Effects of Different Strategies of Low Temperature on Egg Hatching of *Dactylogyrus Vastator* (Monogenea: Dactylogyridae)

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Research

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Abstract

Background: The development of dactylogyrids is water temperature dependent, and their eggs fail to hatch below 5°C. The dactylogyrids are supposed to overwinter with adults on hosts or eggs in the water. In the field investigation, however, mean abundance of some *Dactylogyrus* species increases and reaches to a high level in winter, which suggests that the eggs may hatch into infective oncomiracidia in winter. Therefore, effects of low water temperature on egg hatching of *D. vastator* were determined on gills of goldfish (*Carassius auratus*) in laboratory.

Results: The eggs of *D. vastator* hatched and the hatching success was 65.3%, 62.7%, 42.6% and 22.3% when eggs were firstly incubated for 0, 7, 14 and 21 days at 5 °C and then maintained for 15 days at 20 °C. Hatching success in 14 and 21-day group was significantly lower than in 0 and 7-day group. When eggs were directly incubated at 5 °C, eggs failed to hatch within one month. However, the hatching success was 69.8% and 66.7%, respectively, when maintained at 5 °C after12 and 24 h incubation at 20 °C.

Conclusions: Egg incubation for more than two weeks at low temperature had significant impacts on hatching success of *D. vastator* at room temperature. But low temperature had little effects on hatching success when eggs are firstly exposed to suitable temperature range for a short time. Once the embryonic development of eggs is activated, egg hatching will continue regardless of the low temperature. *D. vastator* eggs laid in late autumn are able to hatch in winter.

Background

Monogeneans, belonging to the parasitic Platyhelminth, are usually found on gills, fins and scales of fish, and transmit rapidly because of the direct life cycle and short-generation time [1]. Hooks and hamuli of the opisthaptor penetrate deep into the gill lamellae and produce erosion and inflammation of the epithelium of the primary and secondary lamellae [2]. Some blood-feeding monogeneans on gills of fish cause the anemia [3]. Infection of the ectoparasites often cause great losses of farming fish [4, 5] and wild fish [6].

In the field investigation, different monogeneans species display different patterns of seasonal occurrence [7]. Mean abundance of *Pseudodactylogyrus anguillae* in eel *Anguilla anguilla* [8] and *Dactylogyrus ctenopharyngodonis* on grass carp (*Ctenopharyngodon idellus*) [9] is high in summer and autumn, which are warmth-loving species [7]. Whereas, the number of *D. lamellatus* peaks in late winter and spring [9], which is cold-loving type. Under laboratory condition, development of monogeneans is water temperature dependent [10, 11, 12].

The monogenean *Dactylogyrus vastator* Nybelin, 1924 (Dactylogyridae) is common on gills of common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) [13, 14, 15]. Heavy infection with *D. vastator* results in host death[16, 17]. The egg hatching and the development of *D. vastator* are also shown to be temperature-dependent [1, 18]. Bychowsky (1957) supposes that the adult individuals survive the winter on host and no egg laying and hatching occurs in winter [1]. Actually, mean abundance of *D. lamellatus* on grass carp [9] and *D. vastator* on goldfish (unpublished data) increases and reaches to a high level in winter. This phenomenon suggests that egg hatching maybe occur in winter. Although many researches focus on effects of temperature on development of monogeneans [10, 11, 12, 18, 19, 20, 21, 22], few involve the effect of temperature change on development of monogeneans to simulate the scenario of seasonal variation. Therefore, effects of different strategies of low water temperature on egg hatching of *D. vastator* were investigated on gills of goldfish in laboratory.

Methods

Collection of *Dactylogyrus vastator*

Goldfish infected with *Dactylogyrus vastator* were obtained from our lab [23] and reared in a 40 L glass tank indoor. The water temperature was maintained at around 20 °C and the goldfish were fed daily on commercial pellet feed.

Subsequently, the gills of the goldfish were removed and examined for *D. vastator* under a stereomicroscope. *D. vastator* was collected from the gills using fine needles. Based on body size and color, the matured individuals were selected and placed in 24-well culture plates for egg hatching experiments.

The effect of low water temperature on egg hatching

Eggs laid in the 24-well plate were collected immediately and randomly distributed to 7 groups with 3 replicates per group. At least 60 eggs were included in each group.

To determine whether the duration of egg hatching at low temperature affected hatching success, eggs were incubated for 0 (control), 7, 14 and 21 days at 5 °C, and then transferred to maintain for 15 days at 20 °C. To determine whether the eggs deposited in late autumn can hatch during winter, eggs were exposed to 20 °C for 0 (control), 12 and 24 h, and then maintained for one month at 5 °C.

