

Invited Review

Cryptobiosis and its control in North American fishes

Patrick T.K. Woo*

Department of Zoology and Axelrod Institute of Ichthyology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Received 9 October 2000; received in revised form 4 January 2001; accepted 4 January 2001

Abstract

Cryptobiosis is caused by the haemoflagellates *Cryptobia bullocki* and *Cryptobia salmositica*. These parasites infect food fishes (e.g. flounders, salmon) on both the Atlantic and Pacific coasts of North America and clinical signs of the disease include anaemia, and abdominal distention with ascites. The virulent factor in salmonid cryptobiosis, caused by *C. salmositica*, is a secretory metalloprotease (200 kDa). Fish mortality may be up to 100% in the absence of treatment, consequently strategies have been developed to protect them from disease/mortality. A single dose of a live vaccine protects fish for at least 2 years, and it is via the production of complement-fixing antibodies, enhanced phagocytosis and cell-mediated cytotoxicity. Inhibition of the parasite's cysteine protease by a monoclonal antibody reduces multiplication, infectivity and survival of the parasite. Consequently, the recombinant cysteine protease (49 kDa) of the parasite will be tested as a potential vaccine. The trypanocidal drug, isometamidium chloride (1.0 mg/kg), is effective (therapeutic and prophylactic) against *C. salmositica* in chinook salmon. Its efficacy is significantly enhanced if it is conjugated either to a monoclonal antibody or to polyclonal antibodies from immune fish. Selective breeding of *Cryptobia*-resistant brook charr (innate resistance to infection) is possible, and the resistant factor(s) is controlled by a dominant Mendelian locus. In these resistant charr the parasite is lysed via the alternate pathway of complement activation (innate immunity to infection). There are also *Cryptobia*-tolerant charr, fish that are susceptible to infection but have no clinical disease (innate resistance to disease). In these fish, one of the natural anti-proteases, alpha2-macroglobulin, neutralises the metalloprotease secreted by *C. salmositica*. Production of transgenic *Cryptobia*-tolerant salmon is an option to vaccination and or chemotherapy. Also, transgenic pathogen-tolerant animals may be an alternate strategy against other pathogens where the disease mechanism is similar to cryptobiosis. © 2001 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Haemoflagellates; *Cryptobia*; Cryptobiosis; Vaccination; Innate resistance; Therapy

1. Introduction

Cryptobiosis is caused by a biflagellated protozoa (*Cryptobia*, Kinetoplastida). The parasite is elongated and has two flagella which originate from the anterior end; the anterior flagellum is free while the recurrent flagellum is attached to the body and ends as a posterior free flagellum. Its kinetoplast is large and it is anterior to the nucleus which is located at the anterior part of the organism. There are at least 52 nominal species (five from the gills and body surface, seven from the digestive system, and 40 from the blood); most species are not well studied and as far as we know are not known to cause disease. Species that inhabit the blood (haemotozoic) are normally transmitted indirectly by blood sucking leeches, while those in the digestive system and body surface have direct transmission (Woo, 1994).

Two haemotozoic *Cryptobia* cause disease and mortality in fish of economic importance in North America. *Crypto-*

bia (*Trypanoplasma*) *bullocki* (Section 1) is in flounders, hogchoker, and croaker on the Atlantic coast while *Cryptobia* (*T.*) *salmositica* (Section 2) infects all species of salmon and sculpins on the Pacific coast (Woo and Poynton, 1995). The focus of the present discussion is on the disease, and on the development of strategies against salmonid cryptobiosis.

2. Cryptobiosis and protective strategies

2.1. *Cryptobia* (*T.*) *bullocki*

Cryptobia bullocki was first described from the winter flounder (*Pseudopleuronectes americanus*) in New Hampshire (Strout, 1965). It has subsequently been reported in other estuarine and inshore marine fishes from New Brunswick to Georgia and also in the northern Gulf of Mexico. In some localities, the parasite is very common (70–100%) in commercially important flounders that use estuaries as nursery grounds. These include winter and summer flounders (*P. americanus* and *Paralichthys dentatus*) along the

* Corresponding author. Fax: +1-519-767-1656.
E-mail address: pwoo@uoguelph.ca (P.T.K. Woo).

Atlantic coast, and southern flounder (*Paralichthys lethostigma*), hogchoker (*Trinectes maculatus*) and croaker (*Micropogonias undulatus*) in the southern Atlantic coast to the northern Gulf of Mexico. Also, smooth flounder (*Liopsetta putnami*) in New England and hogchoker and summer flounder in the Chesapeake Bay are commonly infected. The vector is the marine leech (*Calliobdella vivida*) and the leech is only present from mid-October until early April when they deposit their cocoons and die (Strout, 1965; Laird and Bullock, 1969; Daily, 1978; Newman, 1978; Becker and Overstreet, 1979; Bureson and Zwerner, 1982).

The disease develops slowly in summer flounders, and the clinical signs are anaemia, splenomegaly, abdominal distention with ascites and sluggishness (Bureson, 1982). Ascites is evident at about 5 weeks after infection and there is haemorrhaging into the ventral musculature. Ulcerative and haemorrhage lesions are in the abdominal cavity, necrosis of the intestine and oedema in the stomach are common (Newman, 1978). Very little is known about host immune response and there is neither chemotherapy nor a vaccine against the pathogen (Woo and Poynton, 1995).

2.2. *Cryptobia (T.) salmositica*

The parasite was initially described from the blood of coho salmon (*Oncorhynchus kisutch*) in Washington State (Katz, 1951). It has since been reported from all species of Pacific *Oncorhynchus* spp., *Salmo trutta*, *Prosopium williamsoni*, and seven species of *Cottus*. *Cryptobia salmositica* is in fish from northern California to southern British Columbia, Vancouver Island, and southwestern Alaska (Wales and Wolf, 1955; Becker and Katz, 1965a; Katz et al., 1966; Bower and Margolis, 1984). In streams and rivers on the Pacific coast the parasite is transmitted indirectly by a freshwater leech (*Piscicola salmositica*) (Becker and Katz, 1965b). Under certain laboratory (Woo and Wehnert, 1983) and hatchery conditions (Bower and Margolis, 1983), it can also be transmitted directly between fish in the absence of the leech vector. Once in the fish, the parasite multiplies readily (Woo, 1978) in the blood and in the absence of treatment the mortality can be 100% in some stocks and species of salmon (Woo and Poynton, 1995).

