The osmotic relationship of the swimbladder nematode *Anguillicola crassus* with seawater eels

R. S. KIRK[1,2]*, D. MORRITT[1], J. W. LEWIS[1] *and* C. R. KENNEDY[2]

[1] *School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK*
[2] *School of Biological Sciences, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK*

(*Received 18 May 2001; revised 16 August 2001; accepted 5 September 2001*)

SUMMARY

The study was designed to test the hypothesis that the majority of individuals in a population of *Anguillicola crassus* can tolerate seawater by osmoconformation with the blood plasma of the eel host. The osmolality of the pseudocoelomic fluid of pre-adult and adult *A. crassus* was compared to the osmolality of eel plasma after eels were maintained in laboratory freshwater for 2 weeks (short-term transfer) or 3 months (long-term transfer) or in natural seawater for 2 h (acute transfer), 2 weeks or 3 months. The majority of *A. crassus* (at least 90% of the tested population) osmoconform with their hosts in seawater within ±30 mOsm/kg of host osmolality. Some pre-adults and adults (15–21% of the total population) were unable to withstand osmotic stress resulting in vacuolation of the hypodermis and intestinal wall, and cuticular detachment. The reasons for variation in the tissue tolerance of *A. crassus* to increased osmolality of host plasma are unknown and are not related to maturity, sex or conditions in the swimbladder. Osmoconformation in the majority of the population, however, enables parasites to survive and reproduce for long periods in seawater eels. The adaptation of *A. crassus* to its euryhaline host has played an important part in the rapid spread of the swimbladder nematode in populations of the European eel.

Key words: *Anguillicola crassus*, nematode, swimbladder, eel, osmoconformation, seawater.

INTRODUCTION

The rapid spread of the swimbladder nematode, *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974, through European eel, *Anguilla anguilla* L., populations in Europe and Northern Africa can be attributed to the activities of the eel trade (Koops & Hartmann, 1989) and the excellent colonization abilities of the parasite (Kennedy & Fitch, 1990). It is also likely that the natural vagility of the eel host and its tolerance of different salinities have facilitated movement of the parasite. Eels are capable of active osmotic and ionic regulation of their body fluids while moving through salinity gradients. This ability enables juvenile yellow eels to live in fresh or tidal waters and migrate between these environments (Tesch, 1977) or to live solely in marine waters (Tsukamoto, Nakai & Tesch, 1998) until they mature into silver eels and migrate to spawning areas near the Sargasso Sea (Tesch, 1977). Thus *A. crassus* could be disseminated by long-shore movement of eels and across marine barriers if it is capable of tolerating osmotic changes in the blood plasma of the eel host.

Adult *A. crassus* live in the liquid/gaseous environment of the swimbladder lumen and feed on the capillary network. Their exposure to osmotic changes in the external environment is therefore buffered by the osmoregulatory capacity of the host. Short-term transfer (3 week) studies on seawater-acclimated eels suggested that *A. crassus* shows very limited regulatory abilities and depends on its eel host for osmoregulation (Scholz & Zerbst-Boroffka, 1994). Long-term population biology experiments showed that about 90% of *A. crassus* infrapopulations were able to survive and reproduce in seawater eels for up to 6 months. The remaining 10% of the parasite population exhibited tissue damage throughout the experimental period (Kirk, Lewis & Kennedy, 2000*a*). The present study was designed to test the hypothesis that the majority of individuals in an *A. crassus* population can osmoconform in response to osmotic changes in eel blood plasma when their hosts are subject to acute, short-term and long-term transfer to seawater. The nature of ultrastructural damage that occurs in individuals unable to tolerate these changes was investigated by transmission electron microscopy.

MATERIALS AND METHODS

Specimen collection and maintenance

One hundred European yellow eels were collected by electro-fishing freshwater localities in Devon and East Anglia, UK with a previous history of *A. crassus* infection. On transfer to the laboratory, the eels were

* Corresponding author: School of Life Sciences, Kingston University, Kingston upon Thames, Surrey KT1 2EE. Tel: +020 8547 2000 X2732. Fax: +020 8547 7562. E-mail: R.Kirk@kingston.ac.uk

DOI: 10.1017/S0031182001001299 Printed in the United Kingdom

Table 1. Osmolality of pseudocoelomic fluid (PF) of *Anguillicola crassus* and eel plasma (EP) in response to acute, short-term and long-term transfer to laboratory freshwater (LFW) and seawater (SW) (Laboratory freshwater = dechlorinated tap water at 20 mOsm/kg. Seawater = natural seawater from Poole Harbour, Dorset at 1000 mOsm/kg. 20 eels/treatment group.)

		Median osmolality (mOsm/kg) and [variance]			
Experimental regime	Treatment	PF from *A. crassus*	EP from eels with current infections* of *A. crassus*	EP from eels with previous infections† of *A. crassus*	EP from uninfected eels
Short-term transfer to LFW	2 weeks in LFW	305·0 [85·1] $n = 35$	307·0 [76·3] $n = 7$	305·0 [159·3] $n = 8$	310·0 [47·3] $n = 5$
Long-term transfer to LFW	3 months in LFW	328·5 [41·9] $n = 18$	300·0 [37·6] $n = 8$	305·5 [63·7] $n = 8$	311·5 [83·0] $n = 4$
Acute transfer to SW	2 hours in 100 % SW‡	348·0 [803·3] $n = 33$	348·0 [133·5] $n = 14$	340·5 [60·3] $n = 4$	344·5 [84·5] $n = 2$
Short-term transfer to SW	2 weeks in 100 % SW‡	311·0 [336·9] $n = 31$	331·0 [26·6] $n = 11$	329·8 [27·9] $n = 8$	330·0 [−] $n = 1$
Long-term transfer to SW	3 months in 100 % SW‡	325·0 [64·4] $n = 28$	321·0 [70·1] $n = 13·0$	325·0 [25] $n = 3$	323·0 [62] $n = 4$

