ELSEVIER

Comparative Biochemistry and Physiology Part B 122 (1999) 173–180

CBP

Humoral immune parameters in Atlantic cod (*Gadus morhua* L.) I. The effects of environmental temperature

Bergljót Magnadóttir [a,*], Halla Jónsdóttir [a], Sigurður Helgason [a], Björn Björnsson [b], Trond Ø. Jørgensen [c], Lars Pilström [d]

[a] *Institute for Experimental Pathology, University of Iceland, Keldur v/Vesturlandsveg, IS-112, Reykjavík, Iceland*
[b] *The Marine Research Institute, Skúlagata 4, P.O. Box 1390, IS-121, Reykjavík, Iceland*
[c] *The Norwegian College of Fishery Science, University of Tromsø, N-9037, Tromsø, Norway*
[d] *Department of Medical Immunology and Microbiology, Uppsala University, S-751 23, Uppsala, Sweden*

Received 26 May 1998; received in revised form 29 October 1998; accepted 23 November 1998

Abstract

The effects of environmental temperature on certain humoral immune parameters in Atlantic cod (*Gadus morhua* L.) were studied. Serum samples were collected from captive cod, of wild origin, kept at different temperatures for 12 months. It was found that immunoglobulin and natural antibody levels increased with increasing temperature whereas the total serum protein concentration, anti-protease activity, iron concentration, unsaturated and total iron binding capacity decreased with increasing temperature. Haemolytic activity and percentage iron saturation also tended to decrease with increasing temperature although this was not statistically significant. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Anti-protease; *Gadus morhua*; Haemolysin; Immunoglobulin; Iron; Lysozyme; Natural antibodies; Temperature

1. Introduction

Atlantic cod (*Gadus morhua* L.) is an economically important fish species in the North Atlantic and its ecology as well as its physical and biological characteristics are well documented [23,36]. A further impetus to its importance has come with the suggestion by the International Council for Exploration of the Sea (ICES) that cod should be used as a model for predicting the effects of climatic change on the marine life of the North Atlantic [16] as well as with the growing concern for its survival in the North Sea [9]. It is thus important to examine the effects environmental changes may have on the immune system of cod and hence its ability to combat diseases.

Fish have been found to exert a varied, non-specific [1,3] and specific [11,24,35], immune response to different antigens. Several studies have shown that seasonal and circadian changes in temperature and salinity [1,5,19,39] affect the immune system of fish, as do antibiotics and detrimental environmental factors such as pollutants [2,24]. Certain pathogens may also modulate or suppress the response to antigenic stimuli [24,28].

Although bony fish have generally only one type of immunoglobulin (IgM), many species can exert an adequate humoral antibody response against various antigens [14,18,22]. However, attempts to induce antibody response in cod, for example by immersion or injection vaccination with killed pathogenic bacteria, have been unsuccessful although giving protection against infec-

Abbreviations: Asa, Aeromonas salmonicida ssp. achromogenes; BSA, bovine serum albumin; ECP, extracellular product; Glyc, glycogen; LPH, Limulus polyphemus hemocyanin; Pf, Pseudomonas fluorescens; ssDNA, single stranded DNA; Thyr, thyroglobulin; TNP, trinitrophenyl.

* Corresponding author. Tel.: +354-567-4700; fax +354 567 3979; e-mail: bergmagn@rhi.hi.is.

0305-0491/99/$ - see front matter © 1999 Elsevier Science Inc. All rights reserved.
PII: S0305-0491(98)10156-6

tion [11,35]. Another aspect of the cod's immune system is the presence of a relatively high level of natural or non-specific antibodies in serum [31]. So far nothing has been found that satisfactorily explains the lack of specific response. Although cod has a limited antibody repertoire it can distinguish related antigenic determinants [11], and the genetic organisation of the variable domains is similar to that of other teleost species [4].

The objective of this study was to examine if and how certain humoral factors of cod, which are considered of immunological importance, are influenced by the environmental temperature.

2. Materials and methods

2.1. Fish and sampling

Wild cod, initial mean weight of 600 g, captured off the south-west coast of Iceland, were kept in three circular fibreglass tanks, 3 m in diameter and 0.8 m deep, supplied with a flow-through of sea water, salinity 32 ppm. Following acclimatisation at 7°C, the temperature in two of the tanks was gradually changed to either 1 or 14°C and the fish maintained at these temperatures for 12 months. A natural photo period was provided, aided by an electric bulb (60 W) over each tank connected to a light detector outside. The fish were fed to satiation on capelin (*Mallotus villosus*) 4 days a week and shrimp (*Pandalus borealis*) 2 days a week. Each group initially comprised 33 fish, tagged at the base of a dorsal fin.

