

A method to determine which nutrient is limiting for plant growth in estuarine waters—at any salinity

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Abstract

A method, utilising overlaid graphs for nutrients vs salinity, was developed in order to determine which nutrient is *limiting* for plant growth in estuarine waters—at any salinity. Dissolved inorganic nitrogen ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) and *o*-phosphate (PO_4^-) are the main forms of N and P that are readily bio-available for plant growth in waters and these have a *Redfield atomic ratio* of N:P = 16:1 (i.e. aquatic plants absorb N and P in the average ratio of 16 atoms of N to 1 atom of P). Graphs are prepared for (i) DIN vs salinity and (ii) *o*-phosphate vs salinity with the vertical scales for DIN and *o*-phosphate set at a ratio of N:P = 16:1; when these graphs are overlaid on each other then the lowermost trendline denotes the *limiting* nutrient for plant/algal growth—at any salinity. The graphs also indicate the extent by which one or other of the nutrients is *limiting*—at any salinity. Furthermore, if there is a transition from P to N limitation somewhere along the salinity gradient, then this occurs at the salinity where the trendlines intersect. The concept was applied to three estuaries in the southeast of Ireland and the results show that, in all of these circumstances, P is the *limiting* nutrient throughout—except for the higher salinities (i.e. salinities $\geq 30\text{‰}$), where either (i) N and P may become equally *limiting* at salinity $\approx 35\text{‰}$ or (ii) N may become *limiting* at salinity $\geq 30\text{‰}$. Overlaid nutrients vs salinity graphs were also used to demonstrate that, in the estuaries in southeast Ireland, carbon (as dissolved inorganic carbon, $\text{DIC} = \text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$) is not the *limiting* nutrient—at any salinity.

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1. Introduction

Eutrophication is defined as “*the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned*” (CEC, 1991). Therefore, it is important to be able to determine which is the *limiting* nutrient for plant/algal growth in waters—including estuarine waters and covering the full range of salinity—especially where the waters are eutrophic or where there is a risk that they might become eutrophic.

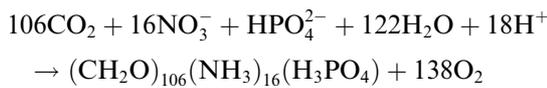
1.1. Nutrient balance and phytoplankton production

Justus Von Liebig (1803–1873), regarded as the founder of the chemical fertiliser industry, was the first to realise that the addition of a single fertiliser will increase crop yield in agriculture if a particular soil can deliver all the other necessary nutrients and *Liebig's Law of the Minimum* remains today a central concept in agriculture (Liebig, 1840). The same principle applies to aquatic systems and, where factors such as light and temperature are favourable, then the nutrient in shortest supply relative to the requirements of the aquatic plants will limit their growth—this is called the *limiting* nutrient (Redfield et al., 1963; Doering et al., 1995).

Aquatic plants (phytoplankton, macroalgae and macrophytes) absorb nutrients in specific proportions during

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photosynthesis and growth. The proportions in which carbon, nitrogen and phosphorus (C, N and P) are taken up by aquatic plants from seawater were determined from the elementary composition of biomass by Redfield (1934) and Fleming (1940)—they established the atomic ratios for the principal elements present in organic matter as C:N:P = 106:16:1—this is referred to as the *Redfield atomic ratio* and it is regarded as the ideal balance between these nutrients for algal production. The proportions and amounts of nutrients absorbed by aquatic plants from water vary between species; however, the overall average composition of aquatic plant tissue is C₁₀₆H₂₆₃O₁₁₀N₁₆P (i.e. atomic ratio of C:N:P = 106:16:1)—in addition to other trace elements. A general equation for photosynthesis in unpolluted waters is as follows:



In polluted waters, ammonium (NH₄⁺) may also play a part in providing nutrient for plant tissue (EEA, 1999). If the ratio of nutrients in water deviates significantly from the *Redfield atomic ratio* of C:N:P = 106:16:1 then one of these nutrients could become *limiting* for growth.

N and P are the nutrients that are commonly referred to as being potentially *limiting* in estuarine and coastal waters (EEA, 1999; US EPA, 2001). In general, the *limiting* nutrient for plant growth in freshwater ecosystems is usually attributed to P; whereas in coastal waters the *limiting* nutrient is often attributed to N (EEA, 1999; Ryther and Dunstan, 1971; Fisher et al., 1992; Nixon et al., 1996), however this is not necessarily the case in all circumstances (Howarth, 1988; Krom et al., 1991). The possibility that carbon might be the *limiting* nutrient in estuarine waters is not usually considered and data in this regard is scarce; however the present study also examines the limiting potential of C in the estuaries in southeast Ireland (see Sections 2.1 and 3.1 below).

All of the nutrients incorporated during photosynthesis are in ionic forms; dissolved inorganic nitrogen (DIN = NO₃⁻ + NO₂⁻ + NH₄⁺) and *o*-phosphate (PO₄⁻ = dissolved inorganic phosphorus = DIP) are the main forms of N and P that are readily bio-available for plant growth in waters—including estuarine waters (EEA, 1999). Organic forms of N and P may also be present, however with the exception of urea and free amino acids, these are not considered to be immediately available for plant growth (Antia et al., 1991). Nitrite (NO₂⁻) is a transition phase in the oxidation–reduction between ammonium (NH₄⁺) and nitrate (NO₃⁻)—therefore, NO₂⁻ is included in the DIN group of bio-available N.

Redfield (1958) reported that N and P are present in the oceans in the average proportion of N:P = 15:1 which is very nearly the same ratio as the average

requirement for phytoplankton growth. High salinity water in the Irish Sea also has a nutrient ratio N:P ≈ 15:1 (data presented by Gillooly et al. (1992), included 24 water samples from the Irish Sea with salinity ≥ 34.5‰; these had an average atomic ratio of N:P = 14.9:1). The foregoing indicates that in marine waters, including some waters at the seaward end of estuaries, the N:P ratio for phytoplankton (N:P = 16:1) and water (N:P = 15:1) are almost in balance and therefore N and P are equally *limiting* (also referred to as *dual limitation*)—this *coincidence* was first noted by Harvey (1926) in the English Channel. However Redfield et al. (1963) reported that, in coastal waters and in the surface of the open ocean, the N:P ratio can depart widely from average oceanic values. Redfield also reported that *the concentrations of P found in the waters of the Pacific and Indian Oceans are more than twice those found in the North Atlantic*. Therefore the N and P concentrations and the N:P ratios in coastal waters at the mouth of estuaries may vary considerably.

