Hatchery crashes among shellfish research hatcheries along the Atlantic coast of the United States: a case study at Horn Point Laboratory oyster research hatchery

Matthew W. Gray[1]*, Stephanie Alexander[1], Brian Beal[2]; Tom Bliss[3]; Colleen A. Burge[4]; Jacob Cram[1]; Michael De Luca[5]; James Dumhart[6]; Patricia M. Glibert[1]; Michael Gonsior[7]; Andrew Heyes[7]; Klaus Huebert[1]; Vyacheslav Lyubchich[7]; Katherine McFarland[8]; Matt Parker[9]; Louis Plough[1]; Eric Schott[10]; Lisa Wainger[7]; Gary H. Wikfors[8]; Ami Wilbur[11]

1. Horn Point Laboratory, University of Maryland Center for Environmental Science, 2020 Horns Point Road, Cambridge, MD 21613, USA
2. Downeast Institute, 39 Wildflower Lane, Beals, ME 04611, USA
3. Marine Extension Service, University of Georgia, Shellfish Research Laboratory, 20 Ocean Science Circle, Savannah, Georgia 31411, USA
4. Institute of Marine and Environmental Technology, University of Maryland Baltimore County, 701 E Pratt Street, Baltimore, MD 21202, USA
5. Aquaculture Innovation Center, Rutgers University, 3920 Bayshore Drive, Cape May, NJ 08204, USA
6. Maryland Department of Natural Resources, Piney Point Aquaculture Center, P.O. Box 150, 17996 Piney Point Rd, Piney Point, MD 20674
7. Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, 146 Williams St. Solomons, MD 20688, USA
8. NOAA Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Avenue, Milford, CT 06460, USA
9. University of Maryland Extension, 6707 Groveton Drive, Clinton, MD 20735, USA
10. IMET, University of Maryland Center for Environmental Science, 701 East Pratt St., Baltimore, MD, 21202, USA
11. Shellfish Research Hatchery, Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K Moss Lane, Wilmington, NC 28409

*Contact information: mgray@umces.edu; +1 410.221.8348

Citation:
M.W. Gray, S.T. Alexander, B.F. Beal, T. Bliss, C.A. Burge, J.A. Cram, M. De Luca, J. Dumhart, P.M. Glibert, M. Gonsior, A. Heyes, K.B. Huebert, V. Lyubchich, K. McFarland, M. Parker, L.V. Plough, E.J. Schott, L.A. Wainger, G.H. Wikfors, A. E. Wilbur (2022). Hatchery crashes among shellfish research hatcheries along the Atlantic coast of the United States: A case study at Horn Point Laboratory oyster research hatchery. *Aquaculture* 546:737259
https://doi.org/10.1016/j.aquaculture.2021.737259

Abstract

Shellfish hatcheries have become an increasingly important component of aquaculture production in the United States. Although the industry has been advancing technologically over time to stabilize production and supply, many hatcheries suffer regularly from bouts of stalled or failed production, termed crashes. Crashes are widely acknowledged to occur and are considered a persistent problem in the industry but also an understudied phenomenon in the field of shellfish aquaculture that warrants greater investigation. Furthermore, there are few thorough reports on production variability from established hatcheries. To help fill the data gap and initiate a broader discussion on the causes of hatchery crashes, we provide testimonials from research hatchery managers across the Atlantic Coast about their experiences with crashes. As a case study, we report on long-term production trends (2011-2020) at Horn Point Laboratory's oyster hatchery, which included persistent production failure during the 2019 season. During the 2019 season, larval assays were conducted to determine drivers of production failure; however, no clear culprits were identified. Machine learning was used to help characterize production variability and hindcast the specific conditions when the hatchery's production was most efficient. Microbial community structure of larval associated microorganisms was shown to differ between a crash and non-crash time-point. We highlight the ubiquity of hatchery crashes along the Atlantic Coast of the US, the range of severity at which crashes can occur, and the difficulty of identifying the underlying causes of crashes, even at world-class research facilities. Collectively, we conclude that more research, data sharing, and cross-institution collaboration are needed to prevent crashes, and to develop mitigation strategies to maintain high levels of consistent shellfish aquaculture production.

Keywords: hatchery, bivalve, production, *C. virginica*, seed supply, larvae, machine learning

1.1 Introduction

The United States is the 3rd largest global consumer of seafood and imported $402 million (155 million pounds) of bivalves in 2019 to meet domestic demand for oysters, mussels, scallops, and clams (USDA, 2019). To reduce these trade deficits and relieve harvest pressure on wild stocks, federal (e.g. NOAA, USDA) and state agencies have promoted shellfish production along all US coastlines. Although regional production rates may vary greatly (NOAA, 2016) because of a variety of factors (environmental conditions, regulations, supply chains, etc.), larval supply underpins cultured production of oysters nationally (Ekstrom et al., 2015). Aside from a few distinct bays (e.g. Willapa Bay, WA), hatchery production of oyster larvae and seed has become increasingly important to fuel domestic aquaculture production (Barton et al., 2015) in the US and plays an important role for enhancing wild fisheries and restoration efforts in many coastal bays and estuaries (Hornick and Plough, 2019). Decades of research on larval diets, water treatment, and

culture practices have led to large improvements in the production rates and efficiencies of shellfish hatcheries (Elston et al., 1981; Helm and Millican, 1977; Lewis et al., 1988; Robert and Gérard, 1999; Urban Jr and Langdon, 1984). These facilities are now responsible for reliably supplying high-quality larvae and seed to a growing aquaculture industry.

Although hatchery staff may be highly skilled, most facilities still experience periods of poor larval growth or mass mortality events, termed crashes, which have been a perpetual problem in the industry (Walker, 2017). Crashes are recognized as a world-wide industry phenomenon not unique to oysters but have been observed among a variety of cultivated shellfish species (Jones, 2006). Crashes may occur over short timescales with entire batches (tens of millions) of larvae lost overnight. Upon inspection of tanks in the morning after a crash, managers may observe larvae to be thickly coated with bacteria or filled with ciliates, making it difficult to determine if these microorganisms were the cause of the crash or opportunistic invaders that exploited the compromised batch (Estes et al., 2004). Aside from bacteria, there are a wide variety of potential chemical contaminants or other biological agents that could quickly degrade culture conditions, such as toxic or inhibitory metals, organic or inorganic chemicals coming from natural or anthropogenic sources (e.g. terrestrial agriculture), and poor water quality (see mini-review by Jones 2006). Furthermore, all of these sources have several opportunities to disrupt hatchery production, as they can affect broodstock conditioning, algal culture, or the larvae themselves in culture tanks. Without effective diagnosis, developing mitigation or avoidance strategies can be a daunting task. Indeed, hatchery staff rarely have the time or resources to investigate the causes of crashes in their facilities. Instead, the most common approach to coping with crashes is to simply dump ‘bad water’, clean tanks and other equipment, and hope that tanks can be filled with ‘good water’ so that production can resume. Bad water that reduces larval growth and survival, such as from coastal upwelling of acidic water, can linger in coastal margins for a long time (i.e. weeks to months) and potentially re-enter the hatchery repeatedly over the production season (Barton et al., 2012).

Crashes are costly as they result in loss of revenue while wasting labor and other production resources. These losses can reverberate throughout the industry. Larvae and seed supply is already among the top concerns among some growers (Langston, 2015; Lukenbach et al., 2008), and weak hatchery production only exacerbates these concerns and may deter new entrants into the industry. The ‘Seed Crisis’ observed in the western USA oyster industry is a notable example. During the late 2000s, commercial hatcheries struggled to produce oyster larvae for years, which ultimately resulted in an estimated industry loss of $110 million in the Pacific Northwest (Ekstrom et al., 2015).

Although crashes in shellfish hatcheries are acknowledged to occur and considered common, they also represent one of the unspoken failures in current shellfish cultivation. Crashes remain under-investigated and mostly unexplained. A major impediment to exploring crashes is the understandable lack of willingness among private hatcheries to divulge production failures. Indeed, through our informal surveying of the private industry, we found that many hatcheries were interested in learning how to avoid or mitigate crashes but were reluctant to share data or publicly discuss production failures occurring at their facilities. In contrast to private hatcheries, shellfish research hatcheries, which also suffer from crashes, produce larvae for a variety of purposes other than aquaculture (e.g., research, restoration, fisheries enhancement) and often carry the mandate of sharing information with the public.

Here we highlight the spatial and temporal variability of crashes among a network of research hatcheries along the Atlantic Coast of the US (**Fig. 1**). The University of Maryland Center for Environmental Science (UMCES) Horn Point Laboratory oyster hatchery (HPLOH) is the largest hatchery supplier of oyster larvae and seed on the Atlantic Coast. Annually, it produces billions of pediveliger larvae, typically between the months of April and September, for a variety of purposes in the Chesapeake Bay, including oyster sanctuary restoration, replenishment of the public fishery, and supplying eyed larvae for the rapidly growing aquaculture industry in Maryland and Virginia (UMCES Horn Point Laboratory, 2020).

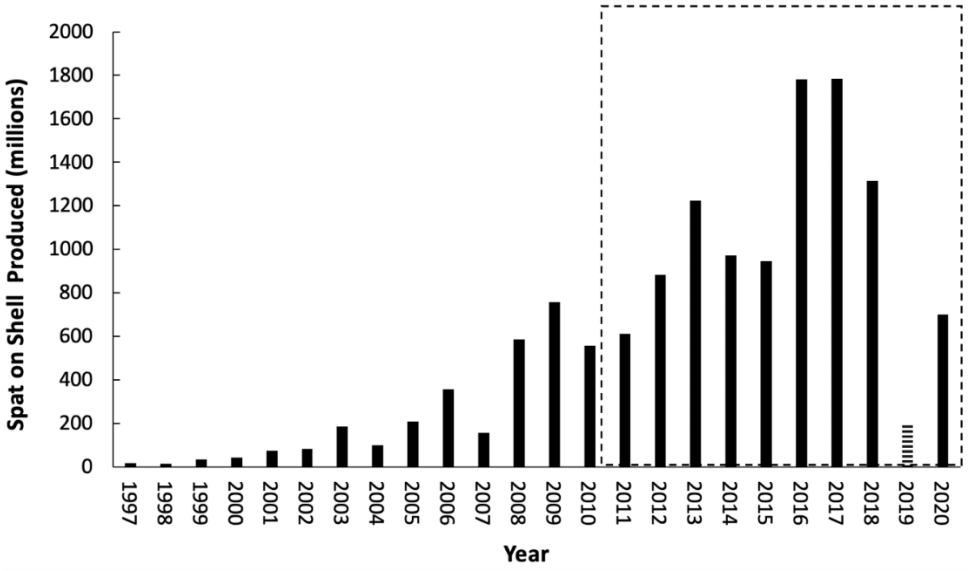


Figure 1. Total production of spat on shell at the University of Maryland Center for Environmental Science Horn Point Laboratory oyster hatchery over time. Period with available hatchery records is denoted by dashed box. Persistent production failure during 2019 is highlighted with horizontal stripes. Reduced production in 2020 was due to the COVID-19 pandemic and depressed demand and related production challenges.

At these large production scales (mean batch size = 80 million eyed larvae; $300/million diploid wild larvae), a single crash results in approximately $24,000 in lost revenue. This loss estimate excludes accounting for ancillary expenses associated with production, such as wasted labor and other resources (e.g., seawater, electricity, algae and reagents). Although the HPLOH is fully equipped and staffed with highly experienced personnel, some of whom hold advanced degrees in science, periodic crashes of oyster larvae have occurred annually.

Most recently, during the 2019 production season, HPLOH was unable to produce larvae for ⅓ of the production season (late May to early August). Harmful algal blooms in the Chesapeake Bay have been suspected as frequent contributors to hatchery crashes. Other factors such as harmful bacteria, organic pollutants, poor water quality, and low salinity events also may impair production, given the hatchery's location within the mesohaline portion of this bay. Although production failure was linked to historic rainfall and low salinity conditions for months preceding the 2019 season (examined and described below), no clear causes or factors have yet been identified to explain the prolonged crashes in 2019.

146 Aside from HPLOH, production crashes also vary greatly among our network of research
147 hatcheries along the Atlantic Coast (**Fig. 2**). For brevity, we have not included site-specific
148 environmental details, which would show differences among facilitates due to their latitude,
149 proximity to the ocean, surrounding land use, etc; however, it is important to acknowledge that
150 despite these differences, some forms of crashes have occurred at all the listed facilities (**Table 1**).
151 Interestingly, the closest hatchery to HPLOH (co-located in the mesohaline portion of Chesapeake
152 Bay) also suffers from periodic crashes but at asynchronous periods at HPLOH. Starting furthest
153 away from HPLOH, the Downeast Institute (DEI; Maine) has difficulty culturing microalgae in
154 August and many times in January/February. Oyster larvae production problems occur periodically
155 at other times of the year and can lead to entire losses of larval batches, which seem to be linked
156 to disease outbreaks (i.e. *Vibrio* spp). DEI hypothesizes that these crashes are associated with
157 strong winds that suspend pathogens embedded in sediments that are pumped into the hatchery.
158 A new threat to production at DEI is a marine fungus that creates 'pink blobs' which, after
159 appearing on tank walls, are followed by complete loss of larvae within 2-3 days. Crashes at DEI
160 slow aquaculture research progress and delay delivery of oysters and clams purchased by local
161 growers and communities that use DEI seed to manage their stocks. Rutgers University's
162 Aquaculture Innovation Center (AIC; New Jersey) typically observes crashes annually for 2-4
163 weeks during late May to early June driven by a mysterious cause. Crashes of oyster larvae and
164 juveniles at AIC represent a substantial burden on annual seed production upon which many
165 commercial farmers rely. During the entire 2018 season (March through July), AIC had persistent
166 low larval survival (< 10%), crippling production despite numerous attempts to triage and prevent
167 crashes (including extra cleaning, water filtration, probiotics, etc.). The precise causes of the
168 season-long crash at AIC were never determined. After thorough cleaning in the 2018 offseason,
169 AIC reported few production issues in 2019. University of Georgia's Shellfish Laboratory (UGA)
170 was created in 2015 to help revive the state's oyster aquaculture industry through research and
171 extension. This research laboratory has also been a valuable resource regionally, supplying seed

to South Carolina after a local, private hatchery ceased production. For several weeks in August of 2017, UGA noticed late-stage (7-9 days post fertilization) larval mortality. Late-stage mortality is less common than early-stage mortalities in hatcheries but is particularly costly because of the amount of resources invested prior to failure. NOAA's Northeast Fisheries Science Center (NEFSC; Connecticut) has stable production, but reported relatively slow larval growth and poor setting after July annually. This facility does not supply oysters to the local shellfish industry but instead uses these larvae for research purposes. University of North Carolina Wilmington's Shellfish Research Hatchery has experienced production swings historically and episodic crashes during 2010-2013. Since this time, however, they have had stable and reliable production, which they linked to optimizing temperatures for larval growth and survival within their hatchery. These anecdotal observations illustrate how hatchery crashes are common, originate from many potential sources, and remain largely unresolved.

