

REPRODUCTION AND SURVIVAL UNDER DIFFERENT WATER TEMPERATURES OF *GYRODACTYLUS MEXICANUS* (PLATYHELMINTHES: MONOGENEA), A PARASITE OF *GIRARDINICHTHYS MULTIRADIATUS* IN CENTRAL MEXICO

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ABSTRACT: Gyrodactylid population growth may depend on abiotic variables such as temperature. We tested the survival and reproductive rate of *Gyrodactylus mexicanus*, a parasite infecting fins of *Girardinichthys multiradiatus*, at 3 different water temperatures, 10–13, 19–22, and 24 °C. The temporal sequence of birth and age at death of each parasite isolated from the hosts was recorded through at least 8 generations. Our results showed that the average number of offspring per parasite was 2.0 when averaged across all temperatures. However, the generation time was negatively correlated with temperature. The innate capacity for increase (r_m) was positively correlated with water temperature: from 0.29 parasite/day at 13 °C to 0.48 parasite/day at 24 °C. These data confirm that water temperature has a direct influence on parasite population dynamics. The current study represents the first contribution to understanding the population ecology of the monogenean *G. mexicanus* in central Mexico.

Species of *Gyrodactylus* von Nordmann, 1832, are ectoparasites that inhabit the skin, fins, and gills of a great variety of marine, freshwater, and brackish water fishes. Over 400 valid species of the genus are currently recognized worldwide, and they are considered as one of most invasive fish parasites due to their viviparous mode of reproduction and exponential growth rate (Cable and Harris, 2002; Harris et al., 2004).

In Mexico, only 9 species of *Gyrodactylus* have been described, most from the Neotropics. Three of these species occur on Mexican poeciliid fish (*Gyrodactylus bullatarudis* Turnbull, 1956, *Gyrodactylus jarocho* Rubio-Godoy, Paladini, García-Vásquez, and Shinn, 2010, and *Gyrodactylus xalapensis* Rubio-Godoy, Paladini, García-Vásquez, and Shinn, 2010). Three other species occur on introduced cichlids (*Gyrodactylus cichlidarum* Paperna, 1968), on characids (*Gyrodactylus neotropicalis* Kritsky and Fritts, 1970), and carp (*Gyrodactylus sprostonae* Ling, 1962). Another 3 species (*Gyrodactylus elegans* von Nordmann, 1832, *Gyrodactylus lamothaei* Mendoza-Palmero, Sereno-Uribe, and Salgado-Maldonado, 2009, and *Gyrodactylus mexicanus* Mendoza-Palmero, Sereno-Uribe, and Salgado-Maldonado, 2009) occur on endemic goodeines (see Mendoza-Franco et al., 1999; Salgado-Maldonado et al., 2001; Mendoza-Palmero et al., 2007, 2009; Rubio-Godoy et al., 2010).

Gyrodactylids are viviparous and produce in most cases a single offspring at each birth. Sexually mature monogenoids give birth to the first progeny after 1 day, a second after 5–6 days, and a third after 10–12 days. This reproductive pattern has been observed throughout at least 12 generations (Cable and Harris, 2002). Some gyrodactylids are cold-water stenotherms (Malmberg, 1973). The water temperature affects both the birth and mortality processes of *Gyrodactylus* spp., which determine the population growth (Jansen and Bakke, 1991). As a consequence, infection rates and population dynamics are related to seasonal and geographic variation. *Gyrodactylus mexicanus* is the only species found thus far at Chicahuapan Lake in central Mexico, where it infects the body surface (fins and skin) of a species of goodein (Mendoza-Palmero et al., 2009). In the Neotropical region of Mexico, the water temperature is relatively constant during the whole year, in contrast to water bodies located in the Neartic region, where there is marked seasonal variation in water

temperature. The present study was designed to determine the effect of water temperature on the survival and reproductive rate of *G. mexicanus*.

MATERIALS AND METHODS

The goodeids used in the present experiments, *Girardinichthys multiradiatus*, were caught using hand dip nets in Chicahuapan Lake, which is located about 26 km west of Toluca, Estado de México (19°10'N, 99°29'W, 2670 m). Fish were maintained alive at room temperature in aquaria. To remove all ectoparasites, individual fish were treated with a 1:4,000 formalin solution for 1 hr and examined using stereomicroscopy daily for 1 wk to guarantee they were free of monogenoids (Lester and Adams, 1974).

