Journal of Wildlife Diseases, 24(3), 1988, pp. 456–460

HELMINTHS OF STOCKED RAINBOW TROUT (*SALMO GAIRDNERI*) WITH SPECIAL REFERENCE TO *CLINOSTOMUM COMPLANATUM*

Alexander J. Szalai and Terry A. Dick
Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

ABSTRACT: Rainbow trout (*Salmo gairdneri*) stocked into a small prairie dug-out were examined for helminths at harvest time. Five species of helminths were found (*Diplostomum* sp., *Diplostomulum scheuringi*, *Clinostomum complanatum*, *Crepidostomum farionis* and *Pomphorhynchus bulbocolli*); *C. farionis* and *P. bulbocolli* mature in fish while the remaining species utilize fish-eating birds as definitive hosts. Examination of stomach contents indicated that all invertebrate hosts required for the life-cycles of these helminths were present in the dug-out. The most conspicuous and prevalent helminth was *C. complanatum* as fish were "wormy" and unmarketable due to the presence of high numbers of metacercariae. Metacercariae of *C. complanatum* were recovered from most organs of rainbow trout. The low survival of stocked fish suggests that *C. complanatum* may cause some host mortality, but the condition of infected and uninfected fish was similar. It appears that a community of ichthyoparasites can become established in a population of stocked fish in a single growing season in north temperate regions.
Key words: *Salmo gairdneri*, rainbow trout, *Clinostomum complanatum*, yellow grub, helminths, stocking, aquaculture.

INTRODUCTION

The importance of parasites associated with high density stocking of fish is an increasing problem in freshwater aquaculture. It could be predicted that parasites that are generalists with little or no host specificity would be of major concern. However, there are few documented cases of rapid increases in numbers of these types of parasites. Furthermore, there is little information on the types of parasites that can become established in dug-outs stocked with fish.

This paper reports the helminths recovered from a population of rainbow trout stocked into a dug-out and documents the severity of infection of these fish with *Clinostomum complanatum* or the "yellow grub."

MATERIALS AND METHODS

The site of the stocking program was a small (approximately 1 ha), shallow (2 m maximum) dug-out fed by surface runoff in southeastern Manitoba, Canada (96°30′W, 56°00′N). The Brokenhead River flows to within 2 km of the dug-out and harbours populations of great blue herons (*Ardea herodias*) and belted kingfishers (*Ceryle alcyon*). The dug-out was constructed in 1977. Prior to stocking there were no fish in the dug-out. Fish were stocked in 1985 and again in 1986. Fingerling rainbow trout for stocking were obtained from the Rockwood Experimental Fish Hatchery (Gunton, Manitoba, Canada R0C 1H0) where fingerling rainbow trout were reared in tanks using filtered well-water and fed a diet of commercial fish food (Grower Pellets for Salmonids Martins 84G, Martin Feed Mills, P.O. Box 130, Elmira, Ontario, Canada N3B 3A2). An extensive examination of rainbow trout by the authors since 1983 has not found any fish from the hatchery stock infected with parasites, including helminths. In 1985 the stocking program was a success since 490 fish were recovered and none were infected with parasites. In 1986 the stocking effort failed and the few fish that did survive were "wormy."

Trout were collected from the dug-out with gill-nets (3.8 cm mesh) set overnight in September 1986. Fish were either examined fresh or frozen within 6 hr of capture for later processing. The fork-length (L) and round weight (W) were recorded and the gastrointestinal (GI) tract removed from each fish. The GI tract was examined externally, opened longitudinally, scraped and the contents were examined. The skin, fins, eyes, kidneys, swimbladder, gall bladder, heart, spleen, liver and brain were examined also. The number and species of helminths found, their sites of infection and the stomach contents were recorded for each fish. Fillets were removed, divided into four sections (anterior epaxial, anterior hypaxial, posterior epaxial, and posterior hypaxial sections), and the flesh teased apart to locate metacercariae. The remaining axial skeleton was examined for parasites using transmitted light. Fulton's condition factor (K =

TABLE 1. Helminths of 43 rainbow trout from a dug-out in Manitoba, Canada.

