptobia* (one from Europe and three from North America). They confirmed the four *Cryptobia* are very closely related, and that '…neither tree topology, nor nucleotide differences, nor the type of RNA editing supports a generic distinctiveness for *Trypanoplasma*'. Based on kDNA structure and small-subunit rRNA gene sequences, Dolezel, Jirku, Maslov & Lukes (2000) concluded differently; they suggested '…the subgeneric status of *Trypanoplasma* within the genus *Cryptobia*, as proposed by Woo (1994), is inadequate, and that the genus *Trypanoplasma*…be considered valid'. More recently Callahan, Litaker & Noga (2002),

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supported the conclusion of Wright *et al.* (1999). It is evident that more studies, especially on the non-haematozoic *Cryptobia*, are required to resolve this systematic problem.

As noted earlier, it is accepted that the haematozoic and non-haematozoic species in fish are very closely related (Woo & Wehnert 1983; Nohynkova 1984). A few non-haematozoic species (e.g. *C. branchialis*, *C. iubilans*) are known to cause disease while others (e.g. *C. stilbia*, *C. dahli*) are not pathogenic. Very little is known about the biology of non-haematozoic species from fish (those on the body surface and in the digestive system), hence molecular and biological studies of these species will be rewarding. They will help to determine the relationships between haematozoic and non-haematozoic species and also to clarify the status of *Trypanoplasma*. Until non-haematozoic species have been studied it is more prudent to assign all the species to *Cryptobia* and this has always been the approach taken by this author.

Cryptobia (*Trypanoplasma*) *salmositica*, geographical distribution, prevalence: percentage of infected animals in a population

Cryptobia (T.) salmositica (Fig. 1) was first described from the blood of coho salmon, *O. kisutch* (Walbaum), in Washington State, USA (Katz 1951). It is an elongated flagellate with a prominent kinetoplast and a nucleus at the anterior end. Two flagella arise at the anterior end; the anterior flagellum is free while the recurrent flagellum is attached to the body and extends beyond it as a free flagellum. In stained specimens, the body length is about 15 μm, body width about 2.5 μm, anterior flagellum length about 16 μm and posterior free flagellum length about 9 μm (Katz 1951).

The parasite has a cytostome and two flagella that arise from a flagellar pocket. Paterson & Woo (1983) found contractile (pulsatile) vacuoles in parasites from the blood of fish and this was most unexpected. The vacuole is located at the base of the flagellar pocket and is associated with the post-flagellar pit. Vacuoles in both systole and diastole were seen. Although this organelle has been found in non-haematozoic parasites it has not been reported from haematozoic *Cryptobia* (Brugerolle, Lom, Nohynkova & Joyon 1979). In general, ultrastructural features in *C. (T.) salmositica* are similar to those of *C. (T.) borreli* from the blood of cyprinids (Brugerolle *et al.* 1979), *C. dahli* from the stomach of fish (Khan, Moyles & Bal 1980) and *C. vaginalis* from leeches (Vickerman 1977).

Wales & Wolf (1955) found the parasite (misidentified as *Cryptobia (T.) borreli*) in six species of teleosts in northern California. The parasite was found in large numbers in tissues of salmonids at the Mt Shasta Hatchery, USA and it was associated with high mortality in salmonids (Leitritz 1960). The haemoflagellate has since been found in all species of Pacific salmon, *Oncorhynchus* spp., and numerous other teleosts in freshwater streams from northern California to south-western Alaska, USA (Woo 1987; Woo & Poynton 1995). Woo (1987) suggested the parasites found naturally in cyprinids and suckers are probably not *C. salmositica* as these fish have their own *Cryptobia* which are not infective to salmonids. In addition, these fish cannot be experimentally infected with *C. salmositica* and under *in vitro* conditions the parasite is lysed in the blood of these fishes via the alternative pathway of complement activation (Wehnert & Woo 1980).

The parasite was first found in coho fingerlings in 1954 in a coastal stream in Oregon, USA (Davison, Breese & Katz 1954). Its prevalence in young salmon varied from 3 to 21% between years and geographical areas (Becker & Katz 1966). Wood (1979) found the parasite in coho and chinook salmon <60 days old and fingerlings had detectable infections in late autumn and winter. Many downstream migrants (normally year old fish) in early spring were infected and experimental studies showed infected presmolt salmon retained their infections when transferred to salt water and mortality was not reduced in sea water (Bower & Margolis 1985). In the Fraser River drainage, British Columbia, Canada, mature salmon that return from the marine environment to spawn have detectable infections within 5 days of returning to fresh water, and the longer they are in fresh water the higher their parasitaemias. The prevalence of the infection in returning salmon is low in September but increases to about 100% in December (Bower & Margolis 1984b). These increases are related to increased numbers of leeches in November (Becker & Katz 1965b, 1966; Bower & Margolis 1984b). The parasite is considered an important pathogen of salmon in semi-natural and intensive salmon culture facilities on the west coast of North America (Bower & Thompson 1987).

The torrent sculpin, *Cottus rhotheus* (Smith), is considered an important reservoir host of *C. salmositica* although this will have to be confirmed with

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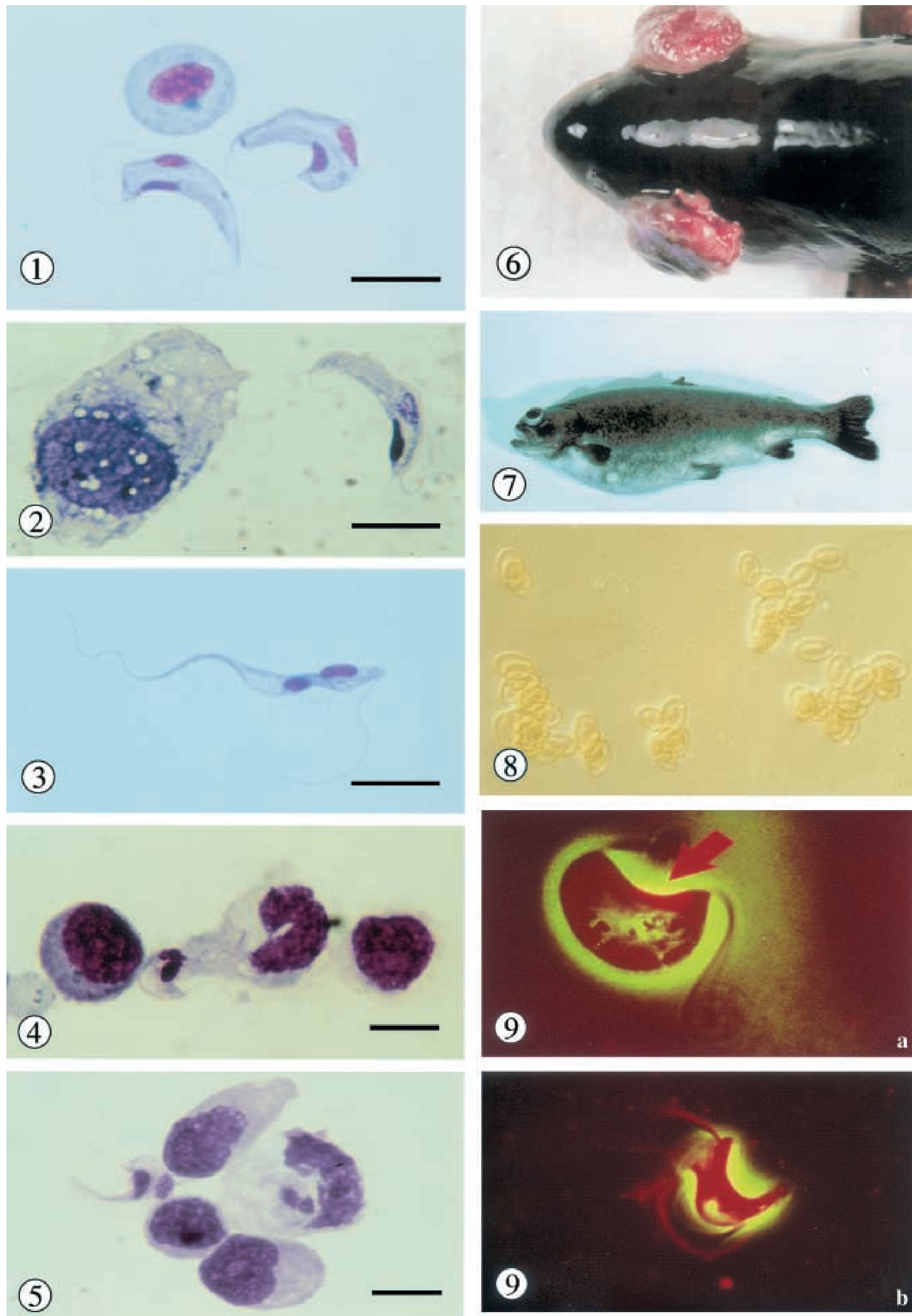

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careful cross-transmission studies. The DNA probe developed by Li & Woo (1996) can be used to confirm the identity of isolates from the *Cryptobia*-tolerant sculpins. According to Becker & Katz (1966) the overall prevalence was high (60%) in hatchery streams in Washington State, and lowest (27%) in small fish (< 65 mm). Parasitaemias were high in small fish and they decreased in the larger fish. In a coastal river in British Columbia, prevalences of the parasite ranged from 8 to 95% in *Cottus aleuticus* Gilbert (Bower & Margolis 1984b). In small sculpins (< 40 mm) the prevalence was low between August and November but was highest in April. This seasonal trend was also seen in large sculpins (> 40 mm) in which prevalence was also higher than in small fish.

Indirect and direct transmission of the pathogen between fish

The freshwater leech, *P. salmositica*, becomes infected after feeding on infected fish and large numbers of parasite are present in the crop of the leech 7–8 days after an infective blood meal. The parasite is transmitted when the infected leech feeds again (Becker & Katz 1965b). There are no indications the parasite is in the proboscis sheaths of infected leeches. Woo (unpublished data) has found numerous slender *Cryptobia* in the proboscis sheath of leeches removed from infected salmon from Vancouver Island, Canada. The flagellates are morphologically similar to the 'slender' ectoparasitic form of *C. salmositica* (Fig. 2) and a couple of the isolates are infective and cause disease when inoculated experimentally into rainbow trout, *O. mykiss* (Walbaum). Development of *C. salmositica* in, and transmission by, leeches need more careful laboratory studies.

Woo & Wehnert (1983) found the ectoparasitic form of the parasite (Fig. 2) in mucus on the body surface of trout about 6 weeks after the parasite was inoculated into the fish. This ectoparasitic form is infective when inoculated into fish. It is very likely the parasite is released onto the body surface through a blister near the abdominal pore of infected fish (Bower & Margolis 1983). Woo & Wehnert (1983) showed that 67–80% of uninfected trout became infected if allowed to mix freely with infected trout held in the same tank. If uninfected fish were 'downstream' of infected fish (in the same tank) but separated by a wire screen, about 50% became infected. They suggested ectoparasitic forms were transported to uninfected fish via mucous strands and the parasite entered the lining of the gills or oral cavity. Biochemical studies on the parasites (Zuo & Woo 1997a, 1998a) showed that it has two proteases, a cysteine protease (49, 60, 66 and 97 kDa) and a 200-kDa metalloprotease (Fig. 10). The metalloprotease is a histolytic enzyme (Fig. 11) and is an important virulence factor. It is probably also involved in direct transmission of the pathogen by first causing lesions on the skin so that the parasite becomes ectoparasitic and then allowing them to penetrate mucous membranes of uninfected fish (e.g. enters via the gill filaments when mucous strands are trapped on the gills).

