

1 Black gill increases the susceptibility of white shrimp, *Penaeus setiferus*

2 (Linnaeus, 1767), to common estuarine predators

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24 **Abstract**

25

26 Parasites can kill hosts directly, but also indirectly, by enhancing susceptibility to environmental  
27 factors and biotic interactions. In the United States South Atlantic Bight region of the northwest  
28 Atlantic Ocean, white shrimp (*Penaeus setiferus*) support a substantial commercial fishery and  
29 are also valuable prey for many marine and estuarine species. Since the late 1990s, a condition  
30 known as black gill has been observed in penaeid shrimp in the South Atlantic Bight. In this  
31 region, black gill is typically caused by an apostome ciliate that elicits an innate immune  
32 response in shrimp, manifested through the melanization of gill tissues, which impedes  
33 respiratory functions and hemolymph ion regulation. The objective of this study was to  
34 determine if black gill subjects shrimp to higher rates of predation by red drum (*Sciaenops*  
35 *ocellatus*), spotted seatrout (*Cynoscion nebulosus*), and blue crab (*Callinectes sapidus*). A series  
36 of simultaneous prey choice mesocosm experiments was conducted, during which single-species  
37 predators were able to consume shrimp that were both symptomatic and asymptomatic of black  
38 gill over a four-hour period. Predator species were 1.4 to 3.0 times more likely to consume  
39 symptomatic shrimp than asymptomatic shrimp. The hinderance of shrimp physiology and  
40 escape responses due to gill melanization likely increases the vulnerability of shrimp to  
41 predation. This study emphasizes that mortality from parasitic infections is not always direct and  
42 that black gill may have a significant impact on penaeid shrimp through secondary, or indirect,  
43 mortality.

44

45 Keywords: predator-prey interaction, parasitism, secondary mortality, parasite-host dynamic,  
46 shrimp black gill, *Penaeus (Litopenaeus) setiferus*

47

48 **1. Introduction**

49 Parasites can directly affect host fitness by altering growth rates (Fernandez and Esch, 1991),  
50 hindering reproductive development (Astete-Espinoza and Caceres, 2000), or transmitting  
51 diseases (Dobson and Hudson, 1986). Parasites can also impact their hosts indirectly by causing  
52 behavioral modifications in prey species that increase their susceptibility to predation (Lafferty  
53 and Morris, 1996; Kunz and Pung, 2004; Brinton and Curran, 2015). For example, the cestode  
54 *Polypocephalus* sp. increases shrimp activity upon infection and thus susceptibility to predation  
55 by the ultimate host of the cestode, likely a skate or ray (Carreon and Faulkes, 2014). Other  
56 parasites affect the physiological functions of their hosts, which can also ultimately lead to  
57 increased host vulnerability (Hudson et al., 1992). Because indirect effects of parasites and  
58 disease must be considered in a multispecies, community context, they can be more difficult to  
59 discern than direct effects (Wood et al., 2007; Dunn et al., 2012). These types of interactions are  
60 known to have negative effects on commercially important species (Shirakashi et al., 2008).

61 White shrimp (*Penaeus setiferus*; Linnaeus, 1767) are both economically and ecologically  
62 important in estuarine and coastal environments of the U.S. South Atlantic Bight region of the  
63 northwest Atlantic Ocean. In addition to supporting an extensive commercial fishery in the  
64 southeastern U.S. (Gillet, 2008; NMFS, 2017), white shrimp, in their migrations between salt  
65 marsh and offshore habitats, contribute substantially to the diets of many vertebrate and  
66 invertebrate species (Overstreet and Heard, 1978; Hettler, 1989; Scharf and Schlight, 2000). In  
67 South Carolina and Georgia, United States (U.S.), white shrimp landings have decreased in  
68 recent years (NMFS, 2017), and the decline may be attributed partially to a parasitic infection  
69 directly affecting shrimp gills (Gambill et al., 2015; Frischer et al., 2017, 2018).

