

EFFECT OF CONCURRENT INFECTIONS OF LERNAEOCERA BRANCHIALIS (COPEPODA) AND TRYPANOSOMA MURMANENSIS (PROTOZOA) ON ATLANTIC COD, GADUS MORHUA

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EFFECT OF CONCURRENT INFECTIONS OF *LERNAEOCERA BRANCHIALIS* (COPEPODA) AND *TRYPANOSOMA MURMANENSIS* (PROTOZOA) ON ATLANTIC COD, *GADUS MORHUA*

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ABSTRACT: A study was initiated to ascertain the prevalence, effect and interaction of the adult stages of the parasitic copepod, *Lernaecera branchialis*, on Atlantic cod concurrently infected with a hematozoan, *Trypanosoma murmanensis*, by comparing condition (K) factor, organ somatic indices, hematological values and lipid concentrations of the liver from infected and uninfected fish of comparable length. Prevalence of the copepod varied from 9 to 21% and was highest in fish examined on the southern coast of Newfoundland. Body condition and blood values were significantly lower in young cod infected with two parasites whereas only K-factor was altered in two size classes parasitized by one copepod. Although lipid concentrations were similar in three length groups of fish without and infected with *L. branchialis*, the pooled values were significantly greater among the infected group. Mortality occurred only in juvenile cod infected with the copepod and *T. murmanensis* concurrently. Cod which survived dual infections had lower K-factor and blood values than controls or fish harboring single, parasitic infections. It is concluded that an infection with an adult *L. branchialis* does not impair the health of fish in this area of the northwestern Atlantic Ocean, but the presence of *T. murmanensis* concurrently can potentiate its effect.

INTRODUCTION

Concurrent infections with several species of parasites, including the copepod, *Lernaecera branchialis* (L.), have been reported previously in codfishes (Scott, 1909; Sproston and Hartley, 1941; Dollfus, 1953; Kabata, 1958). The adult stages of species of *Lernaecera* are blood feeders and evidence from studies on parasitized haddock, *Melanogrammus aeglefinus* (L.), and whiting, *Merlangius merlangus* (L.), in the North Sea indicates that infected fish weigh less, and hemoglobin and lipid concentrations are lower than in uninfected fish of the same size (Mann, 1952, 1970; Kabata, 1958; van den Broek, 1978; Hislop and Shanks, 1979). There is a report that *L. branchialis* reduced body condition in Atlantic cod, *Gadus morhua* L., in European waters to "... skin and bone ..." through loss of blood (Scott,

1909). Two or more adult parasites produced more pronounced effects. Retardation of gonadal development has been observed also in haddock parasitized by *L. branchialis* (Kabata, 1958; Hislop and Shanks, 1979).

Atlantic cod in the northwestern Atlantic Ocean are infected naturally also with *L. branchialis* and a blood protozoan, *Trypanosoma murmanensis* Nikitin (Templeman, 1963; Khan et al., 1980). Templeman et al. (1976) studied the distribution and prevalence of the copepod in different length groups of fish and deduced that it delayed sexual maturity. Experimental studies on *T. murmanensis* provided evidence of blood changes and mortality especially in young cod (Khan, 1977b, 1985). High prevalences of the hemoflagellate infection were reported in cod from some areas of the northwestern Atlantic (Khan et al., 1980). Both parasites can therefore occur concurrently in cod. Little information, however, exists on the role of dual parasitic infections in fish, es-

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pecially when both induce blood loss. The present study was designed to ascertain the prevalence and effect of *L. branchialis* in naturally infected cod and also the changes associated with infections of the copepod and *T. murmanensis* concurrently in subadult and adult cod.

MATERIALS AND METHODS

Cod, 10–16 cm in length (year class 0⁺), were caught by seine whereas larger fish (28–60 cm) were obtained from cod traps set 20–30 m deep in Conception Bay, located on the northeastern coast of Newfoundland in June and October 1983–1984. Fish examined at the two other locations were taken either by pair trawling (Fortune Bay, southern coast) or gill net (St. Georges Bay, western coast) set at 110–130 m. At necropsy all fish were measured; total body length, gutted (carcass) and organ weights were recorded from fish infected with adult *L. branchialis* and from uninfected cod of comparable length. Specimens of *L. branchialis* have been deposited in the National Museum of Canada, Ottawa, and assigned numbers NMC-C-1985-138 and -139. Cod sampled in October were gutted prior to examination. Therefore, only length and gutted weights were recorded. The fish sampled were divided into six length groups, viz., <23, 24–29, 30–35, 36–41, 42–47 and >48 cm as these correspond approximately to year classes 2⁺, 3⁺, 4⁺, 5⁺, and 6⁺, respectively. Cod originating from Conception Bay become mature at 6⁺ years and consequently were grouped together with the exception of one sample. Data were recorded from 25 fish in both the infected and uninfected groups in each size class.

