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Analysis of hydrobiological responses to anthropogenic and natural influences in a lagoon system in the Gulf of California

by

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Abstract

The hydrological characteristics and the rate of organic matter supply from coastal lagoons help to understand their responses to nutrient input by anthropogenic and natural sources. The aim of this study was to determine the hydrologic and trophic status of the El Rancho-Empalme lagoon system in a semiarid region in the Gulf of California, Mexico. This lagoon system consists of two geomorphological subsystems which are affected by nutrient inputs from shrimp farm effluents and coastal upwelling. Sampling was conducted over the course of one year and included measurements of temperature, salinity, and dissolved oxygen, surface water grab samples to analyze nitrite, nitrate, ammonia, orthophosphate, and chlorophyll *a*. The trophic status was assessed using the TRophic IndeX (TRIX). The subsystems El Rancho and Empalme had a similar hydrological behavior throughout the year, reflecting a good exchange of water, materials and energy. The TRIX index showed oligotrophic state during spring, summer and autumn, and a mesotrophic state in winter. Nutrient inputs from shrimp farm effluents were not responsible for trophic status increases, however, coastal upwelling in the region plays an important role in the growing rate of seasonal supply of organic matter to these coastal lagoons.

Key words: subtropical coastal lagoons, hydrobiology, trophic state, Gulf of California

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Introduction

Coastal lagoons act as buffers at the land-sea interface, providing valuable ecosystem services such as nutrient recycling, decomposition of organic matter and removal of pollutants. Lagoons are the regions of restricted exchange, subject to anthropogenic impact that may result in problems such as eutrophication which is a process defined as an increase in the rate of supply of organic matter to an ecosystem (Nixon 1995). The eutrophication in coastal water bodies have had many contributions to specific topics about causes, nutrient recycling, limiting nutrients, reference conditions, association with other types of impact (*e.g.* climate change), strategies for monitoring, modeling and adaptive management (Cloern 2001; Howard & Marino 2006; Andersen & Conley 2009). An important observation is to consider eutrophication not only as a simple problem of pollution by nutrients, but actually as an increase in the supply of organic matter to a given ecosystem, this implies that eutrophication is primarily a change in the energy base that can spread through the ecosystem in various ways and produce a variety of changes (Nixon 2009). Estuaries and coastal lagoons are susceptible to eutrophication because:

1) they are usually rich in nutrients from the atmospheric deposition, rivers, runoff from rainfall, groundwater, sea and internal recycling,

2) they are recipients of nitrogen and phosphorus loads from anthropogenic sources, and

3) their geomorphic characteristics result in varying water exchange rates, but their connection with oceans is usually limited (Scavia & Liu 2006; Painting et al. 2007).

Recent evidence of complex responses of coastal ecosystems to nutrient reduction requires management goals and policies to be reconsidered to include nonlinear responses and thresholds (Duarte 2009).

Different hydrologic responses of estuaries and coastal lagoons are controlled by various factors associated with ecogeomorphology (salt and water balance, residence time, tidal range, climate, hydrology, geomorphology, geologic origin) and their natural and anthropogenic sources of fresh water and nutrients (Wolfe & Kjerfve 1986; Eyre 1998).

In this context, the hydrological characteristics and the rate of supply of organic matter from estuaries and coastal lagoons are fundamental to understand the responses of these ecosystems to the nutrient input from anthropogenic and natural sources (de Jonge et al. 2002; Giordani et al. 2009).

A subtropical lagoon system composed of two subsystems located in a coastal area where shrimp farming is carried out and coastal upwelling processes occur with a seasonal pattern was targeted in this study. One of the subsystem (El Rancho) directly receives an effluent from shrimp farming and the other subsystem (Empalme) is connected with the sea.

The El Rancho-Empalme lagoon system is representative of a group of coastal lagoons located in the north of the Tropic of Cancer along the eastern coast of the Gulf of California, which are characterized by arid or semi-arid climate, scarcity of fresh rainwater (<300 mm yr^{-1}), annual variations in temperature (16-32°C) and salinity (~ 30-40 PSU), and permanent opening to the sea. This zone in the Gulf of California represents a top priority in the Marine Spatial Planning Program due to ecological importance and ecosystem services provided by these lagoons to fisheries, shrimp farming, tourism, and conservation (SEMARNAT 2006) and a better understanding of ecohydrological processes in these lagoons is necessary for environmental management. The aim of this study was to understand the role of nutrient inputs by anthropogenic and natural sources in the hydrological behavior and trophic status of this arid subtropical lagoon system.

