QUANTIFICATION OF NITRATE SOURCES AND SINKS USING A WATER QUALITY NETWORK
IN MORRO BAY ESTUARY, CALIFORNIA

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by
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ABSTRACT

Quantification of nitrate sources and sinks using a water quality network in Morro Bay Estuary, California

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Using an instrumented water quality network in Morro Bay Estuary, California from 2007 to 2010 (15 min sampling frequency), this study addressed the two objectives of constructing a nitrate budget and assessing the influence of sampling frequency on water quality parameters. These two objectives led to the submission of an original report of research (Appendix A) and a note (Appendix B) to peer-reviewed journals.

The first objective was to characterize the high spatial and temporal variation in physical parameters and nitrate concentrations and to construct a nitrate budget quantifying sources and sinks of nitrate from the ocean, streams, and groundwater, as well as biological processes in the Estuary. Morro Bay Estuary was found to be a non-eutrophic system and a mean net exporter of nitrate, 327.15 t yr^{-1} . Fifty-four percent of the nitrate export was attributed to nitrate sources and internal biological processing. Nitrate loading from streams contributed 37 % to the export of nitrate (124.01 t yr^{-1}), while groundwater nitrate loading supplied a conservative estimate of 46 % of the exported nitrate (153.92 t yr^{-1}), with a neap tide enhancement of the discharge. Denitrification, *Zostera marina*, and benthic macroalgae assimilation of nitrate were the dominant internal biological processes for removal and retention, but were only 35% of the total nitrate budget.

The second objective was to investigate the impact of sampling frequency and sampling location on understanding dynamics in water quality by degrading a year time series of seven parameters from three water quality monitoring stations to sampling frequencies ranging from 15 minutes to 28 days. In Morro Bay Estuary, the semi-diurnal tidal cycle was the maximum component frequency driving the variability of temperature, turbidity, and dissolved oxygen concentrations. For these parameters, asymptotes were reached and sampling frequencies greater than six hours did not explain the additional variation in the parameters sampled. Whereas, salinity, turbidity, and nitrate concentrations lacked an asymptote, and decreased sampling frequencies led to increased estimated error. Sampling water quality parameters every 28 days can lead to mean annual difference of 30 – 140 % from 15 minute sample annual mean. We recommend sampling frequencies should be selected to oversample the tidal signal to at least hourly

frequencies to capture diel cycles and episodic events that contribute significantly to understanding the variability in the estuarine physical and biological dynamics.

Keywords: Estuaries, eutrophication, Morro Bay Estuary, nitrate, nutrient budgets, sampling frequency, sensor networks, monitoring, *Zostera marina*

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APPENDIX A

Quantification of nitrate sources and sinks in Morro Bay Estuary, California.

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Abstract

Using an instrumented water quality network in Morro Bay Estuary, California from 2007 to 2010 (15 min sampling frequency), we characterized the high spatial and temporal variation in physical parameters and nitrate concentrations and constructed a nitrate budget quantifying both sources and sinks of nitrate from the ocean, streams, groundwater, as well as biological processes in the Estuary. Morro Bay Estuary was found to be a non-eutrophic system and a mean net exporter of nitrate, 1.36 t day^{-1} , with variability across multiple time and space scales. Oceanic exchange due to mixed semi-diurnal tidal forcing and mixing contributed 90 % of the nitrate exchange in Morro Bay Estuary. The remaining exported nitrate was attributed to freshwater sources that were equally split between streams and groundwater and contributed on average 2.76 % to the total bay water volume. Fifty-four percent of the nitrate export was attributed to nitrate sources and internal biological processing. Nitrate loading from streams contributed 37 % to the export of nitrate (124.01 t yr^{-1}) while groundwater nitrate loading supplied a conservative estimate 153.92 t yr^{-1} or 46 % exported nitrate, with a neap tide enhancement of the discharge. Denitrification and *Zostera marina* and benthic macroalgae assimilation of nitrate were the dominant internal biological processes for removal and retention but were only 35% of the total nitrate budget. These data provide insight to the dynamic nature of estuarine environments and illustrate the need for the continued watershed monitoring of stream and groundwater sources in order to evaluate the impact of management actions on nitrate loading in these systems.

Keywords: Estuaries, eutrophication, Morro Bay Estuary, nitrate, nutrient budgets, sensor networks

Introduction

Estuaries are a dynamic interface between freshwater inflows and coastal ocean systems and are among the most productive marine ecosystems. As transitional zones, estuaries receive and process large loadings of land-based nutrients and pollutants entering by the way of surface runoff and groundwater discharge delivered from urban centers and agricultural watersheds (Chapin et al. 2004; Paerl 2006; Sieyes et al. 2008). In the past century, estuaries have been subject to increasing anthropogenic pressures, such as coastal population growth, changes in land-use practices, and elevated freshwater nutrient inputs (Howarth et al. 1996; NRC 2000; Lotze et al. 2006; Paerl 2006). Nitrogen, in the inorganic form of nitrate (NO_3^-), is

an essential limiting factor controlling estuarine primary productivity (Caffrey et al. 2002). Nitrogen over-enrichment, a condition that occurs when loading exceeds rates required to sustain desirable primary productivity (Paerl 2006), often leads to excessive primary production and eutrophication. Over time, eutrophication can lead to secondary consequences such as chronic decreases in dissolved oxygen, changes in species composition, and negative feedbacks for seagrass beds and marine organisms (Nixon 1995; Nixon et al. 1996; Hauxwell et al. 2003; Lotze et al. 2006). Secondary consequences from persistent nitrogen over-enrichment are capable of altering ecosystem dynamics, in turn reducing economic and intrinsic benefits of an estuary (NRC 2000; Robson et al. 2008).

In response to concerns of eutrophication, nutrient budgets are an essential first step in quantifying the temporal and spatial magnitude and variability of nutrient inputs and exports to an estuary (Nixon 1995; Mortazavi et al. 2000; NRC 2000) and have been used for many of the major estuaries of the Atlantic East coast and Gulf coast, such as: the Chesapeake Bay, Neuse Estuary, Narragansett Bay, Apalachicola Bay. These examples have well-established nutrient budgets (Nixon et al. 1996; Mortazavi et al. 2000; Whittall et al. 2003). However, proportionally fewer studies of nutrient budgets and nitrogen dynamics studies have been conducted on small estuaries located on the Pacific West coast (Page et al. 1995; Boyle et al. 2004; Chapin et al. 2004). In fact, Boynton et al. (1995) suggested nutrient budgets should be constructed for a range of estuarine systems with different characteristics in size, morphology, and flushing rates to further our understanding of ecosystem-scale nutrient dynamics and to guide resource management actions in estuaries. Previous studies have also been limited in sampling frequency, duration, or both, making it difficult to distinguish the seasonal impacts of episodic events that dominate estuarine dynamics (Chapin et al. 2004).

Estuaries along the coast of central and southern California are characterized by wet winters and long periods (~ 8 months) without measurable precipitation. Periods of upwelling, deep ocean water are a significant contributor to nitrate sources in these estuaries (Boyle et al. 2004; Chapin et al. 2004; Caffrey et al. 2007). Other significant nitrate contributors are freshwater runoff (Boyle et al. 2004; Chapin et al. 2004; Caffrey et al. 2007) and submarine groundwater discharge enhanced during neap tides (Oberdorfer et al. 1990; de Sieyes et al. 2008). Nitrate sink processes are denitrification and burial in sediments (Seitzinger 1987; Caffrey and Kemp 2000; Caffrey et al. 2003), uptake by *Zostera marina* (Pedersen and Borum 1992;

Perdersen and Borum 1993; Caffrey and Kemp 2000; MacGlathery et al. 2007), and uptake by phytoplankton and macroalgae (Pennock 1987; Pedersen and Borum 1996; MacGlathery et al. 2007). These sources and sinks of nitrate are tightly connected by complex physical, chemical, and biological interactions at multiple spatial and temporal scales.

The majority of nutrient and water quality monitoring programs conduct sampling on weekly to monthly intervals (Glasgow and Burkholder 2000; NRC 2000; Chapin et al. 2004). This can result in under-sampling of the high frequency and episodic events that dominate the environmental variability of estuaries and contribute to a large percentage of the annual nitrate budget (Chapin et al. 2004; Jannasch et al. 2008). Instrumentation provides the capability to monitor changes in estuaries over long-term deployments with high sampling frequencies (Chapin et al. 2004; Johnson et al. 2007) and can capture the critical elements of the hydrologic cycle, nitrogen cycle, and associated ecosystem processes in estuaries (Jannasch et al. 2008).

Here, we highlight results from a network of water quality stations that were installed in Morro Bay Estuary, California in 2007 and operated over a three-year period. The objectives of this study were to characterize spatial and temporal variation in physical parameters and nitrate concentrations and to construct a nitrate budget for the Estuary. This study was part of a larger initiative by the San Luis Obispo Science and Ecosystem Alliance (SLOSEA; see www.slosea.org) designed to improve our understanding of estuarine dynamics and present conditions that could be used to evaluate existing management approaches, specifically in Morro Bay Estuary.

Materials and Methods

Study Area

Morro Bay Estuary is a natural embayment on the central coast of California, approximately 160 km south of Monterey Bay and 100 km north of Point Conception (Fig. 1). The Morro Bay Watershed (194 km²) is comprised of the Chorro Creek basin (116 km²) and the Los Osos Creek basin (78 km²) (Fig. 1). Roughly 50 % of the watershed is utilized for agriculture rangeland, 30 % is conserved land, 10 % is cropland, and the remaining 10 % is urban (MBNEP 2000). The semi-arid, Mediterranean climate of the central California coast is characterized by drought and low-flow conditions during the dry season and intense, intermittent precipitation and high-flow conditions in the wet season with an average rainfall of 45

cm (Page et al. 1995; Largier et al. 1997). The two stream inputs are Chorro and Los Osos Creeks, which merge on the east side of the Estuary and then converge with the main channel. In addition to surface flows, Morro Bay Estuary receives freshwater inputs from groundwater sources and runoff from the coastal towns: City of Morro Bay and Los Osos-Baywood Park (US Army Corps of Engineers Los Angeles District 2003).

Morro Bay Estuary is approximately 6.5 km long in the north-south direction and 2.8 km wide in the east-west direction at its maximum width, with a single entrance to coastal ocean of Estero Bay (Ji et al. 2001). The main transport mechanism for water is tidal exchange with a tidal range of 2 m (US Army Corps of Engineers Los Angeles District 2003). At high tide inundation, the area of the Estuary is 9.3 km². The bay is predominantly flat and shallow with a mean water depth of 0.5 – 1 m and with a main shipping channel of a 4 – 9 m depth. At low tide, approximately 60 % of the bay area emerges as tidal mudflats and exposed *Z. marina* beds (Ji et al. 2001).

