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EFFECT OF DECREASED OXYGEN CONTENT ON EELS *(Anguilla anguilla)* INFECTED BY *Anguillicola crassus* (NEMATODA: DRACUNCULOIDEA)

K. MOLNÁR

Veterinary Medical Research Institute, Hungarian Academy of Sciences, H–1581 Budapest, P. O. Box 18, Hungary

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The survival chances of eels with *Anguillicola crassus* infection of varying intensity and with varying pathological changes were studied in an experimental system devoid of fresh oxygen supply. Eels most severely affected by anguillicolosis died first, while those with less expressed pathological lesions tolerated sublethal oxygen levels for a longer time. Findings were similar at 20–21 °C and at 27–28 °C; at 27–28 °C, however, the fish required a higher oxygen content to survive. The experiments demonstrate that *Anguillicola* infections substantially impair the eels' natural resistance and, under unfavourable environmental conditions, may lead to their death.

Key words: Eel, *Anguillicola crassus*, oxygen deprivation, pathogenicity

Since in the late 1980's the nematode *Anguillicola crassus* Kuwahara, Niemi et Itagaki, 1974 was introduced to Europe, dozens of papers have discussed its rapid spread, development and prevalence. However, relatively few researchers have dealt with the parasite's effect on the fish organism and with its pathogenicity. Of the latter, Boon et al. (1989, 1990*a*, 1990*b*) and Höglund et al. (1992) studied the effect exerted by the parasite on the eel's blood composition, while Sprengel and Lüchtenberg (1991) reported that parasite-infected eels were characterized by a reduced swimming speed. The observation of Thomas and Ollevier (1992), i.e. that more severely infected eel specimens are more easily sucked in by the water-pipes of power stations than those with a less intensive infection, also belongs to this category. Only a single work (Molnár et al., 1993) furnishes detailed information about the histopathology of *Anguillicola* infection, but data on the helminths' pathogenic effect exerted on the swimbladder and on occasional mortalities can be found also in the works of Egusa (1979), Hartmann (1987), Liewes and Schaminee-Main (1987), Mellergaard (1988), Boon et al. (1989), van Willigen and Decker (1989),

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Haenen et al. (1989), Banning and Haenen (1990) and Kamstra (1990). Similar data concern infections of eels with *A. globiceps* (Yamaguti, 1935) and with *A. novaezelandiae* (Sarti et al., 1985). To date, only one paper (Molnár et al., 1991) has been published on mass mortality caused by *A. crassus*.

In the summer of 1991, a mass and selective mortality occurred among eels of Lake Balaton in Hungary. This mortality recurred, though in a less severe form, in 1992. About 200 tons of eel died in 1991 and 40 tons in 1992. Our research team attributed this mortality to the extremely severe *Anguillicola* infection and the resulting swimbladder damage. As this opinion was received sceptically by Hungarian specialists unfamiliar with fish pathology, we conducted experiments to determine the influence exerted by adverse environmental conditions on the survival rate of eels whose physiological capacity and natural resistance had been impaired by *Anguillicola* infection. As a first step, the survival chances of eels affected with *Anguillicola* infection of varying intensity were studied in an environment of low oxygen content.

Materials and methods

Eels (*Anguilla anguilla* L.) derived from Lake Balaton and naturally infected by *A. crassus* were used in the experiments. The eels were 17–72 cm long and had been kept in the laboratory for a few days before the experiment. Each treatment group comprised 10 fish. The fish used in the individual experiments had always been derived from the same place and, as far as possible, they were of nearly the same size. The experimental fish specimens were placed in 10-litre aquaria filled with water to repletion and covered with a glass plate to prevent fresh oxygen supply. During the experiments, water temperature and oxygen content were recorded quarter-hourly. Water temperature was measured with a common internal water thermometer. Oxygen content of the water was measured with "Aquacheck", an instrument developed by the Radelkis Co-operative (Budapest) and routinely used in the Hungarian farm-pond practice for measuring water pH and oxygen level. The instrument measures the percentage of oxygen dissolved in water. From that value, the concentration of dissolved oxygen (in mg/litre) can be calculated with the help of a table if water temperature is known. Two temperature ranges were used. In ex-

periments conducted in the spring, water temperature of the aquaria was adjusted to 20–21 °C while in the summer experiments water temperature was kept at 27–28 °C. Eels that died of anoxia were removed from the water in the order and at the time of death. The oxygen level measurable at the time of death was determined. If the last two eels of a group did not die shortly after the others, they were killed with an overdose of the hypnotic MS-222.

The dead eels were then subjected to full parasitological dissection. Particular attention was paid to the swimbladder lesions. The number and size of worms found in the swimbladder, the developmental stage and number of larvae burrowing in the swimbladder wall, the thickness of the swimbladder wall, the status of its mucous membrane, the occurrence of foci, pigment deposits or abscesses in the swimbladder wall, and the quantity and quality of fluid released from disrupted worms present in the swimbladder lumen were recorded.

