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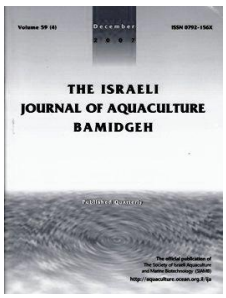

Influence of Temperature and Parasite Intensity on Egg Production and Hatching of the Monogenean *Dactylogyrus extensus*

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Abstract

Dactylogyrus extensus is a species of monogenetic flukes of the family Dactylogyridae that infests cultured as well as wild fish. Understanding its reproductive biology would help control its population. Temperature and parasite intensity influence egg production and larvae hatching. Therefore, we investigated the effect of these parameters on egg production *in vivo* and *in vitro* of *D. extensus* taken from the gills of *Cyprinus carpio* at 10°C and 17°C, and on larvae hatching at 10°C, 17°C, and 25°C. Temperature significantly effected both egg production and larvae hatching. A significantly greater number of eggs was produced at 17°C than at 10°C, but the hatching rate was higher and more rapid at 25°C. Egg production *in vitro* was relatively higher than *in vivo*. Eggs were resistant to cold water and larvae development was inhibited at 2-3°C. However, when the temperature was increased, larvae development commenced and hatching was observed.

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Introduction

Monogeneans are economically important agents of fish disease in aquaculture. They can multiply rapidly in high-density aquaculture environments due to their direct, single host life cycle (Turgut and Akın, 2003). Temperature affects the *in vivo* and *in vitro* reproductive biology of monogeneans (Paperna, 1963; Prost, 1963; Nilakarawasan, 1993; Tubbs et al., 2005; Mooney et al., 2006, 2008) while the intensity of the parasite infection affects egg production and larvae development (Combes, 1972; Jackson and Tinsley, 1988).

The monogenean parasite *Dactylogyrus extensus* Mueller and Van Cleave 1932 occurs on gills of young and adult fish and its pathogenic role in aquaculture is well known (Prost, 1963). Understanding its egg production, development time, larval hatching, and temperature tolerance can be helpful in controlling the parasite population.

The objective of this study is to examine the effect of temperature and parasite intensity on *in vivo* and *in vitro* egg production and larval hatching of *D. extensus*.

Materials and Methods

Specimens of *Cyprinus carpio,* infected by *D. extensus*, were obtained from a fish farm. The host fish were 7.2-11.2 cm (avg 8.97 cm) and kept in 15-l aerated tanks for acclimatization for 4 days at 10°C or 17°C.

Effect of temperature on egg production in vitro. The egg-laying rate was determined at 10°C and 17°C *in vitro* with worms detached from the gills of the carp. Four parasites were transferred to each of three replicate glass embryo dishes (3 cm diameter) with aquarium water at 10°C or 17°C and kept up to 12 h. Care was taken to select only active parasites and inactive or apparently damaged worms were discarded. Laid eggs were collected and counted every hour for 6 h. The worms were left for a further 6 h and any further laid eggs were counted. Standardized Test Procedures (STP) and Dunn's test were used to test the effect of temperature on egg production at 10°C and 17°C.

Effect of temperature on egg production in vivo. Seven fish were kept in each of three 500-ml aerated containers for each temperature (10°C and 17°C) in total darkness for 48 h. Water was changed every 12 h and filtered through a 31.5-µm nylon mesh. Eggs were counted under a dissection microscope. After 48 h, the fish were killed and the gills were removed and examined for the presence of the monogenean. A flat preparation of *D. extensus* was fixed with ammonium-picrate glycerine (Malmberg's fixative) and used to determine the maturity of each parasite. Immature and mature worms were differentiated by the stage of development of the ovary and copulatory organs. STP and Dunn's test were used to test the effect of temperature on egg production at 10°C and 17°C.

Effect of temperature on larval hatching. The effect of temperature on larval hatching was observed using 44, 52, and 64 eggs at 10°C, 17°C, and 25°C, respectively. Eggs laid *in vivo* were collected and approximately five eggs were placed in each of three replicate embryo dishes containing aquarium water at the same temperature as the tank in which the eggs were laid. Larvae hatching was observed under a dissection microscope every 4 h.

Resistance of eggs to low temperature. Parasites were collected from carps at 10°C and allowed to lay eggs *in vivo* as described above. Four eggs were kept in each of three embryo dishes in darkness in a refrigerator at 2-3°C for seven days. Water was changed every 12 h. The embryo dishes were transferred to an incubator at 17°C and larval development and hatching rate were observed as described above.