Some estuaries might also change from *P limiting* to *N limiting* at different times—for example: Fisher et al. (1992) reported a change from P limitation for algal growth in Chesapeake Bay, USA, during high river flows in spring to N limitation during periods of low river flows in summer; Lee et al. (1996) also reported that, in Hiroshima Bay, Japan, the growth of phytoplankton is limited mainly by N, but with P limitation at times.

Estuaries are dynamic ecosystems that contain a mixture of freshwater and seawater—P may be *limiting* at one end of the estuary with N *limiting* at the other end; with a transition from P to N limitation somewhere along the salinity gradient. Doering et al. (1995) also found, in studies using a simulated salinity gradient where the *limiting* nutrient shifted from P to N, that coincident with the shift in *limiting* nutrients was a shift in the N:P ratio of nutrient supply from greater than the Redfield ratio of 16 to less than 16. This supports the concept that the overall average N:P ratio in estuarine plants/algae is approximately N:P = 16:1.

Nutrient addition bioassays have been commonly used for assessing nutrient limitation in lagoons, coastal and ocean waters (Schluter, 1998; Carlsson and Graneli, 1999; Holmboe et al., 1999; Ault et al., 2000); however nutrient ratios in water have also been used to provide this information (Goldman et al., 1979; Doering et al., 1995; Alonso-Rodriguez et al., 2000; Havens, 2000; Wu and Chou, 2003). The present study uses the nutrient ratio approach for determining nutrient limitation in estuarine waters.

It should be kept in mind that factors other than nutrient supply might also have a *limiting* effect on plant growth in estuaries. Also, in estuaries, salinity largely controls the distribution of particular organisms, including plants, by excluding those species that are adapted

mainly to full freshwater or to full marine conditions (McLuskey, 1971). The relationship between nutrients and plant production in estuaries is further complicated by the natural cycles of the living plants and by climatic and weather conditions; plant growth processes also have a time factor. Furthermore, plants need sunlight for photosynthesis, which in turn can be affected by the transparency or turbidity of the water or by cloud cover. Water temperature can also have a significant effect on algal production (Kocum et al., 2002).

Besides being a direct measurement of the salt content of estuarine waters, salinity is also used to calculate the freshwater/seawater mix or the dilution of freshwater with seawater. In the present study salinity is expressed as parts per thousand (‰) where full seawater $\approx 35\text{‰}$ (i.e. $\approx 35,000$ mg/l total dissolved solids).

2. Materials and methods

The method to determine which is the *limiting* nutrient for plant growth in estuarine waters, at any salinity, is based on nutrient ratios in water and utilises overlaid graphs for nutrients vs salinity. Water samples are taken at various locations throughout the estuary and at various stages of the tide—in order to cover the full range of salinity. The water samples are analysed for NO_3^- , NO_2^- , NH_4^+ , PO_4^- and salinity (notes: $\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$; if the cadmium reduction analytical procedure is used then the parameters $\text{NO}_3^- + \text{NO}_2^-$ are measured in combination—see Section 2.3.2). Graphs are prepared for (i) DIN vs salinity and (ii) *o*-phosphate vs salinity—with the scales for DIN and *o*-phosphate on the vertical axes set at a ratio of N:P = 16:1 (i.e. the scales for N and P in the graphs are set so that they are proportional to the average rate at which these nutrients are absorbed by aquatic plants during growth—for example, maximum scales of $320 \mu\text{mol/l}$ N and $20 \mu\text{mol/l}$ P). When these graphs are overlaid or superimposed on each other, then the lowermost trendline denotes the *limiting* nutrient for plant growth—at any salinity. Furthermore, if there is a transition from P to N limitation somewhere along the salinity gradient then this occurs at the salinity where the trendlines intersect. The graphs also indicate the extent by which one or other of the nutrients is *limiting*—at any salinity. The method is illustrated in Fig. 1 using six hypothetical situations and Fig. 1 also serves as an overall *précis* of the method.

Ammonium (NH_4^+) and nitrite (NO_2^-) are usually present in very low concentrations in unpolluted estuarine waters relative to the nitrate (NO_3^-) fraction; therefore, in some nutrients vs salinity graphs, it may be practicable to use only NO_3^- instead of DIN. However, $\text{NO}_3^- + \text{NO}_2^-$ are usually measured in combination and samples may need to be analysed for NH_4^+ regardless.

Furthermore, NH_4^+ may be more readily bio-available for plant growth than NO_3^- (Kocum et al., 2002).

Estuaries are dynamic ecosystems and, for reasons of practicality, water samples from various locations throughout an estuary are usually taken over a time period, this can lead to a degree of scatter in the data points on the nutrient/salinity graphs—because of the ongoing exchange of nutrients between water and plants during the natural processes of photosynthesis and phytoplankton growth and decay; furthermore there is an ongoing exchange of CO_2 between water and air. Nutrient concentrations in estuarine waters may also vary with the seasons and with weather conditions among others; for example, nitrates concentrations in rivers discharging to the estuaries in southeast Ireland are significantly higher in winter—when river flows are also generally highest (Neill, 1989). Therefore, in order to improve correlation (i.e. to minimise scatter) on the nutrient vs salinity graphs, it is recommended that the monitoring data displayed in each graph is gathered in as short a period as possible—preferably over a tidal cycle. If surveys are carried out at various times of the year then the data for each survey should be plotted on separate graphs.

In some estuaries, and especially those that are in close proximity to a major ocean, the N:P ratios for phytoplankton and for full salinity water may be almost in balance (i.e. N:P = 16:1 for phytoplankton and N:P \approx 15:1 for seawater) (Redfield et al., 1963). In estuaries where N and P are equally *limiting* at the seaward end, the trendlines for N and P on the overlaid nutrients vs salinity graphs tend to converge at salinity $\approx 35\text{‰}$ (as illustrated in Fig. 1b).

