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Diversity and abundance of epibiota on invasive and native estuarine gastropods depend on substratum and salinity

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Abstract. Our understanding of variation in epibiota communities remains incomplete. This study relates such variability to multiple concurrent environmental factors. Specifically we determined the relative importance of salinity, depth, wave exposure, habitat and 'shell type' (shell type combined species, size, morphology and mobility traits) for community structure of sessile epibiota on gastropods in the Swan River Estuary, Australia. We quantified distribution, biofouling patterns, and detailed epibiota community structures on gastropod species in the estuary – the native *Nassarius pauperatus* and *Bedeva paiva* and the invasive *Batillaria australis*. The invasive *Batillaria* was much more abundant, and more biofouled, than any of the native species, thereby supporting orders of magnitude more epibiota in the estuary. Generalised linear models were used to partition variation in richness and abundance of epibiota among the above listed factors. Of the five factors were only shell type and salinity significant in 9 of 14 models. These results highlight (1) that a single invasive species can alter epibiota communities on a large system-wide scale, (2) an overwhelming importance of shell type and salinity in explaining estuarine epibiota communities, and (3) that additional environmental factors need to be included in future studies to improve predictive models of distribution for epibiota communities.

Additional keywords: *Batillaria*, biodiversity, epibiosis, fouling, *Gracilaria*, invasive species.

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Introduction

Benthic life is prolific in estuarine ecosystems, where abiotic and biotic hard surfaces are used as substratum by a variety of sessile species competing for space (Wahl 1989, Anderson and Underwood 1994; Vasconcelos *et al.* 2007; Harder 2009). Epibiosis (life on another living organism) is a direct consequence of this competition for space, and settlement and growth of epibiota species on other organisms (the 'basibiont' or 'host') is widespread in both intertidal and subtidal habitats (Davis and White 1994; Wahl and Mark 1999; Creed 2000).

Research has shown that the distribution, abundance and composition of epibiota species depend on the size and behaviour (Becker and Wahl 1996; Creed 2000; Gribben *et al.* 2009; Wernberg *et al.* 2010), species identity and shell morphology (Sandford 2003; Thyrring *et al.* 2013), and anti-fouling mechanisms (Wahl 1989; Wahl *et al.* 2010) of the host. Additionally, it has been shown that epibiota communities can change seasonally (Davis and White 1994; Sandford 2003) and are affected by interspecific competition between solitary and colonial species (Jackson 1977), external grazing pressure (Buschbaum 2000) and various habitat characteristics (e.g. tidal zones) (McLean 1983; Bell 2005). Many factors associated with the host and the external abiotic and biotic environment are therefore likely to influence epibiota in estuaries, but most studies have only focussed on one or a few factors at a time (see above references). Consequently, there is little understanding of the relative importance of individual factors influencing epibiotic community structure.

Estuaries are ecotones between marine and freshwater habitats. In estuaries, gradients in salinity, light, temperature hydrodynamic forces and physico-chemical conditions vary at small and large spatio-temporal scales. In general, such fluctuating environments restrict species richness (Mclusky and Elliott 2004). Estuaries are dominated by soft sediments, and hard substrates (suitable for colonisation by sessile organisms) are often limited to mollusc shells (Creed 2000; Olabarria 2000). Epibiosis may therefore be a particularly important process in

Fig. 1. Map of the 13 study sites in the Swan River Estuary. Dotted lines divide the estuary into three regions sampled and classified by their annual lowest salinity: *Outer estuary* (annual lowest salinity >30‰), *Central estuary* (salinity >5‰) and *Inner estuary* (salinity >1‰). *Outer estuary*: 1: Gilbert Fraser (0.40); 2: Leeuwin (0.16); 3: Chidley Point* (0.35); 4: Freshwater Bay (3.49). *Central estuary:* 5: Point Resolution (1.15); 6: Charles Court* (4.06); 7: J.H Abrahams (2.04); 12: Jeff Joseph (3.32); 13: Point Walter (2.63). *Inner estuary:* 8: Matilda Bay (2.60); 9: Mills Point* (4.97); 10: Como* (4.88); 11: Heathcote (3.89). Sites used to estimate shell abundance and degree of fouling are denoted by asterisks. No seagrass beds at Matilda Bay. Effective fetch showed in parentheses after site names. Insert map: Location of the Swan River Estuary, Perth, Western Australia. The thick black line shown along the eastern coastline of Australia corresponds to the native distribution of *Batillaria australis*.

estuaries, for example, compared to rocky reefs, where epibiota can also occupy abiotic surfaces. That is, shells in estuaries can facilitate entire sessile communities that would otherwise be non-existent or very rare (Knott *et al.* 2004; Harder 2009).

The Swan River Estuary is the largest estuary in Western Australia (Fig. 1) and is, like other estuaries, characterised by strong environmental gradients (Brearley 2005). The Swan River Estuary is therefore a good model system to study the relative importance of multiple environmental factors on epibiotic community structures. Seagrass beds, dominated by the small stress-resistant and fast growing species, *Halophila ovalis*, are widely distributed in the otherwise sandy and muddy sediments (Brearley 2005). The non-indigenous invasive gastropod *Batillaria australis* is, together with two native gastropods (*Nassarius pauperatus* and *Bedeva paiva*), abundant throughout most of the Swan River Estuary, providing most of shell substrates available for colonisation by sessile epibiota (Thomsen *et al.* 2010*b*).

Our objective was to characterise epibiota communities on seven shell types of various sizes (cf. Table 1) with four environmental conditions, including habitat types (seagrass *v.* mudflat), water depth, salinity and wave exposure. More specifically, we hypothesised that epibiota communities would be richer and more abundant (1) on large shells with more space and time for colonisation (compared to small shells), (2) on 'live/moving' shells that are more likely to remain at the sediment surface (compared to dead shells), (3) near the mouth of the Swan River Estuary where salinity stress is smallest (compared to upstream sites), (4) at shallow depth with more light and reduced risk of becoming buried by sediments (compared to deep sites), (5) at protected sites with lesser risk of epibiota being dislodged (compared to exposed sites), and (6) in seagrass beds that also support an epibiota community (compared to 'barren' mudflats). Furthermore, partitioning variation in epibiota community structure based on their host shell type and the external environment, within a single analytical framework, allowed us to rank test factors according their relative importance.

