Physiological status of wild European eels (*Anguilla anguilla*) infected with the parasitic nematode, *Anguillicola crassus*

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SUMMARY

The effect of the parasitic swimbladder nematode, *Anguillicola crassus*, on the physiological status of wild European eels (*Anguilla anguilla*) was investigated during an 18 month survey (February 1995 until September 1996), and compared with that of wild uninfected eels collected over the same time-period. Despite the occurrence of up to 15 blood-feeding adults in the swimbladder lumen of the infected eels and as many as 25 additional larvae in the swimbladder wall, there were no major differences in hormonal, metabolic or osmoregulatory status of the 2 groups of eels. Wild European eels appear to adapt to chronic parasitism with *Anguillicola crassus*. The possible adverse effects of additional simultaneous stressors are discussed.

Key words: *Anguillicola crassus*, *Anguilla anguilla*, stress, cortisol.

INTRODUCTION

Anguillicola crassus (Kuwahara, Nimi & Itagaki, 1974) originates from South East Asia where it is endemic in the swimbladders of its native definitive host, the Japanese eel, *Anguilla japonica*. The adult nematodes are found with the lumen of the swimbladder, while L_3 and L_4-larvae occur in the swimbladder wall where they feed on host swimbladder tissue. The adult nematodes feed on the blood of the eel, but generally cause no serious damage to the host (Egusa, 1979).

A. crassus was introduced into Europe during the early 1980s, presumably via the importation of live infected eels (Belpaire, De Charleroy & Grisez, 1989). In Europe, *A. crassus* has transferred definitive hosts to the European eel, *Anguilla anguilla*. Prevalence level and mean intensity of infection are much higher in this host than in *A. japonica* and pathological effects have been observed which have not previously been reported for the Japanese eel. These include the swimbladder becoming inflamed, fibrous and thickened (Van Banning & Haenen, 1990). Sprengel & Luchtenberg (1991) reported a reduction in the swimming speed of the European eel, even when infected with only 1 nematode and Würtz, Taraschewski & Pelster (1996) reported a decrease in the oxygen content of swimbladder gas of infected eels.

Freshwater fish respond to most stressful stimuli with a predictable pattern of primary endocrine and resultant physiological changes, the typical stress response generally being independent of the type of stressor (Wendelaar Bonga, 1997). The initial elevation of plasma cortisol concentrations after release *de novo* from the corticosteroid tissue, and the resultant rise in plasma glucose concentrations, are the most widely used indicators of stress in fish (Waring, Stagg & Poxton, 1992; Brown, 1993; Wendelaar Bonga, 1997). Cortisol, by virtue of its catabolic properties, mobilizes stored food reserves, thereby enabling the fish to cope with increased energy demands (Paxton, Gist & Umminger, 1984). Although the stress responses of fish have been widely studied (Schreck, 1982; Pickering & Pottinger, 1989; Carragher & Rees, 1994; Cech *et al.* 1996), there have been very few studies of fish harbouring parasites (Laitinen, Siddall & Valtonen, 1996). In the present study, the physiological status of wild European eels infected with *A. crassus* was compared with that of clean uninfected eels over an 18 month period.

MATERIALS AND METHODS

Fish

Eels were sampled from 2 sites in the Southwest of England at monthly intervals whenever possible from February 1995 until September 1996. A minimum of 10 yellow eels (sex not determined) was collected on each occasion from Slapton Ley nature reserve, a site known to be infected with *A. crassus*, and the R. Otter, at that time an uninfected site. On all occasions, eels were caught by electrofishing with

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a hand-held pulse d.c. unit with a peak power output of 2·8 kW. Eels could not be collected from either site in March and July 1995 or March 1996 and from the R. Otter in February, May, June and November 1995, due to technical difficulties. Eels were transported back to the laboratory and held, unfed, in 80-litre containers in the freshwater aquarium which is maintained at 10±1 °C on a 12 h light:12 h dark photo-period throughout the year. After 2 weeks, eels were transferred to individual 10-litre, partially blacked-out tanks of aerated freshwater so that they could be blood sampled individually avoiding the effects of repetitive disturbance by netting. Blood samples were collected 2 weeks later. The 4 week period between capture and blood sampling ensured recovery from the stress of electrofishing and transportation (Gollock, Kennedy & Brown, unpublished data).

