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ARTICLE

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Abstract
Since 1991, the number of penaeid shrimp occurring in Virginia waters of Chesapeake Bay has steadily increased, prompting an interest in developing a fishery. Although development of a shrimp fishery in the Chesapeake Bay region could bring economic benefits, the fishery may be hampered by the presence of a disease syndrome known as shrimp black gill (sBG). The objectives of our study were to (1) describe the spatial distribution and abundance patterns of shrimp in Chesapeake Bay, (2) relate relative abundance of shrimp to habitat characteristics, and (3) determine the presence and seasonality of sBG to better understand disease dynamics in the region. Subadult penaeid shrimp were collected monthly from Virginia waters by trawl from 1991 to 2017, and individuals were identified to species and counted. White shrimp *Litopenaeus setiferus* were the most numerous species captured, followed by brown shrimp *Farfantepenaeus aztecus* and pink shrimp *F. duorarum*. Shrimp were captured primarily from July to December. White shrimp were the only species that exhibited visible signs of sBG, which was first observed in October 2016 (13.4% prevalence); the condition continued into November and recurred the following year. Shrimp with visible signs of gill disease were examined by microscopy, histology, and PCR assay and were diagnosed with infections of a histophagous apostome ciliate, presumably *Hyalophysa lynni*. Any impacts of sBG on shrimp survival or marketability should be considered in fishery management plans to ensure sustainability of the resource.

Penaeid shrimp have been observed in Chesapeake Bay waters as far back as 1880, occurring in some of the earliest fisheries surveys undertaken by the U.S. Fish Commission (Baird 1882). However, the relative abundance of penaeid shrimp was very low, and catches in fishery-independent surveys were not recorded until a notable increase in catch was observed in 1991 (Geer et al. 1993). In the southeastern USA, penaeid shrimp support a valuable commercial fishery, with a dockside value of more than US$436 million in 2016 (NMFS 2018). Three species of penaeid shrimp support the commercial fishery, including white shrimp *Litopenaeus setiferus*, brown shrimp

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Farfantepenaeus aztecus, and pink shrimp *F. duorarum*. White shrimp comprised 57% of the 2016 shrimp landings, followed by brown shrimp at 39%, and the remaining 4% consisted of pink shrimp (NMFS 2018).

The increase in abundance of penaeid shrimp in Virginia waters and resulting interest from commercial fishers prompted the Virginia Marine Resources Commission (VMRC) to study the potential for developing a fishery in Virginia's coastal waters. Although trawling has been prohibited inside Chesapeake Bay since 1989 (Code of Virginia 1989), VMRC established an experimental shrimp trawl permit in 2017 and 2018 in Virginia's coastal waters. In 2019, six watermen were permitted to harvest penaeid shrimp in Virginia's state waters.

Although the development of a shrimp fishery in the Chesapeake Bay region could bring economic benefits to coastal fishing communities, the sustainability and marketability of shrimp may be hampered by the presence of a disease syndrome known as shrimp black gill (sBG). Penaeid shrimp captured off Georgia first exhibited symptoms of the disease as early as 1996, and sBG is now found in shrimp from Georgia, South Carolina, and as far north as Chesapeake Bay (Frischer et al. 2017; Fowler et al. 2018). Shrimp black gill disease is caused by the histophagous apostome ciliate *Hyalophysa lynni*, which infects the gills and induces a melanization response by the host that results in necrotic, melanized gill tissues (Frischer et al. 2017, 2018; Landers et al. 2020). The prevalence of sBG infections appears related to increasing temperatures and decreasing dissolved oxygen conditions during summer (Frischer et al. 2017; Fowler et al. 2018). The effects of sBG on shrimp health, survival, and mortality may be significant because damaged gills likely impair respiration in penaeid shrimp (Frischer et al. 2018) as well as in other decapods with similar infections (Johnson and Bradbury 1976; White et al. 1985; Burnett and Burnett 2015). A decrease in metabolic rate and oxygen uptake due to sBG infection, along with prolonged heat stress, could increase the vulnerability of penaeid shrimp to predation (Fowler et al. 2018; Gooding et al. 2020) or may lead to a more severe disease condition (Shields 2019; Shields and Huchin-Mian 2020).

Adult penaeid shrimp spawn in the coastal ocean, and shrimp postlarvae migrate to low-salinity regions inside bays and estuaries to grow (Williams 1955; Wenner and Beatty 1993). Young shrimp can be found in a variety of substrates and salinities, although they prefer oligohaline and mesohaline regions (Wenner and Beatty 1993). As shrimp grow, they move from shallow tidal creeks and low-salinity regions into high-salinity areas toward the mouth of bays and inlets, where they reside as subadults (Weymouth et al. 1933). Our study represents the first effort to quantify penaeid shrimp abundance in the Virginia portion of Chesapeake Bay, focusing primarily on the subadult stage, and to investigate the distribution and prevalence of sBG disease. The objectives of our study were to (1) describe the spatial distribution and abundance patterns of three penaeid shrimp species in the lower Chesapeake Bay region; (2) relate relative abundance of penaeid shrimp to the coastal abundance of penaeid shrimp and to habitat characteristics (i.e., water temperature, salinity, freshwater flow, and submerged aquatic vegetation [SAV]); and (3) determine the presence and seasonality of sBG in populations of shrimp in Chesapeake Bay to better understand the disease dynamics of this syndrome in the region.

METHODS

Subadult penaeid shrimp were collected from Chesapeake Bay waters and the James, York, and Rappahannock River subestuaries by the Virginia Institute of Marine Science (VIMS) Juvenile Fish Trawl Survey (hereafter, "VIMS trawl survey") from 1991 to 2017. We used a 9.1-m, four-seam, semi-balloon otter trawl with a body made from 38.1-mm stretch mesh and a 6.4-mm-mesh liner. Monthly samples consisted of 5-min tows at approximately 4.63 km/h (2.5 knots) at each site. Following each tow, surface and bottom water temperature, depth, salinity, and dissolved oxygen were measured with a hand-held YSI 650 meter. In each year, the survey sampled 111 stations monthly from May to November (22 stations in each subestuary and 45 stations in the bay); 105 stations monthly in December, February, and April (22 stations in each subestuary and 39 stations in the bay); and 66 stations monthly in January and March (only the subestuaries were sampled). All penaeid shrimp caught by the trawl survey were identified to species and counted, and a random subsample (up to 30 individuals) from each tow was measured (TL; from the rostrum tip to the end of the tail). Shrimp with visible signs of sBG (i.e., visible melanized nodules in gill tissues) were recorded as showing signs of the disease; several of these shrimp were placed in coolers on ice and then brought to VIMS for further examination and diagnostics (see below).

Habitat associations and spatial and temporal distributions.— Habitat associations, coarse spatial distribution (e.g., region), and temporal occurrence of shrimp were examined for each species through boosted regression tree (BRT) analysis using the "dismo" package in R (Elith et al. 2008; R Core Team 2018). Boosted regression trees provide an ensemble modeling approach that relies on machine learning to identify relationships between the abundance (numbers) of shrimp and conditions from which they were captured (Elith et al. 2008). Regression trees are nonparametric and provide reliable descriptions of species–habitat relations (Brodie et al. 2020) by using cross validation of the resulting tree models. Habitat

variables considered for the shrimp BRTs included depth and bottom water conditions (temperature, salinity, and dissolved oxygen). Region (i.e., Chesapeake Bay, James River, York River, or Rappahannock River), year, and month were also included in BRTs to examine the influence of spatial and temporal factors. Counts of shrimp were modeled in BRTs assuming a Poisson distribution. The proportion of observations used for model building is called the bag fraction and must be specified by the analyst; the bag fraction was held at 0.75, and the remainder (0.25) of the observations was used to perform the cross validation of the trees. Two BRT model parameters—tree complexity (TC) and learning rate (LR)—control the manner in which trees are grown; TC refers to the level of interactions possible between predictor variables, and LR refers to the contribution of each tree to the overall model. Slower LRs (<0.01) are preferred (Elith et al. 2008). The LR and TC parameters are set by the analyst and should be optimized for the particular data set under study (Elith et al. 2008). We evaluated multiple values for TC (1, 2, and 3) and LR (0.001, 0.005, 0.0075, 0.01, 0.02, 0.03, 0.04, and 0.05) and selected values that produced at least 1,000 trees and resulted in the lowest estimated cross-validated deviance and the highest cross-validated correlation (Elith et al. 2008). Model parameters that produced the lowest cross-validated deviance were an LR of 0.0075 for white, brown, and pink shrimp; a TC of 3 for white shrimp; a TC of 1 for brown shrimp; and a TC of 2 for pink shrimp. Results from the BRT analyses were used to inform models for standardizing abundances for further analyses.

