Intestinal coccidiosis of anadromous and landlocked alewives, *Alosa pseudoharengus*, caused by *Goussia ameliae* n. sp. and *G. alosii* n. sp. (Apicomplexa: Eimeriidae)

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**A B S T R A C T**

Anadromous alewives, *Alosa pseudoharengus*, have experienced significant population level declines caused by factors including habitat destruction. Alewives occur in two different life histories, anadromous and landlocked forms. The landlocked alewife evolved from ancestral anadromous populations, resulting in an exclusively freshwater and phenotypically unique form. The occurrence of parasites in a host is linked to the environment, making alewives an ideal model to compare parasitology within a single species with contrasting life histories. Currently, little information exists on the presence and impacts of parasites in these fish populations; the present study sets out to better understand coccidiosis within the threatened anadromous populations and to understand how coccidian parasites compare in both life history forms. The intestinal coccidian, *Goussia ameliae* n. sp., was described infecting the pyloric cecum of 76% and 86% of young-of-the-year and adult anadromous alewives, respectively, from the Maurice River, New Jersey, USA. The coccidian was found in landlocked alewife populations with a prevalence of 92% and 34% in YOY and adult fish, respectively. An analysis of the small subunit 18S ribosomal RNA gene of *G. ameliae* from both life history forms demonstrated that the coccidian had 100% sequence identity, confirming the same parasite species in both forms. Though genetic analysis demonstrated *G. ameliae* to be identical, some differences were observed in sporulation and morphology of the parasite within the two populations. The sporocysts in anadromous populations were shorter and wider, and sporulation timing differed from that of landlocked fish. These differences may either be attributed to differences in the host type or to the sporulation environment. Lastly, alewives from landlocked populations were frequently co-infected with a second coccidian species in the posterior intestine, which occurred at a lower prevalence. This species, *G. alosii* n. sp., was described based on morphological characters of the sporulated oocysts in fresh parasitological preparations.

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1. Introduction

The alewife, *Alosa pseudoharengus*, is an anadromous fish native to the east coast of North America, whose populations have dramatically declined throughout their range. Alewives are a critical component of aquatic and terrestrial ecosystems, being a major source of forage for predatory game fish in freshwater and marine environments, as well as for coastal birds (Hall et al., 2012). They have also been demonstrated to be important agents for nutrient transport in aquatic ecosystems (West et al., 2010). Alewife populations are estimated to have declined by more than 98%, from a maximum catch in 1956 of 16,148 metric tons to a low of 7.5 metric tons in 2006 (Limburg and Waldman, 2009). It is believed that habitat loss caused by damming has been a major factor for the decline of this species, but other factors, including overfishing, pollution, climate change, and increased predation, have also contributed to their declines (Limburg and Waldman, 2009; Hall et al., 2010). In 2006, river herring were identified as a “Species of Concern” (NOAA, 2009), and in 2012, a comprehensive stock assessment by the Atlantic States Marine Fisheries Commission led to a coast-wide closure of commercial and recreational fishing for the two species of river herring, *A. pseudoharengus* and *A. aestivalis* (ASMFC, 2012).

The declines in alewife populations have brought to the forefront a need to better understand the biology of this species. While climate change and habitat loss have had direct impacts on alewife populations, it is also possible that these stressors can have indirect negative impacts to fish by benefiting parasite replication or disease transmission (Marcogliese, 2008). However, little
information is known about the impacts of infectious diseases, including parasites, in alewives, making it difficult to obtain a full picture of the factors involved in population declines or failed recovery of this species. The negative impacts of diseases and parasites of other clupeid species have been studied in more depth. For example, ichthyophthiriasis is a parasitic disease implicated in the mortality of wild Atlantic herring, *Clupea harengus*, and Pacific herring, *C. pallasi* (Rahimian and Thulin, 1996; Marty et al., 1998; Kocan et al., 1999). It has been hypothesized that the failed population recovery of Pacific herring stocks in Alaska following the Exxon Valdez oil spill can be partially attributed to diseases including ichthyophthiriasis (Marty et al., 1998; Hershberger et al., 2002).

Coccidians are a group of parasites common in marine fishes, though with little information available on their diversity and impacts on fish populations. Coccidians are apicomplexan parasites that cause intestinal or extraintestinal infections (Dykova and Lom, 2007). Though little is known about the impacts of coccidiosis in fish hosts, it is often assumed that infections cause little disease under natural conditions, unless the host–parasite–environmental balance is disturbed (Davies and Ball, 1993). There have been reports of suspected mortality caused by coccidians in cultured marine fish; *Goussia kuehne* was believed to cause mortality in Asian seabasses, *Lates calcarifer*, due to low daily water exchange rates (Gibson-Kueh et al., 2011; Szekely et al., 2013). Additionally, coccidian infections have been suggested to reduce body condition of wild fish. Infections caused by *Goussia* sp. in the liver have been linked to poor body condition in wild blue whiting, *Micromesistius poutassou* (Abollo et al., 2001), and Atlantic herring (Morrison and Hawkins, 1984).

In alewives, reports of parasitic infections in general are sparse (Muzzall, 1994). Reports of coccidian infections in clupeid fishes appear to be limited to descriptions of the liver coccidian, *G. clupearum*, and a coccidian in the testis (Morrison and Hawkins, 1984; Morrison and Marryatt, 2012). An intestinal coccidian has been reported within the pyloric ceca of the Pacific herring, *C. pallasi*, by histology, though this species was not fully characterized (Marty et al., 1998). With current information lacking on the parasites of depressed anadromous alewife populations, the intention of the present study was to document and characterize coccidian parasites infecting this species.