Blood sampling

To minimize potential diurnal effects, all fish were blood sampled between 13.00 and 15.00 h. Infected and uninfected eels were anaesthetized in freshwater containing Benzocaine (Sigma, 2·5 g/litre) for 1 min. A blood sample was rapidly withdrawn from the caudal vasculature, using a 2 ml syringe (Plastipak, Type B-D) and 23G or 25G needle (Microlance) heparinized using ammonium heparate (28 mg/ml), and dried in a 40 °C oven. Blood sampling was always completed within 5 min from initial disturbance (and usually within 3 min) to avoid distortion of physiological parameters simply due to the effects of handling and blood sampling.

Epidemiology

After blood sampling, eels were examined for the presence of *A. crassus*; this procedure was performed on all eels to ensure that eels which had been classified as uninfected were actually free from infection. Length and weight of all fish were recorded. All *A. crassus* nematodes found in the swimbladder lumen were counted, measured, weighed (wet wt) and sexed. The swimbladder wall was inspected for larval stages of *A. crassus* and numbers recorded. The mean *A. crassus* intensity and abundance of infection were calculated for individual fish and prevalence was calculated for the entire group of fish sampled.

Physiological parameters

Haematocrit was measured immediately after the blood sampling. Heparinized microhaematocrit capillary tubes were partially filled with blood, one end sealed with Critoseal (Monoject Scientific), centrifuged (Clandon TH21 haematocrit program: at 15250 ***g*** for 7·5 min) and the percentage packed cell volume was measured using a microhaematocrit reader. The remainder of the blood sample was centrifuged at 11000 ***g*** (MSE, MicroCentaur) for 3 min. The plasma was aliquoted for radioimmunoassay of plasma cortisol, assay of plasma glucose, and determination of plasma osmolality and sodium and chloride concentrations. Plasma osmolality was measured by freezing point depression (Camlab micro-osmometer) within 6 h. The remaining plasma aliquots were stored at −20 °C until analysis.

Plasma cortisol was measured by an established radioimmunoassay (Brown, Edwards & Whitehead, 1989). In the present studies, intra-assay coefficient of variation was 6·26 %($n = 5$), inter-assay coefficient of variation 18·9 % ($n = 10$) and minimum detectable limit 2·16 ng/ml. Extraction efficiency, determined by addition of radiolabelled cortisol to a pooled plasma sample, was 85·7±1·5 % ($n = 16$). Assay results were not corrected for extraction efficiency.

Plasma glucose concentrations were measured using a glucose oxidase/peroxidase enzymatic assay kit (Boehringer–Mannheim). Plasma chloride concentrations were measured by electrochemical titration (Corning 920 chloride analyser). Plasma sodium concentrations were measured by flame emission spectroscopy (Perkin–Elmer SP9 atomic absorption spectrophotometer).

Statistical analyses

Data for each physiological parameter from both sites were normally distributed. Data for each parameter, at each site, were tested using separate one-way ANOVAs followed by Tukey tests to identify months where data were significantly different from other months. Significance was accepted when $P < 0{\cdot}05$. Monthly data for each parameter in Slapton Ley eels and R. Otter eels were compared using Mann–Whitney *U*-tests, followed by Bonferroni sequential rejection tests to avoid type-1 errors (Rice, 1989). Correlation analyses between *A. crassus* loads (total number of adults and larvae; adults in lumen; larvae in swimbladder wall), weight of adult nematodes and physiological parameters for individual fish were performed using standardized residual (Excel 7.0), to remove any effect which eel weight may have had on the analyses.

