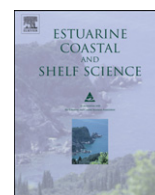




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Elucidating terrestrial nutrient sources to a coastal lagoon, Chincoteague Bay, Maryland, USA

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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 ($\delta^{15}\text{N}$) 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 $40.0 - 73.2 \mu\text{M}$). 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.

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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.

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'Hotspots' of elevated terrestrially derived total nitrogen (TN) ($51.1 \pm 1.0 \mu\text{M}$), total phosphorus (TP) ($4.20 \pm 0.16 \mu\text{M}$), and $\delta^{15}\text{N}$ values in macroalgae ($8.0 \pm 0.3\text{‰}$) and oyster gills ($8.4 \pm 0.3\text{‰}$) were previously identified (Fertig et al., 2009) within the CB embayment Johnson Bay (JB) ($38^{\circ}3'N$, $75^{\circ}20'W$). Yet elevated nutrient and $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 ($^{14}\text{NH}_3$ is slightly more volatile than $^{15}\text{NH}_3$) resulting in enriched ^{15}N (Cline and Kaplan, 1975; Kendall,

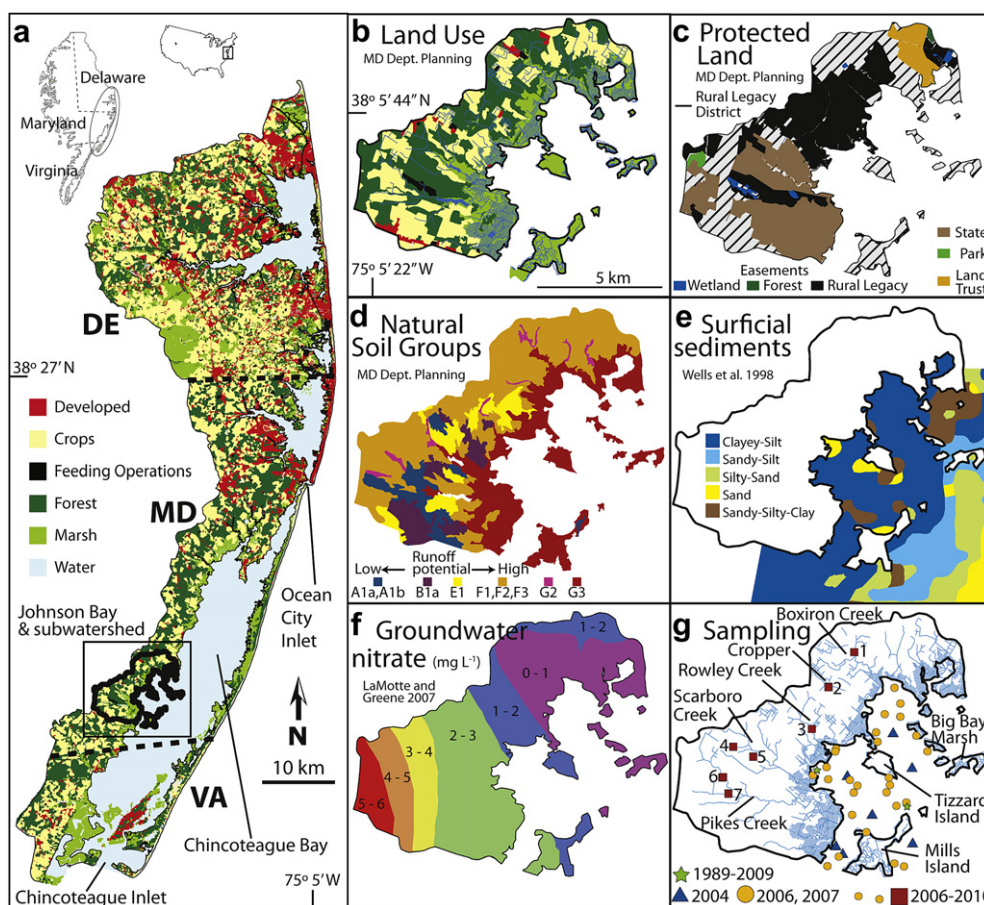


Fig. 1. Location of JB within CB and land use within the watersheds of these mid-Atlantic coastal lagoons along Delmarva Peninsula (a) and the JB sub-watershed (b). Land subject to protections and conservation (c). Natural soil groups (d) and sediments (e) within JB sub-watershed and bay, respectively. 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 $\delta^{15}\text{N}$ 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 $\sim 35,000$ 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 $\delta^{15}\text{N}$ and terrestrially derived nitrogen to JB and 2) Placing JB eutrophication and nutrient monitoring data into historical and