Water Quality Network

The water quality network consisted of three strategically located, fixed water quality stations and one mobile survey station (Fig. 1). Each station supported physical, chemical, and biological sensors selected to best tolerate marine conditions (Jannasch et al. 2008). The first station, BS1 (35° 20' 1.6794" N, 120° 50' 50.28" W), was installed on 21 December 2006 in the southern end of the Estuary at a depth of 0.72 m below mean lower-low water (MLLW). BM1 (35° 22' 15.2394" N, 120° 51' 32.04" W) near the mouth of the Estuary was installed on 4 April 2007 and was mounted to a pier at a depth of 0.83 m below MLLW to capture the flux between Morro Bay Estuary and Estero Bay. CM1 (35° 20' 17.1594" N, 120° 49' 56.64" W) was installed on 26 November 2007 downstream of the confluence of Chorro and Los Osos Creeks. CM1 water quality sensors were originally deployed at a fixed depth, 1 m above the bottom of the channel; however, due to accessibility CM1 was reconfigured as a floating sensor array on 29 September 2009.

Each fixed station was composed of an array of water quality and data transfer sensors. Further details about these stations and a full list of deployed sensors are readily available at www.slosea.org. Relevant to this study, each of the three fixed station and the one survey station deployed a Satlantic *In Situ* Ultraviolet Spectrophotometry (ISUS-X) nitrate sensor (Johnson and Coletti 2002), a Sea-Bird CTD (SIP-

37) sensor, and a WET Labs Combination Chlorophyll Fluorometer and Turbidity Sensor (ECO-FLNTUS). BM1 had a Nortek Continental 2D Current Profiler (ADCP) fixed 3.76 m below MLLW. The ADCP had an acoustic frequency of 470 kHz, a 3.6° tilt upwards, and a profiling range of 100 m with 25 bins spaced 4 m apart. BS1 had a Novalynx rain gauge deployed on 2 October 2009. The rain gauge was located above the influence of shadows and wind modifying structures at 5.58 m above MLLW.

At each fixed station, the instruments sampled every 15 minutes for a two minute sampling interval. Each parameter was averaged over the two minute period, and then the mean was assigned a timestamp to the nearest 15 minute period. Each value was subjected to threshold processing based on sensor ratings and physical limits to ensure quality assurance and quality control (QA/QC). Missing data were a result of transmission error, instrument malfunction, instrument calibration and repair, or QA/QC process. To minimize the effect of bio- and geo-fouling over the entire duration of this study, each station was cleaned once a month and more frequently during high growth seasons. Additionally, all sensors were factory calibrated and updated each year.

To enhance understanding of the spatial dynamics and connectivity between the stations, a mobile survey station compiled with the identical sensors was deployed on missions throughout Morro Bay Estuary. The sensors were deployed in a cage at a fixed depth of 0.2 m below the surface. Surveys occurred at slack tide and low wind conditions to minimize the influence of transient features and wind-driven mixing effects. The boat was driven at a constant 1.5 knots to minimize the production of bubbles and the mixing of the water. This study focused on two surveys, 16 October 2009 and 19 May 2010, hereafter respectively named Roving 1 and Roving 2, to characterize freshwater and circulation spatial dynamics (Fig. 2). Roving 1 occurred during a high tide of 1.74 m above MLLW and three days after the first rain event of the season with 4 cm of rainfall. Roving 2 occurred during a low tide of -0.09 m below MLLW and was categorized as post-wet season since the last rain event happened 27 days prior to the survey.

For this study, the time series was divided into water years due to the defined wet and dry seasons driven by the Mediterranean climate. A water year was defined as 1 October of previous year to 30 September of the following year. The entire study period ranged from 4 April 2007 to 1 June 2010, and thus encompassed the full water years of 2008 and 2009 and partial coverage for 2007 and 2010.

Nitrate Sources

This study examined four sources of nitrates into Morro Bay Estuary: stream, submarine groundwater discharge, atmospheric deposition, and nitrification. The freshwater nitrate sources, specifically stream and groundwater discharge, were quantified using the water quality stations while nitrification and atmospheric deposition loads were estimated based on previously published rates. Quantification of freshwater nitrate sources required calculation of freshwater nitrate concentrations, freshwater volumes, and freshwater nitrate loads. The location of CM1 was selected to capture stream inputs while BS1 was located to best capture groundwater inputs.

The high sampling frequency of tide, salinity, and nitrate concentrations resulted in capturing unique signals attributed to stream and submarine groundwater discharge sources. Decreases in salinity were assumed to be attributed to input from freshwater sources. Decreases in salinity (a conservative property) were assumed to be a resultant of freshwater input, and thus elevated nitrate concentrations were attributed to the freshwater source.

The freshwater nitrate concentrations were quantified for time periods displaying patterns of increased nitrate concentrations corresponding with decreased salinity and tide. The freshwater nitrate concentration analysis conducted was identical for the selected time series at BS1 and at CM1. Of the time periods selected, the daily higher-high tide was found and then the corresponding maximum salinity (S_H) and minimum nitrate concentrations (N_H) within a window 1.5 hours before and after the higher-high tide. Next, the daily lower-low tide was found and the corresponding minimum salinity (S_L) and the maximum nitrate concentrations (N_L) within a window of 1.5 hours before and after the lower-low tide. In both cases, the window was applied as a control for the effects of tidal hysteresis. After finding the salinity and nitrate values associated with high and low tide, Eq. 1 was used to find the freshwater nitrate ratio (R).

$$R = \left(\frac{S_H}{S_L} \right) / \left(\frac{N_H}{N_L} \right) \quad (\text{Eq. 1})$$

Once the freshwater nitrate ratio was determined, Eq. 2 was used to estimate the freshwater nitrate concentration (F_N).

$$F_N = R * N \quad (\text{Eq. 2})$$

Each station lacked a complete time series of daily measurements. To construct a complete time series of daily measurements, daily freshwater nitrate concentrations were linearly interpolated. Linear interpolation of freshwater nitrate concentrations accounted for rain events, elevated stream volume, and baseline flows.

Stream volumes and stream nitrate loads were estimated from stream discharge measurements from stage height data from two stream gauges in the Chorro Creek basin located on Chorro Creek (35° 21' 11.36" N, 120° 47' 20.36" W) and San Luisito Creek (35° 21' 21" N, 120° 47' 06" W). The Chorro Creek gauging station measured flow from a 56.38 km² drainage area, and the San Luisito Creek gauging station measured flow from a 21.45 km² drainage area. The stream gauges were maintained by the SLO County Water Resources, Division of Public Works and were sampled every 15 minutes (MBNEP 2010b). Stream discharges recorded at the Chorro Creek and San Luisito Creek stream gauges were only a subset of the entire stream flow into Morro Bay Estuary. Additional inputs came from San Bernardo Creek sub-watershed and the Los Osos Creek Basin. Since San Bernardo Creek sub-watershed is similar in size and in land use patterns to San Luisito Creek sub-watershed, San Luisito Creek discharge was doubled. Based on historical data, Los Osos Creek basin contributed 23 % of the annual stream flow of Morro Bay Watershed (SLO County Public Works Department 2005). To approximate a total of the Morro Bay Watershed stream volume, the entire Chorro Creek basin stream discharge was scaled up to account for Los Osos Creek basin contribution. Quantified stream nitrate concentrations were multiplied with stream volume to produce stream nitrate loads.

In contrast to stream volumes, groundwater volumes were estimated by indirectly calculating the total freshwater contribution in Morro Bay Estuary. This consisted of a three-step process: estimate the mean daily volume, calculate the daily total freshwater volume, and find the groundwater volume of the total freshwater volume.

First, to estimate the mean daily volume, a tidal prism was developed from a three-dimensional circulation and transport numerical model based on Delft3D-FLOW, and the prism was used to analyze the vertically integrated tidal circulation in Morro Bay Estuary (Hibler et al. 2008; Deltares 2009). The model was developed using a geographical coordinated system. Key assumptions associated with the model were that horizontal circulation dominated over vertical motions, tides dominated the circulation in the Bay, fluid stresses were appropriately treated as Reynolds stresses, and a k- ξ turbulence model was appropriate for

estimating the sub-grid scale dissipation of momentum. The tidal prism was developed using predicted XTides for October 2009 at a cross section near the mouth and BM1. The Bay volume was computed based on an initial volume estimate, $5 \times 10^7 \text{ m}^3$, derived from a circulation model developed by Tetra Tech (Ji et al. 2001). Water flux simulations produced a tidal prism to estimate the water volume of the entire estuary (MB_v) using BM1 tidal height (T_{BM1}) with:

$$MB_v = 8,656,077,468.67 * T_{BM1} - 1,116,325,624.26 \quad (\text{Eq. 3})$$

The mean daily volume of Morro Bay Estuary was calculated from the tidal prism based on BM1 tidal measurements.

Second, the calculation of the daily freshwater contribution relied on several assumptions. Salinity measurements at BM1 were assumed to be representative of the salinity for the entire volume of the estuary. Just as with the freshwater nitrate concentration calculations, it was assumed decreases in salinity were attributed to input from freshwater sources and proportional to the freshwater contribution to the total volume. The first step in finding the daily freshwater contribution was to quantify the percent difference in salinity between high and low tide at BM1. For the periods selected, the daily higher-high tide and the corresponding maximum salinity were within a window of one hour before and after the high tide. Next, the daily lower-low tide and the corresponding minimum salinity were found within a window of one hour before and after the low tide. The two hour window was applied to control for the influence of tidal hysteresis. Periods when salinity at low tide exceeded salinity at high tide were characterized as indicators of evaporation and hyper-saline conditions (Largier et al. 1997). The second step was to calculate the total freshwater contribution. The daily total freshwater contribution was calculated by multiplying the daily percent difference and daily mean volume.

Third, the contribution of freshwater sources to the total freshwater water contribution was calculated by accounting for the measured freshwater inputs: stream and rainfall volumes. Rainfall volumes were based on measured precipitation from the BS1 rain gauge and were supplemented by a rain gauge located at the Los Osos Landfill (35° 19' 21.03" N, 120° 48' 07.64" W) prior to BS1 rain gauge installation. The difference between total freshwater and measured freshwater inputs was assigned as the remaining freshwater source contribution and attributed as the daily groundwater volume. Since estimated groundwater discharge was relatively constant, the annual averages of daily groundwater discharge were

calculated and further applied to the derived groundwater nitrate concentrations to provide groundwater nitrate load estimates.