RESULTS

The mean intensity of both adult and larval stages of *A. crassus* was highly variable over the study period (Fig. 1). The highest mean intensity of both adult and larval *A. crassus* occurred in February 1996 while the lowest intensity was in May (1996 for adults, 1995 for larvae). Prevalence was more stable, ranging from 70 to 100 %.

Correlation analyses did not reveal any significant correlations between *A. crassus* infection (number of

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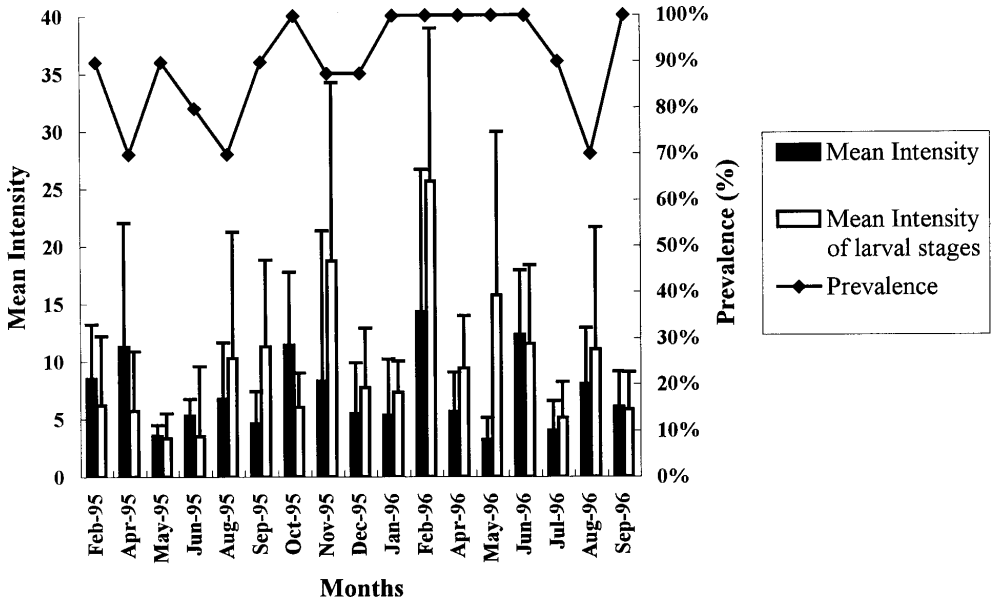

Fig. 1. Mean intensity of adult and larval stages ± S.E. and prevalence of *Anguilla crassus* in wild Euorpean eels from Slapton Ley from February 1995 to September 1996.