Both the adult and juvenile specimens of *Anguillicola* and the 3rd and 4th stage larvae occurring in the swimbladder wall were detected with the help of a stereomicroscope; however, only the stages found in the swimbladder lumen were collected. Since the size of eels used in the different experiments was not uniform and the worms represented different stages of development, the number of worms found in the swimbladder is a less suitable indicator of the severity of infection. Therefore, we also used the term "quantity of worms" to indicate the extent to which the swimbladder was filled with worms. In cases marked *** the swimbladder was either completely filled with worms or it was at least ¾ full, in cases marked ** it was filled approximately to half, while the score * represented cases when only a few adult worms or several juvenile stages were present in it. The wall thickness of the swimbladder was measured in mm. In the majority of cases this value represented the wall thickness of the organ cut through in the middle in transverse direction. In some cases, however, only one or both ends of the swimbladder were thickened. In those cases wall thickness was given as a mean value. Wall thickness values exceeding 3 mm corresponded to severe, those between 1.5 and 3 mm to moderate, while values between 1.0 and 1.5 mm to mild thickening. Although a more than >0.3 mm wall thickness of the swimbladder can usually be attributed to infection, swimbladder walls less than 1 mm thick were considered thin in this study. The data are summarized in Tables 1 and 2.

Table 1

Oxygen deficiency tolerance of eels affected by *Anguillicola crassus* infection of varying severity at 20—21 °C

No. and date of exp.	Order of deaths	Length of eel (cm)	Time of death[1]	O_2 content of water (mg/l)[2]	Swimbladder lesions						Worms in swimbladder wall		Larvae in swimbladder wall		Severity grade[3]
					Swimbladder wall thickness (mm)				Other lesions						
					3–7	1.5–3	1–1.5	<1	Focus in wall	Fluid in lumen	number	quantity	4th stage	3rd stage	
	1-2	24	3'40	0.99	*						-	-	-	-	1
	1-2	30	3'40	0.99			*				11	**	3	2	2
I	3	43	4'10	0.99		*				*	3	*	2	-	3
26.2	8-9	36	7'10	0.99			*			*	-	-	3	-	9
	8-9	31	7'10	0.99				*			-	-	-	-	10
	10	45	7'50	0.99			*				1	*	4	2	8
	1-2	35	4'00	1.11			*				-	-	-	-	2
	1-2	25	4'00	1.11				*			1	*	1	-	3
II	3	44	4'50	1.01				*			10	*	3	5	1
27.3	8	30	5'40	1.01				*			-	-	-	-	9
	9	33	7'00	1.01				*			-	-	2	-	8
	10	50	killed	1.01				*			-	-	-	-	10
	1	78	5'00	1.33		*					30	***	20	10	2
	2	78	5'20	1.33		*				*	9	**	100	20	3
III	3	68	6'30	1.05		*				*	38	***	35	70	1
07.5	8	62	10'20	0.90			*		*		-	-	-	-	8
	9-10	56	killed	0.90				*			-	-	-	-	9-10
	9-10	58	killed	0.90				*			-	-	-	-	9-10

Table 1 continued

No. and date of exp.	Order of deaths	Length of eel (cm)	Time of death[1]	O_2 content of water (mg/l)[2]	Swimbladder lesions						Worms in swimbladder wall		Larvae in swimbladder wall		Severity grade[3]
					Swimbladder wall thickness (mm)				Other lesions						
					3–7	1.5–3	1–1.5	<1	Focus in wall	Fluid in lumen	number	quantity	4th stage	3rd stage	
	1	70	2'00	1.15	*						68	***	10	80	1
	2	72	5'10	1.06		*					56	***	40	15	2
IV	3	72	7'15	0.89		*					13	*	6	14	3
11.5	8	70	9'30	0.89			*				2	*	4	10	6
	9-10	66	killed	0.89				*			6	*	2	20	7-8
	9-10	68	killed	0.89			*				4	*		30	7-8
	1	73	4'30	1.06	*						59	***	20	115	1
	2	51	6'00	0.97	*						-	-	5	200	2
V	3	61	6'15	0.97	*				*		-	-	-	-	3
12.5	8	65	8'20	0.89			*		*		7	-	15	20	7
	9-10	69	killed	0.89			*		*		-	-	10	10	8
	9-10	59	killed	0.89				*			-	-	-	-	10

[1] Time of death after oxygen deprivation
[2] O_2 content of water at time of death (mg/l)
[3] Severity grade by gross pathological findings

Table 2

Oxygen deficiency tolerance of eels affected by *Anguillicola crassus* infection of varying severity at 27—28 °C