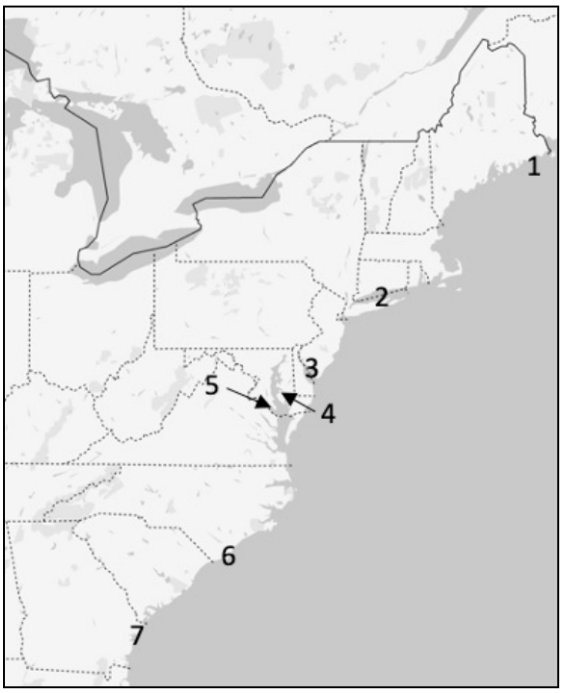


Figure 2. Atlantic research hatchery network member locations: 1) Downeast Institute (ME), 2) NOAA Northeast Fisheries Science Center (CT), 3) Rutgers University's Aquaculture Innovation Center (NJ), 4) UMCES Horn Point Laboratory, 5) MD DNR Piney Point Hatchery, 6) UNC Wilmington's Hatchery (NC), 7) University of Georgia's Shellfish Laboratory (GA).

In this study, we sought to examine the phenomenon of crashes at HPLOH by mining the detailed, long-term hatchery production dataset at HPLOH. Since 2011, HPLOH has monitored and recorded a vast array of production data including broodstock conditioning, food ration, and production rates. These production data alone are interesting as there are few examples of long-term hatchery-scale shellfish production data that have been published and available for scrutiny by scientists or the public. In addition to production data, a series of larval assays and other investigations were conducted by the hatchery staff and other researchers during short-term crashes (2018) and persistent crashes (2019). These studies provide insight into the complexities limiting identification of drivers of hatchery crashes so that mitigation strategies may be developed. We conclude this work by identifying primary knowledge gaps surrounding hatchery crashes and their impact on the shellfish aquaculture industry. We also, however, offer a path forward for future investigations and highlight emerging technology for improving hatchery performance or increasing flexibility to maintain production in the face of adverse environmental conditions.

Table 1. Shellfish research hatcheries in our study network with their specific location, crash symptom, and suspected culprit. HPLOH refers to the Horn Point Laboratory Oyster Hatchery.

Facility Name	Location	Crash Symptoms	Culprit
Horn Point Laboratory Oyster Hatchery	Cambridge, Maryland	Periodic crashes of new batches or slowed growth Prolong failure in 2019.	Unknown
Downeast Institute	Beals, Maine	Periodic crashes of new batches; complete loss of all larvae	Benthic pathogens/fungus
Rutger's Aquaculture Innovation Center	Cape May, New Jersey	Periodic crashes in late May Persistent failure in 2018	Unknown
Piney Point Oyster Hatchery	Piney Point, Maryland	Periodic, asynchronous failure with HPLOH	Unknown
University of Georgia	Savanah, Georgia	Late-stage mortality in 2017	Unknown
NOAA Northeast Fisheries Science Center	Milford, Connecticut	Slow larval growth and poor setting in July	Unknown
University of North Carolina	Wilmington, North Carolina	Production swings from 2010-2013	Suboptimal temperatures

2.1 Methods

We used a variety of methods to examine hatchery crashes at HPLOH, including oyster larvae developmental assays, microbial community analysis and sequencing, meta-analysis of production records, and a suite of exploratory water-quality analyses. Statistical analysis of production trends also was performed to examine production bottlenecks and to identify environmental and culture conditions that drive production yield (defined below).

2.2 Study site

HPLOH is located adjacent to the Choptank River (38°35’06.92N, 76°05’08.45”W), which is the largest tributary of the Chesapeake Bay on the eastern shore and located in the mesohaline portion of the Chesapeake Bay.

2.3 Choptank River water quality data

Archived real-time water quality data, used to examine how bay conditions affect hatchery production retrospectively, were obtained from the Goose Reef Buoy (GRB) located in the main stem of Chesapeake Bay (Maryland Dept. of Natural Resources Station XEF3551) (**Fig. 3**). GRB has been collecting data at hourly intervals since 2008. Water-quality variables monitored by GRB dissolved oxygen, water temperature, salinity, pH, chlorophyll, and turbidity. Long-term (1986-2020), monthly water-quality data were downloaded from a fixed monitoring station located upstream from the hatchery (Maryland Dept. of Natural Resources Station ET5.2, Choptank River). These data were used to examine how 2019 water conditions differed from historical trends.

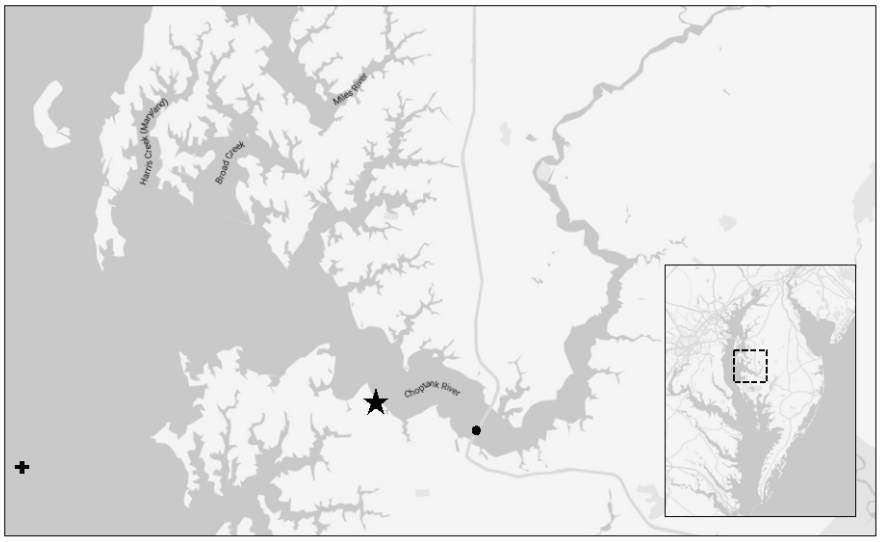


Figure 3. Location of Horn Point Laboratory oyster hatchery on the Choptank River (star) in the mesohaline portion of the Chesapeake Bay. Water quality monitoring stations: Maryland Dept. of Natural Resources Station on the Choptank River (circle) and the Goose Reef Buoy in the main stem of the Chesapeake Bay (cross).

2.4 HPLOH hatchery water-quality data and larval culture

Within the hatchery, the staff measures water temperature, salinity, and pH daily with a handheld sonde (YSI pro1030). Since 2016, the hatchery crew has been monitoring (with intermittent data gaps) alkalinity from weekly titrations (n = 193; SI Analytics TitroLine 6000) during the production season. Aragonite saturation state (Ωar) was determined using carbonate system variables (pH and alkalinity), for seawater temperature and salinity, and entered into carbonate calculating software (CO2SYS). For the past 3 years, the hatchery staff has used carbonate data and additions of Na_2CO_3 to alter tank chemistry to ensure tank Ωar and pH are above 1 and 8, respectively. Larval cultures are reared at 28-30ºC and maintained by the air temperature of the hatchery itself. Salinity of larval cultures ranged throughout the season; however, a minimum salinity of 10 ppt is maintained with additions of artificial salt mix (Crystal Sea MarineMix). Afterward, there is no further manipulation of larval culture water quality.

2.5 Production data

Long-term hatchery production data (2011-2019) were obtained from HPLOH hatchery data archives. These records include a wide variety of information around the broodstock, spawning, and larval production process in the hatchery. For brevity, we list most but not all data that were collected to illustrate the detail of these records as well as list the numerous variables that initially were considered relevant to our investigation.

- ***Broodstock data***: origin of broodstock (river and bar location); temperature, salinity, and pH information (min, max, average) during conditioning.
- ***Spawning data***: stimulus used to initiate spawning, length of time for first female to release eggs, length of time for first male to release sperm, number of oysters used for spawn, sex ratio, % females spawned, average shell height of females in spawn, eggs produced, fecundity (average number of eggs per spawned female); subjective visual assessments: general condition (0 - watery and transparent to 4 - full body filling shell cavity with visible organs); gonadal index (0 - no visible gonadal development to 4 - follicles clearly defined to engorged); egg quality (0 - no eggs to 4 - very well developed); sperm mobility (0 - no sperm to 4 - sperm present and extremely active).
- ***Production data***: total eggs added to tank, age at first tank drain, survival to prodissoconch I (D-hinge), days to first eyed veliger stage, total eyed veligers produced. Additional notes within production data explicitly stated if a batch crashed, was discarded, or combined with another batch because of slow growth.

Every spawn and resulting brood were tracked continuously through the entire production process unless it crashed or was combined and mixed with another batch of larvae. For our investigation, we excluded mixed-broods to avoid confounding factors among batches. This enabled us to link hatchery production of larvae back to the specific environment and husbandry under which they were cultured, spawned, and their parents were conditioned.

275 Long-term production data were analyzed for two performance metrics: production yield and
276 production rate. Yield, with its potential range from 0 (crash) to 100% (all eggs produced
277 competent larvae and no mortality observed), described the ability of the hatchery to usher larvae
278 through the production process and was defined as the ratio of outputs to inputs:

279

280 $$Yield\ (\%) = (Eyed\ larvae\ produced\ /\ Eggs\ added\ to\ tank) \times 100\% \quad (1)$$

281

282 Production rate was defined as the day at which larvae first reached the pediveliger stage, a
283 production metric routinely recorded by the hatchery. All production data from 2019 were analyzed
284 separately from long-term analysis of production trends at HPLOH attributable to anomalous,
285 persistent crashes observed throughout most of this season.

286 *2.6 Larval developmental assays during 2019 crashes*

287 Complete production failure was observed for approximately 8 consecutive weeks in the middle of
288 the 2019 season (late May to late July), representing the worst season in this facility's history. The
289 hatchery staff conducted 8 larval developmental assays and other water quality analyses to identify
290 drivers of production failure. During each trial, adult oysters were spawned according to standard
291 hatchery protocols, and fertilized eggs were stocked in triplicate in 1,000-L conical tanks at 10
292 larvae per mL. Tanks were drained and cleaned at 24 h post-fertilization and every 2-3 days
293 thereafter. The need to quickly identify crashes and complete lack of production led to most trials
294 being assessed qualitatively as either ‘crashed’ or ‘survived’ among treatments within each assay.
295 Daily rations of food consisted of standard, live hatchery diets, which varied in composition over
296 time and were scaled commensurately with culture age (Kuang et al., 2003), unless otherwise
297 noted. Algal food cultures at HPLOH are created from pure stocks of monocultures which are
298 grown in Choptank River water that has been filtered in series through sand filters then 1-μm string

299 cartridge filters, which is then either ozonated (large tanks in greenhouses) or autoclaved (flasks
300 and carboys).

301 The following section describes the purpose and treatments for each assay. Here, we group these
302 assays by common factors (**Table 2**). At the outset of the 2019 production season, low Choptank
303 River salinity (< 50% historic average) was observed, and early-season larval culture was
304 unsuccessful. The HPLOH responded by adding salt to adult conditioning systems and larval
305 production tanks to achieve minimum salinities (10 ppt). When the standard artificial seawater
306 (Crystal Sea®) failed to improve production yield, an assay was developed to examine if larval
307 development could be improved using a regionally-mined, fine-grained sodium chloride (Mix-n-Fine®)
308 (assay 1). Later, it was hypothesized that poor condition of broodstock attributable to abnormally-
309 low salinities during the spring of 2019 might explain poor oogenesis and larval production. To
310 address this, broodstock from saltier portions of the lower Chesapeake Bay (Manokin River; 15-18
311 ppt) were spawned, and resulting larvae were monitored during a larval assay (assay 2). Low river
312 salinity also was suspected to degrade equality/physiology of microalgae fed to larvae. At this time
313 the salinity within the algal system was 6.5. Algal cultures were inspected microscopically for
314 presence of HAB or other undesirable microorganisms by the hatchery algologist, but none were
315 observed. Nevertheless, an assay was developed to examine larval development in response to
316 algae with greater salt content (11 ppt), prefiltering water supplying algal cultures (charcoal or
317 string cartridge filter or ozonation) + algae + salt, and Reed Shellfish Diet® + prefiltered water on
318 larval development were evaluated (assay 3). Furthermore, there was concern that the automated
319 feeding system at HPLOH, which directly pumped food from the greenhouse to algal tanks, may
320 have been a source of contamination. To tease apart these potential competing sources of
321 contamination, hand feeding trials that included a salinity component (6.5 and 11 ppt) were added
322 to this assay (assay 4).

