Estuarine, Coastal and Shelf Science 116 (2013) 1–10

Contents lists available at SciVerse ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Elucidating terrestrial nutrient sources to a coastal lagoon, Chincoteague Bay, Maryland, USA

B. Fertig[a,*], J.M. O'Neil[b], K.A. Beckert[b], C.J. Cain[c], D.M. Needham[b,1], T.J.B. Carruthers[a,2], W.C. Dennison[a]

[a] Integration and Application Network, University of Maryland Center for Environmental Science, Annapolis, MD, USA
[b] Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, MD, USA
[c] Maryland Coastal Bays Program, National Estuary Program, Ocean City, MD, USA

ARTICLE INFO

Article history:
Received 22 November 2011
Accepted 9 August 2012
Available online 21 August 2012

Keywords:
coastal lagoons
nitrogen
anthropogenic factors
water quality
land use
Maryland
Chincoteague Bay

ABSTRACT

Long-term non-linear ecosystem-scale changes in water quality and biotic communities in coastal lagoons have been associated with intensification of anthropogenic pressures. In light of incipient changes in Johnson Bay (an embayment of Chincoteague Bay, Maryland-Virginia, USA), examination of nitrogen sources was conducted through synoptic water quality monitoring, stable nitrogen isotope signatures ($\delta^{15}N$) of *in situ* bioindicators, and denitrification estimates. These data were placed in the context of long-term and broader spatial analyses. Despite various watershed protection efforts, multiyear summer time studies (2004–2007) suggested that high levels of terrestrially derived nutrients still enter Johnson Bay. Total nitrogen concentrations in Johnson Bay were 132% the concentrations in the broader Chincoteague Bay during the late 1970s (mean 2004–2007 was 40.0 − 73.2 μM). Comparing total nitrogen concentrations in Johnson Bay to St. Martin River (consistently the most eutrophic region of these coastal bays), Johnson Bay has increased from 62.5% to 82.5% of the concentrations in St. Martin River during the late 1970s. Though specific sources of nitrogen inputs have not yet been definitively identified, the long-term increase in total nitrogen concentrations occurred despite increased and continued conservation and protection measures. We suggest that investigating nutrient sources can reveal potentially ineffective nutrient policies and that this knowledge can be applied towards other coastal lagoons.

1. Introduction

Coastal lagoons along Delmarva Peninsula (Mid-Atlantic, USA) including Chincoteague Bay (CB) are undergoing ecosystem-scale changes due to anthropogenic stressors (Hager, 1996; Kennish and Paerl, 2010). Collectively, nonlinear trends in nutrient concentrations and water quality (Wazniak et al., 2007), changes in primary production (Goshorn et al., 2001; Harris et al., 2005; Orth et al., 2010), increasing frequency of harmful algal blooms (Trice et al., 2004; Tango et al., 2005; Glibert et al., 2007), and reductions in benthic communities (Tyler, 2007) were seen in CB.

'Hotspots' of elevated terrestrially derived total nitrogen (TN) (51.1 ± 1.0 μM), total phosphorus (TP) (4.20 ± 0.16 μM), and $\delta^{15}N$ values in macroalgae (8.0 ± 0.3‰) and oyster gills (8.4 ± 0.3‰) were previously identified (Fertig et al., 2009) within the CB embayment Johnson Bay (JB) (38°3′N, 75°20′W). Yet elevated nutrient and $\delta^{15}N$ values, indicative of potential human and/or animal wastes (Kendall, 1998; Fry, 2006) in this shallow coastal lagoon are incongruous with the intensity of associated land uses. JB's sub-watershed (9935 ha) within that of CB is dominated by forest and wetland (cumulatively 66.5% watershed area) and is relatively undeveloped (Fig. 1a,b). Furthermore, JB is generally less degraded, in terms of nutrient concentrations, than other mid-Atlantic coastal lagoons (Dennison et al., 2009).

Enriched $\delta^{15}N$ values in dissolved inorganic nitrogen (DIN) and tissues of bioindicator species can be indicative of human and/or animal wastes (Kendall, 1998), but interpretation must be balanced against alternative processes e.g. denitrification (which favors uptake of ^{14}N) or ammonia volatilization ($^{14}NH_3$ is slightly more volatile than $^{15}NH_3$) resulting in enriched ^{15}N (Cline and Kaplan, 1975; Kendall,

* Corresponding author. Present address: Institute of Marine and Coastal Sciences, Rutgers University, The State University of New Jersey, 71 Dudley Rd., New Brunswick, NJ 08903, USA.
E-mail address: fertig@marine.rutgers.edu (B. Fertig).
[1] Present address: Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA.
[2] Present address: Secretariat of the Pacific Regional Environment Programme, Apia, Samoa.

0272-7714/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ecss.2012.08.013

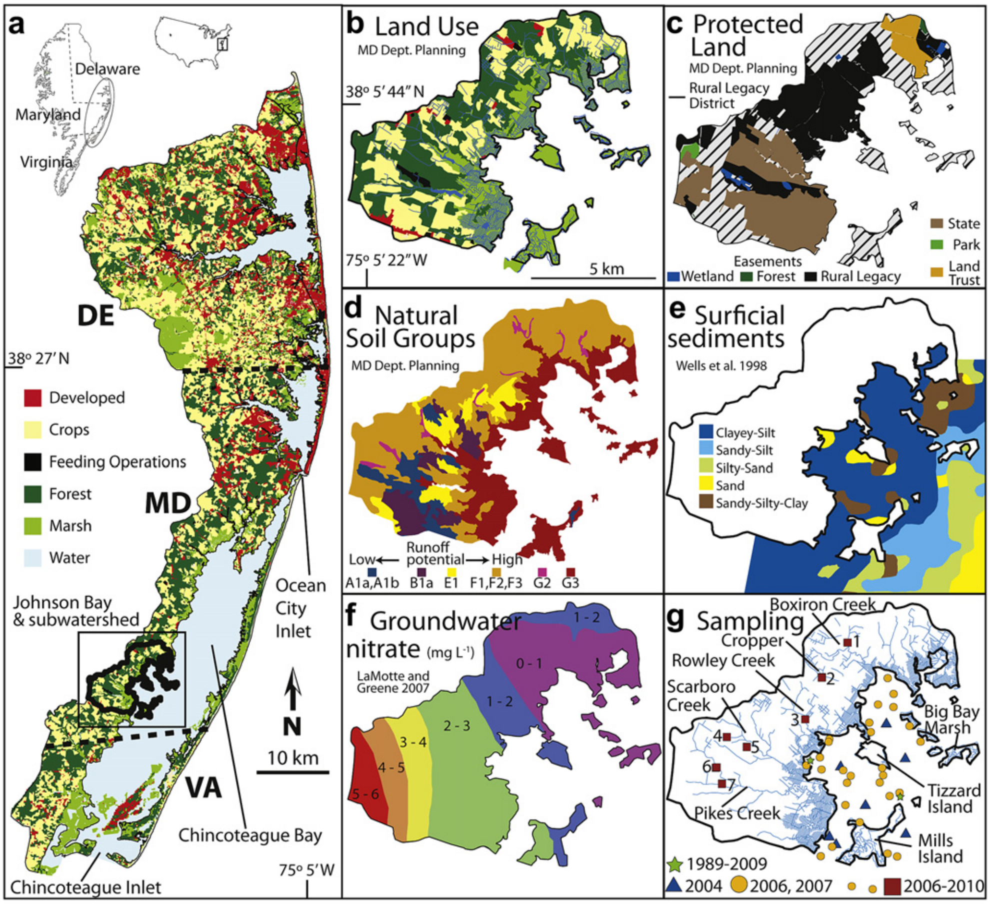

Fig. 1. Location of JB within CB and land use within the watersheds of these mid-Atlantic coastal lagoons along Delmarva Peninsula (a) and the JB sub-watershed (b). Land subject to protections and conservation (c). Natural soil groups (d) and sediments (e) within JB sub-watershed and bay, respectively. Groundwater nitrate (mg L^{-1}) in JB sub-watershed (f). Fixed stream water quality monitoring stations (red squares) sampled in spring (April) 2006–2010 while randomized JB stations sampled in summer 2004 (June) and 2006–2007 (May and July) (g). Data in panels a, b, c, d, from Maryland Department of Planning (2010); panel e from Wells et al., (1998); panel f from LaMotte and Green (2007).

1998; McClelland and Valiela, 1998; Fry, 2006). Wastewater treatment plants employ denitrification, and animal manure fertilizers readily volatilize, elevating $\delta^{15}N$ signatures. However, these processes are not necessarily associated with human and/or animal wastes, or may occur prior to nitrogen entering aquatic ecosystems.

Coastal lagoons along the Delmarva Peninsula have a gradient of land use intensity that decreases north-south (e.g. poultry production, crop agriculture, and residential development) within 6 km to the shoreline, which can drive ecosystem change (Boynton et al., 1996; Hager, 1996; Jordan et al., 1997; Stanhope, 2003). CB (encompassing JB) has TN loads and concentrations intermediate with respect to coastal lagoons of the Delmarva Peninsula (Fig. 2; K. McGlathery, W. Ullman pers. comm.). Septic systems are prevalent in the watershed (Souza et al., 1993). Human population doubled between 1980 and 2000 to ~35,000 people (Hager, 1996). Increases in point source discharges and changes to nutrient loadings from diffuse sources (Boynton, 1993; Dillow et al., 2002) are associated with land use changes, leading in many cases to eutrophication (Souza et al., 1993; Boynton, 1993, 1996; Nixon et al., 2001; Wazniak et al., 2007; Fertig et al., 2009). Groundwater is an important nutrient transport mechanism for these lagoons (Valiela et al., 1990; Aravena et al., 1993; Dillow and Greene, 1999; Miller and Ullman, 2004; Dillow and Raffensperger, 2006) due to low relief, high permeability soils and aquifers, and deeply incised baseflow-dominated streams in the watershed (Hays and Ullman, 2007).

