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Publication details
Published version available from:
http://dx.doi.org/10.1080/13235818.2017.1279472
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Running Head: Temporal variation in heterobranch sea slugs

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Abstract
Assemblages of heterobranch sea slugs are notoriously variable in space and time which has sometimes led to their exclusion from broader studies of patterns of biodiversity. This variability may also result in a failure to detect underlying spatial and temporal patterns. Assemblage data (species abundances) from three intertidal sites over a period of 13 months, were used to compare the conclusions drawn from three different analytical approaches - a non-specific test for differences among months, and tests of cyclicity, and seriation, using specific model matrices. While no significant difference was detected for the non-specific test (i.e. concluding no significant temporal variation), the data were significantly correlated to both model matrices with the cyclicity model outperforming the seriation model at two of the three sites. The study also highlighted the spatial variability of assemblages over a scale of just a few kilometres. Wider testing using previously published data sets confirmed the utility of these models for exploring specific hypotheses about patterns of temporal change.

Key Words: subtropical eastern Australia, intertidal, rocky shore, stochastic corkscrew
Introduction
With estimates of more than 6,000 species worldwide (Behrens & Hermosillo 2005; Yonow 2008), and distributions that include most marine habitats (García & Bertsch 2009), heterobranch sea slugs (formerly known as the opisthobranchs) comprise an important component of marine communities. Despite this, and in comparison to other speciose groups of marine taxa, there have been remarkably few studies of their ecology (Todd 1981; Marshall and Willan 1999; Gosliner & Draheim 1996). There is little doubt that this relates to the notoriety of the group for being rare in space and time (Marshall and Willan 1999), and having boom-and-bust patterns of abundance (Rudman and Willan 1998), both of which may add unwanted noise to broader ecological data sets (Smith 2005). However, noise in this context may simply arise because patterns are complex and therefore difficult to detect. Some of this variability is undoubtedly accounted for by the limited life-span of most taxa (< 1yr) (Thompson 1976; Nybakken 1978; Wells and Bryce 1993; Marshall and Willan 1999; Rudman and Willan 1998) and the fact that populations are primarily controlled by food availability (i.e. bottom-up processes). Thus, variation in food availability and any of the factors that influence recruitment, may have a substantial effect on the composition of assemblages at different sites and different times (Ros 1978). For these reasons, it is unsurprising that past studies of spatial and temporal variation have often failed to detect consistent patterns. For example, using data collected over a 40-month period from Californian rocky shores, Nybakken (1978) found no significant temporal variation and this was interpreted to indicate that populations are temporally stable. Similar studies from the Mediterranean (Domenech et al. 2002; Ros 1978), the North Sea (Aerts 1994), Mexico (Angulo-Campillo 2005) and Taiwan (Su et al. 2009) showed a range of outcomes with some documenting seasonal cycles of species richness, and abundance for some species, and others showing a lack of clear pattern (Su et al. 2009). However, a number of these studies simply examined trends without supporting these with statistical analyses.

Here, we posit an alternate hypothesis to explain the lack of consistency in published assessments of temporal variation of sea slug abundance – that, at least in some cases, the analytical methods were too non-specific to detect real patterns. We explore this for a 13-month data set by using statistical tests that assess the type of temporal variation within the data set – from non-specific analysis of difference between months, to unidirectional change (seriation) or clear seasonal patterns (cyclicity). Tests of cyclicity generate significant statistical outcomes if the following conditions are met: i) assemblages that are close together
in time are more similar than those that are more temporally distant; and ii) assemblages show seasonal convergence. In contrast, tests of seriation provide a strong fit if only the first condition is met. Having explored hypotheses associated with these models for our data, we also re-evaluate data from some previous studies to see if these more targeted analyses alter their original conclusions.

