

Transmission experiments to determine the relationship between *Sphaerospora* sp. from Atlantic salmon, *Salmo salar*, and *Sphaerospora truttae*: a revised species description for *S. truttae*

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Key words: Myxosporea, Myxozoa, *Sphaerospora*, fish parasites, salmonids

Abstract. Extrasporogonic stages of *Sphaerospora* sp. from the kidneys of Atlantic salmon (*Salmo salar* L.) were successfully transmitted via intra-peritoneal injection to naive Atlantic salmon and brown trout (*Salmo trutta* L.). Rainbow trout (*Oncorhynchus mykiss* Walbaum) could not be infected in this way. Transmitted extrasporogonic stages continued their development to form sporogonic stages and mature spores in the kidney tubules. Extrasporogonic stages, sporogonic stages and mature spores of the parasite in both experimentally infected hosts were morphologically identical to the equivalent stage in naturally infected Atlantic salmon, although minor differences were seen in spore dimensions. A farm-based exposure experiment confirmed the susceptibility of brown trout to the salmon *Sphaerospora*. These results are consistent with the view that the salmon *Sphaerospora* is *Sphaerospora truttae* Fischer-Scherl, El-Matbouli et Hoffmann, 1986. The parasite is redescribed according to the guidelines of Lom and Arthur (1989) since details of extrasporogonic stages, the ultrastructure of extrasporogonic and sporogonic stage development, and of the parasite's epidemiology are known from Atlantic salmon but not from other reports.

The secure identification of many myxosporean species is problematic for a variety of reasons, including a lack of information on host specificity, geographical range, tissue specificity, epidemiology and ultrastructural development. In some cases, even basic details of spore morphology, dimensions and variation are lacking. Comparison between similar or closely related species is therefore difficult since many have been poorly characterised in the past (Lom and Arthur 1989). These problems are further compounded by an historic inability to experimentally transmit myxosporeans.

Kidney infecting species of the genus *Sphaerospora* Thélohan, 1892 have been reported to show a high degree of host specificity, with many species known only from a single host (Shulman 1966, El-Matbouli and Hoffmann 1992). Most species have been described from cyprinids (Arthur and Lom 1985) in which, even when spore forms are very similar, extrasporogonic stages exhibit marked morphological differences which can be considered species specific (Lom et al. 1985, Baska and Molnár 1988). However, this situation has led to the restricted host specificity of *Sphaerospora* spp. being used in itself as a justification for erecting new species.

Problems of host, geographical and tissue specificity could be explored by cross infection experiments. These are becoming possible as laboratory-based life cycles involving oligochaete alternate hosts releasing

actinosporean stages become established (Markiw and Wolf 1983, El-Matbouli et al. 1992). However, the presence of extrasporogonic stages in some species, especially in the genus *Sphaerospora*, has enabled successful direct fish-fish transmissions of a number of species including *Sphaerospora renicola* Dyková et Lom, 1982 (Molnár 1984, 1988, Molnár and Kovács-Gayer 1986a) and PKX (Clifton-Hadley et al. 1984, Kent and Hedrick 1985).

McGeorge et al. (1994) described a *Sphaerospora* sp. in juvenile freshwater Atlantic salmon from Scottish fish farms; this parasite has an extrasporogonic stage in the blood and kidney interstitium, and a sporogonic stage in the kidney tubules. Its sporogonic stages and spores are broadly similar in morphology and dimensions to those of *Sphaerospora truttae*, a species first described by Fischer-Scherl et al. (1986) from brown trout, *Salmo trutta* L., in Germany. *S. truttae* has since been recorded from grayling, *Thymallus thymallus* L. (Walter et al. 1991). However, a lack of information on the ultrastructure of *S. truttae* development, and especially on whether extrasporogonic stages were present in the life cycle, precluded McGeorge et al. (1994) from assigning the salmon *Sphaerospora* to *S. truttae*. The epizootiology of *S. truttae* infection has been reported by McGeorge et al. (1996).

The presence of extrasporogonic stages in the life cycle of salmon *Sphaerospora* provided a means of

attempting experimental infections. The aims of the present study were therefore: to determine whether the parasite was able to infect a range of salmonids by means of exposure experiments and experimental transmissions; to determine the extent of morphological variation seen in the parasite between different hosts; and to arrive at a specific identification.

MATERIALS AND METHODS

(a) Natural exposure

A small number of 0⁺ brown trout, the progeny of returning sea trout, were transferred to a freshwater hatchery on the Northwest coast of Scotland as part of an unrelated study. These fish weighed ~20 g on transfer, had been raised on bore-hole hatchery water and thus had not been previously exposed to infection. Each year class of juvenile Atlantic salmon at the farm to which these trout were transferred were known to become infected by *Sphaerospora* in their first summer (McGeorge et al. 1994). Six fish from this population were sampled and examined for the presence of *Sphaerospora* in October 1992 after their first summer at the farm. Unfortunately, for husbandry reasons, other species of fish could not be permitted to be exposed on site in this way.

