

Predicting ecological regime shift under climate change: New modelling techniques and potential of molecular-based approaches

Richard STAFFORD^{1*}, V. Anne SMITH², Dirk HUSMEIER³, Thomas GRIMA⁴, Barbara-ann GUINN⁴

¹School of Applied Sciences, Bournemouth University, BH12 5BB, UK

²School of Biology, University of St Andrews, St Andrews, KY16 9ST, UK

³School of Mathematics and Statistics, University of Glasgow, G12 8QW, UK

⁴Department of Life Sciences, University of Bedfordshire, LU1 3JU, UK

Abstract Ecological regime shift is the rapid transition from one stable community structure to another, often ecologically inferior, stable community. Such regime shifts are especially common in shallow marine communities, such as the transition of kelp forests to algal turfs that harbour far lower biodiversity. Stable regimes in communities are a result of balanced interactions between species, and predicting new regimes therefore requires an evaluation of new species interactions, as well as the resilience of the ‘stable’ position. While computational optimisation techniques can predict new potential regimes, predicting the most likely community state of the various options produced is currently educated guess work. In this study we integrate a stable regime optimisation approach with a Bayesian network used to infer prior knowledge of the likely stress of climate change (or, in practice, any other disturbance) on each component species of a representative rocky shore community model. Combining the results, by calculating the product of the match between resilient computational predictions and the posterior probabilities of the Bayesian network, gives a refined set of model predictors, and demonstrates the use of the process in determining community changes, as might occur through processes such as climate change. To inform Bayesian priors, we conduct a review of molecular approaches applied to the analysis of the transcriptome of rocky shore organisms, and show how such an approach could be linked to measurable stress variables in the field. Hence species-specific microarrays could be designed as biomarkers of *in situ* stress, and used to inform predictive modelling approaches such as those described here [*Current Zoology* 59 (3): 403–417, 2013].

Key Words Regime shift, Phase shift, Alternative stable state, Intertidal, Food web, Resilience

While the net, global effect of anthropogenic climate change on physical properties such as temperature are well documented in scientific literature (Peters and Lovejoy, 1994; IPCC, 2007), the magnitude of these effects are less certain, and the local effects are less certain still, and most likely spatially and temporally variable (Peters and Lovejoy, 1994; Gilman et al., 2010). Given the uncertainty of changes to physical environments, changes in biological characteristics of ecological communities are highly uncertain and current modelling approaches closely match biological distributions to the physical predictions, with little regard for other biological processes such as species interactions (e.g. climate envelope modelling—Pearson and Dawson, 2003; Gilman et al., 2010).

Physiological changes to climate-induced stressors,

such as temperature, have been conducted for many species. In invertebrates, studies such as lethal temperature, heat coma temperatures or changes to heart rate in response to increasing temperatures have been conducted on many species, both in the laboratory, and in field conditions (reviewed by Somero, 2002). However, the wealth of possible data given by modern genomic techniques, such as studying gene expression using microarrays, has generally been confined to the laboratory (Kammenga et al., 2007; van Straalen and Roelofs, 2012). While the lack of knowledge of gene function hinders field-based studies on most invertebrates (van Straalen and Roelofs, 2012), examining changes along stress gradients (i.e. latitudes) gives an indication of how overall levels of gene expression may vary *in situ* to physical conditions, such as climate (Place et al.,

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* Corresponding author. E-mail: rstafford@bournemouth.ac.uk

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2008). Determining such changes in gene expression in measurably significantly different microhabitats would therefore provide a mechanism of predicting sub-lethal physiological stress caused by climate-induced factors, such as temperature, or indeed, any other physiological stress. Such investigations could predict which species may suffer most from any changes.

Given any species exists as part of an ecological community, interacting with prey, predators, parasites and competitors, changes in the physiology, ecology or distribution of any species will have some degree of community level effect. Predicting changes in communities, and resultant changes to ecosystem services, is therefore a complex task (Peters and Lovejoy, 1994). Shallow marine ecosystems are well studied, and ecologically and economically important (Peterson and Lubchenco, 1997). For example, many shallow marine ecosystems contain economically important fish species, either directly, or as juveniles in nursery grounds. Furthermore, subtidal reefs, such as kelp forests and coral reefs are important to tourism, through SCUBA diving (Peterson and Lubchenco, 1997). These ecosystems also are some of the most threatened by climate change, with rising temperatures being attributed to aspects of coral bleaching and kelp death (e.g. Mumby, 2007).

Many shallow marine and intertidal ecosystems demonstrate alternative regimes or ecological stable states (Lewontin, 1969; Sutherland, 1974; May, 1977; Harrold and Reed, 1985; Knolton, 1992; Konar and Estes, 2003; Petraitis and Dudgeon, 2004; Mumby, 2007), where communities are robust to small perturbations and disturbances, and population sizes are relatively stable, (although seasonal cycles can occur in some instances). Large disturbances, however, create rapid 'regime shifts', for example, the reduction of high diversity kelp forest to largely barren algal turf (Harrold and Reed, 1985; Konar and Estes, 2003). In general, post regime-shift states are not only highly stable themselves, but, compared to pre-regime shift states, are also 'poorer' both in biodiversity and provision of ecosystem services (Walker and Salt, 2006). Recently an examination of these rapid changes by Dudgeon et al. (2010) has suggested the use of the term 'alternative stable state' for where two or more possible stable community structures exist with the same physical environment (e.g. temperature), and 'phase' or 'regime shift' to be used where only one community exists at a given level of environmental variables (such as temperature). This means an invasive species may create a new alternative stable state, where as a disturbance such as climate

change increasing temperatures will create a new regime. Both systems, however, rely on positive feedback to keep communities stable until a 'tipping point' occurs (Dudgeon et al., 2010). The methods used in this study apply equally to both classifications; however, since the technique depends on the concept of stability of communities, we have used the term 'stable state' throughout.

With some degree of knowledge about current species interaction strengths in the community and given the robustness of community structure when in a stable state, it is possible to build and parameterise stable state models, based on localised computational search strategies (Stafford et al., 2008). Briefly, these involve searching multidimensional space for optima, as per many existing computational optimisation techniques such as genetic or evolutionary algorithms (e.g. Holland, 1992). However, in this situation it is necessary to find local optima, rather than the global optimum value – a much computationally simpler process. Equally, given a known disturbance to any one or multiple species in the community, it is possible to apply similar stochastic localised optimisation techniques to predict new potential stable states or regimes (Stafford et al., 2008). Hence, although we apply this technique under the context of climate change, it is equally applicable to any disturbance event that affects population sizes or interactions between species.

