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Effect of salinity on hatching, survival and infectivity of *Anguillicola crassus* (Nematoda: Dracunculoidea) larvae

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ABSTRACT: The effect of salinity on hatching, larval survival and infectivity of *Anguillicola crassus* was studied under experimental conditions using eggs obtained from naturally infected eels. Egg hatching rate, second-stage larval survival and larval infectivity were maximal in fresh water and declined with increase in salinity. Larvae survived up to 100 d in fresh water, 70 d in 50% sea water and 40 d in 100% sea water. Infectivity experiments demonstrated that salinity influenced transmission success throughout the life cycle by decreasing total infectivity of the larval population *in utero* within female *A. crassus* and when larvae were free-living in the aquatic environment. Infectivity was age-dependent in relation to salinity. Larvae were infective to intermediate and paratenic hosts for up to 80 d in fresh water, 21 d in 50% sea water and up to 8 d in 100% sea water. The data confirm field observations that infection levels decrease with an increase in salinity. The study contributes to experimental verification of the colonization abilities of *A. crassus* and supports the hypothesis that *A. crassus* can be disseminated and transmitted in brackish water. The importance of regular monitoring and stringent hygiene practices in the transportation of eels is emphasised.

KEY WORDS: *Anguillicola crassus* · Parasitic nematode · Eels · Swimbladder · Salinity · Egg hatch · Larval survival · Infectivity

INTRODUCTION

Environmental factors are of crucial importance in the infection dynamics of parasites and the subsequent expression of pathogenic potential. They are particularly relevant to parasites of migratory fish that are physiologically adapted to tolerate changes in the salinity of the aquatic environment in order to spawn. The nematode *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 is a pathogenic parasite in the swimbladder of catadromous eels, now reported in the European eel *Anguilla anguilla* (L.), Japanese eel *Anguilla japonica* Temminck & Schlegel and American eel *Anguilla rostrata* (LeSueur) (Moravec & Škoríková 1998). Experimental studies by Kirk et al. (2000) have shown that *A. crassus* can survive and reproduce in eels in estuarine and marine simulated conditions and therefore could survive during the spawning migration of eels to the Sargasso Sea.

Anguillicola crassus is ovoviviparous. The mature eggs *in utero* contain fully formed, motile second-stage larvae that are released into the eel swimbladder through the vulva of the female nematode. Examination of the intestine of infected eels (J. Shears pers. comm.) has confirmed that most eggs leave the swimbladder via the pneumatic duct, pass down the intestinal tract and hatch in the water, although a small number hatch in the swimbladder. The second-stage larvae attach to the substratum and undulate to stimulate predation by a wide range of invertebrate intermediate hosts (Thomas & Ollevier 1993). After consumption by a suitable host, the larvae migrate to the haemocoel and develop into third-stage larvae that are infective to a variety of paratenic hosts (fish, amphibians, insect

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larvae and snails) and to eel definitive hosts, via predator-prey transmission (Moravec & Konecny 1994, Moravec & Škoríková 1998). Thus the eggs and second-stage larvae are subject to the influence of abiotic factors such as salinity.

Previous research has shown that increased salinity reduces the hatching rate of eggs, decreases survival time of second-stage larvae and subsequent infectivity to copepod intermediate hosts (de Charleroy et al. 1989, Kennedy & Fitch 1990). The life cycle of *Anguillicola crassus* has been transmitted in 50 and 100% sea water in the laboratory, but infection levels in 100% seawater hosts were very low (Kirk et al. 2000). The present study was designed to quantitatively determine whether salinity only influences the free-living stages in the aquatic environment or has an additional influence on larvae *in utero* of female *A. crassus* inhabiting the swimbladder of seawater eels. The effect of salinity on transmission success in relation to larval age was investigated.

MATERIALS AND METHODS

Naturally infected eels were collected by electrofishing from Slapton Ley, Devon, United Kingdom (n = 180, mean length 371.2 mm ± SD 106.4; mean weight 93.4 g ± 70.4) and divided randomly into 3 groups of 60 eels. One group was maintained in fresh water (dechlorinated tap water) as controls and a second group were kept in 50% sea water (50:50 dechlorinated water/natural sea water at 500 mOsm kg^{-1} l^{-1}; 17‰ NaCl). The remaining eels were maintained in 100% sea water (natural sea water from Poole Harbour, Devon, at 950 to 1000 mOsm kg^{-1} l^{-1}; 33‰ NaCl). Seawater eels were subjected to step-wise acclimatisation to salt water over 1 wk. The water was aerated and filtered by Fluval pumps.

