Effects of infection with *Anguillicola crassus* and simultaneous exposure with Cd and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) on the levels of cortisol and glucose in European eel (*Anguilla anguilla*)

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SUMMARY

To investigate whether the stress response of European eels infected with *Anguillicola crassus* is influenced by environmental pollutants, experimentally infected eels were exposed to Cd and/or to 3,3′, 4,4′, 5-pentachlorobiphenyl (PCB 126). Serum cortisol and glucose concentrations of these eels were monitored over a period of 103 days and were compared with data from infected, unexposed eels as well as with data from uninfected eels. Additionally, the levels of cortisol were correlated with concentrations of *Anguillicola*-specific antibodies. All eels showed an initial increase of the cortisol levels until day 63. This general elevation of plasma cortisol is most likely due to handling stress, as all eels were repeatedly netted and afterwards inoculated with a feeding tube. At the end of the exposure period eels which were infected and those which were infected and simultaneously exposed to Cd and PCB showed significantly higher levels than the controls. The general course of serum glucose levels in eels resembled that of cortisol. Accordingly, Spearman correlation analysis revealed that an increase in serum cortisol concentrations is correlated with rising levels of glucose. With respect to immune-endocrine interactions a significant negative correlation between cortisol and anti-*A. crassus* antibodies was found. Our data show that *A. crassus* is the most potent stressor for European eels among the treatments tested within this study. This is important in terms of ecotoxicological studies as the main effects are caused by parasites rather than chemicals. Accordingly, effects of parasites on the physiological homeostasis of organisms must be considered in ecotoxicology. From the parasitological point of view our results suggest that probably as part of an unbalanced host-parasite interaction *A. crassus* evokes a strong cortisol response in *A. anguilla*, thereby suppressing the immune response which in turn enables the parasite to establish. The parasite-induced stress response in the newly adopted European eel might be one of the factors which contributes to the extremely effective colonizing strategy of *A. crassus*.

Key words: stress response, cortisol, glucose, *Anguillicola crassus*, *Anguilla anguilla*, parasites as stressors, PCB, Cd.

INTRODUCTION

Stress directed against a wide variety of intrinsic or extrinsic stimuli, commonly referred to as stressors, is the summary of reactions of the respective organism to re-establish its physiological homeostasis (Wedemeyer, Barton and McLeay, 1990; Wendelaar Bonga, 1997; Kloas, 1999). The stress response of organisms including fish can be divided into 3 phases (Wendelaar Bonga, 1997; Kloas, 1999). The primary response is characterized by an activation of brain centres leading to a massive release of catecholamines and cortisol. As secondary response effects of the stress hormone release on blood and tissue levels could be observed resulting, for example, in increased oxygen uptake, disturbance of hydromineral balance and mobilization of energy reserves. Tertiary responses manifest usually after a prolonged exposure to stress at the level of organisms or even populations. Accordingly, the dominant roles of cortisol, as primary messenger and of glucose as a secondary metabolite during a stress response in teleostean fish are generally recognized (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Kloas, 1999). Stressors which may evoke a stress response in fish comprise different challenges such as extreme changes in the physical environment (e.g. temperature, salinity; see Barton and Iwama, 1991), human interference (e.g. handling, crowding, netting; see Barton and Iwama, 1991), intra- and interspecific interactions (e.g. competition for food, sexual partners, predation, parasites; see Wendelaar Bonga, 1997) and water

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pollution (e.g. Folmar, 1993). Despite the variety of factors that are known to elicit a stress response, very little information is available on the role of parasites as stressors (e.g. Wendelaar Bonga, 1997; Kelly, Kennedy and Brown, 2000; Sures, Knopf and Kloas, 2001; Sures *et al*. 2002; Gollock *et al*. 2004; Klar and Sures, 2004; Gollock, Kennedy and Brown, 2005).

