TITLE

Oyster hatchery breakthrough of two HABs and potential effects on larval eastern oysters (*Crassostrea virginica*)

AUTHORS

Sarah K. D. Pease[a], Kimberly S. Reece[a], Jeffrey O'Brien[a], Patrice L. M. Hobbs[a], Juliette L. Smith[a*]

[a] Virginia Institute of Marine Science, William & Mary, P.O. Box 1346, Gloucester Point, VA 23062, USA

skpease@vims.edu, kreece@vims.edu, jcobrien01@email.wm.edu, ptrice@vims.edu, jlsmith@vims.edu

[*] Corresponding author

ABSTRACT

Harmful algal bloom (HAB) dinoflagellate species *Karlodinium veneficum* and *Prorocentrum cordatum* (prev. *P. minimum*) are commonly found in Chesapeake Bay during the late spring and early summer months, coinciding with the spawning season of the eastern oyster (*Crassostrea virginica*). Unexplained larval oyster mortalities at regional commercial hatcheries prompted screening of oyster hatchery water samples for these HAB species. Both HAB species were found in treated hatchery water during the oyster spawning season, sometimes exceeding bloom cell concentrations (≥ 1,000 cells/mL). To investigate the potential for these HAB species, independently or in co-exposure, to affect larval oyster mortality and activity, 96-h laboratory single and dual HAB bioassays with seven-day-old oyster larvae were performed. Treatments for the single HAB bioassay included fed and unfed controls, *K. veneficum* at 1,000; 5,000; 10,000; and 50,000 cells/mL, *P. cordatum* at 100; 5,000; 10,000; and 50,000 cells/mL. Subsequently, the 1,000 cells/mL *K. veneficum* and 50,000 cells/mL *P. cordatum* treatments were combined in a co-exposure treatment for the dual HAB bioassay. At all cell concentrations tested, *K. veneficum* swarmed oyster larvae and caused significant larval oyster mortality by 96 hours ($Karlo_{1,000}$: 21 ± 5% $Karlo_{5,000}$: 93 ± 2%; $Karlo_{10,000}$: 85 ± 3%; $Karlo_{50,000}$: 83 ± 5%, SE). In contrast, there was no significant difference in larval oyster mortality between the control treatments and any of the *P. cordatum* treatments by 96 hours. By 24 hours, larval oysters were significantly less active (immotile) in the presence of either HAB species as compared to control treatments (e.g., $Karlo_{1,000}$: 37.8 ± 4.1%; $Proro_{100}$: 47.3 ± 7.4%; Fed: 10.8 ± 3.2%; Unfed: 10.1 ± 4.9%, SE). In the dual HAB bioassay, larval oyster mortality associated with 1,000 cells/mL *K. veneficum* (44 ± 9%, SE) was not changed by the addition of 50,000 cells/mL *P. cordatum* (55 ± 7%, SE), demonstrating that *K. veneficum* was primarily responsible for the observed mortality. This study

demonstrated that even low cell concentrations of *K. veneficum* and *P. cordatum* are harmful to larval oysters, and could contribute to reductions in oyster hatchery production through impacts on this critical life stage.

KEYWORDS

Harmful algal bloom, *Karlodinium veneficum*, *Prorocentrum cordatum*, oyster larvae, hatchery, Chesapeake Bay, *Crassostrea virginica*, aquaculture

1. INTRODUCTION

In the late spring to early summer months, the harmful algal bloom (HAB) species *Prorocentrum cordatum* (prev. *P. minimum*, Velikova and Larsen 1999) and *Karlodinium veneficum* are common in the Chesapeake Bay (Li et al. 2000, Glibert et al. 2007, Marshall and Egerton 2009). The mixotrophic dinoflagellate *K. veneficum* (Li et al. 1999, Adolf et al. 2006a) produces karlotoxins (KmTxs), a class of bioactive compounds with hemolytic, cytolytic, ichthyotoxic, and allelopathic effects (Deeds et al. 2002, Kempton et al. 2002, Adolf et al. 2006b, Place et al. 2012, Dorantes-Aranda et al. 2015, Yang et al. 2019). This species is perhaps best known for causing finfish kills (Goshorn et al. 2004); however, it has also been shown to have harmful effects on zooplankton (Adolf et al. 2007, Waggett et al. 2008, Yang et al. 2019) and shellfish, including, blue mussels (*Mytilus edulis*, Nielsen and Strømgren 1991, Galimany et al. 2008), hard clams (*Mercenaria mercenaria*, Place et al. 2008), and some life stages of oysters (*Crassostrea virginica* and *C. ariakensis*, Glibert et al. 2007, Brownlee et al. 2008, Place et al. 2008, Stoecker et al. 2008, Lin et al. 2017). Another mixotrophic dinoflagellate known to have harmful effects on a variety of aquatic organisms, including finfish, shellfish, and zooplankton, is *P. cordatum* (reviewed in Heil et al. 2005). Exposure to *P. cordatum* has been shown to produce highly-variable effects amongst shellfish species and life stages, including juvenile bay scallops (*Argopecten irradians irradians*) and hard clams, as well as larval, juvenile, and adult oysters (reviewed in Wikfors 2005). From their work on oyster embryos exposed to *P. cordatum* lysates and filtrates, Wikfors and Smolowitz (1995) concluded that the harmful effects of *P. cordatum* likely relied on exposure to live *P. cordatum* cells rather than a released bioactive compound.

Chesapeake Bay blooms of *K. veneficum* and *P. cordatum* spatially and temporally overlap with the spawning season of the eastern oyster (*C. virginica*; Glibert et al. 2007, Place et

al. 2008). Previous research has focused on the potential effects of these HAB species on oyster wild-stock recruitment, aquaculture, and restoration (Tango et al. 2005, Glibert et al. 2007, Place et al. 2008, Stoecker et al. 2008). Little research has focused on the impacts of these HABs within the context of oyster hatcheries (Luckenbach et al. 1993). Acute exposure to these HAB species has harmful effects on many early oyster life stages (Luckenbach et al. 1993, Wikfors and Smolowitz 1995, Glibert et al. 2007, Brownlee et al. 2008, Place et al. 2008, Stoecker et al. 2008, Lin et al. 2017). All but one of these studies (Wikfors and Smolowitz 1995) focused on acute HAB exposure within 2 days post-fertilization, or after the oysters were at least 14 days old. The veliger life stage, lasting 2 days post-fertilization to 14 - 16 days, requires further investigation. During this time, larvae are free-swimming and typically feed on particles smaller than 10 µm (Fritz et al. 1984). Oyster hatchery production relies on the health and survival of this critical larval life stage.

Unexplained larval mortality events at regional oyster hatcheries have raised concerns over the possibility of *K. veneficum* and *P. cordatum* breakthrough into hatcheries (Luckenbach et al. 1993, Tango et al. 2005). "Breakthrough," in the context of this study, is the introduction of HAB cells and/or HAB-associated toxins into hatchery water (i.e., when water treatment processes at the hatchery fail to remove or degrade HAB cells or HAB-associated toxins from the incoming source water). Furthermore, breakthrough of live *K. veneficum* has been previously reported in a finfish hatchery in the Maryland portion of the Chesapeake Bay, where it was associated with finfish mortalities (Deeds et al. 2002). The first objective of the current study was to screen water samples from a commercial oyster hatchery in the lower Chesapeake Bay to detect and quantify any breakthrough of live *K. veneficum* and/or *P. cordatum*.

89 To better prepare hatchery managers to appropriately mitigate a breakthrough event of
90 either or both of these HAB species, a better understanding of the interactions between these
91 HAB species and larval oysters is needed. The second objective of the current study was to
92 assess the potential harmful effects of *K. veneficum* and *P. cordatum* on oyster veliger larvae.
93 Seven-day-old oysters were used in a series of 96-h larval oyster bioassays in the laboratory. The
94 bioassays were designed to measure larval oyster mortality and changes in larval oyster motility
95 during acute, static exposure to one or both of these naturally co-occurring HAB species.
96 Treatments consisted of a range of cell concentrations, representative of cell concentrations
97 documented in natural blooms of these HAB species and hatchery breakthrough events observed
98 during this study. Additionally, the potential effects of *K. veneficum* on a hatchery-relevant
99 beneficial food source, *Pavlova pinguis* were explored.

2. MATERIAL & METHODS

2.1 In-hatchery HAB screening

During the oyster spawning season, water samples were collected from inside an oyster hatchery and screened for the HAB species *K. veneficum* and *P. cordatum* to document any hatchery breakthrough. Water grab samples of 100-mL were collected inside of Oyster Seed Holdings, LLC, a commercial oyster hatchery in the lower Chesapeake Bay, Virginia, USA (37.4937, -76.3037). Hatchery staff collected water samples at-will throughout the commercial oyster spawning season (March through June). Samples were collected in 2014 and 2016, and delivered to the Virginia Institute of Marine Science (VIMS) for detection and quantification of *K. veneficum* and *P. cordatum* (see section 2.7); the hatchery did not provide samples for analysis in 2015. Three types of water samples were collected from within the hatchery at various stages along the water treatment process: 1) mixed-media – incoming water that was filtered to contain only particles less than 20-30 µm using mixed-media mechanical filtration, 2) filtered water – the mixed-media water that was further filtered to contain only particles less than 1-10 µm, and 3) feed algae – incoming water that was treated with mixed-media mechanical filtration, with additional 1-µm filtration, and used to cultivate beneficial feed algae through nutrient amendment. Mixed media or filtered water were treated for the purpose of oyster culturing, while feed algae samples were taken directly from the active algal culture bags within the hatchery. All water samples were collected in duplicate to run parallel analyses (see section 2.7). Water samples were received from the hatchery in various combinations of sample type and on random dates throughout the spawning seasons; this allowed for a screening of *K. veneficum* and *P. cordatum* for the specific dates and sample types collected. See section 2.7 for in-hatchery HAB cell enumeration methods.

123 2.2 Experimental design of bioassays

124 A series of 96-h, static, single and dual HAB bioassays were done in the laboratory to
125 assess acute and combined effects of *K. veneficum* and *P. cordatum* on larval oysters (**Table 1**).
126 Bioassays were carried out in 24-well tissue culture plates (Falcon®, Corning Inc., Corning,
127 New York, USA), with ten replicate wells per treatment. Treatments were made by diluting algal
128 cultures with treated hatchery water (**Table 1**). Estuarine York River water was treated by the
129 ABC hatchery using a sequence of two sand filters, a 20-µm cartridge filter, a diatomaceous
130 earth filter, a UV sterilizer, and a 1-µm filter bag. Treated water was then sterile-filtered through
131 a 0.2-µm Polycarp 75 TC filter (GE Whatman®, Sigma-Aldrich, St. Louis, Missouri, USA) and
132 was pre-aerated to ensure oxygen saturation. Treated water conditions were measured once at the
133 start of each bioassay with a YSI meter equipped with pH and polarographic dissolved oxygen
134 (DO) sensors (YSI Pro Plus Multiparameter Instrument, Yellow Springs, Ohio, USA). Sensors
135 were calibrated on the first day of the single HAB bioassay, 12 days before the dual HAB
136 bioassay; pH was calibrated using a 3-point calibration with standard buffers. Initial water
137 conditions for the single HAB bioassay were 20.0 ° C, 8.34 mg/L DO, salinity 13.83, pH 7.92,
138 and for the dual HAB bioassay were 19.8 ° C, 7.71 mg/L DO, salinity 13.87, pH 7.81.

