ADDITIONAL OBSERVATIONS ON THE DEVELOPMENT AND TRANSMISSION OF *TRUTTAEDACTNITIS PYBUSAE* ANDERSON, 1992 (SEURATOIDEA: CUCULLANIDAE) OF THE BROOK LAMPREY, *LAMPETRA APPENDIX* (DEKAY, 1842)

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Summary:

Spawning brook lampreys *(Lampetra appendix)* in a small stream in Ontario, Canada were invariably infected with *Truttaedactnitis pybusae* Anderson, 1992 (Seuratoidea: Cucullanidae). The gut in spawning lampreys in May degenerates and relatively few eggs of *T. pybusae* are passed into the spawning area. In prespawning adult lampreys collected in April, the gut was intact and eggs could readily pass into the lamprey beds containing larval lampreys (ammocoetes). It is suggested this is the source of the eggs which eventually produce larvae which infect a high percent of the older ammocoetes. Attempts to infect rainbow trout *(Oncorhynchus mykiss)* with third-stage larvae from the liver of ammocoetes were unsuccessful. Some adult worms intubated into the stomach of *O. mykiss* attached to the gut wall and persisted in diminishing numbers for about three weeks. *T. pybusae* is apparently an independent parasite of *Lampetra appendix* and only an incidental parasite of trout which might consume spawning lamprey containing adult worms.

KEY WORDS : transmission, *Truttaedactnitis pybusae, Lampetra appendix,* lamprey.

MOTS CLÉS : *biologie,* Truttaedactnitis, Lampetra appendix, *lamproie.*

Résumé : OBSERVATIONS NOUVELLES SUR LA BIOLOGIE DE *TRUTTAEDACTNITIS PYBUSAE* ANDERSON, 1992 *(SEURATOIDEA: CUCULLANIDAE)* PARASITE DE LA LAMPROIE, *LAMPETRA APPENDIX* (DEKAY, 1842).

Les lamproies (Lampetra appendix) *en période de reproduction d'un petit ruisseau d'Ontario, Canada, se trouvent toutes parasitées par* Truttaedactnitis pybusae *Anderson, 1992 (Seuratoidea : Cucullanidae). Au mois de mai, l'intestin des lamproies en période de reproduction dégénère et relativement peu d'œufs de* T. pybusae *sont évacués sur les frayères. Par contre, chez les lamproies adultes récoltées en avril, avant la période de reproduction, l'intestin est intact et les œufs du parasite peuvent facilement atteindre les lits contenant les ammocètes. Nous supposons que ce sont ces lamproies qui constituent la source des larves qui infectent un fort pourcentage des ammocètes âgées. Des tentatives d'infestation expérimentale de la truite arc-en-ciel* (Oncorhynchus mykiss) *avec des larves du troisième stade provenant du foie d'ammocètes ont échoué. Quelques vers adultes introduits par intubation dans l'estomac d'*Oncorhynchus mykiss *se sont fixés à la paroi intestinale et ont survécu en nombre de plus en plus faible pendant environ trois semaines.* T. pybusae *est donc vraisemblablement un parasite spécifique de* Lampetra appendix *et parasite seulement accidentellement la truite par suite de l'ingestion de lamproies en période de reproduction contenant des vers adultes.*

INTRODUCTION

The development and transmission of the species of *Truttaedactnitis* (Seuratoidea: Cucullanidae) of brook lamprey, *Lampetra appendix* (= *L. lamottenii*) in North America were first studied by Pybus *et al.* (1978). Adults of the nematode were found only in the intestine of spawning adult lampreys captured the second and third weeks of May; after spawning the lampreys die. Larvae identified as third stage were commonly found in the liver of ammocoetes and transformers. Pybus *et al.* (1978) concluded that during transformation of the ammocoetes to adults, third-stage larvae in the degenerating liver moved to the intestine where they mated and produced eggs. Eggs deposited in the intestine were excreted into the water and carried to ammocoete beds where they would embryonate to first-stage larvae in 18-20 days at 13 °C or in 8-10 days at 22 °C. The larvae would hatch from the eggs and be ingested by filter-feeding ammocoetes. The larvae invaded the liver of the ammocoetes and remained there in the third-stage until the lamprey transformed into adults. The authors concluded that the nematode was exclusively a parasite of the brook lamprey and that it used the ammocoete as an intermediate host and the adult lamprey as a definitive host. The parasite has never been identified from teleosts in North America.