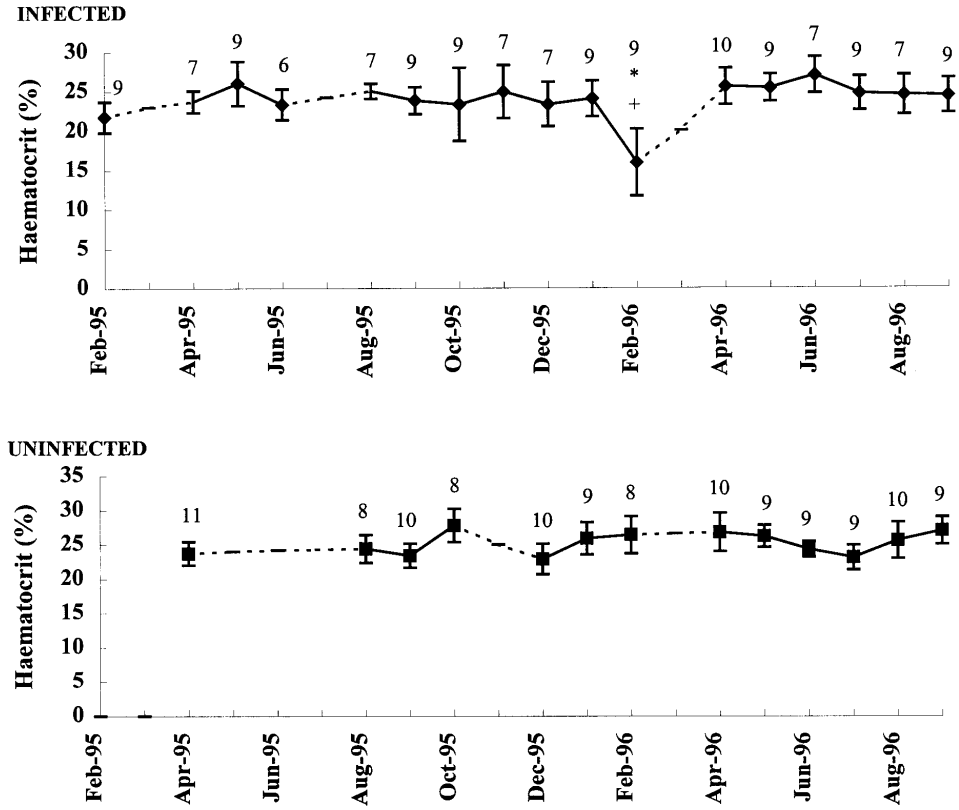

Fig. 2. Mean monthly haematocrit values (± S.E.) for infected (Slapton) eels and uninfected (R. Otter) eels. Numbers above error bars indicate number of eels in relevant group. Dotted lines indicate months when fish were not sampled. * Indicates significant difference ($P < 0{\cdot}01$) from other months at the same site. + Indicates significant difference ($P < 0{\cdot}001$) between infected and uninfected eels sampled that month.

nematodes in swimbladder lumen, number of larvae in swimbladder wall, total number of adults and larvae, total weight of adult nematodes) and any physiological parameter. With 2 exceptions, probability values exceeded 0·11, on most occasions exceeding 0·5. The 2 exceptions were plasma glucose correlated against total number of adults and larvae (where $P < 0{\cdot}08$) and plasma glucose correlated against the number of *A. crassus* in the swimbladder lumen (where $P < 0{\cdot}07$).

Figures 2–4 and Table 1 show physiological data for eels sampled over the 18-month study period.

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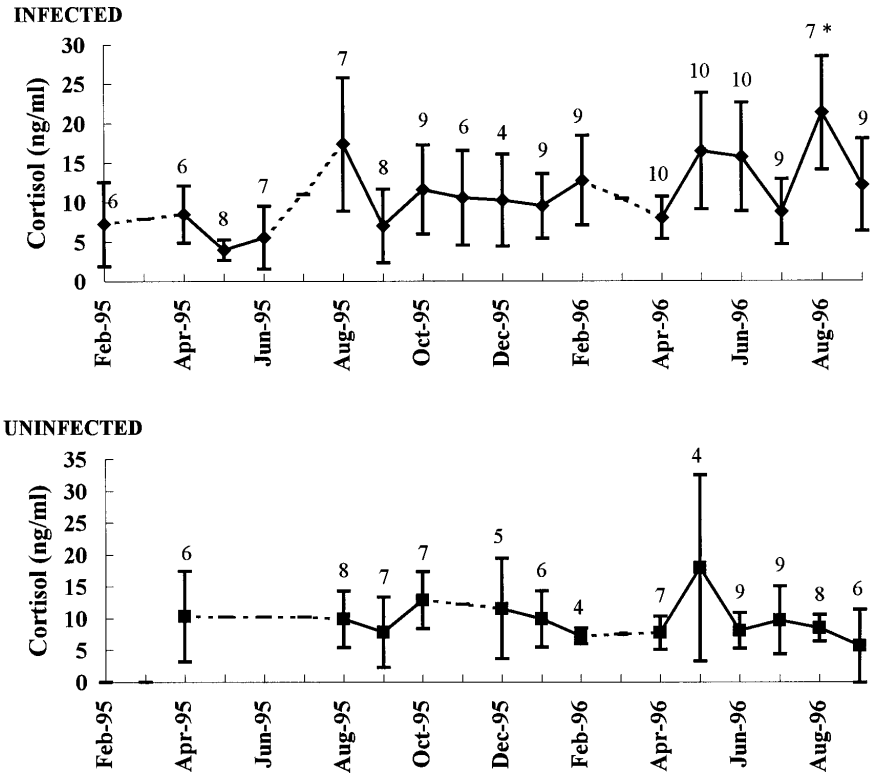

