Infection prevalence, seasonality and host specificity of actinosporean types (Myxozoa) in an Atlantic salmon fish farm located in Northern Scotland

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Abstract. A total of 28,387 oligochaetes belonging to the families Tubificidae, Lumbriculidae, Naididae and Enchytraeidae were examined at regular intervals for actinosporean infections from October 1996 to August 1998 from a freshwater salmon farm in Northern Scotland. A total of 21 types of actinosporeans belonging to seven collective groups synactinomyxon (three types), aurantiactinomyxon (four types), echinactinomyxon (five types), raabeia (six types), triactinomyxon (one type), neoactinomyxum (one type) and siedleckiella (one type) were found. Synactinomyxon type 1, echinactinomyxon type 1 and raabeia type 4 were most abundant. The overall infection prevalence of oligochaetes was 2.9%. Aurantiactinomyxon, synactinomyxon and neoactinomyxum were most common in summer and autumn. Raabeia was most common in spring and summer and echinactinomyxon in winter and spring. Siedleckiella was found only in spring and triactinomyxon in all seasons except winter. A positive relationship between water temperature and the number of actinosporean types released was observed. Most actinosporean types were found in only one host species.

Despite the importance of the actinosporeans as alternate stages in the life cycle of myxosporeans, surprisingly little is known about their ecology and there have been only a few studies focused on the ecological associations of the actinosporean parasites and their annelid hosts (MacKinnon and Adam 1924, Janiszewska 1955, Hamilton and Canning 1987, McGeorge et al. 1997, El-Mansy et al. 1998a,b, Xiao and Desser 1998, Negredo and Mulcahy 2001).

Infections of oligochaete worms with actinosporean parasites in wild environments occur at low prevalence rates, although they may be rather higher in fish farms. The prevalence rates of 0.33% recorded from the river Thames in England (MacKinnon and Adam 1924), 1% in a lake in Canada (Xiao and Desser 1998) and 2% in an Irish river (Negredo and Mulcahy 2001) can be compared with maximum prevalence rates of 4.75% in trout farms in England (Hamilton and Canning 1987), 4.1% in goldfish culture ponds in Japan (Yokoyama et al. 1991), 1% in an Atlantic salmon farm in Scotland (McGeorge et al. 1997) and up to 55% in cyprinid culture ponds in Hungary (El-Mansy et al. 1998a). At the individual actinosporean type level where this has been recorded, the infection prevalence rates recorded are generally very low (<1%) from all environments. However, Negredo and Mulcahy (2001) report prevalence rates of up to 23.8% for individual actinosporean types.

Recent studies have also shown that seasonal variations in prevalence of infections of oligochaetes exist, at least as measured by spore release (Yokoyama et al. 1993, El-Mansy et al. 1998a,b, Xiao and Desser 1998, Negredo and Mulcahy 2001). Raabeia, aurantiactinomyxon and echinactinomyxon were mainly found in summer and autumn, whilst neoactinomyxum was present mainly in autumn and winter (Yokoyama et al. 1993, El-Mansy et al. 1998a,b). A positive relationship between the number of actinosporean types released and the water temperature between May and September in a Canadian lake was shown by Xiao and Desser (1998). In May, when the average water temperature was 12°C, spores belonging to five different actinosporean types were released, whilst in July and August, when the water temperature averaged 23°C, sixteen different actinosporean types were released.

Actinosporean types mostly seem to be very specific to the oligochaete host and mixed infections of actinosporean types in an individual oligochaete are apparently very rare (Styer et al. 1992). There are a few instances where a single actinosporean type is released from more than one oligochaete host species. McGeorge et al. (1997) found their synactinomyxon "B" type to be released by *Tubifex tubifex* (Müller, 1774) and *Lumbriculus variegatus* (Müller, 1774), whilst Xiao and Desser (1998) reported three triactinomyxon types occurring in two oligochaete species and El-Mansy et al. (1998a) also found that two triactinomyxon types and a raabeia type were released from two species of oligochaetes.

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It is important to understand the ecology of actinosporeans in relationship to the biology and possible control of myxosporeans in fish. This paper describes the prevalence rates in the oligochaete hosts, seasonality of prevalence and host specificity of 21 types of actinosporeans from the settlement pond of a freshwater Atlantic salmon (*Salmo salar* L.) farm located in the extreme north of Scotland.