Recently, 4 studies report on the effects of *Anguillicola crassus* on the stress response of eels (Kelly *et al*. 2000; Sures *et al*. 2001; Gollock *et al*. 2004, 2005). This swim-bladder nematode might be a serious threat to the European eel (Sures and Knopf, 2004*a*) due to a variety of pathological effects, which may even lead to mass mortalities (Molnár, Székely and Baska, 1991). Thickening of the swim-bladder wall, inflammation, infiltration of white blood cells, fibrosis and changes in the epithelial cells are the most frequent histopathological changes in infected swim-bladders (Van Banning and Haenen, 1990; Molnár *et al*. 1993; Molnár, 1994; Molnár, Szakolczai and Vetési, 1995; Würtz and Taraschewski, 2000). Additionally, infestation with *A. crassus* appears to make the fish more susceptible to secondary bacterial infections (Van Banning and Haenen, 1990). In aquaculture, heavily infected European eels show reduced growth and increased mortality (Køie, 1991). Due to the pathogenicity of the nematode, a few studies have been conducted on the effects of *A. crassus* on the physiological homeostasis of its host. Kelly *et al*. (2000) have performed a field study on eels being naturally infected with *A. crassus*. Although the authors found elevated glucose levels in infected eels as compared with uninfected conspecifics, neither a primary nor a tertiary response was reported due to the parasite. Sures *et al*. (2001) investigated cortisol levels as part of the primary stress response during an experimental infection of naïve eels with larvae of *A. crassus*. The results showed that the application of infective third-stage larvae (L_3) resulted in a significant increase of cortisol levels. The period of time at which elevated cortisol values were observed was consistent with the time of larval development and the appearance of adult *A. crassus*. Thus, there is a stress response to the larval and young adult stages, but no chronic response to older adults. Sures *et al*. (2001) concluded that infection of eels with *A. crassus* larvae may be a considerable stressor, especially in combination with different environmental factors like water temperature, pH, oxygen concentration, pollution and inter-individual relationships.

The aim of the present study was to investigate whether the stress response, as measured by serum cortisol and glucose levels of eels infected with larvae of *A. crassus* is affected by environmental pollutants like metals and/or PCBs. Therefore eels were experimentally infected with *A. crassus* and simultaneously exposed to Cd in the water and to 3,3′, 4, 4′, 5-pentachlorobiphenyl (PCB 126) via the food.

Table 1. Experimental design

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

Serum cortisol and glucose concentrations were monitored over a period of 103 days. Due to the intimate contact between the endocrine and immune system in fish (both are located in the inter-renal cells of the head kidney) the levels of cortisol determined in the present study were compared with data on the immune response of the same eels that were published recently (Sures and Knopf, 2004*b*).

MATERIALS AND METHODS

To evaluate single and combined effects of a parasitosis and exposure with environmental pollutants on the stress response, eels were experimentally inoculated with infective larvae of *Anguillicola crassus* and/or exposed to Cd via the water and/or fed PCB 126-containing food, according to the main routes of uptake. To follow the stress response of fish, serum levels of cortisol and glucose were analysed on several occasions during the experimental period of 103 days. Infection of the eels, blood sampling and force feeding of control and PCB 126-containing food was finished within 2 to 3 min after the eels were caught by net, to minimize the effects of handling on the physiological parameters. For all procedures fish were not sedated which was in accordance with local animal welfare regulations.

Experimental design

Uninfected European eels (*Anguilla anguilla*) with a length of $41{\cdot}5 \pm 4{\cdot}0$ cm (mean ± S.D.) and a weight of 101 g ± 22 g (mean ± S.D.) were obtained from an eel farm (Limnotherm, Bergheim, Germany) known to be free of *A. crassus* (see Sures, Knopf and Taraschewski, 1999; Sures *et al*. 2001). The absence of *A. crassus* was confirmed by necroscopy of 12 eels. Eels were allowed to acclimatize for 2 months prior to the experiment. The experimental design is summarized in Tables 1 and 2. Eels were randomly divided into 8 groups with 8 eels each (Table 1). Half the groups of eels were infected with larvae of *A. crassus* and/or orally exposed to PCB 126 and/or kept in

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.

