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Relationship Between Water Temperature and Immune Response of Grass Carp (*Ctenopharyngodon idellus* C. et V.)

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Abstract

The relationship between immune response of grass carp (*Ctenopharygodon idellus*) and water temperature was investigated according to changes in antiserum neutralization titer (ANT) and percent relative protection (PRP) of fish immunized with killed cell-cultured vaccine against Fish Reovirus (Vaccine CFRV). Experimental results showed that: 1) the water temperature of 10°C is the critical point in the immunization of grass carp with Vaccine CFRV; 2) the immune response was inhibited below 10°C and enhanced with in-crease in water temperature up to 32°C, after which it decreased; and 3) water tempera-ture at the inductive phase was one of the key factors that determined the occurrence and strength of the immune response.

Introduction

Several environmental and physiological factors affect the immune response of fish, and one of prime importance is temperature of the environment. Nybelin (1935) first proved that production of strain-specific agglutinins for *Vibrio* produced in eel (*Anguilla anguilla*) was temperature-dependent. Later, many studies noted that the immune response of teleost fish, such as *Cyprinus carpio, Carassius auratus, Salmo trutta, Notothenius rossii, Salmo gairdneri* and *Onchorhynchus mykiss,* was markedly influenced by environmental water temperature (Cushing 1942; Bissett 1947, 1948, 1949; Pliszka 1949; Clem and Sigel 1963; Avtalion 1969; Baudouy et al. 1980; Bly and Clem 1992).

Grass carp (*Ctenopharyngodon idellus* C. et V.) is a major pond-cultured fish in China, but hemorrhage of grass carp (HOGC) caused by Fish Reovirus (FRV) has devastated the production of fingerlings. It is difficult to protect grass carp from this disease with medicines, and immunoprophylaxis with killed cell-cultured vaccine against FRV (Vaccine CFRV) plays an increasingly

important role in controlling this disease (Yang et al. 1986, 1989). However, little systematic research has been done on the influence of water temperature on immune response of grass carp. In order to identify the optimum immunization conditions for the vaccine, to enhance the immune efficiency and to supply a theoretical basis for the rational and effective use of this vaccine, the relationship between environmental water temperature and immune response of grass carp was investigated. Antiserum neutralization titer (ANT) and percent relative protection (PRP) of immunized grass carp was measured under different temperature conditions.

Materials and Methods

Grass carp fingerlings, 10 cm in length, were proved HOGC-free by ELISA and maintained in net cages one week before the experiment. Vaccine CFRV from strain FR-854 was prepared and appraised according to Yang et al. (1986, 1989). The titer was determined by measuring the virus titer prior to its inactivation ($lgTCID_{50}ml^{-1}$). Fish were injected intraperitoneally with 0.4 ml of the vaccine (about 4.5 $lgTCID_{50}ml^{-1}$) per fish.

Evaluation of the Immune Response

After vaccinated fish were cultured for some time, PRP $[PRP = (1 - \frac{\%\ \text{mortality in vaccinated}}{\%\ \text{mortality in control}}) \times 100\%]$ was determined by challenging with 6 $lgTCID_{50}ml^{-1}$ of virus, and the ANT (indicated by the reciprocal value of the highest antiserum dilution which protects 50% of cells from cytopathic effect) measured in serum collected from immunized fish to evaluate the immune response (Yang et al. 1986, 1989).

Relationship between Water Temperature and Immune Response

Four experiments were conducted under different conditions of water temperature, and the PRP and ANT of the fish in each group measured.

Experiment 1

At five levels of natural water temperature during different months, 60 fish were immunized with an equal titer of Vaccine CFRV.

Experiment 2

Two groups of 50 vaccinated fish each were maintained in net cages from March to May for 39 d when environmental water temperature was rising; another two groups, with the same number of fish immunized with equal titer

of vaccine, were maintained from October to December for 38 d when environmental water temperature was falling.

Experiment 3

In December, when natural water temperature was below 10°C, three groups of 180 fish each, were immunized at the same time. In Groups A and B, vaccinated fish were cultivated at a controlled water temperature of 20°C for 5 and 10 d, respectively; then in net cages in natural water. In group C, fish were cultivated in a net cage in natural water. But on days 30, 95 and 132, 40 fish were taken out and cultivated at 20°C for 10 d; after which their immune responses were determined.