Materials and methods

The El Rancho-Empalme lagoon system is located on the eastern coast of the Gulf of California (27°54'60" – 27°55'42"N and 110°52'00" – 110°49'50"W) (Fig. 1). The El Rancho subsystem has a surface area of ~8 km^2, average depth of 0.5 m and is connected with the Empalme subsystem by two tidal channels of ~20 m wide and ~2 m in depth each. The Empalme subsystem has an area of ~16 km^2, average depth of 2.3 m and is connected with the open sea by a mouth of up to 0.4 km width. In this lagoon system, the tide is mixed-semidiurnal with an amplitude of 1 m (Valle-Levinson et al. 2001). The weather is of BW(h') type: very dry, very warm and warm

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(García 1988) and the rate of evaporation (2700 mm yr^{-1}) exceeds the rate of precipitation (230 mm yr^{-1}). El Rancho receives effluents from a shrimp farm having 0.44 km^2 in area with an average volume of 31,290 m^3 day^{-1} during the breeding period (April to October). El Rancho also provides water for pond filling as well as for water exchange in ponds and also the shrimp aquaculture effluents are discharged back to El Rancho. The seasonal pattern of winds is southeasterly in summer at 5 m s^{-1} and northwesterly in winter at 8-12 m s^{-1} and the latter induces coastal upwelling from October to March on the eastern coast of the Gulf of California (Lluch-Cota 2000) where the studied lagoon is located.

The El Rancho-Empalme lagoon system, near the Bay of Guaymas, has been blessed with a vibrant commercial fishery that has contributed to the establishment and growth of the cities of Guaymas and Empalme (Arreola-Lizárraga et al. 2004) supporting ~200,000 inhabitants. This area is separated from the cities by lagoons and a terrestrial connection that precisely divides the subsystems of El Rancho and Empalme.

Calculation of the residence time

The sea level values were obtained by tidal prediction using the computer system version 0.53 Mar prepared by CICESE (www.cicese.mx). A bathymetric survey with the GARMIN GPSMAP 188C graphical depth sounder was conducted. The sea levels were measured at the beginning and at the end of bathymetric surveys to correct the effect of the tide. The water volume in El rancho was estimated with the software CivilCAD® (CivilCAD, 2012), cross sections were plotted every 50 m along the length of the lagoon and the hydraulic volume of each section was determined from the seafloor to the level of 0.00, which is defined as the mean sea level. Subsequently, the volumes of the sections were summed.

The water residence time was estimated for water balance and salinity, as described by Gordon et al. (1996) for the Land-Ocean Interactions in the Coastal Zone (LOICZ) project. Salinity measurements were registered (see the section *Measurements of the water quality*), and rates of precipitation and evaporation were obtained from a meteorological station of the National Water Commission located ~3 km east of the El Rancho.

The following general equations were used to calculate the balance:

$$dV/dt = V_Q + V_P + V_G + V_O - V_E + V_R \tag{1}$$

$$d(V_S)/dt = V_P S_P - V_E S_E + V_R S_R + V_X (S_{EMP} - S_{RAN}) \tag{2}$$

$$d(V_Y)/dt = V_R Y_R + V_X (Y_{EMP} - Y_{RAN}) + \Delta Y \tag{3}$$

where,

V_Q – Runoff (or river) flow volume
V_P – Precipitation volume
V_G – Groundwater flow volume (in our case it was assumed to zero)
V_O – “Other” flow volume (in our case it was assumed to zero)
V_E – Evaporation volume
V_R – Residual flow volume
S_{EMP} and S_{RAN} are Empalme and El Rancho salinities, respectively.
S_P, S_E and S_R are average salinity values due to precipitation, evaporation and residual flow between two boundaries, *i.e.* the ocean and the system. ΔY denotes fluxes of non-conservative material (DIN and DIP), Y_R – the average value of the material between two non-conservative borders. Y_{EMP}, Y_{RAN} are the average values of the material and ocean conservative system, respectively.