Effect of parasite infection intensity on egg production. Seven fish were kept for 48 h in each of three 500-ml aerated containers for each temperature: 10°C and 17°C. Water was changed every 12 h and filtered through a 31.5-µm nylon mesh. Eggs and parasites were counted as described above. STP and Dunn's test were used to test the effect of parasite intensity on egg production.

Results

Effect of temperature on egg production in vitro. Mean egg production during 5 h was significantly higher (p<0.005) at 17°C (1.7±1.8 eggs/h/worm) than at 10°C (0.9±1.05 eggs/h/worm). Worms produced the highest number of eggs in the first hour of observation: 1.8±1.7 eggs/worm/h (max four eggs) and 3.5±1.6 eggs/worm/hour (max seven eggs) at 10°C and 17°C, respectively. At both temperatures, the size of the eggs diminished over the period of observation and the number of deformed eggs increased. Moreover, after 5 h, the worms laid eggs without vitelline material.

Effect of temperature on egg production in vivo. The mean egg production over 48 h was significantly higher (p<0.01) at 17°C (0.3-3.4 eggs/worm/h, avg 1.35±0.89) than at 10°C (0.2-0.7 eggs/worm/h, avg 0.4±0.18). All eggs appeared normal and contained vitelline material, and the tanning process was complete.

Effect of temperature on larval hatching. The effect of temperature on larval hatching is shown in Fig. 1. At 10°C, eye spots became visible after 10-12 days of incubation; hatching started after 14 days of incubation and continued for 7 days. The overall hatching rate was 63.46%, however, 4.54% of the developed eggs failed to hatch after 15 days and subsequently degenerated. Nineteen oncomiracidium survived, 79% for less than 24 h and 21% up to 48 h.

At 17°C, eyespots became visible during the second or third day of incubation; hatching started during the fourth day and continued for 7 days. The overall hatching rate was 78.84%, however, 3.85% of the developing eggs failed to hatch, stopped further development after 8 days, and degenerated. Twenty-five oncomiracidium survived, 72% for less than 48 h but 28% up to 78 h.

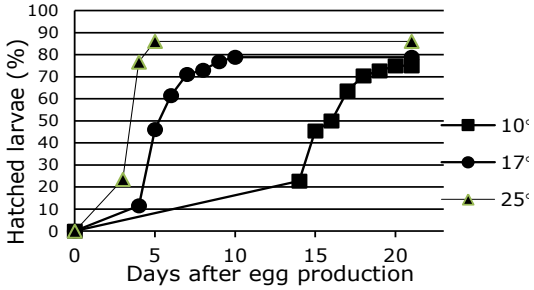

Fig. 1. Percentage of hatched *Dactylogyrus extensus* eggs at 10°C, 17°C, and 25°C.

At 25°C, eyespots became visible on the second day of incubation. Hatching started after 3 days and continued for 3 days. The overall hatching rate was 85.93%. Eighty-five percent of the hatched oncomiracidium remained alive for 9-10 h while 15% survived and remained active, although swimming less rapidly, up to 36 h.

Development was completely inhibited at 2-3°C but, after the incubation temperature was increased to 17°C, commenced. Hatching started after 4 days of incubation and continued for 3 days. Overall hatching was 75%. The oncomiracidium appeared normal and survived approximately 48 h.

Effect of parasite intensity of infection on egg production. There was very weak correlation between the total mature parasite burden and egg production in *in vivo* experiments at both 10°C and 17°C (Fig. 2).