The N:P ratio in individual plant species may vary significantly from the *Redfield ratio* of N:P = 16:1 (Boynton et al., 1982; Atkinson and Smith, 1983; EEA, 1999; Lapoint et al., 1992; Blanchard and Montagna, 1992). Therefore, if a particular species is being considered (e.g. in case of an algal bloom); then the N:P ratio on the overlaid nutrients vs salinity graph/s may need to be adjusted appropriately. However, it should be borne in mind that estuaries are complex ecosystems and that individual plant species do not exist in isolation. The present paper refers to ambient algal or plant growth in estuaries and the Redfield atomic ratio of N:P = 16:1 is an overall average for plant nutrient requirements (EEA, 1999; Doering et al., 1995).

There are various references in the literature to differences in nutrient requirements per unit of biomass between various aquatic plant species; in general, larger perennial plants have lower nutrient requirements per unit of biomass when compared to phytoplankton; and phytoplankton are more susceptible to transient or seasonal changes in nutrient availability than the perennial macroalgal species (Pedersen and Borum, 1996). However the concept outlined in the present paper is based on the ratio of plant nutrient uptake

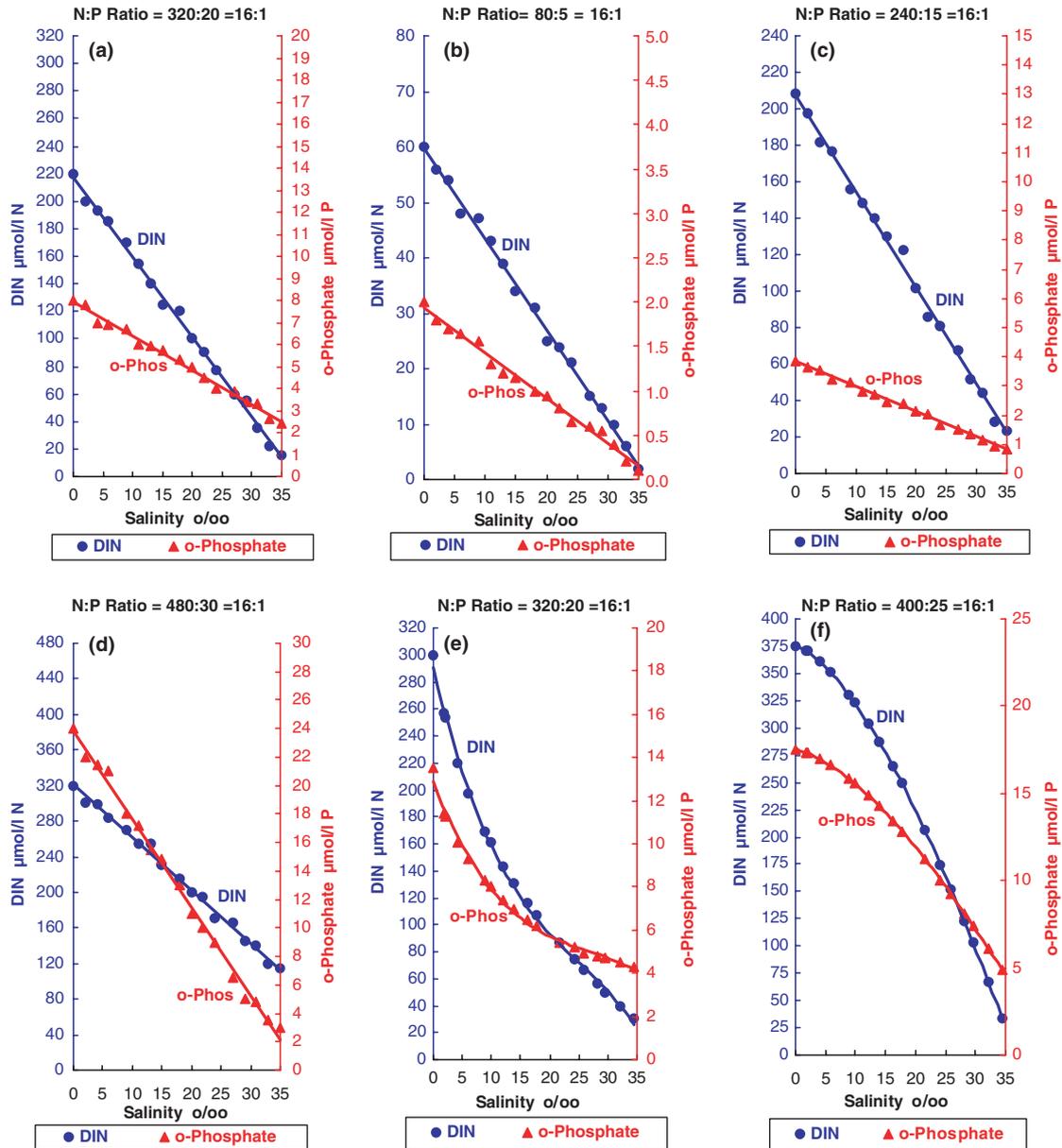


Fig. 1. The six hypothetical situations illustrated above serve as a *précis* to demonstrate how the *limiting* nutrient for plant growth may be determined—in any estuary and at any salinity from freshwater to full seawater (0–35‰). DIN ($= \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) and *o*-phosphate (PO_4^-) are the forms of N and P that are most readily bio-available for growth in aquatic plants. The *Redfield atomic ratio* is N:P = 16:1 (i.e. on average, aquatic plants absorb N and P in the approximate ratio of 16 atoms of N to 1 atom of P). Therefore, when the DIN and *o*-phosphate results are expressed as micromoles per litre ($\mu\text{mol/l}$)*, then the average ratio for plant nutrient uptake from waters is approximately 16 $\mu\text{mol/l}$ N to 1 $\mu\text{mol/l}$ P. Consequently, if the scales for DIN and *o*-phosphate on the vertical axes of the overlaid graphs are set at a ratio of N:P = 16:1 (i.e. the scales on the vertical axes are set so that they are proportional to the average rate at which N and P are absorbed by plants during photosynthesis), then the lowermost trendline denotes the *limiting* nutrient for plant growth—at any salinity. The graphs also indicate the extent by which one or other of the nutrients is *limiting*—at any salinity. Furthermore, if there is a transition from P to N limitation somewhere along the estuary, then this occurs at the salinity where the trendlines intersect. For example, in Fig. 1a, there is a transition from P to N limitation at salinity = 27‰, with *o*-phosphate *limiting* at salinity < 27‰ and DIN *limiting* at salinity > 27‰. * $\mu\text{mol/l N} = [(\text{mg/l N}) \times 1000]/14$ and $\mu\text{mol/l P} = [(\text{mg/l P}) \times 1000]/31$ where 14 and 31 are the atomic weights of N and P respectively.

and not on the absolute nutrient requirements per unit of biomass.