To determine lipid concentrations, pieces of liver were removed from a number of uninfected and infected cod of three size groups and kept on ice until frozen at –60 C. Selection of the infected groups was based on the presence of the copepod. The fish were collected in July 1983 at one location on the northeastern coast. Lipid analysis was performed subsequently by the method of Bligh and Dyer (1959) as outlined by Dey et al. (1983).

Fish used for experimental studies were obtained from Conception Bay. Juvenile cod were held for 3 mo and adults 6 mo before use. Prior to experimentation, all cod were screened twice at weekly intervals for hemoprotozoans by examining two wet mount preparations (each about 50 fields, with a 10× objective). Infected fish were discarded. Groups of cod, both uninfected and naturally infected with *L. branchi-*

alis, were exposed subsequently to the leech vector, *Johanssonia arctica* (Johansson) (five/fish). These leeches, reared in the laboratory, were infected by feeding on cod harboring *T. murmanensis* (Khan, 1977a). Two other groups of cod, with and without infections of *L. branchialis*, were also exposed to an equal number of uninfected leeches. Each group of fish was held subsequently in a 3,000-liter tank, through which seawater flowed at 0 or 10 C. Adult cod (49–62 cm) were held for 8 wk at 0 C while the immature fish (48–48 cm) were kept for 7 wk at 10 C. An additional experiment (five fish/group) was designed to determine the effect of dual infections on cod (30–40 cm) harboring the remnants of the cephalothorax and either one or two adult *L. branchialis* at 0 C. The cod were fed to satiation freshly thawed caplin, *Mallotus villosus* (Muller), twice or thrice weekly at 0 and 10 C, respectively, but were deprived of food 24 hr before necropsy. The length and weight of each fish were recorded before the experiments commenced and at the conclusion, total length, body and organ weights as well as blood parameters were determined.

The effect of the parasite on its host was determined by comparing condition (K) factor [$100 \times \text{total body weight (g)}/\text{length}^3 \text{ (cm)}$] and organ somatic indices (s.i., % organ/total body weight) of the various length groups of infected and uninfected fish. Blood parameters included hemoglobin, hematocrit and total plasma protein. The data were analyzed by a two-way ANOVA for significant differences within each group with the respective controls. Mean and standard errors were determined also for all values.

RESULTS

Prevalence of *Lernaeocera branchialis*

Prevalence of adult *L. branchialis* (egg-string stage) infections in cod from three areas are shown in Table 1. Prevalences were similar during the month of June between the St. George's (11%) and Conception Bay's (9%) samples, but were considerably higher (21 and 17%) in fish examined at Fortune Bay during June and October, respectively. Although the prevalence of infection in fish was higher consistently in the 30–35-cm group than in the others from the northeastern coast, no trend was apparent among the samples taken from the southern or western coasts.

TABLE 1. Prevalence of *Lernaeocera branchialis* on Atlantic cod examined at the south (Fortune Bay), northeast (Conception Bay) and west (St. Georges Bay) coasts of Newfoundland during June and October 1983–1984.

Size class (cm)	South		Northeast		West
	June	Oct.	June	Oct.	June
<23	0/35 ^a	—	1/94	—	—
24–29	11/80	—	23/166	—	—
30–35	6/37	13/56	15/81	12/60	2/17
36–41	21/65	10/78	18/125	6/74	2/33
42–47	77/324	18/58	54/545	4/75	8/56
>48	101/498	22/115	53/604	25/343	20/194
Total	216/1,039	53/307	164/1,615	47/552	32/300
% Infected	21	17	10	9	11

^a No. infected/no. examined.

About 5% of the cod from Fortune and 1% from Conception and St. George’s bays harbored two or three parasites. An additional 182 cod, 10–14 cm in length (year class 0⁺), were also examined in July in Conception Bay, but none was infected with *L. branchialis*. Similarly, none of 479 cod, <12 cm in length, was infected in an inlet adjacent to Conception Bay during 1982–1983, but 9% (six) of 70 fish, 15–21 cm long (year class 1⁺), harbored the copepod. Twelve additional species of fish, which included pleuronectiforms and perciforms, were examined for *L. branchialis*, but only one of 242 American plaice, *Hippoglossoides platessoides* (Fabricius) harbored an infection.

