THE INFLUENCE OF ESTUARINE WATER QUALITY ON COVER OF BARNACLES AND *ENTEROMORPHA* SPP[1].

Glenn COURTENAY[1,5], William GLADSTONE[2,3], Marcus SCAMMELL[4], Renée KIDSON[4] and Julie WOOD[4]

1: co*E*co, 40 Hillcrest St, Terrigal, 2260 NSW Australia

2: School of Environmental and Life Sciences, University of Newcastle, PO Box 127, Ourimbah NSW 2258, Australia

3: Present address: Department of Environmental Sciences, University of Technology, Sydney, PO Box 123, Broadway, 2007 NSW Australia

4: Sydney Water Corporation, PO Box 399, Parramatta, 2124 NSW Australia

5: Author for correspondence: Tel/fax: +61 2 4385 6632; email: courtenay@exemail.com.au

[1] This is the accepted version of the manuscript that was published in Environmental Monitoring and Assessment 175:685–697 (2011).

Abstract. The influence of ambient water quality on the settlement of barnacles and the green alga *Enteromorpha* spp. to an artificial substratum in the estuaries of Sydney, Australia, was investigated to test the efficacy of both groups of organisms as indicators of changes in water quality due to urban stormwater runoff and/or sewage overflows. Wooden settlement panels were immersed for four months on 17 occasions between 1996 and 2005 at 11 locations known to vary in water quality parameters (conductivity, total uncombined ammonia, oxidised nitrogen, total nitrogen, filterable phosphorus, total phosphorus, faecal coliforms and chlorophyll-*a*) and ambient meteorological conditions (total rainfall, maximum rainfall). Water quality data were collected during the time that the settlement panels were deployed. Cover of barnacles was highly variable among locations (range 1.2 – 55.2%). Hierarchical partitioning found that chlorophyll-*a*, total phosphorus and total nitrogen had significant independent positive effects on barnacle cover. Together, these variables explained 26% of the variation in barnacle cover. Mean cover of *Enteromorpha* spp., however, did not vary significantly among locations suggesting that other potentially more important factors are influencing its settlement and growth. The results of this study suggest that barnacle cover is likely to be a useful indicator of some components of water quality.

Additional Keywords: estuary, indicator, settlement, urbanised catchments, water quality

Introduction

Urbanised estuaries are subjected to numerous anthropogenic stressors which are expected to intensify with increasing population growth and density. The impacts that many urbanised estuaries are experiencing include destruction of habitats, alteration to freshwater flow and deterioration of water quality due to nutrient enrichment and sewage inputs (Kennish 2002, Van Dolah et al. 2008; DiDonato et al. 2009). Urban wastewater inflows and sewage effluent, for example, may result in domination of benthic infaunal assemblages by particular species (sometimes exotic and invasive), increased variability in intertidal assemblages, changes to community biomass and reductions in settlement of macroalgal species (Nordby and Zedler 1991; Saiz-Salinas and González-Oreja 2000; Courtenay et al. 2005). Additionally, anthropogenic nutrients and organic matter may cause eutrophication and localised depletion of oxygen (Paerl et al. 1998).

In addition to high temporal and spatial variability in water quality parameters in estuaries, physical and chemical variables lack the responsiveness to assess ecosystem change (Cohen and Fong 2006). Since monitoring of water quality parameters only provides a 'snapshot' of environmental conditions at the time of sampling, biological assessment has been identified as a key tool for developing a better understanding of ecosystem processes and for assessing changes in aquatic ecosystems and achievement of management goals and water quality objectives. Estuarine ecosystems evolve over time where multiple processes, such as the addition, growth, decline and elimination of populations, may interact at multiple scales to produce the observed communities (Thrush et al. 2000; Cadotte and Fukami 2005). The condition of an ecosystem is therefore dynamic and requires a long period of observation to reveal the magnitude of these dynamics (Cadotte et al. 2005). Consequently, the natural complexity and variability of estuarine ecosystems will need to be addressed in the design of monitoring programs by integrating water quality monitoring and biological processes.

Estuarine biota, for example, continuously sample the surrounding water, responding to the biologically available nutrients and integrating conditions over time. Furthermore, juvenile organisms may show increased sensitivity to altered water quality which may be sublethal to adult organisms (Fairweather 1991). A pelagic phase is a feature of the early life history of many aquatic organisms and the transition from this pelagic phase to a more stationary or site-attached benthic stage is termed 'settlement'. The magnitude of settlement is spatially and temporally variable (Caffey 1985; Tremblay et al. 2007) due to variation in physical transport processes such as upwelling and local wind patterns, the numbers and behaviour of larvae in the water column, the availability of space for colonisation, the magnitude of settlement cues and water quality conducive to settlement and survival of larvae (Tamburri et al. 1996; Olivier et al. 2000; Browne and Zimmer 2001; Ellien et al. 2004). Since settlement is very site-specific and may change rapidly in response to disturbance, analysis of the magnitude of settlement and early growth of the juvenile stages may potentially indicate a more current picture of the environmental conditions and may provide the first indication of adverse changes in the estuary due to pollution input (Fairweather 1991; Lotze et al. 2000).

Numerous studies have demonstrated the influence of water quality on suspension feeders and algae. For example, settlement of barnacles is enhanced by sewage discharge and high phytoplankton concentrations (Scammell and Besley 1995; Sanford and Menge 2001). Food concentration has a clear impact on barnacle larvae survival and growth (Hentschel and Emlet 2000) as nutrient limitations contribute to lower energy stores, inferior juvenile competitive abilities and consequently, for the intertidal barnacle, *Balanus amphitrite*, for example, poor settlement success, survival and growth (Jarrett and Pechenik 1997; Thiyagarajan et al. 2005; Tremblay et al. 2007). Barnacles, however, have not been assessed for their potential as indicators of urban runoff and sewage-related contamination. Under natural conditions, settlement and early growth of *Enteromorpha* spp. is influenced by the effect of freshwater runoff on salinity, light availability and nitrogen-phosphorus ratios (Martins et al. 2001). Spores of *Enteromorpha* spp. are extremely responsive to variation in ambient nutrient concentrations and preferentially settle in areas that are nutritionally favourable for growth (Fletcher and Callow 1992; Worm and Lotze 2006; Sousa et al. 2007). Sewage discharge, for example, enhances recruitment of *Enteromorpha* spp. (Bellgrove et al. 1997; Kamer and Fong 2001; Cohen and Fong 2004). *Enteromorpha* spp. proliferates in areas such as estuaries where nutrient inputs may be episodic, such as stormwater flows during high rainfall events (Barr and Rees 2003; Fong et al. 2004; Worm and Lotze 2006). Furthermore, Cohen and Fong (2005) found accumulation of nitrogen in *E. intestinalis* tissue was predictable over a range of estuarine nitrogen concentrations. Consequently, *E. intestinalis* is a potential indicator organism to assess changes in eutrophication (Fong et al. 1998; Cohen and Fong 2006; Worm and Lotze 2006). Therefore, it may also be useful as a biological indicator of water quality.