The weight and length of the fish was recorded every 3 months. At the end of the 12-month period sex and sexual maturity was determined. Blood samples were collected at the end of the experiment from a caudal vessel from 11–12 surviving fish from each group. The blood was allowed to clot at room temperature (22°C) for 1–2 h and then at 4°C overnight. Serum, collected after centrifugation at $750 \times g$ for 10 min, was divided into several aliquots and stored at -20°C.

2.2. Protein concentration

A protein assay kit (Pierce, USA) was used for protein estimation following the manufacturer's instructions. The kit was based on the analytical method described by Bradford [7] with bovine serum albumin (BSA) used as the standard protein.

2.3. Immunoglobulin (IgM) concentration

The total immunoglobulin (IgM) concentration in cod serum was measured using the capture-ELISA described by Pilström and Petersson [31] and Israelsson and co-workers [17], with minor modifications to incubation times. Rabbit polyclonal anti-cod-IgM antibodies (prepared by L. Pilström) were used for the capture and mouse monoclonal anti-cod-IgM antibodies (prepared by L. Pilström), followed by alkaline phosphatase conjugated rabbit anti-mouse-Ig antibodies (Dako, Denmark), were used for the detection of captured serum IgM. Optical density (OD) was read at 405 nm.

Purified cod IgM, isolated from pooled serum from several captive cod of wild origin, was used as a standard, the concentration of which was estimated by measuring the absorbance at 280 and 310 nm, using the following expression: 1 mg cod IgM ml^{-1} has (A280 − A310) = 1.115 [31].

2.4. Natural antibody activity

The antibody activity in cod serum, against eight antigens, was measured using the double sandwich-ELISA previously described [24].

The following antigens were used (see list of abbreviations): TNP–BSA (containing 21 TNP residues per BSA molecule), LPH (Sigma, USA), ssDNA (from calf thymus, Sigma, USA), Thyr (bovine, Sigma, USA), BSA (Sigma, USA), Glyc (from *Mytilus edulis*, Sigma, USA), ECP-Asa and ECP-Pf. The last two antigens were prepared from bacteria, isolated from Icelandic salmon, by the modified overlay method [13].

Microtrays (MaxiSorp, Nunc, Denmark) were coated with 100 μl $well^{-1}$ of antigen, diluted to 10 μg ml^{-1} in coating buffer, except TNP–BSA, which was diluted to 5 μg ml^{-1}. Blocking of residual sites was with 0.1% skimmed milk powder in coating buffer (0.05 M carbonate buffer, pH 9.6 (tablets, Sigma, USA)) instead of BSA which was a modification of the original method [24]. Sera were tested in a 1/100 dilution and incubated overnight at 4°C. Mouse polycolonal anti-cod-IgM antibodies (prepared by B. Magnadóttir), followed by alkaline phosphatase conjugated rabbit anti-mouse-Ig antibodies, were used for the detection of bound cod antibodies.

2.5. Haemolytic activity

The haemolytic activity of serum was measured using a modification of the method described by Yano [38] for measuring complement activity by the alternative pathway. The target cells were sheep red blood cells (s-RBC) and the reaction buffer was complement fixation test buffer containing Mg^{2+} and Ca^{2+} (prepared from tablets, Oxoid, UK), with 0.1% gelatine (CFT-G) added.

s-RBC (stored in Alsever's solution, 1:1) were washed three times in saline and finally in CFT-G. A 1% suspension was prepared in CFT-G, adjusted so that 100 μl lysed with 3.4 ml of H_2O gave an OD reading of 0.740 at 414 nm against a blank of distilled water.

Test serum was diluted 1/50 in CFT-G and 250 μl incubated with 100 μl of 1% s-RBC suspension for 90 min. at 22°C, mixing from time to time. Blank and 100% lysis control contained CFT-G in place of serum.

Following the incubation, 3.15 ml of saline (0.85% NaCl) was added to each test sample and the blank and 3.15 ml of distilled water to the 100% lysis control. The suspensions were centrifuged for 10 min at 750 g and the OD of the supernatant read at 414 nm, subtracting the blank. The percentage haemolysis was calculated from the OD value of the 100% lysis control.

2.6. Lysozyme activity

A turbidimetric assay was used [29] with some modifications. On a 96 well microtray 100 μl of serum in four 2-fold serial dilutions, starting with 1/10 dilution, was mixed with 100 μl of a suspension of *Micrococcus lysodeikticus* (0.4 mg ml^{-1}, Sigma, USA). OD against a buffer blank was read at 540 nm at 0, 5, 10, 20 and 40 min. Four incubation temperatures were tested, 4, 10, 22 and 37°C, and three buffer systems, 0.05 M phosphate buffer, pH 5.7 and 6.2 and 0.01 M phosphate buffered saline, pH 7.4. Sea bass (*Dicentrarchus labrax*) serum was used as a positive control. A unit of lysozyme activity was defined as the amount of serum causing a decrease in OD of 0.001 min^{-1}.