* Eels with current infections of *A. crassus* were infected with live parasite stages in the swimbladder wall and lumen.
† Eels with previous infections of *A. crassus* contained encapsulated parasite stages in the swimbladder wall. See text for further details.
‡ Following 1 week in 50 % seawater.

randomly divided into 5 groups of 20 individuals. Two groups were maintained as controls and were examined after short-term or long-term transfer to laboratory freshwater. Three groups were examined after acute, short-term or long-term transfer to seawater as described in Table 1. Laboratory freshwater and seawater were prepared and maintained as described by Kirk *et al.* (2000*a*). The osmolality of freshwater was maintained at 20 mOsm/kg and seawater at 1000 mOsm/kg. Eels were fed on strips of carp in freshwater and 50 % seawater, but refused to feed in 100 % seawater.

Eel sampling procedure

Osmolality of eel plasma was determined using standard methods. Each eel was anaesthetized by benzocaine (20 ml/litre of water) and terminally blood sampled by severance of the tail and extraction of blood from the caudal vein using a heparinized syringe without a needle. The blood was placed in a 1 ml vol. microcentrifuge tube and centrifuged at 12000 r.p.m for 2 min (MSE Microcentaur, Sanyo, UK) to separate the plasma which was pipetted into a clean tube. The osmolality of duplicate 10 μl eel plasma samples was measured using a vapour pressure osmometer (Wescor 5500, Logan, Utah, USA). The swimbladder of each eel was removed and examined for parasites.

Parasite sampling procedure

Pre-adult and adult *A. crassus* were removed from the swimbladder lumen of each infected eel and examined by low-power microscopy for determination of sex and anatomical deformities. 'Damaged' nematodes were categorized as those individuals that were slow-moving with a disrupted cuticle and/or internal changes. The length and width of each nematode were measured to the nearest mm and adherent debris removed. A sample of 10 nematodes was taken for further examination by transmission electron microscopy and the remainder were each transferred to a siliconized watch-glass containing immersion oil (Sigma Scientific, 125 centistrokes viscosity). The swimbladders were refrigerated and later examined by light microscopy for 3rd- and 4th-stage juveniles in the swimbladder wall in order to record the prevalence and mean intensity of infection.

Osmolality of nematode pseudocoelomic fluid was determined by use of a nanolitre osmometer using techniques developed by Morritt & Spicer (1996). The cuticle behind the pharynx of each nematode was carefully cut using a pair of fine dissecting scissors so that the pseudocoelomic fluid emerged from the wound as a distinct droplet under the oil. A sample of the pseudocoelomic fluid was aspirated into a siliconized glass microcapillary and duplicate 10–20 nl subsamples transferred to apertures in the sample stage of a direct-reading nanolitre osmometer (Clifton Technical Physics, Hartford, NY, USA) containing a more viscous immersion oil (Cargilles Type B, 1250 centistrokes viscosity). The osmolality of samples was determined in mOsm/kg water. Mean values of duplicate samples were used for data analysis. The 2 osmometers were regularly calibrated against Wescor NaCl standards and distilled water

https://doi.org/10.1017/S0031182001001299 Published online by Cambridge University Press