70 Over the past few decades, a condition known as black gill has been observed at high  
71 prevalence levels in white shrimp in the South Atlantic Bight (Gambill et al., 2015; Fowler et al.,  
72 2018), peaking in the fall with an average peak prevalence of 48% (SCDNR, unpubl.). This  
73 condition can manifest as the melanization of gill tissues as an innate immune response to gill  
74 irritants elicited across the subphylum Crustacea (Burnett and Burnett, 2015) due to a variety of  
75 pathogens, parasites, contaminants, and nutrient deficiencies (Lightner, 1985; Cerenius et al.,  
76 2010; Frischer et al., 2017). In South Carolina and Georgia, black gill is specifically linked to an  
77 immune response caused by an apostome ciliate (Frischer et al., 2017). While black gill has been  
78 hypothesized to have contributed to the decline of the shrimp fishery in Georgia (Frischer et al.,  
79 2018), the effects of black gill on shrimp populations have yet to be mechanistically determined.

80 Impaired physiological functions in shrimp that are symptomatic of black gill suggest that  
81 these shrimp may experience increased rates of mortality due to predation (Frischer et al., 2018).  
82 Melanized nodules, which can be associated with black gill, can form on the gills of shrimp and  
83 obstruct respiratory function and ion regulation (White et al., 1985; Martin et al., 2000; Burnett  
84 and Burnett, 2015). Limited respiratory function can increase direct mortality of shrimp,  
85 particularly under hypoxic or anoxic conditions and at higher temperatures (Lightner et al., 1975;  
86 Delves-Broughton and Poupard, 1976). The impairment of respiratory functions due to nodules  
87 has been previously reported in Pacific white shrimp (*Penaeus vannamei*; Boone, 1931;  
88 Scholnick et al., 2006), and blue crabs (*Callinectes sapidus*; Rathbun, 1896; Burnett et al., 2006;  
89 Thibodeaux et al., 2009). Burnett and Burnett (2015) also demonstrated that nodules on the gills  
90 can negatively affect cardiovascular activity and metabolism in several crustacean species. These  
91 physiological responses can potentially lead to increased vulnerability to predation.

92 In the wild, shrimp are important prey for many predator species in estuarine environments  
93 (Pearson, 1929; Bass and Avault Jr., 1975; Overstreet and Heard, 1978; Laughlin, 1982). The  
94 objective of this study was to test the hypothesis that shrimp that are symptomatic of black gill  
95 are more susceptible than asymptomatic shrimp to predation by three abundant predator species  
96 in estuarine environments of the southern U.S. The selected predators, each representing  
97 different feeding modes, included two recreationally valued finfish species, red drum (*Sciaenops*  
98 *ocellatus*; Linnaeus, 1766), and spotted seatrout (*Cynoscion nebulosus*; Cuvier, 1830), as well as  
99 the commercially and recreationally important blue crab. While red drum and blue crabs are both  
100 benthic feeders, red drum typically hunt in groups and blue crabs tend to be more solitary.  
101 Spotted seatrout, also schooling fish, generally forage in the pelagic zone (Llansó et al., 1998).  
102 Each of these species, while using various feeding methods, consume penaeid shrimp (Pearson,  
103 1929; Bass and Avault Jr., 1975; Alexander, 1986; Fujiwara et al., 2016). Black gill may induce  
104 high secondary mortality, potentially leading to broad ecological and fishery implications, even  
105 if the parasite exerts low direct mortality on its host. By determining if black gill increases the  
106 susceptibility of shrimp to predation, this study expands knowledge of black gill effects in a  
107 community context, which is an important step toward understanding larger scale impacts of  
108 black gill on shrimp populations.