Fig. 3. Mean monthly plasma cortisol concentrations (± S.E.) for infected (Slapton) eels and uninfected (R. Otter) eels. Numbers above error bars indicate number of eels in relevant group. Dotted lines indicate months when fish were not sampled. * Indicates significant difference from other months at the same time.

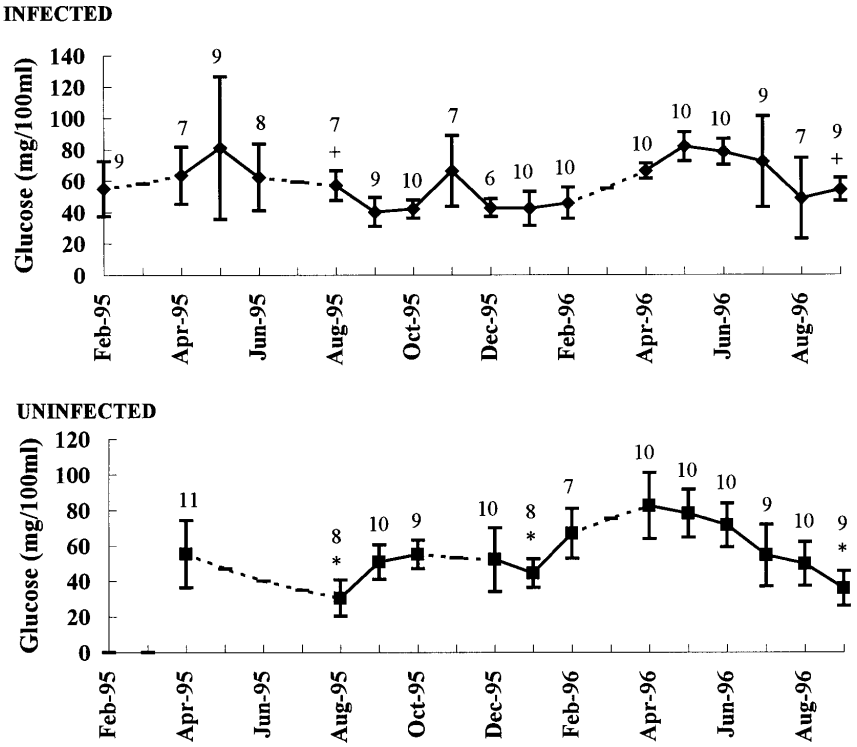

Fig. 4. Mean monthly plasma glucose concentrations (± S.E.) for infected (Slapton) eels and uninfected (R. Otter) eels. Numbers above error bars indicate number of eels in relevant group. Dotted lines indicate when fish were not sampled. * Indicates significant difference ($P < 0{\cdot}01$) from April, May and June 1996. + Indicates significant difference between infected and uninfected eels.

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Table 1. Plasma osmolality (mOsm/kg) and sodium and chloride concentrations (mM) of eels infected with *Anguilla crassus* and uninfected eels sampled between Feb. 1995 and Sept. 1996

(Number of fish shown in parentheses. * Values significantly different between infected and uninfected eels in that month; †, value for that month significantly different from that for other months at the same site.)

