International Journal for Parasitology: Parasites and Wildlife 22 (2023) 113–120

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Reproductive strategies of the parasitic flatworm *Thaparocleidus vistulensis* (Siwak, 1932) (platyhelminthes, monogenea) infecting the European catfish *Silurus glanis* Linnaeus, 1758

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ARTICLE INFO

Keywords:
Thaparocleidus vistulensis
Host-specific monogenean
Silurus glanis
European catfish infection dynamics
Life cycle
Hatching rates
Survival rates

ABSTRACT

The life cycle of *Thaparocleidus vistulensis* (Siwak, 1932), a host-specific monogenean parasite of European catfish (*Silurus glanis* Linnaeus, 1758), was investigated by detailed observation of infection dynamics, egg development, hatching rate and *in vitro* survival rates of the parasite at different life stages at 23 °C. A total of 30 naive fingerlings were infected in three exposure trials by co-habitation with donor fish carrying adult parasites. Two fish were dissected every two days during the 10-day experimental period to explore the development of larvae and juvenile parasites on the host gills. Freshly laid eggs by adult monogeneans were collected and observed daily under a light microscope until hatching. A total of 445 eggs were collected and distributed into wells of 96-well microtiter plates containing filtered fish tank water to determine their hatching rates. A similar method was used to investigate the survival rates of isolated parasites at different developmental stages (larvae, juveniles, and adults). *T. vistulensis* populations on the European catfish in fish tanks increased markedly within ten days, dependent on the severity of the initial infection levels of the donor fish. The first eggs hatched three to four days after oviposition, and the hatching rate peaked on the fifth day (89.7%). The survival rate for freely swimming oncomiracidia without host was 7.4% after five days, whereas isolated juvenile and adult parasites showed a higher dependence of host contact (survival rates three days post-isolation of 0.9% and 1.6%, respectively). The data allows prediction of parasite-host dynamics and may improve control of gill-disease in cultured European catfish stocks in fish farms.

1. Introduction

Monogenean gill parasites in siluriform fish, including the European catfish *Silurus glanis* Linnaeus, 1758, belong mostly to the genus *Thaparocleidus* Jain, 1952 (Dactylogyridea: Monopisthocotylea). The three congeneric species, *T. siluri*, *T. vistulensis, and T. magnus,* were described from the European catfish by Zandt (1924), Siwak (1932), and Bychowsky and Nagibina (1957), respectively. They have previously been assigned to the genera *Ancyrocephalus* Creplin (1839), *Ancylodiscoides* Yamaguti (1937), *Parancylodiscoides* Akhmerov (1964), and *Silurodiscoides* Gussev (1985), before Lim et al. (2001) revised the list of known dactylogyridean monogenean genera and transferred them to the genus *Thaparocleidus,* which was erected based on studies of Indian host fishes (Jain, 1952). In contrast to Europe, where only *Silurus glanis* represents the Old World silurids, a wide range of species within the genus *Thaparocleidus* can be found on a richness of silurid species in the Far East and India. Of the latter, Pandey et al. (2003) re-described the *T. wallagonius* the type-species of the genus, and proposed junior synonym names for some others.

Thaparocleidus vistulensis is the most pathogenic gill monogenean infecting European catfish. It is abundant in natural waters, where it may cause mortalities in juvenile host populations, and in intensively farmed fish all host sizes may be affected resulting in severe disease (Molnár et al., 2019). Due to the evolutionary advantage of their direct

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https://doi.org/10.1016/j.ijppaw.2023.09.010
Received 15 August 2023; Received in revised form 26 September 2023; Accepted 26 September 2023
Available online 29 September 2023

life cycle, without intermediate hosts, the monogenean populations can increase exponentially on the host (Buchmann and Bresciani, 2006) and cause high morbidity and mass mortality in confined environments, such as fish farms, leading to significant economic losses for aquacultural enterprises (Thoney and Hargis, 1991). Basic knowledge and understanding of host dynamics of these parasites may assist the development of management tools, which can be used to control the disease (Buchmann, 1988a). The present study investigated the infection dynamics and life cycle parameters (oviposition, egg development, hatching, and survival of larvae, developing juvenile and adult parasites) for *T. vistulensis* currently eliciting disease in recirculated catfish farms. Based on the observed dynamics, we suggest future control by use of life cycle management.

