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# Serological evidence of an antibody response in farmed southern bluefin tuna naturally infected with the blood fluke *Cardicola forsteri*

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**Abstract** In this study, adaptive immune response was investigated in farmed southern bluefin tuna, *Thunnus maccoyii*, infected with a sanguinicolid *Cardicola forsteri*. A cohort ($Cohort_{2005}$) of southern bluefin tuna was sampled between March 2005 and August 2006. Samples were taken at the transfer of wild caught tuna to sea cages and then at regular intervals. Parasite intensity, abundance and prevalence data were recorded. An ELISA was developed to detect and quantify an antibody response against the blood fluke in southern bluefin tuna serum. Intensity and prevalence of the blood fluke were shown to peak in May 2005 at 10.9 flukes per infected fish (SE = 1.72) and 97.5% prevalence and then decreased to low prevalence (10%) and intensity (1.0). There were no significant changes in prevalence or intensity in 2006. Antibody titres and seroprevalence increased from 1.37 U $\mu l^{-1}$ and 10% at transfer in March 2005 to reach a peak in December 2005 of 25.86 U $\mu l^{-1}$ (SE = 6.26 U $\mu l^{-1}$) and 66.66%. No significant changes were observed in antibody titres for the same cohort of fish during 2006. Parasitological and serological values from $Cohort_{2005}$ were compared to a 2006 cohort ($Cohort_{2006}$) in March 2006 and August 2006 to determine if prior infection in $Cohort_{2005}$ elicited any protection against infection in 2006. Although significant differences were not observed in intensities between cohorts it was shown that $Cohort_{2005}$ had significantly lower abundances and prevalences of blood fluke infection than $Cohort_{2006}$. Although there was no significant difference in mean antibody titres between cohorts in March 2006, the mean antibody titre

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of Cohort$_{2006}$ was significantly greater than that of Cohort$_{2005}$ in August 2006. No significant differences were observed in seroprevalence. This is one of the few studies to demonstrate the development of acquired resistance in fish against a parasite in an aquaculture environment under natural infection conditions.


## Introduction

Southern bluefin tuna, *Thunnus maccoyii*, is a commercially important aquaculture species. The industry is based on the capture of 2—3 year old wild fish which are purse-seined in the Great Australian Bight, moved into towing pontoons and towed to the farming zone in the Spencer Gulf of South Australia where they are transferred into sea cages. On-growing in sea cages occurs for a period of 2—8 months before southern bluefin tuna are exported as frozen or fresh product to Japan for the premium sashimi market. The southern bluefin tuna farming industry is economically significant, producing 9993 tonnes worth AUD160 million in 2006 (T.B.O.A., pers. com.).

Although wild southern bluefin tuna are infected with a range of parasites (see review in Ref. [1]) heavy infections are rarely observed in southern bluefin tuna aquaculture [2]. This could be a consequence of a well developed host immune system in southern bluefin tuna [3]. Of the parasites that are of concern, a sanguinicolid, *Cardicola forsteri*, is a cause of significant gill pathology [4,5]. Sanguinicolids are parasites of marine and freshwater fish [6]. Most species establish in the heart, bulbus arteriosus, ventral aorta, or branchial vessels, although distributions within the cephalic or dorsal vessels are not uncommon [7]. Once established, the adult fluke lays eggs which travel to the gills where they lodge. Here the eggs hatch and break out of the gill as free living miracidia. These miracidia infect an intermediate host into which they penetrate to undergo asexual reproduction as rediae and/or sporocysts to produce infective cercariae. The intermediate host of *C. forsteri* is as yet unknown. Bivalves and polychaetes have been reported to be intermediate hosts for some species of marine sanguinicolids [6,8]. Cercariae emerge from the intermediate host and actively search for the definitive host, a fish. The cercariae penetrate the skin of the host and juvenile flukes attempt to reach the circulatory system in which they undergo a migration to a final site where they mature [6]. For *C. forsteri* the final site is the heart [9]. In southern blue fin tuna this parasite can reach 100% prevalence with heavy burdens in the first 2 months of growout [10]. However, toward the end of the growout season (6—8 months) low intensities and prevalences are observed. A previous study has shown a specific response against the blood fluke in farmed southern bluefin tuna using Western blot analysis [11]. It is not known whether this antibody response in the tuna has a protective role or how it is affected by infection dynamics. Little is known about specifics of the immune responses directed at marine sanguinicolids [12].

In this study, an enzyme-linked immunosorbent assay (ELISA) was developed to detect and quantify antibodies against the blood fluke. ELISA is believed to be the best method to measure specific antibody titres in fish and is a widely used, sensitive and reliable monitoring tool for the detection and quantification of specific humoral antibody responses to a variety of fish pathogens [13—16]. The development of such serological tests is important for risk assessment in disease management strategies [17]. Serology has a number of advantages over direct detection of parasite pathogens. Diagnostically, serological assays offer the potential to demonstrate exposure to given parasites long after the parasite may be detected [18].

The aim of this study was to investigate the antibody response of southern bluefin tuna against *C. forsteri* infection. We approach this issue by addressing the following questions: (1) what is the relationship over time between parasite burden and antibody titres? (2) Is there an antibody response that could lead to resistance against re-infection in an aquaculture environment?

## Methods and materials

### Experimental fish and study design

A cohort of southern bluefin tuna (Cohort$_{2005}$) was sampled (hearts and blood) at various stages over a 16 month period during 2005 and 2006 from one company's lease site. Wild *T. maccoyii* were captured by purse-seine in the Great Australian Bight (map reference 33° 27′S, 132° 04′E) on 19th February 2005 and towed to the Spencer Gulf farming zone over a period of approximately 6 weeks in a towing pontoon. Tuna were transferred from the tow pontoon to four sea cages for farming on 5th April 2005. Two hundred and twenty tuna were transferred into each sea cage and 10 tuna were sampled at this time representing 4.5% of the total population for that sea cage. During the growout period, 10 tuna were sampled from each of the four cages on 30th May, 11th July, and 22nd August. Following a harvest in August, all the remaining tuna of the four cages were moved to a single cage. Thirty tuna were then sampled from this cage on 6th December, 10 tuna sampled on 7th March, 14th March, 31st March and then 30 tuna sampled on 15th August. A total of 220 research tuna were examined. In addition, tuna from the 2006 intake of wild fish (Cohort$_{2006}$) were sampled for comparison with Cohort$_{2005}$ to determine if prior infection in the remaining southern bluefin tuna from Cohort$_{2005}$ elicited any protection against re-infection in 2006. Twenty tuna from a different company were examined on 24th March (37 days post transfer) and 10 tuna on 28th March (41 days post transfer). Twenty tuna from the same company farming the Cohort$_{2005}$ were sampled on 18th August (154 days post transfer). A total of 50 Cohort$_{2006}$ tuna were examined. The sampling times and number of fish were determined in collaboration with the industry. The number of southern bluefin tuna sampled