Experiment 4

From August to October, 1,075 vaccinated fish were maintained in natural water with an average temperature of 29°C (range 24-35°C). On days 5, 10, 15, 20, 30, 60 and 80 after vaccination, the ANT was determined, and the results compared with groups A and B in Experiment 3.

Controls, unvaccinated fish and the corresponding numbers of the test groups in every experiment were arranged simultaneously as indicated above.

Results

The Immune Response at Different Environmental Water Temperatures

Fig. 1 presents the results of immunization tests at the average water temperature of 7.0, 14.4, 19.4, 26.9 and above 32°C. When environmental water temperature was below 10°C (average 7±1.9°C, range 4-9°C), the immune response of grass carp to Vaccine CFRV was inhibited, as indicated by low ANT [11.6±3.1(3)] and PRP [20.2±8.8(3)%]. However, as temperature increased to above 10°C (average 14.4±2.8°C; range 10-21°C), ANT and PRP rose noticeably and separately reached 73.6±28.1(3) and 79.0±12.9(3)%, respectively, which was significantly higher than before ($P<0.01$). As temperature increased, ANT and PRP also increased gradually. When the average temperature, approached 19.4±4.2°C and range 11-26°C, high levels of ANT [129.5±44.4(2)] and PRP [91.6±12.0(2)%] were obtained. At an even higher temperature, there was no enhancement of the immune response of grass carp to Vaccine CFRV. The ANT and PRP of vaccinated fish at 26.9±2.4°C (average) or 21-31°C (range) were 114.1±2.5(2) and 93.4±9.4(2)%, respectively, not significantly different from those at 19.4±4.2°C ($0.8>P>0.7$ and $P>0.9$). When average temperature rose to 32°C (range 30-37°C), ANT and PRP fell to 66.4±11.3(3) and 73.8±19.7(3)%, respectively, both of which are different from those at 26.9±2.4°C at the significance level of $0.2>P>0.1$.