2. Materials and methods

2.1. Source of fish and parasites

European catfish naturally infected with *Thaparocleidus vistulensis* served a source of parasites for the experiments. They were transported from a commercial fish farm in Hungary and subsequentlymaintained in 50 L flow-through system tanks at Veterinary Medical Research Institute, Budapest, Hungary (HUN-REN VMRI). On arrival, the infected catfish were anaesthetised with clove oil (Javahery et al., 2012), and small samples of the gills were taken using surgical scissors at the first gill arch to confirm the presence of parasites and obtain a rough estimate of their abundance (Gussev, 1983 modified by Bognár et al., in press). Infection was then maintained by co-habitation according to the method of Hutson et al. (2018) with modifications. To ensure a controlled infection of donor fish we added additional oncomiracidia to cohabitation tanks with naïve fish. These infective larvae were produced from parasite eggs settled on glass Petri dishes (with nylon mesh attached to wooden frames), which were placed in tanks with the heavily infected fish from the farm. The eggs were subsequently hatched, whereby oncomiracidia could be used for infection of naïve fish to be used as donor fish in the subsequent infection trials. These were conducted by placing the donor fish with naive juvenile fish, which were hatched and reared in closed non-infected systems (commercial farm). Absence of the monogenean in these fish were confirmed by parasitological examination before experimental start. All infection and experimental studies were performed at 23 ± 1 °C. All the life cycle stages of parasites were collected and maintained in filtered (22 μm pore size) fish tank water.

2.2. Infection dynamics

A total of 30 European catfish fingerlings with a body weight of 6.35 ± 1.84 g and body length of 9.53 ± 1.16 cm were used in three exposure studies. Juvenile fish (n = 10/trial) were cohabited with infectious donor fish (n = 1/trial) (with different infection levels – roughly estimated by the Gussev 1983 method). The estimated number of monogeneans on the gills of donor fish for the First Trial, Second Trial, and Third Trial were <100, <200, and >500, respectively. To determine the intensity of *T. vistulensis* infection throughout the course of infection, two fish were sacrificed every two days during the 10-day experimental period. The fish were weighed and euthanized by decapitation. The branchial baskets of the sacrificed fish were removed from both sides, and the gill arches were separated. The worms found on all lamellae were counted individually under a stereo microscope (Olympus SZ40, Olympus Optical, Tokyo, Japan) at 6.7–40× magnification. The number of parasites found was recorded, and photos were taken using a high-resolution microscope equipped with a digital camera (Olympus DP74).

2.3. Parasite egg development

Monogeneans were isolated from the gill filaments using modified insect pins under a stereo microscope. Gravid specimens of *T. vistulensis* were observed releasing eggs when they were gently disturbed by vigorous shaking in a cavity block containing filtered water from a fish tank. Thereafter, the eggs were randomly distributed in groups (n = 5–10) on concave glass microscope slides, 200 μL of filtered water from the fish tank was added, a cover-slip applied whereafter the slide was stored at 23 ± 1 °C in a humid chamber to prevent evaporation. The time of oviposition was set to "Time 0". The time periods are indicated below as hours post-oviposition (hpo) or days post-oviposition (dpo). The eggs were observed daily under a compound microscope (Olympus BX53) until hatching. Any changes in their morphology were documented and photographed using a high-resolution digital camera (Olympus DP74) mounted on a compound microscope.

2.4. In vitro hatching rates

The hatching rate was determined as an important parameter of the egg development process. *Thaparocleidus vistulensis* eggs were collected as previously described on glass Petri dishes by placing them overnight into tanks of heavily infected fish stock. A total of 445 eggs were collected with a modified butterfly needle and glass Pasteur pipette and immediately distributed into the wells of a 96-well microtiter plate, each containing 100 μL of filtered fish tank water, and subsequently observed daily under a stereo microscope. No water exchange or agitation was applied (Whittington and Kearn, 1988). Hatching rates were recorded daily. These time periods will be expressed below as days post-oviposition (dpo). Hatching success (the percentage of hatched eggs of all the observed eggs) was determined by counting the number of empty egg shells with an open operculum. The experiment was terminated 24 h after the last eggs hatched.

2.5. In vitro survival rates

In these trials, the *in vitro* survival rates of different life cycle stages of *T. vistulensis* (larvae, developing juvenile- and adult monogeneans) (without host) were observed. To achieve this, oncomiracidia (time periods are indicated below as days post-oviposition (dpo)) were obtained by hatching eggs in glass Petri dishes, while developing juvenile (4–6 days post-infection (dpi)) and adult (>10 dpi) flukes were collected directly from the separated gills arches with modified insect pins and butterfly needle. Developing juvenile parasites (n = 204), adults (n = 153) and oncomiracidia (n = 135) were transferred into the wells of a 96-well microtiter plate and maintained at 23 ± 1 °C. The wells contained 50–100 μL of filtered (22 μm pore size) fish tank water. Parasites that showed signs of trauma or died within 1 h of disposition were discarded and excluded from the experiment. The microtiter plates were held under a stereo microscope, and the activity of the flukes, including death was observed and recorded daily. Active flukes were defined as those that swam (for oncomiracidia), showed normal movements with their anterior end, or exhibited a regular vigorous longitudinal contraction. These parasites were transparent and not swollen. Moribund flukes rarely moved spontaneously, but responded to stimulation (when gently touched with the side of a dissecting needle) with slow contractions. Immobile worms, which occasionally were swollen, and repeatedly failed to respond to small nudges with a needle (Grano-Maldonado et al., 2011), were considered dead.