The host species used in the experimental life cycles were selected on the criteria of susceptibility to *Anguillicola crassus* infection and availability from uninfected sources. All field sites were regularly monitored for the presence of *A. crassus* and were uninfected at the time of the experiment. Laboratory-cultured freshwater copepods *Acanthocyclops vernalis* (Fischer) were used as the freshwater intermediate hosts. Euryhaline calanoid copepods *Eurytemora affinis* (Poppe) were collected by plankton sampling in the Exe Estuary, Devon, and were utilized as seawater intermediate hosts. The susceptibility of *E. affinis* to infection was confirmed by Kirk et al. (2000). Three-spined sticklebacks *Gasterosteus aculeatus* L. (n = 242, mean length 30.3 mm; ± SD 5.7; mean weight 0.3 g ± 0.2) and minnows *Phoxinus phoxinus* L. were hand-netted from brooks in Exeter, Devon, and Egham, Surrey, and were used as paratenic and surrogate hosts respectively. Uninfected yellow eels (n = 32, mean length 333.4 ± SD 64.2; mean weight 57.6 ± 38.7) were collected by electro-fishing from the River Clyst and River Otter, Devon, for use as definitive hosts. Numbers of eels were limited due to samples required for monitoring and climatic conditions during sampling. All host animals were maintained in aerated, filtered fresh water, 50 or 100% sea water and were prohibited food 24 h before exposure to infection.

Influence of salinity on *in utero* larval infectivity. Infectivity of larvae from freshwater and seawater eels was directly compared by completion of the life cycle in fresh water at 15°C ± 2. *Anguillicola crassus* eggs were recovered from the swimbladder of 5 eels maintained in fresh water, 50 and 100% sea water at 2 wk intervals for up to 12 wk. The eggs were hatched in fresh water at room temperature and used for experimental infections within 24 h. Female *Acanthocyclops vernalis* with a minimum length of 1.0 mm were individually exposed to a dose of 3 second-stage larvae in 2 ml of filtered pond water $well^{-1}$ in plastic multi-well plates for 24 h. Five replicate groups of 30 copepods were exposed for each maintenance salinity and time interval. They were maintained in a 50:50 mixture of dechlorinated water and filtered pond water seeded with a *Chlamydomonas* sp. culture. Preliminary maintenance experiments demonstrated that over-feeding with *Chlamydomonas* sp. almost totally prevented intraspecific predation within the *A. vernalis* population over 1 mo. The copepods were screened for infection by live microscopic examination at 21 d post infection (p.i.). Infected *A. vernalis* were fed to individual sticklebacks at a dose of 10 larvae $fish^{-1}$ in 300 ml of fresh water for 24 h. The sticklebacks were killed by vertebral severance and examined by dissection for larvae at 14 d p.i. The swimbladder of each infected stickleback was inverted by means of a dorsal incision so that the larvae on the outer surface were enfolded within the swimbladder wall. The swimbladder was then pipetted into the intestine of dead minnows killed by vertebral severance. This surrogate host infection technique enabled enumeration of the larval dose fed to eels and was used as an alternative option to quantitative oral administration methods adopted by other workers (e.g. Boon et al. 1990, Haenen & van Banning 1991). Minnows were used as surrogates because captive eels demonstrated a preference for feeding on minnows in comparison to sticklebacks (authors' unpubl. obs.). Eels were individually confined in 10 l of fresh water and offered larvae in minnows at a dose of 10 larvae eel^{-1}. The eels were examined for infection by dissection at 2 mo p.i. Only larvae originating from donor eels maintained for 6 and 12 wk in experimental salinities were used for infections.

Hatching rate and larval survival. *Anguillicola crassus* eggs were recovered from the swimbladder of naturally infected eels kept in fresh water, 50% sea water and 100% sea water for 12 wk as previously described. One hundred eggs containing motile second-stage larvae were pipetted from each eel swimbladder lumen and divided into groups of 10 in 20 ml of medium in petri dishes. Eggs were maintained at 10°C ± 2 in previously aerated, autoclaved media, according to the maintenance regime of the host. Pond water and natural sea water were used for production of media. Hatching rate and subsequent larval survival were recorded every 2 d and the medium was changed in each petri dish every 2 wk. Larvae were judged to be dead when they failed to respond to a stream of water from a pipette.