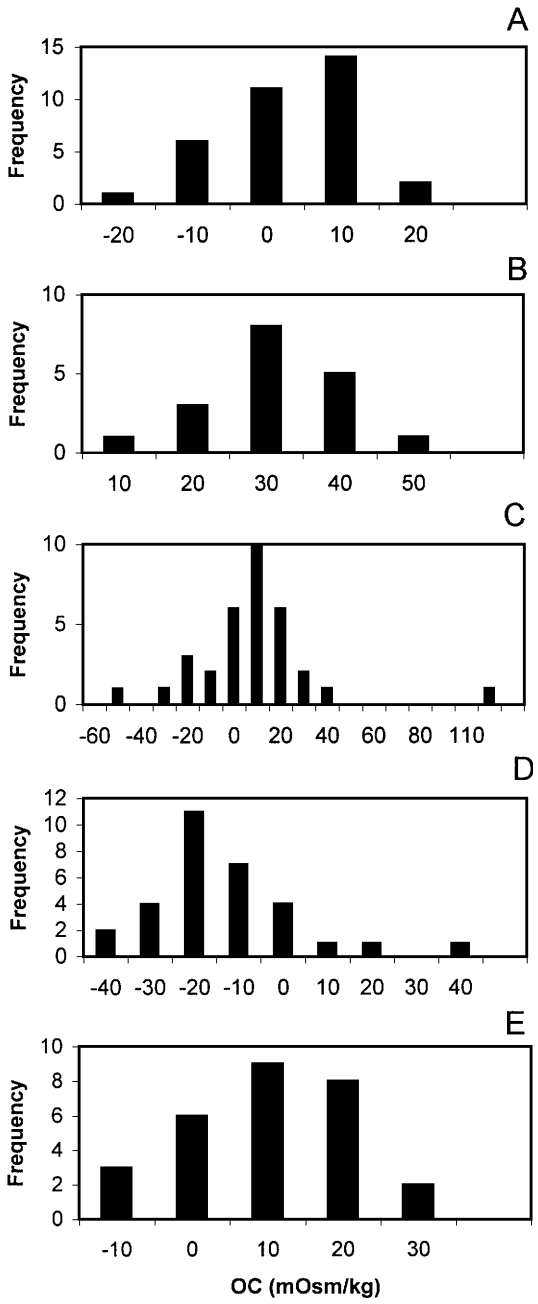

Fig. 1. Osmotic capacity (OC) (mOsm/kg) of *Anguillicola crassus* in European eels after (A) Short-term (2 weeks) transfer to laboratory freshwater; (B) long-term (3 months) transfer to laboratory freshwater; (C) acute transfer (2 h) to seawater; (D) short-term (2 weeks) transfer to seawater and (E) long-term (3 months) transfer to seawater. OC, difference between the pseudocoelomic fluid osmolality of each nematode with the plasma osmolality of its host.

and also against each other. Discrepancy between osmometers was always < 5 mOsm/kg. *A. crassus* is an extremely fragile nematode with a small pseudocoelom that is in close proximity to digestive and reproductive organs and therefore any samples of pseudocoelomic fluid contaminated with intestinal or gonadal materials were discarded. An attempt was made to measure the osmolality of 4th-stage juveniles and severely damaged adults during trial studies, but the pseudocoelomic fluid was contaminated in most specimens.

Statistical analysis

Distribution of the data was tested using the Kolmogorov-Smirnov test for goodness of fit to normality (SPSS). Some values were found to depart from normality with a high variance : mean ratio and therefore the non-parametric Mann-Whitney test was employed to analyse the results. Significance was accepted for $P \leqslant 0{\cdot}05$. Individual osmotic response to increased salinity was investigated by recording the osmotic capacity for each nematode. The index was calculated by determining the difference between the osmolality of the pseudocoelomic fluid of each nematode with the plasma osmolality of its eel host. The strength of the relationship between intensity of infection with juveniles (an expression of swimbladder condition), age of the nematodes (measured by the surrogate parameters length and body index (length × width)) and degree of osmoconformation was tested using Spearman Rank Correlation. Infection terminology is defined according to the recommendations of Bush *et al.* (1997).

Transmission electron microscopy

Ten pre-adult or adult *A. crassus* from the parasite population of each experimental group were processed for transmission electron microscopy to examine the ultrastructure of the body wall and intestine. The samples from freshwater eels consisted of undamaged individuals only because no damaged nematodes were recovered. The sample from each group of seawater eels consisted of 5 undamaged and 5 damaged individuals for comparison. To ensure maximum penetration of primary fixative, nematodes were microwaved in the primary fixative (3 % glutaraldehyde and 4 % formaldehyde in 0·1 M PIPES buffer at pH 7·2) at 46 °C, cut into pieces, changed into fresh fixative and microwaved again to 46 °C. The fixative was again changed and the specimens were fixed at room temperature for 1 h. They were rinsed in 0·1 M PIPES buffer and then post-fixed in 1 % osmium tetroxide in 0·1 M PIPES buffer. After 2 buffer rinses, the specimens were dehydrated through an ethanol series, placed in propylene oxide, embedded in 50 : 50 propylene oxide/Spurr resin for 24 h, Spurr resin for 24 h and then embedded in fresh Spurr resin and polymerized at 60 °C for another 24 h. Ultra-thin gold sections were mounted on grids, double-stained in uranyl acetate and lead citrate and viewed using a Zeiss transmission electron microscope 109 operating at 60 kV. No changes in ultrastructure were detected

https://doi.org/10.1017/S0031182001001299 Published online by Cambridge University Press

Table 2. Prevalence and intensity of *Anguillicola crassus* in eels exposed to acute, short-term and long-term transfer to laboratory freshwater (LFW) and seawater (SW) and number of damaged individuals according to light and transmission electron microscopy (Laboratory freshwater = dechlorinated tap water at 20 mOsm/kg. Seawater = natural seawater from Poole Harbour, Dorset at 1000 mOsm/kg. 20 eels/treatment group.)

Experimental regime	Treatment	Prevalence (%)*	Mean intensity and [intensity range] of *A. crassus* infection		Proportion of damaged pre-adults and adults (%)
			Total parasite population	Pre-adults and adults	
Short-term transfer to LFW	2 weeks in LFW	35	21·6 [6–47] $n = 151$	6·4 [2–15] $n = 45$	0
Long-term transfer to LFW	3 months in LFW	40	7·9 [5–13] $n = 63$	3·5 [1–6] $n = 28$	0
Acute transfer to LFW	2 hours in 100 % SW†	70	10·3 [5–23] $n = 144$	3·1 [1–7] $n = 43$	18·6
Short-term transfer to SW	2 weeks in 100 % SW†	55	9·4 [6–20] $n = 103$	3·8 [2–8] $n = 42$‡	21·4
Long-term transfer to SW	3 months in 100 % SW†	65	7·0 [3–16] $n = 91$	3·0 [1–6] $n = 40$‡	15·4

* Eels with current infections of *A. crassus* were infected with live parasite stages in the swimbladder wall and lumen. The values are the prevalence (%) of current infections of *A. crassus* in each treatment group.
† Following 1 week in 50 % seawater.
‡ Some damaged adults had to be discarded due to sample contamination during measurement of osmolality, but they are included in the proportion of damaged individuals.

between control nematodes fixed by microwave technique and routine methods (Taraschewski, Renner & Mehlhorn, 1988), except greater penetration of fixative in microwaved specimens.

