Northward Expansion of Bopyrid Isopod Parasites in Daggerblade Grass Shrimp in Cape Cod, MA

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Executive Summary

The daggerblade grass shrimp, *Palaemonetes pugio*, is among the most abundant species of shrimp inhabiting estuaries along the East Coast of the United States. They play a crucial trophic role as detritivores, transferring detrital energy to higher trophic levels, and are prey for many commercially and ecologically important species. They also enhance macrophyte biomass through epiphyte grazing and feces deposition, increasing light and nutrient availability for the vegetation. Due to the vital role they play in these estuarine environments, any change in their population has the potential to alter community composition and disrupt ecosystem functioning.

Daggerblade grass shrimp are the definitive host of bopyrid isopod *Probopyrus pandalicola*, an ectoparasite that infects the branchial chamber of the shrimp. The primary effects of the parasites on their host are decreased energy availability and sexual sterilization of the female hosts. The bopyrid isopod has been reported in daggerblade grass shrimp in the Southeastern U.S. at rates ranging from .001% to 5.7% but has not been documented north of Maryland.

The goal of the project was to determine the prevalence of *P. pandalicola* parasitized *P. pugio* and potential spatiotemporal differences in Cape Cod, Massachusetts after receiving personal observations of bopyrid isopods parasitizing grass shrimp at Long Pasture Wildlife Sanctuary in 2019. We sampled at five different sites along Cape Cod in August 2021 to enumerate bopyrid isopod parasitization. Bopyrid isopods were found at four of the five sites, with prevalences ranging from .03% to 17%, the highest prevalence being at Long Pasture Wildlife Sanctuary in Cummaquid, MA. After August sampling, we focused on the Cummaquid site, resampling in October 2021 and March 2022 to evaluate potential spatiotemporal variations in bopyrid parasitization. Findings in this study indicate a northward expansion of *P. pandalicola*. Key findings include:

1. This is the first study to document a population of bopyrid isopod parasitizing daggerblade grass shrimp in a novel host population north of Maryland.

2. March had the highest prevalence of bopyrid isopods (17% ± 2.81%), followed by August (14% ± 3.41%) and October (8% ± 1.35%). These are at times orders of magnitude higher than reported bopyrid parasite prevalence in the literature.
Previously, the highest prevalence of bopyrid isopods in daggerblade grass shrimp was 5.7% in South Carolina.

3. There was a significant relationship between shrimp abundance and parasite abundance, potentially indicating a density-dependent relationship.

4. There was a significant relationship between salinity and parasitization, with higher parasite prevalence in lower salinities. Such a relationship has climate change implications as heavier rainfall predicted in the northeast can lower salinity at our sampling locations.

5. There weren’t significant differences between sampling seasons or individual transects at Long Pasture Wildlife Sanctuary.

Based on this research, we are working on establishing a long-term monitoring program with Long Pasture Wildlife Pasture to manage this population of bopyrid isopods.
1. Introduction

1.1 Study Species

Daggerblade grass shrimp, *Palaemonetes pugio*, are among the most abundant shrimp species inhabiting salt marshes and estuaries along the East Coast and Gulf of Mexico (Welsh, 1975; Anderson, 1985). They play a critical trophic role as an intermediate species. Depending on their life stage and food availability, they act as opportunistic omnivores or detritivores. As detritivores, they recycle detrital material, transferring energy to higher trophic levels (McCall and Rakocinski, 2007; Welsh, 1975). Epiphyte grazing by grass shrimp enhances macrophyte biomass by increasing light access, thus inhibiting dieback, and stimulating growth through excretion, bioturbation, and direct feces deposition near macrophyte roots (McCall and Rakocinski, 2007). Grass shrimp are also important prey for many commercially and ecologically important invertebrates, fish, and birds like white shrimp *Penaeus setiferus* (Kneib and Knowlton, 1995), summer flounder *Paralichthys dentatus* (Manderson et al., 2000), and mummichog *Fundulus heteroclitus* (Welsh, 1975). Grass shrimp are also used in toxicity assays as a bioindicator for environmental health, as they are sensitive to many environmental contaminants (Key et al., 2006). Due to the many roles they play in these environments, changes to their population can change estuarine ecosystem structure and functioning.