Annual indices of abundance.— Indices of abundance for each species were calculated using generalized linear models (GLMs), with habitat covariates informed by the BRT results and assuming a negative binomial distribution of shrimp counts. The GLM-based annual index of abundance for white shrimp was based on September–December catches from sites in the James, York, and Rappahannock rivers because white shrimp were infrequently captured in the main stem of the bay (only 7.6% of all white shrimp were from bay stations). The annual index of abundance for brown shrimp was based on August–December catches from sites in the bay and the James and York rivers (only 4% of brown shrimp were captured in the Rappahannock River). Pink shrimp were the least abundant species and were found primarily in the James River and bay sites from September through December. As indicated by the BRT results, habitat covariates for all shrimp GLMs included bottom temperature, salinity, and depth; annual indices of abundance for each species were estimated from 1991 to 2017.

We also used GLMs to investigate factors that may affect the annual abundance indices of shrimp in Chesapeake Bay (the same indices calculated above were used

as the response variable). For example, habitats with SAV serve as nursery areas for juvenile shrimp (Williams 1955; Murphey and Fonseca 2014), so the annual extent of SAV in Chesapeake Bay (https://www.vims.edu/research/units/programs/sav/reports/index.php) was examined in the GLMs. We considered average minimum bottom water temperature during the previous winter from main-stem bay stations in Virginia (Chesapeake Bay Program's Water Quality Database, 1991–2017; https://www.chesapeakebay.net/what/data) as a proxy for regional conditions, as cold temperatures (<8°C) inhibit overwinter survival of adults in the coastal ocean and thus may affect the number of juveniles that were produced and available to our survey the following summer (Etzold and Christmas 1977; DeLancey et al. 2008). We also included cumulative annual freshwater flow into the bay from U.S. Geological Survey gauges (James River: 02037500; Chickahominy River: 02042500; Pamunkey River: 01673000; Mattaponi River: 01674500; and Rappahannock River: 01668000) to account for annual variation in salinity. Finally, we included an annual index of shrimp abundance from the south Atlantic region from the South Carolina Department of Natural Resources' Southeast Area Monitoring and Assessment Program (SEAMAP) survey (SEAMAP-SA Data Management Work Group 2016). The SEAMAP survey produces an annual index of abundance from three cruises during spring, summer, and fall along the southeastern U.S. coast stretching from northern Florida to North Carolina. We recognize that the SEAMAP survey may not correspond with the shrimp life cycle in Chesapeake Bay; however, we include the SEAMAP data as a proxy to examine any potential relationship between the relative abundance of adult penaeid shrimp on the continental shelf and catches of subadults in Chesapeake Bay. Multiple models that included the SEAMAP index of adult abundance along the coast and various habitat characteristics were evaluated to identify the variables that were most important in predicting juvenile and subadult shrimp abundance in Chesapeake Bay. All predictor variables were standardized prior to model fitting to allow us to directly compare their importance in explaining variation in shrimp relative abundance in Chesapeake Bay (Schielzeth 2010). Models were fitted using the R packages "statmod" and "tweedie." Models were compared using Akaike's information criterion corrected for small sample size (AIC$_c$; Burnham and Anderson 2002).

Histology.— Shrimp were kept on ice or under refrigeration for approximately 12–18 h prior to dissection. In November 2017, seven shrimp (six from the York River and one from Chesapeake Bay) were measured (TL), sexed, and photographed. The branchial gills of a few selected animals were excised and examined at 40–400× magnification using an Olympus BX51 light microscope (Olympus, Inc., Center Valley, Pennsylvania). The

remaining gills were fixed in Bouin's solution (Fisher Scientific). Gill samples were then processed through a standard histological series (Shields et al. 2012). Sections were cut at 5–6 μm and stained with Harris's hematoxylin and eosin (Humason 1979). Prepared microslides were evaluated at 40–1,000× magnification using an Olympus 51 BMX compound microscope and images captured using an Olympus DP73 camera and cellSens software. Only contrast and brightness levels were adjusted as needed on photographs and only for presentation purposes.

Molecular detection of the shrimp black gill ciliate.— A previously developed diagnostic PCR assay (Frischer et al. 2017) was used to screen a subset of shrimp gill samples for the presence of the presumptive sBG-causing ciliate. Gill tissues from 15 white shrimp displaying sBG symptoms (light melanization: $n = 9$; moderate: $n = 5$; dark: $n = 1$) in November and December 2017 were removed and placed into 95% ethanol. Prior to DNA extraction, gill tissues were immersed in molecular-grade water for 60 min to allow for removal of residual ethanol. The DNA was extracted using a Qiagen Blood and Tissue Kit (Qiagen, Valencia, California) following the manufacturer's instructions for animal tissues. All DNA samples were eluted in 100 μL of Qiagen AE buffer, quantified using a Nano-Drop 2000 spectrophotometer (Thermo Scientific, West Palm Beach, Florida), and stored at −20°C.

Amplification reactions were carried out in a S1000 Thermal Cycler (Bio-Rad Laboratories, Hercules, California) using a Qiagen PCR Core Kit. Each reaction contained 50–100 ng of genomic DNA, 1× PCR buffer, 200 μM of each dNTP, 0.5 μM of each primer (Hyalo-18SF-754 and Hyalo-18SR-952; Frischer et al. 2017), 0.5 units of *Taq* DNA polymerase, and sterilized deionized water to a final volume of 20 μL. Thermal cycling conditions were as described previously (Frischer et al. 2017). Aliquots of 10 μL of the amplification products were visualized using 2% (weight/volume) agarose gel electrophoresis, stained with ethidium bromide, and viewed under an ultraviolet light source.

Sequencing and analysis of sequence data.— The remaining contents from six individual PCR assays that produced intense single-reaction products of approximately 198 bp in size were purified using a QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol. Purified PCR products were bi-directionally sequenced using the Big Dye Terminator Kit (Applied Biosystems, Foster City, California) with the original amplification primers (Hyalo-18SF-754 and Hyalo-18SR-952) and one-eighth the recommended concentration of Big Dye. The sequencing reaction products were precipitated using ethanol and sodium acetate and re-suspended in 16 μL of Hi-Di formamide; 10 μL of each were electrophoresed on an ABI 3130 Prism Genetic Analyzer (Applied Biosystems). Forward and reverse sequencing reactions were imported into

Sequencher version 5.1 for trimming of sequences and creation of consensus sequences. Consensus sequences were aligned in MacVector version 12.5.1 (MacVector, Inc., Apex, North Carolina) and compared to those deposited in GenBank using Basic Local Alignment Search Tool (BLAST) searches.

RESULTS

Penaeid Shrimp and Habitat Associations in Virginia

The number of penaeid shrimp captured in Virginia varied annually and by species, with white shrimp being the most numerous shrimp species captured across years ($N = 17,822$), followed by brown shrimp ($N = 1,209$) and pink shrimp ($N = 354$). The size range and average size of shrimp were similar among species (white shrimp: size range = 35–195 mm TL, mean = 112.7 mm; brown shrimp: 35–190 mm TL, mean = 114.4 mm; pink shrimp: size range = 38–166 mm TL, mean = 98.3 mm). Penaeid shrimp were captured primarily from July to December, although shrimp occurred in trawl survey catches throughout the year (Figure 1). White shrimp were more abundant in subestuaries, whereas brown shrimp were more abundant in the bay and in the James and York River subestuaries (Figure 2). Pink shrimp were encountered most often in the lower James River and the bay (Figure 2).