Table 2. Suspected problems and hatchery responses during persistent production failures during the 2019 production season at Horn Point Laboratory. Water filtration assay (5) includes Standard Filtered Water (SFW), Charcoal Filtered Water (CFW); assay 3 includes ozonated water (OZ) as a pre-treatment to algae production.

Assay number	Suspected Problem	Hatchery Response	Result
1	Salt quality?	**Larval developmental assay:** Mix-n-Fine® Salt vs Crystal Sea®	Crash
2	Broodstock impacted by low salinity?	**Larval developmental assay:** Obtain broodstock from saltier portion of the bay	Crash
3	Salinity impacting food quality?	**Larval developmental assay:** algal paste vs algae + salt vs algae + filtered seawater + salt vs DI + salt + algae, OZ water treatment	Crash
4	Automated feeder contaminated	**Larval developmental assay:** Hand feeding 2L bottles with various diets, salted or unsalted water, Choptank or foreign water (Wachapreague, MD).	Crash
5	Water filtration not sufficient?	**Larval developmental assay:** SFW vs CFW	Crash
6	Lab contamination?	**Larval developmental assays:** reciprocal broodstock and larval transfers - VIMS vs HPL	HPL broodstock spawned at VIMS produce competent larvae; Spawning and early larval culture at HPL Problematic, 3-day old larvae from VIMS survive at HPL.
7	Choptank River water bad?	**Larval developmental assay:** Choptank CFW vs Deal Island CFW	Choptanks CFW: low initial survival (6%) and crashed at day 8 Deal Island CFW: high initial survival, moderate survival (44%) at day 8
8	Chemical contamination in Choptank River?	Water samples sent to MD Spectral laboratory	No harmful chemicals detected
9	Presence of adverse *Vibrio* spp.?	Water and larval samples sent to USDA for Vibrio plate streaking	No *Vibrio* detected (highly unusual)
10	Missing beneficial bacteria?	**Larval developmental assay:** probiotic study using proprietary blend of Bacillus spp.	No significant difference on production compared to control (no dose); Coincidently, Choptank River salinity naturally increase and crashes ceased.
11	Bacterial community altered among crashed and non-crashed 2018 samples?	**Larval development assay: DNA sequencing**	Microbiota of the larvae from the crash were statistically dissimilar from those obtained during normal production

326 Various potential sources of larval culture seawater also were examined. The standard filtered
327 water (SFW) supplying larval tanks consisted of Choptank River water passed through sand filters
328 followed by cartridge filters to remove particles >1µm. The first assay compared larval development
329 under SFW to the same water with additional filtration through activated charcoal (CFW) (assay 5).
330 It was thought that one or a combination of these filters would remove harmful algae/bacteria or
331 toxins that may be present in the SFW. Considering the facility may be contaminated from a variety
332 of sources (i.e. water, tanks, food, etc.), reciprocal transfers of broodstock and spawned larvae
333 between HPLOH and the Virginia Institute of Marine Science (VIMS) were performed and tested
334 under a separate assay (assay 6). Concerned that a contaminant was coming in through the water
335 filtration system, water was brought into HPLOH from 50 km south of the hatchery in the mainstem
336 of the bay (Deal Island, MD) (assay 7).

337 For more detailed analysis of water quality, hatchery water was analyzed for chemical
338 contaminants and *Vibrio* pathogens. HPLOH is located in a rural environment and is surrounded by
339 active, large farms (corn, soybean, wheat). Previous reports have indicated that insecticides,
340 herbicides, and fungicides enter into the Choptank River at different times during the farming
341 season, with the highest concentration of herbicides in surface water found between May and
342 August (Kuang et al., 2003). SFW and CFW samples were sent to Maryland Spectral Services
343 (Baltimore, MD) in June of 2019 (assay 8) and analyzed for > 70 semivolatile organics (EPA
344 methods 8270D via GC/MS) and > 20 chlorinated pesticides (EPA 80801B via GC/ECD). The
345 analysis included scans for many persistent organic pollutants consistent with EPA protocols,
346 including pesticides that are long-lived and were typically used by the agriculture industry.

347 To examine for the presence of harmful bacteria, larvae and water samples were sent to USDA,
348 ARS in Dover, DE, for microbial analysis (assay 9). The media used for testing and quantifying
349 *Vibrio* levels was Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS; Difco, BBL brand). This agar
350 inhibits most non-*vibrios* by the inclusion of high salt levels. Many *Vibrio* spp. grow on it, including
351 *V. coralliilyticus* and *V. tubiashii*. For assays, 0.1 mL of water or algal/water sample was spread

over the entire surface of a freshly-prepared TCBS plate and incubated overnight at 26°C. For larval samples, a few hundred larvae were crushed in an empty petri dish followed by addition of 0.1 mL of sterile seawater, and the entire volume was tested by spread plating on the TCBS agar similarly to water and algal samples.

Toward the end of the failure period, the hatchery staff attempted to use a blend of proprietary *Bacillus* spp. probiotics (Quality Bacteria Laboratories; St. Louis, MO) to improve water quality (assay 10). Uncertain of how to adapt the use of probiotics to ameliorate culture conditions within tanks, the standard dose (8 g/m^3), ½ dose (4 g/m^3), 2x dose (16 g/m^3), and a control (0 g/m^3) were evaluated for their effects during larval developmental assays. Although larval samples from crashed and non-crashed cultures were not preserved from the 2019 production season, similar samples were archived from 2018 during larval diet studies at HPLOH using larvae produced from wild broodstock and two selectively bred lines (LOLA and NEH). Here we present the microbial community structure associated with larvae from crashed samples (acute mortality of larval experimental and control treatments) and non-crashed samples (high survival of all larvae) (assay 11). Both studies were designed initially to study the effects of starvation on larval gene expression of several strains, so the differences in crashed and non-crashed samples were compared to the differences between strains (wild and selectively bred lines), and between fed and non-fed larvae, and across three replicate treatments per group. Crashed and non-crashed microbial communities were analyzed using amplicon sequencing of the 16S and 18S ribosomal RNA Gene (Supplemental Materials).

2.7 Statistical analysis

Several parametric statistical tests, including linear and non-linear regression, analysis of variance (ANOVA) and t-tests, were used to examine general aspects of interannual hatchery production and failures. Assumptions of normality and homoscedasticity were checked using Shapiro-Wilk's

test and Levene's test, respectively. Regression models were selected using the lowest AICc values.

Detailed production analysis was performed using regression trees. A total of 20 variables were considered as predictors of hatchery production yield and hatchery production rate (**Table 3**). These factors cover the full range of production from broodstock collection, conditioning, spawning, and larval culture. Furthermore, water quality conditions found in the main stem of the Chesapeake Bay at the Goose Reef Buoy was also considered. Although a total of 497 un-mixed broods were cultured to competency during 2011-2020 production seasons (2019 season data excluded), a total number of 144 broods were available for most of the analysis as there were incomplete records for some of the variables. When some of the variables were removed from the model as not statistically significant, the sample size was reassessed to include additional observations, with complete records for the variables remaining in the model.

To study the effects of these variables in a data-driven way, potentially incorporating nonlinearities and interactions or combined effects of the variables, and to identify important predictors of the crashes, we applied a machine learning method termed "random forest" (Breiman, 2001). The basic element of random forest is a classification and regression tree (Breiman et al., 1984) (CART), which recursively partitions the records of hatchery efficiency into homogeneous subsets, using values of the predictors. R package *ranger* was used to apply the random forest methods (Wright et al., 2020), and the Boruta algorithm was used to select relevant predictors (Kursa and Rudnicki, 2010, 2020, Degenhardt et al., 2019). Predictive performance of individual CARTs and random forests was evaluated using 10-fold cross-validation, which was repeated 20 times for enhanced stability of the results (i.e., accuracy measures of model predictions were calculated 200 times on random hold-out data, then averaged).

400 **Table 3**. Factors considered during CART and Regression Trees analysis. Significant predictors are listed in decreasing order of importance from top
401 to bottom. Quadrant 1 refers to the relationship of production yield over production rate (Fig. 7c) when the hatchery is most efficient.

Predictors of Production			Significant Predictors		
Production Stage	**All factors considered**	**Units or condition**	**Production Yield**	**Production Rate**	**Quadrant 1**
Broodstock Collection	Year Broodstock Collected	year	Week of Year	Average Conditioning pH	Survival to D-hidge
	Broodstock Source Location	oyster bar name	Survival to D-hidge	Broodstock Source Location	Avg. Conditioning Salinity
	River System of Collected Broodstock	river name	GRB Oxygen	Survival to D-hidge	GRB Oxygen
Conditioning	Avg. Conditioning Salinity	ppt	GRB Chlorophyll	Tank Buffering	Average Conditioning pH
	Avg. Conditioning Temperature	C	GRB Turbidity	Avg. Shell Height of Females	GRB Temperature
	Average Conditioning pH	NBS units	Fecundity	Avg. Shell Height of Males	Year Broodstock Collected
	Stimulation Used	yes or no	Year Broodstock Collected	GRB Temperature	GRB Oxygen
Spawning	Sex Exposed to Stimulant	male or female	Avg. Conditioning Salinity		Week of Year
	Avg. Shell Height of Males	mm	Gonadal Index		Fecundity
	Avg. Shell Height of Females	mm	GRB Temperature		GRB Salinity
	Fecundity	millons of eggs/female	Avg. Shell Height of Males		Tank Buffering
	Gonadal Index	1 to 4	Avg. Shell Height of Females		Avg. Shell Height of Females
Larval Culture	Week of Year	week	Average Conditioning pH		
	Survival to D-hidge	%	River System of Collected Broodstock		
	Tank Buffering	buffered/unbuffered	GRB Salinity		
Environmental Data	GRB Oxygen	mg/l	Avg. Conditioning Temperature		
	GRB Turbidity	NTU			
	GRB Salinity	ppt			
	GRB Chlorophyll	µg/l			
	GRB Temperature	C			

402

Microbial community structure was compared between the crash and non-crash studies and treatments therein by applying redundancy analysis to determine if samples differed significantly between the crash and non-crash study and to compare these differences to those driven by larval strain and feeding status (fed *vs* starved).

3.1 Results

3.2 Water quality conditions in the Choptank River

HPLOH is located in the mesohaline portion of the Chesapeake Bay; therefore, we thought it was important to first report on the conditions found in the Choptank River to orient readers that are used to working in more marine environments that have less water quality variability and greater carbonate buffering.

Water quality variables (oxygen, pH, salinity, Secchi depth, and temperature) varied greatly over seasonal scales (**Fig. 4**). Here we report on the range of means spanning > 30 years of water quality monitoring on the Choptank River (MDNR Station ET5.2) adjacent to the hatchery. Average (standard deviation) oxygen, pH, salinity, Secchi depth, and temperature were 8.25 (3.02) mg/L, 7.74 (0.11) units, 9.93 (1.25) ppt, 1.02 (0.22) m, 15.1 (9.16) ºC, respectively.

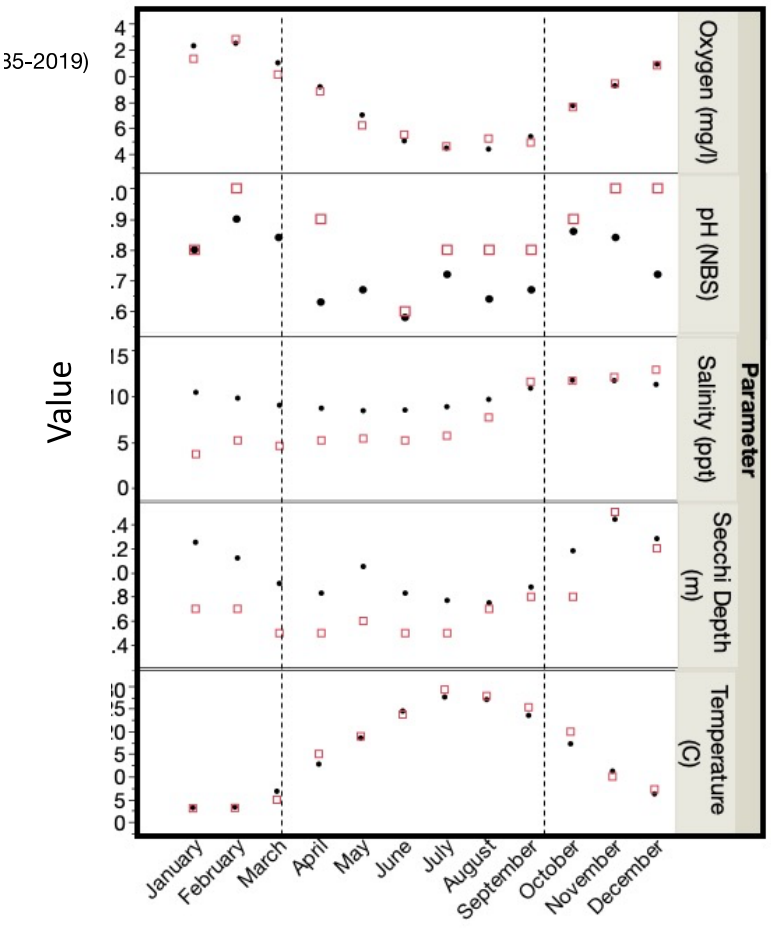


Figure 4. Monthly average (1986-2020) water quality conditions in Choptank River, MD (filled circles) and similar water quality data from 2019 (open squares). Hatchery season represented as area between dashed lines. No differences among y-axis units. Data source: Maryland Dept. of Natural Resources station ET5.2

Average alkalinity measured at HPLOH between 2016-2020 was 825.70 (110.36) uequl/L. The average Ωar was 0.33 (SD:0.24). A strong non-linear relationship between river water Ωar and pH was observed (R^2 = 0.92), while a weaker linear relationship was found between Ωar and salinity (R^2 = 0.11; **Fig. 5**).