2.7. Anti-protease activity

A modification of the method described by Ellis [10] was used. 20 μl of serum were incubated with the same volume of standard trypsin solution (Sigma T-7409, 1000–2000 BAEE, 5 mg ml^{-1}) for 10 min. at 22°C. To this was then added 200 μl of 0.1 M phosphate buffer, pH 7.0 and 250 μl 2% azocasein (Sigma A-2765) and incubated for 1 h at 22°C. Then 500 μl of 10% trichloro acetic acid (TCA) was added with mixing and incubated for 30 min. at 22°C. The mixture was centrifuged at $6000 \times g$ for 5 min. 100 μl of the supernatant was transferred to a 96 well non-absorbent microtray (Nunc, Denmark) containing 100 μl of 1 N NaOH per well. The OD was read at 450 nm. The blank was phosphate buffer in place of serum and trypsin and the reference sample was phosphate buffer in place of serum. The percentage inhibition of trypsin activity compared to the reference sample was then calculated for each serum sample.

2.8. Iron binding capacity

The total iron content (TI, μg ml^{-1}) and unsaturated iron binding capacity (UIBC, μg ml^{-1}) of cod serum were determined using a kit (Sigma no. 565) based on the method described by Persijn et al. [30]. The manufacturer's procedure was followed with the modifications that all volumes were reduced by a factor of 10 [20].

Total iron binding capacity (TIBC μg ml^{-1}) was calculated as the sum of TI and UIBC and the per cent saturation was calculated as (TI/TIBC) × 100.

A precipitate sometimes formed when cod serum was mixed with the TI buffer reagent (Sigma no. 565-1). This precipitate, which did not contain iron, was removed by centrifuging at $6000 \times g$ for 10 min. and the analysis continued using the supernatant.

2.9. Data handling

The StatView™ analysis system for Macintosh was used for all statistical analysis. The data showed non-parametric distribution. Mann–Whitney U test was used for comparative analysis, the criterion for significance was set at $P < 0.05$. Spearman Rank Correlation test was used for correlation analysis and ρ-values exceeding [0.5] were considered of practical significance ($P < 0.0025$).

3. Results

3.1. Health status, growth and maturity

After the 12-month period 4 (12.5%), 2 (6%) and 16 (48.5%) of the 33 fish, kept respectively at 1, 7 and 14°C, had died. The death rate at 14°C was gradual over the 12-month period, levelling off during the final months. The pathological changes most frequently seen in the moribund cod at 14°C were gill lesions caused by the parasite *Loma morhua*. In some cod which died granulomatous changes in various organs were seen, generally due to *L. morhua*, the fungus *Ichthylophonus hoferi*, and occasionally these were caused by the bacteria *Aeromonas salmonicida* ssp. *achromogenes*. Tissue changes observed in some of the survivors were attributed to *I. hoferi* and *L. morhua*.

Fish kept at 1°C increased in length over the 12-month period by a factor of about 1.2 and approximately doubled their weight. Fish kept at 7 and 14°C on the other hand increased their length by a factor of 1.5 and nearly quadrupled their weight (Fig. 1). The difference in growth between fish kept at 7 and 14°C was not statistically significant whereas the difference between these and fish kept at 1°C was significant.

In the fish sample taken at 1°C there were five males and six females, at 7°C there were six of each sex and at 14°C there were eight males and four females. All of the fish at 7°C and 94% of the fish at 14°C were sexually mature at the end of the 12-month period but only 54% of the fish kept at 1°C.

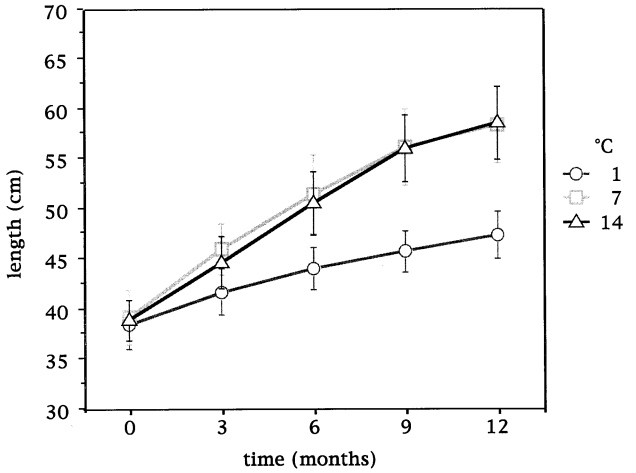

Fig. 1. The growth rate of cod kept at 1, 7 or 14°C for 12 months. The mean length (cm) and standard deviation are shown.

3.2. Protein concentration

The serum protein concentration decreased with increasing temperature (Fig. 2). The mean values, which were close to the median values shown in Fig. 2. were 46.3, 43.3 and 39.1 mg ml^{-1} for fish kept at 1, 7 and 14°C respectively. Only the difference between fish kept at 1 and 14°C was statistically significant.