The haematocrit centrifuge technique (Woo, 1969; Woo and Wehnert, 1983) is a sensitive diagnostic technique; it can detect live *Cryptobia* in the blood of fish as early as a week after infection (e.g. Sitja-Bobadilla and Woo, 1994). Also, infections can be detected using the antigen-capture ELISA (Verity and Woo, 1996). It is just as sensitive as the haematocrit centrifuge technique but it uses a monoclonal antibody (mAb-007) to detect soluble parasite antigen.

2.2.1. Salmonid cryptobiosis and mechanism of the disease

(i) The clinical signs of the disease in *Oncorhynchus* spp. include exophthalmia, splenomegaly (spleen may be enlarged six–eight times by volume), a microcytic and

hypochromic anaemia, general oedema, abdominal distention with ascites fluid (Woo, 1979), anorexia (Li and Woo, 1991), erythrocytes are anti-globulin positive (Thomas and Woo, 1988), and immunodepression (Jones et al., 1986).

Histopathological lesions in juvenile rainbow trout appear at about 3–4 weeks after p.i. (Fig. 1); this is the start of acute disease. However, the onset of cryptobiosis varies as it is dependent on the size of the infective dose and on the susceptibility of species or stocks of fish (Woo and Poynton, 1995). Microscopic lesions include reticular hyperplasia in the spleen, mononuclear cell infiltration, endoperivascularitis, tissue necrosis and extravascular localisation of parasites in various organs. At acute disease (usually about 4–6 weeks p.i.) there is severe anaemia, tissue necrosis, infiltration of pleomorphic cells, parasite phagocytosis, proliferative glomerulonephritis, depletion of haematopoietic tissues, and thrombosis in the eye. At 7–9 weeks postinfection, the disease becomes chronic and there is remission of the anaemia (Bahmanrokh and Woo, 1994).

(ii) Acutely infected trout are susceptible to environmental hypoxia partly because of the severe anaemia and high parasitaemia (Woo and Wehnert, 1986). Also, their metabolism and swimming performance are significantly reduced (Kumaraguru et al., 1995), and the bioenergetic cost of the disease is very considerable to infected fish. These result in retarded growth as there are significant reductions in food consumption, dry weight and energy gained, energy concentration, and gross conversion efficiency (Beamish et al., 1996).

(iii) The cysteine protease (49, 60, 66 and 97 kDa) and

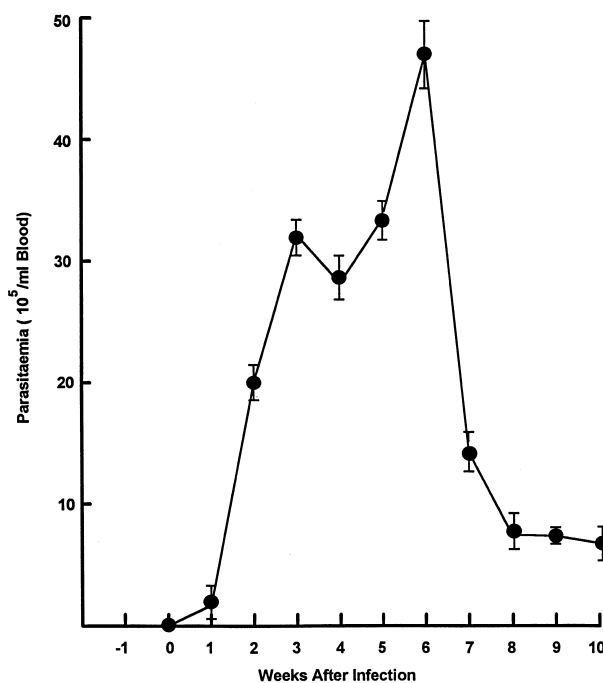


Fig. 1. Parasitaemia (mean \pm SE) in the blood of rainbow trout experimentally infected with *Cryptobia salmositica* (From Verity and Woo, 1996).

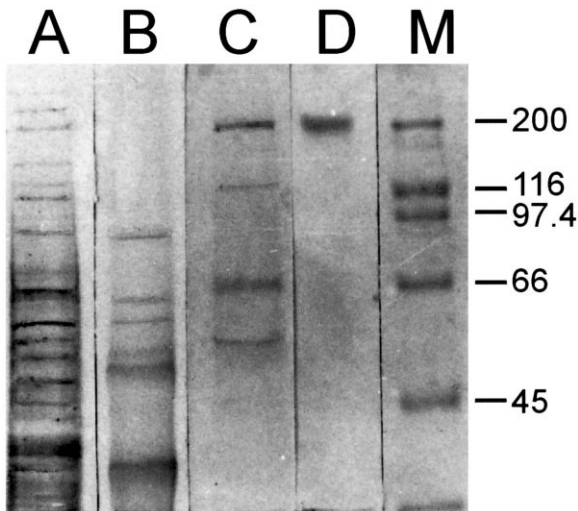


Fig. 2. Purification of cysteine protease and metalloprotease from *Cryptobia salmositica*. Lane A, crude cell lysate; B, partially purified cysteine protease from DEAE-agarose column; C, metalloprotease from DEAE-agarose column; D, purified metalloprotease from Sephacryl S-300 column; M, molecular weight markers (kDa) (Modified from Zuo and Woo, 1997d).

metalloprotease (200 kDa) of *C. salmositica* (Zuo and Woo, 1997a) have been isolated and purified (Fig. 2; Zuo and Woo, 1997d). They have high proteolytic activities against azocasein, haemoglobin and fibrinogen. Also, the metalloprotease has high activity against azocoll and gelatin but low activity against albumin while it is the reverse for the cysteine protease. The optimal pHs for the metalloprotease and cysteine protease are 7.0 and 5.0 respectively (Zuo and Woo, 1997d; 1998a). The cysteine (thiol) protease of *C. salmositica* has similar properties (e.g. mol. wt., substrate specificity, inhibitor sensitivity, optimal pH) as that of *Trypanosoma cruzi* and *Trypanosoma brucei* (Zuo and Woo, 1998a). The enzyme is in both pathogenic and non-pathogenic strains and species of *Cryptobia* spp. (Zuo and Woo, 1997a). Its optimal activity is at acidic pH and has high enzymatic activity against albumin (Zuo and Woo, 1998a); these along with results of the effects of its inhibition by a monoclonal antibody (see Section 2.2.2) indicate that the enzyme is important for intracellular protein catabolism which results in the release of amino acids for parasite protein synthesis.