Integrating long-term monitoring data enables assessment of historical and spatial context and enhances our understanding of the complex transport and processing pathways across the land–sea interface for nitrogen sources available to moderately eutrophic coastal lagoons (Scanes et al., 2007). Water quality monitoring, nutrient source identification, and microbial recycling datasets within JB are assembled and integrated to examine eutrophication and changes, in the context of longer-term and broader spatial analyses. Specifically, this paper addresses these issues with two main goals: 1) Discussing potential sources of elevated bioindicator $\delta^{15}N$ and terrestrially derived nitrogen to JB and 2) Placing JB eutrophication and nutrient monitoring data into historical and

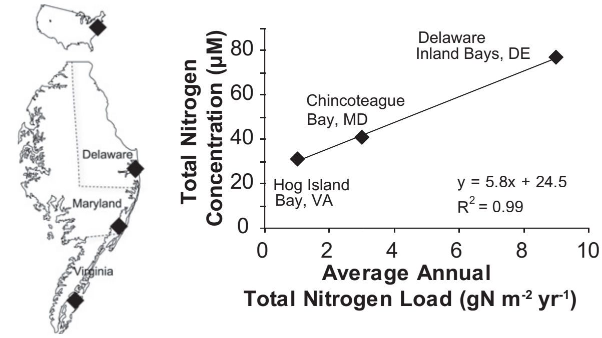

Fig. 2. CB in context of other coastal lagoons along Delmarva Peninsula with respect to TN concentration vs. average annual TN load. Hog island, VA data courtesy of K. McGlathery (http://www.lternet.edu/sites/vcr/) and Delaware Inland bays data courtesy of W. Ullman.

spatial context relative to these mid-Atlantic coastal lagoons to track its ecosystem trajectory.

2. Methods

2.1. Study location and dataset description

Johnson Bay (JB) (38°3′N, 75°20′W) is a small (23 km^2 water surface area) coastal estuarine lagoon (Fig. 1a–g) midway along Chincoteague Bay (CB) (extending from 38°15′N, 75°12′W in the north to 37°54′N, 75°25′W in the south) between inlets at either end of Assateague Island (38°19′N 75°05′W and 37°52′N 75°25′W). JB and CB are shallow (2 m mean depth) and non-stratified with slow flushing (estimated ~63 days, Pritchard, 1960; Lung, 1994; modeled 83.0–96.6 days, Wang, 2009) and a small tidal range (generally $<$ 1.0 m, Allen et al., 2007). Salinities range 0.0–0.2 in inflowing streams, (e.g. Boxiron, Rowley, Scarboro, and Pikes Creeks) and agricultural ditches, to 30–35 in the bays. In addition to overland sources (Lung, 1994; Schwartz, 2003; Dillow and Raffensperger, 2006; Schmidt et al., 2007), groundwater is an important but slow (>50 years) freshwater and nutrient transport mechanism (Andres, 1992; Dillow and Greene, 1999; Dillow et al., 2002; Krantz et al., 2004; Manheim et al., 2004; Bratton et al., 2004, 2009; LaMotte and Greene, 2007).

Synoptic water quality monitoring conducted in JB in 2004, 2006, and 2007 were integrated with monthly long-term (1989–2009) water quality datasets (Table 1). Long-term water quality was monitored at two stations (7: 38°4′29″N 75°21′46″W and 14: 38°03′42″N 75°19′25″W) in JB (Sturgis, 2001). Springtime (April) stream water quality dataset collected by the Maryland Coastal Bays Program (Fig. 1g, Table 1). Synoptic bay water quality monitoring dates in 2006 were not selected *a priori* for association with precipitation, yet twelve events (0.3–53.3 mm; Fertig et al., 2009) between the 2006 surveys made 2006 a particularly wet year.

2.2. Synoptic water quality monitoring

Synoptic sampling included physical (salinity, temperature, pH, dissolved oxygen concentration, and Secchi depth), chemical (TN, TP, nitrate + nitrite ammonium, and phosphate), and biological (bacteria and virus abundances, chlorophyll *a* and phaeophytin concentrations, $\delta^{15}N$ values in seston, macroalgae, and oyster gill, and oyster gill %N and C/N ratio) metrics. Total nutrient concentrations include both dissolved and particulate fractions.

Table 1
Datasets available for JB. MDP = Maryland Department of Planning, DESPC = Delaware Office of State Planning Coordination, VADF = Virginia Department of Forestry, NA = Not Applicable.

Dataset	Source	# Locations	Years
Synoptic water quality	Fertig et al., 2009, this study	28	2004, 2006, 2007
Long-term water quality	Sturgis, 2001	2	1989–2009
Historical water quality	Boynton, 1973, Fang et al., 1977a,b	3	1970, 1975–1976
Stream water quality	This study	7	2006–2010
Microbial abundance	This study	3	2007
Denitrification	This study	3	2007
Nitrogen uptake	This study	3	2007
Stable isotope	Fertig et al., 2009, this study	28	2004, 2006, 2007
Nutrient loading	Cerco et al., 1978, Jacobs et al. 1993	NA	1975–1976, 2004
Land use/land cover	MDP 2010, DESPC 2010, VADF 2010	NA	2002

Standard water quality monitoring methodology and physical metrics were measured in surface and bottom water with a pre-calibrated YSI water quality probe UMCES-HPL Analytical Services analyzed TN and TP (unfiltered surface water) and DIN (GF/F filtered surface water) according to standard methods (D'Elia et al., 1977; Solórzano and Sharp, 1980a,b; Valderrama, 1981; Parsons et al., 1984; Kerouel and Aminot, 1987; Sharp et al., 1995;Arar, 1997; Clesceri et al., 1998). Water samples for nitrogen uptake rates, and bacteria, virus abundances, and sediment samples for measurable denitrification analysis were collected at three stations forming an inshore-offshore transect (Fig. 3a–e). Urea methods (Revilla et al., 2005) were modified by using microplate analysis and a spectrophotometer equipped with a low volume plate reader. Unfiltered, duplicate 50 mL bacteria and virus samples were preserved with 1% formalin; epifluorescent enumeration occurred by SYBR Green staining (Noble and Fuhrman, 1998; Patel et al., 2007). Chlorophyll *a* and phaeophytin collected on GF/F filters were extracted and measured (Arar, 1997). Seston $\delta^{15}N$ were analyzed at UC Davis Stable Isotope Facility. For definition, $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 10^3$, where $R = {}^{15}N/{}^{14}N$ (Fry, 2006). Deployment of macroalgae and oysters for $\delta^{15}N$ values, %N, and C/N ratio is described elsewhere (Costanzo et al., 2001; Fertig et al., 2009, 2010). Oyster gill data (initially 7.8 ± 0.8‰ in 2006) are reported here. UC Davis Stable Isotope Facility analyzed stable isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) against NIST 1577 and atmospheric N_2 standards (±0.2‰ reproducibility).

2.3. Nitrogen uptake

Relative rates of nitrogen uptake were measured (Glibert and Capone, 1993) to determine which nutrients favor proliferation of phytoplankton. To triplicate surface water samples, ^{15}N substrates (NH_4^+, NO_3^{2-} urea) were added at ~10% ambient concentrations. Incubations were conducted under ambient light and temperature for ~0.5 − 1 h. Samples were subsequently filtered onto pre-combusted GF/F filters, dried, and analyzed using mass spectrometry and rates of uptake were calculated (Glibert and Capone, 1993).

2.4. Measurable denitrification

Presence or absence of measurable and potential denitrification in sediments (top 1 cm) was determined using acetylene inhibition techniques (Balderston et al., 1976; Yoshinari and Knowles, 1976; Sørenson, 1978). Triplicate sediment slurries were created with an equal volume of sediment and water. Calcium carbide generated acetylene (C_2H_2) was added (15% of gas phase volume) to arrest the multi-step denitrification process at the production of the N_2O intermediary. N_2O from slurry headspace (0.1 ml) was measured after 24 h with a GC-ECD (Shimadzu), then spiked with 100 μM KNO_3 then measured after 24 h under non-limiting nitrate conditions. Though newer techniques are available (Kana et al., 1994), acetylene inhibition can provide a minimum measurable denitrification rate, even though it may underestimate actual rates due to inhibition of nitrification and coupled nitrification-denitrification.