3. Results

3.1. Infection dynamics

Following experimental exposure of European catfish to *T. vistulensis,* we observed a significant increase in the number of gill flukes over the 10 d observation period. The intensity was dependent on the initial infection level of the donor fish (Fig. 1). Fish were affected by the infection level and showed equilibrium disturbances (position upside

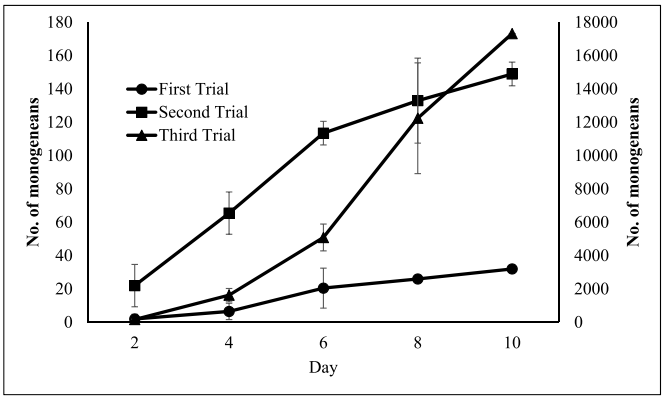

Fig. 1. Average infection dynamics of *Thaparocleidus vistulensis*. The First Trial and Second Trial refer to the primary axis (left side), while the Third Trial refers to the secondary axis (right side).

down in a near vertical position on the 10th day of co-habitation in the Third Trial), where the estimated number of monogeneans attached to the gills were up to 17,000–18,000 individuals. These fish clearly exhibited pathological changes of the gill structure, whereas the lower infection of the fish in the First and Second Trials showed fewer gross pathological changes of the filaments. Thus, the Third Trial showed gills heavily populated by monogeneans (Fig. 2AB), which was associated with various levels of colour changes (from anaemic via pale colour to places to areas with bright red appearance) and lamellar fusion (Fig. 2C and D). Mature monogeneans with an egg inside the body could be observed from 8 to 10 dpi for all trials (Fig. 2C and D).

3.2. Egg development

Eggs were spheroidal in shape; 72.35 ± 5.03 μm (range: 65.40–82.78) in length and 56.20 ± 4.14 μm (range: 47.46–62.44) in width, with a short and hooked polar filament 42.31 ± 6.87 μm (range: 55.07–31.06) facilitating attachment to substrate (n = 30). Mature monogeneans deposited eggs individually. The viable eggs were light brown, while the infertile eggs were darker and sometimes deformed. The development of eggs until the first hatching 3 to 4 dpo showed a uniform pattern. Shortly after oviposition, the eggs, were full of vitelline material (Fig. 3A) and the embryo could not be distinguished (Fig. 3B). Vitelline material was displaced to the periphery of the egg when an organized embryonic mass could be seen inside the egg with a central circle (Fig. 3C). After 24 hpo, the embryo became visible in the center of the egg, surrounded with dispersed vitelline material (Fig. 3D), which gradually decreased during the period of observation. The progressive development of the embryo was detected (Fig. 3E) within 48 hpo. Before the larva was fully developed, two pairs of primordial eyespots were visible as small and scattered accumulation of pigment that gradually condensed into well-defined eyespots. The appearance of a primordial hamulus was also positioned between 48 and 72 hpo of development (Fig. 3F and G). During this time, primordia of aligned ciliated cells were visible (anterior, lateral, and posterior). The anterior part (head) of the oncomiracidium was directed towards the operculum, while the posterior part (haptor) folded backwards. The movement of larva in the egg was limited in this phase. After 72 hpo, the sclerotized structures (central anchor and marginal hooklets) were completely formed (Fig. 3H and I). In this phase, the lively movement of the larva were observed, with the oncomiracidium retracting and elongating in the egg. Ciliary beating was seen in the anterior, lateral-, and posterior ciliated cell groups. Prior to hatching vigorous movements of the oncomiracidium along with the ciliated activity accelerated until the operculum opened, whereafter the