The purified metalloprotease is inhibited by metal-chelating agents and excess of zinc ions, but is activated by calcium ions. (Zuo and Woo, 1998a). It lyses fish erythrocytes under in vitro conditions (Zuo and Woo, 2000) by digesting the proteins in erythrocyte membranes (Fig. 3; Zuo and Woo, 1997d). This enzyme is an important contributing factor to the anaemia in infected fish (Zuo and Woo, 1997c). It is the haemolysin that was identified earlier as one of the causes of the anaemia in salmonid cryptobiosis (Thomas and Woo, 1988).

The parasite metalloprotease is also collagenolytic as it readily degrades various types of collagens (type I, IV and

V) and laminin (Zuo and Woo, 1997d). Its in vitro secretion by the pathogen is significantly increased in the presence of either type I or IV collagen (Zuo and Woo, 1998b). Since it is a secreted histolytic enzyme it contributes to the disease, and consequently the severity of the disease in *Oncorhynchus* spp. is related to the parasitaemia (Woo, 1979).

(iv) The piscine immune system is depressed during cryptobiosis. Its humoral response to sheep erythrocytes or to another pathogen, *Yersinia ruckeri*, was significantly reduced. Consequently, vaccination against yersiniosis was unsuccessful in infected rainbow trout (Jones et al., 1986). Anorexia contributes to the immunodepression (Thomas and Woo, 1992); however, it is also beneficial to infected fish as it lowers the plasma protein which reduces parasitaemia (Li and Woo, 1991), and subsequently the severity of the disease (Woo, 1979).

2.2.2. Protective strategies

2.2.2.1. Vaccination In general, not all infected fish die from the disease; mortality is dependent on fish stocks and species (Woo and Poynton, 1995). Those that recover are protected on challenge and this protection is via humoral (e.g. Jones and Woo, 1987; Li and Woo, 1995) and cell-mediated (e.g. Thomas and Woo, 1990; Feng and Woo, 1996a) responses. However, the bioenergetic cost of the disease to fish is very significant (Beamish et al., 1996). Two vaccines are being developed to protect fish from salmonid cryptobiosis. The choice of the vaccine is partly dependent on the size of the fish and whether they are in streams or in hatcheries.

The pathogen was attenuated by in vitro culture and has been used as a vaccine. It circulates in the blood of fish for at least 6 months, does not cause disease but protects them

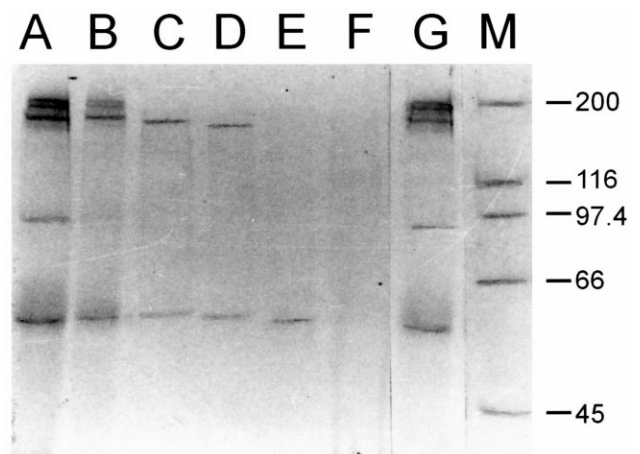


Fig. 3. In vitro digestion of erythrocyte membrane proteins (EMP) by purified metalloprotease (MP). Lane A, EMP + MP for 0 h; B, EMP + MP for 0.5 h; C, EMP + MP for 1 h; D, EMP + MP for 1.5 h; E, EMP + MP for 2 h; F, EMP + MP for 2.5 h; G, EMP + phosphate buffered saline for 2.5 h; M, molecular weight markers (kDa) (From Zuo and Woo, 1997d).

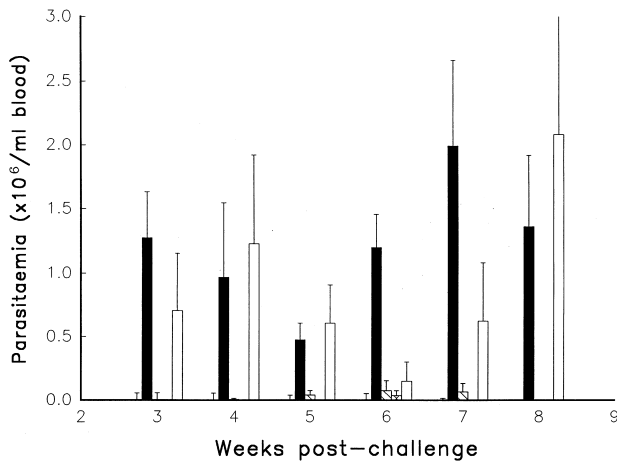


Fig. 4. Parasitaemias in the blood of vaccinee and challenged rainbow trout maintained either in fresh water (right diagonal bars) or in sea water (left diagonal bars) or transferred from fresh to sea water (cross bars); infected fish (controls) maintained in sea water (open bars) and in fresh water (solid bars) (From Li and Woo, 1997).

from disease (Woo and Li, 1990). The avirulent strain has been cloned. A single dose of the vaccine protects rainbow trout for at least 24 months and this is via the production of complement-fixing antibodies, enhanced phagocytic activity of parasites, and cell-mediated cytotoxicity (Li and Woo, 1995). The vaccine is also effective when fish, vaccinated in fresh water are transferred and held in sea water (Fig. 4). Production of complement-fixing antibodies in vaccinated fish (held in either fresh or sea water) increases rapidly and significantly after parasite challenge (Fig. 5; e.g. Li and Woo, 1995; 1997). The vaccine has also been used in studies to better understand factors (e.g. age, dietary ascorbic acid, thymectomy) that may affect the acquisition of protective immunity by salmonids (e.g. Ardelli et al., 1994; Sitja-