A clear disadvantage of this technique is that many possible new stable states can be predicted, and evaluating which is the most likely can be little more than guess work, without data to verify the predictions (Stafford et al., 2008). As such, further approaches to evaluate changes in stable state are used in this study alongside the stable state optimisation approach. Firstly resilience of the post regime-shift stable state is examined, since new stable states are also known to be difficult to restore to their former state (Knolton, 1992; Petraitis and Dudgeon, 2004; Walker and Salt, 2006). This is likely to mean that resilient positions are more likely to be found than non-resilient stable positions by stochastic search algorithms. Secondly, Bayesian belief networks are used in parallel to this optimisation process to allow a statistical quantification of prior knowledge as a probability of whether a species will increase or decrease in abundance (Hammond and Ellis, 2002; Hosack et al., 2008). These probabilities can be refined by including 'observations' on the likely response of a species to climate change, which could be based on existing data. For example, heat coma or lethal temperatures could be used for very vulnerable species, or gene ex-

pression data may inform of relative *in situ* stress levels (see previously).

This study builds on the concepts developed in Stafford et al. (2008) for predicting new stable states, and applies the results to a ‘typical’ rocky shore community as could be affected by climate change (but in practice, affected by any community or population level disturbance). It considers a much more complex community than previously examined, which is likely to result in a far greater number of post regime-shift stable states. As such, community resilience and Bayesian belief networks are used to inform decisions on post regime-shift community structure. Although very much preliminary work, we also review studies on differential gene expression of rocky shore organisms from different microhabitats, and examine how these data could be incorporated into these predictive models.

1 Materials and Methods

1.1 Rocky shore model

Rocky shores are coastal marine ecosystems, which show stable or seasonally stable community structure, exhibiting several different stable states depending on physical or biological properties (e.g. Petraitis and Dudgeon, 1999; 2004). They are extensively studied in terms of their ecology, with many *in situ* experiments having been conducted to determine grazing, predation and competitive interactions between species (reviewed by Little et al., 2009). For the purposes of this study, a simplified version of a rocky shore community is extracted from what commonly exists in the UK. Grazing, competitive and predatory interactions are all included. The rocky shore model should be considered representative of a real rocky shore community, but not wholly accurate in every detail. As such, results should be considered as indicative of what may occur through climate change, rather than true predictions. The reasons for this approximate model, rather than an exact simulation, are due to the data requirements of the model, and further research to make the approach predictive is given in the discussion.

The rocky shore model considers three species of grazing gastropod, which graze on epilithic photosynthetic biofilm. Due to their larger size, limpets are the competitively superior gastropod on the mid shore of many rocky shore ecosystems, but co-exist alongside grazing topshells and littorinids (Fig. 1). Competition occurs between all of these three species. Predatory interactions occur from both dogwhelks, a predatory gastropod mollusc, and starfish. Some degree of competi-

tion occurs between these species, but starfish will also prey on dogwhelks (Fig. 1).

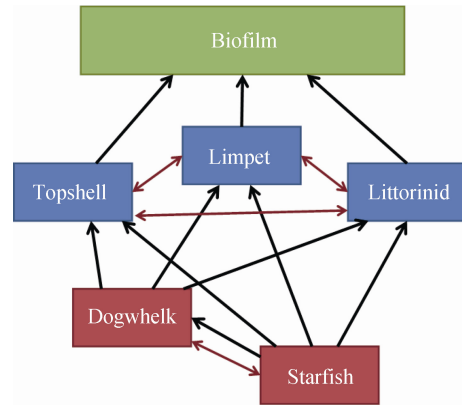


Fig. 1 Community interaction web, showing trophic interactions between species in black (arrow points from predator to prey) and competitive interactions in red

A series of equations are used to realise the rocky shore model in R (R core development team, 2012). At each timestep of the model, grazing/predatory interactions occur, competitive interactions occur and growth of the population (through recruitment) occurs. Each of these types of interaction is simulated by the following equations, where initial values for population, activity and constants for each species are given in Table 1.

Grazing and predation, of species X on species Y:

$$\text{Pred}_{X \rightarrow Y} = a \cdot \text{Activity}_X \cdot \text{Population}_X \cdot \text{Population}_Y,$$

where activity relates to the time spent active on the shore during a tidal cycle, and a is the predation coefficient.

Competition between species X and species Y:

$$\text{Comp}_{X \leftrightarrow Y} = b \cdot \text{Population}_X \cdot \text{Activity}_X \cdot c \cdot \text{Population}_Y \cdot \text{Activity}_Y,$$

where b is the competition coefficient of species Y, and c is the competition coefficient of species X.

Growth of biofilm:

$$\text{Growth}_{\text{biofilm}} = d \cdot \text{Population}_{\text{biofilm}} \cdot \text{Activity}_{\text{biofilm}},$$

where d is a growth coefficient, activity relates to the time spent active on the shore during a tidal cycle, and population is a measure of biofilm density.

Growth of Animals:

$$\text{Growth}_X = e \cdot \sum \text{Pred}_{\rightarrow X},$$

where all the predatory or grazing interactions for a single species are summed.

Change in population of species X:

$$\text{Population}_{X,t+1} = \text{Population}_{X,t} + \text{Growth}_X - \sum \text{Comp}_X - \sum \text{Pred}_{\rightarrow X},$$

Table 1 Initial parameters of the model used in the mathematical equations

	Biofilm	Topshell	Limpet	Littorinid	Dogwhelk	Starfish
Initial Population	5	10	10	10	10	3
Activity	20	20	20	20	15	10
a	n/a	0.001 on biofilm	0.004 on biofilm	0.0015 on biofilm	0.001 on all grazers	0.001 on all animals
b / c	n/a	0.002 on limpet 0.003 on littorinid	0.005 on topshell and littorinid	0.004 on limpet and topshell	0.003 on starfish	0.01 on dogwhelk
d	0.1	n/a	n/a	n/a	n/a	n/a
e	n/a	0.1	0.1	0.1	0.1	0.1

Population figures relate to relative biomass of organisms – but are indicative figures, rather than exact measurements from real shores, and hence no units are given.

Where the sum of predatory interactions on species *X* and competitive interactions involving species *X* are subtracted from the growth.

The simulation runs for 10 timesteps, and the stability of the system is determined by summing the percentage change of the population of each species between the first and last timestep. Ten timesteps were used as preliminary results showed that systems stable for this length of time remained stable for much longer periods. Given the high computational demands of optimisation (multiple individuals over multiple generations, replicated multiple times – see below), reducing the number of timesteps in the model resulted in much faster optimisation and performance of the technique.

1.2 Parameterisation and optimisation of the rocky shore model

The rocky shore model above was parameterised with best guess estimates. For example, competition coefficients were generated relative to each other, based on prior experimental knowledge (Table 1). Population sizes were again based on limited prior knowledge of biomass or energetic turnover of the species in question. However, as detailed previously, the parameters are indicative, rather than representing any definite quantity of the actual system. Over 10 timesteps, several populations became extinct, hence the model did not represent a stable state. To parameterise the rocky shore model into a stable state, a form of evolutionary algorithm was used, deliberately designed to find localised optima, rather than the normal goal of finding global optima. Note, the evolutionary algorithm is simply an optimisation technique used on the identified population sizes and interaction parameters, and does not imply evolution or adaptation of individuals or populations of rocky shore organisms in any way.

