

Copepod consumption by amphibians and fish with implications for transmission of *Dracunculus* species

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ABSTRACT

Parasitic nematodes in the genus *Dracunculus* have a complex life cycle that requires more than one host species in both aquatic and terrestrial habitats. The most well-studied species, *Dracunculus medinensis*, is the causative agent of human Guinea worm disease (dracunculiasis). There are several other *Dracunculus* species that infect non-human animals, primarily wildlife (reptiles and mammals). The classic route of *D. medinensis* transmission to humans is through the ingestion of water containing the intermediate host, a cyclopoid copepod, infected with third-stage larvae (L3s). However, many animal hosts (e.g., terrestrial snakes, dogs) of other *Dracunculus* sp. appear unlikely to ingest a large number of copepods while drinking. Therefore, alternative routes of infection (e.g., paratenic or transport hosts) may facilitate *Dracunculus* transmission to these species. To better understand the role of paratenic and transport hosts in *Dracunculus* transmission to animal definitive hosts, we compared copepod ingestion rates for aquatic species (fish, frogs [tadpoles and adults], and newts) which may serve as paratenic or transport hosts. We hypothesized that fish would consume more copepods than amphibians. Our findings confirm that African clawed frogs (*Xenopus laevis*) and fish consume copepods, but that fish ingest, on average, significantly higher numbers (68% [34/50]) than adult African clawed frogs (36% [18/50]) during a 24-h time period. Our results suggest that amphibians and fish may play a role in the transmission of *Dracunculus* to definitive hosts. Still, additional research is required to determine whether, in the wild, fish or frogs are serving as paratenic or transport hosts. If so, they may facilitate *Dracunculus* transmission. However, if these animals simply act as dead-end hosts or as means of copepod population control, they may decrease *Dracunculus* transmission.

1. Introduction

Dracunculus species are a group of subcutaneous parasitic nematodes that can infect a diversity of reptiles and mammals (Cleveland et al., 2018). The transmission of *Dracunculus* nematodes to the definitive host occurs through the ingestion of intermediate hosts (cyclopoid copepods) harboring third-stage larvae (L3s) (Fedchenko, 1870). Copepods become infected when they ingest first-stage *Dracunculus* larvae (L1s) that are released into water bodies by gravid female worms that have emerged from vertebrate hosts (Muller, 1971; Cleveland et al., 2018).

The most well-studied species in this genus, *Dracunculus medinensis*

(human Guinea worm), causes significant morbidity in patients in the remaining endemic regions of Sub-Saharan Africa (Cairncross et al., 2002). Although eradication efforts have been extremely effective at decreasing the number of human cases (from an estimated 3.5 million cases in 1986 to only 27 cases in 2020), some countries are experiencing increasing numbers of *D. medinensis* infections in animals (primarily, dogs [*Canis lupus familiaris*] and cats [*Felis catus*] in Chad, and dogs and baboons [*Papio anubis*] in Ethiopia), with the highest increase seen in dogs from Chad (Ruiz-Tiben and Hopkins, 2006; Cleveland et al., 2019; Guagliardo et al., 2020). In 2020, Chad reported only 12 human cases, but there were infections reported in 1507 dogs, 61 cats, and 2 African

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wildcats (*Felis lybica*) (World Health Organization [WHO], 2021). Despite years of increasing numbers of infected dogs and cats, 2020 dog infection totals in Chad were lower than 2019 dog infection totals (1507 infections vs. 1935) and human case numbers have also decreased (12 in 2020 vs. 49 in 2019) (WHO, 2020). This may be due to increased numbers of interventions aimed at decreasing transmission among dogs.

A recent study showed that copepod ingestion by domestic dogs was relatively low when lapping water and that the numbers of copepods consumed were unlikely high enough to maintain currently reported levels of Guinea worm transmission among dogs in Chad (Garrett et al., 2020). In order to attain Guinea worm eradication, it is necessary to investigate alternative *Dracunculus* transmission routes, in particular, the possible role of aquatic animals as paratenic or transport hosts (Eberhard et al., 2016b; Cleveland et al., 2017). Studies have shown that tadpoles are experimentally susceptible to infection with *Dracunculus insignis* (a parasite of many mammal hosts in North America), *Dracunculus ophidensis* (a parasite of common garter snakes [*Thamnophis sirtalis*]) from North America, and *D. medinensis* from Africa (Brackett, 1938; Crichton and Beverley-Burton, 1977; Eberhard and Brandt, 1995; Eberhard et al., 2016b; Cleveland et al., 2017). Anurans can also retain infections with *Dracunculus* larvae through metamorphosis and transmission can occur to appropriate definitive hosts when infected amphibians are ingested (Eberhard and Brandt, 1995; Eberhard et al., 2016b). Natural infections of amphibians with *D. insignis* and *D. medinensis* L3s have also been reported (Eberhard et al., 2016a; Cleveland et al., 2019, 2020).

Neither *D. insignis* nor *D. medinensis* larvae have been recovered from wild fish (Eberhard et al., 2014; Cleveland et al., 2019, 2020). No *D. medinensis* larvae have been recovered from experimentally infected fish, but *D. insignis* larvae have been recovered from a low number of experimentally infected fish; although, even in infected fish, the larval recovery was low (0.6–2.0%) (Crichton and Beverley-Burton, 1977; Eberhard et al., 2016b; Cleveland et al., 2019, 2020). However, the experimental transmission of *D. insignis* and *D. medinensis* using fish as short-term transport hosts was successful (Cleveland et al., 2017). These findings supported the continued investigation into the role of fish in *Dracunculus* transmission, despite their apparent inability to serve as paratenic hosts.