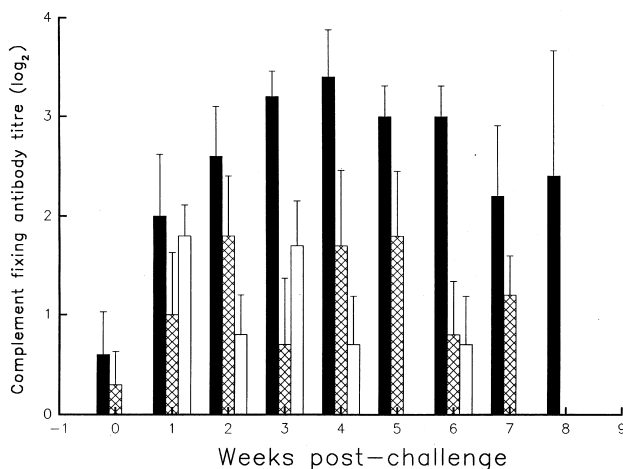


Fig. 5. Titres (mean \pm SE) of complement fixing antibody in the blood of vaccinated and challenged rainbow trout maintained in sea water (open bars), in fresh water (solid bars), and transferred from fresh to sea water (cross bars) (From Li and Woo, 1997).

Bobadilla and Woo, 1994; Li and Woo, 1995; 1997; Feng and Woo, 1996a; Li et al., 1996; Staines and Woo, 1997).

The vaccine strain has remained avirulent and protective for about 12 years, and it does not cause disease because it no longer produces metalloprotease (Zuo and Woo, 1997a). Vaccination does not affect growth of juvenile fish nor has detectable bioenergetic cost to them (Beamish et al., 1996). Consequently, it is a viable protective strategy especially for fish in streams and rivers where the vaccine can be delivered (and hopefully maintained in the wild fish population) by the leech vector. Details of this strategy have been discussed earlier (Woo, 1992).

The second approach is to use a recombinant cysteine protease (49 kDa) to vaccinate young fish before they are released from hatcheries to waters that may have infected leeches and fish. An IgG1 monoclonal antibody (mAb-001) produced against a surface glycoprotein agglutinates *C. salmositica* but does not fix complement to lyse it. Parasites exposed to the antibody do not multiply and they die in a culture medium that normally supports rapid multiplication (Feng and Woo, 1996b). Its epitope consists of a polypeptide, a carbohydrate and probably a phospholipid. It likely has a phosphatidylinositol residue which anchors the polypeptide to the parasite surface membrane, and it is post-translationally modified (Feng and Woo, 1998). The glycoprotein (200 kDa) has high mannose components and it appears as a single band in the vaccine strain and as a doublet in the pathogenic strain (Feng and Woo, 2001). As indicated earlier, the pathogen has both metalloprotease and cysteine protease (see Section 2.2.1) and the antibody (mAb-001) cross reacts with the carbohydrate moieties of these enzymes to inhibit their enzymatic activities (Zuo et al., 1997). MAb-001 also significantly inhibits in vitro oxygen consumption and multiplication in *C. salmositica* exposed to the antibody (Hontzeas and Woo, unpublished). The antibody is also therapeutic when inoculated i.p. into infected juvenile rainbow trout with high parasitaemias (Fig. 6; Feng and Woo, 1997). These studies confirm that cysteine protease is an important metabolic enzyme (Zuo and Woo, 1997a, 1998a) and its inhibition by mAb-001 is detrimental to the parasite and its survival.

The recombinant cysteine protease (49 kDa; Fig. 7) been produced in its 'active' form (Hontzeas and Woo, unpublished) and studies will be conducted to determine its efficacy as a vaccine, the duration of protection and the best delivery system (e.g. by immersion or inoculation). It may also be a good candidate vaccine against other infectious organisms whose cysteine proteases are similar (e.g. in structure, function) to that of *C. salmositica*.

2.2.2.2. Chemotherapeutic and immuno-chemotherapeutic strategies. The trypanocidal drug, isometamidium chloride (Samorin), as in pathogenic mammalian trypanosomes, rapidly concentrates in the mitochondrion of *C. salmositica* and lyses the parasite under in vitro conditions (Ardelli and Woo, 2001a). The drug is effective against *C.*

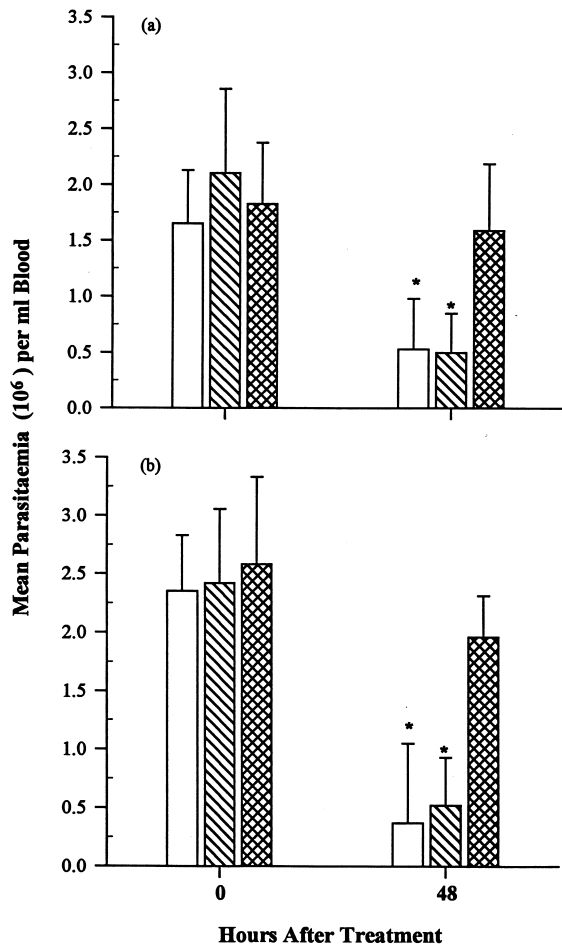


Fig. 6. Parasitaemias (3 weeks after infection) in the blood of juvenile rainbow trout at 48 h after i.p. injection of either monoclonal antibody (mAb-001) or polyclonal antibodies (from recovered trout); (a) - each fish was initially injected i.p. with 2000 parasites, (b) - each fish was initially injected i.p. with 20000 parasites. MAb injected fish (open bars), polyclonal antibodies injected fish (left diagonal bars), and saline injected fish (cross bars) (From Feng and Woo, 1997).