Eggs were observed under an inverted microscope every 12 h. The empty eggs with open opercula were recorded as the number of hatching success. Hatching success was expressed as the proportion of the number of empty eggs out of the total number of eggs incubated.

Statistical analysis

A Chi-square test was used to evaluate whether there were significant differences in egg hatching success among the different temperature groups. Analyses were performed using the program SPSS 13.0.

Results

The eggs hatched when eggs initially incubated for 0, 7, 14 and 21 days at 5 °C and then maintained for 15 days at 20 °C. The hatching success of eggs declined with the increasing days at 5 °C (Table 1). Hatching success in the groups of 14 and 21 days (42.6% and 22.3%) was significantly lower than the groups of 0 and 7 days (65.3% and 62.7%) ($P < 0.05$). The hatching success incubated for 21 days was significantly lower than that of incubation of 14 days ($P < 0.05$).

No egg hatched after one month of incubation at 5 °C. However, the hatching success of eggs was 69.8% and 66.7%, respectively when eggs were firstly incubated for 12 and 24 h at 20 °C and then maintained for one month at 5 °C (Table 1). The hatching success show no significant differences with tthat of direct incubation at 20 °C (65.3%) ($P < 0.05$).

Discussion

Development of monogeneans is usually water temperature dependent [1, 12, 19]. Hatching times are inversely related to water temperature [10, 21]. Hatching success increases with the rise of water temperature [18, 24]. Eggs fail to hatch at extremely low water temperature [10, 24]. In the present study, eggs of *D. vastator* also failed to hatch when directly incubated at the water temperature of 5 °C. However, the eggs exposed to low temperature hatched when subsequently maintained at 20 °C, and the hatching success decreased with the duration of cold exposure (7-21 days at 5 °C).

These results suggested that a short period of cold shock (within one week) had limited impact on egg viability, whereas long-term exposure to low temperature had serious impacts on embryonic development. Eggs may be arrested at low temperature but retained high viability; their development resumed and hatching occurred after the period normally required at this higher temperature [25]. Eggs of *Pseudodactylogyrus bini* did not hatched when incubated for 10 days at 5 °C, but hatched with 75.5% hatching success after being transferred to room temperature [26]. The eggs of *Heterobothrium okamotoi* did not hatch after 23 days of incubation at 10 °C, but high hatching rates were detected when transferred to 15 °C [22]. The eggs of *Protopolystoma orientalis* and *P. xenopodis* apparently retained a relatively high viability when exposed to 5 °C for a short period (18 h) and then incubated at 25 °C [24]. But all eggs of *P. xenopodis* were found to have died after incubation for 3 months at 10 and 12 °C [27]. More than three weeks of incubation at 5 °C had significant impacts on hatching success of *Diplectanum aequans* eggs before incubation at room temperature [19]. Our results revealed that 2 weeks of exposure to 5 °C appeared to have significant impacts on the hatching success of. *D. vastator*, and the effects of low temperature on egg viability were dependent on exposure time.

Although viability of eggs of *D. vastator* was related to exposure time to low temperature, hatching success was hardly affected at low temperature after short-term incubation at room temperature. This result suggested that eggs of *D. vastator* laid in late autumn could hatch in winter. Generally, monogeneans survived winter mainly as adults on host or eggs in the water (Bychowsky 1957). As a rule, reproduction does not take place during winter for freshwater monogeneans [1]. When directly incubated at low temperature, eggs did not hatch for *Diplectanum aequans* [10], *Pseudodactylogyrus bini* [26], *Heterobothrium okamoto* [22], *Protopolystoma orientalis* and *P. xenopodis* [24, 27].

In the present study, however, a high hatching success was detected when eggs incubated at 5 °C after 12-24 h incubation at 20 °C. This result indicated that short exposure to moderate temperatures activated embryonic development of the eggs of *D. vastator*, and the developing eggs eliminated the effects of low temperature and continued hatching. The eggs of *Entobdellu soleue* are originally colourless inside the reproductive adults, but become darker in the uterus or after laying [28]. The change in colour of the *E. soleue* eggs corresponds with the hardening of the egg shell [29]. Hatching success was significantly higher at day 35 than at day 6 when treated with freshwater and formalin since the harder egg shell was less flexible and more impermeable [30]. Therefore, the eggshell of monogeneans had the capability to protect the developing embryo and unhatched larva from detrimental osmotic effects [31]. Based on this result, some eggs of *D. vastator* laid in late autumn were supposed to hatch during winter.