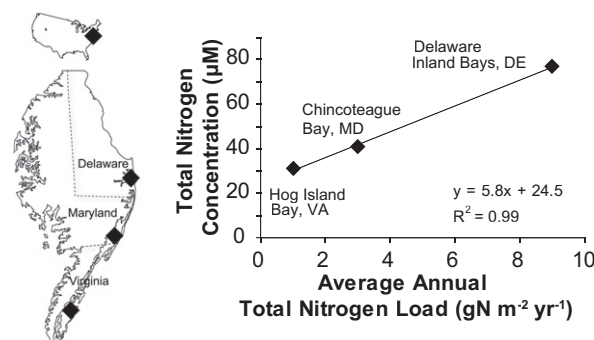


Fig. 2. CB in context of other coastal lagoons along Delmarva Peninsula with respect to TN concentration vs. average annual TN load. Hog island, VA data courtesy of K. McGlathery (<http://www.lternet.edu/sites/vcr/>) and Delaware Inland bays data courtesy of W. Ullman.

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 km² 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, $\delta^{15}\text{N}$ 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	Boynnton, 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 $\delta^{15}\text{N}$ were analyzed at UC Davis Stable Isotope Facility. For definition, $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$, where $R = {}^{15}\text{N}/{}^{14}\text{N}$ (Fry, 2006). Deployment of macroalgae and oysters for $\delta^{15}\text{N}$ values, %N, and C/N ratio is described elsewhere (Costanzo et al., 2001; Fertig et al., 2009, 2010). Oyster gill data (initially $7.8 \pm 0.8\text{‰}$ 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 ($\pm 0.2\text{‰}$ 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, ${}^{15}\text{N}$ substrates (NH_4^+ , NO_3^- 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; Sørensen, 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

