Individual and combined effects of cadmium and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) on the humoral immune response in European eel (*Anguilla anguilla*) experimentally infected with larvae of *Anguillicola crassus* (Nematoda)

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SUMMARY

The individual and combined effects of cadmium (Cd) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) on the antibody response of fish against metazoan parasites were tested. Eels experimentally infected with the swim bladder nematode *Anguillicola crassus* were exposed to sublethal concentrations of Cd and PCB 126. Cd was added to the water resulting in an effective concentration of 21·7 ± 12·8 μg/l (mean ± S.D.). PCB 126 was applied orally at a dose of approximately 100 ng PCB 126 per g body weight. At the end of the experiment, 76 days post-infection (p.i.), eels were found to be infected with 2–3 worms. Immunoblot analyses revealed that the body wall of adult worms was the most suitable crude antigen, and was subsequently used for an ELISA to evaluate the immune response of *A. anguilla* under various conditions. A significant increase of *Anguillicola*-specific antibodies in the peripheral blood was first detected 61 days p.i., indicating that it was not the invasive larvae but the adult worms which elicit the antibody response. The presence of Cd in the concentrations applied did not appear to modulate the production of antibodies. In contrast, the exposure to PCB 126 resulted in a complete suppression of the antibody response. A similar effect was also found for the combined exposure of the infected eels to Cd and PCB 126. A suppressed immune response, as demonstrated here, may be the reason why hosts exposed to environmental pollution became often much more easily infected than unexposed conspecifics.

Key words: *Anguilla anguilla*, pollution, cadmium, PCB 126, parasites, antibody response, immunosuppression.

INTRODUCTION

All species of the genus *Anguillicola*, Yamaguti 1935, inhabit the swim bladder of eels (genus *Anguilla*) in various regions of the world (Taraschewski *et al.* 1987; Moravec & Taraschewski, 1988). *A. crassus* originates from East Asia where it parasitizes *Anguilla japonica* and also cultivated European eels (*A. anguilla*) (Nagasawa, Kim & Hirose, 1994). Soon after its first report in Germany *A. crassus* successfully invaded the European continent (Moravec, 1992) and now shows a prevalence of nearly 100% and mean intensities of approximately 5–8 adult worms per eel (e.g. Sures *et al.* 1999*a*; Sures & Streit, 2001). Water temperature and salinity are factors limiting the parasite's distribution (Höglund *et al.* 1992; Knopf *et al.* 1998; Kirk, Lewis & Kennedy, 2000*a*; Kirk, Kennedy & Lewis, 2000*b*). Recently, *A. crassus* was also found in North America (Fries & Williams, 1996; Barse & Secor, 1999) and in North Africa (Maamouri *et al.* 1999). The rapid spread of *A. crassus* was assisted by human interference (Belpaire *et al.* 1989; Kennedy & Fitch, 1990) and the excellent colonizing ability of *A. crassus* (Kennedy & Fitch, 1990). For example, at least 30 fish species (Haenen & Van Banning, 1991; Thomas & Ollevier, 1992; Moravec & Konecny, 1994; Székely, 1994, 1995, 1996; Sures, Knopf & Taraschewski, 1999*b*) and also aquatic snails and insects, as well as amphibians (Moravec, 1996; Moravec & Škoriková, 1998) are known to serve as paratenic hosts which support the spread of *A. crassus* in various biotopes.

Due to the pathological effects comprising histopathological changes of the swim bladder wall (Van Banning & Haenen, 1990; Molnár *et al.* 1993; Molnár, 1994; Molnár, Szakolczai & Vetési, 1995; Würtz & Taraschewski, 2000) as well as alterations of the host's physiology (Würtz, Taraschewski & Pelster, 1996; Kelly, Kennedy & Brown, 2000; Sures, Knopf & Kloas, 2001) special interest has been shown in the immune response of eels infected with this parasite (Buchmann, Pedersen & Glamann, 1991; Höglund & Pilström, 1995; Haenen *et al.* 1996; Nielsen & Buchmann, 1997; Knopf *et al.* 2000*a*, *b*). The humoral

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immune response of eels against *A. crassus* following experimental infection was recently described in detail by Knopf *et al.* (2000*a*). Anti-*A. crassus* antibodies were first observed 8 weeks post-infection, and appeared to be independent of the number of infective 3rd-stage larvae (L_3).

