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APPLIED STUDIES

The nutrient footprint of a submerged-cage offshore aquaculture facility located in the tropical Caribbean

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The effect of effluent generated by a commercially scaled offshore (~13 km) finfish aquaculture facility in the tropical Caribbean on the water column and benthic nutrients and chlorophyll-*a* is described. Water column samples were collected up- and downstream of the site at various times between 2012 and 2018. Typically, no significant difference in dissolved oxygen, chlorophyll-*a*, particulate organic carbon, particulate organic nitrogen, nitrate + nitrite, and total dissolved nitrogen concentration was observed in the water column between the up- versus downstream samples. Similarly, sediment samples were collected at various times between 2012 and 2018. Samples were collected at up- versus downstream locations and analyzed for benthic carbon, nitrogen, and chlorophyll-*a* content. Some of the collected data demonstrates a trend toward sediment enrichment within the vicinity of the farm. These data are of interest to stakeholders concerned with the expansion of offshore aquaculture in the United States and other countries. To our knowledge, this is the first report of its kind from a commercially scaled aquaculture facility utilizing offshore submersible-cage technologies.

KEYWORDS

effluent, environmental impacts, nutrients, offshore aquaculture

1 | INTRODUCTION

On June 26, 2018, Bill S.3138 was introduced in the U.S. Senate. This bill is titled Advancing the Quality and Understanding of American Aquaculture Act (2018) (or AQUAA Act) and creates a regulatory system for the permission and operation of marine aquaculture facilities in the United States Exclusive Economic Zone (EEZ). While there have

been previous efforts to establish a permitting system for offshore aquaculture in the U.S. EEZ, and while there are outstanding procedural and political issues left unresolved by the AQUAA Act (Lester, Gentry, Kappel, White, & Gaines, 2018), industry observers anticipate that companies will begin applying for permits to establish offshore aquaculture facilities in U.S. waters in the near future.

To some degree, these are unsurprising developments. The United States currently runs a significant trade deficit in seafood and is dependent on imports to meet demand. In 2017, the United States imported seafood worth more than $21 billion, approximately 90% of the country's total supply. In the same year, U.S. exports of seafood were worth only $5.4 billion (NOAA, 2018). Moreover, global demand for seafood has increased for decades, and developing countries such as China and India are likely to consume a larger percentage of their own production over time, a trend that will inevitably lead to tighter supplies for U.S. importers (Kearney, 2010). Given these realities, a desire by U.S. policymakers and business leaders to increase the domestic supply of seafood is both rational and predictable.

In addition to the economic rationale, a suite of technologies has been developed in recent years that allows for the conduct of aquaculture in more distant, high-energy, offshore marine waters. Not only does this obviate some of the spatial requirements of more traditional land-based or near-shore aquaculture systems (Lester et al., 2018), offshore aquaculture also has several potential environmental advantages. A move by fish farmers into the offshore would allow effluents to be discharged into larger reservoirs, with potentially shorter residence times, allowing for a greater degree of "dilution" of aquaculture effluents by natural waters, thus limiting the environmental impact of the discharged nutrients. There is some theoretical support for this idea, and several studies conducted at more conventional near-shore farms located in the highly oligotrophic eastern Mediterranean have provided some empirical support as well (Neofitou & Klaoudatos, 2008; Pitta et al., 2009; Pitta, Apostolaki, Tsagaraki, Tsapakis, & Karakassis, 2006).

In spite of increasing demand by consumers and theoretical arguments for lesser environmental harm, there is also reason for concern regarding the development of a new aquaculture industry in the U.S. EEZ. In addition to the usual litany of environmental problems associated with large-scale aquaculture (e.g., escapism, fish meal and fish oil consumption, antibiotic use, etc.) (Holmer, 2010), some worry that even truly offshore aquaculture, if practiced at a sufficient scale, could generate a nutrient flux capable of causing problems in the offshore region that have traditionally been associated with near-shore fish farming (e.g., eutrophication, harmful algae blooms, etc.) (Adams et al., 2009; Food and Water Watch, 2011; Sarà et al., 2011).

Stakeholders interested in the issue of aquaculture have thus been left with a chicken-and-egg dilemma: short of modeling and analysis of imperfect (i.e., near-shore) analogs, there is no way to be certain that commercially scaled offshore aquaculture systems will not generate unacceptable nutrient-related environmental impacts. Without operational offshore facilities, there is no ability to collect the sort of real-world data that would allow for empirically based regulatory decision-making to occur. Recently, an opportunity to address this knowledge gap presented itself. In the Republic of Panama, a large offshore aquaculture facility was permitted and constructed. This farm has been fully operational since 2009 and, to our knowledge, is the world's first truly offshore (i.e., ~13 km from the shoreline) aquaculture facility operating at a commercial scale. Currently, this farm has 22 individual cages ≥6,400 m^3. As many as 20 of these cages are in operation at any given time, and the farm is on track to produce more than 1,400 m.t. of fish this year. This farm is a rich source of data relevant to decision makers and stakeholders in the aquaculture community, especially those interested in the future of offshore aquaculture in the Gulf of Mexico and other tropical and subtropical environments.

In this article, we describe the results of environmental monitoring work conducted at this facility since 2012. This monitoring occurred in two distinct phases. The first phase consisted of two intensive 10-day study periods that occurred in 2012 and 2013. The first phase was part of a grant-funded research project conducted by the University of Miami and other collaborators. During the first phase of this monitoring, we used LaGrangian (or dynamic) monitoring techniques to compare a suite of biogeochemical characteristics of water and sediment samples collected upstream (and thus unaffected by) and downstream of the aquaculture facility. The second phase of the research described in the article was conducted by the farm itself as part of an expanded environmental monitoring program,

with technical assistance provided by the authors and the University of Miami. The second phase consisted of additional sampling required to comply with government and third-party certification body requirements. This sampling focused on sediment total organic carbon (TOC) and water column ammonia (NH_4^+) levels around the farm in 2017 and 2018.

2 | MATERIALS AND METHODS

2.1 | Study location and site description

This study occurred at an offshore aquaculture facility dedicated to the production of cobia (*Rachycentron canadum*). Cobia is a large pelagic fish whose natural distribution is circumtropical, with the probable exception of the eastern Tropical Pacific (Franks & Brown-Peterson, 2002). Cobia exhibit rapid growth and other characteristics desired by aquaculturists (Benetti et al., 2010). Cobia is a relatively new fish to the aquaculture industry. Research into aquaculture techniques for cobia began in the 1970s in the United States (Hassler & Rainville, 1975), and large-scale production began in the 1990s (Liao et al., 2004). Today, the total annual production of cobia is less than 50,000 tons globally, most of which occurs in China (FAO, 2013).

The farm site is located on the Atlantic coast of Panama in the Costa Arriba region. The site is approximately 13 km offshore, in depths ranging from 55 to 65 m. At the beginning of phase one (2012), the farm site was occupied by 16 Ocean Spar "Sea-Station" cages, each ~ 6,400 m^3. During the 2013 sampling, there were 21 cages on site. By 2017, the number of cages on site had grown to 22. The farm has been operating continuously since 2010, although the data collected in this article are the first large-scale effort to collect data on effluent impacts. Future additions to the farm are planned, although the cages intended for use in the future are considerably larger (14,400 m^3) and will be located on a new site approximately 1 km to the west of the current site.

