PHENOLOGY OF ROCK POOL MOSQUITOES IN THE SOUTHERN APPALACHIAN MOUNTAINS: SURVEYS REVEAL APPARENT WINTER HATCHING OF *Aedes japonicus* AND THE POTENTIAL FOR ASYMMETRICAL STAGE-SPECIFIC INTERACTIONS

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ABSTRACT. The North American rock pool mosquito, *Aedes atropalpus*, has reportedly decreased in abundance following the introduction of *Ae. japonicus japonicus* to the USA, but the specific mechanisms responsible for the reduction remain unclear. Thus, there is a need for field studies to improve our knowledge of natural rock pool systems where both species co-occur. We sampled rock pool invertebrates over a 12-month period along the Chattooga River at a high-elevation site (728 m) near Cashiers, NC, and at a lower-elevation site (361 m) near Clayton, GA. We identified 12 orders of macroinvertebrates representing at least 19 families and 5 mosquito species. *Aedes j. japonicus* was present year-round at both sites. We observed overwintering *Ae. j. japonicus* larvae in pools with water temperatures as cold as 3°C and detected apparent winter egg hatching in water below 10°C. *Aedes atropalpus* was rarely encountered at the high-elevation site but was highly abundant in the summer months at the low-elevation site. Late-stage *Ae. j. japonicus* larvae inhabited pools in March 2019 when *At. atropalpus* first appeared in the same pools, creating the potential for asymmetrical stage-specific interactions. Our observations provide evidence of overwintering and early hatching of *Ae. j. japonicus* in the southeastern climate. Further study of the importance of stage-dependent competition and winter egg hatching of diapausing *Ae. j. japonicus* eggs is warranted.

KEY WORDS *Aedes atropalpus, Ae. japonicus*, ecology, *Ochlerotatus*, relative abundance

INTRODUCTION

Native and invasive mosquitoes are among the diverse assemblages of aquatic macroinvertebrates that develop within rock pools (Jocque et al. 2010, Byrd et al. 2019). In the USA, 2 common rock pool mosquitoes are the native *Aedes atropalpus* (Coquillett) and an invasive species, *Ae. japonicus japonicus* (Theobald). *Aedes atropalpus* is a facultatively autogenous species of no recognized public health importance (Shaw and Maisey 1956, O’Meara and Krasnick 1970, Telang and Wells 2004, Scholte et al. 2009), whereas *Ae. j. japonicus* is a potential vector of endemic pathogens including West Nile and La Crosse viruses (Turell et al. 2001, Sardelis et al. 2002, Schaffner et al. 2009, Turell et al. 2013, Harris et al. 2015). There is evidence that the establishment of *Ae. j. japonicus* in the USA has coincided with a decrease in *At. atropalpus* abundance (Andreadis et al. 2001, Scott et al. 2001, Bevins 2007, Andreadis and Wolfe 2010, Armistead et al. 2012, Kaufman and Fonseca 2014). The reported displacement presents a scenario where an invasive vector of endemic pathogens is displacing a native nonvector. Because of the public health relevance of *Ae. j. japonicus*, research efforts have sought to advance the understanding of the species’ ecology and ability to vector diseases within the USA, but relatively few studies have focused on its ecological role in natural rock pool systems (Armistead et al. 2012, Hardstone and Andreadis 2012, Kaufman and Fonseca 2014, Reuss et al. 2018).

Understanding the impacts of invasive mosquitoes on native and established species is challenging (Juliano and Lounibos 2005). Invasive species may reduce or displace established populations, as demonstrated when *Ae. aegypti* (L.) populations declined in the parts of the USA following the invasion of *Ae. albopictus* (Skuse) (O’Meara et al. 1995a). Conversely, native species may also act as barriers to invasion, as revealed by the inability of *Ae. albopictus* to occupy water-holding bromeliad axils in area of Florida where native *Wyeomia* spp. use them for larval development (O’Meara et al. 1995b). The impact of the *Ae. j. japonicus* invasion on US rock pool communities remains unclear, and a lack of historical data prevents us from truly understanding its influence on *Ae. atropalpus* populations. Andreadis and Wolfe (2010) found that the relative abundance of *At. atropalpus* decreased at a tire disposal site in Connecticut from 1987 (preinvasion) to 2005, while their oldest rock pool collections were from 1999, after the establishment of *Ae. j. japonicus*. Other reports have documented that *Ae. j. japonicus* is found in high relative abundances in its invasive range and prefers temperate water for larval development (Kaufman and Fonseca 2014, Reuss et al. 2018, Montarsi et al. 2019). Although *Ae. j.
japonicus is the dominant mosquito in many rock pools in the eastern USA, Ae. atropalpus remains abundant in many rock pools exceeding 30°C (Andreadis and Wolfe 2010, Byrd et al. 2019) in the southeastern Appalachian region. Competition experiments with Ae. j. japonicus and Ae. atropalpus have yielded ambiguous results and have not shown Ae. j. japonicus to be a stronger larval competitor (Armistead et al. 2008, Hardstone and Andreadis 2012). It is possible that Ae. j. japonicus competitively excludes Ae. atropalpus in temperate pools, so that the native species may only persist at higher temperatures in its southernmost geographic range. It is alternatively conceivable that Ae. atropalpus acts as a competitive barrier to Ae. j. japonicus in warm pools and has historically been less abundant in, or even avoided, colder ones (Day et al. 2021).

