

Research Note

Specificity of the Monogenean *Gyrodactylus tularosae* Kritsky and Stockwell, 2005, to Its Natural Host, the White Sands Pupfish (*Cyprinodon tularosa* Miller and Echelle 1975)

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ABSTRACT: We examined host specificity of a recently described parasite (*Gyrodactylus tularosae* Kritsky and Stockwell, 2005) to its natural host, the White Sands pupfish (*Cyprinodon tularosa* Miller and Echelle, 1975), compared with a closely related congener, the sheepshead minnow (*Cyprinodon variegatus*). In each of 8 replicates, 1 uninfected *C. tularosa* and 1 uninfected *C. variegatus* were exposed to 3 *C. tularosa* infected with *G. tularosae* over a 4-d exposure trial. Focal fish were subsequently isolated for 5 d to evaluate infection persistence. Experiment-wide fluke infection prevalence was 100% for *C. tularosa* throughout the experiment. Prevalence on *C. variegatus* increased to 100% by d 3 of exposure but declined in isolation to 50% on the last day of the experiment. Fluke intensity was significantly higher for *C. tularosa* than *C. variegatus* throughout the experiment. Following isolation, parasite intensity declined for both species. These data suggest that *G. tularosae* prefers its native host, *C. tularosa*, but it may be able to use *C. variegatus* as a transient host.

KEY WORDS: *Cyprinodon tularosa*, *Cyprinodon variegatus*, endemism, Gyrodactylidae, *Gyrodactylus tularosae*, host specificity, pupfish, transient host, Tularosa Basin

Host specificity of *Gyrodactylus* species has important implications for actively managed fish

species, because many gyrodactylids have been reported to be pathogenic (Thoney and Hargis, 1991; Bakke and Harris, 1998). For example, *Gyrodactylus salaris* can impact a variety of native salmonid populations when introduced with fish released from aquaculture (Malmberg, 1993; Bakke and Harris, 1998). Although species of *Gyrodactylus* have been reported from members of a multitude of fish families (Bakke et al., 2002; Zietara and Lumme, 2002), most research on host specificity and pathogenicity has focused on a small number of gyrodactylid species such as *G. salaris* and *Gyrodactylus turnbulli* (Scott, 1982; Scott and Anderson, 1984; Bakke et al., 1990, 1991, 1999; Leberg and Vrijenhoek, 1994). Pupfishes (Cyprinodontidae) serve as host to a number of gyrodactylid species (Hargis, 1955; Mizelle and Kritsky, 1967; Williams and Rogers, 1971), but host specificity and potential pathogenicity have not been evaluated for these gyrodactylid species.

A recent parasite survey revealed *Cyprinodon tularosa* (Miller and Echelle, 1975) to be infected with only 1 monogenean parasite, an undescribed gyrodactylid (Stockwell, unpublished data). This monogene was subsequently described as *Gyrodactylus tularosae* (Kritsky and Stockwell, 2005). *Cyprinodon tularosa* is a New Mexico state-listed threatened species that belongs to the *Cyprinodon variegatus* complex of pupfishes (Echelle and Echelle, 1992) and is sister to a clade that includes *C. variegatus* (Echelle and Echelle, 1992; Echelle et

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al., 2005). *Cyprinodon tularosa* has been isolated from *C. variegatus* for ca 2 million yr (Echelle et al., 2005), which may be sufficient time for host specificity of gyrodactylids to develop (Zietara and Lumme, 2002). Here, we evaluate host specificity of *G. tularosae* to *C. tularosa* by conducting a challenge experiment including both *C. tularosa* and *C. variegatus*.

Cyprinodon tularosa is endemic to the Tularosa Basin in New Mexico, U.S.A. Native populations occur at Malpais Spring and Salt Creek on White Sands Missile Range (WSMR). Furthermore, 2 recently established populations occur at Mound Spring on WSMR and Lost River on Holloman Air Force Base; both introduced populations were established by translocation of fish from Salt Creek between 1967 and 1973 (Stockwell et al., 1998; Pittenger and Springer, 1999). *Cyprinodon tularosa* is the only fish species in each of these habitats. *Gyrodactylus tularosae* has been observed on fish from all populations of *C. tularosa* (Stockwell and Vinje, unpublished data).