salmositica when it is inoculated i.m. into infected rainbow trout and chinook salmon. It is both therapeutic and prophylactic in chinook salmon (Fig. 8), and its level in the blood peaks at 2 weeks after i.m. inoculation (Ardelli and Woo, 1999, 2001b). All juvenile chinook salmon treated with 1.0 mg/kg at 3 weeks p.i. had significantly lower parasitaemias and all treated fish survived the disease while mortality was 100% in untreated fish. A higher dose (2.5 mg/kg) eliminated the parasite from the blood in 30% of adult chinook salmon while parasitaemias were significantly reduced in the remaining fish (Ardelli and Woo, 2001b). An antigen-capture ELISA was developed to monitor the drug level in *Oncorhynchus* spp. (Ardelli and Woo, 2000).

The effectiveness of the drug is significantly enhanced after it is conjugated to either a monoclonal antibody (mAb-001; see Section 2.2.2.1) or to polyclonal antibodies from immune fish (Ardelli and Woo, unpublished). This

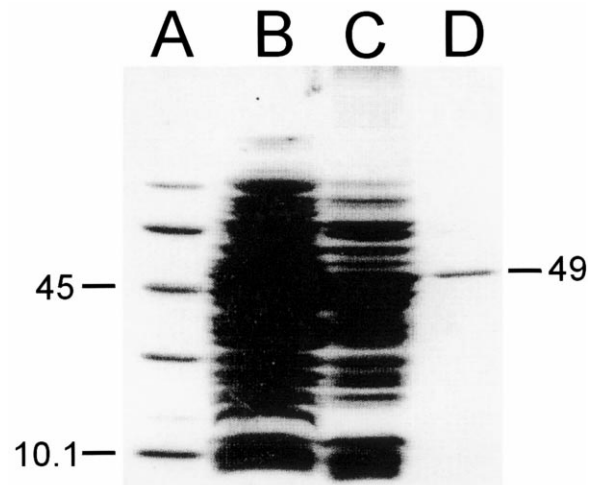


Fig. 7. Purified recombinant cysteine protease (49 kDa) of *Cryptobia salmositica*. Lane A, molecular weight markers; B, cell lysate of uninduced *Escherichia coli*, containing gene insert (no IPTG); C, induced *E. coli*, containing gene insert (with IPTG); and D, purified cysteine protease (From Hontzeas and Woo, unpublished).

immuno-chemotherapeutic strategy is designed to control and manage outbreaks of cryptobiosis in brood (sexually

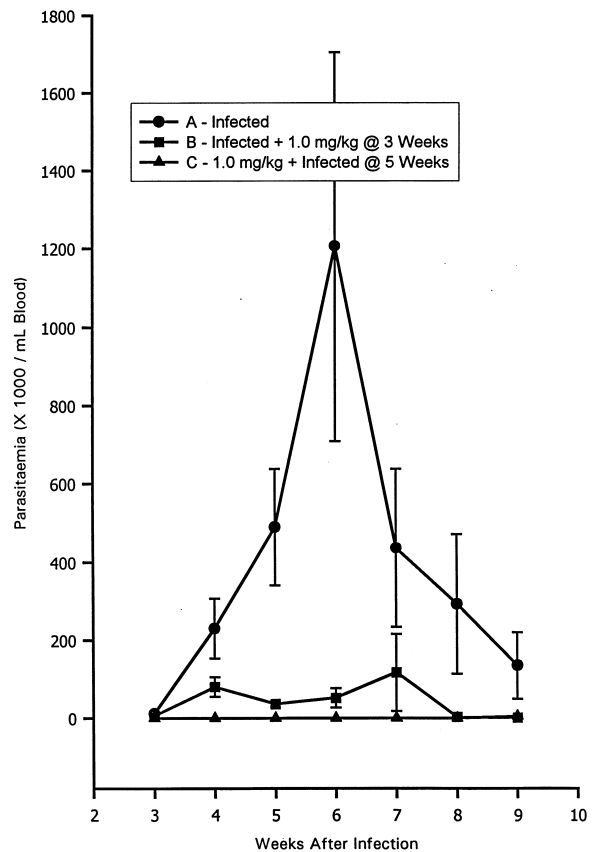


Fig. 8. Parasitaemias in the blood of adult chinook salmon injected i.m. with isometamidium chloride (1.0 mg/kg body weight) at 3 weeks after infection, or treated prophylactically with the drug and infected 5 weeks later (From Ardelli and Woo, 2001b).

mature) fish held in salmon culture facilities on the Pacific coast. For example, in the Soleduck hatchery (Washington State) about 50% of chinook salmon broodstock annually die from cryptobiosis (Woo, 1998). There was a recent outbreak of the disease with more than 50% mortality in chinook salmon undergoing sexual maturation in sea netpens. These fish were held in cages off Vancouver Island, British Columbia, and it was assumed they were infected in fresh water as smolts prior to transfer to sea water (Kent, 1998).

One of the advantages of using an antibody conjugated drug is that the drug will attach specifically to the pathogen; this will greatly reduce the amount of drug needed, the side effects of chemotherapy, and the accumulation of the drug in animals. The last factor is an important consideration if treated animals are subsequently for human consumption (Woo, 1995). Also, decreasing the amount of drug may also slow down the development of drug resistance by the pathogen and drug tolerance by animals. This approach has not been used against infectious organisms; it obviously is more expensive to implement than straight chemotherapy, but it may be a viable option under certain circumstances.

2.2.2.3. Innate protection. Innate immunity is seldom considered a viable strategy to protect animals from infectious organisms. There are two forms of innate protection; the first is resistance to infection and the other is innate protection from disease in infected animals. Both forms have been studied and are part of an on-going program to better understand and to exploit innate immunity to protect fish from *Cryptobia* and cryptobiosis.