Parasite[a]	Intensity[b]	Prevalence	Status[c]
Diplostomum sp. (m)	1.0 ± 0.0	2	PR
Diplostomulum scheuringi (m)	26.4 ± 6.0	23	NHR
Clinostomum complanatum (m)	58.8 ± 10.8	100	NHR
Crepidostomum farionis (a)	2.0 ± 0.0	5	PR
Pomphorhynchus bulbocolli (i)	4.3 ± 0.5	56	NHR

[a] (m), metacercariae; (a), adult; (i), immature.
[b] Mean ± standard error.
[c] PR, previous record; NHR, new host record.

W/L^3; Ricker, 1975) was determined for all infected rainbow trout collected from the dug-out and compared to K values for uninfected trout of similar age obtained from the Rockwood Hatchery. All parasites were removed, heat killed in distilled water (70 C), fixed in formalin–acetic acid–alcohol for 24 hr and stained with Semichon's acetocarmine. The range, prevalence, relative abundance and intensity of infection were determined (Margolis et al., 1982) for each species of parasite and the distribution of metacercariae among the four regions of the body musculature was tested using chi-square analysis. The Shapiro-Wilk statistic (*SW*; Shapiro and Wilk, 1965) was computed to test if parasite frequency distribution was normal and the relationship between body condition and the number of *C. complanatum* per fish was tested using simple linear regression. Means and standard errors are given where known.

Specific identity of *Clinostomum complanatum* was verified in a series of laboratory experiments. Metacercariae from the flesh of rainbow trout were surgically implanted into the abdominal cavity of anesthetized Swiss-Webster Crl COBS CFW mice (Charles River Breeding Laboratories, Wilmington, Massachusetts 01887, USA) and the incision sutured (Dowsett, 1967). Four days later the mice were sacrificed, mature worms collected and morphometric measurements made. A set of representative helminth specimens has been deposited in the National Museum of Natural Sciences (Ottawa, Ontario, Canada K1A 0MB; Accession numbers NMCP 1987-2640 to NMCP 1987-2649).

RESULTS

Forty-three rainbow trout (L = 204.2 ± 3.6 mm; W = 134.4 ± 9.1 g) from a total of 1,000 stocked were recovered from the dug-out. Five helminth species (Table 1) were found, but *Crepidostomum farionis* and metacercariae of *Diplostomum* sp. were rare. *Diplostomulum scheuringi*, *Clinostomum complanatum* and *Pomphorhynchus bulbocolli* are new records for this host.

Clinostomum complanatum was the most important parasite found, it occurred in all fish examined at the highest intensity of infection and was normally distributed among hosts ($SW = 0.71$, $P < 0.01$). Measurements of *C. complanatum* recovered from the peritoneal cavity of mice agreed with those of Dowsett (1967). Metacercariae were found throughout the body of the fish (Table 2) and were occasionally concentrated at the base of fins. There was no significant difference between the number of *C. complanatum* in the anterior epaxials and hypaxials and posterior epaxials and hypaxials (Table 2) ($\chi^2 = 4.06$, df = 3, $P > 0.05$). There was no linear relationship between body condition and the density of *C. complanatum* per infected host ($Y = 0.014 - 0.000005X$, $r = 0.054$, $P = 0.19$) and Fulton's K-factor for infected rainbow trout (1.41 ± 0.08) did not differ significantly from the condition factor for uninfected rainbow trout from the same stock but reared in a different area (1.39 ± 0.07) (*t*-test, $P > 0.05$).

Amphipods (mostly *Gammarus* sp.) were present in 81% of the rainbow trout stomachs examined. Gastropods (mostly *Helisoma* sp.) were found in 45% of the trout. Fingernail clams (*Musculium* sp.) and nymphs of Ephemeroptera were in 19% and 3% of rainbow trout stomachs, respectively. Coleoptera (84%), *Daphnia* sp. (29%), Diptera (23%), nymphs of Tri-

TABLE 2. Sites of metacercariae of *Clinostomum complanatum* in rainbow trout.