White, brown, and pink shrimp exhibited different responses to habitat characteristics found in Chesapeake Bay according to species-specific BRT results. We found that 64% of the deviance for white shrimp was explained by depth, temperature, salinity, dissolved oxygen, region, and month, whereas 37% of the deviance was explained by the model covariates for brown shrimp and 20% of the deviance was explained for pink shrimp (Table 1). The abundance of white shrimp was positively related to salinities greater than 8 psu, well-oxygenated waters, warm temperatures (>15°C), and depths to 20 m. The abundance of brown shrimp was positively related to salinities greater than 20 psu, well-oxygenated waters, and depths greater than 10 m. Brown shrimp abundance was also positively related to water temperatures greater than 10°C. The abundance of pink shrimp was positively related to depths greater than 15 m and salinities greater than 20 psu. However, the BRT results for pink shrimp habitat associations were less reliable than those for white and brown shrimp because those for pink shrimp were based on ensembles of only 450 trees, and this was likely due to the limited number of pink shrimp captured.

Patterns in Relative Abundance (Generalized Linear Model Results)

The species composition of penaeid shrimp in Chesapeake Bay has been inconsistent since 1991. Annual
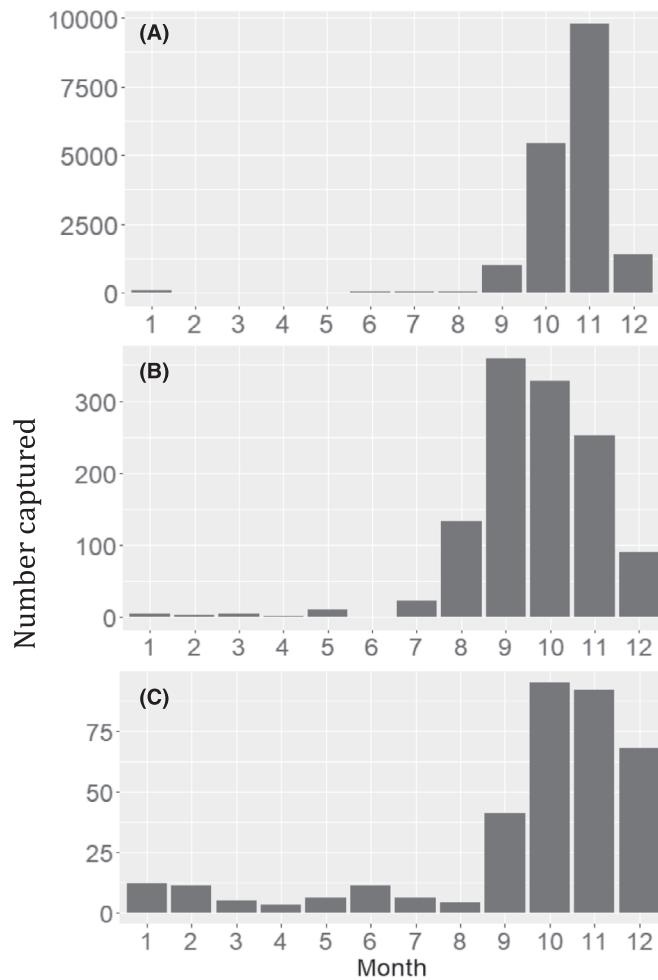
FIGURE 1. FNumber of shrimp captured in Virginia waters of Chesapeake Bay by month (1 = January, 2 = February, and so forth) for all years (1991–2017) combined: **(A)** white shrimp ($N = 17{,}822$), **(B)** brown shrimp ($N = 1{,}209$), and **(C)** pink shrimp ($N = 354$). Note the differences in y-axis scales.
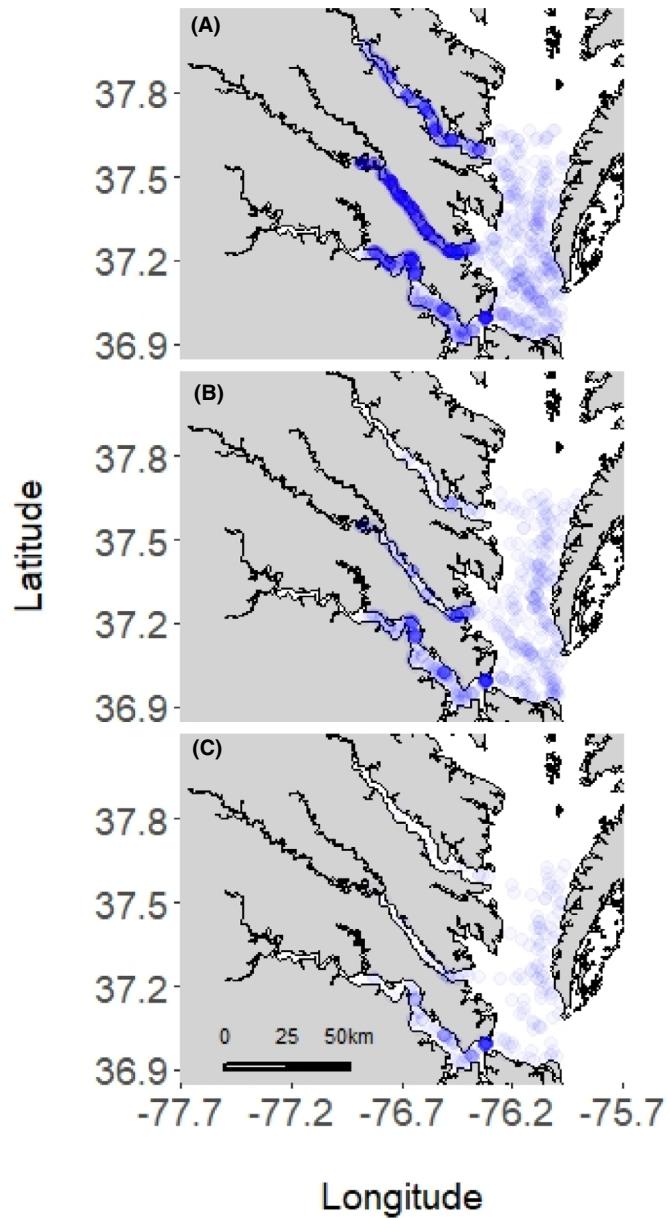

FIGURE 2. Distribution of shrimp captured by the Virginia Institute of Marine Science trawl survey in Chesapeake Bay from all strata and months, 1991–2017: **(A)** white shrimp, **(B)** brown shrimp, and **(C)** pink shrimp. Darker colors indicate greater catches.

indices of brown shrimp abundance in the early 1990s were generally higher than more recent observations (except 2007; Figure 3B). The annual index of abundance for white shrimp increased since 1991, and the highest abundance was observed in 2016 (Figure 3A). The annual index of abundance for pink shrimp was lower than that of brown or white shrimp, with peaks occurring throughout the time series but at much lower levels than were observed for the other two shrimp species (Figure 3C). In the south Atlantic, relative abundance estimates from the SEAMAP survey indicated higher abundance of white and brown shrimp in recent years but no obvious pattern for pink shrimp (Figure 4). The annual index of abundance for white shrimp from the VIMS trawl survey was positively correlated with the SEAMAP white shrimp index (Spearman's rho = 0.65, $P < 0.001$), whereas the

VIMS trawl survey's annual index of brown shrimp abundance was negatively correlated with the SEAMAP index for brown shrimp (Spearman's rho = −0.55, $P = 0.04$). There was no correlation between the VIMS trawl survey's annual abundance index for pink shrimp and the SEAMAP index for pink shrimp (Spearman's rho = 0.18, $P = 0.39$).

All GLMs investigating variation in annual indices of abundance for the three shrimp species were supported by

TABLE 1. Summary statistics for boosted regression tree (BRT) models of the numbers of white, brown, and pink shrimp captured in Virginia from 1991 to 2017. Estimated cross validation (CV) deviance values are shown (SEs in parentheses).