Estimates of the water balance, salt balance were performed for each season.

Calculation of the water balance

Equation 1 describes the volume of water conservation. For El Rancho, the balance between the input and output fresh-water flows in the system must be equal to the volume stored within the system.

$$V_R = -V_P + V_E \tag{4}$$

In the study period, the supply of shrimp effluents into El Rancho occurred exclusively in summer and autumn.

$$V_R = - V_P - V_Q + V_E \tag{5}$$

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Calculation of the salt balance

Equation 2 describes the salt added or removed from the water circulation system. Processes that affect the salt concentration include not only V_R, but also the exchange of water with the net flow of water. The mixture (V_X) is the mixing flow between Empalme and El Rancho (i.e. VX is an equal magnitude flow in both directions between two subsystems mostly forced by tides and wind). The salt balance was calculated assuming the steady state:

$$V_X = V_R S_R / (S_{SYS} - S_{LAG}) \qquad (6)$$

S_R is the residual flow associated with the salinity and represents the average salinity El Rancho (S_{RAN}) and Empalme (S_{EMP}).

The water and salt balance described together the advective and mixing processes between El Rancho and Empalme.

Measurements of the water quality

Water quality sampling was conducted three times a week every season: (February), spring (March), summer (June) and autumn (November) at 5 sampling sites in El Rancho and 8 sampling sites in Empalme (Fig. 1).

In situ measurements of temperature, salinity, and dissolved oxygen (Hydrolab DS5X, Hach, Loveland, CO) were made at each station. Water samples were collected between 07:00 and 13:00 h in 1 l plastic bottles at 30 cm below the surface to measure nitrite, nitrate, ammonium, orthophosphate, and chlorophyll *a*. Samples were transported on ice to a laboratory for analysis. Nutrient concentrations were determined by chemical methods (Strickland & Parsons 1972). Samples for chlorophyll *a* were collected by filtration through Whatman GF/C glass fiber filters (Whatman International, Maidstone, Kent, UK) extracted with 90% v/v acetone and spectrophotometrically measured (Parsons et al. 1984).

Calculation of the TRIX

The Trophic Index (TRIX) based on the pooled effect of oxygen saturation, nitrite, nitrate,

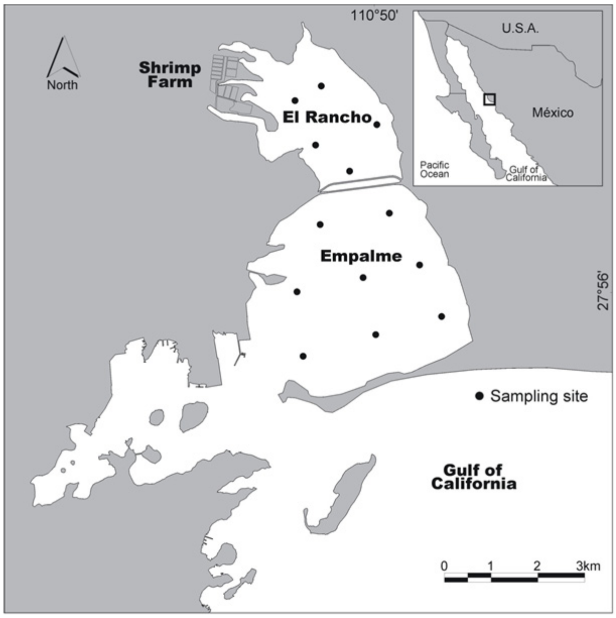

Figure 1

Map showing the location of the El Rancho-Empalme lagoon system. The dots indicate the sampling sites. The map shows the location of the shrimp farm which delivers effluents to El Rancho

ammonium, orthophosphate, and chlorophyll *a* was used to assess the water body trophic state according to Vollenweider et al. (1998). The index is given by:

$$TRIX = [\log 10 (Chl\ a \times D\%O \times N \times P) + 1.5] \times 1.2$$

where Chl *a* is chlorophyll *a* ($\mu g\ l^{-1}$), D%O is oxygen as an absolute deviation (%) from saturation, N is dissolved inorganic nitrogen $N{-}NO_3{+}NO_2{+}NH_4$ (μM), and P is the total phosphorus $P\text{-}PO_4$ (μM). TRIX was scaled from 0 to 10, covering a range of four trophic states (0.1 – 2.5 oligotrophic; 2.6 – 5 mesotrophic; 5.1 – 7.5 eutrophic; 7.6 – 10 hypertrophic).