Aedes j. japonicus has a long active season, and seasonally is among the earliest mosquitoes to appear in containers in its invasive range (Andreadis et al. 2001, Burger and Harry 2008, Kaufman et al. 2012, Kaufman and Fonseca 2014). Females lay diapausing eggs that overwinter in the embryonic phase as a response to shortened days and low temperatures (Bova et al. 2019), and overwintering Ae. j. japonicus larvae have also been observed (LaCasse and Yamaguti 1950, Armistead et al. 2012). Scott (2003) observed overwintering Ae. j. japonicus larvae swimming below ice in January 2003 and observed egg hatching in March at 5°C. Scott also demonstrated that the species could develop to adulthood at temperatures as cold as 10°C, and predicted that larvae would overwinter in habitats that do not freeze solid. Reuss et al. (2018) established that larvae from a German population of Ae. j. japonicus could temporarily survive at temperatures as low as 0°C. The cold tolerance of Ae. j. japonicus and its early arrival into containers each year likely increases the number of generations produced per season and may provide an advantage over native species (Kaufman and Fonseca 2014). Aedes atropalpus females also lay diapausing eggs as an overwintering trait (Anderson 1968, Kalpage and Brust 1974, Beach 1978), but to our knowledge the effects of winter temperatures on diapausing eggs and larval development have not been extensively studied in the native species. In some regions, Aedes j. japonicus may gain an advantage over Ae. atropalpus by colonizing rock pools early in the year and remaining active until late in the fall, with the potential for asymmetric larval competition or intraguild predation by late-stage Ae. j. japonicus larvae on Ae. atropalpus hatchlings (Edgerly et al. 1999, Lounibos et al. 2003).

The seasonality of US rock pool mosquitoes and other coincident taxa remains largely undescribed, limiting the potential for informed hypotheses of ecological interactions among the relevant taxa. Rock pools on the Chattooga River in the southeastern USA are particularly intriguing for investigating the distributions and relative abundances of Ae. atropalpus and Ae. j. japonicus. Near the river’s headwaters in Cashiers, NC, rock pool water temperatures are low, and Ae. atropalpus is found in reduced abundances as compared with a downstream lower elevation site near Clayton, GA, where temperatures are much warmer and Ae. j. japonicus is less commonly encountered during the summer months (Byrd et al. 2019). Here we describe the phenology of rock pool inhabitants, with a primary emphasis on mosquito taxa, at both sites.

MATERIALS AND METHODS

We sampled rock pools at 2 sites along the Chattooga River. The Bull Pen (BP) rapid site is located in Jackson County, NC (35.015934°, –83.126434°), with an elevation of 728 m, and the Bull Sluice (BS) rapid site is in Rabun County, GA (34.817710°, –83.303943°), along the Georgia–South Carolina border at an elevation of 361 m (Fig. 1). We haphazardly selected 8 rock pools at BP and BS (Table 1), but only routinely sampled 6 pools at BS after the 1st sampling event due to logistical (time) constraints. At both sites we selected pools comprising a variety of sizes (volumes) and distances (both horizontally and vertically) from the river that were safely accessible at normal river levels as the pools were immediately adjacent to river hazards (Class IV–V rapids). The sampling period ran from November 2018 until November 2019, with the initial objective of sampling monthly at both sites. At BP we resampled 8 pools across 10 sampling events (dates) for a total of 80 individual rock pool observations. At BS, 8 pools were sampled on the 1st sampling event and 6 were resampled on 8 sampling events for a total of 56 observations.

We developed a standardized sampling protocol to maintain consistency throughout the study period. Prior to sampling, we measured the length, width, depth, and distance between the rock pool water surface and the lip of the rock (where water would overflow) with an imperial tape measure to the nearest 10th of an inch (later converted to centimeters) and used an Oakton pH tester model WD-35634-30 (Vernon Hills, IL) to measure the temperature of the water in each pool. To sample the macroinvertebrate fauna, we performed 10 sweeps with a fine-mesh aquarium net (3.5 × 2.5 × 0.5 inches), with a single sweep defined as moving the net swiftly through the water for approximately 25 cm. The first 3 sweeps were near the water surface with the net fully submerged. The following 3 sweeps were conducted at a middle depth and the next 3 at the deepest depth of the pool without collecting substrate; a final sweep was performed to collect substrate at the bottom of the pool. We counted macroinvertebrates after each sweep and identified them in the field to the lowest practical taxon. Except for mosquitoes, we held captured invertebrates in a separate tray and returned them to the pools after completing the final sweep. In
sampling events prior to May 2019, mosquitoes were identified in the field and returned to the pools. After May 2019, we transported the mosquito larvae to the laboratory alive to facilitate accurate and timely identification, as the mosquito larval abundance considerably increased during the warmer months. In both cases, 2nd–4th instars were identified to species microscopically with morphological charac-