The Ocean Spar Sea Station's 6,400 m^3 cages consist of a 24 m-tall central spar oriented vertically in the water column and surrounded by an exterior rim with a diameter of approximately 35 m that circles the spar at its midpoint. Netting is stretched from the top of the spar, down around the rim, and back to the bottom of the spar, creating a three-dimensional space that resembles two cones joined at their bases (Figure 1). Cages are moored with multipoint moorings secured within an anchor grid and are maintained in a submerged position, although they are surfaced occasionally for maintenance or harvesting. When submerged, the cages rest with the top of the spar more than

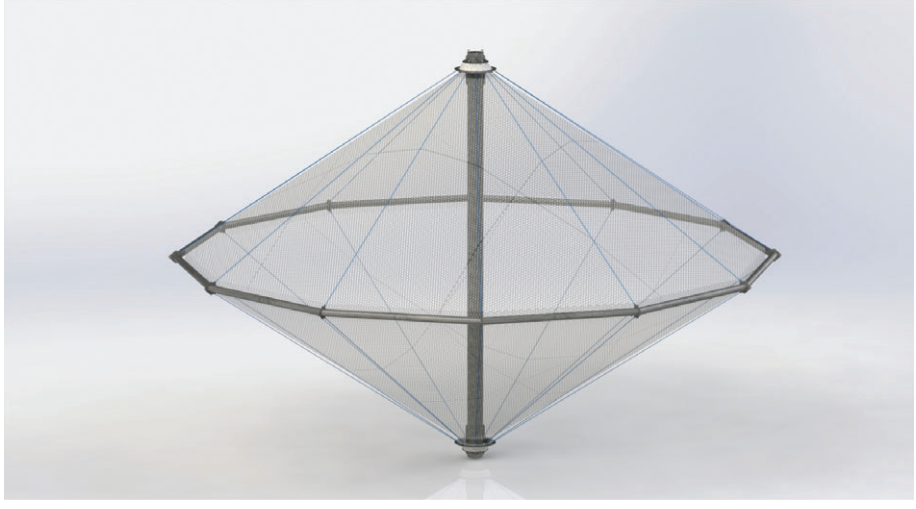

FIGURE 1 A 6400 m^3 Sea Station cage (Solidworks 2013 render by Richard Pasma, OceanSpar Inc.)

10 m below the surface, which can be submerged to greater depths. The entire group of cages is moored in two separate mooring grids. In this article, the group of cages in these two mooring grids is referred to collectively as the "cage field."

Cages are tended to by a small fleet of service boats, including feed boats, harvest boats, and maintenance boats. Divers work in and around the cages on a daily basis. Pelletized feed is provided to the fish via a pumping system that delivers feed to the fish through extended hoses connected to feed boats. Feed is provided to the cages once daily at a rate of <3% of biomass per day. Cages are generally stocked so that the final density at harvest remains below 25 kg/m^3. The target harvest size of the fish is 4–5 kg. The total biomass in the cages at the end of 2012 was 571,907 kg, and the total biomass in the cages at the end of 2013 was 919,917 kg. Since then, the biomass on the farm has risen steadily and reached 1,360,000 kg at the end of 2017.

The economic feed conversion ratio (eFCR) on the farm throughout the study period has gradually declined and, today, is between 2.5 and 3.0, where the eFCR describes the feeding efficiency of animal production operations. The eFCR is the total amount of feed provided to a cohort of fish divided by the amount of whole, wet-weight biomass of that cohort. The eFCR is not modified to account for fish escape, mortality, or any other form of crop loss that occurs prior to harvest.

The local climate at the farm site is typical for its location within the Inter-Tropical Convergence Zone (ITCZ). Strong trade winds blow generally from the north to the south and bring precipitation to the area as they reach the mountainous isthmus and shed moisture. Shifts in the ITCZ generate a wet season that runs generally from May to December and a drier season from January until April (Jackson & D'Croz, 1997). Farm staff reports that estimated wave heights at the site are typically <1 m, although they can reach 4–5 m under severe conditions. During the sampling described in this manuscript, researchers experienced all of these conditions, including estimated wave heights of 4–5 m.

Surface currents at the site have been measured by farm staff using a variety of different current meters and run alongshore in a predominantly eastward direction (although, occasionally, currents will run westward) at speeds between 0.05 and 0.7 m/s. These measurements were consistent with conditions observed during the sampling described here. Vertical current profiles were not available during our 2012 or 2013 sampling campaigns; however, in 2015, current measuring devices were installed by farm management. Resulting data indicate that currents are relatively consistent within the upper 30 m of the water column and then decrease with depth but retain the same direction of flow. The 2012 sampling occurred during windy (~10–25 km/hr) and rainy conditions, with seas of up to 3 m. Currents during the 2012 sampling were variable. The 2013 samples were taken during weather characterized by very hot (~30°C) and calm (≤5 km/hr) conditions, with no rain. Sampling during 2017 and 2018 occurred in all weather and current conditions, including dry and rainy season conditions.

2.2 | Phase 1 sample collection

During phase one of this project, sampling work was conducted by a team from the University of Miami. The phase one sampling protocol was designed to evaluate whether the aquaculture facility affected the biogeochemical characteristics of local waters and sediments. Water column samples were collected for the analysis of dissolved oxygen (DO), total dissolved nitrogen (TDN), nitrate + nitrite (NO_3^- + NO_2^-), chlorophyll-*a* (chl-*a*), particulate carbon (PC), and particulate nitrogen (PN) concentrations. The water column sampling strategy included collecting samples at one upstream location and three downstream locations for each sampling "run" (Figure 2). Sampling locations were chosen based on the trajectory of a Coastal Ocean Dynamics Experiment design drifter (Davis, 1985) released to track surface currents around the cage site. Prior to sampling, the drifter was deployed within the cage field and followed with the research boat in order to determine the current direction. Once the drifter confirmed the current direction, it was retrieved, and the research boat was moved to the upstream side of the cage field where an "Upstream Station" was chosen. The precise location of the Upstream Station varied from run to run but was always within 75 m of the cages on the upstream side of the cage field. In order to collect samples at the Upstream Station, the research