Site	*n*	Intensity[b]	%[c]	Comments
Gills/mouth	43	16.2 ± 2.5	10.18	On branchiostegals
Swimbladder	43	11.0 ± 1.6	8.11	On surface membrane
Heart	43	2.0 ± 0.0	0.05	Encysted in pericardium
Kidneys	43	4.3 ± 0.6	2.22	Within tissues
Eyes/brain	43	16.1 ± 2.7	11.98	Within vitreous humor
Intestine	43	1.6 ± 0.1	0.24	In lumen or encysted
Left AE[a]	41	7.7 ± 1.7	7.99	Between myomeres
Left AH[a]	41	7.7 ± 1.3	9.02	Between myomeres
Left PE[a]	41	7.3 ± 1.9	9.41	Between myomeres
Left PH[a]	41	8.3 ± 1.6	8.16	Between myomeres
Right AE[a]	8	6.7 ± 2.9	7.22[d]	Between myomeres
Right AH[a]	8	9.5 ± 5.0	9.36	Between myomeres
Right PE[a]	8	7.9 ± 3.9	8.18	Between myomeres
Right PH[a]	8	7.1 ± 2.9	7.90	Between myomeres

[a] A, anterior; P, posterior; E, epaxial; H, hypaxial.
[b] Mean ± standard error.
[c] Relative abundance. For definition see Margolis et al. (1982).
[d] Values estimated where complete data not available.

choptera (16%), nymphs of Ephemeroptera (7%), nymphs of Plecoptera (3%) and unidentified plant material (10%) also were found as food items.

DISCUSSION

Our results indicate that a community of icthyoparasites can become established in a population of stocked fish in a dug-out which was not connected to any other aquatic system. Although all stages of the life-cycles of the helminths recovered were not evaluated, it is obvious that all hosts necessary for the transmission of *Diplostomum* sp., *Crepidostomum farionis, Diplostomulum scheuringi* and *Clinostomum* sp. were present. Three of the five helminths recovered (metacercariae of *Clinostomum complanatum, Diplostomulum scheuringi* and *Diplostomum* sp.) normally mature in piscivorous birds. These helminths must have been introduced into the dug-out in bird faeces as a normal route of transmission and subsequently have become established in the invertebrate hosts and then in the rainbow trout since there is no route for fish immigration to the dug-out nor were indigenous fish species present.

The high prevalence of *P. bulbocolli* is surprising considering the fish were uninfected when stocked, there were no indigenous fish hosts in the dug-out, and none of the worms recovered from the rainbow trout were gravid. This suggests introduction into the dug-out through the "contaminated" faeces of piscivorous birds. Perhaps the high prevalence of *P. bulbocolli* is due to the "seeding" of the dug-out with large numbers of parasite eggs a few times by a small number of birds, or by many birds perhaps in a flock, visiting the dug-out. Whatever the process of introduction of *P. bulbocolli* it was sufficient to raise intensities of this helminth in the invertebrate hosts in one season or through its accumulation in infected overwintering invertebrates such that >50% of the rainbow trout were infected.

Clinostomum sp. has been recovered from migratory rainbow trout, but prior to our study it was not known if non-migratory stocks of rainbow trout were susceptible (Uzmann and Douglas, 1966). However, there is an anecdotal report of this helminth in rainbow trout from Manitoba (Dickson, 1964). The life-cycle of *Clinostomum* sp. is well documented. Briefly, *Clinostomum* sp. is transmitted through planorbid snails (*Helisoma* sp.), various fishes as intermediate hosts, and great blue herons (*Ardea herodias*), gulls (Laridae), cormorants (*Phalacrocorax* sp.) and belted kingfishers (*Ceryle alcyon*) as definitive hosts (Hunter and Hunter, 1934; Uzmann and Douglas, 1966). Whether the presence of *Helisoma* sp. in the diets of rainbow trout and the presence of metacercariae in most internal organs are related and therefore suggestive of a second route of infection, remains to be proven.