A landlocked form of alewife, which spends its entire life in freshwater, has been derived from the ancestral anadromous form (Palkovacs et al., 2008). Anadromous alewives gained entry into lakes through canals or fish transfers, and have adapted to the freshwater environment, thus losing their anadromous life history stage. It is believed that the natural and anthropogenic damming of lake outlets has caused many independent populations of anadromous alewives to diverge into landlocked forms through parallel evolution over the past 300–5,000 years (Palkovacs et al., 2008; Jones et al., 2013). One example of this is in the Laurentian Great Lakes, where alewives were first detected in 1873 and are believed to have been introduced through the Erie Canal and/or through fish transfers. Whenever method of their introduction, massive reproduction and establishment of this species has caused a major shift in the Great Lakes ecosystem (Fuller et al., 2014). Though considered the same species, landlocked and anadromous alewives do not generally cohabitate the same water bodies, thus interbreeding among populations is unlikely (Palkovacs et al., 2008). Divergence of anadromous alewives into a solely freshwater niche led to major phenotypic changes that distinguish the derived landlocked form from the anadromous form, including earlier age and smaller size at maturity, slower adult growth, and reduced fecundity (Gross, 1951; Graham, 1956). Further, Jones et al. (2013) have demonstrated that body form is different in landlocked and anadromous alewives, and is associated with population-level dietary patterns. The fast adaptation and evolution of the alewife to the freshwater environment has been shown to be a result of large regulatory modifications, rather than coding changes (Czesny et al., 2012), and phenotypic changes, such as reduction in gill-raker spacing to adapt to feeding on small plankton found in freshwater, occurred over a short time period (Palkovacs et al., 2014).

The ecology of parasites is directly related to the host environment, and the fast adaptation of the landlocked alewife to a new ecological niche would likely impact the ecology of parasites in this species. Clearly, landlocked alewives have not adjusted to all aspects of life in freshwater lakes, since massive kills have been reported in landlocked alewife populations during the winter months (O’Gorman and Schneider, 1986). Winter mortality is believed to be related to a loss of homeoviscous adaptation (Snyder and Hennessey, 2003) since landlocked alewives cannot escape the coldwater temperatures as effectively as can the anadromous forms in the marine environment. Lepak and Kraft (2008) also propose that in overwintering landlocked populations, a combination of immunosuppression and poor condition leaves fish more vulnerable to disease. The intent of the present study was to identify and compare coccidian parasites in anadromous and landlocked alewife forms.

Anadromous alewives in New Jersey, USA, belong to the mid-Atlantic stock (Palkovacs et al., 2014). They enter streams to spawn in early February, with peak spawning occurring during mid-April through May. The post-spawned adults return to the ocean in mid-June and the hatched larvae remain in freshwater, with emigration of juveniles occurring from July to mid-November (Corbett and Allen, 2012). Based on surveys done in the 1970s, it was concluded that of the 132 spawning runs of alewife and blueback herring, *A. aestivalis*, in the state, nine have already become extinct due to habitat loss and barriers blocking fish passage (Zich, 1978). New Jersey has one of the earliest reported landlocked populations of alewives in Lake Hopatcong, the state’s largest lake spanning about 10 km² in area, with reports of alewives dating back to 1850 (Gross, 1951). The introduction of alewives in Lake Hopatcong was attributed to the old Morris canal that connected the Delaware River to the lake (Gross, 1951) for means of transporting coal, iron, and zinc across the state. The landlocked alewife population in Lake Hopatcong has been completely isolated from extant anadromous forms since the beginning of the 1900s due to the blockage of canal and river passages to and from the lake. Currently, Lake Hopatcong supports a large population of landlocked alewives, which are the main forage for a variety of game fish. The lake also supports a commercial bait fishery that supplies alewives for fisherman throughout the region. The ecological importance of both anadromous and landlocked populations of alewives in the environment and their importance in supporting large predatory fish led us to better characterize coccidian parasites encountered within this species.

2. Materials and methods

2.1. Fish collection and sampling

2.1.1. Adult anadromous alewives

Adult alewives were collected on April 16, 2014 from the Maurice River, Millville, NJ, during their spawning migration. Samples were collected in conjunction with an annual survey of river herring conducted by the Bureau of Marine Fisheries, N.J. Division of Fish and Wildlife (NJFW) (project led by H. Carberry). Briefly, gill nets were set in the river, left for approximately 1 hour, and checked for river herring. A total of 28 adult alewives (mean fork length = 253 mm, SD = 10.86, n = 28) were collected and kept in a water reservoir until being transported back to shore by boat. The water temperature during fish collection was 13 °C and the salinity ranged from 0 to 0.1 ppt. In the field, fish were euthanized using tricaine methanesulphonate (MS-222) buffered with sodium bicarbonate and...
2.1.2. Young-of-the-year anadromous alewives

Young-of-the-year (YOY) alewives were collected on August 4, 2014 from the same location that adults were collected in conjunction with the Bureau of Marine Fisheries, NJFW. Collection was done by beach seine from a boat; live fish were transported into a live well with flowing water on the boat, and transported back to shore. Water temperature during time of fish collection was 25.7 °C and salinity ranged from 0.1 to 2.1 ppt. A total of 33 fresh YOY fish (mean fork length = 68.1 mm, SD = 5.4 mm, n = 30) were preserved for histological processing. Fish were euthanized with MS-222 buffered with sodium bicarbonate, cut along the ventral surface, and whole fish were fixed in 10% NBF for 48–72 h. Additionally, about 100 live fish were collected for parasitological analysis. These fish were transported back to the laboratory in a live fish transport truck in two 300 gallon tanks containing freshwater with added salt and supplied with compressed oxygen bubbled into the water. The total transport time was approximately four hours. Upon arrival to the laboratory, fish were transferred and maintained in a 100 gallon rectangular tank with supplemental aeration and filtration until the fish were assessed in the laboratory for parasites; holding time was no longer than 48 hours. Temperature in the tank was maintained between 24 °C and 26 °C.