Between August 1996 and May 2005, artificial substrata were deployed on 17 occasions in the estuaries around Sydney, Australia, to assess the influence of estuarine water quality on the cover of barnacles and *Enteromorpha* spp. The following hypothesis was tested: cover of barnacles and *Enteromorpha* spp. are greater in areas of high nutrient concentrations and are not independently influenced by rainfall. If a relationship can be established between barnacles and *Enteromorpha* spp. cover and high nutrient concentration, measurement of settlement and early growth of barnacles and *Enteromorpha* spp. may be a potential indicator of variation in ambient water quality and may yield insight into the effect of urban stormwater and sewage-related contamination on intertidal settlement processes in estuaries and, consequently, estuarine health.

Methods

Study Locations

Prior to the commencement of sampling, results from previous water quality studies of Sydney estuarine locations were considered to select study locations (AWT Ensight 1996). Eleven intertidal locations in the estuaries of the Sydney region were selected within Iron Cove, Lane Cove River, Cooks River, Port Jackson, Middle Harbour and Port Hacking (Figure 1). These locations were selected due to their historically high, but variable, levels of faecal coliforms, chlorophyll-*a*, total phosphorus, total nitrogen, oxidised nitrogen and/or total uncombined ammonia, relative to accepted guidelines (AWT Ensight 1996). To negate the confounding influence of reduced salinity due to dilution by stormwater and sewage-associated disturbances, only locations with a median salinity greater than 27‰ (approximately 42 $mS.m^{-1}$ conductivity, adjusted to 25ºC; UNESCO 1980) were included in the study. Reference locations were not included in this study due to the absence of available water quality data for suitable locations. Water quality data collection at potential reference locations had been discontinued prior to the commencement of this study because recorded concentrations of most variables at these locations were usually close to lower detection limits (AWT Ensight 1996).

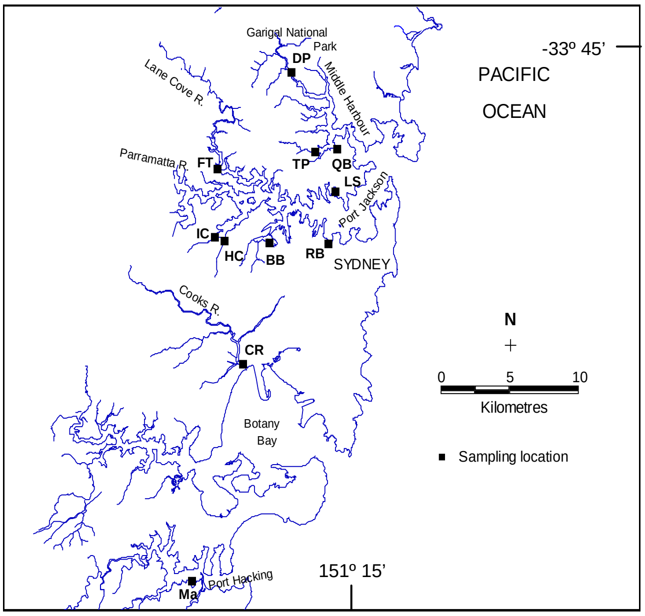

Fig. 1 Sydney estuarine intertidal settlement sampling locations: Davidson Park (DP), Tunks Park (TP), Quakers Hat Bay (QB), Little Sirius Cove (LS), Fig Tree Bridge (FT), Iron Cove Creek (IC), Hawthorne Canal (HC), Blackwattle Bay (BB), Rushcutters Bay (RB), Cooks river (CR) and Maianbar (Ma).

Field Sampling

At each location, untreated hardwood fence palings (1.8 m long by 0.1 m wide and hereafter called 'settlement panels') were deployed for 4 months on 17 occasions between August 1996 and May 2005. The settlement panels were deployed at the lowest growing height of established mangroves, or at a similar tidal height, and were forced vertically into the mud leaving approximately 1.5 m exposed to the tidal range. At each location, two settlement panels were placed adjacent to each other at each of two randomly selected sites separated by approximately 100 m. Pilot studies indicated that barnacle larvae (predominantly *Balanus* spp. but with a number of other species belonging to the genera *Elminius* and *Hexaminius*) and spores of *Enteromorpha* spp. were ubiquitous in the plankton at all study locations (Scammell and Besley 1995). Panel deployment coincided with times of the year (June to February) when the greatest numbers of *Balanus* spp. larvae were likely to be present based on studies of barnacle reproduction in the Sydney region (Wisely and Blick 1964; Egan and Anderson 1986). The 4 month deployment period was selected as a balance between obtaining sufficient settlement (such that differences could be measured if they exist) and limiting the effects of competition and other ecological processes so that they would not interact with the effects associated with anthropogenic disturbance on settlement.

In the laboratory, replicate quadrats (10 cm length x 1 cm width) were measured at 0, 2, 20, 22, 40, 42, 60, 62, 80 and 82 cm height from the mud line on each side of each panel. Cover of algae was estimated visually as a percentage of a quadrat covered by algae. The number of barnacles in each quadrat was counted and the diameter of the five barnacles nearest to one end of each quadrat was measured. The average diameter of the five barnacles was used to calculate the average barnacle size and this value was multiplied by the density of barnacles within a quadrat to estimate barnacle cover. Results obtained during the pilot study indicated that the consistent measurement of barnacle cover in a quadrat in this way was representative of cover in the rest of that quadrat. Subsequently, percentage cover for each taxon on each panel was estimated by averaging the cover measured in each quadrat on each side of each panel (i.e. n = 40 quadrats).