109

## 110 **2. Materials and methods**

### 111 *2.1. Experimental mesocosm system*

112 To quantify whether predators differentially consumed shrimp with and without black gill, a  
113 simultaneous prey choice mesocosm experiment was conducted, utilizing four outdoor  
114 mesocosms (3.7 m diameter, 0.6 m water depth) at the South Carolina Department of Natural

115 Resources (SCDNR) Marine Resources Research Institute in Charleston, South Carolina. The  
116 mesocosms were supplied with raw, flow-through water from Charleston Harbor and were  
117 continuously aerated. All standpipes were surrounded by taller mesh-covered pipes to prevent  
118 predator or prey escape. Every three to four days, excess waste material and sediment were  
119 siphoned from the mesocosms. Temperature in each mesocosm was monitored throughout the  
120 experiment using HOBO Pendant Data Loggers (model: UA-001-08, Bourne, Massachusetts).  
121 Dissolved oxygen, salinity, and temperature were measured once in each mesocosm during all  
122 trials using a YSI Pro 2030 water quality meter.

123

## 124 2.2. *Collection and holding of experimental animals*

125 All trials were conducted during August, September, or October 2017 and 2018, periods  
126 when black gill is generally most prevalent in the natural environment (Fowler et al., 2018).  
127 White shrimp were collected from the Ashley River and Charleston Harbor throughout the study  
128 by towing a 4.6- or 6.1-m otter trawl for five to 10 minutes. Shrimp ranged from 80-100 mm  
129 total length (TL), a size consumed by all three predator species used in this study. Prior to each  
130 trial, all shrimp were acclimated for one to two days in a holding tank (1.8 m diameter) with flow  
131 through water and aeration as described for experimental mesocosms above. All predator species  
132 were also collected in and around Charleston Harbor (Table 1). Predator size was selected to  
133 reflect the size class most likely to encounter and feed on shrimp during the fall (Anweiler,  
134 personal communication, February 26, 2018). Finfish predators were collected by hook-and-line  
135 and during trammel net surveys conducted by the SCDNR Inshore Fisheries Section. All blue  
136 crabs were collected using crab pots baited with Atlantic menhaden (*Brevoortia tyrannus*;  
137 Latrobe, 1802). Upon collection, all finfish were placed into oxygenated containers immediately

138 and remained there for transport. Blue crabs were transported in crab baskets under damp burlap,  
139 which effectively kept them calm and prevented them from desiccating.

140 For each species, predators were stocked into mesocosms based on the total length of each  
141 individual to ensure that each mesocosm contained a relatively equal biomass of predators.  
142 Predators were then allowed time to acclimate to holding conditions. Acclimation time was  
143 determined based on the robustness of the predator (e.g., spotted seatrout required more time to  
144 settle and begin feeding than red drum or blue crab). Prior to beginning the trials, red drum were  
145 acclimated for four days, spotted seatrout were acclimated for 34 days, and blue crabs were  
146 acclimated for two days.

147

### 148 2.3. *Experimental design*

149 Each predator species was tested separately. For the purposes of this study, individual  
150 mesocosm tanks were considered replicates of each predator species evaluated. Trials, defined as  
151 a set of two to four concurrent replicates, were conducted on three separate dates for each of the  
152 predator species, resulting in a total of nine replicates for red drum, 10 replicates for spotted  
153 seatrout, and 12 replicates for blue crabs. During periods of acclimation and time between trials,  
154 predators were fed mummichogs (*Fundulus heteroclitus*; Linnaeus, 1766) or white shrimp with  
155 clipped pleopods to ensure that these food items were not mistaken for experimental shrimp.  
156 Prior to each trial, predators were starved for approximately three days. The number of predators  
157 introduced to each mesocosm in all but one case was  $>1$  to allow for possible group hunting  
158 techniques, but low ( $<10$ ), to minimize interference among them (Griffen and Byers, 2006; Table  
159 A1).

160 The same predator individuals were used throughout the experiment, apart from replacing a  
161 small number of deceased animals between trials (five spotted seatrout and five blue crabs). No  
162 predator mortalities occurred during the trials. Each mesocosm with red drum contained three  
163 predators. Of the 10 mesocosms with spotted seatrout, six mesocosms contained three predators  
164 each. The other four mesocosms contained a lower number of predators (n=1-2) due to predator  
165 mortalities between successive trials. Over the course of the blue crab trials, the number of crabs  
166 per mesocosm was reduced from nine (n=4 replicates) to eight (n=4 replicates) to five (n=4  
167 replicates) to minimize the confounding effects of intraspecific agonistic behaviors among crabs  
168 on shrimp consumption (Table A1). Only blue crabs with both claws and all legs intact were  
169 introduced into mesocosms. There was also some variation in predator number due to crab  
170 mortalities that occurred between trials in four mesocosms.

