Trematode prevalence and richness in ponds in Teton County, Wyoming

Michele Larson and Amy Krist
University of Wyoming, Department of Zoology and Physiology
Laramie Wyoming

Trematodes have complex life cycles with infective stages in multiple hosts including species of conservation concern. We conducted a field survey of seven ponds in Teton County, Wyoming for trematode prevalence and richness. We collected snails from each location and determined trematode prevalence by shedding snails and determined trematode richness by dissection of infected snails. At each location we also measured temperature, conductivity, and dissolved oxygen. We found trematode prevalence varied by location and infection rates of 15% or lower in most snail taxa. Snails in the families Physidae and Lymneaidae, the two most common families in our survey, were more often infected with trematodes than other snails. We found four different trematode cercarial types: echninostome cercaria, furcocercous cercaria, monostome cercariae, and xiphidiocercaria, which translates into 11 possible families of trematodes. Although we found significant differences in abiotic factors among locations, none of the abiotic factors that we examined predicted trematode prevalence or richness. We found levels of trematode prevalence that are similar to reports in other locations. Prior research also indicates that trematode infections are usually not fatal to waterfowl, but that young birds are more susceptible to infections. We advise waterfowl managers to inoculate captive bird population to reduce trematode and other parasitic worm infections. We also advise against the use of molluscides to reduce trematode infection in wildlife as this strategy is not effective and may create long term problems for waterfowl.

Figure 1. Swans at Teton Science School Pond (left) and Swan Lake (right).

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Abstract

Trematodes have complex life cycles with infective stages in multiple hosts including species of conservation concern. We conducted a field survey of seven ponds in Teton County, Wyoming for trematode prevalence and richness. We collected snails from each location and determined trematode prevalence by shedding snails and determined trematode richness by dissection of infected snails. At each location we also measured temperature, conductivity, and dissolved oxygen. We found trematode prevalence varied by location and infection rates of 15% or lower in most snail taxa. Snails in the families Physidae and Lymneaidae, the two most common families in our survey, were more often infected with trematodes than other snails. We found four different trematode cercarial types: echninostome cercaria, furcocercous cercaria, monostome cercariae, and xiphidiocercaria, which translates into 11 possible families of trematodes. Although we found significant differences in abiotic factors among locations, none of the abiotic factors that we examined predicted trematode prevalence or richness. We found levels of trematode prevalence that are similar to reports in other locations. Prior research also indicates that trematode infections are usually not fatal to waterfowl, but that young birds are more susceptible to infections. We advise waterfowl managers to inoculate captive bird population to reduce trematode and other parasitic worm infections. We also advise against the

use of molluscides to reduce trematode infection in wildlife as this strategy is not effective and may create long term problems for waterfowl.

Introduction

Trematodes (flukes) are a diverse group of parasitic flatworms that infect multiple hosts in their complex life cycle. Snails are the first intermediate host for most trematodes while vertebrates are always the definitive host (Olsen 1974, Poulin and Cribb 2002). Many species of trematodes also have second intermediate hosts, which can be invertebrates and vertebrates, depending on the species of trematode. For many trematode species the first intermediate hosts (snails) are often genus or species specific while the second intermediate and definitive hosts are typically much less specific (Horak and Kolarova 2005).

The life cycle of most trematodes begins when adult flukes in the vertebrate host release eggs. The first intermediate host (usually snails) becomes infected by either directly consuming encapsulated miracidia, a larval transmission stage, or by penetration by swimming miracidia. Within the snail host multiple rounds of asexual reproduction of the larval trematode occurs resulting in the production of larval transmission stages, called cercaraie, that are released into the water. Many thousands of cercariae can be released by a single infected snail per day (Seppala et al. 2007). In life cycles with second intermediate hosts, the cercariae find and infect the second intermediate host (usually fish, amphibians, bivalves, amphipods, or insect larvae) and encyst as metacercariae in the tissue of the second intermediate host. To complete the life cycle, encysted metacercariae are ingested by the definitive host, travel within the definitive host to a target tissue and begin reproducing sexually. For life cycles without second intermediate hosts, cercariae encyst on vegetation that is consumed by the definitive host, are consumed by the definitive host or directly penetrate the definitive host tissues (Poulin and Cribb 2007, Horak and Kolarvoa 2011).

Trematodes are associated with reduced fitness and disease. Trematode infections of snails (first intermediate hosts) often castrate the snail thereby eliminating evolutionary fitness (Horak and Kolarova 2011) and lowering snail population growth (Brown et al 1988). In some cases trematode infections can cause snail death (incompatibility of trematode to snail; Zbikowski and Zbikowska 2009). In vertebrate hosts, trematodes cause diseases including Schistosomiasis (in birds and mammals, including humans), which can be fatal (Horak and Kolarova 2011). Humans can also contract cercarial dermatitis ("swimmer's itch") from bird schistosomes (Lindblade 1998, Horak and Kolarova 2011).

