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SCCWRP Technical Report 934

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Prepared for:
The California Environmental Protection Agency
State Water Resources Control Board
(Agreement Number 13-098-250)

June 2016
Technical Report 934

ACKNOWLEDGEMENTS

This report was produced under California State Water Board contracts to the Southern California Coastal Water Research Project (Agreement Number 12-109-150 and 13-098-250). Thanks to the California Coastal Records Project for use of its photos depicting the six estuary sites.

EXECUTIVE SUMMARY

Intermittently Closed and Open Lakes and Lagoons (ICOLLs), a class of estuaries that experience periodic or prolonged closure of their tidal inlets in times of low river flow, are among the most susceptible to anthropogenic eutrophication. Water quality objectives (WQOs) are needed to protect these habitats, which are critical for their support of salmonids, migratory birds, as well as threatened and endangered species, from the effects of nutrient pollution and eutrophication. While the wealth of scientific literature supports the use of algal biomass and dissolved oxygen (DO) to assess eutrophication, specific thresholds that distinguish protective versus levels at risk of adverse effects are difficult to derive. Policy decisions on numeric goals can be supported in part based on an understanding of the deviation from "reference" conditions when data prior to degradation are available. No studies exist to document the natural background levels of DO, macroalgae and phytoplankton biomass in minimally disturbed "reference" ICOLL estuaries along the Pacific West Coast, particularly during a closed tidal inlet condition, considerated the "critical condition."

Here, we report on a study to document the natural background concentrations of DO, macroalgal biomass (dry weight), and phytoplankton biomass (measured as *chl-a*) for six "minimally disturbed" ICOLL estuaries across California during the growing season. The goals of the study were to: 1) quantify the temporal and spatial variability in macroalgal biomass, phytoplankton *chl-a*, and DO relative to established benchmarks and 2) document the relationship between these eutrophication indicators of interest and major factors that can influence their variability.

Across ICOLL estuaries, algal biomass, a major symptom of eutrophication, was generally low throughout most of the study period. The 75th percentile of phytoplankton biomass, measured as water column chlorophyll-a (chl-a), was ~20 mg m^{-3}, a result consistent with estuaries at low risk of toxic cyanobacteria blooms (< 30 mg m^{-3}) and with conditions that provide full support for contact recreation use. Similarly, the 75th percentile of peak biomass across estuaries was ~7 g dw m^{-2}, a value that is consistent with previous estimates of natural background levels of algal biomass on intertidal flats in California estuaries in an open tidal inlet condition (< 13 g dw m^{-2}).

Hypoxia (< 2.8 mg L^{-1}) was a common occurrence in bottoms waters of ICOLL reference estuaries and most estuaries spent a considerable amount of time below existing water quality objectves. Four of six estuaries experienced hypoxia in bottom waters greater than 10% of the time, with some ranging up to 50% of the time. With the exception of Topanga and San Onofre, which experienced extended periods of hypoxia lasting > 24 hours, hypoxia in ICOLL reference estuaries was of high frequency but generally short duration, tending to last several hours. Using the State Water Board policy guidance on listing of State surface waters (7-day mean of daily DO minima), only five of six would be listed as impaired based on a WARM beneficial use WQO of 5 mg L^{-1} ; all would be listed as impaired based on a COLD beneficial use WQO of 7 mg L^{-1} (Table 4). DO data distributions illustrate ICOLL estuaries spend as much as 92% of their

time below 5 mg L^{-1} during the "growing" season. This suggests the need for improved science supporting interpretation of existing DO WQO.

All six ICOLL estuaries were subject to the same seasonal hydrology with reduced freshwater inflow during the summer months, and associated seasonal closure of the mouth. However, the estuaries exhibited a variety of responses to closure in terms of water levels, temperature, salinity and stratification. The vertical structure and temporal variability in dissolved oxygen levels observed in these small coastal lagoons are primarily explained in terms of the physical conditions – specifically mouth closure, freshwater inflow, wave overwash, wind exposure, stratification and light penetration. The strongest and most persistent hypoxia is observed when a lower saltwater layer is trapped by a closed/perched mouth and below strong stratification. Other low-oxygen occurrences are associated with inflows (but rare and not severe) or with the minima in shallow waters that exhibit large day-night fluctuations.

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INTRODUCTION

Nutrient over-enrichment has led to multiple ecosystem impairments of the world's estuaries (Nixon 1995; Paerl 1997; Cloern 2001; Diaz and Rosenberg, 2008; Bricker et al. 2008). Among the most vulnerable to eutrophication are Intermittently Closed and Open Lakes and Lagoons (ICOLLs), a form of wave-dominated estuary, common in Mediterranean-climate regions that experience periodic or prolonged closure in times of low river flow (Largier and Taljaard 1991). With prolonged mouth closure, these estuaries become vulnerable to nutrient overenrichment and eutrophication. Mouth closure increases the residence time of surface waters, enabling the proliferation of primary producers, particularly when enriched with urban and agricultural nuisance flows during the growing season. These conditions put ICOLLs at risk for extensive harmful algal blooms, low dissolved oxygen (DO), and their effects on other primary producers and consumer organisms. Excessive blooms have a variety of negative effects on estuaries including: 1) increasing frequency of water column and sediment hypoxia and heightening heterotrophic bacterial activity, resulting in poor water quality and increased frequency of diseases (Diaz 2001), 2) alteration of biogeochemical cycling, more rapid nutrient regeneration (Tubbs and Tubbs 1980; Raffaelli, Limia et al. 1991; Wennhage and Pihl 1994; Bolam, Fernades et al. 2000), 3) shading or smothering of seagrass, shellfish beds and other important habitats (Nelson 2009, Young 2009), 4) decreased recruitment and survival of benthic invertebrates and reduced carrying capacities for fishes and shorebirds (e.g., Raffaelli 1999; Thomsen and McGlathery 2006; Nezlin, Kamer et al. 2009), 5) poor aesthetics and an increase in odors relating to the decomposition of organic matter and increased sulfide production, and 6) subsequent changes in both trophic and community structure of invertebrates, birds and fishes (Raffaelli et al. 1989, 1991; Bolam et al. 2000). Water quality goals are needed to protect these habitats, which are critical for their support of salmonids, migratory birds, as well as threatened and endangered species, from the effects of nutrient pollution and eutrophication.

Identifying specific water-quality goals for nutrients in ICOLLs has proven difficult, however, because ecological responses to nutrients are complex and variable. Effects of nutrient pollution are often better diagnosed if focused on the ecological response of the estuary to this enrichment. Indicators such as DO, phytoplankton biomass as *chl-a,* and macroalgal biomass are frequently used in the assessment of eutrophication and to support regulatory goals (Bricker et al. 2003; Zaldivar et al. 2008; Harding et al. 2014, McLaughlin et al. 2013, Sutula et al. 2016). They can be linked specifically to nutrient inputs and have well-established links to adverse effects on aquatic life (see Sutula 2011 for comprehensive review). While the wealth of scientific literature supports the use of such indicators to assess eutrophication, specific thresholds that distinguish protective versus levels at risk of adverse effects are difficult to derive. Policy decisions on numeric goals can be supported in part based on an understanding of the deviation from "reference" conditions when data prior to degradation are available (Sutula et al. 2014, Andersen et al. 2010, 2015). Notably, while in California some regionally-focused studies have been published to date documenting patterns in dissolved oxygen, primary producers, and their

relationship to eutrophication (Central Coast, CLEAP 2008; South Coast, McLaughlin et al. 2014), no studies exist to document the natural background levels of DO, macroalgae and phytoplankton biomass in minimally disturbed "reference" ICOLL estuaries along the Pacific West Coast.

In Mediterranean-climate ICOLL estuaries around the world, the timing, duration and frequency of mouth closure regulates eutrophication processes via control of the relative abundance of primary producer communities. ICOLLs are host to a range of naturally-occuring primary producers, from phytoplankton and cyanobacteria (Knoppers et al. 1991, Perissinotto et al. 2000, Froneman 2004, Oczkowski and Nixon 2010), seagrass (both eelgrass, and brackish submerged aquatic vegetation and their epiphytes (Lukatelich et al. 1987, Viaroli et al. 1997, Silva and Asmus 2001), and macroalgae (Whitfield 1988, Cummins et al. 2004, Odebrecht et al. 2010, Kjerfve 1986). Dramatic changes in physicochemical variables (salinity, turbidity, light) that control the relative abundance of these primary producers may occur over very short time frames and can also greatly vary from year to year, depending on the timing and magnitude of freshwater inputs relative to mouth closure (McLaughlin et al. 2014). When open, ICOLL estuaries tend to be dominated by intertidal habitat, well flushed, with intermediate to marine salinity ranges (McLaughlin et al. 2014, CLEAP 2008) and support primary producers in proportions typically found in the intertidal habitats of other perennially tidal river mouths and lagoons (macroalgal mats and MPB). As the mouth restricts and is closed however, water levels rise, and the habitat becomes more lacustrine, promoting the growth of drift macroalgae, phytoplankton, cyanobacteria, and brackish water submerged aquatic vegetation (SAV). In eutrophic ICOLLs during prolonged mouth closure, cyanobacteria can proliferate (Magrann et al. 2015), exposing both estuarine and marine organisms to cyanotoxins (Miller et al. 2010). During mouth closure, drift and floating macroalgal mats can have maximum impact, as they are relegated to movement within the system and are limited in their ability to be flushed out (Whitfield 1988), shading seagrass and microphytobenthos (Whitfield 1988; Cummins et al. 2004), promoting hypoxia (Sutula et al. 2004, McLaughlin et al. 2014) and low aquatic diversity (Knoppers et al. 1991, Herrera-Silveira and Morales-Ojeda 2010, Mutchler et al. 2010). It is in this condition that ICOLLs typically exhibit the most severe eutrophication symptoms. Thus it is critical that documentation of natural background concentrations of phytoplankton and macroalgae encompass periods in which ICOLLs are in a prolonged period of closure.

The timing, duration and frequency of mouth closure versus the magnitude of freshwater input also regulate physical and biological controls on DO. Hypoxia is a consequence of the balance of atmospheric oxygen diffusion to surface waters, the *in situ* production of oxygen by primary producers during daylight hours, and the consumption of oxygen via respiration, decaying organic matter and other biogeochemical processes that consume oxygen within surface waters and sediments (Diaz and Rosenberg 1995, Diaz 2001). DO exhibits temporal variability on diurnal, tidal, lunar, and seasonal timescales, in asssociation with biological production and respiration and physical processes such as tidal mixing, wave overtopping, and stratification. In

ICOLLs, stratification can occur during physical conditions where the estuaries "trap salt" (Largier et al. 1991). Hypoxic water can occur as stratified water prevents the oxygenated surface water from mixing downward and respiration in the water and sediment depletes oxygen faster than it can be replenished. Although this hypoxia can be naturally occurring in ICOLLs, the discharge of nutrients that promotes organic matter accumulation and other oxygen-demanding materials can increase the level of bottom water hypoxia. Beyond acute and chronic effects on survival and reproduction, this bottomwater hypoxia can cause habitat compression for pelagic species, including salmonids, forcing foraging to occur in the upper water column where there is increased risk of predation. Thus it is critical to document the spatial (vertical and horizontal) and temporal aspects of the natural background concentrations of hypoxia in ICOLLs, particularly during periods of prolonged closure in order to better inform ICOLL water quality goals and management actions.

Here, we report on a study to document the natural background concentrations of DO, macroalgal biomass (dry weight), and phytoplankton biomass (measured as *chl-a*) for six "minimally disturbed" ICOLL estuaries across California during the growing season. The intent is that this information supports decisions on thresholds to assess status and trends of water quality in California ICOLLs as required by both scientists and managers (SWRCB 2015; Sutula et al. 2016). Our goals are: 1) quantify the temporal and spatial variability in macroalgal biomass, phytoplankton chl-a, and DO and 2) document the relationship between these eutrophication indicators of interest and major factors that can influence their variability, specifically mouth status, temperature, salinity, stream and estuarine ambient nutrient concentrations.

METHODS

Geographic Context

The California coast extends from the Smith River (41.46°N) to the U.S.-Mexico border (32.53°N; Fig. 1). Along this 1700 km coastline, a temperate climate exists north of Cape Mendocino with a moderate Mediterranean climate to the south. Average annual air temperatures and rainfall range from 15°C and 967 mm of rainfall in the north to 19°C and 262 mm in the south. Rainfall along the coast is concentrated from fall through late spring. This study captured conditions of moderate to extreme drought, with rainfall from October 1st – April 30th ranging from 45% below "normal" (i.e. long-term running average) in South Coast to 17% below normal in North Coast 2014-15.

Within California, ICOLL estuaries are the most numerous of all estuarine classes in the state, representing nearly 85% of the nearly 400 coastal confluences inventoried (Sutula 2011). ICOLL estuaries are usually shallow (< 2 m), with reduced tidal action during time periods when the sand bar restricts or eliminates surface tidal exchange, typically during periods of low freshwater input (Largier and Taljaard 1991). Although the diversity and configuration of habitats within

ICOLLs can be quite high, most are characterized by a central channel dominated by sandy sediments, with fringing SAV (e.g., *Ruppia* spp., *Zostera* spp.) and emergent wetland habitats that increasingly grow into the channel over the course of the summer dry season.

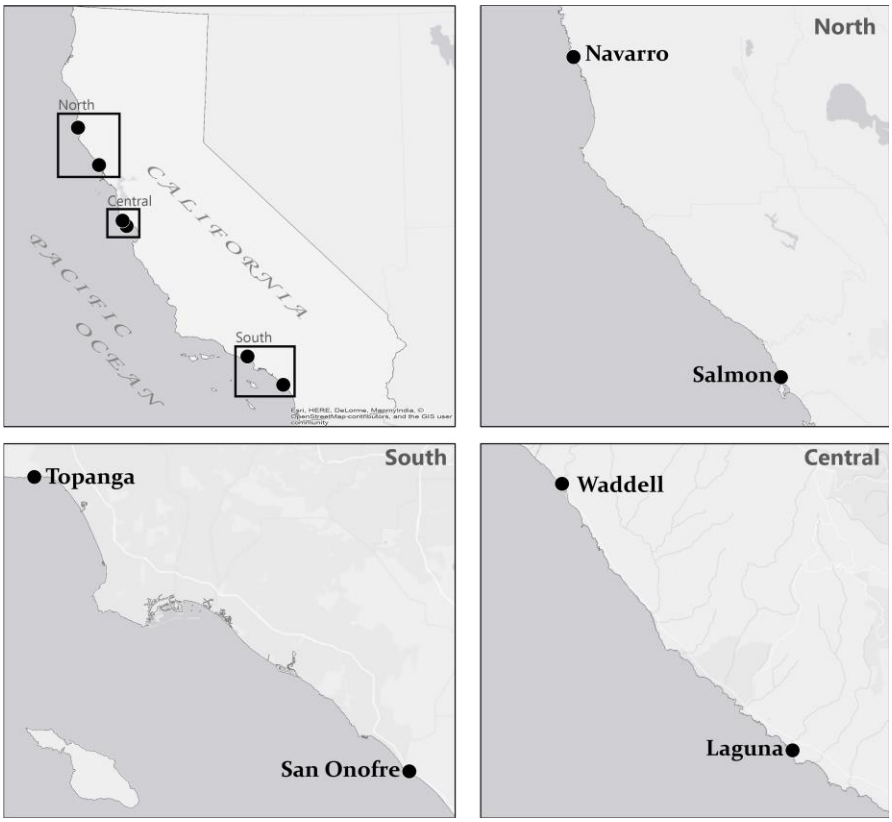

Figure 1. Map of coastal California showing locations of ICOLL estuaries in three regions.