2.1.3. Landlocked alewives from Lake Hopatcong

Sample collection was coordinated with a commercial bait operation on the lake. Fish were collected through the night by the commercial bait operator by attracting them with lights on a barge in various locations in the lake and seining the fish. The fish were transported back to shore by boat and maintained in flow-through tanks for no longer than 72 h prior to sampling. On August 21, 2014, a total of 88 fish composed of 50 1+ year olds (mean total length = 118 mm, SD = 5.97, n = 50) and 38 YOY (mean total length = 72 mm, SD = 6.52, n = 38) were preserved for histological analysis. Fish were euthanized with buffered MS-222. This was followed by making a ventral incision in the fish and fixing the whole fish in 10% NBF for 48–72 hours for histological processing. Ages were estimated based on previous growth rate data for this species in Lake Hopatcong. It has been reported for Lake Hopatcong that alewives reach about 92 mm in total length in their first year and two-year old fish reach about 127 mm total length, with growth slowing dramatically after reaching sexual maturity at about 2–3 years (Bochenek, 1981). Fresh parasite preparations and molecular samples were taken from heavily infected fish based on the screening of 40 freshly euthanized fish that were collected either on August 21, 2014 or October 15, 2014.

2.2. Fresh preparations of coccidia

Twenty YOY anadromous alewives and 40 adult landlocked alewives were processed during separate times in the laboratory. Fish were euthanized with an overdose of buffered MS-222 and the anterior intestine was dissected under a Zeiss Stemi 2000C stereo-microscope. Intestinal mucus was separated from the intestine and fecal material; wet mounts were prepared of the intestinal mucus and analyzed under a Nikon Eclipse E600 light microscope and photographed with a microscope-mounted Jenoptik, ProgRes SpeedXT Core3 digital camera. If a mucus preparation contained coccidial stages, then the remaining mucus was transferred to a conical tube containing tap water supplemented with 200 U penicillin/ml, 200 μg streptomycin/ml, and 0.5 μg amphotericin B/ml (Lonza, Walkersville, MD, USA), and maintained for 48 hours in the dark at room temperature to induce sporulation. For samples from anadromous alewives, 5 PPT NaCl was added to the water to more closely simulate the brackish environment from which the fish were sampled. Measurements of coccidian oocysts and sporocysts were done on fresh parasite preparations using the Jenoptik imaging software. For both oocysts and sporocysts, the lengths at the longest axis and the perpendicular short axis were taken. Additionally, the length/width relationship was calculated for the oocysts and sporocysts.

For statistical analysis, a two-tailed t-test was used to compare parasite dimensions, including length, width, and length/width relationship between all coccidia types. Five samples from each anadromous and landlocked fish that were heavily infected with coccidia were transferred to 1.5 ml centrifuge tubes, centrifuged at 6,000 RPM for 1 min, the supernatant was removed, and the pellet was frozen at −80 °C until further molecular analysis. Prior to freezing the intestinal samples from landlocked alewives for molecular analysis, the coccidians were first sporulated and examined with a light microscope to ensure that a single coccidian species was present. Only samples containing a single apparent species were used for molecular analysis.

2.3. Histopathology

Following fixation, all adult fish tissue samples were trimmed to include the anterior pyloric cecum and the posterior portions of the intestinal tract. For YOY fish, cross sections were prepared through the entire fish, starting at the level of the gill and sectioned back to the level of the posterior intestine. Tissues were routinely processed for histology, including embedding in paraffin, and 4 μm thick sections were cut and stained with hematoxylin and eosin (H&E). Serial sections were cut in areas necessary for further analysis. If proper interpretation of gastro-intestinal samples was not possible due to post-mortem autolysis, then the sample was not included in the analysis. A prevalence of coccidial infection was documented when typical coccidial stages were observed in either the pyloric cecum or intestine.

2.4. Molecular analysis

2.4.1. DNA extraction, polymerase chain reaction, and DNA sequencing

Coccidia samples that contained a single apparent species, as determined by morphological assessment, were selected for analysis. DNA was extracted using the QiAmp DNA mini QIAcube kit (Qiagen) according to the manufacturer’s instructions. Polymerase chain reactions were run in 50 μl volumes, consisting of 3 μl extracted DNA, 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each primer, 1.25 U Taq polymerase (Invitrogen), topped up with molecular grade water. Two sets of primers specific for coccidia were used for each sample; the 18E (5′-CTTTCGCAGTAGTTCGTC) reverse primers were used to amplify the brackish environment from which the fish were sampled. Measurements of coccidian oocysts and sporocysts were done on fresh parasite preparations using the Jenoptik imaging software. For both oocysts and sporocysts, the lengths at the longest axis and the perpendicular short axis were taken. Additionally, the length/width relationship was calculated for the oocysts and sporocysts.

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3. Results

3.1. Coccidia in anadromous alewives

Seven out of 20 fresh parasite preparations from intestinal mucus of YOY alewives had moderate to heavy infections with *G. ameliae* n. sp (full species description below and in section 3.5). The heavily infected individuals had an excess of mucoid material within the gastrointestinal tract, which was not observed in individuals with light or unapparent infections. The fresh mucus samples contained both unsporulated and sporulated oocysts, with four of the samples containing a large proportion of sporulated oocysts directly in the fresh preparation from freshly euthanized fish. There did not appear to be a difference in the number of sporulated oocysts in the samples following 48 hours of incubation in tap water with added salt (5 ppt) and antibiotics. In wet mounts, all of the oocysts and sporocysts appeared to be a single species, *G. ameliae*, based on uniform morphological characteristics.

The unsporulated oocysts (length = 13.11 μm ± 1.21 × width = 10.82 μm ± 0.70, n = 20) were slightly oval in shape (L/W = 1.21 μm ± 0.10, n = 20) and were frequently found in aggregates (Fig. 1A). Oocysts in the fresh samples occurred both sporulated and some in the process of sporulation (Fig. 1B,C). Sporocysts contained abundant sporocyst residuum. The oocyst wall was very thin, and although the oocysts generally had a spheroid shape, the oocyst wall was irregular (Fig. 1C); no oocyst residuum was observed. Measurements for oocysts and sporocysts are summarized in Table 1. Histological detection of the coccidian was predominantly in the pyloric cecum with a prevalence of 76% and 86% in YOY and adult fish respectively (Table 2). Severe infections occurred in 14% of the adult fish sampled, while none of the YOY fish were severely infected. Severe infections were characterized by dense colonization of various parasite stages throughout the intestinal epithelium covering about 20% or more of the epithelial surface of the pyloric cecum (Fig. 1D), and intestinal epithelial necrosis and sloughing were associated with the release of oocysts. Early parasite stages included meronts and developing merozoites, which were more rarely observed in the tissues (Fig. 1E). The predominant parasite stages included macrogamonts, microgametocytes, and unsporulated oocysts at an epicellular position within the epithelium of the pyloric cecum (Fig. 1F). The infection was associated with excess mucoid material within the intestinal lumen containing parasite stages and sloughed intestinal epithelial cells, loss of intestinal surface due to the large numbers of parasites in an epicellular position, and multifocal areas of intestinal necrosis associated with sloughing of parasites (Fig. 1G). Aggregates of sporulated oocysts were also observed within intestinal lesions (Fig. 1H).