Knowing the detailed characterization of the eel's antibody response against *A. crassus*, factors suppressing the immune response are of great interest, as eels are known to live in polluted waters (Tesch, 1999). It is commonly accepted that the immune response of organisms can be impaired due to exposure with immunosuppressive chemicals (Bols *et al.* 2001). Among a variety of environmental pollutants, heavy metals such as cadmium (Cd) and polychlorinated biphenyls (PCB) have direct immunomodulatory effects (Thuvander, 1989; Arkoosh *et al.* 1998*a*, *b*; Zelikoff, 1998). Cd is still released into the environment, especially from metal processing industries (Merian, 1991) and has been reported to cause immunosuppression as well as immunostimulation (Schulte *et al.* 1994; Zelikoff, 1998). PCBs were manufactured for industrial purposes, such as dielectric fluids in transformers, and as paint additives and are still present in the environment due to their high persistence (Safe, 1994). Coplanar congeners such as PCB 126 are especially known to affect the immune response of fish (Regala *et al.* 2001).

Most studies dealing with immunotoxicity of xenobiotics are based on challenges with viral and bacterial pathogens or synthetic antigens rather than dealing with parasitic infections (Hoole, 1997). This is not surprising since the ability to infect fish with a known number of parasites has been achieved only with a few metazoan parasite species like *Diplostomum* sp. and *Sanguinicola inermis* (Hoole, 1997). However, infective stages (L_3) of *A. crassus* can be grown in the laboratory, which allows an exact number of worms to be given by experimental infection with a feeding tube (Haenen, Van Wijngaarden & Borgsteede, 1994; Knopf *et al.* 1998; Sures *et al.* 2001).

The aim of the present study was to identify the individual and combined effects of Cd and PCB 126 on the antibody response of eels experimentally infected with larvae of *A. crassus*. Antigens were prepared from different tissues of *A. crassus* and characterized with SDS–PAGE and immunoblotting. The body wall antigen of adult worms was found to be the most suitable antigen and was subsequently used in an ELISA to evaluate the immune response of *A. anguilla* under various conditions.

MATERIALS AND METHODS

Source and treatment of eels

Uninfected European eels (*Anguilla anguilla*) with a length of 41·5 ± 4·0 cm (mean ± S.D.) and a weight of 101 ± 22 g (mean ± S.D.) were obtained from an eel farm (Limnotherm, Bergheim, Germany) known to be free of *Anguillicola crassus* (see Knopf *et al.* 1998; Sures *et al.* 2001). The absence of *A. crassus* was confirmed by necroscopy of 12 eels.

Table 1. Experimental design

Group	*n**	*A. crassus*†	Cd-exposure	PCB 126 exposure
Control	5	−§	−	−
Inf	6	+	−	−
Inf and Cd	7	+	+	−
Inf and PCB	6	+	−	+
Inf and Cd and PCB	7	+	+	+

* Number of living eels at the end of the experiment.
† Each eel was inoculated with 10 L_3 of *A. crassus*.
§ Eels were sham-infected with 100 µl of RPMI 1640.

The experimental design is summarized in Tables 1 and 2. Eels were randomly divided into 5 groups with 8 eels each (Table 1). Four groups of eels were infected with larvae of *A. crassus*. Three groups of these infected eels were additionally orally exposed to 3,3′,4,4′,5- pentachlorobiphenyl (PCB 126) and/or kept in water with Cd (Table 2). One group of eels was used as control and these eels were sham infected, by dosing with 100 µl of RPMI-1640 medium and were not exposed to chemicals. Eels were maintained individually in 40 l-tanks equipped with a polypropylene tube serving as a hiding place. The water was aerated and kept at a constant temperature of 20 °C. Due to the Cd exposure a flow-through system could not be used, but 80% of the water was replaced weekly. Eels were force fed once weekly with pellet food at a rate of 0·5 g per eel. Prior to the experiment, fish were allowed to acclimatize for 2 months.