Influence of salinity and larval age on infectivity. Approximately 20 000 eggs were removed from the swimbladder of 5 naturally infected eels maintained in fresh water, 50% sea water and 100% sea water. Eggs were hatched as described above and the second-stage larvae were placed into groups of 500 in 1 l of aerated, autoclaved media, according to the maintenance regime of the host. Live larvae were tested for infectivity immediately after removal from the swimbladder and at 10 time intervals throughout the survival curve in each medium as shown in Table 2 ('Results'). Freshwater larvae were transmitted through a freshwater life cycle as previously described. Seawater larvae were transmitted through a 50 or 100% seawater cycle via *Eurytemora affinis* and seawater-acclimatised sticklebacks and eels using methodology given in Kirk et al. (2000). Thirty intermediate hosts were exposed at each time interval.

Statistical analysis. The terms prevalence, mean abundance and mean intensity are used according to recommendations by Bush et al. (1997). A metapopulation is defined as all of the infrapopulations sampled from a given host species (Bush et al. 1997). Distribution of the data was shown to depart from normality (Shapiro-Wilk Test) and therefore non-parametrics tests (SPSS) were employed to analyse the data. Significance was accepted for $p \leq 0.05$.

RESULTS

Influence of salinity on *in utero* larval infectivity

Third-stage larvae were fully developed in the haemocoel of *Acanthocyclops vernalis* by 21 d p.i. They were liberated from the second-stage cuticular sheath and were coiled within the cephalothorax or abdomen. Infected *A. vernalis* were readily discernible by their yellow-orange colouring and slower movement than uninfected individuals. Prevalence and mean abundance of *A. crassus* infection in copepod hosts were significantly higher using larvae from freshwater eels compared to 50 and 100% seawater eels (Mann-Whitney Test, $p < 0.05$) (Fig. 1). In most cases, prevalence and mean abundance were significantly higher using 50% seawater-derived larvae than 100% sea water larvae (Mann-Whitney Test, $p < 0.05$, except 8 wk and 12 wk). Mean intensity showed no significant difference between experimental groups, and there was no relationship between infection parameters and maintenance time of eels in experimental media (Kruskal-Wallis Test, $p > 0.05$).

Third-stage larvae were recovered from the outer surface of the swimbladder of sticklebacks at 14 d p.i. The number of sticklebacks exposed to infection was governed by the availability of larvae from copepods and was therefore always lower in seawater infections. Differences in sample size can bias data and limit statistical analysis. Thus prevalence appeared to be similar between experimental groups (Fig. 2). Mean abundance and mean intensity, however, were usually significantly higher using freshwater-derived larvae

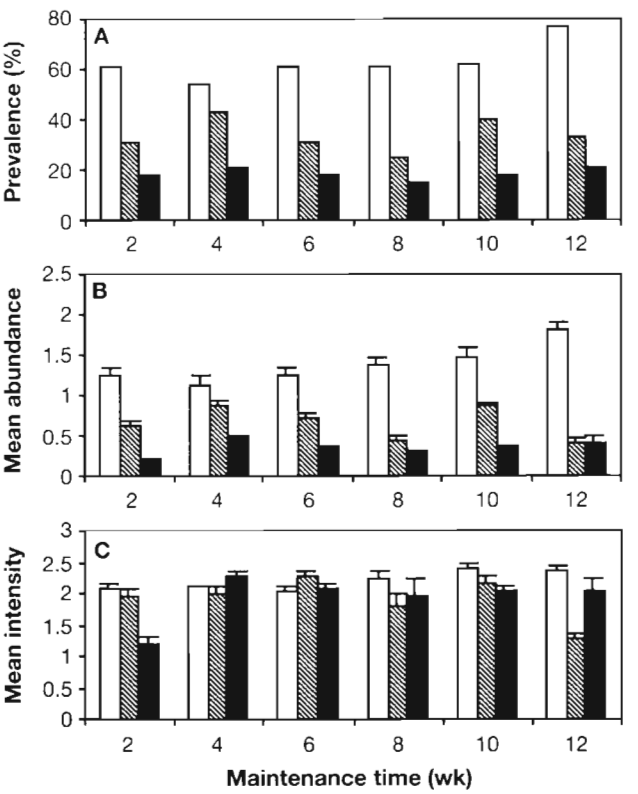

Fig. 1. (A) Prevalence, (B) mean abundance and (C) mean intensity of *Anguillicola crassus* in *Acanthocyclops vernalis* in fresh water using larvae derived from eels maintained in fresh water (□), 50% sea water (▨) and 100% sea water (■). Results are expressed as a mean + SE for 150 copepods per time period