Water samples were collected manually at each of the eleven locations and analysed for total phosphorus (P), filterable phosphorus (FP), total nitrogen (N), oxidised nitrogen (NOx), total uncombined ammonia (TUA), chlorophyll-*a* (Chl-*a*), faecal coliforms (FC) and conductivity. Water samples were collected monthly during the period of deployment and mean values for each parameter were calculated. When a wet weather event occurred during panel deployment, data collected during the event was combined with monthly dry weather data to calculate the mean. Fourteen wet weather events, triggered when at least 25 mm of rainfall was recorded over a 24-hour period, occurred while panels were deployed during the study period. Prior to water sampling, each sampling container was rinsed with 70% ethanol and then with an aliquot of sample water. Water sampling was conducted by plunging the mouth of the sample container into the water to a depth of approximately 50 cm to avoid introducing surface scum into the container. The container was pointed into the current and away from hands to minimize contamination. Since composite samples are more useful for determining the average concentration of a system, two replicate samples were collected approximately 20 m apart at each site and composited in a separate container. After collection, the composited sample was initially poured into the FC sample bottle and then the other labelled sample bottles were filled to the level recommended by the National Association of Testing Authorities (NATA) accredited laboratory. All sample bottles were then stored on ice and returned to the Sydney Water Laboratory for processing and analysis. Determinants and methods for sample analysis are described in "Standard Methods for the Examination of Water and Wastewater" by APHA (1998). All sampling equipment was prepared according to these standards. Monthly rainfall data was collected at the rainfall station nearest to each sampling location and were provided by the Bureau of Meteorology (Figure 2). These data were used to calculate maximum monthly and total rainfall for each 4 month period of panel deployment.

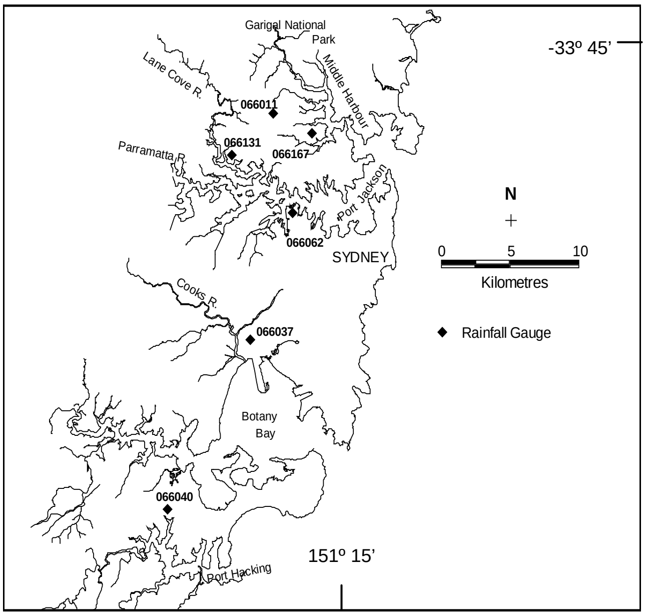

Fig. 2 Sydney rainfall gauges accessed during the intertidal settlement study: Chatswood (066011), Sydney Airport (066037), Miranda (066040), Sydney Observatory (066062) Riverview (066131) and Northbridge (066167).

Statistical Analyses

Prior to testing for a relationship between water quality variable and cover of algae and barnacles, a one-factor analysis of variance (ANOVA) was used to test whether mean cover of barnacles and algae differed significantly among locations. The coverage of each taxon at each location was calculated as the mean of the two site means (which were each calculated from the mean of the two settlement panels in each site). However, individual panels or all four panels were occasionally damaged or destroyed. Approximately 77% of panels were retrieved without damage or loss.

Retrieval of panels during the study was lowest at Little Sirius Cove (53%) and greatest at Hawthorne Canal (96%). Panels were retrieved on most occasions from Hawthorne Canal (*n*=17) and on the least occasions from Little Sirius Cove (*n*=13). Consequently, mean cover for each sampling occasion was calculated using the remaining panels, if any. Data used for the one-factor ANOVA were the mean covers for each taxon at each location for each sampling occasion. Variances for barnacles were heterogeneous and transformation did not make them homogenous; however ANOVA is robust to departures from the assumption of homogeneity of variances for sample sizes of the magnitude used in these analyses (Underwood, 1997). A regression-type approach was used to test for significant relationships between water quality and mean cover of barnacles and algae. Data recorded from all sampling occasions were combined for analysis. The water quality data for each location was the mean of the four composite samples collected during the period of deployment (or five composited samples if a wet weather event was experienced during the period of deployment) with the exception of FC which was calculated as the geometric mean (Hunter 2002). Initial, exploratory analysis (by box plots and scatter plots) of the raw data for the water quality variables revealed that most had skewed distributions with several outliers. Log_{10}-transformation of TUA, NOX, N, FP, P, Chl-*a* and FC removed right-skewness and most outliers. Transformation of conductivity data by reflection followed by square-root (Quinn and Keough 2002) corrected the left-skewed distribution. Raw data for total rainfall and maximum rainfall were used because they were near-normal with no outliers. Water quality variables were recorded in different units and were therefore standardized (by subtracting the mean and dividing by the standard deviation) prior to analysis (Quinn and Keough 2002). Spearman rank correlation coefficients were calculated for all pairwise combinations of water quality variables to check for the likelihood of multicollinearity.

Hierarchical partitioning (Chevan and Sutherland 1991; MacNally 2000; 2002) was used to identify the water quality variables that made a significant, independent contribution to variation in the cover of barnacles. Hierarchical partitioning assesses all possible explanatory models (i.e. 2^{10} in this case where 10 was the number of water quality variables tested and meteorological variables collected) that relate the dependent variable (in this case, cover of barnacles) to each water quality variable singly and in combination, and partitions the contribution of each water quality variable to explained variation into its independent contribution and its joint contribution with other variables. This method of identifying important water quality variables was used in preference to other methods (e.g. stepwise multiple regression) because extensive multicollinearity in the water quality variables meant that it would be impossible to distinguish variables that were independently important from variables that were unimportant but correlated with another variable that was itself important (Quinn and Keough 2002).

The statistical significance of each water quality variable's independent contribution (I_{obs}) to explained variation was determined by randomizing (n=1000) the data matrix to produce a distribution of I values. An I_{obs} is regarded as being significant (and therefore capable of explaining a significant amount of variation) when it is extreme i.e. greater than the 95 percentile of the randomized values. The results of the hierarchical partitioning for each water quality variable are expressed as *Z*-scores ([observed – mean{randomizations}]/SD{randomizations}) with statistical significance based on the upper 95% confidence limit of $Z \geq 1.65$ (MacNally 2002). The hier.part and rand.hp packages in the R Statistical package (available as freeware) were used for the hierarchical partitioning and statistical testing respectively (Walsh and MacNally 2004). The variables that were identified as being independent contributors to variation in cover of barnacles were then used to develop explanatory models using multiple regression (with SPSS v.16).

Results

Water Quality

All water quality and meteorological variables were significantly positively correlated with one another. All correlations were significant at α=0.01 and therefore there is little chance of a type 1 error in the 45 correlations. The largest correlations (i.e. R_s>0.80) were between P, TUA and NOx; N, FP and FC; NOx, TUA and N; and between total and maximum rainfall.