Infection of eels and detection of Anguillicola crassus

Second-stage larvae (L_2) were collected from the swim bladder lumen of eels naturally infected with *A. crassus*. These larvae were fed to wild-caught planktonic copepods. After 20 days at 20 °C the infective 3rd-stage larvae (L_3) were isolated from the intermediate hosts by the method described by Haenen *et al.* (1994). The larvae were stored in RPMI-1640 medium (Sigma, Taufkirchen, Germany) containing 0·2% kanamycin at 4 °C until use. For the experimental infection 10 L_3 were counted and suspended in approximately 100 µl of RPMI-1640. This suspension was introduced into the stomach of unsedated eels, using a feeding tube (diameter 1·5 mm, length 12 cm).

At the end of the experiment, eels were dissected and swim bladders were examined macroscopically and microscopically for adult and larval *A. crassus*.

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Table 2. Time schedule of the experiment

Day	Blood sampling	Infection with *A. crassus**	Cd-exposure†	PCB-exposure§
0	X			
27	X	X		
46	X			
54			Start	X
63	X			
75				X
88	X			
103	X		End	

* Each eel was inoculated with 10 L_3 of *A. crassus*.
† Cd-concentration in the water: $21{\cdot}7 \pm 12{\cdot}8$ μg/l.
§ Each eel was inoculated with approx. 10 μg PCB 126.

Exposure

According to the main uptake routes of the substances used, PCB 126 was administered orally whilst Cd was given into the water. A stock solution of 1 g/l Cd^{2+} was prepared from $Cd(Cl)_2$ (Merck, Darmstadt) dissolved in distilled water and was subsequently used to prepare exposure concentrations of 50 μg/l. Cd exposure started on day 54 and continued for 7 weeks until day 103 (see Table 2). The tank water was replaced weekly and Cd was dosed accordingly. To evaluate real Cd concentrations in the tanks, 15 water samples were taken from each tank through time and analysed by electrothermal atomic absorption spectrometry. The mean Cd concentration over time was found to be $21{\cdot}7 \pm 12{\cdot}8$ μg/l (mean $\pm$ S.D.) for the exposed eels whereas the Cd level in the tanks of the unexposed eels was $2{\cdot}9 \pm 1{\cdot}7$ μg/l (mean $\pm$ S.D.).

Because chlorinated biphenyls are lipophilic (Rice *et al*. 1998; Quabius *et al*. 2002), PCB 126 was administered via the food. After preparing a stock solution of 5 mg PCB 126 (AccuStandard Europe, Niederbipp, Switzerland) in 20 ml of ethanol (96%, Roth, Karlsruhe, Germany), 1·6 ml of this solution was mixed with 10 ml of pellet food and 10 ml of water. Following the evaporation of ethanol overnight, each eel was force fed with 0·5 ml of mushy food using a 1-ml syringe fitted with a feeding tube according to the infection procedure. Considering a mean weight of 101 g, the applied dose was approximately 100 ng PCB 126 per 1 g body weight. Fish which were not exposed to PCB 126 were also force fed pellet food which was prepared using the same volume of ethanol without addition of PCB. After a first application of PCB 126 on day 54, the procedure was repeated on day 75 (see Table 2).

Sampling of sera

Blood samples of 150 μl were drawn from the caudal vein of unsedated eels every 3 to 4 weeks. Samples were collected on 2 occasions prior to infection and 3 times prior to chemical exposure (Table 2). Blood was allowed to clot for 20 min at 20 °C and subsequently centrifuged for 5 min at 2000 ***g***. Sera were collected and stored at −70 °C.

Preparation of crude antigen

Crude antigen extracts were prepared from adult *Anguillicola crassus* and infective larvae (L_3) as described by Knopf *et al*. (2000*b*). Adult nematodes collected from naturally infected eels were washed in phosphate-buffered saline, pH 7·2 (PBS) and dissected. The worms were divided into body wall, female reproductive tract comprising eggs with L_2, and intestinal content. For immunoblotting these parts of adult nematodes and L_3 were suspended in sample buffer containing 50 mM Tris, 10% (v/v) glycerol, 7% (w/v) sodium dodecyl phosphate (SDS), 1 mM EDTA, 1 mM phenylmethyl sulfonylfluoride (PMSF) and 3% (w/v) dithiothreitol (DTT), heated for 3·5 min at 95 °C and centrifuged for 20 min at 16 000 ***g***. The resulting supernatants were used as crude antigens.