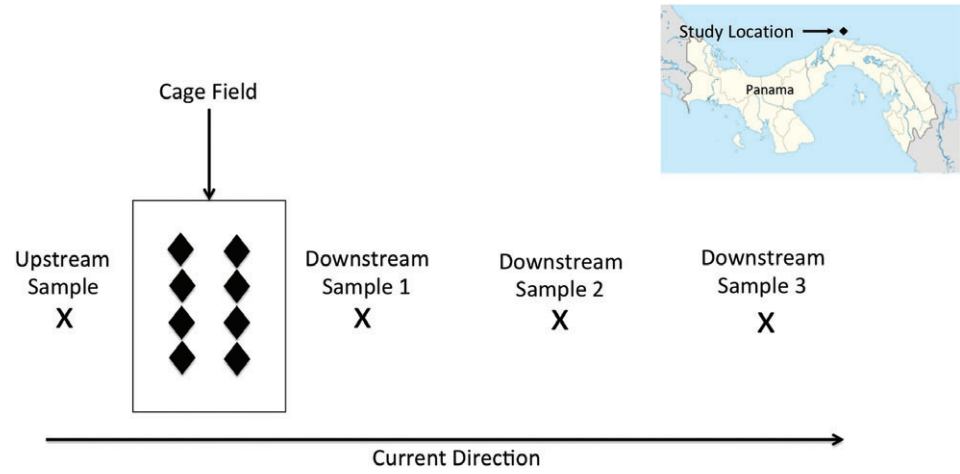

FIGURE 2 Water column sampling scheme relative to position of cage field

boat was moored directly to one of the "crown" buoys that were secured to the mooring grid's outer anchors. Once samples and measurements were collected at the Upstream Station, the drifter was released on a trajectory that allowed it to pass through the cage field and then drift downstream with the prevailing currents. Downstream 1 station was located at the point where the drifter cleared the cage field on the downstream side of the cage site and was always within 75 m of the cages. Stations Downstream 2 and Downstream 3 were located at approximately 1-hr intervals along the drifter trajectory downstream of the cage site (Figure 2). Distances between Stations Downstream 1, Downstream 2, and Downstream 3 varied with the current and ranged from a few hundred meters to more than a kilometer. Sampling began approximately 2 hr after farm crews had begun daily feedings.

The drifter was built at the University of Miami and consisted of four sails arranged in an "X" shape around a central mast. Each sail was approximately 50 cm in width and 50 cm in height. The mast was constructed of 75 cm of 2″ polyvinyl chloride pipe, and the spars were made from 0.5″ outer diameter fiberglass rods. The fiberglass rods were inserted in the mast (top and bottom) via holes drilled into the spar at right angles to one another. Sleeves sewed into the sails (top and bottom) allowed them to be affixed to the rods. The spar was ballasted with two 16-oz lead weights at the bottom, and four 12″ all-purpose styrofoam buoys were attached to the top spars, ensuring that the drifter maintained a vertical orientation while submerged in the water column.

At each sampling station, individual water samples were taken at 5, 15, 30, and 60 m. Samples were collected from the sampling vessel using a hand-operated 10 L Niskin bottle (General Oceanics, Miami, FL) that was closed with a messenger. Depths were measured using premeasured lengths of line attached to the Niskin bottle. Individual samples from each station and depth were stored in 10-L high-density polyethylene (HDPE) carboys in a cooler until returning to land where they were processed within 6 hr of collection. In addition to collecting individual water samples, in situ DO, temperature, and salinity measurements were made at each station using a Seabird SBE 43 DO sensor (Seabird Electronics, Bellevue, WA) attached to a SBE M19 conductivity, temperature, depth (CTD) unit (Seabird Electronics, Bellevue, WA). The DO sensor and CTD unit were deployed by hand immediately after the water samples were collected. The units were deployed by hand and then allowed to descend to the bottom at ~0.3 m/s. The DO sensor and CTD unit were retrieved at the same approximate speed. The CTD unit and DO sensor were factory calibrated prior to deployment (Seabird Electronics, Bellevue, WA).

Similar to the water column, sediment samples were collected to evaluate the effects of the aquaculture facility on sediment biogeochemical parameters. Sampling was divided into three zones: a near zone (Z1), within 50 m of the cage field; an intermediate zone, between 50 and 150 m from the cage field (Z2); and a far zone, between 150 and 500 m from the cage field (Z3). Each zone was further subdivided on the east and west side of the cages so that each

zone had an east and west subzone (Z1E, ZIW, Z2E, Z2W, Z3E, Z3W) (Figure 3). Currents at the site ran primarily to the east, making the eastern subzones the downstream zones, while the western subzones were the upstream zones. Finally, samples were collected from a control site in a location (1 km to the north) that was unaffected by the effluent from the cage field because of the prevailing current direction (Figure 3).

Sediment samples were collected with an 8.2-Liter Ponar grab sampler (Wildlife Supply Company, Yulee, FL). Immediately upon opening the grab sampler, 1 cm of the top layer of sediment was collected into a plastic bag and stored on ice in a cooler for sediment chl-*a* analysis onshore. In addition, ~200 mL of sediment was collected into plastic bags and stored on ice in a cooler until returning to land, where samples were stored frozen at −20°C for shipment back to the United States.

2.3 | Phase 2 sample collection

During phase 2 of this work, samples were collected for additional sediment and water column analysis. Monitoring began in early 2017 and was performed by farm employees assisted by personnel from the University of Miami. Sediment sampling was conducted using the same scheme used in 2013, but Zone 3 was eliminated from consideration, leaving Z1E, Z2E, Z1W, and Z2W (Figure 3). The control site utilized in 2017 was the same site utilized during the 2013 sampling work. As during Phase 1, currents at the site ran primarily to the east, making the eastern subzones the "downstream" zones, while the western subzones were the upstream zones. Samples in the second phase of the monitoring program were collected using a 20″ Heavy KB-Core Sampler (Wildlife Supply Company, Yulee, FL) with plastic core liners. The corer was deployed from a work boat outfitted with an electric winch. When cores were retrieved, the top 2.5 cm of the core was extruded from the core liners, collected into plastic bags, and stored on ice in a cooler until returning to land. All samples were delivered on ice to Aquatec Laboratories in Panama City, Panama within 24 hr of collection, where they were analyzed for TOC.

Water samples for ammonia (NH_4^+) analysis were also collected beginning in 2017. These samples were collected once a month from within submerged cages at midcage depth (approximately 20 m) and at a control site approximately 1 km south of the cage field. Water samples were collected using a hand-operated 10-L Niskin Bottle

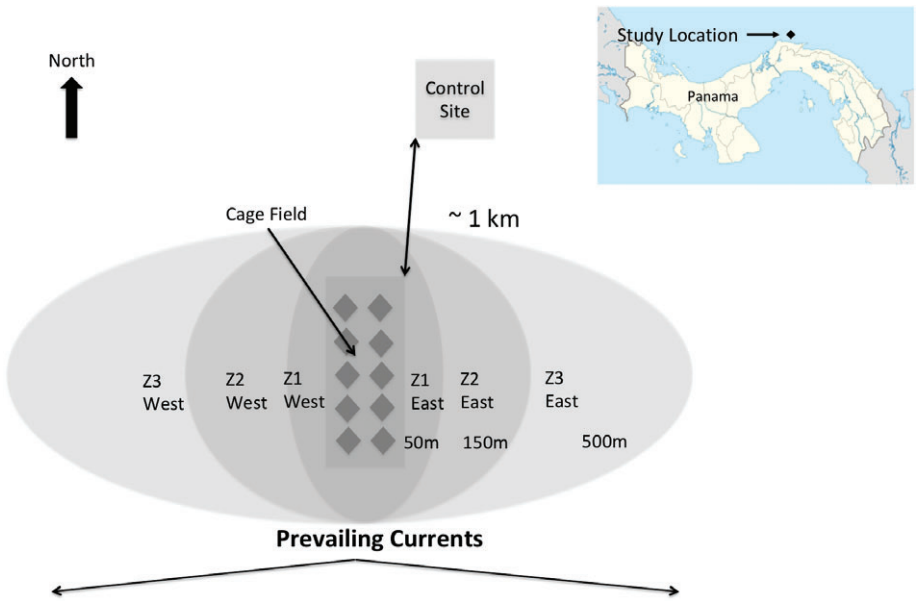

FIGURE 3 Sediment sampling scheme relative to cage field. Sites in "Zone 1", "Zone 2", and "Zone 3" are ~ 50 m, 150 m, and 500 m east and west of the cage field, respectively. Control site represented by "C"

(General Oceanics, Miami, FL) that was carried by a diver for samples taken in the cages and operated from the sampling vessel for samples taken at the control site.