139 Each replicate well was loaded with 1 mL of treatment before approximately 10, actively
140 swimming, 7-day-old, larval oysters were added. This larval density was chosen as it was within
141 the range of typical hatchery stocking densities for oysters of this age (4-15 larvae/mL, Castagna
142 et al. 1996). During the 96-h bioassays, well plates were kept in a Percival AL36L4 incubator
143 (Percival Scientific, Perry, Iowa, USA; 19.1 ± 0.5 ºC, 39 ± 7 µmol/m^2/s, 14:10 hour light-dark
144 cycle) with the lids on, except during daily observations of larval oyster motility, behavior, and
145 mortality (see section 2.4). No water changes were performed, no algal additions were made, and

146 no larval oysters were removed throughout the 96 hours. For all bioassays, 1-mL glass
147 microbeaker inserts (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) were used
148 inside of all wells to reduce chemical-surface interactions that could artificially reduce dissolved
149 bioactive compounds in the treatments, e.g., adsorption of lipophilic toxins to plastic well plates.
150 In the single HAB bioassay, treatments consisted of each HAB species at a variety of cell
151 concentrations (*K. veneficum*: 1,000; 5,000; 10,000; and 50,000 cells/mL; *P. cordatum*: 100;
152 5,000; 10,000; and 50,000 cells/mL). The lowest cell concentrations were chosen to reflect the
153 order of magnitude of 2014-2016 average cell concentrations from samples with co-occurring *K.*
154 *veneficum* and *P. cordatum* from the VIMS state HAB monitoring samples (n = 261, 1150 ± 317
155 cells/mL standard error [SE] and 389 ± 132 cells/mL SE, respectively). The cell concentrations
156 tested were within the range of cell concentrations observed in natural blooms of these two
157 species in the Chesapeake Bay (1,000 – 100,000 cells/mL for both species, Marshall and Egerton
158 2009). Control treatments included Unfed controls, i.e., no algae present, and Fed controls.
159 Unfed controls were included to account for any changes in larval oyster behavior or mortality
160 due to malnourishment. Past studies have found that oyster larvae can tolerate days to weeks
161 without algae, and may feed off of other nutriment during these times (Kennedy 1996) such as
162 bacteria and heterotrophic flagellates (Baldwin and Newell 1991). Fed control oysters received
163 only 25,000 cells/mL of *P. pinguis*, a typical cell concentration used to feed hatchery oyster
164 larvae (see section 2.5).
165 In the dual HAB bioassay, larval oysters were co-exposed to 1,000 cells/mL *K. veneficum*
166 and 50,000 cells/mL *P. cordatum* to assess potential combined effects of these co-occurring
167 HAB species. These cell concentrations were selected based on results from the single HAB
168 bioassay to maximize the potential of detecting a combined effect on larval oyster mortality

within 96 hours. In the single HAB bioassay, high cell concentration treatments of *K. veneficum* (>1,000 cells/mL) were determined to be too lethal for assessing combined effects over 96 hours. On the other hand, larval oyster mortality was so low for *P. cordatum* treatments that the highest *P. cordatum* cell concentration treatment was used in the dual HAB bioassay. To control for variability between oyster spawns and changing water conditions, relevant controls and single HAB bioassay treatments (Fed; Unfed; $Karlo_{1,000}$; $Proro_{50,000}$) were re-run for direct comparison with the co-exposure treatment.

2.3 Feed algae study

To assess potential effects of *K. veneficum* on *P. pinguis*, *P. pinguis* cell concentration data were compared between *P. pinguis* treatments (25,000 cells/mL) with or without *K. veneficum* (1,000 cells/mL), in the absence of larval oysters, over 96 hours (**Table 1**). During the dual HAB bioassay, 12 additional wells without larval oysters were made up with each of these two algal treatments. Every 24 hours, the contents of three wells from each treatment were transferred into separate 1.5-mL low retention microcentrifuge tubes, fixed with 10% neutral buffered formalin (Pharmco-Aaper, Brookfield, Connecticut, USA), and stored at 4 ºC. At the start of the study, triplicate 1-mL samples of the initial algal treatments were collected and stored as just described. *Pavlova pinguis* was enumerated using a hemocytometer and light microscopy (Olympus CX31 or CX41, Olympus Corp., Shinjuku, Tokyo, Japan).

2.4 Larval oyster metrics

During both bioassays, the well plates were removed daily from the incubator for assessment of larval oyster mortality and behavioral observation of oysters by light microscopy (Olympus CKX53 or IX50 inverted microscopes, Olympus Corp., Shinjuku, Tokyo, Japan). To capture novel behavior of interest, discovered after the start of the bioassays, still images and

short videos were collected using Infinity Analyze 6.5.4 (Lumenera Corp., Ottawa, Ontario, Canada) or CellSens Standard 1.12 (Olympus Corp., Shinjuku, Tokyo, Japan) software. Daily assessment of larval oyster mortality in each well consisted of counting *dead* oyster larvae that exhibited no ciliary movement, or that had intact and empty shells; these observations were made at 40X magnification. After all observations and larval oyster mortality assessments were made at 96 hours, 10% neutral buffered formalin (Pharmco-Aaper, Brookfield, Connecticut, USA) was used to fix the wells, allowing for an exact *total* larval oyster count of each well (at the start of the bioassays, wells were loaded with approximately 10 live larval oysters). Cumulative larval mortality (CLM) was calculated at each timepoint for each well using the formula: *CLM = (dead / total) * 100*. These values were used to calculate daily average CLM for each treatment (n = 10), from which the daily average *% surviving* could also be calculated, as *100 - CLM*. The daily average CLM for each treatment were plotted over the 96 hours for the single and dual HAB bioassays.

In the single HAB bioassay, larval oyster motility in each well was assessed every 24 hours using a protocol designed to measure activity/inactivity of larval shellfish species (Yan et al. 2001, Basti et al. 2015). Briefly, when the well plates were removed from the incubator, the plates were gently swirled to cause larvae to stop swimming and sink to the bottom of the wells. After five minutes, the number of non-swimming, *immotile* larvae in each well was recorded. *Immotile* larval oysters were easily distinguished from *dead* larval oysters by ciliary action, visible by light microscopy at 40X magnification. To calculate the percentage of *immotile* larvae in each well at each timepoint, while excluding *dead* larvae, the following equation was used, *% immotile = [(immotile – dead) / (total – dead)] * 100*. These values were used to calculate the average *% immotile* for each treatment at 24 and 96 hours; wells with 0 *% surviving* were

excluded from these calculations. Sample size for average % *immotile* was 10 wells per treatment, except at 96 hours for average % *immotile* values for $Karlo_{5,000}$ (n = 6), $Karlo_{10,000}$ (n = 9), and $Karlo_{50,000}$ (n = 8). These lower sample sizes were due to the exclusion of wells with 0 % *surviving*.

2.5 Larval oyster culturing for bioassays

Oyster larvae (*C. virginica*) were acquired from the Aquaculture Genetics and Breeding Technology Center (ABC) oyster hatchery at VIMS. Oysters were spawned separately for the single and dual HAB bioassays, 12 days apart. The single HAB bioassay spawn had 12 parents and the dual HAB bioassay spawn had 8 parents. For both spawns, ABC used 3-yr-old, diploid, DEBY oysters. The DEBY line of oysters is widely used at oyster hatcheries along the eastern coast of the USA (Dr. Jessica Moss Small, ABC, pers. comm.). Adult oysters were strip-spawned, all eggs were pooled and then split into a number of batches equaling the number of male oysters used in that spawn. Each male's sperm was used to fertilize one batch of eggs and the fertilized embryos were then re-pooled, resulting in a spawn with all possible crosses between parents. The resulting larvae were raised with aeration in 60- or 200-L barrels at 24-28 ºC in the hatchery and were fed a daily diet of *P. pinguis* at 20,000-35,000 cells/mL. Full water changes were performed on day 2, and day 4 or 5, after the spawn. On day 7, oyster larvae were collected on a 63-µm sieve, transferred to new, treated hatchery water, and transported to the laboratory for the bioassays.

2.6 Algal culturing for bioassays and feed algae study

The ABC facility cultured *P. pinguis* using f/2 medium (Fritz Aquatics, Mesquite, Texas, USA, Guillard and Ryther 1962, Guillard 1975) using treated hatchery water. Large batch

237 cultures were held at 20 ºC under constant light. The average salinity of treated hatchery water
238 during the month of the bioassays was 13.6 (Dr. Jessica Moss Small, ABC, pers. comm.).
239 In the laboratory, single-cell isolate, clonal cultures of the HAB species, *K. veneficum*
240 (CCMP 1974, Stoecker et al. 2008), and *P. cordatum* (JA 98-01, Rosetta and McManus 2003),
241 were grown in f/2-Si medium (Guillard and Ryther 1962, Guillard 1975) made with autoclaved,
242 0.22 µm-filtered York River seawater. The salinity of both cultures was approximately 20, as
243 measured with a refractometer. Batch cultures were grown at 19.1 ± 0.5 ºC (SD) under 39 ± 7
244 µmol/m^2/s (SD) light irradiance and a 14:10 hour light-dark cycle (see section 2.2). For the
245 single HAB bioassay, the *K. veneficum* culture used to begin the bioassay was in the stationary
246 phase (the growth phase in which the cell population has stabilized), while the *P. cordatum*
247 culture was in stationary phase leading up to the bioassay but was diluted with new medium the
248 day before the bioassay. For the dual HAB bioassay, both HAB cultures were in the stationary
249 phase at the start of the bioassay.
250 2.7 In-hatchery HAB cell enumeration
251 In 2014, *P. cordatum* was quantified in samples preserved with Lugol's solution
252 (Carolina Biological Supply Company, Burlington, North Carolina, USA), using a 1-mL
253 Sedgewick-Rafter slide and light microscopy (100X magnification, Olympus 1X51 with
254 Olympus DP73 digital camera, Center Valley, Pennsylvania, USA). All quantification of *K.*
255 *veneficum* in 2014 and 2016, as well as *P. cordatum* in 2016, was completed using quantitative
256 real-time PCR (qPCR).
257 In preparation for qPCR, samples were filtered through 3-µm Isopore™ membrane filters
258 (Millipore Corp., Darmstadt, Germany) for DNA extraction and qPCR analysis. Filters were
259 placed in 5-mL centrifuge tubes, frozen at -20 °C until DNA was extracted using the QIAamp ®

Fast Stool Mini Kit (QIAGEN©, Germantown, Maryland, USA) according to the manufacturer's protocol with the following modifications. Instead of using only 200 µL of the lysate, the entire sample was carried through the extraction protocol. The reagent volumes were increased in subsequent steps to maintain the proper ratio of sample to reagents. A "blank" extraction (reagents only) was included with each set of samples to ensure there was no contamination. Extracted DNA samples were stored at -20 °C until they could be quantified using qPCR.