RESULTS

Each experimental group was composed of eels with current infections of *A. crassus*, eels with previous infections of *A. crassus* and uninfected eels (Table 1). Eels with a current infection contained 3rd- and 4th-stage juveniles of *A. crassus* in the swimbladder wall and pre-adult and adult nematodes in the swimbladder lumen. The condition of the swimbladder varied according to the intensity of juveniles and duration of infection. When the juvenile infrapopulation was low and the swimbladder showed few pathological changes, the intra-luminal stages were bathed in a moist, gas-filled environment characteristic of uninfected swimbladders. When the juvenile infrapopulation was high and the swimbladder showed clinical signs of anguillicolosis such as a swollen and fibrotic wall with haemorrhaged tissue, the infra-luminal stages were surrounded by digested blood, cell debris, eggs and 2nd-stage juveniles. Eels with a previous infection of *A. crassus* had a thickened, fibrotic swimbladder wall with intensive pigment accumulation and remains of encapsulated parasite stages. The lumen of the swimbladder was reduced or collapsed and typically filled with a black mass of digested blood and debris. No other macroparasites were found in the swimbladders of eels.

Comparison of values in Table 1 shows that eels with current infections exhibited the same trends in plasma osmolality as previously infected and uninfected eels when transferred to seawater, although low sample sizes of uninfected eels prevent statistical comparison. The blood of all eels was hyper-osmotic to freshwater and hypo-osmotic to seawater. Median osmolality of infected eels did not change significantly after 3 months in laboratory fresh water (Mann–Whitney $U = 20·5$, $P = 0·4$). Acute transfer to sea water caused a significant elevation in the median osmolality of infected eels ($U = 0·0$, $P = 0·0$), accompanied by an increase in variance. Median osmolality then decreased significantly after 2 weeks in seawater ($U = 13·0$, $P = 0·0$) and showed a further significant decrease after 3 months ($U = 9·0$, $P = 0·0$), associated with an increase in variance.

The majority of tested pre-adult and adult *A. crassus* demonstrated an iso-osmotic relationship with eel hosts in fresh water and sea water (Table 1, Fig. 1). After short-term transfer to laboratory freshwater, there was no significant difference in median osmolality between the nematodes and their hosts ($U = 116·5$, $P = 0·8$). Frequency distribution of osmotic capacity shows that the parasites maintained their osmolality within ± 20 mOsm/kg of the

https://doi.org/10.1017/S0031182001001299 Published online by Cambridge University Press