**Materials and methods**

*Geographical setting*

The Solitary Islands Marine Park (SIMP), on the mid-north coast of New South Wales (NSW) provides an ideal location for studies of temporal variability in shallow subtidal assemblages as it protects an area where tropical and temperate marine biomes overlap (Smith and Simpson 1991; Harriott et al. 1994; Malcolm et al., 2010). The coastal morphology of the SIMP is generally characterised by long sandy beaches interrupted by rocky headlands which provide an enduring and persistent, mostly greywacke, substratum for establishment and retention of sessile biota (Korsch 1980). Coralline algae comprise the dominant benthic cover in tidal pools (Smith, 2005), which, where deeper (>1m), often support larger phaeophyta such as *Sargassum* spp. C. Agardh, 1820 and *Ecklonia radiata* (C. Agardh) J. Agardh, 1848 (Smith and Simpson 1991; Davie 1998). A range of sessile benthic fauna is also present, including various ascidians, cnidarians and encrusting *Porifera* (Smith and Simpson 1991; Smith 2005).

*Study design and field methods*

Three separate mainland rocky reef sites (Bare Bluff, Low Reef and Woolgoolga Headland – Fig. 1) were selected for survey based on their ease of access, proximity to each other, and the presence of suitable intertidal habitat (shallow and deep tidal pools, sections of boulder field – Smith 2005). Surveys were conducted on low tides ≤0.45 m during calm seas, in an area of approximately 200 m² at each site. An identical survey route and search method was employed for each monthly survey over a period of 13 months (March 2014 to March 2015, inclusive).

<Figure 1 about here>
Preliminary, timed studies identified that a 2-hr search period was sufficient to document the majority of species (i.e. cumulative species richness reached asymptote) and this was adopted as the standard replicate for subsequent surveys. Observations were recorded on waterproof data sheets and each individual was photographed in the field using an Olympus TG-3 compact camera in a waterproof housing. The final data thus comprised a species/abundance matrix for each site at each time. Sampling was conducted in accordance with a permit issued by NSW Department of Primary Industries (SIMP 2014/001).

**Statistical analysis**

Patterns among samples were initially visualised using non-metric multidimensional scaling (nMDS) of Bray-Curtis similarities based on square-root transformed data. Cyclicity is likely to be evident in terms of changes in population sizes (i.e. species with seasonal peaks in abundance) as well as in assemblage structure (seasonal presence/absence of species, many of which are rare). Using untransformed data biases the analysis in favour of abundance-driven cyclicity, whereas a strong transformation (e.g. fourth-root) biases the analysis towards patterns driven by the presence or absence of rare species. The square-root transformation provides a compromise that allows both to contribute to the overall assessment of cyclicity.

We tested for a non-specific temporal effect using a one-way PERMANOVA in which samples from each headland were the replicates for each month: 999 permutations were specified for all PERMANOVA analyses. We conducted more specific tests of cyclicity and seriation using the RELATE routine using *a priori* defined cyclical and seriation matrices (Clarke and Gorley 2006). The degree to which variation in assemblage data conformed to the two models was measured using a Spearman Rank Correlation (ρ) (Clarke and Gorley 2006). As the nMDS revealed very obvious differences in the assemblage structure at each sampling site, further tests of cyclicity and seriation were performed separately for data from each site.

Given that most previous evaluations of temporal variation in heterobranch assemblages have focused on summary variables rather than multivariate assemblage patterns, we also analysed temporal variation for species richness (S – total number of species) and total abundance (N). In this case, we used the Euclidean Distance measure in the calculation of similarities amongst samples.
**Wider testing on past datasets**

To test how the use of these analytical models might affect the conclusions of previous work, where possible, we extracted data from publications and used seriation and cyclical analyses to explore their inherent temporal variation. We could not conduct tests for a general temporal effect as data were either unreplicated or, in some cases, summarised as a monthly average where the study duration was >1 yr.

Ros (1978) provides four years of data from the Catalonian coast collated into a single semi-quantitative measure for each month. The study identified seasonal patterns that were primarily related to increases in abundance in spring and summer. We re-analysed the untransformed semi-quantitative data for each species.