For ELISA, body walls of adult *A. crassus* were sonicated in Sarcosyl-TE-buffer (10 mM Tris, 1 mM ethylenediamine tetracetic acid disodium salt (EDTA), 2% *N*-lauroylsarcosine-sodium salt, pH 8·0) and centrifuged for 20 min at 16 000 ***g***. The resulting supernatant was used as crude antigen.

Immunoblot

The immunoblotting procedure was described in detail by Knopf *et al*. (2000*a*). Briefly, SDS–PAGE was performed under reducing conditions in the Phast-System (Pharmacia LKB, Uppsala, Sweden) with purchasable homogenous gels containing 12·5% polyacrylamide (Pharmacia LKB, Uppsala, Sweden). Semi-dry Western blotting on a polyvinyldiene fluoride (PVDF) membrane (Pall Gelman Sciences, Roßdorf, Germany) was also performed in the Phast-System.

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Strips of the membrane were successively incubated with eel sera diluted 1 : 100 in PBS containing 0·05% Tween 20 (PBS-T), with mouse anti-eel Ig (WEI 1, Van der Heijden *et al.* 1995; 1 : 500 in PBS-T) and with sheep anti-mouse IgG conjugated with horseradish peroxidase (AP271, The Binding Site, England; 1 : 1000 in PBS-T). Bands were visualized by adding the chromogenic substrate, diaminobenzidine (DAB, Sigma, Deisenhofen, Germany). For the immunoblotting procedure, sera of 4 eels from previous experiments were used (for details see Knopf *et al.* 2000*a*; Sures *et al.* 2001). Sera of eels were collected prior to experimental infection with larvae of *A. crassus* and, after repeated infection (20 L_3 once a week, for 20 weeks), on day 325 (Knopf *et al.* 2000*a*).

Enzyme-linked immunosorbent assay (*ELISA*)

The ELISA protocol was similar to the procedure published recently by Knopf *et al.* (2000*a*, *b*). Polystyrene microtitre plates (Maxisorp, Nunc, Kamstrup, Denmark) were coated with the body wall antigen extract at a concentration of 1·0 μg/ml in carbonate coating buffer (10 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9·6) and blocked with 1% (w/v) bovine serum albumin (Fluka, Buchs, Switzerland) in PBS containing 0·05% (v/v) Tween 20 (PBS-T-BSA). Eel sera were tested in duplicate at a dilution of 1 : 100 in PBS-T-BSA and incubated for 1 h at 37 °C. As secondary and tertiary antibodies, monoclonal antibodies specific for the heavy chain of eel immunoglobulin (WEI 1, Van der Heijden *et al.* 1995; 1 : 1000 in PBS-T-BSA) and sheep anti-mouse IgG conjugated with horseradish peroxidase (AP271, The Binding Site, England; 1 : 4000 in PBS-T-BSA) were used, respectively. Both antibodies were incubated for 45 min at 37 °C. For each step the wells were filled with a volume of 75 μl and after incubation plates were washed 3 times with PBS-T. As substrate 75 μl of 3,3′,5,5′-tetramethylbenzidine (TMB, Sigma, Steinheim, Germany) was added and the reaction was stopped after 15 min with 50 μl of 0·5 M H_2SO_4. Results were read at 450 nm (Spectra Fluor, Tecan, Crailsheim).

The inter-assay variation was eliminated by relating the measurements to the absorbance value of a defined negative control-serum by calculation of the quotient Q:

$$Q = \frac{\text{Absorbance of test-serum}}{\text{Absorbance of negative control-serum}}.$$

The relative increase of *Anguillicola*-specific antibodies in the eels as shown in Fig. 2 was calculated by dividing Q determined at any time during the experiment by Q determined for the respective eel at day 0.