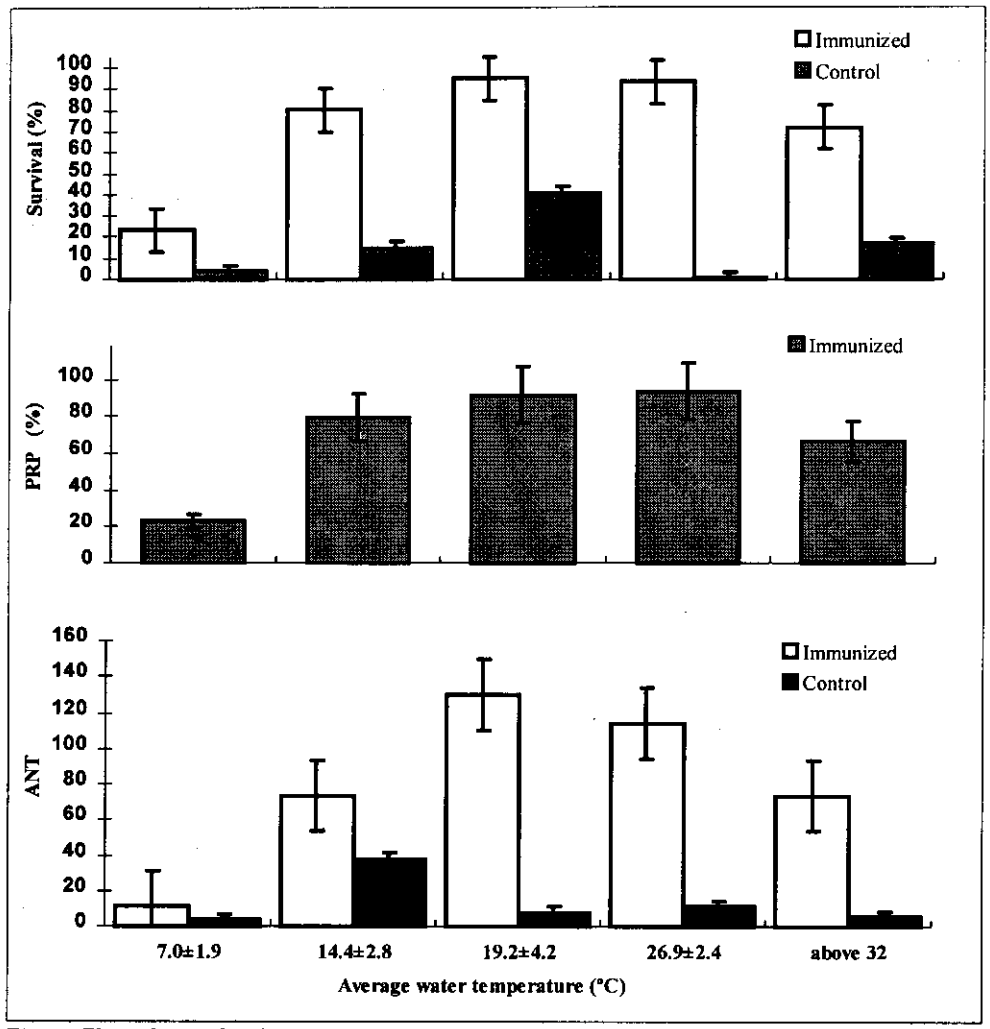

Fig. 1. The relationship between environmental water temperature and the immune response of grass carp.

The Immune Responses at Two Types of Water Temperature

Fig. 2 presents two types of water temperature and the immune response. One tended to increase, the average and range of which, in the first 5 d, were 12.2±1.9°C and 10-15°C, respectively. The other, which tended to decrease, had an average of 19.4±0.9°C, and a range of 18-20°C. In the entire experiment, average water temperature of the decreasing type was 17.3±3.9(39)°C, much higher than that of the increasing type [15.8±2.0(37)°C]. The differences were significant (T test: 0.05>P>0.01). Whether water temperature tended to increase or decrease, fish responded to Vaccine CFRV. The ANT of immunized fish (63.6±36.1[2] for increasing temperature, and 108.5±27.5[2] for decreasing temperature), were notably higher than those of controls (38.0 and 5.0). Their PRP rose to 74.5±10.2(2)% and 83.6±0(2)%, respectively. On the other hand, the immune response of fish at increasing temperatures was lower, analyzed by ANT or PRP.