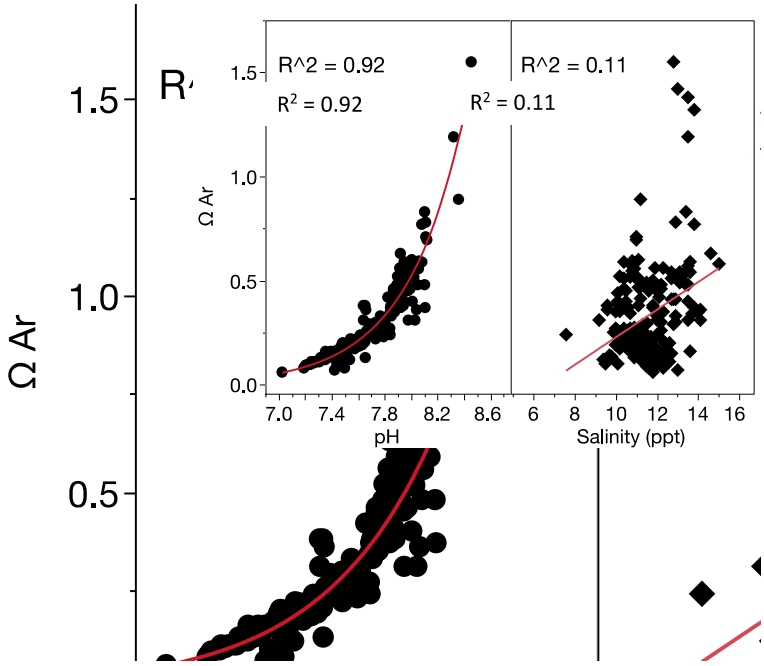


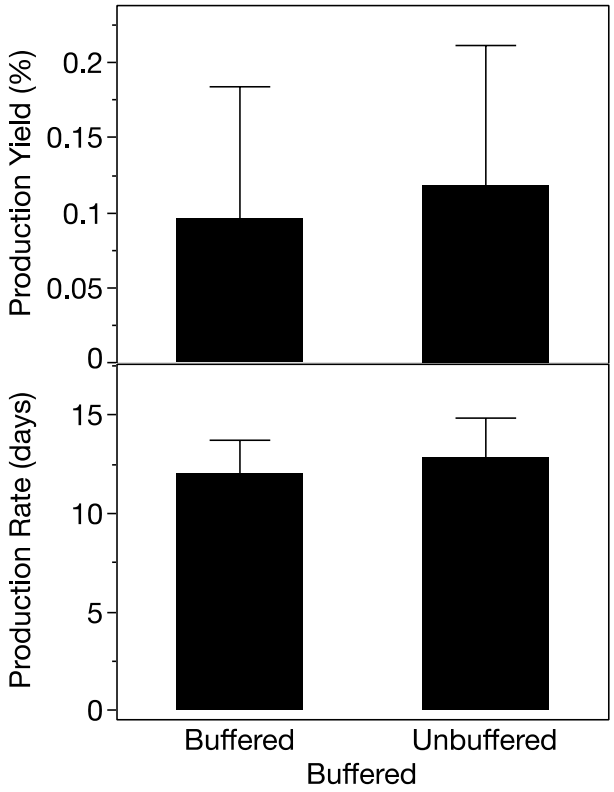
Figure 5. Distribution of aragonite saturation state at HPL between 2016-2020 and outlier box plot. Inset: aragonite saturation state at HPL over pH and salinity from discrete measurements in HPL hatchery during the production seasons from 2016-2020. Data excludes 2019 production season.

HPLOH production trends (2011-2020)

Production has, until recently, trended positively at HPLOH since 2011 and the hatchery has reliably produced millions if not billions of spat on shell annually in recent history. For example, HPLOH consistently produced more than 500 million spat-on-shell since 2011 up until 2019. Maximal production occurred in 2017, with 1.784 billion spat-on-shell produced. Spat production trends have been driven by federally-supported oyster restoration in the Chesapeake Bay.

To better understand inter-annual variation in production yield, we examined average efficiency on several time scales. HPLOH hatchery production yield of eyed-larvae between 2011 and 2020 varied from 0% to 56%. Average production yield during this period was 10.7% (std dev = 9.2%). Average annual production yield (ANOVA $F_{8,486}$ = 10.47, p-value < 0.0001) and production rate (ANOVA $F_{8,412}$ = 9.02, p-value < 0.0001) varied significantly across all seasons; however, there were no clear trends in production yield or rate over time.

445 The effect of manipulating carbonate chemistry was examined by comparing the average
446 production yield and production rate after the hatchery began buffering larval tanks (2016-2020;
447 excluding 2019) to the four previous years (2013-2015) (**Fig. 6a**). A small but significant reduction
448 in yield was detected between buffered (9.6%) and unbuffered (11.8%) batches (t(447) = 2.68, p-
449 value = 0.004). Production rates of buffered batches were found to be significantly quicker (12.02
450 days) than earlier unbuffered batches (12.97 days) (t(298) = 4.49, p-value<0.001) (**Fig. 6b**).



451

452 **Figure 6.** Production yield and production rate at HPL hatchery among unbuffered (2011-2015) and buffered
453 batches (2016-2020). Asterisk indicates significant greater means. 2019 production rate data were excluded
454 from the buffered pooled mean.
455

456 Next, seasonal trends were explored statistically. Aggregating data among all seasons, the
457 following quadratic function relates mean production yield to the week of the year:

458 $$Yield\ (\%)\ =\ a\ +\ b\ \times W\ +c \times W^2 \quad (2)$$

459 where *a*, *b*, and *c* are coefficients (estimated for weekly average data as -0.16, 0.024, and -0.0005,
460 respectively) and *W* is the week of the year. This nonlinear function explained much of the variance
461 in weekly average production yield over time (R^2 = 0.70, **Fig. 7a**). Mean production rate was found
462 to decline significantly and linearly across the production season ($F_{1,27}$ = 53.76 p-value < 0.0001;
463 R^2 = 0.67; **Fig. 7b**). Additionally, mean production rate was nonlinearly and significantly predictive
464 of production yield with the following quadratic function:

465 $$Yield\ (\%) = a + b \times PR + c \times PR^2 \qquad (3),$$

466 where *a*, *b*, and *c* are coefficients (estimated for weekly average data as -1.43, 0.15, and -0.003,
467 respectively) and *PR* is the production rate in days (R^2 = 0.29, **Fig. 7c**). Hatchery crashes were
468 found to have occurred fairly evenly throughout the 2011-2020 production seasons. Crashes were,
469 however, most common in the 35th and 36th week of the year, which corresponds typically to
470 either the last week of August or first week of September (**Fig. 7d**).

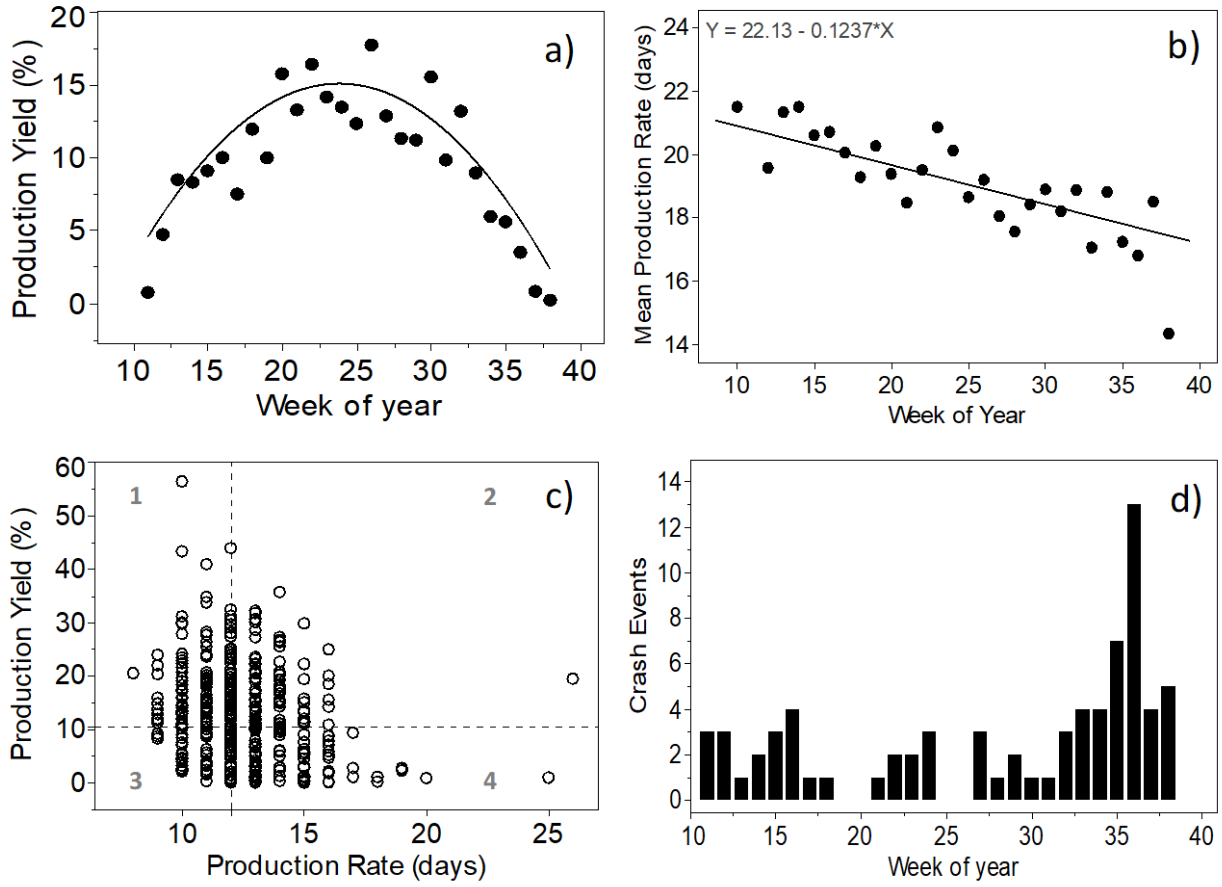


Figure 7. a) Average hatchery production yield over the week of the year; b) mean production rate over week of year; c) mean production yield over production rate divided among four quadrants separated by median values (dashed lines); d) number of crash events per week of the year. All data are derived from long-term (2010-2020) hatchery production data but exclude 2019 data.

3.3 Drivers of production yield and production rate

For predicting hatchery yield, a Boruta algorithm retained 16 of the 20 potential predictors (**Table 3**) as statistically significant (the following categorical variables were removed: the source name, stimulation and its type, and whether the tank was buffered). When tested on out-of-sample data in the cross-validation study, random forest achieved $R^2 = 0.54$, root mean squared error RMSE = 6.6%, and mean absolute error MAE = 5.0%. For comparison, predictive performance of a CART was worse than of random forest, with the $R^2 = 0.36$ being lower and errors being higher (RMSE = 8.0%, MAE = 5.7%). The final random forest for yield was trained on 177 observations and ranked

485 the top-3 predictors in order of decreasing importance as follows: 1) Week of year, 2) Percent
486 survival of eggs to D-hinge, 3) GRB oxygen.

487 For predicting production rate, the same 20 predictors were considered, from which only 7 were
488 retained by Boruta as statistically significant. The cross-validation study provided R^2 = 0.19, RMSE
489 = 1.8 days, and MAE = 1.4 days for the random forest. The final model was trained on 225
490 observations and ranked the predictors as follows: 1) Average conditioning pH, 2) Broodstock
491 source location, 3) Survival of eggs to D-hinge, 4) Tank buffering, 5) Average shell height of
492 females, 6) Average shell height of males, and 7) GRB temperature (**Table 3**).

493 Lastly, we examined how these same 20 factors influenced the distribution of the data when larval
494 yield is regressed over production rate. The relationship between yield and rate can help describe
495 production efficiency at the hatchery scale. Ideally, from a manager's perspective, high yielding
496 broods that are produced quickly most efficiently use fewer resources and are least expensive to
497 produce (**Fig. 7c** quadrant 1). Conversely, low-yielding broods that grow slowly are the most
498 expensive to produce (**Fig. 7c** quadrant 4). Boruta algorithm retained 12 variables as important
499 factors. In the cross-validation study, random forest with these factors showed accuracy 60.2% and
500 average F1 score 0.65. The final random forest model was trained on 148 observations and
501 reported predictors in order of decreasing importance: 1) Survival of eggs to D-hinge, 2) Average
502 conditioning salinity, 3) GRB turbidity, 4) Average conditioning pH, 5) GRB temperature, 6) Year of
503 broodstock, 7) GRB oxygen, 8) Week of year, 9) Fecundity, 10) GRB salinity, 11) Tank Buffering,
504 and 12) Average shell height of spawning females (**Table 3**). Predictive performance of a single
505 CART was again lower than of random forest, with cross-validated accuracy 56.5% and average
506 F1 score of 0.62. Despite lower accuracy, visual analysis of the classification tree (**Fig. 8**) aids in
507 understanding the conditions under which broods end up in one of the four quadrants of hatchery
508 efficiency shown in Figure 7c. For example, broods produced from broodstock collected in 2018
509 and later (right branch with the terminal node 8, corresponds to 27% of the analyzed sample), had
510 the greatest probability, 79%, of appearing in quadrant 4 (i.e. low hatchery efficiency), while the

likelihood of them falling in quadrants 1-3 were just 8%, 8%, and 5%, respectively. For broodstock collected before 2018 (large left branch), water quality variables and fecundity allowed further separation of observations. For example, both nodes 1 and 3 correspond to quadrant 1 and represent a similar share of the sample data (8% and 7%), but the observed probability of quadrant 1 is higher in node 3 (90% vs. 55% in node 1). Node 3 corresponds to high salinity conditions both for broodstock and larvae, while node 1 is formed only considering the larvae conditions.