3.3. Immunoglobulin (IgM) concentration

The serum IgM concentration increased with increasing temperature (Fig. 2). The mean values, which were close to the median values shown in Fig. 2, were 5.3, 10.2 and 12.2 mg ml^{-1} for fish kept at 1, 7 and 14°C respectively. The difference in IgM concentration was significant between cod kept at 1 and 7 or 14°C but not between cod kept at 7 and 14°C. When expressed as a percentage of the serum protein the same trend and statistical significance was observed, the mean values being 11.8, 24.5 and 32.7% at 1, 7 and 14°C respectively.

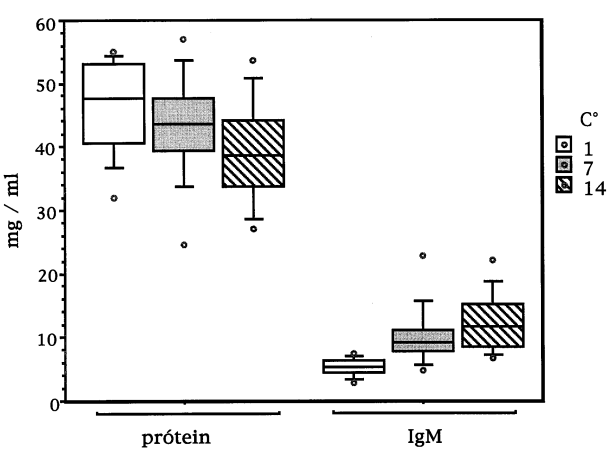

Fig. 2. Box plots of serum protein and IgM concentration (mg ml^{-1}) of cod kept at 1, 7 or 14°C for 12 months. The median is the line within each box, the boxes indicate 25–75 percentiles, the whiskers 10–90 percentiles and the circles the extreme values.

3.4. Natural antibody activity

In most cases the natural antibody activity against the eight antigens increased with increasing temperature (Table 1)). This increase was significant between fish kept at 1 and 14°C, with respect to the activity against the antigens, TNP–BSA, LPH, ssDNA, and Thyr. The difference in activity against BSA, Glyc, ECP-Asa and ECP-Pf, on the other hand, was variable and generally not statistically significant (Table 2)). By far the strongest response in all three groups was against TNP-BSA. Most of the fish gave OD readings of >1.0 against TNP–BSA and 42% of the fish kept at 14°C gave readings of >2.0. The response to the other antigens was generally low although some individuals gave OD readings of 0.6–0.7, for example against ECP-Asa, especially fish kept at 14°C. A significant correlation was observed between the binding capacity for LPH, ssDNA and Thyr on the one hand ($\rho > 0.657$) and between the binding capacity for ECP-Asa, ECP-Pf and Glyc on the other hand ($\rho > 0.642$). Only the activity against ssDNA correlated with the IgM concentration ($\rho = 0.596$).

3.5. Haemolytic activity

The serum haemolytic activity was low in all three groups (Fig. 3) and lowest in fish kept at 14°C. The mean values were 34.5, 38.2 and 16.9% for fish kept at 1, 7 and 14°C respectively. The corresponding median values, shown in Fig. 3, were 13.6, 17.3 and 13.9%. This reflects the strongly non-parametric distribution of the data especially for fish kept at 1 and 7°C. As can be ascertained from Fig. 3, few individuals kept at 1 and 7°C showed over 40% lysis whereas the rest gave less than 15% lysis. Only two fish kept at 14°C showed haemolytic activity exceeding 15%. The difference in haemolytic activity was not statistically significant between groups.

3.6. Lysozyme activity

Lysozyme activity was not detected in any of the sera when diluted 1/10 under any of the conditions used. A few serum samples, tested undiluted, were also negative. Sea bass serum had 1280 lysozyme units ml^{-1} at pH 6.2, incubated at 22°C, comparable to reported values of rainbow trout serum [21].

Table 1
Natural antibody activity (OD at 405 nm) of cod kept at three different temperatures for 12 months; the mean values and standard deviation

Activity	Cod kept at 1°C ($n = 11$)	Cod kept at 7°C ($n = 12$)	Cod kept at 14°C ($n = 12$)
TNP-BSA	1.294 ± 0.577	1.436 ± 0.632	1.932 ± 0.539
LPH	0.186 ± 0.104	$0.273 \pm 0{,}332$	0.440 ± 0.251
ssDNA	0.284 ± 0.091	0.534 ± 0.406	0.698 ± 0.377
Thyr	0.150 ± 0.057	0.216 ± 0.244	0.238 ± 0.130
Glyc	0.148 ± 0.081	0.160 ± 0.114	0.198 ± 0.095
ECP-Asa	0.361 ± 0.210	0.244 ± 0.173	0.427 ± 0.292
ECP-Pf	0.265 ± 0.140	0.159 ± 0.146	0.248 ± 0.130
BSA	0.267 ± 0.123	0.231 ± 0.160	0.380 ± 0.219

3.7. Anti-protease activity

The anti-protease activity decreased with increasing temperature (Fig. 3). The mean percentage inhibition values of serum from fish kept at 1, 7 and 14°C were 75, 58 and 45% respectively. This was close to the median values shown in Fig. 3. The difference in anti-protease activity was statistically significant between groups.