MATERIALS AND METHODS

Oligochaete samples were collected from a settlement pond in an Atlantic salmon farm sited in Northern Scotland (58º30'N, 4º40'W). Sampling was carried out every 4 weeks in spring and summer and every 6 weeks in autumn and winter between October 1996 and August 1998.

Gravel and particulate sediments were sieved through 1.5 mm, 1 mm and 500 µm mesh to separate oligochaetes on site as far as possible before placing them in plastic bags. However, mud samples containing oligochaetes were not sieved and the mud collected was put directly into a plastic bag. Oligochaete samples were transferred to the Institute of Aquaculture in aerated river water. After arrival, the contents of the bags were poured into 10- to 25-litre plastic containers supplied with continuous aeration until they were sorted. All the plastic containers were kept at ambient temperature and sorting always took place within 1 day of arrival at the Institute.

Oligochaete worms were separated from the mud substrate by passing the sediment through 1.5 mm, 1 mm and 500 µm mesh. Alternatively, mud samples were placed on a 1.5 mm mesh sieve immersed in dechlorinated tap water up to the level of the mesh of the sieve for at least one hour. Oligochaetes found their way in large numbers into the water in the container through the mesh of the sieves. This method appeared to be the most effective in terms of the number of worms obtained in a short time. Oligochaetes subjected to previous sieving at the settlement pond were re-sieved to separate them from plant debris. The contents of all the sieves were then emptied into shallow basins containing dechlorinated tap water and observed under bright light to detect oligochaetes. A plastic pipette was used to transfer worms into cell-well plates according to the method of Yokoyama et al. (1991). Each well was scanned using a Zeiss Traval 3 inverted microscope for released actinosporeans. Infected worms were identified according to Brinkhurst (1963) while the spores were identified using the keys and diagrams of Janiszewska (1955, 1957), Marques (1984), Lom et al. (1997) and by comparison with other published reports. The actinosporean types found in this study were described in Özer et al. (2002).

RESULTS

During the two-year survey, twenty-one types of actinosporeans belonging to the collective groups synactinomyxon, aurantiactinomyxon, echinactinomyxon, raabeia, triactinomyxon, neoactinomyxum and siedleckiella were found (Table 1).

Prevalence rate of actinosporean infections of oligochaetes

The overall prevalence rate of infection of oligochaetes with actinosporeans was 2.9%. However, it was rather higher in the first year of study than in the second year with prevalence rates of 3.3% and 2.3%, respectively (Table 2). The collective group echinactinomyxon was the most common with an overall prevalence rate of 1.1% over the two-year period, with a rate of 1.4% in the first year and 0.6% in the second year. The collective groups triactinomyxon, neoactinomyxum and siedleckiella were the least common with prevalence rates of ≤0.05%, whilst the collective groups synactinomyxon, aurantiactinomyxon and raabeia had prevalence rates of 0.2-0.7% (Table 2).

In the case of individual actinosporean types, while several types were observed to occur throughout the study period, most were observed only in particular seasons. The highest prevalence rate recorded was 0.9% for echinactinomyxon type 1, followed by synactinomyxon type 1 (0.65%), raabeia type 4 (0.41%) and raabeia type 5 (0.33%). The remainders of the actinosporean types found had prevalence rates of less than 0.2% (Table 1).

Seasonality of actinosporean infections of oligochaetes

The highest number of actinosporean infections of oligochaetes were recorded in summer (June-August) (4.1%), followed by autumn (September-November) (2.9%), spring (March-May) (2.8%) and winter (December-February) (1.6%). Synactinomyxon, aurantiactinomyxon and neoactinomyxum were more common in summer and autumn (Table 3). Raabeia was found in all seasons but was most abundant in spring and summer. Echinactinomyxon was also found in all seasons but was most common in winter and spring. Siedleckiella was observed only in spring although at a low prevalence. Triactinomyxon was found in all seasons except winter, but only at a low prevalence. During the winter, prevalence rates were very low and only four collective groups were found (Table 3).