A second type of coccidian found in the posterior intestine was generally associated with light infections and was rarely observed only with histology. These occurred at a prevalence of 5% and 14% in YOY and adult fish, respectively (Table 2). The infections comprised of spherical early stages within the brush border of the intestinal epithelium (Fig. 2A,B). Macrogamonts were embedded within the epithelial cells and instead of being uniformly oval in shape, often had an indentation and were often slightly wider at the end protruding into the intestinal lumen (Fig. 2C,D). Larger and more elongated sporonts with lightly staining cytoplasm were observed in the intestinal epithelium (Fig. 2D). No fresh preparations were observed of this coccidian type.

3.2. Coccidia in landlocked alewives

Two types of coccidia were found in fresh mucus preparations of landlocked alewives, one which heavily infected the pyloric cecum, *G. ameliae* n. sp. (species description below and in section 3.5), and a second that was associated with the mid-posterior intestine, *G. alosii* n. sp. (species description below and in section 3.5). This similar host tissue tropism was also observed with histology. The prevalence and infection severity of the two coccidians based on histology are summarized in Table 2. The two coccidian species compared in wet mounts and histology had significant morphological differences.

*Goussia ameliae* was the predominant coccidian type in the samples and had slightly ovoid unsporulated oocysts (length = 11.0 μm ± 1.39, width = 9.81 μm ± 0.86, with L/W of 1.12 μm ± 0.92, n = 30) in fresh preparations of mucus (Fig. 3A). Sporulated oocysts very rarely occurred in fresh mucus preparations. Following 48 hours of incubation in tap water with added antibiotics, all of these parasite stages developed into sporulated oocysts (Fig. 3B). The sporulated oocysts had a rounded to slightly ovoid, irregular shape, and the oocysts wall was very thin (Fig. 3B). The oocysts each contained 4 elongated sporocysts (Fig. 3C). Measurements of oocysts and sporocysts are summarized in Table 1. In histology, the earliest detected stage of the parasite were meronts, containing merozoites, slightly embedded in the brush border of the intestinal epithelium within the pyloric ceca (Fig. 3D). Early developmental stages appearing as 2 μm sized basophilic staining spherical bodies embedded in the brush border of the intestinal epithelial cells were found throughout the tissue (Fig. 3E). Macro and microgametocytes were in an epicellular position (Fig. 3F); microgametocytes and rounded unsporulated oocysts were often found sloughed in the lumen of the pyloric cecum (Fig. 3G). Severe infections with the coccidia had a mixture of all developmental stages covering the surface of the intestinal epithelium in the pyloric cecum (Fig. 3H).

*Goussia alosii* was observed in fresh wet mounts of intestinal mucus. In fresh mucus preparations, highly elongated unsporulated oocysts (length = 21.38 ± 190 μm, width = 9.79 ± 0.80 μm, n = 30) occurred in aggregates and were over double the length than width (L/W = 2.19 ± 0.20 μm, n = 30; Fig. 4A). The sporulated oocysts had
a relatively thin, but thicker oocyst wall than G. ameliae. The oocyst wall was smooth and created a uniform elongated and ovoid oocyst shape (Fig. 4B), compared to the thin, irregular oocyst wall of G. ameliae (Fig. 3B). Each sporocyst contained 4 elongated ellipsoidal sporocysts; measurements of oocysts and sporocysts are summarized in Table 1. Similar to the elongated oocyst in this species, the sporocysts were highly elongated with a L/W relationship of 3.34 ± 0.37 μm. In histology, the intestinal tract posterior to the pyloric cecum was infected with coccidia in 49% and 14% of YOY and adult fish, respectively (Fig. 4C; Table 2). In the lower intestine, unsporulated oocysts that were pale staining and elongated were embedded in the intestinal epithelium (Fig. 4D). Mucoid casts in the lower intestinal tract contained macrogamonts and pale staining, elongated unsporulated oocysts. These elongated oocysts (Fig. 4E) appeared to correlate with the ones seen in the wet mounts of the mucus samples.

Table 1

|                  | Oocyst length ± SD (range), (n) | Oocyst width ± SD (range), (n) | Oocyst L/W ± SD (range), (n) | Sporocyst length ± SD (range), (n) | Sporocyst width ± SD (range), (n) | Sporocyst L/W ± SD (range), (n) |
|------------------|--------------------------------|--------------------------------|-------------------------------|-----------------------------------|-----------------------------------|---------------------------------|
| G. amelii ANA     | 15.4 ± 1.8 μm (12.1–19.5), (n = 70) | 12.7 ± 1.6 μm (9.6–16.9), (n = 70) | 1.22 ± 0.12 μm (0.96–1.46), (n = 70) | 6.0 ± 0.4 μm (4.8–7.1), (n = 80) | 4.3 ± 0.4 μm (3.4–5.4), (n = 80) | 1.40 ± 0.13 μm (0.95–1.70), (n = 80) |
| G. amelii LL      | 18.6 ± 1.5 μm (13.8–23.2), (n = 70) | 14.1 ± 1.2 μm (11.7–18.9), (n = 70) | 1.33 ± 0.13 μm (0.97–1.67), (n = 70) | 8.1 ± 0.7 μm (6.3–9.9), (n = 80) | 3.4 ± 0.4 μm (2.4–4.4), (n = 80) | 2.38 ± 0.37 μm (1.71–3.45), (n = 80) |
| G. alosii LL      | 25.3 ± 1.9 μm (21.6–28.8), (n = 25) | 14.1 ± 1.3 μm (11.4–16.2), (n = 25) | 1.80 ± 0.16 μm (1.51–2.13), (n = 25) | 11.8 ± 1.3 μm (9.9–14.1), (n = 25) | 3.6 ± 0.3 μm (2.8–4.3), (n = 25) | 3.34 ± 0.37 μm (2.69–4.20), (n = 25) |