(b) Experimental infections

(i) Donor fish

For details of the seasonality of infection of *Sphaerospora* from Atlantic salmon see McGeorge et al. (1996). All experiments were undertaken when donor fish harbouring the relevant stage of *Sphaerospora* were available, according to epizootiological observations.

Experiments 1–2: Infections with sporogonic stages. Fifteen Atlantic salmon parr of ~40–60 g were obtained in April 1993 from a farm site where *Sphaerospora* infections recur annually. These fish had been on site since March 1992. The kidney tubules of these fish contained large numbers of differentiating pseudoplasmodia and mature spores. Extrasporogonic stages had not been detected in this cohort of fish since early September 1992.

Experiments 3–6: Infections with extrasporogonic stages. Forty Atlantic salmon parr of ~25–35 g were obtained in early August 1993 from the same farm as above. These fish were in their first summer on site, having been transferred as alevins in March 1993. The kidney interstitium, and to a lesser extent blood of these fish contained large numbers of extrasporogonic stages.

(ii) Recipient fish

All experiments. Recipient fish for all experiments were obtained from hatcheries with bore-hole water supplies where *Sphaerospora* had never been recorded. Fish were transferred

to 40 l flow through tanks for seven days on charcoal filtered aerated mains water at 12°C and fed *ad libitum* with a commercial pelleted feed. Prior to each experiment 8 fish were sacrificed and examined to confirm that no myxosporean infections were present.

(iii) Preparation and administration of blood and kidney homogenates

The method given below is one which has been used by the authors over a number of years to successfully transmit PKX extrasporogonic stages from infected kidneys to naive rainbow trout.

All homogenates were prepared fresh, shortly before the commencement of the infection procedure, and retained in sterile universal tubes on ice during the course of the experiment. Kidneys were removed under sterile conditions from donor fish; a small sample of the mid-portion of each kidney was removed and scanned by means of fresh squash preparations and Giemsa stained smears to confirm the presence of sporogonic or extrasporogonic stages, as appropriate. The remainder of each kidney was placed in a pre-weighed sterile universal containing 1 ml of sterile phosphate buffered saline (PBS) at 15°C. PBS was added to give a ratio of approximately 1 g tissue to 10 ml PBS. The kidney was then pressed forcibly through a 100 µm nylon mesh filter with a sterile spatula to disrupt the tissue and create an injectable homogenate.

Blood was obtained from the severed caudal arteries of sacrificed donor fish using heparinised glass capillary tubes. A small subsample of blood was examined fresh and as a Giemsa stained blood film to ensure parasites were present. The remaining blood was pooled and mixed 1 : 1 with PBS to form an injectable homogenate.

Recipient fish were individually anaesthetised using benzocaine. According to the experiment they were IP-injected with 0.4 ml kidney homogenate, 0.4 ml blood suspension, or 0.4–0.6 ml of kidney suspension was introduced into their stomachs via a sterile plastic 1 ml pipette. All injected fish were panjet marked to ensure their future discrimination from control fish. Control and experimental fish for each experiment were held in the same tank, under conditions as detailed in (ii) above.