BRT model results	White shrimp	Brown shrimp	Pink shrimp
Mean total deviance	5.03	0.35	0.12
Mean residual deviance	1.83	0.22	0.10
Deviance explained (%)	63.6	37.1	20.0
Estimated CV deviance	3.84 (0.52)	0.31 (0.03)	0.11 (0.02)
CV correlation	0.13 (0.014)	0.11 (0.02)	0.11 (0.03)
Number of trees	3,250	2,100	450

the data. The model with the most support in explaining the annual index of white shrimp abundance in Chesapeake Bay included average minimum winter water temperature and the SEAMAP index, although most models were plausible (difference in AIC_c [Δ_i] < 7; Table 2). For brown shrimp, three models were supported by the data: one model included the average minimum winter water temperature and the SEAMAP index, another included SAV and the SEAMAP index, and the last model included only the SEAMAP index of abundance (Table 2). The best candidate model (lowest AIC_c) explained 54.1% of the total deviance for white shrimp, 19.8% of the total deviance for brown shrimp, and 31.8% of the total deviance for pink shrimp.

Shrimp Black Gill Visible Presentation and Prevalence

White shrimp with visible signs of sBG were captured from a wide range of salinities (2.1–29.4 psu) throughout the Virginia portion of the bay and its subestuaries. Bottom water temperatures where diseased shrimp were encountered ranged from 7.8°C to 26.1°C, and dissolved oxygen levels ranged from 3.9 to 9.9 mg/L. Shrimp black gill was also prevalent in a wide size range of individuals (Figure 5).

White shrimp infected with late stages of sBG disease exhibited characteristic signs of infection that were macroscopically visible through the carapace overlying the branchial chamber (Figure 6). In Chesapeake Bay, white shrimp were captured throughout the summer and first exhibited macroscopic (externally visible) signs of sBG in October 2016 (13.4% prevalence, $N = 1,308$ individuals examined); the condition continued into November (9.4% prevalence, $N = 3,838$). Macroscopic prevalence in 2017 was similar, with relatively high prevalence levels in October (18.6%, $N = 654$) and a decline in November (1.1%, N
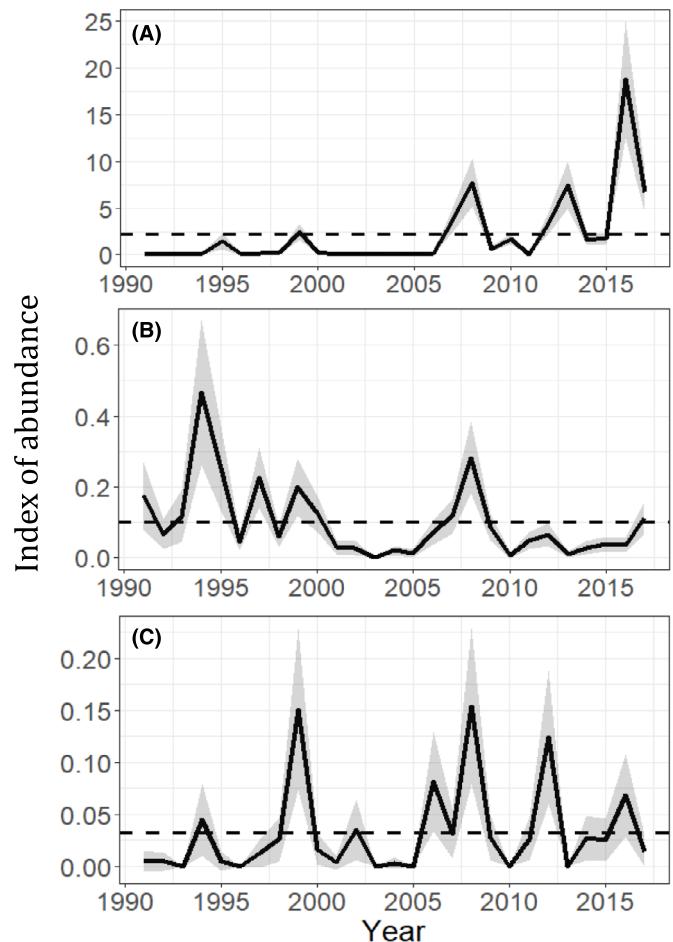

FIGURE 3. Standardized indices of abundance for shrimp captured by the Virginia Institute of Marine Science trawl survey in Chesapeake Bay, 1991–2017: **(A)** white shrimp, **(B)** brown shrimp, and **(C)** pink shrimp. Gray shading denotes the 95% confidence interval. Dotted lines indicate the time series averages (1991–2017). Note the differences in y-axis scales.

$= 1,311$) and December (1.9%, $N = 216$). Only one individual exhibited signs of sBG in September (10%, $N = 10$; Figure 5). Unlike white shrimp, the brown shrimp ($N = 157$) and pink shrimp ($N = 64$) that were captured in fall (September–December) of 2016 and 2017 did not show visible signs of sBG.

Histological Analysis of Shrimp Black Gill in White Shrimp

All of the shrimp sampled for histopathology ($n = 7$) exhibited gross signs of blackened gills, but the extent of damage varied among individuals. The black appearance of the gills was due to intensive melanization of the damaged lamellae. Affected animals showed significant melanization on every gill branchia and in both left and right branchial chambers. In the more advanced cases, the tips of the dendrobranchiate lamellae on the gill branchiae were severely necrotic, melanized, or physically absent due
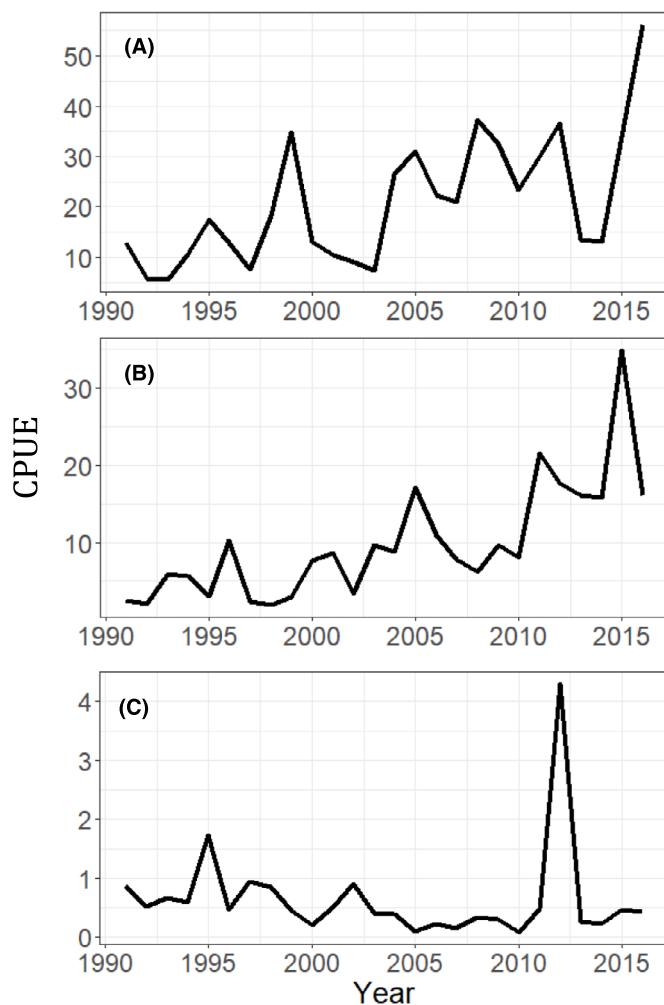
FIGURE 4. Indices of abundance (CPUE; number per hectare) for shrimp captured in the Southeast Area Monitoring and Assessment Program survey, 1991–2016: **(A)** white shrimp, **(B)** brown shrimp, and **(C)** pink shrimp. Note the differences in *y*-axis scales.

to the severity of the damage (Figures 6, 7). In the early or less-advanced cases of the disease, the lamellae retained their characteristic bifurcated, branching morphology but with small, focal lesions exhibiting melanization (Figure 8). In more severe cases, the tips of the lamellae and areas of branching appeared to be "burned off" by the extensive necrosis and melanization, and the condition had coalesced broadly along the damaged branchiae (Figure 6D). Microscopically, affected lamellae had melanized lesions with subjacent necrosis of connective tissues and, in some cases, intensive hemocyte infiltration (Figures 7, 8).

