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Seasonal Variation of Pseudophyllidean cestode, *Diphyllobothrium* spp. Infection in *Cyclops strenuus abyssorum* (Copepoda) in Loch Lomond

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Abstract: The seasonal variation in natural levels of infection with procercoids of pseudophyllidean cestodes, *Diphyllobothrium* spp, and the abundance of *Cyclops strenuus abyssorum* (Copepoda) were studied in Loch Lomond, Scotland, From March 1993, to February 1994. The prevalence of infected copepods was found to increase with the temperature of the water, a peak occurring in June, while relatively low levels were recorded between December and March.

The population density of *C. strenuus abyssorum* also exhibited seasonal variation, being higher during the warmer months. Water temperature in Loch Lomond, near Rowardennan, ranged from 3.2 to 16°C. The lowest water temperature was recorded in January and the highest in July.

Key Words: Infection, *Diphyllobothrium* spp., *Cyclops strenuus abyssorum*

Lomond Gölü'nde *Cyclops strenuus abyssorum* (Copepoda)'da Pseudophyllidean Cestode, *Diphyllobothrium* spp. Enfeksiyonunun Mevsimsel Değişimi

Özet: İskoçya, Lomond Gölü'nde *Diphyllobothrium* spp. ile enfekte olan *Cyclops strenuus abyssorum*'un enfeksiyon seviyesi ve yoğunluğu Mart 1993 ve Şubat 1994 tarihleri arasında incelendi. Enfekte copepodların yüzdesi su sıcaklığı ile arttı, Haziran'da en yüksek değerine ulaşırken, nisbeten düşük değerler Aralık ile Mart arasında tespit edildi. *Cyclops strenuus abyssorum*'un populasyon yoğunluğu da mevsimlere göre değişti; soğuk aylarla karşılaştırıldığında, sıcak aylarda daha yüksek bulundu. Çalışma bölgesinde su sıcaklığı çalışma süresince 3.2 ile 16°C arasında değişti. En düşük değer Ocak ayında ve en yüksek değer Temmuz'da kaydedildi.

Anahtar Sözcükler: Enfeksiyon, *Diphyllobothrium* spp., *Cyclops strenuus abyssorum*

Introduction

Seasonal variation in the intensity of infection in Copepods with *Diphyllobothrium* spp. procercoids.

Plerocercoids of th genus *Diphyllobothrium* are common and widely distributed cestode fish parasites, especially prevalent among salmonid fish in the British Isles (2,3). Plerocercoids of this genus are also commonly found encysted in the stomachs of powan from Loch Lomond (4,5). Moreover, Pasternak *et al.* (6) studied the metabolism and behaviour of the freshwater copepod *Cyclops strenuus abyssorum* in Loch Lomond infected with procercoids considered to be *Diphyllobothrium* spp.

Pseudophyllidean cestodes usally require three hosts (1). For example, *Diphyllobothrium dendriticum* eggs are released by means of birds' faeces and hatch in the water. The coracidia are ingested by copepods and develop in to procercoids within this host. In turn, fish become infected by ingesting copepods carrying procercoids. In the fish, the parasite develops to the plerocercoid stage, which is infective to birds. The length of the embryonic development period varies with respect to external conditions. Completion of development to coracidium takes from 8 days to several weeks, depending on the temperature (1).

Although the seasonal dynamics of the infection of intermediate hosts is an important factor in the cestode life cycle in natural waters, relatively few studies have been carried out in this area. Fish being poikilotherms, the effects of abiotic factors on fish tapeworms are pronounced. The development of pseudophyllidean eggs and coracidia is affected by a variety of environmental factors, the most important of which is water temperature (7). Most infections of fish occur during the summer and early autumn, and most tapeworms reach