Three temperature ranges were selected: 10–13, 19–22, and 24 °C, representing the minimum and maximum temperatures observed in Chicahuapan Lake during the year. In total, 136 monogenoid-free fish were acclimatized (22 fish at 13 °C, 95 at 22 °C, and 19 at 24 °C) under natural day length in dechlorinated water for a minimum of 24 hr prior to each experiment.

To estimate the reproductive rate of *G. mexicanus*, a single monogenoid was attached to an uninfected fish, using the following method. The uninfected fish had been anesthetized with a 1:1,000 solution of acetone (dimethylketone alpha methyl quinoline) and placed near the infected fish, stimulating the monogenoid to pass from fish to fish. The newly infected fish was placed in a container with 250 ml of water and examined daily until the parasites were dead. All observations were made using a stereomicroscope.

A newly produced monogenoid offspring contained an embryo, which distinguished it from the parent whose uterus was empty. A second way to distinguish newborns from the parent was to place the infected fish in a 1:10,000 solution of neutral red for 1 hr before reproduction. The parent monogenoid retains the stain for over 24 hr, and the newborn is unstained (Lester and Adams, 1974).

To maintain a single monogenoid infestation on each experimental fish, the daughter or parent was immediately removed and placed on an uninfected fish using the previously described method. The longevity of the parasite was recorded from day 0 (the first observation of the parasite) to the day that the monogenoid died. The survival was estimated as a percentage of the monogenoids still living from the previous observation, assuming a stable age-structure, a constant growth rate, and absence of inter- and intraspecific competition and migration.

Data obtained from isolated parasites were used to design a life-span table, estimating reproductive rate (in the case of hermaphroditic organisms such as *G. mexicanus*, R_0 , equals the mean number of offspring/parasite), and generation time (G). The innate capacity for increase (r_m) was estimated by the Lotka equation (see Krebs, 1985) as follows: $\exp(-r_m x) l_x b_x = 1$. The generational times were tested with a 1-way ANOVA after confirming normality and homoscedasticity of variances. The life-span table was treated via the Kaplan-Meier method using a graph with cumulative proportion survival and after comparing

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the survival curves at each temperature (10–13, 19–22, and 24 °C). The 3 survival curves were analyzed using a chi-square test. All tests were performed with a significance level of 0.05 and using the software STATISTICA version 7. Vouchers of *G. mexicanus* were deposited in the Colección Nacional de Helminthos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, México City, under numbers 6307–6308, 6745–6748, and 7128.

RESULTS

Of the 136 infected fish, 84.5% of the monogenoids ($n = 115$) established successfully, 20 monogenoids at 10–13, 76 at 19–22, and 19 at 24 °C. The longevity of *G. mexicanus* was up to 17 days. The 3 survival curves at different temperatures were significantly different from each other ($X^2 = 16.43$, $df = 2$, $P = 0.00027$). The survival proportion obtained by the Kaplan Meier equation showed differences between temperatures, i.e., 50% survival was 7 days at 10–13 °C, 6 days at 19–22 °C, and 4 days at 24 °C (Fig. 1). The maximum number of offspring observed from each parasite was 2 for all temperatures, and the mean number of offspring is shown in Figure 2.

The net reproductive rate (R_0) was 3.54 for 10–13, 2.85 for 19–22, and 4.26 for 24 °C. The generation time of the monogenoids decreased with increasing water temperature (Fig. 3) and was significantly different at the different temperatures ($F_{2,15} = 10.780$, $P = 0.00125$). The innate capacity for increase (r_m = parasite/day) improved with higher temperatures (Fig. 4). Based on these values, the population of *G. mexicanus* doubles in size after approximately 3 days at 10–13 °C, 4 days at 19–22 °C, and 5 days at 24 °C, assuming a stable age-structure, a constant growth rate, and absence of inter- and intraspecific competition and migration.