Materials and Methods

Field sampling

All gastropod shells (*B. australis, N. pauperatus, B. paiva*) were collected in late austral spring and early summer, between October and early December 2011 in the Swan River Estuary, Perth, Western Australia (31°59′30.96″S, 115°48′59.82″E; Fig. 1).

Shell size, density and fouling

We quantified size structures, densities and degree of fouling on shells of the three gastropod species from three quadrats (0.058 m^2) haphazardly placed within each of nine sites (Fig. 1). These samples were only collected from seagrass beds because here the three gastropod species were found together in much higher densities compared to adjacent mudflats (Thomsen *et al.* 2010*b*).

Epibiota

We quantified epibiota communities on 3226 gastropod shells collected from 13 sites (each site ~150 × 150 m) distributed throughout the lower estuary (Fig. 1). *B. australis* shells were divided into five types commonly found in the Swan River Estuary (Table 1). At each site shells were collected haphazardly from two habitats (seagrass beds *v.* mudflats, but one site did not have a seagrass bed) and two different depths (~0.5 *v.* 1.5 m). Most shell types were found at most sites, in seagrass beds and sediments, and in shallow and deep waters. However, *B. australis* recruits were only found at sites 1, 2, 4 and 6 (see Fig. 1). Four or five sites were sampled within each of three salinity regions, based on annual minimum salinities obtained from the Swan River Trust (http://www.swanrivertrust.wa.gov.au, accessed 5 May 2014): *Inner estuary* (1‰), *Central estuary* (5‰) and *Outer estuary* (30‰). Within each salinity region, site specific wave exposure was calculated as 'Effective Fetch' (Ruuskanen *et al.* 1999) based on 15 distance measurements from the site centre to the opposite shore. Measurements were made on a Swan River Estuary chart in scale 1 : 25 000. The site specific wave exposure ranged from fully protected (effective fetch = 0) to highly exposed (effective fetch = 5) (see Table S1 available as Supplementary material to this paper).

Laboratory procedures

All shells were carefully brought ashore and to the laboratory to ensure attached species did not break off.

Table 1. Shell types collected in the Swan River Estuary, based on their morphological characteristics and ecological importance
n, number of each shell-type included in the data analysis of epibiota communities

Shell type and species	Life stage	Characteristics	Ecological importance	*n*	Shell area (cm^2)
Covered *Batillaria australis* (Bat–Gra+)	Adult	Shells with attached dense fronds of coarsely branched red alga *Gracilaria comosa*	A relatively common shell type. The large seaweed fronds may create novel micro-habitat on shells	476	7.6
Normal *Batillaria australis* (Bat–Gra–)	Adult	Shells without dense *Gracilaria comosa*	The most common shell type in Swan River	660	7.5
Dead *Batillaria australis* (Bat–Empty)	Adult	Empty shells	A non-moving common shell type; accumulate in massive 'graveyards'	491	7.6
Hermit *Batillaria australis* (Bat–Hermit)	Adult	Shells inhabited by hermit crabs	Different movement than live adult *B. australis* shells	490	7.6
Juvenile *Batillaria australis* (Bat–Small)	Juvenile	Small shell size (<1.3 cm high)	Common but inconspicuous shell type; important component to understand effect of host size and age on epibiota	146	1.6
Bediva pavia (Bed)	Adult	Live shells from the largest native snail	Native snail of similar size as *B. australis*. Third most abundant snail in Swan River	398	5.0
Nassarius pauperatus (Nas)	Adult	Live shell from the smallest native snail	Small native snail, second most abundant in Swan River	565	1.0

Shell size, density and fouling

We measured shell length and width to nearest mm with digital callipers of the first 50 shells of each adult species encountered in the quadrate. Length and width was converted to a univariate shell dimension using Appleton's (1980) formula: Shell Dimension = log shell height × log shell width. We subsequently counted all the randomly collected gastropods and quantified the degree of biofouling – classifying a shell as 'fouled' if a least one epibiota species was found attached.

Epibiota

Attached sessile epibiota were identified and quantified under a dissection microscope at 40× magnification. We estimated percentage cover of encrusting species per shell (e.g. *Ralfsia* sp., *Membranipora* sp.) and counted the number of foliose algae (e.g. *Gracilaria comosa*) and solitary invertebrates (e.g. *Pomatoceros* sp.). The length and width of each shell was measured and converted to shell dimension as described in the previous paragraph.

Statistical analyses

Shell size, density and fouling

One-way ANOVA was used to test if sizes differed between the three gastropod species and two-way ANOVA tested if shell density and degree of fouling varied between species and salinity regions. Homogeneity of variances was evaluated with Barletts and Brown–Forsythe tests and data were square root transformed when necessary to meet assumptions of homogeneity of variances and normal distribution. Finally, Tukey HSD test was used to compare significant treatment effects ($P < 0.05$).

Epibiota

Generalised linear models (GLM) were used to model correlations between the explanatory factors and taxonomic richness and abundance of all epibiota species combined, encrusting species, foliose algae, and solitary invertebrates, and of the abundances of the most common epibiota taxa. Prior to analysis we tested if shell sizes (within each of the 7 shell types) differed between habitats, salinity, water depth and wave exposure (using 3-factorial ANOVA's with wave exposure as co-variate). These tests showed that sizes (of a shell type) were statistically similar between environments (see Table S2 available as Supplementary material to this paper). Data exploration was then carried out following the protocol of Zuur *et al.* (2010). Relationships between co-variates were assessed using boxplots and Pearson's correlation coefficients (Zuur *et al.* 2010). The two variables 'shell type' and 'shell size' showed a high level of collinearity ($r = 0.79$), and we therefore excluded shell size from further analysis to eliminate correlation between co-variates. Cook's plot and boxplots were used to identify outliers and to investigate relationships between variables; as a result we eliminated one extreme value of abundance of *G. comosa* because it would otherwise have made pattern detection in the data more difficult (Quinn and Keough 2002). We found no indication of zero inflation or over-dispersion for richness data, which were therefore analysed using GLM with Poisson distributions. In contrast, abundance data was characterised by over-dispersion (without zero inflation) and was therefore analysed using GLM with negative binomial distributions (Hilbe 2011). Shell type, salinity, wave exposure, habitat and water depth were included as explanatory variables in the full models, and the models were reduced to final best-fit models using Akaike Information Criterion (AIC) with $\Delta AIC < 2$ (Burnham and Anderson 2002).