Daggerblade grass shrimp are hosts for various parasites (Overstreet, 1978; Pung et al., 2002; Overstreet, 1978; Anderson, 1977). They are the definitive host of bopyrid isopod *Probopyrus pandalicola*, an ectoparasite found in female-male pairs in the shrimp’s branchial chambers (Anderson, 1977) (Figure 1). The first isopod to infect the host becomes the female, and as they mature, they become ovigerous under the shrimp’s carapace and release larvae in tandem with grass shrimp molting (Beck et al., 1980). The female isopods ingest hemolymph
from their host, consuming 1-10% of the total energy intake of *P. pugio* (Anderson, 1977). This forces an increase in grass shrimp caloric intake and reduces shrimp respiration, metabolism, and growth (Anderson, 1975; Ludwig, 2009). The bopyrid isopod also sexually sterilizes the female host, preventing reproduction and negatively affecting population size (Sherman and Curran, 2015; Chaplin-Ebanks and Curran, 2007).

Any reduction in grass shrimp reproduction could adversely impact the trophic dynamics and community composition in salt marsh ecosystems, decreasing grass shrimp predator recruitment success (Brinton and Curran, 2015; Chaplin-Ebanks and Curran, 2007). Bopyrid isopods may also affect grass shrimp behavior. Bass and Weis (1999) found that parasitized shrimp may capture significantly fewer prey items and have lower activity levels, influencing community trophic interactions that maintain the balance of bottom-up and top-down controls of the ecosystem. The bopyrid isopod has been reported in grass shrimp in the Southeastern U.S. but has not been documented north of Maryland.

![Figure 1: Male-female pair of Bopyrid Isopod, *Probopyrus pandalicola*, in the branchial chamber of the Daggerblade Grass Shrimp, *Palaemonetes pugio*](image)

1.2 *Project Objectives*

This project aimed to identify and document for the first time the northward expansion of *P. pandalicola* in a novel host population of *P. pugio* after observations were made of grass
shrimp parasitization in Long Pasture Wildlife Sanctuary in Cummaquid, MA (E. Stoner, personal communication, 2019). We used field sampling to detect potential variations in spatiotemporal abundances of *P. pandalicola* in Cape Cod, Massachusetts. The main objectives were to 1) determine if there was an established population of bopyrid isopods parasitizing daggerblade grass shrimp on Cape Cod, 2) identify spatiotemporal differences in bopyrid isopod abundance along Cape Cod and among individual salt pannes at Long Pasture Wildlife Sanctuary in Cummaquid, Cape Cod, and 3) identify environmental conditions affecting bopyrid isopod parasitization.

2. Methods

2.1 Sample Location Descriptions

Five sites were sampled in Cape Cod (Figure 1). Sites were selected based on the following criteria: proximity to grass shrimp habitat (marsh vegetation or seagrass beds), access by foot, and representative distribution along the Cape. The Wellfleet site is located in the creek connecting Silver Spring harbor and Loagy Bay, adjacent to Lieutenant’s Island in Wellfleet, MA. This is the northernmost location and is relatively isolated compared to the other sites. Samples were collected from the creek’s edge directly below the Lieutenant Island Road bridge (41.8936129°N 70.0034407°W) and in the center of the creek 75 meters from the bridge (41.8931992°N 70.0041433°W). The Yarmouth Port site (41.7230402°N 70.2360016°W) is in the Chase Garden Creek Salt Marsh on the bayside of the Cape and was sampled from under the boardwalk connected to Gray’s Beach. This site had high foot traffic, recreational crab traps within the marsh, and a parking lot adjacent to the sampling site. The East Sandwich site (41.7344246°N 70.4254431°W) was sampled from a tide pool in Scorton Creek off of Scorton
Harbor. This site was near Old King’s Highway, a busy two-lane highway, and next to a gravel parking lot. The West Dennis site (41.6510057°N 70.1939541°W) is located behind West Dennis Beach, a popular tourist destination, and is the only location on the south side of the Cape. This location was sampled along the salt marsh edge.