	Osmolality		Chloride		Sodium	
Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
1995						
Feb.	316·2 ± 4·7 (9)	—	128·3 ± 5·9 (9)	—	140·3 ± 7·7 (6)	—
March	—	—	—	—	—	—
April	319·1 ± 8·7 (7)	314·0 ± 4·7 (11)	130·1 ± 8·2 (7)	133·0 ± 3·5 (11)†	131·2 ± 10·7 (7)	133·4 ± 20·6 (5)
May	298·4 ± 11·5 (9)	—	118·6 ± 12·9 (9)	—	137·5 ± 10·9 (7)	—
June	308·5 ± 7·9 (8)	—	119·1 ± 8·2 (8)	—	142·8 ± 12·9 (8)	—
July	—	—	—	—	—	—
Aug.	322·7 ± 11·1 (7)	306·6 ± 5·8 (8)	122·4 ± 5·3 (7)*	99·9 ± 10·2 (8)	140·3 ± 6·5 (7)	141·7 ± 21·8 (5)
Sept.	300·0 ± 7·7 (9)	310·6 ± 5·2 (10)	104·4 ± 7·3 (9)†	108·1 ± 5·4 (10)	130·7 ± 9·7 (8)	131·9 ± 11·9 (6)
Oct.	316·1 ± 10·3 (10)	318·3 ± 5·2 (7)	111·2 ± 10·6 (10)	106·6 ± 15·0 (9)	130·7 ± 12·2 (7)	125·7 ± 18·6 (6)
Nov.	310·0 ± 11·4 (7)	—	105·9 ± 13·8 (7)	—	134·9 ± 11·6 (6)	—
Dec.	327·6 ± 17·4 (7)†	323·0 ± 10·8 (9)	102·7 ± 7·5 (7)†	108·3 ± 5·1 (10)	145·0 ± 8·6 (4)	137·9 ± 15·6 (4)
1996						
Jan.	319·3 ± 4·8 (9)	314·0 ± 4·3 (8)	120·7 ± 5·3 (10)	110·8 ± 4·6 (8)	143·7 ± 3·6 (9)*	125·4 ± 10·3 (6)
Feb.	316·2 ± 4·2 (10)	303·8 ± 7·6 (4)	114·6 ± 5·1 (10)*	95·7 ± 4·9 (6)	141·4 ± 7·9 (9)	116·4 ± 14·9 (3)
March	—	—	—	—	—	—
April	305·2 ± 2·2 (10)*	289·8 ± 13·6 (9)†	108·7 ± 8·8 (10)*	88·9 ± 7·0 (8)	144·4 ± 5·1 (10)	128·8 ± 16·1 (7)
May	283·2 ± 7·1 (10)†	274·4 ± 6·4 (9)†	90·8 ± 8·5 (10)†	74·0 ± 8·8 (6)†	140·5 ± 8·5 (10)	134·4 ± 11·6 (5)
June	308·7 ± 3·8 (10)	306·1 ± 4·4 (9)	119·0 ± 7·5 (10)	109·2 ± 5·6 (10)	149·3 ± 9·7 (10)	131·2 ± 8·6 (8)
July	316·9 ± 14·1 (9)	307·3 ± 4·3 (9)	107·3 ± 16·4 (9)	105·7 ± 7·1 (9)	149·2 ± 7·2 (8)	135·6 ± 9·9 (8)
Aug.	322·3 ± 22·7 (7)	312·8 ± 3·3 (8)	118·2 ± 12·9 (7)	106·6 ± 7·7 (8)	150·9 ± 11·4 (6)	129·7 ± 9·9 (4)
Sept.	307·3 ± 5·1 (9)	316·7 ± 16·1 (9)	103·6 ± 9·1 (10)†	113·5 ± 3·7 (9)	141·1 ± 4·2 (9)	132·8 ± 8·7 (6)
Overall mean	310·9 ± 2·8 (147)	307·5 ± 3·2 (110)	112·9 ± 2·7 (149)	106·4 ± 3·1 (112)	141·0 ± 2·3 (131)	131·5 ± 3·8 (73)

Haematocrit (Fig. 2) was fairly stable throughout the survey both in uninfected and infected eels with an overall mean haematocrit for infected eels of $23{\cdot}8 \pm 0{\cdot}7\,\%$ ($n = 139$) compared to $25{\cdot}1 \pm 0{\cdot}6\,\%$ ($n = 120$) in uninfected eels. In February 1996, however, infected eels had significantly lower haematocrits than in other months ($P < 0{\cdot}01$), and values were significantly lower than those of uninfected eels ($P < 0{\cdot}001$).