A previously published TaqMan® qPCR assay was used to target *P. cordatum* (Handy et al. 2008). To target *K. veneficum*, the current study used a qPCR TaqMan® assay originally developed and optimized at VIMS around 2008-2009 (VA DEQ 2014). This *K. veneficum* assay was chosen because it was, and remains, routinely used in Virginia for official state monitoring of this HAB species. The *K. veneficum* primers were KvITS_242F (5'-TTCGTTGTGTAGTTGTTGACTCG-3') and KvITS_328R (5'-TGCTGACCTAACTTCATGTCTTG -3'), and the probe was Kv_266PR (5'FAM -AGCCTGCTCCAGCTCACGACTCCT-3'TAMRA). These *K. veneficum* primers were tested for cross-reactivity against all phytoplankton species listed in Table 2 of the VA DEQ (2014) report, species all found in lower Chesapeake Bay waters. Control stocks of *K. veneficum* and *P. cordatum* were maintained in culture at VIMS. Cell counts for the control stock cultures were determined by light microscopy. DNA was extracted from a known number of cells to use as positive control material and for generating standard curves through serial dilution of the DNA to achieve a range of cell number equivalents. Samples from the hatchery were run against these standard curves to quantify the cells in the sample. qPCR assays were performed on 7500 Fast, QuantStudio 6, or QuantStudio 3 Real-Time PCR systems (Applied Biosystems™, ThermoFisher, Waltham, Massachusetts, USA) using the following cycling parameters: an initial

denaturation step at 95 °C for 20 s followed by 40 cycles of 95 °C for 3 s to denature and 60 °C for 30 s to anneal and extend. All reactions were performed in duplicate with reagent concentrations for each reaction of 0.9 µM for each primer, 0.1 µM for the probe and 1X concentration of the TaqMan® Fast Advanced Master Mix (Applied Biosystems™, ThermoFisher, Waltham, Massachusetts, USA) in a 10 µL final volume. A subset of qPCR results was cross verified by light microscopy counts (100X magnification, Olympus 1X51 with Olympus DP73 digital camera, Center Valley, Pennsylvania, USA) of Lugol's-preserved (Carolina Biological Supply Company, Burlington, North Carolina, USA) duplicate samples.

2.8 Data analysis and statistics

Differences in larval oyster motility *(% immotile*) between treatments were assessed at 24 and at 96 hours in the single HAB bioassay using Kruskal-Wallis tests followed by post hoc analysis using Dunn tests with the Benjamini and Hochberg (1995) p-adjustment method for multiple comparisons.

Differences in mortality (CLM, see section 2.4) between treatments for the single and dual HAB bioassays were analyzed using separate, linear mixed effects models (LMMs). To account for differences between wells, wells were coded as a random (intercept) factor, nested within treatment. Time, treatment, and the interaction between time and treatment, were coded as fixed factors. LMMs allowed for a time decaying correlation structure in the data, i.e., correlations between data collected in time from the same subject declined as the sampling timepoints got further apart (Liu et al. 2010). The first order autoregressive structure was applied to these models. CLM was arcsine transformed to improve homogeneity of variance of the proportional data (Lin and Xu 2020), which was assessed through residual plots. Models were fitted using a restricted maximum likelihood (REML) approach in R using the "nlme" package

306 (Pinheiro et al. 2020). Least-squares means (LSM) of arcsine-transformed CLM were calculated
307 from the LMM for each treatment within each level of time using the "emmeans" package in R
308 (Lenth et al. 2020). Significant differences between LSM of treatments within each level of time
309 were determined by using the Tukey-Bonferroni method for multiple comparisons (Liu et al.
310 2010). Briefly, Tukey's pairwise comparisons of treatments within each level of time were
311 calculated, and the significance level (α) was adjusted by dividing by the number of levels of
312 time that comparisons were made for, e.g., n = 4, therefore $\alpha = 0.05/4 = 0.0125$.

313 In the feed algae study, *P. pinguis* cell concentration was modeled using a multiple linear
314 regression (MLR) to assess potential effects of *K. veneficum* on *P. pinguis*. The model variables
315 included time (days) and presence/absence of *K. veneficum* (0 = absence, 1 = presence).
316 Scatterplots and correlation tests were used to examine relationships between these model
317 variables and the response, *P. pinguis* cell concentration. Cell concentration (cells/mL) was ln-
318 transformed to normalize the residuals (Shapiro-Wilk's: W=0.99, p=0.95). Collinearity of model
319 variables, and homogeneity of variance of model residuals, were assessed to ensure assumptions
320 of the model were met.

321 Statistical tests were performed in R Studio (2019) using R version 3.6.1. Tests used a
322 significance level (α) of 0.05, unless otherwise noted.

323 **3. RESULTS**

324 3.1 In-hatchery HAB screening

325 During the 2014 and 2016 oyster spawning seasons, both *K. veneficum* and *P. cordatum*
326 were identified and enumerated in water samples collected from within a commercial oyster
327 hatchery (**Table 2**). Both HAB species were found in all three types of treated water in 2014, i.e.,
328 mixed-media, filtered water, and feed algae, and exceeded "bloom" cell concentrations (> 1,000
329 cells/mL) in the former two types. Water treated for feed algae, however, contained lower cell
330 concentrations of both HAB species, rising above 100 cells/mL in May 2014. In 2016,
331 measurable cell concentrations of both HAB species were again present in filtered water, but
332 remained below "bloom" cell concentrations, ranging from 19 – 937 cells/mL *K. veneficum* and
333 3 – 9 cells/mL *P. cordatum*.

334 3.2 Single and dual HAB bioassays

335 3.2.1 *Karlodinium veneficum* swarming behavior

336 In both the single and dual HAB bioassays, *K. veneficum* was observed swarming live
337 and dead oyster larvae (**Fig. 1**), a behavior in stark contrast to the uniform distribution of *P.*
338 *cordatum* (**Fig. 2**, **Supp. Fig. 1**) and *P. pinguis* (**Videos 1** and **2**) swimming cells. This was a
339 novel behavior of interest (see section 2.4) that was captured in photos and videos as well as in
340 the following qualitative observations. Swarming behavior of *K. veneficum* was observed during
341 every observation timepoint in both bioassays, with more larvae swarmed with time. Swarming
342 behavior was observed in all wells with *K. veneficum* and oyster larvae, but not all larvae within
343 each well were swarmed, or were swarmed at the same time. Less *K. veneficum* were associated
344 with highly active larvae (**Videos 1** and **2**), while immotile larvae (see section 3.2.2) were
345 generally swarmed. Some live oyster larvae that were actively being swarmed, exhibited

hindered swimming capabilities (**Video 1**). Live *K. veneficum* were frequently observed inside of the shells of living and dead oyster larvae (**Fig. 1B**, **Video 2**). Swarms of *K. veneficum* appeared to persist around larvae until the larvae died, and the larval shells were emptied of all tissue, at which point in time the empty shells were generally abandoned within 24 hours.

3.2.2 Larval oyster immotility

Immotility and survival of larval oysters was quantified at 24 and 96 hours in the single HAB bioassay (**Table 3**). Larvae were significantly less motile after 24 hours of exposure to *K. veneficum* or *P. cordatum* when compared to controls (Kruskal-Wallis, $\chi^2 = 77$, df = 9, $p < 0.0001$). More specifically, all *K. veneficum* treatments ($Karlo_{1,000}$: 37.8 ± 4.1%; $Karlo_{5,000}$: 70.0 ± 3.6%; $Karlo_{10,000}$: 79.3 ± 3.7%; $Karlo_{50,000}$: 94.0 ± 5.0% SE), as well as the lowest *P. cordatum* treatment ($Proro_{100}$: 47.3 ± 7.4% SE), exhibited significantly higher percentages of immotile larvae than the Fed or Unfed controls at 24 hours (Fed: 10.8 ± 3.2%; Unfed: 10.1 ± 4.9% SE; Dunn, all $p < 0.05$). At 96 hours, there was also a significant difference in the percentage of immotile larval oysters between treatments (Kruskal-Wallis, $\chi^2 = 68$, df = 9, $p < 0.0001$). All *K. veneficum* treatments ($Karlo_{1,000}$: 42.0 ± 6.0%; $Karlo_{5,000}$: 100%; $Karlo_{10,000}$: 100%; $Karlo_{50,000}$: 79.2 ± 11.5% SE), as well as the highest *P. cordatum* treatment ($Proro_{50,000}$: 55.3 ± 6.4% SE), exhibited significantly higher percentages of immotile larvae than the Fed or Unfed controls at 96 hours (Fed: 10.8 ± 4.0%; Unfed: 5.7 ± 2.5% SE; Dunn, all $p < 0.05$).

3.2.3 Larval oyster mortality

In the single HAB bioassay, Fed and Unfed controls exhibited low mortality by 96 hours (Fed: 1 ± 1% standard error [SE]; Unfed: 0%; n = 10 wells per treatment). The $Karlo_{1,000}$ treatment exhibited moderate mortality by 96 hours ($Karlo_{1,000}$: 21 ± 5% SE), and the higher cell concentration *K. veneficum* treatments exhibited high mortality by 96 hours ($Karlo_{5,000}$: 93 ± 2%;

Karlo$_{10,000}$: 85 ± 3%; Karlo$_{50,000}$: 83 ± 5% SE; **Fig. 3A**). Conversely, oyster larvae exposed to all *P. cordatum* treatments exhibited low mortality throughout the single HAB bioassay (**Fig. 3B**).

Within each timepoint (24, 48, 72, 96 hours) in the single HAB bioassay, Fed and Unfed controls and all *P. cordatum* treatments showed no significant difference in mortality (Tukey-Bonferroni: all $p > 0.0125$, **Table 4**). At 24 hours, Karlo$_{10,000}$ and Karlo$_{50,000}$ treatments had significantly higher mortality than all other treatments (Tukey-Bonferroni: all $p < 0.0125$). At 48, 72, and 96 hours, the three highest *K. veneficum* treatments (Karlo$_{5,000}$, Karlo$_{10,000}$, and Karlo$_{50,000}$) had significantly higher mortality than all other treatments (Tukey-Bonferroni: all $p < 0.0125$). At 96 hours, Karlo$_{1,000}$ had significantly higher mortality than the Fed and Unfed controls and *P. cordatum* treatments (Tukey-Bonferroni: all $p < 0.0125$). Well nested in treatment was a significant predictor of mortality ($\lambda_{LR} = 91$, $p < 0.0001$), explaining 0.02% of the difference in mortality after accounting for the effects of time and treatment. The interaction between time and treatment was significant in the model (LMM: $F_{27, 270} = 17$, $p < 0.0001$). Time (LMM: $F_{3, 270} = 115$, $p < 0.0001$) and treatment (LMM: $F_{9, 90} = 103$, $p < 0.0001$) were significant predictors of mortality.

In the dual HAB bioassay, Fed and Unfed controls and the Proro$_{50,000}$ treatment all exhibited low mortality by 96 hours (Fed: 3 ± 2%; Unfed: 0%; Proro$_{50,000}$: 5 ± 2% SE; n = 10 wells per treatment), while the Karlo$_{1,000}$ treatment and the Karlo$_{1,000}$ X Proro$_{50,000}$ treatment both exhibited moderate mortality by 96 hours (Karlo$_{1,000}$: 44 ± 9%; Karlo$_{1,000}$ X Proro$_{50,000}$: 55 ± 7% SE; **Fig. 4**).

At 24 hours in the dual HAB bioassay, there was no significant difference in mortality between any of the treatments (Tukey-Bonferroni: all $p > 0.0125$, **Table 5**). At 48, 72, and 96 hours, the Karlo$_{1,000}$ and Karlo$_{1,000}$ X Proro$_{50,000}$ treatments had significantly higher mortality

than all other treatments (Tukey-Bonferroni: all $p < 0.0125$) with no significant difference in mortality between these two treatments (Tukey-Bonferroni: all $p > 0.0125$). Well nested in treatment was a significant predictor of mortality ($\lambda_{LR} = 123$, $p < 0.0001$), explaining 46.8% of the difference in mortality after accounting for the effects of time and treatment. The interaction between time and treatment was significant in the model (LMM: $F_{12, 135} = 12$, $p < 0.0001$). Time (LMM: $F_{3, 135} = 45$, $p < 0.0001$) and treatment (LMM: $F_{4, 45} = 24$, $p < 0.0001$) were significant predictors of mortality.