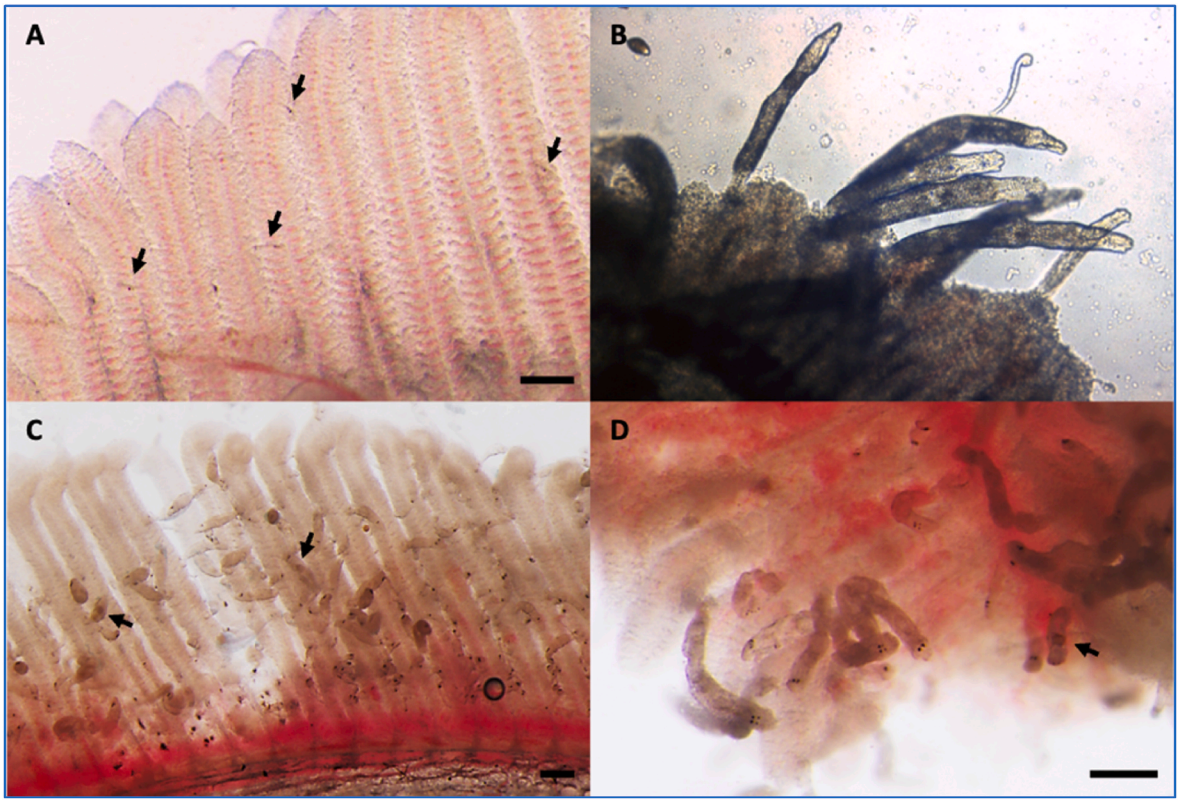

Fig. 2. The gills of infected fingerling European catfish by *T. vistulensis*. (A) Developing *T. vistulensis* attached to the normal gill filaments (arrows) at 2 dpi; (B) Abundance of *T. vistulensis* on the gill at 10 dpi; (C) (D) Sexually mature monogenean with egg inside the body (arrows) situated on the heavily injured gill at 10 dpi. Scale bars represent 200 μm.