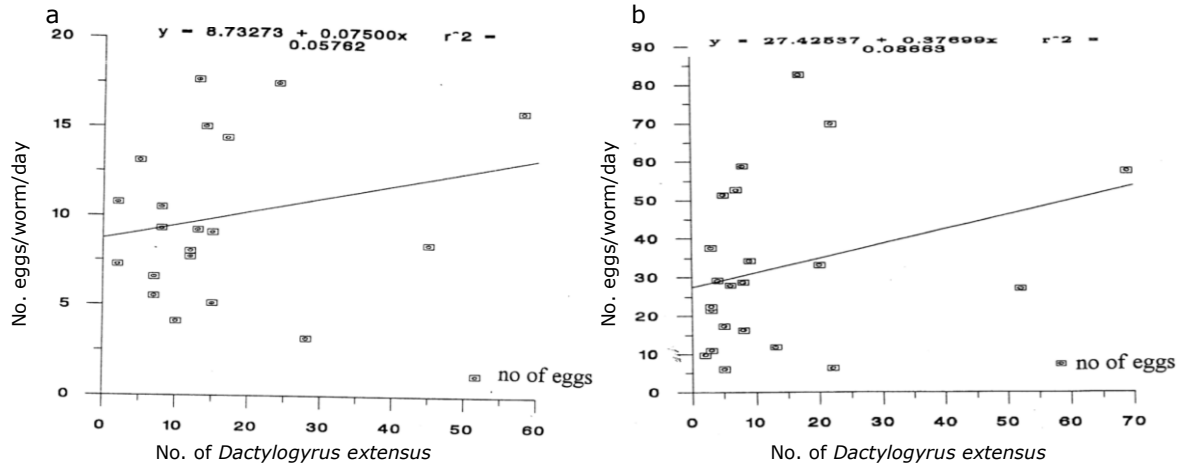

Fig. 2. Correlation between *in vivo* egg production of *Dactylogyrus extensus* from carp and parasite burden at (a) 10°C and (b) 17°C.

Discussion

Temperature had a major effect on egg production, larvae hatching, and the life span of the oncomiracidium. Egg production was significantly higher at 17°C than at 10°C. The worms laid 1.35 eggs/h at 17°C and 0.4 eggs/h at 10°C *in vivo*, compared to 7 eggs/h at 17°C and up to 4 eggs/h at 10°C *in vitro*. Almost all the eggs produced *in vitro* were laid in the first hours after detachment of the parasite from the host. Egg size appears to gradually diminish over the laying period and eggs laid later contained no vitelline material. Presumably, unfavorable *in vitro* conditions caused the higher rate of egg production by detached parasites, i.e., adverse conditions increase fecundity. The diminished egg size and loss of vitelline material suggest exhaustion of the parasite in the absence of a host.

Egg production was measured in *Dactylogyrus vastator* (Izyumova, 1953) and *D. anchoratus* (Prost, 1963). Egg production rates were higher in monogeneans removed from gills than in worms *in vivo*, e.g., *D. vastator* produced 5.1 eggs/worm/h *in vitro*, compared with 9.3 eggs worm/day *in vivo* at 18°C; *D. anchoratus* produced 1.8 eggs/worm/day *in vivo* at 14°C (Prost, 1963).

Temperature has a significant effect on larval hatching in monogeneans and development is generally more rapid at higher temperatures. In the present study, egg development at 25°C was similar to that reported by Prost (1963) but much longer at lower temperatures. Prost (1963) recorded that larval hatching of *D. extensus* eggs takes 3 days at 22-26°C and 8-9 days at 16-17°C. The reason for the discrepancies between the results of our study and those of Prost are unclear but may be related to differences in water quality, seasonality, or age of the parasite. Larval hatching of *D. extensus* eggs takes 6 days at 20°C and 8 days at 16°C (Bauer and Nikolskaya, 1954).

Most monogeneans show a decrease in development time as the temperature increases. Egg development generally ceases at temperatures close to zero, although development of some eggs continues when the temperature is increased (Molnar, 1971; Ogawa, 1998). In our study, *D. extensus* eggs were resistant to cold water and resumed development with 75% hatching success when the temperature was raised. This preliminary result needs further study to determine the effect of adverse conditions on larval development and hatching. Likewise, *Diplectanum aequans* eggs are able to survive at 5°C and resume their life cycle when the temperature rises, behavior that seems to be an adaptation of the parasite to the biological cycle of the host, even though newly-hatched, free-swimming *D. aequans* larvae have little chance of finding a host (Cecchini et al., 2001). Eggs of monogeneans are very resistant to adverse environmental conditions, presumably because of the tanned egg shell (Macdonald and Jones, 1978). Eggs may even pass undamaged through the guts of crustaceans and other small predators. The ability of *Dactylogyrus* eggs to survive cold water conditions is obviously advantageous to the parasite since it allows eggs to overwinter before infecting hosts when environmental conditions become favorable. Such eggs ensure renewed infection of overwintered or newly-hatched fish.

There was a very weak relationship between the number of parasites in infected individuals and egg production due, perhaps, to the small size of our sample. A positive relationship between the number of produced eggs and parasite intensity on hosts was recorded for *Protopolystoma xenopodis*, *Polystoma integerrimum,* and *Cichlidogyrus sclerosus* (Combes, 1972; Tinsley and Owen, 1975; Shaharom-Harrison, 1983; Jackson and Tinsley, 1988). Population density might affect parasite egg production in two ways: intraspecific competition for limited food resources may cause a decrease in egg production while some parasite species might react to unfavorable conditions and high population density by increased fecundity (Combes, 1972; Jackson and Tinsley, 1988).

This study demonstrated the influence of environmental conditions, particularly temperature, on reproduction and larval hatching in *D. extensus*. From here, suitable preventive control measures can be drawn and applied to culture conditions.

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