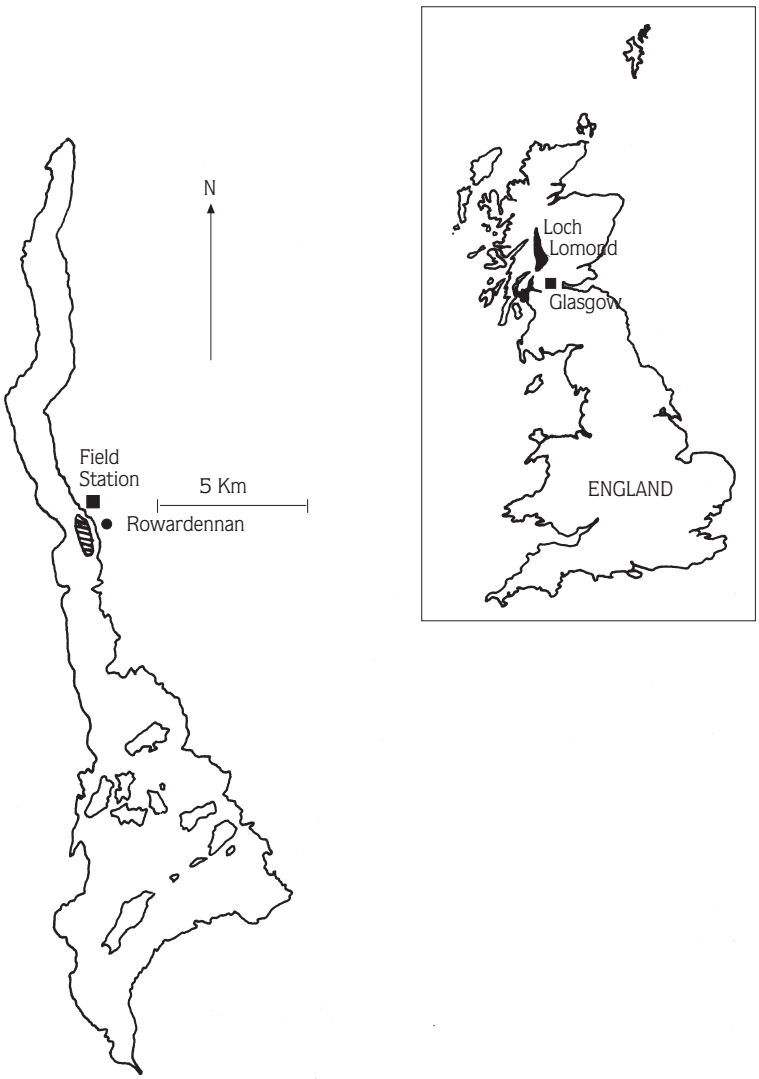

Figure 1. Outline map of Loch Lomond showing sampling area.

sexual maturity and release eggs in late spring and early summer. This is also the time when populations of the invertebrate hosts reach their peak. Differentation into procercoids is also rapid due to high water temperatures and there is sufficient time for infections of fish to occur prior to winter (8). It has been suggested that the critical temperature for the hatching of *Diphyllobothrium latum* eggs is 8°C and that this condition would occur in spring and summer, mainly in the littoral region where the majority of *Diphyllobothrium latum* eggs would accumulate from sewage and animal excreta (9). Previous studies have indicated that natural infections of cyclopoid copepods are greater in summer than in winter (10, 11, 12). For example, Halvorsen (13) found a seasonal variation in the incidence and intensity in infection of female *Eudiaptomus gracilis* with procercoid of *Diphyllobothrium dendriticum.* The infection level was minimum in March, higher in January and April, but maximum in June, July and October. Guttowa (10) examined a total of 1187 specimens from 11 species of Copepoda, finding 40% of *Cyclops strenuus* and 18.5% of *Thermocyclops oithonoides* to be infected with procercoids of *Diphylobothrium latum.* Infection peaked with a prevalence of 52.3% in mid-june, followed by a fall the end of June. In a recent study carried out by Hanzelova (12), infections generally involving a single procercoid of *Proteocephalus* were found in four copepod species, namely, *Eudiaptomus zachariasi, Cyclops vicinus, C. strenuus* and *Mesocyclops albidus.* Two peaks in the intensity of *Proteocephalus neglectus* procercoids in copepods were detected in the summer season. A typical short-term increase in infection intensity was observed in September.

