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Effect of Temperature on Oviposition of *Dacylogyrus extensus* Mueller & Van Cleave, 1932 (Monogenea, Dactylogyridae)

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Abstract

Dactylogyrus extensus is a common, economically significant pathogenic monogenean ectoparasite of *Cyprinus carpio*. It attaches itself to its host by means of an opisthaptor containing two hamuli, a connecting bar and 14 marginal hooks.

Smaller *D. extensus* which are found in higher numbers in fish presumably represent the recently invaded individuals. Large worms are found in lower numbers. *D. extensus* does not need to attach itself to the host tissue to initiate egg laying. The rate of egg laying varies with the environmental temperature. The first eggs that are produced *in-vitro* are of larger size but as oviposition continues the size of the egg becomes smaller.

The mean oviposition rates were 2.03, 1.97, 1.45 $eggs^{-1}worm^{-1}$ at 11°C, 13°C and 19°C respectively. The time taken to lay an individual egg was calculated as 29.60 mins, 30.45 mins and 41.38 mins at 11°C, 13°C and 19°C respectively.

Key words: *Cyprinus carpio, Dactylogyrus extensus, in-vitro,* oviposition rates, temperature.

Introduction

Dactylogyrus extensus is a common pathogenic monogenean ectoparasite of fish. It is usually present on the fish throughout the year but is generally more abundant during spring and summer months than during winter months. It attaches to the host by an opisthaptor, which contains two hamuli, a connecting bar and 14 marginal hooks. Several authors have attempted to measure the oviposition rate of monogenans. There is a significant difference in the rate of oviposition of *D. extensus* between the *in-vivo* individuals and those removed from the gills [1, 2]. The rates of oviposition of detached worms are higher than that of the *in-vivo* individuals. Paperna recorded 29 eggs per worm per day at 24°C for *D. vasator* [3].

An increase in temperature will generally lead to more eggs being produced by monogeneans. However, at extremely high temperatures the egg output may fall. Paperna, Prost and Imada & Muroga found this relationship in *D. vasator,* when the temperature ranged from 20-25°C [3, 4,5] . Paperna working in the tropical range of temperatures with *D. vasator,* found that oviposition rates at low (12°C) and high (37°C) temperatures were significantly lower compared with the rates at 24°C and 28°C [3.6]. There was no pronounced difference between the oviposition rates at 24°C and 28°C. This study focused on the ovipositional rate of *D. extensus* at the selected temperatures (11°C, 13°C and 19°C) based on the population studies, where it showed the higher population number at 17°C .

Materials and Methods

Behaviour During Egg Formation and Oviposition

Dactylogyrus extensus were collected from *Cyprinus carpio* from the aquaria of the Institute of Aquaculture, University of Stirling. Carp gills infected with *D. extensus* were left in a petri dish with aquarium water and viewed under a microscope (x40 magnification). The behaviour of 25 worms was followed, for 6 hours in 15 minutes interval, from production of an egg from the ovary to its explusion to the exterior.

Rate of Egg Laying in *In-vitro*

The egg laying rate of *D. extensus* in *in-vitro* was determined for worms at various temperatures, following their separation from the gills. A fine needle was used to separate the worms from the gills and pasture pipette was used to transfer the worm into another petri dish with aquarium water. Care was taken to select only active worms, for each temperature fresh twenty active worms were used. Those which were inactive or apparently damaged during separation from the site of attachment were discarded. Active worms left in aquarium water in a petri dish were studied during the entire duration of their *in-vitro* life. The egg laying rates were observed at 11˚C, 13˚C and 19˚C and expressed as mean ± standard deviation of mean. The number of eggs laid in each 15 minutes period over a total time of six hours was counted. Parasites were then left for further 6 hours and the number of eggs produced was also counted again. The collected data were analyzed by paired t test for the egg laying rate.

The size of the egg at oviposition was measured under a microscope using an eye piece graticule (x 40 magnification).