We surveyed seven ponds in Teton County, Wyoming for trematode prevalence (infection rates in snails) and richness to determine how widespread and diverse trematode parasites were in this region. The seven ponds are important habitat for waterfowl including trumpeter swans (*Cygnus buccinator*), a Species of Greatest Conservation Need in Wyoming (WGFD 2010). Our investigation of trematodes may aid wildlife managers in reducing the risk of infection in waterfowl and other vertebrate definitive hosts.

Methods

Snail Sampling

We collected snails and determined trematode prevalence and richness for seven ponds in Teton County, Wyoming. We collected snails by hand or using hand sieves and D-framed nets in vegetation along the shore at each pond. We collected snails from as many different habitats (different substrates, undercuts of banks, overhanging vegetation, etc.) along the shore as we could access. When possible, we collected 50-100 snails of each genus at each location. Snails were placed into large buckets after collection and transported to laboratory facilities at the NPS-UW field station (AMK Ranch) in Grand Teton National Park.

To assay incidence of infection of trematodes in all snails, we placed individual snails in 30 ml cups with lake water (obtained from Jackson Lake, Grand Teton National Park, WY) under a 60 watt light bulb for at least two hours. This method causes the release of the trematode transmission stage (cercariae). We dissected all infected snails under a dissecting microscope to confirm infection status and to identify the trematodes. Using compound light microscopy, individual trematode cercariae were identified using Schell (1970) to the family or superfamily level, the lowest level of taxonomy that can be obtained from cercarial morphology.

Sampling Abiotic Factors

Temperature, conductivity, and dissolved oxygen were collected from three locations at each site using YSI series environmental probes. We also collected water samples from the water column for each location for analysis of pH and ion concentrations. We placed all water samples on ice until returning to the research station. We processed water samples for pH immediately upon our return to AMK Ranch using a pH meter (Corning 430). Water samples for anions were frozen while samples for cations were acidified using HCl (0.5 M solution) and refrigerated. Ion water samples were analyzed using ion chromatography (Dionex ICS 5000) upon return to the University of Wyoming.

Data Analyses

For each snail taxa, trematode prevalence is simply the fraction of infected snails in each location (the number of infected snails divided by the total number of snails collected). We determined trematode richness for each snail species and each pond by counting the number of different trematode families present. We also conducted one-way ANOVA's among sites for temperature and dissolved oxygen. Because the conductivity data were non-parametric, we conducted a ranked one-way ANOVA to determine whether conductivity differed among ponds. We conducted linear regressions between abiotic factors and trematode prevalence and richness to determine if any abiotic factors significantly influenced trematode prevalence. We conducted all data analyses using Systat 13 and created all graphs using Sigma Plot 11.0.

Results

Trematode prevalence varied by both species and location (Table 1). Trematode prevalence was lowest (0.88%) at Teton Golf Course Pond and highest (64.7%) at Homestead Pond. Overall, snails in the families Physidae and Lymnaeidae had the highest infection rates, and they were also the most common snails found in the ponds that we sampled.

In only four cases we found trematode prevalence above 20% (Table 1). These high infection levels were found at Homestead Pond, Nature Center Pond, and Swan Lake. However, because of low sample sizes, our estimates of trematode prevalence at Homestead Pond and Nature Center Pond (17 and 19 snails respectively) may not be accurate.

We found a total of four different types of cercariae in our survey. The four cercariae types were echinostome, monostome, furcocercous, and xiphidiocercariae. We were able to further identify the furcocercus cercariae to brevifurcate-apharyngeate cercariae and the xiphidiocercariae to Armatae cercariae (Schell 1970). Echinostome cercariae occur in the family Echinostomatidea; monostome cercariae occur in the families Notocotylideae, Nudacotylidae, and Pronocephalidae; furcocercus cercaria occur in the families Spirorchiidae and Schistosomatidae; and xiphidiocercaria occur in the Auridistomidae, Cephalogonimidae, Ochestosomatidea, Plagiochiidae, and Telorchiidae (Schell 1985).

We found between one to three cercarial types in each pond. All ponds with identifiable cercariae (all ponds but Teton Pines Golf Course Pond and South Park Pond) had furcocercous cercariae (Table 1). Echinostome cercariae were the second most common cercarial type, occurring at four of the five sites with identifiable cercariae (Table 1). We found the remaining two cercariae types, monostomes and xiphidiocercariae, only at a single location. We only found monostome cercariae at Swan Lake and xiphidiocercariae at Homestead Pond (Table 1). At two ponds (Teton Pines Golf Course Pond and South Park Pond) snail released cercariae in cups, but when we dissected the snails, no cercariae were mature enough to identify to type.