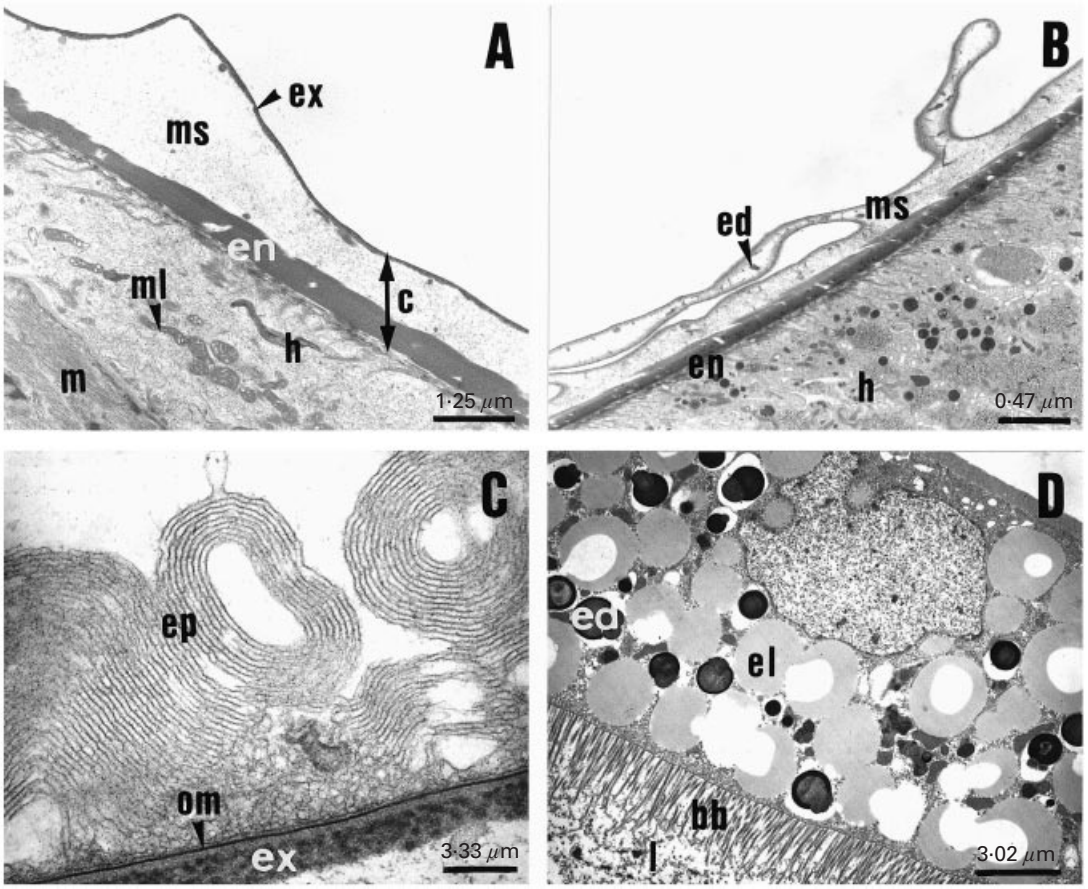

Fig. 2. Electron micrographs of sections through *Anguillicola crassus* from freshwater eels to show (A) the exocuticle (ex), mesocuticle (ms) and endocuticle (en) layers of the cuticle (c), the hypodermis (h) with multilamellate structures (ml) and longitudinal muscle layer (m); (B) folding of the mesocuticle which contains electron-dense inclusions (ed); (C) the multi-layered epicuticle (ep) overlying an osmiophilic membrane (om) and the sublayers of the exocuticle beneath; (D) the intestinal wall with electron-dense and electron-lucent inclusions (el) and the brush border of microvilli (bb). The remains of eel erythrocytes can be seen in the lumen (l).

osmolality of the host (Fig. 1A). After long-term transfer to laboratory freshwater, median osmolality of nematodes was significantly higher than eel hosts ($U = 0{\cdot}0$, $P = 0{\cdot}0$). All nematodes were hyperosmotic to their eel hosts under these experimental conditions, although 67 % were within 30 mOsm/kg of the osmolality of the host (Fig. 1B).

After acute transfer to seawater, median osmolality of nematodes increased in accordance with their eel hosts ($U = 217{\cdot}0$, $P = 0{\cdot}7$). The osmolality of 90 % of these nematodes was within ± 30 mOsm/kg of host osmolality, but variance increased because 3 damaged individuals exhibited much lower or higher osmolality values than their host (Fig. 1C). After short-term transfer to seawater, median osmolality of nematodes was significantly different from eel plasma osmolality ($U = 43{\cdot}5$, $P = 0{\cdot}0$) due to variation in the degree of osmoconformity in the parasite population, although the osmolality of 90 % of this nematode population was within ± 30 mOsm/kg of host osmolality (Fig. 1D). One of the nematodes with an osmolality outside this range (-40 mOsm/kg) exhibited obvious cuticular damage. After long-term transfer to seawater, variation between the parasites and their hosts decreased (Fig. 1E) and there was no significant difference in median osmolality ($U = 119{\cdot}5$, $P = 0{\cdot}8$). The correlation between intensity of juvenile infection, nematode age (body length and body index) and osmotic capacity was very weak (Spearman Rank Correlation $R_s = 0{\cdot}0$–$0{\cdot}2$; $P > 0{\cdot}05$).

Transmission electron microscopy

Values for prevalence and intensity of *A. crassus* infection in each treatment group of eels and the proportion of damaged pre-adults and adults detected by light and transmission electron microscopy are given in Table 2. As described, no damaged nematodes were recovered from eels subject to short-term and long-term transfer to laboratory freshwater. The ultrastructure of *A. crassus* from these freshwater eels corresponds with previous accounts by Taraschewski *et al.* (1988) and Lamah *et al.* (1990). This account, therefore, provides basic

https://doi.org/10.1017/S0031182001001299 Published online by Cambridge University Press