Seasonal occurrence of *D. vastator* also provided some evidences for eggs hatching in winter. In the field investigation, mean abundance of *D. vastator* in *C. auratus* increased in winter and reached a high level in early spring (unpublished data). In addition, the host goldfish tends to shoal at the bottom of water bodies, where the water temperature is higher than 5 °C. So it is possible that the hatched oncomiracidia have the infective ability to find hosts and locomote to the gills of goldfish in winter.

Conclusions

Long-term egg incubation (more than two weeks) at low temperature had negative impacts on hatching success of *D. vastator* at room temperature. But low temperature had little effects on hatching success when eggs are firstly exposed to suitable temperature range for a short time. Since the eggshell of *D. vastator* had the capability to protect the developing embryo and unhatched larva from detrimental osmotic effects, once the embryonic development of eggs is activated, the developing eggs of *D. vastator* can eliminate the effects of low temperature and continue hatching. That is why some eggs laid in late autumn can hatch in winter. Besides the adults on hosts or eggs in the water, hatched oncomiracidia is also a potential pathway to overwinter.

Declarations

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XPZ performed the laboratory work, performed the analysis and wrote the manuscript. WXL designed the experiment and analysed the data. All authors contributed to the interpretation of the findings and approved the final manuscript.

References

1. Bychowsky BE. Monogenetic trematodes, their classification and phylogeny. Moscow: Leningrad: Academy of science. USSR; 1957.
2. Dezfuli BS, Giari L, Simoni E, Menegatti R, Shinn AP, Manera M. Gill histopathology of cultured European sea bass, *Dicentrarchus labrax* (L.), infected with *Diplectanum aequans* (Wagener 1857) Diesing 1958 (Diplectanidae : Monogenea). Parasitol Res. 2007;100 4:707-13.
3. Yoshinaga T, Kamaishi T, Segawa I, Yamano K, Ikeda H, Sorimachi M. Anemia caused by challenges with the monogenean *Neoheterobothrium hirame* in the Japanese flounder. Fish Pathol. 2001;36 1:13-20.
4. Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, et al. Disease and health management in Asian aquaculture. Vet Parasitol. 2005;132 3-4:249-72.
5. Thoney DA, Hargis WJ, Jr. Monogenea (platyhelminthes) as hazards for fish in confinement. Annual Reviews of Fish Diseases. 1991;1:133-53.
6. Shirakashi S, Yamada T, Yamada T, Ogawa K. Infection dynamics of *Neoheterobothrium hirame* (Monogenea) on juvenile olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel), in coastal waters of Japan. J Fish Dis. 2006;29 6:319-29.
7. Chubb JC. Seasonal occurrence of helminths in freshwater fishes Part I. Monogenea. Advances in Parasitology Volume 15; 1977. p. 133-99.
8. Nie P, Kennedy CR. Occurrence and seasonal dynamics of *Pseudodactylogyrus anguillae* (Yin & Sproston) (Monogenea) in eel, *Anguilla anguilla* (L), in England. J Fish Biol. 1991;39 6:897-900.
9. Yang BJ, Zou H, Zhou S, Wu SG, Wang GT, Li WX. Seasonal dynamics and spatial distribution of the *Dactylogyrus* species on the gills of grass carp (*Ctenopharyngodon idellus*) from a fish pond in Wuhan, China. Journal of Parasitology. 2016;102 5:507-13.