Abiotic factors varied among locations (Table 2). Temperature, conductivity, and dissolved oxygen differed among locations (Table 3). Temperature was significantly higher at Teton Science School Pond than the Teton Pines Golf Course Pond, Homestead Pond, and Nature Center Pond (Figure 1). We also found Homestead Pond and Nature Center Pond had significantly lower temperatures than all the other ponds except Teton Pines Golf Course Pond (Figure 1). Conductivity differed among ponds with the highest conductivity (433.82 $\mu S/cm^2$) at South Park Pond and the lowest conductivity (144.23 $\mu S/cm^2$) at Swan Lake (Figure 2). Dissolved oxygen varied by location with a significantly higher level at the Teton Science School Pond than South Park Pond, Oxbow Lake, and Swan Lake (Figure 3). Yet, none of the abiotic factors measured in our survey had any significant influence on trematode prevalence or richness (Table 4).

Discussion

We found high variability in trematode prevalence (0.9-64.7%) by snail genus and location (Table 1). Trematode prevalence in natural waters is highly variable and depends on abiotic factors (temperature, water level, water movement, shore slope, ect.) and biotic factors (snail density, vertebrate host density, etc.) as well as season (Zbikowska and Nowak 2009). In a review of trematode studies in Europe, Zbikowska and Nowak (2009) found trematode prevalence in snails ranging from 1-89.9% depending on region and snail species. Studies from across the world for bird schistosomes in snails found prevalences ranging from 1% -52.4% depending on location (Horak and Kolarova 2011). Adema and colleagues (2009) found trematode prevalence ranging from 0-42% in multiple sites on the Snake River and Polecat Creek in Grand Teton National Park; all infections occurred in Physidae and Lymaeidae snails.

Consistent with Adema et al. (2009), we also found higher infection rates in Physidae and Lymaeidae snails (Table 1) which may reflect the higher abundance and larger size of snails in these families. Trematode prevalence has been correlated to increased snail size (Briers 2003, Graham 2003) because larger snails are often older and therefore have had more time to become infected. However, other factors including nutritional level of snails, temperature, and phenotypic plasticity can result in wide size variation in snails of the same age (Graham 2003). Other factors that could be responsible for increased prevalence with increased snail size include more surface area for miricidia to penetrate snails, increased detection by miricidia, unknown enhancement to survival provided by trematode infections, or decreased immunity to trematodes with increased snail size (Graham 2003).

We found four cercarial types in our survey (Table 1). Adema and colleagues (2009) sampled seven sites in Grand Tetons and found three cercarial types: xiphidocercaria, furcocercous cercaria and echinostome cercariae. These cercarial types use different hosts (Table 5) including snails for first intermediate hosts; snails, clams, larval insects, amphipods, tadpoles, and fishes for second intermediate hosts; and vertebrates of different classes for final hosts (Schell 1985). For schistosomes (furcocercous cercaria), the most common snail hosts are in the families Lymnaeidae, Physidae, and Planorbidae (Horak and Kolarova 2011).

We also found several abiotic factors that varied among locations. Temperature (Figure1), conductivity (Figure 2), and dissolved oxygen (Figure 3) all varied by location, however, no significant relationships were found between any of these three factors and trematode prevalence and richness (Table 4). The lack of any relationship between trematodes and abiotic factors may be attributed to the large range of abiotic factors that trematode hosts are able to tolerate.

Trematode infections in Waterfowl

Avian trematode infections can be diverse and widespread in waterfowl. In France, examination of sixteen different species of waterfowl for schistosome infections found 60% of aquatic birds had intestinal schistosome infections (Jouet et al 2009). In Thailand, Saijuntha and collegues (2013) found echinosome infections in 56.7% of examined free-grazing ducks. However, the parasite load of ducks was low with fewer than 10 adult echinosome worms per duck (Saijuntha et al. 2013). Also, infection by echinosomes may be beneficial because this type of trematode is used for biological control of the pathogenic trematode, the liver fluke *Fasciola gigantic* (Saijuntha et al. 2013).