(iv) Experimental design

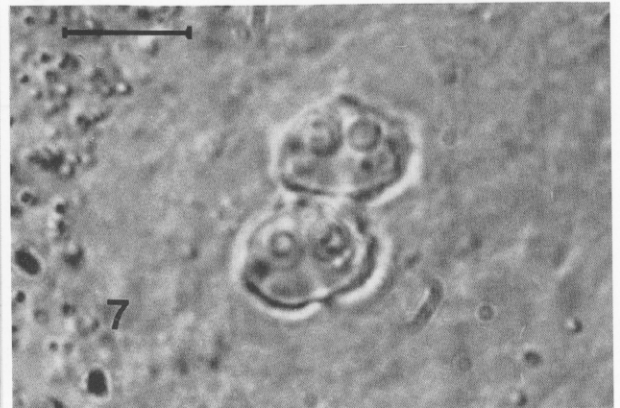
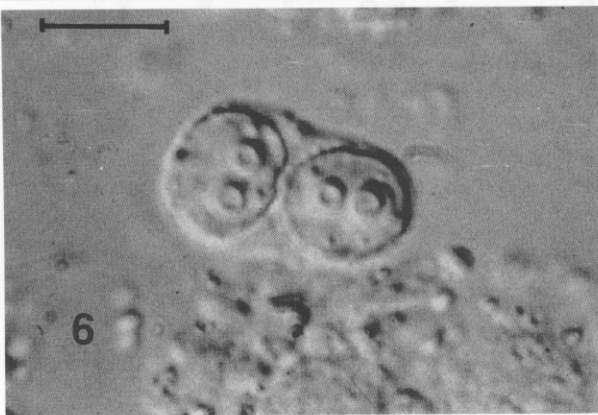
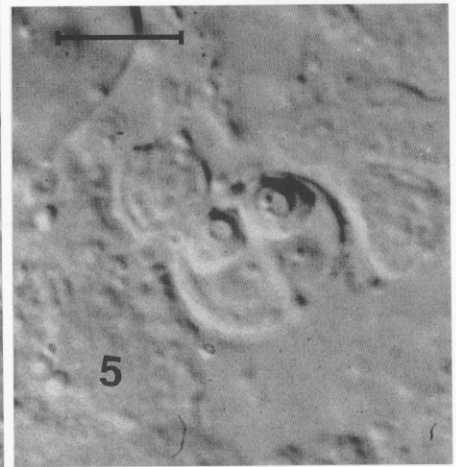
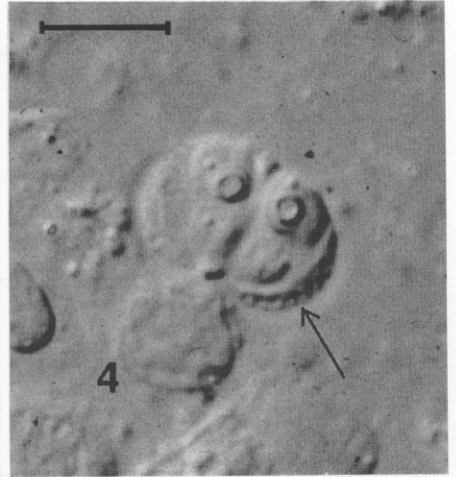
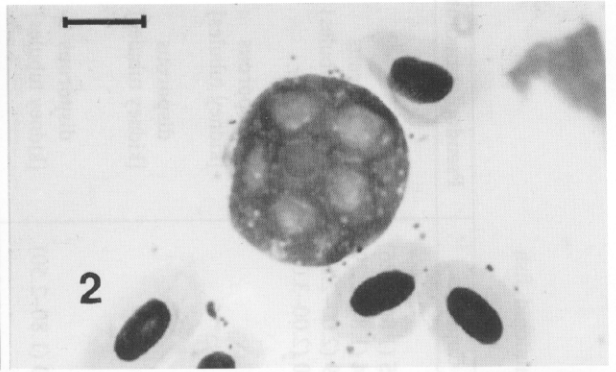
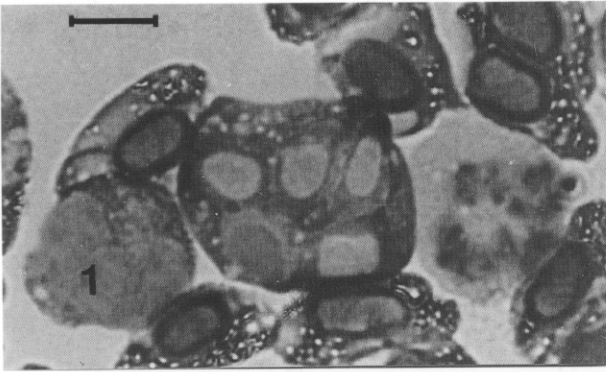
Experiment 1: IP-injection of sporogonic stages to Atlantic salmon. Kidney homogenate from Atlantic salmon, containing numerous pseudoplasmodia, immature and mature spores, was IP-injected into 25 naive 30 g Atlantic salmon on 05.04.93. Fifteen control fish (from the same stock) received an injection of 0.4 ml PBS.

Experiment 2: Oral administration of sporogonic stages to Atlantic salmon. The same homogenate used in experiment 1 was introduced orally by plastic pipette into the stomachs of 25 naive 30 g Atlantic salmon on 05.04.93. Fifteen control fish received an oral administration of 0.4 ml PBS.

Experiment 3: IP-injection of extrasporogonic stages from blood to Atlantic salmon. Blood suspension from extrasporogonic stage-infected Atlantic salmon were IP-injected

Table 1. Comparative spore dimensions for reports of *Sphaerospora/Leptotheca* sp. from salmonid fish