Statistical data analysis

Data of water parameters were further analyzed using the ordination technique and non-metric multidimensional scaling (nMDS). A similarity matrix was constructed from ln(x + 1) transformed values of water parameters (dissolved oxygen, nitrite, nitrate, ammonium, orthophosphate and chlorophyll *a*) using the Euclidian distance coefficient of similarity, and relationships between

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samples were plotted by nMDS to determine whether the El Rancho and Empalme subsystems were similar in terms of water quality during all seasons. The PRIMER 6 statistical software (Primer-E, Ivybridge, UK) was used to perform the analyses.

TRIX values were analyzed by comparing mean values with one-way analysis of variance.

Results

Table 1 shows characteristics and flushing time of El Rancho.

All of the Empalme and El Rancho hydrological variables (dissolved inorganic nitrogen, dissolved inorganic phosphorus and chlorophyll *a*) showed a pattern that tends to differentiate specific conditions for each season. These results are consistent with nMDS that showed stress values ranging from 0.1 to 0.08 for El Rancho and Empalme, respectively (Fig. 2).

In both subsystems, water temperature had an annual range of 18-32°C. Salinity had an annual range of 34.5-41 PSU at El Rancho and 36-38.5 PSU in Empalme. Dissolved oxygen concentrations had an annual range of 3-8 mg l^{-1} (Table 2).

In both subsystems, the average concentrations of dissolved inorganic nitrogen were higher in winter, with ammonium values of (~7 μM) and nitrite + nitrate values (~4 μM), and the lowest in spring, summer and autumn, with ammonium values (<1 μM) and nitrite + nitrate values (<1 μM); orthophosphate concentrations were higher in winter (~2 μM) and lower in spring (~1.5 mM), summer (~1 μM) and autumn (~0.8 μM) (Table 2).

In both subsystems, concentrations of chlorophyll *a* were similar in winter, spring and summer (~1 mg m^{-3}), in autumn the highest concentrations were observed in El Rancho ~3 mg m^{-3} and Empalme ~2 mg m^{-3} (Table 2).

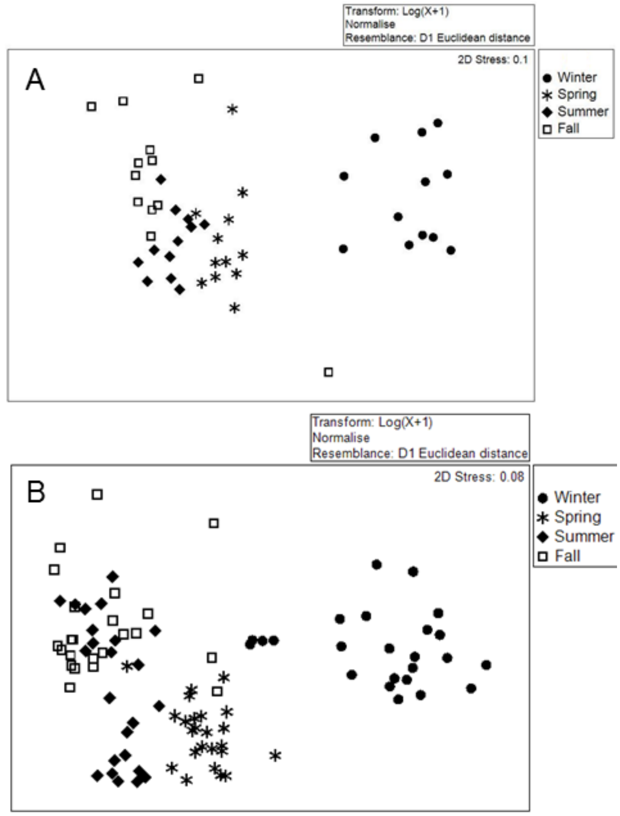

Figure 2

Nonmetric Multidimensional Scaling (nMDS) was used to compare seasonal variation in the groups of samples based on water quality parameters for the El Rancho (A) and Empalme (B) subsystems. Nitrite, nitrate, ammonium, soluble phosphate, and chlorophyll *a*, were included in the multivariate analysis.