Direct transmission was more efficient if heavily infected and uninfected juvenile sockeye salmon, *O. nerka* (Walbaum), were brought together daily in dip nets and held for a brief period out of water (Bower & Margolis 1983). The infection rate was 64–89% when fish were held in fresh water and it increased to 94% in sea water. In addition, the prevalence of infected coho salmon in a hatchery pond rose from 29 to 74% after fish were transferred whilst the prevalence remained the same in fish that were not transferred. It is possible the

Figure 1 *Cryptobia* (*Trypanoplasma*) *salmositica* with red blood cell from an experimentally infected rainbow trout, *Oncorhynchus mykiss* (Giemsa: bar = 10 μm).

Figure 2 *Cryptobia salmositica* in mucus from the body surface of a rainbow trout, *Oncorhynchus mykiss*, 6 weeks after infection; slender form next to an epithelial cell (Giemsa; bar = 10 μm).

Figure 3 Attenuated *C. salmositica* from culture; note it is much more slender than forms from the blood (Giemsa; bar = 10 μm).

Figures 4 & 5 Peritoneal macrophage in the ascites of an experimentally infected rainbow trout, *Oncorhynchus mykiss*; *C. salmositica* (×1000) in the process of being ingested (Giemsa bar = 10 μm) (from Woo 1979).

Figure 6 Exophthalmia in a rainbow trout, *Oncorhynchus mykiss*, with acute experimental infection (from Woo & Poynton 1995).

Figure 7 General oedema with abdominal distension and ascites during acute experimental disease in rainbow trout, *Oncorhynchus mykiss* (from Woo 1979).

Figure 8 Positive Coombs' reaction; red blood cells from an experimentally infected rainbow trout, *Oncorhynchus mykiss* upon addition of anti-trout immunoglobulin.

Figure 9 Phase contrast and fluorescent microscopy of *C. salmositica* after *in vitro* exposure to isometamidium chloride. (a) Exposed to the drug only – note accumulation of isometamidium (in red) in the kinetoplast; (b) exposed to the drug conjugated to polyclonal antibodies – note the drug is found throughout the organism (from Ardelli & Woo 2001d).

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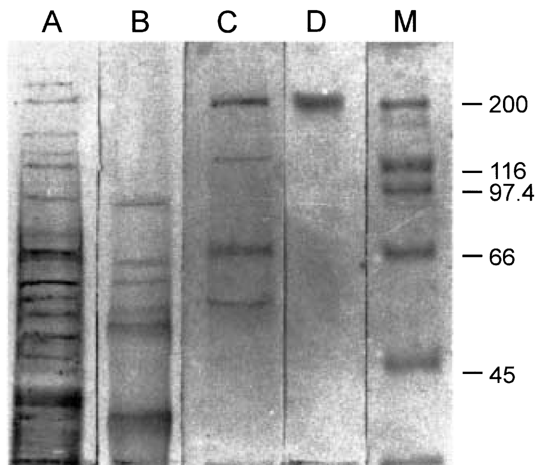

Figure 10 Purification of cysteine protease and metalloprotease from *C. salmositica*. Lane a, crude cell lysate; lane b, partially purified cysteine protease from DEAE-agarose column; lane c, partially purified metalloprotease from DEAE-agarose column; lane d, a single band of purified metalloprotease from Sephacryl S-300 column; M, molecular markers (kDa) (modified from Zuo & Woo 1998a).

increased prevalence in the 'moved' fish was the result of enhanced transmission due to direct contact between infected and uninfected fish during the 'move' and or the result of a 'stress' related relapse in some fish due to the 'move'. This will have to be resolved in further studies. According to Becker & Katz (1965a) there were also earlier reports on outbreaks of the disease in hatcheries where no leeches were found. The direct transmission of *C. salmositica* between fish in hatcheries (see 'The effect of cryptobiosis on fish production') clearly needs more studies and further careful documentation.

Parasite multiplication and detection of infection in fish

Cryptobia salmositica divides rapidly by binary fission in the blood of salmonids. The first indication of division is the production of two new flagella, and this is followed by replication of its nucleus and kinetoplast. Body division is initiated from the posterior end of the parasite, and it is longitudinal and unequal (Woo 1978). Based on the course of infection in rainbow trout, Woo (1979) suggested the parasite might undergo antigenic variation, and recent studies on the immunological response of Atlantic salmon, *Salmo salar* L., to the parasite tend to confirm this suggestion (Ardelli & Woo 2002).

During acute infections, the parasite can readily be detected by microscopic examination of a drop of blood/ascites fluid under a cover-slip ('wet mount' technique). Many infected fish may show one or more clinical signs (e.g. anaemia, anorexia, general oedema, abdominal distension with ascites, exophthalmia) and these may help with the diagnosis of the disease (see 'Cryptobiosis and mechanism of disease'). The parasite is more difficult to detect using 'wet mount' examination at early infection stages and/or during chronic infections. At low parasitaemias the parasite is detected more readily using concentration techniques, for example, the haematocrit centrifuge technique (Woo 1969; Woo & Wehnert 1983). This simple parasitological diagnostic technique is sensitive as it can detect live *Cryptobia* in the blood of some fish as early as a week after infection when it is not detectable using the 'wet mount' technique (e.g. Sitja-Bobadilla & Woo 1994).

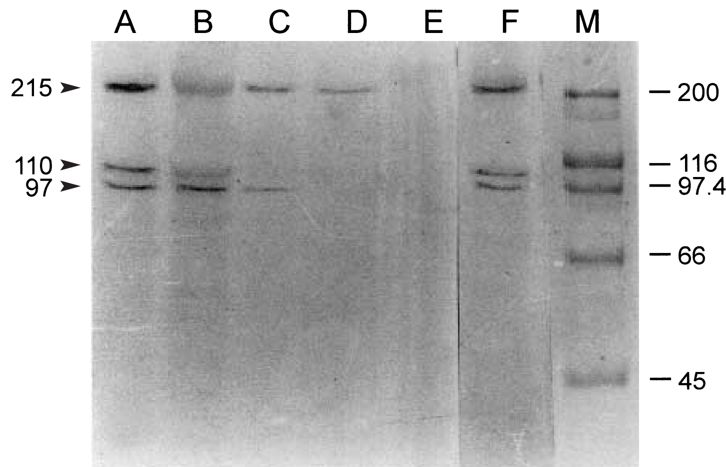

Figure 11 *In vitro* proteolytic degradation of collagen type V by purified metalloprotease. Lanes a–e, collagen incubated with metalloprotease for 0, 2, 4, 6 and 8 h, respectively; lane F, collagen incubated under the same conditions except without the metalloprotease (control); M, molecular markers (kDa) (from Zuo & Woo 1997d).

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Antibodies against the parasite can be demonstrated in the blood of some fish at about 2 weeks after infection (Woo 1990; Sitja-Bobadilla & Woo 1994; Chin & Woo 2002). Woo (1990) described the microscopic immuno-substrate-enzyme technique (MISET) for the early detection of humoral response to the parasite. MISET (Woo 1990), the antibody-capture ELISA (Sitja-Bobadilla & Woo 1994) and the antigen-capture ELISA (Verity & Woo 1996) are as sensitive as the haematocrit centrifuge technique for detection of infections. MISET and antibody-capture ELISA are based on the same basic principle and hence their sensitivities are similar. The antigen-capture ELISA uses a monoclonal antibody (mAb-007; see 'Glycoproteins, surface membrane and metabolism') produced against a major secreted 47 kDa *Cryptobia* polypeptide and it is not specific to *C. salmositica*. The monoclonal antibody cross reacts with antigen(s) in *Cryptobia (T.) bullocki*, a pathogen of flounders on the Atlantic coast in North America, and in the non-pathogenic *Cryptobia (T.) catostomi* in white suckers found in the Great Lakes (Verity & Woo 1996). Li & Woo (1996) also developed a simple species-specific DNA probe (1.2 kbp) to distinguish *C. salmositica* from other species of haematozooic *Cryptobia*.

Glycoproteins, surface membrane and metabolism

Cryptobia salmositica was attenuated by prolonged *in vitro* culture in supplemented minimum essential medium (MEM) (Woo & Li 1990). The avirulent strain (Fig. 3) is smaller and has at least 21 polypeptide bands; there are at least five bands (20–200 kDa) that are either absent or antigenically different from those in the virulent strain (Woo & Thomas 1991). Differences between virulent and avirulent strains were confirmed in subsequent studies. The avirulent strain does not have the 200 kDa metalloprotease (Fig. 10; see 'Cryptobiosis and mechanism of disease'). In addition, the virulent strain has a more negative surface charge (about −15 mV) than the attenuated strain (about −7.9 mV). The major components of the cell membrane include sialic acid residues, phosphate groups and protein glycoconjugates and they probably contribute to the negative surface charge (Vommaro, Attias, Silva Filho, Woo & De Souza 1997). There are also differences in expression of surface carbohydrate residues and glycoproteins on cell membranes of the pathogenic and vaccine strains; specifically, the increased surface carbohydrate residues coincide with its loss of virulence (Feng & Woo 2001). In another study Feng & Woo (1998d) showed the non-pathogenic *Cryptobia catostomi* had more carbohydrates on its surface and had stronger reactions with lectins than the pathogenic *C. salmositica*.

A monoclonal antibody (IgG1; designated mAb-001) was produced against a surface epitope. It agglutinates living *C. salmositica*, and does not activate complement to lyse the parasite. The antibody reduces the survival and infectivity of the parasite (Feng & Woo 1996b). The purified antibody inhibits parasite multiplications and respirations (Hontzeas, Feng & Woo 2001), and significantly reduces cysteine and metalloprotease activities (Zuo, Feng & Woo 1997). The monoclonal antibody is against a 200-kDa glycoprotein. Its epitope (designated Cs-gp200) consists of carbohydrate determinants and conformational polypeptide with internal disulphide bonds. It is a hydrophilic antigen and is secreted by the parasite (Feng & Woo 1998a). Cs-gp200 has an asparagine-bound N-glycosidically linked hybrid-type carbohydrate chain with the minimum length of a chitobiose core unit. It has a phosphatidylinositol residue which anchors the conformational polypeptide (with disulphide bonds) to the surface of the pathogen. The molecule is extensively post-translationally modified (Feng & Woo 1998b). Cs-gp200 has high mannose components and it appears as a doublet in the pathogenic strain and as a single band in the vaccine strain (Feng & Woo 2001).

Another monoclonal antibody mAb-007 (an IgG3 isotype with kappa light chains) is produced against a major 47 kDa polypeptide secreted by both pathogenic (*C. salmositica* and *C. bullocki*) and non-pathogenic (*C. catostomi*) species of *Cryptobia*. This antigenic polypeptide is in the cytoplasm of the parasite and is secreted by both the pathogenic and vaccine strains of *C. salmositica*. It can be detected readily in the blood of fish at 1–2 weeks after infection although the amount is higher in rainbow trout infected with the pathogenic than the non-pathogenic strain. This is in part because the pathogenic strain multiplies readily in trout while the avirulent strain does not (Verity & Woo 1996).