2.4 | Phase 1 sample analysis

For NO_3^-, $+NO_2^-$ and TDN concentrations, water column samples were filtered through a 0.7 μm Whatman GF/F glass microfiber filter and collected into 60-mL acid-washed, sample-rinsed HDPE bottles and immediately frozen at −20°C. These samples were shipped frozen to the University of Miami, United States, where NO_3^- + NO_2^- and TDN concentration were measured using chemiluminescent analysis (Braman & Hendrix, 1989) and persulfate oxidation of TDN to NO_3^- (Solorzano & Sharp, 1980), adapted according to Knapp, Sigman, and Lipschultz (2005), with the resulting NO_3^- measured by chemiluminescence. The chemiluminescent analysis of NO_3^- + NO_2^- was performed using a configuration with a detection limit ~0.05 μM (±0.1 μM 1 *SD*). The concentration of TDN in a sample is the sum of dissolved organic nitrogen + NO_3^- + NO_2^- + ammonium (NH_4^+), and the propagated error for TDN concentration measurements was ±0.5 μM (±0.3 μM 1 *SD*).

In 2013, the water column chl-*a* concentration was analyzed on site in Panama based on methods described in Holm-Hansen and Riemann (1978). Briefly, concentrations were determined by filtering 150 mL of sample through a Whatman GF/F glass microfiber filter with nominal pore size of 0.7 μm. Filters were then placed in a centrifuge tube with 8 mL of methanol, stirred, and frozen for a minimum of 24 hr, after which time, samples were allowed to rise to room temperature in a warm water bath and then centrifuged for 1 min. The supernatant was pipetted off, and the fluorescence of samples was measured using a Turner Designs Trilogy Fluorometer (Turner Designs, San Jose, CA). Immediately after the initial reading, two drops of 10% HCl was added to the test tube, and a second reading was taken, a necessary step for determining phaeopigment concentration. Between each sample, the test tube was rinsed in triplicate with methanol. Chl-*a* concentration was not analyzed in 2012.

In 2013, water column suspended PC and PN concentrations were measured by filtering 1.5 L of sample onto a precombusted (4 hr at 450°C) 25 mm Whatman GF/F glass microfiber filter and then drying the filter in a 50°C drying oven located on site in Panama. Filters were then stored in polycarbonate filter cases and shipped to the United States for analysis. Once in the United States, PC and PN sample filters were pelletized and sent to the UC Davis Stable Isotope Facility for quantification of the PC and PN content by combustion analysis. PC samples were not acidified and so include both particulate organic and inorganic carbon.

In 2013, sediment samples for PC and PN analysis were packaged into 60-mL HDPE bottles, frozen at −20°C, and then shipped from Panama to the United States for analysis. Once in the United States, the samples were thawed and placed in a drying oven at 50°C for approximately 48 hr. Once dry, the samples were weighed into tin capsules and sent to the UC Davis Stable Isotope Facility for analysis.

In 2013, samples intended for sediment chl-*a* analysis were frozen in Panama and shipped to the University of Miami. Analysis of sediment chl-*a* content was conducted by thawing frozen sediment samples and then weighing them and transferring them to preweighed test tubes. Test tubes and samples were then reweighed in order to determine the weight of the sediment sample. After an accurate weight was established for each sample, chl-*a* was extracted in 9 mL of ethanol. Samples were spun in a centrifuge and stored at −20°C. Samples were then brought up to room temperature and centrifuged. Chl-*a* analysis was performed on the supernatant liquid. A Turner Design Trilogy Laboratory Fluorometer with a chl-*a* module was used for the analysis. Analysis was performed using the methods outlined in Holm-Hansen and Riemann (1978).

2.5 | Phase 2 sample analysis

Sediment samples collected during Phase 2 of this research were sent to Aquatec Testing Laboratories in Panama City, Panama, where they were analyzed for TOC according to the United States Environmental Protection Agency method sea water (SW) 9060 A. The SW 9060 A method converts organic carbon to carbon dioxide (CO_2) via

combustion, where it is then measured directly via infrared absorbance or is converted to methane (CH_4) and then measured using a flame ionization detector.

Water samples collected for NH_4^+ analysis in Phase 2 of this research were analyzed at onsite laboratory facilities using a Hach DR 900 Colorimeter (Hach, Loveland, CO). Water samples for NH_4^+ were analyzed using the Ammonia Salicylate method (Hach method 8155).

2.6 | Statistical analysis

The Kruskal-Wallis Rank Sum test for nonparametric data was used to evaluate whether there were significant differences between the nutrient concentrations measured in the water column and benthic samples collected upstream versus downstream of the aquaculture cage field. Given that photosynthesis produces DO, PC, PN, and chl-*a* in marine surface waters, while respiration in subsurface waters consumes DO, PC, and PN and regenerates inorganic nutrients at depth (Emerson & Hedges, 2008), we only compare upstream versus downstream water column measurements at a given depth. We also evaluated the distribution of nutrients, DO, and chl-*a* on density (i.e., sigma theta) surfaces instead of by depth, but this did not affect our results (Supporting Information Table S1).

3 | RESULTS

3.1 | Phase 1: Upstream versus downstream water column nutrient concentrations

The DO concentration in upstream and downstream samples were largely similar, although in some cases, DO concentrations at individual depths were potentially distinguishable from each other. Comparing the upstream versus downstream 2012 DO concentrations using the Kruskal-Wallis test, no significant difference (i.e., $p \geq 0.1$ in all cases) was detected between the samples collected at 5, 15, or 30 m. The average DO concentration in these upper three depths ranged between 6.44 ± 0.01 and 6.47 ± 0.01 mg/L (Figure 4, Table 1). However, in the 2012 60 m samples, the upstream DO (6.49 ± 0.01 mg/L) was significantly higher than the downstream DO (6.46 ± 0.01 mg/L) at the $0.1 \geq p \geq 0.05$ significance level (Figure 4, Table 1). In 2013, the average 5 m upstream DO concentration was 6.32 ± 0.01 mg/L, which was lower than the average downstream DO concentration at 5 m, which was 6.33 ± 0.01 at the $0.1 \geq p \geq 0.05$ significance level. In 2013, the average 15 m upstream DO was 6.32 ± 0.00 mg/L, which was significantly higher than the downstream DO concentration of 6.30 ± 0.02 mg/L, also at the