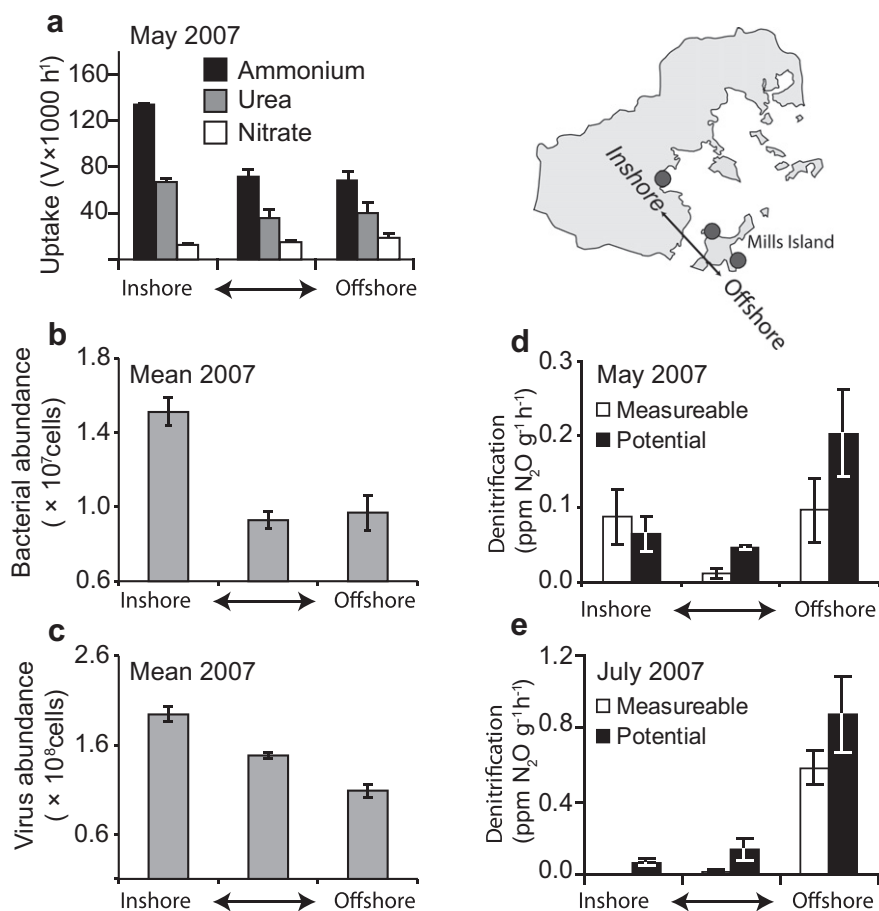


Fig. 3. Rates (\pm se) of relative velocity of nitrogen uptake for ammonium (black), nitrate (white), and urea (grey) along an inshore-offshore transect (a), Mean (\pm se) of bacteria (b) and viruses (c) collected from surface water samples in JB in 2007. Mean (\pm se) of measured (white) and potential (addition of $100 \mu\text{M NO}_3^-$; 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 \pm 1.6 μM in July 2006 to 60.4 \pm 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.

Table 2

Mean (\pm 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)

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 ($0.20 \pm 0.10 \text{ ppm N}_2\text{O g}^{-1} \text{ h}^{-1}$) and potential denitrification ($0.36 \pm 0.11 \text{ ppm N}_2\text{O g}^{-1} \text{ h}^{-1}$) were greater in July 2007 than during May 2007 (measured: $0.06 \pm 0.02 \text{ ppm N}_2\text{O g}^{-1} \text{ h}^{-1}$; potential: $0.09 \pm 0.03 \text{ ppm N}_2\text{O g}^{-1} \text{ h}^{-1}$; 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 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 phosphorus	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)	
	Bacteria abundance	× 10 ⁷ cells	nd	nd	nd	1.2 (0.1; 5)	1.1 (0.1; 5)	
	Virus abundance	× 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)		
Biological	Chlorophyll <i>a</i>	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.