	Host	Method of infection	Length	Thickness	Capsules	Pseudoplasmodium
<i>Sphaerospora</i> sp. (McGeorge et al. 1994) (present study) (present study) (present study)	<i>Salmo salar</i>	natural exposure	7.35 (6.51–8.37)	9.90 (8.84–11.16)	2.35 (1.86–3.26)	disporous [kidney tubules]
	<i>Salmo salar</i>	IP-injection	7.75 (7.00–8.00)	9.59 (9.30–10.30)	2.14 (1.86–2.79)	
	<i>Salmo trutta</i>	natural exposure	7.41 (6.79–7.91)	10.10 (8.84–12.50)	2.39 (2.00–2.50)	
	<i>Salmo trutta</i>	IP-injection	7.13 (6.80–8.20)	10.75 (9.00–12.25)	2.40 (2.00–3.00)	
<i>Sphaerospora truttae</i> (Fischer-Scherl et al. 1986)	<i>Salmo trutta</i>	natural exposure	6.84 (6.58–8.68)	8.81 (8.22–10.11)	–	disporous [kidney tubules]
	<i>Salmo trutta</i>	natural exposure	7.20	10.10	–	disporous [kidney tubules]
<i>Sphaerospora truttae</i> (Lom and Dyková 1989)	<i>Salmo trutta</i>	natural exposure	7.20	10.10	–	disporous [kidney tubules]
	<i>Salmo trutta</i> ; <i>Thymallus thymallus</i>	natural exposure	6.30 (5.00–8.00)	7.80 (7.00–10.00)	2.00 (1.80–2.50)	disporous [kidney tubules]
<i>Sphaerospora krogiusi</i> (Konovalov and Shulman 1965)	<i>Oncorhynchus</i> spp.; <i>Salvelinus alpinus</i> ; <i>Salvelinus leucomenes</i> ; <i>Salmo mykiss</i>	natural exposure	(5.80–7.50)	(7.50–11.50)	(2.70–3.60)	disporous [urinary bladder]
	<i>Coregonus lavaretus</i>	natural exposure	8.20	11.30	1.90	disporous [kidney tubules]
<i>Sphaerospora coregoni</i> (El-Matbouli et al. 1995)	<i>Coregonus lavaretus</i>	natural exposure	8.20	11.30	1.90	disporous [kidney tubules]
	<i>Coregonus autumnalis</i> ; <i>Thymallus thymallus</i>	natural exposure	(7.00–8.40)	(9.80–10.50)	2.80	disporous [kidney tubules/ureters]
<i>Leptotheca subsphaerica</i> (Zaika 1963)	<i>Oncorhynchus nerka</i>	natural exposure	9.10 (8.20–10.20)	10.40 (10.20–11.20)	3.00 (2.90–3.30)	monosporous [kidney tubules]
	<i>Oncorhynchus mykiss</i>	natural exposure	6.00	7.00	2.00	monosporous [kidney tubules]
<i>Sphaerospora oncorhynchi</i> (Kent et al. 1993)	<i>Oncorhynchus mykiss</i>	natural exposure	6.10	7.10	2.00	monosporous [kidney tubules]
	PKX ? (Odening et al. 1988)	natural exposure	6.10	7.10	2.00	monosporous [kidney tubules]
PKX (Hedrick et al. 1988)	<i>Oncorhynchus mykiss</i>	natural exposure	6.10	7.10	2.00	monosporous [kidney tubules]



into 25 naive 20 g Atlantic salmon on 10.08.93. Ten control fish received an injection of 0.4 ml PBS.

Experiment 4: IP-injection of extrasporogonic stages from kidneys to Atlantic salmon. Kidney homogenates from the same infected fish stock used in experiment 3 were IP-injected into 25 naive 21 g Atlantic salmon on 10.08.93. Control fish were those used in experiment 3.

Experiment 5: IP-injection of extrasporogonic stages from kidneys to rainbow trout. Kidney homogenates from Atlantic salmon infected with extrasporogonic stages were IP-injected into 50 naive 18 g rainbow trout on 11.08.93. Fifteen control rainbow trout received an injection of 0.4 ml PBS.

Experiment 6: IP-injection of extrasporogonic stages from kidneys to brown trout. Kidney homogenates from Atlantic salmon infected with extrasporogonic stages were IP-injected into 39 naive 26 g brown trout on 10.08.93. Fifteen control brown trout received an injection of 0.4 ml PBS.

(v) Sampling procedure

Where possible 5 fish were sampled on each sampling date. Experiments 1 and 2 were sampled on days 14, 28, 42, 70 and 120 post injection. All other experiments were sampled at 28, 42, 70 and 120 days post injection. Some experiments were terminated before their full course had run due to depletion of stock as a result of *Aeromonas* infection (see results). Control fish were sacrificed at the termination of each experiment. All fish were examined using standard parasitological procedures for the detection of *Sphaerospora* infection (see McGeorge et al. 1994).

RESULTS

(a) Exposure experiment

All six brown trout sampled from the exposed population at the farm were found to be positive for *Sphaerospora* sporogonic stages. Mature spores were of similar size range to those of salmon *Sphaerospora* (Table 1).

(b) Experimental infections

(i) Controls

All eight fish subsampled from each recipient stock prior to each experiment were uninfected. Experimental control fish were negative for infection at the termination of the study.

(ii) Experimental fish

Experiments 1 and 2: IP-injection and oral administration of sporogonic stages. Fish receiving kidney homogenate containing mature and developing spores, orally or via IP-injection, were negative for myxosporean parasites up to and including day 120 when remaining control and experimental fish were sacrificed.

Experiment 3: IP-injection of extrasporogonic stages from blood to Atlantic salmon. Atlantic salmon injected with blood-borne extrasporogonic stages were negative at all sampling dates for myxosporean infection. This experiment was terminated on day 85 due to elevated mortalities depleting the experimental stock.