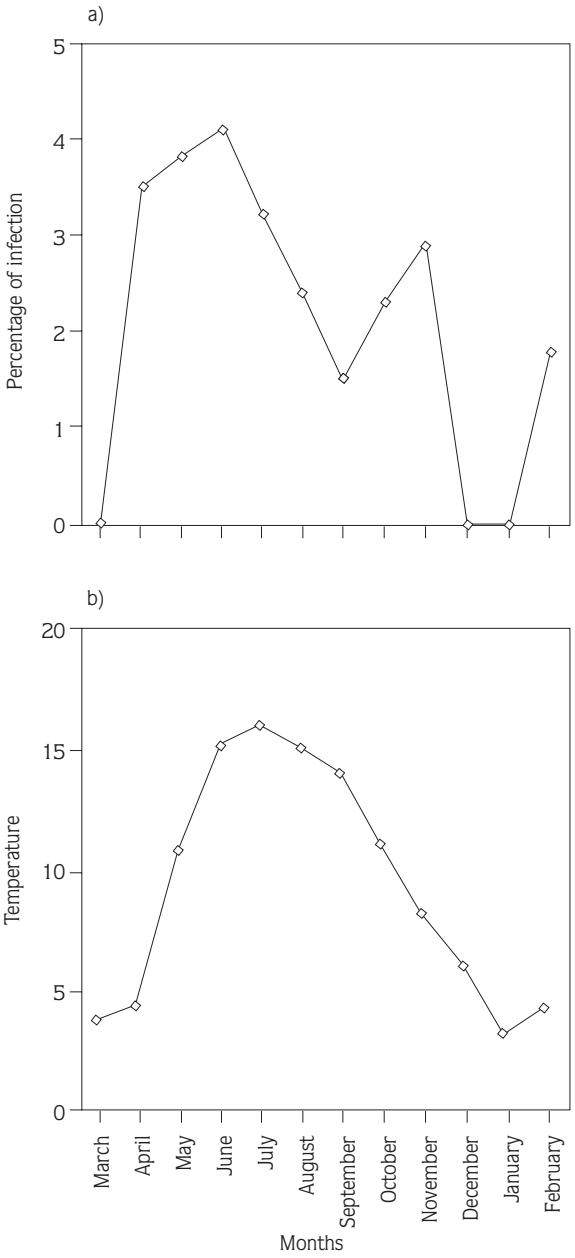

Figure 2. Seasonal changes of (a) the prevalence of infected *Cyclops strenuus abyssorum* and (b) surface water temperature of Loch Lomond at Field Station Bay.

The lack of literature on infection in intermediate cestodes hosts prompted this investigation of the natural levels of infection with procercoids of pseudophyllidean cestodes, *Diphyllobothrium* spp, and seasonal variation in the abundance of copepod species in Loch Lomond.