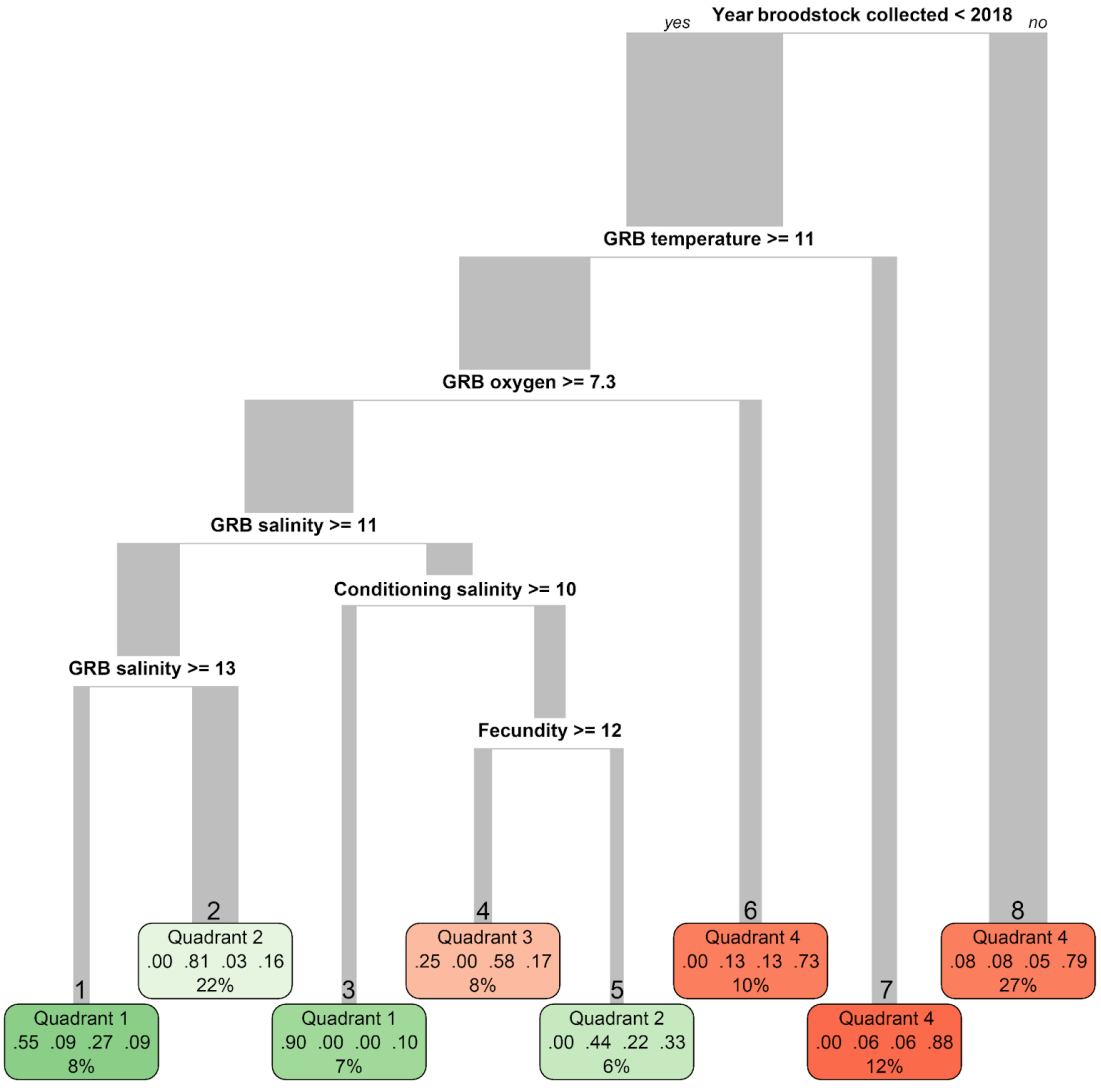


Figure 8. A classification tree separating the observed data (top, starting with all *n* = 144 or 100% of data) by the values of the predictor variables. Branch widths are proportional to the number of observations. The terminal nodes of the tree are numbered and show the predicted quadrant, the predicted probability of each quadrant (color ranges from bright and pale green for quadrants 1 and 2 to pale and bright red for quadrants 3 and 4), and the node size expressed as percentage out of *n*.

Note that the relative importance of factors in CART may differ from the order the factors were used in the tree (**Fig. 8**) and from the importance ranking provided by random forest. Variable importance in CART was quantified by the reduction of the node impurity the variable was able to provide (in classification tasks, node impurity is usually measured using Gini index), and although year of the broodstock was considered a good factor to start the data splitting, other factors provided more substantial reductions of the node impurity, leading to more homogeneous terminal nodes. In the CART in **Fig. 8**, the top-3 important variables were average salinity for the broodstock and larvae, and week of year, and year of the broodstock was ranked sixth. In random forests, importance of variables was quantified differently, as the increase of a tree's prediction error when values of one of the variables was randomly permuted. Such evaluation was done on out-of-bag data that were not used for constructing the tree, then the error metrics were averaged across all trees. Also, because each tree in a random forest was trained on a slightly different (bootstrapped) sample of the data, the random forest results provide a wider range of situations arising from the same sample, with some observations being randomly removed or included several times in a bootstrap sample. Hence, random forest results and specifically rankings of the variable importance were more robust than those of CART.

3.4 Water quality during the 2019 production season

There were several water quality variables that varied significantly during the 2019 production season. Student's t-tests on each variable determined significantly lower annual mean values for salinity ($t_{1,23}$ p-value = 0.0375) and Secchi depth ($t_{1,23}$ p-value = 0.0216) during the 2019 season compared to those from long-term average (1986-2020).

3.5 Larval assays to identify 2019 crash sources

All treatments from larval developmental assays examining the effects of salt supplementation (assay 1), salinity effects on food quality (assay 3), automatic algae feeding system (AAFS) contamination (assay 4), water filtration efficacy (assay 5) were found to have crashed upon inspection of tanks on the first drain (**Table 2**).

Other larval assays provided mixed results. Importing broodstock from Manokin did not permit production to resume readily (assay 2). During reciprocal transfer larval assays (assay 6), it was determined that HPLOH broodstock were successfully spawned at VIMS and their resulting larvae could be reared satisfactorily on this institute's water and algae. HPLOH-spawned larvae transferred to VIMS were not successfully transported and crashed quickly after arriving at the facility. Portions of this same cohort that were left behind at HPLOH, crashed at HPLOH on day 5 post-fertilization. Conversely, 3-day-old larvae that were spawned at VIMS, shipped to HPLOH, reared in CFW, held at salinity of 11 ppt, and fed from the AAFS were raised to settlement. Larvae reared in the foreign water hatched and developed to a greater extent than those reared in standard FSW (assay 7); however, this assay was terminated early as larvae reared in Choptank CFW water crashed by the third tank drain (approximately 6 days post-fertilization).

Outsourced water quality, bacterial analysis, and attempts to improve water quality through probiotics were met with interesting yet puzzling results. Spectral analysis of Choptank River water and FSW did not detect any harmful chemicals (assay 8), indicating no apparent chemical contamination. *Vibrio* analysis did not detect any known harmful *Vibrio* species (assay 9); in fact, no *vibrios* were detected at all. During the course of the probiotics investigation (assay 10), salinity in the Choptank River had increased to > 10 ppt and larvae within all treatments reached the eyed stage at 14 days post-fertilization, rendering the need for probiotics obsolete. The 2x dose of probiotics, however, produced significantly more larvae (2.23 larvae million) than the ½ dose treatment (1.57 million larvae) and was similar to normal dose (1.82 million larvae) and control treatments (1.94 million larvae) by the end of the 14-day study (ANOVA $F_{3,9} = 7.77$ p-value = 0.0172). As Choptank River salinity exceeded the 12 ppt threshold at this time, production

inexplicably resumed and production yields increased markedly (**Fig. 9a**) without revealing precisely what had been driving crashes throughout the summer (**Fig. 9b**). Once production resumed, the hatchery staff extended the season as long as possible and applied greater effort to recover from production loss. As a result, HPLOH produced a total of 962 million eyed larvae in waning time left of the season.

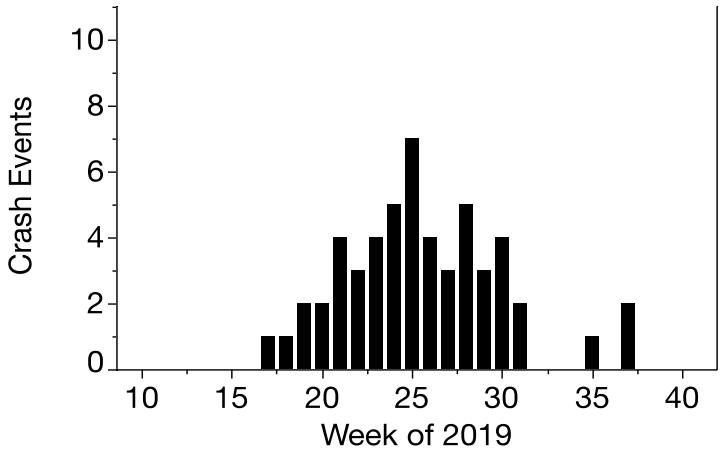


Figure 9. a) Average hatchery production efficiency and b) number of crash events per week over week of the year from 2019 season.

Significant differences in the microbial communities were observed between crashed and non-crashed cultures (assay 11). Redundancy analysis suggested that the species level community

structure variability was statistically significantly related to whether the samples were from the "good" or "crash" project, with 34% of the variance in community structure explained by project ($p < 0.001$). Another 16% of the variability was related to whether the larvae were fed, rather than starved ($p < 0.001$). The pre-treatment time-point appeared to be similar to the "starved" treatment in the crash project, so for analysis purposes, the pretreatment and starved groups were combined. The microbial community structure did not significantly relate to the larval strain ($p = 0.08$), with strain explaining only 4% of the variance. A remaining 45% of the variance in microbial community structure between samples did not appear related to any of our variables (**Fig. 10**).

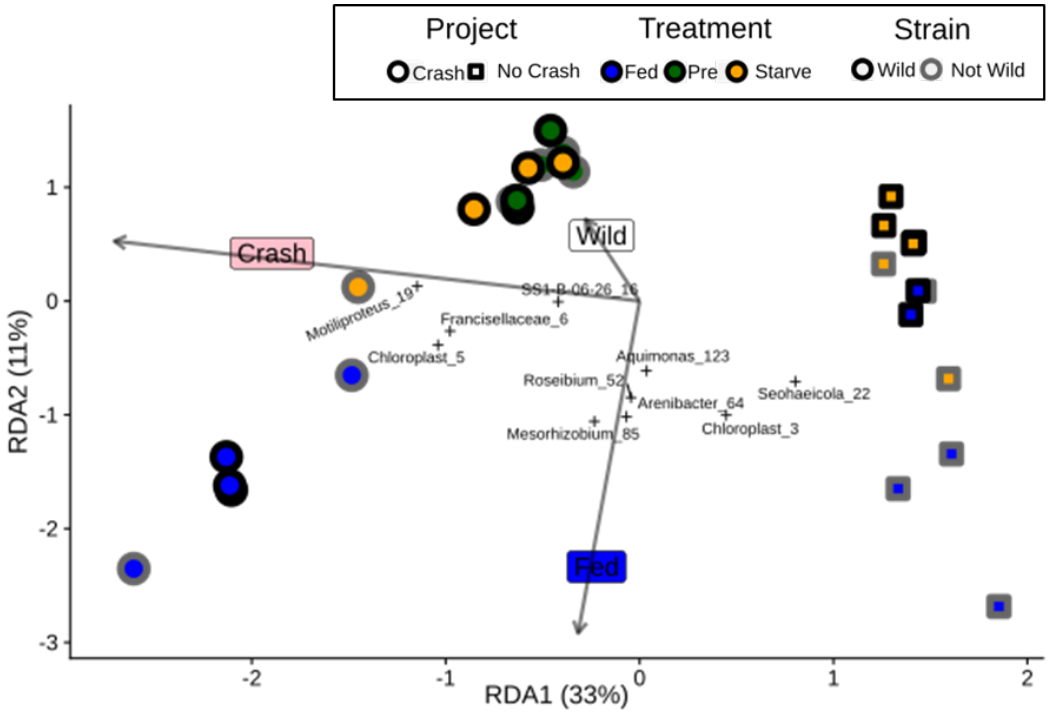


Figure 10. Visualization of the redundancy analysis that compared the relationship between project (Crash vs non-crash), treatment (fed vs starved and pretreatment), and strain (wild vs LOLA (non-crash project) or NEH (crash project)). Large points correspond to tank samples, with shape corresponding to project, border color corresponding to strain, fill color relating to treatment. Samples with points closer together are more similar. The axes indicate the position of each point along each RDA axis returned by the analysis. Vectors indicate gradients on the ordination plot that represent samples that are more typical of the different treatments. For instance, Crash samples generally are lower along RDA1, but non-crash are higher along RDA1. + signs indicate how different ASVs relate to the project, strain and treatment predictor variables. The positions of the points correspond to the different treatments, with points in the direction of a given vector more typical of that predictor variable. Thus, ASVs with points in the upper left-hand quadrant are more commonly found in crash samples. Only the ten ASVs with distance farthest from the origin are shown. Many others are related to variables of interest.

Because so much of the variance in microbial community structure was associated with our variables of interest, it is not possible to identify particular ASVs that predict the variables of interest. Rather, general linear mixed models showed that when allowing for treatment and strain to be excluded as random effects, 155 of 446 observed (63%) of the ASVs were statistically significantly related to "Project" ($P < 0.01$). Similarly, when holding project and strain as random effects, 31% of the ASVs were related to "Treatment" (fed vs starved only) ($P < 0.01$).