10. Cecchini S, Saroglia M, Berni P, Cognetti-Varriale AM. Influence of temperature on the life cycle of *Diplectanum aequans* (Monogenea, Diplectanidae), parasitic on sea bass, *Dicentrarchus labrax* (L.). J Fish Dis. 1998;21 1:73-5.
11. Jackson JA, Tinsley RC. Effects of temperature on oviposition rate in *Protopolystoma xenopodis* (Monogenea: Polystomatidae). Int J Parasitol. 1998;28 2:309-15.
12. Lackenby JA, Chambers CB, Ernst I, Whittington ID. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. Dis Aquat Organ. 2007;74 3:235-42.
13. Li WX, Zou H, Wu SG, Xiong F, Li M, Ma XR, et al. Composition and diversity of communities of *Dactylogyrus* spp. in wild and farmed goldfish *Carassius auratus*. Journal of Parasitology. 2018;104 4:353-8.
14. Ling F, Tu X, Huang A, Wang G. Morphometric and molecular characterization of *Dactylogyrus vastator* and *D. intermedius* in goldfish (*Carassius auratus*). Parasitol Res. 2016;115 5:1755-65.
15. Ogawa K, Egusa S. Six species of *Dactylogyrus* (Monogenea : Dactylogyridae) collected from goldfish and carp cultured in Japan. Fish Pathol. 1979;14:21-31.
16. Paperna I. Dynamics of *Dactylogyrus vastator* Nybelin (Monogenea) populations on the gills of carp fry in fish ponds. Bamidgeh. 1963;5:31-50.
17. Molnar K. Effect of decreased water oxygen content on common carp fry with *Dactylogyrus vastator* (Monogenea) infection of varying severity. Dis Aquat Organ. 1994;20 2:153-7.
18. Zhang XP, Shang BD, Wang GT, Li WX, Yang X, Li ZY. The effects of temperature on egg laying, egg hatching and larval development of *Dactylogyrus vastator*. Acta Hydrobiologica Sinica. 2011;39:1191-7.
19. Cecchini S, Saroglia M, Cognetti-Varriale AM, Terova G, Sabino G. Effect of low environmental temperature on embryonic development and egg hatching of *Diplectanum aequans* (Monogenea, Diplectanidae) infecting European sea bass, *Dicentrarchus labrax*. Fish Pathol. 2001;36 1:33-4.
20. Hirazawa N, Takano R, Hagiwara H, Noguchi M, Narita M. The influence of different water temperatures on *Neobenedenia girellae* (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack *Seriola dumerili* (Carangidae) and the histopathological effect of this parasite on fish skin. Aquaculture. 2010;299 1-4:2-7.
21. Tubbs LA, Poortenaar CW, Sewell MA, Diggles BK. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. Int J Parasitol. 2005;35 3:315-27.
22. Yamabata N, Yoshinaga T, Ogawa K. Effect of water temperature on the egg production and egg viability of the monogenean *Heterobothrium okamotoi* infecting tiger puffer *Takifugu rubripes*. Fish Pathol. 2004;39 4:215-7.
23. Zhang XP, Li WX, Ai TS, Zou H, Wu SG, Wang GT. The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea). Aquaculture. 2014;420-421:302-7.

24. Jackson JA, Tinsley RC, Du Preez LH. Differentiation of two locally sympatric *Protopolystoma* (Monogenea: Polystomatidae) species by temperature-dependent larval development and survival. Int J Parasitol. 2001;31 8:815-21.
25. Jackson JA: Systematic and ecological studies on the helminth parasites of *Xenopus* species. University of London, London, UK.; 1993.
26. Chan BZ, Wu BW. Studies on the pathogenicity, biology and treatment of *Pseudodactylogyrus* for the eels in fish farms. Acta Zool Sinica. 1984;30 2:173-80.
27. Tinsley RC, York JE, Stott LC, Everard ALE, Chapple SJ, Tinsley MC. Environmental constraints influencing survival of an African parasite in a north temperate habitat: effects of temperature on development within the host. Parasitology. 2011;138 8:1039-52.
28. Kearn GC. Observations on egg production in the monogenean *Entobdella soleae*. Int J Parasitol. 1985;15 2:187-94.
29. Llewellyn J. The evolution of parasitic platyhelminths. In: Taylor A, editor. Third Symposium of the British Society for Parasitology. Blackwell: Oxford; 1965. p. 47-78.
30. Svendsen YS, Haug T. Effectiveness of formalin, benzocaine, and hyposaline and hypersaline exposures against adults and eggs of *Entobdella hippoglossi* (Muller), an ectoparasite on Atlantic halibut (*Hippoglossus hippoglossus* L.). Laboratory studies. Aquaculture. 1991;94 4:279-89.
31. Kearn GC. The eggs of monogeneans. Adv Parasit. 1986;25:175-273.

Tables

Table 1. Effect of low water temperature on the hatching success of *Dactylogyrus vastator*. Treatment 1, the eggs were incubated at 5 °C for different days and then maintained for 15 days at 20 °C; Control 1, the eggs were maintained at 20 °C; Treatment 2, the eggs were incubated at 20 °C for 12 and 24 h and then transferred to maintain for 30 days at 5 °C; Control 2, the eggs were maintained at 5 °C.

Groups	Incubation time			No. of eggs	Hatching success (%)
	20 °C	5 °C	20 °C		
Control 1	0	0	15 d	72	65.3
Treatment 1	0	7 d	15 d	61	62.7
	0	14 d	15 d	115	42.6
	0	21 d	15 d	112	22.3
Control 2	0 h	30 d	0	72	0
Treatment 2	12 h	30 d	0	86	69.8
	24 h	30 d	0	93	66.7

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- egghatching.png