3.8. Iron binding capacity

The TI of cod serum from all three groups was < 1 μg ml^{-1}, the UIBC and TIBC values were about 7–10 μg ml^{-1} and the percentage saturation $< 6.5\%$.

The four parameters, TI, UIBC, TIBC and % saturation, were generally lower in fish kept at 14 or 7°C than in fish kept at 1°C (Table 3). With the exception of the % iron saturation the difference between the fish kept at 1 and 14°C was statistically significant. Only UIBC and TIBC were significantly higher in cod kept at 1°C compared to cod kept at 7°C. The % iron saturation was lower in cod kept at 14°C compared to cod kept at 7°C otherwise the difference between these two groups was insignificant (Table 4).

Table 2
Natural antibody activity (OD at 405 nm) of cod kept at three different temperatures for 12 months; comparison of data using the Mann–Whitney U test (P-values)

Activity	1 vs. 7°C	1 vs. 14°C	7 vs. 14°C
TNP-BSA	0.3097	0.0056*	0.0326*
LPH	0.4416	0.0042*	0.0209*
ssDNA	0.0905	0.0003*	0.1333
Thyr	0.8055	0.0363*	0.0939
Glyc	0.9020	0.2679	0.2040
ECP-Asa	0.1480	0.8055	0.0647
ECP-Pf	0.1096	0.9264	0.0734
BSA	0.2679	0.1569	0.0377*

* The critical level of significance was set at $P \leq 0.05$.

4. Discussion

The environmental temperature was considered the prime variable in this study. Other factors were probably also influential such as the size differences between the groups [25] and stress and latent infections in cod kept at 14°C, bearing in mind the high death rate in this group.

Temperature around 7°C is considered to be near the favoured environmental temperature for the North Atlantic cod whereas 1 and 14°C are near its lower and upper limits [23,36]. Compared to cod kept at 7 and 14°C, the growth rate was reduced and sexual maturation delayed in fish kept at 1°C.

The total serum protein concentration was relatively high, compared to that of wild cod of the same size range [25], probably reflecting the abundant and regular feeding under the cultured conditions [23]. The protein level, however, decreased with increasing environmental temperature, especially at 14°C and this was accompanied by an increased immunoglobulin concentration. An overall reduction of serum protein concentration

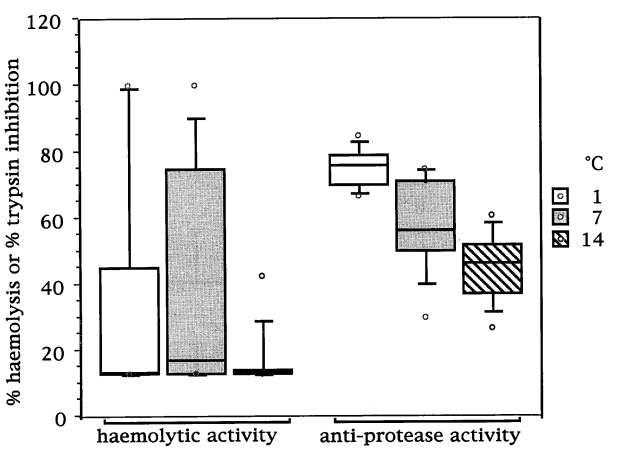

Fig. 3. Box plots of serum haemolytic (% haemolysis) and anti-protease (% trypsin inhibition) activity of cod kept at 1, 7 or 14°C for 12 months. The median is the line within each box, the boxes indicate 25–75 percentiles, the whiskers 10–90 percentiles and the circles the extreme values.

Table 3
The total iron (TI, μg ml^{-1}), unsaturated iron binding capacity (UIBC, μg ml^{-1}), total iron binding capacity (TIBC, μg ml^{-1}) and percentage iron saturation (% I) in serum of cod kept at different temperatures for 12 months; the mean values and standard deviation

	Cod kept at 1°C(n = 11)	Cod kept at 7°C(n = 12)	Cod kept at 14°C(n = 12)
TI	0.58 ± 0.16	0.54 ± 0.20	0.40 ± 0.20
UIBC	8.97 ± 0.85	7.68 ± 0.74	8.13 ± 0.39
TIBC	9.55 ± 0.84	8.22 ± 0.75	8.52 ± 0.43
% I satn.	6.15 ± 1.93	6.43 ± 2.48	4.50 ± 2.39

has been observed in cod and other fish species in association with stress and certain infections [27,28,32]. In these instances reduced serum protein level was at the expense of albumin and serum proteins other than immunoglobulins, resulting in an unchanged or increased relative immunoglobulin level [23,32].