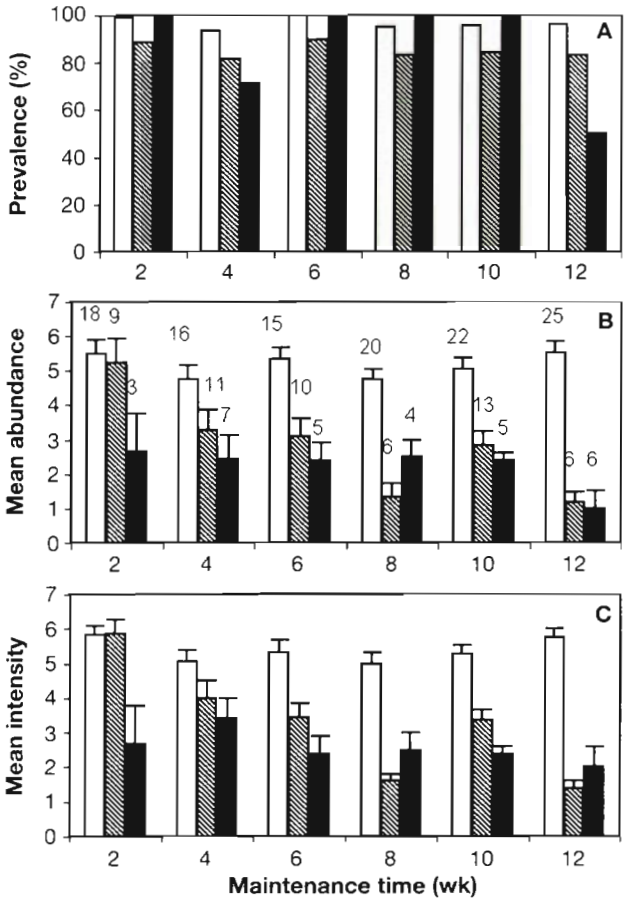

Fig. 2. (A) Prevalence, (B) mean abundance and (C) mean intensity of *Anguillicola crassus* in sticklebacks in fresh water using larvae derived from eels maintained in fresh water (□), 50% sea water (▨) and 100% sea water (■). Standard error bars are given. Numbers in (B) refer to sample sizes

(Mann-Whitney Test, $p < 0.05$, except 2 wk) and generally lowest using 100% seawater-derived larvae (except 8 and 12 wk). Freshwater larvae were successfully transmitted to 9 out of 10 eels via minnows (Table 1). Third-stage larvae developed to fourth-stage larvae, pre-adults or adult worms. Ten to 60% of the original larval dose administered to each eel was recovered. Larvae from donor eels in 50% sea water for 6 wk were transmitted to 2 out of 3 eels and developed to fourth-stage larvae or adults. Thirty to 40% of the original larvae dose was transmitted. The 2 uninfected eels (6 wk in fresh water and 50% sea water) each regurgitated their minnow and probably caused loss of larvae. No successful infections resulted using larvae from donor eels in 50% sea water for 12 wk and all 100% seawater eels.

Hatching rate and larval survival

Hatching rate showed a significant positive correlation with time in all media (Spearman Rank Correlation, freshwater [FW] $r_s = 0.97$; 50% seawater [SW] $r_s = 0.99$; 100% SW $r_s = 0.98$, $p = 0$) and was adversely affected by high salinity (Fig. 3A). There was no significant difference in the rate of egg hatching in fresh water and 100% sea water up to 50% hatch at 10 to 11 d (Wilcoxon Signed Rank Test, $Z = -1.43$, $p > 0.05$). Hatch rate in 50% sea water was significantly slower than in fresh water with 50% hatch achieved at 15 d (Wilcoxon Signed Rank Test, $Z = -2.34$, $p < 0.05$). Hatch rate in 100% sea water then declined, 94.4% of the eggs hatched in 40 d and the remainder degenerated. In fresh water and 50% sea water all eggs hatched, 95% of eggs hatched in 22 d in fresh water, compared to 25 d in 50% sea water. Survival rate of larvae showed a significant negative correlation with time in all media (Spearman Rank Correlation, FW $r_s = -0.99$; 50% SW $r_s = -0.99$; 100% SW $r_s = -0.97$, $p = 0$) (Fig. 3B) and was significantly influenced by salinity (Wilcoxon Signed Rank Test, $Z = -3.92$ to 4.84, $p = 0$). Second-stage larvae survived up to 100 d in fresh water, 70 d in 50% sea water and 40 d in 100% sea water.