3.3 Feed algae study

There was a small, but significant negative effect of *K. veneficum* presence on *P. pinguis* cell concentration (MLR: $F_{1,26} = 4.8$, $p = 0.038$). Cell concentration over time of *P. pinguis,* with and without *K. veneficum,* was modeled by the equation: $Y_{Pav} = 10 - 0.08\,(Karlo) + 0.4\,(Time)$, where Y_{Pav} was the ln-transformed cell concentration (cells/mL) of *P. pinguis*, *Karlo* was the presence/absence of *K. veneficum* (0 = absence, 1 = presence), and *Time* was measured in days (MLR: $F_{2,26} = 308$, $p < 0.0001$, $R^2 = 0.96$). Time was also a significant predictor of *P. pinguis* cell concentration in the model (MLR: $F_{1,26} = 612$, $p < 0.0001$).

4. DISCUSSION

4.1 *Karlodinium veneficum* and oyster larvae

4.1.1 Swarming behavior of *K. veneficum*

This study provided novel documentation of larval oyster mortality resulting from predation by *K. veneficum*, and the swarming behavior of *K. veneficum* that preceded this mortality (**Figs. 1** and **2, Videos 1** and **2**). These observations support recent documentation of aggregations of *K. veneficum* cells around single-celled and metazoan prey items (Yang et al. 2020). The authors of that study proposed this behavior was part of a potential feeding mode, i.e., micropredation, for this mixotrophic HAB species. Swarming of metazoan organisms by dinoflagellates has been previously documented (Spero and Morée 1981, Delgado and Alcaraz 1999, Springer et al. 2002, Berge et al. 2012, Yang et al. 2020), including during laboratory exposures of larval oysters to *Pfiesteria* spp. (Springer et al. 2002, Shumway et al. 2006). Swarming behavior has also been reported in another *Karlodinium* species (Berge et al. 2008, 2012), *K. armiger*, where it was associated with the immobilization, predation, and ultimate death, of copepods (Berge et al. 2012). In the same study, a polychaete trochophore was swarmed and almost entirely consumed within 24 hours. *Karlodinium armiger* can feed on its prey using a peduncle for tube feeding, or through phagotrophy (Berge et al. 2008), similar to *K. veneficum* (Yang et al. 2020). Chemotaxis towards prey items has been reported in *K. armiger* (Poulsen et al. 2011, Berge et al. 2012). Observations from the current study suggest that there was some form of chemosensory attraction of *K. veneficum* to the oyster larvae that may have facilitated the observed swarming.

While KmTxs were not quantified in this study due to limited cell biomass and the lack of a commercially-available standard, their probable involvement deserves some discussion. The

strain of *K. veneficum* used in the current study (CCMP 1974) produces KmTx 1 and KmTx 3 (Brownlee et al. 2008, Stoecker et al. 2008, Adolf et al. 2009, Bachvaroff et al. 2009). Exposure to the same strain of toxin-producing *K. veneficum* caused immotility in 2-week-old oyster larvae (Glibert et al. 2007) and mortality in just-hatched larvae, while a non-toxin-producing strain of *K. veneficum* had no effect on mortality (Stoecker et al. 2008). KmTxs have also been shown to be involved in *K. veneficum* prey-capture by reducing swimming speeds and immobilizing single-celled prey (Sheng et al. 2010). Similarly, KmTxs are thought to have played a role in the observed larval oyster immotility and mortality in the current study. KmTxs are poorly-soluble, leading to the hypothesis that they must be administered within close proximity to the target (Sheng et al. 2010). This would indicate that *K. veneficum* swarming likely preceded any involvement of KmTxs in the current study, and that once swarmed, larval oysters were more likely to experience immobilization or other effects of KmTxs. The precise role of KmTxs in the interaction between larval oysters and *K. veneficum* deserves further study.

The current study demonstrated interactions between *K. veneficum* swarming behavior and larval motility. Larval swimming has been hypothesized to create microscale turbulence capable of preventing or reducing the risk of a dinoflagellate coming into close proximity with an active oyster larva (Springer et al. 2002). This small-scale turbulence surrounding swimming larvae could have interfered with *K. veneficum* swarming behavior and may help explain why some oyster larvae survived. Furthermore, *K. veneficum* was found to preferentially feed on immotile prey (Yang et al. 2020). In the current study, immotile larvae were almost always observed with physically-associated *K. veneficum*. Once *K. veneficum* are physically-associated with a larva, in addition to the potential effects of KmTxs mentioned previously, the associated cells may increase physical drag during swimming attempts by the larva, or the cells may

directly interfere with the larval swimming mechanism. Any of these outcomes further enhance the likelihood of larval immotility and the risk of being swarmed by additional *K. veneficum* cells. As the *K. veneficum* exposure duration or dose (cell concentration) increases, larval oysters experience more close encounters with individual *K. veneficum* cells. When considered in conjunction with previous studies, the current study suggests that without sustained, close proximity between *K. veneficum* and the larval oysters, the effect of *K. veneficum* on the oysters may be minimized. The authors propose that larval immotility ultimately enhances the opportunity for *K. veneficum* to initiate, or advance, an attack on the larvae, initiating a positive feedback loop that facilitates swarming and increases the harmful impact of this HAB species on larval oysters.

4.1.2 *Karlodinium veneficum* and larval oyster mortality

Exposure to *K. veneficum* caused significant larval oyster mortality at all cell concentrations tested, representing cell concentrations observed in hatchery breakthrough (1,000 cells/mL treatment; **Table 2**) and Chesapeake Bay bloom events (5,000 – 50,000 cells/mL treatments; Marshall and Egerton 2009). This supports previous research that has shown other early life stages of the eastern oyster (*C. virginica*) to be vulnerable to harmful effects of *K. veneficum*. Past studies have found that oyster larvae exposed to *K. veneficum* during the first two days of life exhibited deformities (Glibert et al. 2007) and elevated mortality compared to control larvae (Glibert et al. 2007, Stoecker et al. 2008, Lin et al. 2017). Additionally, Glibert and colleagues (2007) found that 14-day-old oyster larvae (pediveligers) stopped swimming after 72 hours of exposure to *K. veneficum*, an effect they hypothesized would lead to larval oyster mortality. The current study provided new information on the effects of *K. veneficum* exposure between these two previously-studied oyster developmental stages, using 7-day-old oyster larvae

476 (veligers). Together, these findings suggest that hatchery breakthrough of *K. veneficum* can lead
477 to significant larval oyster mortality inside of an oyster hatchery. In addition, the mortality
478 associated with the higher *K. veneficum* cell concentrations tested in the current study (5,000 –
479 50,000 cells/mL), is considered ecologically significant and demonstrates severe consequences
480 from acute exposure of oyster larvae to this HAB species (**Fig. 3**).

481 The Fed and Unfed controls showed no significant larval oyster mortality over 96 hours,
482 demonstrating the habitable conditions of this bioassay design. The static nature of these
483 bioassays, however, may have led to declining water quality and subsequent larval stress. Water
484 quality, therefore, cannot be ruled out as a possible confounding, and unmeasured, factor in the
485 mortality metric. These mortality results, however, are highly applicable to static hatchery
486 conditions where larvae are kept in tanks without water flow, moribund or dead larvae are not
487 removed, and the water is changed out every few days. Water quality issues may exacerbate
488 effects of *K. veneficum* exposure, although more research into combined effects of co-stressors in
489 a hatchery setting is needed.

490 This is the first study to test the combined effects of these naturally co-occurring HAB
491 species. During the dual HAB bioassay, there was no change in larval oyster mortality when *K.*
492 *veneficum* was alone or with *P. cordatum*, signifying that *K. veneficum* drove the observed larval
493 oyster mortality in the co-exposure (**Fig. 4**). The results of the dual HAB bioassay also
494 demonstrated that larval oyster mortalities were not explained by high cell biomass or particulate
495 concentrations, i.e., the *K. veneficum* with *P. cordatum* treatment had the highest cell biomass
496 and particulate concentration of all treatments tested.

497 4.2 *Prorocentrum cordatum* and oyster larvae

Exposure to *P. cordatum* was occasionally associated with larval immotility (**Table 3**), indicating that interactions with *P. cordatum* are disruptive to larval oyster behavior. Significant immotility compared to larval oyster controls was observed in the exposures as early as 24 hours, even with *P. cordatum* cell concentrations as low as 100 cells/mL. Reduced motility during exposure to *P. cordatum* has previously been reported in juvenile bay scallops (Li et al. 2012) and in pediveliger Suminoe oysters, *C. ariakensis* (Glibert et al. 2007). The latter study also reported reduced swimming speeds in pediveliger eastern oysters, *C. virginica*, exposed to *P. cordatum*. Reduction in motility of larval oysters may result in reduced grazing, which could in turn reduce larval growth, and ultimately, survival.

While some previous studies have shown *P. cordatum* to cause mortality in early life stages of oysters (Luckenbach et al. 1993, Wikfors and Smolowitz 1995, Glibert et al. 2007), the current study, and a similar study with just-hatched larvae (Stoecker et al. 2008) found no significant effect of *P. cordatum* on larval oyster mortality. The lack of mortality in these studies may be due to insufficient *P. cordatum* exposure duration or dose. Moreover, it could be related to the physiological states of *P. cordatum* culture used (Li et al. 2012). *Prorocentrum cordatum* cultures in decline have been found to produce more harmful effects than culture in other growth phases (Grzebyk et al. 1997, Li et al. 2012), such as stationary phase, as was used in the current study. Regardless, the observed larval immotility indicated a low-level, harmful effect of *P. cordatum* exposure that would have been missed if bioassay metrics had been limited to larval oyster mortality.

4.3 Implications for oyster hatcheries

This is the first documentation of breakthrough of live cells of *K. veneficum* and *P. cordatum* into an oyster hatchery (**Table 2**). Breakthrough occurred during oyster spawning

season, a time of year when these two HAB species co-occur in the Chesapeake Bay (Glibert et al. 2007). Cells of *K. veneficum* (length 9-18 µm, Ballantine 1956) and *P. cordatum* (length 20 µm, Faust 1974) survived mechanical filtration with nominal pore sizes of 1-30 µm, demonstrating that filtration of incoming water, even down to 1 µm, may not be sufficient to keep these HAB species out of hatcheries. HAB cell concentrations inside the hatchery occasionally reached low-bloom cell concentrations (≥ 1,000 cells/mL). In laboratory bioassays, treatments of similar cell concentrations caused significant larval oyster immotility (by *P. cordatum* and *K. veneficum*) and significant larval oyster mortality (by *K. veneficum*). The bioassays in this study used the popular DEBY oysters and future research should explore whether different genetic lines of eastern oyster (*C. virginica*) respond differently to *K. veneficum* or *P. cordatum*.

The current study demonstrated that proximal, acute exposure to *K. veneficum* is a major concern for larval oyster health. Larval oyster mortality was preceded by *K. veneficum* actively swarming the larval oysters. Close proximity may be avoided or minimized when oyster larvae are healthy and actively swimming, when water conditions are turbulent, or, in the case of oyster hatcheries, when frequent full-water changes are performed. Based on the results from the single and dual HAB bioassays, daily full water changes would be advised to minimize the risk of larval oyster mortality due to static exposure to *K. veneficum* during a breakthrough event. At a fish hatchery, potassium permanganate was successfully used to mitigate an incidence of *K. veneficum* breakthrough without any ill effects (Deeds et al. 2002). Testing would be needed to determine the appropriateness of this approach for an oyster hatchery.