Mean conductivity and mean total and maximum monthly rainfall for the deployment period did not vary greatly among locations (Table 1). Mean values for the other variables differed between locations and the greatest mean values for most variables were measured at Tunks Park (adjacent to Northbridge sewage overflow) or Cooks River (downstream from the South Western Suburbs Ocean Outfall System (SWSOOS). Lowest mean values were generally measured at Little Sirius Cove (in Port Jackson) or Maianbar (adjacent to Royal National Park) (Table 1).

Table 1 Locations where mean high and mean low values of environmental variables were recorded during 4 months of deployment in Sydney estuaries for 1996-2005. Data are for total rain recorded during 4 months of deployment (Total Rain), maximum monthly rain (Max. Rain), and means of conductivity (Cond), total uncombined ammonia (TUA), oxidised nitrogen (NOx), total nitrogen (N), filterable phosphorus (FP), total phosphorus (P) and chlorophyll-*a* (Chl-*a*), and geometric mean of faecal coliforms (FC).

Variable	Location (high values)	Location (low values)
Cond	Maianbar (51.5 mS.cm^{-1})	Fig Tree Bridge (42.6 mS.cm^{-1})
Total Rain	Tunks Park (410 mm)	Cooks River (359 mm)
Max. Rain	Little Sirius Cove (213 mm)	Cooks River (184 mm)
TUA	Tunks Park (0.26 mgL^{-1}) Cooks River (0.24 mgL^{-1})	Little Sirius Cove (0.02 mgL^{-1}) Maianbar (0.03 mgL^{-1})
NOx	Cooks River (0.28 mgL^{-1})	Maianbar (0.02 mgL^{-1})
N	Cooks River (0.88 mgL^{-1}) Tunks Park (0.82 mgL^{-1})	Little Sirius Cove (0.20 mgL^{-1}) Maianbar (0.20 mgL^{-1})
FP	Tunks Park (0.054 mgL^{-1}) Cooks River (0.047 mgL^{-1})	Maianbar (0.013 mgL^{-1}) Little Sirius Cove (0.014 mgL^{-1})
P	Tunks Park (0.10 mgL^{-1}) Cooks River (0.09 mgL^{-1})	Little Sirius Cove (0.02 mgL^{-1}) Maianbar (0.02 mgL^{-1})
Chl-a	Hawthorne Canal (7.9 µgL^{-1}) Fig Tree Bridge (7.5 µgL^{-1}) Iron Cove Creek (7.5 µgL^{-1})	Maianbar (1.3 µgL^{-1})
FC	Tunks Park (683 cfu.100 mL^{-1}) Rushcutters Bay (662 cfu.100 mL^{-1})	Maianbar (4 cfu.100 mL^{-1}) Little Sirius Cove (9 cfu.100 mL^{-1})

Patterns of Cover of Biota

Four taxa were observed on settlement panels with cover dominated by barnacles (predominantly *Balanus* spp.) and/or the alga *Enteromorpha* spp. Settlement of *Galeolaria* spp. (Polychaeta) and Bryozoa was also observed but at insufficient frequencies and cover (<1%) to justify inclusion in the data analysis. Variation in mean barnacle cover among locations was greater than the variation in algal cover (Fig. 3a). Mean algal cover was greatest at Iron Cove Creek (40.6%), Blackwattle Bay (37.7%) and Rushcutters Bay (38.4%) which drain older industrial and residential areas of Sydney. Mean algal cover at the remaining seven locations was relatively similar (ranging from 20.6% at Maianbar to 31.2% at Hawthorne Canal) despite the type of catchment they drain. Overall, mean cover of algae did not vary significantly among locations ($F_{10,157}$=1.64, P=0.10) and so was not analysed further for its relationship with water quality variables.

Barnacle cover varied significantly among the 11 locations ($F_{10,157}$=11.69, P<0.001) (Fig. 3b). Hawthorne Canal and Cooks River, which drain older industrial and residential areas of Sydney, had higher mean barnacle cover (55.2% and 51.7% respectively) than other locations. Barnacle cover was least at Quakers Hat Bay (1.5%), Maianbar (1.2%), and Davidson Park (2.2%). Quakers Hat Bay is a small inlet surrounded by residential housing on steep terrain. The latter two locations are surrounded by national parks.