Cryptobia salmositica consumes oxygen under *in vitro* conditions, and parasite motility and oxygen consumption are reduced significantly after the parasite is exposed to sodium azide (Thomas,

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Ballantyne & Woo 1992). However, pre-azide activities are restored once the azide is removed by washing even after 24 h exposure. The parasite produces hydrogen peroxide and some glycolytic products (i.e. lactate and pyruvate), even under aerobic culture conditions. It is also sensitive to acidic pH; hence for large-scale culture of *C. salmositica* the medium has to be buffered as the carbon dioxide it produces changes the pH (Ardelli & Woo 2001c). If its mitochondrion is damaged (e.g. after exposure to the trypanocidal drug isometamidium chloride), oxygen consumption and carbon dioxide production decrease dramatically with concurrent and significant increases in the secretion of glycolytic products (Ardelli & Woo 2001a). This shows it can switch readily from predominantly aerobic respiration to glycolysis. The parasite has its Embden-Meyerhoff pathway enzymes (e.g. hexokinase, fructose-6-biphosphate aldolase, triodephosphate isomerase, glucosephosphate isomerase, etc.) as well as the peroxisomal enzyme, catalase, sequestered in microbodies that are commonly called glycosomes (Ardelli, Witt & Woo 2000). The Kinetoplastida, which includes the *Trypanosoma*, are the only known living organisms where glycolysis is compartmentalized in glycosomes. These microbodies may also contain enzymes for peroxide metabolism and it is presumed the compartmentalization of enzymes facilitates higher rates of glycolysis (Opperdoes 1988).

The parasite does not multiply rapidly in MEM unless foetal bovine serum (FBS) is added to the medium. Multiplication increases with increasing amounts of FBS and reaches a peak number of approximately 9×10^6 parasites mL^{-1}. Glucose is depleted from the medium and the depletion increases with increasing numbers of parasite (Li & Woo 1991a). The optimum temperature for *in vitro* multiplication of virulent and avirulent strains is 10 °C, although the avirulent strain multiples significantly faster than the pathogenic strain (Woo & Thomas 1992). The avirulent strain has been in continuous *in vitro* culture since 1989 (Woo & Li 1990) and is still infective to fish. The pathogenic and avirulent strains have minor nutritional differences especially in utilizations of carbohydrates. The pathogenic strain multiplies more readily in MEM with monosaccharidea supplements, specifically D(−) ribose, D(+) xylose, D(+) galactose, D(+) glucose, D(+) mannose and D(−) fructose. However, the attenuated strain does better with disaccharides, e.g. β-D(+) glucose, α-lactose, maltose and sucrose. The avirulent strain multiplies more readily than the pathogenic strain when L-glutamine and D(−) proline are added to the medium (Ardelli & Woo 2003).

Both pathogenic and avirulent strains have cysteine (thiol) protease (49, 60, 66 and 97 kDa) and the enzyme has been isolated and partially purified (Fig. 10; Zuo & Woo 1997a,d, 1998a). Its optimal pH is 5.0 and it has many similar properties (e.g. substrate specificity, inhibitor sensitivity, optimal pH) to the protease from pathogenic mammalian trypanosomes (Zuo & Woo 1998a). It is a metabolic enzyme and has been detected in pathogenic and non-pathogenic strains and species of *Cryptobia* spp. (Zuo & Woo 1997a). Like the purified metalloprotease (Fig. 10; see 'Cryptobiosis and mechanism of disease') the partially purified cysteine protease has high proteolytic activities against azocasein, haemoglobin and fibrinogen and it also has high enzymatic activity against albumin (Zuo & Woo 1998a). About 80% of its *in vitro* activity is inhibited (Zuo *et al.* 1997) by the monoclonal antibody mAb-001. This antibody agglutinates live parasites and inhibits its *in vitro* multiplication and oxygen consumption (Feng & Woo 1996b; Hontzeas *et al.* 2001). It is therapeutic when injected into fish with acute infections (Feng & Woo 1997b). These studies indicate that cysteine protease is an important metabolic enzyme and is probably involved in intracellular protein catabolism that results in the release of amino acids for protein synthesis and parasite multiplication.

Host–parasite relationships including disease mechanism

Immune response

Humoral response to the parasite is detectable in some fish at about 2 weeks after experimental infection (e.g. Jones & Woo 1987; Woo 1990; Sitja-Bobadilla & Woo 1994; Chin & Woo 2002). Fish that recover from the disease are protected and their antisera contain agglutinating, neutralizing and complement fixing antibodies (e.g. Sitja-Bobadilla & Woo 1994; Li & Woo 1995; Feng & Woo 1997a, 1998c; Ardelli & Woo 2002; Mehta & Woo 2002). Intraperitoneal implantation of cortisol lowers antibody production and this increases parasitaemias in rainbow trout. The

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mortality of infected cortisol-implanted fish is higher than infected fish or cortisol-implanted but not infected (Woo, Leatherland & Lee 1987). In addition, the monoclonal antibody mAb-001 (see 'Glycoproteins, surface membrane and metabolism') that agglutinates the parasite but does not fix complement to lyse it, is also therapeutic and prophylactic against the parasite in fish (Feng & Woo 1997b). Titres of complement fixing antibodies in recovered and vaccinated fish rise significantly after *C. salmositica* challenge (e.g. Li & Woo 1995; Ardelli & Woo 1997, 2002; Feng & Woo 1998c; Mehta & Woo 2002). This classical anamnesis response also confirms that protective immunity is in part due to a humoral response in infected fish.

Long-term thymectomized adult rainbow trout do not show decreased production of complement fixing antibody if infected or vaccinated. However, protective antibody production is reduced in short-term thymectomized fish (Feng & Woo 1997a). Parasitaemias and the production of complement fixing antibodies in thymectomized and intact rainbow trout [injected with rabbit anti-thymocyte serum (RATS) before *Cryptobia* infection] are not significantly different from control fish (infected, but not injected with RATS). Both groups acquire protection on recovery. RATS is not cytotoxic to B-like cells and the protective antigen on *C. salmositica* is thus thymus-independent (Feng & Woo 1998c).

Peritoneal macrophages in ascites of infected trout often have engulfed parasites (Figs 4 & 5; Woo 1979). This was confirmed under *in vitro* conditions with macrophages from the head kidneys of both vaccinated and recovered fish (Li & Woo 1995). Adoptive transfer of leucocytes and plasma from immune fish conferred partial protection in naïve fish (Jones & Woo 1987). Cell-mediated response to the parasite was demonstrated using delayed-type hypersensitivity and the macrophage inhibition migration test (Thomas & Woo 1990a), and enumeration of lymphocyte numbers in intact and thyectomized rainbow trout (Feng & Woo 1996a). Activities of circulating lymphocytes (T cells and B cells) in infected Atlantic salmon was depressed until 4 weeks after infection. In fish injected with the attenuated live *C. salmositica* vaccine the humoral response (i.e. B-lymphocytes) was greater than the cell-mediated response (i.e. T-lymphocytes); the reverse occurred in fish infected with the pathogenic strain (Ardelli & Woo 2002). Respiratory burst activities of macrophages (from head kidneys) from infected rainbow trout were significantly higher than those from vaccinated fish. However, macrophage activity from vaccinated fish rose rapidly after parasite challenge and they were comparable with those in infected fish (Mehta & Woo 2002). This is also a classical anamnesis response.

Cryptobiosis and mechanism of disease

In susceptible fish the parasite multiplies readily in the blood and the severity of the disease (e.g. anaemia) is related directly to the parasitaemia (Woo 1979). The clinical signs of the disease in *Oncorhynchus* spp. include exophthalmia (Fig. 6), splenomegaly (spleen is enlarged six to eight times by volume), a microcytic and hypochromic anaemia, general oedema, abdominal distention with ascites fluid (Fig. 7; Woo 1979), anorexia (Li & Woo 1991b), anti-globulin positive or Coombs' positive erythrocytes (Fig. 8; Thomas & Woo 1988) and significant reduction of blood complement (Thomas & Woo 1989a). Immunodepression during acute disease is probably brought about by the anaemia, anorexia, significant reduction in complement and antigenic competition (Wehnert & Woo 1981; Jones, Woo & Stevenson 1986; Thomas & Woo 1992). However, anorexia is also beneficial to the host in that it lowers the plasma proteins and this reduces the parasitaemias and subsequently the severity of the disease (Li & Woo 1991b).

Although all species of salmonids can be infected by *C. salmositica* their susceptibility to disease and mortality varies greatly between species and stocks of salmon. Putz (1972) showed that the parasite was more pathogenic to coho salmon (100%) than to chinook salmon. However, Bower & Margolis (1984a, 1985) found the reverse, i.e. 100% mortality in chinook with no mortality in coho salmon. These differences are most probably due to the genetics of the fish. A good example is in the susceptibility of sockeye salmon – those from the Fulton River stock (in British Columbia, Canada) had high mortality when injected with about 100 parasites per fish while those fish from the Weaver Creek stock (also in British Columbia) suffered light mortality even when injected with about 10^6 parasites per fish. Mortality of sockeye was consistent within the same fish stock and to different parasite isolates. Susceptibility even varied significantly between families of Atlantic salmon; this variation was correlated to immune response. Families with detectable circulating antibodies at

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3 weeks after infection had significantly lower parasitaemias than those where antibodies were detected at 5 weeks after infection (Chin, Eldridge, Glebe & Woo 2002; Chin & Woo 2002).

Parasitaemias were significantly higher in trout fed a high protein diet (e.g. 3.5 g dL^{-1} plasma protein) than those on a low protein diet (e.g. 1.0 g dL^{-1} plasma proteins) (Thomas & Woo 1990b). As noted earlier, anorexia is beneficial to the infected fish as it lowers plasma proteins and subsequently lowers parasitaemias and reduces the severity of the disease (Li & Woo 1991b). Dietary ascorbic acid had a similar effect; rainbow trout on an ascorbic acid-deficient diet had lower parasitaemias than fish on supplemented diets (Li, Cowey & Woo 1996). Production of antibodies against *C. salmositica* in rainbow trout was depressed in fish on a low protein (19%) or on a pantothenic acid-deficient diet (Thomas & Woo 1990b). Fish on the low protein diet (19%) were less anaemic and had significantly lower parasitaemias than fish on higher protein diets (29 or 38%) as severity of the anaemia is directly related to the parasitaemia (Woo 1979). Red blood cells from 19% protein-diet fish were Coombs' negative (because of low antibody production) while those from the higher protein-diet fish were Coombs' positive 4–6 weeks after infection.

Wehnert & Woo (1981) also showed depression of the humoral response to second antigen (sheep red blood cells) in infected fish on a normal diet during acute cryptobiosis. This was confirmed using both sheep red blood cells and a pathogenic bacterium, *Yersinia ruckeri* (Jones *et al.* 1986). The bacterium is Gram-negative and it causes enteric redmouth disease in salmonids (Horne & Barnes 1999). Fish with acute cryptobiosis did not respond when exposed to a non-lethal dose of *Y. ruckeri* and the mortality rate (~90%) of *Yersinia*-exposed fish was similar to *Yersinia*-naïve fish when they were both exposed to a lethal dose of the bacterium (Jones *et al.* 1986). The immunodepression was not evident in fish that had recovered from cryptobiosis at the time they were exposed to *Yersinia*; these fish were protected from yersiniosis when challenged with the bacterium. Humoral depression to a secondary antigenic stimulation in infected fish may be due to a variety of factors including anorexia, significant reduction in complement antigenic competition, and anaemia.