Generally, all individuals within a susceptible host species can be infected with a pathogen, but the severity of the disease may vary. However some laboratory and hatchery raised brook charr (*Salvelinus fontinalis*) are resistant to *C. salmositica* infection (*Cryptobia*-resistant) while others can be infected (Ardelli et al., 1994; Forward et al., 1995). Little is known about this type of innate protection in animals (e.g. mechanism of protection, inheritance of resistant factor) within a susceptible host species. Fresh plasma from *Cryptobia*-resistant charr lyse the parasite via the alternate pathway of complement activation while those from *Cryptobia*-tolerant charr (susceptible to infection but with no clinical disease) are not cryptobiocidal (Forward and Woo, 1996). This innate resistance to infection is not related to age of the host nor to the route of infection (Table 1) but is controlled by a dominant Mendelian locus. Consequently, it is possible to breed *Cryptobia*-resistant charr by selective breeding; at least one parent has to be resistant to infection, i.e. either heterozygous (e.g. males from families 115, 117, 129) or homozygous (e.g. male from family 108) dominant for the gene (Table 2). If both parents are susceptible to infection (i.e. homozygous recessive), then all the progenies can be infected (Forward et al., 1995). There are no obvious detectable differences between the *Cryptobia*-resistant and *Cryptobia*-tolerant charr. They both grew well and their

Table 1
Susceptibility of laboratory raised brook charr (*Salvelinus fontinalis*) to *Cryptobia salmositica* infection (Modified from Forward et al., 1995)

Number of resistant fish/ number of fish inoculated	Age of fish (year)	Number of families	Route of inoculation
40/85	1	17	i.p.
17/36	2	11	i.p.
15/28	2	6	i.m.

immune system responded (humoral and cell mediated immunity) rapidly to a commercial *Aeromonas salmonicida* vaccine (Ardelli and Woo, 1995).

The second type of innate protection is the absence of disease in infected animals. The parasitaemias in infected *Cryptobia*-tolerant charr are just as high as in *Oncorhynchus* spp. (e.g. rainbow trout), however infected charr do not have cryptobiosis (Ardelli et al., 1994; Forward et al., 1995). This is because the metalloprotease secreted by the pathogen (see Section 2.2.1) is neutralised by the alpha2-macroglobulin (Zuo and Woo, 1997b,c), one of the two natural antiproteases in the blood of animals. In trout, the alpha2-macroglobulin level dropped significantly after infection and the low level of the antiprotease (about 10% activity) coincided with the acute phase of the disease, e.g. anaemia (Zuo and Woo, 1997c; Fig. 9a). Also, the parasite metalloprotease was detected in the blood of trout.

In *Cryptobia*-tolerant brook charr the alpha2-macroglobulin level was higher than in trout prior to infection. After infection the level was lowered to about 40% activity for a short period during high parasitaemias (Fig. 9b); however, it was still significantly higher than in trout and there was no anaemia. Recovery was more rapid as infected charr did not have clinical disease and the parasite metalloprotease was not detected in their blood (Zuo and Woo, 1997c). One long-term goal is to produce transgenic *Cryptobia*-tolerant salmon, which like the *Cryptobia*-tolerant charr, will rapidly

Table 2
Susceptibility to *Cryptobia salmositica* infection in brook charr progenies of resistant males and susceptible females (#1 or #2) (Modified from Forward et al., 1995)

Family (female X male)	Sample size	Number of infected fish	Number of resistant fish
#1 X #108	17	0	17
#2 X #108	22	0	22
#1 X #129	19	14	5
#2 X #129	22	12	10
#1 X #117	20	9	11
#2 X #117	21	10	11
#1 X #115	20	11	9
#2 X #115	22	10	12
Total	163	66	97

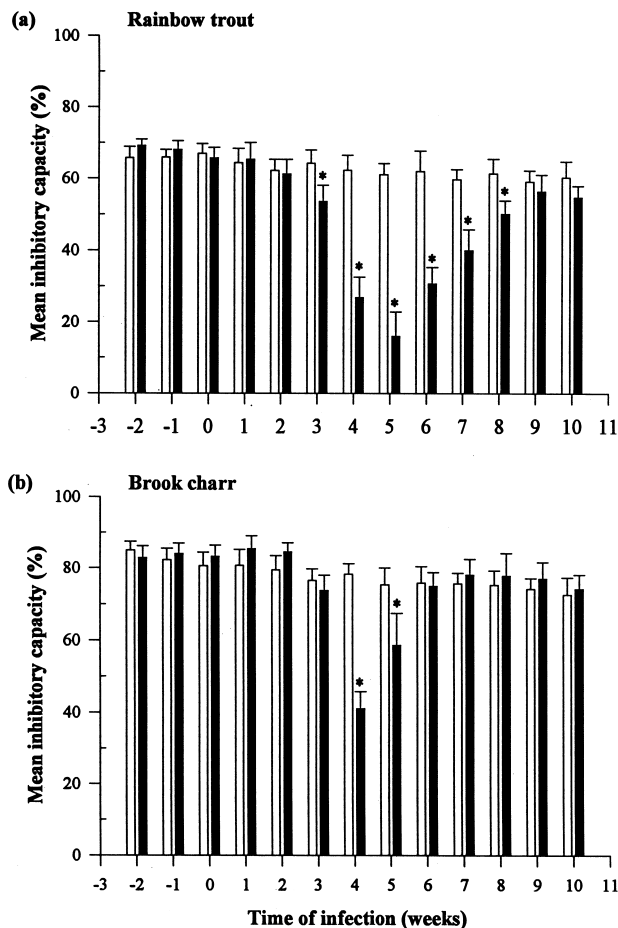


Fig. 9. Inhibitory capacities of alpha2-macroglobulin in the blood of adult rainbow trout (a), and brook charr (b); uninfected (open bars), and infected fish (closed bar); *significantly lower than in uninfected group (From Zuo and Woo, 1997c).

produce alpha2-macroglobulin so that there will not be a need for vaccination or chemotherapy against cryptobiosis.

The use of this strategy to protect animals from an infectious disease is quite novel, and it will likely initiate discussions on the ramifications of applying such a preventive measure. This strategy has at least one obvious advantage, and that is human intervention (e.g. chemotherapy, vaccination) is not required once the transgenic animal is produced. The immune system of the animal can be relied on to effectively control the parasitaemia if the animal gets infected. Innate protection from disease is not well understood nor its mechanism(s) carefully elucidated. It may also operate to protect animals from other infectious diseases for example the 'protection' in reservoir hosts in mammalian trypanosomiasis.