In advanced cases of the disease, few identifiable pathogens were present in the tissues. However, in less-advanced cases, ciliates and ciliate cysts were observed near or within the necrotic lesions. The encysted ciliates—presumptive tomites (the immature form that must infect a new host) or early trophonts (attached, feeding stage of the ciliate parasite)—were approximately 25–30 μm in length, multi-nucleate (macronuclei and micronuclei), and often nestled and presumably encysting within the bifurcations of the lamellae (Figure 8). Ciliates were present on the affected gill lamellae, encysted within a thick, basophilic cyst wall (Figure 8). Remnants of the cyst wall were occasionally observed, and when present they were adjacent to or within necrotic and melanized areas.

Black Gill PCR Prevalence and Ciliate Identity in White Shrimp

The PCR assay produced single-band amplification products using DNA derived from white shrimp displaying discolored gills. However, not all samples that were assessed as infected by gross visible observation were positive by PCR. The PCR assays were positive in 7 of 15 samples that were macroscopically infected. Discrepancies between PCR and visible diagnosis were primarily from visible diagnosis of light infections ($n = 6$), although two shrimp with moderate and heavy infections also contributed to this discrepancy. Alternatively, as the gill samples assessed by the PCR assay were from shrimp captured in November ($n = 11$) and December ($n = 4$) 2017, when the macroscopic prevalence of sBG had declined, the observed discrepancies may be the result of the pathogen's destruction by host defenses or the pathogen exiting the host as part of its life cycle (see Discussion). The DNA sequences obtained from pathogens on the gill tissues of six white shrimp were identical. Searches using BLAST revealed that the 154-bp fragment (after primer removal) was identical to the corresponding region within the nearly complete 18S ribosomal RNA gene sequence from the Georgia sBG ciliate (GenBank Accession Number KX906567), which was determined by Frischer et al. (2017).

DISCUSSION

Shrimp Abundance

To our knowledge, this is the first study to provide relative abundance estimates for the three species of penaeid shrimp in Chesapeake Bay. Prior to 1991, penaeid shrimp were occasionally encountered by the VIMS trawl survey, but such events were infrequent and occurrences were not recorded (Geer et al. 1993). The number of individuals captured during the early 1990s was low: 41 individuals in 1991 (930 tows), 66 individuals in 1992 (982 tows), and 42 individuals in 1993 (915 tows). In more recent years, the number of penaeid shrimp encountered by the trawl was orders of magnitude higher: 5,809 in 2016 (1,224 tows) and 2,363 in 2017 (1,224 tows). The increase in relative abundance of white shrimp in Chesapeake Bay during recent years (since

TABLE 2. Models considered for estimating the relative annual abundance of white, brown, and pink shrimp captured by bottom trawl in Chesapeake Bay (generalized linear model results). Explanatory variables include submerged aquatic vegetation (Bay.grass), freshwater flow (Flow), the Southeast Area Monitoring and Assessment Program index of abundance (SEAMAP), and average minimum bottom water temperature (Avg.min.-temp). The best models, as determined by Akaike's information criterion corrected for small sample size (AIC_c), are highlighted in bold (k = number of model parameters; % Dev exp = percentage of the total deviance explained; Δ_i = difference in AIC_c between the given model and the best model).

| Species	Model	k	% Dev exp	AIC_c	Δ_i
White shrimp	SEAMAP + Bay.grass + Avg.min.temp + Flow	6	54.23	102.82	6.61
	SEAMAP + Bay.grass + Avg.min.temp	5	54.20	99.33	3.12
	SEAMAP + Avg.min.temp	**4**	**54.14**	**96.21**	**0.00**
	SEAMAP + Bay.grass	4	49.34	98.97	2.76
	SEAMAP	3	48.99	96.30	0.09
Brown shrimp	SEAMAP + Bay.grass + Avg.min.temp + Flow	6	21.57	−34.83	8.95
	SEAMAP + Bay.grass + Avg.min.temp	5	21.47	−38.31	5.47
	SEAMAP + Avg.min.temp	4	21.28	−41.41	2.37
	SEAMAP + Bay.grass	4	19.86	−40.94	2.84
	SEAMAP	**3**	**19.80**	**−43.78**	**0.00**
Pink shrimp	SEAMAP + Bay.grass + Avg.min.temp + Flow	6	32.87	−82.61	3.09
	SEAMAP + Bay.grass + Avg.min.temp	**5**	**31.78**	**−85.70**	**0.00**
	SEAMAP + Avg.min.temp	4	20.96	−85.03	0.67
	SEAMAP + Bay.grass	4	17.17	−83.81	1.89
	SEAMAP	3	9.63	−84.40	1.30
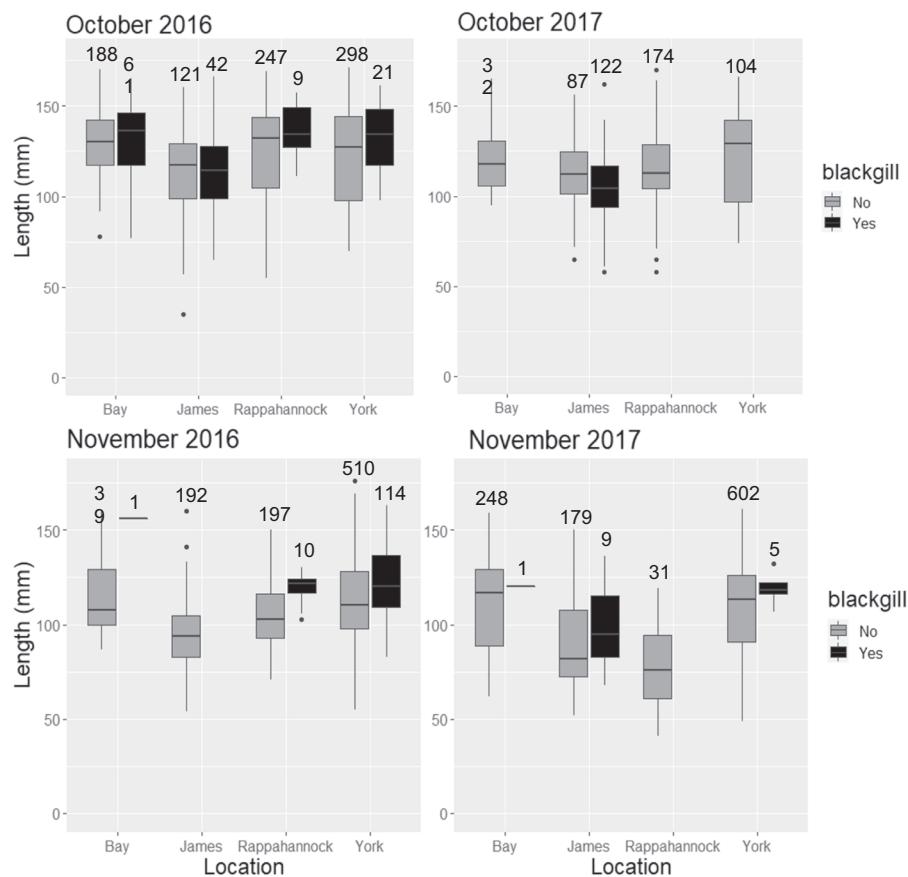
FIGURE 5. Box plots depicting TLs of white shrimp exhibiting macroscopic signs of black gill disease (black) and all other white shrimp (gray) captured in Chesapeake Bay (Bay) and the James, Rappahannock, and York rivers during October and November 2016–2017. The number above each box indicates sample size, horizontal lines show the median, top and bottom of the boxes are the 75th and 25th percentiles, upper and lower whiskers extend from the median to the highest and lowest values within 1.5 times the interquartile range, and outliers are plotted as points.
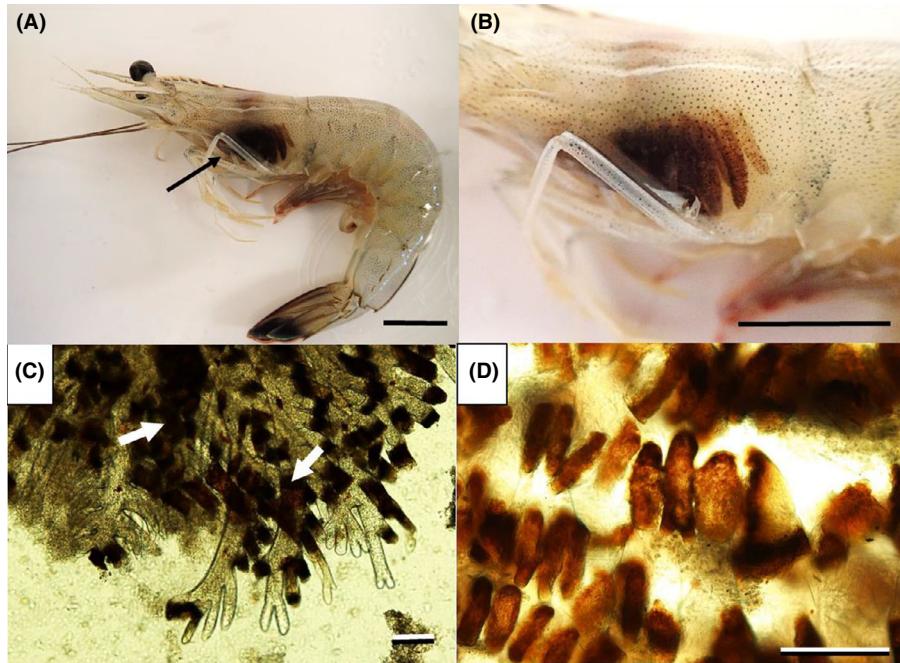
FIGURE 6. **(A)** White shrimp exhibiting a severe case of black gill disease (arrow; scale bar = 15 mm); **(B)** detail of the gill region in situ, showing that all branchiae are affected (scale bar = 15 mm); **(C)** gill branchia from the same animal, showing large accumulations of melanized foci (arrows) dispersed on the dendrobranchiate lamellae (scale bar = 200 μm); and **(D)** high magnification of a squash preparation, showing melanized lamellae along the branchia (scale bar = 100 μm). These characteristics are indicative of a post-heavy infection state because no obvious pathogens are present in the lesions.