Briefly, the algorithm generated an initial 120 parameter sets (or in terms of evolutionary algorithms, a population of 120 individuals), with values for each of

the parameters in table 1, uniformly randomly generated in the range $X \pm (X/3)$, where *X* is the value in table 1.

Each ‘individual’ set of parameters is evaluated over 10 timesteps described above, and the stability of the system (or ‘fitness’ of the individual) is determined by summing the percentage change of the population of each species between the first and last timestep. The best eight individuals (those with the lowest level of change in population sizes, representing the ‘fittest’ individuals in the population) were then copied without any modification in the population in the next generation, and 14 copies of each of these eight individuals were made, with modification, which is $\pm 10\%$ of the current value. This evolutionary algorithm is run for 100 generations, to minimise changes in the population of each species, and the best ‘individual’ or set of parameters selected at the end, to provide parameters which create a more stable system, but still bear resemblance to the initial parameter set. ‘Individuals’ or parameter sets were only considered stable positions if the overall change in the population of all species (the selected ‘fitness’ parameter above) was $< 10\%$ (or an average change per species $< 1.7\%$).

1.3 Possible community change scenarios for most sensitive species

Three different scenarios were considered for community responses to climate change. For example, starfish populations are likely to be reduced in response to warming temperatures, since starfish cannot tolerate high levels of desiccation (Menge, 1972), hence their density will likely decrease in the intertidal zone. Equally, some limpets are shown to have high mortality rates in hot weather, and the population of limpets may reduce (Wolcott, 1973; Williams and Morritt, 1995). However, since climate will affect different species in different ways, we have also included a scenario where biofilm increases in response to climate change. Possible scenarios are given in Table 2, including some simple

Table 2 Scenarios of possible species responses to climate change, used to determine new stable states

	Species 1	Species 2	Species 3
Scenario 1	Limpet population reduction by 35%	-	-
Scenario 2	Starfish population reduction by 50%	-	-
Scenario 3	Starfish population reduction by 50%	Limpet population reduction by 35%	Biofilm population increased by 30 %

scenarios where information on only a single species is known and a more complex scenario, where effects on multiple species are known from prior evidence (e.g. experimental or observational data). However, mainly these are illustrative accounts to understand the dynamics of the rocky shore model, rather than based on knowledge of likely species tolerances to climate change. As such, it is entirely possible that in reality, different changes may occur, or that these changes could arise as a result of a different disturbance (for example disease in starfish).

1.4 Bayesian belief network

A Bayesian belief network was constructed using JavaBayes (Cozman, 2001). Essentially, each species in a community is considered as a node in the belief network graph. Each species can either be ‘increasing’ or ‘decreasing’ in population size. Although in theory other levels can be incorporated into such networks (i.e. increasing a lot, increasing a little, not changing), this simple two level approach reduces the level of ‘prior knowledge’ required from the system, and is commonly used in other belief network studies in ecology (e.g. Hammond and Ellis, 2002).

Bayesian networks are directed acyclic graphs (DAGs). This is consistent with trophic interactions, due to the fact that the effect of the predator on the prey (death) is much stronger than the reverse effect (providing food), and the assumption of directed interactions is thus appropriate. However, the DAG assumption is too restrictive for modelling competition, given that these interactions are intrinsically bidirectional. For example, there is a competitive interaction between limpets and topshells, and since limpets are considered the dominant competitor, there is no reciprocal competition from topshells on limpets indicated in the Bayesian network (Fig. 2). To overcome this issue, the species classed as the superior competitor (i.e. limpets over topshells and littorinids, and littorinids over topshells) is shown to have an effect on the inferior competitor, but reciprocal competition is considered only when assigning ‘prior’ values to the interaction (see example below).

Each node or species has a probability computed from prior knowledge of the network or community, and

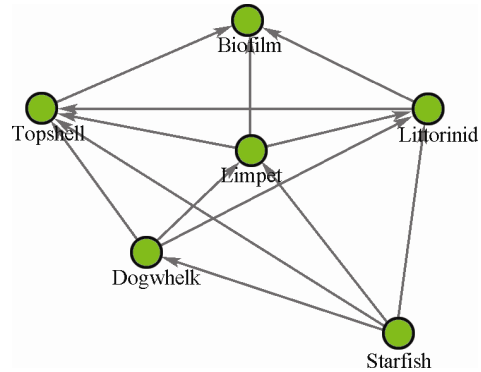


Fig. 2 Bayesian belief network structure, note one way competitive interactions, and only a single link between starfish and dogwhelk

observations on the individual species that comprise the network or community (i.e. known changes to species – summarised in Table 2). For example, the most complex node is probably that of ‘Topshell’ where the probability of the population increasing or decreasing depends on all other animals in the system, either through competitive or trophic interactions, more formally:

$$P(\text{Topshell} \mid \text{Limpet}, \text{Littorinid}, \text{Starfish}, \text{Dogwhelk}),$$

Priors for each of the nodes are based on best guess approaches from known interactions (Table 3). Reciprocal competition is considered in these approaches. For example, the effects of predation on limpets are much less certain than for some of the other grazing species, because when predation favours the other species (e.g. littorinids or topshells – or possibly dogwhelks), then the limpets may increase in population size, despite the increase in predators, due to competitive effects.

Observations are then made on actual species decreases and increases, as per Table 2, where increase in growth or decrease in activity are defined as ‘increase’ or ‘decrease’ respectively. Observations therefore have known levels of probability, and are either certainly increasing ($p_{\text{decrease}} = 0$, $p_{\text{increase}} = 1$) or certainly decreasing ($p_{\text{decrease}} = 1$, $p_{\text{increase}} = 0$).

1.5 Combining the results of each model

For each scenario in table 2, the population sizes of the initially parameterised stable rocky shore model are adjusted, and the optimisation process is re-run to find