Results

Shell size, density and fouling

The average shell surface area of the non-indigenous *B. australis* was 7.25 $cm^2 \pm 0.33$ s.e. ($n = 50$), and significantly larger than the native gastropods *B. paiva* (5.07 $cm^2 \pm 0.14$ s.e., $n = 50$) and *N. burchardi* (1.06 $cm^2 \pm 0.04$ s.e., $n = 50$) (ANOVA: $F_{2,147} = 597.8$; $P < 0.001$; see Table S3 available as Supplementary material to this paper). *B. australis* shells were also

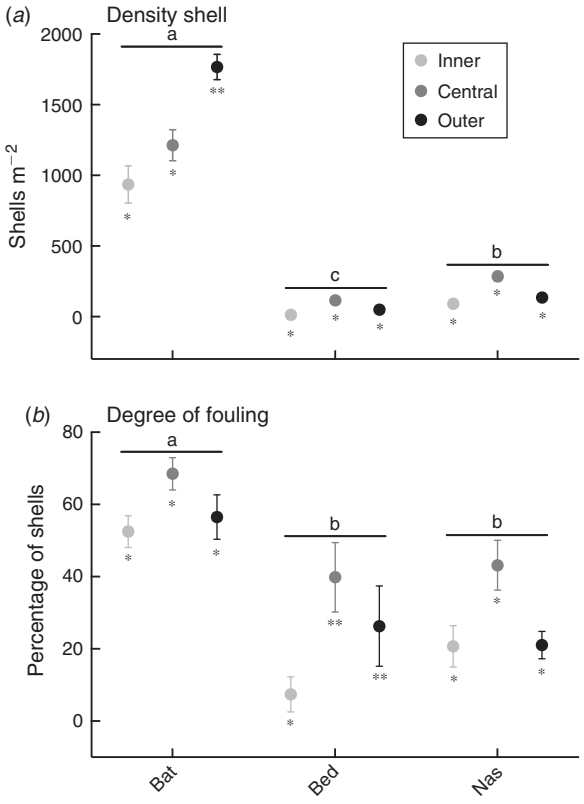

Fig. 2. Shell density (shells m^{-2}) and degree of shells biofouled (percentage of shells with at least one epibiotic taxa) for the three gastropod host-species (*Battilaria australis*, Bat; *Bedeva paiva*, Bed; *Nassarius pauperatus*, Nas) from the three sampled regions (Outer, Central and Inner estuary). Error bars indicate standard error. Different letters indicate a significant difference ($P < 0.05$) among shell types. Different numbers of asterisks indicates a significant difference ($P < 0.05$) among the salinity regions (Inner, Central, Outer).

more abundant (all sub-types combined) with a maximum density of 1767 shells m^{-2} found in the Outer estuary (ANOVA: $F_{2,78} = 153.2$; $P < 0.001$; Fig. 2*a*). The highest density of shells from native gastropods was found in the Central estuary with 285 m^{-2} of *N. burchardi* and 116 m^{-2} of *B. paivae* (Fig. 2*a*). A significantly higher proportion of *B. australis* shells were fouled compared to *B. paivae* and *N. burchardi* in all salinity regions (e.g. the maximal average fouling was 70% of *B. australis* shells (all sub-types combined) in the Central estuary) (ANOVA: $F_{2,78} = 14.74$; $P < 0.001$; Fig. 2*b*).

Epibiota

A total of 10 epibiota taxa were identified on the 3226 gastropod shells examined, represented by three foliose algae (*G. comosa*, *Chaetomorpha linum*, *Grateloupia* sp.), three solitary invertebrates (*Pomatoceros* sp., *Ascidiacea* sp., *Anthozoa* sp.) and four encrusting species (*Ralfsia* sp., *Membranipora* sp., coralline algae sp. 1, coralline algae sp. 2). Generally, shell type and salinity were the most important variables that explained patterns in epibiota richness (Table 2) and abundances of common taxa (Tables 3, 4). The highest taxonomic richness and abundances was found on 'Bat–Gra+' and 'Bat–Hermit' followed by 'Bat–Gra–', and 'Bat–Empty' shells. In comparison, 'Bat–Small', 'Bed' and 'Nas' shells generally had lower richness and abundances (Figs 3, 4, 5).

Table 2. Generalised linear model results partitioning variation in taxonomic richness of all epibiotic taxa combined (model 1), encrusting taxa (model 2), foliose algae (model 3) and solitary invertebrates (model 4) using a Poisson distribution

Only significant explanatory variables are shown. For each model we show degrees of freedom (d.f.), variables deviance (Deviance), likelihood ratio test value (LRT) and significant $P < 0.05$

GLM Models (Poisson distribution)	d.f.	Deviance	LRT	*P*
Model 1 (All taxa ~Shell type + Salinity)				
Explained deviance = 25%				
Shell type	6	4624.6	1119.8	<0.001
Salinity	2	3594.9	90.1	<0.001
Residuals	3217	3504.7		
Model 2 (Encrusting taxa ~Shell type + Salinity + Habitat)				
Explained deviance = 8%				
Shell type	6	2596.0	160.6	<0.001
Salinity	2	2495.9	60.4	<0.001
Habitat	1	2440.1	4.6	0.03
Residuals	3216	2435.5		
Model 3 (Foliose algae ~Shell type + Salinity)				
Explained deviance = 34%				
Shell type	6	3588.2	1221.7	<0.001
Salinity	2	2382.9	16.44	<0.001
Residuals	3217	2366.4		
Model 4 (Solitary invertebrates ~Shell type + Salinity)				
Explained deviance = 10%				
Shell type	6	2224.7	197.6	<0.001
Salinity	2	2059.5	32.4	<0.001
Residuals	3217	2027.1		