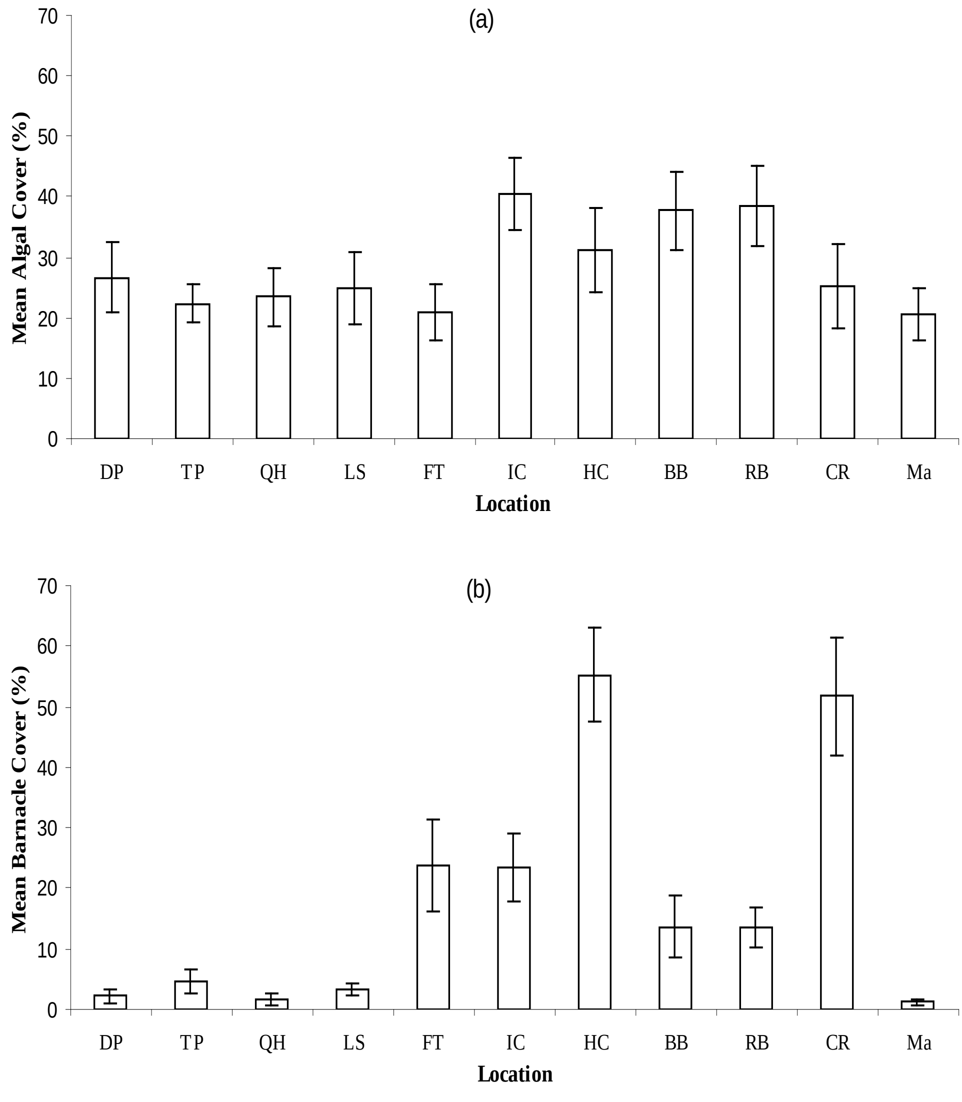

Fig. 3 Mean (± SE) of (a) algal cover and (b) barnacle cover at each sampling location in the Sydney region. Location codes are shown in Figure 1.

Barnacle cover was significantly correlated with all water quality variables, except total rainfall and maximum rainfall during the deployment period. However, hierarchical partitioning found that only three variables had a significant, independent association with barnacle cover: Chl-*a* (Z=21.70), P (Z=4.05) and N (Z=3.66). Chl-*a* had the strongest association, accounting for 54.0% of the total independent effects. The magnitude of the independent contribution of Chl-*a* was similar to its contribution in common with other environmental variables (as evidenced by the large joint contribution in Table 2). Total phosphorus and nitrogen accounted for 9.8% and 9.1% of total independent effects, respectively. Other environmental variables had only minor and non-significant independent associations with variation in barnacle cover. The relationship between barnacle cover and the variables identified by hierarchical partitioning is described by the following multiple regression model: barnacle cover = 17.80 + 5.23 (P) + 13.28 (Chl-*a*) – 3.07 (N), R^2_{adj} = 0.26, $F_{3,164}$ = 20.78 (P < 0.001).

Table 2 Results of hierarchical partitioning of R^2 for the relationship between cover of barnacles and water quality variables in Sydney estuaries from 17 sampling events between August 1996 and May 2005. The contribution of a water quality variable to variation in cover of barnacles is measured as R^2 independently (Ind.) and jointly with other predictor variables (Joint.). % Contribution (Cont.) is the independent contribution of a water quality variable to total variation in the cover of barnacles. Ind. R^2 values have been rounded to 2 decimal places (* P < 0.05). (See Table 1 for abbreviations).

	Barnacle cover		
	R^2		% Cont.
	Ind.	Joint	Ind.
Cond.	0.01	0.04	3.86
Total Rain	0.00	0.00	1.54
Max. Rain	0.01	-0.01	3.60
TUA	0.01	0.03	4.35
NOx	0.01	0.04	4.07
N	0.02	0.07	9.10*
FP	0.01	0.03	3.80
P	0.02	0.07	9.81*
Chl-*a*	0.11	0.16	54.02*
FC	0.01	0.05	5.85

Discussion

Although a number of recent studies have identified changes in biota due to altered water quality (Bellgrove et al. 1997; Calcagno et al. 1998; Hindell and Quinn 2000; Saiz-Salinas and González-Oreja 2000; Courtenay et al. 2005), the impact of sewage and stormwater-related contamination on the settlement and/or early growth of estuarine organisms has received little attention. The present study assessed the potential of several measures of estuarine water quality to influence the cover of green algae and barnacles to an artificial wooden substrate. Despite dominating settlement at eight of the eleven locations, mean cover of algae did not differ among locations. Three variables (chlorophyll-*a*, total phosphorus and total nitrogen), however, had significant, independent associations with barnacle cover. In contrast, total rainfall and maximum monthly rainfall during the deployment period were not identified as being significantly associated with variation in barnacle cover. Furthermore, the hierarchical partitioning suggests that the significant correlations between barnacle cover and the other water quality variables most likely occurred because of the high degree of multicollinearity amongst the water quality variables (Chevan and Sutherland 1991, MacNally 2000). That is, spurious significant correlations are likely to occur when an unimportant water quality variable is itself significantly correlated with an influential water quality variable.

Water quality data indicated that nutrient levels were generally high at all locations relative to accepted guidelines. Furthermore, mean barnacle cover was clearly greater at locations where the highest levels of chlorophyll-*a* were measured. In a related study, conducted in the same estuaries from 1995 to 1999, chlorophyll-*a* was the best single environmental variable that correlated with spatial variation in whole assemblages of intertidal organisms on natural rock surfaces in most years (1997-1999) (Courtenay et al. 2005). Point-source effluent discharge of nutrients has been shown to cause increased primary production of phytoplankton, measured as chlorophyll-*a* concentration (Day et al. 1989; Hargrave 1991; Valiela 1991; Kennish 1992; Mann 2000; Pierson et al. 2002). Starr et al. (1991) found the release of larvae of the barnacle *Semibalanus balanoides* to be directly influenced by phytoplankton abundance. Furthermore, reproductive development of another barnacle, *Balanus amphitrite*, is positively related to chlorophyll-*a* concentration (Desai et al. 2006). Since barnacles feed on phytoplankton and detritus, higher levels of productivity would result in greater rates of growth, reproductive output and settlement of barnacles (Bertness et al. 1991; Sanford and Menge 2001). Overall, the results of the current study support the previous published findings of the potential link between nutrients, chlorophyll-*a* and barnacle settlement, growth and abundance.

Predation, local hydrodynamics, the presence of established recruits and environmental variables can all influence variation in settlement (Keough 1998; Ross 2001; Pech et al. 2002). However, in this study, there was no direct evidence of predation on settlers as no predators were identified in the taxa on the panels. Similarly, another study of estuarine barnacles suggested that predation was an unlikely source of mortality (Ross 2001). Although current velocities vary considerably in magnitude throughout the estuary, sampling locations were selected so that panels would be exposed to similar hydrodynamics. Also, there is little variation in the amplitude and phase of the tidal range throughout the estuary, thus reducing the likelihood that variation in current would be responsible for differences in settlement (Das et al. 2000).