In addition to stream and groundwater nitrate sources, nitrate loading from atmospheric deposition through rainfall and nitrification was approximated based on published rates. Non-stream runoff contributed to incoming nitrate loading, and these sources were not included in this analysis and are assumed too small. Rainfall nitrate concentration of 0.62 mg L^{-1} was applied to rainfall measurements applied across the area of Morro Bay Estuary (Padgett et al. 1999; Fenn et al. 2003). Nitrification rates of $99.21 \text{ mg m}^{-2} \text{ day}^{-1}$ were attributed to yearly intertidal mudflat area estimates (Jenkins and Kemp 1984; Caffrey et al. 2003). Multi-spectral imagery of habitat coverage area was collected by the MBNEP during a November low tide in 2007, 2009, and 2010 with a filter combination of 451-550-640-790 nm (MBNEP 2011). Habitat classification included *Z. marina* beds, plant and algal groups, intertidal mudflats, and water channels of Morro Bay Estuary with aerial coverage of these habitats as estimated by Ocean Imaging, Inc.

Nitrate Sinks

Nitrate sinks considered in this study included the processes of denitrification and sediment burial and the processes of *Z. marina*, macroalgae, and phytoplankton assimilation of nitrate. These processes of nitrate removal were based on published rates. Analogous to nitrification, denitrification and sediment burial, *Z. marina*, and benthic macroalgae uptake rates were applied to MBNEP habitat coverage areas from 2007, 2009, and 2010 (Table 1). Denitrification and sediment burial rates of $142.61 \text{ mg m}^{-2} \text{ day}^{-1}$ were applied to intertidal mudflat areas (Seitzinger 1987; Mortazavi et al. 2000; Caffrey et al. 2003; Chapin et al. 2004). *Z. marina* uptake rates had a seasonal component that fluctuated from $35 \text{ mg m}^{-2} \text{ day}^{-1}$ in the low growing season to $257 \text{ mg m}^{-2} \text{ day}^{-1}$ in the peak growing season (Pedersen and Borum 1992, 1993). *Z. marina* uptake rates were directly applied to the *Z. marina* beds aerial coverage, which was assumed to be a representative estimate for *Z. marina* population biomass. Macroalgae blooms were evident during the MBNEP habitat studies and were found to be composed of *Gracilaria verrucosa*, *Chaetomorpha linum*, *Enteromorpha* sp., and *Ulva* sp. Seasonal assimilation rates, ranging from 1.00 to $6.17 \text{ mg m}^{-2} \text{ day}^{-1}$ (McGlathery et al. 2007), were applied to the previously measured yearly macroalgal coverage.

Nitrate sink through phytoplankton uptake was estimated by first calculating the daily net exchange of chlorophyll *a* at the mouth of Morro Bay. A carbon-to-chlorophyll *a* ratio of 15 was selected

to be representative for the low light estuary system (Banse 1977; Gallegos and Vant 1996). Total carbon exchange was converted to nitrogen by using the Redfield ratio (Mortazavi et al. 2000). Periods lacking chlorophyll *a* measurements were linearly interpolated.

Oceanic Exchange

In order to complete the nitrate budget of Morro Bay Estuary, it was necessary to assess the flux of nitrate loads at the ocean interface. The location of BM1 was selected to capture the flux of water and nitrate at the mouth of the Estuary. Quantification of the bay flux required measuring the average cross-sectional area of the channel bathymetry and the current velocity through the channel cross-section during each tidal cycle. Harmonic analysis of tidal elevation and the description of dominant tidal constituents at BM1 were performed using T-Tide (Pawlowicz et al. 2002).

On 22 January 2008, a channel bathymetry study in collaboration with Tenera Environmental occurred during a 1.92 m high tide. The study focused on the development of a bathymetry profile along a channel cross-section, corresponding to the path BM1 ADCP. The channel bathymetry was measured with a BioSonics, Inc. DTX echo-sounder operating in single beam mode mounted to a 14' Boston Whaler. A 328 m channel cross-section transect was the sample that mostly closely aligns with the ADCP beam path of 100 m. The ADCP beam path corresponded to the maximum channel depth. The channel bathymetry was not re-measured during the course of the study, and it was assumed that the measured bathymetry represented a mean cross-sectional area. In addition to measuring channel bathymetry, vertical profiles of currents across the channel were measured during 25 passes with a Sontek ADP. The vertical profile surveys occurred during an outgoing and incoming spring tide at the maximum transition period. The profiles showed little evidence of vertical structure and therefore were assumed to be well-mixed.

For volumetric analysis, the location of ADCP bins was extended to the channel edges on both sides of the true ADCP bins for a total of 86 bins, each associated with channel bathymetry. The two end channel bins were assigned the current velocity of zero. Current velocity at each of the generated bins was linearly interpolated based on the measured current velocity and channel bathymetry. The bathymetry-interpolation was evaluated by comparing the percentage of total flow underlying each bathymetry bin across during the vertical profiles to the entire BM1 ADCP dataset. The vertical profiles and the BM1 ADCP accounted for 57 % and 54 % of the total flow within the 25 bins of the BM1 ADCP coverage area.

The comparison of the bathymetry-interpolation to the vertical profile produced a mean 0.28 % percent difference between the flows represented in the 61 bins outside the ADCP coverage. The bathymetry-interpolation method was thus assumed to realistically model current velocity across the channel.

Current direction for each of the bins was defined by utilizing the BM1 pressure gradient, ADCP current speed, and ADCP current direction data. The pressure measurements identified the direction of the tidal forcing as either ebb or flood. Ebb and flood tides were assigned using the pressure gradients by finding each nadir and then the zenith between each nadir pair. ADCP current speed was used to capture current momentum. As a result, the nadirs of current speeds were found, and they corresponded to tidal slacks. Current speeds between nadirs were defined as either ebb or flood by alternating and using the pressure designation to begin the pattern. A consistent trend was present in the ADCP current direction data of a gradual transition from ebb to flood but a quick transition from flood to ebb. To account for this phenomenon, if the current speed nadirs were located on a direction value greater than the mean of the ADCP current direction, the first three consecutive directions within a four hour window less than the mean ADCP current direction were found. Under these situations, the ebb period, as defined by the ADCP current speed, was extended up to the first of the three consecutive directions. The direction of the current speed in each bin was designated as ebbing or flooding and was respectively expressed as positive and negative.

With current velocity modeled across the main channel cross-section, the area of each bin was calculated with the water level based on the tide at BM1, thus accounting for the change in total cross-sectional area of the channel due to tide. Each bin area was multiplied by each modeled bin current velocity. The volume flux of each bin was added together to produce a volume flux over the entire channel. To estimate nitrate load import and export at the mouth, measured nitrate concentrations were multiplied by the overall volumetric water transported across the channel to quantify the nitrate load.

Results

Nitrate Sources

The two roving missions provided a characteristic spatial context for freshwater input and circulation in Morro Bay Estuary during wet and dry periods (Fig. 2). The first mission during the wet season was characterized by horizontal stratification from north to south of salinity, temperature, and

nitrate. Salinity ranged from 30.1 – 33.6 psu with an average of 32.7 psu. Nitrate concentrations ranged from 0 – 1.35 mg L⁻¹ with an average of 0.40 mg L⁻¹. Runoff from a 4 cm rain event three days prior to the survey in concert with tidal forcing resulted in the freshwater being trapped in the back bay during high tide (Fig. 2). In the absence of rainfall and runoff, the second mission revealed direct input from Chorro Creek. A low salinity plume coincided with elevated nitrate concentrations where the Chorro Creek channel merges with main channel of Morro Bay Estuary. The average nitrate concentration outside of the plume was 0.37 mg L⁻¹ while the maximum nitrate concentration in the plume was 2.05 mg L⁻¹ (Fig. 2). These spatial surveys provided evidence that freshwater inputs contributed nitrate to the estuary throughout the year during both the wet and dry periods and the distribution of freshwater at any given time in the Estuary was dependent on these inputs and the tidal dynamic.

With this information, the first nitrate source considered in this study was from stream runoff sources in the Morro Bay Watershed. Stream nitrate concentrations were estimated at CM1 and captured the combined signals of Los Osos and Chorro Creeks. Stream nitrate concentrations were directly dependent on rainfall and stream volume. Wet, high flow seasons had significantly elevated stream nitrate concentrations in comparison to dry, low flow seasons ($p_{\alpha=0.05} < 0.00$, $n = 974$). In the dry seasons, the stream nitrate concentrations and nitrate loads decreased to a low flow baseline, mirroring the pattern of the stream volume (Fig. 3).

Over the study period, the total rainfall was 111.06 cm, the total stream volume was 9.39×10^{10} L, the mean daily stream nitrate concentration was 2.93 mg L⁻¹, and the total nitrate load was 372.03 t (Fig. 3). The 2010 water year, even though only partially sampled, had the greatest recorded rainfall and annual stream volume of the three water years. High rainfall, 50.16 cm, and stream volume, 3.48×10^{10} L, were combined with the highest mean nitrate concentration of 4.59 mg L⁻¹ and an annual load of 190.43 t. The greatest stream nitrate concentration measured was 14.57 mg L⁻¹ on 28 February 2010. This peak stream nitrate concentration occurred after two consecutive rain events totaling 1.95 cm. The 2009 water year was characterized by low total rainfall, 20.90 cm, and low annual volume, 2.69×10^{10} L. The 2009 water year also had the smallest daily mean nitrate concentration, 1.93 mg L⁻¹, and the lowest annual nitrate load, 54.68 t. The 2008 water year was characterized by an annual rainfall of 43.00 cm, a total volume of 3.21×10^{10} L, a mean daily stream nitrate concentration of 2.79 mg L⁻¹, and an annual nitrate load of 100.01 t.

In the spring of 2008, there was a unique pattern between stream volume and stream nitrate concentration (Fig. 3). The final rain event for the water year was 4.20 cm from 19 – 24 February 2008 and resulted in a peak stream volume of 1.31×10^9 L. After the final rain event, the stream volume decreased to a dry season baseline flow. There were two periods of increased stream concentrations, 2 – 8 April and 20 April – 13 May, 2008, that did not follow the trend of elevated nitrate concentrations during winter rain events and elevated stream volumes. Without elevated stream volumes, the potential sources of the elevated freshwater nitrate concentrations could only be hypothesized since the sources were unrelated to rainfall runoff. These events however, contributed 10.4 % of the total stream nitrogen load for the 2008 water year and thus were significant.

The second nitrate source was submarine groundwater discharge, which was quantified by estimating the total freshwater contribution in the Estuary and the annual groundwater volume. Results from the tidal prism showed fluctuations in the daily mean of the Morro Bay Estuary volume from 3.82×10^9 – 8.91×10^9 L with a mean daily volume of 6.52×10^9 L over the time series. Over the study, the annual minimum volume consistently occurred between April and May whereas the maximum volumes occurred during late summer to winter months.