Experiment 4: IP-injection of extrasporogonic stages from kidneys to Atlantic salmon. Infections were not detectable on day 14. However, on day 28, both extrasporogonic and sporogonic stages were detected in 4/5 (extrasporogonic) and 2/5 (sporogonic) fish. The time for detectable infection to appear was therefore 14–28 days. Sporogonic stages were subsequently detectable for the rest of the experiment, and were found in 7/8 experimental fish sacrificed at the termination of the study. Extrasporogonic stages were detectable in only the 28 and 42 day samples. Mortalities within the experimentally infected salmon stock began at day 24. Continued increased mortality levels led to the experiment being terminated on day 85.

Experiment 5: IP-injection of extrasporogonic stages from kidneys to rainbow trout. Eight rainbow trout sampled at 28, 42 and 70 days were all found to be

← **Figs. 1–7. *Sphaerospora truttae*.** **Fig. 1.** Extrasporogonic stage with four secondary cells from the kidney of naturally infected Atlantic salmon. Giemsa stained kidney smear; bar = 5 µm. **Fig. 2.** Extrasporogonic stage with five secondary cells from the kidney of experimentally infected brown trout following IP-injection with extrasporogonic stages from Atlantic salmon. Giemsa stained kidney smear; bar = 8 µm. **Fig. 3.** Immature spores of *Sphaerospora truttae* from naturally infected Atlantic salmon. Note the thickness of the spores in the plane perpendicular to the suture; the prominence of the suture line, especially posteriorly (arrow) and the vacuolation of the valve cell. Fresh squash preparation of kidney tissue, Nomarski interference contrast; bar = 10 µm. **Fig. 4.** Immature spore from naturally infected Atlantic salmon. The vacuolation of the valvogenic cell is arrowed. Fresh squash preparation of kidney tissue, Nomarski interference contrast; bar = 10 µm. **Fig. 5.** Maturing spore from naturally infected Atlantic salmon. The spore suture is becoming less prominent and the spore is less thick perpendicular to the plane of the suture than in more immature forms. Fresh squash preparation of kidney tissue, Nomarski interference contrast; bar = 10 µm. **Fig. 6.** Disporous pseudoplasmodium with almost mature spores from naturally infected Atlantic salmon. The outer membrane of the pseudoplasmodial cell is clearly visible (arrow). Note the appearance of slight ridges on the spore valves, perhaps indicative of spore surface striations or ornamentation. Fresh squash preparation of kidney tissue, Nomarski interference contrast; bar = 10 µm. **Fig. 7.** Mature spores from brown trout experimentally infected by IP-injection of extrasporogonic stages from the kidneys of naturally infected Atlantic salmon. Fresh squash preparation of kidney tissue, Nomarski interference contrast; bar = 10 µm.

