Retrospective qualitative analysis of ecological networks under environmental perturbation: a copper-polluted intertidal community as a case study

Rodrigo Ramos-Jiliberto · Leslie Garay-Narváez · Matías H. Medina

Abstract The coast of Chañaral Bay in northern Chile has been affected by copper mine wastes for decades. This sustained perturbation has disrupted the intertidal community in several ways, but the mechanisms behind the observed shifts in local biodiversity remain poorly understood. Our main goal was to identify the species (lumped into trophic groups) belonging to the Chañaral intertidal community that, being directly affected by copper pollution, contributed primarily to the generation of the observed changes in community structure. These groups of species were called *initiators*. We applied a qualitative modelling approach based only on the sign and direction of effects among species, and present a formula for predicting changes in equilibrium abundances considering stress on multiple variables simultaneously. We then applied this technique retrospectively to identify the most likely set of initiators. Our analyses allowed identification of a unique set of four initiators in the studied intertidal system (a group of algae, sessile invertebrates, a group of herbivores and starfish), which were hypothesized to be the primary drivers of the observed changes in community structure. In addition, a hypothesis was derived about how the perturbation affected these initiators. The hypothesis is that pollution affected negatively the population growth rate of both algae and sessile invertebrates and suppressed the interaction between herbivores and starfish. Our analytic approach, focused on identifying initiators, constitutes an advance towards understanding the mechanisms underlying human-driven ecosystem disruption and permits identifying species that may serve as a focal point for community management and restoration.

Keywords Complex systems · Community ecotoxicology · Community matrix · Jacobian · Loop analysis · Press perturbation

R. Ramos-Jiliberto (✉) · L. Garay-Narváez
Centro Nacional del Medio Ambiente, Fundación de la
Universidad de Chile, Av. Larraín, 9975 La Reina,
Santiago, Chile
e-mail: rodrigo.ramos@cenma.cl

M. H. Medina
AVS Chile SA, Imperial 0655, Off. 3A, Puerto Varas, Chile

M. H. Medina
Centro i-mar, Universidad de Los Lagos, Camino Chinquihue
km 6, Puerto Montt, Chile

Introduction

Copper-mine tailings have been dumped for more than 60 years in the coast of Chañaral Bay (26°15′S, 69°34′W) in northern Chile. Since 1978, the observation of beach progradation and changes in the intertidal communities around this site has stimulated different research campaigns which have resulted in dozens of reports assessing several potential effects of the mine tailings. Field and laboratory studies that assessed the effect of this long-term polluting event have reported a persistently high copper concentration in seawater and a strong modification of the intertidal community structure, in which the abundance of several species of macroalgae, benthic herbivores and benthic carnivores around dumping sites have been severely depleted (Medina et al. 2005).

Although these studies have allowed a general assessment of tailing effects, the underlying mechanisms by which this perturbation drives structural changes in local biodiversity remain poorly understood. This is not an uncommon situation in ecology, where the effects of

perturbations are often large but the mechanisms unknown. A serious obstacle for understanding how a stressor alters the structure and functioning of ecological communities lies in the inherent complexity of ecological networks, with many species or groups of species interacting in intricate ways. Thus a given perturbation may affect directly a number of species, but this effect can be transmitted to other members of the community, affecting them indirectly (Fleeger et al. 2003). Therefore, uncovering mechanisms by which mine tailings, or any environmental perturbation have altered the structure of the communities would require determining how the perturbation influences the system, i.e., which species have been directly affected, and how those effects are propagated through the community via indirect effects. For example, if a species A is affected by a pollutant via decreasing birth rate or increasing mortality, this effect could secondarily decrease the population growth rates of A's predators, which would be faced with decreased resources. Likewise, the resources on which A depends will have a weaker top-down control and could increase their abundance. Hence, the direct effect of a pollutant on a single species could propagate to a larger part of the community via chains of effects driven by shifts in abundances of the interacting species (Kasai and Hanazato 1995; Preston 2002; Fleeger et al. 2003). On the other hand, if a pollutant exerts a negative effect on the feeding rate of species A on species B, this will be translated into a decrease in the growth rate of A and an increase in the growth rate of B (Dambacher and Ramos-Jiliberto 2007). Thus, the way a pollutant affects community structure depends on which species and interactions among them are primarily affected by the perturbation agent, and how these effects are propagated through the ecological network via density-mediated and trait-mediated indirect effects (Abrams 1996; Relyea and Hoverman 2006).