171 Before beginning each trial, white shrimp were removed from the holding tank and placed in  
172 mesh baskets inside each mesocosm at the surface. Each basket was stocked with 32 shrimp, 16  
173 each of symptomatic (highly melanized gills) and asymptomatic (showing no visual signs of gill  
174 melanization) shrimp, which were categorized under natural light conditions by the naked eye.  
175 This number of shrimp provided predators with sufficient opportunities to encounter prey  
176 without any confounding effects of overcrowding. Shrimp were acclimated for 30 minutes in the  
177 baskets, after which any dead, moribund, or lethargic shrimp were replaced. Baskets were then  
178 slowly lowered into the tanks, allowing shrimp to swim into the water column simultaneously,  
179 and the trial commenced. After four hours, all mesocosms were drained, predators were removed  
180 and placed into aerated holding bins, and all remaining shrimp were collected and categorized as  
181 either symptomatic or asymptomatic of black gill. Missing shrimp were assumed to have been



182 consumed by the predators. Shrimp molts and unconsumed shrimp carcasses were counted and  
183 recorded.

184

#### 185 *2.4. Stomach content analysis*

186 To verify the assumption that shrimp that were not recaptured at the end of the four-hour  
187 trials had been consumed by the predators in the mesocosms, stomach content analyses were  
188 conducted after one of the experimental trials. Following the end of the last spotted seatrout trial,  
189 each fish was sacrificed, and stomach contents were removed and immediately frozen. Upon  
190 thawing days later, stomachs were opened, and shrimp were removed. The shrimp were  
191 undigested enough to easily determine the number of shrimp in each stomach and to characterize  
192 each shrimp as symptomatic or asymptomatic of black gill. The chronological order of  
193 symptomatic and asymptomatic shrimp consumed was not able to be discerned, because the  
194 orientation of the stomach front to back was not retained on the datasheet during the dissection.

195

#### 196 *2.5. Statistical analyses*

197 To standardize data and to account for differences in the numbers of live predators between  
198 replicates, predation rates were calculated as the number of white shrimp consumed per predator  
199 per hour. Predation rates were compared across predator species to assess variability in rates  
200 using non-parametric Kruskal-Wallis tests where Shapiro-Wilk tests indicated non-normal  
201 distribution of data. To test for differences in the probabilities and odds of each of the three  
202 predator species consuming symptomatic shrimp and asymptomatic shrimp, generalized linear  
203 mixed effects models of shrimp mortality with binomial distributions, logit link functions, and  
204 associated likelihood ratio tests were used. Individual shrimp within each replicate were

205 designated as symptomatic or asymptomatic of black gill (predictor variable) and as consumed or  
206 unconsumed (response variable). To account for the experimental design, replicate was nested  
207 within trial and used as a random effect in each of the models. Probabilities represent shrimp  
208 consumed from each group (i.e., symptomatic and asymptomatic) compared to all shrimp  
209 available within each group. The relative risk of symptomatic shrimp being eaten was calculated  
210 as the ratio of symptomatic to asymptomatic probabilities. Odds represent the number of shrimp  
211 consumed compared to the number of shrimp left unconsumed within each group (i.e.,  
212 symptomatic and asymptomatic). The odds ratio is the odds of symptomatic shrimp being  
213 consumed compared to asymptomatic shrimp for each predator species and can be interpreted as  
214 an effect size. These analyses were conducted in the 'lme4' package in R version 3.5.1 (Bates et  
215 al., 2015; R Core Team, 2018).