Furthermore, both *K. veneficum* and *P. cordatum* were found in feed algae cultured at the hatchery, making feed algae an additional potential source of HABs to hatchery oysters. This

544 study also showed that *K. veneficum* could negatively impact the cell concentration of a common
545 hatchery feed alga (*P. pinguis*). A reduction in hatchery feed algae during a *K. veneficum*
546 breakthrough event could represent another stressor on hatchery oysters, and poses additional
547 challenges for hatchery aquaculturists.

548 This study documented HAB cell breakthrough into an oyster hatchery, harmful effects
549 of two common Chesapeake Bay HAB species on the vulnerable veliger life stage of the eastern
550 oyster (*C. virginica*), as well as potential negative effects of *K. veneficum* on hatchery feed algae.
551 Evidence of oyster hatchery HAB breakthrough of low-bloom cell concentrations, and damage to
552 larval oysters exposed to these HAB species, validate regional concerns and suggest that these
553 HAB species could have contributed to past unexplained larval oyster mortalities at regional
554 hatcheries. The authors recommend that hatcheries continue to monitor treated water and feed
555 algae for HAB breakthrough, especially when water will be used with early oyster life stages.
556 The harmful effects of these two HAB species on larval oysters can likely be mitigated through a
557 combination of monitoring and frequent full water changes.

ACKNOWLEDGEMENTS

The authors thank the Aquaculture Genetics and Breeding Technology Center (ABC), especially Dr. Jessica Moss Small, Amanda Chesler, Lauren Gregg, Karen Sisler, and Dr. Standish Allen, for providing treated hatchery water, larval oysters, *Pavlova pinguis*, and invaluable background information. The authors also express their gratitude to Mike Congrove (Oyster Seed Holdings, LLC) for providing commercial oyster hatchery water samples, Bill Jones (VIMS) for supplying HAB cultures, and Dr. Jeffrey Shields (VIMS) for use of his microscopes. The authors greatly appreciate Dr. Roger Mann, Dr. Kathi Lefebvre, and two anonymous reviewers for their constructive comments on this manuscript. This paper is Contribution No.xxxxxxx of the Virginia Institute of Marine Science, William & Mary.

Funding: This work was supported by the NOAA Sea Grant National Aquaculture Initiative [grant numbers #NA14OAR4170093 and #NA17OAR4170321] to J.L.S. and K.S.R. (VIMS).

571 **REFERENCES**

572 Adolf, J.E., Stoecker, D.K., Harding, Jr., L.W., 2006a. The balance of autotrophy and
573 heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). J.
574 Plankton. Res. 28(8), 737-751. doi:10.1093/plankt/fbl007

575 Adolf, J.E., Bachvaroff, T.R., Krupatkina, D.N., Nonogaki, H., Brown, P.J.P., Lewitus, A.J.,
576 Harvey, H.R., Place, A.R., 2006b. Species specificity and potential roles of *Karlodinium*
577 *micrum* toxin. African J. Mar. Sci. 28(2), 415-419. doi:10.2989/18142320609504189

578 Adolf, J.E., Krupatkina, D.N., Bachvaroff, T.R., Place, A.R., 2007. Karlotoxin mediates grazing
579 by *Oxyrrhis marina* on strains of *Karlodinium veneficum*. Harmful Algae 6, 400-412.
580 doi:10.1016/j.hal.2006.12.003

581 Adolf, J.E., Bachvaroff, T.R., Place, A.R., 2009. Environmental modulation of karlotoxin levels
582 in strains of the cosmopolitan dinoflagellate, *Karlodinium veneficum* (Dinophyceae). J.
583 Phycol. 45, 176-192. doi:10.1111/j.1529-8817.2008.00641.x

584 Bachvaroff, T.R., Adolf, J.E., Place, A.R., 2009. Strain variation in *Karlodinium veneficum*
585 (Dinophyceae) toxin profiles, pigments, and growth characteristics. J. Phycol. 45, 137-153.
586 doi:10.1111/j.1529-8817.2008.00629.x

587 Baldwin, B.S., Newell, R.I.E., 1991. Omnivorous feeding by planktotrophic larvae of the eastern
588 oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 78, 285-301. doi:10.3354/meps078285

589 Ballantine, D., 1956. Two new marine species of *Gymnodinium* isolated from the Plymouth area.
590 J. Mar. Biol. Assoc. UK 35, 467-474. doi:10.1017/S0025315400010316

591 Basti, L., Nagai, S., Go, J., Okano, S., Nagai, K., Watanabe, R., Suzuki, T., Tanaka, Y., 2015.
592 Differential inimical effects of *Alexandrium* spp. and *Karenia* spp. on cleavage, hatching,

593 and two larval stages of Japanese pearl oyster *Pinctada fucata martensii*. Harmful Algae
594 43, 1-12. doi:10.1016/j.hal.2014.12.004
595 Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful
596 approach to multiple testing. J. R. Stat. Soc. B 57(1), 289-300. doi:10.1111/j.2517-
597 6161.1995.tb02031.x
598 Berge, T., Hansen, P.J., Moestrup, Ø., 2008. Feeding mechanism, prey specificity and growth in
599 light and dark of the plastidic dinoflagellate *Karlodinium armiger*. Aquat. Microb. Ecol.
600 50, 279-288. doi:10.3354/ame01165
601 Berge, T., Poulsen, L.K., Moldrup, M., Daugbjerg, N., Hansen, P.J., 2012. Marine microalgae
602 attack and feed on metazoans. ISME J. 6, 1926-1936. doi:10.1038/ismej.2012.29
603 Brownlee, E.F., Sellner, S.G., Sellner, K.G., Nonogaki, H., Adolf, J.E., Bachvaroff, T.R., Place,
604 A.R., 2008. Responses of *Crassostrea virginica* (Gmelin) and *C. ariakensis* (Fujita) to
605 bloom-forming phytoplankton including ichthyotoxic *Karlodinium veneficum* (Ballantine).
606 J. Shellfish Res. 27(3), 581-591. Doi:10.2983/0730-
607 8000(2008)27[581:ROCVGA]2.0.CO;2
608 Castagna, M., Gibbons, M.C., Kurkowski, K., 1996. Culture: application. In: Kennedy, V.S.,
609 Newell, R.I.E., Eble, A.F. (Eds.), The Eastern Oyster: *Crassostrea virginica*. Maryland Sea
610 Grant, College Park, MD, pp. 675-690.
611 Deeds, J.R., Terlizzi, D.E., Adolf, J.E., Stoecker, D.K., Place, A.R., 2002. Toxic activity from
612 cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae)—a
613 dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful
614 Algae 1, 169-189. doi:10.1016/S1568-9883(02)00027-6

615 Delgado, M., Alcaraz, M., 1999. Interactions between red tide microalgae and herbivorous
616 zooplankton: the noxious effects of *Gyrodinium corsicum* (Dinophyceae) on *Acartia grani*
617 (Copepoda: Calanoida). J. Plankton Res. 21(12), 2361-2371. doi:10.1093/plankt/21.12.2361
618 Dorantes-Aranda, J.J., Seger, A., Mardones, J.I., Nichols, P.D., Hallegraeff, G.M., 2015.
619 Progress in understanding algal bloom-mediated fish kills: the role of superoxide radicals,
620 phycotoxins and fatty acids. PLoS ONE 10(7), e0133549.
621 doi:10.1371/journal.pone.0133549
622 Faust, M.A., 1974. Micromorphology of a small dinoflagellate *Prorocentrum marie-lebouriae*
623 (Parke and Ballantine) Comb. Nov. J. Phycol. 10, 315-322. doi:10.1111/j.1529-
624 8817.1974.tb02720.x
625 Fritz, L.W., Lutz, R.A., Foote, M.A., van Dover, C.L., Ewart, J.W., 1984. Selective feeding and
626 grazing rates of oyster (*Crassostrea virginica*) larvae on natural phytoplankton
627 assemblages. Estuaries 7(4), 513-518. doi:10.2307/1352056
628 Galimany E., Place, A.R., Ramón, M., Jutson, M., Pipe, R.K., 2008. The effects of feeding
629 *Karlodinium veneficum* (PLY # 103; *Gymnodinium veneficum* Ballantine) to the blue mussel
630 *Mytilus edulis*. Harmful Algae 7, 91-98. doi:10.1016/j.hal.2007.05.004
631 Glibert, P.M., Alexander, J., Merritt, D.W., North, E.W., Stoecker, D.K., 2007. Harmful algae
632 pose additional challenges for oyster restoration: impacts of the harmful algae *Karlodinium*
633 *veneficum* and *Prorocentrum minimum* on early life stages of the oysters *Crassostrea*
634 *virginica* and *Crassostrea ariakensis*. J. Shellfish Res. 26(4), 919-925. doi:10.2983/0730-
635 8000(2007)26[919:HAPACF]2.0.CO;2
636 Goshorn, D., Deeds, J.R., Tango, P., Poukish, C., Place, A.R., McGinty, M., Butler, W., Luckett,
637 C., Magnien, R., 2004. Occurrence of *Karlodinium micrum* and its association with fish kills

638 in Maryland estuaries. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A.
639 (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida
640 Institute of Oceanography, and Intergovernmental Oceanographic Commission of
641 UNESCO, St. Petersburg, FL, pp. 361-363.
642 Grzebyk, D., Denardou, A., Berland, B., Pouchus, Y.F., 1997. Evidence of a new toxin in the
643 red-tide dinoflagellate *Prorocentrum minimum*. J. Plankton Res. 19(8), 1111-1124.
644 doi:10.1093/plankt/19.8.1111
645 Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith,
646 W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New
647 York, NY, pp. 26-60.
648 Guillard, R. R. L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana*
649 Hustedt and *Detonula confervacea* Cleve. Can. J. Microbiol. 8, 229-239. doi:10.1139/m62-
650 029
651 Handy, S.M., Demir, E., Hutchins, D.A., Portune, K.J., Whereat, E.B., Hare, C.E., Rose, J.M.,
652 Warner, M., Farestad, M., Cary, S.C., Coyne, K.J., 2008. Using quantitative real-time PCR
653 to study competition and community dynamics among Delaware Inland Bays harmful algae
654 in field and laboratory studies. Harmful Algae. 7, 599-613. doi:10.1016/j.hal.2007.12.018
655 Heil, C.A., Glibert, P.M., Fan, C., 2005. *Prorocentrum minimum* (Pavillard) Schiller A review of
656 a harmful algal bloom species of growing worldwide importance. Harmful Algae 4, 449-470.
657 doi:10.1016/j.hal.2004.08.003
658 Kempton, J.W., Lewitus, A.J., Deeds, J.R., McHugh Law, J., Place, A.R., 2002. Toxicity of
659 *Karlodinium micrum* (Dinophyceae) associated with a fish kill in a South Carolina brackish
660 retention pond. Harmful Algae 1, 233-241. doi:10.1016/S1568-9883(02)00015-X