Conceptual Approach and Site Selection

The natural background concentrations of DO, macroalgal and phytoplankton biomass were quantified in six minimally-disturbed "reference" California ICOLLs over the growing season (April-October) during a one-year period. Levels of these eutrophication indicators were measured synoptically with observations of tidal inlet status and exchange (water level, temperature, salinity), freshwater discharge and nutrient loading, and estuarine ambient nutrient concentrations to investigate factors controlling peak periods of algal abundance or hypoxia. Two reference ICOLLs were selected within each of three regions: North, Central, and South Coasts (Table 1, Figures 1 and 2). Within each region, minimally-disturbed is defined as best available, since few watersheds are without some form of human disturbance, and there is a clear gradient in urbanization of coastal watersheds from North to South (Solek et al. 2002). Sites were selected for inclusion in the study based on geographic information system analysis and

field verification of three criteria designed to ensure that sampling would capture natural conditions without influence from any land-based anthropogenic input:

1) Contributing drainage area should be at least 90% undeveloped;

2) Sites should not be within watersheds that have burned during the previous 3 years;

3) Field reconnaissance should reveal no evidence of anthropogenic effects, such as septic tanks, isolated residences, and/or excessive wildlife or human use.

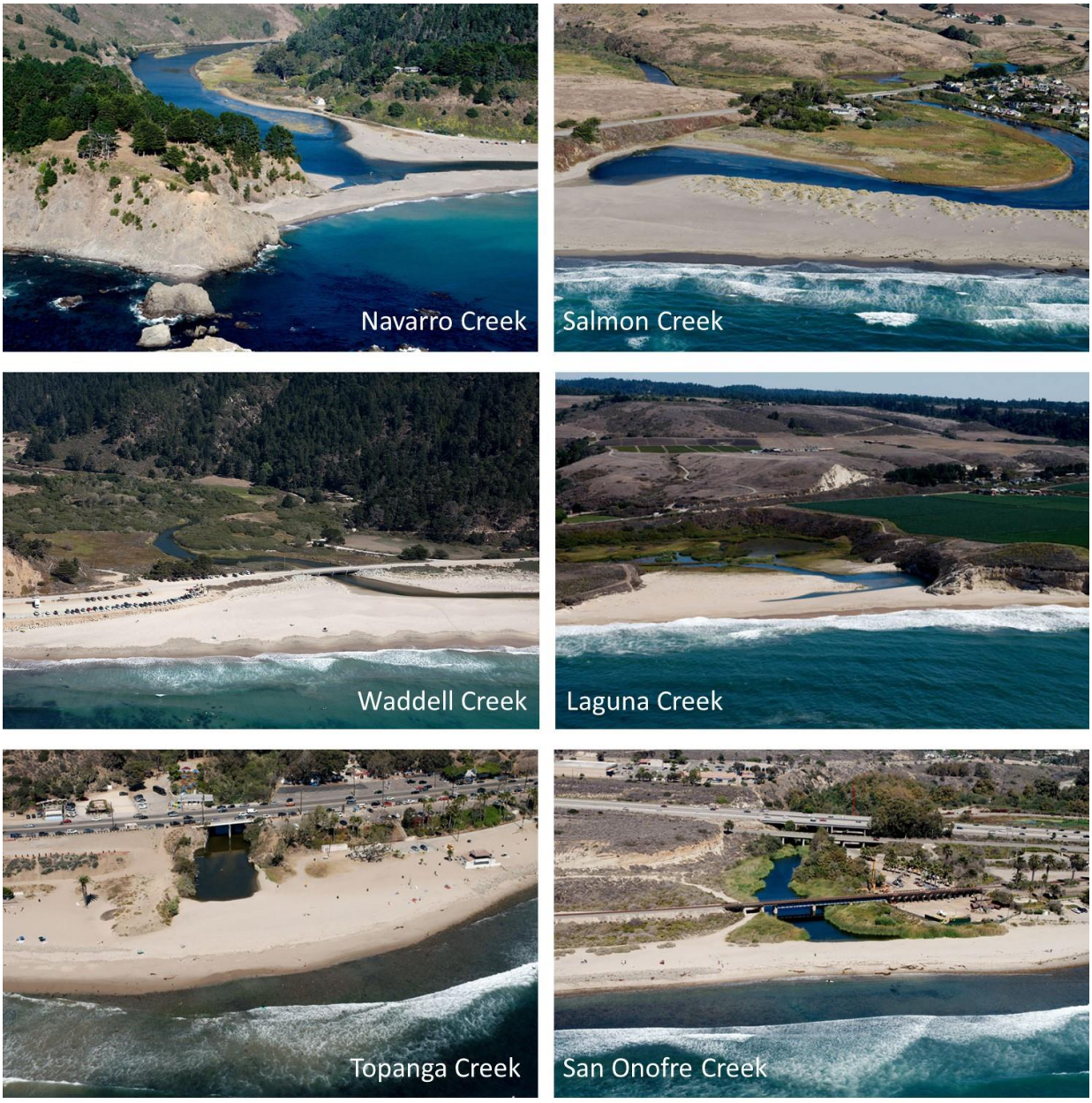

Figure 2. Aerial photographs of the six reference ICOLL estuaries.

Table 1. Location and characteristics of ICOLL reference estuaries.

Estuary Name	Region	Open Water Area (ha)	Latitude	Longitude	Watershed Size (Km^2)	% Open Space
San Onofre	South	0.76	N 33.381492	W 117.578516	110.5	97
Topanga	South	0.31	N 34.038741	W 118.583123	50.9	94.6
Laguna	Central	0.51	N 36.981959	W 122.154706	20.8	97.1
Waddell	Central	14.32	N 37.096149	W 122.278222	62	99.4
Salmon	North	7	N 38.354767	W 123.066863	90.9	96.4
Navarro	North	12.54	N 39.191729	W 123.761392	815.9	98.5

Field Sampling

Field methods consisted of three types of sampling: 1) vertical profiles and continuous monitoring of DO, chl-a fluorescence, temperature, salinity, and water level using moored data sondes and water level loggers, 2) monthly sampling of estuarine surface water and algal biomass, and 3) monitoring of dry weather freshwater discharge and nutrient loads.

DO and Water Column Physio-Chemistry. A data sonde continuously monitoring DO, water level and physiochemistry was deployed at one site/depth through the monitoring period. Water column physiochemical parameters and water level were measured continuously using a YSI 6600 data sonde at South Coast sites. At central and northern California sites, salinity and temperature were measured with a SeaBird SBE-37 microCAT while chlorophyll fluorescence and turbidity were measured with a WetLabs sensor. Dissolved oxygen was measured with a PME miniDOT optical sensor. All sensors were treated with anti-fouling tape and calibrated at a minimum of once monthly. Sondes were deployed at one location in each segment, in bottom water (30 cm from the sediment surface), with a 15-minute sampling interval.

In addition, in each estuary on a monthly to bimonthly frequency, vertical "profile data" on temperature, salinity, oxygen, and chlorophyll fluorescence were obtained at 15 stations (3 stations on each of 5 cross-estuary transects A through E, with A near the sea and E the furthest landward in the estuary).

Macroalgal Biomass, Phytoplankton *chl-a,* and Estuarine Ambient Nutrient Concentrations. Discrete water column samples of macroalgal biomass, phytoplankton *chl-a,* and ambient nutrient concentrations were sampled at 15 stations evenly distributed in a grid format. This sampling was conducted on monthly to bimonthly basis during the growing season (March – October in South Coast and May-November in Central and North Coast), with a temporal offset of sampling reflecting peak periods of insolation and temperature. Macroalgal biomass was collected as a bio-volume (grams per cubic meter of water) using a specially designed sampler to comprehensively sample biomass throughout the vertical profile of the water

column, above a prescribed surface area of estuary sediment. Water column depth at each sampling location was recorded and multiplied by the surface area to generate water column volume. Macroalgae samples will be rinsed lightly in the field to eliminate excess sediment and organisms, stored in plastic bags on ice, and transported to the laboratory for processing within 24 hours of collection. Water column *chl-a* samples were filtered on 0.7 μm Whatman GF/F filters and stored inside foil wrappers. The filtrate was collected into tripled rinsed HDPE sample bottles for analyses of dissolved inorganic and total dissolved nitrogen and phosphorus. An additional set of filters were collected for particulate nitrogen (PN) and phosphorus (PP) estuarine ambient concentrations. Chl-a, and estuarine particulate and dissolved nutrients were immediately frozen for subsequent analysis within 28 days of collection.

Riverine Nutrient Concentrations and Water Level. River water level and discrete nutrient concentrations were measured throughout the growing season. Continuous water level was recorded with a HOBO data logger (Onset Corp.) as a measure of river flow rate. Creek particulate and dissolved inorganic and organic nutrient samples were collected and processed as was done for the estuarine ambient nutrient discrete samples.

Laboratory Analyses

In the laboratory, macroalgal samples were cleaned of macroscopic debris, mud and animals, and sorted to genus level. Excess water was shed from each sample, which was then weighed wet, and dried at 60°C to a constant weight, then weighed dry. Macroalgal biomass was reported as dry weight (g dw) per unit area of the sampling device (0.71 m^2) and per unit volume sampled (area multiplied by water depth).

Chl-a samples were analyzed using EPA 445 on a Turner Designs fluorometer. Fluorescence measurements were calibrated to chlorophyll-a concentrations using least squares regression of daily averaged data probe measurements and discrete concentration data collected on the same day.

Dissolved inorganic nutrients including ammonium (NH4), nitrate+nitrite (NO3+NO2), and soluble reactive phosphorus (SRP) were assayed by flow injection analysis using a Lachat Instruments QuikChem 8000 autoanalyzer. Total and total dissolved nitrogen and phosphorus (TN, TP, TDN, and TDP) were via persulfate digestion followed by analysis of automated colorimetry (Alpkem or Technicon) for nitrate-N and orthophosphate-P (Koroleff 1985).

Data Analyses

Four types of descriptive analyses were conducted to summarize the status and trends in data on the six ICOLL reference estuaries:

(1) Analysis of physical and physiochemical profile and continuous data. In each estuary, vertical "profile data" on temperature, salinity, oxygen, and chlorophyll fluorescence were

plotted in “waterfall plots” to visualize data obtained at roughly monthly intervals at the 15 stations. Time series plots of sensors deployed at one site/depth through the monitoring period were generated to identify high-frequency temporal patterns in estuarine physical forcing.

(2) Dry weight monthly-bimonthly macroalgal biomass was normalized over the surface area of the collection device, then averaged over approximately 15 points sampled to yield a mean value for each sampling event. The peak value, mean and range for each estuary was then summarized.

(3) Water column chl-a (phytoplankton). By estuary, the median and range of discrete chl-a samples from each sampling event were summarized. Across estuaries, the 75th percentile of summertime median values was calculated. Chl-a fluorescence in profile and continuous sonde data was used to observe trends in bloom conditions over time, but not to report on chl-a concentrations.

(4) The magnitude, frequency and duration of DO concentration in the six estuaries over the growing season was summarized with respect to established DO water quality criteria or benchmarks. DO benchmarks of 5.0 and 7.0 mg L^{-1} represent established water quality objectives for non-salmonid and salmonid waters (WARM and COLD, respectively). DO concentrations of 5.8, 5.0, 4.0, and 2.8 mg L^{-1} represent benchmarks for the protection of aquatic life in European Estuarine waters, where 5.8 mg L^{-1} and 5.0 mg L^{-1} represents benchmarks protective of chronic effects to salmonid and non-salmonid species, respectively; 4.0 and 2.8 mg L^{-1} represent benchmarks for acute effects to salmonid and non-salmonid species, respectively (Best et al. 2009; Bailet et al. 2014). Finally, policies and rationale for assignment of WARM, COLD, or SPAWN beneficial use designation are not consistent across Regional Water Boards and therefore difficult determine whether salmonid or non-salmonid WQO would apply. Therefore, we utilized California Water Resources Control Board guidance for listing of impaired waters to determine the percentile of time in which ICOLL reference estuaries met DO WQO of 5 and 7 mg L^{-1}, based on the 7-day running mean of DO daily minima (SFRWQCB, 2015).

RESULTS AND DISCUSSION

Influence of Freshwater Discharge and Ocean Exchange on Estuarine Physiochemistry

All six estuaries were subject to the same seasonal hydrology with reduced freshwater inflow during the summer months, and associated seasonal closure of the mouth. However, the estuaries exhibited a variety of responses to closure in terms of water levels, temperature, salinity and stratification. These variations on a theme are related to watershed size, freshwater inflow, lagoon depth and area, and ocean-lagoon interactions.

The vertical structure and temporal variability in dissolved oxygen levels observed in these small coastal lagoons are primarily explained in terms of the physical conditions – specifically mouth closure, freshwater inflow, wave overwash, wind exposure, stratification and light penetration. Oxygen levels do not exhibit a simple seasonal cycle in concert with growth of macro-algae and/or phytoplankton, but rather exhibit changes in daily mean and the strength of day-night fluctuations that can be related to changes in mouth state (i.e., lack of tidal exchange) and stratification in addition to algal levels (primarily phytoplankton layers) and light availability. The strongest and most persistent hypoxia is observed when a lower saltwater layer is trapped by a closed/perched mouth and below strong stratification. Other low-oxygen occurrences are associated with inflows (but rare and not severe) or with the minima in shallow waters that exhibit large day-night fluctuations. Details with respect to particular estuaries are given in the subsequent sections.

Navarro River Estuary

The Navarro Estuary is the northernmost system monitored and also has the longest estuary, the largest watershed, and the strongest river inflow. Prior to the seasonal closure of the mouth of the Navarro Estuary on 14 April, there were two brief back-to-back closure events (each about a week), breaching naturally on 26 March and again on 7 April due to significant river inflow (Figure 3). During April, the water level rose by 2 m in the lagoon and formed a closed, but over-flowing mouth (i.e., perched lagoon) with a steady water level through May. As the river flow decreased, the estuary water level dropped in June and July, reaching a new steady state and exhibiting a fully closed mouth in late summer and fall (August-November). Winter rains filled the estuary in early December and the mouth breached on 12 December, following heavy rains and during a major wave event.