Taxonomic richness

We found significant effects of shell type and salinity on total epibiota richness (Model 1: Explained deviance = 25%; $P < 0.001$; Table 2). Most taxa were found on 'Bat–Gra+', followed by 'Bat–Hermit', 'Bat–Gra–' and 'Bat–Empty' shells, with more species found on shells from the Outer estuary compared to shells from the Inner estuary (Fig. 3*a*, salinity effect could not be evaluated for 'Bat–Small' from the Inner estuary because we did not find this shell type here). Richness of encrusting species was affected by shell type, salinity and habitat (Model 2: Explained deviance = 8%; $P < 0.001$; Table 2). 'Bat–Gra+' and 'Bat–Hermit' shells had the highest richness, whereas no differences were found among 'Bat–Empty', 'Bat–Small', 'Nas' and 'Bed' (Fig. 3*b*). Richness was higher on shells from the Outer estuary, compared to Inner estuary shells for all shell types (Fig. 3*b*). Richness of foliose algae was significantly affected by shell type and salinity (Model 3: Explained deviance = 34%; $P < 0.001$; Table 2); 'Bat–Gra+' had highest richness, and significantly fewer species were found on 'Bat–Gra–' and 'Bat–Hermit' shells in the Inner estuary (Fig. 3*c*). Taxonomic richness of solitary invertebrates were also affected by shell type and salinity (Model 4: Explained deviance = 10%; $P < 0.001$; Table 2). Richness was

Table 3. Generalised linear model results partitioning variation in abundances of all epibiotic taxa (model 5), encrusting taxa (model 6), foliose algae (model 7) and solitary invertebrates (model 8) using a negative binomial distribution

Only significant explanatory variables are shown. For each model we show degrees of freedom (d.f.), variables deviance (Deviance), likelihood ratio test value (LRT) and significant $P < 0.05$

GLM Models (Negative binomial distribution)	d.f.	Deviance	LRT	*P*
Model 5 (all taxa ~Shell type + Salinity)				
Explained deviance = 6%				
Shell type	6	3462.7	129.5	<0.001
Salinity	2	3446.5	113.3	<0.001
Residuals	3217	3333.1		
Model 6 (Encrusting taxa ~Shell type + Salinity)				
Explained deviance = 3%				
Shell type	6	2261.3	36.8	<0.001
Salinity	2	2269.6	45.2	<0.001
Residuals	3217	2224.4		
Model 7 (Foliose algae ~Shell type + Salinity)				
Explained deviance = 27%				
Shell type	6	2261.3	36.8	<0.001
Salinity	2	2269.6	45.2	<0.001
Residuals	3217	2224.4		
Model 8 (Solitary invertebrates ~Shell type + Salinity)				
Explained deviance = 16%				
Shell type	6	1954.3	129.5	<0.001
Salinity	2	1787.3	82.5	<0.001
Residuals	3217	1704.8		

generally higher in the Outer estuary than Inner estuary on 'Bat–empty', 'Bat–Gra–', 'Bat–Gra+', 'Bat–Hermit' and 'Bed', but significant effects was only found for 'Bat–Hermit' (Fig. 3*d*).

Group abundances

We found significant effects of shell type and salinity on total epibiota abundances (Model 5: Explained deviance = 6%; $P < 0.001$; Table 3). Highest abundances were found on shell type 'Bat–Gra+', 'Bat–Hermit' and 'Bat–Gra–' (Fig. 4*a*). Significantly higher epibiota abundances were found on all shell types in the Outer estuary compared to shells from the Inner estuary (Fig. 4*a*). Abundance of encrusting taxa was also only affected by shell type and salinity (Model 6: Explained deviance = 3%; $P < 0.001$; Table 3). 'Bat–Hermit' shells had significant higher epibiota cover compared to 'Bat–Small', whereas no differences were found among the other shell types (Fig. 4*b*). Furthermore, cover was higher on shell types (except 'Bat–Small') in the Outer estuary compared to the Inner estuary (Fig. 4*b*). Abundance of foliose algae was also significantly affected by shell type and salinity (Model 7: Explained deviance = 27%; $P < 0.001$; Table 3). 'Bat–Gra+' had the highest abundance of foliose algae, and there was significantly more foliose algae on 'Bat–Gra+' and 'Bat–Gra–' in the Outer than Inner estuary (Fig. 4*c*). Abundances of solitary invertebrates were also affected by shell type and salinity (Model 8: Explained deviance = 16%; $P < 0.001$; Table 3), with highest abundances on 'Bat–Hermit' shells in the Central and Outer estuary (Fig. 4*d*).

Table 4. Generalised linear models partitioning variation in abundance of the most common epibiotic species found in the Swan River Estuary including *Gracilaria comosa* (model 9), *Chaetomorpha linum* (model 10), *Grateloupia* sp. (model 11), *Pomatoceros* sp. (model 12), *Ralfsia* sp. (model 13) and *Membranipora* sp. (model 14) using a negative binomial distribution

Only significant explanatory variables are shown. For each model we show degrees of freedom (d.f.), variables deviance (Deviance), likelihood ratio test value (LRT) and significant $P < 0.05$

GLM Models (Negative binomial distribution)	d.f.	Deviance	LRT	*P*
Model 9 (*Gracilaria comosa* ~Shell type + Salinity)				
Explained deviance = 6%				
Shell type	6	3459.5	133.4	<0.001
Salinity	2	3437.5	111.4	<0.001
Residuals	3212	3326.1		
Model 10 (*Chaetomorpha linum* ~Shell type + Salinity + Depth + Wave exposure)				
Explained deviance = 19%				
Shell type	6	1729.4	254.0	<0.001
Salinity	2	1503.9	28.5	<0.001
Depth	1	1483.1	7.8	0.005
Wave exposure	1	1481.5	6.2	0.01
Residuals	3215	1475.4		
Model 11 (*Grateloupia* sp. ~Shell type + Wave exposure)				
Explained deviance = 25%				
Shell type	6	980.7	239.5	<0.001
Wave exposure	1	747.2	5.9	0.01
Residuals	3218	741.25		
Model 12 (*Pomatoceros* sp. ~Shell type + Salinity)				
Explained deviance = 16%				
Shell type	6	1909.3	243.5	<0.001
Salinity	2	1749.5	83.8	<0.001
Residuals	3217	1665.8		
Model 13 (*Ralfsia* sp. ~Shell type + Salinity + Wave exposure)				
Explained deviance = 5%				
Shell type	6	2012.3	51.5	<0.001
Salinity	2	2006.3	45.5	<0.001
Wave exposure	1	1967.0	6.2	0.01
Residuals	3216	1960.8		
Model 14 (*Membranipora* sp. ~Shell type + Salinity + Wave exposure)				
Explained deviance = 13%				
Shell type	6	596.1	41.0	<0.001
Salinity	2	581.9	26.7	<0.001
Wave exposure	1	568.8	13.6	<0.001
Residuals	3216	555.2		