661 Kennedy, V.S., 1996. Biology of larvae and spat. In: Kennedy, V.S., Newell, R.I.E., Eble, A.F.
662 (Eds.), The Eastern Oyster *Crassostrea virginica.* Maryland Sea Grant College, University of
663 Maryland System, College Park, MD, pp. 371-422.
664 Lenth, R., Buerkner, P., Herve, M., Love, J., Riebl, H., Singmann, H., 2020. emmeans:
665 Estimated marginal means (aka Least-squares means). R package version 1.5.1.
666 https://cran.r-project.org/web/packages/emmeans/index.html
667 Li, A., Stocker, D.K., Adolf, J.E., 1999. Feeding, pigmentation, photosynthesis and growth of the
668 mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquat. Microb. Ecol. 19, 163-176.
669 doi:10.3354/AME019163
670 Li, A., Stoecker, D.K., Coats, D.W., 2000. Spatial and temporal aspects of *Gyrodinium*
671 *galatheanum* in Chesapeake Bay: distribution and mixotrophy. J. Plankton Res. 22(11),
672 2105-2124. doi:10.1093/plankt/22.11.2105
673 Li, Y., Sunila, I., Wikfors, G.H., 2012. Bioactive effects of *Prorocentrum minimum* on juvenile
674 bay scallops (*Argopecten irradians irradians*) are dependent upon algal physiological status.
675 Botanica marina 55, 19-29. doi:10.1515/bot.2011.123
676 Lin, C., Accoroni, S., Glibert, P.M., 2017. *Karlodinium veneficum* feeding responses and effects
677 on larvae of the eastern oyster *Crassostrea virginica* under variable nitrogen: phosphorus
678 stoichiometry. Aquat. Microb. Ecol. 79, 101-114. doi:10.3354/ame01823
679 Lin, L., Xu, C., 2020. Arcsine-based transformations for meta-analysis of proportions: pros,
680 cons, and alternatives. Health Science Reports 3(3), 1-6. doi:10.1002/hsr2.178
681 Liu, C., Cripe, T.P., Kim, M.O., 2010. Statistical issues in longitudinal data analysis for
682 treatment efficacy studies in the biomedical sciences. Mol. Ther. 18(9), 1724-1730.
683 doi:10.1038/mt.2010.127

684 Luckenbach, M.W., Sellner, K.G., Shumway, S.E., Greene, K., 1993. Effects of two bloom-
685 forming dinoflagellates, *Prorocentrum minimum* and *Gyrodinium uncatenum*, on the growth
686 and survival of the eastern oyster, *Crassostrea virginica* (Gmelin 1791). J. Shellfish Res. 12,
687 411–415.
688 Marshall, H.G., Egerton, T.A., 2009. Increasing occurrence and development of potentially
689 harmful algal blooms in Virginia tidal rivers. In: Fay, P. (Ed.), 2009 Virginia Water Research
690 Conference: Water Resources in Changing Climates. Virginia Water Resources Research
691 Center and Rice Center for Environmental Life Sciences, Richmond, VA, pp. 89-101.
692 Nielsen, M.V., Strømgren T., 1991. Shell growth response of mussels (*Mytilus edulis*) exposed to
693 toxic microalgae. Mar. Biol. 108, 263-267. doi:10.1007/BF01344341
694 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2020. nlme: Linear and nonlinear
695 mixed effects models. R package version 3.1.149. https://cran.r-
696 project.org/web/packages/nlme/nlme.pdf
697 Place, A.R., Brownlee, E.F., Nonogaki, H., Adolf, J.E., Bachvaroff, T.R., Sellner, S.G., Sellner,
698 K.G., 2008. Responses of bivalve molluscs to the ichthyotoxic dinoflagellate *Karlodinium*
699 *veneficum*. In: Moestrup, O. (Ed.), Proceedings of the 12th International Conference on
700 Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental
701 Oceanographic Commission of UNESCO, Copenhagen, pp. 5-8.
702 Place, A.R., Bowers, H.A., Bachvaroff, T.R., Adolf, J.E., Deeds, J.R., Sheng, J., 2012.
703 *Karlodinium veneficum*—The little dinoflagellate with a big bite. Harmful Algae 14, 179-
704 195. doi:10.1016/j.hal.2011.10.021

705 Poulsen, L.K., Moldrup, M., Berge, T., Hansen, P.J., 2011. Feeding on copepod fecal pellets: a
706 new trophic role of dinoflagellates as detritivores. Mar. Ecol. Prog. Ser. 441, 65-78.
707 doi:10.3354/meps09357
708 Rosetta, C.H., McManus, G.B., 2003. Feeding by ciliates on two harmful algal bloom species,
709 *Prymnesium parvum* and *Prorocentrum minimum*. Harmful Algae 2, 109-126.
710 doi:10.1016/S1568-9883(03)00019-2
711 Sheng, J., Malkiel, E., Katz, J., Adolf, J.E., Place, A.R., 2010. A dinoflagellate exploits toxins to
712 immobilize prey prior to ingestion. PNAS 107(5), 2082-2087. doi:10.1073/pnas.0912254107
713 Shumway, S.E., Burkholder, J.M., Springer, J., 2006. Effects of the estuarine dinoflagellate
714 *Pfiesteria shumwayae* (Dinophyceae) on survival and grazing activity of several shellfish
715 species. Harmful Algae 5, 442-458. doi:10.1016/j.hal.2006.04.013
716 Spero, H.J., Morée, M.D., 1981. Phagotrophic feeding and its importance to the life cycle of the
717 holozoic dinoflagellate, *Gymnodinium fungiforme*. J. Phycol. 17, 43-51. doi:10.1111/j.0022-
718 3646.1981.00043.x
719 Springer, J.J., Shumway, S.E., Burkholder, J.M., Glasgow, H.B., 2002. Interactions between the
720 toxic estuarine dinoflagellate *Pfiesteria piscicida* and two species of bivalve molluscs. Mar.
721 Ecol. Prog. Ser. 245, 1-10. doi:10.3354/meps245001
722 Stoecker, D., Adolf, J.E., Place, A.R., Glibert, P., Meritt, D., 2008. Effects of the dinoflagellates
723 *Karlodinium veneficum* and *Prorocentrum minimum* on early life history stages of the
724 eastern oyster (*Crassostrea virginica*). Mar. Biol. 154, 81–90. doi:10.1007/s00227-007-
725 0901-z

726 Tango, P.J., Magnien, R., Butler, W., Luckett, C., Luckenbach, M., Lacouture, R., Poukish, C.,
727 2005. Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake
728 Bay. Harmful Algae 4, 525-531. doi:10.1016/j.hal.2004.08.014
729 [VA DEQ] Virginia Department of Environmental Quality, 2014. Quality assurance project plan
730 (QAPP) for James River chlorophyll-α study. Special Study #14098. Retrieved from
731 https://www.deq.virginia.gov/Portals/0/DEQ/Water/WaterQualityStandards/James%20River%20C
732 hl%20A%20Study/SAP_Reports/QAPP_JR_CHLa_Study_with_signatures.pdf
733 Velikova, V., Larsen, J., 1999. The *Prorocentrum cordatum/Prorocentrum minimum* taxonomic
734 problem. Grana 38, 108-112. doi:10.1080/00173139908559219
735 Waggett, R.J., Tester, P.A., Place, A.R., 2008. Anti-grazing properties of the toxic dinoflagellate
736 *Karlodinium veneficum* during predator-prey interactions with the copepod *Acartia tonsa*.
737 Mar. Ecol. Prog. Ser. 366, 31-42. doi:10.3354/meps07518
738 Wikfors, G.H., 2005. A review and new analysis of trophic interactions between *Prorocentrum*
739 *minimum* and clams, scallops, and oysters. Harmful Algae 4, 585-592.
740 doi:10.1016/j.hal.2004.08.008
741 Wikfors, G.H., Smolowitz, R., 1995. Experimental and histological studies of four life-history
742 stages of the eastern oyster, *Crassostrea virginica*, exposed to a cultured strain of the
743 dinoflagellate *Prorocentrum minimum.* Biol. Bull. 188, 313–328. doi:10.2307/1542308
744 Yan, T., Zhou, M., Fu, M., Wang, Y., Yu, R., Li, J., 2001. Inhibition of egg hatching success and
745 larvae survival of the scallop, *Chlamys farreri*, associated with exposure to cells and cell
746 fragments of the dinoflagellate *Alexandrium tamarense*. Toxicon 39, 1239-1244. doi:
747 10.1016/S0041-0101(01)00080-0

748 Yang, H., Hu, Z., Xu, N., Tang, Y.Z., 2019. A comparative study on the allelopathy and toxicity
749 of four strains of *Karlodinium veneficum* with different culturing histories. J. Plankton Res.
750 41(1), 17-29. doi:10.1093/plankt/fby047
751 Yang, H., Hu, Z., Shang, L., Deng, Y., Tang, Y.Z., 2020. A strain of the toxic dinoflagellate
752 *Karlodinium veneficum* isolated from the East China Sea is an omnivorous phagotroph.
753 Harmful Algae. 93, 101775. doi:10.1016/j.hal.2020.101775

Table 1

Response variables and treatments for the single and dual HAB bioassays, and the feed algae study.

Experiment	Response variables	Treatments[a]	Algal Species	Cell Concentration (cells/mL)
Single HAB Bioassay	Larval oyster mortality	Fed (Pav)[b]	*Pavlova pinguis*	25,000
		Unfed[b]	None	0
	Behavioral observations	Karlo 1,000	*Karlodinium veneficum*	1,000
		Karlo 5,000		5,000
	Larval oyster immotility	Karlo 10,000		10,000
		Karlo 50,000		50,000
		Proro 100	*Prorocentrum cordatum*	100
		Proro 5,000		5,000
		Proro 10,000		10,000
		Proro 50,000		50,000
Dual HAB Bioassay	Larval oyster mortality	Fed (Pav)[b]	*Pavlova pinguis*	25,000
		Unfed[b]	None	0
	Behavioral observations	Karlo 1,000[b]	*Karlodinium veneficum*	1,000
		Proro 50,000[b]	*Prorocentrum cordatum*	50,000
		Karlo 1,000 X Proro 50,000	*Karlodinium veneficum*	1,000
			& *Prorocentrum cordatum*	50,000
Feed Algae Study	*P. pinguis* cell concentration	Fed (Pav)[b]	*Pavlova pinguis*	25,000
		Karlo 1,000 X Fed (Pav)	*Karlodinium veneficum*	1,000
			& *Pavlova pinguis*	25,000

[a] Pav = *Pavlova pinguis*, Karlo = *Karlodinium veneficum*, Proro = *Prorocentrum cordatum*, numbers represent cell concentrations in cells/mL

[b] Control treatments within each experiment

Table 2

Karlodinium veneficum and *Prorocentrum cordatum* detected in samples from a commercial oyster hatchery in the lower Chesapeake Bay, Virginia, USA.

Date		Sample Type	Cell Concentration (cells/mL) *Karlodinium veneficum*[a]		*Prorocentrum cordatum*[b]	
2014	May 12	Mixed-media	506		579	
		10 µm-filtered water	30		< 1	
		Feed algae	< 1		< 1	
	May 19	Mixed-media	60		3,630	*
		10 µm-filtered water	67		2,940	*
	May 20	10 µm-filtered water	144		192	
		Feed algae	167		716	
	May 28	Feed algae	< 1		< 1	
	June 4	Mixed-media	15		11	
		10 µm-filtered water	9		8	
		Feed algae	< 1		< 1	
	June 13	Mixed-media	1,094	*	767	
		Feed algae	0		0	
2016	March 11	10 µm-filtered water	19		7	
	March 24	10 µm-filtered water	185		3	
	April 11	1 µm-filtered water	937		9	
	April 18	1 µm-filtered water	163		6	

[a] All samples were quantified using quantitative real-time PCR

[b] 2014 samples were quantified using cell counts by light microscopy, 2016 samples were quantified using quantitative real-time PCR

* bloom cell concentration (> 1,000 cells/mL)

Table 3

Immotility and survival of larval oysters in the single HAB bioassay.