2.3. Cryptobiosis and trypanosomiasis

There are similarities between piscine cryptobiosis and mammalian trypanosomiasis, and they include the mechanism of anaemia (e.g. Thomas and Woo, 1988). Woo (1998)

earlier suggested that it might be rewarding to re-examine the mechanism of trypanotolerance in reservoir hosts (e.g. wild animals). If the mechanisms of pathogen-tolerance in the two diseases are similar, then there may be an alternate strategy to chemotherapy against animal trypanosomiasis. This may include identification of the gene(s) related to high alpha2-macroglobulin production and/or activity in trypanotolerant wild animals, and the subsequent production of transgenic trypanotolerant domestic animals. Since these transgenic animals will not suffer from trypanosomiasis it is expected their immune system will likely be more effective in controlling parasitaemias. This approach is worth consideration especially when there are (a) evidence of drug resistant parasites in the field, (b) no new drugs being developed, and (c) no vaccines available at this time.

The technology for producing transgenic animals is well established, but it is still costly and time consuming to produce a breeding population of transgenic animals. Since fish reach sexual maturity much earlier (usually 1–3 years after hatching) and significantly higher fecundity (hundreds of eggs per fish) it will take less time and will perhaps be easier to have a reproducing colony of transgenic fish than mammals.

3. Conclusions

Piscine cryptobiosis is in food fishes on both the Atlantic and Pacific coasts of North America. Mortality of infected fish may reach 100% in the absence of human intervention (e.g. vaccination, chemotherapy). We have been able to quite successfully exploit both innate and acquired immunity to protect susceptible fish from salmonid cryptobiosis, and hopefully the best is yet to come.

On a more personal note, I would like to thank the organisers for the invitation to again participate in this 'virtual trypanosome meeting'. I have always maintained a keen interest in trypanosomes and trypanosomiasis, and this 'cross pollination' has been useful to us in studying cryptobiosis. I hope that some of our experience and/or approaches against piscine cryptobiosis may be of interest to colleagues working on mammalian trypanosomiasis.

References

- Ardelli, B.F., Woo, P.T.K., 1995. Immune response of *Cryptobia*-resistant and *Cryptobia*-susceptible *Salvelinus fontinalis* to an *Aeromonas salmonicida* vaccine. *Dis. Aquat. Org.* 23, 33–38.
- Ardelli, B.F., Woo, P.T.K., 1999. The therapeutic use of isometamidium chloride against *Cryptobia salmositica* in rainbow trout (*Oncorhynchus mykiss*). *Dis. Aquat. Org.* 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. *Dis. Aquat. Org.* 39, 231–6.
- Ardelli, B.F., Woo, P.T.K., 2001a. The in vitro effects of isometamidium chloride (Samorin) on the piscine hemoflagellate *Cryptobia salmositica* (Kinetoplastida, Bodonina). *J. Parasitol.* 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*). Parasitol. Res. 87, 18–26.
- Ardelli, B.F., Forward, G.M., Woo, P.T.K., 1994. Brook charr (*Salvelinus fontinalis*) and cryptobiosis: a potential salmonid reservoir host for *Cryptobia salmositica* Katz 1951. J. Fish Dis. 17, 567–77.
- Bahmanrokh, M., Woo, P.T.K., 1994. The histopathology of cryptobiosis in juvenile *Oncorhynchus mykiss* (Walbaum). 8th Int. Cong. Parasitol. 2, 434.
- 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. Dis. Aquat. Org. 25, 1–8.
- Becker, C.D., Katz, M., 1965a. Infections of the haemoflagellate *Cryptobia salmositica* Katz 1951, in freshwater teleosts of the Pacific coast. Trans. Am. Fish Soc. 94, 327–33.
- Becker, S.D., Katz, M., 1965b. Transmission of the haemoflagellate *Cryptobia salmositica* Katz 1951, by the rhynchobdellid leech vector. J. Parasitol. 51, 95–99.
- Becker, C.D., Overstreet, R.M., 1979. Haematzoa of marine fishes from the northern Gulf of Mexico. J. Fish Dis. 2, 469–79.
- Bower, S.M., Margolis, L., 1983. Direct transmission of the haemoflagellate *Cryptobia salmositica* among Pacific salmon (*Oncorhynchus* spp.). Can. J. Zool. 61, 1242–50.
- Bower, S.M., Margolis, L., 1984. Distribution of *Cryptobia salmositica*, a haemoflagellate of fishes in British Columbia and the seasonal pattern of infection in a coastal river. Can. J. Zool. 62, 2512–8.
- Burreson, E.M., 1982. Trypanoplasmosis in flounder along the Atlantic coast of the United States. In: Anderson, D.P., Dorson, M., Dubourget, P.H. (Eds.). Les Antigenes des Micro-organismes Pathogenes des Poissons, Collection Fondation Marcel Merieux, France, pp. 251–60.
- Burreson, E.M., Zwerner, D.E., 1982. The role of host biology, vector biology and temperature in the distribution of *Trypanoplasma bullocki* infections in the lower Chesapeake Bay. J. Parasitol. 68, 306–13.
- Daily, D.D., 1978. Marine fish hematozoa from Maine. J. Parasitol. 64, 361–2.
- 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. Parasitol. Res. 82, 604–11.
- Feng, S., Woo, P.T.K., 1996b. Biological characterization of a monoclonal antibody against a surface membrane antigen on *Cryptobia salmositica* Katz 1951. J. Fish Dis. 19, 137–43.
- Feng, S., Woo, P.T.K., 1997. The therapeutic and prophylactic effects of a protective monoclonal antibody (MAb-001) against the pathogenic haemoflagellate *Cryptobia salmositica* Katz 1951. Dis. Aquat. Org. 28, 211–9.
- Feng, S., Woo, P.T.K., 1998. Biochemical characterization of an epitope on the surface membrane antigen (Cs-gp200) of the pathogenic piscine hemoflagellate *Cryptobia salmositica* Katz 1951. Exp. Parasitol. 88, 3–10.
- Feng, S., Woo, P.T.K., 2001. Cell membrane glycoconjugates on virulent and avirulent strains of the haemoflagellate *Cryptobia salmositica* (Kinetoplastida). J. Fish Dis. 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*. Parasitol. Res. 82, 238–41.
- 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, 337–45.
- 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. J. Fish Dis. 10, 395–402.
- Jones, S.R.M., Woo, P.T.K., Stevenson, R.M.W., 1986. Immunosuppression in *Salmo gairdneri* caused by the haemoflagellate, *Cryptobia salmositica*. J. Fish Dis. 9, 931–8.
- Katz, M., 1951. Two new hemoflagellates (genus *Cryptobia*) from some western Washington teleosts. J. Parasitol. 37, 245–50.
- Katz, M., Woodey, J.C., Becker, C.D., Woo, P.T.K., Adams, J.R., 1966. Records of *Cryptobia salmositica* from sockeye salmon from the Fraser River drainage and from the State of Washington. J. Fish. Res. Bd. Canada 23, 1965–6.
- Kent, M.L., 1998. Protozoa and Myxozoa. In: Kent, M.L., Poppe, T.T. (Eds.). Diseases of Seawater Netpen-Reared Salmonid Fishes, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, Canada, pp. 49–67.
- 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). J. Fish Dis. 18, 297–305.
- Laird, M., Bullock, W.H., 1969. Marine fish haematzoa from New Brunswick and New England. J. Fish. Res. Bd. Canada 26, 1075–102.
- Li, S., Woo, P.T.K., 1991. Anorexia reduces the severity of cryptobiosis in *Oncorhynchus mykiss*. J. Parasitol. 77, 467–71.
- 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. Vet. Immunol. Immunopathol. 48, 343–53.
- 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. J. Fish Dis. 20, 369–74.
- 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*. Dis. Aquat. Org. 24, 11–16.
- Newman, M.W., 1978. Pathology associated with *Cryptobia* infection in a summer flounder (*Paralichthys dentatus*). J. Wildlife Dis. 14, 299–304.
- 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. J. Fish Dis. 17, 399–408.
- Staines, G.J., Woo, P.T.K., 1997. Immunization of susceptible chinook salmon, *Oncorhynchus tshawytscha*, against cryptobiosis. Can. Soc. Zool. Bulletin 28, 123–4.
- Strout, R.G., 1965. A new hemoflagellate (genus *Cryptobia*) from marine fishes of northern New England. J. Parasitol. 51, 654–9.
- 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*. J. Fish Dis. 11, 425–31.
- Thomas, P.T., Woo, P.T.K., 1990. In vivo and in vitro cell-mediated immune responses of *Oncorhynchus mykiss* against *Cryptobia salmositica* (Sarcocystidophora: Kinetoplastida). J. Fish Dis. 13, 423–33.
- Thomas, P.T., Woo, P.T.K., 1992. Anorexia in *Oncorhynchus mykiss* infected with *Cryptobia salmositica* (Sarcocystidophora: Kinetoplastida): its onset and contribution to the immunodepression. J. Fish Dis. 15, 443–7.
- 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). J. Fish Dis. 19, 91–109.
- Wales, J.H., Wolf, H., 1955. Three protozoan diseases from trout in California. Calif. Fish Game 41, 183–7.
- Woo, P.T.K., 1969. The haematocrit centrifuge technique for the detection of trypanosomes in blood. Can. J. Zool. 47, 921–3.
- Woo, P.T.K., 1978. The division process of *Cryptobia salmositica* in experimentally infected rainbow trout (*Salmo gairdneri*). Can. J. Zool. 56, 1514–8.
- Woo, P.T.K., 1979. *Trypanoplasma salmositica*: experimental infections in rainbow trout, *Salmo gairdneri*. Exp. Parasitol. 47, 36–48.
- Woo, P.T.K., 1992. Immunological responses of fish to parasitic organisms. Ann. Rev. Fish Dis. 2, 339–66.