water with Cd (Table 2). To analyse the levels of cortisol and glucose blood was drawn every 3–4 weeks from the caudal vein of each individual eel. Therefore, eels had to be kept individually at 20 °C in a tank with a volume of 40 l. The water was aerated and equipped with a polypropylene tube serving as hiding place. Due to the metal exposure a flow through system could not be used, but 80% of the water was replaced weekly in all groups (Cd was added for the Cd-exposed eels accordingly). Eels were force fed once weekly pellet food at a rate of 0·5 g per eel. In order to avoid an increase in stress parameters due to the physical treatment, force feeding as well as the renewal of water was performed after blood collection. During the experiment a few eels died in all groups for unknown reasons. Upon post-mortem dissection none of them showed signs of bacterial or parasitological infections except for those eels that were experimentally infected with *A. crassus*. Eels which died during the experiment were excluded from the study.

Experimental infection of eels

Eels were experimentally infected with infective third-stage larvae (L_3) of *A. crassus*. L_3 were obtained by feeding second-stage larvae (L_2) collected from the swim-bladder lumen of naturally infected eels to planktonic copepods, mainly comprising *Thermocyclops cf. crassus* and *Mesocyclops leuckarti* (Knopf *et al.* 1998). The third-stage larvae were isolated 20 days p.i. from the intermediate hosts by the Potter method described by Haenen, Van Wijngaarden and Borgsteede (1994) and stored in RPMI-1640 medium (Sigma, Deisenhofen, Germany) containing 0·2% Kanamycin at 4 °C until application. Ten L_3 were counted in a round-bottomed 98-well plate and suspended in approximately 100 μl of RPMI-1640 medium. This suspension was introduced into the stomachs of each eel, using a 1-ml syringe fitted with a 12 cm length of 1·5 mm diameter plastic tubing. Those groups of eels which were not infected with L_3 were sham-infected, by dosing with 100 μl of RPMI-1640 medium to treat all eels in a similar manner. Infection of the eels was finished within 2 min after the eels were caught by net, to minimize the effects of handling on the physiological parameters.

Exposure of eels

According to the main uptake routes of the substances used, PCB 126 was administered orally whilst Cd was given into the water. Chemical exposure was started 4 weeks after infection of eels. This period of time allows the larvae to reach the swim-bladder and start growing (Knopf *et al.* 1998). 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, which is close to environmental Cd levels and only 10-fold higher than the limit for drinking water (Stoeppler, 1984). Cd exposure started on day 54 and continued for 7 weeks until day 103 (see Table 2). Monitoring of Cd in the water (water samples were collected every other day during the exposure period) by electrothermal atomic absorption revealed a concentration of $21{\cdot}7 \pm 12{\cdot}8$ 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, PCB 126 was administered via the food (see e.g. Quabius, Balm and Wendelaar Bonga, 1997). After preparing a stock solution of 5 mg PCB 126 (AccuStandard Europe, Niederbipp, Switzerland) in 20 ml ethanol (96%, Roth, Karlsruhe, Germany), 1·6 ml of this solution was mixed with 10 ml pellet food and 10 ml of water. Following evaporation of the 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 administered dose was approximately 100 ng PCB 126 per 1 g body weight, which was the same dose used by Quabius *et al.* (1997). Fish which were not exposed to PCB 126 were also force fed pellet food which was prepared using the same volume of ethanol without insertion of PCB. After a first application of PCB 126 on day 54 the procedure was repeated on day 75 (see Table 2).

Determination of cortisol

Blood samples of 150 μl were drawn from the caudal vein of unsedated eels every 3–4 weeks. Blood was allowed to clot for 20 min at 20 °C, centrifuged for 5 min at 2000 ***g*** and sera were collected and stored at −70 °C until analysis by a radioimmunoassay (RIA). Cortisol was extracted by adding 1000 μl of 96% ethanol to 40 μl of serum, vortexing, centrifuging for 2 min at 10 000 ***g***, and the organic phase including