Pybus *et al.* (1978) identified the worms in brook lampreys in Ontario as *T. stelmioides* (Vessichelli, 1910), originally described in *Lampetra planeri* in Italy and now regarded as a synonym of *Truttaedactnitis truttae* (Fabricius, 1794) (= *Cucullanus truttae* of some European authors), a parasite of salmonids in Europe which

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apparently uses the brook lamprey *(Lampetra planeri)* as an intermediate host (Moravec, 1979, 1980, 1994). Third-stage larvae of *T. truttae* are found encapsulated on the intestine of the ammocoete whereas larvae of the species in Canada occur only in the liver. Also larvae of *T. truttae* are smaller than those found in brook lampreys in Canada and were reported to develop to adulthood in trout (Moravec, 1979, 1980). For these, and other reasons, Anderson (1992) thought it prudent to regard the form in brook lampreys in Ontario as a species distinct from that in brook lampreys in Europe and he proposed the name *T. pybusae*. The current hypothesis to explain the transmission of *T. pybusae* is based on the assumption that brook lampreys can shed sufficient numbers of eggs while spawning to infect ammocoetes elsewhere in the stream. This assumption requires further consideration because it is well known that the gut of adult brook lampreys degenerates and forms constriction, a process which might reduce the likelihood of nematode eggs being excreted in numbers by spawning lampreys. In addition, the possible role in transmission of trout which might consume infected spawning lampreys needed further study as well as the infectivity to teleosts of larvae found in the liver of ammocoetes.

MATERIALS AND METHODS

The study was conducted at Blue Springs Creek (latitude 43°36'N, longitude 80°05'W), a tributary of the Eramosa River draining eventually into Lake Ontario, Ontario, Canada. The site was used by Pybus *et al.* (1978) in the earlier study of *Truttaedactnitis pybusae*. Ammocoetes and prespawning adults of *Lampetra appendix* were collected using a portable electrofishing unit at various times during the spring and summer. Spawning adults were easily captured by dip nets the second and third weeks of May. Lamprey were transported in ample water to the laboratory at the University of Guelph. Some were maintained alive for varying periods of time in tanks with a constant flow of well water at 14-15 °C and ample air.

Prior to dissection, lampreys were anaesthetized by immersion in a 0.2 % solution of tricaine methane sulphonate (MS222, Kent Laboratories Ltd.). During dissections of adult lampreys, special attention was paid to the structure of the intestine, the location of nematodes and the presence of nematode eggs in the gut.

Spawning adult lampreys were placed in a large funnel (diameter maximum 30 cm) filled with well water. Rubber tubing and a clamp prevented water from escaping from the funnel and air was bubbled into the water. At various times the sediment was examined for eggs of *T. pybusae*.

Rainbow trout *(Oncorhynchus mykiss)* maintained at 11 °C in well water were given larvae and adult *T. pybusae* in the following ways:

A. *Third-stage larvae in livers of ammocoetes:* portions of lamprey containing the liver were placed in the fish tank after the fish (25-30 cm in length) had been deprived of food for several days. Alternatively, the livers of infected ammocoetes were intubated into the stomachs of trout (17-18 cm in length) anaesthetized with MS222, using a modified Pasteur pipette.

B. *Adult worms from the intestine of spawning lampreys:* the worms were placed in saline, counted and divided into four parts each of which was intubated into an anaesthetized trout (30-31 cm in length) with the modified Pasteur pipette. Fish were fed 5-GR Trout pellets (Martin Feed Mills, Elmira, Ontario) each day and were later examined for the presence of *T. pybusae*.