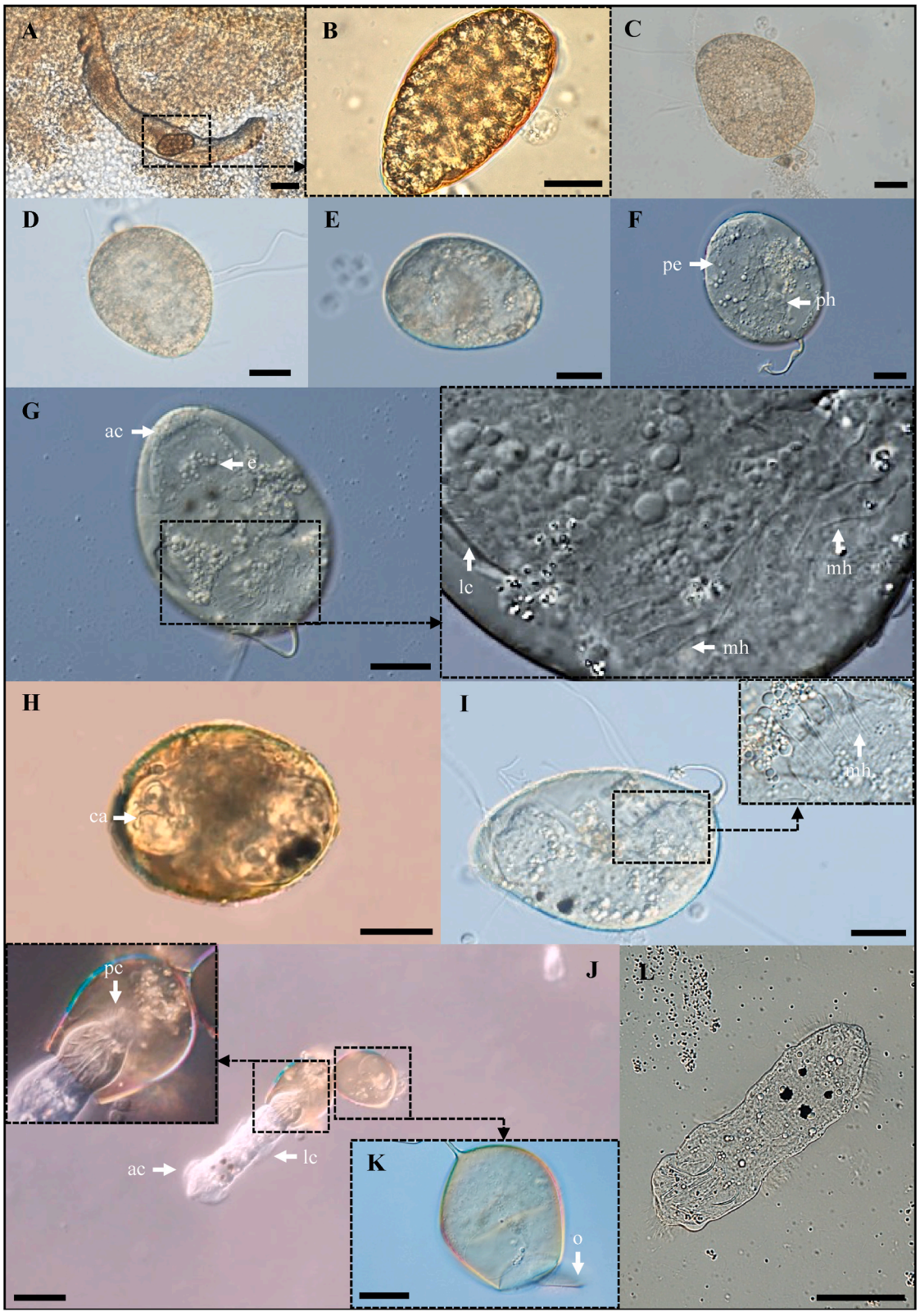

Fig. 3. Light micrographs of egg development of *T. vistulensis*. (A) Adult T. vistulensis with an egg inside its body; (B) egg right after oviposition; (C) Egg after 6 hpo; (D) Egg after 24 hpo; (E) (F) Eggs between 24 and 48 hpo: (E) The whole embryo, (F) Larva with primordia of scattered pigment of eyespots and primordia of hamulus; (G) Eggs between 48 and 72 hpo: Developing larva with marginal hooklets and ciliated cells, ventral view; (H) (I) Eggs after 72 hpo: (H) Developed larva before eclosion with anchors and (I) marginal hooklets, lateral view; (J) Moment of eclosion; (K) Empty egg shell with opened operculum; (L) Recently hatched oncomiracidium. Abbreviations: ac, anterior cilia; ca, central anchor; e, eyespot; lc, lateral cilia; mh, marginal hooklets; o, operculum; pc, posterior cilia; pe, primordial eyespot; ph, primordia of hamulus. Scale bars represent 20 μm except for (A), (J), and (L) 50 μm.

larva could escape by contraction of the body and propulsion by the cilia (Fig. 3J). Empty eggs shells were observed between 72 and 96 hpo (Fig. 3K), and the number of free swimming oncomiracidium emerged in the water column (Fig. 3L).

3.3. *In vitro hatching rates*

The average time for *Thaparocleidus vistulensis* egg hatching was 3 d and the over-all hatching success was 89.7%. The first hatching was detected on 3 dpo at a rate of 12.5% whereafter hatching ended at 84% on 5 dpo (Fig. 4). Most of the eggs hatched between 3 and 4 dpd, resulting in a 72.2% increase in cumulative hatching success, which raised only a further 5% by 5 dpo. No newly hatched eggs were observed after 5 dpo. The unhatched eggs were stuck at some stage of the developmental process, and their colour changed to dark brown.

3.4. *In vitro survival rates*

Isolated parasites (larvae, developing juveniles, adults) were observed *in vitro*, without host. The survival rates of the monogeneans at different life cycle stages differed considerably. While the oncomiracidia could survive for up to 5 days (7.4%), the developing juveniles and adult monogeneans stayed alive for up to 3 days (0.9% and 1.6%, respectively) (Fig. 5). The mobility rate gradually decreased over time and the monogeneans were considered dead when they showed no physical response to an external stimulus. Sometimes their bodies were swollen or opaque white.

4. Discussion

Life cycle studies aiming at revealing the reproductive potential of monogeneans on fish provide a basis for understanding infection processes in fish farms and may eventually lead to development of management and control methods. Due to the amazing adaptability of monogeneans to abiotic and biotic factors over time it is advised to perform these studies for specific systems at specific time periods. Thus, Molnár (1968, 1980) previously published well-supported data on the infection dynamics of *Thaparocleidus vistulensis* and its life cycle parameters, but some deviation was found in the present study focusing on development of embryo in the egg, hatching rate, hatching success, and survival rates without host. It has previously been shown that the main abiotic factors influencing the monogenean parasite population are water temperature, light intensity and salinity (Bauer et al., 1973; Buchmann 1988b; Gannicott and Tinsley, 1997). The main biotic factor is the host species, but in addition the microbiota in the fish farm filters and water phase may affect the life cycle significantly (Buchmann and Bresciani, 2006). We showed that the *T. vistulensis* population on European catfish in fish tanks has a marked propagation potential and may

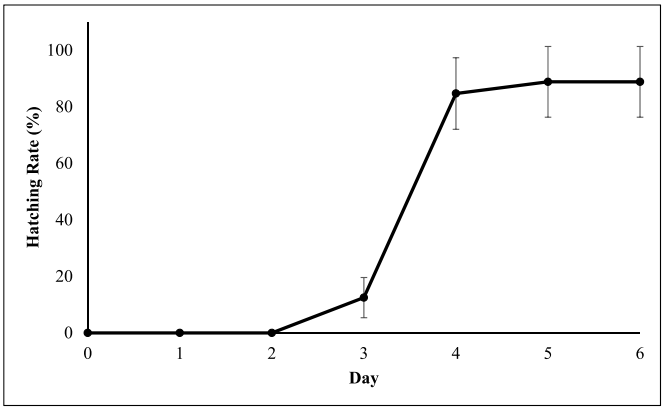

Fig. 4. Average hatching rates of *T. vistulensis* larvae.