Treatments[a]	24 hours			96 hours		
	Average ***% surviving***	**Average** ***% immotile***[b]		**Average** ***% surviving***	**Average** ***% immotile***[b]	
Fed (Pav)	100.0 (0.0)	10.8 (3.2)		99.1 (0.9)	10.8 (4.0)	
Unfed	100.0 (0.0)	10.1 (4.9)		100.0 (0.0)	5.7 (2.5)	
Karlo 1,000	98.1 (1.3)	37.8 (4.1)	*	79.0 (4.8)	42.0 (6.0)	*
Karlo 5,000	90.9 (2.8)	70.0 (3.6)	*	6.6 (1.9)	100.0 (0.0)	*
Karlo 10,000	85.0 (3.6)	79.3 (3.7)	*	15.5 (2.8)	100.0 (0.0)	*
Karlo 50,000	78.0 (4.6)	94.0 (5.0)	*	16.9 (4.6)	79.2 (12.9)	*
Proro 100	100.0 (0.0)	47.3 (7.4)	*	96.3 (2.7)	14.2 (4.3)	
Proro 5,000	99.0 (1.0)	33.2 (5.6)		96.1 (2.2)	13.6 (4.8)	
Proro 10,000	100.0 (0.0)	17.9 (4.7)		99.0 (1.0)	8.9 (2.4)	
Proro 50,000	100.0 (0.0)	18.1 (2.6)		92.9 (3.0)	55.3 (6.4)	*

Values indicate the treatment average with standard error given in parentheses.
Sample size was n = 10 wells per treatment, except for average *% immotile* values at 96 hours for $Karlo_{5,000}$ (n = 6), $Karlo_{10,000}$ (n = 9), and $Karlo_{50,000}$ (n = 8), due to the exclusion of wells with 0 *% surviving*.
[a] Pav = *Pavlova pinguis*, Karlo = *Karlodinium veneficum*, Proro = *Prorocentrum cordatum*, numbers represent cell concentrations (cells/mL)
[b] Percentage of surviving larval oysters that were immotile
*Value significantly different from the Fed and Unfed control *% immotile* values (Dunn, $\alpha = 0.05$)

Table 4

Least-squares means of arcsine-transformed cumulative larval mortality in the single HAB bioassay.

Treatments*	24 hours	48 hours	72 hours	96 hours
Fed (Pav)	0.00 a	0.00 c	0.00 e	0.03 h
Unfed	0.00 a	0.00 c	0.00 e	0.00 h
Karlo 1,000	0.06 a	0.08 c	0.22 e	0.44 i
Karlo 5,000	0.24 a b	0.49 d	0.74 f	1.37 j
Karlo 10,000	0.35 b	0.53 d	0.99 g	1.19 j
Karlo 50,000	0.46 b	0.66 d	0.83 f g	1.20 j
Proro 100	0.00 a	0.04 c	0.07 e	0.09 h
Proro 5,000	0.03 a	0.06 c	0.08 e	0.11 h
Proro 10,000	0.00 a	0.00 c	0.03 e	0.03 h
Proro 50,000	0.00 a	0.03 c	0.06 e	0.19 h

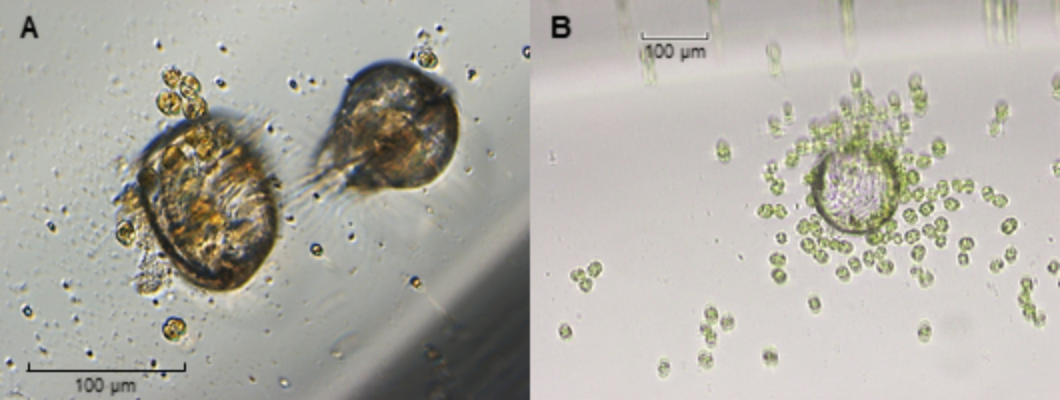
Standard error = 0.047 for all reported least-squares means values.
Letters denote groups of treatments that were or were not significantly different from one another based on Tukey's post hoc pairwise comparisons calculated within each level of time (Tukey-Bonferroni adjusted $\alpha = 0.0125$).
* Pav = *Pavlova pinguis*, Karlo = *Karlodinium veneficum*, Proro = *Prorocentrum cordatum*, numbers represent cell concentrations (cells/mL)

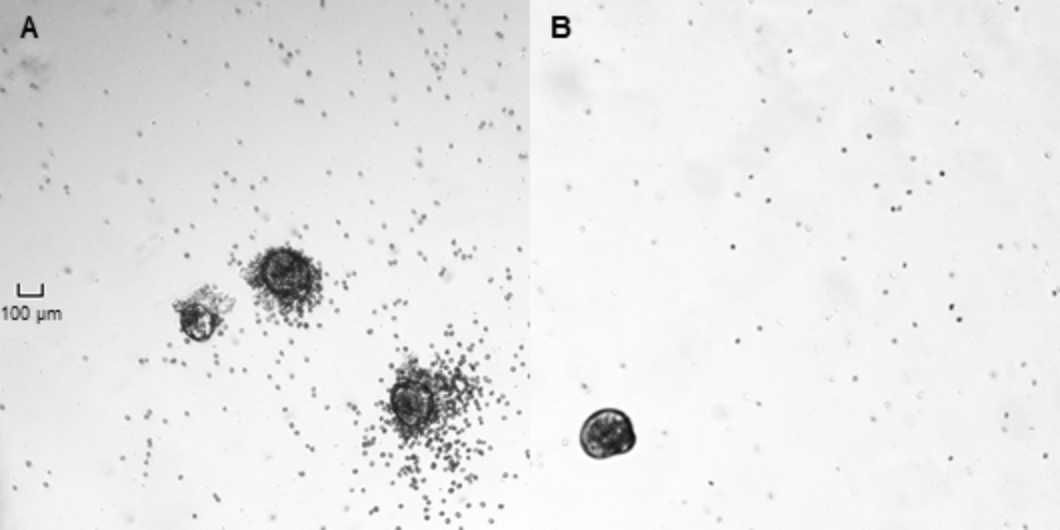
Table 5

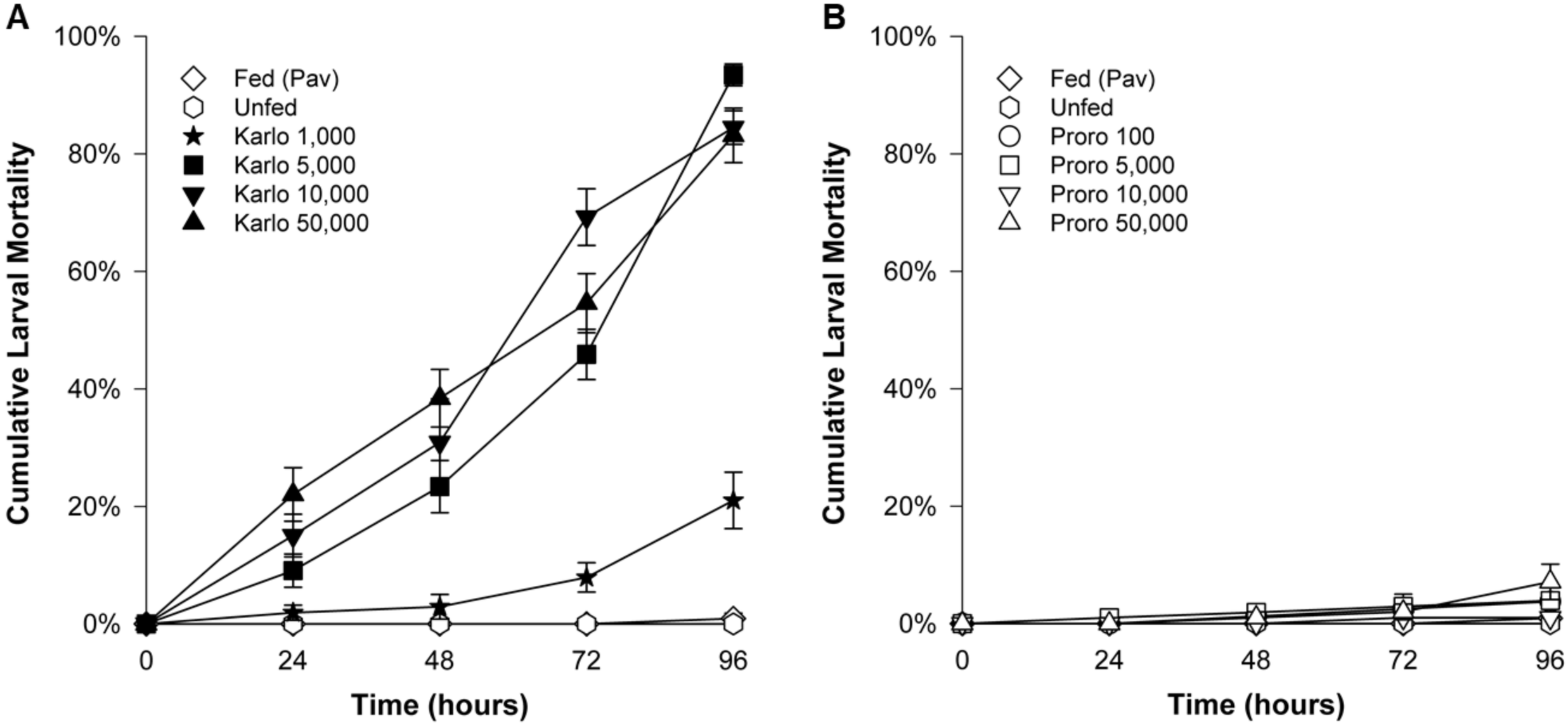
Least-squares means of arcsine-transformed cumulative larval mortality in the dual HAB bioassay.