Effects of *Lernaeocera branchialis*

Adverse effects of *L. branchialis* were observed mainly in juvenile cod infected with two or more parasites. In addition to a significantly lower ($P < 0.05$) K-factor (Table 2), gills of the fish were pale and hemoglobin values (2.6 ± 0.3 g%) were depressed in contrast to controls (4.3 ± 0.3 g%) of comparable length. Among two other groups of juvenile fish (30–35- and 36–41-cm classes) infected with a single parasite, only K-factor was lower than in corresponding controls while hemoglobin values were similar. No apparent change was observed in the subadult (42–47-cm)

or adult (>48-cm) groups. The weights of the liver, heart, spleen and alimentary tract in fish parasitized by a single copepod were not significantly different from uninfected fish of comparable lengths in

TABLE 2. A comparison of condition (K) factor (gutted) and liver somatic index (s.i.) among different size groups of Atlantic cod without (U) or infected (I) with one or two adult parasitic copepods, *Lernaeocera branchialis*, taken from Conception Bay in summer (groups I–IV) and autumn (groups V–VII), 1983.

Group		Mean length (cm)	K-factor	Liver s.i.
I ^a	U	33.3 ± 0.4	0.92 ± 0.03	3.5 ± 0.3
	I	31.8 ± 1.7	0.82 ± 0.02 ^b	3.1 ± 0.3
II	U	38.8 ± 0.4	0.85 ± 0.01	4.5 ± 0.3
	I	39.3 ± 2.0	0.78 ± 0.02 ^b	5.1 ± 0.4
	I ^c	39.1 ± 1.7	0.77 ± 0.03 ^b	3.8 ± 0.6
III	U	43.6 ± 0.3	0.75 ± 0.03	4.5 ± 0.3
	I	43.5 ± 0.4	0.71 ± 0.04	4.9 ± 0.5
IV	U	54.8 ± 0.8	0.73 ± 0.02	4.5 ± 0.2
	I	53.7 ± 0.9	0.73 ± 0.01	4.2 ± 0.4
V	U	42.6 ± 0.4	0.74 ± 0.01	
	I	43.1 ± 0.7	0.76 ± 0.02	
VI	U	55.1 ± 0.6	0.72 ± 0.01	
	I	54.2 ± 0.8	0.74 ± 0.02	
VII	U	63.7 ± 0.7	0.70 ± 0.07	
	I	63.5 ± 1.3	0.70 ± 0.02	

^a 25 fish examined from each of the uninfected and infected groups.

^b Significant differences between treatments in two-way ANOVA ($P < 0.05$).

^c Infected with two *L. branchialis*.

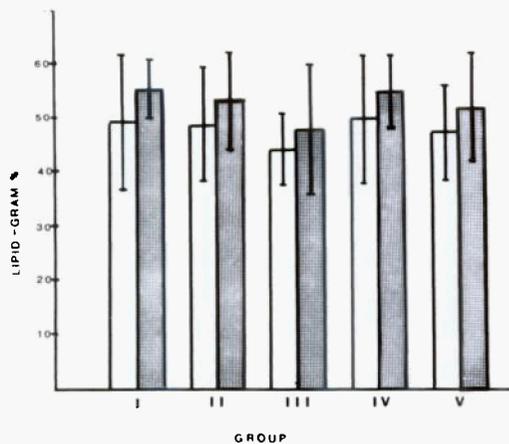


FIGURE 1. Total concentration of liver lipids in different length groups of cod without and infected (shaded) with one *Lernaecera branchialis*. Bars indicated 95% confidence intervals. The concentrations in groups I to III are similar, but are significantly different in the pooled samples (groups IV and V). I = immature (30–39 cm, $n = 16$); II = subadult (41–45 cm, $n = 12$); III = adult (>48 , $n = 25$); IV = total immatures and subadults ($n = 28$); V = total sample ($n = 53$).

all size groups. Among the three length groups (V–VII) examined in autumn, K-factor was similar between infected and uninfected fish.

Lipid concentrations in the liver of cod infected with one *L. branchialis* did not differ significantly from concentrations in uninfected fish of comparable length (Fig. 1). However, the pooled values in the infected groups were significantly higher ($P < 0.05$) than in control fish.

Effects of *Lernaecera branchialis* and *Trypanosoma murmanensis*

The effect of *L. branchialis* and *T. murmanensis* was studied as single or dual infections in subadult and adult cod (Table 3). The most noticeable effects occurred in subadult cod infected concurrently with the two species of parasites. Two cod in this group succumbed to the infections. The gills were pale and hemo-

TABLE 3. Effect of single or dual infections of *Lernaecera branchialis* and *Trypanosoma murmanensis* on condition (K) factor, organ somatic indices (s.i.) and blood values of subadult and adult Atlantic cod held at 10 and 0 C, respectively. Means and standard errors are presented for those factors which differed significantly ($P < 0.05$) between control and infected groups.

