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RAINBOW TROUT (*SALMO GAIRDNERI*) STOCKING AND *CONTRACAECUM* SPP.

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ABSTRACT: A stocking program with rainbow trout (*Salmo gairdneri*) at High Rock Lake, Manitoba failed due to infections with large numbers of *Contracaecum* spp. larvae. Nematode larvae in the intestinal tract, body cavity and musculature made the fish unmarketable. A combination of experimental infections of rainbow trout and pelicans (*Pelecanus erythrorhynchos*), observations on the behavior of fish-eating birds, and numbers of larval *Contracaecum* spp. in minnow species led to the following conclusions. The introduction of rainbow trout attracted large numbers of fish-eating birds, particularly pelicans. Concurrent predation by rainbow trout on fathead minnows (*Pimephales promelas*), five-spined sticklebacks (*Culaea inconstans*), and nine-spined sticklebacks (*Pungitius pungitius*), concentrated the parasites. The combined increase in densities of the introduced fish host and fish-eating birds, and the short life cycle of the parasite, increased the numbers of parasites in rainbow trout over a season and in the indigenous minnow species between years. Numbers of larvae in the indigenous minnow species declined when stocking of rainbow trout was stopped and use of the lake by fish-eating birds, particularly pelicans, returned to normal levels.

Key words: Rainbow trout, stocking, *Contracaecum* spp., nematode larvae, pelicans, *Salmo gairdneri*.

INTRODUCTION

Nematodes belonging to the genus *Contracaecum* are frequently associated with fish and fish-eating birds (Hoffman, 1967; Huizinga, 1971; Barus et al., 1978). Apparently, parasites of this group are not very host-specific in fish (Hoffman, 1967). Additionally, their range of fish hosts and transmission dynamics are not well understood (Margolis and Arthur, 1979). This is particularly important in aquaculture and stocking programs because there is little information on the interrelationships among fish stocking (numbers and species), feeding behavior of fish-eating birds and *Contracaecum* spp. This paper reports the results of a rainbow trout stocking program, the transmission of *Contracaecum* spp. and the effects of *Contracaecum* spp. on the success of the program at High Rock Lake, Manitoba, Canada.

MATERIALS AND METHODS

Study area: High Rock Lake (HRL), the site of the stocking program, is a small (545 ha), shallow (1.0–2.5 m deep) lake situated in the interlake region of Manitoba (51°25′N, 97°40′W). There are numerous small lakes in the region with substantial breeding populations of double-crested cormorants (*Phalacrocorax auritus*) and white pelicans (*Pelecanus erythrorhynchos*).

Fish species: A new stock of rainbow trout was introduced each yr because rainbow trout were unable to survive through the winter. The size and numbers of rainbow trout stocked into HRL are listed in Table 1. These fish were collected and harvested with gill nets (5.0 cm stretched mesh) and were examined for nematodes during cleaning. All minnow species survived overwinter. Minnow species were collected with minnow traps. Necropsy procedures included an examination of the heart, pericardial cavity, the entire digestive tract (lumen and serosal surface), liver, gonads, body cavity and musculature of all minnow species and 230 rainbow trout. Rainbow trout were examined for nematode larvae at monthly intervals from July until harvesting. All larvae recovered from the minnow species were counted and, based on the counts of larvae from rainbow trout, total estimates for all rainbow trout were made: 1 = 1 larva; 2 = ≥10 larvae; 3 = >100 larvae (Table 1). The commercial fishermen slit open the intestine so that estimates included those larvae in the intestine, along the viscera, in the body cavity and in the musculature. A factor of 10

TABLE 1. Rainbow trout stocking program for High Rock Lake, Manitoba and *Contracaecum* spp. All stocked fish were between 8 and 10 cm in length and weighed 30–40 g.

Year	Number stocked	Number re-covered	$\bar{x}$ weight/ fish (g)	*Contracaecum*[a] prevalence/ range of intensity
1980	10,000	1,600	500	0/0
1981	80,000	5,000	130	100/1–10
1982	50,000	3,600	200	100/10–100
1983	3,000	3	195	100/10–100
1984	3,000	30	210	100/10–100
1985	0	0	—	—

[a] At harvesting (October–November); prevalence and intensity is based on the commercial operator recording presence or absence and range in numbers of *Contracaecum* spp.

for these estimates was used to account for the error in estimates made by the fishermen and because the larval problem was most serious when larval numbers were ≥100 larvae per fish.