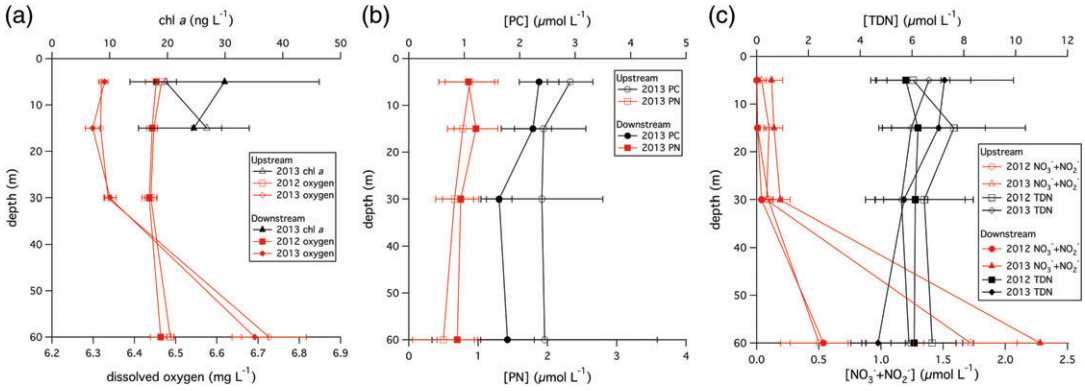

FIGURE 4 Water column chlorophyll-*a* (triangles) and dissolved oxygen (squares and diamonds) (a), particulate carbon (circles) and nitrogen (squares) (b), and total dissolved nitrogen (squares and diamonds) and NO_3^- + NO_2^- (circles and triangles) (c) for upstream (open symbol) and downstream (filled symbol) sampling locations in 2012 and 2013

TABLE 1 Average (± 1 *SD*) water column measurements

	$NO_3 + NO_2$ (μM)			
	2012		2013	
Depth (m)	Upstream	Downstream	Upstream	Downstream
5	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.12 ± 0.09
15	0.01 ± 0.02	0.01 ± 0.02	0.10 ± 0.04	0.14 ± 0.07
30	0.09 ± 0.11	0.04 ± 0.09	0.08 ± 0.05	0.19 ± 0.08
60	0.50 ± 0.23	0.54 ± 0.34	1.72 ± 0.38	2.28 ± 1.02
	TDN (μM)			
	2012		2013	
Depth (m)	Upstream	Downstream	Upstream	Downstream
5	6.0 ± 1.4	5.8 ± 1.4	6.6 ± 1.6	7.3 ± 2.7
15	7.6 ± 2.8	6.2 ± 1.5	6.0 ± 1.1	7.0 ± 1.8
30	6.5 ± 1.9	6.1 ± 1.9	5.6 ± 1.0	5.7 ± 0.8
60	6.8 ± 1.6	6.1 ± 1.9	5.9 ± 1.8	4.7 ± 1.1
	Oxygen (mg/L)			
	2012		2013	
Depth (m)	Upstream	Downstream	Upstream	Downstream
5	6.47 ± 0.01	6.45 ± 0.03	6.32 ± 0.01	6.33 ± 0.01
15	6.45 ± 0.00	6.44 ± 0.01	6.32 ± 0.00	6.30 ± 0.02
30	6.44 ± 0.02	6.44 ± 0.02	6.34 ± 0.01	6.34 ± 0.01
60	6.49 ± 0.01	6.46 ± 0.02	6.73 ± 0.09	6.69 ± 0.03
	Chl-*a* (μg/L)			
	2012		2013	
Depth (m)	Upstream	Downstream	Upstream	Downstream
5	N/A	N/A	19.7 ± 1.9	29.9 ± 16.4
15	N/A	N/A	26.8 ± 2.6	24.6 ± 9.6
	PCsusp (μM)			
	2012		2013	
Depth (m)	Upstream	Downstream	Upstream	Downstream
5	N/A	N/A	2.91 ± 0.41	2.35 ± 0.36
15	N/A	N/A	2.43 ± 0.76	2.24 ± 0.34
30	N/A	N/A	2.40 ± 1.10	1.63 ± 0.23
60	N/A	N/A	2.45 ± 2.03	1.78 ± 0.48
	PNsusp (μM)			
	2012		2013	
Depth (m)	Upstream	Downstream	Upstream	Downstream
5	N/A	N/A	0.88 ± 0.36	0.86 ± 0.43
15	N/A	N/A	0.78 ± 0.22	0.97 ± 0.32
30	N/A	N/A	0.66 ± 0.27	0.75 ± 0.26
60	N/A	N/A	0.50 ± 0.44	0.70 ± 0.29

Note. TDN: total dissolved nitrogen.

0.1 ≥ p ≥ 0.05 significance level. However, in 2013, the 30 m upstream and downstream DO concentrations, both 6.34 ± 0.01 mg/L, were not distinguishable from each other. Similarly, the upstream and downstream DO concentrations collected at 60 m in 2013, 6.73 ± 0.09 and 6.69 ± 0.03 mg/L, respectively, were not significantly different from each other (Figure 4, Table 1).

The concentration of chl-*a* was measured in samples collected at 5 and 15 m in 2013. The 5 m average upstream chl-*a* concentration, 19.7 ± 1.9 ng/L, was significantly lower than the downstream 5 m chl-*a* concentration of 29.9 ± 16.4 ng/L at the 0.1 > p > 0.05 significance level. However, in the 15 m samples, there was no difference in the upstream versus downstream chl-*a* concentration (26.8 ± 2.6 and 24.6 ± 9.6 ng/L, respectively) (p > 0.1) (Figure 4, Table 1).

Samples were collected at all depths in both 2012 and 2013 for NO_3^- + NO_2^- and TDN concentration measurements. The NO_3^- + NO_2^- concentration of samples from 5, 15, and 30 m were typically <0.2 μM and, at 60 m, increased to ~0.5 μM in 2012 and to ~2.0 μM in 2013 (Table 1). In 2012, the upstream versus downstream NO_3^- + NO_2^- concentrations were not significantly different at any depth. In 2013, the Kruskal-Wallis test indicated that the upstream 5 and 30 m NO_3^- + NO_2^-concentrations were significantly lower than the downstream samples at the 0.025 > p > 0.01 significance level. However, the upstream versus downstream samples at 15 and 60 m did not show significant differences in their NO_3^- + NO_2^- concentrations (i.e., p > 0.1). TDN concentrations were relatively constant with depth in the water column and ranged from 4.7 ± 1.1 to 7.6 ± 2.8 μM (Figure 4, Table 1). There was no evidence of significantly different TDN concentrations in upstream versus downstream samples at any depth in either year.

Water column PC concentrations were the highest in surface waters and decreased with depth, ranging from 2.91 ± 0.41 μM in upstream 5 m samples to 1.78 ± 0.48 μM in downstream samples collected at 60 m (Figure 4, Table 1). Similarly, the water column suspended PN concentrations were the highest in surface waters and decreased with depth, ranging from 0.88 ± 0.36 μM in 5 m upstream samples and decreasing to 0.50 ± 0.44 μM in 60-m upstream samples (Figure 4, Table 1). Analysis of the water column suspended PC concentrations from 2013 using the Kruskal-Wallis test indicated that the 5 m and 30 m 2013 upstream samples had higher PC concentrations than the downstream samples at the 0.1 > p > 0.05 significance level but that the means of the 15- and 60-m sample PC concentrations were indistinguishable. The Kruskal-Wallis test was unable to identify a significant difference in the PN concentration in upstream versus downstream samples at 5, 15, 30, or 60 m (p > 0.1) (Figure 4, Table 1).