A gyrodactylid-free, captive-reared stock of *C. variegatus* was provided by a commercial supplier (Aquatic Biosystems Inc., Ft. Collins, Colorado, U.S.A.) and was most likely originally derived from a wild population near Slidell, Louisiana, U.S.A. (S. Kellman, personal communication). Individuals of *C. tularosa* were captured with minnow traps at Salt Creek (cable crossing site; 33°11'N; 106°26'W; Sierra County, New Mexico, U.S.A.). For field inspections, 10 fish were collected on 3 June 2001, killed by double pithing, and inspected for presence of *G. tularosae* on the caudal, anal, dorsal, and pectoral fins. Prevalence was 100%.

Subsequently, collections of live *C. tularosa* made on 9 June 2001 and 25 June 2001 were transported to North Dakota State University (NDSU) for exposure experiments. After transport, all fish from the 9 June 2001 collection were not infected with *G. tularosae*. This apparent loss of infection (from 3 June 2001) was presumably a result of exposure to Instant Ocean® during transport to NDSU. Because of this observation, fish were subsequently maintained in a solution created to mimic the ionic composition of Salt Creek water (in grams of reagent per liter of deionized water: 0.054 NaHCO₃, 0.192 KCl, 1.502 MgSO₄, 1.695 CaSO₄, and 6.577 NaCl).

The exposure experiment was initiated on 16 July 2001. The experiment was conducted using 8 aquaria containing 37.5 liters of artificially created Salt Creek water. Each experimental tank received 3 infected individuals of *C. tularosa* (25 June collection; source

fish) and 2 uninfected focal fish; 1 *C. tularosa* (9 June collection) and 1 *C. variegatus*. Each replicate of 3 source fish had between 60 and 70 parasites on their fins. Focal fish were each marked with a diagnostic caudal fin clip, with all clips of equal surface area. Fish were fed ad libitum and maintained on a 16:8-h light/dark cycle at ca 25°C.

Parasite inspections were conducted at 48, 72, and 96 hr after experiment initiation. Focal fish were anesthetized in MS-222 (80 mg/liter) and inspected for parasites on the anal, caudal, dorsal, and both pectoral fins by using a dissecting microscope (×6.3–40). Subsequently, each fish was returned to its aquarium.

After the parasite count at 96 hr, each focal fish was isolated in a separate 3.7-liter aquarium to evaluate infection persistence in isolation. Fish were reexamined for parasites at 72, 96, and 120 hr postisolation.

Parasite prevalence (sensu Bush et al., 1997) for each species was determined at each sampling period by counting the number of infected focal individuals across all 8 replicates. Parasite intensity (i.e., the number of parasites on an individual host; Bush et al., 1997) also was determined for each focal fish during each sampling period. To evaluate parasite intensity, we conducted analyses for the exposure and isolation segments of the experiment separately. We conducted repeated measures analysis of variance (ANOVA) on the raw data and also on ranked data. This approach has been advocated for situations that call for nonparametric repeated measures approaches (Conover, 1999) and is robust if the results from both analyses are similar. Both analyses revealed the same effects of species on parasite intensity, but the time effect was only significant for the analyses of raw data because of the loss of information associated with transforming raw data into ranked data. For brevity, we report only the repeated measures ANOVA on raw data.

No fish died during the course of the experiment. A notable difference was detected in prevalence for both pupfish species during both the exposure and isolation portions of the experiment (Fig. 1). For *C. tularosa*, parasite prevalence rose to 100% at the first sampling period (48 hr) and remained at 100% for the duration of the experiment, including the entire period of isolation (Fig. 1). By contrast, parasite prevalence for *C. variegatus* rose to only 60% in the first 48 hr, 100% during the subsequent 48 hr, and gradually dropped during the isolation period to 50% (Fig. 1).