No. and date of exp.	Order of deaths	Length of eel (cm)	Time of death[1]	O_2 content of water (mg/l)[2]	Swimbladder lesions						Worms in swimbladder wall		Larvae in swimbladder wall		Severity grade[3]
					Swimbladder wall thickness (mm)				Other lesions						
					3–7	1.5–3	1–1.5	<1	Focus in wall	Fluid in lumen	number	quantity	4th stage	3rd stage	
VI 03.8	1	36	1'00	2.24	*				*		-	-	-	-	1
	2-3	23	1'15	1.60							-	-	-	-	2
	2-3	32	1'15	1.44							1	*	-	-	3
	8	39	2'20	1.44				*			7	*	-	2	8
	9	27	2'20	1.44				*			-	-	1	10	10
	10	50	4'00	1.44				*			4	*	7	7	9
VII 06.8	1-2	29	1'05	2.24	*						-	-	-	-	1
	1-2	29	1'05	2.24	*				*		-	-	-	-	2
	3	31	1'45	1.76	*						-	-	-	-	3
	8	38	3'00	1.60							-	-	-	-	8
	9	33	4'00	1.60			*				1	*	-	-	9
	10	35	killed	1.60				*			-	-	-	-	10
VIII 08.12	1-2	43	1'30	2.56	c						-	-	-	-	1
	1-2	47	1'30	2.56	*						-	-	-	-	2
	3	44	1'35	2.27	*					*	-	-	-	-	3
	8	53	4'00	2.27			*				-	-	1	-	9
	9	51	4'10	2.27				*			3	**	4	8	8
	10	50	4'20	2.27			*				-	-	-	-	10

Table 2 continued

No. and date of exp.	Order of deaths	Length of eel (cm)	Time of death[1]	O_2 content of water (mg/l)[2]	Swimbladder lesions						Worms in swimbladder wall		Larvae in swimbladder wall		Severity grade[3]
					Swimbladder wall thickness (mm)				Other lesions						
					3–7	1.5–3	1–1.5	<1	Focus in wall	Fluid in lumen	number	quantity	4th stage	3rd stage	
IX 13.8	1	23	2'05	2.40		*					1	*	-	-	2
	2	24	2'03	2.24	*						2	*	1	-	1
	3	49	2'35	2.24		*					-	-	-	-	3
	8	17	2'40	2.24			*				-	-	-	-	8
	9	23	3'30	2.24				*			-	-	-	1	10
	10	41	3'40	2.24			*				-	-	1	-	9
X 31.8	1-2	19	1'10	2.40		*					-	-	-	-	3
	1-2	28	1'10	2.40			*				6	**	4	-	2
	3	19	1'20	2.30		*					-	-	1	-	4
	8	30	2'10	2.30				*			2	*	-	-	8
	9	30	killed	2.30				*			-	-	-	2	9
	10	31	killed	2.30				*			-	-	-	-	10

[1] Time of death after oxygen deprivation
[2] O_2 content of water at time of death (mg/l)
[3] Severity grade by gross pathological findings

Two different rankings were used for evaluating the results. The chronological order of death was determined and the cases were ranked by severity according to the gross pathological findings. In determining the severity grade, less importance was attached to the number of worms and larvae present. The grade of severity was determined primarily on the basis of swimbladder wall thickness, the number of foci present in the swimbladder wall and the quantity of fluid observed in the lumen of the swimbladder. In cases marked *** and **, and also in the case of intensive larval infection, however, the number of worms or larvae was also taken into consideration when establishing the severity scores. Although 10 eels were always examined, only data on the three eels that died first and those of the last 3 survivors are included in the tables for a better demonstration of the differences.

Although a few *Pseudodactylogyrus* monogeneans were often found on the gills of the experimental eels, and *Eimeria anguillae, Myxidium giardi* and *Myxobolus portucalensis* infections of low intensity were also observed in the intestine, parenchymal organs and on the fins, respectively, no substantial parasitosis was present which could have disturbed the experiments. In the majority of the dissected eels, *Aeromonas* and *Pseudomonas* bacteria were isolated from the swimbladder; with the exception of one fish, however, these bacteria did not produce clinical signs.

Results

The data presented in Tables 1 and 2 indicate that there is a rather close correlation between the severity of *Anguillicola* infection and toleration of hypoxic environment by the eels. In 9 out of the 10 experiments, the three fish judged to be the most severely affected on the basis of the gross pathological findings were among those three eels which died first. In 8 out of the 10 experiments, the three fish considered to be the least affected survived for the longest time in the hypoxic environment.

Table 1 shows that at a water temperature of 20–21 °C the eels will die if the oxygen content is 0.89–1.15 mg/l. However, significant differences are demonstrable between the less affected and the more severely affected specimens in the time of tolerating this low oxygen content. In the case of an oxygen content higher than that (1.33 mg/l) only two fish with

extremely severe infection died at the above temperature (Table 1, experiment 3).