No ASV from the *Vibrio* genus occurred frequently enough to pass quality filtration cutoffs. Although some vibrios were occasionally quantified at very low amplicon copy numbers in some samples, none were present consistently across replicates in any group, and none were present at least twice in 20% of our samples; therefore, they were excluded from our analysis.

4.1 Discussion

Detailed analysis of shellfish hatchery production trends is not common in the research literature as many hatcheries are private entities and may not want to publicize their production process or production problems for competitive reasons. Our disclosure of hatchery production problems across the network of Atlantic research hatcheries contrasts this sentiment as it was our intention to highlight crashes as an important topic of future investigation to bolster shellfish aquaculture. Another major objective of this research was to share long-term hatchery production trends of a mature hatchery staffed with seasoned personnel that have kept exceptional records of the production process. Our analysis revealed quantitative information about the ‘when and how’ HPLOH production was most efficient, which can be used in the future to improve hatchery performance and move closer towards optimization.

4.2 Study site, water quality and larval production at HPLOH

The HPLOH resides in the mesohaline portion of the Chesapeake Bay, which experiences water quality characteristics, such as salinities (10 ppt), pH (7.7), and Ωar (0.33), that would be considered adverse to larval production. The low Ωar in the Choptank River is attributable to a combination of eutrophication, microbial-respired primary production, and low salinity (Kemp et al., 2005). Laboratory studies have shown clearly that the calcification rates of juvenile *C. virginica* from the mesohaline portion of Chesapeake Bay decline with pH (Waldbusser et al., 2010). From an aquaculture perspective, the carbonate chemistry found regularly in the Choptank River represents extreme values most hatcheries would try to avoid. For example, Whiskey Creek Shellfish Hatchery (Netarts Bay, OR) found that 53% of relative production of *Crassostrea gigas* was correlated to Ωar that larvae experienced during the first 24 hours of production and a Ωar > 1.75 was needed for positive growth of the production brood. Conversely, the average Ωar at HPLOH (0.33) would elicit 100% production failure at Whiskey Creek (Barton et al., 2012), demonstrating that carbonate chemistry thresholds for hatchery production are likely regionally- and species-dependent. Historically, however, the HPLOH conditioned, spawned, and cultivated larvae in undersaturated river water regularly without aid of any additional carbonate buffering. Based upon the growing body of evidence that larvae development and growth responds to □ar, the hatchery has used Na_2CO_3 since 2016 to improve larval development. Interestingly, we only detected a moderate improvement in the rate of production attributable to buffering and actually detected a small but significant increase in yield when batches were unbuffered. (**Fig. 6**).

Our study suggests that hatchery production of *C. virginica* can be maintained despite the low alkalinity and relatively acidified conditions found in the mesohaline portion of the Chesapeake Bay. It is not clear, however, how production at HPLOH has overcome low Ωar to achieve reliable production of *C. virginica*. Acidification stress imposes energetic burdens on numerous processes of the developing larvae, including homeostasis, calcification, and development (Gray et al., 2017; Kurihara, 2008; Waldbusser et al., 2015b). The high-quality and high-volume diets that larvae

receive in hatcheries may allow them to cope with energetic costs of undersaturated carbonate conditions (Gibbs et al. 2021; Thomsen et al 2013). Alternatively, other evolutionary factors may be at play. Some oysters in Chesapeake Bay may be more adapted to conditions of coastal acidification. Broodstock for the HPLOH is collected from local tributaries fed by the undersaturated water of the Choptank. Yet, unpublished spatfall data from Maryland Dept. of Natural Resources show these same tributaries (Harris Creek and Broad Creek) have experienced consistent, albeit irregular, recruitment over the past 34 years. In general, others have found populations naturally exposed to low pH/low Ωar may be much more adapted to fluctuating or extreme acidification stress than populations from more buffered and stable environments (Pansch et al., 2014). The Pacific oyster *C. gigas* has repeatedly shown vulnerability to low pH/low Ωar (Kurihara et al., 2007; Timmins-Schiffman et al., 2013; Waldbusser et al., 2015a), but populations from the Yellow Sea which are exposed seasonally to pH values between 7.7 to 7.4 appear resistant to acidification stress at larval and post-larval stages (Ginger et al., 2013). Similarly, in short-term studies, *C. virginica* that originated from Saint-Simon Bay (Brunswick, Canada) with natural acidification, produced larvae with greater survival under acidified conditions (pH = 7.46) using standard hatchery protocols (Clements et al., 2020).

Cultivating larvae in the mesohaline portion of the Chesapeake Bay provides both advantages and disadvantages not found in saltier regions of this estuary. For one, the low salinity waters are thought to have lower annual recruitment but also represent a refuge from some forms of disease pressure (MSX and Dermo) (Mann and Powell, 2007; Mccollough et al., 2007). Although adult oysters grow rapidly in the highly productive waters of the Choptank River, salinity can drop rapidly with intense or prolonged rainfall, pushing salinity well below the threshold where adults will not actively feed and grow (~5 ppt) (Casas et al., 2018). For Maryland, 2018 was the wettest year on record (Hopkins et al., 2020). It is likely that the low salinity in the fall of 2018 and first half of 2019 greatly reduced the overwintering condition and gonadal development of broodstock used in the 2019 production season.

4.3 Experimental larval assays during persistent crashes

The subsequent series of larval assays provided little explanation as to what environmental insult(s) drove the persistent crashes at HPLOH throughout most of the 2019 production season. Indeed, the only conclusion was that failed production was more complicated than a simple salinity issue (**Table 2**). Factors that were also ruled out include salinity negatively affecting food, artificial salt quality, automated feeder contamination, pesticides/toxicology, and broodstock quality. Indeed, reciprocal transfer experiments revealed broodstock derived from HPLOH could produce competent larvae when reared at another facility. Conversely, 3-day old larvae brought to and reared at HPLOH were able to develop. These data suggest the culprit driving crashes at HPLOH was most lethal when larvae were exposed to it during the first hours or days after fertilization (the trochophore stage through prodissiconch I). Previous toxicological studies as well as other hatchery production studies mentioned above have confirmed this early, unshelled larval life-stage to be most vulnerable to environmental perturbations (Barton et al., 2012; His et al., 1999; Ragg et al., 2019).

Although some assays enabled us to rule out certain suspects and identify important windows of exposure to insults, others left us with more questions than answers. Interestingly, hatchery water and cultured larvae that were assayed for *Vibrio* spp., which may cause hatchery production failure (Prado et al., 2005; Richards et al., 2015; Sugumar et al., 1998; Ushijima et al., 2018), came back not only negative for harmful *Vibrio* spp. (e.g., *V. tubiashii* or *V. coralliilyticus*) but also revealed the absence of all *Vibrio* spp detectable on this agar medium. Such an outcome is highly unusual, especially during periods of major mortalities and suggests that whatever was suppressing larval growth may have also suppressed growth of *Vibrio* spp. One *Vibrio* that does not grow well on TCBS agar is *V. hollisae*, which can also infect shellfish, so this species could have influenced mortalities (Gary Richards – US Dept. of Agriculture, personal communication). Vibrios, however, are not the only bacterial pathogens that can elicit larval oyster mortalities, so other pathogens should be considered in future investigations. For example, the Ostreid herpesvirus 1, a highly

709 virulent pathogen of the Pacific oyster can also infect and kill multiple bivalve species (Arzul et al.,
710 2017); only one study to date, conducted in 2002 has tested for OsHV-1 in larval *C. virginica*
711 (Burge et al., 2017; Friedman et al., 2005). Comorbidity from suboptimal culture conditions
712 combined with bacterial processes represents a new and important avenue worth exploring. Coffin
713 et al. (2021) recently found endogenous, exploitative bacteria can accelerate mortality of bivalves
714 under adverse culture environmental conditions.

715 Experimental assays conducted to examine some of the more common factors thought to influence
716 production were neither comprehensive nor exhaustive. For example, we considered low-salinity
717 harmful algal species (e.g. *Prorocentrum minimum* and *Karlodinium veneficum*) or the toxic
718 chemicals they exude could have compromised food quality or larval production. HAB events
719 commonly overlap spatially and temporally with the oyster spawning season (Glibert et al., 2007; Li
720 et al., 2015), have been increasing in frequency in Chesapeake Bay, and pose a threat to
721 environmental health and aquatic life (Li et al., 2015), including early-life stages of *C. virginica*
722 (Brownlee et al., 2008; Glibert et al., 2007). We did not, however, explicitly test for the presence of
723 these species or substances. Rather, we simply examined how various filtration systems (sand
724 filtration, ozonation, activated charcoal, etc.), thought to be capable of removing the HAB species
725 and their organic chemicals (Falconer et al., 1989; Gonçalves and Gagnon, 2011; Newcombe and
726 Nicholson, 2004), modified culture production. Additionally, we explored if harmful algae could
727 have contaminated HPLOH algal cultures, which are supplied with sand-filtered (effective filtration
728 of ~50 μm particles) river water, by comparing growth and survival rates of larvae fed standard
729 diets versus live algal diets from pure cultures diluted with pre-filtered river water or algal pastes.
730 As no filtration system improved production outcomes, we assumed HABs were not present and
731 did not interfere with larval production. Additionally, contamination of algal stocks with HABs would
732 likely have been recognized by the HPLOH algologist, who monitors and maintains cultures. We
733 recognize this is an incomplete analysis, but even large research hatcheries, such as HPLOH,

734 have limited resources that at times limit lines of inquiry when preliminary findings suggest pivoting
735 and exploring other avenues.

736 The observation that microbiota differed significantly between the two projects in which larvae grew
737 normally or crashed (**Fig. 10**) could suggest a possible relationship between the microbiota and
738 hatchery crashes. Additionally, among crashed larvae, we observed starved larvae survived for
739 longer periods of time and had microbiota that were more similar to non-crashed larvae,
740 suggesting a possible three way interaction between larval digestion, the microbiota, and the
741 mortality dynamics associated with the crash. Notably our observations of statistically detectable
742 differences between crash and non-crashed larvae contrasted with Ramachandran et al's (2018)
743 previous analysis, which showed no detectable bacterial community variability between tanks with
744 high and low survival rates within the same facility. In contrast, it was more similar that of Timmins-
745 Schiffiman (2021) in which a pH driven mortality event in a hatchery was shown to covary with
746 bacterial community variability. A main caveat to this finding is that, although treatment tanks were
747 replicated, and there were multiple treatments within our two experiments, we sampled only during
748 one crash event, and one non-crash event. Future studies should sample at multiple crash and
749 non-crash time points. In particular, time series analysis would allow examination of changes in the
750 microbiota that precede a crash, rather than just co-occur with crashes. Another caveat, is that we
751 have not yet examined the community structure of viruses associated with the larvae, and viruses
752 may affect both larval health and the microbiota.

753 If our observations prove robust in subsequent analysis, several different factors could relate
754 microbial community structure to the health of their larval hosts. Some microbiota are oyster
755 pathogens (Richards et al., 2015; Travers et al., 2015), and so there could be some element of
756 these vastly different communities that affects host health. Microbiota also modulate the immune
757 systems of many host species (Chu and Mazmanian, 2013; Geva-Zatorsky et al., 2017), and could
758 make the hosts susceptible to pathogenesis (Croswell et al., 2009) by organisms that we do not
759 test for, such as viruses. Conversely, the health of the larvae may affect the microbiota rather than

760 *vice versa*. Indeed, the presence of decomposing oysters, present to some degree in the crash
761 samples, could create a refuge for a very different microbiota than those of living hosts. It is also
762 possible that during the crash there is a problem with the larval food, and that dysbiosis could
763 occur as the hosts struggle to process this food. The observation that the microbiota of larvae
764 collected during a crash were dissimilar from larval microbiota during a time of normal production
765 suggests the possibility that microbiota associated with larvae could relate to, or even drive these
766 crashes. With only two time points, however, it is not possible to deconvolve normal temporal
767 variability from crash effects. Accordingly, studies that investigate the microbiota at multiple time
768 points, both during crashes and times of normal production, are warranted.

769

770 *4.4 Long-term production trends*

771 Examining the variation in long-term trends in HPLOH production yield does indicate there are both
772 hatchery and environmental factors that have important effects. Larval survival to the first water
773 change 24 hr after stocking is a major production bottleneck at HPLOH and likely other hatcheries
774 because of the highly sensitive nature of the embryo to early prodissococh life-stages. This may
775 represent a critical time period that the hatchery staff could focus on, as fully shelled larvae are
776 more resistant and resilient to suboptimal culture conditions. Many additional contributors to larval
777 production occur prior to the spawn, indicating environmental conditions during broodstock
778 conditioning is of critical importance (Utting and Millican, 1997). Environmental factors we found to
779 be significant for broodstock conditioning and resultant larval production were average salinity, pH,
780 and temperature, as well as source and year the broodstock was collected. Female fecundity and
781 gonadal index also were found to be significant endogenous factors that were predictive of larval
782 performance and hatchery production yield. Each of these environmental factors could conceivably
783 be manipulated by the hatchery staff to improve production outcomes. Similarly, the hatchery staff
784 could explore how these environmental factors or others (e.g. diet) improve fecundity, which has

also been shown to be positively correlated with production yield and rate. Alternatively, breeders may want to focus on working with larger, more fecund individuals to improve production. Indeed, time series analysis, in which larvae are archived continuously over one or more growing seasons with crash and non-crash periods would allow researchers to identify microbial trends that precede, rather than simply coincide with crashes.