In addition to specimens of *L. appendix* collected in Blue Springs Creek in 1994-1996, formalized specimens consisting of a few prespawning and spawning adults collected from Blue Springs Creek in April of 1980 were available for study.

RESULTS

Examination of spawning lampreys for adults and eggs of *T. pybusae* in 1994 and 1995

In 1994 spawning began on May 6 and ceased about May 18. On May 6 five spawning lampreys were placed in water in a large funnel and the sediment was drawn off and examined 24 hours later. A single active male worm and some 20 eggs of *T. pybusae* were found in the sediment. Worms were found in all five lampreys at necropsy. In three lampreys, the intestine was enlarged immediately behind the liver and this region contained most of the worms and many eggs in a yellowish, mucus-like material. In one lamprey worms were distributed in all regions of the intestine and in another most worms were found in the posterior third of the intestine. The anus appeared to be patent in all five lampreys and eggs were noted in the region of the anus in two specimens. Eleven days after spawning began (May 17) five lamprey captured on May 8 and maintained in the laboratory were placed in water in a large funnel and the sediment was examined 24 hours later. A considerable amount of brownish sediment was present and about 24 eggs of *T. pybusae* and a single male were found in this sediment. At necropsy, all five lampreys were

infected with 15-35 ($\bar{x}$ = 35) worms. Most worms were found in an enlarged part of the gut immediately behind the liver in mucus-like material containing numerous eggs of *T. pybusae*. The gut seemed to be open at the anus but it was generally reduced in diameter posteriorly.

Three additional lampreys collected while spawning on May 6 were also examined on May 17. The mid-intestine in one lamprey was filamentous and the lumen closed and the anterior region immediately behind the liver was enlarged and contained 25 worms and many eggs in mucus-like material. The lumen of the gut appeared to be open in the remaining two lampreys which contained 19 and 40 worms, mainly in an enlarged region of the gut immediately behind the liver where there were also large numbers of eggs.

In 1995 spawning began on May 7 and ceased about May 18. However, two prespawning adults were obtained by electroshocking on May 3. Both lampreys were infected, one with five males and nine females, the other with 10♂♂ and 10♀♀ of *T. pybusae*. The gut in both lampreys was apparently intact and eggs were found along its entire length. In one of the lampreys, worms extended throughout the gut, in the other, some adult worms were found in the anal region but most worms occurred in an enlarged region behind the liver.

Six spawning lampreys were collected on May 15 and kept at 14 °C in the laboratory. These six lampreys died on the following dates (number of lamprey in parentheses): May 19 (1), May 21 (2), May 22 (1), May 23 (2). Worms occurred in all these lampreys, mainly in an enlarged region behind the liver. Collectively 45♀♀ and 39♂♂ were recovered with a mean and range of 17 (6-26). In three lampreys the gut was collapsed or reduced to a fine filament. Eggs were numerous around the adult worms.

After death of lampreys, the worms remained alive in the carcass (at 14 °C) for about four days and some eggs embryonated in the gut to the early larval stage. Many eggs taken from the gut of decomposing lampreys 2 and 4 days after death embryonated normally at room temperature giving rise to larvae 450 (416-492) µm in length and with lateral alae. Eggs from lampreys dead for 5-6 days degenerated and failed to embryonate.

EXAMINATION OF PRESPAWNING AND SPAWNING ADULTS COLLECTED IN 1980

Eight prespawning adult lampreys were collected by electroshocking at Blue Springs Creek in April 1980 (Table I). These specimens were preserved in 10 % formalin. All the specimens were infected with adult *T. pybusae*. The worms were gravid but more eggs were noted in the anterior uterus than in the posterior uterus. The worms were consistently well distributed along the gut, i. e. there was no accumulation of worms and eggs behind the liver (see above). Eggs were observed in the gut of four of the lampreys.