At BM1, 301 days during the study had conditions where salinity at high tide was less than salinity at low tide, which suggests increased evaporation conditions and decreased freshwater inflows during dry summer months (Largier et al. 1997). The observations occurred between late April and early December for each year of the study, consistent with this interpretation.

The majority of detectible freshwater influx occurred during the wet season, late December to early April (Fig. 4). The daily total freshwater contribution ranged from 0.001 – 54.49 % of the mean Morro Bay volume, with an overall mean of 2.76 %. The largest daily total freshwater contribution, 54.49 %, took place on 22 December 2008 and coincided with a storm event producing 6 cm of rain and a daily maximum stream volume of 8.51×10^7 L. The total freshwater contribution sources were computed since the stream volumes were directly measured. Stream volumes accounted for 1.56 – 99.19 % of the daily freshwater contribution with a daily mean of 47.21 % (Fig. 4). The groundwater contribution ranged from 0.46 – 98.44% with an annual average of 52.79 %. The average daily groundwater volume was 2.81×10^8

L. Mean daily groundwater volume, partitioned by water year, ranged from 2.46×10^8 L in 2009 to 5.16×10^8 L in 2007.

While salinity at BM1 indicated evaporative conditions during the dry season, the same conditions were rarely present at BS1. There was a minimal freshwater signal present during low tides. This condition continued to provide evidence that groundwater contribution of freshwater and nitrate was continual throughout the year in the back bay. By contrasting stream nitrate concentrations, groundwater nitrate concentrations had a narrower range of concentrations, $0.62 - 10.23 \text{ mg L}^{-1}$. The average groundwater nitrate concentration was $1.45 \pm 0.78 \text{ mg L}^{-1}$, with no statistical differences between years (Fig. 9). The relative consistency of daily nitrate concentrations between the years and between the rain and dry seasons suggested stream flow and surface runoff had little influence on the groundwater nitrate concentrations calculated at BS1. Daily groundwater loads ranged from $0.19 - 3.15 \text{ t}$, with a mean daily nitrate load of 0.48 t (Fig 5; Table 3). The annual nitrate loads varied from $105.06 - 195.84 \text{ t}$, with a mean annual load of 150.05 t . While stream nitrate concentrations were on average greater than groundwater nitrate concentration, nitrate loading from groundwater was 2.6 times higher than nitrate loading from the streams.

In addition to streams and groundwater, rainfall accounted for a small proportion of the freshwater contribution and nitrate loading into Morro Bay Estuary. When rainfall events occurred, rainfall accounted for a mean of 8.04% with a range of $0.09 - 38.42 \%$ of the entire freshwater budget. Annual nitrate loading rates from rainfall ranged from $0.68 - 2.89 \text{ t}$ (Fig. 9; Table 3). Nitrification was estimated to supply a similar amount of nitrate to Morro Bay Estuary as stream and groundwater sources. Annual nitrification loads ranged from $78.07 - 161.38 \text{ t}$ with an average of 114.75 t (Fig. 9; Table 3).

Nitrate Sinks

Nitrate sinks in the Estuary were considered as the combined processes of denitrification, sediment burial, and uptake of nitrate by *Z. marina*, macroalgae, and phytoplankton. Denitrification and sediment burial removed $164.95 \pm 56.10 \text{ t yr}^{-1}$ (Fig. 9; Table 3). Denitrification was estimated to remove more nitrate than was produced by nitrification, and thus there was a net sink of nitrate in the estuary. Taking into consideration the decrease in *Z. marina* cover during the study, the nitrate uptake varied from $5.84 - 23.64 \text{ t}$

yr⁻¹. Macroalgae nitrate sink was 63 % greater than *Z. marina* sink, resulting from larger coverage and higher assimilation rates. Macroalgae blooms removed $28.16 \pm 16.29 \text{ t yr}^{-1}$ (Fig. 9; Table 3).

As expected, high chlorophyll *a* concentrations were from the early spring and summer months at all three stations, with the seasonal maximum between May to July ($4.4 - 6.8 \mu\text{g L}^{-1}$). The mean chlorophyll *a* concentration over the remaining months was $0.78 \pm 1.02 \mu\text{g L}^{-1}$. There was a constant annual net export of phytoplankton from Morro Bay Estuary, with a net uptake of nitrate at 3.61 t yr^{-1} (Fig. 9; Table 3).

Oceanic Exchange

Volumetric water analysis across the channel near the mouth of Morro Bay Estuary revealed the importance of two temporal scales of variation between tidal currents: fortnightly variability in current magnitude and tidal asymmetry between ebb and flood. Harmonic analysis of the tidal elevation revealed the three dominant tidal constituents as M_2 , the principal lunar semidiurnal, K_1 , the principal lunisolar, and O_1 , the lunar diurnal constituents. The M_2 constituent drives the strong spring/neap fortnightly cycle, and the combination of K_1 and O_1 results in strong diurnal inequality between high and low tides (Table 2). The form number was 0.94, which indicates a mixed, mainly semidiurnal tidal regime (Nidzieko 2009). Larger tides and current velocities were concurrent with the winter and summer solstices, producing larger inequalities between spring and neap. Due to the phase lag of diurnal constituents relative to the semidiurnal constituent phase by an average of 35.98° , higher-highs were consistently followed by lower-low water (Nidzieko 2009). In addition, this phase lag produced an ebb-dominant asymmetry where the duration of ebbing water is shorter than that of flooding water. To counteract the phase lag, a difference in current speed was present where the mean ebb current speed was 0.307 m sec^{-1} and the mean flood current speed was 0.299 m sec^{-1} .

The current ebb dominance resulted in an annual net export of water out of Morro Bay Estuary. The mean flooding volume was $3.04 \times 10^8 \text{ L}$ while the mean ebb volume was $2.94 \times 10^8 \text{ L}$. The daily sum volume across the entire channel exhibited a pattern reflecting the fortnight variability. During the spring periods, there was a decreased volumetric export with a larger flux of water into the Estuary. There was increased volumetric export during the neap periods with an overall smaller import of water. The fortnight variability pattern, caused by the strong M_2 tidal constituent, was the dominant trend in the daily sum

volume. This pattern was slightly interrupted during periods of high rainfall and peak stream flow, where volumetric water export was dominant. The mean daily volumetric flux through the main channel had variability between the study periods, with a mean daily exporting flux of 1.40×10^9 L.

The volumetric flux across the channel was the primary driver of the trends in the directionality of nitrate load flux, and over the study, there was a net export of nitrate from Morro Bay Estuary. The mean BM1 nitrate concentration was 0.78 ± 0.38 mg L⁻¹, with a maximum of 3.68 mg L⁻¹. A net average 1.36 ± 2.72 t day⁻¹ of nitrate was exported to Estero Bay (Fig. 7). Similar to that of water volume, the largest mean daily nitrate flux occurred in 2009 with an export of 1.70 t day⁻¹. The smallest mean daily flux occurred in 2008 with an export of 0.87 t day⁻¹.

In addition to the fortnight signal, multiple sources were responsible for episodic and non-tidal increasing in nitrate concentration; two drivers of nitrate flux were coastal upwelling and watershed runoff. Evidence of coastal upwelling occurred from 3 – 9 June 2008. During this time period, there was a decrease in measured temperature from 14 to 11.24 °C and a simultaneous increase of two pulses from nitrate from 0.35 to 1.01 mg L⁻¹ (Fig. 8). These changes coincided with a net import of 3.80 t (0.63 t d⁻¹) of nitrate. This upwelling event resulted in the utilization of nitrate for primary production with an increase in chlorophyll *a* concentrations in the estuary from 2.36 to 6.95 µg L⁻¹ (Fig. 8). Similar coastal upwelling events were captured annually between April to July, as consistent with California Current upwelling patterns (Huyer 1983).

In addition to coastal upwelling, the other non-tidal nitrate source documented at BM1 came as a result of watershed runoff events occurring in the wet season. Watershed runoff was captured from 13 – 27 January 2010, when a 25.99 cm rain event transpired. The water temperature was relatively constant at 13.2 °C. The nitrate concentrations increased from 0.69 to 1.57 mg L⁻¹, with a concurrent decrease in salinity from 33.31 to 25.16 psu. This runoff event resulted in a net export of 45.86 t and a maximum daily export of 13.2 t (Fig. 7).

The fortnight variability combined with seasonally episodic events led to differences between the magnitude of net nitrate export during the dry and wet seasons. From the summer of 2007 through the winter of 2009, the pattern featured increased export during the winter and decreased export during the summers. The summers of 2007 and 2008 ranged from 0.82 – 0.65 t day⁻¹, respectively, while the winters

of 2008 and 2009 ranged from 1.41 – 1.45 t day⁻¹, respectively. Beginning in the summer of 2009, there was a reversal in the established seasonal flux pattern with 1.59 t day⁻¹ exported in the summer of 2009, as consistent with winter fluxes. A slight decrease in export occurred in the winter of 2010 and was followed with the largest net export in the summer of 2010, 1.40 and 2.28 t day⁻¹, respectively.

Discussion

Estuarine nutrient dynamics are driven by multiple and often non-linear processes that occur at overlapping frequencies and time scales. Tidal forcing, upwelling events, mixing, biological productivity, seasonality, and freshwater watershed inputs are intersecting processes that influence the magnitude of nutrients imported to an estuary, exported to the coastal ocean, and processed within an estuary (Chapin et al. 2004; Caffrey et al. 2007; Jannasch et al. 2008). Spatial and temporal variability of nutrient dynamics presents challenges to scientists and resource managers when they attempt to effectively sample and quantify trends in order to address consequences from increased anthropogenic pressures and eutrophication.

Our results quantified the changing nitrate fluxes over multiple years and illustrated the importance of high temporal sampling in capturing the frequent episodic events that dominated the sources and sinks of nitrate. Morro Bay Estuary was determined to be a nitrate source through two independent methods: the quantification and estimation of nitrate sources and sinks within the Bay and the calculation of the net oceanic exchange at the mouth. On average, Morro Bay Estuary exported 327.15 t yr⁻¹ of nitrate (Fig. 9; Table 3). Fifty-four percent of the measured export loads were attributed and quantified to nitrate sources and sinks in the Estuary (Table 3). Given the variability in the system over multiple years, the ability of this approach to quantify the mean internal nitrate sources and sinks on the same order of magnitude as the measured oceanic exchange is significant. To our knowledge, the comprehensive quantification of nitrate sources and sinks has not been documented by other estuarine nutrient mass balance studies, which have relied on a considerable number of assumptions due to limitations in sampling frequency, sampling duration, or both (Nixon et al. 1996; Mortazavi et al. 2000; Chapin et al. 2004).