There was considerable variation in plasma cortisol concentrations for eels from either site at any one sampling point (Fig. 3). Mean cortisol for infected eels sampled throughout the survey was $11{\cdot}1 \pm 1{\cdot}5$ ng/ml ($n = 134$) compared to $9{\cdot}5 \pm 1{\cdot}5$ ng/ml ($n = 86$) for uninfected eels. During the summer months infected eels tended to show higher cortisol values than those of uninfected eels but in any one month no statistically significant difference was detected.

Plasma glucose concentrations varied throughout the sampling period, both in uninfected and infected eels (Fig. 4). Overall plasma glucose for infected eels was $58{\cdot}9 \pm 4{\cdot}8$ mg/100 ml ($n = 147$) compared to $56{\cdot}2 \pm 4{\cdot}5$ mg/100 ml ($n = 121$) for uninfected eels. Infected eels showed significantly higher glucose concentrations than those of uninfected eels in both August 1995 and September 1996 ($P < 0{\cdot}01$).

Plasma osmolality showed monthly variations and chloride concentrations tended to follow a similar though less pronounced pattern (Table 1). The most striking changes were the significantly depressed plasma osmolalities and chloride concentrations of both infected and uninfected eels sampled in May 1996 ($P < 0{\cdot}01$ compared to all months). In the preceding month, April 1996, plasma osmolarity (but not chloride concentration) of uninfected eels was again significantly lower than in all months except August 1995 and months between February and July 1996 ($P < 0{\cdot}01$ for all comparisons). In contrast, plasma osmolalities were significantly elevated in infected eels in December 1995 (compared to September or May 1995 or May 1996; $P < 0{\cdot}01$). However, plasma chloride concentrations in these infected fish (and also in September, both years) were significantly depressed (compared to values in February and April 1995; $P < 0{\cdot}01$). In April 1995 plasma chloride concentrations of uninfected eels (but not plasma osmolality) were significantly elevated compared to all other months ($P < 0{\cdot}01$). Only in a single month, April 1996, was a significant difference in the plasma osmolality of infected and uninfected eels detected ($P < 0{\cdot}01$). The slightly raised osmolality of infected eels compared to uninfected eels that month was accompanied by elevated plasma chloride concentrations ($P < 0{\cdot}01$). Chloride concentrations of infected eels were also significantly elevated compared to those of uninfected eels in both August 1995 and February 1996 ($P < 0{\cdot}01$).

Plasma sodium concentrations were more stable than plasma chloride (Table 1), although in January 1996 values were significantly higher in infected than in uninfected eels ($P < 0{\cdot}001$).

DISCUSSION

During this study, all stages of the life-cycle of *A. crassus* in its definitive host *A. anguilla* were found at all times of the year, though numbers varied between months. As reported by Thomas & Ollevier (1992) there appeared to be no seasonal cycle in either prevalence or mean intensity of *A. crassus* in the European eel. There were no apparent correlations between loadings of *A. crassus* in the lumen or the larvae in the swimbladder wall and any of the physiological parameters measured. Such correlations would have provided strong evidence for a direct physiological impact of *A. crassus* on the eels. In the absence of any correlations, chronic impacts of *A. crassus* seem likely to be limited. This notion is supported by lack of any statistical evidence of a generalized endocrine stress response in eels infected with *A. crassus*, although the large variation in plasma cortisol at any one sampling point could have prevented detection of subtle effects. The period of parasitism of the wild eels studied is obviously not known. The life-cycle of *A. crassus* has been shown to be completed with 2 months in the laboratory (De Charleroy *et al.* 1990) but may take much longer in the wild. It seems probable that within the period of infection recovery from any acute stress has occurred.