This could be a contributory factor to the difference observed in serum IgM concentration between the groups. Stronger immunological stimuli at higher temperatures might also lead to higher IgM levels. It is worth noting, however, that generally there was an insignificant difference between most of the parameters, including IgM concentration, in cod kept at 7°C, with a 6% death rate, and cod kept at 14°C, with a 48.5% death rate. Another important factor was the size difference between groups. In wild cod the immunoglobulin level increased significantly with increasing size [25]. In this context the IgM level observed in cod kept at 7 and 14°C was close to the values observed in wild cod of the same size whereas the IgM level in cod kept at 1°C was considerably lower. This suggests a suppressive effect on the serum IgM level of cod kept at extremely low temperature for an extended period. Antibody response of fish is quicker at higher temperature [33] but very few studies have examined the influence of environmental temperature on the serum immunoglobulin level of healthy, non-immunised fish. Klesius [19] found no change in the serum immunoglobulin levels in Channel catfish, kept at 10 or 30°C for a period of 30 days. A longer period might, however, have produced a change.

The level of natural antibodies in cod generally increased with increasing temperature. These results therefore paralleled the results for the serum IgM concentration, in spite of the overall poor correlation between IgM concentration and antibody activity. The present results showed that the binding capacity of cod immunoglobulin varied depending on the antigen used. Based on the results of the correlation analysis of antibody activity the antigens tested showed tendency to belong to one of two antigen groups, TNP–BSA, LPH, ssDNA and Thyr or Glyc, BSA, ECP-Asa and -PF. High IgM level and non-specific antibody activity against such antigens as TNP/DNP-haptenated proteins, ssDNA and thyroglobulin are amongst characteristics of mammalian auto-antibodies associated with certain immune diseases [26,37]. The high IgM levels and relatively strong activity against these antigens seen in some fish species [12,26,37], including cod, are believed to indicate similarities to the polyspecific mammalian auto-antibodies and may reflect a primitive regulatory system [37].

Haemolysins are important in the first line of defence of fish against invading pathogens and complement components of the alternative and classical pathways are important haemolysins [3,34]. The haemolytic activity of cod serum was relatively heat resistant, loosing only about 50% activity following incubation at 56°C for 30 min (Magnadóttir, unpublished results). This suggests that typical complement factors of the alternative pathway may not have been the only contributory factor to the haemolytic activity of cod serum. The relatively low haemolytic activity compared to wild cod [25] probably reflects the stress associated with the unnatural conditions of long captivity including regular handling. Reduced levels have been observed during infection, physiological transformations and other stressful periods in other fish species [34].

Lysozyme was not detected in cod serum and other studies have indicated very low levels in cod tissues [21]. Although an important lysin involved in the non-specific defence of several fish species, lysozyme activity appears to be absent in others [3]. The reason for this is not clear.

The anti-protease activity of cod serum was strongly affected by the environmental temperature, the mean activity dropping from about 75% in fish kept at 1°C to 45% in fish kept at 14°C. These levels, especially in fish kept at 1°C, were relatively high compared to wild cod [25]. Protease inhibitors are of importance in delaying

Table 4
The total iron (TI, μg ml^{-1}), unsaturated iron binding capacity (UIBC, μg ml^{-1}), total iron binding capacity (TIBC, μg ml^{-1}) and percentage iron saturation (% I) in serum of cod kept at different temperatures for 12 months; comparison of data using the Mann–Whitney U test (*P*-values)

	1 vs. 7°C	1 vs. 14°C	7 vs. 14°C
TI	0.4602	0.0247*	0.1489
UIBC	0.0019*	0.0046*	0.0941
TIBC	0.0026*	0.0046*	0.2482
% I satn.	0.9264	0.0648	0.0496*

* The critical level of significance was set at $P \leq 0.05$.

or inhibiting pathogens which produce toxic proteases [3,6]. Several proteins may contribute to the anti-protease activity of fish serum and both α1 antitrypsin- and α2 macroglobulin have been identified in fish serum and tissue [6,15]. These were not distinguished in this study.