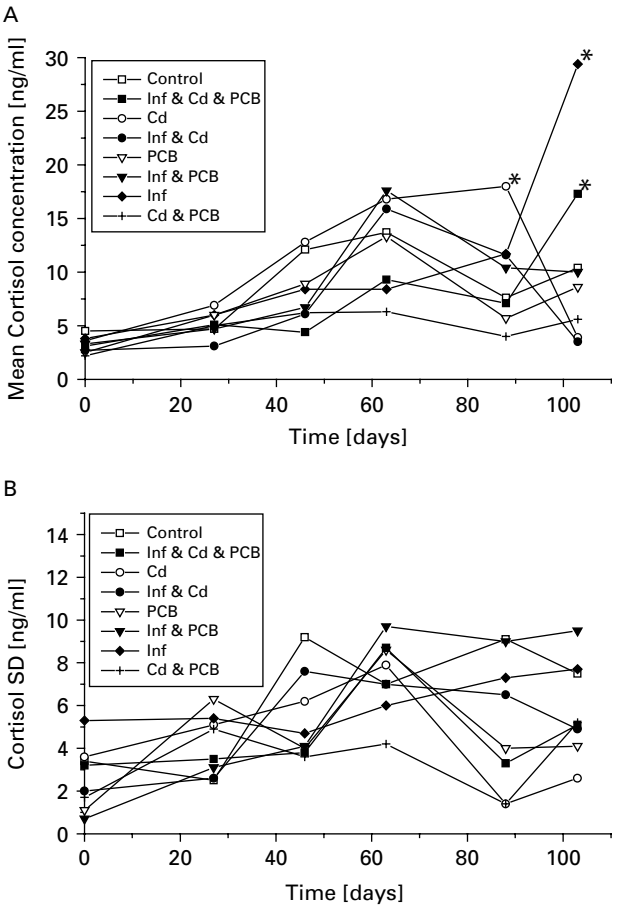

Fig. 1. Course of mean (A) and standard deviation (B) of cortisol concentrations in eels following different treatments. *Significant ($P \leqslant 0{\cdot}05$) difference from controls (U-test).

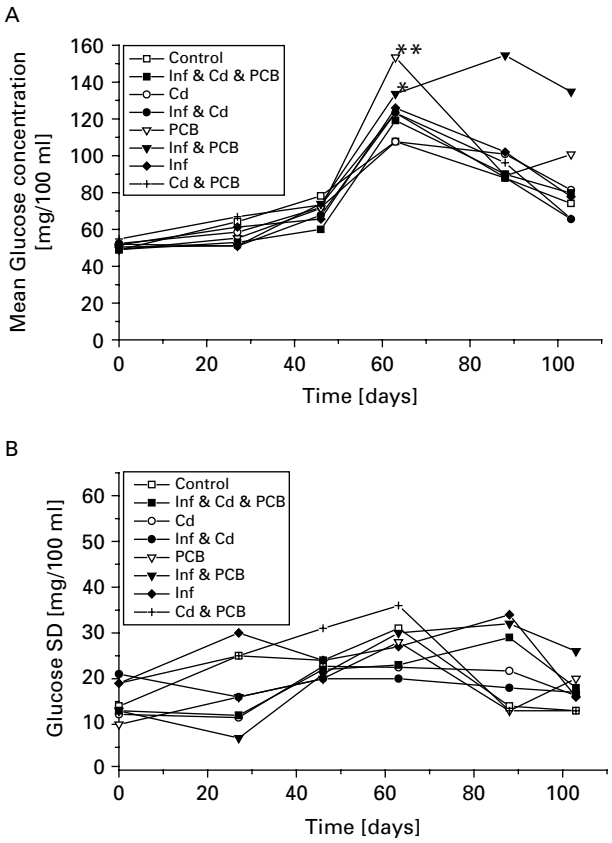

Fig. 2. Course of mean (A) and standard deviation (B) of glucose concentrations in eels following different treatments. Significant difference from controls (U-test), with *$P \leqslant 0{\cdot}05$ and **$P \leqslant 0{\cdot}01$.