Although there is experimental evidence that frogs may serve as paratenic hosts and fish may serve as transport hosts, the importance of different host species in the *Dracunculus* life cycle are unknown; a better understanding may be gained from directly comparing copepod consumption by these animals. We evaluated copepod ingestion by several species of amphibians (frogs, tadpoles, and newts) and small fish. We hypothesized that fish would consume more copepods than amphibians because of general diet preferences and more active feeding behavior (Piasecki et al., 2004; Ibrahim et al., 2015; Ocock et al., 2019). Developing a better understanding of copepod consumption by these potential host species is important, as it may offer insight into *Dracunculus* transmission dynamics, as well as inform future research pertaining to Guinea worm eradication.

2. Materials and methods

2.1. Copepods

Copepods used in this study were from lab-reared colonies of *Macrocyclops* species. Species identification was determined through morphology and sequence analysis of a portion of the cytochrome c oxidase 1 (COI) gene (Pennak, 1963; Folmer et al., 1994). The colony was derived from wild-caught copepods obtained from ponds in Athens, Georgia, USA, and maintained at the University of Georgia's Aquaculture Biotech Environmental Lab (ABEL) in Athens, Georgia, USA.

2.2. Study animals (amphibians and fish)

Five species of fish, ranging in size from three to 12 cm in length, were included in the study: channel catfish [*Ictalurus punctatus*], Congo tetra [*Phenacogrammus interruptus*], mosquitofish [*Gambusia affinis*], featherfin catfish [*Synodontis eupterus*], and Nile and blue hybrid tilapia [*Oreochromis aureus* x *Oreochromis niloticus*]. All fish were captive-bred and commercially sourced except tilapia, which were captive-bred at the University of Georgia. Eight species of tadpoles were included in this study: African clawed frog (*Xenopus laevis*), American bullfrog (*Lithobates [Rana] catesbeianus*), Cope's gray treefrog (*Hyla chrysoscelis*), Cuban treefrog (*Osteopilus septentrionalis*), Fowler's toad (*Anaxyrus [Bufo] fowleri*), green frog (*Lithobates [Rana] clamitans*), pickerel frog (*Lithobates [Rana] palustris*), and wood frog (*Lithobates [Rana] sylvaticus*). Adult African clawed frogs and one species of adult newt (eastern newt [*Notophthalmus viridescens*]) were also included in this study. All amphibians (except African clawed frogs, which were sourced from Xenopus Express, Brooksville, FL and Cuban treefrog, which were wild-caught in Florida) were wild-caught in Georgia. Some fish and amphibian species were selected because they (or close relatives) are found in Chad, Africa and may play a role in *D. medinensis* transmission, while other species were selected because they are native to North America and potentially relevant to the transmission of native North American *Dracunculus* species such as *D. insignis*, *D. ophidensis*, or *D. lutrae*.

2.3. Trial setup

Individual feeding trials were conducted in 2-L transparent plastic tanks filled with 1 L of dechlorinated water at 23 °C and outfitted with an oxygenating bubbler in a temperature-controlled room (water temperature: 23±0.15 °C). Fifty copepods were added to each tank, representing an average copepod density in bodies of water in Chad (Garrett et al., 2020). Copepods were allowed to acclimate and disperse for 5 min before adding the aquatic animal to be tested. Trials were conducted using a 12-h day/night light cycle. A total of 294 trial replicates were conducted.

Total length was measured for fish and Gosner stage was determined for tadpoles of all species (Gosner stage is a more accurate measurement than length for tadpoles across different species), except *X. laevis*, before the individual was added to the trial container. One animal was tested per trial container, and time and water temperature were recorded at the beginning and end of each trial.

Each trial was run for 24 h, after which the animal being tested was removed using a large-holed net (to avoid removing copepods). The net and animal were thoroughly rinsed with dechlorinated water to ensure no copepods were inadvertently removed. The rinse water was returned to the trial tank so that any copepods rinsed from animals or nets would be included in the count. Water containing copepods from the feeding trial was poured through a 100-µm filter. The tank was rinsed using dechlorinated water to ensure no copepods remained. Finally, copepods were rinsed from the filter into a Petri dish and enumerated. Control trials were run with the same methods, but no fish or amphibian was added to the container.

2.4. Statistical analyses

Statistical analyses were conducted in R (R Core Team, 2019). We fit an analysis of variance model using the function *aov* (R package *stats* [Chambers et al., 1992]) to determine whether the number of copepods consumed differed between animal types (fish, tadpole, adult African clawed frog, or newt). For all analyses of copepod loss, we included initial water temperature given the potential effects of temperature on fish, amphibian, or copepod activity level. Further, we chose to include initial temperature instead of final or average temperatures because analyses indicated that all three temperatures were highly correlated

(Pearson's correlation > 0.7) and showed quantitatively similar results.