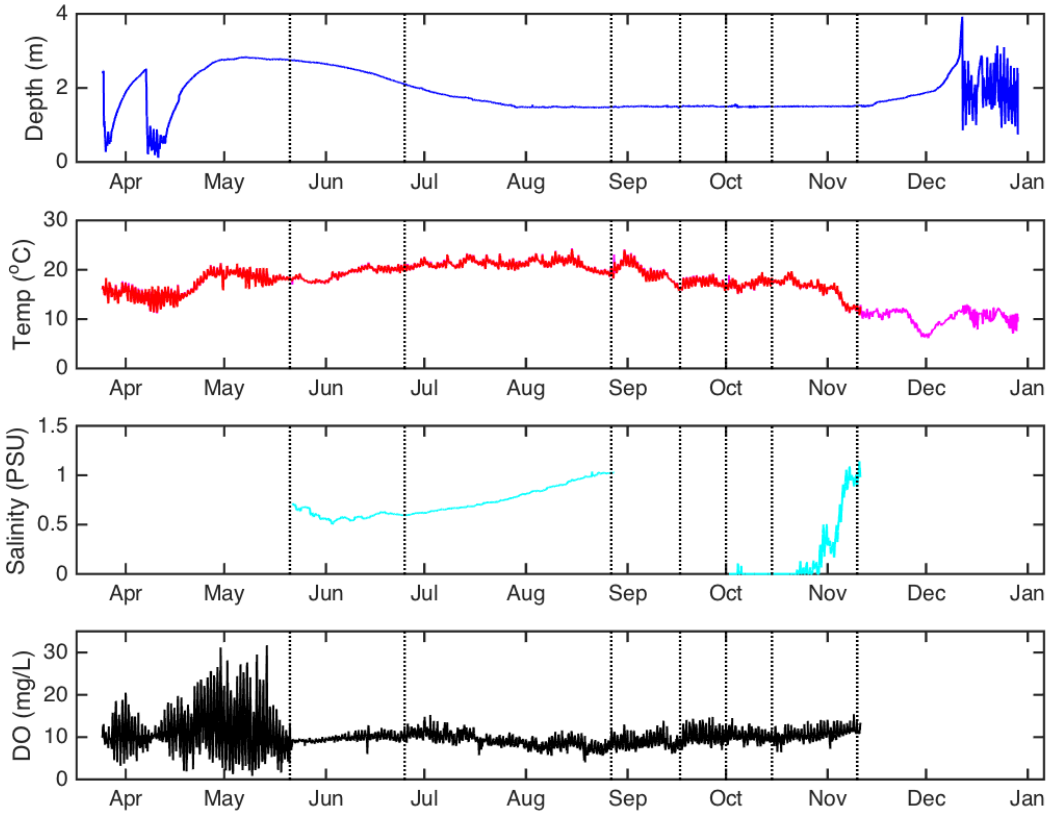

Figure 3. Time-series data in 2015 for Navarro Estuary at site A-3, close to the mouth: depth of sensor (i.e., water level), temperature, salinity and dissolved oxygen. Sensors were fixed with depth sensor ¼ m off bottom and water property sensors ½ m off the bottom. Vertical dashed lines indicate times at which profile data were collected. No salinity data are available for 8/27 to 10/1 due to an instrument malfunction. Water quality sensors were recovered on 10 November in anticipation of heavy rains (the depth-temperature sensor remained on site).

On 21 May, about a month after the April closure, a well-defined lower saline layer was evident in the lagoon below 3-m depth (S_{max} ~ 27 near-bottom at B) – and a freshwater layer above that (Figure 4). In the surface layer, oxygen levels were close to saturation values for the given temperature and salinity values (8.5 to 10 mg/L), while in the deeper saltwater layer oxygen levels dropped to ~ 3 mg/L near-bottom at B. Also higher chlorophyll levels were observed near-bottom. In the halocline, super-saturated oxygen levels were observed due to photosynthesis during the day and isolation from the atmosphere (due to stratification and the absence of mixing). At transect E about 9 km from the mouth, freshwater continued to fill the 6 m deep channel and the water was well oxygenated (~ 10 mg/L).

The time-series data show large day-night fluctuations during the first month of closure, as well as during the brief spring closure (28 March to 7 April, Figure 3). By June, there was no stratification and chlorophyll levels were low everywhere (~1 µg/L, Figure 4), consistent with

the demise of the strong day-night oxygen fluctuations observed in April-May (Figure 3). In June, the whole estuary was fresh (S ~0.5) with just a trace of higher salinities within 0.1m of the bed at a few stations (perhaps interstitial waters that were mixed by the profiling instrument). This vertically mixed, freshwater state persisted through the summer and fall (in spite of the streambed being dry), with oxygen levels close to saturation or higher (given that profile data were obtained during the day). However, on closer inspection there was a weak gradient in salinity from the most landward transect E (S ~0.2) to the most seaward transect A (S ~1.0 to 1.8), suggesting that some small volumes of seawater entered the lagoon at times – presumably by wave overwash at high tide. However, no significant stratification developed and the sensor deployed near-bottom at transect A showed persistently low salinity values (S ~1) and high levels of oxygen (~10 mg/L).

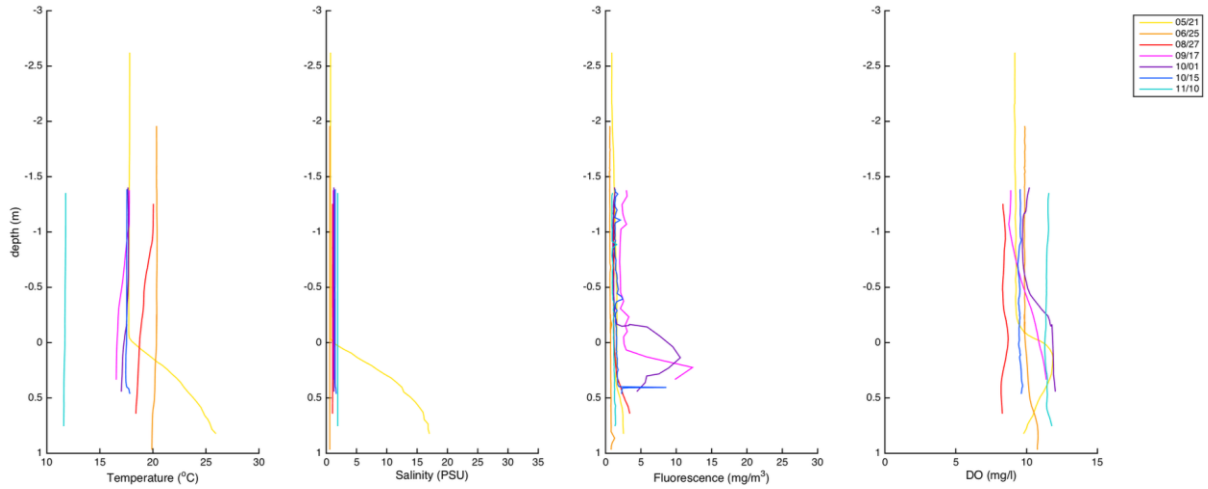

Figure 4. Profile data for temperature, salinity, chlorophyll fluorescence and dissolved oxygen at station A-3 in Navarro Estuary. Data are shown for 7 dates in 2015, with a focus on the late summer and fall when warm and hypoxic conditions are expected: 21 May, 25 June, 27 August, 17 September, 1 October, 15 October, and 10 November. Profiles have been adjusted vertically to account for changes in water level between survey dates – zero on the depth axis refers to the height of the water-level sensor (data plotted in Figure 3).

Salmon Creek Estuary

The Salmon Estuary is similar to the Navarro Estuary in that much of the lagoon is found in a narrow well-defined canyon, but it is shorter (only about 2 km long) and with a smaller watershed. Also in Northern California, it typically receives strong winter rains (although not during the 2014/15 drought). By March, the mouth of the lagoon was already highly constricted and tidal fluctuations in water level in the estuary were weak (0.1 to 0.2 m range) and irregular (Figure 5). Wave-driven sand accretion in the mouth at the end of March led to a brief monotonic rise in water level until 4 April when breaching was followed by rain and enhanced creek flow

on 5 April. Water level in the lagoon increased briefly on 7-8 April due to overwash during large waves at high tide, but the next large-wave event closed the mouth completely (wave heights exceeded 3m from 12 to 18 April). Large waves during spring high tides on 18-20 April led to strong overwash and a rapid rise in lagoon water level (as well as further building of the berm that separates the lagoon from the ocean). The mouth may have remained closed for the season if it weren't for a late-winter rain event on 25 April, which resulted in breaching of the mouth on 27 April. However, waves immediately started to close the mouth again and the seasonal closure was completed on 1 May during spring high tides (1-5 May) and another large-wave event (5-7 May) – again with strong overwash and a rapid rise in water level. The water level remained steady through May and early June, but then fell slowly until September owing to a negative water balance in summer. Wave overwash events occurred through the summer with the largest seawater inflow occurring during spring high tides on 30-31 July (see Figure 5 – also 13-15 June, 1 July, 12-14 July). In the fall, overwash events occurred during large waves, most notably on 28-29 October (see Figure 5 – also 17-18 September, 3-4 October, 11 October, 14-16 November, 25 November). The lagoon water level started rising in October, owing to both wave overwash and creek inflow due to early winter rains. The mouth breached on 11 December following heavy rains (4-13 December) and during very large waves.

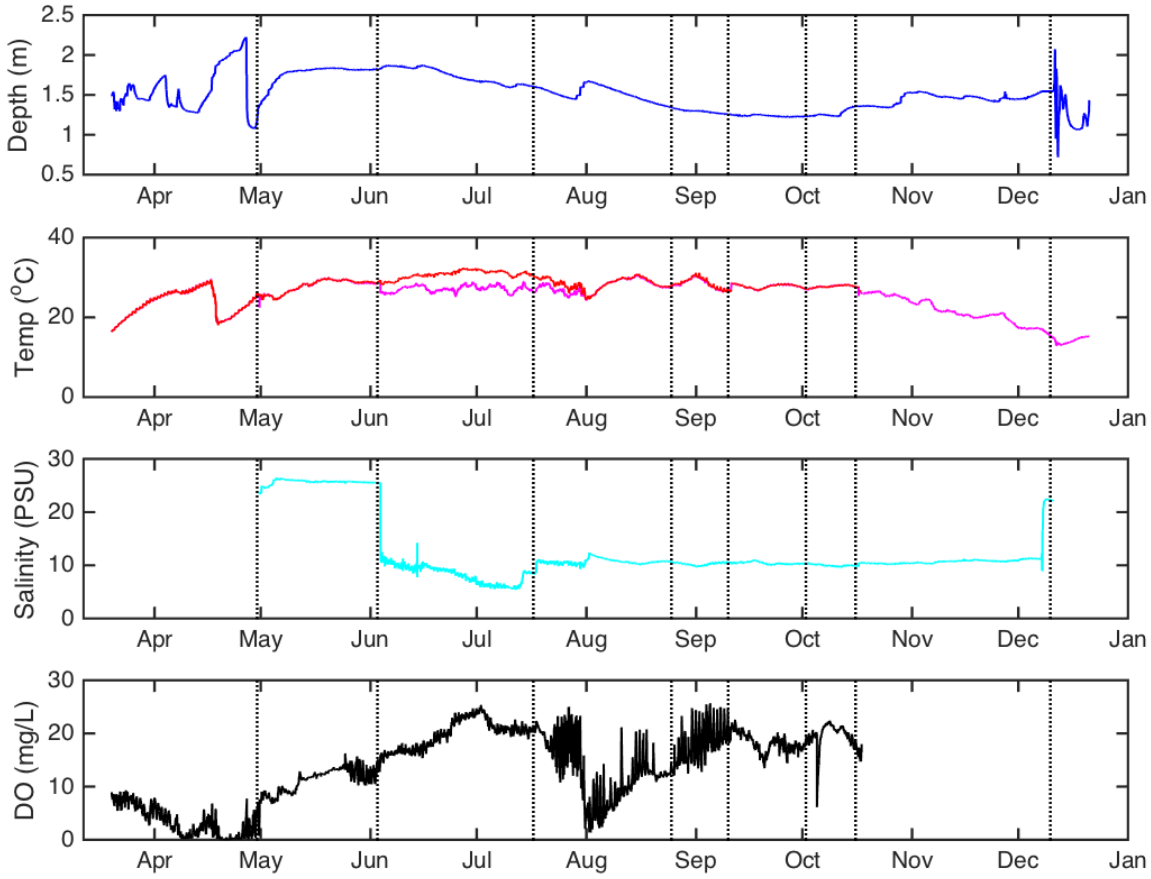

Figure 5. Time-series data in 2015 for Salmon Estuary at site C-3, just east of Highway-1: depth of sensor (i.e., water level), temperature, salinity and dissolved oxygen. Sensors were fixed with depth sensor ¼ m off bottom (also temperature data shown by magenta line) and water property sensors ½ m off the bottom. Vertical dashed lines indicate times at which profile data were collected. Salinity values change markedly when the sensor was swapped as it was in the halocline, so that a small change in the height at which it was fixed can result in a big change in salinity (0.1m height difference may account for salinity difference of 10 – see Figure 6). Water quality sensors were recovered on 10 December during heavy rains (depth-temperature sensor remained).

As the seasonal closure started at the end of April, a large volume of seawater was trapped in the lagoon, evident as a lower saltwater layer in profiles on 30 April (Figure 6). In the outer lagoon (transect B), bottom salinity was ~30. High-salinity water at transect C persisted through May, when sensor was swapped – see time-series data (Figure 5). Profile data in June and July show that the 2-layer structure persisted at transect C and elsewhere with little change at depth (bottom salinity ~25 and halocline ~0.7m above sensor), although the surface layer was increasing in salinity. As observed previously in this system, super-heated water is observed in the halocline, with a maximum observed in profile data in July (T ~33^{o}C) and the persistence of this super-

heated water evident in the time-series data (Figure 5). By August, and continuing through September and October, the 2- layer was not well defined with a continuous gradient in salinity from ~25 at depth to ~10 near surface in the outer estuary (and cooling of the mid-depth temperature maximum) – indicating active vertical mixing. Seawater inflow events are evident, maintaining the saltwater layer – most notably during events on 12-14 July and 30-31 July salinity increased and temperature decreased at the time-series sensors (Figure 5). Meanwhile, at transect E, surface salinities remained low throughout the summer/fall (S ~1), indicating a continual inflow of freshwater although the streambed was dry; weak salinity stratification was observed at depth. In December, a new intrusion of seawater was evident with a halocline at ~0.8m above sensor and bottom-layer salinity ~30.