Settlement of estuarine organisms onto artificial substrata may differ from natural hard surfaces (McGuinness 1989; Anderson and Underwood 1994; Atilla 2000). Nevertheless, artificial substrata such as concrete, tiles and plastic, have been used in numerous studies of settlement (Dean and Hurd 1980; Gombach et al. 1992; Keough 1998; Fairfull and Harriott 1999; Fairweather 1999; Satumanatpan et al. 1999; Atilla 2000; Bulleri and Chapman 2004; Bulleri et al. 2005; Moreira 2006; Worm and Lotze 2006). Although natural variability in settlement may render the process unreliable as a single indicator of anthropogenic impact (Fairweather 1999), settlement of larvae to artificial substrata may provide a measure of the existing and potential conditions (Dean and Hurd 1980; Gombach et al. 1992; Keough 1998; Fairfull and Harriott 1999; Fairweather 1999; Satumanatpan et al. 1999; Atilla 2000).

Heterogeneity and complexity of an artificial substrate can also significantly influence settlement in an epibenthic, subtidal assemblage. Pech et al. (2002) showed that abundance of settlers was higher on panels with small scales of heterogeneity and intermediate orders of complexity and that abundance of *Balanus* sp. decreased with increasing complexity. However, in this study, the untreated hardwood fence palings deployed as settlement panels were all sourced from the same distributor and it is expected that the characteristics of all panel surfaces were relatively similar. Therefore, it is unlikely that differences in small-scale surface heterogeneity were responsible for the observed differences in settlement.

Relating physical and chemical water quality data to ecological impacts is not precise (Loeb 1994; Lopez and Dates 1998; Jones et al. 2001; Cohen and Fong 2006) and intense (and expensive) water quality sampling is required to make appropriate conclusions (Fry et al. 2003). This study, however, suggests that the use of cover of encrusting organisms on artificial settlement substrata is a potentially useful indicator of relative differences in nutrient levels in estuaries. The analyses of the settlement of barnacles and *Enteromorpha* spp. on artificial substrata in Sydney's estuaries has confirmed that correlation exists between high levels of nutrients and secondary production demonstrated by enhanced settlement cover of barnacles in Sydney's estuaries. The relationship between water quality and algal settlement in Sydney estuaries is less clear suggesting that there are other factors influencing the settlement and growth of *Enteromorpha* spp.

The finding that cover of barnacles is influenced by nutrient levels highlights the great potential for further research into the application of this technique in other contexts such as before-after-control-impact assessments of changes in water quality management (e.g. infrastructure development). While the relationships demonstrated here between cover of barnacles and water quality variables were based on water quality data collected at intervals over the sampling period, more detailed research on these relationships could involve water quality data collected continuously over the period of deployment of settlement panels and a re-examination of their influence on cover of barnacles and *Enteromorpha* spp. Further research of this sort could also usefully address questions relating to sampling design (e.g. optimal number of replicate samples, relevance of small-scale spatial variation), effect sizes, and statistical power.

Summary

Ecological responses to water quality impacts within estuarine systems are difficult to predict. Nevertheless, the cover of barnacles in Sydney Harbour and surrounding estuaries appears to be influenced by the quality of runoff from urbanised catchments. The monitoring of physical and chemical water quality parameters, however, only provides a 'snapshot' of environmental conditions. While measurement of cover on artificial substrata does not provide a definitive measure of nutrient concentrations, the results of this study suggest that barnacle cover may be a useful indicator of some components of water quality. For comprehensive monitoring of estuarine health, the settlement panel approach could be used concurrently with measurement of intertidal rock and mangrove tree assemblages to quantify the complex relationship between eutrophication and secondary production in estuaries (Courtenay et al. 2005).

Acknowledgements Thanks to Sydney Water Corporation for access to extensive water quality data and to numerous Australian Water Technologies (AWT) staff for field assistance. The authors thank the reviewers for constructive suggestions that improved the quality of this manuscript.

References

Anderson, M. J., & Underwood, A. J. (1994). Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage. *Journal of Experimental Marine Biology and Ecology*, 184, 217-236.

APHA [American Public Health Association, American Water Works Association and Water Pollution Control Federation] (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. Washington DC: American Public Health Association.

AWT - Ensight (1996). Biological Indicators Pilot Studies – May 1994 to December 1995. Prepared for Clean Waterways Programme, Sydney Water Corporation.

Atilla, N. (2000). Meiofaunal Colonization of Artificial Substrates in an Estuarine Embayment. *Marine Ecology,* 21, 69-83.

Barr, N. G., & Rees, T. A. V. (2003). Nitrogen status and metabolism in the green seaweed *Enteromorpha intestinalis*: an examination of three natural populations. *Marine Ecology Progress Series*, 249, 133-144.

Bellgrove, A., Clayton, M. N., & Quinn, G. P. (1997). Effects of secondarily treated sewage effluent on intertidal macroalgal recruitment processes. *Marine and Freshwater Research*, 48, 137-46.

Bertness, M. D., Gaines, S. D., Bermudez, D., & Sanford, E. (1991). Extreme spatial variation in the growth and reproductive output of the acorn barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series*, 75, 91-100.

Browne, K. A., & Zimmer, R. K. (2001). Controlled Field Release of a Waterborne Chemical Signal Stimulates Planktonic Larvae to Settle. *The Biological Bulletin*, 200, 87-91.

Bulleri, F., & Chapman, M. G. (2004). Intertidal assemblages on artificial and natural habitats in marinas on the north-west coast of Italy. *Marine Biology*, 145, 381-391.

Bulleri, F., Chapman, M. G., & Underwood, A. J. (2005). Intertidal assemblages on seawalls and vertical rocky shores in Sydney Harbour, Australia. *Austral Ecology,* 30, 655–667.

Cadotte, M. W., & Fukami, T. (2005). Dispersal, spatial scale, and species diversity in a hierarchically structured experimental landscape. *Ecology Letters*, 8**,** 548–557.

Cadotte, M. W., Drake, J. A., & Fukami T. (2005). Constructing nature: laboratory models as necessary tools for investigating complex ecological communities. *Advanced Ecological Research*, 37, 333–353.

Caffey, H. M. (1985). Spatial and temporal variation in settlement and recruitment of intertidal barnacles. *Ecological Monographs*, 55, 313-332.

Calcagno, J. A., López Gappa, J., & Tablado, A. (1998). Population dynamics of the barnacle *Balanus amphitrite* in an intertidal area affected by sewage pollution. *Journal of Crustacean Biology***,** 18, 128-137.

Chevan, A., & Sutherland, M. (1991). Hierarchical Partitioning. *American Statistician*, 45, 90-96.