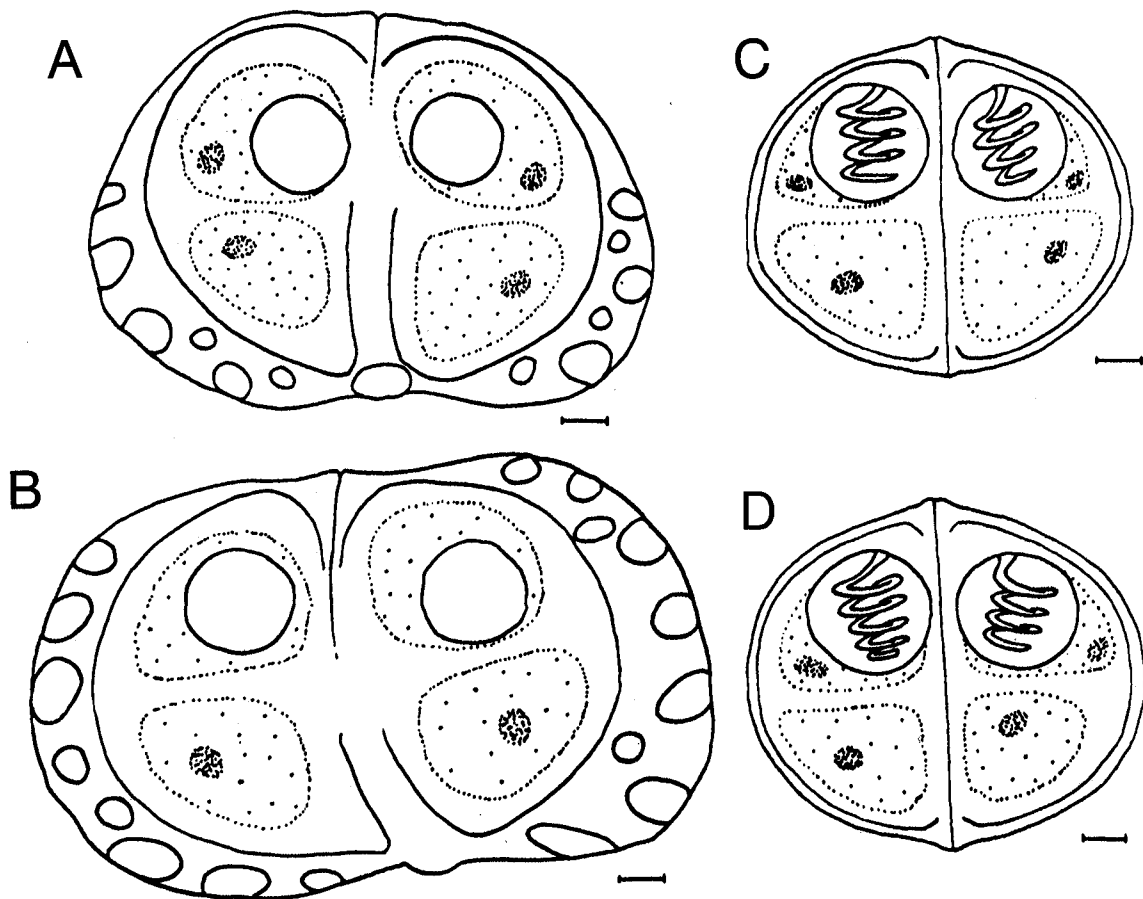


Fig. 8. Drawings of immature (A, B) and mature (C, D) spores of *Sphaerospora truttae* to illustrate the marked differences between them. Bar = 1 µm.

negative for myxosporean infection. The experiment was terminated on day 117 when the remaining 16 fish were found to be negative for infection. Ten mortalities during the study were all negative for infection.

Experiment 6: IP-injection of extrasporogonic stages from kidneys to brown trout. Extrasporogonic and sporogonic stages were first detected in brown trout experimentally injected with extrasporogonic stages on day 28 post-infection. Both life cycle stages were present in the samples taken at 42 and 63 days. High levels of mortality amongst experimentally infected brown trout began on day 31, leading to a premature termination of the experiment on day 63.

Aeromonas salmonicida, the causative agent of furunculosis, was isolated from all experimentally infected stocks, although rainbow trout experienced fewer mortalities (10 over the course of the experiment). PBS-control fish suffered no mortality. The donor farm has endemic furunculosis, and it is probable that this disease was transmitted to the experimental stock with the injected kidney homogenate. In future experiments, antibiotics would be added to the homogenate to prevent such infection.

(c) Comparative parasite morphology

Extrasporogonic stages from experimentally infected Atlantic salmon and brown trout appeared to be identical morphologically to those from naturally infected Atlantic salmon. All shared the same staining characteristics and appearance, with no parasite deviating morphologically from the range of developmental forms found in naturally infected Atlantic salmon at the farm from which the donor fish were obtained (Fig. 1). However, the full range of secondary cell number seen in such natural infections (up to 120) was not seen in experimentally infected fish, the latter containing only up to 50 secondary cells. The intensity of infection in experimentally infected fish was lower than that seen in the equivalent donor fish.

Table 1 compares spore dimensions of experimentally infected Atlantic salmon and brown trout (experiments 4 and 6, respectively) and brown trout from the exposure experiment, with the measurements from naturally infected Atlantic salmon of McGeorge et al. (1994). Mature spores produced in experimentally infected Atlantic salmon and brown trout were of similar

size and morphology to those from naturally infected stocks. Table 1 also compares the measurements from the present study with other reports of *S. truttae* and similar *Sphaerospora*-like myxosporean infections of salmonids.

Immature and mature spores varied markedly in dimensions and morphology. Immature spores were considerably broader (thickness) and deeper (length) than when mature, commonly by 30–50%. The sutural ridge was more prominent, especially towards the posterior of the spore, and the developing polar capsules were much further apart than when mature. Immature spore valves were much thicker and were extensively vacuolated posteriorly; the spore coat of each spore as it matured was often seen to pull away posteriorly from the cytoplasm of the pseudoplasmodium. Mature spores were smaller, more rounded and compact (Figs. 1, 2).

DISCUSSION

In the past, much information on myxosporean specificity has been obtained from exposure experiments, either by exposing fish at infective sites (Hoffmann and Putz 1969, Sanders and Fryer 1970, O'Grodnick 1979), or by holding a variety of fish species in pond polyculture (Molnár and Kovács-Gayer 1986b). In the present study brown trout exposed at a farm where Atlantic salmon become infected with *Sphaerospora* also become parasitised.

The results of the transmission experiments indicated that spores were not directly transmissible either orally or via IP-injection. However, extrasporogonic stages were successfully transmitted to naive salmon and brown trout by the IP-injection of extrasporogonic stages present in the kidneys of donor salmon. Both the extrasporogonic K-swimbladder and C-blood stages of *S. renicola* (Molnár 1984, 1988, Molnár and Kovács-Gayer 1986a), and extrasporogonic PKX stages in the kidney interstitium and blood (Clifton-Hadley et al. 1984, D'Silva et al. 1984, Kent and Hedrick 1985, Clifton-Hadley and Feist 1989) have been transmitted in a similar fashion between host fish. In the present study, transmission via the IP-injection of blood was not successful. This might be due to the relatively low intensity of *Sphaerospora* extrasporogonic stages in the blood compared to the kidney of the naturally infected fish used as donors.