Oxygen at the time-series site drops to anoxic levels during perched conditions in early April and again during the brief closure in late April – both events terminated by breaching and flushing (Figure 5). Although no profile data are available during these short-lived anoxic events, the profile data during 2-layer conditions in early summer exhibit under-saturated oxygen below the halocline (mostly at depths greater than 1.5 m where light levels were lower), but the lowest value observed was ~3 mg/L. During 2-layer stratification, super-saturated oxygen levels were observed in the halocline, which is the depth at which the time-series sensor was located. Low oxygen levels at this sensor were also observed following the large seawater inflow event at the end of July. This sudden decline in oxygen may have been due to a decline in oxygen levels in the lower layer as well as due to a rise in the halocline depth so that the sensor changed to a depth below the halocline. Chlorophyll remained low at all times, mostly below 10 µg/L.

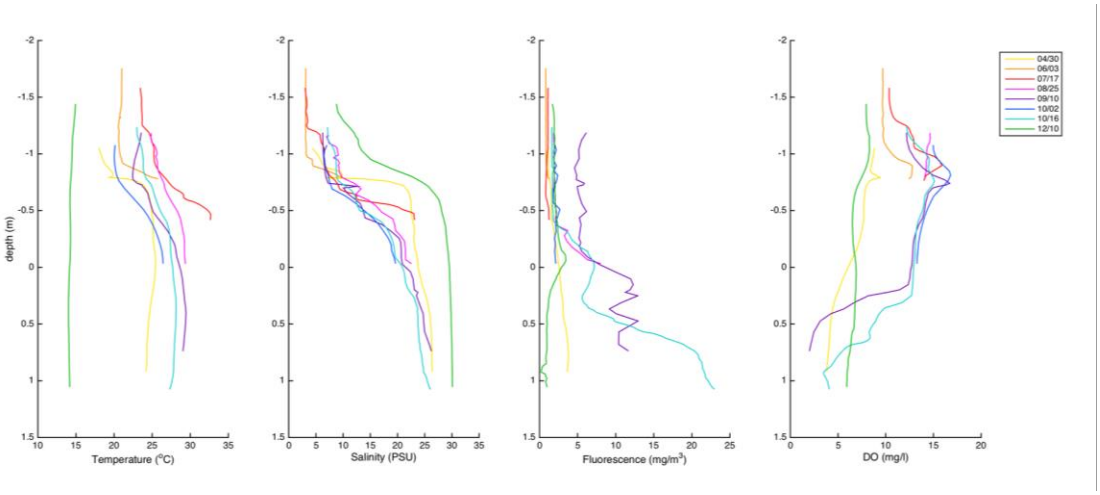

Figure 6. Profile data for temperature, salinity, chlorophyll fluorescence and dissolved oxygen at station C-3 in Salmon Estuary. Data are shown for 8 dates in 2015, with a focus on the late summer and fall when warm and hypoxic conditions are expected: 30 April, 3 June, 17 July, 25 August, 10 September, 2 October, 16 October, and 10 December. Profiles have been adjusted vertically to account for changes in water level between survey dates – zero on the depth axis refers to the height of the water-level sensor (data plotted in Figure 5).

Waddell Creek Estuary

The Waddell Estuary is smaller than Salmon Estuary (about 1.5 km long) and fringed by marsh and riparian vegetation. In late May the lagoon mouth was shallow, only allowing tidal inflow during spring high tides – and then it closed during wave forcing on 1 June. Seawater overwash continued for a few days so that the lagoon water level rose quickly. The lagoon continued to fill slowly and breached on 18 June, after which a perched state was again observed (water level low, but without tidal fluctuations or inflow). Weak tidal inflows are evident during spring tides and big waves at the end of June, which also acted to deposit sand and close the mouth on 30 June. The mouth stayed closed until November (end of data), with constant water level after mid-July only interrupted by wave overwash events at the end of July, end of August, on 11-12 October and late October (same events as observed in Salmon Estuary). Saline waters remained in the estuary, replenished by seawater overwash (Figure 7: salinity increase and temperature decrease) – most notably at the end of July, in mid-October and in late October.

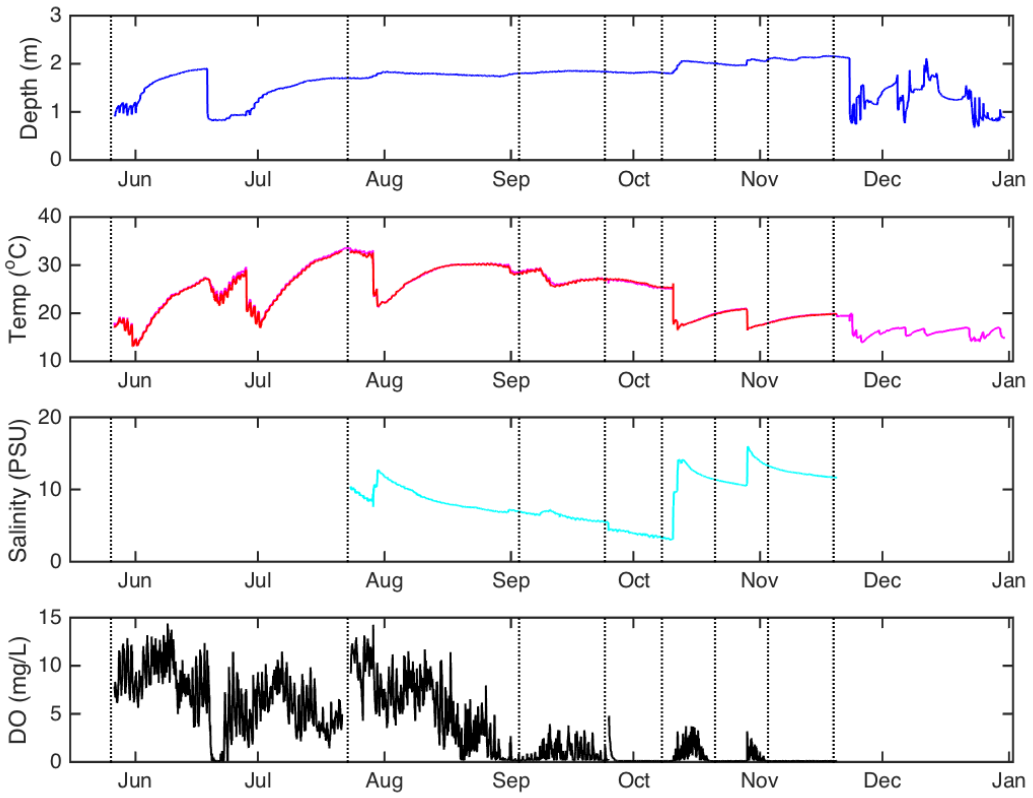

Figure 7. Time-series data in 2015 for Waddell Estuary at site B-3, just east of Highway-1: depth of sensor (i.e., water level), temperature, salinity and dissolved oxygen. Sensors were fixed with water property sensors 1 m off the bottom. The jump in oxygen values when the sensor was swapped in late July is likely due to a small change in depth of the sensors, closer to the oxygen maximum. Vertical dashed lines indicate times at which profile data were collected. Sensors were recovered on 19 November in anticipation of heavy rains.

During the perched conditions in late May, a saltwater layer was observed at all sites, beneath a ¼-m deep freshwater layer. In the halocline, between ¼ and ½ m, oxygen levels were high (~13mg/L) and water was warm (20^{o}C). At the most landward transect E, low oxygen levels were observed below the thermocline (minimum of ~3mg/L at 0.6m) as well as higher chlorophyll levels (10-20μg/L). A brief anoxic event was observed at the sensors following the breach in mid-June.

Similarly, a saltwater layer was trapped following the seasonal closure at the beginning of July (Figure 8). By 23 July, a 1 m thick freshwater layer has developed, overlying saline water that increased linearly in salinity (maximum salinity ~24 at bottom on transect B, ~2 m below surface). This lower layer was super-heated (maximum temperature ~ 34^{o}C at depth on transect B), exhibited higher chlorophyll (5-20 μg/L within 0.5m of bed), and oxygen levels were super-saturated in the upper halocline (maximum ~17 mg/L at -0.6m on transect B). The fixed sensors at B-3 were in the lower halocline, showing temperatures > 30^{o}C and high oxygen levels, which exhibit some day-night fluctuation.

The intrusion of seawater at the end of July, was observed as an increase in salinity, decrease in temperature and decrease in oxygen. Through August this lower layer was trapped and not replenished, resulting in a slow decline of oxygen levels at the sensor – becoming anoxic in early September. A small overwash and/or mixing event in early September appears to have ventilated the bottom layer, with low levels and day-night fluctuations continuing through September. Profile data show the persistence of the saltwater layer in the lower water column – only observed on transects A and D on 3 September, but at all stations on 24 September after the overwash event. Chlorophyll levels were higher in late September, presumably due to phytoplankton imported from the sea during the overwash event. On 24 September anoxic levels were observed near-bottom where light is low – and this was observed again in October and November profile data (with anoxia at shallower levels on transect D and E where shading of the channel reduced light penetration). However, there were other ventilation events due to seawater inflow during overwash events in mid-October and again in late October – and a weakening of stratification associated with mixing of saline waters into the surface layer (Figure 8).

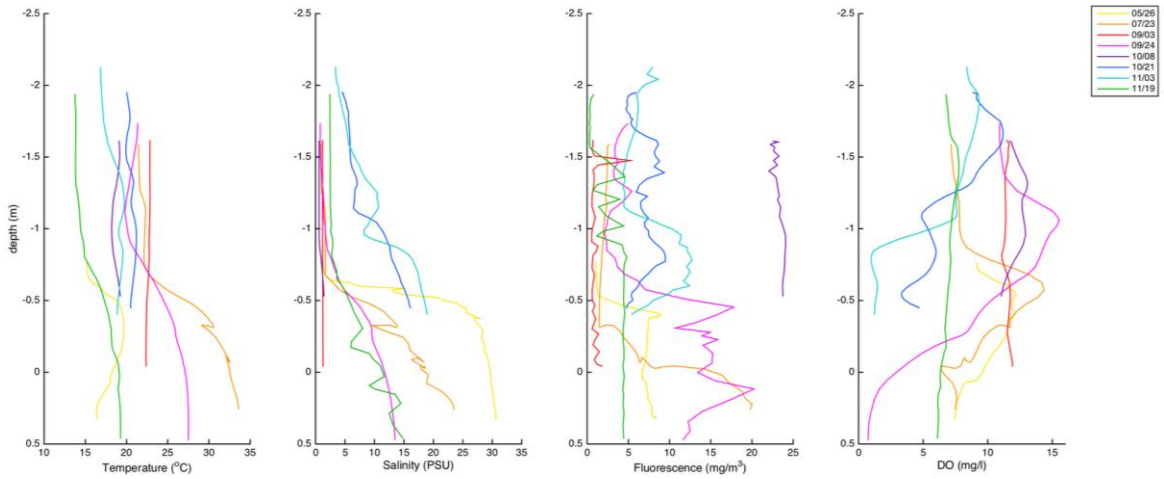

Figure 8. Profile data for temperature, salinity, chlorophyll fluorescence and dissolved oxygen at station B-3 in Waddell Estuary. Data are shown for 8 dates in 2015, with a focus on the late summer and fall when warm and hypoxic conditions are expected: 26 May, 23 July, 3 September, 24 September, 8 October, 21 October, 3 November and 19 November. Profiles have been adjusted vertically to account for changes in water level between survey dates – zero on the depth axis refers to the height of the water-level sensor (data plotted in Figure 7).

Laguna Creek Estuary

The Laguna Estuary is small (<1 km in length) and entirely west of Highway-1. The seasonal closure started on 1 June (Figure 9) and persisted until early December; this was preceded by a brief spring closure (3-14 May) and shoaling of the mouth channel without closure during big waves 30-31 March, 18-19 April, and 1-4 May. When instruments were deployed on 12 March a trickle outflow was observed, but prior to and following this day it appears that the mouth state was highly variable, with daily closure/perching and breaching during spring tides evident in the water level record (e.g., 23-25 March, 5-7 April, 21-23 April and 16-19 May). During neap tides the lagoon water level remained low and the water column in the lagoon was very shallow (< 1 m in most places) – however, some tidal seawater intrusions are evident as cold-water pulses at the time-series sensor. Stronger seawater intrusions are seen in salinity data during spring tides in mid-May (immediately after the breach) and at the end of May (before and during the closure). Following both closures (3 May and 1 June), the water level in the lagoon rises rapidly due to wave overwash during spring high tides – and the lagoon is filled with mostly seawater.

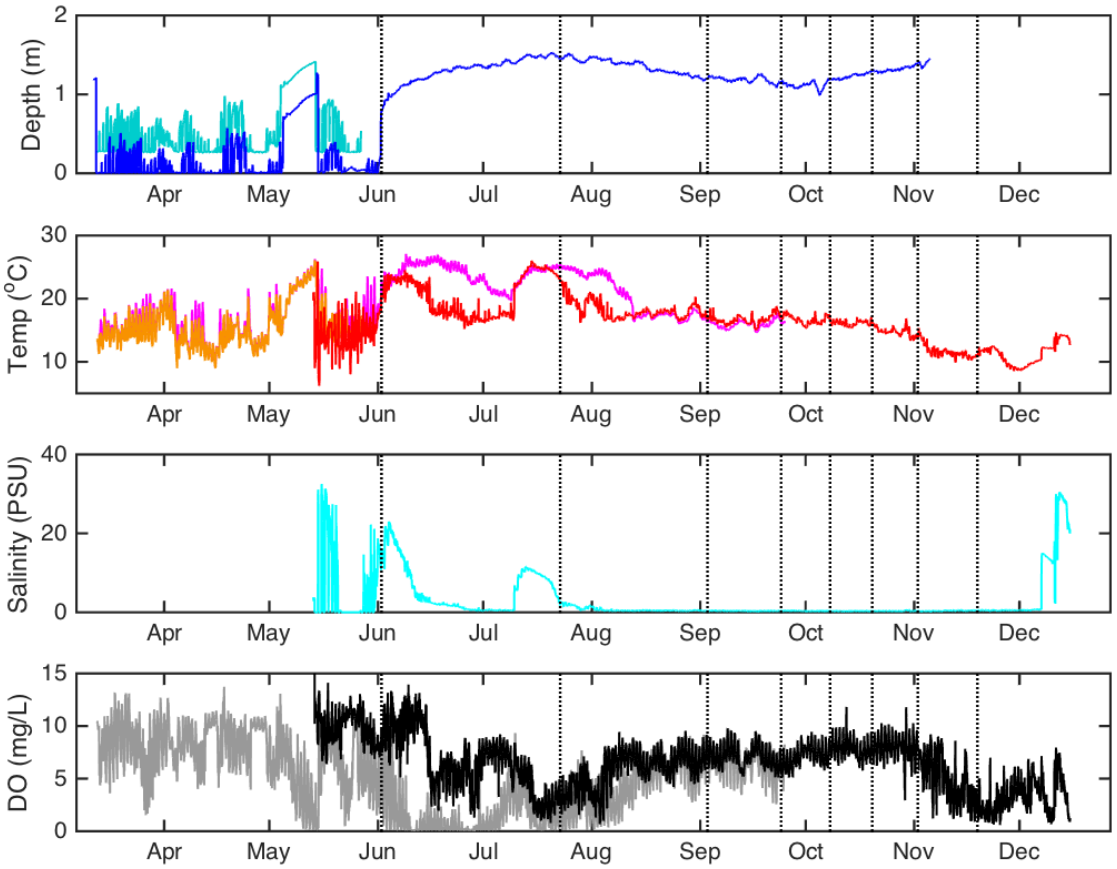

Figure 9. Time-series data in 2015 for Laguna Estuary, initially at site E-2 (turquoise line for depth, magenta and orange lines for temperature, grey line for oxygen) and later with overlap at site D-1 (blue line for depth, red line for temperature, turquoise line for salinity, black line for oxygen): depth of sensor (i.e., water level), temperature, salinity and dissolved oxygen. Sensors were fixed about ½m off the bottom. Vertical dashed lines indicate times at which profile data were collected.