Influence of salinity and larval age on infectivity

Infectivity and thus the size of the metapopulation in intermediate and paratenic hosts declined with larval

Table 1. Infection of freshwater eels with *Anguillicola crassus* at 2 mo p.i. using larvae derived from eels maintained in fresh water (FW), 50 and 100% sea water (SW) for 6 and 12 wk

Maintenance regime of eels	No. of eels exposed	Exposure dose (larvae eel^{-1})	Prevalence (%)	Mean intensity (Range)	No. fourth-stage larvae	No. pre-adults	No. adults
6 wk FW	5	10	80.0	2.5 (1–6)	7	1	2
12 wk FW	5	10	100.0	2.0 (1–2)	8	2	0
6 wk 50% SW	3	10	66.7	3.5 (3–4)	5	0	2
12 wk 50% SW	1	7	0.0	0.0	–	–	–
6 wk 100% SW	1	10	0.0	0.0	–	–	–
12 wk 100% SW	1	6	0.0	0.0	–	–	–

Table 2. Decline in metapopulation size of *Anguillicola crassus* in intermediate and paratenic hosts in relation to salinity and larval age

Larval age (d)	Total no. larvae available from copepods (n = 30)	No. sticklebacks exposed	Total no. larvae available from sticklebacks
Fresh water	*Acanthocyclops vernalis*		
0	40	4	15
10	34	3	12
20	29	2	9
30	24	2	10
40	25	2	10
50	20	2	7
60	18	1	2
70	13	1	2
80	12	1	1
90	2	1	0
100	0	0	0
50% sea water	*Eurytemora affinis*		
0	21	2	9
7	20	2	6
14	10	1	2
21	10	1	1
28	13	1	0
35	7	1	0
42	6	1	0
49	5	1	0
56	0	0	0
63	0	0	0
70	0	0	0
100% sea water	*Eurytemora affinis*		
0	11	1	3
4	10	1	2
8	8	1	2
12	5	1	0
16	6	1	0
20	3	1	0
24	0	0	0
28	0	0	0
32	0	0	0
36	0	0	0
40	0	0	0

age and increase in salinity (Tables 2 & 3). Prevalence and mean intensity of third-stage larvae in intermediate hosts were significantly negatively correlated with larval age (Spearman Rank Correlation, FW $r_s = -0.97$ and -0.76; 50% SW $r_s = -0.97$ and -0.83; 100% SW $r_s = -0.91$ and -0.94, $p = 0$) and adversely affected by salinity. Larvae were infective to copepods for up to 90 d in fresh water, 49 d in 50% sea water and 20 d in 100% sea water (Fig. 4). Similarly, mean intensity of infection in stickleback hosts was significantly negatively correlated with larval age (Spearman Rank Correlation, FW $r_s = -0.89$; 50% SW $r_s = -0.91$; 100% SW $r_s = -0.92$, $p = 0$) (Fig. 5) and adversely influenced by salinity. Prevalence was not age dependent due to