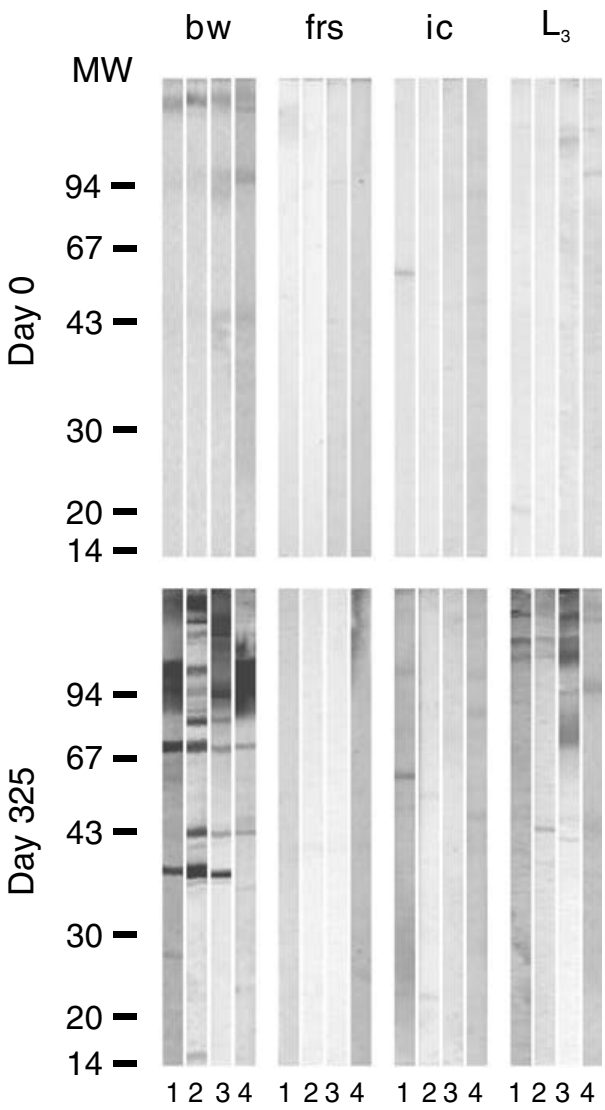

Fig. 1. Immunoblot analysis of *Anguillicola crassus* antigens with sera from 4 experimentally and repeat-infected eels (lanes 1–4), taken prior to infection (day 0) and 325 days after the first infection. bw, Body wall from adults; frs, female reproductive system; ic, intestinal content, (L_3) 3rd-stage larvae. Molecular weight markers (MW) are indicated in kDa.

Statistical analysis

The time of onset and the intensity of the antibody response were compared between different treatments using the Kruskal–Wallis Test and the U-test with a significance level of $P \leqslant 0{\cdot}05$.

RESULTS

Recovery of Anguillicola crassus

The prevalence of *Anguillicola crassus* in the experimentally infected eels ranged between 67 to 88%, with mean intensities of 2–3 worms (adults and L_4). Statistical analysis revealed no significant effects of the treatment on the recovery rate of the nematode. Thus, the infection success, as well as the individual

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Table 3. Levels of *Anguillicola*-specific antibodies in the eels (mean ± S.D.) in relation to initial values at the beginning of the experiment

Group	Day 0	Day 27	Day 46	Day 63	Day 88	Day 103
Control	1·00 ± 0·00	1·02 ± 0·40	1·00 ± 0·66	0·99 ± 0·65	1·01 ± 0·67	0·89 ± 0·43
Inf	1·00 ± 0·00	0·95 ± 0·04	0·95 ± 0·22	0·91 ± 0·28	1·43 ± 0·58	1·90 ± 0·52
Inf and Cd	1·00 ± 0·00	1·18 ± 0·52	1·19 ± 0·34	0·93 ± 0·13	1·20 ± 0·47	1·68 ± 0·51
Inf and PCB	1·00 ± 0·00	0·95 ± 0·27	0·95 ± 0·22	0·79 ± 0·26	1·05 ± 0·17	0·95 ± 0·06
Inf and Cd and PCB	1·00 ± 0·00	0·90 ± 0·26	0·84 ± 0·09	0·86 ± 0·16	0·90 ± 0·14	0·94 ± 0·23