Table 1. Bull Pen rock pool measurements taken on January 12, 2019, and Bull Sluice rock pool measurements taken on January 15, 2019; the river level on January 15, 2019 was 2.45 ft (river flow: approximately 1,250 ft³/sec) at the US Geological Survey Chattooga (Clayton, GA) gauge.

| Site       | Pool no. | Length (cm) | Width (cm) | Depth (cm) | Height to river (cm) |
|------------|----------|-------------|------------|------------|----------------------|
| Bull Pen   | 1        | 63.5        | 44.5       | 71         | 61                   |
|            | 2        | 81          | 79         | 85         | 76                   |
|            | 3        | 150.5       | 69         | 69         | 76                   |
|            | 4        | 55          | 27         | 34         | 30.5                 |
|            | 5        | 63.5        | 40.6       | 40.6       | 91                   |
|            | 6        | 61          | 35.5       | 66         | 91                   |
|            | 7        | 47          | 28         | 15         | 84                   |
|            | 8        | 71          | 46         | 23         | 30.5                 |
| Bull Sluice| 1        | 85          | 76         | 28         | 366                  |
|            | 2        | 163         | 90         | 9          | 396                  |
|            | 3        | 84          | 27         | 26         | 183                  |
|            | 4        | 56          | 52         | 34         | 122                  |
|            | 5        | 155         | 71         | 23         | 91                   |
|            | 6        | 99          | 56         | 20         | 122                  |
|            | 7¹       | 92          | 70         | 18         | Not measured         |
|            | 8¹       | 110         | 94         | 23         | Not measured         |

¹ Pools only sampled once at the start of the study.
ters using a dichotomous key (Harrison et al. 2016). First instars of *Ae. j. japonicus* and *Ae. atropalpus* cannot be reliably distinguished with morphological characters, so we reared them in the laboratory to later developmental stages at 27°C with a photoperiod of 16 h light and 8 h dark in an environmental growth chamber (Thermo Scientific model pr505755L; Waltham, MA) prior to identification. First-stage mosquito larvae were held for at least 72 h before identification. Similarly, pupal collections were held until eclosion, and the resulting adults were frozen within 72 h after emergence for identification.

We used R version 3.6.1 (R Core Team, Vienna, Austria) in RStudio version 1.2.5019 (RStudio Team, Boston, MA) to calculate mean abundances with standard errors of mosquito species on each sampling date. We used the R package *cooccur* version 1.3 (Griffith et al. 2016) to analyze co-occurrence of mosquito species with a probabilistic model allowing for pairwise comparisons of species and to calculate standardized effect sizes (expected co-occurrence minus observed co-occurrence frequency) (Veech 2013). An effect size <1 implies a negative association, while an effect size of 0 implies no association, and an effect size >0 implies a positive association between 2 species. We obtained ambient temperature data from a weather station in Mountain Rest, SC, approximately midway between the 2 sampling sites (US Climate Data: https://www.usclimatedata.com) and obtained daily mean river height from a river gauge placed near the Bull Sluice rapid maintained by the United States Geological Survey (http://waterdata.usgs.gov [Site: 02177000]).

### RESULTS

We collected 5 mosquito species over the course of the sampling period: *Ae. atropalpus*, *Ae. j. japonicus*, *Culex territans* (Walker), *Cx. restuans* (Theobald), and *Anopheles punctipennis* (Say). Mosquitoes were the most abundant macroinvertebrate family encountered during the study and accounted for more than 88% of the enumerated taxa (Table 2). We encountered several potential mosquito predators, including species from order Odonata, and families Veliidae, Gerridae, Notonectidae, Elmidae, Chaoboridae, and Dytiscidae (Miura and Takahashi 1988, Ohba et al. 2011, Saha et al. 2012, Roux et al. 2015). We found no statistical evidence of co-occurrence (positive or negative) between mosquitoes and any predacious taxa. The relative abundances of mosquito species differed among months and between the 2 sites (Fig. 2). Bull Pen pools contained more mosquitoes in November 2018, January 2019, and November 2019 (Table 3 and Fig. 2A), but BS pools contained more mosquitoes on average from May to August (Table 4 and Fig. 2B). Mean water temperature at BP was lowest in January (7.55°C) and highest in September (22.24°C) (Fig. 2A). Mean water temperature (on the sampling date) at BS was lowest in January (4.11°C) and highest in July (34.44°C) (Fig. 2B).