Plasma cortisol levels were not significantly different between infected and uninfected fish, however plasma levels of T3, T4 (thyroxine), protein and glucose concentration, and liver glycogen were much lower in infected trout. There were no indications of recovery even during the chronic phase of the disease. These hormonal and metabolic changes clearly show that infected fish were under considerable physiological stress (Laidley, Woo & Leatherland 1988).

Acutely infected trout are susceptible to environmental hypoxia partly because of the severe anaemia and high parasitaemias that occludes small blood vessels (Woo & Wehnert 1986). The metabolism and swimming performance of infected trout are also significantly reduced (Kumaraguru, Beamish & Woo 1995) and the bioenergetic cost of the disease is very considerable to infected fish. These are contributing factors to the retarded growth as there are significant reductions in food consumption, dry weight and energy gained, energy concentration and gross conversion efficiency. However, the attenuated strain has no detectable bioenergetic cost to juvenile trout (Beamish, Sitja-Bobadilla, Jebbink & Woo 1996).

Haemodilution, splenomegaly (Woo 1979; Laidley *et al.* 1988), destruction of red blood cells (Thomas & Woo 1988, 1989b) and depletion of the haemopoietic tissues (Bahmanrokh & Woo 2001) contribute to the anaemia. Thomas & Woo (1988, 1989b) showed that the destruction of red blood cells is via the secretion of a 'haemolysin' by *C. salmositica* and the formation of immune complexes on red cells. Red blood cells with immune complexes on their surfaces are Coombs' positive. However, not all Coombs' positive cells are lysed because of the significant depletion of complement in infected fish (Thomas & Woo 1989b). A metalloprotease (200 kDa) has been isolated from pathogenic *C. salmositica.* The enzyme has been purified and its optimal activity is pH 7.0 (Zuo & Woo 1997a,d, 1998a). It has high proteolytic activities against azocasein, haemoglobin, fibrinogen azocoll and gelatine but low activity against albumin and the activity is inhibited by metal-chelating agents and excess of zinc ions, but is activated by calcium ions (Zuo & Woo 1997d, 1998a). It lyses fish red cells under *in vitro* conditions (Zuo & Woo 2000) by digesting the proteins in erythrocyte membranes (Zuo & Woo 1997d) and consequently is an important contributing factor to the anaemia in infected fish. The enzyme is the 'haemolysin' identified earlier as one of the causes of the anaemia in salmonid cryptobiosis (Thomas & Woo 1988, 1989a). The

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metalloprotease (Fig. 11) readily degrades collagens (types I, IV and V) and laminin (Zuo & Woo 1997d). It is secreted by the pathogen under *in vitro* conditions and its secretion is significantly increased in the presence of either type I or IV collagens and or its breakdown products (Zuo & Woo 1998b). As it is a secreted histolytic enzyme the metalloprotease contributes to the development of histological lesions (see below) in infected fish. Consequently, the severity of the disease in *Oncorhynchus* spp. is directly related to the parasitaemia (Woo 1979).

The pathology of cryptobiosis includes focal haemorrhages, congestion of blood vessels, occlusion of capillaries with parasites and oedomatous changes in kidney glomeruli (Putz 1972). Bahmanrokh & Woo (2001) showed a generalized inflammatory response with severe lesions in connective tissues and in the reticulo-endothelial system. Briefly, micro-lesions appear in the liver, gills, and spleen at 1–2 weeks after infection and these are followed by endovasculitis with mononuclear cell and extravascular parasite infiltrations. The anaemia and necrosis in the liver, kidney, and depletion of haematopoietic tissues are in part responsible for mortality during acute disease. Regeneration and replacement of necrotic tissues, especially haematopoietic and reticular tissues, occur during recovery, usually 7–9 weeks after infection.

Acid phosphatase (ACP) is found in both membrane-bound and in the water soluble fractions of the parasite. However, the pathogenic strain has significantly higher total ACP activity, but this decreases on prolonged culture in MEM; this decrease may be associated with its loss of virulence. The membrane-bound ACP in the pathogenic strain is more resistant to the ACP inhibitor, sodium tartrate (Zuo & Woo 1996). Increased carbohydrate residues on the surface membrane coincide with non-pathogenicity in *Cryptobia catostomi* (Feng & Woo 1998b) and in the attenuated strain of *C. salmositica* (Feng & Woo 2001). Consequently, it is also tempting to suggest that increased expression of carbohydrate residues on the *C. salmositica* vaccine strain and reduced ACP may contribute to its loss of virulence. Further studies are needed to more fully understand the mechanism of disease.

The effect of cryptobiosis on fish production

There have been only a few documented serious outbreaks of cryptobiosis in hatcheries in the United States and Canada. It is most likely some of these outbreaks were built up over time through direct transmission of the pathogen (see 'Indirect and direct transmission of the pathogen between fish'). This could be after infected leeches or fish were introduced into the facility via the water system. Wood (1979) summarized three outbreaks that occurred in 1972 and 1973 and they involved young chinook salmon in three localities on the Pacific coast in the United States. Briefly, the fish had massive numbers of *Cryptobia* in their blood, were anaemic and some had abdominal distension and generalized oedema. Parasites were also found on the body surface and in the ascites of some fish. Mortality was very high in juvenile chinook salmon while coho salmon in the same or adjacent ponds were not affected. P.F. Chapman (personal communication, 1993) described another outbreak of the disease in chinook salmon in a hatchery in Washington State, USA. The outbreak began in December 1992 and peaked in February 1993. Infected fish had the typical clinical signs of the disease and 65 000 fish were involved with peak mortality of 0.1% per day in February. The total mortality was about 50% and mortalities due to cryptobiosis had been recorded prior to the outbreak but they were less severe.

High mortalities were reported in post-spawning rainbow trout and prespawning chinook salmon in North America (Wales & Wolf 1955; P.F. Chapman, personal communication, 1993), and prespawning pink salmon, *O. gorbuscha* (Walbaum), in the former Soviet Union (Makeyeva 1956). More recent reports include outbreaks with about 50% annual mortality of chinook salmon broodstock in a hatchery in Washington State (L. Peck, personal communication, 1994), and with high mortality in Caspian salmon, *Salmo trutta* L., in a hatchery in the Republic of North Osetia-Alania, Russia (P. Golovin, personal communication, 1966).

Outbreaks of the disease also occur in sea cages; in 1997 the parasite caused significant morbidity and mortality in smolts and preharvest chinook salmon in a hatchery on Vancouver Island, Canada. There was a small mortality spike (about 1%) in post-smolts in the first 10–15 weeks after transfer to salt water. Re-emergence of the disease as a significant cause of morbidity and mortality occurred later in preharvest fish (Woo, unpublished data). According to the hatchery management, the outbreak was confined to fish exposed to unfiltered surface water and did not appear to be linked to handling.

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Mortality also seemed to be associated with age and major stressors such as marine mammal harassment. Another outbreak in preharvest chinook salmon occurred in the same site in 2001. *Cryptobia* were in large numbers in the blood and ascites fluid of moribund fish and clinical signs (e.g. exophthalmia, anaemia, anorexia) were evident in many fish. Fish mortality varied between cages (e.g. 3.3–24.9%); they were transferred to sea cages in August–September 1999 and the parasite was detected in the blood of some fish while they were in fresh water in the hatchery. Parasites from moribund fish were morphologically similar to *C. salmositica* and these isolates caused clinical disease in experimentally infected rainbow trout. The attenuated *C. salmositica* strain protected them against the isolates (Woo, unpublished data). As the pathogen is normally transmitted by freshwater leeches it is suggested that these marine outbreaks were initiated because of relapse in some infected fish, and the pathogen was rapidly transmitted directly to other fish, for example during weighing when fish were brought into direct contact in nets (see 'Indirect and direct transmission of the pathogen between fish').

Besides mortality the indirect effects of cryptobiosis on fish reproduction are not known. In the Fraser River drainage system, British Columbia, the parasite was in the blood of some sexually mature Pacific salmon as early as 5 days (in November) after their return to fresh water and parasitaemias in many fish were very high at the time of spawning (Bower & Margolis 1984b). As the severity of the disease is related to the parasitaemias (see 'Cryptobiosis and mechanism of disease') acute cryptobiosis may affect the reproductive capacity of salmon by affecting the viability of gametes, by causing lesions in organs including the gonads that in turn may affect the production of reproductive and related hormones. The hormonal changes may affect reproductive behaviour, quality and quantity of gametes, and the development and survival of the embryos and yearlings.

Protective strategies

Immunological strategies

The defensive mechanisms of teleosts are well developed and effective against infectious organisms (e.g. Van Muiswinkel 1995; Gudding, Lillehaug, Midtlyng & Brown 1997). The protection involves both non-lymphoid (e.g. epithelial barriers, transferring, C-reactive protein, complement, lectins) and lymphoid (humoral and cellular response) components.

Innate immunity

Natural or innate immunity is basically non-lymphoid and includes resistance to infection (pathogen-resistant animal) and resistance to disease (pathogen-tolerant animal). In the present discussion, a distinction is made between the two types of resistance although this is seldom made in the literature. Natural immunity is not often exploited to protect animals from infectious organisms.

Many teleosts (e.g. cyprinids) cannot be infected by *C. salmositica* and fresh plasma from these *Cryptobia*-resistant fish lyse the parasite via the alternative pathway of complement activation (Wehnert & Woo 1980). Some laboratory raised brook charr, *Salvelinus fontinalis* (Mitchill), are also refractive to infections (*Cryptobia*-resistant fish) while others can be infected but do not have clinical disease (*Cryptobia*-tolerant fish) (Ardelli, Forward & Woo 1994). Both juvenile *Cryptobia*-resistant and *Cryptobia*-tolerant charr have similar growth rates and respond equally well (both humoral and cell-mediated immunity) to a commercial *Aeromonas* vaccine (Ardelli & Woo 1995). Under *in vitro* conditions the plasma of refractive charr lyse the parasite via the alternative pathway of complement activation (Forward & Woo 1996). This is innate resistance to infection and is controlled by a dominant Mendelian locus. The protection is inherited, hence it is now possible to breed brook charr that are naturally resistant to *Cryptobia* infection (Forward, Ferguson & Woo 1995).

In infected *Cryptobia*-tolerant charr their parasitaemias were just as high or higher than those in *Oncorhynchus* spp.; however, they did not suffer from cryptobiosis (Ardelli *et al.* 1994). These infected *Cryptobia*-tolerant charr were innately resistant to the disease. As indicated earlier (see 'Cryptobiosis and mechanism of disease'), the secreted metalloprotease is an important contributing factor to disease (e.g. Zuo & Woo 1997a,d). In infected *Cryptobia*-tolerant brook charr the metalloprotease secreted by *C. salmositica* was effectively neutralized by α_2 macroglobulin, a natural anti-protease in the blood. The amount of α_2 macroglobulin was higher in brook charr than in trout prior to infection and it remained high during the

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infection (Zuo & Woo 1997b,c). Neutralization of metalloprotease by α_2 macroglobulin was demonstrated under both *in vivo* and *in vitro* conditions (Zuo & Woo 1997b,c,d). As *Cryptobia*-tolerant brook charr did not suffer from clinical disease, their immune system readily controlled the infection and they recovered much more rapidly than trout from the infection. Consequently, an option to control cryptobiosis is to produce transgenic *Cryptobia*-tolerant salmon (Woo 2001). These transgenic salmon, like *Cryptobia*-tolerant charr, will rapidly produce α_2 macroglobulin to neutralize metalloprotease and there will not be a need for further human intervention (e.g. vaccination and/or chemotherapy). This is innate resistance to disease and the production of pathogen-tolerant animals will be a novel approach to management of an infectious disease.