Lamprey No.	Date collected	Length body (cm)	Number of worms Male	Female	Eggs in intestine	Comments on female worms
1	April 11-20	14.5	14	14	+	Eggs in both uteri, most in anterior. Worms distributed in intestine. Gut complete to anus.
2	April 11-20	15.5	7	14	–	Eggs in both uteri in some specimens, most in anterior. Some worms with eggs in anterior uterus. Gut complete.
3	April 11-20	16.5	1	2	–	Eggs in both uteri, most in anterior. Gut complete.
4	April 11-20	16.4	14	11	+	Eggs in both uteri, most in anterior.
5	April 11-20	16.0	14	12	–	Eggs in both uteri but few and mainly in anterior.
6	April 28	16.0	7	9	+++	Eggs in both uteri, mainly anterior. Gut complete and opening at anus. Numerous eggs in gut.
7	April 28	16.6	13	13	-	Eggs rare in both uteri, mainly anterior. Gut complete and opening at anus.
8	April 28	15.5	11	18	++	Eggs in both uteri, mainly anterior. Eggs (12) present in gut near anus.

Table I. — Results of examining prespawning adult brook lampreys collected April 11-20 and April 28, 1980 at Blue Springs Creek.

Spawning in 1980 at Blue Springs Creek started on May 7-8 and five spawning lampreys were collected and preserved. The following worms were found in these lampreys: 1) 12♀♀, 11♂♂; 2) 4♀♀, 6♂♂; 3) 15♀♀, 11♂♂; 4) 8♀♀, 7♂♂; 5) 6♀♀, 5♂♂. All the female worms were gravid. Careful dissection revealed that the anus was patent and that nematode eggs were found on the rectal mucosa near the anus in two of the lampreys. In some female worms, eggs were equally numerous in both uteri. In two lampreys worms were in a dilation behind the liver.

Attempts to infect trout with third-stage larvae from the liver of ammocoetes

Thirty-three ammocoetes (length $\bar{x}$ = 9.5, range 6.9-16.2 cm) were collected. The livers of 18 were examined for larvae and 50 % were infected. Ten larvae, fixed in hot glycerine-alcohol and cleared gradually in pure glycerine were 1.2 (0.9-1.4) mm in length and had prominent lateral alae extending from the cephalic region to the tail. Because of their delicacy and tendency to become damaged during dissection it was decided to give sections of the body containing the liver of the remaining 15 lampreys to four trout (30 cm in length) from which food had been withheld for one week. The fish, kept at 10-11 °C, ingested the sections of lamprey and were then fed regularly with trout food, but when examined 20, 30, 40 and 56 days later no worms were found.

The livers from six large (11.5-17.5 cm) ammocoetes were intubated intact into the stomach of six rainbow trout (17-18 cm in length) kept at 10-11 °C. The fish were fed regularly with trout food. No worms were found at necropsy 30 days later.

Attempts to infect trout with adult *T. pybusae*

Eight lampreys collected 11 days after spawning began in 1994 contained a total of 199 adult male and female worms. The worms were placed in saline and as rapidly as possible divided into four portions each of which was intubated into the stomach of an anaesthetized trout 30-31 cm in length kept at 10-11 °C.

At necropsy the following was found:

1. 6 days post-infection, 6♀♀ and 4♂♂ were found attached to the posterior end of the intestine. The nematodes were active in saline and females passed eggs. In three females, eggs were approximately equal in number in both uteri. In two females, eggs were more numerous in the anterior uterus than in the posterior uterus.

2. 14 days post-infection, 4♀♀ and 2♂♂ were found attached to the anterior half of the intestine. They were active in saline and deposited eggs. In two of the worms, eggs were present in equal numbers in both uteri. In two worms, eggs were more numerous in the anterior uterus than the posterior uterus.