#### Bull Pen site

*Aedes j. japonicus* was present on every sampling event and was encountered in 82.5% of the individual pool observations (Table 3). The abundance of *Ae. j. japonicus* did not follow a clear seasonal trend, with the species being most abundant in May and February, and then peaking again in August (Table
Fig. 2. Monthly mosquito species relative abundances and average rock pool temperatures at (A) Bull Pen (BP) and (B) Bull Sluice (BS). Temperatures were taken at time of sampling and are not indicative of the temperature for the entire day or month. Five species were encountered at both sites, but Aedes atropalpus was rarely found at BP while being highly abundant at BS in the summer. Aedes j. japonicus was present each month at both sites but was more abundant at BP in the summer.
### Table 3. Mean mosquito abundance at Bull Pen (Chattooga River).

| Date (mean water temperature [°C]) | All mosquito species | *Aedes atropalpus* (SE); proportion of pools encountered | *Ae. j. japonicus* (SE); proportion of pools encountered | *Culex territans* (SE); proportion of pools encountered | *Cx. restuans* (SE); proportion of pools encountered | *Anopheles punctipennis* (SE); proportion of pools encountered |
|-----------------------------------|----------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| Nov. 5, 2018 (13.24)              | 18.80 (± 5.83)       | 16.80 (± 5.33); 4/5                                | 1.80 (± 0.73); 4/5                                   | 0.20 (± 0.20); 1/5                                   | 0                                                    |
| Jan. 15, 2019 (7.55)              | 8.0 (± 2.68)         | 8.0 (± 2.68); 7/8                                  | 0                                                    | 0                                                    | 0                                                    |
| Feb. 17, 2019 (7.79)              | 60.25 (± 10.87)      | 60.25 (± 10.87); 8/8                               | 0                                                   | 0                                                    | 0                                                    |
| Apr. 27, 2019 (14.18)             | 21.75 (± 5.34)       | 21.25 (± 5.30); 8/8                                | 0.38 (± 0.18); 3/8                                   | 0                                                    | 0                                                    |
| May 20, 2019 (18.30)              | 135.50 (± 23.31)     | 93.38 (± 17.73); 8/8                               | 5.37 (± 2.21); 6/6                                   | 32.75 (± 12.32); 7/8                                 | 0.13 (± 0.13); 1/8                                   |
| Jun. 22, 2019 (19.97)             | 21.88 (± 15.28)      | 3.58 (± 2.27); 5/8                                 | 14.75 (± 9.87); 6/8                                  | 2.00 (± 2.00); 1/8                                   | 0                                                    |
| Jul. 31, 2019 (19.70)             | 43.87 (± 19.93)      | 36.13 (± 20.26); 8/8                               | 3.25 (± 1.42); 5/8                                   | 3.75 (± 3.20); 3/8                                   | 0                                                    |
| Aug. 31, 2019 (17.75)             | 74.75 (± 33.61)      | 40.50 (± 32.01); 6/8                               | 32.13 (± 14.16); 7/8                                 | 34.38 (± 39.50); 6/8                                 | 11.625 (± 9.07); 5/8                                 |
| Sep. 28, 2019 (22.24)             | 133.13 (± 77.63)     | 4.00 (± 2.06); 3/8                                 | 81.625 (± 37.11); 8/8                                | 34.38 (± 39.50); 6/8                                 | 11.625 (± 9.07); 5/8                                 |
| Nov. 8, 2019 (10.85)              | 11.00 (± 3.21)       | 11.00 (± 3.21); 7/8                                | 0                                                    | 0                                                    | 0                                                    |

### Table 4. Mean mosquito abundance at Bull Sluice (Chattooga River).

| Date (mean water temperature [°C]) | All mosquito species | *Aedes atropalpus* (SE); proportion of pools encountered | *Ae. j. japonicus* (SE); proportion of pools encountered | *Culex territans* (SE); proportion of pools encountered | *Cx. restuans* (SE); proportion of pools encountered | *Anopheles punctipennis* (SE); proportion of pools encountered |
|-----------------------------------|----------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| Nov. 3, 2018 (9.38)               | 11.75 (± 6.52)       | 1.25 (± 0.77); 3/7                                  | 0.50 (± 0.33); 2/7                                   | 9.13 (± 6.6); 6/7                                    | 0.88 (± 0.88); 1/7                                   | 0                                                    |
| Jan. 12, 2019 (4.11)              | 2.0 (± 0.93)         | 0                                                    | 2.0 (0.93); 3/6                                      | 0                                                    | 0                                                    | 0                                                    |
| Mar. 23, 2019 (15.60)             | 252.0 (± 82.63)      | 0.50 (± 0.22); 3/6                                  | 38.33 (± 16.70); 5/6                                 | 21.83 (± 6.62); 6/6                                  | 9.67 (± 6.07); 3/6                                   | 0.33 (± 0.33); 1/6                                   |
| May 10, 2019 (23.75)              | 215.00 (± 55.94)     | 75.00 (± 24.19); 5/6                                | 43.83 (± 31.68); 6/6                                 | 29.67 (± 8.85); 6/6                                  | 0.17 (± 0.17); 1/6                                   | 0.17 (± 0.17); 1/6                                   |
| Jun. 12, 2019 (25.30)             | 187.83 (± 65.20)     | 119.33 (± 43.81); 5/6                               | 0.83 (± 0.31); 4/6                                   | 7.67 (± 1.78); 5/6                                   | 0.14 (± 0.14); 1/6                                   | 0                                                    |
| Jul. 11, 2019 (34.44)             | 111.29 (± 58.10)     | 101.71 (± 58.98); 6/6                               | 0.29 (± 0.18); 2/6                                   | 6.57 (± 4.05); 4/6                                   | 0                                                    | 0                                                    |
| Aug. 24, 2019 (29.41)             | 177.50 (± 65.08)     | 159.67 (± 58.03); 6/6                               | 0.83 (± 0.65); 2/6                                   | 9.17 (± 2.57); 6/6                                   | 0                                                    | 0.17 (± 0.17); 1/6                                   |
| Sep. 14, 2019 (27.42)             | 80.50 (± 35.47)      | 45.83 (± 32.64); 6/6                                | 2.17 (± 1.38); 2/6                                   | 7.83 (± 3.77); 5/6                                   | 0                                                    | 0                                                    |
| Nov. 3, 2019 (12.13)              | 4.50 (± 2.13)        | 2.00 (± 1.29); 2/6                                  | 2.67 (± 1.14); 3/6                                   | 0                                                    | 0                                                    | 0                                                    |
3 and Fig. 2B). We were unable to sample in December 2018 because of a flooding event that occurred on our scheduled sampling date. *Aedes j. japonicus* larvae were encountered in pools with water as cold as 4.8°C in January and 7.1°C in February, below the reported minimal developmental temperature for the species (Reuss et al. 2018). During the winter months (i.e., December, January, and February), the mean and median *minimum* ambient temperatures approached freezing, but mean and median *maximum* temperatures were warmer and within the reported larval developmental range of *Ae. j. japonicus* (Table 5). Most of the *Ae. j. japonicus* larvae encountered in January and February were early instars, but late instars were also observed (Fig. 3A). The decreased abundance of *Ae. j. japonicus* observed in the June collection may be, in part, to high water levels that occurred immediately prior to our sampling (Table 4); many of the sampled pools were observed to be flooded on June 18 when attempts to sample were unsafe.