4.5 Random forest analysis of production yield and production rate

Random forest analysis allowed us to obtain a data-driven model of relationships between the hatchery production yield and environmental conditions. Without pre-specifying the form of individual relationships or the form and number of interaction effects, the method chose variables by their ability to iteratively differentiate levels of yield. At the same time, predictive importance of the variables in the random forest was assessed by permuting *individual* variables, therefore, *joint* importance of the variables was not assessed. Although random forest is more robust to collinearity of variables than a usual regression (because variables are randomly subset at each new tree split, and collinear variables may be randomly removed), the selected relevant variables may still be collinear or redundant. Hence, random forest facilitated selection of the most important variables to focus on but future research is required to obtain a deeper understanding of the underlying mechanisms and interactions.

The random forest techniques have been successfully implemented in a number of related tasks, such as predicting oyster norovirus outbreaks (Shamkhali Chenar and Deng, 2017), mussel growth (Bergström et al., 2015), and water temperature for marine aquaculture (Otsuka et al., 2018). Although the sample of about 200 observations allowed us to study a number of variables and test their statistical significance, the power of machine learning techniques emerges with large volumes of data. For example, effects at relatively extreme combinations of values are important for predicting production crashes, but are now estimated using only few corresponding observations

and thus bear a large uncertainty. Exploring larger samples would allow us to study the effects of different factors in more detail and improve the random forest model for decision making in oyster aquaculture. Nevertheless, results for our random forest analysis may be immediately useful for the managers in two major ways. First, the data-driven assessment of factors can allow managers to prioritize and focus on manipulating specific broodstock and culture conditions (i.e. salinity and percent survival to D-hinge, respectively) to achieve greater yields at faster rates. Second, estimated relationships are also data-driven and are not restricted to parametric linear functions as is usually done in statistical regression analysis. Those relationships can be studied for the presence and location of change points, thresholds, and combined effects responsible for the best possible outcomes. Indeed, a portion of analysis included partial dependence plots (Supplementary Materials **Fig. 11 - 14**) that display the conditions under which each important factor influences a given brood's yield and production rate. These can be used directly by a hatchery to begin ‘tuning’ production towards higher yields produced at quicker rates.

4.6 Prospective and Summary

As a high-volume, state-of-the-art research hatchery, HPLOH has been producing larvae while at the same time keeping fastidious notes that were immediately digitally archived. Aside from gross production statistics (i.e. larvae produced, number of spawns, etc.), these data were collected for the purpose of improving hatchery performance. When record keeping began, it was impossible to know which data would be important to record and help guide hatchery optimization. As a result, these records are by no means perfect and one lesson learned was that the data management at HPLOH could be improved with some regular QAQC, which could prevent data gaps and prevent inconsistencies that create confusion when gathering and analyzing data. Furthermore, this first analysis indicates only the direction for future studies that may improve production at HPLOH (e.g. broodstock conditioning, initial culture conditions). Therefore, we wish to highlight several points

concerning data collection and future directions of research on hatchery production and crashes elsewhere.

First, our statistical analysis of past production points to factors that the hatchery staff can manipulate and investigate further to improve future production. To develop mitigation strategies and avoid sudden downturn in production, however, forecasting should be a major technological goal. We could imagine monitoring precipitation and predicting declines in river salinity prior to freshwater arrival, at which time the hatchery could store “good” water as a possible avoidance strategy.

Second, the hatchery culture conditions and environmental data used as possible predictors of production are commonly monitored within shellfish hatcheries throughout the US. There is a long list of biological and chemical factors that are not observed routinely but could be vital for better understanding of production trends. For example, after extensive production monitoring and testing at Whiskey Creek Hatchery, Ωar was identified as a key driver of larval production. Since that time, this hatchery expanded its carbonate chemistry monitoring by permanently installing a Burkolator®, enabling them to record with high temporal resolution all carbonate variables. Perhaps because of increasing HAB event frequency in Chesapeake Bay, greater monitoring of HAB species and toxins through more sophisticated means, such as a Flocytobot (McLane®, East Falmouth, MA), may be important in the future. Furthermore, salient environmental drivers may change over time coincident with shifts in climate change, alteration in local land use, or other anthropogenic factors.

Third, although there are bound to be some common factors that govern production among many or all shellfish hatcheries (e.g. survival to D-stage, temperature, algae, etc.), local conditions and site-specific environmental factors are also likely to be important. As a result, most hatcheries that strive to optimize production will likely have to collect a wide variety of production information and local environmental data. Afterward, and with careful analysis, specific production factors may emerge such that collection efforts can be fine-tuned and paired-down. Nevertheless, we also

encourage greater communication among hatcheries to share information on short- and long-term production problems and mitigation strategies used to overcome them. Such information may be valuable for saving time and effort when trying to diagnose problems and reducing the expenses of delayed production.

Fourth, our study of production trends at HPLOH was greatly improved by the breadth of data available to us. This is certainly a consequence of the regularity with which data are gathered across the production process, digitized, and stored. Furthermore, the HPLOH has a dedicated staff member who maintained and collated these data. Many hatcheries, research hatcheries included, collect data but these data often are written on paper, lack organization or consistent notation, contain significant and lengthy data gaps, and are not centralized or digitized. We recognize that not all hatcheries have the means to dedicate personnel to data acquisition, but we hope that this study demonstrates the value of collecting a variety of data to help hatcheries audit production processes and move toward optimization.

Fifth, developing new culture technology that hatchery staff can pivot to and resume production when ‘bad water’ is persistent, such as recirculating aquaculture systems (RAS), may be an important avoidance strategy in the future. These systems may allow production to proceed, albeit at a smaller scale, by using entirely artificial conditions that are less reliant on the fluctuating conditions of natural water bodies. As such, there appears to be growing interest in using RAS across all stages of shellfish production (Frias and Segovia, 2010; Qiu et al., 2017; Ramos et al., 2021).

Sixth, due to the complexities with identifying crash sources, efficiently and reliably responding to crashes may require managers to send samples from productive and unproductive periods to a multidisciplinary team (biologists, chemists, pathologists, microbiologists, metagenomists, etc.) that is capable of quickly analyzing and identifying contaminants so that appropriate mitigation procedures can be developed. Rapid response in collection and preservation or analysis is crucial

during a crash as the presence and stability of chemical constituents might be short. Data from periods of high larvae productivity would enable for quick comparisons and establishments of contaminant thresholds as some potentially harmful compounds will occur naturally and vary in concentration. Additionally, because sources of crashes occur over time, such analysis may have been repeated by the entire team many times per season. Indeed, the authors of this paper first came together in early 2019 to form a proposal that would readily analyze the inorganic/organic chemistry, biogeochemistry, bacterial and viral communities, algal toxins, etc. of crashed oyster larvae across all research hatcheries listed herein. We strongly suggest that this approach be examined more to both explore the variety of insults that disrupt production and how these vary over space and time. Eventually, it is our hope that new technology could be developed that can rapidly predict the arrival of such insults, diagnose impacted facilities, or at least eliminate candidate culprits.

Finally, the economics of hatchery crashes and the consequences on the private shellfish industry as a whole are not well understood and cannot be explored until more hatcheries share data for examination by economists. Private hatcheries may be unwilling to share production problems with researchers or other industry members (i.e. competitors), perhaps fearing how this information may shape their brand; however, a major benefit of detailing crashes and investigating their drivers with research hatcheries was the eagerness of members to share production details to solve an industry-wide problem. Although precise economic information remains scarce, it is certain that production delays cause the profitability of hatcheries, and the growers they supply, to suffer. This is especially true for new or smaller hatcheries that may lose three to four weeks of production, if they rely on single batches that crash toward the end of the two-week production process (i.e. spawn to set). This time loss may be another significant cost, considering the entire production season often lasts only 12-20 weeks. In some locations, hatcheries have closed or been brought to the brink of failure following persistent crashes that can create a bullwhip effect on supply chains, threatening growers and market availability of oysters (Weiss, 2008).

Culture of *Crassostrea virginica* larvae has advanced greatly, albeit slowly, since its humble beginnings in the late 19th century. A primitive understanding of oyster biology and lack of technology that was needed to prepare and maintain cultures hampered development of effective culture systems (see review by Kennedy 2014). After continued investigation and study through the early and mid-20th century, methods for oyster culture had become reliable, arguably culminating in the first comprehensive manual for cultivation of bivalve larvae by Loosanoff and Davis (1963). Since then, bivalve larval culture has continued to advance. Nevertheless, sporadic larval crashes have been a consistently sore topic within the industry. There has been limited motivation for researchers to invest effort in understanding these failures, arguably because they are currently accepted as a common and unavoidable cost of production that is rarely reported; however, we should not accept larval failures or crashes as a major bottleneck that constrains overall shellfish aquaculture production. As in other industries, especially in the agricultural sector, we must identify the weakest links in the production process and fix these to improve production reliability and greater profitability. Shellfish aquaculture should be treated no differently. To meet domestic and international goals of increasing supply of shellfish, which is thought of as the most ecologically sustainable source of animal protein (Shumway et al., 2003), we argue hatchery crashes must be addressed.

Acknowledgements:

This research was sponsored by UMCES Horn Point Laboratory. We thank A. Hollins for assistance with the microbiome sequencing.

References

Arzul, I., Corbeil, S., Morga, B., Renault, T., 2017. Viruses infecting marine molluscs. J. Invertebr. Pathol., Invertebrate Viruses and the Food Chain 147, 118–135. https://doi.org/10.1016/j.jip.2017.01.009

Barton, A., Hales, B., Waldbusser, G.G., Langdon, C., Feely, R.A., 2012. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. Limnol. Oceanogr. 57, 698–710.

Barton, A., Waldbusser, G.G., Feely, R.A., Weisberg, S.B., Newton, J.A., Hales, B., Cudd, S., Eudeline, B., Langdon, C.J., Jefferds, I., others, 2015. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography 28, 146–159.

Bergström, P., Lindegarth, S., Lindegarth, M., 2015. Modeling and predicting the growth of the mussel, *Mytilus edulis*: implications for planning of aquaculture and eutrophication mitigation. Ecol. Evol. 5, 5920–5933. https://doi.org/10.1002/ece3.1823

Breiman, L., 2001. Random forests. Machine Learning 45(1), 5–32. https://doi.org/10.1023/A:1010933404324

Breiman, L., Friedman, J.H., Olshen, R.A., Stone, C.J., 1984. Classification and Regression Trees. Taylor & Francis, New York.

Brownlee, E.F., Sellner, S.G., Sellner, K.G., Nonogaki, H., Adolf, J.E., Bachvaroff, T.R., Place, A.R., 2008. Responses of *Crassostrea virginica* (Gmelin) and *C. ariakensis* (Fujita) to bloom-forming phytoplankton including ichthyotoxic *Karlodinium veneficum* (Ballantine). J. Shellfish Res. 27, 581–591. https://doi.org/10.2983/0730-8000(2008)27[581:ROCVGA]2.0.CO;2

Burge, C.A., Shore-Maggio, A., Rivlin, N.D., 2017. Ecology of emerging infectious diseases of

Invertebrates, in: Ecology of Invertebrate Diseases. John Wiley & Sons, Ltd, pp. 587–625. https://doi.org/10.1002/9781119256106.ch16

Casas, S.M., Lavaud, R., La Peyre, M.K., Comeau, L.A., Filgueira, R., La Peyre, J.F., 2018. Quantifying salinity and season effects on eastern oyster clearance and oxygen consumption rates. Mar. Biol. 165, 90. https://doi.org/10.1007/s00227-018-3351-x

Chu, H., Mazmanian, S.K., 2013. Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat. Immunol. 14, 668–675. https://doi.org/10.1038/ni.2635

Clements, J.C., Carver, C.E., Mallet, M.A., Comeau, L.A., Mallet, A.L., 2020. CO2-induced low pH in an eastern oyster (*Crassostrea virginica*) hatchery positively affects reproductive development and larval survival but negatively affects larval shape and size, with no intergenerational linkages. ICES J. Mar. Sci. https://doi.org/10.1093/icesjms/fsaa089

Coffin, M.R.S., Clements, J.C., Comeau, L.A., Guyondet, T., Maillet, M., Steeves, L., Winterburn, K., Babarro, J.M.F., Mallet, M.A., Haché, R., Poirier, L.A., Deb, S., Filgueira, R., 2021. The killer within: Endogenous bacteria accelerate oyster mortality during sustained anoxia. Limnol. Oceanogr. n/a. https://doi.org/10.1002/lno.11798

Croswell, A., Amir, E., Teggatz, P., Barman, M., Salzman, N.H., 2009. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric salmonella infection. Infect. Immun. 77, 2741–2753. https://doi.org/10.1128/IAI.00006-09

Degenhardt, F., Seifert, S., Syzmczak, S., 2019. Evaluation of variable selection methods for random forests and omics data sets. Briefings in Bioinformatics. Oxford Academic. Brief. Bioinform. 20, 492–503.