Next, we examined fish and tadpoles separately. Specifically, we fit separate analysis of variance models to assess whether species of fish and tadpoles differed in the number of copepods lost. In analyses of fish species, in addition to initial water temperature, we included the length of the fish to account for any potential effects of animal size. In tadpole species analyses (excluding African clawed frog tadpoles, which do not have Gosner stage data), we included initial temperature as well as Gosner stage (ranging from 25 to 42) to account for potential differences between tadpole stages. Additional analysis of copepods consumed by tadpole species and initial temperature was conducted to include all tadpole species, but excluded Gosner stage. We employed Tukey post-hoc contrasts (R package *multcomp* [Hothorn et al., 2008]) to determine which animal types or species differed from the others.

Ethical approval and informed consent

All animal collections, housing, and experiments were reviewed and approved by the University of Georgia's Institutional Animal Care and Use Committee (A2018 01–010).

3. Results

3.1. Copepods consumed by species

Fish consumed the most copepods during trials. An average of 69% ($34.4 \pm 1.4/50$) of copepods were consumed in fish trials compared to 36% ($18.2 \pm 2.6/50$) in African clawed frog trials, 17% ($8.4 \pm 1.0/50$) in newt trials, and 16% ($8.0 \pm 0.4/50$) in tadpole trials. A small number of copepods were lost in control trials (11% [$5.3 \pm 0.9/50$]) (Fig. 1).

3.2. Statistical analysis of results

Animal type (fish, tadpole, African clawed frog, or newt) significantly impacted mean copepods consumed ($p < 0.001$), as did initial water temperature ($p = 0.001$); although, the correlation between water temperature and copepods consumed was weak (Pearson's correlation < 0.08) (Fig. 1; Table 1). Tukey post-hoc contrasts indicated that fish and African clawed frogs consumed statistically different numbers of copepods from each other, as well as from tadpoles and newts ($p < 0.05$) (Fig. 1). There was no significant difference in copepod consumption between tadpoles and newts or copepod loss during control trials ($p > 0.05$) (Fig. 1). When analyzing adult African clawed frogs or newts separately, there was no significant effect of water temperature on

copepods consumed ($p > 0.05$).

Analysis of copepods consumed by fish indicated significant differences by fish species, but no effect of water temperature or fish size (Table 2; Fig. 2). Tukey post-hoc contrasts indicated that all fish species consumed similar numbers of copepods, except Congo tetra, which consumed fewer copepods than all other fish species ($p < 0.05$).

Analysis of copepods consumed by tadpole species indicated no significant difference in copepods consumed by tadpole species (excluding African clawed frogs), Gosner stage, or water temperature (Table 3; Fig. 3). Tukey post-hoc contrasts indicated no significant differences in the number of copepods consumed by tadpole species ($p > 0.05$) (Fig. 3). When additional analysis was performed including all tadpole species and water temperature, results similarly indicated no significant differences in the number of copepods consumed by tadpole species ($p > 0.05$) (Table S1).

3. Discussion

The objective of this study was to determine copepod ingestion by several species of amphibians and fish in order to better understand their potential roles in the transmission of *Dracunculus* species. Our data indicate that both fish and adult African clawed frogs consume high numbers of copepods under experimental conditions. Temperature had a negligible impact on copepods eaten during trials across all species tested.

The most important consumers of copepods in this study were fish, as all species tested ingested a significant number of copepods (average 69% [$34/50$]). Although a previous study found *Dracunculus* L3s in tissues (unknown tissue type as visceral organs, skeletal muscles, and skin were combined) of a low number of experimentally infected fish species (i.e., rainbow trout [*Oncorhynchus mykiss*], common shiner [*Notropis cornutus*]), fish have been more successful in serving as transport hosts than as paratenic hosts in the laboratory (Crichton and Beverley-Burton, 1977; Eberhard et al., 2016b; Cleveland et al., 2017). As of yet, no *Dracunculus* larvae have been recovered from wild fish (Cleveland et al., 2019, 2020). However, in previous surveys for *Dracunculus* larvae in fish, there was a focus on examining muscle tissue for the presence of larvae; therefore, future studies would benefit from examining the gastrointestinal contents of fish to further evaluate their potential to perform as transport hosts in the wild (Cleveland et al., 2019).

Several species of small fish consumed large numbers of copepods in this study, providing further support that small fish may play a role in the transmission of *Dracunculus* spp. to animals, including of *D. medinensis* to dogs in Chad. In Chad, it was noted that many villages