Swans also show variable infection rates by trematodes. In healthy tundra swan (*Cygnus columbianus*) examined during hunting season in Nevada and New Mexico, 92% (12/13) showed infections by the trematode *Allobilharzia visceralis* (a trematode that can cause avian schistosomiasis; Brant 2007). However, the low parasite load and the lack of internal lesions or granulomas in the liver indicated that the swans were unlikely to suffer morbidity due to the trematode infection (Brant 2007). Pennycott (1998) attributed mute swan (*Cygnus olor*) deaths in Scotland to lead poisoning and parasitic infections. Three of fourteen individuals had high enough parasite loads (a combination of trematodes, acathocephalans and nematodes) to cause death (Pennycott 1998). Eight of the swan deaths were attributed to lead poisoning (Pennycott 1998). For the majority of deaths, immature birds die of parasite infections while adult swans died of lead poisoning (Pennycott 1998). In the Netherlands, mute swans (*C. olor*) were infected with *Trichobilharzia sp*., a shistosome trematode that resulted in death due to obliterative endophlebitis (van Bolhuis et al 2004). However, high levels of copper and lead in the swan's tissues made clear diagnosis of cause of death difficult to determine (van Bolhuis et al. 2004). The trematode, *Sphaeridioterma globules*, was found to cause ulcerative hemorrhagic enteritis in mute swans (*C. olor*) in New Jersey (Roscoe and Huffman 1982). From 1977 - 1980, seventy-six mute swans died on Lake Musconetcong, NJ, with the majority of deaths occurring in winter months (Roscoe and Huffman 1982).

Implications for management

Management of trematode infections in wildlife is complex due to the many hosts these organisms use as well as the numerous abiotic factors that can influence each life stage and host of trematodes. For management of waterfowl populations, the majority of deaths due to trematode infections occur in young birds with immature immune systems (Pennycott 1998, van

Bolhuis et al 2004, Horak and Kolarova 2011) and in the winter months most likely due to increased stress and lower food quality/quantity (Pennycott 1998). High densities of waterfowl can increase the risk of infectious disease transmission including trematode infections (Pennycott 1998). Additionally, high levels of parasitism in swans may have been related to lower water levels, allowing swans to access vegetation that may have contained more parasites (Pennycott 1998).

To address these concerns, we recommend the administration of anti-helminth drugs to captive populations of swans and other waterfowl to reduce the transmission of trematodes and parasitic worms among birds. We also advise improvements to water quality in ponds and lakes as an indirect method of reducing prevalence of trematodes. Lakes, streams, and rivers with high nutrient levels (eutrophication) may accelerate snail growth and result in higher prevalence of trematodes (Johnson et al. 2007, Horak and Kolarova 2011). Reductions in nutrients may reduce parasite loads on vertebrate hosts as well as improve water quality.

We do not recommend the use of molluscides in the lakes and ponds in Teton County as these are unlikely to result in long term declines in trematodes and could cause long-term problems for waterfowl. The highly migratory nature of most waterfowl makes the eradication of snails and other mollusks from a small number of waterbodies a less than viable option in controlling trematode infections in waterfowl. The majority of migratory flyways (Mississippi Flyway, neoarctic-Hawaiian flyway, Paleartic-African flyway, etc.) contain numerous waterbodies with high levels of trematode prevalence (Horak and Kolarova 2011) making eradication of snails from a limited number of ponds an ineffective management strategy for reducing trematode infections in migratory bird populations. Also, snails are able to recolonize lakes and ponds during flooding events and by traveling on waterfowl. Eggs and newly hatched juvenile gastropods can attach securely to duck's feet and feathers and be dispersed to nearby ponds for up to 10 km (Boag 1986). Therefore, the eradication of snails from lakes and ponds would at best only be a temporary solution to reducing trematode infections in waterfowl.

Eradiation of snails would also be problematic because snails constitute a large proportion of the diet of waterfowl. The diet of redheads, *Aythya americana*, consisted of 18% gastropods and was the second most important food source for these ducks after shoalgrass (*Halodule wrightii*; Michot et al. 2008). Gastropods contributed 91.7% of greater scaup (*Aythya marila*), 86.2% of lesser scaup (*Aythya affinis*), and 27.5% of long-tailed ducks (*Clangula hyemalis*) autumn diets in Lake Ontario (Ross et al. 2005). Many birds supplement their diet with calcium-rich foods during egg laying (Reynolds and Perrins 2010). Petrie (1996) found increased consumption of gastropods by reproductively active female red-billed teal (*Anas erythrorhyncha*). The high calcium and protein levels in gastropod tissues may be important for egg production and bone health in female waterfowl (Petrie 1996, Scheuhammer et al. 1997).

Acknowledgments

We thank Susan Patla with the Wyoming Game and Fish for arranging access to private ponds and for assistance in field sampling. We are also grateful to Daniel Greenwood for field assistance and to Janet Dewey for analysis of water ion concentrations. Our study was financed by the Meg and Bert Raynes Wildlife Fund. All research was conducted under the permission of the Wyoming Game and Fish Department (Chapter 33 Permit #718) and Grand Teton National Park (Research permit #GRTE-2014-SCI-0039).

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Table 1. Trematode prevalence and richness for first intermediate hosts (snails) collected from seven ponds in Teton County, Wyoming.

