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ASSESSING DIETARY FACTORS THAT INFLUENCE BRANCHIOPOD CYST VIABILITY RATES FOLLOWING MALLARD DIGESTION AND EXCRETION

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Abstract

Waterfowl can passively transport propagules through internal and external mechanisms. Internal transport, called endozoochory, occurs when propagules are consumed, carried and excreted in a new location. For this to be successful in waterfowl, propagules must be able to survive avian digestive processes. Gizzard size is the main contributor to mechanical digestion in birds and can change in response to diet variations. Potential for endozoochoric dispersal has been studied extensively with plant seeds, but research on branchiopod eggs is limited. The goal of this study was to quantify the viability rate of fairy shrimp eggs that were consumed and excreted by mallard ducks and identify the effects of diet on that rate. Six mallards were divided on two different diets and fed two rounds of 200 fairy shrimp eggs each. Their excrement was collected for hatching trials, but no hatched fairy shrimp were identified. The results indicate that internal transport of fairy shrimp by mallards may be lower than previously expected.

Introduction

Seasonal and isolated bodies of water can be cultivated into thriving ecological communities when new species, such as plants and invertebrates, are introduced into the habitat. For this to occur, the propagules of these organisms, seeds and eggs, are translocated by external vectors. One of these means of dispersal is by waterfowl. Many species of waterfowl make seasonal migrations that span long distances in addition to their daily flight patterns between water bodies (Brochet et al., 2009; Kleyheeg et al., 2017). This provides an extensive opportunity for the dissemination of propagules. Waterfowl can transport these propagules either externally, on wings or feet, or internally by ingestion and excretion (Brochet et al., 2010b). These are known as exozoochory and endozoochory, respectively. For successful endozoochoric dispersal to take place, the seeds or eggs must be able to survive the avian host's digestive mechanisms and remain viable upon excretion. Numerous studies have identified propagules of plant and invertebrate species that have this capability, as well as factors that affect the potential viability (Leeuwen et al., 2012; Farmer et al., 2017; Sánchez et al., 2007). Gut morphology, specifically gizzard size, and time inside the intestines, have been shown to have significant effects on the viability of ingested seeds, but these processes have been less studied on invertebrate eggs (Figuerola & Santamaría, 2002; Kleyheeg et al. 2016; Malone, 1965; Sánchez et al., 2007; Soons et al., 2008).

The mallard duck, *Anas platyrhynchos,* is the most numerous and widespread dabbling duck in the world (Mundkur, 2012), and therefore, has served as a model for waterfowl-mediated propagule dispersal studies. Residing primarily in the Northern Hemisphere, the mallard's diet varies seasonally based on available food sources (DuBowy, 1988; Olsen et al., 2011; Dessborn et al., 2011). In an observational study, DuBowy noted that dabbling ducks partake in higher carnivorous behavior in the spring and summer, while mostly foraging with a high fiber diet in the winter months (DuBowy, 1988). To efficiently combat these frequent changes in diet, the mallard has a high degree of plasticity in digestive organ size, with changes taking place in as little as 10 days on a new diet (Miller, 1975; Soons et al., 2008; Jarman et al., 2020). Kleyheeg et al. found these diet-induced physiological responses to have a significant effect on seed recovery rates, with the highest retrieval (mean 19.8%) in mallards accustomed to an animal-based diet, and lowest (mean 4.3%) in those on plant-based diets (Kleyheeg et al., 2018). Similarly, duration spent in the intestines was found to be inversely related to seed viability (Soons et al., 2008), and timeliness of digestion was found to be slower in ducks fed a higher fiber diet (Malone, 1965).

The endozoochory phenomenon is not limited to plant seeds. Certain freshwater invertebrates are also able to undergo identical processes for dispersal (Sánchez et al., 2007; Brochet et al., 2010a; Leeuwen, 2012; Rogers, 2014b). Anostraca is an order under the class Branchiopoda, which includes freshwater crustaceans known as fairy shrimp. Fairy shrimp are abundant in seasonal pools and wetlands across North America (Aguilar et al., 2017; Rodgers, 2014a&b) and produce highly resistant eggs that are capable of surviving freezing temperatures, desiccation, and avian digestion (Dodson & Frey, 2001; Leeuwen, 2012; Rodgers, 2014b). For this reason, it is presumed that waterfowl play an important role as a vector of translocation for fairy shrimp, however, the magnitude by which this may occur is still unknown. The purpose of this study was to identify the potential for fairy shrimp translocation via mallard endozoochory by quantifying the viability rate of ingested eggs, and to identify the effects of seasonal diet changes on egg viability. It was predicted that there would be a lower viability rate for the eggs that passed through the ducks on an herbivorous diet.