Taxonomic abundances

The red alga *G. comosa* was significantly affected by shell type and salinity (Model 9: Explained deviance = 6%; $P < 0.001$; Table 4). *G. comosa* was most common on 'Bat–Gra+' followed by 'Bat–Hermit' shells, but were rare on 'Bat–Small', 'Bed' and 'Nas' (Fig. 5*a*). There was a (non-significant) trend of more *G. comosa* attached to shells in the Outer estuary (Fig. 5*a*). The green alga *C. linum* was affected by shell type, salinity, depth and wave exposure (Model 10: Explained deviance = 19%; $P < 0.001$; Table 4), but the two latter factors accounted for very little of the likelihood ratio test (LRT). *C. linum* was most abundant on *B. australis* shells

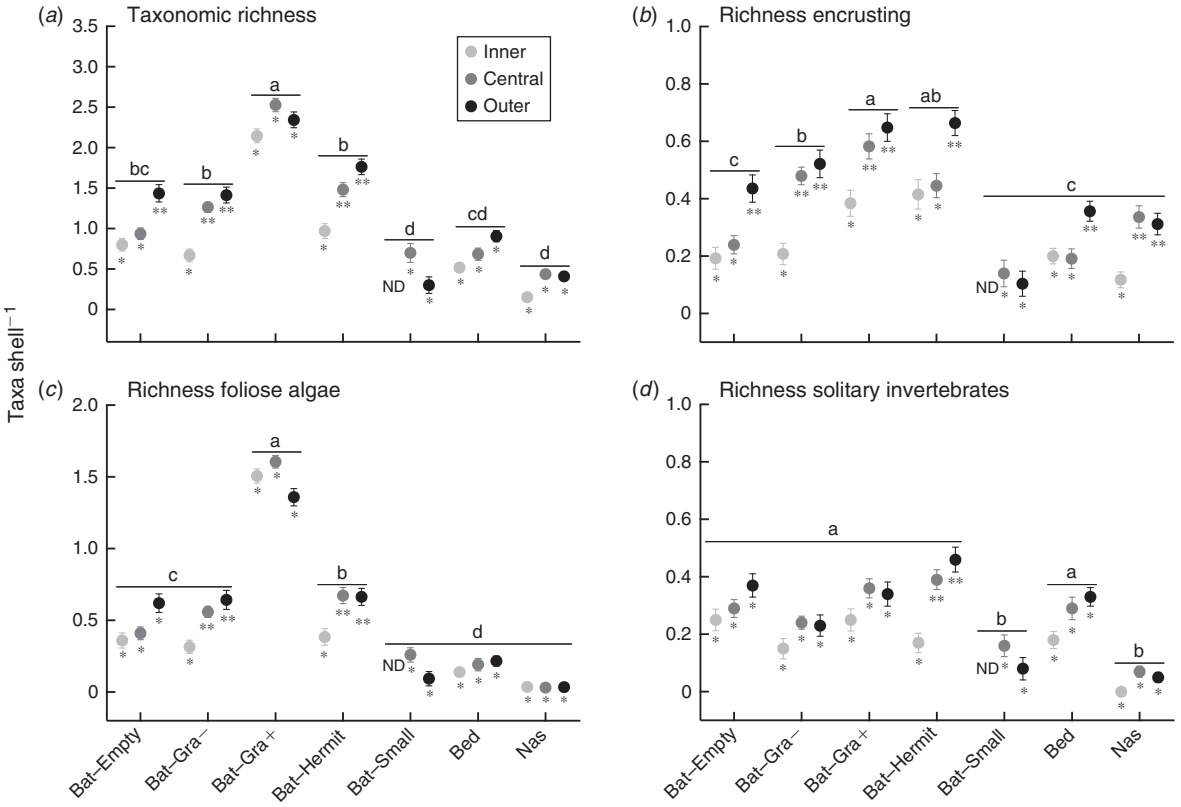

Fig. 3. Taxonomic richness of epibiota attached to *Battilaria australis* (Bat), *Bedeva paiva* (Bed) and *Nassarius pauperatus* (Nas) shell types in the Swan River Estuary. Taxonomic richness of (*a*) All epibiotic taxa combined; (*b*) Encrusting taxa; (*c*) Foliose algae and (*d*) Solitary invertebrates found on seven shell types. Error bars indicate standard error. Different letters indicate a significant difference ($P < 0.05$) among shell types. Different numbers of asterisks indicates a significant difference ($P < 0.05$) among the salinity regions (Inner, Central, Outer). No *N. pauperatus* shells were found in the Outer region.