After closure and once overwash is completed, the saltwater layer in the lagoon exhibited salinity ~22 and temperature ~22ºC (Figure 9; also profiles for 2 June in Figure 10). This layer is immediately eroded by mixing, and salinities decrease; but radiative heating continues as long as the salinity stratification is strong enough to preclude strong vertical mixing (maximum of 26ºC). By mid-June the saltwater layer is weak and eventually absent in early July, with vertical mixing and temperatures dropping to 20ºC. In mid-July (9-12 July) a new seawater intrusion occurs, with non-zero salinity persisting at the sensor through July – and evident in profile data for 23 July (Figure 10). From August to early December salinity is zero, indicating a well-mixed freshwater lagoon. Water levels drop through August and September, then start to rise in October with rain and inflow. The lagoon is breached in early December and seawater intrusions are observed at the sensors.

Low oxygen concentration is observed at the time-series sensor during stratification periods, when sub-surface water is retained. This effect is mild during neap tides in March-April (e.g., 25

March) and May, and strongest about a week after mouth closures and seawater intrusion in July – nocturnal anoxic conditions were observed at the more shaded sensor at E-2 in early May, early June and mid-July (Figure 9). As stratification weakens in mid-June and late July, oxygen is replenished at depth – similar to what was observed in Scott Creek estuary by Nylen (2014). Anoxia is also ended by ventilation due to seawater inflow (mid-May, early July).

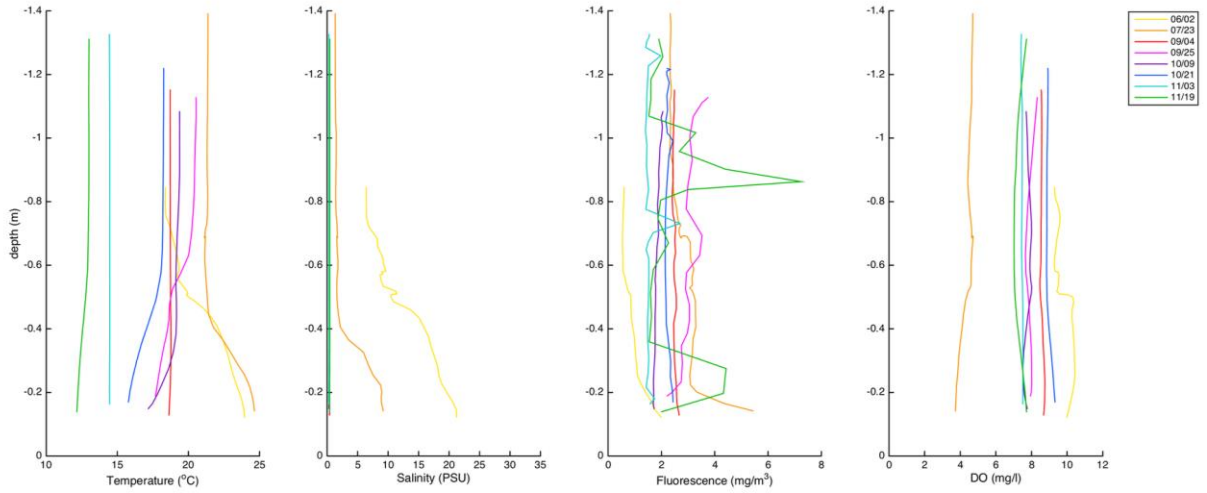

Figure 10. Profile data for temperature, salinity, chlorophyll fluorescence and dissolved oxygen at station D-1 in Laguna Estuary. Data are shown for 8 dates in 2015, with a focus on the late summer and fall when warm and hypoxic conditions are expected: 2 June, 23 July, 4 September, 25 September, 9 October, 21 October, 3 November and 19 November. Profiles have been adjusted vertically to account for changes in water level between survey dates – zero on the depth axis refers to the height of the water-level sensor (data plotted in Figure 9).

Profile data show strong stratification on 2 June following closure (bottom salinity ~25 at depth of ~1 m on transects A, C and E), with lower oxygen levels developing below the halocline (oxygen ~7 mg/L on transect E, where light availability less) and also moderate chlorophyll levels (Figure 10). Similar conditions are observed on 23 July due to recent seawater intrusion, but with more advanced deoxygenation (oxygen ~3 mg/L at depth in inner estuary). The transition to a freshwater, oxygenated lagoon in August is confirmed by profile data in September. The lagoon is well mixed and well oxygenated, but with warmer, saltier and more oxygenated water near the mouth (indicating a continuing inflow of cool freshwater near transect E). In late September and October a cold bottom layer is observed, with lower salinity indicating a hyperpycnal freshwater inflow characteristic of early winter – but oxygen levels remain close to saturation. In November, the water column is again well mixed and remains well oxygenated. Throughout the summer, chlorophyll levels were low (only occasionally exceeding 10 μg/L).

Topanga Creek Estuary

The Topanga Estuary in southern California is very small (< ½ km in length) and straddled by Highway-1. Field data were collected from November 2013 to October 2014, capturing a year with dry-winter conditions and very similar to 2015. For the entire study period the streambed remained dry, but freshwater flow persisted in the creek where it flowed over bedrock further upstream (and presumably entered the lagoon as shallow groundwater); also weak freshwater inflow originated from nearby urban development and road construction. Although the sandbar remained intact for the entire study period, lagoon water appears to have seeped out through the sandbar maintaining a steady water level in the lagoon (variation of ~½ m over a year, Figure 11).

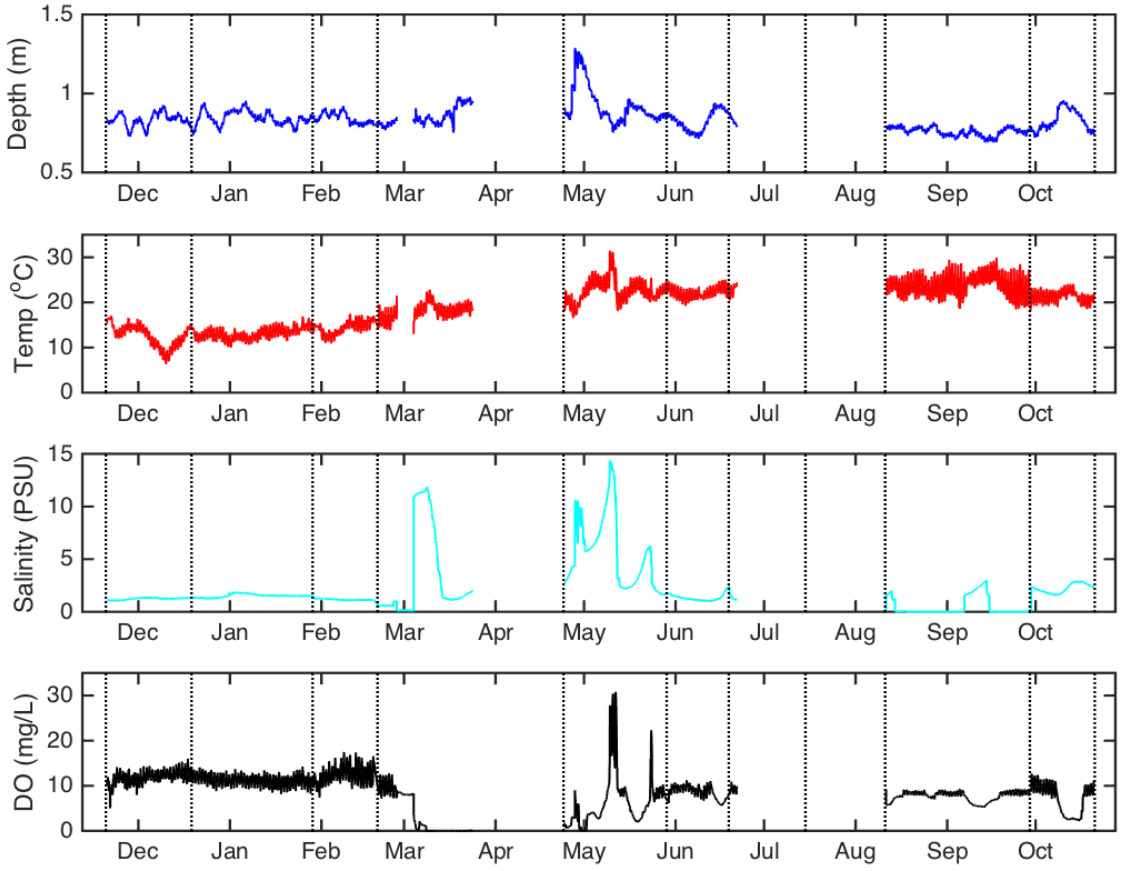

Figure 11. Time-series data in 2014 (starting November 2013) for Topanga Estuary at a site on the west edge of Highway-1 bridge (near station E-1): depth of sensor (i.e., water level), temperature, salinity and dissolved oxygen. Sensors were fixed about ⅓ m off the bottom. Vertical dashed lines indicate times at which profile data were collected. Sensors were recovered on 21 October in anticipation of rain.

Through the winter, lagoon salinity remained low and oxygen levels near saturation with a weak day-night fluctuation. On 27 February and again on 3 March, brief dramatic drops in water level indicated short-lived breaching events, although the mouth closed again immediately. During the

second event, seawater intruded and salinity at the sensor increases to 12 while temperature drops from 22 to 14°C. During the following days, oxygen dropped to zero and remained anoxic while the stratification persisted through March. Although no time-series data are available in early April, profile data from 24 April show a saltwater layer below 0.4m with salinity ~15, but without hypoxia or anoxia at depth (Figure 12). However, by the end of the month, anoxic conditions were again observed at the sensor site (Figure 11). In mid-May and late May, oxygen values are seen to rise to super-saturated values at the sensor concurrent with peaks in salinity, which were presumably associated with seawater inflow; although missed by infrequent profile data in Topanga, hyper-oxic conditions are often observed in the halocline in comparable estuaries (e.g., Waddell). In profile data from May, June and July, the lagoon conditions were well mixed and waters were well oxygenated. Profile data obtained on 11 August again show a saltwater layer starting below 0.4 m and with bottom salinity ~13 (and up to 20 at deeper sites where bottom depth is ~0.7 m). Although lower than surface values, oxygen values in this deeper layer were close to saturation (~8 mg/L). Chlorophyll was high throughout the water column >20 µg/L. Profile data in September and October showed a return to mixed conditions and well-oxygenated water at all depths (Figure 12).

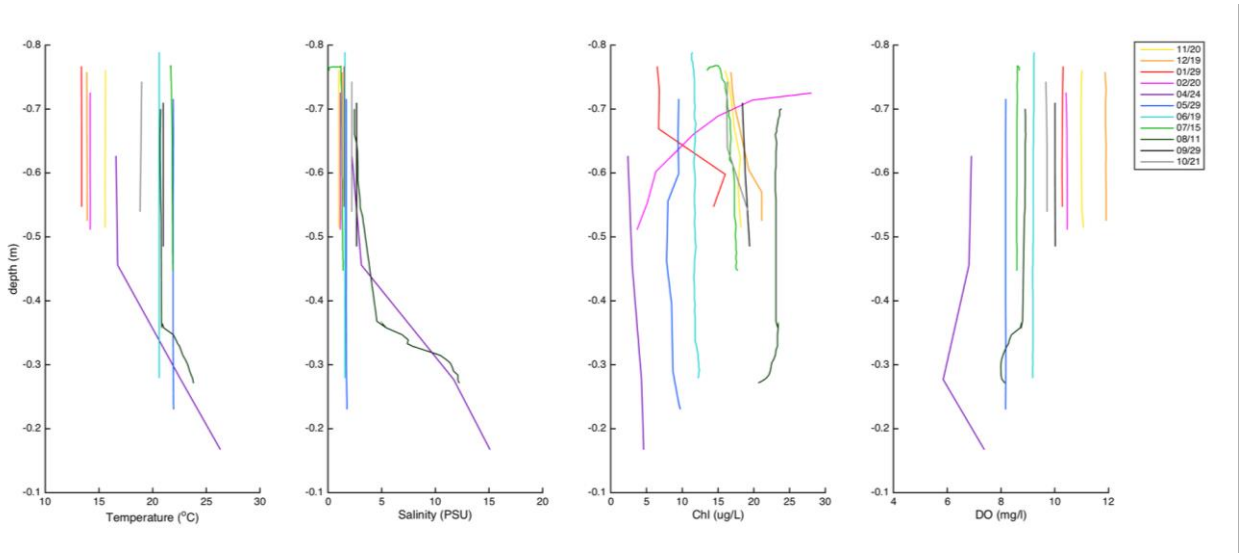

Figure 12. Profile data for temperature, salinity, chlorophyll fluorescence and dissolved oxygen at station A-2 in Topanga Estuary. Data are shown for 11 dates in 2013/14: 20 November, 19 December, 29 January, 20 February, 24 April, 29 May, 19 June, 15 July, 11 August, 29 September, and 21 October. Profiles have not been adjusted vertically to account for changes in water level between survey dates.