Seasonal Variation in Copepod Abundance

The copepod species known to exist in Loch Lomond

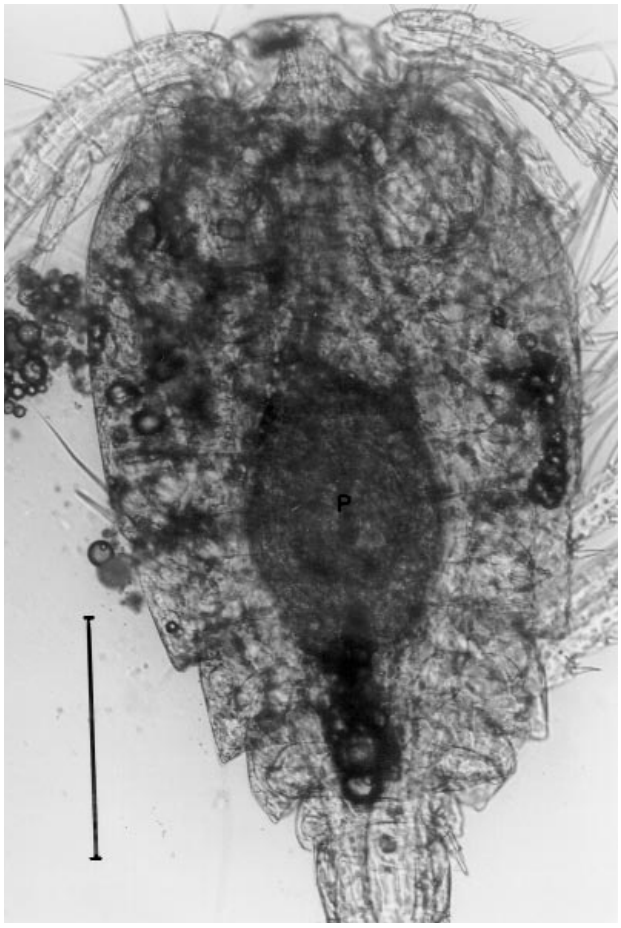

Figure 3. The Cyclops strenuus abyssorum infected with procercoid of Diphyllobothrium spp. (p) procercoid. Bar reperesents 0.3 mm.

are *Cyclops strenuus abyssorum, Diaptomus gracialis* and *Mesocyclops leuckarti* (14).

The copepod community varies not only from year to year but also during the course of a single year. Hanzelova (12) observed that different species were dominant at different times of the year in Dobsina Dam, Czechoslovakia. In years having a regime of decreasing annual temperature, with a cool spring and warm autumn, *Eduiaptomus zacharias* dominated, while a regime of decreasing anual temperature favoured the development of copepods of the genus of *Cyclops.* Hanzelova also recorded a positive correlation between the number of copepods and water temperature. It was reported that in the second half of th second year of the study two of the copepod species (*Cyclops bohater* and *Mesocyclops crassus)* that occured in the lake had not been observed there previously.

Materials and Methods

Study Area

Loch Lomond lies just north of Glasgow (Figure 1), at 56°05' N and 04°35', and has the largest surface area (7112.5 ha) of any of the British lakes (excluding Ireland). It is of glacial origin, which is clearly indicated by the steep-sided narrow and deep upper area. The sampling area chosen for the study is situated near the University Field Station at Rowardennan (Figure 1).

Sample collection

Samples of zooplankton were collected with nets (frame diameter 250 mm; length 0.5 m; mesh 250 µm) from the surface water of Loch Lomond, at the University Field Station near Rowardennan, each month from March, 1993, to February, 1994. The nets were pulled a distance of approximately 500m (repeated 5 times) and samples were poured into 400ml jars. Copepods were counted in 5ml samples, and the number of species in the whole sample and the number of each species per cubic metre wehere then estimated as follows.

$V = \pi.(r)^2.h$

Where:

V = total volume of water filtered

r = half,Diameter of circle of plankton nets

h = distance which nets were pulled

$$N = \frac{400 \times n}{5}$$

Where:

n = mean individual number of zooplankton species in 5ml

N = individual numbers of zooplankton species in 400ml, consequently in total volume of water filtered.

Quantitative plankton samples were collected simultaneously in order to establish the infection level, especially when the number of copepods was low.*Cyclops* species were identified by using the keys in Harding and Smith (15) and individual copepods were examined under light microscope for infection status. Water temperature (°C) was recorded at the time of sampling. Living copepods had to be immobilised to facilitate examination with a compound microscope. This was achieved by placing a copepod on a cavity slide, withdrawing the water and adding a drop of carbonated water to the slide, thereby anaesthetising the copepods.

Table 1. Annual aspects of *Cyclops strenuus abssorum* infected with procercoids of *Diphyllobothrium* spp. in Loch Lomond near Rowardennan.

Months	No of cyclops	Infected	Percentage of	Water temperature
Marc	116	-	-	3.7
April	141	5	3.5	4.3
May	413	16	3.8	10.7
June	290	12	4.1	15.1
July	273	9	3.2	16.0
August	333	8	2.4	15.0
September	534	8	1.5	13.9
October	210	5	2.3	11.0
November	136	4	2.9	8.2
December	38	-	-	6.1
January	63	-	-	3.2
February	55	1	1.8	4.3

Table 2. The numbers of zooplankton species per cubic metre in Loch Lomond near Rowardenan.