The TRIX indicated a similar trophic status in the subsystem El Rancho and Empalme, with higher values (mesotrophic) in winter and lower values (oligotrophic) in spring, summer and autumn (Fig. 3).

Table 1

Characteristics of the El Rancho subsystem, type and surface water inputs-outputs

Parameters	Unit	Non-variant values	Winter	Spring	Summer	Autumn
Area	km²	7.78				
Depth	m	0.50				
Average system volume	m³	3,890,000				
Precipitation	m^3 day $^{-1}$		-	156	-	1,299
Evaporation			30,007	40,012	56,428	27,051
Contribution from shrimp farm			-	-	31.3	31.3
Contribution from the sea			30,007	39,857	54,461	25,752
Residence time	days		0.9	0.4	1.6	0.5

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Table 2

Seasonal variability in water quality of the El Rancho-Empalme lagoon system

	Units	Spring								Summer							
		El Rancho				Empalme				El Rancho				Empalme			
		Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max
Temperature	°C	19.6	0.28	19.04	19.94	20.38	0.34	19.87	21.25	30.15	1.33	28.5	32.24	31.16	1.26	29	33.94
DO	mg l^{-1}	6.26	0.53	5.65	7.07	6.84	0.5	6.02	7.66	4.21	0.83	3.19	5.83	4.76	1.06	3.03	7.14
Salinity	PSU	38.55	0.44	37.95	39.56	37.93	0.24	37.48	38.43	38.41	1.25	36.73	40.78	35.45	3.09	29.87	39.4
NO_2+NO_3	µM	0.38	0.09	0.24	0.49	0.35	0.13	0.08	0.76	0.15	0.05	0.09	0.25	0.1	0.05	0.05	0.22
NH_4		0.52	0.3	0.2	1.12	0.49	0.48	0.1	2.44	0.51	0.35	0.07	1.16	0.46	0.49	0.01	2.3
PO_4		1.4	0.24	0.91	1.73	1.63	0.19	1.2	1.97	0.96	0.2	0.69	1.44	0.84	0.19	0.55	1.26
Chl *a*	mg m^{-3}	1.06	1.65	0.02	5.92	0.57	0.42	0.02	1.64	0.77	0.75	0.04	2.73	1.25	1.2	0	3.29

	Units	Autumn								Winter							
		El Rancho				Empalme				El Rancho				Empalme			
		Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max
Temperature	°C	24.07	1.75	22.45	26.5	23.84	2.15	20.22	26.98	18.43	0.65	17.29	18.99	19.76	0.89	18.57	22.22
DO	mg l^{-1}	37.18	0.48	36.77	37.96	37.19	0.24	36.71	37.61	6.33	0.42	5.72	7.24	6.55	0.66	5.46	8.21
Salinity	PSU	6.4	2.01	0.25	7.79	6.97	0.52	6.06	7.86	37.26	1.06	35.38	38.19	37.44	0.59	36.04	38.19
NO_2+NO_3	µM	3.15	2.31	0.08	0.66	2.09	1.41	0.02	0.81	4.48	1.47	2.48	6.32	3.89	1.76	1.19	6.4
NH_4		0.85	0.82	0.01	3	0.83	0.97	0.02	3.55	7.58	2.61	4.6	11.42	6.5	2.7	3.12	13.56
PO_4		0.9	1.16	0.45	4.56	0.64	0.24	0.44	1.29	2.22	0.38	1.75	3.05	2.07	0.47	1.29	3.05
Chl *a*	mg m^{-3}	3.15	2.31	0.63	7.68	2.09	1.41	0.32	6.51	1.48	1.25	0.13	4.1	0.86	0.41	0.26	1.84