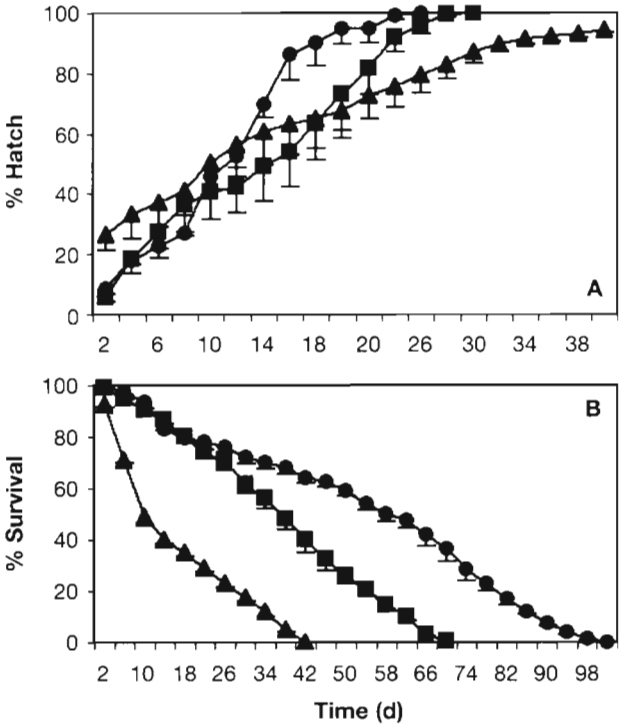

Fig. 3. (A) Hatching rate of eggs and (B) survival rate of larvae of *Anguillicola crassus* in fresh water (●), 50% sea water (■) and 100% sea water (▲). Results are expressed as mean % egg hatch or larval survival – SE for 500 eggs or lavae per time period

Table 3. Infectivity of *Anguillicola crassus* larvae to eels at 2 mo p.i. in relation to salinity and larval age (1 eel exposed at each time period)

Larval age (d)	Exposure dose	Mean intensity	No. fourth-stage larvae	No. pre-adults (no adults)
Fresh water				
0	10	3	2	1
10	10	2	2	0
20	9	1	1	0
30	10	1	1	0
40	10	1	1	0
50	7	0	0	0
60	2	0	0	0
70	2	0	0	0
80	1	0	0	0
50% sea water				
0	9	2	2	0
7	6	0	0	0
14	2	0	0	0
21	1	0	0	0
100% sea water				
0	3	0	0	0
4	2	0	0	0
8	2	0	0	0

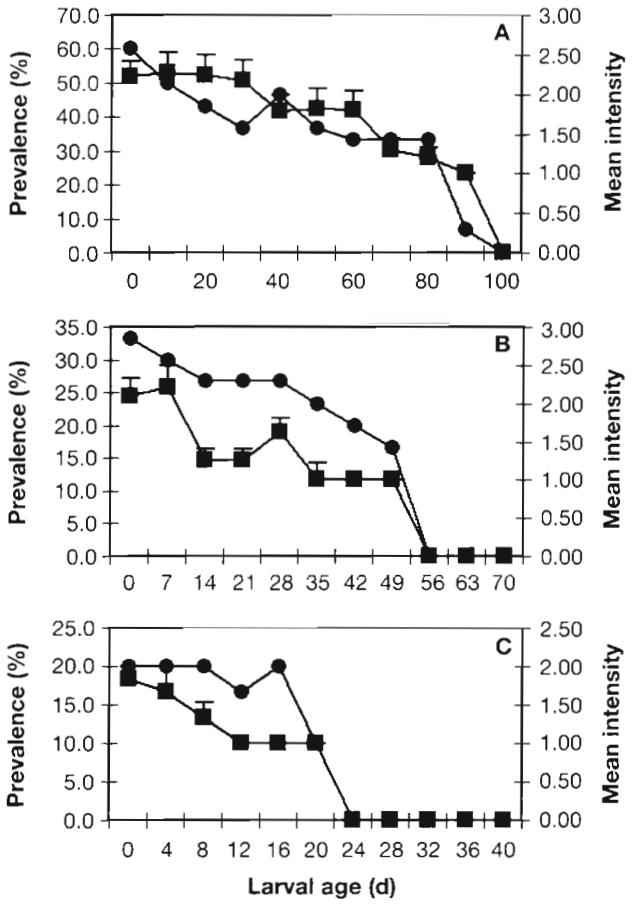

Fig. 4. Relationship of prevalence (●) and mean intensity (■) of *Anguillicola crassus* in intermediate hosts to salinity and larval age. (A) Infection of *Acanthocyclops vernalis* in fresh water, (B) infection of *Eurytemora affinis* in 50% sea water and (C) infection of *E. affinis* in 100% sea water. Results are expressed as a mean + SE for 30 copepods per time period