This strategy will probably initiate discussions on the ramifications of applying such a preventive measure against infectious organisms. The obvious advantage is that other human interventions are not required once the transgenic animal is produced. The immune system can be relied on to effectively control the infection, especially when there is no clinical disease in the animal. There are many similarities in the pathophysiology of piscine cryptobiosis and mammalian trypanosomiasis. Woo (2001) suggested that it might be rewarding to re-examine the mechanism of trypanotolerance in reservoir hosts of pathogenic mammalian trypanosomes. If the mechanisms of pathogen-tolerance in the two diseases are similar, then the production of trypanotolerant zebu cow in Africa is worth serious consideration. Trypanosomiasis in domestic animals in Africa is not under control and there are no new trypanocidal drugs being developed. It is also unlikely because of antigenic variations that there will be vaccines available in the near future.

Acquired immunity

Fish that recover from cryptobiosis are protected from challenge (e.g. Jones & Woo 1987; Li & Woo 1995) and this obviously is an impetus for the development of an acquired protective strategy. As the immunity in recovered fish is non-sterile we assume the protection is for the life of the fish.

Laboratory studies showed that infected fish lost their *Cryptobia* infections or there was no mortality when the water temperature was raised from about 10 to 20 °C (Woo, Wehnert & Rodgers 1983; Bower & Margolis 1985). Woo (1987) suggested modification of this approach might be a useful tool. Bower & Evelyn (1988) confirmed that infected juvenile sockeye salmon acclimatized to 20 °C survived while all infected fish at 10 °C died from acute cryptobiosis. All temperature acclimatized infected fish survived a parasite challenge at 10 °C while all infected non-acclimatised fish died.

As indicated earlier (see 'Glycoproteins, surface membrane and metabolism') the avirulent strain produces low parasitaemias in fish, does not cause disease, but protects fish from disease when they are challenged with the pathogen (Woo & Li 1990). The avirulent strain has remained infective, non-pathogenic and protective for well over a decade. It has been used extensively in studies on host–parasite relationships, and also for comparative studies on the biology of the pathogen and to elucidate the disease mechanism in cryptobiosis (see 'Systematics and biology of *Cryptobia (T.) salmositica*' and 'Host–parasite relationships including disease mechanism'). It has been cloned and its biology studied (e.g. Woo & Thomas 1991, 1992; Zuo & Woo 1996, 1997a; Feng & Woo 1998d, 2001). A single intraperitoneal injection of the live vaccine protects 100% of fish (juveniles or adults) from disease and mortality. In rainbow trout the protection lasts for at least 24 months and this protection is via the production of complement fixing antibodies, enhanced phagocytic activities of macrophages, and both antibody independent- and antibody-dependent cytotoxicity (Li & Woo 1995). Thymectomy (Feng & Woo 1997a) and absence of dietary ascorbic acid (Li *et al.* 1996) do not affect the acquisition of protection in vaccinated fish. The vaccine is also effective when fish vaccinated in fresh water are transferred and held in sea water. Production of complement fixing antibodies in vaccinated fish (either in fresh or sea water) increases rapidly and significantly after parasite challenge (Li & Woo 1995, 1997). Vaccination does not affect growth of juvenile fish or has detectable bioenergetic costs to juvenile fish (Beamish *et al.* 1996). Consequently, it is a viable protective strategy, especially in streams and rivers where this live vaccine can be delivered and maintained in the wild fish population by the leech vector. Details of this vaccine delivery strategy have been discussed earlier (Woo 1992).

Another approach to vaccination is to use a recombinant cysteine protease. As indicated earlier (see 'Glycoproteins, surface membrane and

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metabolism') cysteine protease is an important metabolic enzyme in the parasite and a monoclonal antibody (mAb-001) inhibits its enzymatic activities and is protective. Briefly, the antibody agglutinates the parasite, inhibits its multiplication and oxygen consumption, and is also therapeutic when injected into fish with acute infection (see 'Glycoproteins, surface membrane and metabolism'). The recombinant protease has been produced and work is in progress to assess its efficacy and protective period as a vaccine. Immersion of fish in the vaccine is an ideal delivery system as it is less stressful on fish and a more efficient delivery system.

Many pathogens change their surface epitopes to evade the immune response in hosts, consequently a surface epitope-based vaccine may not be protective against all isolates of a pathogen. An enzyme-based vaccine (e.g. cysteine) has a couple of obvious advantages in that it will probably protect against all isolates because it is unlikely metabolic enzymes are dissimilar in different isolates. It may also be protective against pathogens which change their surface epitopes to evade the host immune system and antibodies against an enzyme-based vaccine may also inhibit enzymatic activities in unrelated pathogens. Thus an enzyme-based vaccine would be a specific but broad spectrum vaccine.

Therapy

Chemotherapy and immuno-chemotherapy

Although there have been extensive studies on the chemotherapy of pathogenic mammalian trypanosomes (e.g. Peregrine 1990) little is known about the effectiveness of drugs against *Cryptobia*. Although trypanosomes are closely related to *Cryptobia*, many trypanocidal drugs (e.g. suramin, berenil and antrycide) have no detectable effects on *C. salmositica* under *in vitro* conditions (Woo, unpublished data). Although a combination of antibiotics (penicillin, streptomycin and amphotericin B) has no effects on trypanosomes it affects the *in vitro* viability of *C. salmositica*. Culture forms are more susceptible than blood forms, and Trypan blue protects *Cryptobia* from the antibiotics. However the antibiotic combination does not have a therapeutic effect in infected fish (Thomas & Woo 1991).

Crystal violet, a triphenylmethane dye, is trypanocidal and it is routinely added to blood in many South American blood banks to prevent transmission of *Trypanosoma cruzi* (Docampo, Moreno, Gadelha, De Souza & Cruz 1988; Docampo & Moreno 1990). Under *in vitro* conditions, crystal violet is also effective against *C. salmositica* (Ardelli & Woo 1998). Low concentrations of the dye inhibits multiplication of *C. salmositica*, lowers its infectivity to fish, and causes ultrastructural lesions on mitochondrial and nuclear membranes. However, the therapeutic dose causes high fish mortality (74%) when it is injected into juvenile rainbow trout, consequently it is not a viable option because of its high toxicity (B.F. Ardelli & P.T.K. Woo, unpublished data).

Isometamidium chloride (Samorin), like crystal violet also lyses *C. salmositica* under *in vitro* conditions. It is widely used as a therapeutic drug against trypanosomiasis in domestic animals (Kinabo, Bogan, McKellar & Murray 1989), and is the only available prophylactic drug against bovine trypanosomiasis (Kinabo & Bogan 1987). Samorin (1.0 mg kg^{-1} weight) injected intramuscularly reaches peak levels in the blood of salmon at 2–3 weeks after injection (Ardelli & Woo 2000). The drug was therapeutic against *C. salmositica* in rainbow trout during pre- and post-clinical periods, but was not effective during acute disease as it modified the surface eptitopes of the parasite so that they were not lysed by complement fixing antibodies (Ardelli & Woo 1999). It was more effective in infected Atlantic salmon, and at a higher dose (2.5 mg kg^{-1}) the infection was eliminated in 30% of adult fish and significantly reduced the parasitaemias in the remaining fish. All infected juvenile chinook salmon treated with isometamidium (1.0 mg kg^{-1}) at 2–3 weeks after infection survived the disease while 100% of untreated infected fish died. The drug also has prophylactic value and did not affect fish growth, food consumption, blood complement levels and haematocrit values (Ardelli & Woo 2001b).

Isometamidium chloride accumulates very rapidly in the kinetoplast of *C. salmositica* (Fig. 9a), causes condensation of kinetoplast DNA and formation of vacuoles and swelling of mitochondrial cristae (Fig. 12). Oxygen consumption and carbon dioxide production decrease after drug exposure with a very significant increase in the secretion of glycolytic products (lactate and pyruvate). The parasite switches from aerobic respiration to glycolysis after its mitochondrium is damaged by the drug (Ardelli & Woo 2001a). *In vitro* exposure to the drug reduces the

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infectivity of the parasite to fish and changes the surface glycoprotein antibody receptor sites (epitopes) of the parasite. This alteration of surface epitopes explains the protection of some parasites from lysis by complement fixing antibodies in infected rainbow trout (Ardelli & Woo 1999). Further work is needed to better understand the effects of the drug on the pathogen.

Chemotherapy is 'selective' toxicity, and this is an important consideration in treating brood fish. Woo (1995) suggested conjugation of a drug to a parasite-specific antibody – essentially to produce a 'guided missile'. This approach will significantly increase the cost of treatment and it is not expected that this strategy will be used routinely. As the drug will be administered at a lower dosage, other obvious advantages will include reduced accumulation of the drug in host tissues and the lower risk of development of drug-resistance by the pathogen. Reduced accumulation in host tissues is an important factor if the fish are for human consumption. This approach may be an option for treating certain