Little is known regarding the levels of *Clinostomum* sp. in salmonids but the prevalence and intensity reported for other fish hosts are low and the number of metacercariae and the health of fish appears uncorrelated (Elliot and Russert, 1949; Hazen and Esch, 1978). Our study clearly showed that rainbow trout are highly susceptible to *C. complanatum*, but this is not surprising because Hoffman (1970) stated that *C. complanatum* is capable of infecting most freshwater fish in North America. Furthermore, *Clinostomum complanatum* invaded most organs of rainbow trout and while metacercariae were reported from the body musculature and the viscera of other fish species (Margolis and Arthur, 1979), neither the presence of these worms in the brain, eyes, kidneys and heart nor the high numbers of metacercariae per fish have been reported previously for this helminth. We did not observe a correlation between the condition factor of the surviving fish and the intensity of *Clinostomum* sp. infections for rainbow trout and the condition factors were similar for infected and uninfected fish. This was surprising because host mortality due to heavy infections of *Clinostomum* sp. has been reported for other fish (Lo et al., 1985).

The high density of introduced fish, the sporadic feeding by piscivorous birds (blue herons were seen at the dug-out intermittently) and the presence of suitable invertebrate hosts allowed parasites to become established and to increase in rainbow trout. Rainbow trout tend to attract piscivorous birds due to their feeding and swimming behaviour (Dick et al., 1987). Consequently, *Clinostomum* sp. will remain a problem for rainbow trout stocking programs where this parasite is indigenous in local fish populations and when the snail and piscivorous bird hosts are present.

ACKNOWLEDGMENTS

The authors thank Otto Mehle, Ken Rowes and Andrew G. Dwilow (Freshwater Institute, Winnipeg, Manitoba) for bringing this parasite problem to our attention. This research was funded in part by a National Science and Engineering Research Council of Canada Postgraduate Scholarship to AJS and by an NSERC operating grant to TAD.

LITERATURE CITED

DICK, T. A., M. H. PAPST, AND H. C. PAUL. 1987. Rainbow trout (*Salmo gairdneri*) stocking and *Contracaecum* sp. Journal of Wildlife Diseases 23: 242–247.

DICKSON, I. A. 1964. Parasites of some fishes in Manitoba. M.S. Thesis. University of Manitoba, Winnipeg, Manitoba, Canada, 161 pp.

DOWSETT, J. A. 1967. The maturation of *Clinostomum complanatum* in some mammalian hosts. M.S. Thesis. University of Manitoba, Winnipeg, Manitoba, Canada, 65 pp.

ELLIOT, A. M., AND L. S. RUSSERT. 1949. Some conditions characteristic of a yellow perch population heavily parasitized by *Clinostomum marginatum*. The Journal of Parasitology 35: 183–190.

HAZEN, T. C., AND G. W. ESCH. 1978. Observations on the ecology of *Clinostomum marginatum* in largemouth bass (*Micropterus salmoides*). Journal of Fish Biology 12: 411–420.

HOFFMAN, G. L. 1970. Parasites of North American freshwater fishes. University of California Press, Berkeley, California, 486 pp.

HUNTER, G. W., AND W. S. HUNTER. 1934. The life cycle of the yellow grub of fish, *Clinostomum marginatum* (Rud.). The Journal of Parasitology 20: 324.

LO, C.-F., S.-C. CHEN, AND C.-H. WANG. 1985. The study of *Clinostomum complanatum* (Rud. 1814). V. The influences of metacercariae of *Clinostomum complanatum* on fish. Fish Pathology 20: 305–312.

MARGOLIS, L., AND J. R. ARTHUR. 1979. Synopsis of the parasites of fishes of Canada. Bulletin of Fisheries Research Board of Canada 199: 1–269.

———, G. W. ESCH, J. C. HOLMES, A. M. KURIS, AND G. A. SCHAD. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). The Journal of Parasitology 68: 131–133.

RICKER, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191: 1–382.

SHAPIRO, S. S., AND M. B. WILK. 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591–611.

UZMANN, J. R., AND J. DOUGLAS. 1966. *Clinostomum marginatum* in steelhead trout (*Salmo gairdneri*) and cutthroat trout (*Salmo clarki*) in a western Washington lake. Transactions of the American Microscopical Society 95: 35–38.

Received for publication 13 August 1987.