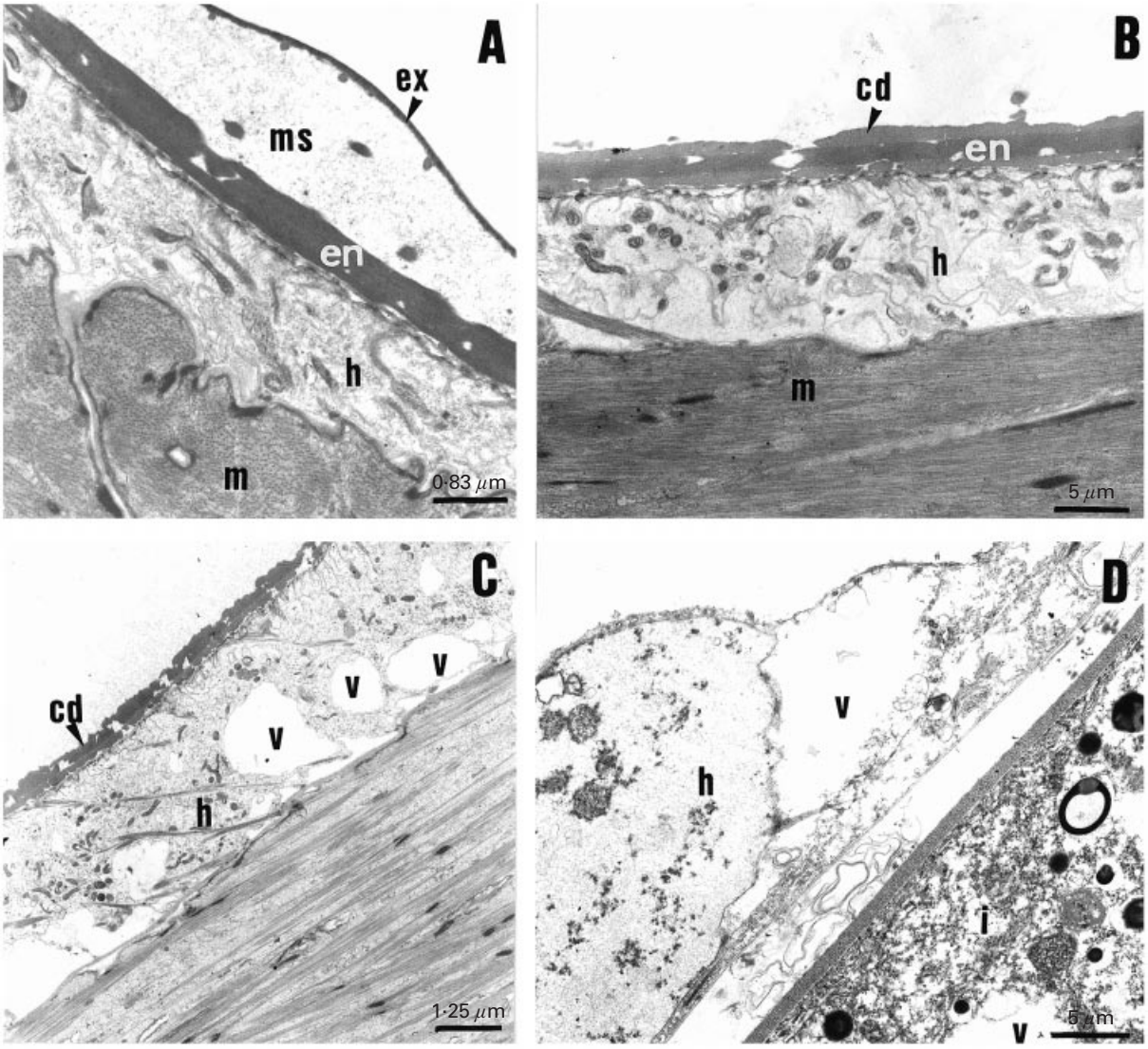

Fig. 3. Electron micrographs of sections through *Anguillicola crassus* from seawater eels. (A) The majority of *A. crassus* are undamaged after acute (2 h), short-term (2 weeks) and long-term (3 months) transfer to seawater. The exocuticle (ex), mesocuticle (ms), erndocuticle (en), hypodermis (h) and musculature (m) exhibit no changes in ultrastructure. A minority of nematodes show (B) cuticular detachment (cd); (C) vacuolation of the hypodermis (v); (D) vacuolation of the hypodermis and intestinal wall (i).

details on the features most relevant to the present study and adds further details on the cuticle. The body wall of pre-adult and adult *A. crassus* from freshwater eels was composed of 3 main tissue layers: cuticle, hypodermis and muscle (Fig. 2A). According to Maggenti (1979) the cuticle can be further subdivided into 4 layers designated the epi-, exo-, meso- and endocuticle. This nomenclature is adopted with previous terminology by Lee (1965) given in parentheses to aid comparison with previous studies. The cuticle had a wrinkled appearance due to irregular extensions of the mesocuticle (median zone) (Fig. 2B). The outermost surface, the epicuticle, was made up of a loose, multi-layered network of filaments, overlying a densely stained osmiophilic membrane (Fig. 2C). The epicuticle formed coils that appeared to be shed from the surface. The thickness of the epicuticle, therefore, varied greatly in thickness from approximately 0·2 to 2·0 μm. The exocuticle (cortical zone) was about 0·2 μm wide and was composed of an outer narrow sublayer of homologous grainy material overlying an inner sublayer with areas of electron-dense granules (Fig. 2C). The mesocuticle (median zone) appeared to be gelatinous in nature with amorphous electron-dense inclusions within a translucent matrix (Fig. 2B). The endocuticle (basal zone) consisted of 3 layers of fibres and was situated over a basal membrane adjoining the hypodermis (Fig. 2B).

The syncytial hypodermis between the dorsal, ventral and lateral cords was rich in multilamellate structures, Golgi tubules and multivesicular bodies (Fig. 2A) and was separated from the platymyarian muscle cells by a basal membrane. The intestinal wall consisted of a single layer of columnar cells separated by desmosomes with an internal brush

https://doi.org/10.1017/S0031182001001299 Published online by Cambridge University Press

border of microvilli (Fig. 2D). Electron-dense and electron-lucent inclusions in the intestinal wall resembled inclusions inside eel erythrocytes in the intestinal lumen.

Most of the undamaged nematodes from each group of seawater eels showed no differences in the ultrastructure of the body wall and intestine in comparison with nematodes from freshwater eels (Fig. 3A). Two individuals from the short-term transfer to the seawater group, which had appeared undamaged according to light microscope examination, showed vacuolation of the hypodermis, but not other changes (Fig. 3C). All damaged specimens from seawater eels exhibited cuticular detachment along part or most of the body wall and vacuolation in the hypodermis and intestinal wall (Fig. 3B–D, Table 2). Presence of tissue changes was not linked to maturity or sex of nematodes.

DISCUSSION

Juvenile yellow eels can live in a wide range of salinities from inland lakes (Tesch, 1977) to saline lagoons (Kennedy *et al*. 1997) and marine waters (Tesch, 1977). They are able to adapt to short-term fluctuations in the space of a tidal cycle in estuaries or to longer-term changes in salinity during seasonal migrations between coastal and fresh waters because they are powerful homeosmotic regulators, able to hyper-regulate in freshwater and hypo-regulate in seawater (Kirschner, 1979; Jobling, 1995). It is very important, therefore, that *A. crassus* is able to tolerate changes in the osmolality of the blood plasma of the host.