216

### 217 **3. Results**

#### 218 *3.1. Water quality*

219 The temperature records collected continuously throughout the study by the HOBO Pendant  
220 Data Loggers did not differ substantially between mesocosms. Additional water quality  
221 measurements (i.e., temperature, salinity, and dissolved oxygen) taken with the YSI Pro 2030  
222 during each trial changed slightly over time throughout the study but did not differ considerably  
223 between mesocosms and remained well within tolerance ranges for predator and prey species  
224 (Table A1). Temperature ranged from 26.0°C to 29.9°C (mean=27.8°C). Salinity ranged from  
225 21.8 to 29.0 (mean=25.9). Dissolved oxygen ranged from 4.9 mg/L to 5.9 mg/L (mean=5.5  
226 mg/L). Calculated percent oxygen saturation (which incorporated salinity, temperature, dissolved  
227 oxygen, and barometric pressure) ranged from 73.3% to 87.78% (mean=79.9%).

228

229 *3.2. Predation rates*

230 All three predator species used in this study were more likely to consume symptomatic  
231 shrimp than asymptomatic shrimp. Throughout the study, red drum consumed 40.6% of shrimp  
232 available (n=288), spotted seatrout consumed 36.6% of shrimp available (n=320), and blue crabs  
233 consumed 11.2% of shrimp available (n=384). Red drum consumed a greater number of  
234 symptomatic shrimp than asymptomatic shrimp in eight of the nine replicates. In total, red drum  
235 consumed 71 symptomatic shrimp and 46 asymptomatic shrimp. Spotted seatrout consumed  
236 greater numbers of symptomatic shrimp than asymptomatic shrimp in five of 10 replicates,  
237 ultimately consuming a total of 68 symptomatic shrimp and 49 asymptomatic shrimp. Blue crabs  
238 consumed more symptomatic shrimp than asymptomatic shrimp in nine of 12 replicates,  
239 consuming a total of 32 symptomatic shrimp and 11 asymptomatic shrimp. Throughout the  
240 experiment, there were only two instances when predator species consumed more asymptomatic  
241 shrimp than symptomatic shrimp in the mesocosms. In one mesocosm, spotted sea trout  
242 consumed seven symptomatic shrimp and eight asymptomatic shrimp, and in another mesocosm,  
243 blue crab consumed zero symptomatic shrimp and one asymptomatic shrimp.

244 The predation rate on shrimp by blue crabs was significantly lower ( $0.13 \text{ shrimp predator}^{-1}$   
245  $\text{hr}^{-1}$ ) than red drum ( $1.03 \text{ shrimp predator}^{-1} \text{ hr}^{-1}$ ) and spotted seatrout ( $1.2 \text{ shrimp predator}^{-1} \text{ hr}^{-1}$ ;  
246  $p$ -values  $<0.001$ ). Mean predation rates were greater for symptomatic shrimp than asymptomatic  
247 shrimp (Table 2). Across all predator species, symptomatic shrimp were more likely to be  
248 consumed than asymptomatic shrimp (Figure 1) with the minimum odds ratio for each species  
249 consistently  $>1$  (Table 3). Based on these data, relative risks suggested that symptomatic shrimp  
250 were 1.5 and 1.4 times more likely to be consumed over a shrimp asymptomatic of black gill by

251 red drum and spotted seatrout, respectively. In the blue crab trials, symptomatic shrimp were 3.0  
252 times more likely to be consumed over an asymptomatic shrimp. Across all predator species,  
253 symptomatic shrimp were an average of 2.0 times more likely to be consumed than  
254 asymptomatic shrimp. Of the total number of shrimp consumed (n=277) by all predators  
255 throughout the study, 61.7% were symptomatic of black gill and 38.3% showed no visible  
256 symptoms of black gill.

257

### 258 3.3. *Stomach content analysis*

259 All shrimp that had been released into the mesocosms during the last of the spotted seatrout  
260 trials were either recovered unconsumed at the end of the trial or found in the stomachs of the  
261 eight spotted seatrout. Additionally, the total number of symptomatic and asymptomatic shrimp  
262 found in the stomachs matched the number of shrimp that were expected to have been consumed  
263 based on the recoveries of live shrimp. Together, these results support the assumptions that 1) all  
264 shrimp that were not recovered at the end of the experiment were consumed by predators in the  
265 mesocosms, and 2) shrimp classified as symptomatic before the experiment were also classified  
266 as symptomatic at the end of the experiment.