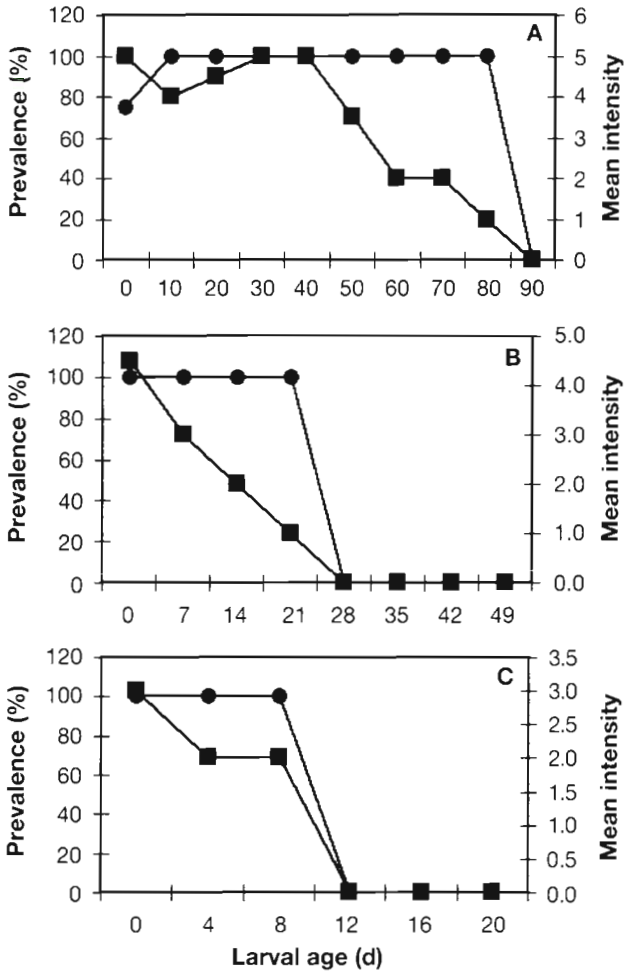

Fig. 5. Relationship of prevalence (●) and mean intensity (■) of *Anguillicola crassus* in paratenic hosts to salinity and larval age. (A) Infection of freshwater sticklebacks, (B) 50% seawater sticklebacks and (C) 100% seawater sticklebacks

sequential reduction in sample size as a consequence of declining metapopulations (Table 2). Third-stage larvae were infective to paratenic hosts up to 80 d in fresh water, 21 d in 50% sea water and 8 d in 100% sea water. Freshwater larvae aged up to 40 d were infective to freshwater eels (Table 3). Larvae in 50% sea water quickly lost infectivity to eels after 7 d. Eel infections in 100% sea water were not successful. Failure by older larvae to infect the definitive host in all media may be influenced by the low number of larvae available from stickleback infections.

DISCUSSION

When the eggs and larvae of *Anguillicola crassus* are released into the external environment, they are known to be subject to the adverse influence of density-independent abiotic factors such as salinity (de Charleroy et al. 1989, Kennedy & Fitch 1990). The present study confirms the importance of salinity and demonstrates that this factor influences transmission success to hosts throughout the life cycle by decreasing total infectivity of the larval population *in utero*, as well as in the external environment. Lower values of prevalence and mean abundance of infections using larvae from seawater eels compared to freshwater eels suggest that less viable larvae were available from seawater hosts. Mean intensity values in intermediate hosts were similar, indicating that some larvae from seawater eels were equally as viable as those from freshwater eels and capable of development to third-stage larvae. The effect of sea water on larval infectivity *in utero* is likely to be related to the osmotic and ionic stress placed upon female parasites after transfer of the host into water of increased salinity. Scholz & Zerbst-Boroffka

(1994) reported that exposure of infected eels to sea water caused considerable mortality of *A. crassus* because the parasite depends almost completely on the osmotic and ionic regulation of the environment carried out by its host. Kirk et al. (2000) noted damage to up to 10% of the parasite metapopulation in eels maintained in sea water for 12 wk, although many individuals were still able to develop and mature. It is probable that the viable larvae originate from the female nematodes that retain an iso-osmotic relationship with their host. The variation in the ability of individual *A. crassus* to osmo-regulate in response to short- and long-term exposure to sea water has been investigated in a further study.

The timing of the pre-patent period reported by previous workers varies according to the maintenance temperature of the life cycle and the size of the infected eels. The freshwater pre-patent period at 15°C ± 2 in this laboratory (second-stage larva to young adult) is estimated to be just over 3 mo (13 wk) via intermediate and paratenic hosts. Third-stage larvae migrate through the intestinal wall and body cavity of eels, moult into the fourth-stage larvae in the swimbladder wall and develop into intra-luminal adults within 2 mo. Transmission of the life cycle in 50% sea water appears to proceed more slowly since an adult was not recovered until 2.5 mo after experimental infection of eels by Kirk et al. (2000). The results in the present study correspond with those of Haenen & van Banning (1991), who force-fed eels (360 mm long) with infected swimbladders from naturally infected ruffe and detected adults after 2 mo p.i. at 18 to 24°C. Moravec et al. (1994) demonstrated that the entire cycle (egg to egg) is completed in about 3 mo at 20 to 22°C when infected copepods are fed directly to small eels (80 to 160 mm). The surrogate host method of infection has been shown to be successful, resulting in a maximum recovery of 60% of larvae from the original larval dose. Recoveries of 25 to 48% are typical using oral administration techniques (Boon et al. 1990, Knopf et al. 1998).