Months	*Cyclops abyssorum*	*Cyclops leucarti*	*Diaptomus gracilis*
Marc	23	-	2946
April	32	-	623
May	173	228	45
June	111	496	158
July	85	731	167
August	27	56	119
September	276	-	80
October	52	189	1975
November	150	7	1025
December	36	-	1306
January	8	10	55
February	6	8	287

Statistical Analyisis

Differences in prevalence of *Diphyllobothrium* spp. infection in *Cyclops strenuus abyssorum* between seasons were investigated by the X^2 test. Prevalence of infection was correlated with water temperature using the Spearman Rank Correlation Coefficient. Changes in abundance of *Cyclops* species between monthly sample in the year was investigated by One-Way ANOVA.

Results

Prevalence of *Diphyllobothrium* spp. in *Cyclops strenuus abyssorum*

The numbers of *C. str. abyssorum* infected with

Diphyllobothrium spp. are given in Table 1. The annual cycle of the infection level is shown in Figure 2a.

Overall, 68 out of 26902 (2.61%) *Cyclops strenuus abyssorum* were found to harbour the procercoid stage of *Diphyllobothrium* spp. (Figure 3). The water temperature at the sampling side ranged from about 3-4 °C between January and April, reaching a seasonal high of 16 °C during June, July and August (Figure 2b). The prevalence of a *Diphyllobothrium* spp. in copepods increased with water temperature (Spearman Rank Correlation, $R_s = 0.729$, $n = 12$, $p < 0.01$), reaching its maximum in early spring when the water temperature was rising steeply. The maximum prevalence of infection was recorded in June at 4.1%, and the infection was either low or undetectable from December to March ($\chi^2 = 15.45$, $df = 5$, $p < 0.01$). No *Cyclops strenuus abyssorum* was found to be infected with more than one procercoid (Figure 3).

Abundance of Species in the Copepoda Community

The numbers of copepoda species per cubic metre each month between March 1993 and February 1994 are given in Table 2. Overall, the small calanoid copepod *Diaptomus gracilis* (Sars) was found to be the most abundant species in the zooplankton for the most of the year. *Cyclops strenuus abyssorum* (Sars, and *C. leuckarti* (Claus) were found to be common members of the plankton community in late spring and summer until mid-autumn.

Cyclops strenuus abyssorum occurs in plankton throughout the year, but its abundance changes with the season (Table 2; one-way Anova; $F_{11,48} = 463.62$, $p < 0.001$). In general, the number of individuals was higher during the warm months ($x^2 = 24.57$, $df = 5$, $p < 0.001$). A seasonal peak occurred in September and the second highest number was recorded late spring, with the lowest levels in January and February.

Discussion

Infections of *Diphylloborthrium* spp. in First Intermediate Host and Effects of Temperature on the Life-Cycle of Pseudophyllidean Cestodes and Abundance of Copepods

Cyclops strenuus abyssorum serves as the potential first intermediate host of *Diphyllobothrium* spp. in Loch Lomond. After leaving the egg shell the larva, now called a coracidium, swims by means of its beating cilia. For further development the coracidium must be swallowed by the first intermediate host, a copepod (16). Soon after being eaten, the coracidium loses its ciliated epithelium and immediately begins to attack the wall of the midgut with its six tiny hooks and penetrates the copepod's body cavity. The larva begins to grow there and on the 10th or 11th day the cercomer begins to form. Differentiation is completed on the 14th day, when the parasite enters the procercoid phase.

The onset of the infection process is dependent on two factors: the time of discharge of eggs into water and the effect of water temperature on rate of procercoid development (17). Change in temperature regime and the presence of extraneous substances in the natural environment affect the parasite fauna of animals associated with the aquatic environment or modify seasonal cycles of occurrence and development of helminths (12).

The abundance of cyclopoids, the prevalence and intensity of infection with the procercoid and the rate of development of the cestode depend on ecological and climatic conditions. Annual changes in the pattern of copepod infection with *Diphyllobothrium* spp. were primarily dependent on climatic factors (11, 12). According to Kuhlow (9) and Halvorsen (13), *Diphyllobothrium* spp. eggs hatched in 8 days at 23°C, 10-11 days at 20°C and 48 days at 7°C. There was no development at 0°C.