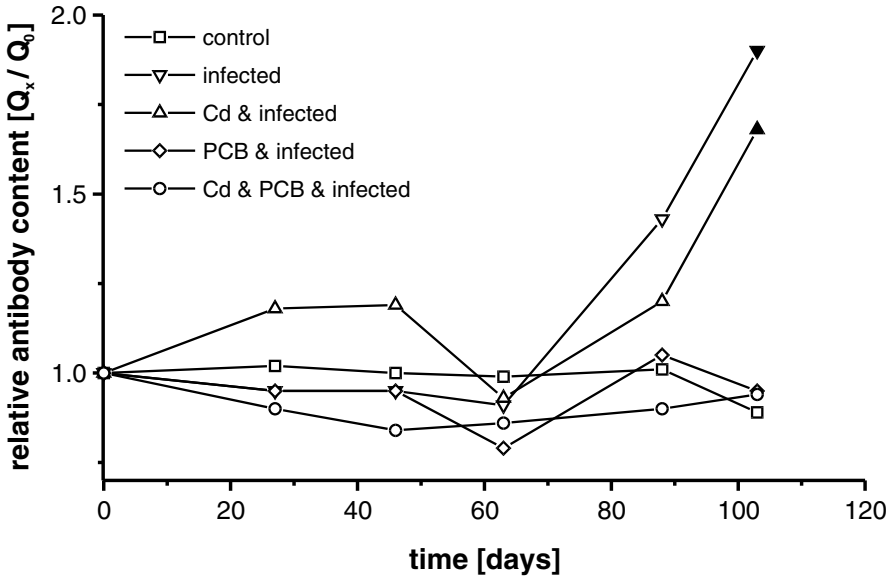

Fig. 2. Time-course of the antibody response of eels inoculated with 10 L_3 on day 27 as measured by ELISA using a crude antigen from the body wall of adult *Anguillicola crassus*. Closed symbols: significant difference from controls (*U*-test, $P \leqslant 0{\cdot}05$); open symbols: no significant difference from controls.

development of *A. crassus*, was not affected by exposure of the eels to Cd and/or PCB 126.

Immunoblotting

Immunoblotting was performed to demonstrate the suitability of the body wall extract as crude antigen in the subsequent ELISA. The most diversified pattern of antigen bands was obtained using the body wall antigen preparation with the sera collected on day 325 (Fig. 1). Strong bands were detected in the high molecular weight range at 71 kDa and higher. Additionally, further antigens of a lower molecular weight appeared at about 43 and 38 kDa. No or only weak reactions were detected with proteins from the female reproductive system and the intestinal content. Using the crude antigen prepared from L_3, two antigens with a molecular weight of approximately 100–110 kDa and around 43 kDa were detected. However, the bands detected appeared to be similar to, but weaker than, those found with the body wall antigen preparation. Prior to infection, sera of the same eels failed to show a positive reaction with any of the antigen preparations (Fig. 1). Because of the conspicuous strong and diversified antigen–antibody reaction found for the adult worm body wall extract, this antigen preparation was used for the subsequent ELISA procedure.

Time-course of the antibody response

The intra-assay coefficient of variation for the ELISA procedure was found to be 4·8% ($n = 80$), whilst the inter-assay variance was 13·1% ($n = 12$).

The antibody response of all groups of eels is summarized in Fig. 2 and in Table 3. At the end of the experiment (day 103), the level of *Anguillicola*-specific antibodies in the peripheral blood was significantly higher in the eels which were only infected, and in eels infected and simultaneously exposed to Cd, compared with the controls (Fig. 2). Between these two groups, no significant differences appeared and increasing antibody levels were first detected at day 88, which is 61 days p.i. However, on day 88 the antibody levels of both groups were not found to be significantly different from the control levels. The controls, inoculated with RPMI-1640, and the remaining groups of eels showed no increased extinction values over the entire experimental period of 103 days. Table 3 shows that the antibody levels of all

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three groups were very similar. Thus, administration of PCB 126, either alone or in combination with Cd, suppresses the immune response of eels. In contrast, exclusive exposure of eels to Cd at an aqueous concentration of approximately 22 μg/l does not reduce the production of *A. crassus*-specific antibodies.

DISCUSSION

Immunoblot analyses with sera from eels experimentally infected with *A. crassus* revealed that the antigen extract from the body wall of adult *A. crassus* is the most suitable antigen preparation to detect the antibody response against this parasite. Strongest antigen–antibody reactions and most antigens were detected in this antigen preparation. This confirms previous results in which serum antibodies of eels naturally infected with *A. crassus* showed comparable strong reactions with antigens from body wall preparations of the adult nematodes (Knopf *et al.* 2000*b*). These authors investigated a variety of other possible crude antigens but concluded from their immunoblot analyses that best results can be expected from antigens associated with the body wall of adult *A. crassus*. Although L_3 are in close contact with the host tissues and are thus directly exposed to the host's immune system while migrating into the swim bladder wall, there was only a weak reaction with proteins of these larvae and no L_3-specific antigens could be detected. Thus, our results support the finding that it is not the invading L_3 but the adult parasites, most likely excretory–secretory antigens derived from their body wall, which elicit the production of antibodies in European eels.