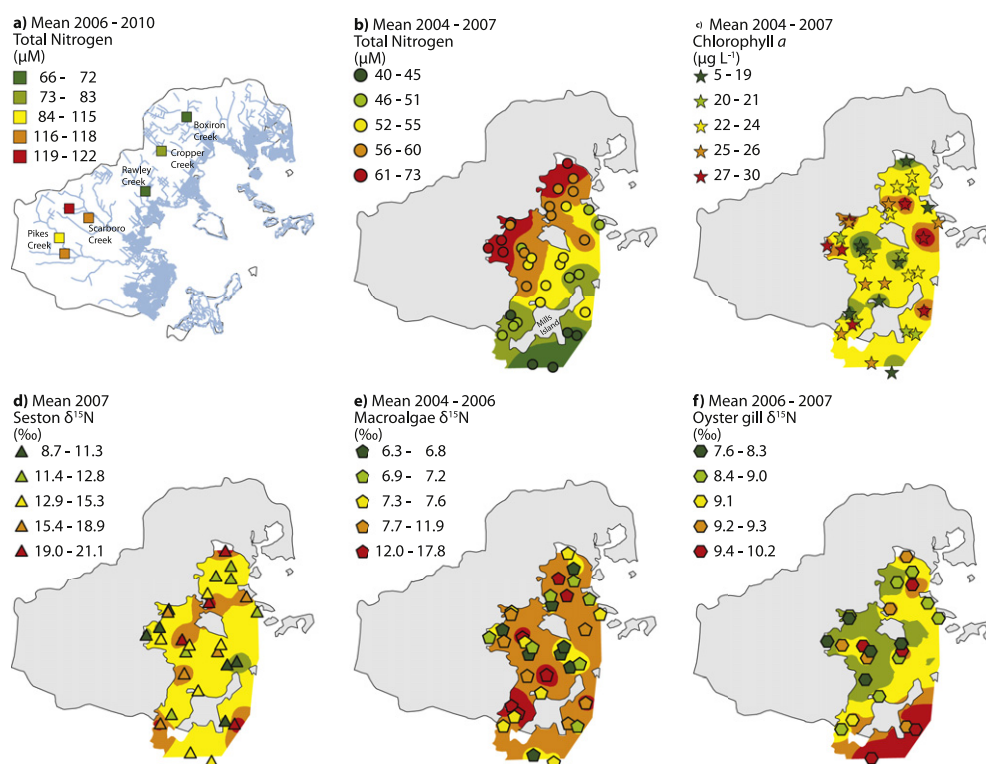


Fig. 4. Mean 2006–2010 total nitrogen concentrations in streams within the JB watershed (a). Mean and interpolated JB 2004–2007 total nitrogen concentrations (b), 2004–2007 chlorophyll *a* (c), 2007 seston δ¹⁵N values (d), 2004–2006 macroalgae δ¹⁵N values (e), and 2006–2007 oyster gill δ¹⁵N values. Interpolation conducted by inverse distance weighting.

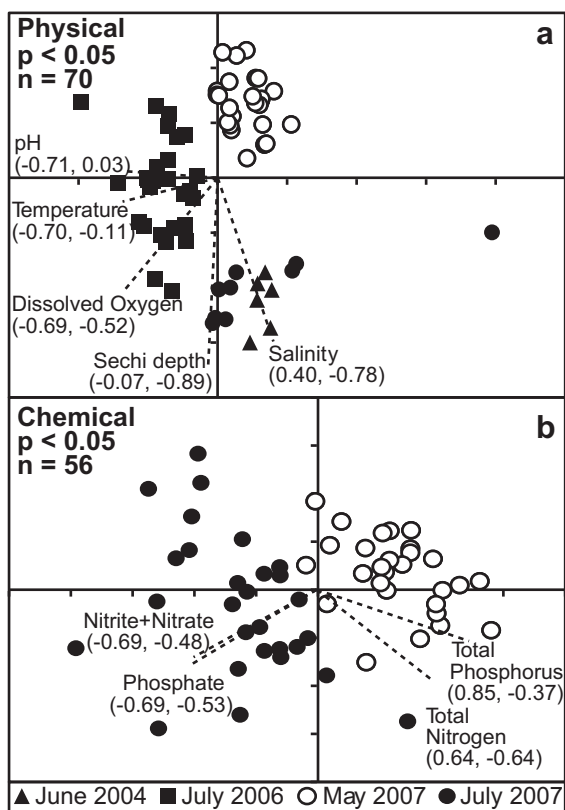


Fig. 5. Non-parametric multidimensional scaling analysis for physical (a) and chemical (b) variables measured in JB for records with no missing data during June 2004 (black triangles), July 2006 (black squares), May 2007 (white circles) and July 2007 (black circles). Significance level (p value) and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

Low $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{15}\text{N}\text{-NH}_4^+$ signatures of -4 to $+4\text{‰}$ (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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values. Though higher measurable denitrification rates in JB were co-located with elevated oyster $\delta^{15}\text{N}$ values (Fig. 3d,e; Fertig et al., 2009), oyster $\delta^{15}\text{N}$ values were much lower than groundwater nitrate $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $15.5\text{ g N m}^{-2}\text{ yr}^{-1}$), St. Martin River (232% from 45.1 to $104.7\text{ g N m}^{-2}\text{ yr}^{-1}$), and Newport Bay (120% from 17.4 to $20.9\text{ g N m}^{-2}\text{ yr}^{-1}$), 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 $1.2\text{ g N m}^{-2}\text{ yr}^{-1}$, respectively)