It can be assumed that procercoids may occur in all months of the year in natural waters, but the incidence of the procercoid is likely to be minimal during the cold months and maximal during the warm months. In colder areas the definitive hosts have seasonal migrations (16). It is clear that these hosts would be infected and release eggs of parasites into the ecosystem only on a restricted seasonal basis. In more temperate regions, however, especially in coastal areas, Laridae may be present all year. In the Hamburg area, Kuhlow (9) found 5.2% of *Larus ridibundus* infected with *D. ditremum* in the winter months. However, from November to January, no *Diphyllobothrian* adults were found, but infection occurred in February (6%), March and April (about 20%). The reason for the appearance of adult worms during these latter months was related to the presence of plerocercoids in *Gasterosteus aculeatus,* and the fact that these fish become available as food for the gulls from February onward.

The low levels of *Diphyllobothrium* infections in *Cyclops strenuus abyssorum* during cold months in Loch Lomond may therefore be due to low water temperature and also the lack of definitive hosts, as a consequence of the availability of parasite eggs. Hanzelova *et al.* (17).

showed that the intensity of infection of *Proteocephalus neglectus* in its first intermediate hosts did not exceed a value of one procercoid. Only in four *Eudiaptomus zachariasi* and in one *C. vicinus* were two procercoids observed. Similarly, in this study, no *C. str. abyssorum* was found to be infected with more than one plerocercoid of *Diphyllobothrium* spp. This may be due to the low numbers of infective coracidia in Loch Lomond's water.

The findings reported here agree with data presented by Watson and Lawler (18), Sergeeva and Freze (19) and Sysoev (11) in that copepod infection with procercoids of *D. latum, D. dendriticum, D. ditremum, Triaenophorus nodulosus, Schistocephalus pungitii, Proteocephalus filicollis* and *Bothriocephalus gowkongensis* occur in high numbers in plankton during the warm period of the year. Sergeeva and Freze (19) identified the procercoid in copepods by examining the definitive and second intermediate hosts. Infections of *D. dendriticum* and *D. ditremum* in those hosts encouraged them to conclude that procercoids in the copepod from Karelia Lake are of *D. dendriticum* and *D. ditremum.* Thus the seasonal pattern of abundance and level of infection in *C. str. abyssorum* with *Diphyllobothrium* spp. in Loch Lomond can be explained in terms of seasonal patterns of temperature changes.

Seasonal Changes in Copepod Abundance

The results of previous studies on seasonal cycles of cyclopoid copepods in Loch Lomond have been variable. For example, Chapman (20) affirmed that *Cyclops strenuus abyssorum* was absent from winter plankton. By constrast, *C. str. abyssorum* was recorded by Maitland *et al.* (21) and Pomeroy (21) throughout the winter in the plankton, although in low numbers. Differences in abundance of the other zooplankton species are also evident in the data recorded by Slack (4), Maitland *et al.* (21) and Pomeroy (22). The abundance of *Daphnia* was found to be variable between surveys, with low numbers recorded by Pomeroy (22). *Holopedium gibberum* was a relatively common seasonal member of the plankton in the loch in the 1970s and 1980s, although not recorded by Slack (4), or Chapman (14,20). The periodicity of the larger Cladocera in the loch is distinct. Numbers of zooplankton taken in hauls in May, 1985, were particullary low compared to May numbers for the previous two years (22). It can be concluded that abundance of zooplankton may vary from year to year, as well as within the year. Seasonal changes in the abundance of cyclopoid copepods can also be explained by alterations in the ecological and climatic conditions in the lake. Adalsteinsson (23) found seasonal variations in the abundance and frequency of crustaceans in Lake Myvatn. The herbivorous cyclopoids *Paracyclops fimbriatus* and *Eucyclops serrulatus* peaked in June and July-August, respectively. Cladocerans peaked in July-August and carnivorous *Cyclops abyssorum* had peaks in May, June and September. *Megacyclops viridis* peaked in July-August. In agreement with most previous studies the abundance of zooplankton species was found to be high during the warm season and low during the cold season.

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