The first detection of *Anguillicola*-specific antibodies by ELISA at day 88 (61 days p.i.) in the present study resembles the results of Knopf *et al.* (2000*a*) who found an immune response 8 weeks post-infection. In contrast, following an intraperitoneal injection of homogenized larval or adult *A. crassus*, antibody production occurred within 5–12 days (Knopf, 1999). Since the development of *A. crassus* from the L_3 stage into the adult worm takes at least 50 days at a water temperature of about 20 °C (Haenen *et al.* 1996; Knopf *et al.* 1998; Sures *et al.* 1999*b*), it could be assumed that the migrating L_3, despite its intimate contact with the host tissues, is not sufficiently immunogenic to elicit a detectable antibody response. The onset of the antibody response, about 8 weeks p.i., seems rather to be elicited by the first adult worms appearing in the swim bladder lumen.

Furthermore, Knopf *et al.* (2000*a*) demonstrated that the onset and the intensity of the antibody response was independent of the number of infective larvae inoculated, but individual eels showed great differences in the course of the antibody response. A high heterogeneity in the antibody response was also found in the present study, which may be due to the genetic variability of eels. As breeding of eels is not possible so far, the eels used in our experiment were caught from a natural population of glass eels, which were subsequently grown in an eel farm. Qualitative and quantitative differences in the individual immune response against parasites are well known also from mammals and it emerges that differences in the susceptibility or resistance against a variety of parasitic infections result from the genetic variability within the host population (Wakelin, 1996).

Not only the post-infection interval at which first antibodies against the parasite were detected, but also the height of the relative antibody content in the present study was similar to the data presented by Knopf *et al.* (2000*a*). The authors have monitored the immune response of eels over a period of 325 days and found highest values for the antibodies at approximately 150 to 200 days post-initial infection. As the present experiment was finished on day 76 p.i., a considerably lower increase of antibodies could be expected. However, eels inoculated with *A. crassus* larvae only and those eels being additionally exposed to cadmium were found to have approximately 1·5 to 2 times higher antibody levels than the uninfected controls and those groups of eels which were exposed to PCB 126. Due to the similarities between the antibody response of infected eels in the present study and those of the previous investigation a further increase of the antibody content in the sera of infected eels could be expected if the experiment would have been prolonged.

Concerning the immunomodulatory effects of the chemicals tested it can be seen that Cd did not obviously affect the antibody production of eels against *A. crassus*, whereas PCB 126 was found to prevent any humoral immune response. Previous studies have shown that exposure to Cd may modulate cell-mediated and humoral immune response in fish. This comprises increased phagocytic activity and chemiluminescence in macrophages (Elsasser, Roberson & Hetrick, 1986; Zelikoff, 1993; Lemaire-Gony, Lemaire & Pulsford, 1995; Zelikoff, 1998), alterations in leucocyte numbers and lymphocyte activity (Murad & Houston, 1988; Ghanmi *et al.* 1989) and antibody-producing cells (O'Neill, 1981; Anderson *et al.* 1989; Thuvander, 1989). It is known from the literature that immunomodulatory effects of Cd are dependent on the sensitivity of the species tested, the time and route of exposure and the concentration applied (Sanchez-Dardon *et al.* 1999). Using aqueous Cd levels of 6 μg/l and 60 μg/l no effect on the immune response was found for *Oryzias latipes* which were intraperitoneally infected with *Yersinia ruckeri* whereas concentrations of 600 μg/l caused an immunosuppression (Zelikoff, 1998). There are also indications that the humoral immune response may be stimulated on exposure to Cd (Hoole, 1997). Robohm (1986) noted that the

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antibody response in *Morone saxatilis* exposed to Cd at a concentration of 10 μg/l was enhanced and Thuvander (1989) observed an increase in antibody response to the O-antigen of *Vibrio anguillarum* when rainbow trout were exposed to 3·6 μg/l Cd. From the information available it appears that the Cd concentrations applied in the present study were not high enough to suppress the immune response of European eels. Furthermore, as it is known that eels are able to withstand environmental pollution and tend to accumulate heavy metals to a very high degree (e.g. Barak & Mason, 1990; Sures, Taraschewski & Jackwerth, 1994) it seems likely that this species is not sensitive enough to show alterations in its immune response at low levels of Cd pollution.