ANA, anadromous; LL, landlocked; SD, standard deviation.
3.3. Comparison of morphology and measurements of coccidia

To compare *G. ameliae* from the pyloric cecum of the anadromous and landlocked alewife populations, measurements of fresh sporulated oocysts and sporocysts were compared. The oocyst wall was similar, both thin and irregular, from both population types. The oocyst length, width, and length/width relationship were all significantly different in the anadromous and landlocked alewives (*p < 0.001*). The ranges in the oocyst dimensions appeared to be similar, although the landlocked populations had slightly larger...

### Table 2

|                     | Prevalence G. ameliae | Severe Inf G. ameliae | Prevalence (Int) | Severe infection (Int) |
|---------------------|-----------------------|-----------------------|-------------------|-----------------------|
| Anadromous YOY      | 25/33; 76% 0/33; 0%   | 2/33; 5%              | 0/33; 0%          |                       |
| Anadromous adult    | 24/28; 86% 4/28; 14%  | 4/28; 14%             | 0/28; 0%          |                       |
| Landlocked YOY      | 34/37; 92% 8/37; 22%  | **18/37; 49%**        | **5/38; 13%**     |                       |
| Landlocked adult    | 17/50; 34% 0/50; 0%   | **7/50; 14%**         | **0/50; 0%**      |                       |

YOY, young-of-year; Inf, infection; Int, posterior intestine; *uncharacterized coccidian; **Goussia alosii.*

### Fig. 2.

Histology of coccidia infection in the intestine of anadromous alewives, stained with H&E, bar = 10 μm. (A,B) Spherical early developmental stages (arrowheads) within the brush border of the intestinal epithelium; (C) macrogamonts (arrowhead) (notice the notches nearly midway through the parasite, embedded within the surface of the intestinal epithelium); (D) macrogamonts with notches (arrowhead) and unsporulated elongated oocysts (arrows) within the intestinal epithelium.

### Fig. 3.

*Goussia ameliae* from landlocked alewives, bar = 10 μm. (A–C) Wet mounts of fresh coccidia preparations with (A) unsporulated oocysts and (B,C) sporulated oocysts containing four elongated sporocysts. (D–H) Histology documenting the development of the coccidian in the pyloric cecum, stained with H&E. (D) Meronts containing merozoites within the brush border on the surface of the intestinal epithelium; (E) early developmental stages (arrowheads) embedded within the brush border; (F) macrogamonts with an epicellular position on the intestinal epithelium; (G) microgametocytes (arrowhead) and unsporulated oocysts (arrow) which have sloughed from the epithelial surface; (H) severe coccidiosis with various developmental stages occupying most of the surface of the intestinal epithelium.
oocyst means (Table 1). The sporocysts appeared morphologically different from each other. The sporocyst length, width, and length/width measure were all significantly different from each other ($p < 0.001$), with the anadromous form having shorter and wider sporocysts compared to the landlocked form, which have slender and elongated sporocysts (compare Figs 1C and 3C). Figures 5A and 5B demonstrate morphological differences in the sporulated oocysts of *G. ameliae* from the two population types, based on line drawings.

The two coccidians, *G. ameliae* and the less prevalent *G. alosii*, were compared to each other in fresh preparations to detect morphological differences in sporulated oocysts. The most notable difference was that *G. ameliae* had a thin, irregular oocyst wall, whereas *G. alosii* had a thicker and smooth oocyst wall with a uniform ovoid shape. Comparing the dimensions, the oocyst length and length/width measurements were significantly different ($p < 0.001$), with *G. alosii* being more elongated and larger than *G. ameliae*. There was no difference noted in the width of the oocysts ($p = 0.84$). There was a clear difference in the length and length/width relationship in the sporocysts, with *G. alosii* being significantly longer than *G. ameliae* ($p < 0.001$). There was no overlap in the ranges

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**Fig. 4.** *Goussia alosii* from the intestine of landlocked alewives, bar = 10 μm. Wet mount of (A) highly elongated unsporulated oocysts and (B) sporulated oocysts with a thicker oocyst wall making up a very regular oval shape containing four highly elongated sporocysts. (C–E) Histology of coccidial stages in the intestine; (C) various stages of coccidia with an epicellular position within the intestinal epithelium; elongated unsporulated oocysts (arrows) found within the (D) intestinal epithelium and (E) within mucoid casts in the intestinal lumen.

**Fig. 5.** Line drawings of sporulated oocysts of *Goussia ameliae* from (A) anadromous and (B) landlocked alewives and (C) *G. alosii* sampled from landlocked alewives, bar = 5 μm.
of length of these sporocysts (Table 1). There was no difference detected in the width of these two types of sporocysts ($p = 0.30$). Figures 3B,C and 4B show the morphological differences between G. ameliae and G. alosii in landlocked alewives; Figs 5B and 5C illustrate the differences of sporulated oocysts of the two species based on line drawings.

3.4. Sequencing and phylogenetic analysis of coccidia

The small subunit 18S ribosomal RNA gene was analyzed from G. ameliae in anadromous and landlocked alewives. A 1,746 bp consensus DNA sequence was generated from both the landlocked and anadromous alewives. An analysis of sequences obtained from three separate fish samples from anadromous alewives and three separate fish samples from landlocked alewives demonstrated 100% homology in the consensus sequences between all samples, demonstrating that the parasite from anadromous and landlocked fish is the same species, G. ameliae. The 1,746 bp DNA sequence was deposited in GenBank under the accession number KP411007.