Results

Behaviour During Egg Formation and Oviposition

Dactylogyrus extensus inhabiting the gills of carp showed a searching behaviour with contraction and relaxation of body wall musculature resulting in extending and shortening of the body. Occassionally immature worms changed their site of attachment by looping in a leech like fashion over the gills; this was not observed in the adults.

In-vitro, the egg producing adult *D. extensus* remained stationary for about 3-5 minutes before the ovum is deposited into the ootype where it remained for 1 – 2 minutes. The ovum then moves forward, along with the vitelline cells deposited around it and enters into the ovovitelline duct as a rather undefined mass. At this stage the worm showed an alternative extension and contracting behaviour, resembles food searching like movment, enable the egg mass to move along the reproductive tract. After another 4 minutes the movement stops and the egg mass is rotated within the ootype for about 2-3 minutes. During this rotation, the mass

acquires a shape closely resembling that of the future egg. It appears as a fairly rigid structure and yellow in colour. After 6 minutes the egg enters the uterus. The egg now has a well defined shape, with increased rigidity and becomes fully tanned. During this process the worm shows alternate searching and resting behaviour. The muscular contractions and relaxations involved in the searching behaviour seem to aid in pushing the developing egg from the reproductive tract to the exterior. The time taken by the egg to pass through the uterus is comparatively longer (5-6 minutes) than the rest of the process. On occasion, an egg was observed to remain in the ootype for up to 15 minutes. During this time no further eggs entered the ootype. Once the egg has reached the genital pore, the worm shows vigorous movements including bending its anterior body against the posterior part for 2-3 minutes to push the egg out of the worm. After the expulsion of the egg, the body straightens and starts to show the typical searching behaviour.

Rate of Egg Laying *In-vitro*

The mean oviposition rates were 2.027(±1.45), 1.97(±1.11) and 1.45(±1.12) eggs hour^{-1} worm^{-1} at 11°C, 13°C and 19°C respectively. The data reveled that a higher proportion of eggs are laid during the early part of the observation period. No eggs were laid between 6 and 12 hours.

The average oviposition rate at three different temperatures shows a parallel decreasing trend (Fig :1). The oviposition rate at 19°C was significantly different at 13°C and 11°C, while there was no significant difference between 11°C and 13°C at probability 0.05.

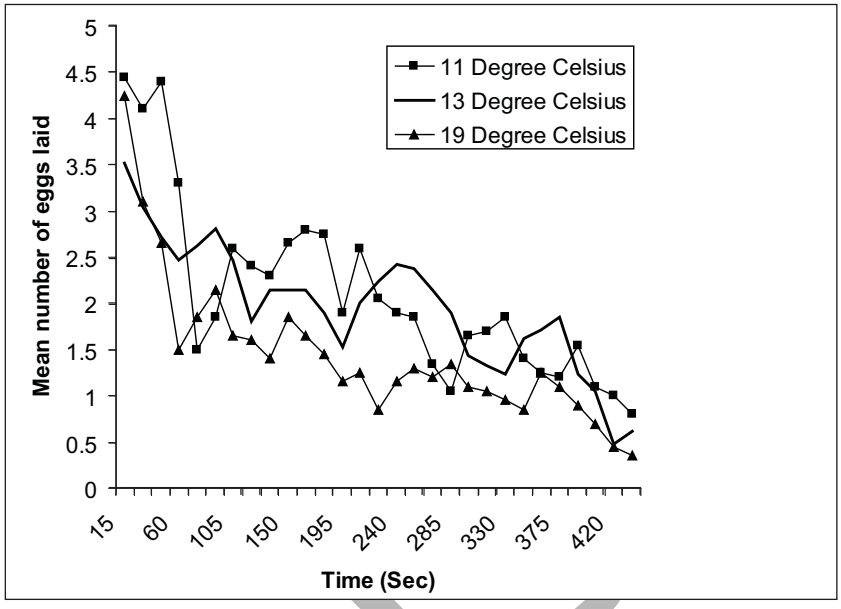

Figure 1: The variation in oviposition of *D. extensus* with time at three different temperatures.