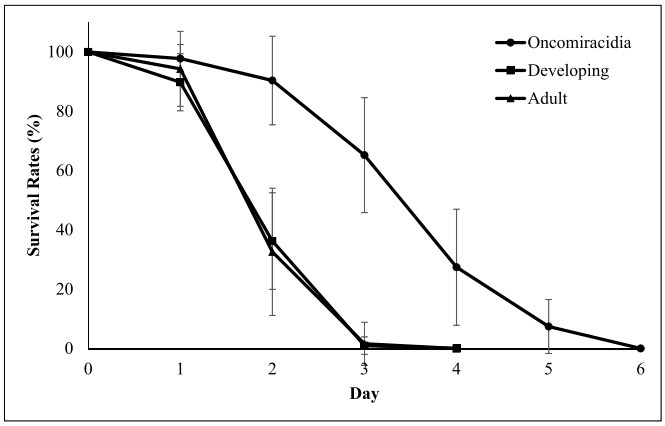

Fig. 5. Average *in vitro* survival rates of *T. vistulensis* at different life stages.

reach devastating levels within 10 days, depending on the severity of the initial infection of the donor fish. This observation should be kept in mind when developing management protocols in fish farms at risk. Quarantine periods of fish before introduction into fish farms is a feasible way to prevent infection (Bauer et al., 1973). The pathology induced by the infection and the associated clinical signs were previously reported by Molnár (1980) but we here indicate that these reactions differ considerably between fish. Thus, it may be suggested that selection and breeding of fish strains with lower susceptibility to a parasitic disease (Jaafar et al., 2020) may be a future approach to reach better health in catfish farms.

An important epidemiological parameter is the generation time. Molnár (1968) attempted to specify the time for a *T. vistulensis* individual to reach maturity and noted adults 14 dpi. Our observation indicated that some of the monogeneans became sexually mature and produced eggs after 8 dpi, which is a highly important factor to consider when forecasting propagation of a parasite population in the farm and for determination for the correct time point for control method installment. Previous studies on *Pseudodactylogyrus* spp. Parasites in recirculated eel farms also showed that adults developed within 7–8 days (Buchmann 1988b).

The reproductive strategies of monogeneans causing diseases in finfish aquaculture have been well summarized by Hoai (2020). Unfortunately, apart from a study by Molnár (1968), there is a little comparative information on reproductive strategies for *Thaparocleidus* species. After oviposition of a mature *T. vistulensis*, the eggs adhere to substrates and hatch to produce a free-swimming ciliated larva. The life cycle of *T. vistulensis* (egg to egg-laying adult) is completed approximately within 13–15 days at 23 ± 1 °C, which corresponds well with Molnár (1968), who showed that the entire life cycle took around 13–14 days at 20–23 °C. The developmental data align very well with other dactylogyrid monogeneans and inspiration for future control methods may be found. One approach is to remove parasite eggs or larvae from the fish farm system. The average size of the eggs (72.35 μm in length and 56.20 μm in width) observed in this study at 23 °C was morphologically consistent with those recorded by Molnár (1968) (67 μm in length and 52 μm in width) at 18 °C, while the average length of the filament was 42.31 μm and 41 μm, respectively. Nevertheless, the average size of the eggs of *T. vistulensis* was smaller than those of *T. gomtius* (84 μm in length and 75 μm in width) and of *T. sudhakari* (87 μm in length and 59 μm in width), which infected the catfish *Wallago attu* (Verma et al., 2017). However, by using the obtained information on egg and larval size it is possible to develop filtration systems in recirculated fish farms. Mechanical filters with a mesh size of e.g. 40 μm can catch both eggs and oncomiracidia and thereby reduce the infection pressure (Buchmann and Bresciani, 2006).