Table 3 Prior values for the Bayesian belief network

Node	Limpet	Littorinid	Topshell	Dogwhelk	Starfish	P(Node) ↑
Biofilm	↑	↑	↑	n/a	n/a	0.01
Biofilm	↑	↑	↓	n/a	n/a	0.15
Biofilm	↑	↓	↑	n/a	n/a	0.20
Biofilm	↑	↓	↓	n/a	n/a	0.50
Biofilm	↓	↑	↑	n/a	n/a	0.50
Biofilm	↓	↑	↓	n/a	n/a	0.20
Biofilm	↓	↓	↑	n/a	n/a	0.80
Biofilm	↓	↓	↓	n/a	n/a	0.99
Topshell	↑	↑	n/a	↑	↑	0.01
Topshell	↑	↑	n/a	↑	↓	0.25
Topshell	↑	↑	n/a	↓	↑	0.20
Topshell	↑	↑	n/a	↓	↓	0.30
Topshell	↑	↓	n/a	↑	↑	0.10
Topshell	↑	↓	n/a	↑	↓	0.20
Topshell	↑	↓	n/a	↓	↑	0.20
Topshell	↑	↓	n/a	↓	↓	0.45
Topshell	↓	↑	n/a	↑	↑	0.30
Topshell	↓	↑	n/a	↑	↓	0.55
Topshell	↓	↑	n/a	↓	↑	0.50
Topshell	↓	↑	n/a	↓	↓	0.80
Topshell	↓	↓	n/a	↑	↑	0.45
Topshell	↓	↓	n/a	↑	↓	0.70
Topshell	↓	↓	n/a	↓	↑	0.80
Topshell	↓	↓	n/a	↓	↓	0.95
Littorinid	↑	n/a	n/a	↑	↑	0.02
Littorinid	↑	n/a	n/a	↑	↓	0.10
Littorinid	↑	n/a	n/a	↓	↑	0.20
Littorinid	↑	n/a	n/a	↓	↓	0.50
Littorinid	↓	n/a	n/a	↑	↑	0.50
Littorinid	↓	n/a	n/a	↑	↓	0.70
Littorinid	↓	n/a	n/a	↓	↑	0.80
Littorinid	↓	n/a	n/a	↓	↓	0.90
Limpet	n/a	n/a	n/a	↑	↑	0.30
Limpet	n/a	n/a	n/a	↑	↓	0.40
Limpet	n/a	n/a	n/a	↓	↑	0.60
Limpet	n/a	n/a	n/a	↓	↓	0.80
Dogwhelk	n/a	n/a	n/a	n/a	↑	0.35
Dogwhelk	n/a	n/a	n/a	n/a	↓	0.65
Starfish	n/a	n/a	n/a	n/a	n/a	0.50

The final column gives the probability of the named node increasing in size of population, given the increases or decreases shown.

new stable community states. The computational optimisation approach provides a unique combination of population sizes and parameters for each run of the process (in this case, the process is run 100 times, so 100 predictions are produced). To assess resilient stable states, we use a simple clustering dendrogram on standardised values of the different population sizes of the six species considered. Specifically, we use the 'hclust' function in R, on the standardised distance matrix calculated using the 'daisy' function in the 'cluster' library

– which used Euclidian distance as a measure of overall difference in community. Similar clusters were considered as the same stable state, as minor changes in behaviour, population sizes and interactions do occur within a given 'stable state' in most ecological systems (reviewed by Walker and Salt, 2006). In this case, we arbitrarily used a distance of 5 units as a cut-off for similarity, largely because this gave around 8 large clusters per scenario, allowing a good comparison of possible stable states with the Bayesian network predic-

tion. Only when five or more possible post disturbance communities fell within a cluster were they considered a resilient community, as the stable states should be able to slightly change parameter settings and remain stable. As such, one potential stable state from each cluster was identified as a ‘typical’ representation of this state which was one of the pair with the closest distance between them.

The population size values for individuals from within each cluster were compared with the predictions of the Bayesian network. For example, where the Bayesian network indicated that the population of a species was likely to increase with a probability of 0.7, for a given scenario, the possible stable states were examined, and a value of 0.7 given for that species if the value had increased from the baseline population and 0.3 if it has decreased from the baseline population. The overall likelihood of the newly parameterised rocky shore model, based on the Bayesian network predictions, was calculated as the product of all six species predictions, and the highest overall value was considered the best fit with the Bayesian network, this value is presented in the results as the ‘product of probabilities’.

1.6 Determining significant differences in *in situ* stress in marine molluscs in different microhabitats

Determination of *in situ* stress in relation to temperature, desiccation and biological factors such as competition may provide a species specific set of biomarkers to inform of sub-lethal effects in organisms. Hence, once biomarkers are identified, examining them in other individuals may inform of *in situ* stress acting on organisms and provide a mechanism for determining likely future response to this stress, even at a very basic level such as determining whether the population is likely to decrease if conditions provide more stress to that species than to others. As such, molecular responses, such as gene expression, known to be linked to stress (rather than knowledge of the exact role of the gene), can be used to inform predictive models, especially simple to parameterise models such as Bayesian networks – where high levels of stress can be used to make ‘observations’ on particular populations (i.e. they are likely to decrease).

A first stage of this process is to determine if biomarkers can be linked to significantly different microhabitats in terms of levels of stress. To determine if significant differences in microhabitats exist for a marine mollusc, *Littorina saxatilis* were examined on man-made breakwaters at Rottingdean Beach (50.80169° N, 0.05636° W) in Sussex, in August 2012. A number of parameters were collected on physical and biological

conditions at the three collection sites on the beach consisting of two sides of a rock breakwater (site 1 and 2), and two sites on a concrete jetty approximately ~200m away from the other sites (sites 3 and 4, Table 4). To facilitate comparison with other microhabitats where the species is known to occur, the site was also compared to data collected from Hartlepool (County Durham UK, 54.69907 °N, 1.17751 °E, Site 5) as part of a separate study (Stafford, 2002).

These conditions were then compared to a previous comprehensive study on *Littorina saxatilis* distribution (Stafford, 2002) to produce a 1–10 marking scheme for each of the characteristics (Table 5), where 1 represented a low value of a parameter and 10 a value close to the maximum recorded over a wide range of shores in the UK.

To calculate the weighted values, the following assumptions were used:

1) Typical exposure from wave action was calculated from data of historic swell height averaged over all months of the year (1.3 m in Sussex and 1.35 m in County Durham). This was compared to data from the Outer Hebrides, demonstrating the most exposed location and largest average swell height (3.1 m) in the UK (data from MetCentral Ltd).

Table 4 Characteristics of the sites from where *Littorina saxatilis* were collected

	Site 1	Site 2	Site 3	Site 4	Site 5
Shore height (+ C.D) (m)	8.3	6.3	6.2	6.3	12.3
<i>L. saxatilis</i> density (m ²)	130	90	110	120	20
<i>M. neritoides</i> density (m ²)	55	0	0	25	860
% crevice	8	6	8	10	6
Aspect	E	W	E	W	NE
Mean aggregation size (n, both species combined)	17	4	2	4	45

Table 5 Adjusted characteristics of *Littorina saxatilis* for each location

	Site 1	Site 2	Site 3	Site 4	Site 5
Shore height	7	5	5	5	10
Temperature	6	8	6	8	2
Exposure	2	4	2	4	4
Crevice	4	3	4	5	3
Interspecific Competition	1	0	0	1	10
Intraspecific Competition	5	3	4	4	1

2) For south coast (Sussex) location the predominant wind and swell direction is SW. Therefore a reduction of 40% in exposure was used for the site facing SE, which was also consistent with observations on breaking wave height at the locations. NE is the predominant swell direction in County Durham, so no adjustment was made for site 5.

3) The south-east of England (Sussex) was shown to have an average annual temperature of 7°C compared to a coastal maximum average of 8°C (Cornwall) and minimum of 3°C (N.E. Scotland) in the UK (data from metoffice.gov.uk), County Durham had average annual temperatures of 5°C.

4) Heat absorption (and hence rock temperature) of east facing walls is 20% lower than that of west facing walls, based on data from previous studies (Nakamura and Oke, 1967). In this case, northeast facing walls were conservatively considered the same as east facing walls.