Phylogenetic analysis demonstrated that G. ameliae grouped with other fish Goussia species in a Goussia clade which forms separately from other fish coccidians, including Eimeria and Calyptospora species (Fig. 6). The closest identities to G. ameliae were G. pannonica, G. janeae, G. szekelyi, and G. koertingi with 97% identity and 96–99% query sequence coverage. The other species that appear to fit into the fish Goussia clade, Coccidia lutjanus and E. leucisci, had 96% and 95% sequence identity, respectively.

3.5. New taxa species description

Goussia ameliae n. sp. (Figs 1,3,5A,5B)

Family: Eimeriidae Minchin, 1903.

Genus: Goussia Labbe, 1986.

Type host: Alosa pseudoharengus (Wilson, 1811), common name, alewife, a species of river herring; parasite found in both anadromous and landlocked alewives, present in both young-of-the-year and adult fish.

Other host: unknown.

Type locality: Maurice River, New Jersey, USA in anadromous fish; Lake Hopatcong, New Jersey, USA in landlocked forms.

Other localities: unknown.

Type material: Histological sections, blocks, and photomicrographs are catalogued at the New Jersey Division of Fish and Wildlife Fish Pathology Laboratory, Oxford, NJ 07863, USA. Extracted DNA is stored at −80 °C in the New Jersey Department of Agriculture, Animal Health Diagnostic Laboratory. The small subunit 18S ribosomal DNA sequence has been deposited in GenBank under accession number KP411007.

Parasite description: In fresh preparations, unsporulated oocysts were rounded to ovoid in shape (Figs 1A and 3A). Sporulated oocysts with smooth, thin, irregular oocyst wall. Oocyst residuum, micropyle and polar granules absent. Oocysts contain 4 ellipsoidal sporocysts with abundant sporocyst residuum; steida body not observed in sporocysts using light microscopy. Two elongated sporozoites closely opposed to each other within each sporocyst and...
partially covered with sporocyst residuum. Dimensions of sporocysts varied between anadromous and landlocked forms after in vitro sporulation (see Table 1), possibly due to incomplete sporulation or differing sporulation dynamics of anadromous form. Line drawings of sporulated oocysts are shown from anadromous (Fig. 5A) and landlocked (Fig. 5B) fish. Measurements summarized in Table 1.

In histology, meronts containing 10–15 elongated merozoites (n = 9) arranged radially in landlocked alewives and 6–7 merozoites (n = 4, Fig. 3D) in anadromous forms (Fig. 1E). Early developmental stages were small (about 2 μm) spherical to slightly elongated, basophilic staining structures in brush border of intestinal epithelium. Macro and microgamonts were spherical to slightly ovoid shaped and occurred in an epicellular position in intestinal epithelium. Unsporulated oocysts were slightly ovoid or spherical and occurred in an epicellular position or sloughed in the intestinal lumen. Sporulation of oocysts occurred in lumen of intestinal tract of anadromous fish, while sporulation was rare in intestine of landlocked forms. Exogenous sporulation occurred in the landlocked forms.

Infection site: Anterior intestine, predominantly pyloric cecum.

Prevalence: In anadromous alewives, prevalence was 76% and 86% in YOY and adult spawning fish, respectively. In landlocked forms, prevalence was 92% and 34% in YOY and adult fish, respectively (see Table 2).

Pathology: Heavy parasite infection on surface of intestinal epithelium covering a large percentage of the intestinal epithelium. Infection was associated with focal necrosis and sloughing of intestinal epithelium. Mucoid casts containing parasite stages were observed in heavily infected individuals.

Etymology: The species was named after Amelia, the senior author’s niece whose birth coincided with the completion of the present study.

**Goussia alosii n. sp.** (Fig. 4, 5C)

Family: Eimeriidae Minchin, 1903.

Genus: **Goussia** Labbe, 1986.

Type host: **Alosa pseudoharengus** (Wilson, 1811), landlocked form, YOY and adult fish.

Other hosts: unknown.

Type locality: Lake Hopatcong, New Jersey, USA.

Other localities: unknown.

Type material: Photomicrographs of fresh preparations, histological sections and blocks are catalogued at the New Jersey Division of Fish and Wildlife Fish Pathology Laboratory, Oxford, NJ 07863, USA.

Parasite description: Unsporulated oocysts elongated and ovoid in shape (length = 21.38 ± 1.99 μm, width = 9.79 ± 0.80 μm, n = 30) (Fig. 4A). Oocyst wall in sporulated oocysts is thin, but thicker than that of **G. ameliae**. Oocyst wall smooth and regular, forming an ovoid shape (Fig. 4B). Micropyle, oocyst residuum and polar granule absent. Each oocyst with four highly elongated sporocysts that are over 3× as long as they are wide (Fig. 4B). Refer to Table 1 for measurements of oocysts and sporocysts; line drawing of sporulated oocysts in Fig. 5C. In histology, parasite stages found in intestinal epithelium with early stages, macrogamonts, and microgamonts localized in an epicellular position (Fig. 4C). The intestinal epithelium is concaved in areas where the parasite is positioned and an apparent membrane covers the macrogamonts. Elongated, unsporulated oocysts in the intestinal epithelium in an epicellular position or deeper within the epithelium (Fig. 4D) and within fecal casts (Fig. 4E).

Infection site: Mid to lower intestinal tract, found posterior to pyloric cecum.

Prevalence: 49% in YOY fish and 14% in adult fish (1+ year old). Dual infection with the more prevalent **G. ameliae** often occurred.

Pathology: Majority of infections were light, with little to no pathology associated. Few heavy infections were characterized by a heavy covering of epicellular parasite stages throughout the intestinal epithelium. Sloughing of intestinal epithelium with unsporulated oocysts forming mucoid casts in the intestinal lumen.

Etymology: The name is derived from the genus of the host **Alosa**, from which the coccidid was found.