2.5. Dataset assembly, integration, limitations, and statistical analysis

Characterization of the JB watershed included land use/land cover data from Maryland (2002; Maryland Department of Planning, 2010), Delaware (2002; Delaware Office of State Planning Coordination, 2010), and Virginia (2005; Virginia Department of Forestry, 2010). Raster land use datasets from

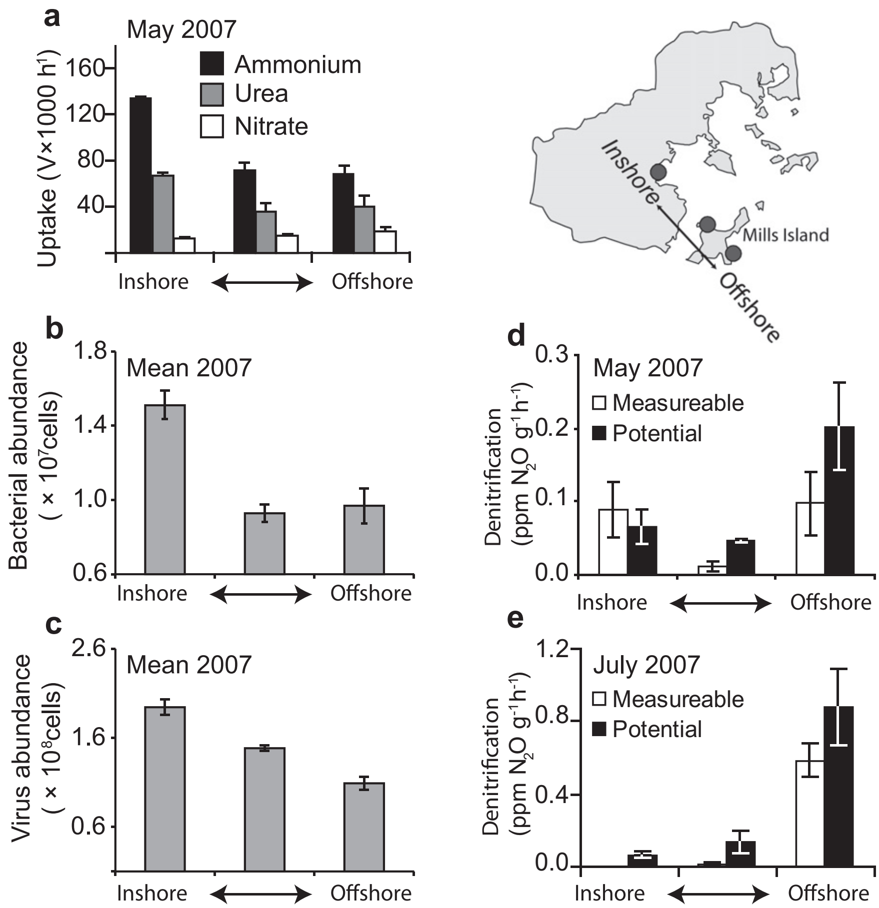

Fig. 3. Rates (±se) of relative velocity of nitrogen uptake for ammonium (black), nitrate (white), and urea (grey) along an inshore-offshore transect (a). Mean (±se) of bacteria (b) and viruses (c) collected from surface water samples in JB in 2007. Mean (±se) of measured (white) and potential (addition of 100 μM NO_3^-; black) denitrification rates analyzed by acetylene inhibition techniques from triplicate sediment (top 1 cm) samples collected May 2007 (c) and July 2007 (d).

Virginia were converted to polygons and dissolved by aggregated land use, which was used to summarize and align land use classifications from all three states (Fig. 1a). Loading calculations were conducted based upon land use aggregations (Boynton, 1993). GIS layers (ESRI ArcMap 9.2) of protected lands (Rural Legacy boundary and easements, State and Land Trust lands, wetland and forest easements, and parks) were provided by Worcester County Government (2010), clipped to the boundaries of the JB watershed (HUC12), and recalculated for polygon areas (ArcMap 9.2, ESRI Inc.).

Long-term water quality monitoring datasets during springtime (April) in freshwater streams (Maryland Coastal Bays Program) were compared to historical (Boynton, 1973; Fang et al., 1977a,b; Cerco et al., 1978) and long-term estuarine datasets available for JB (Maryland Department of Natural Resources, National Park Service Assateague Island National Seashore, Maryland Coastal Bays Program). Stream water quality data (salinity, TN, TP, nitrate, ammonium, phosphate, and chlorophyll *a*) were available as follows: Boxiron Creek (station 1) 2007–2010; Cropper (station 2) 2006–2010; Rowley Creek (station 3) 2006, 2009–10; Scarboro Creek (station 4) 2007–2010; Scarboro Creek at E.A.Vaughn Wildlife Area (station 5) 2006, 2008–10; Pikes Creek (station 6) 2006, 2008–2010; Pikes Creek (station 7) 2006–7, 2009–10; National Park Service Assateague Island National Seashore collected precipitation data.

2.6. *Statistical analyses*

Verified normally distributed datasets not requiring transformation (proc univariate, SAS), tests of ANOVA with Tukey–Kramer adjustments (proc mixed, adjust = Tukey, SAS) were performed separately on physical, chemical, and biological variables to identify differences between sampling times in 2004, 2006, and 2007. Non-parametric multidimensional scaling analysis (MDS) was conducted using Euclidean distances (proc distance, method = euclid, SAS) for datasets with no missing records of physical and chemical variables to further ordinate temporal patterns (proc mds, SAS), regress variables against the first two dimensions (proc reg, mtest/details, SAS) to determine which variables explained most variation, and correlate variables with these two dimensions (proc corr, SAS) to derive coordinates for MDS plots.

Mean data were spatially interpolated using inverse distance weighting (ESRI ArcMap 9.2).

3. Results

3.1. *Land use*

Gradients of intense land use and land cover are observable across the watersheds of mid-Atlantic coastal lagoons. Intense development has occurred in northern regions, particularly along the barrier island beaches extending north, starting at Fenwick Island - the location of Ocean City, MD (Fig. 1a). Residential development and canal estates are characteristic of development associated with diffuse source runoff. The watershed of CB has remained largely forested with intact wetlands, especially surrounding JB (Fig. 1b). This is, in part, a result of various levels of protection provided by the Worcester County Government, including the Rural Legacy program which includes easements (68.7% of the JB watershed) for open rural areas (1736 ha), state-owned wildlife areas (1329 ha), land trusts (237 ha), wetlands (88 ha), parks (30 ha), and forests (19 ha), with an additional 1011 ha (20.2% of the JB watershed) designated for future easements (Fig. 1c, Worcester County Government, 2010). Soils in the watershed are characterized as susceptible to runoff, with the wetlands adjacent to the bay considered to be highly susceptible (Fig. 1d, Maryland Department of Planning, 2010). The majority of the sediments within JB are Clayey-Silt and transition towards Sandy-Silt, Silty-Sand, and Sand along an eastward gradient (Fig. 1e, Wells et al., 1998). Groundwater nitrate concentrations within the watershed of JB are highest in the southwestern portion (Fig. 1f, LaMotte and Greene, 2007), coinciding with the location of development along the Maryland-Virginia state border and near a poultry production facility situated near Scarboro Creek headwaters (Fig. 1b, g).

3.2. Water quality

Nutrient concentrations – in tributary streams and JB – were dominated by organic fractions compared to dissolved inorganic fractions. In watershed streams ammonium and nitrate comprised 1–17% and 1–76% of the TN respectively (Table 2), while in JB ammonium and nitrate contributed only 0–14% and 0–2%, respectively (Table 3). Correspondingly, phytoplankton uptake in JB was greatest for ammonium, intermediate for urea, and lowest for nitrate (Fig. 3a). TN concentrations in tributary streams were high, ranging from 47 to 218 μM and had highest mean values across years in creeks near poultry production and development – Scarboro Creek and Pikes Creeks, respectively (Table 2, Figs. 1b and 4a). In JB, highest mean TN concentrations (56–73 μM) were close to the mainland and decreased (low values ranging 40–45 μM) towards and beyond Mills Island along a general linear gradient when averaged 2004–2007 for each sampling location (Fig. 4b). Such spatial patterns were generally consistent over time, and mean TN concentrations across JB ranged from 50.7 ± 1.6 μM in July 2006 to 60.4 ± 1.4 μM in May 2007 (Table 2). Similarly, phytoplankton uptake rates of dissolved nitrogen species were highest inshore-offshore (Fig. 3a).

Concentrations of physical (dissolved oxygen, Secchi depth, salinity, temperature, and pH) and chemical (TN, TP, nitrate + nitrite, and phosphate) parameters varied temporally (Fig. 5a,b) but not spatially within JB. Data grouped by sampling time in multidimensional scaling analysis plots. Salinity was higher in June 2004 and July 2007 than either July 2006 or May 2007 (Fig. 5a). Dissolved oxygen, temperature, and pH were higher in July 2006 than May 2007 (Fig. 5a). DIN (nitrate + nitrite) and phosphate was higher in July 2007 than in May 2007 (Fig. 5b). Both multidimensional axes significantly related ($p < 0.05$) to all physical parameters except the *x*-axis did not relate to Secchi and the *y*-axis did not relate to pH.

3.3. Microbial responses

Bacteria ($1.14 \times 10^7 \pm 6.69 \times 10^5$) and virus ($1.55 \times 10^8 \pm 8.46 \times 10^6$) abundances in the water column (Table 3) were high and did not significantly differ between sampling months, but decreased with distance from shore (Fig. 3b,c). In contrast, rates of measured denitrification (0.20 ± 0.10 ppm N_2O g^{-1} h^{-1}) and potential denitrification (0.36 ± 0.11 ppm N_2O g^{-1} h^{-1}) were greater in July 2007 than during May 2007 (measured: 0.06 ± 0.02 ppm N_2O g^{-1} h^{-1}; potential: 0.09 ± 0.03 ppm N_2O g^{-1} h^{-1}; Fig. 3d,e). Furthermore, denitrification was measurable furthest offshore, and only measurable close to shore in May 2007 but not July 2007 (Fig. 3d,e) though variability was high relative to observations. A similar pattern was found for potential denitrification – as measured after nitrate addition (Fig. 3d,e). Bacterial and virus abundances in the water column were positively related (Spearman coefficient $r = 0.69$, $p < 0.01$).