Experiment no.	Variable (mean and standard error)	Cod group no.			
		I ^a	II	III	IV
I ^b (subadult)	Initial weight (g)	550 ± 43	493 ± 39	793 ± 24	508 ± 63
	Final weight (g)	737 ± 52	651 ± 34	785 ± 21	498 ± 67
	Final length (cm)	45.4 ± 1.2	41.7 ± 0.8	46.6 ± 0.5	41.4 ± 1.8
	K-factor	0.79 ± 0.02	0.89 ± 0.02 ^d	0.79 ± 0.02	0.69 ± 0.02 ^c
	Liver s.i.	3.9 ± 0.4	5.4 ± 0.5 ^d	2.7 ± 0.3	2.0 ± 0.3 ^c
	Total plasma protein (g%)	49.1 ± 1.5	47.2 ± 4.8	48.9 ± 0.8	32.3 ± 1.8 ^c
	Hematocrit (%)	25.8 ± 1.0	22.8 ± 2.2	23.4 ± 2.1	18.4 ± 1.5 ^c
	Hemoglobin (g%)	4.4 ± 0.2	4.2 ± 0.6	4.1 ± 0.5	2.8 ± 0.3 ^c
II ^c (adult)	Initial weight	1,301 ± 72	1,206 ± 60	1,151 ± 80	1,276 ± 69
	Final weight	1,333 ± 66	1,281 ± 71	1,106 ± 86	1,231 ± 85
	Final length	53.5 ± 0.6	52.3 ± 0.8	51.5 ± 1.0	53.0 ± 1.0
	K-factor	0.86 ± 0.02	0.89 ± 0.02	0.79 ± 0.03 ^f	0.80 ± 0.02 ^f
	Total plasma protein	55.0 ± 2.0	53.2 ± 1.0	49.0 ± 2.3	41.1 ± 2.9 ^f
	Hematocrit	28.3 ± 1.2	27.1 ± 1.1	26.0 ± 1.4	24.1 ± 0.9 ^f
	Hemoglobin	5.4 ± 0.3	5.2 ± 0.3	5.0 ± 0.3	4.5 ± 0.2 ^f

^a I = control; II = *L. branchialis*; III = *T. murmanensis*; IV = *L. branchialis* and *T. murmanensis*.

^b 10 fish/group; 2 died in group IV.

^c Significantly different from controls.

^d Significantly greater than groups I, III and IV.

^e 15 fish/group.

^f Significantly different from *L. branchialis* group.

globin concentrations (<2.0 g%) were depressed while parasitemias of the trypanosome infection were $\sim 1 \times 10^5$ /ml of blood. However, the parasitemias in surviving fish were too few to estimate accurately at the time the experiment was terminated. Control and *L. branchialis*-infected cod increased in body weight by 34 and 32%, respectively, while fish harboring *T. murmanensis* or dual infections decreased by 1 or 2%, respectively, from their initial weights. Condition factor, liver s.i. and blood values were significantly lower in surviving cod harboring dual infections than in controls or fish infected with *L. branchialis* (Table 3).

Since it appeared that dual infections of *L. branchialis* and *T. murmanensis* impaired fish health, an additional experiment was conducted to ascertain the effect of either one or two adult copepods on juvenile cod following infection with the trypanosome. A third group of fish harbored the remnants of the cephalothorax while a fourth was uninfected. Each group consisting of five fish was infected subsequently with *T. murmanensis*. One cod in each of the groups harboring either one or two adult copepods and concurrently infected with the trypanosome died 5–6 wk later. Parasitemias in these fish varied from $2\text{--}5 \times 10^5$ organisms/ml of blood. Following necropsy of the surviving fish 7 wk after infection, a mean decrease of 10, 17 and 29% was noted for K-factor, liver s.i. and hemoglobin, respectively, in cod harboring one adult copepod whereas 18, 25 and 54%, respectively, occurred in the fish-group infested with two *L. branchialis*. No significant alterations were observed in the groups of cod without *L. branchialis* or infected with the remnants of the cephalothorax.

Among adult cod, K-factor in the groups infected with *T. murmanensis* and with dual infections was significantly lower than that of the *L. branchialis* group (Table 3). Moreover, blood values also decreased in

these two groups in contrast to controls or the cod harboring the parasitic copepod.