Date	Collection Location	Taxon of Snail	Snails -Total # Collected	Snails -Total # Infected	Trematode Prevalence	Trematode Richness	Trematodes Present*
7/15/2014	Teton Pines	Family Physidae	113	1	0.88%	1	UND
		Family Lymneaidae	34	0	0%	0	—
		Genus *Gyraulus*	28	0	0%	0	—
7/15/2014	Teton Science School Pond	Family Physidae	47	2	4.26%	2	ECH; FUR
		Family Lymneaidae	80	9	11.25%	1	FUR
		Genus *Gyraulus*	1	0	0%	0	—
8/5/2014	Homestead Pond	Family Physidae	94	9	9.60%	1	ECH
		Family Lymneaidae	17	11	64.70%	2	FUR; XIPH
8/5/2014	Nature Center Pond	Family Physidae	19	6	31.60%	1	FUR
		Genus *Gyraulus*	109	10	9.20%	1	UND

8/5/2014	South Park Pond	Family Physidae	131	20	15.30%	1	UND
		Family Lymnaeidae	1	0	0%	0	—
		Genus *Gyraulus*	1	0	0%	0	—
8/14/2014	Oxbow Lake	Family Physidae	21	3	14.20%	1	FUR
		Family Lymnaeidae	67	6	8.95%	1	ECH
		Genus *Gyraulus*	1	0	0%	0	—
		Genus *Radix*	1	0	0%	0	—
8/16/2014	Swan Lake	Family Physidae	116	28	24.10%	3	MONO; ECH; FUR
		Family Lymnaeidae	1	0	0%	0	—
		Genus *Planorbella*	54	12	22.20%	1	ECH
		Genus *Gyraulus*	2	0	0%	0	—

*Trematode cercariae found at ponds: ECH = Echinostome cercariae , FUR = Furcocercous cercariae, MONO = Monostome cercariae, and XIPH = Xiphidiocercariae. Trematodes that could not be identified shown as UND.

Table 2. Abiotic factors for seven ponds in Teton County, Wyoming.

Location	Mean Temperature (ºC)	Mean Conductivity (μS/cm^2)	Mean Dissolved Oxygen (%)	pH	IONS (ppm) Na	K	Ca	Cl	PO_4	SO_4
Teton Pine's Golf Course Pond	15.73	144.23	46.97	7.61	7.69	0.87	17.32	2.74	22.56	6.93
Teton Science School Pond	22.27	347.22	80.07	8.16	13.59	1.89	38.19	6.47	0.00†	44.98
Homestead Pond	14.90	208.15	43.83	8.13	9.30	1.98	24.83	2.67	0.78	7.17
Nature Center Pond	14.07	336.85	47.27	7.97	12.02	2.27	39.82	5.07	0.00†	35.39
South Park Pond	19.60	433.82	39.93	10.32	35.52	5.52	33.63	38.52	0.00†	29.39
Oxbow Lake*	19.97	134.78	42.07	7.64	10.58	1.56	12.89	5.41	6.08	9.74
Swan Lake*	19.67	116.73	26.10	7.57	7.86	3.26	13.87	2.18	0.00†	1.20

* locations within Grand Teton National Park.

† ion values that were not detectable.

Table 3. ANOVA table for abiotic factors among pond locations. Bold numbers indicate significant ANOVA results.

Variable	Degrees of freedom	Mean Square	F-Ratio	p-value
Temperature	6	28.81	11.25	**<0.01**
Conductivity*	6	126.0	130.67	**<0.01**
Dissolved Oxygen	6	806.75	4.63	**<0.01**

*Ranked one-way ANOVA conducted due to non-parametric data.

Table 4. Regression table for trematode prevalence and diversity for each abiotic factor. Bold numbers indicate significant regressions.

Linear Regression for Trematode Prevalence

Variable	Degrees of freedom	Mean Square	F-Ratio	p-value	R^2	AIC
Temperature	1	632.53	1.626	0.26	0.25	65.26
Conductivity	1	1.26	0.002	0.96	0.00	67.22
Dissolved Oxygen	1	121.25	0.247	0.64	0.05	66.89
pH	1	2.14	0.004	0.95	0.00	67.22
Sodium (Na)	1	76.83	0.154	0.71	0.03	67.02
Calcium (Ca)	1	33.22	0.065	0.81	0.01	67.14
Potassium (K)	1	2.12	0.004	0.95	0.00	67.22
Chloride (Cl)	1	107.32	0.217	0.66	0.04	66.93
Phosphate (PO^4)	1	650.13	1.686	0.25	0.25	65.19
Sulfate (SO^4)	1	96.17	0.194	0.68	0.04	66.96