Rueda et al. (2008) conducted monthly sampling of molluscs associated with seagrass beds in Spain over a 25-month period and reported seasonal variation in abundance. We extracted the data for heterobranchs (11 species) and used the abundance data provided in the supplementary material supplied with the paper. Analytical procedures were as for our dataset for individual sites.

In the Pescadores, Taiwan, Su et al. (2009) recorded contiguous monthly presence/absence data sets from August 2006 to December 2007. Their analyses comprised simple summaries of species richness and diversity (Shannon’s diversity index and equitability): while they concluded that there were “no obvious trends in temporal changes”, this was not formally tested.

**Results**

A total of 1,614 individual heterobranchs from 94 species was recorded during the 39, two-hour surveys conducted for the study. Ten species were dominant, accounting for 64% of total observations (Table 1, Fig. 2). At the other end of the sighting frequency and abundance scale, 48% of species were observed three times or less.

The primary insight gained from nMDS was that patterns of assemblage structure were very different among the sampling sites (no overlap in Fig. 3). When temporal trajectories were added, there was also a strong suggestion of cyclical variation at each site (Fig. 4). The non-specific tests for differences in temporal patterns (one-way PERMANOVA) was not significant ($P(\text{perm}) = 0.438$). Despite the obvious differences in assemblage structure among
sites, temporal variation in the total data showed a significant fit to both the cyclicity ($\rho = 0.414, P = 0.001$) and seriation ($\rho = 0.099, P = 0.021$) models, although the correlation coefficient for the latter was low. As there were obvious differences in assemblage patterns among sites (Fig. 3), tests were repeated for each site separately. With the exception of the analyses for Bare Bluff, the cyclicity model provided a better fit to the patterns of assemblage variation (Table 2).

The non-specific, one-way PERMANOVA revealed a significant temporal effect for both summary variables ($S - P(\text{perm}) = 0.010$; $N - P(\text{perm}) = 0.003$). Temporal trends were found to significantly fit both models (Table 1), however, the seriation model slightly outperformed the cyclicity model but the correlation coefficient was relatively low for both sets of tests.

Wider testing

Ros (1979). Our analyses detected highly significant cyclicity ($\rho = 0.479, P = 0.001$) and seriation ($\rho = 0.434, P = 0.001$). In this case, the data were a slightly better fit to the seriation model. Whilst Ros noted seasonal trends and suggested that this reflected similar variability amongst the opisthobranch food sources, we note that the presence of significant seriation suggests that the assemblage, whilst exhibiting cyclicity, also undergoes a temporal shift in species composition.

Rueda et al. (2008). Using square-root transformed abundance data, we found no significant correlation to the seriation model ($\rho = -0.007, P = 0.475$) and a low, but significant correlation to the cyclicity model ($\rho = 0.149, P = 0.016$).

Su et al. (2009). Our analyses revealed significant cyclicity ($\rho = 0.158, P = 0.049$) but not seriation ($\rho = 0.098, P = 0.160$). However, the correlation coefficient for cyclicity was relatively low. This finding contrasts with the paper’s conclusion of no obvious trends in species composition.

Discussion

This study supports past observations of high levels of variability in sea slug assemblages, both over distances of a few kilometres, and a 13-month temporal scale. However, it has also provided important insight into the nature of this variation. Thus, while patterns show clear
and significant cyclicity, there are coincidental changes in assemblage patterns that result in a significant fit to a seriation model. This is best visualised as a corkscrew trajectory through multidimensional space driven by seasonal cycles of some dominant species (Fig. 2) and a changing identity (and/or abundance) of other species over time. This pattern is driven by sporadic recruitment, and it is conceivable that seriation trajectories might reverse depending on the species pool at any single point in time. Thus, we hypothesise that the trajectory over longer periods of time may be visualised as a “stochastic corkscrew”. There is little doubt that this pattern arises because of the short life-cycles of many species, the fact that some taxa show strong seasonality (e.g. the Aplysiidae – e.g. Nimbs et al., 2016), and the temporal (both intra and inter-annual) variability in recruitment for most. Clearly, as it is based on only 13 months of data, this is speculative. However, it provides a clear model, and therefore hypothesis, against which longer data sets can be compared. While recent work documenting temporal and diel variation in sea slug assemblages at Port Stephens, NSW (Larkin, unpublished data) over a similar time period (13 months) provides support for the model, wider testing over longer time periods is required.