cortisol was carried over in open glass vials. Overnight the ethanol was allowed to evaporate. The following day, 1000 μl of 5% ethanol were added to each sample to redissolve the cortisol again. The sample was divided into 3 aliquots each containing 300 μl of extract, to which 100 μl of tritium-labelled cortisol (3000 cpm) and 500 μl of cortisol antiserum (dilution 1 : 10 000 in lysozyme buffer B) were added according to Kloas, Reinecke and Hanke (1994). These samples were then kept on ice for about 3 h. To isolate the antibody-hormone complex 100 μl of dextran-activated carbon suspension was added. After centrifugation, the supernatant containing the antibody-hormone complexes, was transferred into scintillation vials and filled with scintillation solution (Ultima Gold; Packard, Dreieich, Germany). A liquid scintillation counter (Tri Carb 1900 T; Packard, Dreieich, Germany) was used according to Kloas *et al.* (1994).

Determination of glucose

Plasma glucose concentrations were determined using the Glucose/GOD-Perid method (Boehringer Mannheim, Mannheim, Germany) according to Werner, Rey and Wielinger (1970).

Statistical analysis

Mean concentrations ($\overline{X} \pm$ S.D.) of cortisol (ng/ml serum) and glucose (mg/100 ml serum) are presented in Figs 1 and 2. Cortisol concentrations between all groups were analysed for each sampling time with the Kruskal-Wallis test. The Mann-Whitney *U*-test was used to test for significant differences between each group of infected eels and the control if significant differences between all groups were confirmed by the Kruskal-Wallis test. Significance was accepted when $P < 0{\cdot}05$. Spearman's rank correlation coefficient was calculated to test for significant associations between the levels of cortisol and glucose. Additionally, data published recently on the presence of specific antibodies against *A. crassus* in the blood of eels from the same experiment (Sures and Knopf, 2004*b*) were also tested for associations with the levels of cortisol and glucose gained in the present study.

RESULTS

Recovery of Anguillicola crassus

The prevalence of *A. crassus* in the experimentally infected eels ranged between 67 and 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 development of *A. crassus* was not affected by exposure of eels to Cd and/or PCB 126.

Serum cortisol concentrations in eels

Mean initial cortisol concentrations for each treatment ranged between 2·2 ng/ml and 4·5 ng/ml (Fig. 1). These levels increased continuously for all groups until day 63 until which mean cortisol levels were found to range between 6·3 ng/ml to 17·6 ng/ml. However, statistical analysis revealed no significant differences between the treatments until day 63 (Kruskal-Wallis-test, $P>0{\cdot}05$). On day 88, cortisol levels decreased in all groups of eels except for the group that was exposed to Cd only. These fish showed significantly higher serum cortisol levels than the untreated controls (*U*-test, $P\leqslant 0{\cdot}05$) on day 88, but subsequently levels dropped down to a concentration below that of the control group on day 103. Only those groups of eels which were infected and those which were infected and simultaneously exposed to Cd and PCB showed again an increase in cortisol concentrations with significantly (*U*-test, $P\leqslant 0{\cdot}05$) higher levels than the controls at day 103. Compared with initial levels, these eels were found to have on average 8 (infected only) and 6 (infected and exposed to Cd and PCB) times higher serum cortisol concentrations at the end of the exposure period. On day 103, cortisol concentrations of most groups were approximately twice the initial values, with levels ranging between 5·6 ng/ml and 10·4 ng/ml. These data suggest that mainly infection with *A. crassus* but also – to a lesser extent – exposure to Cd may elicit a stress response in eels.

Serum glucose in eels

The general course of serum glucose levels in eels resembled that of cortisol (Fig. 2). Again, the values increased 2- to 3-fold from an initial range between 49 mg/100 ml and 55 mg/100 ml until day 63 without showing any significant differences (Kruskal-Wallis-test, $P>0{\cdot}05$) between treatment groups. On day 63, however, significant differences (Kruskal-Wallis-test, $P\leqslant 0{\cdot}01$) occurred between the glucose levels of the treatment groups (Fig. 2) with significantly higher glucose concentrations for those eels which were exposed to PCB (*U*-test, $P\leqslant 0{\cdot}01$) and those eels that were PCB exposed and similarly infected with *A. crassus* (*U*-test, $P\leqslant 0{\cdot}05$). Although the mean glucose level of the PCB-exposed and *A. crassus*-infected eels remained higher compared with the other treatment groups, which showed decreasing glucose levels, no significant differences (Kruskal-Wallis-test, $P>0{\cdot}05$) could be detected on days 88 and 103. At the end of the experiment the glucose concentration was 2·7 times higher compared with initial values for the PCB-exposed and *A. crassus*-infected eels, 2 times higher for the PCB-exposed eels and $1{\cdot}5\pm 0{\cdot}2$ times higher for all other treatment groups (Fig. 2).