(except 'Bat–Small') with the highest densities found in the Outer estuary (Fig. 5*b*). The alga *Grateloupia* sp. was the only taxon not affected by salinity, but it was affected by shell type and wave exposure (Model 11: Explained deviance = 25%; $P < 0.001$; Table 4), being most common on 'Bat–Hermit' and 'Bat–Gra+' shells (Fig. 5*c*). Wave exposure only accounted for a low LRT compared to shell type (5.9 *v.* 239.5). The tube building annelid *Pomatoceros* sp. was significantly affected by shell type and salinity (Model 12: Explained deviance = 16%; $P < 0.001$; Table 4). Highest densities were found on 'Bat–Hermit', 'Bat–Gra+', 'Bat–Dead' and 'Bed' (Fig. 5*d*), and for 'Bat–Hermit' shells, with lowest density in the Inner estuary (Fig. 5*d*). The encrusting brown alga *Ralfsia* sp. was significantly affected by shell type, salinity and wave exposure (Model 13: Explained deviance = 5%; $P < 0.001$; Table 4). Highest cover were found on 'Bat–Hermit' and 'Bat–Gra+' followed by 'Bat–Dead' and 'Bat–Gra–' (Fig. 5*e*). Percentage cover per shell of *Ralfsia* sp. was higher on shells from the Outer than Inner estuary (Fig. 5*e*), and on shells from wave protected sites compared to exposed sites (see Table S4 available as Supplementary material to this paper). Finally, *Membranipora* sp. was affected by shell type, salinity and wave exposure (Model 14: Explained deviance = 13%; $P < 0.001$; Table 4). 'Bat–Gra–', 'Bat–Gra+' and 'Bat–Hermit' shells had the highest cover (Fig. 5*f*). In contrast to other taxa, cover of *Membranipora* sp. were generally highest in the Central estuary, although only significant on 'Bat–Gra–', 'Bat–Gra+' and 'Bat–Hermit' (Fig. 5*f*) and on shells from wave exposed sites (see Table S5 available as Supplementary material to this paper).

Discussion

It is important to understand how environmental factors influence epibiota communities to better understand general processes that affect biodiversity in estuarine ecosystems. Here, we documented significant relationships between biogenic substrates, multiple environmental conditions and taxonomic richness and abundance of shell-associated epibiota in the Swan River Estuary, Western Australia. More specifically, we found that shell type and salinity were the most important factors (explaining most of the data variability in GLM models) affecting richness and abundances across epibiota taxa and form groups.

In the Swan River Estuary, the most abundant shell substrata for epibiota communities were provided by only three gastropod species; the native *N. pauperatus* and *B. paivae* and the non-indigenous *B. australis*. Of these species, *B. australis* shells were both more heavily fouled and were 13 times more abundant than the native species. Indeed, *B. australis* shells occurred in

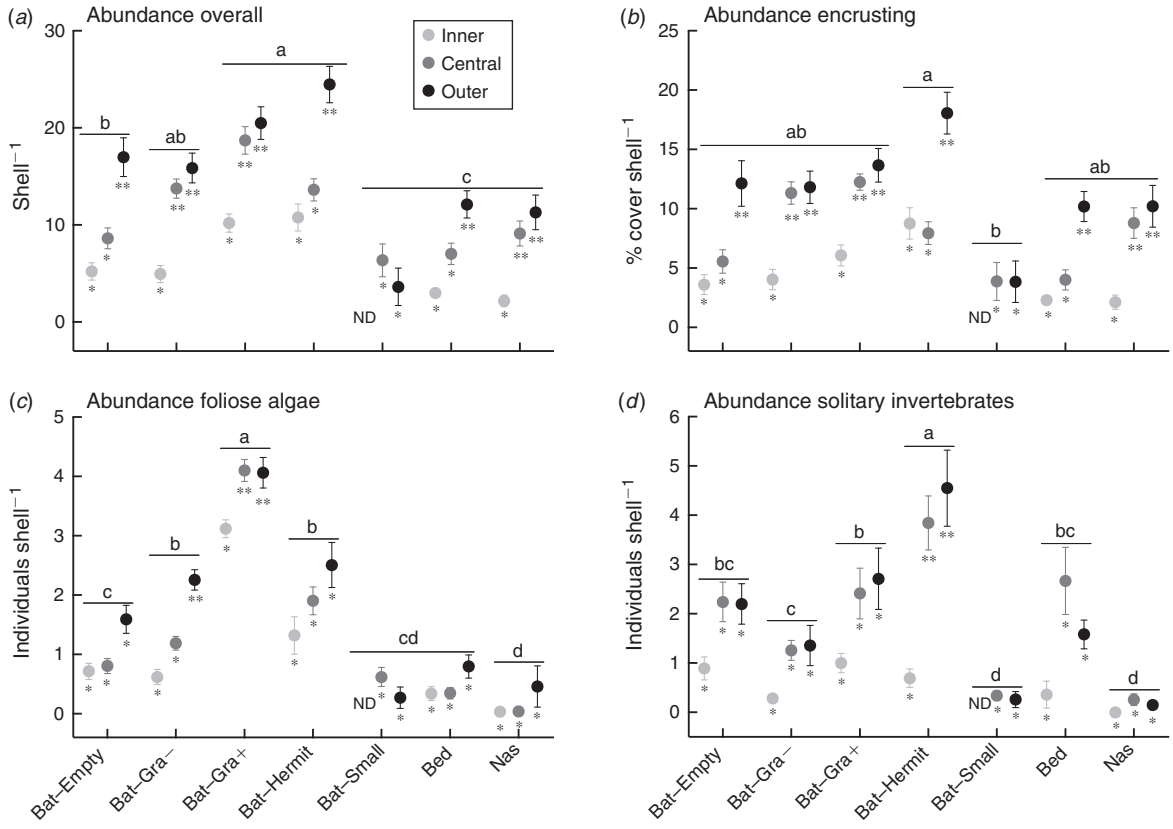

Fig. 4. Abundance of epibiota attached to *Battilaria australis* (Bat), *Bedeva paiva* (Bed) and *Nassarius pauperatus* (Nas) shell types in the Swan River Estuary. Abundance of (*a*) All epibiotic taxa combined; (*b*) Encrusting taxa; (*c*) Foliose algae and (*d*) Solitary invertebrates found on seven shell types. Error bars indicate standard error. Different letters indicate a significant difference ($P < 0.05$) among shell types. Different numbers of asterisks indicates a significant difference ($P < 0.05$) among the salinity regions (Inner, Central, Outer). No *N. pauperatus* shells were found in the Outer region.

densities exceeding 1700 shells m^{-2}, more than twice the density reported in 2007 (Thomsen *et al.* 2010*b*), suggesting a continued rapid population expansion over the last few years. The invasive gastropod is thereby orders of magnitude more important as a biogenic habitat former throughout the estuary, compared to all native shell formers combined.

We also found that taxonomic richness and abundance of the shell-associated epibiota were significantly correlated with shell type, salinity, habitat, water depth and wave exposure, although only shell type and salinity were consistently significant in all models (and explaining most of the data variability in the models, salinity excepted in model 11).