3.2 | Phase 1: Upstream versus downstream benthic chl-a and PC, PN concentrations

Sediment samples were collected in 2013 to evaluate whether the aquaculture facility affected benthic chl-*a*, PC, and PN content. When the benthic PC content from samples collected at all seven sampling locations (Z1E, Z1W, Z2E, Z2W, Z3E, Z3W, and control) was compared, the Kruskal-Wallis test was unable to identify distinct populations (i.e., $p \geq 0.1$ in all cases); the same was true for evaluation of the PN and chl-*a* content of samples from all seven locations. When we compared the mean PC, PN, and chl-*a* content of a smaller set of sampling locations, there was stronger evidence of a difference between the sampling locations. For example, when the PC content from the control site was compared with the PC content of samples collected at Z1E and Z1W, the Kruskal-Wallis test indicated that, at the $p \leq 0.075$ level of significance, there is evidence to suggest that at least two of the populations are different (Figure 5). As the "downstream" Z1E site has the highest PC content, 38.6 ± 5.3 mg C (g sed)$^{-1}$, and the control and "upstream" Z1W site had similar concentrations (28.1 ± 2.5 and 28.1 ± 0.3 mg C [g sed]$^{-1}$, respectively), we conclude that the downstream site has significantly higher benthic PC content than the control or upstream locations (Table 2). When evaluating the benthic PN content, we again compared samples from the control site with those collected at the Z1E and Z1W sites and found that the test statistic indicated that, at the p < 0.061 level of significance, there is evidence to suggest that at least two of the populations are different (Figure 5). Similar to the effect of the aquaculture cage field on benthic PC content, the sample collected at Z1E, immediately downstream of the cage field, had the highest benthic PN content (1.8 ± 0.4 mg N [g sed]$^{-1}$) compared to the control and Z1W sites, both of which had benthic PN contents of 1.3 ± 0.1 mg N [g sed]$^{-1}$) (Figure 5, Table 2). However, the evidence was less compelling that the aquaculture cage field significantly affected benthic chl-*a* content. While the mean benthic chl-*a* content at the sites immediately upstream (0.3 ± 0.2 μg chl-*a* [g sed]$^{-1}$) and downstream (0.4 ± 0.3 μg chl-*a* [g sed]$^{-1}$) of the cage field were higher than at the control site (0.3 ± 0.0 μg chl-*a* (g sed)$^{-1}$), the means were not significantly different

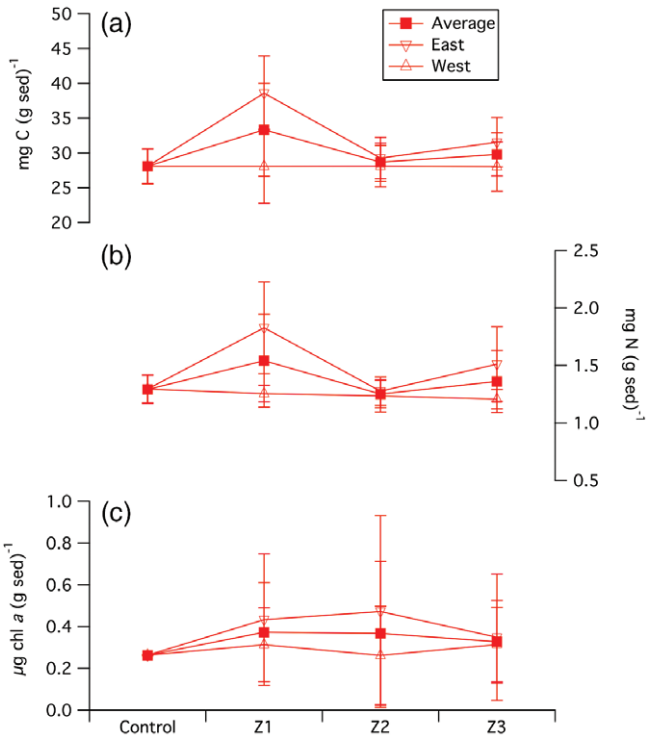

FIGURE 5 Mean benthic carbon content (a), nitrogen content (b), and chlorophyll-*a* content (c) at all sites (filled squares), at sites west of the cage field (open triangles), and at sites east of the cage field (open inverted triangles)

from each other (i.e., $p > 0.1$) (Figure 5). Similarly, while the means of the benthic chl-*a* samples collected at Z2E and Z2W show the greatest difference (i.e., 0.5 ± 0.5 and 0.3 ± 0.2 μg chl-*a* (g sed)$^{-1}$, respectively) (Table 2), because of the high SD associated with these measurements, when these samples were compared with those from the control site, the Kruskal-Wallis test could not discriminate between the means of the populations (i.e., $p \geq 0.1$).

3.3 | Phase 2: Water quality and sediment monitoring results

In Phase 2, sediment sampling was conducted at two locations to the west of the cages (Z1W and Z2W) as well as at two locations to the east of the cages (Z1E and Z2E) and at a "control" sampling location to the north of the cages (Figure 3). This sampling scheme was identical to the scheme used in 2013, but the third zone (Z3E and Z3W) was eliminated from analysis (Figure 3). In April 2018, inclement weather and equipment failures prevented the sampling of Z1W and Z2W. When the median TOC levels from samples were collected on a single date at all five sampling

TABLE 2 The 2013 average (± 1 *SD*) benthic measurements

Location	C content (mg C/g sed)	N content (mg N/g sed)	Chl-*a* content (μg chl-*a*/g sed)
Control	28.08 ± 2.52	1.29 ± 0.12	0.26 ± 0.00
Z1W	28.08 ± 0.78	1.25 ± 0.07	0.31 ± 0.18
Z1E	38.58 ± 5.33	1.83 ± 0.40	0.43 ± 0.31
Z2W	28.10 ± 2.99	1.23 ± 0.14	0.26 ± 0.23
Z2E	29.25 ± 2.98	1.28 ± 0.12	0.47 ± 0.46
Z3W	28.04 ± 1.49	1.21 ± 0.08	0.31 ± 0.18
Z3E	31.54 ± 3.54	1.51 ± 0.33	0.35 ± 0.30