Methods

Study Organisms and Diets

Six captive and domestic mallard ducks (*Anas platyrhynchos*) were housed at Windy Hill Farm in Johnstown, Ohio, including four females and two males. Each duck was approximately two years of age at the time of the study. The ducks were separated randomly into two groups of three for their diet assignment; one group fed a carnivorous pellet, the other fed an omnivorous pellet. In coordination with the BGSU attending veterinarian, to ensure proper and balanced diets for both groups, the diet assignments included premade Mazuri branded pellet feeds. The feeds used were intended to mimic the seasonal dietary variations that wild mallards undergo. For the more carnivorous and higher protein diet of the spring and summer months, Mazuri Diving Duck pellets were used. For the omnivorous diet of the winter, Mazuri Waterfowl Maintenance pellets supplemented with 10% Producer’s Pride Scratch Grains containing corn, milo, and oats, were used (see Appendix A). The ducks were given their assigned pellet available ad libitum as they roamed freely in an outdoor fenced area for three weeks before the first egg-feeding trial. After the feeding trial, the ducks began a transition to switch diets between the groups. For five days, the two pellets were mixed in 1/5 increments increasing each day. This allowed for a smooth transition as each duck changed to eating the diet that it was not previously fed (the carnivorous pellet group was switched to omnivorous, and vise-versa). After three weeks of the second diet, a second egg-feeding trial took place.

First Round of Feeding

Eggs of the Red tail fairy shrimp, genus *Streptocephalus,* were acquired from an online retail vendor (arizonafairyshrimp.com). The eggs were separated from the sediment under a stereo microscope and sorted into 1.5mL microcentrifuge tubes. Two hundred eggs were added to 7 tubes, for a total of 1400 eggs in the first trial. The eggs were mixed with ground duck feed pellets. After the ducks had been on the assigned diet for three weeks, they were individually hand-fed the mix of fairy shrimp eggs and ground pellets. Immediately after the feeding, each duck was placed in a 1 square foot mesh-wire bottom cage with a plastic 14-quart storage tote underneath to collect droppings. Ducks were provided food and water ad libitum during this period. A control group of 200 eggs was subjected to the same environment in a nearby 14-quart tote, but were not fed to ducks. The ducks were maintained in the individual cages for 24 hours to allow the eggs to pass through their digestive systems. After 24 hours, the ducks were returned to their outdoor enclosure. The residue left on the cages was washed with distilled water into the storage totes below. After this, the contents of the totes were washed into quart sized containers and sealed to take to the lab.

Within 3 hours of excrement collection, the contents of each quart container were washed through three sieves with mesh sizes of- 1.00-mm, 500-µm, and 106-µm, in succession. The contents of the 106-µm sieve were sent through a vacuum filtration process and stored in a labeled petri dish. All 6 duck excrement samples, as well as the control were subjected to this procedure. To hatch the processed excrement, the samples were taken back to Windy Hill Farm, and each sample was placed in a quart sized container filled with 500mL distilled water. The containers were located underneath a 4-foot 36-watt fluorescent grow light (Sylvania Grolux) and next to an eastward-facing window. The number of hatched fairy shrimp in each container was counted by eye while using a flashlight twice daily for ten days.

Second Round of Feeding

The second feeding trial used the same procedure as the first, except that the ducks were allowed to roam about in their enclosure for 30 minutes immediately after being fed the fairy shrimp eggs, but before being placed in the mesh bottom cages. Additionally, the hatching trial took place in the lab with a 75-watt LED grow light (Osunby LED Grow Light) rather than fluorescent.

Results

Out of the 2400 total redtail fairy shrimp eggs that were fed to mallards, no viable hatchlings were identified in either of the hatching trials. However, the control group for the first trial contained 63 hatched fairy shrimp, and the control group for the second trial contained 53 fairy shrimp. The combined average hatch rate from the controls was 29%. No dietary comparison was able to be completed due to the lack of hatched fairy shrimp from mallard excrement.