It is pertinent to note that, at least for the tropically affiliated species, recruitment may be dependent on larval supply via the East Australian Current (EAC). This current is highly variable in terms of its influence across the continental shelf within the region (Malcolm et al., 2011) and there is considerable support for the hypothesis that this drives broader community structure from nearshore to offshore locations (Harriot et al., 1994; Malcolm et al., 2010; Harrison and Smith, 2012). Sporadic, EAC-mediated delivery of larvae has also been suggested as one of the key drivers for variation in specific molluscan populations (e.g. tridacnid and lithophagin bivalves– Smith, 2011a, b). With predictions that the EAC will strengthen under climate change, it is likely that population dynamics for many species may also change and there is already evidence of this through range extensions for a number of heterobranch sea slug species (Nimbs et al., 2015, 2016; Nimbs and Smith, 2016).

What is also apparent from this small study is that the actual patterns are highly spatially constrained such that assemblages at sites that are only a few kilometres apart support very different assemblages in terms of species and their abundance. The patterns of change over time are also site dependent, with assemblages from Bare Bluff best fitting a seriation model while those from the other two sites best fitting a cyclicity model. Some of these differences can undoubtedly be explained by the differences in food and habitat availability at each site. For example, Bare Bluff supports a sheltered rocky slot (sheer sides, ~1m wide, 8m long) that
supports a relatively stable benthos including sponges and soft corals that persisted throughout the study. As a result, a suite of 6-8 species was found on almost every sampling occasion. In contrast, habitats at the other sites were macroalgal dominated, relatively exposed to wave action, and showed obvious changes in benthic cover over the duration of the study (pers. obs., MJN and SDAS). These observations provide some support, albeit anecdotal, for the suggestion that heterobranch assemblages are strongly regulated by bottom-up processes (Thompson 1976; McDonald and Nybakken 1997; Rudman 1998; Rudman and Willan 1998; Cobb and Willan 2006).

These results highlight the importance of considering exactly what is being evaluated in assessments of temporal variation. Specific tests of models of discrete types of variation clearly provide a much more effective way of evaluating temporal change and help to identify patterns in apparently noisy data. This is borne out by the re-evaluation of previously published data sets. In one case (Su et al. 2009), a significant cyclical pattern was detected from presence/absence data despite the fact that the study concluded there was no clear pattern. In the dataset from *Zostera* seagrass beds (Rueda et al. 2008), there was no evidence of seriation but clear evidence of cyclical patterns, although the very low or zero abundances at some sample times led to a weak, though significant correlation coefficient ($\rho = 0.149$).

Interpretation of the final dataset (Ros, 1978) was partly confounded by the fact that the monthly data represent averages across 4 years and so much of the real variability was lost. Nevertheless, the strong and significant fit to both the cyclicity and seriation models indicate the utility of the method for addressing specific hypotheses (in this case, general seasonal patterns over the 4-yr study period).

While the application of hypothesis-based succession models is novel in the context of heterobranch sea slugs, these types of tests have now been successfully applied to a range of temporal marine datasets, including bacteria, benthic communities and fish (e.g. Giarrizzo and Krumme 2007; Wernberg and Goldberg 2008; Davis et al. 2016; Xu and Xu 2016). In this case, use of these models has provided insight into trajectories of change in sea slug assemblages and direction for future work to further explain temporal and spatial variation in this charismatic and popular group of molluscs.
Acknowledgements
This work forms part of a 3rd-year undergraduate project by MJN. We sincerely thank Dr Richard Willan for his assistance throughout the project. We also thank Dr Paul Somerfield for guidance about cyclicity testing in the PRIMER package. Kathryn James assisted with the production of the figures. Academic, operations and administrative staff and other students of the National Marine Science Centre and Marine Ecology Research Centre at Southern Cross University also provided support for this project. The work was undertaken under NSW Department of Primary Industries Research Permit #SIMP 2014/001. Prof Andy Davis and an anonymous reviewer provided helpful comments that improved the manuscript.
References

http://doi.org/10.1093/mollus/60.2.129


Table 1. Ranked list of the 10 dominant heterobranch species from 39 two-hour surveys conducted from March 2013 and March 2015 on three rocky headlands in the Solitary Islands Marine Park, NSW.