In the five experiments summarized in Table 2 water temperature was adjusted to 27–28 °C. In that case, deaths occurred at an oxygen content of 1.44–2.56 mg/l. Also in that case, eels with a markedly thickened swimbladder wall died at the highest oxygen values (2.56 mg/l).

Only experiments no. 4 and 5 seem not to fit in this picture: namely, in these experiments eels with a less severely damaged swimbladder died earlier than fish judged to have more severe lesions. It should be mentioned, however, that these eels showed the signs of incipient bacterial infection. The "first three" data of experiment 9 also differ from the results expected: here the fish judged to be the most severely affected died later than the three others following it in the order of severity. In that experiment, however, severity grades were difficult to establish because of the lower intensity of infection and the less expressed swimbladder lesions.

Discussion

No unequivocal data are available on the physiological effect and pathogenicity of anguillicolosis in eels. At the same time, Sprengel and Lüchtenberg (1991) have reported that the presence of already a few worms markedly reduced the swimming speed of eels. Thus, helminth infection affects the physiological capacity of the fish. The results of the present experiments demonstrate that this effect can sometimes be very substantial and may impair the eel's natural resistance to such an extent that the fish becomes vulnerable to environmental stress factors and eventually dies.

The experiments have confirmed our earlier statements (Molnár et al., 1993) that the severity of damage is determined by the infection-induced thickening of the swimbladder wall rather than by the number of worms present in the swimbladder. Eels having a thick swimbladder wall and infected by large numbers of worms can be considered the most severely affected. A stage almost as severe as that is spoken of when worms and larvae are no longer present in the swimbladder as a result of the host reaction, but the swimbladder shows anatomical deformities and physiological dysfunctions resulting from the infection. According to the results

obtained by Molnár et al. (1993), infection by larvae and worms, the appearance of a fluid containing blood and worm debris in the swimbladder lumen as a result of worm disruption, the subsequent serous and fibrous thickening of the swimbladder wall, and the formation of foci around the necrotic larvae should be considered the stages of the same process. Within that process, the stage characterized by the absence of worms from, and the presence of severe lesions in the swimbladder represents a phase when the course of the disease is decided: either regeneration of the swimbladder will take place or the fish will die. According to Kamstra (1990), disruption and full atrophy of the swimbladder are common in the final phase of the disease. In that case, stress factors probably do not play a role, and the eel dies even in the absence of external factors impairing its natural resistance. However, at the peak of infection or at the time of helminth elimination, when the chances of regeneration and death are approximately equal, the presence of adverse external conditions markedly influence the chances of survival, as it has been shown in these experiments.

Practically no eel free from *Anguillicola* infection can be found in Lake Balaton. Therefore, in my experiments the eels free from worms and larvae represented specimens which had recovered from infection and were not yet affected by reinfection. At the same time, in the rivers of Western and Northern Europe characterized by a lower intensity of infection, the negative fish include both eels which have never been infected and those which have recovered from infection, are not infected by helminths but show lesions caused by the helminthosis. If fish of such diverse history are used in experiments as controls, this may lead to false results. Presumably this is why Boon et al. (1989, 1990*b*) and Höglund et al. (1992) found only minor differences between the infected and the control fish in haematocrit and serum protein profiles. Similarly, Möller et al. (1991) observed only minor differences between infected and control eels in body condition and liver somatic index. From the tables of their paper it is apparent, however, that the highest standard deviation was observed for the control group in both the body condition factor and the liver somatic index. This can probably be attributed to the fact that the authors evaluated the intensity of *Anguillicola* infection merely by the number of worms present, disregarding the pathological changes of the swimbladder, and — besides the yet uninfected fish — included in the "control" group also the eels representing the most advanced stage of infection, i.e. those which had just got rid of

worms and showed various stages of swimbladder regeneration. Studies performed by Boon et al. (1990*a*) using carefully selected controls, however, show that under experimental conditions a significant change in haematocrit and plasma protein values can be observed in eels infected by larvae.

The present experiments clearly demonstrate that *Anguillicola* infection markedly impairs the eels' physiological capacity and natural resistance. In the presence of any stress factor harmless to healthy fish, this infection increases the chances of mortality. (In our experimental model, high temperature and hypoxia were used as stress factors). The data obtained support our view that the mass mortality that occurred among eels of Lake Balaton in 1991 and 1992 was basically due to intensive, clinically apparent *Anguillicola* infection. This does not amount to saying that hypoxia certainly played a role in the observed mortality. Artificially produced oxygen-deficient environment only simulated a possible stress factor which may develop in the lake at any time. In these experiments, at a higher water temperature the fish became affected even if the oxygen content was higher. This is suggestive of a close correlation between temperature as a stress factor and the summer mortality of eels.

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