Bird observations: Birds were identified and observed daily from the time of rainbow trout introduction until these birds migrated south in September. Our estimates on flock sizes are based on actual counts and then presented as a range in flock size throughout the summer months. A range for bird numbers is given for the following reason. Cormorants in the spring arrived in flocks of 50, 75, or >100. They stayed two days or less. Consequently, estimates in the spring were based on several flocks of varying sizes using the lake for short periods of time. Alternatively, pelicans frequented the lake throughout the spring and summer, so our estimates were based on weekly counts.

Experimental infections: Rainbow trout (Nasqually strain) weighing 151–244 g and 235–287 mm fork length were maintained in the laboratory at 12 C and at a 12-hr photoperiod. These fish were fed to satiation with trout grower number 4 (Martins 84G, Martin Feed Mills, Elmira, Ontario, Canada). They were exposed to larval *Contracaecum* spp. in two ways; (1) fish were anaesthetized with Ethyl m-Aminobenzoate Methanesulfonate (Kent Laboratories Ltd., Vancouver, Canada) and 8 larvae/fish were placed into the stomach with a stomach tube and (2) sticklebacks and fatheads collected from HRL were placed in a tank with rainbow trout and observed daily until all minnows were consumed (5–6 days). An estimate of numbers of larvae in the minnows presented to the rainbow trout was based on the 1983 (Table 2) sample since this was the year that experimental infections were performed. Calculations were as follows: Prevalence and intensity for 1983 in fathead minnows were 15.6% and 1.6, respectively, and for sticklebacks they were 36.8% and 1.4, respectively; experiment 1 (Table 2) $(27 \times 0.156 \times 1.6) + (18 \times 0.368 \times 1.4) = 15.9$ (rounded to 16 larvae). Fish were necropsied at designated times (Table 3).

All animals used for experimental studies were maintained under a photoperiod of 12 hr. Hamsters (*Mesocricetus auratus*), and rats (Sprague-Dawley outbred) were maintained at 21 ± 2 C and given lab chow (Ralston Purina Co., Canada) and water ad libitum. Eggs of pelicans, ring-billed gulls (*Larus delawarensis*), and herring gulls (*Larus argentatus*) were incubated in a forced-air incubator at 39 ± 1 C and 65 ± 5% relative humidity for 2–3 wk. After hatching the birds were maintained at 33–37 C. Young gulls were fed according to the methods of Bober and Dick (1983). The young pelicans were fed three times daily on a food mixture produced by a local mink farmer. The food was warmed to 37 C and fed by hand to the pelicans along with substantial volumes of warm water (at least 30–50 ml per feeding).

Hamsters and rats were lightly anaesthetized with ether and the larval nematodes inoculated with a stomach tube. The reason for these experiments was to determine if a mammalian host could become infected because Deardoff and Overstreet (1980) imply that some *Contra-*

TABLE 2. *Contracaecum* spp. larvae in fathead minnows and sticklebacks from High Rock Lake, Manitoba.

Year	Fathead minnows			Sticklebacks[a]		
	n	Prevalence	Intensity	*n*	Prevalence	Intensity
1982	64	14.8	—	—	—	—
1983	230	15.6	1.6 ± 1.2	228	36.8	1.4 ± 0.7[b]
1984	143	23.1	1.3 ± 0.8	9	11.0	1.0
1985	346	7.2	1.1 ± 0.3	281	1.6	1.1 ± 0.4

[a] Sticklebacks of both species were combined.
[b] Mean ± standard deviation.

TABLE 3. Experimental infections of *Contracaecum* spp. in rainbow trout.[a,b]

Experiment number	Number rainbow trout	Number parasites administered	Duration in days	Number fish infected/ intensity[d]
I[a]	5	27 FH (16)[c] 18 S	11	2/1.5 ± 0.7
II[a]	3	40 S (21)[c]	30	1/1.0 ± 0
III[b]	3	8 larvae/fish	8	2/2.0 ± 1.4

[a] Fed on fathead minnows (FH) and sticklebacks (S).
[b] Infected with larvae dissected from FH and S.
[c] Number of parasites given (estimates based on the 1983 sample).
[d] Mean ± standard deviation.

caecum spp. may be infective to man. Birds were infected by placing larvae in a small amount of mink food and placing the mixture in the back of the mouth of each bird. Controls were uninfected birds and mammals (2 per species) that were treated in the same way, except that they were not given larvae. Ten larval nematodes were given to each of four hamsters, four rats, six ring-billed gulls and four herring gulls. Two pelicans were given 15 and 25 larvae, respectively.