5) Maximum density of *Littorina saxatilis* and *Melarharpe neritoides* combined can be as high as 900m⁻². Maximum density of *Littorina saxatilis* was 300 m⁻² (Stafford, 2002).

6) Maximum recorded shore height of each species >

12 m +C.D. (Stafford, 2002).

7) Maximum crevice density is ~ 20 % of the rock surface (Stafford, 2002).

For each site the percentage contribution of each factor (e.g. shore height) was calculated. These percentages were used in a bootstrapped Principal Component Analysis (following the procedure in Stafford et al., 2012a) to determine significant differences in micro-habitat between sites.

2 Results

2.1 Bayesian belief network

Initially, the Bayesian belief network was altered using ‘observations’ of what was predicted to happen to individual species, or groups of species, as a result of climate change (as per Table 2). Specific increases or decreases were applied for these ‘observed’ species nodes (posterior probabilities for increases in population size set at 0 for limpets and starfish in scenarios 1 and 2 respectively, and 0, 0 and 1 for increases to limpets, starfish and biofilm respectively for scenario 3) and the resultant changes in posterior probability for the unobserved species are given in Table 6.

Table 6 Results of scenarios of Bayesian belief network – data are for posterior probabilities of a given node increasing (given observations on population sizes of certain species in the community), values in bold indicate ‘observed’ values set at either 1 (increasing) or 0 (decreasing)

	Biofilm	Topshell	Littorinid	Limpet	Dogwhelk	Starfish
Control	0.43	0.39	0.46	0.52	0.5	0.5
Scenario 1	0.51	0.54	0.69	0	0.66	0.52
Scenario 2	0.41	0.45	0.50	0.54	0.65	0
Scenario 3	1	0.73	0.55	0	0.86	0

2.2 Parameterisation and optimisation of the rocky shore model

The initial rocky shore model and parameters presented in Table 1 did not produce a stable state model, but a model where populations of biofilm, topshells and limpets collapsed, and littorinid and dogwhelk populations increased dramatically over 10 timesteps (Table 7). Parameterisation of the rocky shore model (using the local optimisation process described previously) to find a stable state model, similar to the initial model produced a range of similar models (examples in Table 7). The Bayesian network also predicted changes to population sizes for the initial ‘control’ population, and the rocky shore model with best fit from the Bayesian network (the highest product of probabilities) was chosen as model 2, below. Technically, model 4 had a higher product of probability, but

was not biologically realistic, given the absence of biofilm, the only real food source for the community. The population sizes for each species in model 2 were all within 10% of initial values, and the parameters of this rocky shore model also made logical sense (Table 8). As such, the parameters and initial values of model 2 were used as a control from which to predict new stable states. While some parameter values appear dramatically different from the initial values, they are compensated for by other values. For example, where ‘activity’ of a species has decreased as compared to the initial values, its other parameters relating to competition or predation may have increased. As such, the rocky shore model is overall biologically similar to the initial, non-stable, model. However, formulation of mathematical models for community interactions is considered in the discussion.

Table 7 Results of optimisation runs for initial parameterisation. Fitness values and population sizes given after 100 generations of selection of 10 cycles of the model

	Cumulative probability	Biofilm	Topshell	Littorinid	Limpet	Dogwhelk	Starfish
Initial values	n/a	5	10	10	10	10	3
Non-parameterised*	n/a	0	0	192.5	0	23.0	5.05
Model 1	0.012	5.02	10.6	9.94	10.07	9.76	3.07
Model 2	0.018	5.07	9.45	9.01	10.33	10.08	3.05
Model 3	0.017	5.05	9.96	8.85	9.88	9.91	3.00
Model 4*	0.023	0.00	7.70	6.44	7.56	10.40	3.67
Model 5	0.016	4.99	11.0	8.78	10.11	10.00	3.08

*Agreement was highest with the Bayesian network for Run 4 (based on cumulative probability value), but this model was not biologically meaningful. Model 2 was selected as most realistic scenario – after studying the model parameters in Table 8.

Table 8 Parameters of optimum model from Table 7 above

Parameter name	Initial Parameters	Model 2
Activity of Biofilm	20.0000	8.0131
Activity of Topshell	20.0000	18.7967
Activity of Limpet	20.0000	6.2907
Activity of Littorinid	20.0000	6.2821
Activity of Dogwhelk	15.0000	1.7940
Activity of Starfish	10.0000	2.0628
Topshell Grazing	0.0010	0.0010
Limpet Grazing	0.0040	0.0068
Littorinid Grazing	0.0015	0.0022
Limpet -> Topshell	0.0050	0.0023
Topshell -> Limpet	0.0020	0.0008
Littorinid -> Topshell	0.0040	0.0041
Topshell -> Littorinid	0.0030	0.0009
Limpet -> Littorinid	0.0050	0.0018
Littorinid -> Limpet	0.0040	0.0018
Starfish -> Dogwhelk	0.0100	0.0090
Dogwhelk -> Starfish	0.0030	0.0025
Dogwhelk prey Topshell	0.0010	0.0004
Dogwhelk prey Limpet	0.0010	0.0007
Dogwhelk prey Littorinid	0.0010	0.0004
Starfish prey Limpet	0.0010	0.0005
Starfish prey Topshell	0.0010	0.0003
Starfish prey Littorinid	0.0010	0.0006
Starfish prey Dogwhelk	0.0010	0.0007
Biofilm Growth	0.1000	0.0937
Topshell Growth	0.1000	0.1026
Limpet Growth	0.1000	0.1097
Littorinid Growth	0.1000	0.0703
Dogwhelk Growth	0.1000	0.2015
Starfish Growth	0.1000	0.0551

2.3 Post disturbance regime prediction and robustness

The clustering of the post disturbance rocky shore models is shown in Fig. 3, each scenario has eight clusters or post disturbance stable states with five or more predictions in it. Tables 9–11 show representative values for each of these clusters, for each of the different disturbance scenarios respectively. The closest agreement between the Bayesian network and the stable state is highlighted in bold.

2.4 Significant differences in microhabitats

Differences in microhabitats were found, with site 1 being significantly different from site 2, both in Sussex, and the site in County Durham being significantly dif

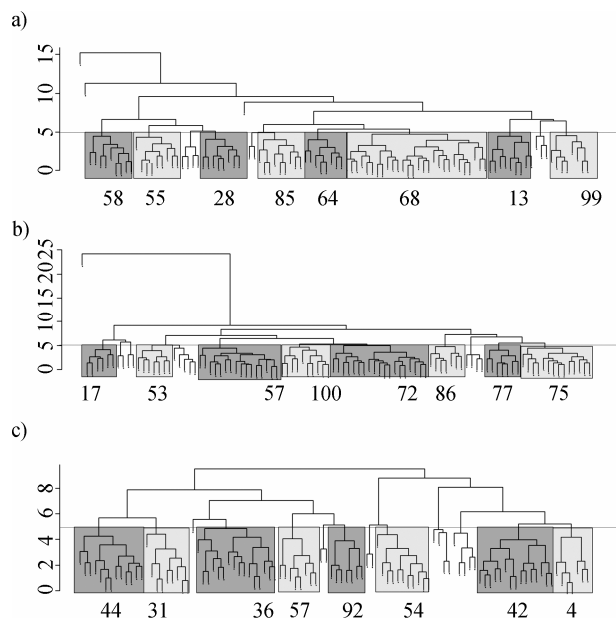


Fig. 3 Dendrograms showing similar clusters of communities for (A) Scenario 1, (B) Scenario 2 and (C) Scenario 3 Similarity was based on clusters being less than 5 units apart, and only clusters with five or more members are shaded above (shading is for distinguishing clusters, and not of any other relevance). Numbers refer to position of replicate which are investigated further in tables 9–11.