**4. Discussion**

This is the first study to characterize intestinal coccidiosis in anadromous and landlocked alewives. Although this study documented two coccidian species in the intestinal tract, **G. ameliae** was the predominant species, being associated with a higher prevalence and heavier infection intensity. The developmental cycle of **G. ameliae** was similar to that of other eucoccidian coccidians in fish, such as **G. janae** (Lukes and Dykova, 1990), **G. pannonica** (Molnar, 1989; Lukes, 1992), and G. koertingi (Baska 1997), in which developmental stages were intracellular, though occurring in the extracytoplasmic region in the microvillar zone of enterocytes. Epicellular development has been seen in other apicomplexan parasites including gregarines and cryptosporidium, apparently benefitting the parasites by providing a mechanism to evade the immune response, acquire nutrients, and aid in transmission (Valigurova et al., 2008; Dumenil, 2011; Valigurova, 2012). The epicellular developmental stages in **G. ameliae** included merogony, gamogony, and sporogony occurring in an epicellular position. In some **Goussia** species, the number of merozoites developing within the meronts was reported for species. For example, **G. janae** was reported to have 4–10 merozoites per meront (Lukes and Dykova, 1990) and 8 merozoites per meront were reported in **G. pannonica** (Molnar, 1989). The number of merozoites per meront in **G. ameliae** was variable, with the anadromous populations having 6–7 merozoites and the landlocked populations containing 10–15 merozoites per meront. These may be underestimated considering that these counts came from meronts within a single section plane in histology. The size of oocysts and sporocysts in **G. ameliae** is similar to the ranges that have been reported for other eucoccidian **Goussia** species, such as **G. janae** (Lukes and Dykova, 1990), **G. pannonica** (Molnar, 1989), *G. koertingi* (Baska 1997), G. molnarcia (El-Mansy, 2008), and G. szekelyi (Molnar, 2006). Phylogenetic analysis has placed **G. ameliae** closely related to other fish **Goussia** species, particularly **G. pannonica**, **G. janae**, and **G. koertingi**, which also share morphological characteristics, such as epicellular development and similar parasite dimensions. Ninety-seven percent identity also occurred between **G. ameliae** and G. szekelyi, though G. szekelyi develops within the apical plasm of the cell instead of a truly epicellular position (Molnar, 2006). The phylogenetic separation of **Goussia** species infecting fish from other coccidian clades has been previously reported by Molnar et al. (2012).

Additionally, in this study a second coccidian species, **G. alosii**, was characterized from landlocked fish by morphology alone. With major morphological differences in **G. alosii**, it was clearly a separate species from **G. ameliae**. **Goussia alosii** was found predominantly in the intestine, whereas **G. ameliae** occurred predominantly in the pyloric cecum. The oocyst wall was smooth compared to the irregular oocyst wall of **G. ameliae** and dimensions of oocysts in **G. alosii** were much larger than that reported for **G. ameliae**. In fact, the oocysts of **G. alosii** were larger than most other reports in **Goussia** species, with the exception of G. kuehae, which was reported to have very large oocysts measuring about 37.9 × 29.3 μm (Szekely et al., 2013). The oocyst size of **G. alosii** was intermediate between that of **G. ameliae** and the large oocysts of G. kuehae. Although this coccidian type was not characterized from fresh parasite preparations of anadromous fish, histology demonstrated developmental stages of an intestinal coccidian that is unique from **G. ameliae** and similar to the elongated sporont of **G. alosii** in the intestine, suggesting a similar type of coccidian in the anadromous fish. Future sequence
Epicellular coccidians have been reported to have a seasonal pattern in which they have the highest prevalence in the spring (Lukes et al., 1992). Work by Lukes and Dykova (1990) demonstrated that sporulation of _G. janae_ oocysts is temperature dependent, with sporulation occurring predominantly at 10 °C. Additionally, _G. molnarczus_ has been demonstrated to have a seasonal occurrence, with highest infection prevalence in the spring and lowest in November (El-Mansy, 2008). The present study demonstrated that _G. ameliae_ is highly prevalent in anadromous fish during the sample periods (April–August). The difficulty of sampling fish from the marine environment, where fish spend the majority of their lives, did not allow for determination of parasite prevalence in the marine life phase. Interestingly, in anadromous alewives, the prevalence and severity of infection were heavier in adult spawning fish compared to YOY fish. This is in contrast to what was observed for landlocked fish, in which the highest prevalence and infection intensity were in YOY fish and prevalence/infection intensity decreased in older fish. It may be expected that adult fish would be more resistant to infection. A study by Steinhagen et al. (1998) demonstrated that resistance was acquired following a primary exposure to the intestinal coccidian _G. carpelli_ in common carp, _Cyprinus carpio_. The authors showed that a strong adaptive immune response prevents reinfection, which was effective even under immunosuppressive conditions (Steinhagen et al., 1998). This adaptive response has not been demonstrated for _G. ameliae_ and it would be expected that _G. ameliae_ would stimulate a less efficient adaptive immune response because of its epicellular localization, while _G. carpelli_ invades more deeply into the intestinal epithelium, thus likely inducing heavier systemic immune responses. A possible explanation for the increased prevalence and infection intensity in anadromous adults is that the adults may be more heavily stressed due to osmoregulatory changes and spawning stress, making them more susceptible to infections.