Table 3. Spearman correlation coefficients (r) and levels of significance (*P*) for the significant relationships between the concentrations of cortisol, glucose and specific antibodies in the serum of eels

x – y	R	*P*
[Cortisol] – [Glucose]	0·3152	<0·001
[Cortisol] – [Antibodies]	−0·2321	<0·001
[Antibodies] – [Glucose]	−0·0677	>0·05

Immuno-neuroendocrine relationships

The same eels which were used in the present study were also investigated in respect of their humoral immune response against *A. crassus* (for details see Sures and Knopf, 2004*b*). Therefore, we have performed Spearman correlation analysis in order to test for significant associations between the levels of cortisol, glucose and specific antibodies in the serum of eels (Table 3). Two associations were found to be highly significantly (Spearman, $P\leqslant 0{\cdot}001$) correlated: a positive relationship occurred between the levels of glucose and cortisol, whereas cortisol and antibody levels are negatively associated. Accordingly, an increase in serum cortisol concentrations is correlated with rising levels of glucose and similarly with decreasing levels of anti-*A. crassus* antibodies. No correlation was found between the levels of glucose and antibodies.

DISCUSSION

The dominant role of plasma corticosteroids, mainly cortisol, as primary messengers of a stress response in teleostean fish is generally recognized (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Kloas, 1999). Mean basal levels for cortisol in eels between 2·2 ng/ml and 4·5 ng/ml in the present study resemble results of an earlier study where 3·9 ng/ml cortisol was described as the initial level (Sures *et al.* 2001) with a decrease in the range of 2 to 12 ng/ml mentioned by Gilham and Baker (1987) and Gollock *et al.* (2004). However, even cortisol levels of 50 ng/ml were found for unstressed control eels (Teles, Santos and Pacheco, 2004). Similarly, basal glucose levels were also comparable to concentrations reported for eels in other studies (Gollock *et al.* 2004).

Elevation of plasma cortisol was recorded for all groups of eels around day 63, irrespective of the treatment. This increase of cortisol concentrations is most likely due to handling stress, as all eels were

previously inoculated with a feeding tube for either infection or sham-infection purposes. It is already known from the literature that aquaculture practices such as netting, handling, transport, etc. evoke a stress response in fish (Wendelaar Bonga, 1997). Therefore, one could expect a similar initial increase in plasma cortisol concentrations as all groups of eels were physically treated in the same manner. Additionally, aquaculture practices were performed during the whole exposure period which might explain that cortisol levels at the end of the exposure period were found to be twice as high as at the beginning of the experiment. In addition to this handling stress, another increase of plasma cortisol resulting in significantly higher concentrations for the infected eels and those eels which were infected and exposed to both chemicals was detected at the end of the experiment. Compared to a previous study on the cortisol release of eels infected with *A. crassus* (Sures *et al.* 2001), data of the current experiment showed a clearer and more pronounced stress response. However, the period of time at which elevated cortisol concentrations were found is comparable between both studies and resembles the time of larval development and the appearance of adult *A. crassus*. In contrast, wild eels chronically infected with *A. crassus* showed no major differences in plasma cortisol from those of uninfected eels (Kelly *et al.* 2000). Therefore the results of the present study strengthen the hypothesis that the eel's stress response is mainly directed against the larval and young adult stages, but not against older adults (Sures *et al.* 2001). From the parasite's point of view an increase in cortisol concentrations might aid or even allow the survival of *A. crassus* inside its host. Spearman correlation analysis has shown that cortisol levels are significantly negatively correlated with the concentration of anti-*A. crassus* antibodies. Therefore, it appears likely that the parasite evokes a clear stress response, i.e. an increase in cortisol concentrations, which then suppresses the host's humoral immune response. It is already known from the literature that cortisol has strong immunosuppressive effects (Wendelaar Bonga, 1997; Weyts *et al.* 1999). Accordingly, it could be part of the extremely effective colonizing strategy of *A. crassus* to induce stress in its newly adopted final host and rely on the resulting immunosuppression for its own successful infestation.