Table 9 Example results from Scenario 1

	Cumulative Probability	Biofilm	Topshell	Littorinid	Limpet	Dogwhelk	Starfish
Initial values	n/a	5.07	9.45	8.98	6.71	10.08	3.05
Run 58	0.025	2.96	6.34	12.36	6.45	7.65	2.02
Run 55	0.025	3.20	5.56	9.06	7.02	6.33	2.10
Run 28	0.012	5.34	6.24	4.73	7.65	8.83	2.57
Run 85	0.030	4.97	9.58	9.68	7.83	7.01	2.36
Run 64	0.011	4.05	8.09	8.68	6.19	9.25	2.58
Run 68	0.026	5.13	6.60	9.65	6.42	9.00	2.63
Run 13	0.012	4.35	8.55	8.60	6.29	6.90	3.40
Run 99	0.031	5.15	9.59	9.49	9.91	8.29	2.57

A number of different stable states from clusters in Fig. 3a are identified – run 99 selected as best match to Bayesian network, based on highest cumulative probability.

Table 10 Example results from Scenario 2

	Cumulative Probability	Biofilm	Topshell	Littorinid	Limpet	Dogwhelk	Starfish
Initial values	n/a	5.07	9.45	8.98	8.98	10.08	1.53
Run 17	0.034	5.50	6.80	12.70	7.38	11.10	1.78
Run 53	0.034	5.43	6.09	8.73	6.34	11.13	2.09
Run 57	0.031	3.70	6.38	9.00	9.81	8.98	1.64
Run 100	0.031	4.53	8.15	4.59	10.75	9.25	1.41
Run 72	0.049	4.45	8.81	10.72	7.55	10.17	1.40
Run 86	0.021	6.43	7.68	7.90	9.23	9.82	1.65
Run 77	0.026	4.44	6.22	7.62	7.89	5.93	1.34
Run 75	0.018	5.10	5.90	8.35	7.30	9.50	1.87

A number of different stable states from clusters in Fig. 3b are identified – run 72 selected as best match to Bayesian network.

Table 11 Example results from Scenario 3

	Cumulative Probability	Biofilm	Topshell	Littorinid	Limpet	Dogwhelk	Starfish
Initial values	n/a	6.59	9.45	8.98	6.71	10.08	1.53
Non-parameterised*							
Run 44	0.000	4.87	5.34	8.77	6.25	9.74	1.55
Run 31	0.017	6.71	6.54	7.92	6.66	8.17	1.38
Run 36	0.104	6.98	7.08	7.80	6.76	10.66	1.99
Run 57	0.128	6.27	8.94	10.76	6.26	11.77	1.16
Run 92	0.000	4.77	6.36	7.21	6.58	11.10	0.69
Run 54	0.017	5.33	7.54	8.11	7.75	9.09	1.61
Run 42	0.000	6.47	6.50	9.73	9.32	6.27	1.57
Run 4	0.283	7.58	9.59	7.24	5.32	10.70	1.91

A number of different stable states from clusters in Fig. 3c are identified – run 4 selected as best match to Bayesian network.

ferent from all sites is Sussex (Fig. 4). Differences are determined by overlap of the bootstrapped 95% Confidence radii, as indicated by the size of the bubble (see Stafford et al., 2012a for further details).

2.5 Review of transcriptome methods applied to rocky shore organisms

Rocky shore ecology has long been a strongly field based discipline, whereas ecological genomics has had a

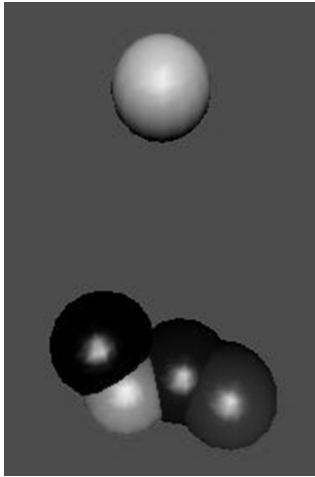


Fig. 4 Bootstrapped PCA, where each bubble represents a single site (bottom left to right = site 1, site 3, site 4, site 2, top = site 5)

Significant differences exist between site 1 and site 2, and between site 5 and all other sites, as defined by overlap of the bubbles.

strong laboratory-based tradition, largely because controlled conditions are required to understand the role of expressed genes (Kammenga et al., 2007; van Straalen and Roelofs, 2012). However, several rocky shore species have been subjects of transcriptome analysis, including microarrays specifically developed for mussels (*Mytilus* spp. Gracey et al., 2008; Place et al., 2008) and the dogwhelk (*Nucella lapillus* Pascoal et al., 2013). Studies on *Littorina littorea* have also been conducted, using human microarrays, which demonstrated > 18% cross reactivity (Larade and Storey, 2009). All of these species show significant changes in gene expression in relation to ‘stress’ in its broadest terms (Table 12). However, the studies carried out in field conditions, both on intertidal mussels, identified temporal changes associated with desiccation and temperature during the tidal cycle (Gracey et al., 2008), yet failed to identify spatial trends, such as latitudinal trends in gene expression (Place et al., 2008).

Table 12 Overview of transcriptome studies conducted on rocky shore invertebrates

Species	Location	Scientific name	Analysis type	Type of stress	Overview of results	Reference
Dogwhelk	North Wales, UK	<i>Nucella lapillus</i>	Custom microarray	Endocrine disruptors	Lab results of exposure to TBT tested against field results from different levels of TBT exposure.	Pascoal et al. (2013)
Mussel	NW America (US and Canada)	<i>Mytilus californianus</i>	Custom microarray	Natural field conditions over large geographical area	Differences between site, but no latitudinal pattern. 12% of probes showed 2 fold changes between some sites.	Place et al. (2008)
Mussel	California	<i>Mytilus californianus</i>	Custom microarray	Natural field conditions over tidal cycle	Distinct gene expression profiles for moderate and severe heat stress at low water.	Gracey et al. (2008)
Periwinkle	Canada (lab based study)	<i>Littorina littorea</i>	Human microarray	Anoxia (lab based)	>10 % of activated probes showed 2 fold changes in regulation.	Larade and Storey (2009)

While much work in gene expression focusses on understanding the roles of the genes up-or down-regulated, the fact that significantly different microhabitats, in terms of likely environmental stress can be identified (see previously), and gene expression changes with different levels of stress, then quantifying overall changes in gene expression between ‘typical’ and ‘stressful’ habitats, or even the time from emersion taken to enter a heat or desiccation induced low tide stress phase, presents a mechanism to identify changing stress levels on the shore, as a result of environmental change, and a possible mechanism to inform Bayesian networks about likely changes to community structure.