2007) coincided with the observed increased in relative abundance of this species along the southeastern U.S. coast, suggesting favorable conditions for production of white shrimp at a regional scale. Unlike the patterns observed for white shrimp, brown shrimp annual indices from the VIMS trawl survey and the SEAMAP survey exhibited an inverse pattern (see Figures 3B, 4B). If brown shrimp in Chesapeake Bay originate from the coastal population sampled by SEAMAP, then the inverse relationship we observed suggests that local biotic or abiotic conditions in Chesapeake Bay and Virginia coastal waters play a role in regulating the recruitment and population size of brown shrimp in Chesapeake Bay. An additional consideration is that the SEAMAP index may not be coupled to the life cycle of shrimp in Chesapeake Bay. We would need a survey of adult shrimp outside of the bay to link to recruitment of subadults inside the bay, and we are unaware of any such data. Pink shrimp exhibited variable abundance in Chesapeake Bay at an order of magnitude lower than that of brown shrimp and two orders of magnitude lower than that of white shrimp. Low and variable annual abundance of pink shrimp suggests that recruitment into the bay is also low and highly variable.

The observed differences in patterns of abundance among the three species may result from variations in stock sizes, spawning season, postlarval survival, recruitment periods, migration success, habitat suitability, or a combination of one or more of these factors. For example, the low relative abundance of pink shrimp is consistent with the smaller size of the coastal stock (as determined by the SEAMAP survey) as well as the shorter spawning duration of pink shrimp. Spawning in this species occurs during a 3-month period, compared with the longer, 5-month spawning period of white and brown shrimp (Williams 1969). In addition, Chesapeake Bay is at the northern edge of the distribution of pink shrimp, which exhibit a center of abundance near northern Florida (Williams 1984). Given this distribution, pink shrimp abundance in Chesapeake Bay is expected to be lower than that of brown or white shrimp, which are found in high abundance along the Atlantic coast as far north as North Carolina and occur at lower levels of abundance further north (Williams 1984).

The relatively large stock sizes and longer spawning durations of white and brown shrimp may allow greater postlarval recruitment of these two species to Chesapeake Bay but does not explain the order-of-magnitude difference in abundance between subadult white shrimp and brown shrimp observed in Chesapeake Bay. The numbers of postlarval white shrimp that recruit to Chesapeake Bay
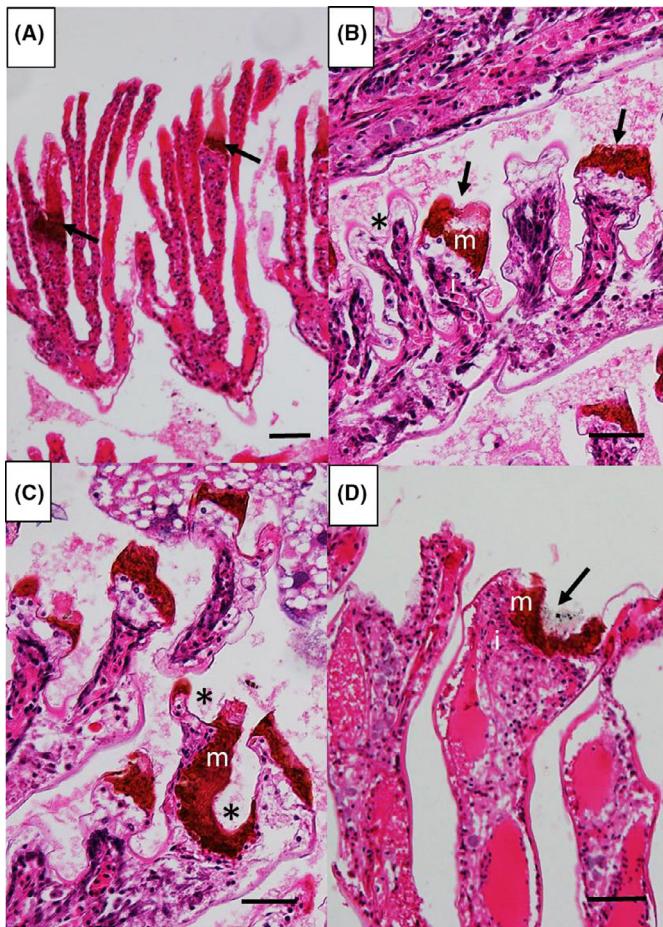
FIGURE 7. Histological sections of affected gills from white shrimp exhibiting a late stage of infection with black gill disease. **(A)** Gill branchiae with normal lamellae and lightly infected lamellae with little melanization (arrows) are shown (scale bar = 200 μm). **(B)** Gill lamellae showing the characteristic lesions associated with the disease are depicted (m = melanization; i = hemocyte infiltration; arrows = damage to the tips of the lamellae). Normal lamellae are bifurcated twice, near the base and near the tips (*). The bifurcation is frequently lost due to disease pathology and incipient melanization (scale bar = 100 μm). **(C)** Extensive melanization is visible at the base of a bifurcation (*), with focal areas of melanization (m) that are presumptive sites of infection (scale bar = 100 μm). **(D)** A cup-like lesion (arrow) with incipient melanization and hemocyte infiltration (i) is apparent (scale bar = 100 μm).