The total iron content (TI) of cod serum was relatively low (about 0.5 μg ml^{-1}) compared, for example, to salmonids (about 1 μg ml^{-1}) [8,20]. The iron binding capacity (UIBC), on the other hand, was higher than in salmonids (approximately 8 μg ml^{-1} compared to about 4 μg ml^{-1}). Hence, the percentage iron saturation of cod serum was comparatively low [8,20]. The relatively high iron binding capacity of cod indicates either higher levels of transferrin or transferrin-like molecules than in salmonid serum or a molecule with different valency. The strong iron binding capacity, also seen in wild cod [25], suggests that the ability to bind iron may be an important non-specific defence mechanism in cod. Hypoferremia or a decrease in normal total iron and saturation levels is a feature of the acute phase response of higher vertebrates and has been demonstrated in rainbow trout [8]. The reduced iron saturation level seen in cod kept at 14°C may therefore be an indication of an acute phase reaction to this extreme temperature condition [8].

A study on Channel catfish has indicated that non-specific components like phagocytes were more resistant to low temperatures than specific components like lymphocytes [1].

Similar phenomenon was seen in the present study: Non-specific immune components, especially the anti-protease activity, were more evident at low temperatures than at high temperatures whereas the parameters associated with the specific immune capacity of fish, like immunoglobulin and natural antibody level, were more prominent in cod at higher temperatures.

Acknowledgements

The authors thank the staff of the Marine Research Institute in Reykjavik and Grindavík for their assistance. The Nordic Council of Ministers, Copenhagen, was the major financial contributor to this project (nr. 66080200). The work was also supported by grants from the Icelandic Research Council, Reykjavík and the Research Fund of the University of Iceland, Reykjavík.

References

[1] Ainsworth AJ, Dexiang C, Waterstrat PR, Greenway T. Effect of temperature on the immune system of Channel catfish (*Ictalurus punctatus*)—I. Leucocyte distribution and phagocyte function in the anterior kidney at 10°C. Comp Biochem Physiol 1991;100A:907–12.

[2] Albergoni V, Viola A. Effects of cadmium on catfish, *Ictalurus melas*, humoral immune response. Fish Shellfish Immunol 1995;5:89–95.

[3] Alexander JB, Ingram GA. Noncellular nonspecific defence mechanisms of fish. Annu Rev Fish Dis 1992;49:79.

[4] Bengtén E, Strömberg S, Pilström L. Immunoglobulin VH regions in Atlantic cod (*Gadus morhua* L.): Their diversity and relationship to VH families from other species. Develop Comp Immunol 1994;18:109–22.

[5] Bly JE, Clem LW. Temperature and teleost immune functions, review article. Fish Shelfish Immunol 1992;2:159–71.

[6] Bowden TJ, Butler R, Bricknell IR, Ellis AE. Serum trypsin-inhibitory activity in five species of farmed fish. Fish Shellfish Immunol 1997;7:377–85.

[7] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye binding. Anal Biochem 1976;72:248–54.

[8] Congleton JL, Wagner EJ. Acute-phase hypoferremic response to lipopolysaccharide in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol 1991;98A:195–200.

[9] Cook RM, Sinclair A, Stefánsson G. Potential collapse of North Sea cod stocks. Nature 1997;385:521–2.

[10] Ellis AE. Serum antiproteases in fish. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, van Muiswinkel WB, editors. Techniques in Fish Immunology. Fair Haven, N.J: SOS, 1990:95–9.

[11] Espelid S, Rødseth OM, Jørgensen TØ. Vaccination experiments and studies of the humoral immune response in cod, *Gadus morhua* L., to four strains of monoclonal-defined *Vibrio anguillarum*. J Fish Dis 1991;14:185–97.

[12] Gonzalez R, Charlemagne J, Mahana W, Avrameas S. Specificity of natural serum antibodies present in phylogenetically distinct fish species. Immunol 1988;63:31–6.

[13] Guðmundsdóttir BK, Hastings TS, Ellis AE. Isolation of a new toxic protease from a strain of *Aeromonas salmonicida* subspecies *achromogenes*. Disease Aquat Org 1990;9:199–208.

[14] Guðmundsdóttir BK, Jónsdóttir H, Steinthórsdóttir V, Magnadóttir B, Guðmundsdóttir S. Survival and humoral antibody response of Atlantiv salmon (*Salmo salar* L.) vaccinated against *Aeromonas salmonicida* subsp. *achromogenes*. J Fish Dis 1997;20:351–60.

[15] Hjelmeland K. Proteinase inhibitors in the muscle and serum of cod (*Gadus morhua*). Isolation and characterization. Comp Biochem Physiol 1983;76B:365–72.

[16] ICES. New cod and climate study interests ICES scientists. ICES Information Copenhagen 1991;18:1–2.

[17] Israelsson O, Petersson A, Bengtén E, Wiersma EJ, Andersson J, Gezelius G, Pilström L. Immunoglobulin concentration in Atlantic cod, *Gadus morhua* L., serum and cross-reactivity between anti-cod antibodies and immunoglobulins from other species. J Fish Biol 1991;39:265–78.