Certain types of plant and algae also require other nutrients for growth in addition to N and P—such as silicon (essential for cell walls or frustules in diatoms) or

iron (Fe) or vitamin B₁₂; these other nutrients tend to be species-specific in their *limiting* properties and do not normally limit the total amount of plant biomass production; also, estuarine waters tend to contain higher levels of Si and Fe than seawater—since land is the

major source of these elements (EEA, 1999). However there are exceptions, Wu and Chou (2003) reported that, in the polluted subtropical estuary of the Tamsui river in Taiwan, Si is the primary *limiting* factor for phytoplankton growth and that the elemental composition for phytoplankton collected in that estuary had a mean nutrient ratio of Si:N:P = 16.5:25.5:1. Therefore, if instances should arise where multiple nutrients need to be considered as possibly *limiting*, then it may be necessary to use an overlaid nutrients vs salinity graph with three or more Y-axes (i.e. a separate Y-axis for each nutrient). Then, provided that the scales for the individual nutrients on the various Y-axes are set so that they are proportional to the rate at which the nutrients are absorbed by the plant/s, the concept still applies and the lowermost trendline on the multiple nutrients vs salinity graph will denote the *limiting* nutrient for plant growth—at any salinity.

2.1. Concentrations of inorganic carbon

In aquatic systems, including estuaries, carbon (as CO₂) is also absorbed from water by plants during photosynthesis. However CO₂ is present in chemical equilibrium in water along with carbonic acid, bicarbonate and carbonate as follows:



As CO₂ is consumed during photosynthesis the equilibrium readjusts and more CO₂ is produced. Therefore dissolved inorganic carbon (DIC = CO₂ + H₂CO₃ + HCO₃⁻ + CO₃²⁻) is the form of carbon that is readily bio-available for aquatic plant growth. Alkalinity in river waters consists largely of H₂CO₃ + HCO₃⁻ + CO₃²⁻ (in addition to OH⁻ at pH > 8.3); therefore rivers with low alkalinities are also likely to be low in DIC.

In order to determine if DIC is a limiting nutrient for plant growth in estuarine waters—at any salinity, water samples from throughout the estuary are analysed for DIC (see Section 2.3.6) in addition to DIN, *o*-phosphate and salinity. The data are then presented on overlaid graphs for nutrients vs salinity; however, in this circumstance there are three Y-axis—one each for DIC, DIN and *o*-phosphate and with their scales set at the Redfield atomic ratio of C:N:P = 106:16:1. Therefore the lowermost trendline on the multiple nutrients vs salinity graphs denotes the *limiting* nutrient for plant growth—at any salinity.

2.2. Non-conservative behaviour of nutrients in estuaries

Where a nutrient (or any substance) passes through an estuary without undergoing any reaction, except for dilution of freshwater with seawater, then the nutrient is said to act conservatively and a graph of that nutrient

vs salinity is a straight line (in the case of N and P, the straight line is usually inverse and it is also known as the theoretical or ideal dilution line). However if the nutrient reacts by physical, chemical or biological processes, as it passes through the estuary then the nutrient is said to behave non-conservatively and the graph deviates from a straight line and becomes curved (Burton and Liss, 1976):

- (i) The curve is inward where the nutrient is removed from the system—for example by phytoplankton growth; sedimentation; denitrification (see Fig. 1e).
- (ii) The curve is outward if more of the nutrient becomes available—for example from effluent discharges; or from recycling from sediments; or from the decay and breakdown of plant and animal material; or from nitrogen fixation (see Fig. 1f).
- (iii) Estuaries are complex ecosystems with many variables; more complex graphs may also be produced where reactions take place mainly at one end of the salinity range and trends can also be affected by seasonal, climatic and weather conditions.

This concept can be useful for investigating the removal of nutrients from estuarine waters by living plants or in investigating possible increases in nutrients due to effluent discharges, etc. However, irrespective of whether the trendlines on the nutrient vs salinity graphs are straight (conservative) or curved (non-conservative); the *limiting* nutrient can still be determined from the lowermost trendline—provided that the axes ratio is N:P = 16:1 (as in Fig. 1e and f).

2.3. Analytical procedures used at the EPA Laboratory, Kilkenny

Analysis of estuarine waters can be more problematic than either freshwaters or marine waters—because salinities vary from 0 to 35‰ and each sample can have a different salinity. Where possible the analytical methods used at the EPA laboratory in Kilkenny are based on the US *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998). Documented quality control (QC) procedures are followed; the quality control system requires that at least one QC standard, a duplicate test sample and a blank be analysed with each batch of samples (and in that order); the maximum batch size is 20 samples. QC charts are kept.

2.3.1. Salinity

Salinity measurements are carried out using a conductivity/salinity meter (APHA, 1998). The instrument used is a WTW—model LF 538 (lab) or LF 197 (field).

2.3.2. Nitrate + Nitrite ($\text{NO}_3^- + \text{NO}_2^-$)

These combined parameters are also referred to as total oxidized nitrogen. The cadmium reduction method (APHA, 1998) is the widely recommended procedure for estuarine waters and this has been adapted in the Kilkenny laboratory for use with an automated discrete analyser (Konelab30™). Nitrate is reduced to nitrite by passing the sample through a cadmium reduction column, the nitrite (reduced nitrate plus original nitrite) then reacts with sulphanilamide followed by *N*-(1-naphthyl)ethylenediamine dihydrochloride to produce a magenta colour which absorbs light at a wavelength of 520 nm.