In contrast to Cd, the application of PCB 126 had a clear immunosuppressive effect. All groups of eels treated with polychlorinated biphenyls either alone or in combination with Cd had antibody levels similar to the uninfected control eels. There is evidence from the literature that certain commercial PCB mixtures are immunotoxic to fish (Rice & Schlenk, 1995). Among the 209 congeners of PCBs, those with coplanar structures receive the most attention due to their toxicity, which seems to be related to a structural similarity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic and prototype halogenated aromatic hydrocarbon (HAH) (Regala *et al*. 2001). The degree of immunotoxicity of PCBs correlates with the degree of binding affinity to the cytosolic aryl hydrocarbon receptor (Ahr) (Kafafi *et al*. 1993), which is a well-described transcription factor for a variety of gene products, including cytochrome P 450 1A (Hahn & Stegemann, 1994). Due to its similarity with TCDD (equivalent factor of 0·1), PCB-126 should exhibit TCDD-like toxicities, but at a lower level (Regala *et al*. 2001; Kafafi *et al*. 1993). HAH were described to show a moderate suppression of antibody response in rainbow trout (Spitzbergen *et al*. 1986*a*, *b*) whereas, due to its lower toxicity, PCB-126 was not found to affect the antibody response of channel catfish, *Ictalurus punctatus*, to *Edwardsiella ictaluri* (Rice & Schlenk, 1995). In contrast, Regala *et al*. (2001) found a suppression of the antibody response in channel catfish against *Vibrio anguillarum* following intraperitoneal injection of *I. punctatus* with 1 mg/kg PCB-126, whereas the application of 0·01 mg/kg PCB-126 did not reduce the antibody responses. Accordingly, the immunotoxicity is clearly related to the PCB-126 concentrations applied. In mammals, PCB-126 and other planar HAHs are known to be potent suppressors of the antibody response (Holsapple *et al*. 1991). In the present study a dietary exposure of 0·1 mg/kg PCB-126 was sufficient to prevent the immune response of eels against *A. crassus*.

Studies addressing the question as to how environmental pollution affects the immune status of aquatic animals are of increasing interest (e.g. Aaltonen *et al*. 2000; Grinwis *et al*. 2000; Van Loveren *et al*. 2000; Bols *et al*. 2001). Because the immune and the endocrine systems are closely related in fish (Weyts *et al*. 1999) and both systems play an essential role in the maintenance of homoeostasis, the stress and immune response should not be regarded separately. Chronic stressors like pollution may cause a slight but chronic elevation of cortisol levels (reviewed by Barton & Iwama, 1991) and immunosuppression in fish is often associated with increased plasma cortisol levels (e.g. Wendelaar Bonga, 1997; Weyts *et al*. 1999). The results presented in this article are part of a series of experiments in which also plasma cortisol levels were determined in eels (Sures, 2002). Correlation analysis of the cortisol concentrations and antibody levels revealed that high plasma cortisol levels in eels were associated with low antibody levels. However, not only pollution but also parasitic infections may cause stress in fish as recently demonstrated for eels infected with *A. crassus* (Sures *et al*. 2001). Eels were found to elicit a stress response during larval development and initial appearance of adult *A. crassus*, but no chronic response to older adults as confirmed by Kelly *et al*. (2000).

From our results and the studies cited above it emerges that investigations aimed at the combined effects of parasite infections and pollution are very important to understand their interaction and effects of these 'stressors' on the physiological homeostasis of the host. It is commonly accepted that parasitized hosts show a higher susceptibility to environmental pollutants than uninfected conspecifics (reviewed by MacKenzie *et al*. 1995; Lafferty, 1997; Sures, 2001, 2003). Similarly, hosts which were exposed to pollutants often became much more easily infected than unexposed fish (MacKenzie *et al*. 1995; Lafferty, 1997). Due to insufficient knowledge on possible antagonistic or synergistic effects of parasitic infections and environmental factors on the physiological status of the hosts, further studies are necessary.

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