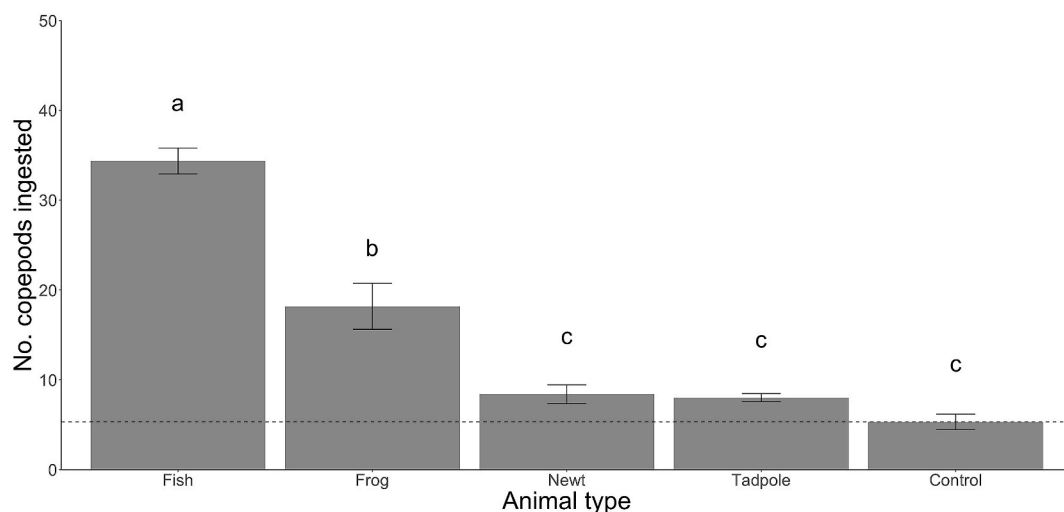


Fig. 1. Average copepods ingested by animal type during the feeding trial. Bars represent average copepods ingested, error bars represent standard error, and dotted line shows average copepod loss in control trials. Significant differences ($p < 0.05$) determined by Tukey post-hoc contrasts are indicated by 'a', 'b', and 'c'.

Table 1

Fixed-Effects ANOVA results of copepod ingestion (from 50 copepods/1 L at start) by animal type (fish, frog, tadpole, newt, or control [no animal]) and initial water temperature during 24-h feeding trials.

Predictor	Sum of Squares	df	Mean Square	F	p	partial η^2	partial η^2 90% CI [LL, UL]
(Intercept)	5268.01	1	5268.01	77.16	.000		
Animal type	40043.71	4	10010.93	146.62	.000	.68	[.63, .71]
Initial temperature (C°)	717.39	1	717.39	10.51	.001	.04	[.01, .08]
Error	18981.31	278	68.28				

Note. LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively.

Table 2

Fixed-Effects ANOVA results of copepod ingestion (from 50 copepods/1 L at start) by fish species, fish length, and initial water temperature during 24-h feeding trials.

Predictor	Sum of Squares	df	Mean Square	F	p	partial η^2	partial η^2 90% CI [LL, UL]
(Intercept)	1317.27	1	1317.27	11.81	.001		
Fish species	3617.89	4	904.47	8.11	.000	.34	[.15, .44]
Fish length (mm)	248.32	1	248.32	2.23	.141	.03	[.00, .13]
Initial temperature (C°)	248.88	1	248.88	2.23	.140	.03	[.00, .13]
Error	6913.84	62	111.51				

Note. LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively.

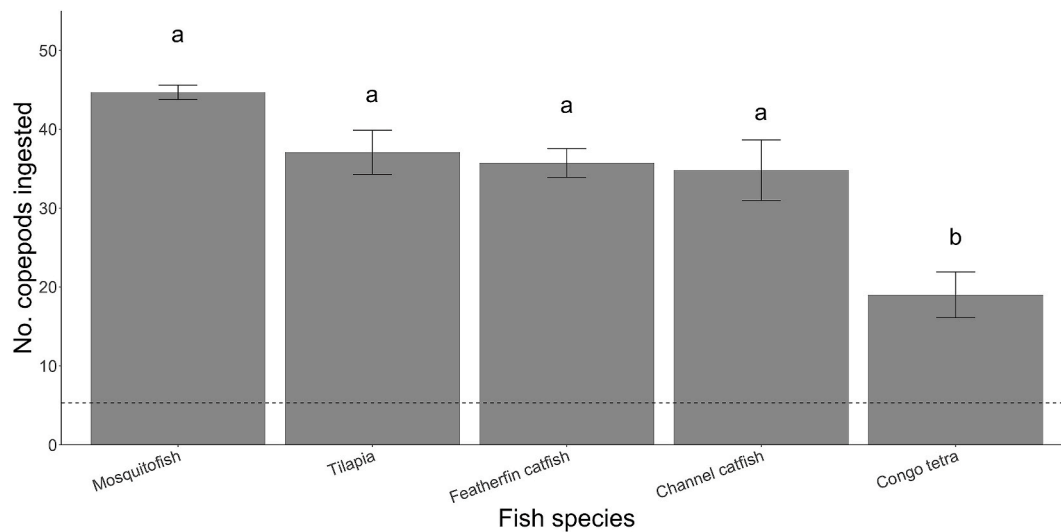


Fig. 2. Average copepods ingested by fish species during the feeding trial. Bars represent average copepods ingested, error bars represent standard error, and dotted line shows average copepod loss in control trials. Significant differences ($p < 0.05$) determined by Tukey post-hoc contrasts are indicated by 'a' and 'b'.

Table 3

Fixed-Effects ANOVA results of copepod ingestion (from 50 copepods/1 L at start) by tadpole species (excluding African clawed frog), Gosner stage, and initial water temperature during 24-h feeding trials.

Predictor	Sum of Squares	df	Mean Square	F	p	partial η^2	partial η^2 90% CI [LL, UL]
(Intercept)	42.91	1	42.91	1.41	.237		
Tadpole species	147.68	6	24.61	0.81	.564	.04	[.00, .06]
Initial temperature (C°)	4.38	1	4.38	0.14	.705	.00	[.00, .03]
Gosner stage	28.15	1	28.15	0.93	.338	.01	[.00, .05]
Error	3982.37	131	30.40				

Note. LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively.

experiencing high Guinea worm transmission are fishing villages where small fish and fish viscera are fed to or scavenged by dogs and cats, and that increased fish consumption is positively correlated with the likelihood of dog infection (Eberhard et al., 2014; McDonald et al., 2020; Richards et al., 2020). Because fish consumed high numbers of copepods during our 24-h trials, it is likely that they would concentrate high numbers of copepods in their gastrointestinal tract after feeding (also concentrating *Dracunculus* larvae inside the fish if those copepods were infected). Fish containing high numbers of concentrated Guinea worm

larvae would pose an infection risk to the dogs or other predators of these fish and could result in high worm burdens in these animals. The transit time of food in the gastrointestinal tract of a fish (the length of time that the L3s would remain after feeding on infected copepods) would depend on numerous factors, including species, diet, and temperature (Uscanga et al., 2010).