RESULTS

Larvae were identified to the genus *Contracaecum* on the basis of the excretory pore emptying near the ventral interlabium (Deardorff and Overstreet, 1981). Definitive identification to species was not possible without male specimens, but our egg producing adult female worms (recovered from pelicans experimentally infected with larvae recovered from fathead minnows and sticklebacks) most closely resembled *C. microcephalum* according to the key by Barus et al. (1978). This is based on size of females (26.7–33.3 mm in length) and the shape of the lips with interlabia tips not bifurcate but distinctly rounded. Specimens are deposited in the National Museum of Natural Science, Ottawa K1A 0M8, Canada as unmounted specimens: NMCP 1986-0078 (2 larvae from sticklebacks), NMCP 1986-0079 (3 larvae from rainbow trout), NMCP 1986-0080 (1 gravid female).

Fauna of High Rock Lake: The minnow species and their proportions were determined from randomly set minnow traps: fathead minnow, *Pimephales promelas*, 58%; nine-spined stickleback, *Pungitius pungitius*, 38%; five-spined stickleback, *Culaea inconstans*, 2%; central mudminnow, *Umbria limi*, 2%; yellow perch, *Perca flavescens*, 1%.

Fish-eating birds frequented the lake and included double-crested cormorants, white pelicans, ring-billed gulls, American bitterns (*Botaurus lentiginosus*), common terns (*Sterna hirundo*), great blue herons (*Ardea herodias*), and common loons (*Gavia immer*). Only cormorants and pelicans arrived in consistent and predictable flock sizes during the stocking program. Flocks of pelicans arrived on the lake in early July (1980) although rainbow trout were introduced in May. During 1981–1984 when rainbow trout were stocked pelicans arrived shortly after the introduction of rainbow trout in May and frequented the lake in large flocks until mid-August to early September. Flock size of pelicans varied from a few birds to >100 and it is likely that these birds came from several breeding colonies in the vicinity. In the case of pelicans our estimates of flock size were 10–100 for 1980, 10–100 for 1981–1984 and 2–3 for 1985. It should be noted that flocks of 2–3 pelicans during 1985 were observed much less frequently (once or twice per wk) than flocks of greater size (a daily occurrence) in the years when rainbow trout were stocked. The estimates of flock sizes of 50 to >100 cormorants in May and late August to early September for 1980–1985 were similar. However, there were more large flocks in August and September and they stayed longer. The size of cormorant flocks was similar between yr, but their stay on the lake especially in late August and September was extended when rainbow trout were present. Both groups of birds were observed feeding extensively on rainbow trout and minnow species of fish. In 1985 no rainbow trout were stocked, very few fish-eating birds were noted and flocks of cormorants visited the lake briefly in late August and early September 1985.

Parasite numbers: Larvae of *Contracaecum* spp. were not recovered from rainbow trout harvested in 1980 (Table 1). In subsequent years 1981–1984 all rainbow trout harvested were infected with nematodes, ranging in number from 10 to 100 per fish (Table 1). The numbers of larvae increased from 1–2 per fish in July to ≥10 by late September and early October. These larvae were in the stomach, the lumen of the intestine (in some heavy infections the anus was blocked), partially penetrated through the intestinal wall, in the body cavity, and coiled within the body wall musculature. Worms located in the musculature were generally associated with the epaxial muscles or near the juncture of epaxial-hypaxial muscles. They could usually be located in a dark hemorrhagic area 3–6 mm in diameter.

By July the stocked rainbow trout were consuming small fathead minnows and a few sticklebacks. This was the time when parasites were first noted in the stocked rainbow trout. The prevalence and intensities of *Contracaecum* spp. in these hosts are given in Table 2. Sticklebacks of both species were combined because they were considered as a food item and no significant differences in prevalence and intensity of *Contracaecum* spp. between five-spined and nine-spined sticklebacks were found. *Contracaecum* spp. numbers were highest in fathead minnows during 1982–1984 and lower in 1985. A similar pattern was observed for sticklebacks for 1983–1984 with a decline in 1985. The proportion of mudminnows in the fish population was low, but those infected (5%) had numbers ranging from 1 to 12 nematodes per fish. Mudminnows are larger than fatheads and sticklebacks and were not observed in the diet of rainbow trout.

Experimental infections: Experimental infections of rainbow trout showed that they can acquire *Contracaecum* spp. through predation (Table 3) and that up to 25% of the larvae survive transmission. Larvae recovered from the experimentally infected rainbow trout were found in the same sites in the fish (Table 3) as those from natural infections and larvae were of a similar size. Larvae recovered from fatheads, sticklebacks, and rainbow trout (natural and experimental) from the complete necropsies were third stage and were similar to those described by Huizinga (1966). There was no evidence of molting or further differentiation of the larvae.