2.3.3. Ammonium (NH_4^+)

The manual phenate method (APHA, 1998) has been adapted to run on an automated discrete analyser (Konelab30™). Ammonium reacts with hypochlorite and phenol catalysed with sodium nitroprusside to form indophenol—an intensely blue compound. The colour is measured at 640 nm.

2.3.4. Dissolved inorganic nitrogen (DIN)

DIN is calculated from $\text{NO}_3^- + \text{NO}_2^-$ and NH_4^+ where $\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$.

2.3.5. *o*-Phosphate (PO_4^-)

The manual ascorbic acid/molybdate method (APHA, 1998) has been adapted to run on an automated discrete analyser (Konelab30™). Ammonium molybdate and potassium antimonyl tartrate react with *o*-phosphate in acid medium to form phosphomolybdic acid—this is reduced by ascorbic acid to intensely coloured molybdenum blue. The colour is measured at 880 nm.

2.3.6. Dissolved inorganic carbon (DIC):

$$(\text{DIC} = \text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-})$$

DIC is determined using a high temperature total organic carbon (TOC) analyser (model Dohrmann DC-190 TOC)—the instrument is operated in inorganic carbon mode bypassing the furnace. Samples must be filtered—especially if there is a possibility that they may contain any sediment of limestone origin. The sample is acidified in the inorganic reactor and $\text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$ are converted to CO_2 . The CO_2 is then purged from the sample with oxygen and passes via a dehumidifier directly into an infrared detector.

3. Results—application of the method to three estuarine systems in southeast Ireland

The method for determining which nutrient is *limiting* for plant growth was applied to three estuarine systems in the southeast of Ireland—shown in Fig. 2. The three

estuaries differ considerably in terms of catchment area, tidal range and freshwater flow (Table 1). In terms of freshwater flow; the Suir/Barrow/Nore estuary is the second largest in Ireland and it is complex in that there are three main rivers discharging into it (the rivers Suir, Barrow and Nore); the Slaney estuary is medium in size while the Colligan estuary is small. There are a number of population centres on each estuary.

The data presented in Figs. 3–5 were collected during estuarine surveys in 2002 and 2003 and in each case the surveys covered the salinity range from 0‰ to >34‰—separate nutrients vs salinity graphs were prepared for each sampling date. Also, there are three main rivers discharging into the Suir/Barrow/Nore estuary along with a number of smaller tributaries; consequently there was a degree of scatter for DIN and *o*-phosphate among the freshwater samples in this instance and that led to a skewing of the trendlines on the graphs—therefore, in the case of Fig. 3, only those samples with salinity >0‰ are included in the graphs. All trendlines shown in the graphs were computer generated using a Microsoft Excel 2000 spreadsheet.

The nutrient/salinity graphs in Figs. 3–5 are generally linear—signifying that the N and P nutrients in all three estuaries behave largely conservatively (i.e. DIN and *o*-phosphate are generally inversely proportional to salinity—indicating that these nutrients are not removed from the system in significant quantities by phytoplankton growth). This may reflect the fact that phytoplankton growth in the estuaries in southeast Ireland is limited by lack of light due to the high turbidity caused

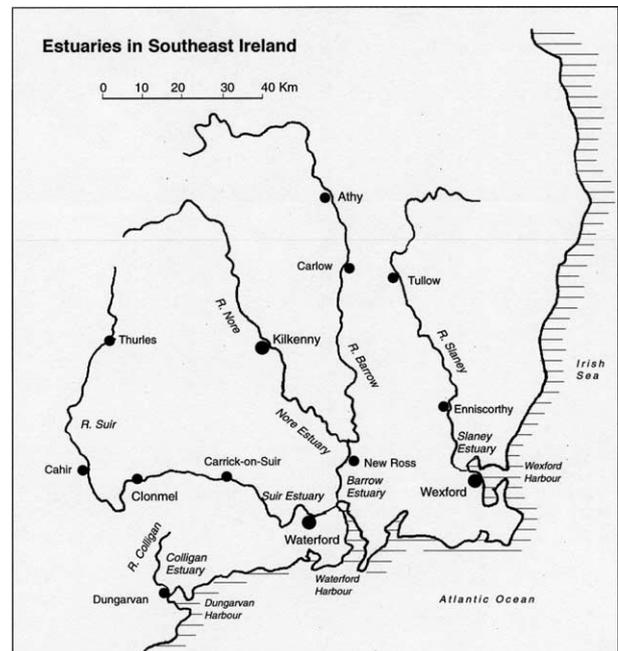


Fig. 2. Estuaries and harbours in southeast Ireland.

Table 1
Summary of the characteristics of three estuarine systems in southeast Ireland

Estuary/harbour	Suir/Barrow/Nore estuary and Waterford harbour	Slaney estuary and Wexford harbour	Colligan estuary and Dungarvan harbour
Size—in terms of freshwater flow	Large	Medium	Small
Freshwater catchment area (km ²)	9300	1860	144
Main riverine inputs	Suir, Barrow and Nore	Slaney	Colligan
Mean freshwater flow (m ³ /s)	130	30.5	5
Mean tidal range (m)			
Springs	3.7	1.5	3.6
Neaps	3.3	0.9	3.3
Max extent of tide inland (km)			
Freshwater	60	36	8.5
Saline water	37	30	8
Estimated mean depth (m) (at low water)	5	1	3
Mean tidal prism (m ³)	380 × 10 ⁶	49 × 10 ⁶	68 × 10 ⁶
Surface area of estuary/harbour (km ²)	80	34	31
Mean freshwater alkalinity (mg/l CaCO ₃)	212	66	26
Latitude and longitude	52°08'N, 6°58'W	52°19'N, 6°24'W	52°05'N, 7°34'W
Discharge to	Celtic Sea/Atlantic Ocean	Irish Sea	Celtic Sea/Atlantic Ocean