Linear Regression results for Trematode Diversity

Variable	Degrees of freedom	Mean Square	F-Ratio	p-value	R^2	AIC
Temperature	1	0.01	0.013	0.91	0.00	21.93
Conductivity	1	0.68	1.019	0.36	0.17	20.65
Dissolved Oxygen	1	0.18	0.233	0.65	0.05	21.63
pH	1	0.89	1.423	0.29	0.22	20.20
Sodium (Na)	1	1.17	2.076	0.21	0.29	19.52
Calcium (Ca)	1	0.19	0.248	0.64	0.05	21.61
Potassium (K)	1	0.10	0.122	0.74	0.02	21.78
Chloride (Cl)	1	1.29	2.366	0.19	0.32	19.24
Phosphate (PO^4)	1	1.12	1.949	0.22	0.28	19.64
Sulfate (SO^4)	1	0.45	0.632	0.46	0.11	21.11

Table 5. Possible hosts for trematode cercarial types (Olsen 1974) found in our survey.

Cercarial Type	Trematode Families	First Intermediate Hosts	Second Intermediate Hosts	Definitive Hosts
Echinostome cercariae	Echinostomatidae	Snails	Snails, tadpoles, fingernail clams, catfish and bullheads	Reptiles, Birds and Mammals
Furcocercus cercariae	Spirorchiidae	Snails	None	Turtles
	Schistomatidae	Snails	None	Birds and Mammals
Monostome cercariae	Notocotylidae	Snails	Encyst on Vegetation	Birds and Mammals
	Nudacotylidae	Snails	Encyst on Vegetation	Mammals
	Pronocephalidae	Snails	Encyst on Vegetation	Birds and Mammals
Xiphidiocercariae	Auridistomidae	Snails	Tadpoles	Turtles
	Cephalogonimidae	Snails	Tadpoles	Fish, Amphibians, and Reptiles
	Ochetosomatidea	Snails	Tadpoles	Snakes
	Plagiochiidae	Snails	Snails and Larval aquatic arthropods (insect larvae and crustaceans)	All Vertebrate classes
	Telorchiidae	Snails	Tadpoles	Amphibians and Reptiles