A series of descriptions have been published on embryonic development of various monogenean species, but none of them concerned

members of the genus *Thaparocleidus*. Nevertheless, information on other monogeneans, including *Heterobothrium ecuadori* (Grano-Maldonado et al., 2011), *Sparicotyle chrysophrii* (Repullés-Albelda et al., 2012), and *Dawestrema cycloancistrium* (Maciel et al., 2017), suggests that the main process during egg development of *T. vistulensis* follows a similar pattern to that described for others. This comprises appearance of the primordial hooks and eyespots, followed by the ciliated wreath and finally the operculum for the eclosion of the oncomiracidium (Repullés-Albelda et al., 2012). This process is consistent with the results of the present study where the primordia of scattered pigment of eyespots and the hamulus became visible before the aligned ciliated cells appeared, and vigorous larval movement began. Unhatched, infertile eggs of *T. vistulensis* turned dark brown, in agreement with *D. cycloancistrium* described by Maciel et al. (2017), but in contrast to *H. ecuadori* (Grano-Maldonado et al., 2011), where the eggs became transparent or colorless. The eggs of *T. vistulensis* hatched spontaneously in the same but filtered water that was used for maintaining of the fish and the process included intense larval movements exerting a direct pressure on the operculum. Once the operculum opened, eclosion occurred with the help of stretching and retraction of the body, and propulsion by the cilia. The ciliary wreath covered the anterior, lateral, and haptoral zones of the oncomiracidia, as in other monopisthocotyleans as described by Whittington et al. (2000). In some cases, the larva hatched immediately after manipulation of the egg. Some studies suggested hatching to be hampered by environmental factors like clarity, darkness, shade or the presence of the host (Tinsley and Owen, 1975; Whittington and Kearn, 1988; Gannicott and Tinsley, 1997). Direct predation on eggs by invertebrates in the biofilm microbiota or even anaerobic zones in fish tank filters were suggested to inhibit the *Pseudodactylogyrus* egg development (Buchmann, 1988a).

The hatching dynamics of *T. vistulensis* eggs determinated in the present study agrees with the previously published result of Molnár (1968) and showed great similarity with several other freshwater and marine water monopisthocotyleans species (Table 1). In contrast, the reproductive period of polyopisthocotyleans lasts longer on average (Gannicott and Tinsley, 1998a, 1998b). However, environmental factors, especially temperature, could significantly influence the duration of life-cycle and the reproductive pattern of parasites (Tinsley, 2004; Whittington and Chisholm, 2008). Kearn (1986) emphasized that the duration of development of most monogenean eggs is shortened at higher temperatures. Temperature has also been shown to influence hatching success, and there is probably an optimal hatching temperature for each parasite species (Ogawa, 1988; Gannicott and Tinsley, 1998a; Tubbs et al., 2005). Therefore, further systematic *in vitro* studies on the

Table 1
Aquaculture disease-causing monogeneans and their reproductive strategies.

Subclasses/Species	Host	Macro Habitat	Micro Habitat	Hatching time		Oncomiracidia longevity		Reach sexual maturation		Reference
				dpo	°C	dph	°C	dpi	°C	
Monopisthocotyleans										
Thaparocleidus vistulensis	European catfish (*Silurus glanis*)	Freshwater	Gills	3–5	23 ± 1	5	23 ± 1	8	23 ± 1	Present study
T.vistulensis	European catfish (*Silurus glanis*)	Freshwater	Gills	2.5–3 6–6.5	20–25 15–17	1–1.5	20–21	14	20–23	Molnár (1968)
Pseudodactylogyrus bini	European eel (*Anguilla*)	Fresh and Brackish water	Gills	3–6	20–25	5–6 h	19–26	–	–	Chan and Wu (1984) Buchmann (1988b)
P. anguillae	European eel (*Anguilla*)	Fresh and Brackish water	Gills	2–4.5	20–25	3–5 h	20–25	7–9	25–28	Golovin and Shukhgalter (1979) Buchmann (1990)
Diplectanum aequans	Sea bass (*Dicentrarchus labrax*)	Marine water	Gills	3–6	20–25	–	–	–	–	Cecchini (1994)
Benedenia seriolae	Japanese Yellowtail (*Seriola quinqueradiata*)	Marine water	Skin, fins	5	23	1	–	14	22	Kearn et al. (1992b)
Neobenedenia girellae	Japanese flounder (*Paralichthys olivaceus*)	Marine water	Young: Body, skin, fins. Adult: Skin, mouth, eye regions	5–6	25	–	–	10–11	25	Bondad-Reantaso et al. (1995)
Neobenedenia sp.	Barramundi (*Lates calcarifer*)	Marine water	Skin	–	–	37 h	25	–	–	Militz et al. (2014)
Dactylogyrus extensus	Common carp (*Cyprinus carpio*)	Freshwater	Gills	3	22–25	1–2	25	6–7	24–25	Prost (1963) Turgut (2012)
D. aristichthys	Bighead carp (*Hypophthalmichthys nobilis*)	Freshwater	Gills fillaments	2	30	2–11 h	17–23	11–13	17–23	Musselius (1968)
D. vastator	Common carp Goldfish	Freshwater	Gills	2–3	24–28	<1	24–28	10	24–28	Bauer et al. (1973)
Dawestrema cycloancistrium	Piracucu (*Arapaima gigas*)	Freshwater	Gills, skin	3–4	28–32	50–58 h	24–27	–	–	Maciel et al. (2017)
Polyopisthocotyleans										
Heteraxine heterocerca	Japanese Yellowtail (*Seriola quinqueradiata*)	Marine water	Gills	5	23	–	–	–	–	Kearn et al. (1992a)
Discocotyle sagittata	Rainbow trout (*Oncorhynchus mykiss*)	Freshwater	Gills, mainly secondary gill lamellae	20	18	26 h	22	–	–	Gannicott and Tinsley (1998a), (1998b)
Heterobothrium okamotoi	Tiger puffer (*Takifugu rubripes*)	Marine and Brackish water	Branchial cavity wall, gill fillaments	5.3–11.8	15–25	4–9	15–25	–	–	Ogawa (1998)
H. ecuadori	Bullseye pufferfish (*Sphoeroides annulatus*)	Marine water	Gills	7–10	23 ± 1	4–7	21 ± 1	–	–	Grano-Maldonado et al. (2011), (2015)