San Onofre Creek Estuary

The San Onofre Estuary in southern California is also very small (<½ km in length) and west of Highway-1, although crossed by a railway bridge. As with Topanga, the streambed was dry for the entire study period, but shallow groundwater inflow is expected given that there is a flow in

the creek upstream of the sandy coastal plain. This inflow is corroborated by the persistent near-zero salinity observed in the lagoon from November 2014 to August 2015 (Figure 13). The absence of tidal variability in water level, temperature or salinity in the lagoon confirms the isolation of the lagoon from the ocean during the monitoring period. Only one strong stratification period was observed (profile data on 23 December 2014), with a saltwater layer below 0.7 m and bottom salinity ~8, and one weak stratification period (profile data on 16 October 2014) when the whole water column was saline (prior to data shown in Figure 13). At other times, the lagoon was more comparable with a freshwater wetland. Lagoon waters warm from 10°C in winter to 25°C in late summer.

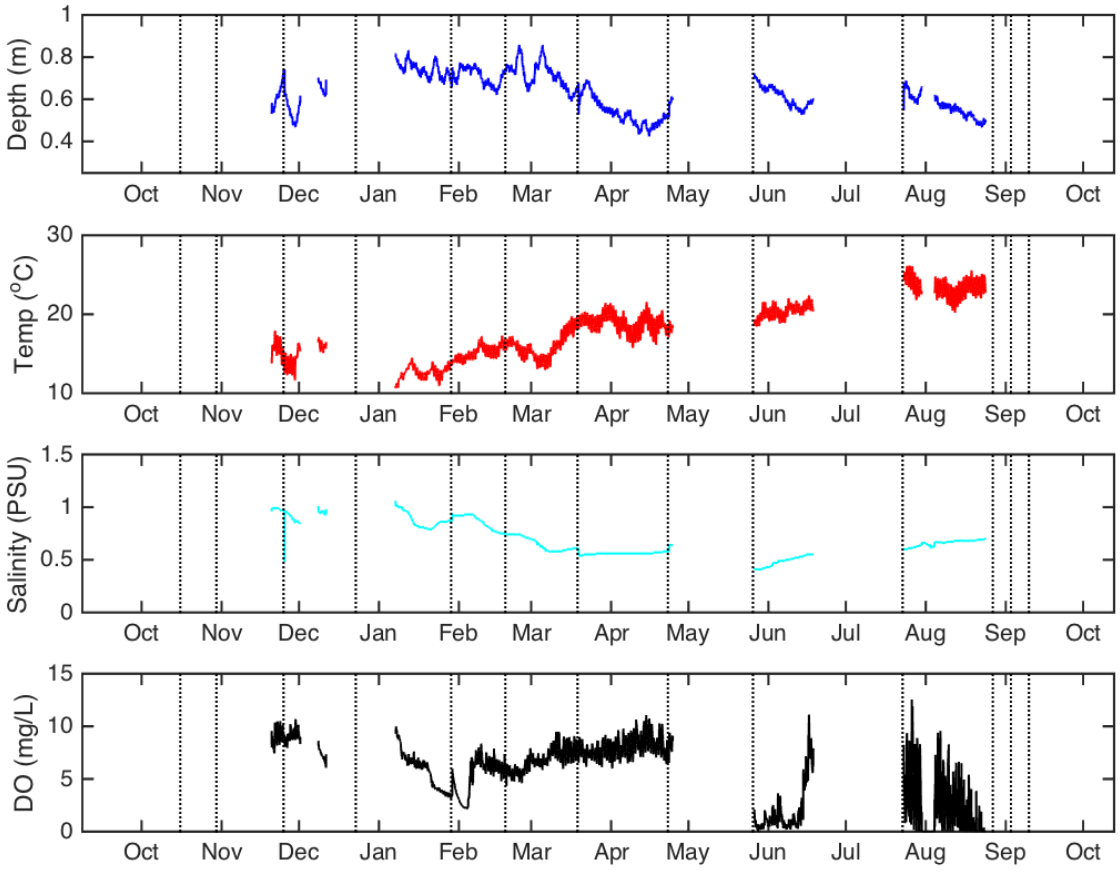

Figure 13. Time-series data in 2015 (starting November 2014) for San Onofre Estuary at site on west edge of railway bridge (near B-3): depth of sensor (i.e., water level), temperature, salinity and dissolved oxygen. Sensors were fixed about ⅓ m off the bottom. Vertical dashed lines indicate times at which profile data were collected. Sensors were recovered on 27 August.

Oxygen levels are close to saturation much of the year, owing to a well-mixed water column with strong air-water oxygen fluxes. However, in profile data from 23 December, oxygen levels are low and drop to ~3 near-bottom at several sites. Although well mixed, lower oxygen values were also seen in profile data from 26 May, 27 August and 10 September (levels of 4-5 mg/L), although not at the site for which data plotted in Figure 14. In addition, the time-series data show

declining levels in late January and early February, although briefly interrupted by a ventilation event at the end of January (increase in oxygen coincided with a small increase in salinity and an increase in water level). More marked is the period of hypoxia/anoxia in late May and early June during dropping water level in the lagoon.

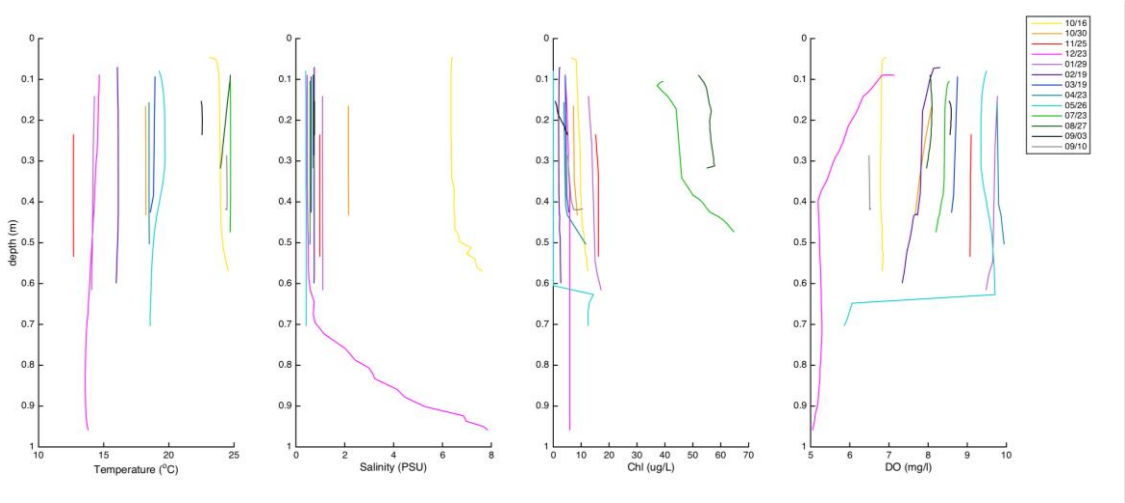

Figure 14. Profile data for temperature, salinity, chlorophyll fluorescence and dissolved oxygen at station B-2 in San Onofre Estuary. Data are shown for 13 dates in 2013/14: 16 October, 30 October, 25 November, 23 December, 29 January, 19 February, 19 March, 23 April, 26 May, 23 July, 27 August, 3 September, and 10 September. Profiles have not been adjusted vertically to account for changes in water level between survey dates.

Temporal Trends in Phytoplankton Biomass (*chl-a*)

Across ICOLL estuaries, (phytoplankton) *chl-a* was generally low throughout most of the study period, with the 75th percentile of median discrete *chl-a* ~ 20 mg m^{-3}. For ICOLL estuaries, whose hydrology is lacustrine during periods of closed tidal inlet conditions, no directly comparable set of *chl-a* endpoints can be found in the literature. Median chl-a of < 20 mg m^{-3} is consistent with estuaries of low-moderate eutrophication risk, according to the NOAA ASSETS framework (Bricker et al. 2003), though this framework is more appropriate for large enclosed estuaries and bays. Use of the concept of a "summer time median or mean" for ICOLLs has similar applicability for lake water quality criteria and those numbers can be compared to our study findings, with ample caveats. Yuan et al. (2014) found low probability of exceeding cyanotoxin concentrations (< 5%) at *chl-a* of 14 mg m^{-3} in lakes sampled one-time through the US EPA National Lakes Assessment. Walker (1985, 1987) found that in reservoirs ~20 mg m^{-3} algal blooms will occur 15-20 percent of the time, and suggested a threshold of 20 mg m-3 as the maximum allowable level consistent with full support of contact recreation use. WHO guideline for protection of human health against cyanobacteria blooms designates 30 mg m^{-3} as the thresholds for moderate or greater risk (WHO 1993).

Regional differences in *chl-a* were apparent. The mean of discrete *chl-a* samples found in the North and Central Coast was significantly lower than that of South Coast ICOLLs (p-value – 0.002), where peak concentrations reached 58 – 161 mg m^{-3} *chl-a* (Table 2, Figure 15). *Chl-a* concentrations as captured by *in situ* fluorescence show the ICOLLs to be generally characterized by episodic blooms that occur periodically throughout the season for a week or two, rather than one extended productive period. The only exception to this was in San Onofre Creek Estuary, where a cyanobacteria bloom coincided with the drawdown of estuarine surface water at the end of the dry season, further compounded by high water temperatures and no tidal flushing, a consequence of an extended period of drought (Figures 15-17).

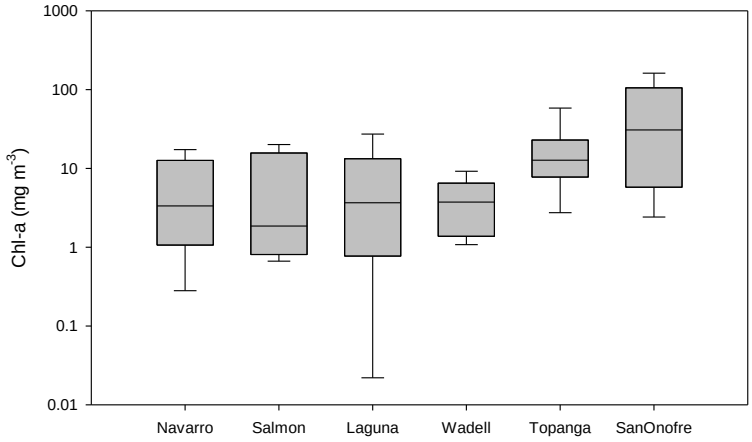

Figure 15. Box and whiskers plot of growing season phytoplankton *chl-a* in the six ICOLL reference estuaries.

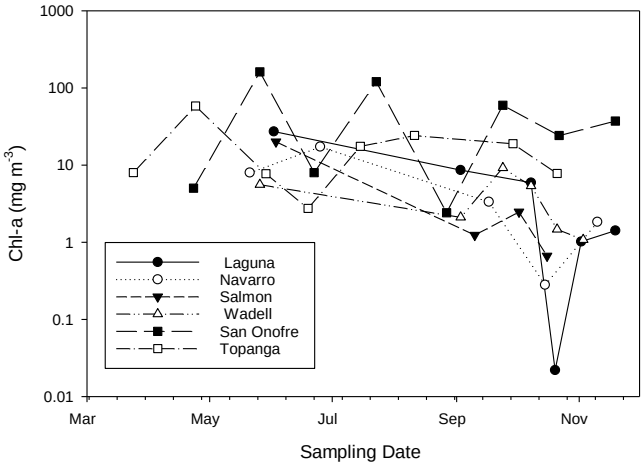

Figure 16. Time series of discrete *chl-a* measurements in the six ICOLL reference estuaries during the growing season.

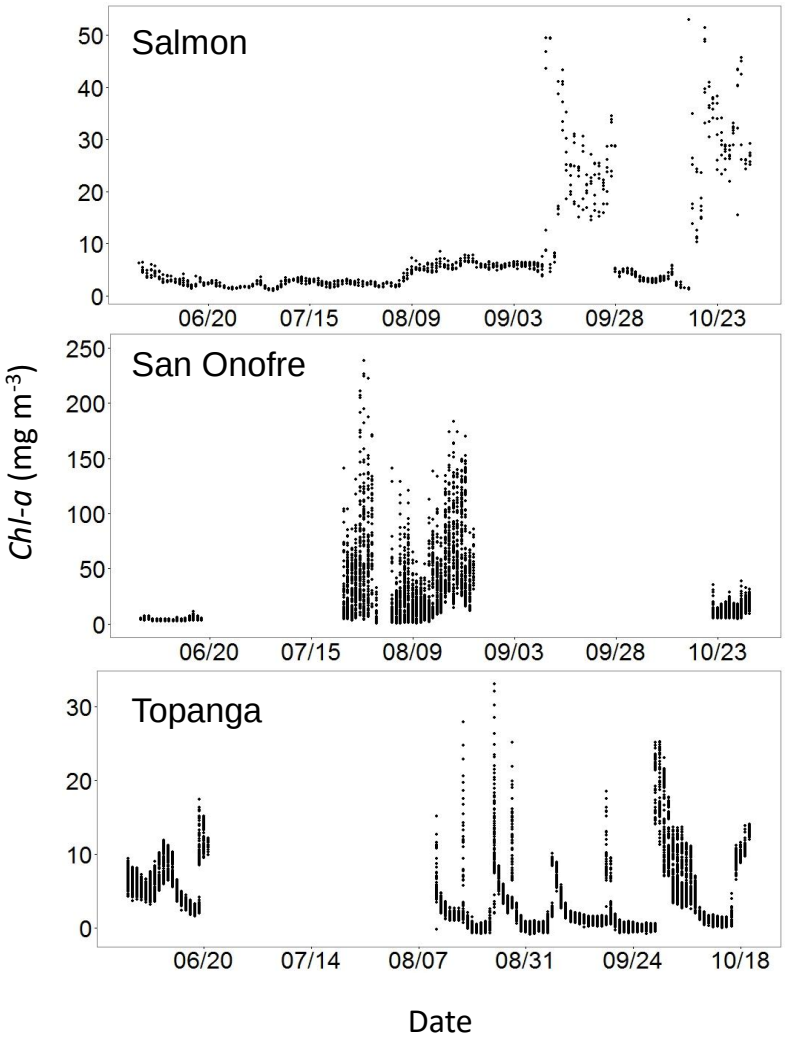

Figure 17. Time series of continuous *chl-a* fluorescence in ICOLL reference estuaries during the growing season.

Temporal Trends in Macroalgal Biomass

All estuaries were generally low in macroalgae across all regions, with the 75th percentile of peak biomass across estuaries ~ 7 g dw m^{-2} (Table 2). This peak biomass is consistent with previous estimates of reference levels of algal biomass on intertidal flats in California estuaries in an open tidal inlet condition (< 13 g dw m^{-2}; Sutula et al. 2014). It is also consistent with a consensus of experts of European Union estuaries that reference levels of macroalgal are generally < 10 g dw m^{-2} (Scanlan et al. 2007, Sutula et al. 2016). The primary producer community in two of the six estuaries were dominated by phytoplankton and had no measurable macroalgal biomass at any time (Laguna Creek and Topango Canyon). Of those remaining, macroalgae in North and Central Coast ICOLLs peaked late season (September – October), while macroalgae in South Coast ICOLLs did not exhibit a single, strong seasonal peak (Figure 18).