267

## 268 4. Discussion

269 The effects of parasites on host behavior and endurance are important to consider when  
270 examining predator-prey interactions. Parasites can alter or slow the locomotion of their hosts,  
271 which can lead to decreased feeding behaviors and movement (Belgrad and Griffen, 2015) and  
272 increased susceptibility to predation (Hoogenboom and Dijkstra, 1987; Poulin, 2010; Gehman  
273 and Byers, 2017). It can be advantageous for parasites to increase host susceptibility to predation

274 by the parasite's definitive host, but there is also evidence of non-trophically linked parasites  
275 changing the behavior of their hosts (Poulin, 2010). For example, Shirakashi et al. (2008) found  
276 that the invasive monogenean *Neoheterobothrium hirame* (Ogawa, 1999), a parasite of olive  
277 flounder (*Paralichthys olivaceus*; Temminck and Schlegel, 1846), caused no direct mortality to  
278 the host, but rather altered flounder behavior by increasing activity level, hindering burrowing  
279 success, and decreasing swimming endurance that ultimately led to increased host susceptibility  
280 to predation. The decline of flounder populations in Japan has been partially attributed to the  
281 predation-enhancing effects of this monogenean (Shirakashi et al., 2008). Some predators may  
282 be indifferent to infections carried by prey (Hulscher, 1973), especially if the predator feeds  
283 indiscriminately or if the prey is already easily caught or exhibits minimal reaction to the  
284 parasite. This study suggests that in a community context, a non-trophically linked parasite (i.e.,  
285 black gill) might increase prey (i.e., shrimp) mortality by increasing susceptibility to predation.

286 In this study, white shrimp symptomatic of black gill were 1.4 to 3.0 times more likely to be  
287 consumed than asymptomatic shrimp by three common and abundant predator species within  
288 estuarine and coastal marine habitats in the southeastern U.S. The mechanism for the increased  
289 susceptibility to predation of symptomatic shrimp may be due to the physiological limitations  
290 associated with the immune response during infection, including restricted respiration and  
291 cardiovascular functions, as documented in other crustaceans symptomatic of black gill (Burnett  
292 and Burnett, 2015). Frischer et al. (2018) demonstrated reduced escape responses and physical  
293 endurance in white shrimp with melanized gills. Additionally, these researchers found that  
294 asymptomatic shrimp spent more time moving forward on a treadmill apparatus, while  
295 symptomatic shrimp displayed exhaustion behaviors more quickly and more frequently. Burnett  
296 et al. (2006) found similar results in blue crabs with damaged gills. Aggregated hemocyte

297 nodules on blue crab gills led to reduced capacity to perform actions demanding a higher oxygen  
298 supply, including predator evasion (Burnett et al., 2006). Similarly, Thibodeaux et al. (2009)  
299 reported that blue crabs with impaired gill function consumed oxygen at a lower rate during and  
300 after periods of increased activity. Therefore, it is possible that behavioral differences  
301 attributable to lower stamina between symptomatic and asymptomatic shrimp contribute to  
302 differential mortality. Although mortality rates of shrimp were not evaluated in control tanks  
303 (i.e., those lacking predators) during this study, consumption rates of symptomatic and  
304 asymptomatic shrimp should sufficiently reflect comparative susceptibility of predation, rather  
305 than any differential mortality of symptomatic shrimp following handling, due to the short  
306 duration of the trials, the stable survivorship of shrimp in holding tanks before initiating the  
307 trials, and equal handling of symptomatic and asymptomatic shrimp.