Ekstrom, J.A., Suatoni, L., Cooley, S.R., Pendleton, L.H., Waldbusser, G.G., Cinner, J.E., Ritter, J., Langdon, C., van Hooidonk, R., Gledhill, D., Wellman, K., Beck, M.W., Brander, L.M., Rittschof, D., Doherty, C., Edwards, P.E.T., Portela, R., 2015. Vulnerability and adaptation

of US shellfisheries to ocean acidification. Nat. Clim. Change 5, 207–214. https://doi.org/10.1038/nclimate2508

Elston, R., Leibovitz, L., Relyea, D., Zatila, J., 1981. Diagnosis of vibriosis in a commercial oyster hatchery epizootic: Diagnostic tools and management features. Aquaculture 24, 53–62. https://doi.org/10.1016/0044-8486(81)90043-0

Estes, R.M., Friedman, C.S., Elston, R.A., Herwig, R.P., 2004. Pathogenicity testing of shellfish hatchery bacterial isolates on Pacific oyster *Crassostrea gigas* larvae. Dis. Aquat. Organ. 58, 223–230. https://doi.org/10.3354/dao058223

Falconer, I.R., Runnegar, M.T.C., Buckley, T., Huyn, V.L., Bradshaw, P., 1989. Using activated carbon to remove toxicity from drinking water containing cyanobacterial blooms. J. AWWA 81, 102–105. https://doi.org/10.1002/j.1551-8833.1989.tb03170.x

Frias, R., Segovia, M., 2010. Gonad development of the Japanese oyster *Crassostrea gigas* in a recirculating system: first step toward the development of conditioning and maturation protocols. J. Shellfish Res. 29, 303–308. https://doi.org/10.2983/035.029.0204

Friedman, C.S., Estes, R.M., Stokes, N.A., Burge, C.A., Hargove, J.S., Barber, B.J., Elston, R.A., Burreson, E.M., S.Reece, K., 2005. Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes. Dis. Aquat. Organ. 63, 33–41. https://doi.org/10.3354/dao063033

Geva-Zatorsky, N., Sefik, E., Kua, L., Pasman, L., Tan, T.G., Ortiz-Lopez, A., Yanortsang, T.B., Yang, L., Jupp, R., Mathis, D., Benoist, C., Kasper, D.L., 2017. Mining the human gut microbiota for immunomodulatory organisms. Cell 168, 928-943.e11. https://doi.org/10.1016/j.cell.2017.01.022

Gibbs, M.C., L.M. Parker, E. Scanes, M. Byrne, W. A. O'Conner, P.M. Ross. Energetic lipid responses of larval oysters to ocean acidification. Mar. Poll. B. 168, 112441

Ginger, K.W.K., Vera, C.B.S., R, D., Dennis, C.K.S., Adela, L.J., Yu, Z., Thiyagarajan, V., 2013. Larval and post-larval stages of pacific oyster (*Crassostrea gigas*) are resistant to elevated CO2. PLOS ONE 8, e64147. https://doi.org/10.1371/journal.pone.0064147

Glibert, P.M., Alexander, J., Meritt, D.W., North, E.W., Stoecker, D.K., 2007. Harmful algae pose additional challenges for oyster restoration: impacts of the harful algae *Karlodinium veneficum* and *Prorocentrum minimum* on early life stages of *Crassostrea virginica* and *Crassostrea ariakensis* J. Shellfish Res. 26, 919–925.

Gonçalves, A.A., Gagnon, G.A., 2011. Ozone application in recirculating aquaculture system: an overview. Ozone Sci. Eng. 33, 345–367. https://doi.org/10.1080/01919512.2011.604595

Gray, M.W., Langdon, C.J., Waldbusser, G.G., Hales, B., Kramer, S., 2017. Mechanistic understanding of ocean acidification impacts on larval feeding physiology and energy budgets of the mussel *M*ytilus *californianus*. Mar. Ecol. Prog. Ser. https://doi.org/10.3354/meps11977

Helm, M.M., Millican, P.F., 1977. Experiments in the hatchery rearing of Pacific oyster larvae (*Crassostrea gigas* Thunberg). Aquaculture 11, 1–12.

His, E., Beiras, R., Seaman, M.N.L., 1999. The assessment of marine pollution-bioassays with bivalve embryos and larvae. Academic Press, pp. 1–178.

Hopkins, K.G., Bhaskar, A.S., Woznicki, S.A., Fanelli, R.M., 2020. Changes in event-based streamflow magnitude and timing after suburban development with infiltration-based stormwater management. Hydrol. Process. 34, 387–403. https://doi.org/10.1002/hyp.13593

Hornick, K.M., Plough, L.V., 2019. Tracking genetic diversity in a large-scale oyster restoration program: effects of hatchery propagation and initial characterization of diversity on restored vs. wild reefs. Heredity 123, 92–105. https://doi.org/10.1038/s41437-019-0202-6

Jones, J.B., 2006. Why won't they grow?–Inhibitory substances and mollusc hatcheries. Aquac.

Int. 14, 395–403.

Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., others, 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Mar. Ecol. Prog. Ser. 303, 1–29.

Kennedy, V.S., 2014. Technological Constraints During the First 40 Years of Eastern Oyster *Crassostrea virginica* Aquaculture. Rev. Fish. Sci. Aquac. 22, 55–72. https://doi.org/10.1080/10641262.2013.822464

Kesarcodi-Watson, A., Kaspar, H., Lategan, M.J., Gibson, L., 2008. Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274, 1–14.

Kuang, Z., McConnell, L.L., Torrents, A., Meritt, D., Tobash, S., 2003. Atmospheric deposition of pesticides to an agricultural watershed of the Chesapeake Bay. J. Environ. Qual. 32, 1611–1622. https://doi.org/10.2134/jeq2003.1611

Kurihara, H., 2008. Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275–284. https://doi.org/10.3354/meps07802

Kurihara, H., Kato, S., Ishimatsu, A., 2007. Effects of increased seawater pCO2 on early development of the oyster *Crassostrea gigas*. Aquat. Biol. 1, 91–98.

Kursa, M.B., Rudnicki, W.R., 2010. Feature selection with the Boruta package. J Stat Softw 36, 1–13.

Kursa, M.B., Rudnicki, W.R., 2020. Boruta: Wrapper Algorithm for All Relevant Feature Selection. R package version 7.0.0. https://CRAN.R-project.org/package=Boruta

Langston, A.L., 2015. 2015 AQ Summit: Identified R&D Needs Report. DigitalCommons@UMaine, Annual Maine Aquaculture R&D and Education Summits.

Lewis, T.E., Garland, C.D., O'brien, T.D., Fraser, M.I., Tong, P.A., Ward, C., Dix, T.G., McMeekin,

T.A., 1988. The use of 0.2-μm membrane-filtered seawater for improved control of bacterial levels in microalgal cultures fed to larval Pacific oysters (*Crassostrea gigas*). Aquaculture 69, 241–251.

Li, J., Glibert, P.M., Gao, Y., 2015. Temporal and spatial changes in Chesapeake Bay water quality and relationships to *Prorocentrum minimum*, *Karlodinium veneficum*, and CyanoHAB events, 1991–2008. Harmful Algae 42, 1–14. https://doi.org/10.1016/j.hal.2014.11.003

Loosanoff, V.L., Davis, H.C., 1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1, 1–136.

Lukenbach, M., Lipton, D., Webster, D.W., Abel, S., Zinn, T., Leggett, T., Rhodes, E., Sellner, K.G., 2008. A framework for native oyster aquaculture development in Maryland (No. 08–166). Chesapeake Research Consortium, Edgewater, MD.

Mann, R., Powell, E.N., 2007. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. J. Shellfish Res. 26, 905–917.

Mccollough, C.B., Albright, B.W., Abbe, G.R., Barker, L.S., Dungan, C.F., 2007. Acquisition and progression of *Perkinsus marinus* infections by specific-pathogen-free juvenile oyster (*Crassostrea virginica* Gmelin) in a mesohaline Chesapeake Bay tributary. J. Shellfish Res. 26, 465–477.

Newcombe, G., Nicholson, B., 2004. Water treatment options for dissolved cyanotoxins. J. Water Supply Res. Technol.-Aqua 53, 227–239. https://doi.org/10.2166/aqua.2004.0019

NOAA, 2016. Annual Landings [WWW Document]. Commer. Fish. Stat. URL https://www.st.nmfs.noaa.gov/commercial-fisheries/commercial-landings/annual-landings/index (accessed 9.19.16).

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' Community Ecol. Package Version 2, 1–295.

Otsuka, T., Kitazawa, Y., Ito, T., 2018. Multiple water-level seawater temperature prediction method for marine aquaculture, in: Mouhoub, M., Sadaoui, S., Ait Mohamed, O., Ali, M. (Eds.), Recent trends and future technology in applied intelligence, Lecture Notes in Computer Science. Springer International Publishing, Cham, pp. 366–371. https://doi.org/10.1007/978-3-319-92058-0_35

Prado, S., Romalde, J.L., Montes, J., Barja, J.L., 2005. Pathogenic bacteria isolated from disease outbreaks in shellfish hatcheries. First description of *Vibrio neptunius* as an oyster pathogen. Dis. Aquat. Organ. 67, 209–215. https://doi.org/10.3354/dao067209

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. https://doi.org/10.1093/nar/gkm864

Qiu, T., Qi, J., Zheng, J., Liu, Y., 2017. Design and performance of a recirculating aquaculture system for oyster larval culture. Aquac. Res. 48, 5699–5706. https://doi.org/10.1111/are.13392

Ragg, N.L.C., Gale, S.L., Le, D.V., Hawes, N.A., Burritt, D.J., Young, T., Ericson, J.A., Hilton, Z., Watts, E., Berry, J., King, N., 2019. The effects of aragonite saturation state on hatchery-reared larvae of the Greenshell mussel *Perna canaliculus*. J. Shellfish Res. 38, 779–793. https://doi.org/10.2983/035.038.0328

Ramachandran, P., Reed, E., Commichaux, S., Strain, E., Depaola, A., Rikard, S., Ottesen, A., 2018. Characterization of the Microbiota of Oyster Larvae (Crassostrea virginica) and Tank Water from an Aquaculture System with High and Low Larval Survival Rates. Genome Announc 6. https://doi.org/10.1128/genomeA.00597-18

Ramos, C. de O., da Silva, F.C., Gomes, C.H.A. de M., Langdon, C., Takano, P., Gray, M.W., de Melo, C.M.R., 2021. Effect of larval density on growth and survival of the Pacific oyster

Crassostrea gigas in a recirculation aquaculture system. Aquaculture 540, 736667. https://doi.org/10.1016/j.aquaculture.2021.736667

Richards, G.P., Watson, M.A., Needleman, D.S., Church, K.M., Häse, C.C., 2015. Mortalities of Eastern and Pacific oyster larvae caused by the pathogens *Vibrio coralliilyticus* and *Vibrio tubiashii*. Appl. Environ. Microbiol. 81, 292–297. https://doi.org/10.1128/AEM.02930-14

Robert, R., Gérard, A., 1999. Bivalve hatchery technology: The current situation for the Pacific oyster *Crassostrea gigas* and the scallop Pecten maximus in France. Aquat. Living Resour. 12, 121–130. https://doi.org/10.1016/S0990-7440(99)80021-7

Shamkhali Chenar, S., Deng, Z., 2017. Environmental indicators of oyster norovirus outbreaks in coastal waters. Mar. Environ. Res. 130, 275–281. https://doi.org/10.1016/j.marenvres.2017.08.009

Shumway, S.E., Davis, C., Downey, R., Karney, R., Kraeuter, J., Parsons, J., Rheault, R., Wikfors, G., 2003. Shellfish aquaculture–in praise of sustainable economies and environments. World Aquac. 34, 8–10.

Sugumar, G., Nakai, T., Hirata, Y., Matsubara, D., Muroga, K., 1998. Vibrio splendidus biovar II as the causative agent of bacillary necrosis of Japanese oyster *Crassostrea gigas* larvae. Dis. Aquat. Organ. 33, 111–118. https://doi.org/10.3354/dao033111

Thömsen, J., I. Casties, C. Pansch, A. Körtzinger, F. Melzner. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. Global Change Bio. 19: 1017-1027.

Timmins-Schiffman, E., O'Donnell, M.J., Friedman, C.S., Roberts, S.B., 2013. Elevated pCO2 causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*. Mar. Biol. 160, 1973–1982.

Timmins-Schiffman, E., White, S.J., Thompson, R.E., Vadopalas, B., Eudeline, B., Nunn, B.L.,

Roberts, S.B., 2021. Coupled microbiome analyses highlights relative functional roles of bacteria in a bivalve hatchery. Environmental Microbiome 16, 7. https://doi.org/10.1186/s40793-021-00376-z

Travers, M.-A., Boettcher Miller, K., Roque, A., Friedman, C.S., 2015. Bacterial diseases in marine bivalves. J. Invertebr. Pathol., Pathogens and Disease Processes in Marine Molluscs 131, 11–31. https://doi.org/10.1016/j.jip.2015.07.010

UMCES Horn Point Laboratory, 2020. About Horn Point Oyster Hatchery | Horn Point Lab Oyster Hatchery. URL http://hatchery.hpl.umces.edu/overview/about-horn-point-oyster-hatchery/ (accessed 7.24.20).

Urban Jr, E.R., Langdon, C.J., 1984. Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. Aquaculture 38, 277–291.

USDA, 2019. Aquaculture Data [WWW Document]. Aquac. Trade Data. URL https://www.ers.usda.gov/data-products/aquaculture-data.aspx (accessed 12.30.19).

Ushijima, B., Richards, G.P., Watson, M.A., Schubiger, C.B., Häse, C.C., 2018. Factors affecting infection of corals and larval oysters by *Vibrio coralliilyticus*. PLOS ONE 13, e0199475. https://doi.org/10.1371/journal.pone.0199475

Utting, S.D., Millican, P.F., 1997. Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effect on egg quality and larval viability. Aquaculture, Proceedings of the fish and shellfish larviculture symposium LARVI ’95 155, 45–54. https://doi.org/10.1016/S0044-8486(97)00108-7

Waldbusser, G.G., Hales, B., Langdon, C.J., Haley, B.A., Schrader, P., Brunner, E.L., Gray, M.W., Miller, C.A., Gimenez, I., 2015a. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nat. Clim. Change 5, 273–280.

Waldbusser, G.G., Hales, B., Langdon, C.J., Haley, B.A., Schrader, P., Brunner, E.L., Gray, M.W., Miller, C.A., Gimenez, I., Hutchinson, G., 2015b. Ocean acidification has multiple modes of action on bivalve larvae. PloS One 10, e0128376.

Waldbusser, G.G., Voigt, E.P., Bergschneider, H., Green, M.A., Newell, R.I.E., 2010. Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuaries Coasts 1–11.

Walker, T., 2017. Seed supply a challenge for North American oyster producers. Hatch. Int.

Weiss, K.R., 2008. A warning from the sea. latimes. https://www.latimes.com/local/la-me-oysters13-2008jul13-story.html

Wright, M.N., Wager, S., Probst, P., 2020. ranger: A Fast Implementation of Random Forests. URL https://CRAN.R-project.org/package=ranger, R package version 0.12.1