[18] Jones SRM, Palmen M, van Muiswinkel WB. Effects of inoculum route and dose on the immune response of common carp, *Cyprinus carpio* to the blood parasite, *Trypanoplasma borreli*. Vet Immunol Immunopathol 1993;36:369–78.

[19] Klesius PH. Effect of size and temperature on the quantity of immunoglobulin in Channel catfish, *Ictalurus punctatus*. Vet Immunol Immunopathol 1990;24:187–95.

[20] Langston AL, Bricknell IR, Ellis AE. Iron binding capacity of peripheral blood leucocyte lysates from Atlantic salmon (*Salmo salar* L.). In: Barnes AC, Davidson GA, Hiney MP, McIntosh D, editors. Methodology in Fish Diseases Research. Aberdeen, UK: Fisheries Research Services, 1998:111–6.

[21] Lie Ø, Evensen Ø, Sørensen A, Frøysadal E. Study on lysozyme activity in some fish species. Disease Aquat Org 1989;6:1–5.

[22] Leiro J, Toranzo AE, Estevez J, Lamas J, Barja JL, Ubeira FM. The humoral immune response of turbot to recently isolated pathogenic Enterococcus strains. Cross-reactivity with other Gram-positive bacteria. Vet Microbiol 1996;48:29–39.
[23] Love RM. The Chemical Biology of Fishes. London: Academic Press, 1970.
[24] Magnadóttir B, Guðmundsdóttir S, Guðmundsdóttir BK. Study of the humoral response of Atlantic salmon (*Salmo salar* L.), naturally infected with *Aeromonas salmonicida* ssp. *achromogenes*. Vet Immunol Immunopathol 1995;49:127–42.
[25] Magnadóttir B, Jónsdóttir H, Helgason S, Björnsson B, Jørgensen TØ, Pilström L. Humoral immune parameters in Atlantic cod (*Gadus morhua* L.), II: The effects of size and seasonal factors. Comp Biochem Physiol. 1999; 122.
[26] Marchalonis JJ, Hohman VS, Thomas C, Schluter SF. Antibody production in sharks and humans: a role for natural antibodies. Develop Comp Immunol 1993;17:41–53.
[27] Melingen GO, Stefansson SO, Berg A, Wergeland HI. Changes in serum protein and IgM concentration during smolting and early post-smolt period in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.). Fish Shellfish Immunol 1995;5:211–21.
[28] Møyner K, Røed KH, Sevatdal S, Heum M. Changes in non-specific immune parameters in Atlantic salmon, *Salmo salar* L., induced by *Aeromonas salmonicida* infection. Fish Shellfish Immunol 1993;3:253–65.
[29] Parry RM, Chandau RC, Shahani RM. A rapid and sensitive assay of muramidase. Proc Soc Exp Biol Med 1965;119:384–6.
[30] Persijn J-P, van der Slik W, Riethorst A. Determination of serum iron and latent iron-binding capacity (LIBC). Clin Chim Acta 1971;35:91–8.
[31] Pilström L, Petersson A. Isolation and partial characterization of immunoglobulin from cod (*Gadus morhua* L.). Develop Comp Immunol 1991;15:143–52.
[32] Rehulka J. Erythrodermatitis of carp, *Cyprinus carpio* (L.): An electrophoretic study of blood serum protein fraction levels. Acta Vet Brno 1993;60:187–97.
[33] Rijkers GY, Frederix-Wolters EMH, van Muiswinkel WB. The immune system of cyprinid fish. Kinetics and temperature dependence of antibody-producing cells in carp (*Cyprinus carpio*). Immunology 1980;41:91–7.
[34] Sakai DK. Repertoire of complement in immunological defence mechanisms of fish. Annu Rev Fish Dis 1992:223–247.
[35] Schrøder MB, Espelid S, Jørgensen TØ. Two serotypes of *Vibrio salmonicida* isolated from diseased cod (*Gadus morhua*); virulence, immunological studies and vaccination experiments. Fish Shellfish Immunol 1992;2:211–21.
[36] Scott WB, Scott MG. Atlantic fishes of Canada. Can Bull Fish Aquat Sci 1988.
[37] Vilain C, Wetzel M-C, Du Pasquier L, Charlemagne J. Structural and functional analysis of spontaneous anti-nitrophenyl antibodies in three cyprinid fish species: Carp (*Cyrinus carpio*), goldfish (*Carassius auratus*) and tench (*Tinca tinca*). Develop Comp Immunol 1984;8:611–22.
[38] Yano T. Assays of hemolytic complement activity. In: Stolen JS, Fletcher TC, Anderson DP, Kaattari SL, Rowley AF, editors. Techniques in Fish Immunology. Fair Haven, NJ: SOS, 1992:131–41.
[39] Zapata AG, Varas A, Torroba M. Seasonal variations in the immune system of lower vertebrates. Immunol Today 1992;13:142–7.