3. 22 days post-infection, a single ♂ was attached to the posterior quarter of the intestine.

4. 30 days post-infection, no worms were present.

DISCUSSION

The results confirm the findings of Pybus *et al.* (1978) that eggs of *Truttaedactnitis pybusae* are passed into the stream by spawning brook lampreys. It is difficult, however, to account for the small numbers of nematode eggs being passed since large numbers of eggs were often present in the gut of the spawning lampreys. These eggs, however, were found mainly around adult worms in a markedly dilated region of the gut immediately behind the liver; in some individuals this dilation extended to about a quarter of the length of the gut. Eggs and worms in these dilations were in a yellowish, mucus-like material not present in posterior regions of the gut. The gut of lampreys is ciliated (Hardisty, 1979) and possibly cilia activity is largely absent in the degenerating gut of most spawning lamprey and this would account for the relatively small numbers of eggs passed and their accumulation around parent worms. In addition, in some lampreys the central or posterior regions of the gut had collapsed and this would have prevented the passage of eggs to the anal region. A few adult worms were passed by the spawning lampreys but these would have been the result of movements of the worms themselves.

The fate of the few eggs passed into the riffle area where spawning takes place is uncertain although one might speculate that they would be carried along with sediment to ammocoete beds downstream since they are smooth shelled and do not adhere to objects in the environment.

Pybus *et al.* (1978) did not examine prespawning adult lampreys in Blue Spring Creek. Because of weather conditions, ice and high water levels, they are difficult or impossible to collect in March and April in the creek. Pybus *et al.* (1978) noted that 9 of 14 transformers from Big Creek, Ontario (latitude 42°50'N; longitude 80°30'W) flowing into Lake Erie contained adult *T. pybusae*. They did not give the date of collection although it was probably in March or April during the spring thaw. They also did not report if these transformers were passing eggs of *T. pybusae* or if the latter were gravid. The information indicates, however, that

the nematode reaches adulthood well before lampreys are ready to spawn.

In the present study, two prespawning adult lampreys were collected four days before spawning commenced (May 7) in Blue Springs Creek. Both contained mature *T. pybusae* and in one the worms were distributed along the length of the gut which was of uniform diameter whereas in the other most worms were in a dilation behind the liver. Eggs were present along the entire length of the gut of both specimens.

All eight prespawners collected April 11-28, 1980 contained gravid nematodes and had a complete gut with no dilations or constrictions. Relatively few eggs were noted in the gut of two prespawners suggesting that eggs were passing through the gut possibly because of residual ciliary activity. These eggs would be deposited directly on beds containing ammocoetes, a process which probably takes place for at least 2-3 weeks during the maturation of the lampreys. Thus, the source of the eggs responsible for the high percentage of larval *T. pybusae* in ammocoetes in Blue Springs Creek (see Pybus *et al.*, 1978) could be from late transformers and certainly prespawning adults living in close association with ammocoetes, rather than spawning adults depositing a few eggs in a riffle area in the stream.

Attempts to infect trout with third-stage larvae of *T. pybusae* were unsuccessful. Moravec (1979, 1980, 1994) reported that larvae of *T. truttae* found encapsulated on the intestine of European brook lamprey *(Lampetra planeri)* developed to adulthood when fed to brown trout *(Salmo trutta)*. The infective larva of *T. pybusae* occurs only in the liver of ammocoetes of *Lampetra appendix* and is longer than that of *T. truttae* (i. e. 1.1-1.4 µm, Pybus *et al.* 1978; 1.2-1.4 µm, this study *versus* 840-924 µm, Moravec, 1979).

Some adult *T. pybusae* intubated into rainbow trout persisted for some two-three weeks and remained gravid for at least 2 weeks. *T. pybusae* has never been reported in teleosts in North America and appears to be essentially a parasite of the brook lamprey. Teleosts which might predate infected spawning lampreys could acquire transient infections which might help to disperse eggs in the environment but this has not yet been demonstrated.

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