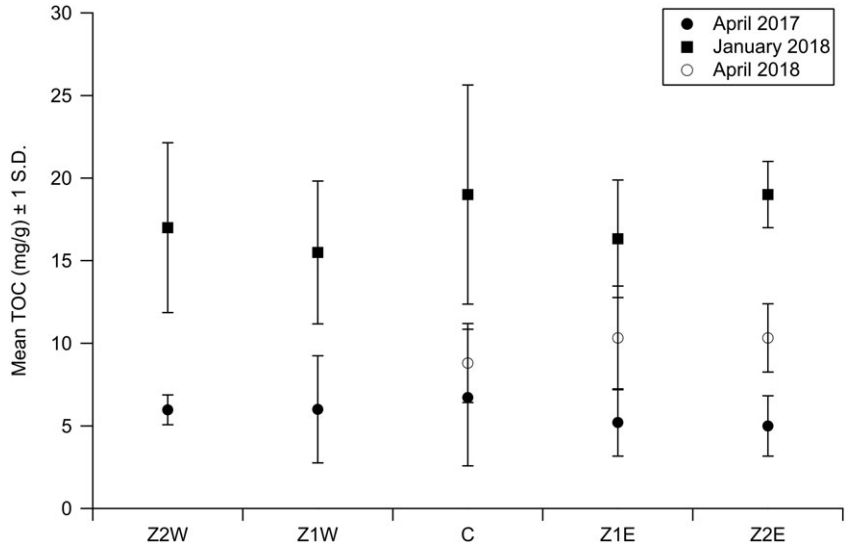

FIGURE 6 Mean benthic total organic carbon concentrations (mg/g) (+1 *SD*) at sampling locations beneath aquaculture cages sampled in April 2017 (filled circles), January 2018 (filled squares), and April 2018 (open circles)

locations (or three locations in the case of the April 2018 sampling session), the Kruskal-Wallis test was unable to identify distinct populations (i.e., $p \geq 0.1$ in all cases) (Figure 6).

When median TOC levels in each zone were analyzed over time, however, they showed significant variation. In ZIE, the April 2017 median TOC (5.4 mg/g) was significantly different ($p = 0.0004$) from the January 2018 median TOC (15.5 mg/g) but not significantly different from the April 2018 median TOC (10.0 mg/g). In Z2E, the April 2017 median TOC (5.3 mg/g) was significantly different from both the median TOC in January 2018 (19.0 mg/g) and April 2018 (13.0 mg/g) ($p = 0.0002$). In the control location, the April 2017 median TOC (6.0 mg/g) was significantly different from the January 2018 median TOC (19.0 mg/g) ($p = 0.015$) but was not different from the April 2018 median TOC (9.0 mg/g). In Z1W, the April 2017 median TOC (7.4 mg/g) was significantly different than the January 2018 median TOC (14.0 mg/g) ($p = 0.02$). In Z1W, the April 2017 median TOC (5.9 mg/g) was significantly different from the January 2018 median TOC (14.5 mg/g) ($p = 0.02$) (Figure 6, Table 3).

Water column samples had ammonia levels that ranged from below the detection limit of the analytical method up to 0.43 ppm inside the cages. At the control site, ammonia levels ranged from below the detection limit of the analytical method to 0.53 ppm. While the relatively limited number of water samples precluded statistical analysis, there was no trend evident in the data (Figure 7).

4 | DISCUSSION

While continued monitoring will be necessary to evaluate the long-term effects on the benthic and water column ecosystems, the data reported here indicate that the net effect of the nutrients emitted by the aquaculture facility in coastal Panama has been minimal over the duration of the time that monitoring has occurred.

TABLE 3 Mean benthic TOC concentrations (± 1 *SD*) at individual sampling locations

	Z2W	Z1W	C	Z1E	Z2E
January 2017	5.97 (0.9)	6.00 (3.24)	6.72 (4.13)	5.20 (2.04)	4.99 (1.83)
January 2018	17.0 (5.14)	15.5 (4.32)	19.0 (6.64)	16.3 (3.56)	19.0 (2.0)
April 2018			8.80 (2.39)	10.33 (3.14)	10.33 (2.07)

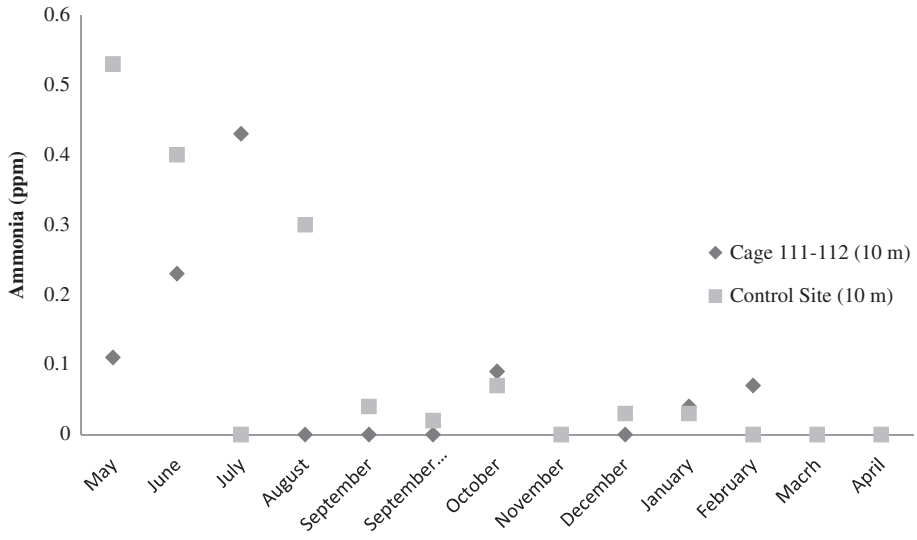

FIGURE 7 Measured ammonia (NH_4^+ levels (ppm) within cages (dark gray diamond) and at the control site 1 km away from cage (light gray square) from May 2017 to April 2018

In Phase 1, while water column parameters, such as slightly higher NO_3^- + NO_2^- and TDN concentrations, in downstream versus upstream 2013 samples (Figure 4, Table 1) potentially recorded the signature of aquaculture effluent, the differences between upstream and downstream samples were typically not significant. This lack of a significant impact on the water column from the aquaculture cages was, in some ways, unexpected. Cobia (*R. canadum*) have relatively high rates of nitrogen excretion and oxygen consumption (Feeley, Benetti, & Ault, 2007). In addition, elevated levels of ammonia (Belias, Bikas, Dassenakis, & Soullos, 2003; Huang, Huang, Meng, Hsieh, & Chen, 2012; Neofitou & Klaoudatos, 2008; Pitta et al., 2006; Pitta, Karakassis, Tsapakis, & Zivanovic, 1999; Sanderson, Cromey, Dring, & Kelly, 2008; Wildish, Keizer, Wilson, & Martin, 1993) and reduced levels of DO (Johansson et al., 2006; Wu, Lam, MacKay, Lau, & Yam, 1994) in the immediate vicinity of cages have been reported in a number of different aquaculture settings. Despite this, there was no consistent evidence of higher nutrient concentrations or reduced DO concentrations in the data.