Dpo, days post-oviposition; dph, days post-hatching; dpi, days post-infection.

correlation of fecundity of *T. vistulensis* eggs with temperature of should be performed to understand its importance in the life-cycle.

The successful hatching of larvae and finding a suitable host are crucial factors for infection and the continuity of the life cycle (Kearn, 1973; Grano-Maldonado et al., 2011). The average survival ability for oncomiracidia of *T. vistulensis* without host was 5 dpo at 23 ± 1 °C, which predicted a relatively long period of infection. This poses a significant risk and should be considered when forecasting the parasite population increase in the fish farm. In systems with water contact between different fish sizes (ages) it is crucial for spreading and sustaining the parasite population because the incubation period of European catfish eggs at a similar temperature (21–23 °C) averages only 2.5–3 days (Molnár, 1968). The survival rate of the oncomiracidia in this study was higher than in most other monogeneans with the exception of the species *Heterobothrium okamotoi* and *H. ecuadori* (Table 1).

Once the oncomiracidia successfully attached to a host, they lost their ciliated cells, developed to the post-larval stage and eventually matured to the adult stage. In the present study, both developing juvenile and adult *T. vistulensis* showed a similar pattern of *in vitro* survival rate. The survivability of developing monogeneans also can have a significant impact on the host, especially in high stocking density farmed fish such as European catfish. According to our observation, both the developing juvenile and adult *T. vistulensis* could crawl by a vermiform movement on at the bottom of the vessel. This leech-like movement enables immature monogeneans clinging to the body surface to reach the preferred site for permanent settlement (Reed et al., 2012). It means that the parasites can actively crawl longer distances to reach the gills of the host. This peculiarity also provides them with the opportunity to find a new host in the rearing system where fish are in close contact, such as the European catfish, which is known for its sedentary behavior (Copp et al., 2009; Brevé et al., 2014; Slavík et al., 2014) with high site fidelity (Carol et al., 2007) and usually stay at the bottom of the pond/tank. Documented data on the effective transmission of live adult monogeneans between hosts can also be found in the literature (Hutson et al., 2018). The survival rate of mature *T. vistulensis* is as important as that of developing monogeneans, since detached parasites also produce eggs. Since the ability of monogenean parasites to anchor firmly to the host gills is usually quite strong, due to their well-developed haptors consisting of central hooks and marginal hooklets, the displacement is relatively rare (Khang et al., 2016). Under specific circumstances, however, they can become detached by external disturbances such as strong water currents or violent movements by the host fish (Kearn, 2014). It can here be added that the high *T. vistulensis* infection levels observed in catfish in this study was associated with severe inflammation. Therefore we suggest that extensive immune reaction in host gills can induce detachment of the parasites, which could survive in the fish tank for a short time and deliver parasite eggs. To date, several *in vitro* studies have been conducted on the fecundity of mature monogeneans (Tubbs et al., 2005; Turgut, 2012; Hirazawa et al., 2010; Mooney et al., 2008; Maciel and Alves, 2020) showing that the adult parasites could survive for some time and produce eggs even after they have been removed from the gills. Nevertheless, *in vitro* studies for assessing adult parasite fecundity are debated, as starvation of monogeneans separated from their host leads to a progressive decline in egg production rate and quality (Whittington, 1997; Mooney et al., 2008). Therefore, in order to complete the data set for epidemiological models in a fish farm system, it is necessary to perform egg deposition rate studies with adult parasites on the host fish as previously shown for *Pseudodactylogyrus* on eel gills (Buchmann 1988b, 1990).

In conclusion, knowledge of the reproductive strategies of the monogenean species *T. vistulensis* is important, especially for the development of specific prophylactic and therapeutic methods in intensive aquaculture. Forecasting parasite population increases and development of epidemiological models are needed in modern fish farming and studies on basic life cycle parameters as presented in this study are basic elements of this process. The knowledge gained in the present study can therefore provide a valuable basis for the control of *T. vistulensis* in cultured European catfish stocks in fish farms.

Declaration of interests

None.

Funding

This project was funded by the European Union's Horizon 2020 research and innovation program under Marie Sklodowska-Curie grant agreement No. 956481.

Conflict of interest and authorship conformation form

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript.

Acknowledgments

The authors acknowledge the Institute for Veterinary Medical Research, Budapest, Hungary, for the facilities provided for the study. The authors thank Mr. Gergely Lajos Zöldi for maintaining fish in the laboratory.

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