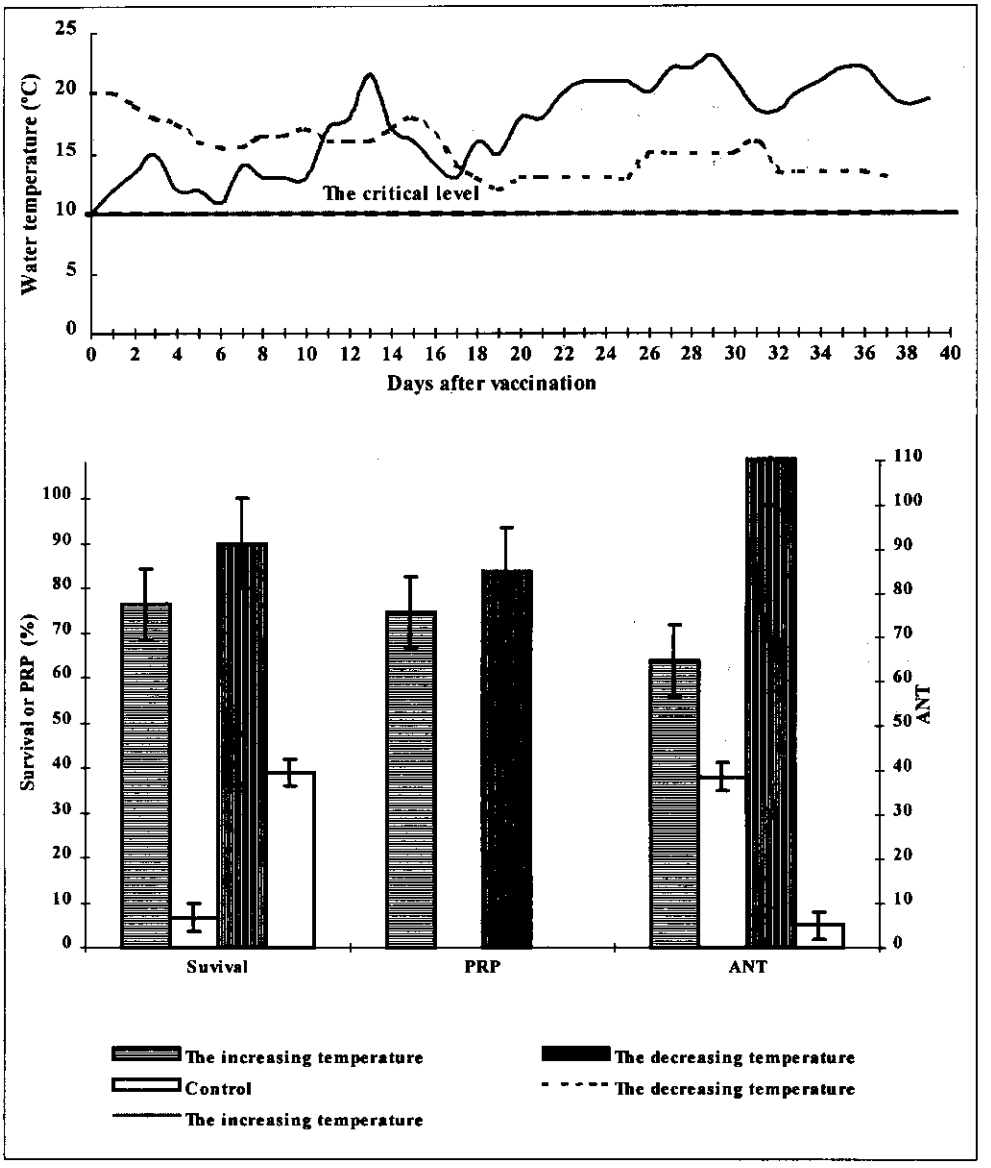

Fig. 2. The relationship between two different water temperature regimes and the immune response.

Influence of Water Temperature in the Inductive Phase on Immune Response

It can be seen from Fig. 3 that daily natural water temperature was below 10°C and the average merely 6.3±1.4°C before day 82; it began to increase and reached 15.7°C on day 101. Under this natural water temperature, the ANT of immunized fish in groups A and B, which were cultivated at 20°C for a short time, went to 140.4±9.1(2) and 122.8±7.4(2) on day 10, then attained a peak value of 270.2±25.7(2) and 197.3±76.2(2) on day 20. On day 30, ANT fell slightly, but maintained a high level. On day 40, ANT decreased to 147.7±6.2(2) for group A, and 137.7±56.7(2) for group B. ANT fluctuated at this level until