4. Discussion

4.1. Sources of N and P inputs and their location in space

Pinpointing sources of nitrogen and sources of elevated $\delta^{15}N$ values in Chincoteague Bay (CB; encompassing Johnson Bay, JB) is difficult due to its intermediate stage of degradation (Fig. 2) and mixed land use (Fig. 1a,b). In comparison, elevated nitrogen loading and concentrations in Delaware Inland Bays have been clearly attributed to anthropogenic sources in their highly developed watershed, while nutrients or high $\delta^{15}N$ values in Hog Island Bay (VA) can be attributed to nutrient recycling and microbial processing due to the lack of human development. Yet identification of specific sources of terrestrially derived nutrient sources in CB (and JB) remain elusive for future investigations.

Spatial configurations and juxtaposition of multiple datasets (Table 2, Fig. 1 and 4) provide some evidence that elevated nutrients are terrestrially derived, as is the case in other studies of temperate estuaries (De Wit et al., 2005; Gonzales et al., 2008; Rodriguez–Rodriguez et al., 2011). Despite temporal (but not spatial) distinctions in physical and chemical data (Fig. 5a,b), the temporally averaged spatial patterns of TN concentrations (Fig. 4a,b), dissolved nitrogen uptake rates (Fig. 3a), and chlorophyll *a* concentrations (Fertig et al., 2006) in JB were consistently higher west and north of Mills Island compared to south and east. This spatial pattern implies that nitrogen entered JB from diffuse terrestrial sources (i.e. Rural Legacy easements, which do not prohibit agriculture, Fig. 1c), or legacy nutrients re-suspended after entrainment in the shallow, poorly flushed area of JB (Wang, 2009).

Possibly, elevated $\delta^{15}N$ values south of Mills Island could be explained by transport of human and/or animal wastes (e.g. septic sources) via water circulation. Yet specific nutrient sources (e.g. agricultural runoff from Rural Legacy easements) cannot be conclusively determined due to conflicting indications obtained from different spatial data. In contrast to TN concentrations in JB

Table 2
Mean (±standard error) stream nutrient (ammonium, nitrate, TN, phosphate, and TP) concentrations for stations within the JB watershed. Data collected yearly (*n*; 2006–2010) in spring (April) by C. Cain; Maryland Coastal Bays Program.

Station	Stream Name	*n*	NH_4^+ (μM)	NO_3^- (μM)	TN (μM)	PO_4^- (μM)	TP (μM)
1	Boxiron Creek	4	8.1 (1.0)	19.4 (4.9)	72.1 (6.9)	1.02 (0.17)	2.52 (0.57)
2	Cropper Creek	5	7.5 (2.6)	24.0 (2.5)	83.4 (10.2)	2.28 (0.62)	6.05 (2.19)
3	Rowley Creek	3	3.1 (0.5)	3.5 (1.3)	66.1 (1.8)	0.29 (0.02)	1.03 (0.10)
4	Scarboro Creek	4	2.5 (0.5)	62.9 (39.4)	121.8 (36.7)	0.58 (0.26)	1.45 (0.64)
5	Scarboro Creek	4	2.6 (0.2)	42.6 (25.2)	116.7 (15.1)	0.68 (0.34)	1.28 (0.49)
6	Pikes Creek	4	2.2 (0.8)	59.6 (16.7)	114.6 (20.0)	0.80 (0.15)	2.48 (0.59)
7	Pikes Creek	4	3.1 (1.6)	51.3 (17.0)	118.1 (19.4)	0.87 (0.16)	2.28 (0.44)

Table 3
Mean, standard error (SE), and sample size (n) values for physical, chemical, and biological variables measured during surveys of JB in June 2004, May and July 2006, and May and July 2007. If No Data are available, 'nd' is listed in the cells.

		Variable	Unit	June 2004 mean (SE; n)	May 2006 mean (SE; n)	July 2006 mean (SE; n)	May 2007 mean (SE; n)	July 2007 mean (SE; n)
Physical	Surface	Temperature	°C	21.0 (0.2; 6)	20.3 (0.2; 28)	29.3 (0.2; 28)	23.9 (0.2; 28)	27.7 (0.3; 27)
		pH		7.9 (0.0; 6)	7.7 (0.0; 28)	7.9 (0.0; 28)	7.8 (0.0; 28)	7.7 (0.1; 9)
		Salinity	ppt	29.5 (0.3; 6)	31.6 (0.1; 28)	26.6 (0.2; 28)	26.4 (0.1; 28)	32.1 (0.1; 27)
		Dissolved oxygen	mg L^{-1}	5.52 (0.07; 6)	nd	5.59 (0.18; 28)	2.97 (0.08; 27)	5.38 (0.15; 28)
	Bottom	Temperature	°C	21.1 (0.2; 6)	20.1 (0.1; 28)	28.8 (0.1; 28)	23.8 (0.2; 28)	28.0 (0.2; 28)
		pH		7.9 (0.0; 6)	7.7 (0.0; 28)	7.8 (0.0; 28)	7.8 (0.0; 28)	7.4 (0.2; 9)
		Salinity	ppt	29.6 (0.3; 6)	31.6 (0.1; 28)	26.7 (0.2; 28)	26.4 (0.1; 28)	32.1 (0.0; 26)
		Dissolved oxygen	mg L^{-1}	5.42 (0.08; 6)	nd	5.04 (0.15; 28)	2.94 (0.09; 27)	4.75 (0.16; 28)
Chemical		Secchi depth	m	0.5 (0.0; 7)	0.4 (0.0; 28)	0.4 (0.0; 28)	0.3 (0.0; 28)	0.4 (0.0; 28)
		Total nitrogen	µM	50.9 (1.4; 7)	51.5 (1.4; 28)	50.7 (1.6; 28)	60.4 (1.4; 28)	54.2 (2.0; 38)
		Total phosporus	µM	3.03 (0.26; 7)	3.27 (0.10; 28)	5.14 (0.17; 28)	3.53 (0.06; 28)	2.85 (0.10; 38)
		Ammonium	µM	nd	nd	nd	0.47 (0.11; 28)	1.97 (0.21; 38)
		Nitrite + Nitrate	µM	nd	nd	nd	0.21 (0.02; 28)	0.35 (0.03; 38)
		Nitrite	µM	nd	nd	nd	0.10 (0.02; 5)	0.14 (0.01; 15)
		Phosphate	µM	nd	nd	nd	0.27 (0.03; 28)	1.20 (0.06; 38)
Biological		Bacteria abundance	$\times 10^7$ cells	nd	nd	nd	1.2 (0.1; 5)	1.1 (0.1; 5)
		Virus abundance	$\times 10^8$ cells	nd	nd	nd	1.6 (0.2; 5)	1.4 (0.1; 5)
		Virus:Bacteria ratio		nd	nd	nd	13.9 (2.2; 5)	13.4 (1.5; 5)
		Chlorophyll *a*	µg L^{-1}	20.7 (3.6; 7)	6.8 (0.6; 27)	34.7 (2.3; 28)	27.8 (1.2; 28)	18.8 (0.8; 36)
		Phaeophytin	µg L^{-1}	8.2 (1.3; 7)	34.2 (2.3; 27)	10.7 (1.3; 28)	5.4 (1.8; 28)	8.8 (1.6; 34)
		Seston δ^{15}N *ppt*		nd	nd	nd	nd	13.9 (0.5; 38)
		Macroalgae %N	%	1.0 (0.1; 7)	1.5 (0.1; 28)	2.8 (0.1; 28)	nd	nd
		Macroalgae δ^{15}N	ppt	14.7 (1.2; 7)	7.9 (0.4; 28)	6.8 (0.2; 28)	nd	nd
		Oyster gill %N	%	nd	nd	8.8 (0.3; 10)	nd	9.6 (0.6; 21)
		Oyster gill C/N ratio		nd	nd	3.9 (0.1; 10)	nd	5.1 (0.0; 21)
		Oyster gill δ^{15}N	ppt	nd	nd	8.4 (0.3; 10)	nd	9.1 (0.1; 21)

(Fig. 4b), those in streams (Table 2) and groundwater nitrate concentrations (Fig. 1f) were highest in southwestern portions of the JB watershed rather than the northern portion, and this spatial mismatch may influence dominant nitrogen sources to JB. To fully judge the contributions of different sources a mass balance of loadings and fate would be necessary, but is confounded by open exchange and potential nutrient flow between JB and the rest of CB.