The detached parasites during this study produced eggs (n=3264) of 72.55 x 65.38 µm during the initial *in-vitro* egg laying period and then the size of the eggs gradually decreased to 45.88 x 29.84 µm with time.

Discussion

Dactylogyrus extensus does not need to be attached to host tissue to initiate egg laying process. The process is very similar to that in *Entobdella soleae* [7]. Though the ootype is not clearly evident, the area where the ovovitelline complex is rotated can be regarded as ootype. *E. soleae* eggs expelled from ootype are rigid and slightly tanned whereas the eggs of *D. extensus* are fully tanned, but the whole process of tanning occurs during the passage through the uterus. The laying of fully tanned eggs is obviously an adaptation for the survival of the species in hostile environment, as the tanned eggs are less likely to be damaged by environmental hazards such as extreme cold or warm temperatures, pH, less dissolved oxygen etc [8]. The time taken to lay an egg by *D. extensus* seems to be longer than the other related monogenans. Time taken to

lay an individual egg by *Enterogyrus globidiscus* is 20 – 30 minutes [9], by *Cichlidogryus sclerosus* it was approximately 45 minutes [10]. Estimates on the egg output of dactylogyrid monogeneans differ depending on whether the parasites were attached to the host or not. A higher *in-vitro* oviposition rates than in the *in-vivo* has been reported for *D. extensus* by Izjumova [11] and Lyaiman [1] and for *Dactylogyrus anchoratus* by Prost [4]. Adverse condition *in-vitro* may induce the worms to lay more eggs as an attempt to increase the chances of transmission to other new hosts. Detached parasites being unable to replenish their body resources by feeding, might be expected to produce fewer eggs than attached parasites. However, Prost reported that the attached *Dactylogyrus anchoratus*, laid fewer eggs than detached parasites [4]. The detached parasites during this study produced eggs of 72.55 x 65.38 µm during the initial *in-vitro* egg laying period and then the size of the eggs gradually diminished to 45.88 x 29.84 µm later in the period of observation. If the available reserves for egg production remain fixed throughout the life of the worm it can produce a smaller number of bigger eggs or larger number of smaller eggs. The production of smaller eggs allows the production of greater number of eggs. An interesting question is whether the viability remains the same or low for the eggs laid during the period of observation. Tinsley summarized that most monogenans deposit less than 100 eggs per 24 hours and may deposit fewer than 5 eggs per hour [12]. Paperna reported that the *in-vivo* oviposition rates vary from 7.2 to 48 eggs per hour in *D. vastator* [13]. Following transfer of infected fish to a new experimental environment, egg laying stopped but from the fourth hour after transfer the number of eggs laid increased steadily. In this study, *D. extensus* laid 7.068 ± 4.1729 eggs a day *in-vitro*. During the initial period after separation from gills *D. extensus* may have the eggs laid which may have been formed inside the ootype before separation from the gill.

It has been found that in *D. extensus* attached to the host, the egg output had increased with the age of the parasite for over a period of 10 days [11]. High fecundity of the parasites is associated with high rate of transmission between the hosts in order to compensate the loss due to high mortality [14]. Fecundity is influenced by several other factors too.

The egg output of the individual parasite is decreased with increase in population density and it is often assumed that this is due to an intra-specific competition for limited food resources [14]. *E. soleae* continues to grow after it attains sexual maturity [16] and egg production increases with the increase in size of the worm [17]. Bychowsky described the different shapes of monogenan eggs and their correlation with the form of the ootype [18]. Kearn noted that the egg of *E. soleae* remains a few minutes or longer in the ootype even after the cessation of the ootype movements and during this period egg shell hardens and take up the shape of egg mould [7]. The results of the present study indicate that the eggs of *D. extensus* remain 1-2 minutes in the ootype after the cessation of ootype movements.

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