This stress induction due to infection with *A. crassus* is affected by the simultaneous application of chemicals. The presence of Cd as well as of PCB 126 (both given alone) significantly reduces the stress response of infected eels. Although it was already shown that inter-renal stress responsiveness of fish is impaired by dietary exposure to PCB 126 (Quabius *et al.* 1997), there is some evidence from the literature that Cd exposure elicits at least a transient stress response in fish (Pratap and Wendelaar Bonga, 1990; Gill *et al.* 1993; Tort *et al.* 1996). A transient increase of plasma cortisol was also found for those eels which were only exposed to Cd. Moreover, the period of time at which higher cortisol levels were found for these eels corresponds very well with the period of cortisol increase described by Pratap and Wendelaar Bonga (1990) for tilapia. It is, however, surprising that there is obviously no additional effect on the cortisol release between Cd exposure and *A. crassus* infection. Interestingly, the group of eels which were infected and exposed to both chemicals showed also increased cortisol concentrations which were, however, not significantly different from the levels of those eels which were infected only. Although it was anticipated that the coincidence of all 3 treatments could result in the highest cortisol concentrations of all groups of eels, it appears that the cortisol-releasing effects of Cd are outweighed by a PCB-induced impairment. Finally, the effects of *A. crassus* infection are mainly reflected by the plasma cortisol levels.

In general, the elevation of plasma cortisol was significantly correlated with an increase in plasma glucose. However, this clear association was not evident for the 2 groups of eels that elicited the strongest cortisol response. A similar phenomenon was recently described by Gollock *et al.* (2004, 2005) who failed to show an elevation in plasma glucose in eels, despite evidence of activation of both corticosteroid and adrenergic systems. From their results (Gollock *et al.* 2004, 2005) the authors suggested that the increased glucose turnover in infected eels compared to uninfected conspecifics could be the reason for the absence of increased plasma glucose levels. Our results are in line with those of Gollock *et al.* (2004 and 2005) and support the hypothesis that cortisol-induced glucose levels might be used for non-specific stress responses caused by higher energy demands due to increased motility and respiratory activity.

In conclusion, it appears that mainly larval *A. crassus* must be considered as stressors for European eels among the treatments tested within this study. The stress induction due to the nematode might be of special importance for the infection success of *A. crassus* in its newly acquired final host *Anguilla anguilla*. Recently, it was shown that the recovery of living *A. crassus* following experimental infection is significantly higher in *A. anguilla* as compared to the original host *A. japonica* (Knopf and Mahnke, 2004). Additionally, the development of the nematodes was shown to be significantly slower in *A. japonica* than in *A. anguilla*. These differences can be attributed to a more efficient immune response in Japanese eels that have had a long co-evolution with *A. crassus*, resulting in a balanced host-parasite relationship. Probably, as part of an unbalanced host-parasite interaction, *A. crassus* evokes a strong cortisol response in *A. anguilla*, thereby suppressing the immune response which in turn enables the parasite

to establish. Further studies investigating the cortisol and immune response of European and Japanese eels experimentally infected with *A. crassus* are highly appreciated to demonstrate whether the lower recovery of *A. crassus* in *A. japonica* coincides with lower cortisol levels than in *A. anguilla*.

Additionally, our results are important for ecotoxicological studies as they show that the main effects with respect to the stress response are not due to chemical exposure, but are rather the result of parasites. Unfortunately, the possible effects of parasites on the physiological homeostasis of organisms have only seldom been recognized, to date, in ecotoxicological investigations (see e.g. Sures, 2004).

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