Parasite intensity showed significant positive growth for both species throughout the exposure trial

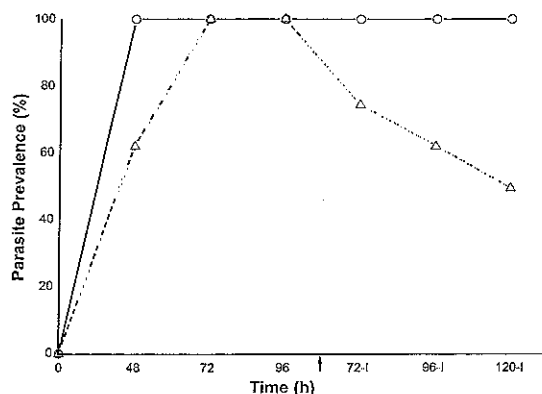


Figure 1. *Gyrodactylus tularosae* prevalence across 8 replicates and 7 time periods during the challenge experiment for *Cyprinodon tularosae* (circles) and *Cyprinodon variegatus* (triangles). Arrow indicates isolation of focal fish from source fish.

($F_{[2,28]} = 20.56$; $P < 0.001$), but intensity was higher for *C. tularosae* than for *C. variegatus* ($F_{[1,14]} = 11.70$; $P = 0.004$; Fig. 2). During isolation, there was a significant decline in parasite intensity for both fish species ($F_{[2,28]} = 3.99$; $P = 0.030$), but parasite intensity was consistently higher for *C. tularosae* than for *C. variegatus* ($F_{[1,14]} = 30.10$; $P < 0.001$; Fig. 2).

Although it may be possible for *G. tularosae* to infect *C. variegatus*, its population growth is higher on its natural host, *C. tularosae*. This finding of host specificity is not surprising, given that these 2 fish species have been isolated from each other for at least 2 million yr (Echelle et al., 2005). Additionally, our results are likely conservative, because previous exposure to parasites can decrease susceptibility to subsequent infection in fish (Scott and Anderson, 1984). Most wild-captured individuals of *C. tularosae* most likely had prior exposure to *G. tularosae*, because our field inspections indicated a high prevalence at the collection site (see above). In contrast, the *C. variegatus* used in this experiment had not previously encountered *G. tularosae*.

Our data suggest that *C. variegatus* may be able to act as a transient host for *G. tularosae*. Similarly, *G. salaris* successfully infected rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) but did not persist as they did on their natural host, Atlantic salmon (*Salmo salar*) (Bakke et al., 1991; Jansen and Bakke, 1995).

In both species of *Cyprinodon*, parasite intensity increased during exposure to an infected host but decreased after isolation. This finding is consistent with other work that has shown similar patterns of infection (Cusack, 1986). The decline in parasite numbers may indicate either a host immune response

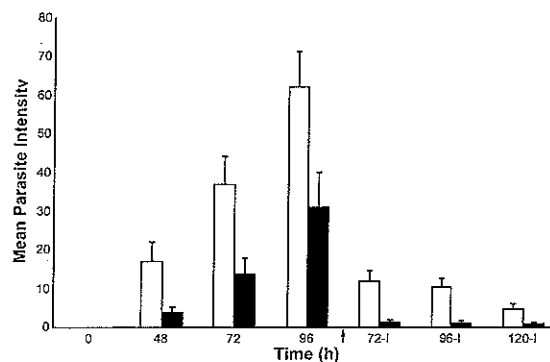


Figure 2. Mean *Gyrodactylus tularosae* intensity (± 1 SEM) is shown for each time period during the challenge experiment for *Cyprinodon tularosae* (open bars; $n = 8$) and *Cyprinodon variegatus* (solid bars; $n = 8$). Arrow indicates isolation of focal fish from source fish.

or source-sink pattern in parasite population dynamics (also see Bakke et al., 1991).

Future research should attempt to examine the mechanisms of parasite infection and fish susceptibility with additional pupfish species. Because many cyprinodontids are endangered, the potential impact of parasites on pupfish is of particular concern for the conservation of these rare fish species.

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