Table 2. Growing season maximum macroalgal biomass in minimally disturbed "reference" ICOLL estuaries.

Estuary	Macroalgal Biomass		Phytoplankton Blomass (mg *chl-a* m^{-3})		
	Mean	Range	Mean	Range	75th Percentile
Navarro River	2.8	0 – 11.3	6.2	0.3 - 11.3	6.6
Salmon Creek	1.1	0 – 5.4	6.1	0.7 - 5.4	14.1
Laguna Creek	0	0	7.4	0.02 - 27.3	6.6
Waddell Creek	1.3	0 - 3.1	4.1	1.0 - 9.2	5.6
Topanga Creek	0	0	19.1	2.7 - 58.5	22.9
San Onofre Creek	0.4	0.08 - 1.9	52.5	2.4 – 161.9	98.1

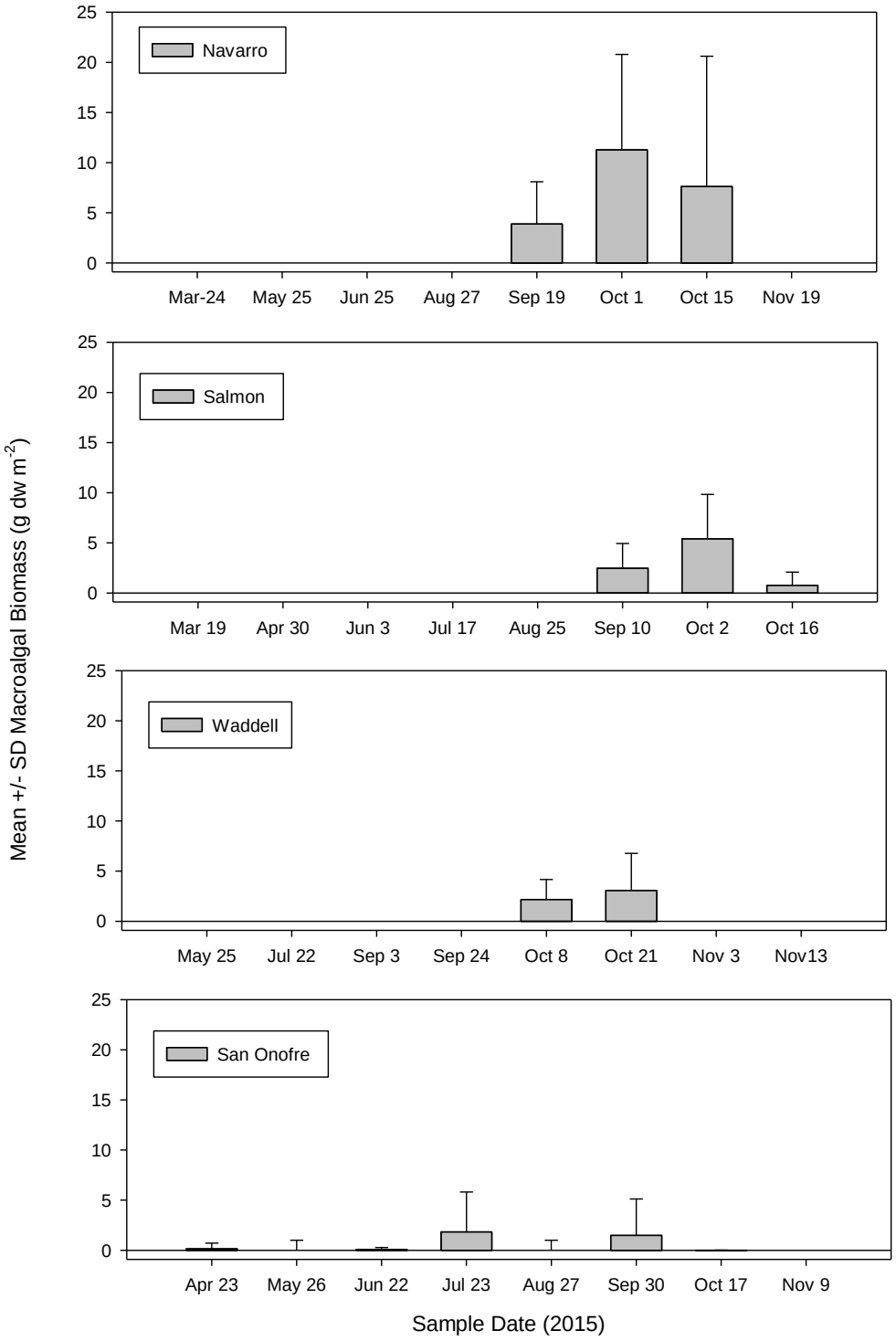

Figure 18. Mean ± SD macroalgal biomass from 15 sampling points within estuary by sampling period. No macroalgal biomass was measured on dates in which no data are shown. No macroalgal biomass was measured in Topanga and Laguna Creeks.

Natural Background Concentrations of DO Relative to Existing Benchmarks

Hypoxia (i.e., low DO concentration) as a stressor differs from chemical toxicants in that it can occur naturally. Hypoxia is a consequence of the balance of atmospheric oxygen diffusion to surface waters, the in situ production of oxygen by primary producers during daylight hours, and the consumption of oxygen via respiration, decaying organic matter and other biogeochemical processes that consume oxygen within surface waters and sediments (Diaz 2001). Examination of the continuous DO concentration data in ICOLL reference estuaries illustrates hypoxia is a common occurrence in ICOLL bottoms waters and most estuaries spend a considerable amount of time below existing water quality objectves. Navarro River estuary was the only ICOLL in which DO was consistently above 7 mg L^{-1}.

The response of aquatic organisms to low DO will depend on the intensity of hypoxia, duration of exposure, and the periodicity and frequency of exposure (Rabalais et al. 2002). In general, these adaptations are well-developed in epibenthic and burrowing animals that commonly experience hypoxia, but poorly developed in animals that inhabit well-oxygenated environments such as the upper water column. However, these are all short-term strategies and will not enable the organism to survive during extended hypoxic periods (e.g., 4.0 mg L^{-1} for salmonids and 2.8 mg L^{-1} for non-salmonid organisms). Sublethal effects may also occur; for example, reduced motor activity from mild hypoxia may make the animal more vulnerable to predators, or decrease its growth or reproduction. In this study, four of six estuaries experienced hypoxia (< 2.8 mg L^{-1}) in bottom waters greater than 10% of the time, with some ranging up to 50% of the time (Table 3). With the exception of Topanga and San Onofre, which experienced extended period of hypoxia lasting > 24 hours, hypoxia in ICOLL reference estuaries was of high frequency but generally short duration, tending to last several hours (Figure 19). Extended DO records in South Coast estuaries illustrate that hypoxia occurrence have considerable seasonal (San Onofre) and interannual (Topanga) variability.

Table 3. Percent of time DO in each of the reference estuaries falls within ranges associated with DO benchmarks. Data are shown for bottom waters during the April 1- October 31 period unless otherwise designated.

Percent of Time Within Each DO Range	Navarro	Salmon	Waddell	Laguna (Bottom)	Laguna (Surface)	Topanga (2014)	Topanga (2009)	San Onofre (Summer)	San Onofre (Winter)
7+	92.9	81.3	27.0	53.1	42.7	63.6	18.1	32.4	42.4
5.8-7	4.7	2.0	8.4	22.0	31.3	10.2	14.7	4.6	33.8
5-5.8	0.9	1.3	4.0	8.5	14.2	6.5	10.3	3.1	8.1
4-5	0.7	1.9	4.8	5.5	7.2	3.0	10.6	3.5	4.9
2.8-4	0.6	2.7	7.7	5.8	3.2	5.9	16.1	5.9	7.7
0-2.8	0.2	10.8	48.1	5.0	1.4	10.8	30.2	50.6	3.2

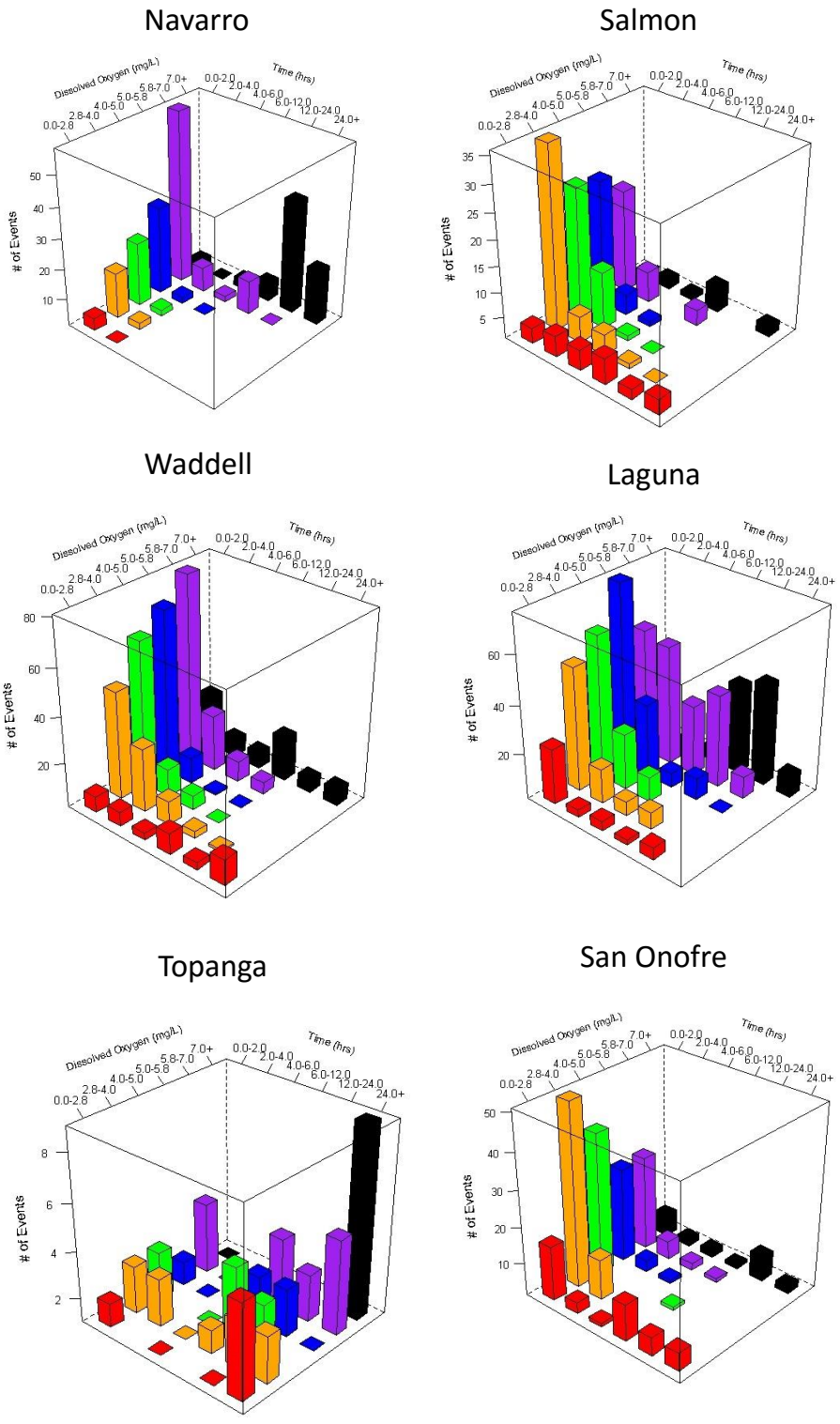

Figure 19. Frequency and duration of DO concentrations in each of the six ICOLL estuaries. Y-axis represents the number of events in which DO in a specified concentration range lasted on timescales of hours to days.

Using the State Water Board policy guidance on listing of State surface waters (7-day mean of daily DO minima), only five of six would be listed as impaired based on a WARM beneficial use WQO of 5 mg L^{-1}; all would be listed as impaired based on a COLD beneficial use WQO of 7 mg L^{-1} (Table 4). DO data distributions illustrate ICOLL estuaries spend as much as 92% of their time below 5 mg L^{-1} during the "growing" season. Given this result, it may be more expedient for Regional Water Boards to consider reviewing the science supporting interpretation of existing DO objectives, in favor of approaches such as the Virginia Province approach (USEPA 2000), which combines both DO concentration and exposure time into the criteria, allowing for deviation from chronic criteria at frequencies that are still protective at the population level (Batuik et al. 2009).

Table 4. Statistics representing attainment of existing water quality objectives. The 10th percentile of 7-day average of daily DO minima represent Water Board guidance for determination of impairment. The percentiles at which the 7-day average of daily DO minima reach WQO representative of WARM (5 mg L^{-1}) and COLD (7 mg L^{-1}) beneficial uses are given. The value of 7 mg L^{-1} represents a freshwater target, while 5.8 mg L^{-1} represents a comparable target at full strength marine salinities.

Estuary	10th percentile (mg L^{-1})	Percentile at 5 mg L^{-1}	Percentile at 5.8 mg L^{-1}	Percentile at 7 mg L^{-1}
Navarro	5.2	9.6	13.4	29.8
Salmon	1.0	18.1	19.2	20.7
Waddell	0.1	72.4	80.3	90.8
Laguna (Bottom)	2.5	28.3	53.0	82.5
Laguna (Surface)	3.4	41.6	73.5	92.2
Topanga (2014)	3.3	20.2	26.6	47.6
Topanga (2009)	0.8	91.5	95.0	97.9
San Onofre (Winter)	3.5	20.2	41.5	81.9
San Onofre (Summer)	0.1	73.3	74.7	81.3

Estuarine Ambient Nutrient Concentrations

Eutrophication in estuaries is strongly linked to an increase in nutrient and organic matter inputs (Pinckney et al. 2001); ambient TN and TP can often explain the variability phytoplankton biomass (Boynton et al. 2008, Smith 2006, Madden et al. 2010). Overall, TN and TP concentrations were highly variable but low in most ICOLL reference estuaries, though San Onofre Creek Estuary was generally twice the concentration of TN and TP as the other five estuaries (Table 5), consistent with trends in phytoplankton biomass. Dissolved inorganic nitrogen (NO3+NO2, NH4) was generally less than 10% of TN; DON was the dominant fraction of TN. In contrast, the majority of TP was phosphate. Though highly variable, in general the concentrations in streams and rivers that discharge into the ICOLL reference estuaries was lower than TN and TP within the estuary itself (Table 6).

Table 5. Growing season ambient N and P concentrations in minimally disturbed "reference" ICOLL estuaries. All units are in mg L^{-1}.