*Culex territans* was encountered at BP in November 2018 but was not encountered again until April 2019. The species was collected on every sampling event between April and November 2019, appearing in 48.8% of the individual pool observations. The average abundance of *Cx. territans* increased from April to September except for a decrease in July, with the mean abundance peaking sharply in September. *Culex restuans* was encountered in November of 2018 and in May, June, July, and September in 2019, and observed in 27.5% of the individual pool collections. The mean abundance of *Cx. restuans* was the highest in September followed by May. *Anopheles punctipennis* was only collected in May 2018 and in May through July of 2019. The presence of *Cx. territans* peaked in May and remained low but consistent from June through September. *Culex restuans* and *An. punctipennis* were collected less often and in lower abundances than the other species, with *Cx. restuans* being encountered in 10.1% of the individual pool observations and *An. punctipennis* in 5.4%. *Culex restuans* was encountered in November 2018 and in May through July of 2019. The presence of *Cx. territans* was positively associated with that of *Ae. atropalpus* at BS (effect = 0.12, P < 0.001). There were no other significant co-occurrence patterns observed at the site.

**Bull Sluice site**

*Aedes j. japonicus* was present on every sampling event, with its average abundance in 2019 increasing from January until its peak in May 2019 before decreasing sharply for the remainder of the year. *Aedes j. japonicus* was the only mosquito species observed at the site in January, being encountered in pools as cold as 3.0°C. A flooding event in late December 2018 submerged all the BS pools (observed via social media video taken of the Bull Sluice rapid), preventing us from sampling and likely washing out overwintering larvae. All *Ae. j. japonicus* encountered in January at BS were 1st instars, and they were present in lower mean abundances than at BP during the same month (Fig. 3B). Early and late instars of *Aedes j. japonicus* were more abundant than any other species in March and co-inhabited pools with *Ae. atropalpus* 1st instars (Fig. 3C).

*Aedes atropalpus* was collected on every sampling event except for January 2019 and was encountered in 62.5% of the individual pool observations (Table 4). The species was first encountered in March, and its highest average abundance increased from March through June, decreased slightly in July, and peaking in August before steadily declining through November. It was the most abundant species from May through September and was encountered in 53.6% of the individual pool observations.

*Culex territans* was collected at BS on every sampling event except for January and November 2019 and was encountered in 69.6% of the individual pool observations. The mean abundance of *Cx. territans* was positively associated with that of *Ae. atropalpus* at BS (effect = 0.12, P < 0.001). There were no other significant co-occurrence patterns observed at the site.

**DISCUSSION**

We investigated the macroinvertebrate fauna, with an emphasis on mosquito phenology, within riverine rock pools on the Chattooga River at sites near the headwaters in North Carolina and further downstream at the Bull Sluice rapid located on the Georgia–South Carolina border. Studies of vector phenology and field surveys are important for public health biologists and mosquito control professionals as they help determine expected patterns of vector abundance and provide records of native, exotic, and