DISCUSSION

The population biology of the monogenoid *G. mexicanus* on the freshwater fish *Girardinichthys multiradiatus* is described for the first time. The present study confirms that the water temperature strongly affects the population buildup of this species of ectoparasite. The longevity of *G. mexicanus* was up to 17 days and was negatively correlated with the water temperature.

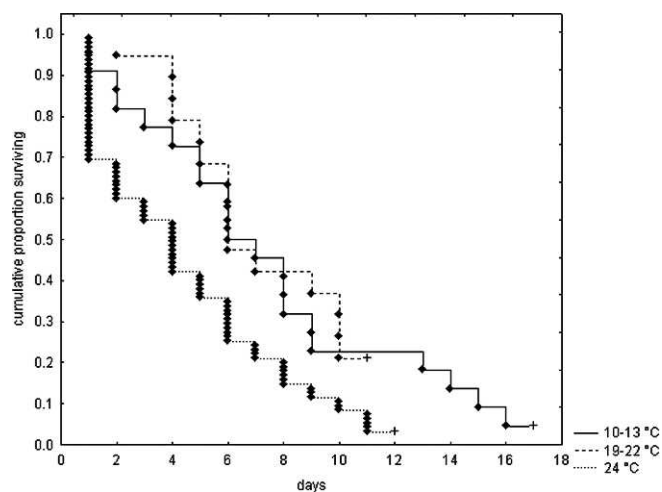


FIGURE 1. Cumulative proportion of *Gyrodactylus mexicanus* surviving at 3 temperatures (10–13, 19–22, and 24 °C) using the Kaplan-Meier method.

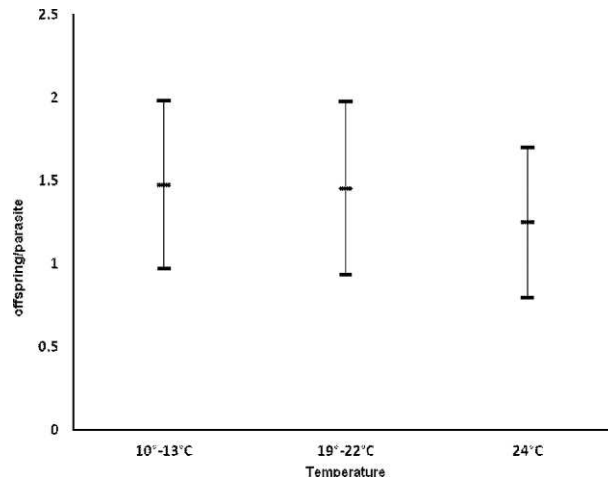


FIGURE 2. Mean number of offspring/parasite for individual isolated *Gyrodactylus mexicanus* on *Girardinichthys multiradiatus* in relation to temperature.

Previous studies with 2 other congeneric species, i.e., *Gyrodactylus derjavini* on rainbow trout and *Gyrodactylus salaris* on Atlantic salmon, indicate a longevity of 35 days for both species (Jansen and Bakke, 1991; Andersen and Buchmann, 1998; Robertsen et al., 2008). Kennedy (1970, 1972, 1985, 1994) suggested that water temperature is an important factor in the maturation, reproduction, longevity, and successful transmission of gyrodactylids.

The number of progeny recorded for *G. mexicanus* was 2 at all temperatures tested, and it is similar to several species in the genus, including *Gyrodactylus alexanderi*, which produces 2 offspring (Lester and Adams, 1974). In contrast, Bychowsky (1957) and Jansen and Bakke (1991) found that the maximum number of offspring produced by other *Gyrodactylus* species was between 3 and 4.

The population growth rate, expressed by the innate capacity for increase (r_m), observed for *G. mexicanus* under laboratory conditions was positive over all temperature ranges (10–13, 19–22, and 24 °C) tested in our experiments, with an optimum value of r_m set at 24 °C. The optimum population growth of *G. mexicanus* is