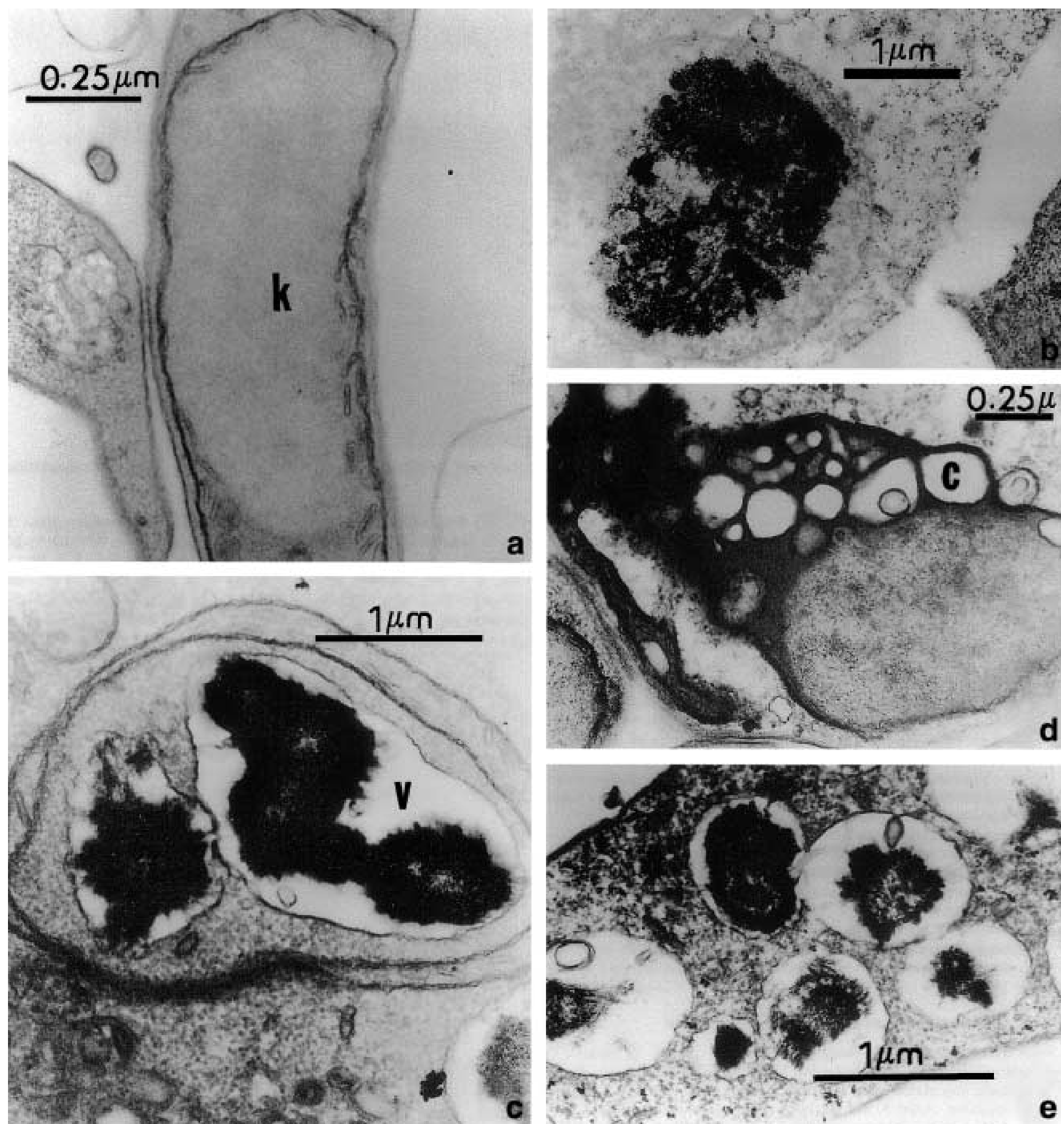

Figure 12 Ultrastructural lesions in *C. salmositica* after *in vitro* exposure to isometamidium chloride: (a) parasite kinetoplast (K) not exposed to the drug; (b) condensation of kinetoplast DNA after exposure to the drug; (c) vacuole (V) formation after exposure to the drug; (d) swelling of mitochondrial cristae (C) after exposure to the drug; (e) vacuole formation in cytoplasm after exposure to the drug (from Ardelli & Woo 2001a).

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types of blood-borne diseases in humans where the cost is not as important as reducing the side-effects of chemotherapy.

Ardelli & Woo (2001d) conjugated isometamidium to polyclonal (antisera from recovered fish – see 'Immune response') and monoclonal (mAb-001 – see 'Glycoproteins, surface membrane and metabolism') antibodies. The conjugated isometamidium occurred throughout the entire parasite (Fig. 9b) while the unconjugated drug accumulated in the kinetoplast (Fig. 9a). Before drug conjugation both antibodies agglutinated living parasites but they behaved differently after drug conjugation. Isometamidium conjugated to polyclonal antibodies lysed the parasite under *in vitro* conditions but parasites were not agglutinated. In contrast, the drug conjugated to mAb-001 did not lyse *C. salmositica* but agglutinated it. Infectivity of the parasite and subsequent parasitaemias in fish after *in vitro* exposure to the isometamidium-polyclonal antibody conjugates were significantly lower than after exposure to other drug antibody combinations, e.g. isometamidium with polyclonal antibodies but without conjugation. Parasitaemias in infected chinook salmon were reduced after injection of the isometamidium-antibody conjugate. These results are encouraging, and indicate that the approach works and that further studies are warranted.

Conclusions

Our concerted effort to better understand *Cryptobia* and cryptobiosis has been challenging but also most satisfying. Over the years I have tried to maintain a balanced research programme with both basic and applied components. This has been quite rewarding and it has also allowed us to devise and/or consider novel approaches to combat pathogens (e.g. an enzyme-based vaccine, immuno-chemotherapy) and to manage infectious diseases (e.g. pathogen-tolerant animals). I hope our research experience and or approaches may be of use and interest to colleagues working on other infectious organisms. On a more personal note, the research has been and still is a very delightful and satisfying experience. I hope the best is yet to come.

Acknowledgements

Over the years I have had the privilege and pleasure of working with a good number of talented and highly dedicated students, postdoctoral fellows and colleagues; consequently much of the research conducted in my laboratory represents our collective efforts, ideas and contributions. This review is dedicated to all my co-workers, past and present. I am also very grateful to my sponsors in Canada, especially the Natural Sciences and Engineering Research Council, Department of Fisheries and Oceans, and AquaNet, Networks of Excellence, for supporting the research.

References

Ardelli B.F. & Woo P.T.K. (1995) Immune response of *Cryptobia*-resistant and *Cryptobia*-susceptible *Salvelinus fontinalis* to an *Aeromonas salmonicida* vaccine. *Diseases of Aquatic Organisms* **23,** 33–38.

Ardelli B.F. & Woo P.T.K. (1997) Protective antibodies and anamnestic response in *Salvelinus fontinalis* to *Cryptobia salmositica* and innate resistance of *Salvelinus namaycush* to the hemoflagellate. *Journal of Parasitology* **83,** 943–946.

Ardelli B.F. & Woo P.T.K. (1998) The *in vitro* effects of crystal violet on the pathogenic haemoflagellate *Cryptobia salmositica* Katz 1951. *Parasite* **5,** 27–36.

Ardelli B.F. & Woo P.T.K. (1999) The therapeutic use of isometamidium chloride against *Cryptobia salmositica* in rainbow trout (*Oncorhynchus mykiss*). *Diseases of Aquatic Organisms* **37,** 195–203.

Ardelli B.F. & Woo P.T.K. (2000) An antigen-capture enzyme-linked immunosorbent assay (ELISA) to detect isometamidium chloride in *Oncorhynchus* spp. *Diseases of Aquatic Organisms* **39,** 231–236.

Ardelli B.F. & Woo P.T.K. (2001a) The *in vitro* effects of isometamidium chloride (Samorin) on the piscine hemoflagellate *Cryptobia salmositica* (Kinetoplastida, Bodonina). *Journal of Parasitology* **87,** 194–202.

Ardelli B.F. & Woo P.T.K. (2001b) Therapeutic and prophylactic effects of isometamidium chloride (Samorin) against the haemoflagellate *Cryptobia salmositica* in chinook salmon (*Oncorhynchus tshawytscha*). *Parasitology Research* **87,** 18–26.

Ardelli B.F. & Woo P.T.K. (2001c) *In vitro* secretion of metabolic end-products by piscine haemoflagellates *Cryptobia salmositica* and *C. bullocki* (Kinetoplastida: Bodonidae) and the relationship of these products to the pH in the medium. *Folia Parasitologica* **48,** 187–191.

Ardelli B.F. & Woo P.T.K. (2001d) Conjugation of isometamidium chloride to antibodies and the use of the conjugate against the haemoflagellate, *Cryptobia salmositica*: an immuno-chemotherapeutic strategy. *Journal of Fish Diseases* **24,** 439–451.

Ardelli B.F. & Woo P.T.K. (2002) Experimental *Cryptobia salmositica* (Kinetoplastida) infections in Atlantic salmon, *Salmo salar* L.: cell-mediated and humoral immune responses against the pathogenic and vaccine strains of the parasite. *Journal of Fish Diseases* **25,** 265–274.

© 2003 Blackwell Publishing Ltd

Ardelli B.F. & Woo P.T.K (2003) *In vitro* nutritional requirements and metabolic products of pathogenic and non-pathogenic strains of *Cryptobia salmositica*: essential carbohydrates and amino acids. *Diseases of Aquatic Organisms* **56,** 49–57.

Ardelli B.F., Forward G.M. & Woo P.T.K. (1994) Brook charr, *Salvelinus fontinalis* (Mitchell), and cryptobiosis: a potential salmonid reservoir host for *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* **17,** 567–577.

Ardelli B.F., Witt J.D.S. & Woo P.T.K. (2000) The identification of glycosomes and metabolic end products in pathogenic and nonpathogenic strains of *Cryptobia salmositica* (Kinetoplastida). *Diseases of Aquatic Organisms* **42,** 41–51.

Bahmanrokh M. & Woo P.T.K. (2001) Relationships between histopathology and parasitaemias in *Oncorhynchus mykiss* infected with *Cryptobia salmositica*, a pathogenic haemoflagellate. *Diseases of Aquatic Organisms* **46,** 41–45.

Beamish F.W.H., Sitja-Bobadilla A., Jebbink J.A. & Woo P.T.K. (1996) Bioenergetic cost of cryptobiosis in fish: rainbow trout (*Oncorhynchus mykiss*) infected with *Cryptobia salmositica* and with an attenuated live vaccine. *Diseases of Aquatic Organisms* **25,** 1–8.

Becker C.D. & Katz M. (1965a) Infections of the haemoflagellate *Cryptobia salmositica* Katz 1951, in freshwater teleosts of the Pacific coast. *Transactions of the American Fisheries Society* **94,** 327–333.

Becker C.D. & Katz M. (1965b) Transmission of the haemoflagellate *Cryptobia salmositica* Katz 1951, by the rhynchobdellid leech vector. *Journal of Parasitology* **51,** 95–99.

Becker C.D. & Katz M. (1966) Host relationships of *Cryptobia salmositica* (Protozoa, Mastigophora) in a Washington hatchery stream. *Transactions of the American Fisheries Society* **95,** 196–202.

Bower S.M. & Evelyn T.P.T. (1988) Acquired and innate resistance to the haemoflagellate *Cryptobia salmositica* in sockeye salmon (*Oncorhynchus nerka*). *Developmental and Comparative Immunology* **12,** 749–760.

Bower S.M. & Margolis L. (1983) Direct transmission of the haemoflagellate *Cryptobia salmositica* among Pacific salmon (*Oncorhynchus* spp.). *Canadian Journal of Zoology* **61,** 1242–1250.

Bower S.M. & Margolis L. (1984a) Detection of infection and susceptibility of different Pacific salmon stocks (*Oncorhynchus* spp.) to the haemoflagellate, *Cryptobia salmositica. Journal of Parasitology* **70,** 273–278.

Bower S.M. & Margolis L. (1984b) Distribution of *Cryptobia salmositica*, a haemoflagellate of fishes in British Columbia and the seasonal pattern of infection in a coastal river. *Canadian Journal of Zoology* **62,** 2512–2518.

Bower S.M. & Margolis L. (1985) Effects of temperature and salinity on the course of infection with the haemoflagellate *Cryptobia salmositica* in juvenile Pacific salmon (*Oncorhynchus* spp.). *Journal of Fish Diseases* **8,** 25–33.

Bower S.M. & Thompson A.B. (1987) Hatching of the Pacific salmon leech (*Piscicola salmositica*) from cocoons exposed to various treatments. *Aquaculture* **66,** 1–8.

Bower S.M. & Woo P.T.K. (1977) Morphology and host specificity of *Cryptobia catostomi* n. sp. (Protozoa: Kinetoplastida) from white sucker (*Catostomus commersoni*) in southern Ontario. *Canadian Journal of Zoology* **55,** 1082–1092.

Brugerolle C., Lom J., Nohynkova E. & Joyon L. (1979) Comparison et evolution des structures cellulaires chez plusieurs especes de bodonides et cryptobiides appartenant aux genres *Bodo*, *Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). *Protistologica* **15,** 197–221.