No tadpole species consumed significantly more copepods than were lost in control trials, suggesting that copepod consumption by tadpoles within this time frame is limited. However, previous studies have shown

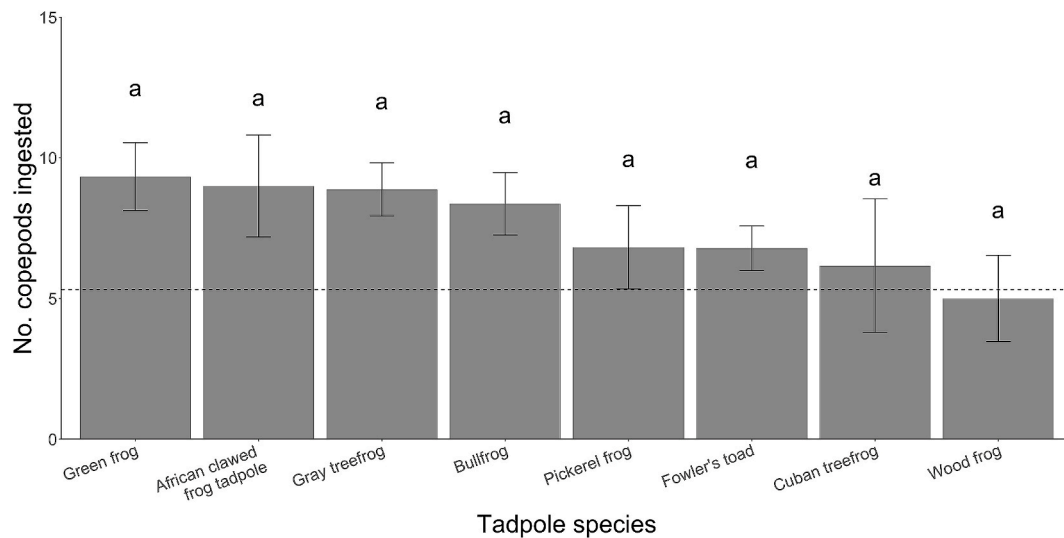


Fig. 3. Average copepods ingested by tadpole species during the feeding trial. Bars represent average copepods ingested, error bars represent standard error, and dotted line shows average copepod loss in control trials. The lack of significant differences ($p < 0.05$) determined by Tukey post-hoc contrasts are indicated by 'a'.

that many species of tadpoles can ingest sufficient numbers of *Dracunculus*-infected copepods to become infected with *D. medinensis*, *D. insignis*, and *D. ophidensis* during infection trials (Brackett, 1938; Eberhard et al., 2016b). In a study by Eberhard et al. (2016b), it was noted that unlike fish, which consumed all copepods that were offered within 24 h, tadpoles did not ingest all offered copepods, even after three days. Another study found that early Gosner stage tadpoles (hind-limb-bud stages) had difficulty ingesting copepods, while later Gosner stage tadpoles (those with hind legs) would ingest copepods readily (Eberhard and Brandt, 1995), although we found no differences in copepod ingestion by different Gosner stages (Fig. 3; Table 3). The short time frame of our study likely underestimates the long-term potential of tadpoles to acquire *Dracunculus* infections.

Dracunculus L3s from infected tadpoles can infect definitive hosts in the laboratory, and natural *D. medinensis* infections have been found in a low percentage (1.4% [4/276]) of wild frogs in Chad (Brackett, 1938; Eberhard and Brandt, 1995; Eberhard et al., 2016b; Cleveland et al., 2019). In North America, *D. insignis* infections were reported from a higher percentage (16.2% [11/68]) of wild frogs surveyed at a site in Georgia with a high prevalence of infection in vertebrate hosts (Cleveland et al., 2020). Our findings indicate that tadpoles do not consume copepods readily under laboratory conditions within 24 h, which, if this holds true in the wild, may explain the low prevalence of *D. medinensis* infections in wild frogs (Cleveland et al., 2019). However, the higher prevalence of *D. insignis* infection found in wild frogs in Georgia, USA suggests potential host and/or spatial variation regarding the role of paratenic hosts in the transmission of different species of *Dracunculus* nematodes (Cleveland et al., 2020). Some of the tadpole species we tested can remain aquatic for months to years (e.g., American bullfrog), while others undergo metamorphosis quickly (e.g., Cuban treefrogs can mature in less than a month) (Casper and Hendricks, 2005; Meshaka, 2005). It may also be possible that some predatory tadpoles could concentrate L3s by ingesting other infected tadpoles. While paratenic host to paratenic host transmission has not been documented for any *Dracunculus* sp., it is worthy of further investigation. A longer time period spent in aquatic life stages when an anuran may ingest copepods, or a predatory diet consisting of other paratenic hosts, may be impacting the frog species that become infected with *Dracunculus* larvae and the difference in the number of L3s they accumulate.