Based on the nitrate mass balance, the magnitude and seasonal variability of nitrate transport was driven by the coastal ocean. On a daily basis, the coastal ocean contributed to 90 % of the nitrate loads entering Morro Bay Estuary. The coastal ocean contribution was regulated by the mixed semi-diurnal

incoming tides (Fig. 9; Table 3). The influx of ocean nitrate was readily exported back to the ocean with nitrate loads from streams and groundwater, except during neap tide periods (Fig. 7). A large spatial gradient in water residence time ranging from hours near the mouth to a month in the back bay is present in Morro Bay Estuary (Ji et al. 2001). The high flushing rates resulted in nitrate source loads being readily transported to Estero Bay, with the Morro Bay Estuary nitrate concentrations remaining near oceanographic concentrations.

The measured net exported nitrate consisted of the remaining 10 % not accounted for by oceanic sources. Of the measured net exported nitrate, the stream contribution was on average 37 % of the total nitrate exported with 124.01 t yr^{-1} (Fig. 9; Table 3), with the magnitude directly dependent on seasonal rainfall and stream volume. The calculated stream nitrate concentrations were the highest of all source concentrations. Yet the highest stream concentrations did not coincide with the largest stream volumes and thus diminished the loading impact. Our estimates of stream nitrate concentration were similar to samples measured by the MBNEP (MBNEP 2010a), with the mean difference in nitrate concentration 0.03 mg L^{-1} between CM1 and the nearest MBNEP sampling site. The minimal rainfall inputs into Morro Bay Estuary (0.55 % of the total budget) contrasts against rainfall inputs of estuaries on the East Coast of the United States where upwards of 30 % of the total nitrate budget can be derived from atmospheric deposition (Fisher and Oppenheimer 1991).

In contrast to stream inputs, the groundwater contribution was a larger and more consistent source of the nitrate to Morro Bay, with 46 % of the net nitrate exported (Fig. 9; Table 3). Annually estimated groundwater nitrate loading was 55 % greater than stream nitrate loading. Over an entire year, the estimated groundwater volume was approximately 6 % greater than the measured volume of freshwater discharge from the streams. The submarine discharge of groundwater contributed a constant nitrate load to Morro Bay Estuary. The groundwater source was slightly enhanced during neap tides, as indicative from the increased net nitrate export at the channel mouth during neap tides. de Sieyes et al. (2008) found that similar heightened submarine groundwater discharge at Stinson Beach, CA during neap tide was due to the relative location of the hydraulic head to the aquatic overhead. During neap tide, the aquifer overhead is lower than the hydraulic head while the hydraulic head is greater than the aquifer overhead in the spring tides.

Groundwater wells in Los Osos have measured nitrate concentrations ranging from 0.4 – 33.6 mg L⁻¹ (Los Osos CSD 2010) while this study found mean groundwater concentrations of 1.47 mg L⁻¹ (Fig. 9). The potential underestimation could be attributed to the diffuse nature of the source and the assumptions made at the point location of BS1. The effect of evaporation, found to occur on a significant number of days during the study, was also not taken into account. If the effect of evaporation was accounted for, then the calculated contribution of groundwater would increase. Given these conservative estimates, results here suggest groundwater discharge is a significant source of nitrates in Morro Bay Estuary. The difference between estimated loading from internal nitrate sources and sinks and the measured nitrate net export (Fig. 9) is likely lower than the groundwater source.

Estimation of nitrate sinks revealed denitrification as the dominant internal process. Nitrification was found to have a comparable nitrate load as stream inputs. Relative to denitrification and nitrification processes, phytoplankton assimilation of nitrate was estimated to have a minimal impact on removing nitrate from the system 3.61 t yr⁻¹ (3.7 %), consistent with conceptual models of shallow systems with moderate eutrophication and/or short water residence times (Ji et al. 2001; McGlathery et al. 2007).

Zostera marina and benthic macroalgae, as estimated by the coverage area and nitrate utilization rates, showed a moderate removal of 28.16 and 15.99 t yr⁻¹, respectively. McGlathery et al. (2007) found a dominant proportion of nitrate utilized in seagrasses is retained in tissue and lost from the system via mass transport while nitrate assimilated macroalgae is returned to the system in days to weeks. Seagrasses settle slowly due to air-filled aerenchyma tissue and thus float to the surface and are exported with the tides. Benthic macroalgae quickly settle to the sediment floor and undergo decomposition. Flindt et al. (2004) concluded that the exclusion of nutrients in plant tissue in mass transport calculations can lead to an overestimate of retention rates by 18 – 60 %, and thus it is critical to estimate *Z. marina* sink of nitrates. Within the Morro Bay Estuary, macroalgae retention of nitrate could be overestimated by 29 % and consequently should lead to improved comparison between estimated loading from internal nitrate sources and sinks and the measured nitrate net export (Fig. 9).

As a result of high flushing rates and the rapid dilution effects on nitrate concentrations (Fig. 2), marine organisms in Morro Bay Estuary are not, on average, exposed to toxic levels of nitrate (88.6 mg L⁻¹; Camargo et al. 2005). Given the higher retention in the back bay, chronic exposure to elevated nitrate may

impose physiological impairment; however further studies are warranted. Morro Bay Estuary nitrate concentrations contrasted with levels of nitrate present in Upper Newport Bay, CA (Boyle et al. 2004), Venice Lagoon (Sfrisco et al. 1989), and the Peel-Harvey estuarine system in Australia (Lukatelich and McComb 1986) have been shown to have dissolved inorganic nitrogen loadings ranging from 1.5 – 161.6 g N m⁻² yr⁻¹ (Table 4). In comparison, Morro Bay Estuary had low to moderate loading conditions of dissolved inorganic nitrogen, as measured by nitrate, with a mean of 28.04 ± 9.36 g N m⁻² yr⁻¹ (Table 4). Based on a ranking by NRC (2000), Morro Bay Estuary exhibited low to moderate eutrophic conditions. Conditions of negative expressions during the study were watershed nitrate source inputs with increased macroalgae and loss of submerged aquatic vegetation. The NRC (2000) recommended that coastal areas currently deemed ‘healthy’ should promote decreasing eutrophic conditions over the next 20 years through resource management practices and initiatives.

Management implications

Results of this study have management implications for Morro Bay Estuary and other estuaries of a similar size and type. The combination of the strategically located stations, the high sampling frequency of the instruments, and SLOSEA’s forum for discussion create an effective triad to collect and share data with resource managers, stakeholders, and decision makers tasked with managing the Morro Bay Estuary and Watershed. Within Morro Bay Estuary, future management should be directed towards initiatives that reduce nitrate contributions from groundwater and stream sources, especially since there is a potential for enhanced eutrophic consequences (Rabalais et al. 2009) due to the predicted 1 m rise in sea level over the next 100 years (Philip Williams & Associates, Ltd 2009).

Over the study period, *Z. marina* bed area coverage declined 42 % from a high of 0.99 km² in 2007 to a minimum of 0.71 km² in 2010 with a concurrent increase of *G. verrucosa* by 538 % (MBNEP 2011). Net declines in *Z. marina* populations are persistent throughout the California coast and worldwide (Larkum et al. 2006). This concurrent trend has also been documented in Tomales Bay, CA with a similar red macroalgal species, *Glacilaria* sp. (Huntington and Boyer 2008). Our results revealed that further management of groundwater and stream nitrate sources can reduce nitrate loading into Morro Bay Estuary and aid in the persistence of *Z. marina* populations.

Groundwater nitrate contribution was found to have a larger impact and more consistent loading than previously known in the Morro Bay Estuary. Homes in Los Osos-Baywood Park, a fringing community along the southern portion of the Estuary (Fig. 1), are on septic systems that are known to seep into the aquifer and the groundwater (Hitt 2009). Increased management of septic systems, specifically of those malfunctioning systems and the density of systems, would likely abet in controlling and reducing nitrogen loading to Morro Bay Estuary. A transition from septic systems to a water treatment plant for Los Osos is currently underway and is scheduled to be on line in 2015. Continued operation of the water quality network through this transition will generate a critical data set to monitor and evaluate changes to the groundwater nitrate contribution to Morro Bay Estuary.

In addition to groundwater sources, continued management of point and non-point sources that result in stream nitrate loading is essential. The Water Quality Control Board has proposed setting the Total Maximal Daily Load (TMDL) of nitrate concentration to 2.5 mg L^{-1} . Had this been enacted and realized during the study period, the stream nitrate loading would have decreased by 79 %. Thus, continued management of groundwater and stream nitrate sources is critical to regulate excess loading of nitrate, which contributed 83 % of the net annual flux into the coastal ocean, and to maintain the intrinsic and economic values of Morro Bay Estuary.

In summary, Morro Bay Estuary was found to be a nitrate source with high spatial and temporal variability. Fifty four percent of the nitrate exported to the coastal ocean was attributed to stream and groundwater nitrate sources and internal biological processing. Nitrate loading from streams contributed 37 % to the export of nitrate (124.01 t yr^{-1}) while groundwater nitrate loading supplied a conservative estimate 153.92 t yr^{-1} or 46 % exported nitrate, with a neap tide enhancement of the discharge. The high sampling frequency approach used here was required to capture the event-scale nitrate dynamics in the Estuary over multiple years. These results highlight the importance of quantifying nutrient budgets in coastal areas to assess sources and sinks and to evaluate the impacts of future management actions in these systems.