Nematodes were not recovered from hamsters, rats, ring-billed or herring gulls. Adult female worms recovered from the proventriculus of two experimentally infected pelicans released shelled eggs into dechlorinated tap water. Male nematodes were not recovered. Two unexposed pelicans were not infected at necropsy.

DISCUSSION

Hoffman (1967) indicated that *C. spiculigerum* probably has no fish host specificity and Margolis and Arthur (1979) compiled an extensive list of fish hosts for *Contracaecum* spp. It was not surprising that we found *Contracaecum* spp. from five fish hosts previously unknown as hosts of *Contracaecum* spp. These hosts included fathead minnow, five- and nine-spined stickleback, central mudminnow, and rainbow trout. What are important, however, are the real and potential effects of these parasites on high density stocking of fish and aquaculture programs.

The life cycles of several *Contracaecum* spp. involve infections acquired by fish through their consumption of infected copepods (Huizinga, 1966, 1967) and through predation of other fish (Thomas, 1944). The introduction of fish species such as rainbow trout which prey on infected minnow species tended to concentrate the parasite. Both rainbow trout and fathead minnows are open water fish. The schooling behavior of fathead minnows, the higher and more consistent prevalences of *Contracaecum* spp. in these fish and their greater proportions in the total fish population indicate that fathead minnows were probably the most important fish species in transmitting larvae to rainbow trout. Larvae of *Contracaecum* spp. were concentrated at one trophic level of the food chain in HRL, but this might not have made the rainbow trout unmarketable if bird populations had remained at their usual levels. A further complication was the introduction of large numbers of rainbow trout that behaved differently from the indigenous fish fauna because they swam closer to the surface. This attracted large numbers of fish-eating birds, particularly white pelicans. Consequently, pelicans began feeding on rainbow trout which were already concentrating the parasites. The pelican infections further concentrated the parasites. The relatively short time required for this nematode to produce eggs (30 days in experimentally infected pelicans) and the possibility that second-stage larvae may directly infect fish (minnows and perhaps rainbow trout) compounded the situation. The result was a large increase in numbers of *Contracaecum* spp. infection in fish during one season and between years. Table 1 shows that it took only 1 yr to establish sufficiently high numbers of *Contracaecum* spp. to establish a 100% prevalence in rainbow trout and 2 yr to increase intensities by a factor of 10. The converse of this is shown in Table 2 where a cessation of stocking with rainbow trout in 1985 decreased the use of the lake by pelicans and other fish-eating birds such that parasite prevalences and intensities declined. The most dramatic decline was in fathead minnows which constitute almost 60% of the minnow population. We believe that pelicans are the key fish-eating bird in the transmission of the parasite in this system because; (1) pelicans were on the lake in large numbers and for a sufficient period of time, (2) third-stage larvae of *Contracaecum* spp. recovered from fish in HRL infected pelicans, and (3) cormorants caught in the commercial nets and necropsied had large numbers of third-stage *Contracaecum* spp. larvae in their proventriculus, but no mature worms. This suggested that the parasites were recently acquired, and that they were in an unsuitable host. Even if cormorants were a suitable host, the worms would not mature until after they left the lake. Therefore, they would play no role in increasing the infective egg stage in HRL by the fall migrants. However, we can not overlook the possibility that cormorants arriving in the spring, even for a brief stop on the lake, contributed infective eggs of *Contracaecum* spp. to the system.

Fish-eating birds in HRL, as of 1985, have returned to near normal feeding densities for small shallow lakes in the interlake region of Manitoba, and large stable breeding colonies of pelicans still persist in the area. Even with a decline in the numbers of *Contracaecum* spp. in the minnow species we expect numbers to stabilize at a prevalence of 5% or slightly less and an intensity of one worm per fish. Consequently, HRL will remain unsuitable for rainbow trout aquaculture in the immediate future.

This study clearly demonstrates the need for more careful assessments of potential aquatic systems for fish aquaculture. Considerably more emphasis should be placed on the density and stability of fish-eating bird populations in the vicinity, the indigenous fish parasite fauna, and the type of life cycle and degree of host specificity of these parasites. A challenging problem for parasitologists is the selection of fish species for stocking programs that do not attract large numbers of fish-eating birds and that do not accumulate a particular species of parasite infective for these birds.

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