Physiological and ultrastructural data in this study support the hypothesis that most *A. crassus* osmoconform in response to acute, short-term and long-term host transfer to seawater without damage to body tissues. Adaptation of eels to seawater during acute transfer (adaptive period) is known to involve physiological modifications to counteract osmotic loss of water and diffusional influx of electrolytes, resulting in a transitory elevation in plasma osmolality and ionic concentrations (Tierney, 1993; Tierney *et al*. 1995). During this adaptive period most tested nematodes (90 %) behaved as osmoconformers with the osmolality of the pseudocoelomic fluid increasing with the osmolality of host plasma, indicating tissue tolerance to osmotic change. One pre-adult and 2 adults were unable to tolerate the rise in plasma osmolality resulting in damage to the body wall and intestine. Acute adaptation of eels to seawater is followed by a longer adjustment period over 1 week eventually leading to a longer-term adaptation (regulatory period). This is a biphasic process involving changes in the osmo- and iono-regulatory mechanisms in the gills, gut and kidney and further lowering of eel plasma osmolality (Jobling, 1995). The majority of tested *A. crassus* (90 %) osmoconformed with their eel hosts after 2 weeks in seawater. All tested individuals showed close osmoconformation with the host after 3 months in seawater, possibly due to mortality of those individuals unable to tolerate changes in plasma osmolality.

Some nematodes experience osmotic stress immediately after their hosts are transferred to seawater, reflected by extensive cuticular detachment and vacuolation of the hypodermal and intestinal tissues. Such severely injured individuals are unlikely to survive for long. Other members of the nematode population may initially osmoconform, but then tissue tolerances to higher osmolality are exceeded within the 2 week period after transfer to seawater. This study shows that 15–21 % of the parasite population from seawater eels exhibited tissue damage thought to be due to osmotic stress. Cuticular detachment is likely to influence the physiology of the nematodes, including development of eggs and juveniles. This may explain why infectivity studies have shown that seawater reduces the transmission success of *A. crassus* to hosts throughout the life-cycle by decreasing the total infectivity of the *in utero* juvenile population, in addition to direct effects on free-living juveniles (Kirk, Kennedy & Lewis, 2000*b*). Transmission electron microscopical examination of the uterus of *A. crassus* from seawater eels would confirm if osmotic stress has an effect on juvenile viability.

Short-term studies by Scholz & Zerbst-Boroffka (1994) have suggested that *A. crassus* is incapable of independent osmoregulation. Osmolality of pseudocoelomic fluid from 7 *A. crassus* removed from eels maintained in artificial sea water (1030 mOsm/kg) for 3 weeks was iso-osmotic with host blood plasma and *in vitro* studies confirmed that *A. crassus* showed no evidence of osmoregulatory mechanisms. Scholz & Zerbst-Boroffka (1994) also noted that osmotic and ionic stress resulted in a marked increase of parasite mortality, although specific mortality data are not given.

The reason why certain individuals in a population deviate from osmoconformity is not clear and is not correlated with age or sex or conditions in the swimbladder. There is scarce reliable information about osmoregulatory mechanisms in nematodes because researchers have found that the physiological condition of a nematode generally declines when it is removed from the host environment and thus measurements are of limited applicability (Fusé, Davey & Sommerville, 1993). The related dracunculoid nematode *Philonema oncorhynchi* Kuitenen-Ekbaum, 1933 in the body cavity of sockeye salmon *Oncorhynchus nerka* (Walbaum) (Lewis, Jones & Adams, 1974) is iso-osmotic with the body fluids of its host. Like *A. crassus*, *P. oncorhynchi* is able to adjust to changes in the osmolality of the body fluids of the host, in this case as the anadromous sockeye

https://doi.org/10.1017/S0031182001001299 Published online by Cambridge University Press

salmon returns to fresh water to spawn. The body wall of *P. oncorhynchi* is clearly important in the iso-osmotic relationship as it is shown to be permeable to water and ions (Lewis *et al*. 1974). Experiments on the anisakid codworm *Pseudoterranova decipiens* (Krabbe, 1878) from Atlantic cod *Gadus morhua* L. have demonstrated that the 3rd-stage juvenile in the muscle appear to be capable of, at least, short-term osmoregulation and that the site of osmoregulation is the body wall (Fusé *et al*. 1993). Investigation of the role of the body wall in uptake of ions could not be undertaken in this study because it was very difficult to isolate the body wall by cylindrical 'sac' preparations involving ligature of dissection without damage to the parasite. The extreme fragility of *A. crassus* is not surprising for a nematode that lives in the swimbladder. Its gelatinous and compressible mesocuticle adapts the nematode for living in a gas/fluid filled space. If the body wall was important in osmoconformation of *A. crassus*, then it would be expected that individuals from a swimbladder contaminated with digested blood and parasite remains would be less capable of osmoconformation than individuals from a 'cleaner' swimbladder, but this difference was not observed.

Fusé *et al*. (1993) suggested that the intestine may be an additional site for osmoregulatory control. If this is the case, then feeding behaviour may be important in osmotic balance of *A. crassus*. There is very little information about the behaviour of *A. crassus* in the swimbladder lumen, but unpublished observations in this laboratory suggest that *A. crassus* pre-adults and adults do not constantly feed on blood and spend some time in the lumen detached from blood vessels. It is a possibility, therefore, that *A. crassus* is able to osmoconform by feeding on eel blood. Many invertebrates that osmoconform achieve tissue tolerance by increasing intracellular osmolality by mobilization of amino acids, thereby balancing extracellular fluid. This reduces the osmotic gradient across cell membranes and maintains constant cell volume (Schmidt-Nielsen, 1998). An animal feeding on host blood proteins, in comparison to a non-blood feeder, will have access to a greater pool of amino acids that could be used in intracellular osmotic regulation. Individual *A. crassus* that are not feeding at the time of transfer to seawater may incur tissue damage due to lack of contact with host blood.