Biofouling typically depends on substrate availability and we therefore expected more species and higher epibiota population abundances on larger than smaller shell hosts (Creed 2000). For example, Wernberg *et al.* (2010) found more epibiota species on large *Turbo torquatus* shells, and Vasconcelos *et al.* (2007) found a higher colonisation score of epibiotic polychaetes on large *Hexaplex trunculus*. Our results support these data; when abundance and richness were evaluated per cm^2 shell we found no differences among shell types (data not shown) but we generally found less species and low abundances associated with small shell types (*N. pauperatus* and small *B. australis*) compared to larger shell types. Importantly, small *B. australis* had much less epibiota than larger conspecifics (including live, empty, and hermit crab-occupied *B. australis* shell types, cf. Figs 3–5) highlighting the importance of substrate availability in explaining variability of host specific epibiota communities. However, the small *B. australis* shells are also younger than the large *B. australis* shells and have therefore had shorter exposure time for settlement of fouling species. Thus, we cannot distinguish if facilitation of epibiota relate more to *host size* or *host longevity* (i.e. substrate availability in space and time respectively), as also noted in other epibiota studies (Creed 2000; Vasconcelos *et al.* 2007; Wernberg *et al.* 2010). Clearly manipulative experiments are needed to separate the relative influence of 'habitat size' *v.* 'habitat longevity' in future epibiota studies.

Epibiota communities can also be modified by the behaviour and movement patterns of the biogenic host (Wahl 1989; Becker and Wahl 1996), e.g. documented in several studies that compared epibiota communities on gastropod shells alive *v.* occupied by hermit crabs (Creed 2000; Bell 2005; Wonham *et al.* 2005). Our data support previous studies as we also found differences between epibiota communities inhabiting live shells, dead shells and shells occupied by hermit crabs. In depositional habitats in Swan River, empty shells are more likely to become buried, live *B. australis* snails are typically partly buried in sediments, but hermit crabs move around on the sediment surface (i.e. their shells

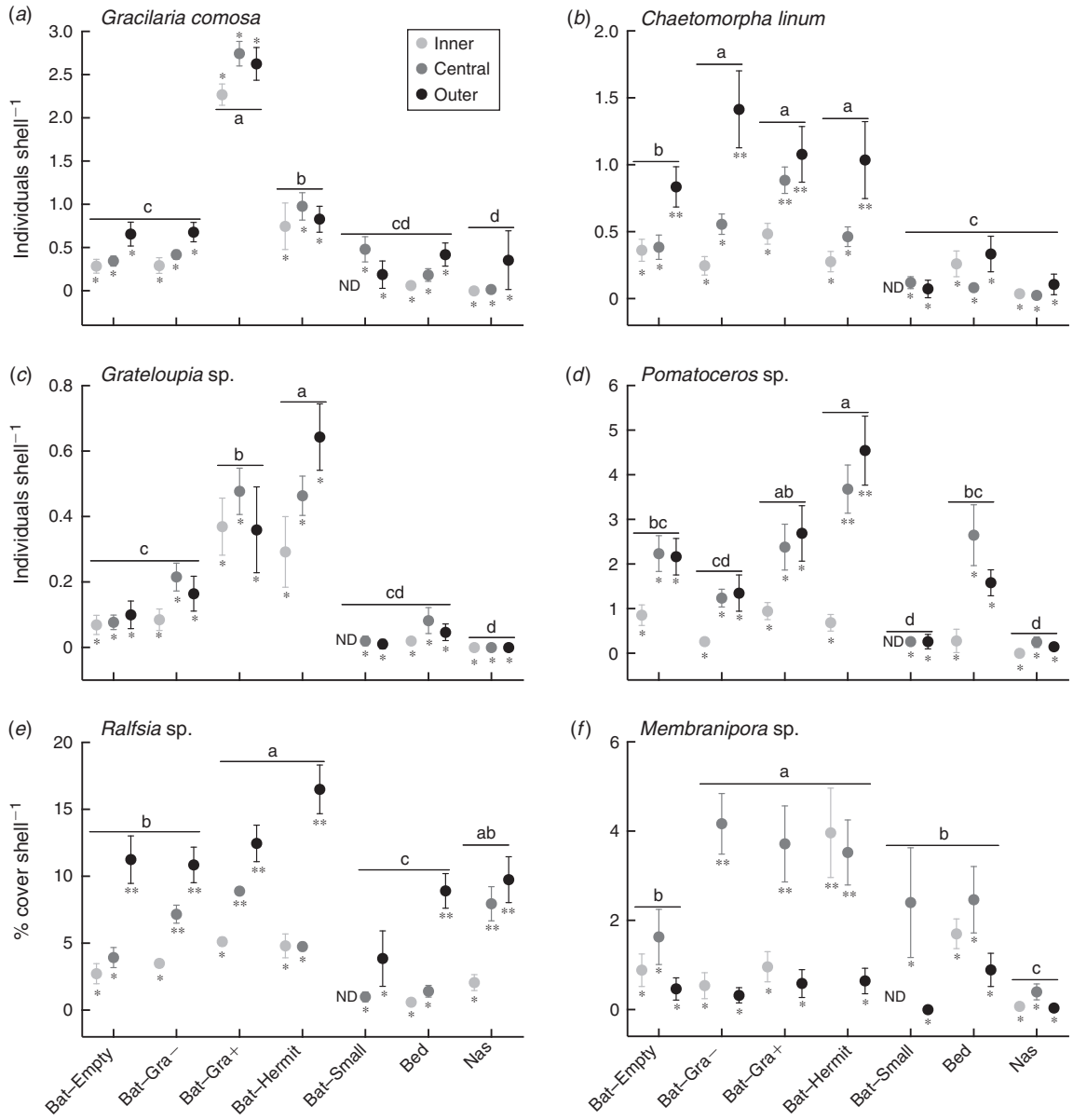

Fig. 5. Abundance of dominant epibiota taxa attached to *Battilaria australis* (Bat), *Bedeva paiva* (Bed) and *Nassarius pauperatus* (Nas) shell types in the Swan River Estuary. Abundance of (*a*) *Gracilaria comosa;* (*b*) *Chaetomorpha linum*: (*c*) *Grateloupia* sp.; (*d*) *Pomatoceros* sp.; (*e*) *Ralfsia* sp. and (*f*) *Membranipora* sp. Error bars indicate standard error. Different letters indicate a significant difference ($P < 0.05$) among shell types. Different numbers of asterisks indicates a significant difference ($P < 0.05$) among the salinity regions (Inner, Central, Outer). No *N. pauperatus* shells were found in the Outer region.

are constantly exposed to epibiota fouling). These differences co-varied with epibiota patterns, as we generally found higher densities, and sometimes also higher richness, on hermit crab shells compared to empty or live *B. australis* shells.