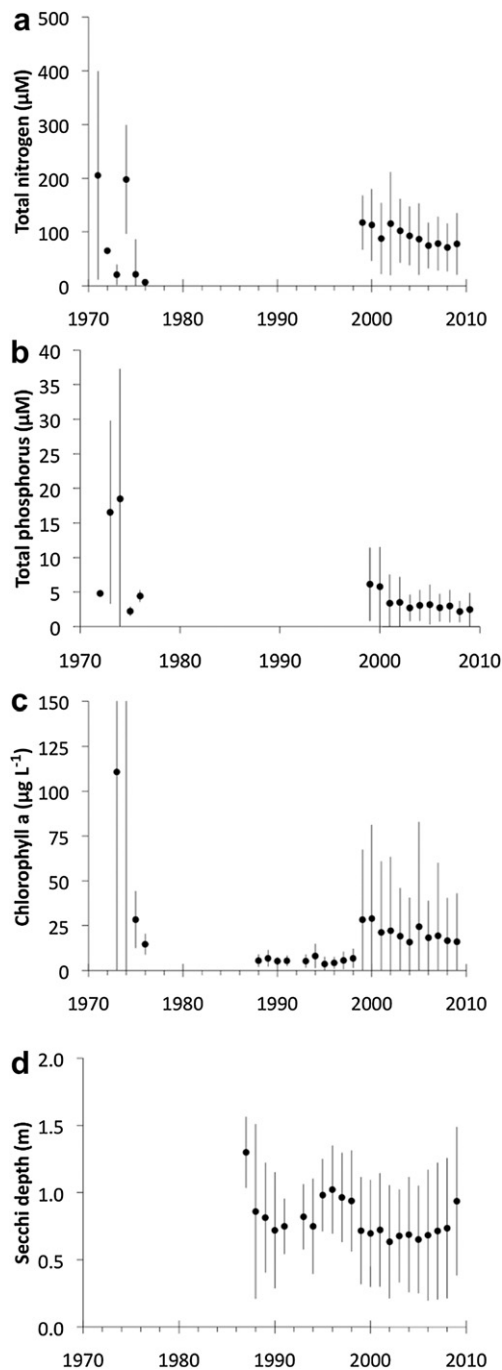


Fig. 6. Assembled long-term datasets plotted as annual average of a) total nitrogen (μM), b) total phosphorus (μM), c) chlorophyll a ($\mu\text{g L}^{-1}$), and d) Secchi depth (m). Circles represent annual means, while lines indicate standard error.

and remained constant in CB ($3.4 \text{ g N m}^{-2} \text{ yr}^{-1}$ in 2002 and $3.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ 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 anophagefferens* 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 $6.9 \text{ g N m}^{-2} \text{ yr}^{-1}$ in 1973 to $8.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ in 2004 while TN concentrations in JB increased from $20.5 \mu\text{M}$ in 1973 to $50.9 \mu\text{M}$ in 2004 (Fig. 7). In comparison, CB increased nutrient loading from 3.1 to $3.4 \text{ g N m}^{-2} \text{ yr}^{-1}$ and TN concentration from 40.5 to $48.2 \mu\text{M}$ 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

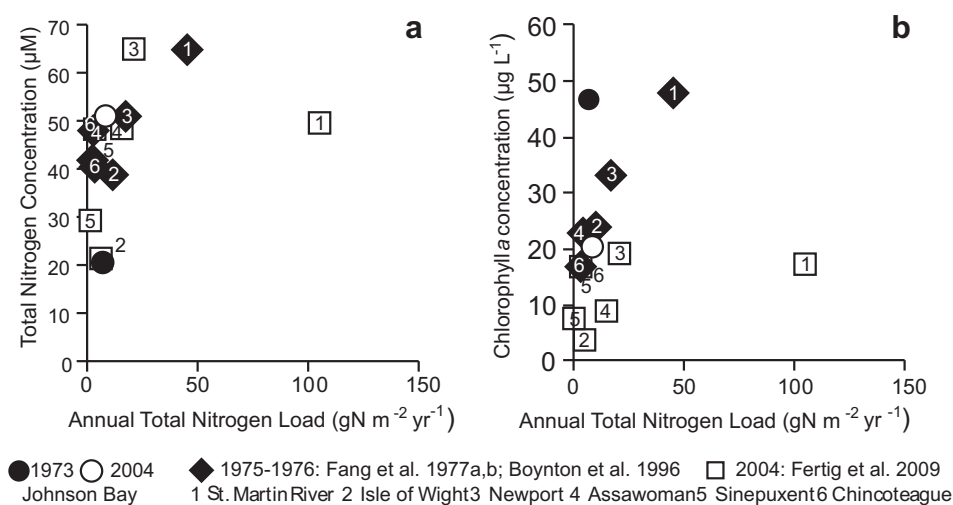


Fig. 7. Comparing historical (1975–1976; Fang et al., 1977a,b; Boynton et al., 1996) and current (2004; Fertig et al., 2009) concentrations of annual total nitrogen load, mean total nitrogen, and mean chlorophyll *a*.

(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.

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