Qualitative modelling of complex systems (Puccia and Levins 1985) is a set of techniques that allow assessing the expected shifts in species abundances after a sustained perturbation enters the system, altering the growth rate of a single species. This approach has the advantage of requiring only the basic topological information of the community under study to predict the direction of change that each species in the community will experience as a consequence of the perturbation. This is especially useful since it is not easy to obtain accurate measures of the strength of interactions among all of the many species that can comprise an ecological community.

In this study we used the available field information for the Chañaral intertidal community to identify the system variables (i.e., species or groups of species) that, being affected by copper pollution, contributed primarily to the generation of the observed changes in community structure. For our purposes, we extended the qualitative analysis

approach to predict the direction of changes in species equilibrium abundances in these areas, considering potential sustained perturbations to the growth rate of multiple species simultaneously. We then applied this technique retrospectively to identify the best hypothesis about the past perturbations that drove the observed changes in community structure.

The identification of components that play a major role in the community response to environmental disruption represents a step forward in understanding the mechanism behind this response. By doing so, this study presents an approach likely to be useful for scientists assessing the impacts and ecological risk of substances released to the environment as well as for policy makers and managers involved in the protection or restoration of ecosystems already under anthropogenic perturbation.

Methods

In performing our analyses, we used previously recorded information from two intertidal communities (Zenteno and Palito) located around Chañaral Bay in northern Chile (Medina et al. 2005). Zenteno was considered a reference site as it does not have a history of metal pollution. Palito, on the other hand, is located 200 m south of the current mine tailings dumping point, which between 1976 and 1989 received ca. 130×10^6 metric tons of tailing. After a sedimentation dam was built inland in 1990, sediment-free wastewaters from the copper mine have been channeled from the dam to this discharge point at a flow rate of 200–250 l/s. At this site, copper has been clearly reported as the main pollutant (Correa et al. 2000). Basic information for the reference (Zenteno) and polluted (Palito) sites is given in Table 1.

Data of species abundance for each study site were compiled from Medina et al. (2005); in addition, five more sampling dates were added to complete a 2 years cycle.

Table 1 General features of the study sites

	Reference (Zenteno)	Polluted (Palito)
Total dissolved copper concentration (min and max)	4.01–7.52 µg/l	8.72–25.64 µg/l
Coordinates	26°51.08′S	26°15.80′S
	70°48.55′W	70°40.63′W
Species richness (intertidal community)	72	42
Trophic groups	7	7

Total dissolved copper was estimated in the water column of each site from two 500 ml representative samples collected from the site shore at each sampling date

Table 2 Species and functional groups composing the trophic groups (TGs) used in this study for modeling the Chañaral intertidal community

TG	Functional groups included	Species	Interactions
A1	Filamentous and foliose algae	Bangia atropurpurea	Grazed by all herbivores
		Ectocarpus sp.	
		Hincksia mitchelliae	
		Chaetomorpha sp	
		Rama novaezelandiae	
		Cladophoropsis sp.	
		Ulva compressa	
		Ulva linza	
		Ulva sp.	
		Porphyra columbina	
		Cyanophycaea	
A2	Corticated and articulated calcareous algae	Codium dimorphum	Grazed by H2
		Codium fragile	
		Gelidium chilense	
		Gelidium sp.	
		Chondrus canaliculatus	
		Ahnfeltiopsis durvillaei	
		Ahnfeltiopsis furcellata	
		Glossophora kunthii	
		Corallina officinalis	
		Corallina sp.	
		Polysiphonia paniculata	
		Centroceras clavulatum	
		Ceramium sp.	
		Scytosiphon tenellus	
		Scytosiphon lomentaria	
		Grateloupia doryphora	
		Petalonia fascia	
A3	Leathery and crustose algae	Rhodoglossum sp.	Grazed by H1 and H2
		Lessonia nigrescens	
		"Green crust"	
		Hildenbrandia lecanellieri	
		Lithothamnium sp.	
		Mesophylum sp.	
		Ralfsia confusa	
		Colpomenia sinuosa	
		Halopteris hordacea	