The largest number of individual types of actinosporeans were found in summer (nineteen types), followed by autumn (thirteen types), spring (ten types) and winter (eight types) (Table 4). In summer, synactinomyxon type 1 had the highest infection prevalence with 1.43%, followed by raabeia type 4 (0.93%), echinactinomyxon type 1 (0.33%) and aurantiactinomyxon type 3 (0.3%). However in winter and spring, echinactinomyxon type 1 was the most common with prevalence rates of 1.09 % and 1.32%, respectively. In autumn, synactinomyxon type 1 had the highest prevalence rate with 1.18%, followed by echinactinomyxon type 1 (0.81%).

Table 1. Overall infection prevalence (%) of each actinosporean type over the two-year study period.

Types of actinosporeans	Host	Prevalence
Synactinomyxon type 1	*Tubifex tubifex* *Lumbriculus variegatus*	0.65
Synactinomyxon type 2	*Tubifex tubifex*	0.02
Synactinomyxon type 3	*Tubifex tubifex* *Lumbriculus variegatus*	0.04
Aurantiactinomyxon type 1	*Tubifex tubifex*	0.09
Aurantiactinomyxon type 2	*Tubifex tubifex*	0.01
Aurantiactinomyxon type 3	*Tubifex tubifex*	0.1
Aurantiactinomyxon type 4	*Tubifex tubifex*	0.01
Echinactinomyxon type 1	*Lumbriculus variegatus*	0.9
Echinactinomyxon type 2	*Tubifex tubifex*	0.05
Echinactinomyxon type 3	*Tubifex tubifex*	0.01
Echinactinomyxon type 4	*Tubifex tubifex*	0.01
Echinactinomyxon type 5	*Lumbriculus variegatus* *Tubifex tubifex*	0.14
Raabeia type 1	Immature	0.007
Raabeia type 2	*Lumbriculus variegatus*	0.01
Raabeia type 3	*Tubifex tubifex*	0.01
Raabeia type 4	*Tubifex tubifex*	0.41
Raabeia type 5	*Lumbriculus variegatus*	0.33
Raabeia type 6	*Tubifex tubifex*	0.003
Triactinomyxon type	Immature	0.003
Neoactinomyxum type	*Tubifex tubifex*	0.003
Siedleckiella type	*Tubifex tubifex*	0.001

Table 2. Infection prevalence (%) of actinosporean collective groups over the two-year study period.

Collective groups	Oct. 1996 to Aug. 1997	Sept. 1997 to Aug. 1998	Overall
Synactinomyxon	0.8	0.6	0.7
Aurantiactinomyxon	0.2	0.2	0.2
Echinactinomyxon	1.4	0.6	1.1
Raabeia	0.7	0.7	0.7
Triactinomyxon	0.006	0.01	0.01
Neoactinomyxum	0.04	0.01	0.03
Siedleckiella	0	0.07	0.03
Overall	3.3	2.3	2.9

Table 3. Seasonal infection prevalence (%) of actinosporean infections of oligochaetes over the two-year study period.

Collective groups	Autumn	Winter	Spring	Summer
Synactinomyxon	1.2	0.03	0.08	1.5
Aurantiactinomyxon	0.2	0.02	0	0.5
Echinactinomyxon	0.9	1.1	1.8	0.5
Raabeia	0.4	0.4	0.8	1.2
Triactinomyxon	0.02	0	0.01	0.01
Neoactinomyxum	0.02	0	0	0.09
Siedleckiella	0	0	0.1	0
Overall	2.9	1.6	2.8	4.1

Table 4. Seasonal infection prevalence (%) of each actinosporean type over the two-year study period.

Types of actinosporeans	Autumn	Winter	Spring	Summer
Synactinomyxon type 1	1.18	0.01	0.05	1.43
Synactinomyxon type 2	0.06	0	0	0.05
Synactinomyxon type 3	0	0.02	0.02	0.1
Aurantiactinomyxon type 1	0.16	0	0	0.21
Aurantiactinomyxon type 2	0	0	0	0.03
Aurantiactinomyxon type 3	0.04	0.02	0	0.3
Aurantiactinomyxon type 4	0.02	0	0	0
Echinactinomyxon type 1	0.81	1.09	1.32	0.33
Echinactinomyxon type 2	0.04	0.02	0.03	0.1
Echinactinomyxon type 3	0.02	0	0	0.02
Echinactinomyxon type 4	0.04	0	0	0.01
Echinactinomyxon type 5	0.04	0.03	0.45	0.03
Raabeia type 1	0	0	0	0.02
Raabeia type 2	0	0	0.01	0.02
Raabeia type 3	0	0	0	0.05
Raabeia type 4	0.46	0.18	0.06	0.93
Raabeia type 5	0	0.26	0.72	0.23
Raabeia type 6	0	0	0	0.01
Triactinomyxon type	0.02	0	0.01	0.01
Neoactinomyxum type	0.02	0	0	0.09
Siedleckiella type	0	0	0.09	0