This investigation provides further verification of the excellent colonization characteristics of *A. crassus*. The ability of *A. crassus* to tolerate small changes in host plasma osmolality enables parasites to survive and reproduce for long periods in seawater eels (Kirk *et al*. 2000*a*). In addition, the free-living stages of the life-cycle are capable of surviving and remaining infective for long periods in a range of salinities (Kennedy & Fitch, 1990; Kirk *et al*. 2000*b*). Thus the superb adaptation of *A. crassus* to its euryhaline host has played an important part in the rapid dissemination of *A. crassus* in European eel populations.

The authors gratefully acknowledge the award of a grant from the Leverhulme Trust that made this work possible. D.M. acknowledges support from the University of London Central Research Fund. The authors are also grateful to the Environment Agency and Phil and Jan Shears (University of Exeter) for collection of eels, to Neil Morley and Gareth Hughes (Royal Holloway) for eel maintenance and to Anton Page and Keira Thorogood (Royal Holloway Electron Microscopy Unit) for assistance with electron microscopy.

REFERENCES

BUSH, A. O., LAFFERTY, K. D., LOTZ, J. M. & SHOSTAK, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology* **83**, 575–583.

FUSÉ, M., DAVEY, K. G. & SOMMERVILLE, R. I. (1993). Osmoregulation in the parasitic nematode *Pseudoterranova decipiens*. *Journal of Experimental Biology* **175**, 127–142.

JOBLING, M. (1995). *Environmental Biology of Fishes*. Chapman and Hall, London.

KENNEDY, C. R. & FITCH, D. J. (1990). Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *Journal of Fish Biology* **36**, 117–131.

KENNEDY, C. R., DI CAVE, D., BERRILLI, F. & ORECCHIA, P. (1997). Composition and structure of helminth communities in eels *Anguilla anguilla* from Italian coastal lagoons. *Journal of Helminthology* **71**, 35–40.

KIRK, R. S., LEWIS, J. W. & KENNEDY, C. R. (2000 *a*). Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. *Parasitology* **120**, 289–295.

KIRK, R. S., KENNEDY, C. R. & LEWIS, J. W. (2000 *b*). The effect of salinity on hatching, survival and infectivity of *Anguillicola crassus* (Nematoda: Dracunculoidea) larvae. *Diseases of Aquatic Organisms* **40**, 211–218.

KIRSCHNER, L. B. (1979). Control mechanisms in crustaceans and fishes. In *Mechanisms of Osmoregulation in Animals* (ed. Gilles, R.), pp. 157–212. John Wiley & Sons, Chichester.

KOOPS, H. & HARTMANN, F. (1989). *Anguillicola*-infestations in Germany and in German eel imports. *Journal of Applied Ichthyology* **1**, 41–45.

LAMAH, TH., FRANZ, M., MEHLHORN, H. & TARASCHEWSKI, H. (1990). Comparison de *Philometra ovata* Zeder, 1803 et d'*Anguillicola crassus* Kuwahara *et al*. 1974 (Nematodes Dracunculoidea): études en microscopie photonique et électronique à transmission. *Annales des Sciences Naturelles, Zoologie, Paris 13th Série* **11**, 123–133. (In French.)

LEE, D. L. (1965). The cuticle of adult *Nippostronglyus brasiliensis*. *Parasitology* **55**, 173–181.

LEWIS, J. W., JONES, D. R. & ADAMS, J. R. (1974). Functional bursting by the dracunculoid nematode *Philonema oncorhynchi*. *Parasitology* **69**, 417–427.

MAGGENTI, A. R. (1979). The role of cuticular strata nomenclature in the systematics of Nemata. *Journal of Nematology* **11**, 94–98.

MORRITT, D. & SPICER, J. I. (1996). Developmental ecophysiology of the beachflea *Orchestia gammarellus* (Pallas) (Crustacea: Amphipoda). I. Female control of the embryonic environment. *Journal of Experimental Marine Biology and Ecology* **207**, 191–203.

SCHMIDT-NIELSEN, K. (1998). *Animal Physiology : Adaptation and Environment*, 5th Edn. Cambridge University Press, Cambridge.

SCHOLZ, F. & ZERBST-BOROFFKA, I. (1994). Osmo- und Ionenregulation des Aalparasiten *Anguillicola crassus* (Nematoda) und seines Wirtes (*Anguilla anguilla*). *Zoologische Beiträge* **35**, 103–117. (In German.)

TARASCHEWSKI, H., RENNER, C. & MEHLHORN, H. (1988). Treatment of fish parasites. 3. Effects of levamisole HCI, metrifonate, fenbandazole, mebendazole, and invermectin on *Anguillicola crassus* (nematodes) pathogenic in the air bladder of eels. *Parasitology Research* **74**, 281–289.

TESCH, F.-W. (1977). *The Eel. Biology and Management of Anguillid Eels*. Chapman and Hall, London.

TIERNEY, M. L. (1993). Endocrine control of osmoregulation in the euryhaline eel, *Anguilla anguilla*. Ph.D. thesis University of St. Andrews.

TIERNEY, M. L., LUKE, G., CRAMB, G. & HAZON, N. (1995). The role of the renin-angiotensin system in the control of blood pressure and drinking in the European Eel, *Anguilla anguilla*. *General and Comparative Endocrinology* **100**, 39–48.

TSUKAMOTO, T., NAKAI, I. & TESCH, W-.V. (1998). Do all freshwater eels migrate? *Nature, London* **396**, 635–636.