Callahan H.A., Litaker R.W. & Noga E.J. (2002) Molecular taxonomy of the Suborder Bodonina (Order Kinetoplastida), including the important fish parasite, *Ichthyobodo necator*. *Journal of Eukaryotic Microbiology* **49,** 119–128.

Chin A. & Woo P.T.K. (2002) Susceptibility and immunological response in families of Atlantic salmon (*Salmo salar*) against *Cryptobia salmositica. 10th International Congress of Parasitology*, Vancouver, Canada (Abstract).

Chin A., Eldridge M., Glebe B.D. & Woo P.T.K. (2002) *Salmo salar* and *Cryptobia salmositica*: variations in susceptibility and humoral response in families of Atlantic salmon to the pathogen. *AquaNet II, Annual General Meeting*, Moncton, Canada (Abstract).

Crawley H. (1909) Studies on blood parasites, II. The priority of *Cryptobia* Leidy 1846 over *Trypanoplasma* Laveran & Mesnil, 1901. *Bulletin of the US Bureau of Animal Industry* **119,** 16–20.

Davison R.C., Breese W. & Katz M. (1954) The hemoflagellate, *Cryptobia salmositica*, in Oregon salmon. *Journal of Parasitology* **40,** 703–704.

Docampo R. & Moreno S.N. (1990) The metabolism and mode of action of gentian violet. *Drug Metabolism Reviews* **22,** 161–178.

Docampo R., Moreno S.N., Gadelha F.R., De Souza W. & Cruz F.S. (1988) Prevention of Chagas' disease resulting from blood transfusion by treatment of blood: toxicity and mode of action of gentian violet. *Biomedical and Environmental Sciences* **1,** 406–413.

Dolezel D., Jirku M., Maslov D.A. & Lukes J. (2000) Phylogeny of the bodonid flagellates (Kinetoplastida) based on small-subunit rRNA gene sequences. *International Journal of Systematic and Evolutionary Microbiology* **50,** 1943–1951.

Feng S. & Woo P.T.K. (1996a) Cell-mediated immune response and T-like cells in thymectomized *Oncorhynchus mykiss* (Walbaum) infected with or vaccinated against the pathogenic hemoflagellate *Cryptobia salmositica* Katz 1951. *Parasitology Research* **82,** 604–611.

Feng S. & Woo P.T.K. (1996b) Biological characterization of a monoclonal antibody against a surface membrane antigen on *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* **19,** 137–143.

Feng S. & Woo P.T.K. (1997a) Complement fixing antibody production in thymectomized *Oncorhynchus mykiss* (Walbaum), vaccinated against or infected with the pathogenic haemoflagellate *Cryptobia salmositica. Folia Parasitologica* **44,** 188–194.

Feng S. & Woo P.T.K. (1997b) The therapeutic and prophylactic effects of a protective monoclonal antibody (MAb-001) against the pathogenic haemoflagellate *Cryptobia salmositica* Katz 1951. *Diseases of Aquatic Organisms* **28,** 211–219.

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Feng S. & Woo P.T.K. (1998a) Characterization of a 200 kDa glycoprotein (Cs-gp2000) on the pathogenic piscine haemoflagellate *Cryptobia salmositica. Diseases of Aquatic Organisms* **32,** 41–48.

Feng S. & Woo P.T.K. (1998b) Biochemical characterization of an epitope on the surface membrane antigen (Cs-gp200) of the pathogenic piscine hemoflagellate *Cryptobia salmositica* Katz 1951. *Experimental Parasitology* **88,** 3–10.

Feng S. & Woo P.T.K. (1998c) *In vitro* and *in vivo* effects of rabbit anti-thymocyte serum on circulating leucocytes and production of complement fixing antibodies in thymectomized *Oncorhynchus mykiss* (Walbaum) infected with *Cryptobia salmositica* Katz, 1951. *Journal of Fish Diseases* **21,** 241–248.

Feng S. & Woo P.T.K. (1998d) Identification of carbohydrates on the surface membrane of pathogenic and nonpathogenic piscine haemoflagellates, *Cryptobia salmositica*, *C. bullocki* and *C. catostomi* (Kinetoplastida). *Diseases of Aquatic Organisms* **32,** 201–209.

Feng S. & Woo P.T.K. (2001) Cell membrane glycoconjugates on virulent and avirulent strains of the haemoflagellate *Cryptobia salmositica* (Kinetoplastida). *Journal of Fish Diseases* **24,** 23–32.

Forward G.M. & Woo P.T.K. (1996) An *in vitro* study on the mechanism of innate immunity in *Cryptobia*-resistant brook charr (*Salvelinus fontinalis*) against *Cryptobia salmositica. Parasitology Research* **82,** 238–241.

Forward G.M., Ferguson M.M. & Woo P.T.K. (1995) Susceptibility of brook charr, *Salvelinus fontinalis* to the pathogenic haemoflagellate, *Cryptobia salmositica*, and the inheritance of innate resistance by progenies of resistant fish. *Parasitology* **111,** 337–345.

Gudding R., Lillehaug A., Midtlyng P.J. & Brown F. (eds) (1997) *Fish Vaccinology.* S. Karger AG, Basel, Switzerland.

Hontzeas N., Feng S. & Woo P.T.K. (2001) Inhibitory effects of a monoclonal antibody (Mab-001) on *in vitro* oxygen consumption and multiplication of the pathogenic haemoflagellate, *Cryptobia salmositica* Katz. *Journal of Fish Diseases* **24,** 391–398.

Horne M.T. & Barnes A.C. (1999) Enteric redmouth disease (*Yersinia ruckeri*). In: *Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections* (ed. by P.T.K. Woo & D.W. Bruno), pp. 455–477. CABI Publishing, Oxon, UK.

Jones S.R.M. & Woo P.T.K. (1987) The immune response of rainbow trout, *Salmo gairdneri* Richardson, to the haemoflagellate, *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* **10,** 395–402.

Jones S.R.M., Woo P.T.K. & Stevenson R.M.W. (1986) Immunosuppression in *Salmo gairdneri* Richardson caused by the haemoflagellate, *Cryptobia salmositica. Journal of Fish Diseases* **9,** 931–938.

Katz M. (1951) Two new hemoflagellates (genus *Cryptobia*) from some western Washington teleosts. *Journal of Parasitology* **37,** 245–250.

Khan R.A., Moyles D. & Bal A.K. (1980) Observations on the fine structure of a flagellated protozoa, *Cryptobia dahli. Proceedings of the Microscopic Society, Canada* **7,** 50–51.

Khan R.A., Lobos V., Garcias F., Munoz G., Valdebenito V. & George-Nascimento M. (2001) *Cryptobia neghmei* sp.n. (Protozoa:Kinetoplastida) in two species of flounders, *Paralichthys* spp. (Pisces: Paralichthydae) off Chile. *Revista Chilena de Historia Natural* **74,** 763–767.

Kinabo L.D.B. & Bogan J.A. (1987) Binding of isometamidium to calf thymus DNA and lipids: pharmacological implications. *Journal of Veterinary Pharmacology and Therapeutics* **10,** 357–362.

Kinabo L.D.B., Bogan J.A., McKellar Q.A. & Murray M. (1989) Relay bioavailability and toxicity of isometamidium residues: a model for human risk assessment. *Veterinary and Human Toxicology* **31,** 417–421.

Kumaraguru A.K., Beamish F.W.H. & Woo P.T.K. (1995) Impact of a pathogenic haemoflagellate, *Cryptobia salmositica* on the metabolism and swimming performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **18,** 297–305.

Laidley C.W., Woo P.T.K. & Leatherland J.F. (1988) The stress response of rainbow trout to experimental infection with the blood parasite, *Cryptobia salmositica* Katz 1951. *Journal of Fish Biology* **32,** 253–261.

Laveran A. & Mesnil F. (1901) Sur les flagelles a membrane ondulante des poissons (genres *Trypanosoma* Gruby et *Trypanoplasma* n.gen.). *Compte Rendu Hebdomadaires des Seances de l'Academie des Sciences, Paris* **133,** 670–675.

Leidy J. (1846) Description of a new genus and species of Entozoa. *Proceedings of the National Academy of Science, Philadelphia* **3,** 100–101.

Leitritz E. (1960) Trout and salmon culture. *California Department of Fish and Game, Fishery Bulletin* **107,** 169.

Li S. & Woo P.T.K. (1991a) *In vitro* effects of fetal bovine serum and glucose on multiplication of *Cryptobia salmositica. Journal of Parasitology* **77,** 151–155.

Li S. & Woo P.T.K. (1991b) Anorexia reduces the severity of cryptobiosis in *Oncorhynchus mykiss. Journal of Parasitology* **77,** 467–471.

Li S. & Woo P.T.K. (1995) Efficacy of a live *Cryptobia salmositica* vaccine, and the mechanism of protection in vaccinated *Oncorhynchus mykiss* (Walbaum) against cryptobiosis. *Veterinary Immunology and Immunopathology* **48,** 343–353.

Li S. & Woo P.T.K. (1996) A species specific *Cryptobia salmositica* (Kinetoplastida) DNA probe and its uses in salmonid cryptobiosis. *Diseases of Aquatic Organisms* **25,** 9–14.

Li S. & Woo P.T.K. (1997) Vaccination of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against cryptobiosis: efficacy of the vaccine in fresh and sea water. *Journal of Fish Diseases* **20,** 369–374.

Li S., Cowey C.B. & Woo P.T.K. (1996) The effects of dietary ascorbic acid on *Cryptobia salmositica* infection and on vaccination against cryptobiosis in *Oncorhynchus mykiss. Diseases of Aquatic Organisms* **24,** 11–16.

Lom J. (1979) Biology of fish trypanosomes and trypanoplasms. In: *Biology of Kinetoplastida*, Vol. 2 (ed. by W.H.R. Lumsden & D.A. Evans), pp. 269–337. Academic Press, New York, USA.

Makeyeva A.P. (1956) On one of the factors of prespawning mortality of pink salmon in rivers. In: *Pacific Salmon*, pp. 18–21. Israel Program for Scientific Translations, Jerusalem,

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1961; Office of Technical Service, US Department of Commerce, Washington, DC, USA.

Mehta M. & Woo P.T.K. (2002) Acquired cell-mediated protection in rainbow trout, *Oncorhynchus mykiss*, against the haemoflagellate, *Cryptobia salmositica*. *Parasitology Research* **88,** 956–962.

Newman M.W. (1978) Pathology associated with *Cryptobia* infection in a summer flounder (*Paralichthys dentatus*). *Journal of Wildlife Diseases* **14,** 299–304.

Nohynkova E. (1984) A new pathogenic *Cryptobia* from freshwater fishes: a light and electron microscope study. *Protistologica* **20,** 181–195.

Opperdoes F.R. (1988) Glycosomes may provide clues to the import of peroxisomal proteins. *Trends in Biochemical Science* **13,** 255–260.

Paterson W.B. & Woo P.T.K. (1983) Electron microscopic observations of the bloodstream form of *Cryptobia salmositica* Katz, 1951 (Kinetoplastida: Bodonina). *Journal of Protozoology* **39,** 431–437.

Peregrine A.S. (ed.) (1990) *Chemotherapy for Trypanosomiasis: Proceedings of a Workshop*. The International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

Putz R.E. (1972) Biological studies on the haemoflagellates *Cryptobia cataractae* and *Cryptobia salmositica*. *Technical Paper, Bureau of Sport Fisheries and Wildlife (USA)* **63,** 3–25.