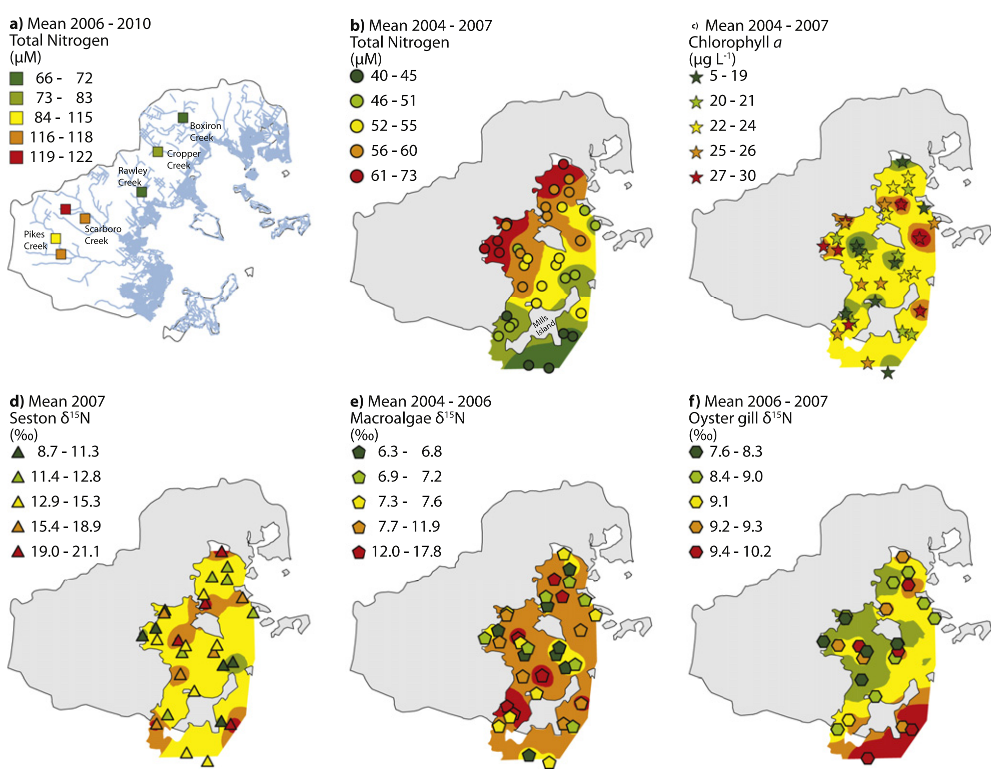

Fig. 4. Mean 2006–2010 total nitrogen concentrations in streams within the JB watershed (a). Mean and interpolated JB 2004–2007 total nitrogen concentrations (b), 2004–2007 chlorophyll *a* (c), 2007 seston δ^{15}N values (d), 2004–2006 macroalgae δ^{15}N values (e), and 2006–2007 oyster gill δ^{15}N values. Interpolation conducted by inverse distance weighting.

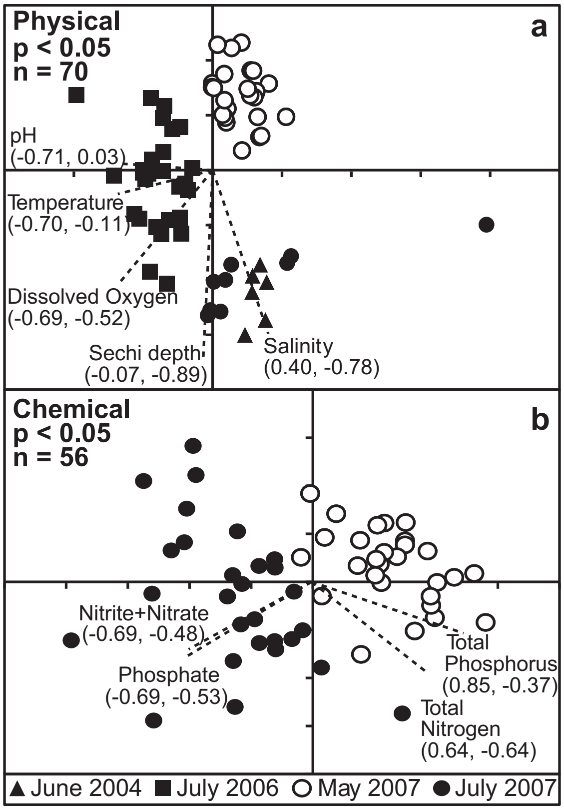

Fig. 5. Non-parametric multidimensional scaling analysis for physical (a) and chemical (b) variables measured in JB for records with no missing data during June 2004 (black triangles), July 2006 (black squares), May 2007 (white circles) and July 2007 (black circles). Significance level (*p* value) and sample size (*n*) are reported. Canonical correlation values for variables and axes are shown as coordinates.

Low δ^{15}N values closer to the shoreline were consistent with the hypothesis of nitrogen sources from agricultural runoff rather than historical poultry production (Beaulac and Reckhow, 1982; Boynton, 1993; Nahm, 2003; Beckert et al., 2011). DIN from synthetic fertilizers that have not been denitrified has $\delta^{15}N{-}NO_3^-$ and $\delta^{15}N{-}NH_4^+$ signatures of -4 to $+4$‰ (Lindau et al., 1989; Kendall, 1998; Vitoria et al., 2004), consistent with observed isotopic values (Table 3) in biological indicators (modified by 3.4‰ per trophic step, Minagawa and Wada, 1984). The nitrogen-recycling hypothesis was consistent with observed higher concentrations of ammonium than nitrate in JB (Table 3) and uptake patterns (Fig. 3a, Mulholland et al., 2004) even though springtime stream nitrate concentrations were greater than ammonium in streams (Table 2).

Sedimentary denitrification, may partially explain elevated oyster δ^{15}N values in JB south of Mills Island (Fertig et al., 2009), since measured and potential sediment denitrification rates displayed similar spatial variability (Fig. 3d,e) to spatial patterns of oyster δ^{15}N values. Though higher measurable denitrification rates in JB were co-located with elevated oyster δ^{15}N values (Fig. 3d,e; Fertig et al., 2009), oyster δ^{15}N values were much lower than groundwater nitrate δ^{15}N values for nitrogen pools that underwent denitrification (Aravena and Robertson, 1998) and 7‰ (fractionation across two trophic levels, Minagawa and Wada, 1984) would be added to these values to estimate oyster δ^{15}N values.

4.2. Impact of eutrophication

Despite management efforts and reduced nitrogen loads in the intervening years, JB underwent a shift in ecosystem response (indicated by chlorophyll *a*) to changes in nutrient concentrations and light regime (Fig. 6a–c). Diffuse loads calculated spatially from current (2002) land use data and loading coefficients (Boynton, 1993) increased from the previous decade (Boynton et al., 1996), for Assawoman Bay (378% from 4.1 to 15.5 g N m^{-2} yr^{-1}), St. Martin River (232% from 45.1 to 104.7 g N m^{-2} y^{-1}), and Newport Bay (120% from 17.4 to 20.9 g N m^{-2} y^{-1}), likely because most of the regional development occurred in these sub-watersheds in the intervening years. In contrast, loading to Isle of Wight Bay and Sinepuxent Bay decreased by roughly 50% (to 5.9 and 1.2 g N m^{-2} y^{-1}, respectively)

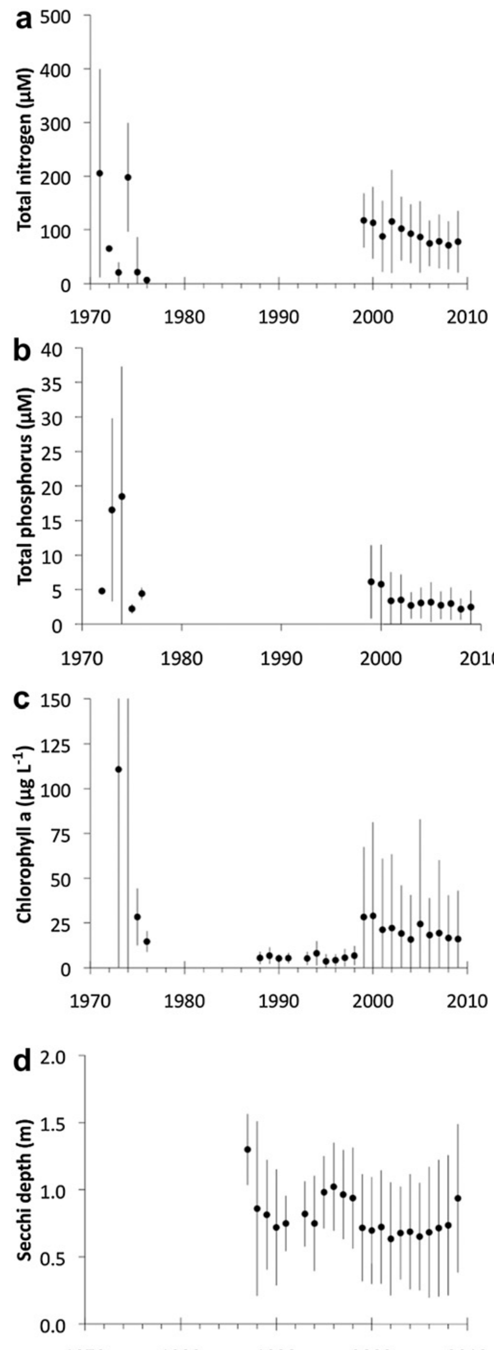

Fig. 6. Assembled long-term datasets plotted as annual average of a) total nitrogen (µM), b) total phosphorus (µM), c) chlorophyll *a* (µg L^{-1}), and d) Secchi depth (*m*). Circles represent annual means, while lines indicate standard error.

and remained constant in CB (3.4 g N m^{-2} y^{-1} in 2002 and 3.5 g N m^{-2} y^{-1} in 1990).