In other respects, however, the lack of a detectable impact on the pelagic environment beyond the cage field agrees with prior research conducted at sites comparable to the one in this study (Alston et al., 2005; Pitta et al., 1999; Pitta et al., 2006; Pitta et al., 2009; Soto & Norambuena, 2004; Vezzulli et al., 2008). Generally, these farms have been located in deep and well-mixed oligotrophic waters (Sarà, 2007). Researchers at these sites have had difficulty observing any measurable effect on the pelagic environment from farm operations at distances beyond a few meters from the cage rims when measuring biogeochemical properties. Vezzulli et al. (2008), for example, analyzed the impact of organic waste generated by a capture-based bluefin tuna (*Thunnus thynnus*) farm on the surrounding environment. The farm in question was located on an exposed site off the SW coast of Italy. The site was <1 km from shore and in ~45 m of water. Analysis of the chl-*a* and organic matter (particulate biopolymeric carbon) samples taken at sampling stations in the immediate vicinity of the cages showed no statistically significant differences relative to a control site (Vezzulli et al., 2008, p. 373). Similarly, the measured biogeochemical properties (e.g., DO) were consistent at both the cage sites and the control sites. In an earlier study, Pitta et al. (2006) studied the effect on the pelagic environment of a group of three commercial sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) farms located in Spain, Italy, and Greece. Each farm produced between 250 and 1,150 m.t. per year. At one farm, a statistically significant spike in nutrients (NH_4^+ and PO_4) was evident at the cage edge relative to a control site at a distance of 500 m when samples were taken at discrete depths (0 m, 10 m, and on the bottom). Integrated water column sampling around and downstream of the farms, however, detected no effect on the biogeochemical variables analyzed, including chl-*a*, PON, POC, NH_4^+, PO_4,

and NO_3^- concentrations (Pitta et al., 2006). These results indicated that mixing and diffusion reduced the analyzed variables to background concentrations very quickly. Alston et al. (2005) monitored the impact of an experimental offshore farm in Puerto Rico culturing mutton snapper (*Lutjanus analis*) and cobia (*R. canadum*). This work utilized an array of fixed point-monitoring stations in and around the two-cage farm site where bimonthly samples were taken and analyzed for NH_4^+, NO_2^- + NO_3^-, and PO_4 in the water column. No statistically significant differences were noted for any monitored water column variable over the course of the study.

Interpreting the sediment data collected here is more difficult. In Phase 1 of our study, the amount of PC and PN in the sediment around the cage site shows a trend toward increased organic loading under the cages relative to the control site (Figure 5). When broken down by subzone, the results are even more suggestive of a trend toward increased organic loading in the benthos because the mean values for benthic PC and PN are highest on the east side of the cage field (ZIE) in the direction of the prevailing current. (Figure 5, Table 2). The observed increase in PC and PN, however, was modest, and the same trend was not observed in the TOC data collected in Phase 2.

In Phase 2, TOC levels in the benthic environment did not appear to vary as a function of the sample location but instead varied significantly over time. TOC levels generally increased across all sample locations, including the control site, between April 2017 and January 2018 and then decreased again across all zones and the control site between January 2018 and April 2018. The fact that these changes occurred across all sampled locations and generally changed in the same sense suggests that seasonal processes, such as Panama's wet-season/dry-season meteorological pattern and/or annual primary productivity cycles, may also be influencing the flux of organic material to the sediments (Aller & Stupakoff, 1996; Gooday, 2002; McKee, Aller, Allison, Bianchi, & Kineke, 2004). More data collection is needed to establish the extent to which TOC levels fluctuate as a result of seasonal processes.

As with the results from the water column sampling, results for the sediment sampling conducted in this study were generally in accord with published results from similar work, most of which have shown that the majority of environmental impacts created by net-pen aquaculture occur in the benthos (Aguado-Giméz & Garciá-Garcia, 2004; Carroll, Cochrane, Fieler, Velvin, & White, 2003; Domínguez, Calero, Martín, & Robaina, 2001; Edgar, Macleod, Mawbey, & Shields, 2005; Kalantzi & Karakassis, 2006; Klaoudatos, Klaoudatos, Smith, Bogdanos, & Papageorgiou, 2006; Mazzola, Mirto, & Danovaro, 1999; Porello et al., 2005; Schendel, Nordström, & Lavkulich, 2004; Soto & Norambuena, 2004).

The data presented here should provide a reason for cautious optimism about emerging offshore aquaculture technologies. Undoubtedly, the release of large amounts of nutrients into marine ecosystems is one of the great worries associated with cage-based aquaculture of any sort. Sarà et al. (2011), for example, correlated increased inputs of N and P from aquaculture with rising chl-*a* concentrations in a large coastal embayment in the Mediterranean. Increased levels of nitrogen and phosphorus may also stimulate blooms of various types of phytoplankton that could be detrimental to the function of a healthy marine ecosystem. Harmful algae blooms are an obvious concern, but there are other possible downstream ecological effects related to persistent nutrient overenrichment, such as regime shifts that lead to outbreaks of cnidarians or other less-desirable species (Richardson, Bakun, Hays, & Gibbons, 2009). It has also been demonstrated that the release of significant quantities of nitrogen and phosphorus without the proportional release of other trace nutrients (e.g., silica) might favor particular groups of phytoplankton over others, thus creating an imbalance at the lower trophic levels of a marine ecosystem (Doering et al., 1989; Justić, Rabalais, Turner, & Dortch, 1995; Nieuwerburgh, Wänstrand, & Snoeijs, 2004; Parsons, Harrison, & Waters, 1978). There is also the possibility that macrofauna in nearby ecosystems might be dislocated because of the input of additional nutrients. Increased epiphyte growth because of nutrient availability, reduced light for photosynthesis because of higher levels of turbidity, and other problems related to aquaculture effluents can be destructive to sensitive benthic ecosystems such as coral reef and seagrass meadows (Marba, Santiago, Diaz-Almela, Alvarez, & Duarte, 2006; Ruiz, Marco-Mendez, & Lizaso, 2009). In short, even with offshore aquaculture, and even given the relatively benign results reported in this study, it is likely that there is an upper level to the carrying capacity of the environment for these systems (Pitta, Apostolaki, Giannoulaki, & Karakassis, 2005; Sarà et al., 2011). At some point, the sustained input of nutrients into a water body, even a very large one, is likely to have unpredictable and potentially harmful impacts.

Nonetheless, nutrients of the sort discharged by aquaculture facilities are not, ipso facto, pollution. N and P lie at the base of the ocean's food web and drive the primary production that, in turn, drives global fisheries production (Ryther, 1969). A growing body of literature supports the notion that large-scale nutrient inputs from aquaculture facilities can have positive effects on fisheries over large (regional) spatial scales (López, Bunke, & Shirai, 2008; Machias et al., 2004, 2005, 2006). These studies correlate the installation of large-scale aquaculture facilities with increases in fish stock biomass, as well as the mean trophic level and aggregate amount of wild fishery landings in a region. These studies suggest that nutrients flow quickly through phytoplankton at the base of the trophic pyramid and up to higher-order consumers (Pitta et al., 2009). In some parts of the aquaculture industry, these nutrient flows are already being exploited to produce additional marketable product via Integrated Multi Trophic Aquaculture techniques (Chopin, Cooper, Reid, Cross, & Moore, 2012; Reid et al., 2010). Finally, it should be noted that the negative effects of aquaculture effluent can be mitigated through conscientious management. Published literature on the subject, for example, indicates that temporary fallowing techniques (i.e., leaving cages empty for a period of time between harvest and restocking) can lessen the effects of nutrient loading on the benthos (Macleod, Moltschaniwskyj, & Crawford, 2006).

As the offshore aquaculture industry grows, questions about the appropriate scale and location of farms will persist (Lester et al., 2018). While no single study can answer all of these questions or resolve the myriad uncertainties surrounding the development of this industry, this study indicates that appropriately sited, commercially scaled offshore aquaculture installations have the potential to operate in a way that produces a relatively small nutrient footprint.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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