Figure 2: Sampling locations in Cape Cod (A) Wellfleet, (B) Yarmouth port, (C) Cummaquid, (D) East Sandwich, (F) West Dennis
2.1.1 Long Pasture Wildlife Sanctuary Description

The Cummaquid site (41.7091601°N 70.2805333°W) is located within the Massachusetts Audubon Long Pasture Wildlife Sanctuary, bordering Barnstable Harbor. This is the only site sampled from salt pannes within the tidal marsh. Long Pasture is part of Mass Audubon, the largest nature-based conservation organization in New England (Mass Audubon, 2022). This was the only site with previous anecdotal evidence of bopyrid presence from May 2019. As a result of the high abundance of parasites found from pannes located at this site in August, 2021, we resampled this site again in October 2021 and March 2022. In total, we sampled 14 pannes though not all pannes were resampled every season due to access constraints (Figure 3). We sampled pannes 6 through 14 in August, 1 through 7 in October, and 2 through 8 in March.

Figure 3 Map of Salt Panne Locations at Long Pasture Wildlife Sanctuary, Cummaquid, MA
2.2 Sampling Procedures

The Wellfleet, Yarmouth Port, East Sandwich, and West Dennis sites were sampled in August 2021 from 3m² quadrats haphazardly placed along the marsh edge. The Cummaquid site was sampled in August 2021, October 2021, and March 2022 using 3m belt transects of the salt pannes. Shrimp were collected using mesh dip nets until three consecutive sweeps yielded fewer than three additional shrimp (following the modified protocol outlined in Hammerschlag-Peyer et al., 2013). Shrimp were bagged and placed on ice in the field and frozen for further processing. Salinity and water temperature were recorded and water samples were collected in clean Nalgene 175ml bottles from each site and individual panne at Long Pasture. Length, width, and depth were recorded for each panne at the Cummaquid site. All sampling took place at low tide.

2.3 Processing Procedures

Shrimp species was confirmed using Anderson (1985) and the presence or absence of Probopyrus pandalicola was visually identified by inspecting the shrimp’s branchial chamber. Female-male pairs of P. pandalicola were easily discernable under the carapace and confirmed in a small subset of shrimp using a dissecting microscope (Figure 4). Shrimp length was measured from the rostrum to the end of the tail, and width was measured below the gills to the nearest millimeter. Parasite length and width were also measured to the nearest millimeter. Wet weight was recorded for all shrimp and bopyrid isopods to the nearest .001g. Orthophosphorus, nitrate nitrite, and ammonia were measured via Flow Injection Analyzer (calibration curve n=7; R²>0.9998). Statistical Analysis was completed using R. Kruskal Wallis nonparametric rank-sum test was used to identify significant differences between months and between individual
pannes at Long Pasture as our data were not normally distributed. A Dunn’s pairwise test was then used to look at which months and which pannes were significantly different from one another. The Bonferroni corrected adjusted p-value was calculated to account for the increased type I error associated with multiple comparisons. Regressions were performed to assess parasite and shrimp abundance, and parasite abundance and water quality.

3. Results

3.1 Total Prevalence

A total of 3410 shrimp were collected from all five sites, 348 of which were parasitized by *P. pandalicola*. The highest prevalence of *P. pandalicola* came from the Long Pasture site, where 2661 grass shrimp were collected across three seasons; 342 grass shrimp were parasitized
by bopyrid isopods. Bopyrid isopods were also found in Sandwich (4 out of 384), Wellfleet (1 out of 282), and Yarmouth Port (1 out of 24). The West Dennis site had 58 grass shrimp, none of which had bopyrid isopods.