Spatial differences between loading to these coastal lagoons generally follow spatial patterns in development and changes to land use (Fig. 1a,b). Yet changes in spatially weighted diffuse source loadings did not greatly impact concentrations of TN or chlorophyll *a* (Fig. 7), and recent chlorophyll *a* concentrations were generally lower than those reported previously (Fig. 7). Changes in TN, and increases in the dissolved organic fraction (including urea) are associated with increases in *Aureococcus anophagefferns* outbreaks (Glibert et al., 2005, 2007). Runoff from diffuse nutrient sources is therefore concluded to be of less importance than other transport pathways, such as groundwater.

Sediment and marsh erosion and associated phosphate release may also contribute to JB eutrophication. Shoreline erosion contributes up to eight times the amount of sediment delivered by streams in this region (Bartberger, 1976) and may account for total suspended solids and low Secchi depth (Table 3). Shoreline erosion contributed >8.5% of TP and TN loads to CB between 1850 and 1989 and more recently contributes 4% of the TN and 9% of the TP (Wells et al., 2002). Spatial patterns of phosphate (2007) suggest erosion; higher concentrations were closer to shoreline. Sediments temporarily serving as a phosphorus sink may release inorganic phosphorus upon influx of organic matter (e.g. a large-scale seagrass die-off in 2008; E. Koch personal communication), associated assimilation of organic phosphorus by bacteria (Clavero et al., 1999), and desorption of adsorbed Fe(III)-bound PO_4^{-3} under anoxic conditions (Froelich, 1988). The widespread distribution of soils with high potential for erosion (Fig. 1d), sediment types (Fig. 1e), low dissolved oxygen (Table 3), high organic content (Table 3), and high bacterial and viral abundances (Fig. 3b,c) of JB fit conditions necessary for summertime PO_4^{-3} release.

Concentrations of bacteria and viruses, and the ratio of bacteria to viruses are within the range of those observed in other coastal ecosystems (Paul et al., 1993; Auguet et al., 2005; Maurice et al., 2011). Strong positive correlation between bacteria and viruses, and the correspondence of bacteria and viruses to nutrient and chlorophyll concentrations is similar to observations elsewhere (Hewson et al., 2001). While correlation of viruses to bacteria suggests ecological linkage, a more detailed analysis of virus production, protistan grazing, and temporal dynamics are needed to understand the influence of each to bacterial and nutrient dynamics in the system.

4.3. Historical context of nutrient loading and eutrophication

Long-term ecosystem changes have been documented (Fig. 6), including TN concentration reductions and subsequent increases (Wazniak et al., 2007) and concurrent increases in seagrass areal coverage in the mid 1980s (Orth et al., 2010) followed by a slowing of the increase and, more recently, declining areal coverage (Orth et al., 2010).

Eutrophication is greater now than historically (Fig. 6a–d), as elsewhere (e.g. Qian et al., 2007). Current proportions of DIN in JB (Table 3) are consistent with historical observations (Boynton et al., 1996), though concentrations are now higher. Nitrogen loading to JB increased from 6.9 g N m^{-2} yr^{-1} in 1973 to 8.5 g N m^{-2} yr^{-1} in 2004 while TN concentrations in JB increased from 20.5 μM in 1973 to 50.9 μM in 2004 (Fig. 7). In comparison, CB increased nutrient loading from 3.1 to 3.4 g N m^{-2} yr^{-1} and TN concentration from 40.5 to 48.2 μM in the same time period, suggesting that JB has been subject to more and increasing loading pressure than the broader system it is a part of. Though CB has been less eutrophic than other regions of Maryland's Coastal Bays (especially St. Martin River), this trend has reversed in recent decades. During the late 1970s, TN concentrations in CB were only 63% that of St. Martin River (Fang et al., 1977a, b). More recent TN concentrations (mean 2004–2007) in CB (Fig. 4b) were 132% that recorded there during the late 1970s, representing an increase to 83% of the TN concentrations in St. Martin River during the late 1970s (Fig. 7; Fang et al., 1977a,b; Boynton et al., 1996).

Water quality in CB has historically been better than other areas of the coastal lagoons in Maryland, as evidenced by low nutrient concentrations (Boynton et al., 1996; Wazniak et al., 2007), intact wetlands along the shoreline with rural and protected land uses in the watershed (Fig. 1a,b,c). Management actions, e.g. designation of Rural Legacy easements, property ownership by a local Land Trust and the State (Fig. 1c), contribute to this characterization. Much of the JB watershed has been under Rural Legacy easement for at least 20 years (Fig. 1c, R. Scrimgeour, pers. comm.).

Nevertheless, examination of data in context of long-term trends identified that JB has undergone ecosystem degradation. Although there is an overall decrease in primary production (phytoplankton), production of bacteria and viruses is high. This could be due to the high ratio of organic vs. inorganic constituents. Conflicting indications from different spatial data and land uses prevented identification of specific nitrogen sources

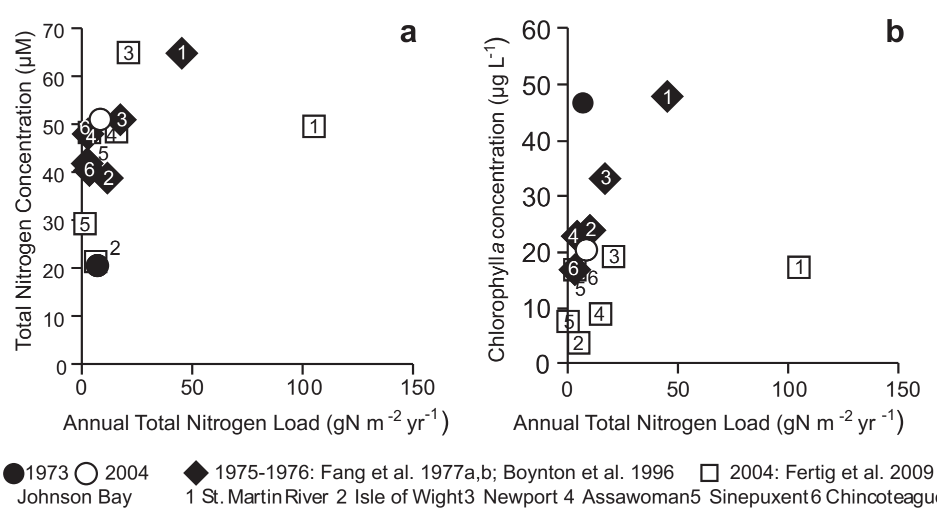

Fig. 7. Comparing historical (1975–1976; Fang et al., 1977a,b; Boynton et al., 1996) and current (2004; Fertig et al., 2009) concentrations of annual total nitrogen load, mean total nitrogen, and mean chlorophyll *a*.

(e.g. agricultural runoff, human and/or animal wastes, sediment erosion, etc.). We can, however, conclude that TN inputs were terrestrially derived and increased over the long-term despite concurrent conservation and protection measures. Therefore, we suggest that these conservation/management measures are not fully effective. As nutrient regulations are being defined, their development may benefit from elucidating sources of nutrient inputs and integrating multiple long-term monitoring datasets available for coastal lagoons such as JB.

Acknowledgements

Funding came from MD Coastal Bays Program Implementation Grants and UMCES-HPL Student Fellowship and Education Committee Student Grant to K. Beckert. UMCES-HPL Analytical Services and UC Davis Stable Isotope Facility conducted chemical analyses. We thank J. Alexander, M. Malpezzi, K. Meyer, E. Nauman, C. Palinkas, C. Schupp, and M. Wright for field/laboratory assistance, and MD-DNR and NPS field crews for data collection. S. Garrison, M. Hall, R. Jesien, B. Sturgis, and C. Wazniak provided long-term data from MD-DNR, MD Coastal Bays Program, NPS Assateague Island National Seashore. S. Hines obtained historical data. A. Dungan provided GIS layers. UMCES contribution 4697.