Salinity was the second most important determinant of epibiota communities in our models. Salinity determine distribution patterns of most estuarine organisms (Middelboe *et al.* 1998; Mclusky and Elliott 2004) because estuarine species are better adapted to marine than freshwater conditions and because salt-water intrusions, and connectivity to the adjacent sea facilitate dispersal of marine species into estuaries (Roegner 2000). Similar patterns have been documented for estuarine epibiota, e.g. Hardwick-Witman and Mathieson (1983) found a decrease in the abundance and richness of epibiota along a salinity gradient into the Great Bay Estuary System (NH, USA). We also documented strong salinity effects on epibiota in the Swan River Estuary; most taxa were more abundant in the high than the low salinity region and this pattern was consistent across shell types. One exception was the bryozoa *Membranipora* sp., which was most abundant in the Central estuary. Some bryozoans are eurythermal and adapted to survive and colonise estuarine ecosystems (Menon and Nair 1972; O'Dea and Okamura1999), potentially explaining why

Membranipora sp. was most abundant in the Central estuary. We did not sample the fresh water streams (constant salinity of ~0–1‰) farther into the Swan Rivers and salinity effects would likely have been even stronger if low salinity areas had been included. However, these areas contain few gastropod hosts, i.e. the salinity threshold of the hosts limited the areas in which we could sample epibiota. We finally note that salinity often co-vary with other environmental factors. For example: flow rates, suspended food particles, water clarity, and sediment grain sizes are typically higher (and nutrient levels lower) near the high-salinity estuary mouth (Mclusky and Elliott 2004; Thomsen *et al.* 2006). Nevertheless, we suggest that salinity generally is more important than these co-variates, although manipulative experiments are needed to verify this hypothesis.

In our initial hypotheses, we suggested that water depth, wave exposure and habitat type (seagrass *v.* mudflats) would, in addition to shell type and salinity, influence epibiota community structures. For example, Barnes and Clarke (1995) found that percentage cover of bryozoan epibiota on the limpet *Nacella concinna* increased with depth, and Rossi *et al.* (2000) found higher abundances of hydroids on their hosts at more sheltered sites. However, in our models, those test factors were rarely significant, and only explained a small proportion of the data variability (i.e. were of no or relative low importance in determining epibiota richness and abundance). There may be several reasons why we found few effects of depth, wave exposure and habitat type. Importantly, *B. australis, N. pauperatus* and *B. paivae* and hermit crabs are active species that move around. Effects of water depth and habitat could therefore be diluted by host movements between habitats and depths. For example, seagrasses are patchily distributed around mudflats, and the living shell types might move in and out of patches to obscure differential settlement patterns of epibiota propagules. Furthermore, currents around seagrass beds and mudflats may mix and disperse propagules to reduce inter-habitat differences in epibiota communities. Finally, waves and currents can entrain both live and dead shell types and move them passively between habitats and depths. Indeed, after storms we have often observed large quantities of *B. australis* on the beach, suggesting passive drift across depth levels. Note also that we compared effects of water depth within a narrow interval (0.5 *v.* 1.5 m). Sampling a larger depth gradient, including shells from shallower and deeper strata, would likely have increased the importance of this test factor. For example, at increasing depth light decrease thereby limiting survival of autotrophic epibiota (Rohde *et al.* 2008). Furthermore, like salinity 'depth' typically co-varies with light levels, wave exposure, currents, turbidity, sediment grain size, re-suspension, etc. Some co-variates might thereby facilitate but other inhibit epibiota communities with increasing depth, and thereby potentially cancel out depth effects. Again, manipulative experiments are essential to test if co-varying factors modify epibiota community structures differently along depth gradients. Finally, wave exposure also only explained little data variability, probably because the Swan River Estuary is fairly protected from waves. Thus, in comparisons to open coastlines, estuarine wave exposure gradients are typically weak and likely to be of less importance in determining epibiotic community structures.

Shell substratum provided by *Battilaria* species has previously been shown to facilitate sessile communities (Chan and Chan 2005; Wonham *et al.* 2005; Thomsen *et al.* 2010*a*; Thomsen *et al.* 2010*b*). Of the different epibiota taxa observed in our study, *G. comosa* is likely to be particular important, because it is common throughout the estuary on different shell types and because it is the only epibiota species that form a large three-dimensional structure. Indeed, *G. comosa*, like other estuarine *Gracilaria* species, can itself facilitate a range of sessile and mobile invertebrates, thereby increasing biodiversity and productivity through cascading habitat formation (Thomsen *et al.* 2010a; Thomsen *et al.* 2012). However, research is needed to better understand processes whereby shell forming hosts directly and indirectly facilitate epibiota and control biodiversity, e.g. by testing if intermediate habitat formers (like *G. comosa*) can have negative effects on other epibiota through competition for nutrients or light or by altering water flow (Tanner 1995; Miller and Etter 2008). However, our data did not indicate negative effects of *G. comosa* on other epibiota, because abundances and richness were generally higher on *B. australis* with, than without, large *G. comosa* fronds.

In summary, our results highlight that shell type and salinity are particularly important in determining community structures of estuarine sessile epibiota. Our models only explained 3–34% of the total data variability, but GLMs are nevertheless a powerful tool to investigate the importance of multiple processes influencing richness and abundance of epibiota communities. We finally suggest that future epibiota studies that test for effects of multiple environmental factors include (1) more explanatory factors in their models, (2) wider ranges of each gradients, (3) manipulative experiments to identify underlying mechanisms and (4) analysis and test of how individual epibiota species affect each other – and the host itself.

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