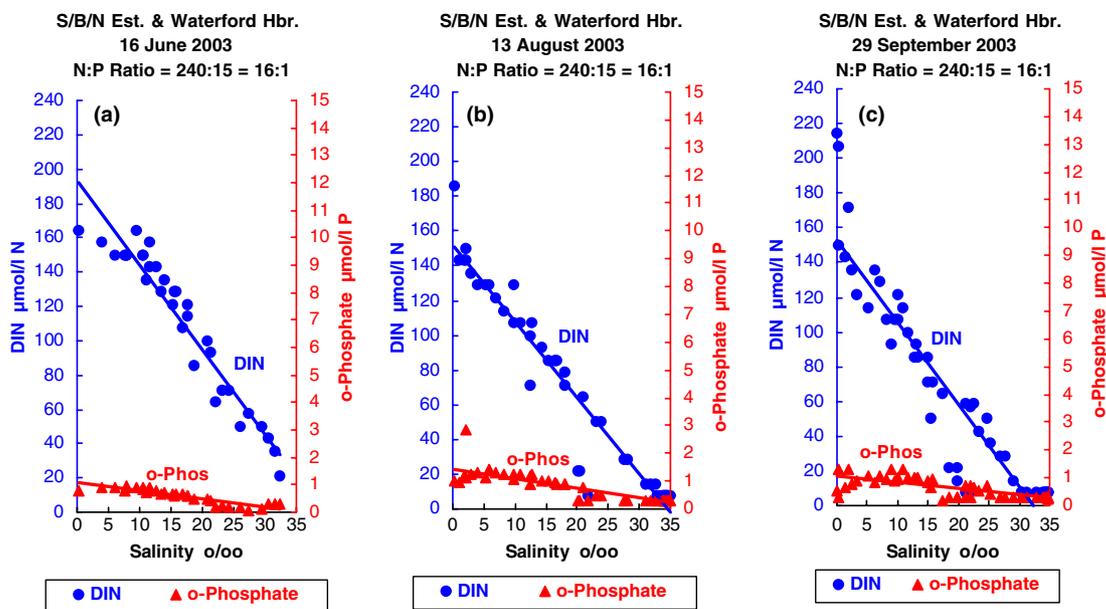


Fig. 3. Overlaid nutrients vs salinity graphs for (i) DIN vs salinity and (ii) *o*-phosphate vs salinity in the Suir/Barrow/Nore estuary and Waterford harbour—June, August and September, 2003. The scales on the vertical axes are set at the Redfield atomic ratio of N:P = 16:1 and therefore, the lower trendline denotes the *limiting* nutrient for plant growth—at any salinity. The graphs indicate that, in this estuary, P is the limiting nutrient throughout—except for the higher salinities (i.e. salinities $\geq 30\text{‰}$), where either (i) N and P may become equally limiting at salinity $\approx 35\text{‰}$ (i.e. the trendlines tend to converge at salinity $\approx 35\text{‰}$) or (ii) N may become *limiting* at salinity $\geq 30\text{‰}$ (i.e. the trendlines for N and P intersect at salinity $\geq 30\text{‰}$).

by the re-suspension of sediments, which in turn is caused by the motion of tide and waves in these moderately shallow estuaries.

The overlaid nutrients vs salinity graphs for the three estuaries/harbours in southeast Ireland (Figs. 3–5) also indicate that:

- (i) In all three estuaries and on all sampling dates, P was the *limiting* nutrient at salinities ≤ 30 .

- (ii) The trendlines for DIN and *o*-phosphate tend to converge with increasing salinity—indicating that the extent by which P is *limiting* tends to diminish with increasing salinity.

- (iii) In six of the graphs (Figs. 3a,b, 4a, 5a–c); P is *limiting* throughout the estuary (i.e. at all salinities $< 35\text{‰}$) and the trendlines for DIN and *o*-phosphate tend to converge at salinity $\approx 35\text{‰}$. This indicates that, in these instances, N and P are

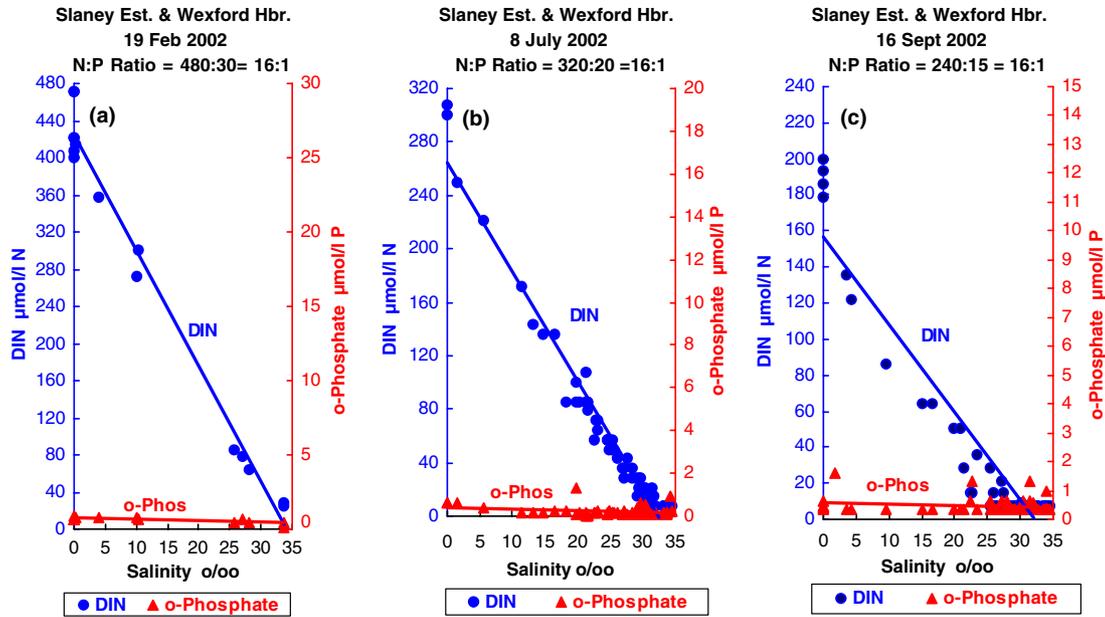


Fig. 4. Overlaid graphs for nutrients vs salinity for the Slaney estuary and Wexford harbour, 2002—showing (i) DIN vs salinity and (ii) *o*-phosphate vs salinity for three sampling dates—and including a limited winter survey. Note that, in this instance, the scales on the vertical axes in (a)–(c) differ (reflecting the fact that nitrate concentrations in the river Slaney are higher during winter); however in each case the N:P ratio for the vertical axes is N:P = 16:1. The graphs indicate that, in the Slaney estuary, P is the limiting nutrient throughout—except for the higher salinities (i.e. salinities $\geq 30\text{‰}$), where either (i) N and P may become equally *limiting* at salinity $\approx 35\text{‰}$ (i.e. the trendlines tend to converge at salinity $\approx 35\text{‰}$) or (ii) N may become limiting at salinity $\geq 30\text{‰}$ (i.e. the trendlines for N and P intersect at salinity $\geq 30\text{‰}$).