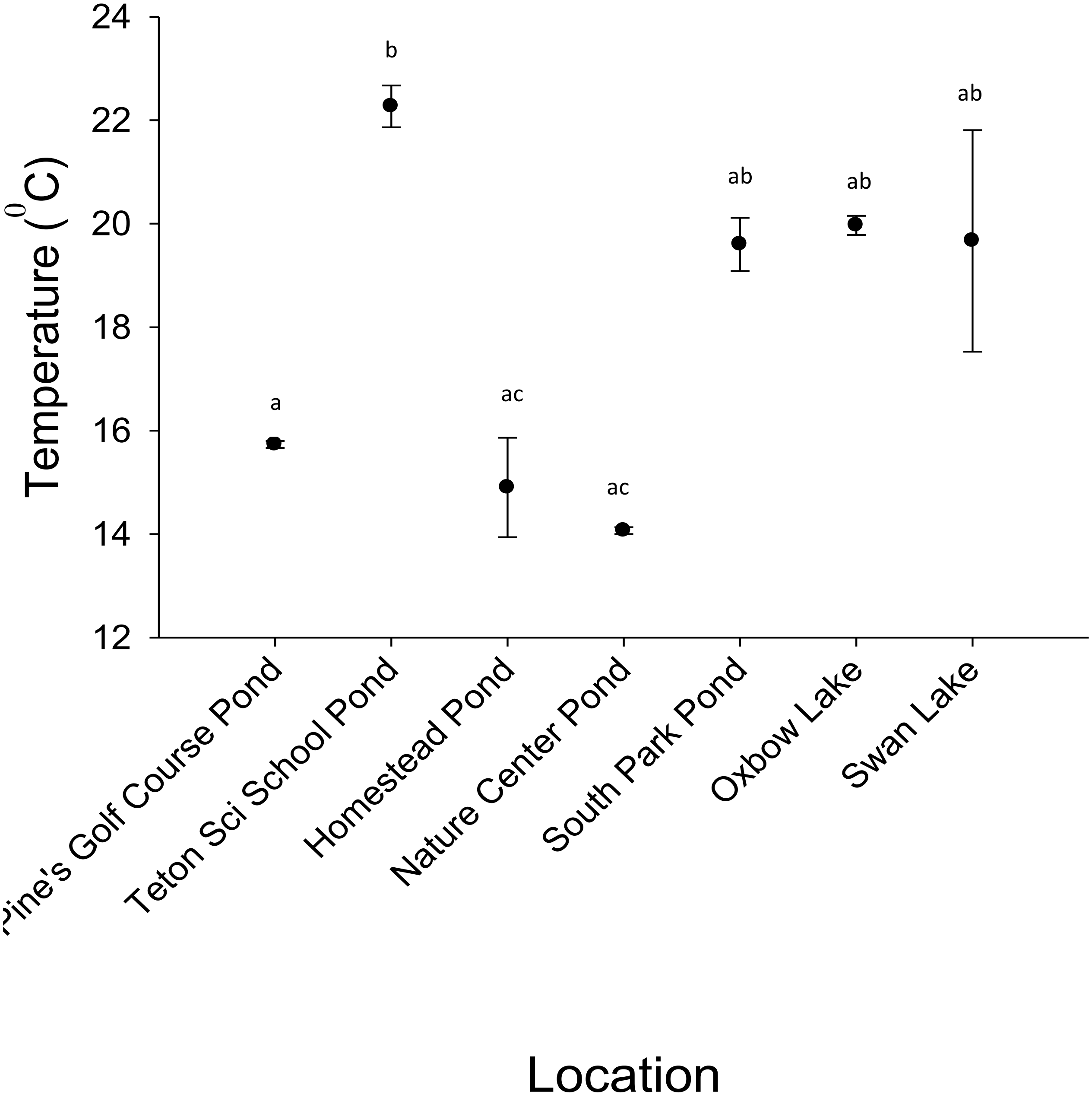

Figure 1. Mean temperature for seven ponds in Teton County, Wyoming. Letters indicate significant differences between locations. Survey occurred between 15 July 2014 to 16 August 2014.

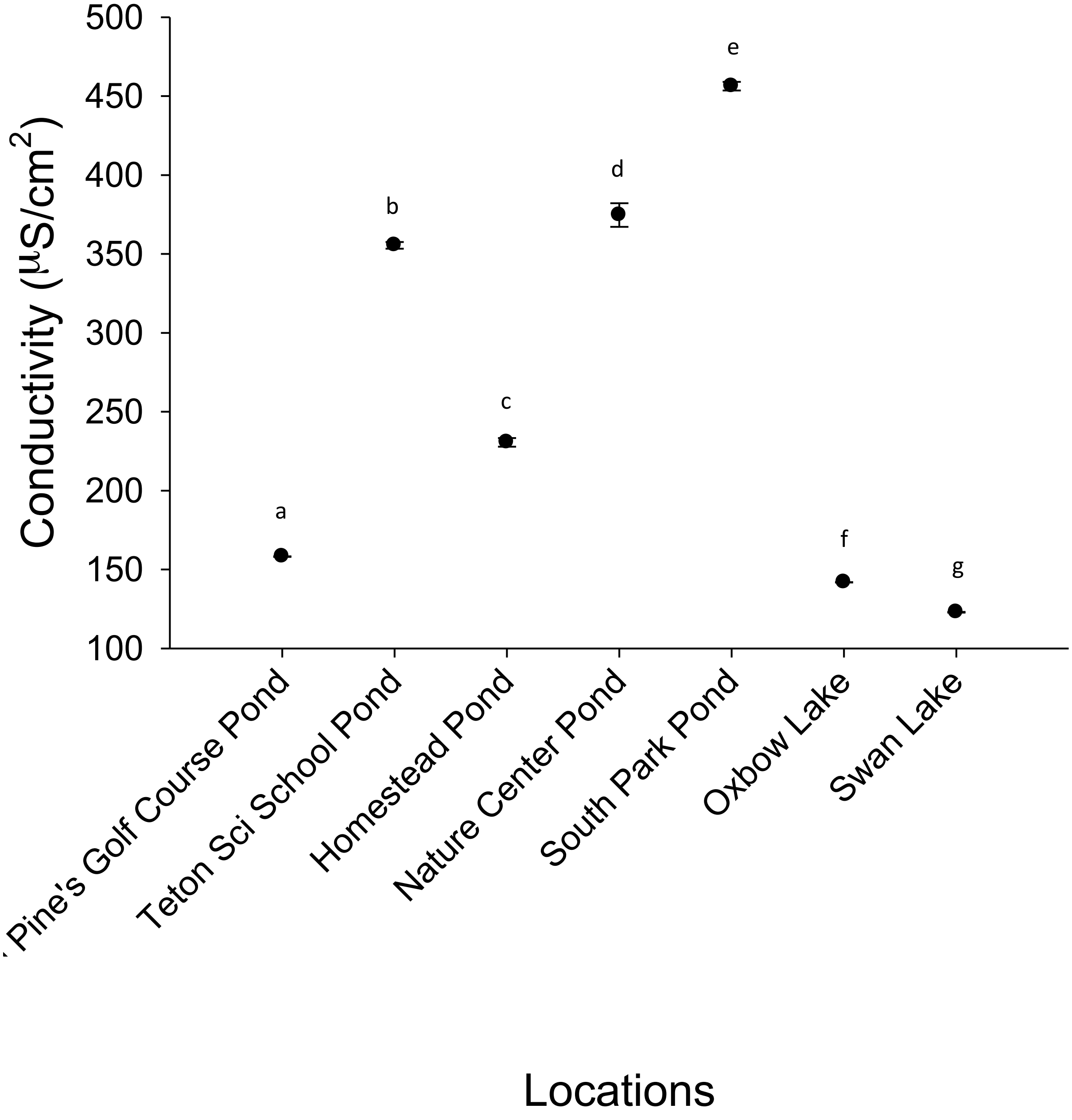

Figure 2. Mean conductivity for seven ponds in Teton County, Wyoming. Letters indicate significant differences between locations. Survey occurred between 15 July 2014 to 16 August 2014.