References

Allen, T.R., Tolvanen, H.T., Oertel, G.F., McLeod, G.M., 2007. Spatial characterization of environmental gradients in a coastal lagoon, Chincoteague Bay. Estuaries and Coasts 30, 959–977.
Andres, A.S., 1992. Estimate of Nitrate Flux to Rehoboth and Indian River Bays, Delaware, Through Direct Discharge Of Ground Water. Open File Report No. 35, Delaware Geological Survey, Newark DE, 39 pp.
Arar, E.J., 1997. Method 446.0 in Vitro Determination of Chlorophylls a, b, c1 + c2 and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry. Cincinnati, Ohio: Revision 1.2 National Exposure Research Laboratory Office of Research and Development. U.S. Environmental Protection Agency.
Aravena, R., Evans, M.L., Cherry, J.A., 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Ground Water 31, 180–186.
Aravena, R., Robertson, W.D., 1998. Use of multiple isotope tracers to evaluate denitrification in ground water: study of nitrate from a large-flux septic system plume. Ground Water 36, 976–982.
Auguet, J.C., Montanié, H., Delmas, D., Hartmann, H.J., Huet, V., 2005. Dynamic of virioplankton abundance and its environmental control in the Charente Estuary (France). Microbial Ecology 50, 337–349.
Balderston, W.L., Sherr, B., Payne, W.J., 1976. Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectormarinus*. Applied Environmental Microbiology 31, 504–508.
Bartberger, C.E., 1976. Sediment sources and sedimentation rates, Chincoteague Bay, Maryland and Virginia. Journal of Sedimentary Petrology 46, 326–336.
Beaulac, M.N., Reckhow, K.H., 1982. An examination of land use – nutrient export relationships. Water Research Bulletin 18, 1013–1024.
Beckert, K.A., Fisher, T.R., O'Neil, J.M., Jesien, R.V., 2011. Characterization and comparison of stream nutrients, land use, and loading patterns in Maryland Coastal Bay watersheds. Water Air Soil Pollution. http://dx.doi.org/10.1007/s11270-011-0788-7.
Boynton, W.R., 1973. Phytoplankton Production in Chincoteague Bay Maryland-Virginia. Masters thesis. University of North Carolina, Chapel Hill, NC.
Boynton, W.R. (Ed.), 1993. Maryland's Coastal Bays: An Assessment of Aquatic Organisms, Pollutant Loadings and Management Options. Chesapeake Biological Laboratory, Solomons, Maryland. Ref. No. [UMCEES] CBL 93–053.
Boynton, W.R., Murray, L., Hagy, J.D., Stokesb, C., Kemp, W.M., 1996. A comparative analysis of eutrophication patterns in a temperate coastal lagoon. Estuaries 19, 408–421.
Bratton, J.F., Böhlke, J.K., Krantz, D.E., Tobias, C.R., 2009. Flow and geochemistry of groundwater beneath a back-barrier lagoon: the subterranean estuary at Chincoteague Bay, Maryland, USA. Marine Chemistry 113, 78–92.
Bratton, J.F., Böhlke, J.K., Manheim, F.T., Krantz, D.E., 2004. Ground water beneath coastal bays of the Delmarva Peninsula: ages and nutrients. Ground Water 42, 1021–1034.
Cerco, C.F., Fang, C.S., Rosenbaum, A., 1978. Intensive Hydrographical and Water Quality Survey of the Chincoteague/Sinepuxent/Assawoman Bay Systems, vol. III. Non-point source pollution studies in the Chincoteague Bay system. Special Scientific Report No. 86. Virginia Institute of Marine Science, Gloucester Point, Virginia.
Clavero, V., Izquierdo, J.J., Fernandez, J.A., Niell, F.X., 1999. Influence of bacterial density on the exchange of phosphate between sediment and overlying water. Hydrobiologia 392, 55–63.
Clesceri, L.S., Greenberg, A.E., Trussell, R.R., 1998. Standard Methods for the Examination of Water and Waste Water. APHA-AWWA-WPCF.
Cline, J.D., Kaplan, I.R., 1975. Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical North Pacific Ocean. Marine Chemistry 3, 271–299.
Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin 42, 149–156.
Delaware Office of State Planning Coordination, 2010. 2002 Land Use/Land Cover Data. http://stateplanning.delaware.gov/info/lulcdata/2002_lulc.shtml.
D'Elia, C.F., Steudler, P.A., Corwin, N., 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnology and Oceanography 22, 760–764.
Dennison, W.C., Thomas, J.E., Cain, C.J., Carruthers, T.J.B., Hall, M.R., Jesien, R.V., Wazniak, C.E., Wilson, D.E. (Eds.), 2009. Shifting Sands: Environmental and Cultural Change in Maryland's Coastal Bays. IAN Press, Cambridge, MD, p. 396.
De Wit, R., Leibreich, J., Vernier, F., Delmas, F., Beuffe, H., Maison, P., Chossat, J.C., Laplace-Treyture, C., Laplana, R., Clave, V., Torre, M., Auby, I., Trut, G., Maurer, D., Capdeville, P., 2005. Relationship between land-use in the agro-forestry system of les Landes, nitrogen loading to and risk of macro-algal blooming in the Bassin d'Arcachon coastal lagoon (SW France). Estuarine. Coastal and Shelf Science 62, 453–465.
Dillow, J.J.A., Banks, W.S.L., Smigaj, M.J., 2002. Groundwater Quality and Discharge to Chincoteague and Sinepuxent Bays Adjacent to Assateague Island National Seashore, Maryland. Water-Resources Investigations Report 02-4029. U.S. Geological Survey, Baltimore, Maryland, USA.
Dillow, J.J.A., Greene, E.A., 1999. Ground-Water Discharge and Nitrate Loadings to the Coastal Bays of Maryland. U.S. Geological Survey. WRIR 99-4167.
Dillow, J.J.A., Raffensperger, J.P., 2006. Estimates of the Loads of Nitrite + Nitrate in the Flow of Bassett Creek to the Maryland Coastal Bays Adjacent to Assateague Island National Seashore, Water Years 2003–2004: U.S. Geological Survey Scientific Investigations Report 2006–5080, 10 pp.
Fang, C.S., Jacobson, J.P., Rosenbaum, A., Hyer, P.V., 1977a. Intensive Hydrographical and Water Quality Survey of the Chincoteague/Sinepuxent/Assawoman Bays, vol. II. Data Report: Intensive Hydrographical and Water Quality. Special Scientific Report No. 82. Virginia Institute of Marine Science, Gloucester Point, Virginia.
Fang, C.S., Rosenbaum, A., Jacobson, J.P., Hyer, P.V., 1977b. Intensive Hydrographical and Water Quality Survey of the Chincoteague/Sinepuxent/Assawoman Bays, vol. I. Study Program. Special Scientific Report No. 82. Virginia Institute of Marine Science, Gloucester Point, Virginia.
Fertig, B., Carruthers, T.J.B., Dennison, W.C., Fertig, E.J., Altabet, M.A., 2010. Eastern oyster (*Crassostrea virginica*) $\delta^{15}N$ as a bioindicator of nitrogen sources: observations and modeling. Marine Pollution Bulletin 60, 1288–1298.
Fertig, B., Carruthers, T.J.B., Dennison, W.C., Jones, A.B., Pantus, F., Longstaff, B., 2009. Oyster and macroalgae bioindicators detect elevated $\delta^{15}N$ in Maryland's Coastal Bays. Estuaries and Coasts 32, 773–786.
Fertig, B., Carruthers, T.J.B., Wazniak, C.E., Sturgis, B., Hall, M.R., Jones, A.B., Dennison, W.C., 2006. Water Quality in Four Regions of the Maryland Coastal Bays: Assessing Nitrogen Source in Relation To Rainfall and Brown Tide. Data Report to Maryland Coastal Bays Program.
Froelich, P.N., 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: a primer on the phosphate buffer mechanism. Limnology and Oceanography 33, 649–668.
Fry, B., 2006. Stable Isotope Ecology, first ed. Springer, New York.
Glibert, P.M., Capone, D.G., 1993. Mineralization and assimilation in aquatic, sediment, and wetland systems. In: Knowles, R., Blackburn, T.H. (Eds.), Nitrogen Isotope Techniques. Academic Press, San Diego, California, pp. 243–272.
Glibert, P.M., Trice, T.M., Michael, B., Lane, L., 2005. Urea in the tributaries of the Chesapeake and coastal bays of Maryland. Water, Air, and Soil Pollution 160, 229–243.
Glibert, P.M., E.Wazniak, C., Hall, M.R., Sturgis, B., 2007. Seasonal and interannual trends in nitrogen and brown tide in Maryland's coastal bays. Ecological Applications 17, S79–S87.
Gonzales, F.U.T., Ilveira, J.A.H.S., Aguirre-Macedo, M.L., 2008. Water quality variability and eutrophic trends in karstic tropical coastal lagoons of the Yucatan Peninsula. Estuarine. Coastal and Shelf Science 76, 418–430.
Goshorn, D., McGinty, M., Kennedy, C., Jordan, C., Wazniak, C., Schwenke, K., Coyne, K., 2001. An Examination of Benthic Macroalgae Communities as Indicators of Nutrients in Middle Atlantic Coastal Estuaries. Maryland Component Final Report 1998–1999. Maryland Department of Natural Resources, Annapolis, MD, USA.
Hager, P., 1996. Worcester County, MD. p. 20–24. In: K. Beidler, P. Gant, M. Ramsay, and G. Schultz (eds.), Proceedings – Delmarva's Coastal Bay Watersheds: Not yet up the Creek. EPA/600/R-95/052. United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, Rhode Island, USA.
Hays, R.L., Ullman, W.J., 2007. Dissolved nutrient fluxes through a sandy estuarine beachface (Cape Henlopen, Delaware, U.S.A.): contributions from fresh groundwater discharge, seawater recycling, and diagenesis. Estuaries and Coasts 30, 710–724.
Harris, L., Granger, S., Nixon, S., 2005. Evaluation of the Health of Eelgrass (Zostera Marina L.) Beds within the Maryland Coastal Bays: A Report to the National Park Service. University of Rhode Island, Graduate School of Oceanography, Narragansett, Rhode Island, USA.

Hewson, I., O'Neil, J.M., Fuhrman, J.A., Dennison, W.C., 2001. Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. Limnology and Oceanography 46, 1734–1746.

Jacobs, F., Bowers, J., Souza, S., Krinsky, B., Seibel, J., 1993. Diagnostic assessments of terrestrial pollutant loadings. In: Boynton, W.R. (Ed.), Maryland's Coastal Bays: An Assessment of Aquatic Organisms, Pollutant Loadings and Management Options. Part 2. Chesapeake Biological Laboratory, Solomons, Maryland, p. 2-1-3-16. Ref. No. [UMCEES] CBL 93-053.

Jordan, T.E., Correll, D.L., Weller, D.E., 1997. Relating nutrient discharges from watersheds to land use and streamflow variability. Water Resources Research 33, 2579–2590.

Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, J.B., Bennett, G.E., Cornwell, J.C., 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Analytical Chemistry 66, 4166–4170.

Kerouel, R., Aminot, A., 1987. Procédure optimisée hors-contaminations pour l'analyze des éléments nutritifs dissous dans l'eau de mer. Marine Environmental Research 22, 19–32.

Kendall, C., 1998. Tracing nitrogen sources and cycling in catchments. In: Kendall, C., McDonnell, J.J. (Eds.), Isotope Tracers in Catchment Hydrology. Elsevier Science B. V, Amsterdam, pp. 519–576.

Kennish, M.J., Paerl, H.W., 2010. In: Coastal Lagoons: Critical Habitats of Environmental Change. CRC Press, p. 568pp.

Krantz, D.E., Manheim, F.T., Bratton, J.F., Phelan, D.J., 2004. Hydrogeologic setting and ground water flow beneath a section of Indian River Bay, Delaware. Ground Water 42, 1035–1051.

LaMotte, A.E., Greene, E.A., 2007. Spatial analysis of land use and shallow groundwater vulnerability in the watershed adjacent to Assateague Island National Seashore, Maryland and Virginia. USA Environmental Geology 52, 1413–1421.

Lindau, C.W., Delaune, R.D., Patrick Jr., W.H., Lambremont, E.N., 1989. Assessment of stable nitrogen isotopes in fingerprinting surface water inorganic nitrogen sources. Water, Air, and Soil Pollution 48, 489–496.

Lung, W.S., 1994. Water Quality Modeling of the St. Martin River, Assawoman and Isle of Wight Bays. Maryland Department of the Environment, Baltimore, Maryland.

Manheim, F.T., Krantz, D.E., Bratton, J.F., 2004. Studying ground water under Delmarva Coastal Bays using electrical resistivity. Ground Water 42, 1052–1068.

Maryland Department of Planning, 2010. Land Use Land Cover (2002). Downloadable Files: http://planning.maryland.gov/ourproducts/mapping.shtml.

Maurice, C.F., Mouillot, D., Bettarel, Y., De Wit, R., Sarmento, H., Bouvier, T., 2011. Disentangling the relative influence of bacterioplankton phylogeny and metabolism on lysogeny in reservoirs and lagoons. The ISME Journal 5, 831–842.

McClelland, J.W., Valiela, I., 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography 43, 577–585.

Miller, D.C., Ullman, W.J., 2004. Ecological consequences of estuarine groundwater discharge at Cape Henlopen, Delaware bay, USA. Ground Water 42, 959–970.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimca Acta 48, 1135–1140.

Mulholland, M.R., Boneillo, G., Minor, E.C., 2004. A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. Harmful Algae 3, 361–376.

Nahm, K.H., 2003. Evaluation of the nitrogen content in poultry manure. World's Poultry Science Journal 59, 77–88.

Nixon, S.W., Buckley, B., Granger, S., Bintz, J., 2001. Responses of very shallow marine ecosystems to nutrient enrichment. Human and Ecological Risk Assessment 75, 1457–1481.

Noble, R., Fuhrman, J., 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquatic Microbial Ecology 14, 113–118.

Orth, R.J., Williams, M.R., Marion, S.R., Wilcox, D.J., Carruthers, T.J.B., Moore, K.A., Kemp, W.M., Dennison, W.C., Rybicki, N., Bergstrom, P., Batiuk, R.A., 2010. Long-term trends in submersed aquatic vegetation (SAV) in Chesapeake Bay, USA, related to water quality. Estuaries and Coasts 33, 1144–1163.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Toronto.

Patel, A., Noble, R.T., Steele, J.A., Schwalbach, M.S., Hewson, I., Fuhrman, J.A., 2007. Virus and prokaryote enumeration from planktonic aquatic environments by epifluorescence microscopy with SYBR Green I. Nature Protocols 2, 269–276.

Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A., Dickson, L., 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Applied and Environmental Microbiology 59, 718–724.

Pritchard, D.W., 1960. Salt balance and exchange rate for Chincoteague Bay. Chesapeake Science 1, 48–57.

Qian, Y., Migliaccio, K.W., Wan, Y., Li, Y., 2007. Trend analysis of nutrient concentrations and loads in selected canals of the southern Indian River Lagoon, Florida. Water, Air, and Soil Pollution 186, 195–208.

Revilla, M., Alexander, J., Glibert, P.M., 2005. Urea analysis in coastal waters: comparison of enzymatic and direct methods. Limnology and Oceanography: Methods 3, 290–299.

Rodriguez-Rodriguez, M., Benavente, J., Alcala, F.J., Paracuellos, M., 2011. Long-term water monitoring in two Mediterranean lagoons as an indicator of land-use changes and intense precipitation events (Adra, Southeastern Spain). Estuarine. Coastal and Shelf Science 91, 400–410.

Scanes, P., Coade, G., Doherty, M., Hill, R., 2007. Evaluation of the utility of water quality based indicators of estuarine lagoon condition in NSW, Australia. Estuarine, Coastal and Shelf Science 74, 306–319.

Schmidt, J.P., Dell, C.J., Vadas, P.A., Allen, A.L., 2007. Nitrogen export from coastal plain field ditches. Journal of Soil and Water Conservation 62, 235–243.

Schwartz, M.C., 2003. Significant groundwater input to a coastal plain estuary: assessment from excess radon. Estuarine Coastal and Shelf Science 56, 31–42.

Sharp, J.H., Benner, R., Bennett, L., Carlson, C.A., Fitzwater, S.E., Peltzer, E.T., Tupas, L.M., 1995. Analyses of dissolved organic carbon in seawater: the JGOFS EqPac methods comparison. Marine Chemistry 48, 91–108.

Solórzano, L., Sharp, J.H., 1980a. Determination of total dissolved nitrogen in natural waters. Limnology and Oceanography 25, 751–754.

Solórzano, L., Sharp, J.H., 1980b. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnology and Oceanography 25, 754–758.

Sørenson, J., 1978. Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Applied Environmental Microbiology 36, 139–143.

Souza, S., Krinsky, B., Seibel, J., 1993. Maryland's Coastal Bays: An Assessment of Aquatic Ecosystems, Pollutant Loadings, and Management Options. Maryland Department of the Environment, Baltimore, Maryland.

Stanhope, J.W., 2003. Relationships between watershed characteristics and base flow nutrient discharges to eastern shore coastal lagoons, Virginia. M.S. thesis. College of William and Mary, Gloucester Point, VA, 158 pp.

Sturgis, B., 2001. Quality Assurance Project Plan for Assateague Island National Seashore's Water Quality Monitoring Program: Chemical and Physical Properties. Assateague Island National Seashore, Berlin, Maryland, USA.

Tango, P.W., Butler, Wazniak, C.E., 2005. Assessment of harmful algae bloom species in the Maryland Coastal Bays. In: Wazniak, C., Hall, M. (Eds.), Maryland's Coastal Bays Ecosystem Health Assessment 2004. Maryland Department of Natural Resources, Tidewater Ecosystem Assessment, Annapolis, Maryland, USA, pp. 8-2–8-32. DNR 12-1202-0009.

Trice, T.M., Glibert, P.M., Van Heukelem, L., 2004. HPLC pigment ratios provide evidence of past blooms of *Aureococcus anaophagefferens* in the coastal bays of Maryland and Virginia. USA Harmful Algae 3, 295–304.

Tyler, R.M., 2007. Effects of coverage by benthic seaweed mats on (northern quahog = hard clam) *Mercenaria mercenaria* in a eutrophic estuary. Journal of Shellfish Research 26, 1021–1028.

Valderrama, J.C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10, 109–122.

Valiela, I., Costa, J., Foreman, K., Teal, J.M., Howes, B., Aubrey, D., 1990. Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. Biodegradation 10, 177–197.

Virginia Department of Forestry, 2010. Virginia Land Use (2005). http://www.dof.virginia.gov/gis/datadownload.shtml.

Vitoria, L., Otero, N., Soler, A., Canals, A., 2004. Fertilizer characterization: isotopic data (N, S, O, C, and Sr). Environmental Science and Technology 38, 3254–3262.

Wang, T., 2009. Numerical Modeling of Eutrophication Dynamics in the Shallow Coastal Ecosystem: A case study in the Maryland and Virginia coastal bays. PhD dissertation. College of William and Mary, School of Marine Science.

Wazniak, C.E., Hall, M.R., Carruthers, T.J.B., Sturgis, B., Dennison, W.C., Orth, R.J., 2007. Linking water quality to living resources in a mid-Atlantic lagoon system, USA. Ecological. Applications 17 (5), S64–S78.

Wells, D.V., Hill, J.M., Park, M.J., Williams, C.P., 1998. The Shallow Sediments of the Middle Chincoteague Bay Area in Maryland: Physical and Chemical Characteristics. (Coastal and Estuarine Geology File Report No. 98-1): Maryland Geological Survey, Baltimore, MD., 104pp.

Wells, D.V., Hennessee, E.L., Hill, J.M., 2002. Shoreline Erosion as a Source of Sediments and Nutrients, Northern Coastal Bays, Maryland (Coastal and Estuarine Geology File Report No. 02-05): Maryland Geological Survey, Baltimore, MD, on Compact Disk (CD-ROM)

Worcester County Government, 2010. GIS layers of protected lands in Worcester County. GIS Data Files.

Yoshinari, T., Knowles, R., 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69, 705–710.