The present study demonstrates that larvae can remain infective for a biologically significant period of time in estuarine (50% sea water) or marine conditions and provides experimental verification of the excellent colonization abilities of *Anguillicola crassus*. The data confirm previous studies by Kennedy & Fitch (1990) that egg hatch and larval survival are deleteriously influenced, but not totally inhibited, by sea water. There was no initial significant difference between egg hatch in fresh water and 100% sea water up to 50% hatch, probably because high osmotic pressure in sea water stimulates hatching. However, the eggs rapidly start to degenerate in sea water and thus subsequent rate of hatch was decreased.

Infectivity of *Anguillicola crassus* was adversely affected by increase in salinity and larval age, confirming field observations that prevalence and intensity of infection decrease with increase in salinity (Taraschewski et al. 1987, Höglund et al. 1992, Pilcher & Moore 1993). For example, Pilcher & Moore (1993) reported that prevalence and intensity of infection in eels in the River Lee at Bow, East London (<1 ppt/Cl) were approximately 2.7 and 1.7 times higher than values in eels at West Thurrock, Thames estuary (10 to 25 ppt/Cl, 30 to 60% sea water) in the tidal Thames catchment area. Similarly, Taraschewski et al. (1987) observed a decrease in infection levels of *A. crassus* towards the open sea in the Elbe estuary.

Anguillicola crassus is known to be exceptional in its ability to disseminate, through the anthropochore activities of the eel trade (Kennedy & Fitch 1990) and probably via the natural movement of eels (Kirk et al. 2000). Although it is a specialist and autogenic species, *A. crassus* utilizes a wide variety of intermediate and paratenic hosts that act as trophic links to eels, from the elver to adult stage. It has a high reproductive potential and can remain infective for long periods in a range of habitat conditions (Kennedy & Fitch, present study). Thus as a successful coloniser, *A. crassus* has been able to infect 3 species of eels in 4 continents (Asia, Europe, Africa and America) (Moravec & Škoríková 1998). The only known limitations to its spread appear to be temperature (Schippers et al. 1991, Höglund et al. 1992, Thomas & Ollevier 1993, Knopf et al. 1998) and salinity (present study). Knopf et al. (1998) have demonstrated that low temperatures (4°C) are detrimental to development and reproduction of *A. crassus* in eels. Their data support the hypothesis by Höglund et al. (1992) that the dissemination of *A. crassus* in Scandinavia will be restricted in cold water temperature regimes. Experimental studies by Kirk et al. (2000) have indicated that *A. crassus* can survive and complete its life cycle in estuarine and seawater laboratory conditions, but transmission in the field will depend on temperature and availability of suitable hosts. Dissemination of infection in coastal waters and estuaries is likely and has probably contributed to the rapid spread of *A. crassus* in Europe. Transmission in brackish habitats is possible where temperatures are not prohibitive and suitable intermediate hosts such as *Eurytemora affinis* (Kirk et al. 2000) and paratenic hosts such as black goby *Gobius niger* L. (Reimer et al. 1994) are available to complete the cycle. Dissemination may also occur when eels undertake short-term movements through marine waters, but transmission during the spawning migration is unlikely due to lack of feeding by silver eels (Tesch 1977) and absence of suitable intermediate hosts (Kirk et al. 2000).

Pathological changes caused by high intensities of *Anguillicola crassus* are thought to result in hydrostatic dysfunction of the swimbladder (Würtz et al. 1996) and reduction in the overall fitness of eels (Sprengel & Lüchtenberg 1991, Molnár 1993), which may impair the success of the spawning migration back to the Sargasso Sea. Thus European eels may be unable to fulfil their reproductive potential in future years. Further dissemination of *A. crassus* will be very difficult to prevent. However, it is crucial for the conservation of eel populations that stringent hygiene practices are adopted in the transportation of eels and that regular monitoring and research on *A. crassus* continues worldwide.

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