3.2 Prevalence at Long Pasture Wildlife Sanctuary

The Cummaquid site was the main focus of this study as it had the highest prevalence. Shrimp were most abundant during our sampling in the warmer months of August (n = 1515, 25.6°C) and an unseasonably warm October (n = 741, 16°C), compared to March (n = 405, 8.3°C). All of the pannes sampled in August and October yielded parasitized shrimp, though October’s shrimp abundance values per panne were consistently lower than the other months. In March, three of the six pannes sampled had shrimp present, and all three yielded parasitized shrimp. March had the highest prevalence of bopyrid isopods (17% ± 2.81%), followed by August (14% ± 3.41%), and October (8% ± 1.35%).

Figure 5 (A) Total number of daggerblade grass shrimp Palaemonetes pugio collected from Long Pasture Wildlife Sanctuary in Cummaquid, MA (B) Percent prevalence of the bopyrid isopod P. pandalicola in daggerblade grass shrimp P. pugio, collected from Long Pasture
Salinity and parasite abundance were found to be negatively correlated (n=18 r = -.53, p < .05). A regression showed that salinity accounts for 38% of the variation in parasite prevalence, with lower salinity values trending toward higher parasite loads (R² = .38, p < .001) (Figure 6). There was no significant relationship between bopyrid prevalence and orthophosphorus, nitrate nitrite, or ammonia. There was a significant relationship between shrimp abundance and bopyrid prevalence, with the regression accounting for 39% of the variability in parasite prevalence (R² = .39, p < .001) (Figure 7). There were no significant differences between individual pannes at Long Pasture (Kruskal-Wallis, X² = 12.577, df = 11, p-value = 0.3219) nor between the different seasons (Kruskal-Wallis, X² = 3.4592, df = 2, p-value = 0.1774). All bopyrid isopods were found in male-female mating pairs, and March had exclusively ovigerous females (Figure 8).
Discussion

4.1 Prevalence

Most notably, this project documents a population of *P. pandalicola* in a novel host population of *P. pugio*, north of its known range. Sampling in Cummaquid revealed a prevalence of *P. pandalicola* considerably higher than previously reported in the literature, infecting 7-18% of shrimp (Table 1). At such high rates, bopyrid parasitism can alter grass shrimp populations by removing a significant portion of females from the reproductive population.

The invasion of another bopyrid species, *Orthione griffenis*, in the pacific northwest is an example of the deleterious effects of high bopyrid prevalence (Whalen et al., 2020). High prevalence caused a significant decline in mud shrimp, *Upogebia pugettensis*, populations. In Oregon, the bopyrid isopod decreased reproduction by 68% over a five-year period (Dumbauld...
et al., 2010). Boots and Sasaki (2002) found that parasites that prevent or reduce reproduction in their host populations can lead to the extinction of the host and in return the parasite itself. High transmission rates, which we have documented in this study, coupled with low host reproduction rates lead to extinction. Although the bopyrid isopod does not cause direct mortality of the daggerblade grass shrimp, it does reduce reproduction, altering population dynamics and ecosystem functioning.

Table 1 Comparison of reports of P. pugio parasitized by P. pandalicola

<table>
<thead>
<tr>
<th>Source</th>
<th>Parasite Prevalence</th>
<th>Total Shrimp</th>
<th>Collection Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheehan et al., 2011</td>
<td>0.002%</td>
<td>3348</td>
<td>Mobile Bay Region, Alabama</td>
</tr>
<tr>
<td>Anderson, 1977</td>
<td>&lt; 3.0%</td>
<td>-</td>
<td>Georgetown, South Carolina</td>
</tr>
<tr>
<td>Chaplin-Ebanks and Curran, 2007</td>
<td>3.10%</td>
<td>21,820</td>
<td>Country Club Creek, Georgia</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td></td>
<td>Moon River, Georgia</td>
</tr>
<tr>
<td></td>
<td>1.80%</td>
<td></td>
<td>Chowan Creek, South Carolina</td>
</tr>
<tr>
<td></td>
<td>1.30%</td>
<td></td>
<td>Harbour Town, South Carolina</td>
</tr>
<tr>
<td>Key et al., 2011</td>
<td>3.17%</td>
<td>14207</td>
<td>Kiawah Island and Wadmalaw Island, South Carolina</td>
</tr>
<tr>
<td>Wilczek et al (in prep)</td>
<td>8-17%</td>
<td>2661</td>
<td>Cummaquid, Massachusetts</td>
</tr>
</tbody>
</table>