The eels studied were held in the laboratory in relatively benign conditions for a total of 4 weeks prior to blood sampling in order to recover from electrofishing, transportation and handling before comparison of the physiological status of *A. crassus*-infected eels with uninfected eels. In the natural environment, where multiple challenges may be simultaneously experienced, it is possible that the effects of the parasite are more marked. The generally higher levels of cortisol in wild *A. crassus*-infected fish during the warm summer months (although not statistically significant) does suggest the possibility of an interactive influence of a secondary stressor such as higher temperature, despite acclimation to chronic parasitism, but further studies are required to explore these trends.

Changes in plasma glucose concentrations in fish are generally taken to result from endocrine responses to stress (Waring *et al.* 1992; Brown, 1993; Wendelaar Bonga, 1997). In the summer months there was a significantly higher plasma glucose concentration in infected eels, although this seemed primarily to reflect lower than average glucose concentrations in uninfected eels during these months, so there is little support for a sustained

endocrine or resultant metabolic response to *A. crassus* parasitism. It is also clear from the data on plasma osmolality and ion composition that, despite the presence of *A. crassus*, eels showed good ionic and osmotic regulation. The cause of the dramatic decrease in plasma osmolality and chloride concentrations in both infected and uninfected eels in May 1996 remains unclear.

An increase in haematocrit is a common physiological effect of catecholamine release in response to acute stress (Mendiola *et al*. 1997; Wendelaar Bonga, 1997). The rise in haematocrit reflects both erythrocyte swelling (Nikinmaa, 1982) and release of splenic stores of erythrocytes (Nilsson, Holmgren & Fange, 1983). However, the blood-feeding activity of *A. crassus* could offset any stress-induced increase in haematocrit, or even depress haematocrit. Boon *et al*. (1989) reported a lack of significant differences in the haematocrit of wild eels infected with *A. crassus* compared to uninfected eels collected from the same site. However, in that study the presence or absence of *A. crassus* was determined simply as the numbers of nematodes currently in the swimbladder lumen. Therefore, eels considered as uninfected 'control' fish might have been infected with larvae in the swimbladder wall or have previously been infected and lost the infection. This seriously limits any conclusions reached. In a later study (Boon *et al*. 1990*b*) the haematocrit of eels infected artificially with high levels of *A. crassus* was reported to be decreased. In our studies, although haematocrit was generally slightly lower in infected eels, the difference was only significant in February 1996. Haematocrit has been reported to be depressed by prolonged starvation (Ince & Thorpe, 1976), and eels are believed to pass through a natural period of starvation from October to April (Sinha & Jones, 1967). Thus the significant depression in haematocrit in February 1996 may reflect the combined effects of *A. crassus*'s sanguivorous activity, together with the winter period of starvation.

Boon reported that the trypanosome, *Trypanosoma granulosum* has a significant effect on blood haematocrit in *A. crassus*-infected eels (Boon, Zuxu & Booms, 1990*a*). Both Slapton Ley and River Otter eels were infected with *T. granulosum*, with between 11 and 100% of the monthly sample of eels infected, but there was no apparent correlation between the presence of trypanosomes, in the presence or absence of *A. crassus*, and the blood haematocrit. Eels from both localities also harboured parasites on the gills and in the intestine (Kennedy, unpublished data). The effects of these parasites are unknown, but would be envisaged to be additive with any effects of *A. crassus*.

From the parameters examined, there is little evidence that chronic *A. crassus* infection adversely affects the physiological status of wild European eels at most times of the year and we assume that the eels can generally adapt to the chronic effects of parasitism. However, the studies were carried out in relatively benign laboratory conditions. In the natural environment, or in aquaculture, eels will experience a wide variety of chronic and acute challenges and further investigations are required to determine the ability to deal with these stressors in addition to the parasite burden. In Lake Balaton (Hungary) in 1991, mass mortality of eels infected with *A. crassus* was concluded to have resulted from high infection rates combined with high temperatures (Molnár, Székely & Baska, 1991). Laboratory investigations of the combined effects of temperature and infection with *A. crassus* are justified.

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