Estuary	TN		TP		NO3+NO2		NH4		PO4	
	Mean (Range)	75thile	Mean (Range)	75thile	Mean (Range)	75thile	Mean (Range)	75thile	Mean (Range)	75thile
Navarro River	0.18 (0.01-0.34)	0.29	0.04 (0.01-0.32)	0.02	0.005 (0.003-0.01)	0.006	0.008 (0.005-0.01)	0.01	0.005 (0.003-0.01)	0.006
Salmon Creek	0.34 (0.20-0.62)	0.45	0.20 (0.03-0.30)	0.11	0.005 (0.002-0.28)	0.043	0.009 (0.004-0.024)	0.009	0.13 (0.0002-0.28)	0.23
Laguna Creek	0.28 (0.12-0.51)	0.42	0.13 (0.07-0.22)	0.19	0.04 (0.007-0.09)	0.06	0.02 (0.008-0.04)	0.02	0.08 (0.01-0.17)	0.11
Waddell Creek	0.27 (0.12-0.50)	0.33	0.08 (0.04-0.19)	0.08	0.009 (0.002-0.03)	0.009	0.013 (0.006-0.04)	0.01	0.03 (0.01-0.04)	0.03
Topanga Creek	0.41 (0.24-0.62)	0.50	0.04 (0.02-0.05)	0.05	0.003 (0.007-0.08)	0.004	0.006 (0.002-0.01)	0.006	0.009 (0.003-0.04)	0.009
San Onofre Creek	1.16 (0.37-2.4)	1.4	0.28 (0.04-0.91)	0.28	0.007 (0.0007-0.002)	0.009	0.01 (0.004-0.03)	0.01	0.09 (0.01-0.44)	0.22
Median ± SD All Estuaries	0.31 ± 0.33	0.43 ± 0.38	0.09 ± 0.10	0.09 ± 0.10	0.006± 0.01	0.021± 0.004	0.01± 0.004	0.01± 0.004	0.01± 0.005	0.07 ± 0.09

Table 6. Growing season N and P concentrations in stream flow to minimally disturbed "reference" ICOLL estuaries. All units are in mg L^{-1}. San Onofre Creek has no surface water stream discharge that was sampleable. Topanga Canyon discharge was sampleable but did not reach the estuary (1 km upstream).

River or Creek	TN		TP		NO3+NO2		NH4		PO4	
	Mean (Range)	75thile	Mean (Range)	75thile	Mean (Range)	75thile	Mean (Range)	75thile	Mean (Range)	75thile
Navarro River	0.11 (0.05-0.32)	0.08	0.03 (0.01-0.07)	0.03	0.01 (0.006-0.03)	0.01	0.01 (0.006-0.03)	0.008	0.007 (0.005-0.01)	0.009
Salmon Creek	0.24 (0.05-0.51)	0.25	0.12 (0.03-0.08)	0.05	0.04 (0.01-0.08)	0.05	0.01 (0.004-0.02)	0.01	0.2 (0.01-0.04)	0.03
Laguna Creek	0.20 (0.05-0.51)	0.16	0.12 (0.08-0.23)	0.11	0.10 (0.03-0.27)	0.07	0.01 (0.005-0.02)	0.01	0.09 (0.04-0.19)	0.09
Waddell Creek	0.21 (0.12-0.33)	0.26	0.07 (0.04-0.08)	0.07	0.08 (0.02-0.16)	0.11	0.01 (0.008-0.02)	0.02	0.04 (0.01-0.06)	0.04
Topanga Creek	0.26 (0.17-0.43)	0.33	0.05 (0.03-0.09)	0.06	0.01 (0.006-0.02)	0.01	0.02 (0.002-0.02)	0.02	0.03 (0.02-0.04)	0.03
Median ± SD All Creeks	0.21 ± 0.05	0.25 ± 0.07	0.07 ± 0.04	0.06 ± 0.03	0.04± 0.04	0.05± 0.04	0.01± 0.004	0.02± 0.004	0.04± 0.007	0.03 ± 0.03

SUMMARY

The natural background concentrations of DO, macroalgal biomass (dry weight), and phytoplankton biomass (measured as *chl-a*) were documented for six "minimally disturbed" ICOLL estuaries across California during the growing season. The goals of the study were to: (1) quantify the temporal and spatial variability in macroalgal biomass, phytoplankton *chl-a*, and DO relative to established benchmarks and (2) document the relationship between these eutrophication indicators of interest and major factors that can influence their variability.

- Across ICOLL estuaries, (phytoplankton) *chl-a* was generally low throughout most of the study period, with the 75th percentile of median discrete *chl-a* ~ 20 mg m^{-3}, a results consistent with estuaries of low risk of toxic cyanobacteria blooms and with conditions that provide full support for contact recreation use.
- All estuaries were generally low in macroalgae across all regions, with the 75^{th} percentile of peak biomass across estuaries ~ 7 g dw m^{-2}, a value that is consistent with previous estimates of natural background levels of algal biomass on intertidal flats in California estuaries in an open tidal inlet condition (< 13 g dw m^{-2}).
- Hypoxia (< 2.8 mg L^{-1}) is a common occurrence in bottoms waters of ICOLL reference estuaries and most estuaries spent a considerable amount of time below existing water quality objectves. Four of six estuaries experienced hypoxia in bottom waters greater than 10% of the time, with some ranging up to 50% of the time. With the exception of Topanga and San Onofre, which experienced extended period of hypoxia lasting > 24 hours, hypoxia in ICOLL reference estuaries was of high frequency but generally short duration, tending to last several hours. Using the State Water Board policy guidance on listing of State surface waters (7-day mean of daily DO minima), only five of six would be listed as impaired based on a WARM beneficial use WQO of 5 mg L^{-1} ; all would be listed as impaired based on a COLD beneficial use WQO of 7 mg L-1 (Table 4). DO data distributions illustrate ICOLL estuaries spend as much as 92% of their time below 5 mg L^{-1} during the "growing" season. This suggests the need for improved science supporting interpretation of existing DO WQO.
- All six ICOLL estuaries were subject to the same seasonal hydrology with reduced freshwater inflow during the summer months, and associated seasonal closure of the mouth. However, the estuaries exhibited a variety of responses to closure in terms of water levels, temperature, salinity and stratification. The vertical structure and temporal variability in dissolved oxygen levels observed in these small coastal lagoons are primarily explained in terms of the physical conditions – specifically mouth closure, freshwater inflow, wave overwash, wind exposure, stratification and light penetration. The strongest and most persistent hypoxia is observed when a lower saltwater layer is trapped by a closed/perched mouth and below strong stratification. Other low-oxygen occurrences are associated with inflows (but rare and not severe) or with the minima in shallow waters that exhibit large day-night fluctuations.

REFERENCES

Bolam, S.G., T.F. Fernandes, P. Read, and D. Raffaelli. 2000. Effects of macroalgal mats on intertidal sandflats: an experimental study. *Journal of Experimental Marine Biology and Ecology* 249: 123-137.

Bricker, S.B., J.G. Ferreira, and T. Simas. 2003. An integrated methodology for assessment of estuarine trophic status. *Ecological Modelling* 169:39-60.

Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae 8*: 21-32.

CLEAP 2008. Central Coast Lagoon Ecological Assessment Project. Prepared for the California Coastal Conservancy by 2nd Nature LLC.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series* 210:223–253.

Cummins, S.P., D.E. Roberts, et al. 2004. Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. *Marine Ecology Progress Series* 266: 77-87.

Diaz, R.J. 2001. Overview of Hypoxia around the World. *Journal of Environmental Quality* 30:275-281.

Diaz, R.J. and R. Rosenberg. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioral responses of benthic macrofauna. *Oceanography and Marine Biology an Annual Review* 33:245-303.

Diaz, R.J. and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. *Science* 321: 926-929.

Froneman, P.W. 2004. Zooplankton community structure and biomass in a southern African temporarily open/closed estuary. *Estuarine, Coastal and Shelf Science* 60:125-132.

Herrera-Silveira, J.A. and S.M. Morales-Ojeda. 2010. Subtropical Karstic Coastal Lagoon assessment, Southeast Mexico: The Yucatan Peninsula Case. In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. 307-333.

Kjerfve, B. 1986. Comparative Oceanography of Coastal Lagoons. In: Estaurine variability ed. D.A. Wolfe. Academic Press. New York. 63-81.

Knoppers, B., B. Kjerfve, and J. Carmouze. 1991. Trophic state and water turn-over time in six choked coastal lagoons in Brazil. *Biogeochemistry* 14:149-166.

Largier, J. L., and S. Taljaard. 1991. The dynamics of tidal intrusion, retention, and removal of seawater in a bar-built estuary. *Estuarine, Coastal and Shelf Science* 33:325-338.

Lukatelich, R.J., N.J. Schofield and A.J. McComb. 1987. Nutrient loading and macrophytes growth in Wilson Inlet, a bar-built southwester Australian estuary. *Estuarine, Coastal and Shelf Science* 24:141- 165.

Magrann, T., M.D.A. Howard, and M. Sutula. 2015. Screening Assessment of Cyanobacteria and Cyanotoxins in Southern California Lentic Habitats. *Environmental Management and Sustainable Development* 4: 91-111.

McLaughlin, K., M. Sutula, L. Busse, S. Anderson, J. Crooks, R. Dagit, and L. Stratton. 2014. A regional survey of the extent and magnitude of eutrophication in Mediterranean estuaries of Southern California, USA. *Estuaries and coasts* 37: 259-278.

Miller, M.A., R.M. Kudela, A. Mekebri, D. Crane, S.C. Oates, et al. 2010 Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. PLoS ONE 5(9): e12576.

Nelson, W.G. 2009. The interaction of epiphytes with seagrasses under nutrient enrichment. IN: Seagrasses and Protective Criteria: A review and assessment of research Status. Office of Research and Development, National Health and Environmental Effects Research Laboratory, EPA/600/R-09/050

Nezlin, N., K. Kamer, J. Hyde, and E. Stein. 2009. Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California. *Estuarine, Coastal and Shelf Science* 82:139-151.

Nixon, S. W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia* 41: 199-219.

Nylen, B. D. 2015. Mouth closure and dissolved oxygen levels in a small, bar-built estuary: Scott Creek, California. MS thesis, University of California Davis, 92pp.

Oczkowski, A.J. and S.W. Nixon. 2010. Lagoons of the Nile Delta. In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. 253-282.

Odebrecht, C., P.C. Abreu, C.E. Bemvenuti, M. Copertino, J.H. Muelbert, J.P. Vierira, and U. Seeliger. 2010. The Patos Lagoon Estuary, Southern Brazil: Biotic Responses to Natural and Anthropogenic Impacts in the Last Decades (1979-2008). In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. 433-455.

Perissinotto, R., D.R. Walker, P. Webb, T.H. Wooldridge, and R. Bally. 2000. Relationships between zooplantkon and phytoplankton in a warm-temperate, semi-permanently closed estuary, South Africa. *Estuarine, Coastal and Shelf Science* 51:1-11.

Paerl, H.W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources *Limnology and oceanography* 42: 1154-1165.

Raffaelli, D. (1999). "Nutrient enrichment and trophic organisation in an estuarine food web." Acta Oecologica 20(4): 449-461.

Raffaelli, D., J. Limia, S. Hull, and S. Pont. 1991. Interactions between the amphipod Corophium volutator and macroalgal mats on estuarine mudflats. *Journal of the Marine Biological Association of the United Kingdom* 71:899-908.

Raffaelli, D., S. Hull, and H. Milne. 1989. Long-term changes in nutrients, weed mats and shorebirds in an estuarine system. *Cahiers de Biologie Marine* 30:259-270.

Sutula, M., K. Kamer, et al. 2004. Sediments as a non-point source of nutrients to Malibu Lagoon, California (USA). Technical Report 441. Southern California Coastal Water Research Project. Westminster, California. 83.

Sutula, M. 2011. Review of Indicators for Development of Nutrient Numeric Endpoints in California Estuaries. Technical Report 646. Southern California Coastal Water Research Project. Costa Mesa, CA.

Sutula, M., L. Green, G. Cicchetti, N. Detenbeck, and P. Fong. 2014. Thresholds of Adverse Effects of Macroalgal Abundance and Sediment Organic Matter on Benthic Habitat Quality in Estuarine Intertidal Flats. *Estuaries and Coasts*. DOI: 10.1007/s12237-014-9796-3.

Sutula, M., K. Kamer, J. Cable, H. Colis, and W. Berelson. 2006. Sediments as a source of nutrients to Upper Newport Bay. Technical Report 482. Southern California Coastal Water Research Project. Costa Mesa, CA.

Sutula, M., Baily H., Poucher S. 2013. Science supporting dissolved oxygen objectives in California Estuaries. Technical Report 684. Southern California Coastal Water Research Project. Costa Mesa, CA.

Sutula, M., P. Fong, and L. Green. 2016b. Synthesis of Science and Proposed Framework for Assessment of Eutrophication in Tidal Flats and Unvegetated Shallow Subtidal Habitat of California Estuaries. Technical Report XXX. Southern California Coastal Water Research Project. Costa Mesa, CA.

Teixeira da Silva, E. and M.L. Asmus. 2001. A dynamic simulation model of the widgeon grass Ruppia maritime and its epiphytes in the estuary of the Patos Lagoon, RS, Brazil. *Ecological Modelling* 137:161- 179.

Thomsen, M.S. and K. McGlathery. 2006. Effects of accumulations of sediments and drift algae on recruitment of sessile organsims associated with oyster reefs. *Journal of Experimental Marine Biology and Ecology* 328:22-34.

Tubbs, C. and J. Tubbs. 1980. Wader and Shelduck feeding distribution in Langstone Harbour, Hampshire. *Bird Study* 27:239-248.

Viaroli, P., M. Baroli, I. Fumagalli, and G. Giordani. 1997. Relationship between benthic fluxes and macrophyte cover in a shallow brackish lagoon. *Water, Air and Soil Pollution* 99:533-540.

Wennhage, H. and L. Pihl. 1994. Substratum selection by juvenile plaice (Pleuronectes platessa L.): impact of benthic microalgae and filamentous macroalgae. *Netherlands Journal of Sea Research* 32:343-351.

Whitfield, A.K. 1988. The role of tides in redistributing macrodetrital aggregates within the Swartvlei estuary. *Coastal and Estuarine Research Federation* 11(3):152-159.

Young, D.R., P.J. Clinton, H. Lee II, D.T. Specht, and T.C. Mochon-Collura. 2009. Aerial measures of estuarine intertidal and shallow subtidal *Zostera marina* coverage. PP 158-172. IN: Lee II, H. and C.A. Brown (eds.) Classification of regional patterns of environmental drivers and benthic habitats in Pacific Northwest estuaries. US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Western Ecology Division. EPA/600/R-09/140

Zaldivar, J.-M., A.C. Cardoso, et al. 2008. Eutrophication in transitional waters: an overview. *Transitional Waters Monographs* 1: 1-78.