Table 5. Seasonal occurrence (%) of each oligochaete family over the two-year study period.

	Autumn	Winter	Spring	Summer	Overall
No. of oligochaetes	4896	7516	7325	8640	28387
Tubificidae	42	20	28	85.1	45.6
Lumbriculidae	58	77.7	70.4	13.6	52.8
Naididae	0	2	0.6	0.1	0.8
Enchytraeidae	0	0.3	1	1.2	0.8

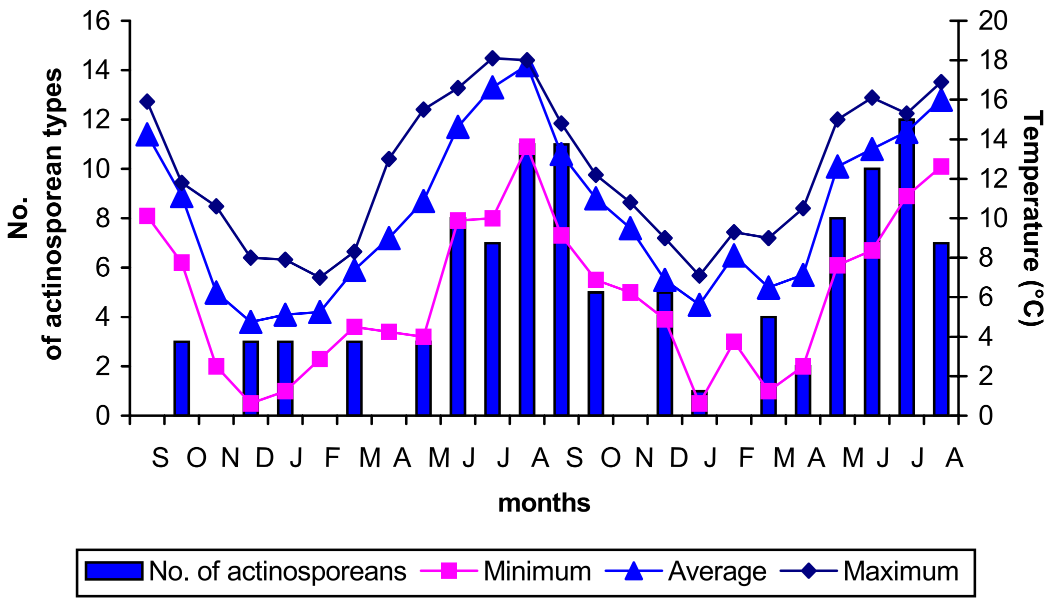

Fig. 1. Monthly minimum, maximum and average water temperatures (°C) and the number of actinosporean types released.

Seasonality of oligochaetes and the host specificity of actinosporeans

In the present study, two tubificids, *Tubifex tubifex* and *Limnodrilus hoffmeisteri* (Claparède, 1862), one lumbriculid, *Lumbriculus variegatus*, one naidid and one enchytraeid were identified. However, *Tubifex tubifex* and *Lumbriculus variegatus* were the most common oligochaete species and were the only species recorded as infected with actinosporeans described in this study (Table 1).

Of the 21 actinosporean types identified, 13 were found only in *T. tubifex*, 3 types only in *L. variegatus*, 2 types only in immature oligochaetes and 3 types in both *T. tubifex* and *L. variegatus* (Table 1). Synactinomyxon type 1 and type 3 were released from two oligochaete species, but the major host for these two types was *T. tubifex*. Conversely, echinactinomyxon type 5 was also released from both oligochaete species, but the major host was *L. variegatus*. Mixed infections of actinosporean types in individual oligochaetes were rather scarce. Synactinomyxon type 1 and raabeia type 4 were both released from an individual *T. tubifex* and synactinomyxon type 1 and echinactinomyxon type 1 from an individual *L. variegatus*.