likely reflect the spawning activity of the shrimp population in the coastal ocean, including North Carolina waters. Here, we note the correlation between white shrimp relative abundance in Chesapeake Bay and relative abundance estimated by the SEAMAP survey, which suggests that the dynamics of the Chesapeake Bay population are coupled with those of the coastal white shrimp population. The order-of-magnitude difference in relative abundance of subadult white and brown shrimp in Chesapeake Bay may be explained by differences in the time of year during which shrimp recruit to estuarine habitats. White

shrimp enter coastal estuaries during spring, summer, and early fall, when water temperatures are elevated, whereas brown shrimp recruit to estuaries in late winter and spring (Williams 1955, 1969). Because of this difference in timing, brown shrimp postlarvae that recruit to Chesapeake Bay from January to March are more likely to encounter water temperatures below their critical threshold for growth (8°C; Etzold and Christmas 1977); thus, brown shrimp that enter Chesapeake Bay in winter may experience reduced growth and potentially lower survival during this period. Due to thermal differences between the times of recruitment and differences in thermal tolerances of the two species, we expect that the proportional survival of postlarval and juvenile white shrimp will be greater than that of brown shrimp. Brown shrimp may also experience unfavorable winds or currents during winter that limit their transport to Chesapeake Bay, resulting in a lower abundance of subadults compared with white shrimp. In addition, behavioral responses of brown shrimp to cold fronts may affect their recruitment to regional estuaries (Rogers et al. 1993).

Our attempt to understand variation in the relative abundance of shrimp in Chesapeake Bay indicates that the coastal abundance of adults and one or more of the habitat characteristics examined may be important for shrimp recruitment and survival in this estuary. Several plausible models were identified for each species; however, the percentage of the deviance explained by these models was somewhat low (maximum deviance explained = 54.1%), suggesting that the covariates we considered were insufficient to model annual variation in shrimp abundance or that low catches hampered the ability to identify models with greater explanatory power. Indeed, the least amount of deviance explained by our models was for brown and pink shrimp, indicating that increasing the sample sizes will be key to understanding the factors driving shrimp abundance in Chesapeake Bay. Furthermore, the VIMS trawl survey is a multispecies survey and is not optimized to sample a single species or group (such as penaeid shrimp); additional targeted sampling aimed at understanding the habitat needs of shrimp in Chesapeake Bay could be incorporated into the survey if the fishery in Virginia becomes established. For example, white shrimp can be found in a wider range of salinities than brown and pink shrimp (Doerr et al. 2016; Zink et al. 2017), and we observed this same pattern in Chesapeake Bay. Brown and pink shrimp prefer higher salinities; thus, their distribution in Chesapeake Bay may be restricted to the lower James River and the bay—and, in the case of brown shrimp, perhaps also in the lower York River. The stratified-random sampling design of the VIMS trawl survey resulted in fewer stations being located in areas where brown and pink shrimp are likely to be found, thus reducing the data available to detect important covariates
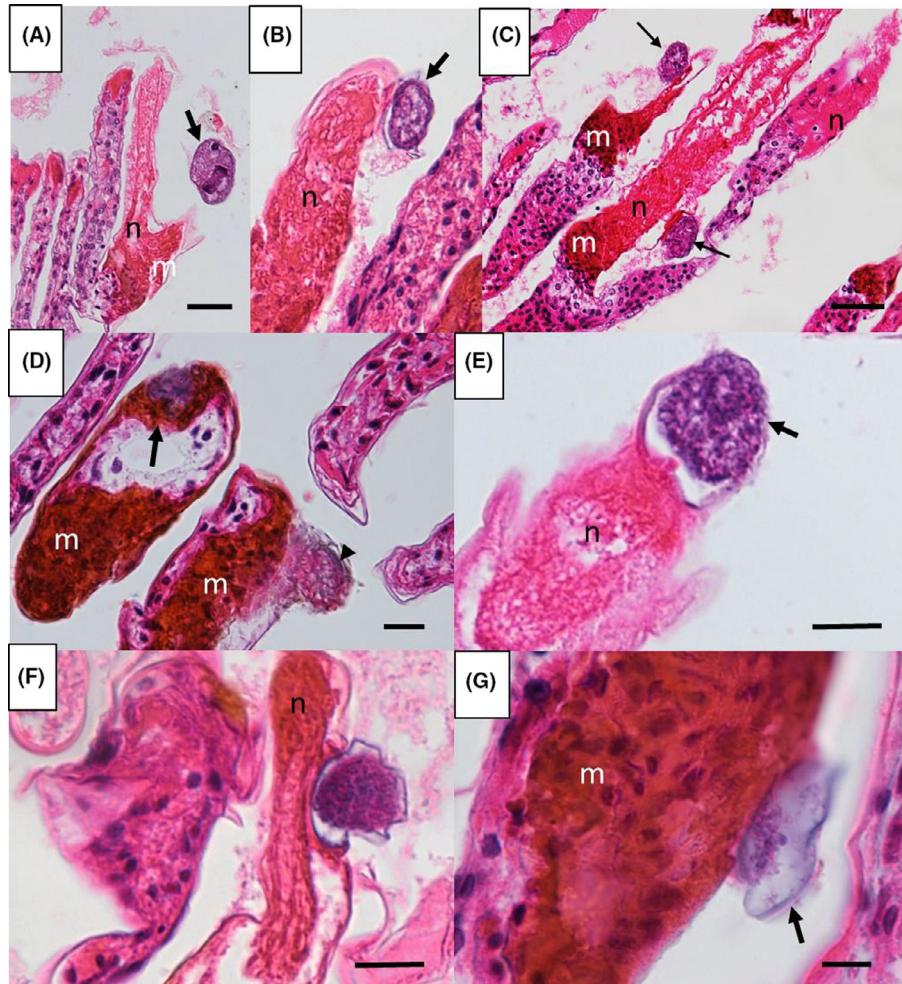
FIGURE 8. Presumptive pathogen in the branchial lamellae of white shrimp exhibiting black gill disease. **(A)** A large ciliate (presumptive agent) free in the branchial chamber (arrow) is depicted. It is adjacent to a necrotic (n) lamella (scale bar = 20 μm). **(B)** An encysted ciliate is shown. Note the necrotic host tissue (n) with loss of cellular organization adjacent to the cyst (scale bar = 20 μm). **(C)** Necrotic (n) and melanized (m) lamellae, with encysted ciliates (arrows) adjacent to affected tissues, are present (scale bar = 50 μm). **(D)** Encysted ciliates (arrow and arrowhead) adjacent to heavily melanized areas on the lamellae are visible. The lamellae are undergoing intense melanization, presumably in response to the tissue damage caused by the ciliate (scale bar = 20 μm). **(E)** Detail showing a tomont encysted on a lamella is presented. The cyst wall is basophilic, as is the encysted ciliate (scale bar = 10 μm). **(F)** The cyst wall of the ciliate is sitting in the "cup" of host material, external to host tissues (scale bar = 10 μm). **(G)** Empty cyst attached to a highly necrotic and melanized area of affected lamella is shown (scale bar = 10 μm).

affecting their abundance. Models developed for these species in their primary fishing regions use environmental covariates to explain and predict shrimp production (e.g., Leo et al. 2016; Fowler et al. 2018), suggesting that a higher intensity of sampling in Chesapeake Bay could be useful for understanding environmentally driven variation in shrimp abundance within this system. In addition, coastal ocean currents and across-shelf transport processes will need to be considered in models of the annual abundance of penaeid shrimp to better explain recruitment of these species to Chesapeake Bay (Wenner et al. 1998, 2005).