African clawed frogs are fully aquatic at all life stages; thus, their copepod ingestion habits are unique to animals with this life history and would not apply to adult terrestrial frogs. It is also unlikely that adult African clawed frogs would lead to high numbers of infections in dogs or

other terrestrial mammals, as aquatic African clawed frogs are not readily accessible prey for these animals. Although several *Xenopus* species are harvested and eaten by people in West Africa, it does not appear they are consumed in high numbers in Chad (Mallon et al., 2015; Cleveland et al., 2019). It is possible, however, that aquatic frog species could play a more substantial role in the transmission of other *Dracunculus* spp. to aquatic definitive hosts (e.g., *D. globocephalus* to snapping turtles [*Chelydra serpentina*] or *D. lutrae* to North American river otters [*Lontra canadensis*]).

We found that adult eastern newts did not consume a significant number of copepods during the 24-h trial period. Larval eastern newts, as well as larval salamanders, can rely heavily on cyclopoid copepods as a food source and adult eastern newts also can ingest copepods (Brophy, 1980; Jarroll, 1980). Eastern newts are hosts for *Sprioxys* sp. nematodes and *Bothriocephalus rarus* cestodes, both of which use cyclopoid copepods as intermediate hosts. Therefore, at some point in the newt life cycle, sufficient numbers of copepods are ingested to allow for the transmission of these parasites (Jarroll, 1980; Muzzall, 1991). Our use of adult newts, which do not consume as many copepods as larval newts, may have led to an incorrect assumption that newts are not involved in *Dracunculus* life cycles, particularly North American *Dracunculus* species, including *D. insignis* (Cleveland et al., 2018). Of note, newts and salamanders are not present in Sub-Saharan Africa, thus would not be involved in the transmission of *D. medinensis* (Channing and Rödel, 2019). Additional insight into the role these animals may play in *Dracunculus* transmission could be gained by comparing copepod consumption across different age or life-stage groups and other salamander species.

One potential limitation of this study is the copepod recounting method. We recorded an average loss of five out of 50 (11%) copepods in control trials. This loss may be attributed to the cannibalistic behavior of adult copepods or copepods that may have become stuck in the 100- μ m filter used for removing copepods from water, although we did thoroughly rinse to avoid copepods sticking to the filter (Toscano et al., 2016). Another potential limitation is that our 24-h trials were not long enough to observe the ingestion of a significant number of copepods by tadpoles. It is also possible that the altered behavior of *Dracunculus*-infected copepods (infectious copepods may become more sluggish and remain lower in the water column) would make them easier for tadpoles to ingest compared to the uninfected copepods which were used in this study (Onabamiro, 1954). In the wild, theoretically other more effective predators of copepods (such as fish) may also eat these "sluggish," infectious copepods before they could be consumed by the

less voracious tadpoles, resulting in fish eating more of the infected copepods on the landscape.

During this study, we were unable to maintain a constant water temperature across all trials. Although it is possible that temperature variation had some impact on the feeding behaviors of animals included in this study, the impact of temperature was not the primary interest of this study and appeared to have a small, if any, effect. Future work could aim to further clarify this relationship. We also only tested small fish (3–12 cm), and it is likely that copepod ingestion would vary between different sizes of fish. For example, researchers have found that Nile tilapia feed on copepods as juveniles but not as adults (Ibrahim et al., 2015). Therefore, our results only apply to the sizes and ages of the fish species that we included in our trials. Further research may be conducted to determine to what degree differences in size or age may impact copepod consumption by different species of fish. We also only tested a limited number of species and may have been able to draw broader conclusions if a greater diversity of animal species were tested. Despite these potential limitations, we believe this work provides relevant and essential information on the potential for *Dracunculus* transmission by paratenic or transport hosts.

3. Conclusions

We found that African clawed frogs and several species of small fish ingest copepods, with fish being the major consumer of copepods in the study. The significance of various species as paratenic and transport hosts in the transmission of *Dracunculus* spp. would vary depending on multiple factors (e.g., the diet of the paratenic, transport, and definitive host). For example, with *D. insignis*, domestic dogs and cats and some wildlife species (e.g., opossum [*Didelphis virginiana*]) are more likely to consume terrestrial prey (frogs), whereas others (e.g., North American river otters) may consume more aquatic prey (fish, tadpoles, and aquatic newts) (Schoonover and Marshall, 1951; Hart et al., 2019; Williams et al., 2018). Even though fish and aquatic frogs may consume large numbers of copepods, if a species is not a viable paratenic or transport host (or even if it is a viable transport or paratenic host but is not a common prey item for definitive hosts of *Dracunculus* spp.), it is possible that ingestion of copepods could actually contribute to decreased transmission overall. Further efforts to understand the interactions between definitive hosts and potential paratenic or transport hosts may elucidate transmission routes of these parasite species to relevant definitive hosts. It is important that these relationships continue to be investigated, especially since primary transmission routes may vary by region (Wilson-Aggarwal et al., 2021). This research contributes to the understanding of *Dracunculus* transmission by highlighting that fish and aquatic frogs are voracious consumers of copepods and that these feeding habits may have an impact on *Dracunculus* transmission.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2021.06.001>.