Cohen, R. A., & Fong, P. (2004). Nitrogen uptake and assimilation in *Enteromorpha intestinalis* (L.) Link (Chlorophyta): using ^{15}N to determine preference during simultaneous pulses of nitrate and ammonium. *Journal of Experimental Marine Biology and Ecology*, 309, 67-77.

Cohen, R. A., & Fong, P. (2005). Experimental evidence supports the use of δ^{15}N content of the opportunistic green macroalga *Enteromorpha intestinalis* (Chlorophyta) to determine nitrogen sources to estuaries. *Journal of Phycology*, 41, 287-293.

Cohen, R. A., & Fong, P. (2006). Using opportunistic green macroalgae as indicators of nitrogen supply as sources to estuaries. *Ecological Applications*, 16, 1405-1420.

Courtenay, G., Gladstone, W., & Schreider, M. (2005). Assessing the Response of Estuarine Intertidal Assemblages to Urbanised Catchment Discharge. *Environmental Monitoring and Assessment*, 107, 375-398.

Das, P., Marchesiello, P., and Middleton, J.H. (2000). Numerical modelling of tide-induced residual circulation in Sydney Harbour. *Marine and Freshwater Research*, 51, 97–112.

Day, J. W., Hall, C. A. S., & Kemp, W. M. (1989). *Estuarine Ecology*. New Jersey: Wiley-Interscience.

Dean, T. A., & Hurd, L. E. (1980). Development in an estuarine fouling community: The influence of early colonists on later animals. *Oecologia*, 46, 295-301.

Desai, D. V., Anil, A. C., & Venkat, K. (2006). Reproduction in *Balanus amphitrite* Darwin (Cirripedia: Thoracica): influence of temperature and food concentration. *Marine Biology*, 149, 1431-1441.

DiDonato, G. T., Stewart, J. R., Sanger, D. M., Robinson, B. J., Thompson, B, C., Frederick Holland, A., & Van Dolah, R. F. (2009). Effects of changing land use on the microbial water quality of tidal creeks. *Marine Pollution Bulletin*, 58, 97-106.

Egan, E. A., & Anderson, D. T. (1986). Larval development of *Balanus amphitrite* Darwin and *Balanus variegates* Darwin (Cirripedia, Balanidae) from New South Wales, Australia. *Crustaceana***,** 51, 188-207.

Ellien, C., Thiébaut, E., Dumas, F., Salomon, J., & Nival, P. (2004). A modelling study of the respective role of hydrodynamic processes and larval mortality on larval dispersal and recruitment of benthic invertebrates: example of *Pectinaria koreni* (Annelida: Polychaeta) in the Bay of Seine (English Channel). *Journal of Plankton Research*, 26, 117-132.

Fairfull, S. J. L., & Harriott, V. J. (1999). Succession, space and coral recruitment in a subtropical fouling community. *Marine and Freshwater Research*, 50, 235-240.

Fairweather, P. G. (1991). Implications of "supply-side" ecology for environmental assessment and management. *Trends in Ecology and Evolution*, 6, 60-63.

Fairweather, P. G. (1999). Determining the 'health' of estuaries: Priorities for ecological research. *Australian Journal of Ecology*, 24, 441-451.

Fletcher, R. L., & Callow, M. E. (1992). The settlement, attachment and establishment of marine algal spores. *British Phycology Journal*, 27, 303-329.

Fong, P., Boyer, K. E., & Zedler, J. B. (1998). Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link). *Journal of Experimental Marine Biology and Ecology*, 231, 63-79.

Fong, P., Fong, J. J., & Fong, C. R. (2004). Growth, nutrient storage, and release of dissolved organic nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. *Aquatic Botany*, 78, 83-95.

Fry, B., Gace, A., & McClelland, J. W. (2003). Chemical indicators of anthropogenic nitrogen loading in four Pacific estuaries. *Pacific Science*, 57**,** 77–101.

Gombach, M., Bressan, G., & Seriani, M. (1992). Microfouling Seasonality in a locality of the Gulf of Trieste (1986-1988). In R. A. Vollenweider, R. Marchetti and R. Viviani (Eds.), *Marine Coastal Eutrophication* (pp. 441-443). Oxford, UK: Elsevier Publishers.

Hargrave, B. T. (1991). Impacts of Man's Activities on Aquatic Systems. In R. S. K. Barnes and K. H. Mann (Eds.), *Fundamentals of Aquatic Ecology* (pp. 245-264). UK: Blackwell Science.

Hentschel, B. T., & Emlet, R. B. (2000). Metamorphosis of barnacle nauplii: Effects of food variability and a comparison with amphibian models. *Ecology***,** 81, 3495-3508.

Hindell, J. S., & Quinn, G. P. (2000). Effects of sewage effluent on the population structure of *Brachidontes rostratus* (Mytilidae) on a temperate intertidal rocky shore. *Marine and Freshwater Research*, 51, 543-51.

Hunter, P. R. (2002). Does calculation of the 95th percentile of microbiological results offer any advantage over percentage exceedence in determining compliance with bathing water quality standards. *Letters in Applied Microbiology*, 34, 283–286.

Jarrett, J.N., & Pechenik, J.A. (1997). Temporal Variation in Cyprid Quality and Juvenile Growth Capacity for an Intertidal Barnacle. *Ecology***,** 78, 1262-1265.

Jones, A. B., O'Donohue, M. J., Udy, J., & Dennison, W. C. (2001). Assessing Ecological Impacts of Shrimp and Sewage Effluent: Biological Indicators with Standard Water Quality Analyses. *Estuarine and Coastal Shelf Science***,** 52, 91-109.

Kamer, K., & Fong, P. (2001). Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis*. *Marine Ecology Progress Series*, 218, 87-93.

Kennish, M. J. (1992). *Ecology of Estuaries: Anthropogenic Effects*. Boca Raton: CRC Press.

Kennish, M. J. (2002). Environmental threats and environmental future of estuaries. *Environmental Conservation*, 29, 78-107.

Keough, M. J. (1998). Responses of settling invertebrate larvae to the presence of established recruits. *Journal of Experimental Marine Biology and Ecology*, 231, 1-19.

Loeb, S. L. (1994). An Ecological Context for Biological Monitoring. In S. L. Loeb and A. Spacie (Eds.), *Biological Monitoring of Aquatic Systems* (pp. 3-7). Boca Raton: Lewis Publishers.

Lopez, C., & Dates, G. (1998). The Efforts of Community Volunteers in Assessing Watershed Ecosystem Health. In D. Rapport, R. Costanza, P. R. Epstein, C. Gaudet, R. Levins (Eds.), *Ecosystem Health* (pp.103-128). UK: Blackwell Science.