Of the 28,387 oligochaete worms examined over the study period, *L. variegatus* was the most common constituting 52.8% of the total number of oligochaetes found, followed by *T. tubifex*, Naididae and Enchytraeidae at 45.6%, 0.8% and 0.8%, respectively (Table 5). *Tubifex tubifex* was the major species found in summer and in the other seasons *L. variegatus* was most abundant.

The relationship between the water temperature and the number of actinosporean types found is shown in Fig. 1. There was a positive correlation between water temperature and actinosporean release. Thus, the smallest number of actinosporean types released was 1 in January when the water temperature was at its lowest level (1°C) and the maximum number was 12 in July when the water temperature reached its highest level of 18°C (Fig 1).

DISCUSSION

The overall prevalence rate of actinosporeans in oligochaetes in the present study was 2.9%. However, for each individual actinosporean type prevalence varied between 0.001% and 0.9%. In the literature, most recorded prevalence rates of actinosporeans in oligochaetes range between 0.1% and 4.75% in fish farms (Hamilton and Canning 1987, Yokoyama et al. 1991). McGeorge et al. (1997) using the same site as in this study found an overall 1% infection prevalence ranging between 0.04% and 0.49% for individual actinosporean types. The data obtained in this study are higher than those of McGeorge et al. (1997) due to the comprehensive sampling covering a two-year period. Xiao and Desser (1998) also found similar results to the present study in the infection prevalences of individual actinosporean types from a Canadian lake.

However, El-Mansy et al. (1998a) found prevalence rates as high as at 98% at particular times of the year. These authors attributed these high prevalence rates to a re-examination of oligochaetes for three months. This method of sampling was used at the beginning of this study, but it did not change the detection rate of actinosporean infections and was abandoned.

The environment studied by El-Mansy et al. (1998a) was a still water polyculture fish pond with a mud substrate which probably harboured large oligochaete populations and with no flushing of released myxospores. This must allow much higher prevalences of infections of oligochaetes than flowing water systems, as in the present study, where there will be a wider dispersal of spores. It must be noted that actinosporean infections are only detected by the release of spores. The overall prevalence rate, including presporogonic stages may be higher.

Some collective groups of actinosporeans were mainly released in a particular season or seasons. Similarities exist for raabeia, aurantiactinomyxon and neoactinomyxum type spores between the present study and those of El-Mansy et al. (1998a) and Yokoyama et al. (1991), although the latter authors found a higher prevalence of echinactinomyxon in summer, rather than in winter and spring as described here.

At an individual actinosporean type level, the prevalence data for aurantiactinomyxon type 1 in the present study can be compared to *Aurantiactinomyxon* sp. 1 of Yokoyama et al. (1993), where, even though the maximum prevalence was higher in the latter (≅ 3% compared to 0.21%), both types peaked in summer and were also present in autumn. However, this contrasts with the very high prevalence rates recorded by Negredo and Mulcahy (2001) for two aurantiactinomyxon types from an Irish river. These authors also recorded relatively high prevalence rates for individual echinactinomyxon and neoactinomyxum types. This may partly reflect the fact that sampling was restricted to spring and summer.

The numbers of actinosporean types released was positively correlated with increased water temperature and the highest number of actinosporean types was released in summer as also reported by Xiao and Desser (1998). A similar correlation was reported by Vincent (see Potera 1997) between the peak presence of the triactinomyxon stage of *Myxobolus cerebralis* and the emergence of larval rainbow trout.

In the present study, of the twenty-one actinosporean types described, thirteen were found only in *Tubifex tubifex*, three in *Lumbriculus variegatus* and two in immature oligochaetes. Only three types were found in more than one oligochaete species. These results are similar to those reported by El-Mansy et al. (1998a) and Xiao and Desser (1998) and show a quite strict host specificity on the part of actinosporeans. At the collective group level, at least in those groups with more than one type found in this study, more than one oligochaete host species was involved, except for members of the collective group aurantiactinomyxon which were found only in *Tubifex tubifex*.

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