References

- Brackett, S., 1938. Description and life history of the nematode *Dracunculus ophidensis* n. sp., with a redescription of the genus. *J. Parasitol.* 24, 353–361.
- Brophy, T., 1980. Food habits of sympatric larval *Ambystoma tigrinum* and *Notophthalmus viridescens*. *J. Herpetol.* 14, 1–6.
- Cairncross, S., Muller, R., Zagaria, N., 2002. Dracunculiasis (Guinea worm disease) and the eradication initiative. *Clin. Microbiol. Rev.* 15, 223–246.
- Casper, G.S., Hendricks, R., 2005. *Rana catesbeiana* Shaw, 1802: American bullfrog. In: Lannoo, M. (Ed.), *Amphibian Declines: the Conservation Status of United States Species*. University of California Press, Berkeley, pp. 540–546.
- Chambers, J.M., Freeny, A., Heiberger, R.M., 1992. Analysis of variance; designed experiments. In: Chambers, J.M., Hastie, T.J. (Eds.), *Statistical Models in S*. Wadsworth & Brooks/Cole, Pacific Grove, California.
- Channing, A., Rödel, M.-O., 2019. *Field Guide to the Frogs & Other Amphibians of Africa*. Struik Nature, Cape Town.
- Cleveland, C.A., Eberhard, M.L., Thompson, A.T., Smith, S.J., Zirimwabagabo, H., Bringolf, R., Yabsley, M.J., 2017. Possible role of fish as transport hosts for *Dracunculus* spp. larvae. *Emerg. Infect. Dis.* 23, 1590–1592.
- Cleveland, C.A., Garrett, K.B., Cozad, R.A., Williams, B.M., Murray, M.H., Yabsley, M.J., 2018. The wild world of Guinea worms: a review of the genus *Dracunculus* in wildlife. *Int. J. Parasitol. Parasites Wildl.* 7, 289–300.
- Cleveland, C.A., Eberhard, M.L., Thompson, A.T., Garrett, K.B., Swanepoel, L., Zirimwabagabo, H., Moundai, T., Ouakou, P.T., Ruiz-Tiben, E., Yabsley, M.J., 2019. A search for tiny dragons (*Dracunculus medinensis* third-stage larvae) in aquatic animals in Chad, Africa. *Sci. Rep.* 9, 375.
- Cleveland, C.A., Eberhard, M.L., Garrett, K.B., Thompson, A.T., Swanepoel, L., Miller, E. A., Stephens, O., Yabsley, M.J., 2020. *Dracunculus* species in meso-mammals from Georgia, United States, and implications for the Guinea Worm Eradication Program in Chad, Africa. *J. Parasitol.* 106, 616–622.
- Crichton, V.F.J., Beverley-Burton, M., 1977. Observations on the seasonal prevalence, pathology and transmission of *Dracunculus insignis* (Nematoda: Dracunculoidea) in the raccoon (*Procyon lotor* (L.)) in Ontario. *J. Wildl. Dis.* 13, 273–280.
- Eberhard, M.L., Brandt, F.H., 1995. The role of tadpoles and frogs as paratenic hosts in the life cycle of *Dracunculus insignis* (Nematoda: Dracunculoidea). *J. Parasitol.* 81, 792–793.
- Eberhard, M.L., Ruiz-Tiben, E., Hopkins, D.R., Farrell, C., Toe, F., Weiss, A., Withers, P. C., Jenks, M.H., Thiele, E.A., Cotton, J.A., Hance, Z., Holroyd, N., Cama, V.A., Tahir, M.A., Moundai, T., 2014. The peculiar epidemiology of dracunculiasis in Chad. *Am. J. Trop. Med. Hyg.* 90, 61–70.
- Eberhard, M.L., Cleveland, C.A., Zirimwabagabo, H., Yabsley, M.J., Ouakou, P.T., Ruiz-Tiben, E., 2016a. Guinea worm (*Dracunculus medinensis*) infection in a wild-caught frog. *Chad. Emerg. Infect. Dis.* 22, 1961–1962.
- Eberhard, M.L., Yabsley, M.J., Zirimwabagabo, H., Bishop, H., Cleveland, C.A., Maerz, J. C., Bringolf, R., Ruiz-Tiben, E., 2016b. Possible role of fish and frogs as paratenic hosts of *Dracunculus medinensis*, Chad. *Emerg. Infect. Dis.* 22, 1428–1430.
- Fedchenko, A.P., 1870. Concerning the structure and reproduction of the Guinea worm (*Filaria medinensis* L.). *Am. J. Trop. Med. Hyg.* 20, 511–523.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Garrett, K., Box, E., Cleveland, C., Majewska, A., Yabsley, M., 2020. Dogs and the classic route of Guinea worm transmission: an evaluation of copepod ingestion. *Sci. Rep.* 10, 1430.
- Guagliardo, S.A.J., Roy, S.L., Ruiz-Tiben, E., Zirimwabagabo, H., Romero, M., Chop, E., Ouakou, P.T., Hopkins, D.R., Weiss, A.J., 2020. Guinea worm in domestic dogs in Chad: a description and analysis of surveillance data. *PLoS Neglected Trop. Dis.* 14, e0008207.
- Hart, S., Spicer, M.M., Wrynn, T., Chapman, T.L., Spivey, K.L., Gall, B.G., 2019. Palatability and predator avoidance behavior of salamanders in response to the Virginia opossum (*Didelphis virginiana*). *Am. Midl. Nat.* 181, 245–258.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biom. J.* 50, 346–363.
- Ibrahim, A.N.A.F., Noll, M.S.M.C., Valenti, W.C., 2015. Zooplankton capturing by Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae) throughout post-larval development. *Zool.* 32, 469–475.
- Jarroll, E.L., 1980. Population dynamics of *Bothriocephalus rarus* (Cestoda) in *Notophthalmus viridescens*. *Am. Midl. Nat.* 103, 360–366.
- Mallon, D.P., Hoffmann, M., Grainger, M.J., Hibert, F., van Vliet, N., McGowan, P.J.K., 2015. An IUCN situation analysis of terrestrial and freshwater fauna in West and Central Africa. In: *Occasional Paper of the IUCN Species Survival*. IUCN, Gland, Switzerland and Cambridge, UK, p. 10. Commission No. 54.
- McDonald, R.A., Wilson-Aggarwal, J.K., Swan, G.J.F., Goodwin, C.E.D., Moundai, T., Sankara, D., Biswas, G., Zingesser, J.A., 2020. Ecology of domestic dogs *Canis familiaris* as an emerging reservoir of Guinea worm *Dracunculus medinensis* infection. *PLoS Neglected Trop. Dis.* 14, e0008170.
- Meshaka Jr., W.E., 2005. *Osteopilus septentrionalis* (Duméril and Bibron, 1841): Cuban treefrog. In: Lannoo, M. (Ed.), *Amphibian Declines: the Conservation Status of United States Species*. University of California Press, Berkeley, pp. 463–465.