<table>
<thead>
<tr>
<th>Species</th>
<th>Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Elysia australis</em></td>
<td>(Quoy &amp; Gaimard, 1832)</td>
</tr>
<tr>
<td><em>Dolabrifera brazieri</em></td>
<td>G. B. Sowerby II, 1870</td>
</tr>
<tr>
<td><em>Mexichromis festiva</em></td>
<td>(Angas, 1864)</td>
</tr>
<tr>
<td><em>Aplysia juliana</em></td>
<td>Quoy &amp; Gaimard, 1832</td>
</tr>
<tr>
<td><em>Glossodoris angasi</em></td>
<td>Rudman, 1986</td>
</tr>
<tr>
<td><em>Tritonia</em> sp. 1</td>
<td></td>
</tr>
<tr>
<td><em>Australaolis ornata</em></td>
<td>(Angas, 1864)</td>
</tr>
<tr>
<td><em>Elysia maoria</em></td>
<td>Powell, 1937</td>
</tr>
<tr>
<td><em>Aplysia argus</em></td>
<td>Rüppel &amp; Leuckart, 1830</td>
</tr>
<tr>
<td><em>Dendrodoris nigra</em></td>
<td>(Stimpson, 1855)</td>
</tr>
</tbody>
</table>

Table 2: Summary of cyclic and serial RELATE tests. The higher value for each set of analyses is shown in bold font and significant results are underlined. S = species richness; N = total abundance.

<table>
<thead>
<tr>
<th>Assemblage</th>
<th>Cyclicity</th>
<th></th>
<th>Seriation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient ($\rho$)</td>
<td>Significance ($\rho$)</td>
<td>Coefficient ($\rho$)</td>
</tr>
<tr>
<td>All sites</td>
<td>0.414</td>
<td>0.001</td>
<td>0.099</td>
</tr>
<tr>
<td>Woolgoolga</td>
<td>0.344</td>
<td>0.004</td>
<td>0.203</td>
</tr>
<tr>
<td>Low Reef</td>
<td>0.507</td>
<td>0.001</td>
<td>0.430</td>
</tr>
<tr>
<td>Bare Bluff</td>
<td>0.391</td>
<td>0.003</td>
<td>0.525</td>
</tr>
<tr>
<td>S All sites</td>
<td>0.170</td>
<td>0.002</td>
<td>0.180</td>
</tr>
<tr>
<td>N All sites</td>
<td>0.132</td>
<td>0.009</td>
<td>0.132</td>
</tr>
</tbody>
</table>
Fig. 1. Location of the three rocky headlands in the Solitary Islands Marine Park. Shading represents land.
Fig. 2. Monthly abundances of ten dominant taxa between March 2014 and March 2015. A. Dolabrifera brazieri, B. Elysia maoria, C. Austraeolis ornata, D. Aplysia juliana, E. Elysia australis, F. Tritonia sp., G. Mexichromis festiva, H. Glossodoris angasi, I. Dendrodoris nigra, J. Aplysia argus. (Note the differences in scale on the y-axis for different plots.)
Fig. 3. Three-dimensional nMDS ordination of square-root transformed monthly data based on Bray-Curtis similarities between March 2014 and March 2015. Squares = Woolgoolga Headland, circles = Low Reef, triangles = Bare Bluff. The size of the symbols represents their relative position in three-dimensional space.
Fig. 4. Two-dimensional nMDS ordinations with trajectory overlays for square-root transformed monthly data based on Bray-Curtis similarities between March 2014 and March 2015.