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Tables

Table 1 Multi-spectral imagery of habitat coverage area (km²) collected during a November low tide in 2007, 2009, and 2010 with a filter combination of 451-550-640-790 nm (MBNEP 2011). Habitat coverage area classified by Ocean Imaging, Inc. Literature denitrification, nitrification, and *Z. marina* and macroalgae assimilation rates applied to respective habitat coverage area

Year	Intertidal mudflats	<i>Z. marina</i> beds	Benthic macroalgae
2007	7.87	1.39	1.58
2008	3.66	1.17	1.18
2009	4.44	0.96	0.78
2010	3.57	0.72	1.76

Table 2 Significant Morro Bay Estuary tidal constituents computed T-Tide (Pawlowicz et al. 2002) from 15-min data at BM1 station (nearest Estuary mouth)

Tidal Constituent	Frequency (sec ⁻¹)	Amplitude (m)	Phase (°)
M2	8.05x10 ⁻²	0.463	173.97
K1	4.18x10 ⁻²	0.349	217.62
O1	3.87x10 ⁻²	0.220	202.28
P1	4.16x10 ⁻²	0.111	213.58
N2	7.90x10 ⁻²	0.106	149.85
S2	8.33x10 ⁻²	0.142	173.97
Q1	3.72x10 ⁻²	0.039	195.12
K2	8.36x10 ⁻²	0.0389	163.03

Table 3 Compilation of annual sources and sinks of nitrate loads in Morro Bay Estuary separated by water year and the mean nitrate load over the study period. Rainfall source loads estimated by applying 0.62 mg L⁻¹ (Padgett et al. 1999; Fenn et al. 2003) to rainfall measurements applied across the area of Morro Bay Estuary. Stream source loads (Fig. 3). Groundwater source loads (Fig. 4; Fig. 5). Nitrification source load estimated with nitrification rates of 1.6 mmol m⁻² day⁻¹ (Jenkins and Kemp 1984; Caffrey et al. 2003) attributed to intertidal mudflat area (Table 1). Denitrification sink loads generated by applying rates of 2.3 mmol m⁻² day⁻¹ (Seitzinger 1987; Mortazavi et al. 2000; Caffrey et al. 2003; Chapin et al. 2004) to

intertidal mudflat area (Table 1). *Z. marina* sink loads had seasonal uptake rates varying from 35 – 257 mg m⁻² day⁻¹ (Pedersen and Borum 1992, 1993) applied to *Z. marina* coverage area (Table 1). Phytoplankton sink load calculated by net exchange of chlorophyll *a* to carbon to nitrogen (Banse 1977; Gallegos and Vant 1996; Mortazavi et al. 2000). Macroalgal sink loads had seasonal assimilation rates ranging from 1.00 – 6.17 mg m⁻² day⁻¹ (McGlathery et al. 2007) applied to macroalgal coverage area (Table 1). Estimated export is the summation of quantified sources and sinks from the Watershed and internal biological processes. Incoming and outgoing loads of oceanic exchange (Fig. 7). Measured export is the net of incoming and outgoing loads

	Water Year	2007 n=273	2008 n=366	2009 n=365	2010 n=244	Mean n=1248
Nitrate Sources	Rainfall	0.68	2.48	1.21	2.89	1.82
	Streams	—	126.92	54.68	190.43	124.01
	Groundwater	195.84	191.16	105.06	108.13	150.05
	Nitrification	78.07	133.20	161.38	86.33	114.75
Nitrate Sinks	Denitrification	-112.23	-191.47	-231.98	-124.10	-164.95
	<i>Zostera marina</i>	-23.64	-16.14	-18.34	-5.84	-15.99
	Phytoplankton	-2.19	-4.75	-5.26	-2.25	-3.61
	Macroalgae	-38.73	-34.23	-22.59	-17.10	-28.16
Sum of Sources and Sinks	Estimated export	97.80	209.17	44.16	238.49	177.92
Oceanic Exchange	Mouth incoming	1583.36	1232.27	3109.79	2675.43	2150.21
	Mouth Outgoing	-1970.06	-1596.59	-3567.62	-3078.19	-2478.11
	Measured export	-86.70	-364.23	-454.83	-402.76	-327.15

Table 4 Annual dissolved inorganic nitrogen (DIN) loading per estuary surface area. *Estimation of DIN for Morro Bay Estuary based on nitrate loads

Estuary	DIN (g N m⁻² yr⁻¹)	Source
Great South Bay, NY	1.5	Nixon et al. 2001
Potomac River, MD, VA	18.2	Boyton et al. 1995; Seitzinger 2000
Narragansett Bay, RI	20.2	Boyton et al. 1995; Seitzinger 2000
Apalachicola Bay, FL	27.4	Mortazavi et al. 2000
*Morro Bay Estuary, CA	28.04 ± 9.36	This study
Tomales Bay, CA	81	Nixon et al. 2001
Norsminde Fjord, Denmark	161.6	Seitzinger 2000

Figures

Fig. 1

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Figure Legends

Fig. 1 Map of the Morro Bay Estuary, CA (9.3 km²) with the location of the San Luis Obispo Science and Ecosystem Alliance's (SLOSEA) three instrumented water quality stations (BM1, BS1, and CM1), two streams (Chorro and Los Osos Creeks), and neighboring towns (Morro Bay and Los Osos-Baywood Park)

Fig. 2 Characteristic spatial responses of freshwater input and circulation during wet and dry periods.

Figures shown are salinity and nitrate concentrations from Roving 1 and Roving 2. Roving 1 occurred on 16 October 2009 during a high tide (1.74 m above MLLW) three days after 4 cm of rainfall. Roving 2 occurred on 19 May 2010 at a low tide (-0.09 m below MLLW) 27 days after the last rain event of the season

Fig. 3 Summary of rainfall (a), stream volume of Chorro and Los Osos Creeks (b), calculated stream nitrate concentrations (c), and stream nitrate loading (d) during the 2008, 2009 and 2010-partial water years.

Stream nitrate concentrations and loading calculated at CM1. Black lines are measured values and grey lines are interpolated values

Fig. 4 Representative time series of freshwater volume percentage in Morro Bay Estuary (a) and the percentage contribution of freshwater sources to total freshwater volume in Morro Bay Estuary (b). Stream sources are grey. Rainfall sources are white. Groundwater sources are black

Fig. 5 Representative time series of groundwater nitrate loading. Groundwater freshwater nitrate concentrations calculated at BS1. The annual groundwater volume estimates were based calculated freshwater source volume in the Estuary (Fig. 4). Black lines are measured values and grey lines are interpolated values

Fig. 6 Difference between percentage of flood and ebb duration in measured and interpolated bins across BM1 channel over the study period. Negative percentages represent ebb dominance, while positive percentages reflect flood dominance

Fig. 7 Daily sum of nitrate flux across the BM1 channel (a). Import is positive and export is negative. The daily sum of volume (b) and tide (c) from 2009 December to 2010 March. (Note: difference in timescales between panel a and panels b and c)

Fig. 8 Representative coastal upwelling event from 3 – 9 June 2008 captured at BM1 by daily temperature (a), nitrate concentrations (b), and chlorophyll *a* concentrations (c)

Fig. 9 Conceptual box model of annual mean sources and sinks of nitrate in Morro Bay Estuary (Table 3). Stream and groundwater sources loads calculated with the water quality stations (Fig. 3; Fig. 4; Fig. 5). Rainfall source loads based on published nitrate concentrations (Padgett et al. 1999; Fenn et al. 2003) applied to measure rainfall amounts. Nitrification source loads based on published nitrification rates (Jenkins and Kemp 1984; Caffrey et al. 2003). Denitrification sink loads based on published denitrification rates (Seitzinger 1987; Mortazavi et al. 2000; Caffrey et al. 2003; Chapin et al. 2004). *Z. marina* sink loads based on published uptake rates (Pedersen and Borum 1992, 1993). Macroalgae sink loads based on published assimilation rates (McGlathery et al. 2007). Nitrification, denitrification, *Z. marina*, and macroalgae rates were applied to annual habitat coverage areas (Table 1). Phytoplankton sink loads calculated by net exchange of chlorophyll *a* to carbon to nitrogen (Banse 1977; Gallegos and Vant 1996; Mortazavi et al. 2000). Estimated export is the summation of quantified sources and sinks from the Watershed and internal biological processes, while measured export is the net incoming and outgoing loads of the oceanic exchange at the BM1 channel (Fig. 6; Fig. 7)

APPENDIX B

Implication of sampling frequency on capturing water quality trends in Morro Bay Estuary, California.

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Abstract

Using an instrumented water quality network in Morro Bay Estuary, California from 2009 to 2010, we investigated the impact of sampling frequency and sampling location on understanding dynamics in water quality. We degraded a year time series of seven parameters from three water quality monitoring stations to sampling frequencies ranging from 15 minutes to 28 days. In Morro Bay Estuary, the semi-diurnal tidal cycle was the maximum component frequency driving the variability of temperature, turbidity, and dissolved oxygen concentrations. For these parameters, asymptotes were reached and sampling frequencies greater than six hours did not explain the additional variation in the parameters sampled. Whereas, salinity, turbidity, and nitrate concentrations lacked an asymptote, and decreased sampling frequencies led to increased estimated error. Sampling water quality parameters every 28 days can lead to mean annual difference of 30 – 140 % from 15 minute sample annual mean. We recommend sampling frequencies should be selected to oversample the tidal signal to at least hourly frequencies. These sampling frequencies capture diel cycles and episodic events that contribute significantly to understanding the variability in the estuarine physical and biological dynamics, such as algal blooms, upwelling events, storm events, wastewater overflows, dredging events, and tsunamis. High frequency sampling methodologies are critical to resolving trends and for continuing management and protection of estuarine ecosystems.

Keywords: Estuaries, sampling frequency, sensor networks, monitoring

Introduction

Estuarine biogeochemistry is driven by complex and often non-linear processes occurring at multiple frequencies. Tidal forcing, upwelling events, mixing, biological productivity, seasonality, and rainfall events are intersecting frequencies influencing abiotic and biotic parameters' signals (Montani et al. 1998; Chapin et al. 2004; Caffrey et al. 2007; Jannasch et al. 2008). The signals can vary on instantaneously to decadal timescales (Johnson et al. 2006; Caffrey et al. 2007). Water column parameters also differ in space, where water residence time, bathymetry, location to freshwater inputs, and habitat type can substantially influence signal variability (Dettmann 2001; Chapin et al. 2004). Although this space and time variability is an issue in all aquatic systems, it is higher in coastal systems (Blackwell et al. 2008) and especially challenging in estuaries where the scales are particularly small (Swaney et al. 2011).

Concern over the past several decades of anthropogenic changes, such as nutrient enrichment, increased frequency of hypoxic events, and changes in food webs in estuaries has led to enhanced monitoring by local, state, and federal agencies as well as the formation of volunteer programs that assess the dynamics and status of estuaries (Nixon et al. 1996; NRC 2000; Meals et al. 2010). A majority of these monitoring efforts sample at weekly to monthly intervals due to a combination of sampling design and resources (Jannasch et al. 2008; Meals et al. 2010). If the primary drivers of system variability are undersampled, such studies can face several challenges in documenting and quantifying trends in estuaries (i.e. water quality) and informing management decisions. Many studies have documented that present sampling efforts fail to capture high frequency and episodic events that contribute to the majority of the annual nutrient loads, sedimentation loads, and primary production (Dickerman et al. 1986; Livingston 1987; Chapin et al. 2004). As Muller et al. (2003) have shown, sampling in drainage pipes every second day versus six times an hour underestimated the loads of NH_4^+ by 550 % and NO_3^- by 22 %. These variations in the watershed drainage have direct downstream impacts on underestimating nutrient loading in estuaries. This underestimation was presented by Chapin et al. (2004) in capturing undocumented, elevated nitrate concentrations during watershed runoff pulses and internal tides from Monterey Canyon into Elkhorn Slough with sensor arrays sampling every hour. Capturing dominant nitrate sources revealed that Elkhorn Slough is a sink for nitrate in the dry season and a source in the wet season (Chapin et al. 2004; Caffrey et al. 2007). In addition, low sampling frequencies can miss intermittent events when regulation standards are exceeded. Leecaster and Weisberg (2001) found as the sampling frequency of bacteriological samples on California beaches decreased from five times a week to once a month, the percentage of samples that exceeded State standards decreased linearly. The inability of sampling designs to accurately capture critical dominant high frequency and episodic events, such as pathogenic bacteria and harmful algal blooms, can have consequential human health and economic effects.