Molnár (1984, 1988) and Molnár and Kovács-Gayer (1986a) found that stages of *S. renicola* became detectable in experimentally injected carp within one to two weeks. The four week period for *Sphaerospora* to become detectable in experimentally infected salmon is similar to that seen in experimental IP-injection studies with PKX (D'Silva et al. 1984, Clifton-Hadley and Feist 1989), and is comparable to that identified as the pre-

patent period before naturally infected salmon show detectable levels of parasites (McGeorge et al. 1996). Many experimentally infected fish were found to have sporogonic stages in the kidney tubules coincident with the first appearance of extrasporogonic stages. This is much sooner for sporogonic stages than would be expected from data obtained for natural infections (McGeorge et al. 1996), where sporogonic stages do not generally appear until 17–31 days after the first appearance of extrasporogonic stages. However, the extrasporogonic stages injected into naive Atlantic salmon in the present study were obtained from donor fish sampled in August, the peak prevalence and intensity period for such stages. This is just prior to the point at which sporogonic stages became detectable in naturally exposed fish (McGeorge et al. 1996). Injected homogenates therefore contained well developed, late extrasporogonic stages containing 'triple formations' of a secondary cell containing two tertiary cells. In natural infections these triple formations, once released from the extrasporogonic primary cell, enter the kidney tubules via the glomerulus where they establish as sporogonic pseudoplasmodia. It seems probable that many injected parasites were therefore already infective for the kidney tubules on injection, and thus sporogonic stages appeared simultaneously with extrasporogonic stages.

The salmon *Sphaerospora* extrasporogonic stages were successfully transmitted to brown trout, in which they continued their development to produce sporogonic stages and spores in the kidney tubules. However, attempts to infect rainbow trout by IP-injection of extrasporogonic stages were unsuccessful, despite the large numbers of parasites contained in the injected homogenate. This indicated that rainbow trout may not be a suitable host for the salmon *Sphaerospora*. Incidental observations have also indicated that rainbow trout are uninfected at a farm site where salmon are infected with *Sphaerospora* in the UK. Fischer-Scherl et al. (1986) found no infections of *S. truttae* in rainbow trout or brook trout in the same German water body in which the parasite was present at a high prevalence in brown trout. Odening et al. (1988) found a *Sphaerospora* sp. in rainbow trout but did not establish its specific identity, although its finding was often coincident with that of PKX, perhaps indicating that it was not related to *S. truttae*.

The results of the exposure experiment, combined with the successful experimental transmission of *Sphaerospora* sp. from salmon to brown trout indicates that the salmon parasite reported by McGeorge et al. (1994) should be considered to be *Sphaerospora truttae*, first described from brown trout in Germany by Fischer-Scherl et al. (1986). A number of features of this species are identical with those of the *Sphaerospora* species

under study. These include disporous development in a salmonid host in Europe, sporogonic stages and spores present in the kidney tubules and Bowman's capsules, and immature spores wider than mature ones. Such changes in spores as a result of maturation appear to be a feature of *Sphaerospora*, having been described from *Sphaerospora araii* Arthur et Lom, 1985, *S. molnari* Lom, Dyková, Pavlásková et Grupcheva, 1983, *S. epinephali* Supamattaya, Fischer-Scherl, Hoffmann et Boonyaratpalin, 1991 and *Leptotheca subsphaerica* Shulman, 1966 as well as from other myxosporean genera (Shulman 1966, Lom et al. 1983, Arthur and Lom 1985, Supamattaya et al. 1991, Athanassopoulou and Sommerville 1993).

Although there are minor differences in mean spore size between different authors (Table 1), spores of *S. truttae* are broadly similar in all recorded hosts including Atlantic salmon. Indeed, spore measurements from brown trout experimentally infected with salmon *Sphaerospora* in the present study differed, though not significantly, from those in the salmon itself. A degree of spore variation within and between individual hosts, sites and organs should be expected and can probably be attributed to phenotypic variation due to factors associated with the physiology or biochemical suitability of the host species concerned (Athanassopoulou and Sommerville 1993). Extrasporogonic stages in experimentally infected brown trout and salmon were identical in morphology to those seen in natural infections of Atlantic salmon, but did not have the same range of secondary cell number seen in natural infections. This is probably simply attributable to the lower intensity of infections found in the experimentally infected fish.

Although Fischer-Scherl et al. (1986) and Walter et al. (1991) reported sporogonic developmental stages of *S. truttae* in renal tubule epithelial cells and in well-defined vacuoles in the kidney interstitium, this was never seen in naturally or experimentally infected fish in the present study. This aspect of *S. truttae* biology requires further study.