There was a significant relationship between parasite prevalence and shrimp abundance, indicating that the larger the shrimp population, the more likely the shrimp are to be parasitized, thus the more likely we are to see potential impacts on the population and marsh food web. There were no significant spatiotemporal differences in parasite abundance at Long Pasture, indicating that the panne location and season do not significantly impact bopyrid parasitization.
4.2 Climate Change Impacts

Although we did not find an effect of poor water quality, these findings may indicate declining ecosystem health as parasite prevalence can be connected to anthropogenic influences (Byers, 2021). In particular, range expansion and high prevalence may be connected to warming waters attributed to climate change. In South Carolina, daggerblade grass shrimp parasitized by bopyrid isopods increased with temperature (Key et al., 2011). Brinton and Curran (2015) recorded higher reproductive rates related to rapid brood development associated with synchronization of grass shrimp molting and bopyrid reproduction. Shortly before the grass shrimp molts, the female bopyrid releases larvae into the host’s respiratory stream. As grass shrimp molting frequency increases with temperature, bopyrid reproduction will likely increase, with a single female producing 3.2 times more larvae in their lifetime (Brinton and Curran, 2015).

In addition, climate change can exacerbate high prevalence rates through increased precipitation in the northeast (Huang et al., 2021). Since the early 1900s, the Northeast has seen the largest increase in heavy precipitation, attributed to anthropogenic climate change (Easterling et al., 2017). Such increases may drive parasitization as our results show a significant relationship between pannes with low salinity and high prevalence rates. Previous studies have mentioned a relationship between bopyrid prevalence and low salinity (Chaplin-Ebanks and Curran, 2007; Sheehan et al., 2011), though this is the first to document a highly significant relationship formally.
4.3 Trophic Implications

Reductions in daggerblade grass shrimp populations due to bopyrid parasitization, as we have seen in the mud shrimp on the West Coast, could result in serious trophic implications. Daggerblade grass shrimp play a crucial role in enhancing various levels of estuarine food webs. They increase macrophyte growth and photosynthesis, allowing energy to enter the system via primary production. The reduction or loss of epiphyte grazing and feces deposition by grass shrimp may constrain macrophyte growth as it is no longer receiving the benefits of light accessibility and limiting nutrients. Grass shrimp are also a pathway for nutrients by making detrital energy available to secondary consumers, and as prey, supporting ecologically and commercially important species.

At Long Pasture Wildlife Sanctuary, declining grass shrimp populations would likely impact the migratory red knot, *Calidris canutus*. The red knot is a threatened shorebird that uses Cape Cod as a foraging stopover location in the fall and spring journey between South America and the Canadian Arctic (Niles et al., 2007). Declines in red knot populations are attributed to the overharvesting of horseshoe crab eggs, a vital food source for the migratory bird (Karpanty et al., 2010). Shrimp serve as a supplemental food source (USFWS, 2014); therefore, if grass shrimp populations declined, it would further reduce food availability for this federally listed species. Monitoring and management of the range expansion of bopyrid isopods in daggerblade grass shrimp is necessary to maintain ecosystem health and functioning.

4.4 Future Work

We are in the process of developing a long-term monitoring partnership with Long Pasture Wildlife Sanctuary. Future studies should evaluate the mechanisms behind this
documented range expansion. We do not know when the bopyrid isopod began infecting
daggerblade grass shrimp on Cape Cod, but we have anecdotal evidence of parasitization in 2019
(E. Stoner, personal observation, 2019). Northward expansion may be the result of both natural
and anthropogenic causes. Without testing sites between Maryland and Cape Cod, we cannot
rule out natural spread through larval dispersal and movement of calanoid copepods, the
bopyrid’s intermediate host, as observed in the poleward expansion of *O.griffenis* (Whalen et al.,
2020).

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USFWS Northeast Region. (2014). *Rufa red knot background information and threats assessment.* [https://doi.org/10.7282/T36M38JS](https://doi.org/10.7282/T36M38JS)