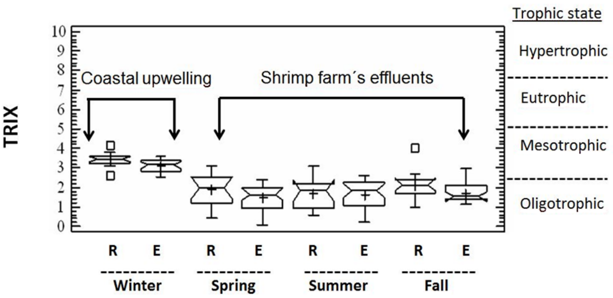

Figure 3

Box and whisker plot of the trophic state by TRIX index for the El Rancho-Empalme lagoon system during seasons. Median, quartiles, ranges, and outliers of data are shown for each event. The plot shows the trophic state levels and the impact of upwelling and shrimp farm effluents.

Discussion

Hydrology and trophic state in the subsystems of El Rancho and Empalme showed a similar pattern throughout the year, mainly due to the good exchange of water between the subsystems, indicated by flushing time <2 days in El Rancho.

Multivariate analysis (nMDS) showed particular hydrological conditions in each season and the TRIX indicated an increased supply of organic matter in winter and this was consistent with the hydrological behavior.

The water temperature ranged from 17 to 34°C in winter and summer (respectively), which is a characteristic phenomenon observed in these semiarid coastal lagoons (Valdez- Holguín 1994; Valenzuela-Siu et al. 2007) and this is explained by coastal water masses characterized by sea surface temperature of 26°C in summer and 17°C in winter in this region of the Gulf of California (Roden & Emilsson 1980), and the influence of air temperature on water temperature in these

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shallow water bodies, because the annual variation in air temperature of this arid region is >14°C (García 1988). In both subsystems, salinity was also characterized by higher values (>35 PSU) compared to the adjacent sea (<35) and low annual variability (35-41 PSU), which is attributed to high rates of evaporation (~3000 mm yr^{-1}) and low rainfall (<300 mm yr^{-1}) occurring in this semiarid region. The shrimp aquaculture effluent did not cause changes in water salinity. In both subsystems, dissolved oxygen concentrations had a pattern with lower values in summer (~4 mg l^{-1}) and the highest values in winter (~8 mg l^{-1}), and the reverse pattern of water temperature, explained by the solubility of the gas, has also been observed in other coastal lagoons in the region, which are well-mixed systems (Valenzuela-Siu et al. 2007).

In winter, nutrient concentrations (nitrite + nitrate, ammonium and phosphorus) were higher in both subsystems, while in spring, summer and autumn – the concentrations were minimal. This explains that the greatest contribution of nutrients in winter is due to the coastal upwelling occurring in this region of the Gulf of California (Lluch-Cota 2000) because this coastal water is rich in nutrients and is incorporated into the lagoon due to the good water exchange with the sea by tidal effects. Other studies in the coastal upwelling zones have documented this source of nutrients, e.g. at Bahia San Quintin, Mexico, nutrient fluxes from the ocean into this body of water were associated with upwelling intensity (Farfán & Alvarez-Borrego 1983); also in Tillamook Bay, Oregon, the USA, it was observed that the seasonal coastal upwelling controls the timing and extent of oceanic delivery of nutrients into the estuary (Colbert & McManus 2003). Our results provide evidence that the adjacent sea is an important seasonal source of nutrients to these coastal lagoons and affects the hydrological conditions and the rate of supply of organic matter. In spring, summer and autumn, the concentration of nutrients is lower, because the rainfall runoff is low (<300 mm yr^{-1}) and the internal recycling is smaller(Gilmartin & Revelante 1978).

The concentrations of chlorophyll *a* observed in winter, spring and summer were similar to average concentrations of <2 mg m^{-3}, whereas in autumn the observed average values were >2 mg m^{-3} and the outliers were >6 mg m^{-3}, which is attributed to the influence of nutrients provided by effluents from the shrimp aquaculture because drainage and harvest of the ponds are performed in the autumn.