| Month   | Median low | Median high | Mean low (SE) | Mean high (SE) |
|---------|------------|-------------|---------------|---------------|
| December | 1.11       | 10.61       | 2.32 (0.76)   | 11.08 (0.63)  |
| January  | 0.00       | 9.39        | 0.60 (0.84)   | 10.08 (0.88)  |
| February | 3.28       | 15.31       | 3.90 (0.78)   | 14.27 (0.93)  |
invasive species. In our study, we found immature mosquitoes in rock pools at both sites on the Chattooga River on every sampling date, including the months of January and February when *Ae. j. japonicus* were present in water colder than the reported minimum temperature for complete larval development (Reuss et al. 2018). Because we think that most of the sampled pools were completely submerged by the river in late December 2018 (Fig. 4), it seems likely that many of the *Ae. j. japonicus* larvae observed in January 2019 were winter hatchlings, though late instars at BP-5 were likely overwintering larvae that survived the flooding event (Scott 2003). We found further evidence suggestive of winter hatching in February and March (Fig. 3A), illustrating the ability of *Ae. j. japonicus* to colonize rock pools earlier in the year than native species. *Aedes j. japonicus* was already abundant in all larval stages in BS rock pools when the first *Ae. atropalpus* hatchlings were encountered in March, creating the opportunity for stage-dependent interactions between species. There is some evidence that competition between early- and late-stage mosquito larvae can have exaggerated impacts on the younger individuals relative to the impacts of same-stage competition, probably because of resource competition, but possibly because of intraguild predation (Edgerly et al. 1999, Lounibos et al. 2003). Future studies of asymmetric stage-based competition may shed light on the ecological interactions between the 2 species. This early colonization of rock pools may allow *Ae. j. japonicus* to decrease the early-season population growth of *Ae. atropalpus* by limiting the developmental success of its 1st generation.

Byrd et al. (2019) found *Ae. atropalpus* in low abundances in BP rock pools, and we encountered the species only once in our study at BP. To claim that *Ae. atropalpus* populations have been reduced at the site, we would need evidence that the species was more abundant there prior to the invasion of *Ae. j. japonicus*. Unfortunately, we are not aware of any historical data to support or refute this assertion. Considering that within the Southern Appalachian region, *Ae. atropalpus* primarily inhabits warm rock pools, it is possible that *Ae. atropalpus*, at least at its southernmost range, has historically been less abundant in more temperate BP pools, leaving considerable suitable habitat for *Ae. j. japonicus*. Indeed, recent work by Day et al. (2021) suggests that *Aedes atropalpus* population growth is considerably depressed at cooler temperatures (e.g., 16–20°C) as compared with warmer pools. The pools at BP were under dense canopy cover, whereas those at BS were largely unshaded. Additional studies exploring the direct and indirect effects of carbon sources, species interactions, and other ecological factors are needed. Transplant “common garden” experiments using natural rock pools and studies of *Ae. atropalpus* ovipositional tendencies would likely be informative. In contrast to BP, *Ae. atropalpus* was the most abundant mosquito from May to September at the BS site, demonstrating that *Ae. atropalpus* remains established within its southeastern range.

As the most abundant macroinvertebrate taxon in the sampled rock pools, mosquitoes are likely important to the broader ecology of some rock pool systems. Our study design was not ideal for the analysis of the interactions between mosquitoes and
their predators or other rock pool inhabitants, but studies with larger sample sizes and finer temporal resolution may reveal important information about the impacts of predators on mosquito communities and the role of predators in shaping the community compositions of rock pools. *Aedes j. japonicus* is more vulnerable to predation by a native tree hole predator than native species (Juliano et al. 2019), but to our knowledge similar studies have not occurred with native rock pool predators. We encountered several potential mosquito predators in our study, including some that appeared at only 1 site, like dragonfly naiads belonging to family Libellulidae (Table 2). Though temperature is a clear correlate with the presence and abundance of *Ae. j. japonicus*, further exploration of the ecological interactions of the invasive mosquito species and native predators could provide a better understanding of the temporal changes in *Ae. j. japonicus* abundances.

We used a metric of co-occurrence to investigate co-occurrence patterns among the mosquitoes encountered during this study, but it is likely that we did not obtain a sufficient sample size or the temporal resolution to observe such patterns accurately. It would be more appropriate to sample many pools on a single sampling event or during a few closely timed events. It would be of interest to know whether *Ae. atropalpus* and *Ae. j. japonicus* demonstrate patterns of co-occurrence or avoidance in rock pools, which could inform the likelihood of competition or competitive exclusion between the species. We found no such pattern in our data at either site. We observed co-occurrences among mosquito species that differed by site. At BP, *Cx. territans* was positively associated with both *Cx. restuans* and *An. punctipennis*. Whereas, at BS, *Cx. territans* was positively associated with *Ae. atropalpus*, but no other species. We did find evidence of positive co-occurrence patterns among other species pairs. However, we believe further study is warranted before emphasizing any co-occurrence relationships.

In the southeastern USA, mosquitoes are present in rock pools year-round, with the invasive *Ae. j. japonicus* being the only species occupying the pools during the winter months. We found that *Ae. j. japonicus* both overwinters and likely undergoes

![Fig. 4. Mean river height (ft) of the Chattooga River near the Bull Sluice rapid (data source: http://waterdata.usgs.gov [Site: 02177000]).](image-url)
winter instars, allowing the species to develop into late instars before \textit{Ae. atropalpus} initially hatch. Only during the warmest (summer) months at the low-elevation site (BS) did \textit{Ae. atropalpus} become more abundant than \textit{Ae. j. japonicus}. At the colder high-elevation site (BP), \textit{Ae. atropalpus} was rarely encountered. These results further implicate the role of temperature as a factor determining southeastern rock pool mosquito community composition. The impacts of flash flooding on the phenology of riverine rock pool inhabitants also remains unclear; this study was not designed to address the immediate or delayed impacts of flooding on rock pool macroinvertebrate phenology. However, in experimental pools, flash flooding is known to result in the loss of food resources such as phytoplankton and zooplankton (Duchet et al. 2017). Thus, further study of the ecological interactions between \textit{Ae. j. japonicus} and \textit{Ae. atropalpus} are warranted and should consider the potential effects of temperature, species interactions, and aperiodic flooding.