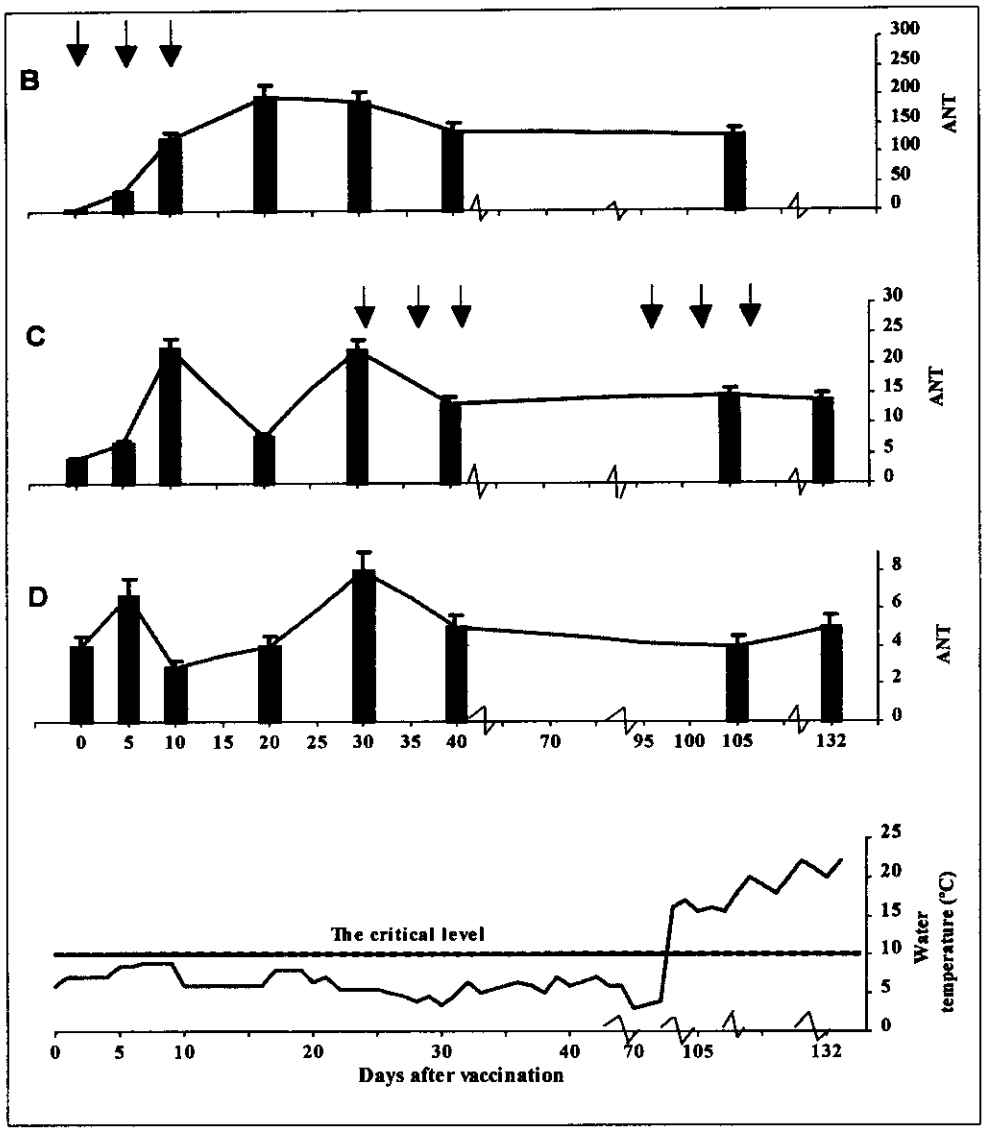

Fig.3. The variation of neutralization antibody of immunized grass carp under different water temperatures. A, B and C for the immunized, D for control. The water temperature in the sections indicated by arrows was 20°C, and in the other sections, the same as environmental water temperature.

day 105. However, fish in group C maintained in natural water of low temperature failed to develop the immune response. The ANT waved at a low level: 22.5±8.5(2), 7.7±3.0(2) and 22.2±0(2) on days 10, 20 and 30, respectively, which was only slightly higher than control. Although some fish were transferred to the controlled water temperature of 20°C on days 30 and 95, and cultivated for 10 d, the ANT was still not enhanced on days 40 [13.30±(2)] and 105 [14.8±3.2(2)]. It was still 13.8±0(2) until day 132, although the natural water temperature had increased to 22°C.

Influence of Water Temperature After the Inductive Phase on Immune Response

When immunized fish were always cultured at an average water temperature of 29°C, their response production phase, peak stage of neutralizing antibodies, and continuity of immunity were basically the same as in groups A and B that were placed above 10°C in the inductive phase and then below 10°C for the remaining period. Their ANT levels were low on day 5 after vaccination. On day 10, they reached peak value at almost the same time. Afterwards, the levels dropped slightly, but remained high on days 80 and 105 (Table 1).

Discussion

Fish are poikilothermic and strictly follow ambient temperature. Their immunologic responsiveness, such as antibody production, as a whole, is influenced by temperature (Dorson 1984); and it is now well established that low environmental temperatures are immunosuppressive in teleost fish (Bly and Clem 1992). The results of this study show that grass carp immunized with Vaccine CFRV and held below 10°C have a weak immune response: both ANT and PRP were low; while those held above 10°C were exactly the reverse. From our research, we believe that there is a critical temperature for immune response in grass carp and we regard this as being 10°C.