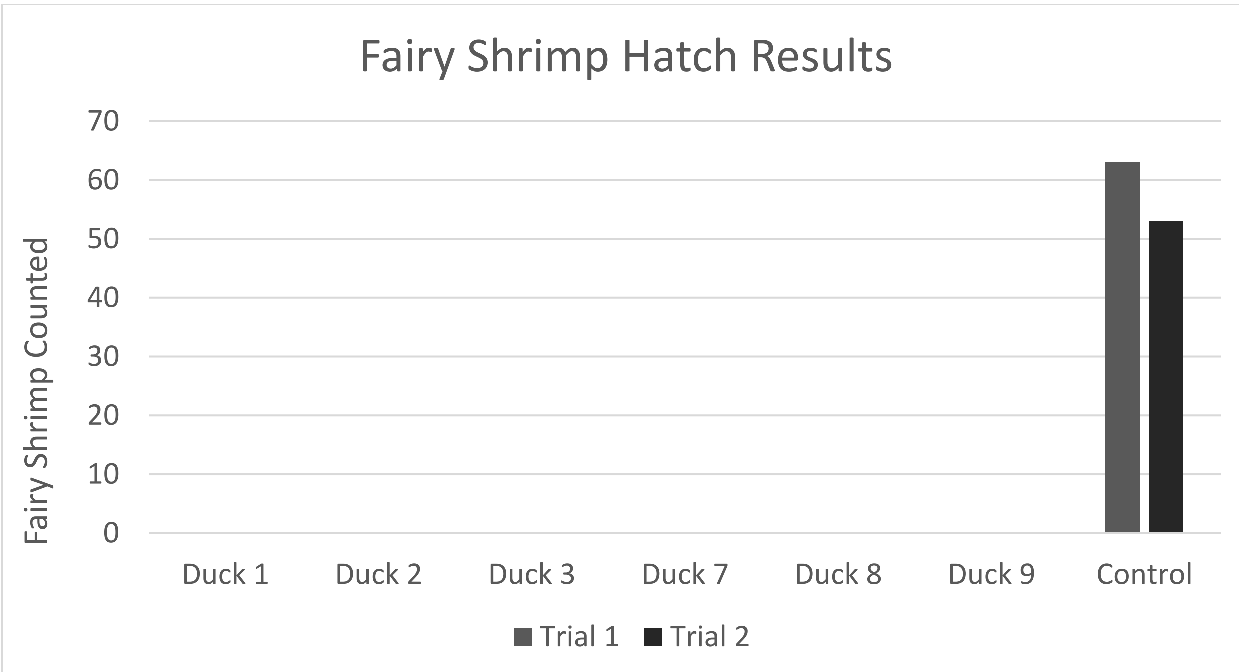

Figure 1. Hatching Results Graph.

Discussion

The lack of fairy shrimp hatching from the mallard excrement suggests that the rate of viability for the *Streptocephalus* eggs, following excretion by domestic mallards, may be lower than what could be observed in this study. Viable fairy shrimp propagules after mallard digestion may still be possible, as numerous studies have identified viable branchiopod eggs in the droppings and intestinal structures of various waterfowl species (Sánchez et al., 2007; Brochet et al., 2010a;

Leeuwen et al., 2012; Malone, 1965; Rogers 2014b). For example, in a 2014 study by C. Rogers, the author collected duck gastrointestinal tract samples from areas known to support Anostracan populations and analyzed the internal stomach contents for branchiopod eggs. In a combination of mallard and bufflehead ducks, he identified 5860 total eggs, 4395 of which were damaged, leaving 1465 in-tact. Out of the 1465 in-tact eggs, he was able to hatch 7 species of Anostraca, totaling 262 individuals (Rogers, 2014b). This corresponds to a 4.48% hatch rate when compared to total collected eggs; however, it cannot be assumed that this accounts for the total number of ingested eggs, as some may have undergone complete digestion or been trapped in areas such as the crop or gizzard. Thus, the rate of viability in excrement may be lower than 4.5%.

Although it cannot explain the differences in hatching between control and fed treatments, it is important to note that Anostraca eggs typically undergo fractional hatching, in which only a portion of the total number of eggs per clutch hatches at a time. Differences in environment, such as temperature and freezing cycles can elicit varying total hatch rates (Rogers, 2014a). This provides a safety net for the population to remain in an area if one generation dies before reproducing (Rogers, 2014a). If the conditions are not ideal for the particular clutch to produce a large hatch, the number of eggs identified as viable may be exiguous. The implications of this fractional hatching make it difficult to assess the actual number of viable Anostraca eggs, rather than just the number that hatch at one particular time. However, the higher hatch rate of the controls in this experiment, mean that fractional hatching cannot completely explain the low viability that was observed.

It is also important to note that in this study, domestic ducks were used, and they were fed preformulated pellets which may not be representative of naturally occurring situations.

Additionally, the eggs of a single genus (and potentially single species) of fairy shrimp were tested. The findings of this study may not entirely reflect occurrences in the natural world. The lack of viable eggs retrieved means that a comparison between the hatch rate produced by carnivorous and herbivorous mallards was not able to be completed. However, this study does indicate that transport of viable propagules of this species of fairy shrimp, internally, by mallards is likely infrequent.