3 Discussion

This study has set out a framework for developing community models with incomplete knowledge of the

system under investigation, especially with regard to model parameters. It has also set out a framework for determining likely post-disturbance communities, as a result of factors including, but not limited, to climate change. Incorporation of predictive Bayesian networks allows the most likely possible ‘stable state’ models to be determined post disturbance, and the consideration of robustness of stable state models (i.e. the existence of similar communities examined through clustering algorithms) makes analysis of likely communities feasible, given the large number of stable states which can exist.

In terms of determining likely changes to species and communities, Bayesian networks provide an opportunity to determine ‘belief’ in events happening, such as a population increasing or decreasing. Such ‘beliefs’ are far simpler to incorporate in predictive models than complex equations and parameters, and such ‘beliefs’

could therefore account for the wealth of molecular data that is increasingly available through approaches such as analysis of the transcriptome of organisms. Understanding how such transcriptomes may alter in response to significantly different microhabitats, and the impact of associated biological and physical stressors of these microhabitats is a first stage to reverse engineering the process to become predictive, and using transcriptome data to inform beliefs about the *in situ* stress acting on certain key species. These belief data can then be used to update probabilities in Bayesian networks, and allow us to consider likely community responses.

The current study provides a basic framework of techniques that can be used for predictive ecology, but it

also highlights a number of areas that need considerable further development. A full process diagram of the framework, linked to a detailed example, is given in Fig. 5. However, as an example of how the process works, a detailed scenario is considered in Table 13.

The development of a simple, robust mathematical model of the real community is fundamental to the success of this approach. The model parameters are of less concern than the underlying mechanisms of the model (e.g. predation and competition), and clear focus needs to be placed on what is required from the prediction – for instance, are population sizes/density of the component species of the community required, or are the relative strengths of organism interaction more important?

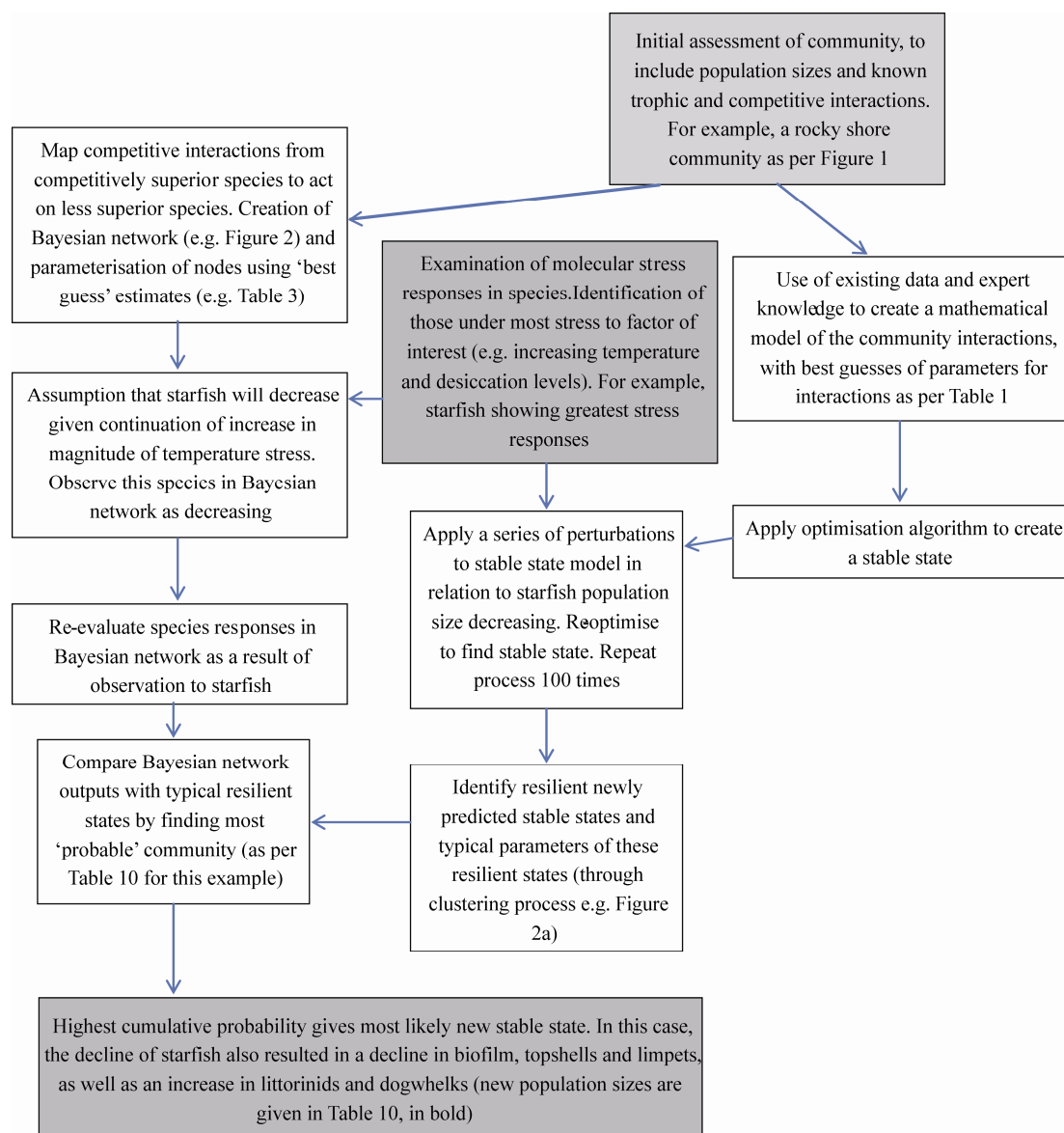


Fig. 5 Diagram of the key processes involved in predicting a new stable state, based on scenario 2 in Table 2 (reduction in starfish number)

Grey boxes indicate the start points and end result of the process. Fig. refers to further detail in tables and text in this study.

Table 13 A simplified and illustrative example of how all component aspects of this paper can be incorporated into an assessment framework

Starfish *Asterias rubens* are examined from a range of microhabitats two weeks into a four week heat wave. Significant differences in microhabitat between an east and west facing breakwater occur after applying bootstrapped principle component techniques. Examination of the causing factors of these significant differences show only temperature and wave action differed between these microhabitats, however, wave action was similar on a breakwater facing north only 2 miles away, and this was not significantly different from either of the other two microhabitats.

Examination of gene expression for starfish in these microhabitats demonstrated that a suite of 36 genes showed increased levels of expression in the west facing microhabitat compared to the east facing. However 8 of these genes also showed differences in expression between the east and north facing habitat.

During the heatwave, 50% mortality of starfish occurred on the west facing microhabitat, but no mortality on the north or east facing microhabitat.