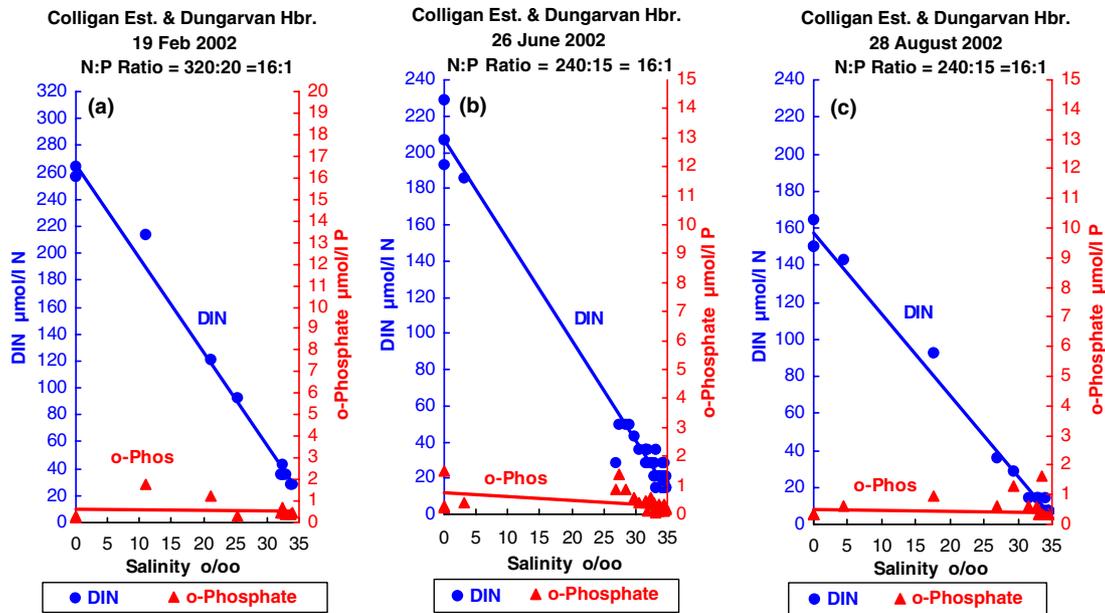


Fig. 5. Overlaid graphs for nutrients vs salinity for the Colligan estuary and Dungarvan harbour, 2002—showing (i) DIN vs salinity and (ii) phosphate vs salinity for three sampling dates—and including a limited winter survey. These graphs indicate that, in the Colligan estuary and Dungarvan harbour for 2002, P is the *limiting* nutrient throughout—except for the seaward end (i.e. salinity $\approx 35\text{‰}$), where N and P appear to be equally *limiting* (i.e. on all three dates, the trendlines for N and P tend to converge at salinity $\approx 35\text{‰}$). There is a relatively small freshwater discharge to this estuary/harbour—this leads to a sharp rise in salinity and hence there are few results for the mid-salinity section.

equally *limiting* in full seawater at the mouth of the estuary (salinity $\approx 35\text{‰}$). Furthermore, in these instances, the N:P ratio in water at the mouth of

the estuary coincides with the average nutrient ratio for phytoplankton growth (N:P = 16:1) and it is also similar to the overall average ratio for

nutrient concentrations in oceanic waters (N:P = 15:1). This finding is not unexpected given the proximity of these three estuaries to the North Atlantic Ocean.

- (iv) In three of the graphs (Figs. 3c, 4b and c), the trendlines intersect at salinities $\geq 30\text{‰}$, indicating that, in these instances, there is a transition from P to N limitation at the seaward ends (the transition from P to N limitation occurs at the salinity where the trendlines cross).

Overall, the overlaid nutrients vs salinity graphs for the three estuaries/harbours in southeast Ireland (Figs. 3–5) indicate that, in all three estuarine systems, P is the *limiting* nutrient throughout—except for the higher salinities at the seaward ends where either:

- (i) N and P may become equally *limiting* at salinity $\approx 35\text{‰}$ (in these cases the trendlines for N and P on the overlaid graphs tend to converge at salinity $\approx 35\text{‰}$) or
- (ii) N may become *limiting* at salinities $\geq 30\text{‰}$.

3.1. Concentrations of inorganic carbon

The possibility that carbon might be the *limiting* nutrient in the estuaries in southeast Ireland was also considered in the present context—and as outlined in Section

2.1. Supplementary surveys were carried out in the three estuaries in September/October 2004 and water samples were analysed for DIC in addition to DIN, *o*-phosphate and salinity. These data are presented graphically in Fig. 6 utilising overlaid nutrients vs salinity graphs; the three nutrients are represented on the Y-axis—with their scales set at the Redfield atomic ratio of C:N:P = 106:16:1—therefore the lowermost trendline on the multiple nutrients vs salinity graphs denotes the *limiting* nutrient for plant growth—at any salinity.

The mean alkalinities for the freshwaters discharging to the three estuaries in the southeast of Ireland are shown in Table 1—the Suir/Barrow/Nore estuary receives high alkalinity water, while the Slaney has moderate alkalinity and the Colligan has low alkalinity.

The graphs in Fig. 6 indicate that:

- (i) Carbon (measured as DIC) is not the *limiting* nutrient in any of the estuaries in southeast Ireland—at any salinity.
- (ii) At the seaward end of all three estuarine systems (i.e. at salinity $\approx 35\text{‰}$), DIC is present at high concentrations relative to DIN and *o*-phosphate. Redfield (1958) and Redfield et al. (1963) found that, in seawater, carbon is present on average at about 10 times the concentration that would be utilised if algal growth were limited by either N or P. The data graphed in Fig. 6 concur with this finding.