Lotze, H. K., Worm, B., & Sommer, U. (2000). Propagule banks, herbivory and nutrient supply control population development and dominance patterns in macroalgal blooms. *Oikos*, 89, 46-58.

MacNally, R. (2000). Regression and model building in conservation biology, biogeography and ecology: the distinction between – and the reconciliation of – 'predictive' and 'explanatory' models. *Biodiversity and Conservation*, 9, 655-671.

MacNally, R. (2002). Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. *Biodiversity and Conservation*, 11, 1397-1401.

Mann, K. H. (2000). *Ecology of Coastal Waters*. UK: Blackwell Science.

Martins, I., Oliveira, J. M., Flindt, M. R., & Marques J. C. (1999). The effect of salinity on the growth rate of the macroalgae *Enteromorpha intestinalis* (Chlorophyta) in the Mondego estuary (west Portugal). *Acta Oecologica*, 20, 259-265.

McGuiness, K. A. (1989). Effects of some natural and artificial substrata on sessile marine organisms at Galeta Reef, Panama. *Marine Ecology Progress Series*, 52, 201-208.

Moreira, J. (2006). Patterns of occurrence of grazing molluscs on sandstone and concrete seawalls in Sydney Harbour (Australia). *Molluscan Research*, 26, 51-60.

Nordby, C. S., & Zedler, J. B. (1991). Responses of Fish and Macrobenthic Assemblages to Hydrologic Disturbances in Tijuana Estuary and Los Peňasquitos lagoon, California. *Estuaries***,** 14, 80-93.

Olivier, F., Tremblay, R., Bourget, E., & Rittschof, D. (2000). Barnacle settlement: field experiments on the influence of larval supply, tidal level, biofilm quality and age on *Balanus amphitrite* cyprids. *Marine Ecology Progress Series*, 199, 185-204.

Paerl, H. W., Pinckney, J. L., Fear, J. M., & Peierls, B. L. (1998). Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River Estuary, North Carolina, USA. *Marine Ecology Progress Series*, 166, 17-25.

Pech, D., Ardisson, P. L., & Bourget, E. (2002). Settlement of a Tropical Marine Epibenthic Assemblage on Artificial Panels: Influence of Substratum Heterogeneity and Complexity Scales. *Estuarine and Coastal Shelf Science***,** 55, 743-750.

Pierson, W. L., Bishop, K., Van Senden, D., Horton, P. R., & Adamantidis, C. A. (2002). Environmental Flows Initiative Technical Report: Environmental Water Requirements to Maintain Estuarine Processes. Environment Australia.

Quinn, G. P., & Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. UK: Cambridge University Press.

Ross, P.M. (2001). Larval supply, settlement and survival of barnacles in a temperate mangrove forest. *Marine Ecology Progress Series*, 215, 237-249.

Saiz-Salinas, J. I., & González-Oreja, J. A. (2000). Stress in estuarine communities: Lessons from the highly-impacted Bilbao estuary (Spain). *Journal of Aquatic Ecosystem Stress and Recovery,* 7, 43-55.

Sanford, E., & Menge, B. A. (2001). Spatial and temporal variation in barnacle growth in a coastal upwelling system. *Marine Ecology Progress Series*, 209, 143-157.

Satumanatpan, S., Keough, M. J., & Watson, G. F. (1999). Role of settlement in determining the distribution and abundance of barnacles in a temperate mangrove forest. *Journal of Experimental Marine Biology and Ecology*, 241, 45-66.

Scammell, M., & Besley, C. (1995). Biological Indicators Pilot Study: Intertidal Settlement – June 1995, Prepared for Clean Waterways Programme, Sydney Water Corporation, Report No. 95/79.

Sousa, A. I., Martins, I., Lillebø, A. I., Flindt, M. R., & Pardal, M. A. (2007). Influence of salinity, nutrients and light on the germination and growth of *Enteromorpha* sp. spores. *Journal of Experimental Marine Biology and Ecology*, 341, 142-150.

Starr, M., Himmelman, J. H., & Therriault, J. (1991). Coupling of nauplii release in barnacles with phytoplankton blooms: a parallel strategy to that of spawning in urchins and mussels. *Journal of Plankton Research*, 13, 561-571.

Tamburri, M. N., Finelli, C. M., Wethey, D. S., & Zimmer-Faust, R. K. (1996). Chemical Induction of Larval Settlement Behavior in Flow. *The Biological Bulletin*, 191, 367-373.

Thiyagarajan, V., Hung, O. S., Chiu, J. M. Y., Wu, R. S. S., & Qian, P. Y. (2005). Growth and survival of juvenile barnacle *Balanus amphitrite*: interactive effects of cyprid energy reserve and habitat. *Marine Ecology Progress Series*, 299, 229–237.

Thrush, S. F., Hewitt, J. E., Cummings, V. J., Green, M. O., Funnell, G. A., & Wilkinson, M. R. (2000). The generality of field experiments: interactions between local and broad-scale processes. *Ecology***,** 81, 399-415.

Tremblay, R., Olivier, F., Bourget, E., and Rittschof, D. (2007). Physiological condition of *Balanus amphitrite* cyprid larvae determines habitat selection success. *Marine Ecology Progress Series*, 340, 1-8.

Underwood A.J. (1997). *Experiments in Ecology: Their Logical Design and Interpretation using Analysis of Variance*. UK: Cambridge University Press.

UNESCO (1980). UNESCO Technical Papers in Marine Science 1978. *Journal of Oceanographic Engineering,* Vol.OE-5, No.1, January 1980.

Valiela, I. (1991). Ecology of Coastal Ecosystems. In R. S. K. Barnes and K. H. Mann (Eds.), *Fundamentals of Aquatic Ecology* (pp. 57-76). UK: Blackwell Science.

Van Dolah, R. F., Riekerk, G. H. M., Bergquist, D. C., Felber, J., Chestnut, D. E., & Fredrick Holland, A. (2008). Estuarine habitat quality reflects urbanization at large spatial scales in South Carolina's coastal zone. *Science of The Total Environment*, 390, 142-154.

Walsh, C., & MacNally R. (2004). The hier.part package' available at http://cran.r-project.org/web/packages/hier.part/ hier.part.pdf. Accessed 6 August 2008.

Wisely, B., & Blick, R. A. P. (1964). Seasonal abundance of first stage nauplii in 10 species of barnacles at Sydney. *Australian Journal of Marine and Freshwater Research*, 15, 162-171.

Worm, B., & Lotze, H. K. (2006). Effects of eutrophication, grazing, and algal blooms on rocky shores. *Limnology and Oceanography*, 51, 569–579.