In addition to capturing dominant events, the sampling design of a study can impact the time needed to statistically document changes, which requires capturing the background variability (Meals et al. 2010). As the number of samples per year decreases, the number of years needed to detect a significant change increases. In fact, Weston Solutions, Inc. (2005) found that a stormwater monitoring program sampling for three events a year would take 17 years to confirm that Cu concentrations were below the

standard while sampling two events a year would take an additional nine years. Statistical lag-time presents a role in estuarine studies and can impede the process of assessing the physical and biological conditions and subsequent management of an estuary.

In light of the biogeochemical dynamics of estuaries and the necessity for monitoring water quality, the question remains as to what sampling frequency is necessary to capture and further estimate estuarine dynamics and trends. Chapin et al. (2004) suggest physical conditions and nutrient concentrations should be monitored long term at an hourly sampling frequency through sensor networks. Sensor networks provide *in situ* instrumentation that sample at high frequencies and capture critical elements of the estuarine and watershed processes (Glasgow and Burkholder 2000; Johnson et al. 2006; Johnson et al. 2007; Jannasch et al. 2008). While there is a growing distribution of sensor networks in estuaries, such as Elkhorn Slough, CA; Yaquina Bay, OR; Penobscot River, ME; Lower Columbia River, OR; and Northwest Arm, Halifax, CA (Jannasch et al. 2008), funding and resource constraints can deter the deployment and continuation of such networks.

A gap still exists in the literature to define the driver for the necessity of high frequency of physical and biological parameters in estuarine study. In an effort to fill this gap, this study investigates the roles of sampling frequency and sampling location in a small estuary. We degraded a year time series of seven parameters from three water quality monitoring stations in Morro Bay Estuary, California to sampling frequencies ranging from 15 minutes to 28 days. By comparing the maximum and standard deviation of the mean absolute differences, we examined the drivers of environmental variability of seven parameters at the station locations to assess how this knowledge can be integrated to a more efficient and effective monitoring of water quality in estuaries. This study was part of a larger initiative by the San Luis Obispo Science and Ecosystem Alliance (SLOSEA; see www.slosea.org) to improve the understanding of estuarine dynamics and to assist current management approaches in Morro Bay Estuary.

Materials and Methods

Study Area

Morro Bay Estuary is a natural embayment on the central coast of California, approximately 160 km south of Monterey Bay and 100 km north of Point Conception (Fig. 1). The Morro Bay Watershed (194 km²) is comprised of the Chorro Creek basin (116 km²) and the Los Osos Creek basin (78 km²) (Fig.

1). The semi-arid, Mediterranean climate of the central California coast is characterized by wet winters (~45 cm) and long periods (~eight months) without measurable precipitation (Page et al. 1995; Largier et al. 1997). The two stream inputs are Chorro and Los Osos Creeks, which merge on the east side of the estuary and then converge with the main channel.

Morro Bay Estuary is approximately 6.5 km long in the north-south direction and 2.8 km wide in the east-west direction at its maximum width with a single entrance to coastal ocean of Estero Bay (Ji et al. 2001). The main transport mechanism for water is tidal exchange with a tidal range of 2 m (US Army Corps of Engineers Los Angeles District, 2003). At high tide inundation, the area of Morro Bay Estuary is 9.3 km². The bay is predominantly flat and shallow with a mean water depth of 0.5 – 1 m and with a main shipping channel with a 4 – 9 m depth. At low tide, approximately 60 % of the estuary area emerges as tidal mudflats and exposed *Z. marina* beds (Ji et al. 2001).

Water Quality Network

The water quality network consisted of three strategically located fixed water quality stations (Fig. 1). Each station supported physical, chemical, and biological sensors selected to tolerate marine conditions (Jannasch et al., 2008). BM1 (35° 22' 15.2394" N, 120° 51' 32.04" W) near the mouth of the estuary was mounted to a pier at a depth of 0.83 m below the mean lower-low water (MLLW) to capture the flux between the estuary and Estero Bay. BS1 (35° 20' 1.6794" N, 120° 50' 50.28" W) was situated in the southern end of the estuary at a depth of 0.72 m below MLLW. CM1 (35° 20' 17.1594" N, 120° 49' 56.64" W) was installed downstream of the confluence of Chorro and Los Osos Creeks and deployed as surface floating.

Each array was comprised of three main components: power supply, water quality instruments, and modem with a remotely located server computer. The power supply contained a 12-volt rechargeable marine-grade battery, a casing for the battery, and a 60-watt solar panel. The water quality instruments included a Satlantic STOR-X data logger, Satlantic *In Situ* Ultraviolet Spectrophotometry (ISUS-X) nitrate sensor (Johnson and Coletti, 2002), Sea-Bird CTD (SIP-37) sensor, WET Labs Combination Chlorophyll Fluorometer and Turbidity Sensor (ECO-FLNTUS), and Aanderaa Oxygen Optode 3835 (Fig. 1). At each fixed station, the water quality instrument sampled every 15 minutes for a two minute sampling interval. With a schedule file, the STOR-X controlled the timing of applying and removing power, acquiring and

packaging data from connected sensors, and communicating with the GSM modem to transmit packaged raw data. The raw data were sent to a California Polytechnic State University's (Cal Poly) Center for Coastal Marine Science (CCMS) server by cell phone connection for storage as a day file and processing. Near-real time data were available for the general public on the SLOSEA website (<http://www.slosea.org/about/dash.php>).

Each parameter was averaged over the two minute burst sampling period every 15 minutes, and the mean was assigned a timestamp to that nearest 15 minute. Each mean value was subjected to threshold processing based on sensor ratings and physical limits to ensure quality assurance and quality control (QA/QC). Missing data were a result of transmission error, instrument malfunction, instrument calibration and repair, or QA/QC process. To minimize the effect of bio- and geo-fouling, each station was cleaned once a month and more frequently during high growth seasons. Additionally, all sensors were factory calibrated and updated each year.

Data Analysis

The effect of sampling frequency was tested by degrading a time series from 1 June 2009 – 31 May 2010 to calculate absolute differences. Water column parameters analyzed from BM1, BS1, and CM1 were temperature, salinity, turbidity, chlorophyll *a* concentration, dissolved nitrate concentration, and dissolved oxygen concentration. Tide was analyzed from only BM1 and BS1, due to station configuration. The water column parameters measured at 15 minute intervals were considered to be the 'true' signal and was defined as the reference for estimates made at different sampling frequencies. The sampling intervals, ranging from 15 minutes to 28 days, with an interval step of 15 minutes, were applied to the measured data starting at the first 15 minute period on 1 June 2009 and sequentially moving to the next 15 minute period till the last 15 minute period on 31 May 2010. The sampling intervals were selected to simulate all possible sampling frequencies conducted by monitoring groups over the period of 28 days. The sampling interval was moved ahead by a 15 minute period at a time and controlled for the effects of the start time of sampling. The reference was defined as the center value within each sampling interval, thus maintaining a two 't'-tailed approach and removing lags at the beginning and ending of the time series. The absolute difference was calculated between the reference and each value within in the sampling interval. Each absolute difference lacked directionality, where the real differences could be greater than or less than the

reference value. At each sampling frequency, each initial parameter was associated with a number of absolute differences depending on the size of the sampling interval. The mean maximum absolute differences and the variance of the mean of the absolute differences were calculated for each parameter.

Results/Discussion

A gradient in the magnitude of the absolute differences between the stations was present for the majority of the parameters. Significant differences were present between the mean maximum absolute differences of all parameters between sites (one-way ANOVA, $p_{\alpha=0.05} < 0.00$, $n=2688$). Overall, parameters from creek station (CM1) had the largest range in absolute differences, while parameters from the center of the bay (BM1) had the smallest maximum and variance of the absolute differences (Fig. 2; Fig. 3; Fig. 4). Maximum absolute differences for salinity, nitrate concentration, and turbidity were greater at the mouth of the estuary (BM1) than BS1 after 4 – 7 days. The standard deviation of absolute differences in chlorophyll *a* concentration at BS1 was greater than at CM1 and BM1 while the standard deviation of absolute differences of nitrate at BM1 was greater in comparison to BS1. The dominant presence of the gradient suggested that the variability increased as the sampling locations were located farther away from the coastal ocean source and closer to the influences of the watershed and freshwater sources.

In addition to a gradient in absolute differences between stations, results indicated that decreasing the sampling frequency resulted in an increased maximum absolute error to an asymptote of all parameters of interest except salinity, dissolved nitrate concentrations, and chlorophyll *a* concentrations at all three stations (Fig. 3; Fig. 4). Sampling at frequencies shorter than the asymptote yielded a more accurate estimation of the parameters during intervals not directly sampled, as well as increased the probability of sampling an episodic event. However, after the curve reached the asymptote, there was little difference in the absolute difference between sampling frequencies (Blackwell et al. 2008). For example, sampling the temperature at BM1 every 15 minutes had a mean maximum absolute difference of 0.1 °C while measuring the temperature every 28 days had a mean maximum absolute difference of 2 °C. Sampling nitrate concentrations at CM1 every 15 minutes had a mean maximum absolute difference of 0.11 mg L⁻¹ whereas sampling once every 28 days had a difference of 2.36 mg L⁻¹ (Fig. 3). In a similar effort, Blackwell et al. (2008) examined critical spatial scales of variability of propeller-driven, autonomous underwater vehicles in near-shore coastal studies. They found that sampling in spatial areas of 10s to 100s of meters provided

scales of variability critical to capturing near-shore dynamics and increasing the confidence in estimating conditions of areas not directly sampled. Thus in the Morro Bay Estuary, sampling at frequencies lower than the asymptote would increase confidence in estimating trends in those parameters exponentially for times not sampled.