DISCUSSION

The present study has provided evidence that the highest prevalence (17–21%) of infection with *L. branchialis* which occurred on the southern coast of Newfoundland was similar to that reported previously (Templeman et al., 1976). Unlike many parasitic copepods, the life cycle of *L. branchialis* is dependent on an intermediate host (Kabata, 1958). Possibly, the larval stages are more abundant in this area on the intermediate host, the lumpfish, *Cyclopterus lumpus* L. Atlantic and Greenland cod, *G. ogac* Richardson and occasionally American plaice (the present study) are final hosts for adult *L. branchialis* in the northwestern Atlantic (Templeman et al., 1976) whereas three final hosts are known in the Barents Sea (Polyanskii, 1955) and at least 17 in the North Sea (Kabata, 1970, 1979).

No marked changes in the present study were observed in juvenile cod infected with one *L. branchialis* except a reduction of body weight. Sherman and Wise (1961) reported also that cod in New England waters were not affected by the copepod. Evans et al. (1983) noted that *L. luscii* (Basset-Smith) had no marked effect on first year bib, *Trisopterus luscus* (L.). However, several studies have provided evidence that lernaeocerid parasites, especially in young fish, retarded growth (Debrosses, 1948; Kabata, 1958; Hislop and Shanks, 1979), caused mortality (Slinn, 1970) or induced pathological changes (Kabata, 1958; Mann, 1970; Natarjan and Balakrishnan Nair, 1977; van den Broek, 1978; Hislop and Shanks, 1979). In young cod and haddock parasitized by *L. branchialis*, weight loss varied from 20 to 38% (Kabata, 1958; Mann, 1970). Since lipid and blood values in haddock harboring immature parasites were higher than in controls, Kabata (1958) surmised that par-

asitized fish compensated initially by increasing their food intake. This resulted in elevated body weight, liver lipid and hemoglobin concentrations, but eventually the metabolic demands of the adult parasites resulted in the associated depressed values. The present study was conducted mainly on cod harboring one adult parasite and the effect of the infection, if deleterious, should have been apparent. However, our studies revealed that liver somatic indices and blood values of parasitized fish of distinct size classes were not different from uninfected cod of comparable length, but pooled lipid concentrations were significantly higher in the infected groups.

Stored liver lipid represents an important energy reserve in cod and is a prerequisite for reproduction. Lipid concentrations were lower in fish infected with *L. branchialis* (Mann, 1952; Kabata, 1958). Consequently, delayed development of the gonads and/or reduced fecundity occurred in fish parasitized by the hematophagous copepods (Kabata, 1958; Natarjan and Balakrishnan Nair, 1977; Hislop and Shanks, 1979). Templeman et al. (1976) observed that prevalences of infection in sexually mature cod in the Newfoundland area were lower than in immature fish of the same length and concluded that the parasite delayed the onset of sexual development. However, our results on *L. branchialis* suggest that the parasite had no apparent effect on lipid concentrations and consequently could not have interfered with the onset of sexual maturity. Additional studies on fecundity are needed to elucidate this point of view.

It is also evident from the present study that cod harboring concurrent infections of *T. murmanensis* and *L. branchialis* were affected adversely in contrast to controls of fish infected with one parasitic species. Since *L. branchialis*-infected cod were unaffected during the experimental period, probably the presence of the try-

panosome in dual infections enhanced the effect of the copepod. Additional evidence in support of this hypothesis became apparent in a recent study which investigated the effect of the parasites on fish growth (Khan, unpubl. data). Ten of 12 subadult cod, harboring concurrently the copepod and a 3-wk-old trypanosome infection, died following a period in adequate water flow. Most of these fish had low hemoglobin concentrations (\bar{x} , 3.9 ± 0.6 g%). No other cod, uninfected or harboring a single parasitic species, were affected although they were held in the same tank (5,500-liter capacity). It is known that *L. branchialis* attaches to the conus arteriosus of cod and partial constriction of this area occurs as a result of the host-parasite interaction (Kabata, 1970). Consequently, the volume of blood flow through the gills and general circulation is reduced. According to Kabata (1958), Mann reported that infected fish consumed less oxygen than uninfected fish. Possibly, mortality was associated with hypoxia induced by anemia, a reduced volume of blood flow through the gills and low concentrations of dissolved oxygen in the water. Other studies on concurrent infections have shown also that some protozoans tend to potentiate the effect of another parasite (Phillips and Wakelin, 1976; Cox, 1977; Bell et al., 1984). We suggest that cod which acquire dual infections concurrently in nature either succumb or, because of morbidity, are more vulnerable to predation or environmental stress. This is a possible explanation for the low prevalence of fish infected with the copepod reported in some offshore locations of the northwestern Atlantic Ocean (Templeman et al., 1976).

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