Since the hatch rate appears to be so low, a similar study with substantially more fairy shrimp eggs may produce significant results. Additionally, other species of branchiopod may exhibit higher resistance abilities, and could be better candidates for endozoochoric dispersal. Similarly, different species of waterfowl may exhibit milder internal environments for the eggs. In this study, the excrement was transported between two distant locations; completing the entire experiment in the lab, cutting down on transportation times, may have an effect. With the above limitations to this study, it is clear that future studies will be necessary to accurately estimate the potential to which branchiopod endozoochory naturally occurs.

Ample evidence outside of this study shows that avian endozoochoric dispersal is a present, naturally occurring phenomenon (Sánchez et al., 2007; Brochet et al., 2010a; Leeuwen, 2012; Rogers, 2014b). Though the hatch rate of waterfowl-ingested branchiopod eggs is seemingly low, there are tens of millions of waterfowl in North America, each of which is a potential vector for the dispersal of organisms. Therefore, endozoochorous dispersal by waterfowl could still play a critical role in the movement of branchiopods.

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Appendix A

Diet nutritional information

Carnivorous Diet:

Mazuri® Diving Duck Diet – Formula Code 561X

Crude protein not less than	28.0%	Calcium not less than	1.95%
Crude fat not less than	5.0%	Calcium not more than	2.45%
Crude fiber not more than	8.0%	Phosphorus not less than	1.20%
Ash not more than	12.0%	Salt not less than	0.25%
		Salt not more than	0.75%
		Sodium not more than	0.55%

Ingredients

Fish meal, wheat middlings, ground wheat, dehydrated alfalfa meal, ground Timothy hay, corn gluten meal, ground corn, porcine meat and bone meal, ground flaxseed, brewers dried yeast, dried plain beet pulp, soybean oil, calcium carbonate, shrimp meal, salt, fish oil, magnesium oxide, l-lysine, choline chloride, pyridoxine hydrochloride, dried Lactobacillus acidophilus fermentation product, dried Lactobacillus casei fermentation product, dried Bifidobacterium thermophilum fermentation product, dried Enterococcus faecium fermentation product, preserved with mixed tocopherols, vitamin D_3 supplement, rosemary extract, d-alpha tocopheryl acetate (form of vitamin E), biotin, menadione sodium bisulfite complex (source of vitamin K), manganous oxide, citric acid (a preservative), vitamin A acetate, zinc methionine complex, zinc oxide, calcium pantothenate, folic acid, nicotinic acid, thiamine mononitrate, riboflavin supplement, copper sulfate, calcium iodate, vitamin B_{12} supplement, basic copper chloride, sodium selenite.

Granivorous Diet:

Mazuri diet with added 10% scratch grains

Mazuri® Waterfowl Maintenance Diet – Formula Code 5642

Crude protein not less than 14.0%	Calcium not less than 0.80%
Crude fat not less than 3.0%	Calcium not more than 1.30%
Crude fiber not more than 4.5%	Phosphorus not less than 0.60%
Ash not more than 7.0%	Salt not less than 0.05%
	Salt not more than 0.55%

Ingredients

Ground Corn, Wheat Middlings, Ground Oats, Dehulled Soybean Meal, Calcium Carbonate, Fish Meal, Soybean Oil, Dicalcium Phosphate, Salt, Biotin, Brewers Dried Yeast, Vitamin D3 Supplement, Menadione Sodium Bisulfite Complex (Vitamin K), Preserved with Mixed Tocopherols, Nicotinic Acid, Rosemary Extract, d-Alpha Tocopheryl Acetate (Vitamin E), Vitamin A Acetate, Choline Chloride, Citric Acid (a Preservative), Manganous Oxide, Zinc Oxide, Calcium Pantothenate, Folic Acid, Copper Sulfate, DL-Methionine, Vitamin B12 Supplement, Riboflavin Supplement, L-Lysine, Calcium Iodate, Sodium Selenite.

Producer's Pride Scratch Grain

Ingredients

Cracked Corn, Milo, Whole Oats.

*Labeling requirements vary from state to state. For an accurate list of ingredients and guaranteed analysis in your region, please refer to the label affixed to the feed product.

Guaranteed Analysis

Nutrient	Analysis
Crude Protein	8% min
Crude Fat	2% min
Crude Fiber	7% max
Calcium	0.01% min
Calcium	0.51% max
Phosphorus	0.2% min