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REFERENCES CITED

Anderson JF. 1968. Influence of photoperiod and temperature on the induction of diapause in \textit{Aedes atropalpus} (Diptera: Culicidae). \textit{Entomol Exp Appl} 11:321–330.

Andreadis TG, Anderson JF, Munstermann LE, Wolfe RJ, Florin DA. 2001. Discovery, distribution, and abundance of the newly introduced mosquito \textit{Ochlerotatus japonicus} (Diptera: Culicidae) in Connecticut, USA. \textit{J Med Entomol} 38:774–779.

Andreadis TG, Wolfe RJ. 2010. Evidence for reduction of native mosquitoes with increased expansion of invasive \textit{Ochlerotatus japonicus} japonicus (Diptera: Culicidae) in the northeastern United States. \textit{J Med Entomol} 47:43–52.

Armistead JS, Nishimura N, Arias JR, Lounibos LP. 2012. Community ecology of container mosquitoes (Diptera: Culicidae) in Virginia following invasion by \textit{Aedes japonicus}. \textit{J Med Entomol} 49:1318–1327.

Armistead JS, Nishimura N, Escher RL, Lounibos LP. 2008. Larval competition between \textit{Aedes japonicus} and \textit{Aedes atropalpus} (Diptera: Culicidae) in simulated rock pools. \textit{J Vector Ecol} 33:238–246.

Beach R. 1978. The required day number and timely induction of diapause in geographic strains of the mosquito \textit{Aedes atropalpus}. \textit{J Insect Physiol} 24:449–455.

Bevins SN. 2007. Establishment and abundance of a recently introduced mosquito species \textit{Ochlerotatus japonicus} (Diptera: Culicidae) in the Southern Appalachians, USA. \textit{J Med Entomol} 44:945–952.

Bova J, Soghigian J, Paulson S. 2019. The prediapause stage of \textit{Aedes japonicus japonicus} and the evolution of embryonic diapause in Aedini. \textit{Insects} 10:222.

Burger JF, Harry D. 2008. Discovery of \textit{Ochlerotatus japonicus japonicus} (Theobald) (Diptera: Culicidae) in southern New Hampshire, U.S.A. and its subsequent increase in abundance in used tire casings. \textit{Entomol News} 119:439–444.

Byrd BD, Sither CB, Goggins JA, Kunze-Garcia S, Pesko KN, Bustamante DM, Sither JM, Vonesh JR, O’Meara GF. 2019. Aquatic thermal conditions predict the presence of native and invasive rock pool \textit{Aedes} (Diptera: Culicidae) in the southern Appalachians, U.S.A. \textit{J Vector Ecol} 44:30–39.

Day CA, Armstrong EG, Byrd BD. 2021. Population growth rates of \textit{Aedes atropalpus} (Diptera: Culicidae) are depressed at lower temperatures where \textit{Aedes japonicus japonicus} (Diptera: Culicidae) are naturally abundant in rock pools. \textit{J Med Entomol} 58:493–497.

Duchet C, Moraru GM, Segov O, Spencer M, Hayoon AG, Blaustein L. 2017. Effects of flash flooding on mosquito and community dynamics in experimental pools. \textit{J Vector Ecol} 42:254–263.

Edgerly JS, Willey MS, Livdahl T. 1999. Intraguild predation among larval treehole mosquitoes, \textit{Aedes albopictus}, \textit{Ae. aegypti}, and \textit{Ae. triseriatus} (Diptera: Culicidae), in laboratory microcosms. \textit{J Med Entomol} 36:394–399.

Griffith DM, Veach JA, Marsh CJ. 2016. cooccurrence: probabilistic species co-occurrence analysis in R. \textit{J Stat Softw} 69:code snippet 2. doi:10.18637/jss.v069.i02

Hardstone AMC, Andreadis TG. 2012. Weak larval competition between the invasive mosquito \textit{Aedes japonicus japonicus} (Diptera: Culicidae) and three resident container-inhabiting mosquitoes in the laboratory. \textit{J Med Entomol} 49:277–285.

Harrison BA, Byrd BD, Sither CB, Whitb PB. 2016. Mosquitoes of the Mid-Atlantic region: an identification guide. Cullowhee, NC: Western Carolina Univ.

Jocque M, Vanschoenwinkel B, Brendonck L. 2010. Invasion biology of \textit{Aedes aegypti}, \textit{Ae. aegypti}, and \textit{Ae. albopictus} (Diptera: Culicidae), in the northeastern United States. \textit{Environ Entomol} 39:641–649.