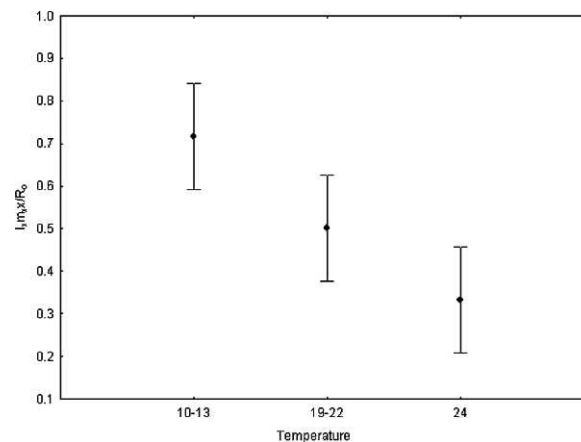


FIGURE 3. Generation time (G) of individually isolated *Gyrodactylus mexicanus* individuals on *Girardinichthys multiradiatus* in relation to temperature.

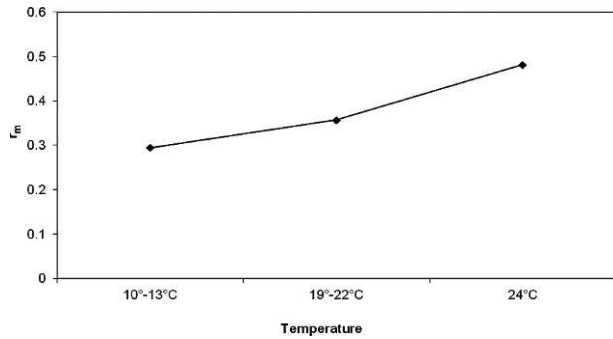


FIGURE 4. Innate capacity for increase (r_m) (parasite/day) for individually isolated *Gyrodactylus mexicanus* specimens on *Girardinichthys multiradiatus* in relation to temperature.

similar to that described previously for *G. salaris*, which is a common parasite of Atlantic salmon (Jansen and Bakke, 1991).

The reproductive pattern of *G. mexicanus* was followed for at least 8 generations at the 3 temperatures tested. In other congeneric species, like *Gyrodactylus wagneri*, 8 generations were also examined (Bychowsky, 1957). For *G. alexanderi*, at least 20 generations were recorded on the three-spined stickleback fish (Lester and Adams, 1974). The number of generations observed for *G. mexicanus* in the current study at 3 temperatures was sufficient for interpreting this aspect of the population dynamics of the parasite under laboratory conditions, i.e., the generation time of *G. mexicanus* decreased with increasing water temperature.

Several field studies have shown that water temperature affects the population biology of gyrodactylids. In a habitat with constant temperature throughout the year, such as in the Neotropics, there are relatively stable infection levels (Bauer and Karimov, 1990). In contrast, in the Neartic region, marked seasonal variations in water temperature are known to impact the population biology of *G. salaris* and *Gyrodactylus callariatis*, respectively (see Appleby, 1996; Appleby and Mo, 1997). It has been proposed that the number of gyrodactylids occurring in natural fish populations will increase at higher temperatures (Harris, 1980). However, the presence of only a small number of parasites in a host population does not necessarily mean that the temperature alone affects parasite growth. More specifically, fish have a body temperature that is essentially the same as that of the surrounding water; their entire physiology, including immune functions, is influenced by environmental temperature (King et al., 2009). Upper and lower temperature limits of the immune response are closely related to the ecological temperature range of each piscine species (O'Neill, 1980; Rijkers et al., 1980).

Other biotic factors, such as the host's reproductive behavior, may also play a crucial role in the transmission of *G. mexicanus*. The most efficient mode of gyrodactylid transmission occurs by direct contact between live hosts (Bychowsky, 1957; Scott and Anderson, 1984; Kamiso and Olson, 1986; Bakke et al., 1992). Reproductive behavior of the host, *Girardinichthys multiradiatus* (involving close contact between male and female), is displayed during the months when the water temperature ranges between 15 to 19°C (Díaz-Pardo and Ortiz-Jimenez, 1986; Macías, 1994). This reproductive behavior should enhance the transmission of *G. mexicanus* among hosts when the water temperature is high.

The current study represents the first attempt to describe the population biology of the monogenoid *G. mexicanus* associated with an endemic goodeid fish, *G. multiradiatus*, in central Mexico. Our investigation of the population dynamics of this monogenoid could be used as a predictive model to better understand the population dynamics of other monogeneans in central Mexico.

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