In general, the phytoplankton biomass observed throughout the year in the El Rancho-Empalme lagoon system is low and this can be explained because the water renewal rates are <2 days in El Rancho (this study) and <8 days in Empalme (Arreola-Lizárraga et al. 2004), which inhibits the growth of phytoplankton biomass (Monbet 2006). The results observed in the El Rancho-Empalme lagoon match the average concentrations of chlorophyll *a* observed in the annual cycle in semiarid subtropical coastal lagoons in this region of the Gulf of California, including systems with no sewage influence and values <5 mg m^{-3} indicating their oligotrophic state, while other lagoons with an input from agricultural and urban wastewater showed values of ~9 mg m^{-3} (Valdez- Holguín 1994; Valenzuela-Siu et al. 2007).

In Mexico, around 90% of shrimp farming has been developed in a coastal zone characterized by deltaic plains and coastal lagoons located in the subtropical-tropical gradient on the States of Sonora (where the El Rancho-Empalme lagoon is located), Sinaloa, and Nayarit, which is sustained mainly by two coastal ecosystem services: water supply and nutrient recycling. However, shrimp farming has an inefficient use of nitrogen (N) and only 20-24% of N content in the food is converted into shrimp biomass, and basically the remaining N is discharged into coastal lagoons and the sea (Magallón-Barajas et al. 2009). One of the key environmental concerns related to shrimp farming is the discharge of waters with high levels of nutrients into adjacent body waters. Nonetheless, the impact of shrimp effluents on the adjacent ecosystems varies and depends on various factors, including the magnitude of the discharge, the chemical composition of effluents, and the specific characteristics of the environment that receives the discharge, such as circulation and dilution rates (Paez-Osuna 2001).

The shrimp farm in El Rancho provides 4,028 kg yr^{-1} of nitrogen and 85 kg yr^{-1} of phosphorus (Hernández-Ibarra 1999). It has operated for 20 years with the same farming surface (0.44 km^2) and our results concerning the nutrient fluxes and trophic state suggest that El Rancho was assimilating and processing the nutrients discharged, but an increase in the shrimp farm area should be evaluated, because eutrophication symptoms may occur as it has been observed in other lagoons of the Gulf of California (Barraza-Guardado et al. 2013).

Based on the TRIX index, the El Rancho-

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Empalme lagoon system is oligotrophic in spring, summer and autumn, while it shows mesotrophic conditions in winter. The oligotrophic conditions that prevail for most of the year can be explained mainly by the water renewal rate (a few days), which is a factor that minimizes the susceptibility of these lagoons to eutrophication as it facilitates the dilution and nutrient flow, and reduces the growth of phytoplankton biomass (Scavia & Liu 2006; Whitall et al. 2007; Garmendia et al. 2012). Our results suggest that the TRIX index was sensitive in detecting changes in the trophic state and was consistent with seasonal hydrologic variability in the lagoon: 1) winter mesotrophic state with the highest concentrations of nutrients and the coastal upwelling as the main source, and 2) spring, summer and autumn with the oligotrophic state with lower concentrations of nutrients from low rainfall, internal recycling, and effluents from shrimp farming. These results are consistent with other arid-subtropical estuaries, such as Shark Bay (Western Australia), which has a very low net production due to low delivery of nutrients to the system (Smith & Atkinson 1983), but they differ from other estuaries indicated by Eyre (1998), e.g. the Mediterranean (dominated by winter floods and summer drought), temperate (lack of well-defined seasonal variation in the flow), transitional (lack of well-defined seasonal variation in the flow and a significant impact of winter storms), and wet and dry tropical and/or subtropical (dominated by summer floods and winter drought) estuaries.

Our results suggest that a combination of different approaches (nutrient budget and trophic state index) may be useful in achieving a more comprehensive understanding of the nutrient dynamics, the trophic state and the responses to nutrient enrichment in these coastal lagoons. The disadvantage of this study is the fact that it covered only one annual cycle and long-term monitoring is required to understand the interannual variations.

Because upwelling is an important process affecting the biogeochemistry in the El Rancho-Empalme lagoon, it is likely that El Niño Southern Oscillation (ENSO) events could have a potentially significant impact on the development of winter trophic state signals. The effect of changes in the offshore upwelling intensity and precipitation during the ENSO events on nutrient biogeochemistry of this lagoon and the other lagoons in the Gulf of California warrants further investigation.

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