Juliano SA, Westby KM, Ower GD. 2019. Know your enemy: effects of a predator on native and invasive container mosquitoes. \textit{J Med Entomol} 56:320–328.

Kalpage KSP, Brust RA. 1974. Studies on diapause and female fecundity in \textit{Aedes atropalpus}. \textit{Environ Entomol} 3:139–145.

Kaufman MG, Fonseca DM. 2014. Invasion biology of \textit{Aedes japonicus japonicus} (Diptera: Culicidae). \textit{Annu Rev Entomol} 59:31–49.
Kaufman MG, Stanuszek WW, Brouhard EA, Knepper RG, Walker ED. 2012. Establishment of Aedes japonicus japonicus and its colonization of container habitats in Michigan. J Med Entomol 49:1307–1317.

LaCasse WJ, Yamaguti S. 1950. Mosquito fauna of Japan and Korea. Part II. Kyoto, Japan: Office of the Surgeon, Headquarters 1 Corps APO 301.

Lounibos LP, O’Meara GF, Nishimura N. 2003. Interactions with native mosquito larvae regulate the production of Aedes albopictus from bromeliads in Florida. Ecol Entomol 28:551–558.

Miura T, Takahashi R. 1988. Predation of Microvelia pulchella (Hemiptera: Veliidae) on mosquito larvae. J Am Mosq Control Assoc 4:91–93.

Montarsi F, Martini S, Michelutti A, Da Rold G, Mazzucato M, Qualizza D, Di Gennaro D, Di Fant M, Dal Pont M, Paleti M, Capelli G. 2019. The invasive mosquito Aedes japonicus japonicus is spreading in northeastern Italy. Parasit Vectors 12:120.

Ohba S, Huynh TTT, Kawada H, Le LL, Ngoc HT, Hoang SL, Higa Y, Takagi M. 2011. Heteropteran insects as mosquito predators in water jars in southern Vietnam. J Vector Ecol 36:170–174.

O’Meara GF, Evans LF, Gettman AD, Cuda JP. 1995a. Spread of Aedes albopictus and decline of Ae. aegypti (Diptera: Culicidae) in Florida. J Med Entomol 32:554–562.

O’Meara GF, Evans LF, Gettman AD, Patteson AW. 1995b. Exotic tank bromeliads harboring immature Aedes albopictus and Aedes bahamensis (Diptera: Culicidae) in Florida. J Vector Ecol 20:216–224.

O’Meara GF, Krasnick GJ. 1970. Dietary and genetic control of the expression of autogenous reproduction in Aedes atropalpus (Coq.) (Diptera: Culicidae). J Med Entomol 7:328–334.

Reuss F, Wieser A, Niamir A, Bálint M, Kuch U, Pfenninger M, Müller R. 2018. Thermal experiments with the Asian bush mosquito Aedes japonicus japonicus (Diptera: Culicidae) and implications for its distribution in Germany. Parasit Vectors 11:1–10.

Roux O, Vautaux A, Roche B, Yameogo KB, Dabiré KR, Diabaté A, Simard F, Lefèvre T. 2015. Evidence for carry-over effects of predator exposure on pathogen transmission potential. Proc R Soc B Biol Sci 282:20152430.

Saha N, Aditya G, Banerjee S, Saha GK. 2012. Predation potential of odonates on mosquito larvae: implications for biological control. Biol Control 63:1–8.

Sardelis MR, Turell MJ, Andre RG. 2002. Laboratory transmission of La Crosse virus by Ochlerotatus j. japonicus (Diptera: Culicidae). J Med Entomol 39:635–639.

Schaffner F, Kaufmann C, Hegglin D, Mathis A. 2009. The invasive mosquito Aedes japonicus in central Europe. Med Vet Entomol 23:448–451.

Scholte EJ, Hartog WD, Braks M, Reusken C, Dik M, Hessels A. 2009. First report of a North American invasive mosquito species Ochlerotatus atropalpus (Coquillett) in the Netherlands, 2009. Eurosurveillance 14:19400.

Scott J. 2003. The ecology of the exotic mosquito Ochlerotatus japonicus japonicus (Theobald 1901) (Diptera: Culicidae) and an examination of its role in the West Nile virus cycle in New Jersey. New Brunswick, NJ: Rutgers Univ.

Scott JJ, Carle FL, Crans WJ. 2001. Ochlerotatus japonicus collected from natural rockpools in New Jersey. J Am Mosq Control Assoc 17:91–92.

Shaw FR, Maisey SA. 1956. The biology and distribution of the rockpool mosquito, Aedes atropalpus. Mosq News 21:12–16.

Telang A, Wells MA. 2004. The effect of larval and adult nutrition on successful autogenous egg production by a mosquito. J Insect Physiol 50:677–685.

Turell MJ, Byrd BD, Harrison BA. 2013. Potential for populations of Aedes j. japonicus to transmit Rift Valley Fever virus in the USA. J Am Mosq Control Assoc 29:133–137.

Veech JA. 2013. A probabilistic model for analysing species co-occurrence. Glob Ecol Biogeogr 22:252–260.