For all parameters at each station, a transition was present for approximately 0.25 days. This transition was less apparent for parameters lacking an asymptote, such as BM1 nitrate concentrations (Fig. 2; Fig. 3; Fig. 4). The transition and asymptote were most clearly defined for the absolute differences for tide at BM1 and BS1. For BM1 and BS1, the change in the maximum absolute differences prior to the asymptote was 1 m while within the asymptote overall change was only 0.6 m (Fig. 2). This pattern was reflective in the average maximum percent differences for the six water quality parameters. For the six defined sampling frequencies, the percent difference between 1 hr and 1 day was the largest increase, which ranged from 1.8 – 3.8 fold increase (Table 1). The defined pattern of the absolute differences for tide suggests that tide was the maximum component frequency regulating the physical and biological parameters in Morro Bay Estuary. The main transport mechanism is a mixed, semidiurnal tidal exchange (US Army Corps of Engineers Los Angeles District 2003), where roughly a high or low tide occurs every six hours. The three dominant tidal constituent are M_2 , K_1 , and O_1 , where M_2 drove the strong spring/neap fortnight cycle and the K_1 and O_1 combination produces a strong diurnal inequality between high and low tides (Weston et al. in review). Similar to Morro Bay Estuary, Lucas et al. (2006) documented that the semidiurnal tides were the dominant driver of intraday variability of physical and the biological parameters in a freshwater tidal lagoon at Mildred Island, CA.

Capturing the tidal signal specifically for temperature, dissolved oxygen concentrations, and turbidity parameters was important to capturing the dominant signal. By capturing the maximum component frequency, further analysis could distinguish between the true measurement and background variability. This could both reduce the statistical lag-time to document a trend (Meals et al. 2010) and sampling-frequency-induced error (Moline and Prèzelin 1997). As shown by Taylor and Howes (1994) for seasonal primary productivity estimates in a temperate, coastal embayment, sampling frequencies closer to the maximum component frequency resulting in the sampling-frequency-induced error were equivalent to the error limit of the analytical limit. In Morro Bay Estuary, the tidal frequency played a critical role when

effectively designing a sampling methodology to capture to the maximum component the frequency driving the physical and biological parameters and to minimize sampling-frequency-induced error.

Almost all analyzed water quality parameters reached an asymptote at roughly six hours, except salinity, nitrate concentrations, and chlorophyll *a* concentrations (Fig. 2; Fig. 3; Fig. 4). Without an asymptote, decreasing the sampling frequency resulted in an increased absolute difference from true measurement. Changes in salinity, nitrate concentration, and chlorophyll *a* concentration were more closely driven by seasonal and episodic events (i.e. upwelling, rainfall discharges) rather than by the consistency of the coastal ocean, and the changes could not be accurately predicted solely by the tidal regime. For these parameters, decreasing sampling frequencies greatly increased statistical lag-time and sampling-frequency-induced error.

In Morro Bay Estuary, decreasing the sampling frequency was found to have direct consequences in quantifying nutrient inputs, assessing the magnitude of phytoplankton blooms, and evaluating the dissolved oxygen conditions. For nitrate concentrations, sampling every 28 days instead of every 15 minutes could result in a maximum difference of $1.4 - 2.7 \text{ mg L}^{-1}$ from a mean nitrate concentration of $1.12 \pm 0.19 \text{ mg L}^{-1}$ (Fig. 3). This would result in a mean percent difference of $91.50 \pm 13.92 \%$ (Table 1). This would directly impact confidence in the estimation of nitrate loads from freshwater non-point sources. Weekly to monthly sampling frequencies could also miss nitrate concentrations exceeding environmental standards ($2.5 - 10 \text{ mg L}^{-1}$). In addition, low sampling frequencies of nitrate concentrations could impact the quantification the oceanic exchange of nitrate since determining directionality of nitrate loads would not be possible (Weston et al. in review). The percent difference between sampling frequencies of 1 hr and 1 day was doubled from 21.44 % to 40.42 %. This is further suggestive the critical nature of capturing the tidal signal with selected nitrate sampling methods.

As to phytoplankton blooms, sampling chlorophyll *a* concentrations every 28 days versus every 15 minutes would result in a mean maximum percent difference of $138.23 \pm 20.66 \%$ from the mean true chlorophyll *a* concentrations ($2.65 \pm 1.20 \text{ mg L}^{-1}$; Fig. 3; Table 1). Depending on the study's objective, the sampling frequency of chlorophyll *a* sampling could vary. If the objective was to estimate net primary production, then a sampling frequency greater than the maximum component frequency is necessary in order to capture the net flux of chlorophyll *a* and the episodic blooms of short duration. This was shown

with a 1.9 fold increase between sampling every hour and once a day (Table 1). In Morro Bay Estuary, annual primary production estimates based on 28 day sampling could conservatively vary from true primary production upwards of 140 % (Table 1). In fact, Taylor and Howes (1994) found that seasonal primary production in near-shore coastal and estuarine ecosystems could be undersampled by 500 % with sampling frequencies each 30 days versus multiple measurements per day. Yet, if a presence or absence of harmful algal blooms was the objective, then a bi-weekly sampling effort would suffice (Cartensen et al. 2007).

Dissolved oxygen concentrations are important to resource managers as a measure of the eutrophic state of an estuary. Our results highlighted that dissolved oxygen concentrations have high temporal variability. Sampling every 28 days versus every 15 minutes showed a mean maximum difference of $5.52 \pm 0.02 \text{ mg L}^{-1}$, where the mean true dissolved oxygen concentration ranged from $8.42 \pm 0.17 \text{ mg L}^{-1}$ (Fig. 3). This led to a 50 % difference in the true dissolved oxygen concentration (Table 1), as concurrently shown by Taylor and Howes (1994). The sampling frequency has a significant role in monitoring the frequency and duration of low oxygen and hypoxic events ($< 2 \text{ mg L}^{-1}$) (Hagy 2004). In the study period, the minimum dissolved oxygen concentrations captured at the three stations varied from 3.2 - 4.8 mg L^{-1} . Oxygen depletion events ranged from 1 to 2 hours and over 1 to 5 days. The rapid events occurred at low tides, indicative of oxygen poor bottom water; the longer events transpired during periods of a constant temperature and increased chlorophyll *a* concentrations (Taylor and Howes 1994; Johnson et al. 2006). If samples were collected every 28 days, there is a strong likelihood that periods of oxygen depletion were entirely missed. In addition to missing low oxygen conditions, diel dynamics regulated by processes, such as primary production and mixing, would fail to be captured, and the realized long term trend would reveal only the seasonal spring and ebb tidal effects based on the sampling times.

In summary, efficient and effective monitoring of estuaries should sample at frequencies that can at best resolve the tidal signal through sensor networks. Monitoring efforts in estuaries that fail to resolve the tidal influence on water quality parameters will be limited in their ability to characterize trends due to inherent variability derived from tides, thus increasing statistical lag time and sampling-frequency-induced error. In estuaries where studies on nutrient loading or salt balance (i.e. Tomales Bay, CA) are occurring in seasonal, low-flow estuaries, similar to Morro Bay Estuary, sampling frequencies should be selected to

oversample the tidal signal at a frequency ranging from every 15 minutes to every hour. This allows for the capturing of intraday and episodic events, such as storm events, accidental wastewater overflows, dredging events, and tsunamis, that contribute significantly to the estuarine physical and biological dynamics. High frequency sampling methodologies are critical to accurately resolving trends and for continued management and protection of estuarine ecosystems.

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Tables

Table 1 Average maximum percent differences between a 15 minute annual mean and six lower sampling frequencies for six water quality parameters (salinity, temperature, dissolved oxygen concentration, turbidity, nitrate concentration, and chlorophyll *a* concentration) from three water quality stations in Morro Bay Estuary, CA

Parameter	Sampling frequencies (%)					
	1 Hour	4 Hours	1 Day	7 Days	14 Days	28 Days
Salinity (psu)	2.23 ± 2.11	7.87 ± 6.82	10.96 ± 8.01	19.00 ± 9.44	24.58 ± 10.16	31.06 ± 8.63
Temperature (°C)	4.03 ± 0.79	15.42 ± 2.46	20.62 ± 4.12	27.82 ± 5.96	30.81 ± 6.59	33.91 ± 7.47
Dissolved oxygen (mg L ⁻¹)	9.09 ± 1.85	26.76 ± 4.25	35.41 ± 5.59	43.77 ± 5.21	46.75 ± 5.04	49.33 ± 4.83
Turbidity (NTU)	28.18 ± 4.69	58.31 ± 12.22	71.23 ± 18.05	84.87 ± 25.97	88.80 ± 28.46	90.59 ± 29.65
Dissolved nitrate (mg L ⁻¹)	21.44 ± 8.20	40.42 ± 18.97	50.61 ± 18.01	71.44 ± 15.32	81.58 ± 14.02	91.50 ± 13.92
Dissolved chlorophyll <i>a</i> (µg L ⁻¹)	45.73 ± 18.56	84.78 ± 27.55	104.98 ± 28.64	128.44 ± 21.92	133.71 ± 20.83	138.23 ± 20.66

Figures

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Figure Legends

Fig. 1 Map of the Morro Bay Estuary, CA (9.3 km²) with the location of the San Luis Obispo Science and Ecosystem Alliance's (SLOSEA) three instrumented water quality stations (BM1, BS1, and CM1), two streams (Chorro and Los Osos Creeks), and neighboring towns (Morro Bay and Los Osos-Baywood Park). Schematic from the BS1 station in the Water Quality Network. (a) Antenna, (b) Solar Panel, (c) GSM

Modem, (d) Battery, (e) Satlantic STOR-X Data Logger, (f) Satlantic ISUS-X Nitrate Sensor, (g) Aanderaa Oxygen Optode 3835, (h) WET Labs ECO-FLNTUS Fluorometer, (i) Sea-BirdSBE-SIP37 CTD

Fig. 2 (a) Average maximum absolute differences between the ‘true’ tidal measurement and each value within a sampling interval ranging from every 30 min to every 28 days. (b) Average variance of the absolute differences between the ‘true’ tidal measurement and each value within a sampling interval ranging from every 30 min to every 28 days. The data set ranges from 1 June 2009 – 31 May 2010 at the water quality stations BM1 (+) and BS1 (□) in Morro Bay Estuary, CA

Fig. 3 Average maximum absolute differences between the ‘true’ parameter measurement and each value within a sampling interval ranging from every 30 min to every 28 days. The parameters evaluated were salinity (psu; a), temperature (°C; b), chlorophyll *a* concentration ($\mu\text{g L}^{-1}$; c), turbidity (NTU; d), nitrate concentration (mg L^{-1} ; e), and dissolved oxygen concentration (mg L^{-1} ; f) from water quality stations BM1 (+), BS1 (□), and CM1 (○) in Morro Bay Estuary, CA

Fig. 4 Average variance of the absolute differences between the ‘true’ parameter measurement and each value within a sampling interval ranging from every 30 min to every 28 days. The parameters analyzed were salinity (psu; a), temperature (°C; b), chlorophyll *a* concentration ($\mu\text{g L}^{-1}$; c), turbidity (NTU; d), nitrate concentration (mg L^{-1} ; e), and dissolved oxygen concentration (mg L^{-1} ; f) from water quality stations BM1 (+), BS1 (□), and CM1 (○) in Morro Bay Estuary, CA