The substantial differences between PKX and sphaerosporosis in salmon were discussed at length by McGeorge et al. (1994). These include much higher numbers of secondary cells in salmon *Sphaerospora*, a comparative lack of host response, ultrastructural differences and disporous sporogonic pseudoplasmodia compared to the monosporous pseudoplasmodia of PKX. However, there are a range of other *Sphaerospora* spp. or *Sphaerospora*-like myxosporeans which have been reported from the urinary systems of salmonids which need to be discriminated from *S. truttae* (Table 1). *Sphaerospora oncorhynchi* Kent, Whitaker et Margolis, 1993 differs from *S. truttae* in that it has monosporous pseudoplasmodia and may be related to PKX (Kent et al. 1993). The spore measurements of *Leptotheca*

subsphaerica Shulman, 1966 and *Sphaerospora krogiusi* Shulman, 1966 overlap with those of *S. truttae*, but *S. krogiusi* appears to be found only in the urinary bladder, whilst *L. subsphaerica* has spore projections not seen in *S. truttae*. The recently described *Sphaerospora coregoni* El-Matbouli, Hoffmann et Kern, 1995 from *Coregonus lavaretus* L. has lateral thickenings of its mature spores which are not seen in *S. truttae* (El-Matbouli et al. 1995). It seems possible from these reports and others (e.g., Hedrick et al. 1988, Odening et al. 1988, Cone and Cusack 1991) that some salmonids are susceptible hosts to a number of different, closely related, but distinct *Sphaerospora*-like species whose distribution may overlap and whose inter-relationships remain largely unknown. Perhaps, in addition, there are some apparently different species whose differences may simply be a result of phenotypic variation according to host.

Confirmation that the salmon myxosporean is *Sphaerospora truttae* adds Atlantic salmon to the established host range of brown trout and grayling (Fischer-Scherl et al. 1986, Walter et al. 1991). It also means that much additional information is now available on *S. truttae*, since details of extrasporogonic stages, the ultrastructure of both extrasporogonic and sporogonic stage development, and of the parasite's epidemiology are known from Atlantic salmon, but not from other reports (McGeorge et al. 1994). As a result, an updated species description for *Sphaerospora truttae* is included here following the guidelines of Lom and Arthur (1989).

***Sphaerospora truttae* Fischer-Scherl, El-Matbouli et Hoffmann, 1986**

Hosts: Brown trout, *Salmo trutta* L.; grayling, *Thymallus thymallus* L.; Atlantic salmon, *Salmo salar* L.

Life stage infected: Freshwater: brown trout (2–4 yrs); grayling (3–4 yrs) [probably any age]; juvenile Atlantic salmon.

Geographical distribution: Reports from Germany, Czech Republic and Scotland. Probably widespread.

Prevalence and seasonality: Atlantic salmon: Prevalence rate of up to 100 % at farm sites in Scotland. Extrasporogonic stages appear in late June/early July and proliferate until September. Sporogonic stages first occur in kidney tubules in early August, with mature spores in September. Spores persist for at least 18 months in fish retained in freshwater, but are lost within four months of transfer to sea water. Brown trout: Prevalence rate of 68.6% [farmed fish], 31.4% [wild fish] (Fischer-Scherl et al. 1986); prevalence rate of 72.3% [wild fish] (Walter et al. 1991). Both reports from Germany. Grayling: Prevalence rate of 85.7 % [wild fish] (Walter et al. 1991) in Germany.

Site of infection: Extrasporogonic stage: Blood and kidney interstitium; occasionally found in blood vessels and sinuses of other organs (e.g. spleen/liver).

Sporogonic stage: Lumen of the kidney tubules. Bowman's capsule. May develop in well-defined vacuoles in the kidney interstitium/kidney tubular epithelial cells (brown trout and grayling only). Occasional spores found in the urinary bladder.

Extrasporogonic stage: 5–60 µm with 1–120 secondary cells. Earliest stage consists of a primary cell containing a single secondary cell in its cytoplasm. Secondary cells may contain 1 or 2 tertiary cells. Released secondary cells containing two tertiary cells appear identical to earliest sporogonic stage (pseudoplasmodium containing two sporoblast cells) in the kidney tubule lumen. For ultrastructural details see McGeorge et al. (1994).

Sporogonic stage: Pseudoplasmodium disporous, round to oval shaped and up to 40 µm in size. Immature spores thicker and longer than when mature. Mature spores are subspherical and may show minor variations in dimen-

sion within individual hosts, between individual hosts and between host species. Range of mean measurements from all reports: length 6.30–7.35 µm; thickness perpendicular to the plane of the suture 7.80–10.10 µm. Slight anterior ridge. There are two uninucleate sporoplasm cells. Polar capsules are round, of equal size (2.00–2.35 µm), do not converge at the apex of the spore and contain a polar filament with four to five coils. Pairs of aberrant spores with three shell valves, polar capsules and sporoplasm cells occasionally found in salmon. For ultrastructural details see McGeorge et al. (1994).

Acknowledgements. The authors wish to thank Maureen Menzies for her technical help and the farms concerned for their co-operation and support during the period of this study. This work was funded by a N.E.R.C. research studentship.

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Received 17 November 1995

Accepted 30 January 1996