308 Poor water quality, specifically hypoxic conditions, may exacerbate stressors on shrimp  
309 symptomatic of black gill. Under natural conditions in southeastern U.S. estuaries, oxygen levels  
310 can be reduced, particularly in warmer months at the benthic boundary layer, producing  
311 conditions that can introduce oxygen-related stress in biota (Lenihan et al., 2001; Wenner et al.,  
312 2004), possibly accentuating the propensity for black gill to compromise anti-predator responses  
313 in infected shrimp hosts. Even in healthy shrimp, hypoxia may cause energy constraints or  
314 restrictions on growth and activity, as well as lead to changes in behavior or a decreased  
315 tolerance to other stressors (Burnett and Stickle, 2001). Fowler et al. (2018), in an analysis of  
316 long-term white shrimp fisheries independent data collected by the SCDNR, suggested that high  
317 salinities and low dissolved oxygen concentrations over at least a month act synergistically to  
318 lower the resistance of shrimp to black gill. The presence of melanized hemocytic nodules in gill  
319 tissues associated with increased immune responses have reduced respiratory function in other

320 decapods (Burnett et al., 2006), so low-oxygen conditions could be especially challenging for  
321 symptomatic shrimp. Oxygen-related stress was likely minimized during this study, during  
322 which dissolved oxygen remained relatively high (~5 mg/L) and above levels that would  
323 generally induce oxygen-related stress in shrimp (Renaud, 1986).

324 Predation on parasitized prey can vary by predator species, which may be partially driven by  
325 the different feeding strategies used by various predators. In this study, although all predators  
326 used different methods of feeding, they all exhibited predation bias toward shrimp with black  
327 gill. Both red drum and spotted seatrout are schooling fish (Wilson and Nieland, 1994;  
328 Handegard et al., 2012). When schooling fish encounter prey, feeding activity can signal to other  
329 fish that prey are in the area (Keenleyside, 1955). Additionally, schooling fish often strike  
330 together, placing more pressure on prey and increasing the likelihood for prey consumption  
331 (Handegard et al., 2012). Handegard et al. (2012) found that spotted seatrout were highly  
332 coordinated while hunting. In contrast, blue crabs in this study appeared to be more territorial  
333 and aggressive towards other crabs, and when in proximity to one another, seemed more focused  
334 on other crabs rather than on shrimp prey (Gooding, personal observation). Additionally, in the  
335 natural environment, blue crabs typically target slow or sessile prey, such as gastropods and  
336 bivalves (Laughlin, 1982; Alexander, 1986; Hines et al., 1990). The finding that all three  
337 predator species, which utilize a variety of feeding methods, preferred symptomatic shrimp over  
338 asymptomatic shrimp suggests that shrimp with black gill are more vulnerable regardless of  
339 predator species.

340 The predators used in this study typically consume prey species that are the most abundant in  
341 their environment (Llansó et al., 1998; Rosas et al., 1994), including shrimp during the summer  
342 and fall (Laughlin, 1982; Overstreet and Heard, 1982; Scharf and Schlicht, 2000). In the South

343 Atlantic Bight, black gill prevalence generally peaks during the fall (Gambill et al., 2015; Fowler  
344 et al., 2018), and high black gill prevalence could lead these and other predators to consume  
345 more shrimp than would be consumed in the absence of black gill. Such a subsidy to predators  
346 could boost their population sizes over time, while also influencing food web structure, possibly  
347 leading to further increased pressure on shrimp and other prey populations (Noonburg and Byers,  
348 2005).

349 If black gill leads to an overall increase in shrimp consumption by predators, this increased  
350 top-down pressure on shrimp populations may be contributing to reduced landings of shrimp in  
351 the commercial trawl industry in the South Atlantic Bight region. A number of other factors may  
352 also be contributing to the decline in shrimp landings over the past few decades, including  
353 reductions in fishing effort, both in terms of the number of vessels shrimping and the number of  
354 hours fished (SEDAR, 2014), and climate (Fowler et al., 2018; Lopes et al., 2018; Oxenford and  
355 Monnereau, 2018; SCDNR, unpubl.). This study suggests secondary mortality resulting from  
356 black gill as another possible factor.