- Woo, P.T.K., 1994. Flagellate parasites of fish. In: Krier, J.P. (Ed.), 2nd Edition. Parasitic Protozoa, Vol. 8. Academic Press, New York, USA, pp. 1–80.
- Woo, P.T.K., 1995. Working with 007. An Aquatic Oasis, University of Guelph Research, Guelph, Canada, p. 20.
- Woo, P.T.K., 1998. Protection against *Cryptobia (Trypanoplasma) salmositica* and salmonid cryptobiosis. Parasitol. Today 14, 272–7.
- 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*. J. Parasitol. 76, 752–5.
- Woo, P.T.K., Poynton, S., 1995. Diplomonadida, Kinetoplastida and Amoebida. In: Woo, P.T.K. (Ed.), Fish Diseases and Disorders, Vol. 1. Protozoan and Metazoan Infections. CAB International, Oxon, UK, pp. 27–96.
- 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. J. Protozool. 39, 334–7.
- Woo, P.T.K., Wehnert, S.D., 1986. *Cryptobia salmositica*: susceptibility of infected trout, *Salmo gairdneri*, to environmental hypoxia. J. Parasitol. 72, 392–6.
- Zuo, X., Woo, P.T.K., 1997a. Proteases in pathogenic and non-pathogenic hemoflagellates, *Cryptobia* spp. (Sarcocystidophora: Kinetoplastida) of fishes. Dis. Aquat. Org. 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, 375–82.
- Zuo, X., Woo, P.T.K., 1997c. The in vivo neutralization of proteases from *Cryptobia salmositica* by alpha2-macroglobulin in the blood of rainbow trout, *Oncorhynchus mykiss* and brook charr, *Salvelinus fontinalis*. Dis. Aquat. Org. 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. Dis. Aquat. Org. 30, 177–85.
- Zuo, X., Woo, P.T.K., 1998a. Characterization of purified metallo- and cysteine proteases from the pathogenic haemoflagellate, *Cryptobia salmositica* Katz 1951. Parasitol. Res. 84, 492–8.
- Zuo, X., Woo, P.T.K., 1998b. In vitro secretion of metallo-protease (200 kDa) by the pathogenic piscine haemoflagellate, *Cryptobia salmositica* Katz, and stimulation of protease production by collagen. J. Fish Dis. 21, 249–55.
- Zuo, X., Woo, P.T.K., 2000. In vitro haemolysis of piscine erythrocytes by purified metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica* Katz. J. Fish Dis. 23, 227–30.
- 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. J. Fish Dis. 20, 419–26.