- Muller, R., 1971. *Dracunculus* and dracunculiasis. *Adv. Parasitol.* 9, 73–151.
- Muzzall, P.M., 1991. Helminth infracommunities of the newt, *Notophthalmus viridescens*, from Turkey Marsh, Michigan. *J. Parasitol.* 77, 366–371.
- Ocock, J.F., Brandis, K.J., Wolfenden, B.J., Jenkins, K.M., Wassens, S., 2019. Gut content and stable isotope analysis of tadpoles in floodplain wetlands. *Aust. J. Zool.* 66, 261–271.
- Onabamiro, S.D., 1954. The diurnal migration of Cyclops infected with the larvae of *Dracunculus medinensis* (Linnaeus), with some observations on the development of the larval worms. *W. Afr. Med. J.* 3, 180–194.
- Pennak, R.W., 1963. Species identification of the fresh-water cyclopoid Copepoda of the United States. *Trans. Am. Microsc. Soc.* 82, 353–359.
- Piasecki, W., Goodwin, A.E., Eiras, J.C., Nowak, B.F., 2004. Importance of Copepoda in freshwater aquaculture. *Zool. Stud.* 43, 193–205.
- R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Richards, R.L., Cleveland, C.A., Hall, R.J., Ouakou, P.T., Park, A.W., Ruiz-Tiben, E., Weiss, A., Yabsley, M.J., Ezenwa, V.O., 2020. Identifying correlates of Guinea worm (*Dracunculus medinensis*) infection in domestic dog populations. *PLoS Neglected Trop. Dis.* 14, e0008620.
- Ruiz-Tiben, E., Hopkins, D.R., 2006. Dracunculiasis (Guinea worm disease) eradication. *Adv. Parasitol.* 61, 275–309.
- Schoonover, L., Marshall, W., 1951. Food habits of the raccoon (*Procyon lotor hirtus*) in North-Central Minnesota. *J. Mammal.* 32 (4), 422–428.
- Toscano, B.J., Rombado, B.R., Rudolf, V.H.W., 2016. Deadly competition and life-saving predation: the potential for alternative stable states in a stage-structured predator–prey system. *Proc. Royal Soc.* 283.
- Uscanga, A., Moyano, F.J., Alvarez, C.A., 2010. Assessment of enzymatic efficiency on protein digestion in the tilapia *Oreochromis niloticus*. *Fish Physiol. Biochem.* 36, 1079–1085.
- Wilson-Aggarwal, J.K., Goodwin, C.E.D., Swan, G.J.F., Fielding, H., Tadesse, Z., Getahun, D., Odiel, A., Adam, A., Marshall, H.H., Bryant, J., Zingesser, J.A., McDonald, R.A., 2021. Ecology of domestic dogs (*Canis familiaris*) as a host for Guinea worm (*Dracunculus medinensis*) infection in Ethiopia. *Transbound. Emerg. Dis.* 68, 531–542.
- WHO Collaborating Center for Research Training and Eradication of Dracunculiasis, Centers for Disease Control and Prevention (CGH), 2020. Guinea Worm Wrap-Up #266.
- WHO Collaborating Center for Research Training and Eradication of Dracunculiasis, Centers for Disease Control and Prevention CGH, 2021. Guinea Worm Wrap-Up #274.
- Williams, B.M., Cleveland, C.A., Verocai, G.G., Swanepoel, L., Niedringhaus, K.D., Paras, K.L., Nagamori, Y., Little, S.E., Varela-Stokes, A., Nemeth, N., Wyrosdick, H., Tucker, A., Deal, L., Gauthier, D., Prouty, S., DeAngelo, C., Marsh, A., Piepgras, D., Cook, L.H., Milliren, K.B., Becker, J.S., Lyons, C., Clark, J., Stumph, J., Borst, M.M., Craig, T., Tucker, K.L., Ward, A., Baird, E.M., Burke, K.A., Camp, J.W., Davis, C.A., Pulaski, C.N., Yabsley, M.J., 2018. *Dracunculus* infections in domestic dogs and cats in North America; an under-recognized parasite? *Vet. Parasitol. Reg. Stud. Reports.* 13, 148–155.