In general, the immunologic responsiveness of fish is enhanced as environmental water temperature rises. On the basis of the results of experiment 1, it can be seen that the immunologic responsiveness of immunized fish generally reaches the highest level when water temperature approaches its optimum growth value, i.e., 20-32°C. Within the range, it does not vary with temperature fluctuation when other factors are constant, such as nutrition, dissolved oxygen content, lighting condition, stress, and so on. Nevertheless, if temperature exceeds optimum growth temperature, immunological responsiveness tends to fall. This indicates that the best immunizing condition for grass carp is around the optimum growth temperature for the species.

Avtalion et al. (1973) claimed that only the induction phase of the primary response is temperature-dependent and not antibody release from plasma cells. As long as the fish has been in contact with the antigen for a short initial period at temperatures above the critical level, antibody production will proceed normally irrespective of environmental temperature. The results of our research are basically in agreement. Experiment 3 shows that when fish in group C were kept at temperatures below the critical level during the inductive phase, the neutralizing antibody in serum was low all along and the response failed to occur even though they were transferred to a temperature above the critical level for 10 d on days 40, 105 and 132, respectively. On the other hand, a short stay at a temperature above the critical level after immunization (group A or B) allowed the fish to continue production of neutralizing antibodies and

Table 1. Comparison of variation of ANT among immunized grass carp under three conditions of water temperature.

Days after vaccination	Immune group in Exp.4	Group A in Exp.3	Group B in Exp.3
5	14.5±1.5(2)[1]	32.0±0(2)	32.0±0(2)
10	108.6±19.5(2)	140.0±9.1(2)	122.8±7.4(2)
15	128.0±0(2)	-	-
20	217.0±39.0(2)	270.2±25.7(2)	197.3±76.2(2)
30	217.0±39.0(2)	183.4±0(2)	186.5±3.5(2)
40		145.7±6.2(2)	137.7±56.2(2)
60	128.0±0(2)	-	-
80	89.1±0(2)	-	-
105	-	139.4±26.2(2)	130.5±56.7(2)
Control	9.1±2.7(2)	6.0±2.7(2)	6.0±2.7(2)

[1]Mean±S.D.(Test numbers).

acquire protection against HOGC. This work suggests that the water temperature during the inductive phase is important in determining immune response in grass carp. Temperature may involve the mechanisms by which the competent lymphocytes are stimulated to become antibody-producing cells, or it may affect the multiplication phase of stimulated and transformed lymphoid blast cells in their production of a clone of plasma cells. Antigen clearance and trapping by immunocompetent cells as well as synthesis and release of antibodies are not inhibited by low temperature. If the temperature during the inductive phase is so low that competent lymphocytes are not stimulated by the antigen, there is no neutralizing antibody occurring in the serum, and the fish have very weak protection, although temperature increases to above the level after the antigen is eliminated. Furthermore, it is demonstrated in experiment 2 that the temperature during the inductive phase is one of the important factors that determine whether the immune response is strong or weak. Higher temperature may accelerate the transformation of immunocompetent cells.

From Table 1, we conclude that the synthesis and release of neutralizing antibodies in immunized grass carp in serum is not affected by temperature during the post-inductive phase of immunization. Whether they are high or low, the time of onset of the response, the time of peak value occurrence and the protective effectiveness for a given period of time, on the whole, showed no difference, and the level of ANT is approximately the same. In addition, the immunological responsiveness of fish in group A kept at a temperature above the critical level for 5 d after immunization is roughly similar to those in group B kept at the same temperature for 10 d. This indicates that the immunization induction phase of grass carp to Vaccine CFRV is no more than 5 d. As long as water temperature remains at 10°C for 5 d after immunization, the optimum result is obtained. Avtalion et al. (1973) found that if carp were immunized at 25°C, then transferred to 12°C at least 8 d after injection of the antigen, then normal antibody response occurred. A comparison between the results of this study and Avtalion et al.'s (1973) indicates that different types of fish and antigens result in different types of immune response.

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