Availability and vulnerability of penaeid shrimp to the trawl gear affect catchability; as such, they are important factors that could affect our estimates of the relative abundance of shrimp in Chesapeake Bay. Pink and brown shrimp tend to bury in sediments during the day, whereas white shrimp bury to a lesser extent (Perez-Farfante 1969; Minello 2017). If those behavioral patterns occur in Chesapeake Bay, then our relative abundance estimates may be biased low because all trawling occurred during daylight hours, when the availability and vulnerability of shrimp to the gear were reduced. However, annual changes in vulnerability are likely to be consistent (assuming no change in the behavior of each species over time) and, thus, would not bias patterns in relative abundance from year to year. Additionally, the VIMS trawl survey does not sample shallow-water habitats (<2.7 m), so our

estimates are relevant only for deeper areas even though postlarval and juvenile shrimp occur in shallow-water habitats (Wenner and Beatty 1993). Furthermore, the size of shrimp ranged from approximately 35 mm TL to a peak length at capture of around 100 mm TL, indicating that gear selectivity, particularly of the smaller sizes, may also affect catchability. If the shrimp fishery becomes established in Virginia, additional surveys in shallow-water habitats that target small individuals could provide a more complete understanding of shrimp distributions and relative abundance.

Continued recruitment and growth of penaeid shrimp populations in Chesapeake Bay alter existing food webs and could affect macrobenthic community structure. For example, a database that examines the diets of Chesapeake Bay predators (VIMS Multispecies Research Group 2018) indicated that penaeid shrimp serve as prey for higher-trophic-level organisms, such as Summer Flounder *Paralichthys dentatus*, Smooth Dogfish *Mustelus canis*, Clearnose Skate *Raja eglanteria*, Weakfish *Cynoscion regalis*, Striped Bass *Morone saxatilis*, Atlantic Croaker *Micropogonias undulatus*, and Black Sea Bass *Centropristis striata*, thereby contributing to food web dynamics in Chesapeake Bay. These predators are abundant during summer and fall in Chesapeake Bay and its subestuaries during the same period in which penaeid shrimp are present at high numbers. Additionally, penaeid shrimp are predators themselves, with high growth rates (36–52 mm/month) in warm waters (Williams 1955). Their fast growth and feeding period coincide with the observed summer/fall minimum in abundance of soft-bottom benthic organisms (Virnstein 1977). The increased predation rate by penaeid shrimp during a period of low relative abundance of the benthic community may reduce the availability of benthic organisms to other species. For example, in caging experiments, white shrimp reduced the abundance and diversity of macrobenthic invertebrates by selectively feeding on high-density patches of soft-bodied prey (Beseres and Feller 2007a, 2007b). Although our density estimates of penaeid shrimp in Chesapeake Bay are low (maximum density observed = 1 individual/m^2) compared with densities used in the caging experiments (25 individuals/m^2; Beseres and Feller 2007a, 2007b), competitive interactions may yet be possible in Chesapeake Bay.

Shrimp Black Gill Disease Syndrome

White shrimp were the only species that exhibited visible signs of sBG in Chesapeake Bay and its subestuaries. Gross signs of the syndrome have not been observed in brown or pink shrimp from the region, but brown shrimp with sBG have been reported from South Carolina and Georgia (Frischer et al. 2017, 2018). Histology, PCR, and sequencing of the amplification products indicate that sBG in shrimp from Virginia have features identical to those of the sBG syndrome reported in shrimp from South Carolina and Georgia. In white and brown shrimp from Georgia and South Carolina, the presumptive agent of the disease is thought to be a newly discovered apostome ciliate, *Hyalophysa lynni* (Landers et al. 2020), which is closely related to a common commensal, *H. chattoni* (Frischer et al. 2017, 2018). Although this may be the case for white shrimp captured in Virginia, we cannot rule out other possible etiologies. For example, Frischer et al. (2017) found that the number of potential false negatives in the molecular diagnostics was quite high: 16.8% of the samples compared visually were found to be negative through PCR, and 19.9% of the histological comparisons failed to indicate the presence of the ciliate that was positively confirmed through PCR. Similarly, the proportion of potential false positives is greater than 12% for visual and histological assessments compared with PCR analyses. Furthermore, investigations with DNA probes using in situ hybridization have not been carried out to conclusively identify and localize the pathogen in the tissues of the shrimp host; however, this needs to be done for confirmation. In addition, the presumptive pathogen appears to be absent from histological samples of late-stage infections. The observed discrepancies between visible detection, histological preparations, and PCR may be the result of the pathogen's destruction by the host defensive response or by the pathogen exiting the host as part of its life cycle—a common occurrence for many marine ectoparasites on crustaceans (Shields et al. 2015). Moreover, another pathogenic ciliate, the histophagous apostome *Synophrya hypertrophica*, is known to infect decapods in high-salinity waters from the region (Johnson and Bradbury 1976) and elsewhere (Lee et al. 2019), but it does not occur at high prevalence levels in shrimp infected with sBG (Frischer et al. 2017). These discrepancies point to the need for more work on determining the etiology of this disease syndrome.

The presumptive agent of sBG in Georgia and South Carolina, *H. lynni*, is found in shrimp from high-salinity waters (Fowler et al. 2018). It is likely that shrimp acquire the infection as postlarvae or juveniles in higher-salinity habitats of the coastal ocean (Frischer et al. 2018). *Hyalophysa chattoni* and *S. hypertrophica* are usually found in hosts living in high-salinity waters (Bradbury 1966; Johnson and Bradbury 1976; Pisani et al. 2008), but these parasites likely survive much lower salinities while in their hosts because they can survive in their cysts (i.e., *Hyalophysa*) or because they are internal parasites (i.e., *Synophrya*) and their hosts are typically osmoregulators in less-saline waters.

Shrimp black gill disease is a seasonal syndrome in shrimp populations within Chesapeake Bay. Macroscopic signs of sBG appear in mid-autumn (October) and decline

through early winter. In more southerly latitudes, sBG appears earlier—as early as July—and prevalence peaks in early autumn (September and October), followed by a decline through November and December (Frischer et al. 2017; Fowler et al. 2018). The onset of infection in shrimp from Chesapeake Bay may be delayed due to the migration of white shrimp from coastal waters into the bay. Accordingly, the VIMS trawl survey may not capture infected postlarval and early juvenile shrimp or detect sBG during summer months due to gear selectivity or shrimp inhabiting regions of the bay that are not sampled. There is also the possibility of a lag in the timing of infection and the timing of macroscopic detection (melanization) of the syndrome; therefore, visible signs of sBG may not be evident in summer and early fall even though the ciliate causing the infection may be present in shrimp at that time (Frischer et al 2017; Fowler et al 2018).

Seasonality, high summer temperatures, high winter temperatures, salinity, and low dissolved oxygen are apparent mediators of sBG, and suitable environmental conditions likely occur in late summer to increase prevalence levels in penaeid shrimp (Fowler et al. 2018). Warming trends in Chesapeake Bay likely explain the immigration of shrimp into the area, and the high salinities and warming conditions found in the lower main stem of the bay likely facilitate the transmission of the pathogen.

Although prevalence of sBG varies seasonally, levels up to 18.6% in white shrimp from Chesapeake Bay were lower than those reported for shrimp from Georgia and South Carolina. In Georgia, up to 45% of shrimp were infected in 2014–2015 (Frischer et al. 2017); in South Carolina, prevalence levels greater than 40% were observed for 7 of 13 years (Fowler et al. 2018). The prevalence levels of sBG and the abundance of shrimp with sBG in Chesapeake Bay have been determined exclusively from macroscopic observation of sBG symptoms. Therefore, our prevalence levels are likely underestimates because the initial stages of ciliate infection do not elicit pathognomonic signs of disease (Frischer et al. 2017). Regardless, these levels indicate a high transmission rate in the region, even in the shrimp population expanding into Chesapeake Bay.

Conclusion

With the increase in shrimp abundance, the development of a penaeid shrimp fishery in Virginia coastal waters is underway. Resource managers will need to balance the potential losses of finfish species due to bycatch mortality against the benefit of a shrimp fishery. Any impacts of sBG on shrimp survival or marketability will have to be considered in fishery management plans to ensure the sustainability of the fishery. The persistence of white shrimp in Chesapeake Bay during the past decade and the documented consumption of shrimp by bay predators indicate that white shrimp have become a component of the food web. Ecosystem models that describe trophic interactions (e.g., Christensen et al. 2009) should include this new prey species to properly reflect the bay's changing food web.

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