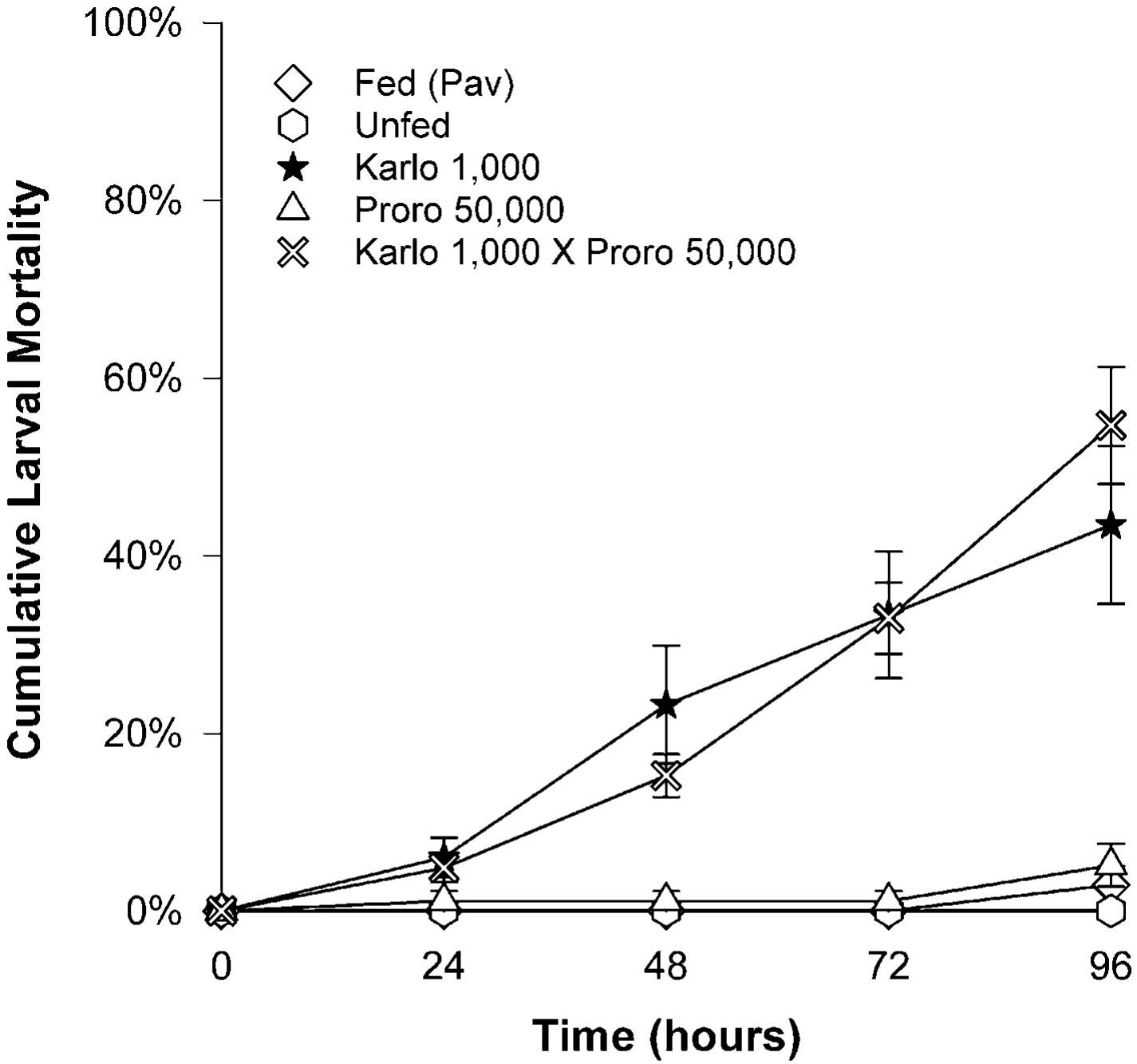
Treatments*	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Fed (Pav)	0.00 a	0.00 b	0.00 d	0.08 f
Unfed	0.00 a	0.00 b	0.00 d	0.00 f
Karlo 1,000	0.18 a	0.44 c	0.56 e	0.67 g
Proro 50,000	0.03 a	0.03 b	0.03 d	0.14 f
Karlo 1,000 X Proro 50,000	0.16 a	0.38 c	0.60 e	0.83 g

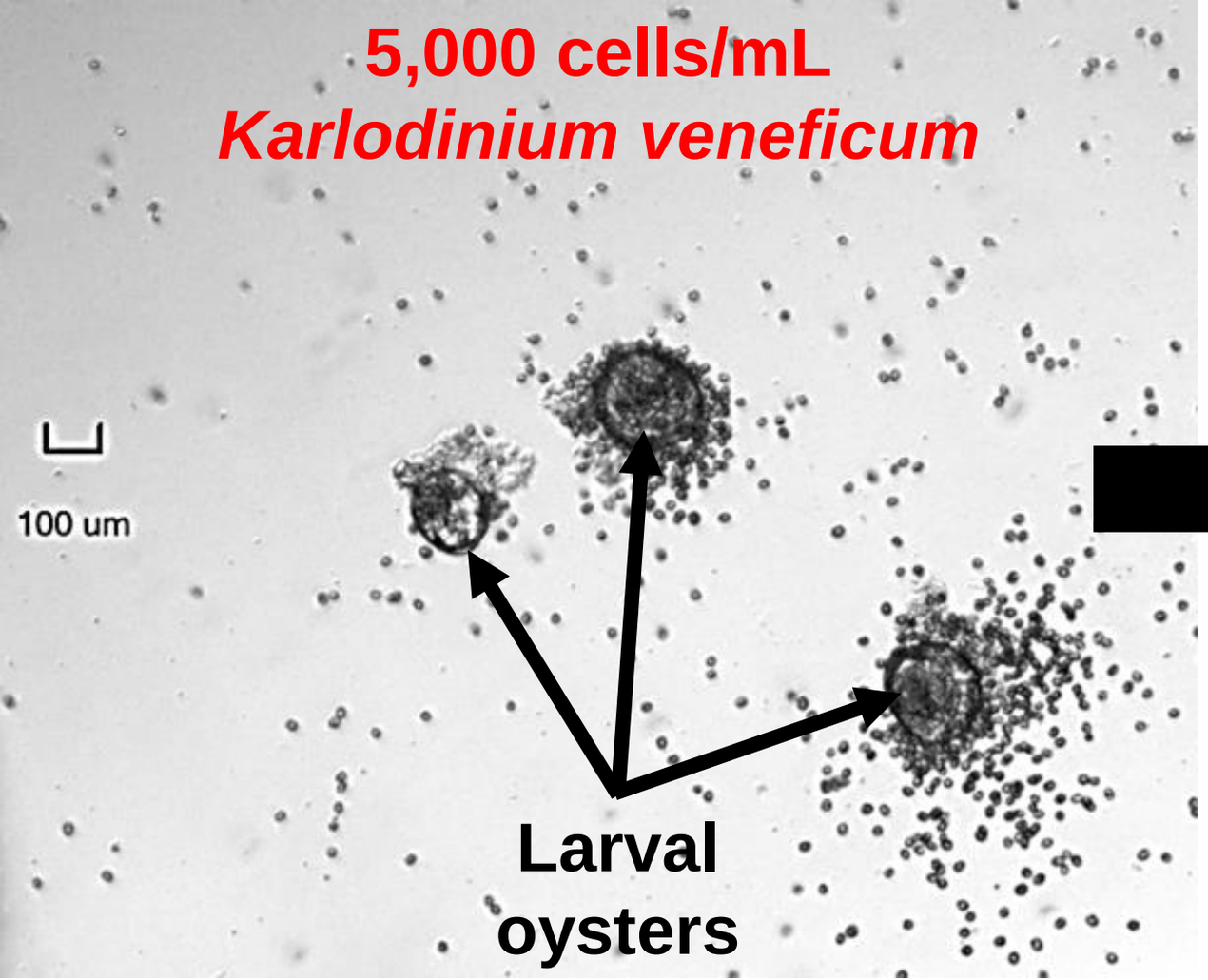
Standard error = 0.056 for all reported least-squares means values.
Letters denote groups of treatments that were or were not significantly different from one another based on Tukey's post hoc pairwise comparisons calculated within each level of time (Tukey-Bonferroni adjusted $\alpha = 0.0125$).
* Pav = *Pavlova pinguis*, Karlo = *Karlodinium veneficum*, Proro = *Prorocentrum cordatum*, numbers represent cell concentrations (cells/mL)

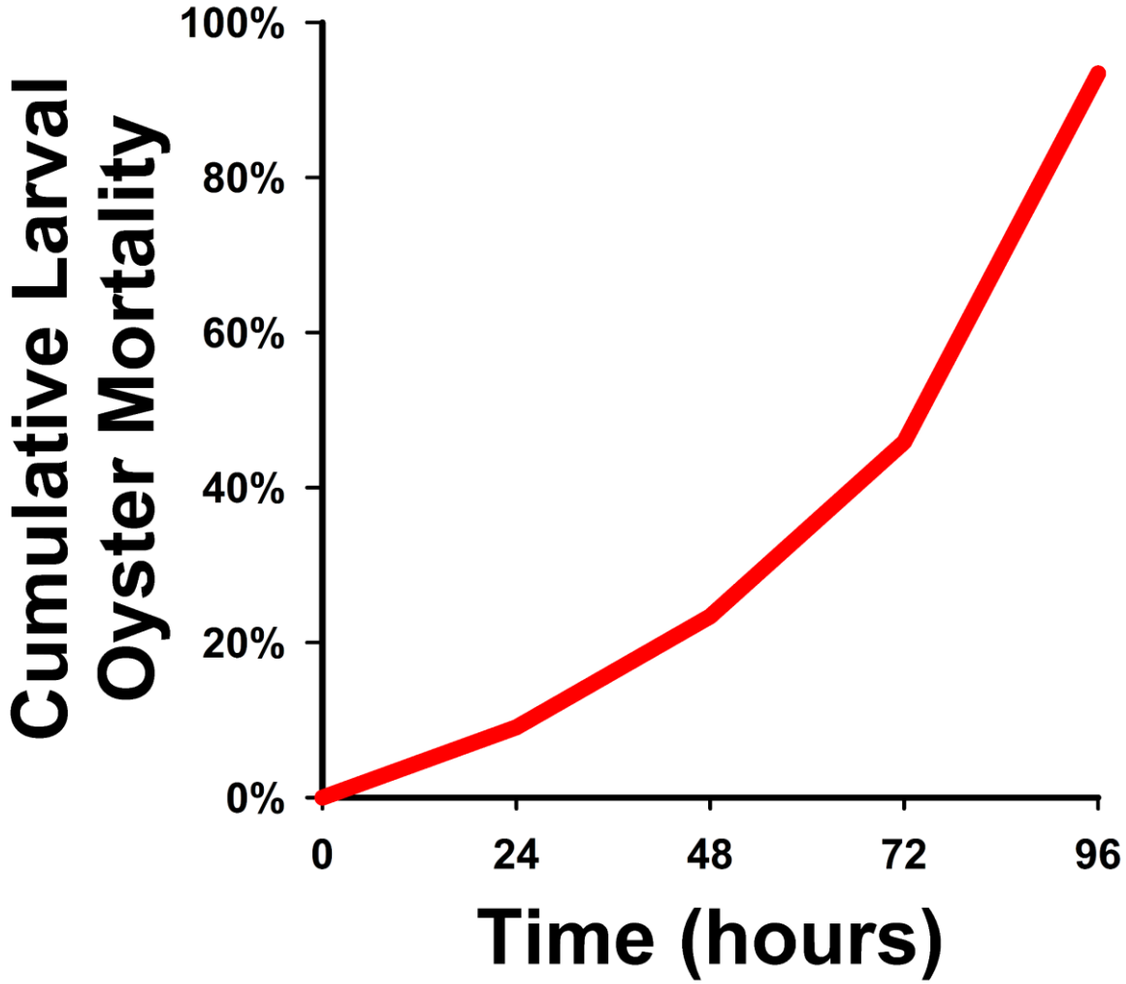

A
100 µm
B
100 µm


A
B
100 µm


A
100%
80%
60%
40%
20%
0%
Cumulative Larval Mortality
Fed (Pav)
Unfed
Karlo 1,000
Karlo 5,000
Karlo 10,000
Karlo 50,000
0
24
48
72
96
Time (hours)
B
100%
80%
60%
40%
20%
0%
Cumulative Larval Mortality
Fed (Pav)
Unfed
Proro 100
Proro 5,000
Proro 10,000
Proro 50,000
0
24
48
72
96
Time (hours)


100%
80%
60%
40%
20%
0%
Cumulative Larval Mortality
Fed (Pav)
Unfed
Karlo 1,000
Proro 50,000
Karlo 1,000 X Proro 50,000
0
24
48
72
96
Time (hours)


5,000 cells/mL
Karlodinium veneficum
100 um
Larval
oysters


Cumulative Larval
Oyster Mortality
100%
80%
60%
40%
20%
0%
0
24
48
72
96
Time (hours)