We conclude, therefore, that the 28 (36-8) genes that showed increased expression in the west facing microhabitat are likely to be a good indication of significant heat stress in the starfish, and if the heat stress continues, may result in significant mortality of the starfish.

A different rocky shore is surveyed in a different year. Population density of different species is measured and from prior knowledge, trophic and competitive interactions are estimated, to form both a Bayesian network and a mathematical model of the relationships between organisms. The mathematical model is then combined with the optimisation algorithm to refine the interaction parameters and create a stable state.

Molecular surveys of starfish show very high levels of expression of the 28 genes identified above during the hottest weeks of the summer. Climate predictions indicate rising temperatures and prolonged heat waves in the next 20 years.

We can therefore predict that starfish are likely to undergo mortality if temperatures continue to rise, so we modify the Bayesian network to indicate they will decrease. We also optimise the mathematical model to find new stable states (given initial stochastic changes to population sizes of starfish).

We examine the stable states in the new mathematical model for resilience, and then compare the resilient states to the predictions of the Bayesian network.

The best fit of the two models is assumed to be the most likely new stable state that will occur on the shore. From the new parameters of the mathematical model, the new population sizes of each species can be determined.

A process diagram of this example is also given in Fig. 5. Note example is illustrative and the molecular data and community considered are speculative.

For example, in the present study, the focus of the prediction was on the population sizes of the component species. A combination of population size, activity of the species and a coefficient determined the levels of competition, predation or grazing between species. Such an approach is logical for rocky shore organisms, as some species are more active over the course of a tidal cycle than others (Little, 1989; Hutchinson and Williams, 2003; Williams and Little, 2007; Stafford et al., 2012b,c). Equally, it is possible that activity levels could drop as a result of increasing stress, without effecting absolute number (especially if the prey supply increased). However, such an approach can be confusing

in terms of the parameters regarding competition and predation. The ‘control’ model, based on the initial parameterisation process showed a number of highly different activity levels to what was initially proposed, and in particular, showed a big, and unrealistic discrepancy in the activity levels of the three grazing molluscs. However, examination of competition and predation coefficients indicated these activity levels were accounted for in the values of the other model coefficients. This is exemplified by the fact that topshells showed far greater activity than limpets in the parameterised rocky shore model, but the topshell \rightarrow limpet completion coefficient was far lower than the initial value given. Removal of parameters such as ‘activity’ may be important if comparisons between species interactions need to be considered as part of the prediction of the model; however, this may effect population estimates, and care to select the best mathematical model through comparison with Bayesian networks are needed. An alternative approach may be to refine the criterion of stability. While stable states are essential for this technique, due to the location of local minima in the search landscape, stability may be defined as something other than change in each species’ population level. Although this approach is intuitive, activity levels and populations sizes can interact, as described above, and stability on criteria such as energy flow (per species) may provide a less ambiguous response, although considerably harder to parameterise though best guess approaches. While mathematically complex concepts of stability exist (e.g. time series measures such as those described in Ives et al. 2003), the behaviour of these during regime shift is rather chaotic (Biggs et al., 2009; Stafford et al., 2011). These measures also reduce the degree of intuition into the results, which a direct measure of community, such as population size across all species, provides. However, this does not mean that alternative measures of stability should not be considered in future studies.

The formation of a traditional and realistic mathematical model of community interactions is highly problematic for most communities. Concepts such as grazing and activity levels of species can be accurately quantified in simple models (e.g. Stafford, 2002), but competition can be difficult to quantify. Experimental studies can indicate its importance, but transforming these results into model parameters can be difficult. The parameterisation process allows ‘best guess’ estimates to be used, and these can be refined to meet the criteria of stability in population sizes. The Bayesian network also requires some knowledge of competition, but

largely this is in terms of ranking species in terms of competitive superiority, rather than exact knowledge of determination of exact model parameters.

Given the framework provided here, the predictive modelling approach next needs to be applied to a range of real ecological systems, which have been extensively studied both pre- and post-regime shift. Of particular interest may be intertidal and shallow marine communities in areas where there are large seasonal changes in community structure (e.g. rocky shore communities in southern China which undergo tropical summers, but temperate winters: Williams, 1993), as these may be better understood. Further experimental measurements can then be taken, as opposed to relying on existing data from historical datasets where regime shift is known to have occurred.

In addition, transcriptome data, such as from RNA microarrays, needs to be collected from organisms in their natural environments, as well as in controlled laboratory conditions (as per Pascoal et al., 2013). Ideally, stress response genes need to be linked to reduction in fitness or near mortality of species, since it is important to determine acute stress from normal biochemical and physiological adaptation to environmental conditions. For example, *Littorina littorea* shows considerable response to anoxia in terms of gene expression on human microarrays (Larade and Storey, 2009), and although anoxia is common in this species, excessive levels of it will decrease fitness, by limiting its energy budget through respiration. Hence early expression of these genes within a tidal cycle will likely mean greater levels of anoxia stress are occurring, and prolonged expression is likely to result in decreased fitness. In this anoxia response, many of these genes that have been up- or down-regulated may be general stress response genes, and analysis of how these genes change occur in response to different microhabitats may provide information on likely stressors to an integrated suite of environmental effects, which will occur in natural habitats. Similarly, given the multitude of possible stressors in natural environments, it is not wholly surprising that trends in gene expression may not follow latitudinal gradients, and emphasis needs to be placed on determining different microhabitats in terms of physical and biological factors. In essence, determining significant differences in microhabitats is akin to a 'natural' experimental manipulation, and analysis of principle components may indicate the key factors that differ between different microhabitats. Hence, as indicated in an idealised example in Table 13, it may be possible to

determine significant differences between habitats in terms of single stressors, such as temperature, and investigate the genes which are differentially over or under expressed in this habitat, whereas inflicting stress in laboratory conditions will stimulate a range of stress response genes. Further focus on the relative importance of different microhabitat factors may need to be considered, however. For example, temperature or desiccation rates may be key physical drivers of stress, yet inter-specific competition between very ecologically similar species, such as *Littorina saxatilis* and *Melarharphe neritoides* may be unimportant when there is strong intraspecific competition. Comparing molecular stress responses to real microhabitats and measureable ecological features of the environment may not only provide information to inform predictive models of susceptible species, but may also indicate the most important drivers of regime shift in a variety of communities.

This study has focussed greatly on climate induced regime shift, but in fact, the modelling techniques here can be used to assess any form of disturbance, and determine not only if new stable states are formed, but what these stable states may be. For example, invasive species (either climate driven or otherwise) can cause large changes in community structure (Pyšek et al., 2012), harvesting of resources (such as through fisheries) can also affect community structure (Magnússon, 2005). The inclusion of molecular data is also not limited to climate effects, but more limited to factors that create internal physiological stress. However, as demonstrated by the uses of microarrays in rocky shore ecology, predictions of the effect of factors such as pollution, anoxia (caused through eutrophication as a result of changes in land use or urbanisation) can all be assessed using these integrated methods.

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