A comparison of the coccidian parasites in anadromous and landlocked fish demonstrated that the predominant coccidian in both forms of alewives was the same species, _G. ameliae_. This was supported by 100% sequence homology in the small subunit 18S ribosomal RNA gene, which has been demonstrated to be a useful gene for determination of taxonomy in apicomplexan parasites (Morrison, 2008). The origin of the parasite in Lake Hopatcong cannot be verified since studies on coccidians of other freshwater species in the lake are lacking. The parasite may have been present in the lake prior to the introduction of alewives, though this is not likely since the alewife is the only clupeid fish species in the lake. Little is known about host specificity in fish coccidia, but several studies suggest that coccidian species generally infect fish within the same genus (Belova and Krylov, 2000) and Goussia species in carp have been shown to be highly host specific (Molnar et al., 2005). With alewives being the only fish in the genus _Alosa_ and only clupeid fish species within the lake, it is likely that the origin of the parasite dates back to the time when ancestral anadromous alewives gained entry into the lake. Since the landlocked populations have been isolated from anadromous forms since the early 1900s, it is likely that the parasite persisted in the derived landlocked populations since that time. This is supported by the fact that landlocked alewife populations have independently evolved and adapted to life in freshwater lakes from the ancestral anadromous forms (Palkovacs et al., 2008). If true, then this demonstrates the success of the parasite in the landlocked environment since its introduction into the lake. With the phenotypic changes that have occurred in landlocked alewives, most notably having less than half the adult size of anadromous fish and a shorter life span (Gross, 1951), it is of interest to understand how these pressures may impact parasite dynamics and the host–parasite relationship. Studies in other parasite species, _Ichthyophonus hoferi_, have demonstrated parasites to adapt to changing host environments (Hersberger et al., 2008). Future studies should more closely compare _G. ameliae_ from these two unique alewife populations. It may be expected that coccidian infections would be increasingly prevalent in landlocked populations in the spring following cold winter temperatures when fish are believed to be immunosuppressed (Lepak and Kraft, 2008). With landlocked populations naturally having low fecundity and small size at spawning, it would be of interest to determine if coccidian infections can impact general body condition or spawning success of fish. In the present study, fish were sampled during the end of the summer, although it would be valuable to sample fish in the spring/early summer when adult fish are spawning to determine if the coccidian is more prevalent during this time of year. Other intestinal and epicellular species of _Goussia_ have been demonstrated to be most prevalent during the spring (El-Mansy, 2008).

Although evidence supports the presence of the same predominant coccidian species, _G. ameliae_, within anadromous and landlocked alewife forms, it was interesting to note some differences in the size and sporulation of the parasite between the two fish forms. These differences were most likely attributed to parasite differences in the host types (anadromous vs. landlocked) or to differences in the _in vitro_ environment leading to differences in sporulation dynamics. In landlocked forms of _G. ameliae_, sporulated oocysts were very rare in fresh intestinal mucus, whereas in anadromous forms sporulated oocysts were a common occurrence. Additionally, following 48 hours of _in vitro_ sporulation, nearly all unsporulated oocysts had sporulated in landlocked forms, whereas in the anadromous form, little difference was noted in the number of sporulated oocysts compared to the fresh preparations. The only difference in the _in vitro_ environment for sporulation was the inclusion of 5 ppt NaCl for the anadromous forms to more closely resemble the brackish environment from which the fish were sampled from. No literature currently exists on the effects of NaCl on sporulation of fish coccidia, although it has been reported that coccidia are especially prevalent in marine and estuarine fishes, or freshwater fish of marine origin (Molnar et al., 2012), suggesting that coccidial sporulation should not be impacted greatly by salinity. In addition to the difference in sporulation dynamics, there was a difference noted particularly in sporocyst size between the two population types. It is possible that oocysts in the anadromous form of _G. ameliae_ were not fully sporulated, thus the final dimensions of oocysts and sporocysts were not attained, though this is not likely since separated sporozoites were observed within the sporocysts, suggesting complete sporulation. It may be that there is some morphological plasticity in the parasite that is apparent when sampled from these two population types. Research in _Eimeria_ from other vertebrates has shown that there can be high polymorphism in oocyst size in single parasite species of the same or closely related host species (Duszynski, 1971; Parker and Duszynski, 1986). Historically, coccidia have been identified by morphological characters of the sporulated oocyst, which generally is completed in 48 hours in fish. The present study demonstrated some morphological differences within the same parasite species sampled from the same host species from unique populations. The isolation of these populations from each other and the contrasting environmental conditions created from landlocked and anadromous populations could drive different selective pressures for the parasite, which could be responsible for some differences noted between the parasites. Further research is required to determine the cause of these sporulation and morphological differences by understanding host and environmental factors that can impact coccidia.

Little is known about the host impacts resulting from heavy infection with this coccidian in spawning or YOY anadromous alewives.
With anadromous alewives severely declining throughout their range in the North Atlantic as a result of environmental factors such as climate change and habitat destruction, it is of interest to understand how parasitism may impact the species. Coccidia have been demonstrated to cause a reduction in the body condition of wild infected fish (Morrison and Hawkins, 1984; Abollo et al., 2001), and these parasites have been problematic in aquaculture when fish are raised in high densities or with poor water exchange (Gibson-Kueh et al., 2011). In the present study, the pathology associated with severely infected hosts suggests that the normal functions of the anterior intestine may be compromised due to the heavy parasite infection and damage to intestinal epithelium. It is common to see heavy parasite infections in asymptomatic fish, thus it is difficult to estimate the impacts parasites have to wild fish. Hemmer et al. (1998) have demonstrated that carp infected with G. carpelli can compensate for coccidial infections by increased epithelial cell turnover in the intestine. Clearly, host responses can compensate for heavy parasite infections, though this would come with an energetic cost. In Pacific herring infected with the parasite Ichthyophonus hoferi, it has been demonstrated that systemic infection was associated with significant energetic costs in fish when the disease did not result in death of the host (Vollenwider et al., 2011). Similarly for heavy coccidial infections, it is likely that the host can compensate for the infections, though it may come at the cost of energy and condition. Based on the high prevalence of this parasite in YOY and adult fish, it is unlikely that significant mortality is occurring in this species, though when estimating the impacts of climate change and habitat loss in this fish species, the impacts and energetic costs of these abundant parasites should be considered. Further research in laboratory experiments would be required to determine the pathophysiological costs of these coccidial infections in alewives.

5. Conclusions

Two intestinal coccidians, Goussia ameliae and G. alosii, infecting alewives have been described for the first time. With anadromous alewife populations threatened throughout their range, and changing habitats adding additional pressure to this species, it is important to understand the parasites that could impact them. The finding of G. ameliae infecting both anadromous and landlocked populations provides an interesting ecological model to understand how a parasite species may adapt to a single host species that has evolved for life in different environments. The present results demonstrated some differences in the sporation dynamics of the parasite in the two alewife population types. Further, the potential of morphological differences in the same coccidial species from unique populations of the same host species demonstrates the importance for providing genetic data in addition to morphology for parasite descriptions.

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