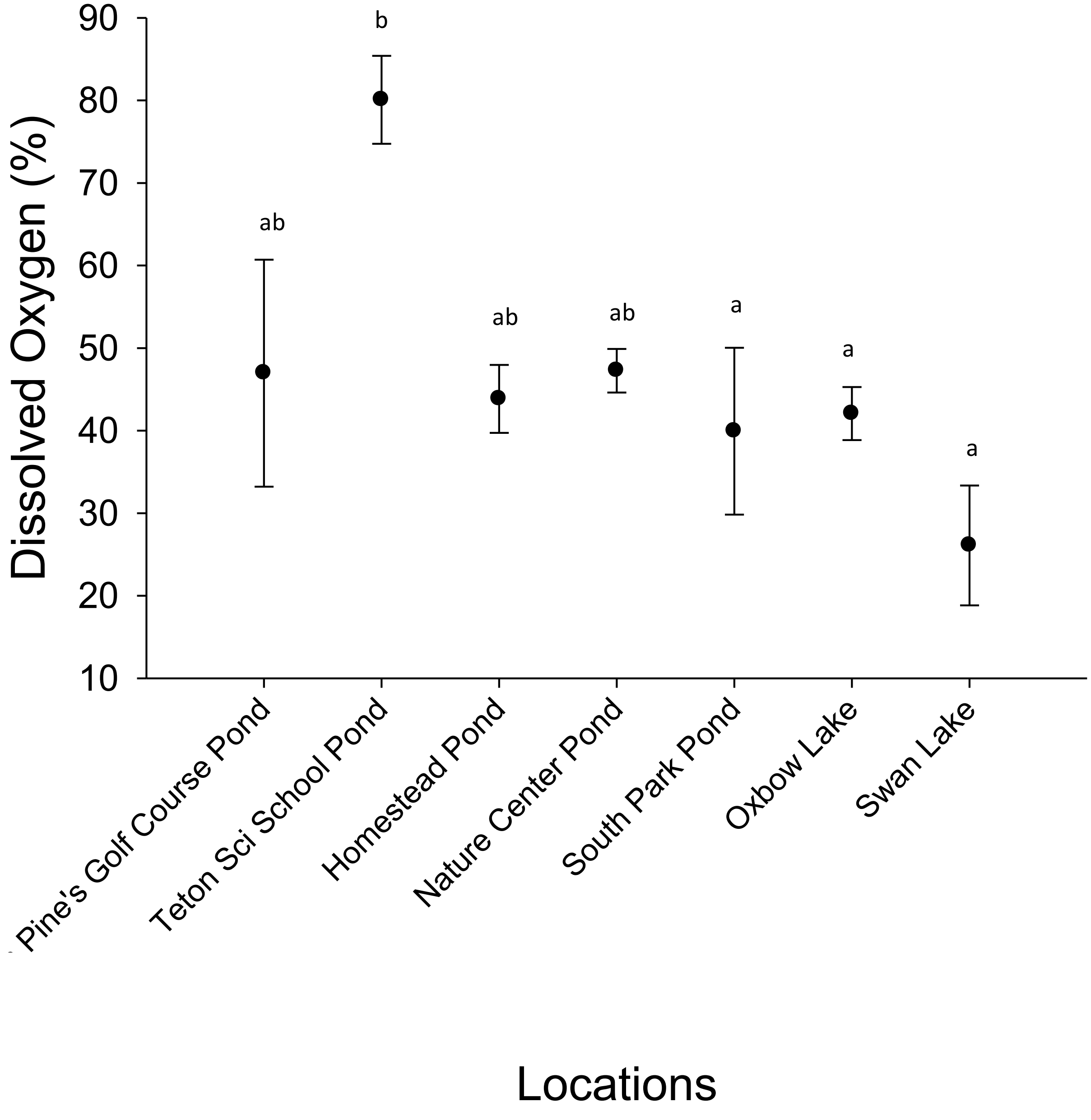

Figure 3. Mean dissolved oxygen levels for seven ponds in Teton County, Wyoming. Letters indicate significant differences between locations. Survey occurred between 15 July 2014 to 16 August 2014.