357

#### 358 *4.1. Conclusion*

359 This study demonstrates that shrimp symptomatic of black gill are more susceptible to  
360 predation by three different common predator species. Secondary mortality resulting from  
361 physiological limitations may exacerbate the effect of black gill on shrimp populations,  
362 potentially contributing to the observed regional decline in landings. The effects of a parasite on  
363 a host may not fully manifest until the host is placed in a community context that includes the  
364 complexity of other biotic and abiotic interactions. To fully understand the impact of black gill or



365 any other infections that may influence additional predator-prey interactions, it is critical to  
366 consider both the direct and indirect effects resulting from these species interactions.

367

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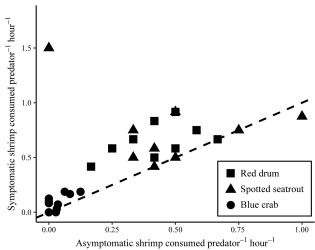
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534

### 535 **Figure captions**

536 Figure 1. Comparison of predation rates (calculated as number of shrimp consumed per predator  
537 per hour) of symptomatic and asymptomatic shrimp for red drum (*Sciaenops ocellatus*), spotted  
538 seatrout (*Cynoscion nebulosus*), and blue crab (*Callinectes sapidus*). Each point represents the  
539 predation rate calculated for each predator species within a single mesocosm over the four-hour  
540 trial period. The dashed line indicates equal consumption of both shrimp conditions.





**Table 1.**

Collection dates, collection locations, experimental dates, number of replicates, and size ranges for each predator species used in this study.

Predator species	Collection dates	Collection locations*	Trial dates	Number of replicates	Size** (mm)
Red drum	21 Jul - 25 Jul 2017	Wando River, Charleston Harbor	28 Sep - 11 Oct 2017	9	265 - 355
Spotted seatrout	27 Jun - 3 Jul 2018	Ashley River, Wando River, Charleston Harbor	28 Aug - 28 Sep 2018	10	283 - 458
Blue crab	21 Sep - 1 Oct 2018	Ashley River	3 Oct - 8 Oct 2018	12	>127

\*Wando River: 32.87°N, 79.87°W; Charleston Harbor: 32.76°N, 79.89°W; Ashley River: 32.82°N, 79.97°W.

\*\*Size ranges for the two finfish species are total length, measured from the mouth to the longest lobe of the caudal fin; crab size refers to the carapace width, measured point-to-point along the lateral spine.

**Table 2.**

Total numbers of symptomatic and asymptomatic white shrimp consumed by each predator species and the average predation rates (shrimp per predator per hour) of prey of each condition for each predator species ( $\pm$ SE).

Predator species	Number of white shrimp offered (symptomatic / asymptomatic)	Number of white shrimp consumed (symptomatic / asymptomatic)	Symptomatic white shrimp consumed predator <sup>-1</sup> hour <sup>-1</sup>	Asymptomatic white shrimp consumed predator <sup>-1</sup> hour <sup>-1</sup>
Red drum	288 (144 / 144)	117 (71 / 46)	0.63 ( $\pm$ 0.09)	0.40 ( $\pm$ 0.07)
Spotted seatrout	320 (160 / 160)	117 (68 / 49)	0.68 ( $\pm$ 0.08)	0.49 ( $\pm$ 0.04)
Blue crab	384 (192 / 192)	43 (32 / 11)	0.10 ( $\pm$ 0.01)	0.03 ( $\pm$ 0.01)

**Table 3.**

Results from generalized linear mixed effects models assessing the probability of an individual white shrimp being consumed by respective predators if symptomatic or asymptomatic of black gill. Odds ratio reports the odds of symptomatic shrimp being consumed compared to asymptomatic shrimp for each species. Confidence intervals (95%) are provided in parentheses.

Species	Symptomatic consumption probability	Asymptomatic consumption probability	Odds ratio	<i>p</i> -value
Red drum	0.49 (0.29 - 0.70)	0.32 (0.24 - 0.41)	2.09 (1.29 - 3.40)	0.004
Spotted seatrout	0.42 (0.22 - 0.64)	0.30 (0.21 - 0.40)	1.70 (1.07 - 2.73)	0.025
Blue crab	0.15 (0.04 - 0.43)	0.05 (0.02 - 0.09)	3.42 (1.70 - 7.40)	<0.001