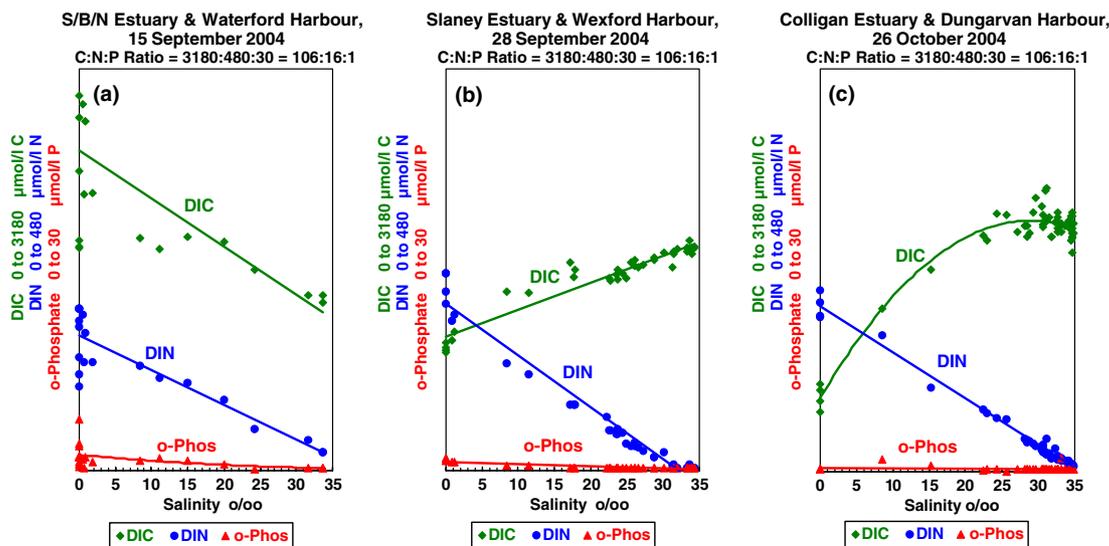


Fig. 6. Nutrients vs salinity graphs for the three estuarine systems in southeast Ireland—including dissolved inorganic carbon (DIC = $\text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$) in addition to DIN (= $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) and *o*-phosphate. The overlaid graphs show (a) DIC vs salinity, (b) DIN vs salinity and (c) *o*-phosphate vs salinity with the scales on the Y-axes set in proportion to the Redfield atomic ratio of C:N:P = 106:16:1 (i.e. the scales on the vertical axes are set so that they are proportional to the average rate at which C, N and P are absorbed by plants during photosynthesis). Therefore, the lowermost trendline denotes the *limiting* nutrient for plant growth—at any salinity. These graphs demonstrate that, in the estuaries in southeast Ireland, carbon (measured as DIC) is not the limiting nutrient at any salinity—even where the alkalinity is low in the freshwater riverine input (as in the Colligan/Dungarvan system). Notes: (a) $\mu\text{mol/l C} = [(\text{mg/l C}) \times 1000]/12$, $\mu\text{mol/l N} = [(\text{mg/l N}) \times 1000]/14$ and $\mu\text{mol/l P} = [(\text{mg/l P}) \times 1000]/31$ where 12, 14 and 31 are the atomic weights of C, N and P respectively. (b) The degree of scatter in (a) at salinity $\approx 0\text{‰}$ is due to fact that three main rivers discharge into this estuary along with a number of smaller tributaries.

- (iii) The DIC concentrations differ at the freshwater ends of the three estuaries (i.e. at salinity $\approx 0\%$). Also, in Fig. 6a the DIC concentration is higher in freshwater than in seawater whereas in Fig. 6b and c the DIC is higher at the seaward ends (i.e. in Fig. 6a the DIC trendline has a negative slope whereas in Fig. 6b and c the DIC slopes are positive). The DIC concentrations at the freshwater ends are generally proportional to the alkalinities (as shown in Table 1).
- (iv) The DIC concentrations also differ at the seaward ends of the three estuarine systems (i.e. at salinities $\approx 35\%$) and DIC at the seaward ends is lower in Fig. 6a than in Fig. 6b or c—this variation may be caused, at least in part, by the uptake of CO_2 by phytoplankton during photosynthesis and growth.
- (v) At the freshwater end of the Slaney and Colligan systems (Fig. 6b and c), the trendlines for DIC dip below those for DIN indicating that, in these instances, C is less available than N as the salinity approaches 0% (although P is still the limiting nutrient in each case).
- (vi) The trendline for DIC in the Colligan system (Fig. 6c) is curved upward—indicating that, in this instance, DIC behaves non-conservatively with more DIC present at mid-range salinities than would be expected from simple mixing of freshwater with seawater.
- (vii) In addition to the DIC that is immediately bioavailable for photosynthesis and plant growth in the estuaries (as graphed in Fig. 6), CO_2 is also available from the atmosphere and this can augment the DIC available for photosynthesis. Therefore it is unlikely that carbon would become limiting in the estuaries in southeast Ireland—even where the freshwater alkalinity is very low (as in the Colligan system).

4. Conclusions

The method outlined is a practical and effective means of determining which nutrient is limiting for plant growth in estuarine waters—at any salinity. Furthermore, if there is a transition from P to N limitation somewhere along the salinity gradient, then the salinity at which the transition occurs is also indicated. The method also indicates the extent by which one or other of the nutrients is limiting—at any salinity. The method was applied to three estuaries in the southeast of Ireland and the results show that, in all of these cases, P is the limiting nutrient throughout—except for the higher salinities, where either (i) N and P may become equally limiting at salinity $\approx 35\%$ or (ii) N may become limiting

at salinity $\geq 30\%$. Overlaid nutrients vs salinity graphs were also used to demonstrate that, in the estuaries in southeast Ireland, carbon (as $\text{DIC} = \text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$) is not the limiting nutrient—at any salinity.

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