Sitja-Bobadilla A. & Woo P.T.K. (1994) An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against the pathogenic haemoflagellate, *Cryptobia salmositica* Katz and protection against cryptobiosis in juvenile *Oncorhynchus mykiss* (Walbaum) inoculated with a live *Cryptobia* vaccine. *Journal of Fish Diseases* **17,** 399–408.

Steinhagen D., Kruse P. & Korting W. (1990) Some haematological observations on carp, *Cyprinus carpio* L., experimentally infected with *Trypanoplasma borreli* Laveran & Mesnil, 1901 (Protozoa, Kinetoplastida). *Journal of Fish Diseases* **13,** 157–162.

Thomas P.T. & Woo P.T.K. (1988) *Cryptobia salmositica*: *in vitro* and *in vivo* study on the mechanism of anaemia in infected rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **11,** 425–431.

Thomas P.T. & Woo P.T.K. (1989a) An *in vitro* study on the haemolytic components of *Cryptobia salmositica*. *Journal of Fish Diseases* **12,** 389–393.

Thomas P.T. & Woo P.T.K. (1989b) Complement activity in *Salmo gairdneri* Richardson infected with *Cryptobia salmositica* and its relationship to the anaemia in cryptobiosis. *Journal of Fish Diseases* **12,** 395–397.

Thomas P.T. & Woo P.T.K. (1990a) *In vivo* and *in vitro* cell-mediated immune responses of *Oncorhynchus mykiss* against *Cryptobia salmositica* (Sarcomastigophora: Kinetoplastida). *Journal of Fish Diseases* **13,** 423–433.

Thomas P.T. & Woo P.T.K. (1990b) Dietary modulation of humoral immune response and anaemia in *Oncorhynchus mykiss* (Walbaum) infected with *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* **13,** 435–446.

Thomas P.T. & Woo P.T.K. (1991) *In vitro* and *in vivo* effects of antimicrobial agents on viability of *Cryptobia salmositica* (Sarcomastigophora: Kinetoplastida). *Diseases of Aquatic Organisms* **10,** 7–11.

Thomas P.T. & Woo P.T.K. (1992) Anorexia in *Oncorhynchus mykiss* infected with *Cryptobia salmositica* (Sarcomastigophora: Kinetoplastida): its onset and contribution to the immunodepression. *Journal of Fish Diseases* **15,** 443–447.

Thomas P.T., Ballantyne J.S. & Woo P.T.K. (1992) *In vitro* oxygen consumption and mobility of *Cryptobia salmositica*, *Cryptobia bullocki* and *Cryptobia catostomi*. *Journal of Parasitology* **78,** 747–749.

Van Muiswinkel W.H. (1995) The piscine immune system: innate and acquired immunity. In: *Fish Diseases and Disorders, Vol. 1: Protozoan and Metazoan Infections* (ed. by P.T.K. Woo), pp. 729–750. CAB International, Oxon, UK.

Verity C.K. & Woo P.T.K. (1996) Characterization of a monoclonal antibody against the 47 kDa antigen of *Cryptobia salmositica* Katz and its use in an antigen-capture enzyme-linked immunosorbent assay for detection of parasite antigen in infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **19,** 91–109.

Vickerman K. (1977) DNA throughout the single mitochondrion of a kinetoplastic flagellate: observations of the ultrastructure of *Cryptobia vaginalis* (Hesse, 1910). *Journal of Protozoology* **24,** 221–233.

Vickerman K. (1978) The free-living trypanoplasma: description of three species of the genus *Procryptobia* n.g. and redescription of *Dimastigella trypaniformis* Sandon, with notes on their relevance to the microscopical diagnosis of disease in man and animals. *Transactions of the American Microscopical Society* **97,** 485–502.

Vommaro R.C., Attias M., Silva Filho F.C., Woo P.T.K. & De Souza W.(1997) Surface charge and surface carbohydrates of *Cryptobia salmositica* virulent and avirulent forms and of *C. bullocki* (Kinetoplastida: Cryptobiidae). *Parasitology Research* **83,** 698–705.

Wales J.H. & Wolf H. (1955) Three protozoan diseases from trout in California. *California Fish and Game* **41,** 183–187.

Wehnert S.D. & Woo P.T.K. (1980) *In vivo* and *in vitro* studies on the host specificity of *Trypanoplasma salmositica*. *Journal of Wildlife Diseases* **16,** 183–187.

Wehnert S.D. & Woo P.T.K. (1981) The immune response of *Salmo gairdneri* during *Trypanoplasma salmositica* infection. *Canadian Society of Zoologists, Bulletin* **11,** 100 (Abstract).

Woo P.T.K. (1969) The haematocrit centrifuge technique for the detection of trypanosomes in blood. *Canadian Journal of Zoology* **47,** 921–923.

Woo P.T.K. (1978) The division process of *Cryptobia salmositica* in experimentally infected rainbow trout (*Salmo gairdneri*). *Canadian Journal of Zoology* **56,** 1514–1518.

Woo P.T.K. (1979) *Trypanoplasma salmositica*: experimental infections in rainbow trout, *Salmo gairdneri*. *Experimental Parasitology* **47,** 36–48.

Woo P.T.K. (1987) *Cryptobia* and cryptobiosis in fishes. *Advances in Parasitology* **26,** 199–237.

Woo P.T.K. (1990) MISET, an immunological technique for the serodiagnosis of *Cryptobia salmositica* (Sarcomastigophoras,

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Kinetoplastida) infection in *Oncorhynchus mykiss*. *Journal of Parasitology* **76,** 389–393.

Woo P.T.K. (1992) Immunological responses of fish to parasitic organisms. *Annual Review of Fish Diseases* **2,** 339–366.

Woo P.T.K. (1994) Flagellate parasites of fish. In: *Parasitic Protozoa*, Vol. 8, 2nd edn (ed. by J.P. Kreier), pp. 1–180. Academic Press, New York.

Woo P.T.K. (1995) Working with 007. In: *An Aquatic Oasis* (ed. by D. Roberts), p. 20. University of Guelph Research, Guelph, Canada.

Woo P.T.K. (1998) Protection against *Cryptobia* (*Trypanoplasma*) *salmositica* and salmonid cryptobiosis. *Parasitology Today* **14,** 272–277.

Woo P.T.K. (2001) Cryptobiosis and its control in North American fishes. *International Journal for Parasitology* **31,** 566–574.

Woo P.T.K. & Li S. (1990) *In vitro* attenuation of *Cryptobia salmositica* and its use as a live vaccine against cryptobiosis in *Oncorhynchus mykiss*. *Journal of Parasitology* **76,** 752–755.

Woo P.T.K. & Poynton S. (1995) Diplomonadida, Kinetoplastida and Amoebida. In: *Fish Diseases and Disorders, Vol. 1: Protozoan and Metazoan Infections* (ed. by P.T.K. Woo), pp. 27–96. CAB International, Oxon, UK.

Woo P.T.K. & Thomas P.T. (1991) Polypeptide and antigen profiles in *Cryptobia salmositica*, *C. bullocki* and *C. catostomi* (Kinetoplastida: Sarcomastigophora) isolated from fishes. *Diseases of Aquatic Organisms* **11,** 201–205.

Woo P.T.K. & Thomas P.T. (1992) Comparative *in vitro* studies on the virulent and avirulent strains of *Cryptobia salmositica* (Sarcomastigophora: Kinetoplastida). *Journal of Fish Diseases* **15,** 261–266.

Woo P.T.K. & Wehnert S.D. (1983) Direct transmission of a haemoflagellate, *Cryptobia salmositica* Katz 1951 (Kinetoplastida: Bodonina) between rainbow trout under laboratory conditions. *Journal of Protozoology* **39,** 334–337.

Woo P.T.K. & Wehnert S.D. (1986) *Cryptobia salmositica*: susceptibility of infected trout, *Salmo gairdneri*, to environmental hypoxia. *Journal of Parasitology* **72,** 392–396.

Woo P.T.K., Wehnert S.D. & Rodgers D. (1983) The susceptibility of fishes to haemoflagellates at different ambient temperatures. *Parasitology* **87,** 385–392.

Woo P.T.K., Leatherland J.F. & Lee M.S. (1987) *Cryptobia salmositica*: cortisol increases the susceptibility of *Salmo gairdneri* Richardson to experimental cryptobiosis. *Journal of Fish Diseases* **10,** 75–83.

Wood J.W. (1979) *Diseases of Pacific Salmon: Their Prevention and Treatment*, 3rd edn. State of Washington, Department of Fisheries, Olympia, Washington, USA.

Wright A.D.G., Li S., Feng S., Martin D.S. & Lynn D.H. (1999) Phylogenetic position of the kinetoplastids, *Cryptobia bullocki, Cryptobia catostomi*, and *Cryptobia salmositica* and monophyly of the genus *Trypanosoma* inferred from small subunit ribosomal RNA sequences. *Molecular and Biochemical Parasitology* **99,** 69–76.

Zuo X. & Woo P.T.K. (1996) Acid phosphatase in pathogenic and nonpathogenic hemoflagellates, *Cryptobia* spp. of fishes. *Journal of Parasitology* **82,** 893–899.

Zuo X. & Woo P.T.K. (1997a) Proteases in pathogenic and nonpathogenic hemoflagellates, *Cryptobia* spp. (Sarcomastigophora: Kinetoplastida) of fishes. *Diseases of Aquatic Organisms* **29,** 57–65.

Zuo X. & Woo P.T.K. (1997b) Natural antiproteases in rainbow trout, *Oncorhynchus mykiss*, and brook charr, *Salvelinus fontinalis*, and the *in vitro* neutralization of fish alpha2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica*. *Parasitology* **114,** 375–382.

Zuo X. & Woo P.T.K. (1997c) The *in vivo* neutralization of proteases from *Cryptobia salmositica* by α2-macroglobulin in the blood of rainbow trout, *Oncorhynchus mykiss* and brook charr, *Salvelinus fontinalis*. *Diseases of Aquatic Organisms* **29,** 67–72.

Zuo X. & Woo P.T.K. (1997d) Purified metallo-protease from the pathogenic haemoflagellate, *Cryptobia salmositica*, and its *in vitro* proteolytic activities. *Diseases of Aquatic Organisms* **30,** 177–185.

Zuo X. & Woo P.T.K. (1998a) Characterization of purified metallo- and cysteine proteases from the pathogenic haemoflagellate, *Cryptobia salmositica* Katz 1951. *Parasitology Research* **84,** 492–498.

Zuo X. & Woo P.T.K. (1998b) *In vitro* secretion of metalloprotease (200 kDa) by the pathogenic piscine haemoflagellate, *Cryptobia salmositica* Katz, and stimulation of protease production by collagen. *Journal of Fish Diseases* **21,** 249–255.

Zuo X. & Woo P.T.K. (2000) *In vitro* haemolysis of piscine erythrocytes by purified metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica* Katz. *Journal of Fish Diseases* **23,** 227–230.

Zuo X., Feng S. & Woo P.T.K. (1997) The *in vitro* inhibition of proteases from *Cryptobia salmositica* Katz by a monoclonal antibody (MAb-001) against a glycoprotein on the pathogenic haemoflagellate. *Journal of Fish Diseases* **20,** 419–426.

Received: 25 November 2002
Accepted: 28 May 2003

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