Table 2 continued

TG	Functional groups included	Species	Interactions
S	Sessile invertebrates. Bivalvia, Cirripedia and Cnidaria	Jhelius cirratus	Preyed by C
		Notochthamalus scabrosus	
		Balanus flosculus	
		Austromegabalanus psittacus	
		Semimytilus algosus	
		Perumytilus purpuratus	
		Phymactis clematis	
		Anthothöe chilensis	
		Phymanthea pluvia	
		Bunodactes sp.	
		Porifera	
		Ectoprocta	
		Spirorbidae	
		Phoronidae	
H1	Invertebrate herbivores with Rhipidoglossan, Taenoglossan and Docoglossan radula	Fissurella crassa	Graze on A1 and A3. Preyed by C
		Fissurella limbata	
		Fissurella maxima	
		Nodilittorina peruviana	
		Nodilittorina araucana	
		Tegula atra	
		Scurra scurra	
		Scurra ceciliana	
		Scurra araucana	
		Scurra bohemita	
		Scurra parasitica	
		Scurra viridula	
		Siphonaria lessoni	
		Trimusculus peruvianus	
H2	Invertebrate herbivores. Polyplacophora and Echinoida	Loxechinus albus	Graze on A1, A2 and A3. Preyed by C
		Tetrapygus niger	
		Enoplochiton niger	
		Acanthopleuraechinata	
		Chiton granosus	
C	Invertebrate Carnivores. Sea snails and sea stars	Concholepas concholepas	Prey on S and all herbivores
		Nucella crassilabrum	
		Heliaster heliantus	
		Meyenaster gelatinosus	
		Stichaster striatus	

Interactions among groups are provided in the last column. Species were those identified in Medina et al. (2005) during samplings of the intertidal zone performed on each studied site every 2 months for 2 years

Both study sites were monitored at low tide (0.23–0.30 m) every 2 months, from July 2002 to July 2004, giving a total of 12 sampling dates. Abundance (i.e., % cover for sessile organisms and ind m^{-2} for mobile invertebrates) was estimated using transects and quadrants. At each site two to three transects, at least 4 m apart, were defined perpendicular to the coast and from high to low in the intertidal gradient. The sampling unit was a 25×25 cm quadrant with a grid of 100 equally spaced intersection points positioned along the transects as many times as it fitted. Algae and sessile invertebrates occurring underneath each intersection point, and all mobile invertebrates inside the quadrant, were identified to the lowest possible taxonomic level. Seventy-two species or morphospecies were found in the reference and polluted sites. These were then grouped into a smaller number of trophic groups (TGs) as system variables. As a first step species were grouped into functional groups according to (Steneck and Watling 1982; Steneck and Dethier 1994; Vasquez et al. 1998). Algae were classified based on their morphological and anatomical characteristics as filamentous, foliose, corticated, articulated calcareous, leathery and crustose (Steneck and Watling 1982). Invertebrate herbivores were grouped by feeding system as: Polychaeta, malacostraca, gastropoda rhipidoglossa, gastropoda taenoglossa, gastropoda docoglossa and polyplacophora (Steneck and Watling 1982; Steneck and Dethier 1994). Filter feeders were additionally grouped as cirripedian filter feeders, bivalve filter feeders and small filter feeders, which contains bryozoans, spirorbids, Phoronida and Porifera. Interactions among functional groups were established according to Dayton et al. (1984), Steneck and Dethier (1994), Vasquez and Buschmann (1997) and Vasquez et al. (1998). As a second step, functional groups were grouped into 7 TGs according to shared consumers and resources, following Steneck and Dethier (1994) and the existing knowledge about ecological interactions among the observed species (Vasquez et al. 1998; see Table 2). The 7 TGs identified constitute the final system variables: A1 (filamentous and foliose algae), A2 (corticated and articulated calcareous algae), A3 (leathery and crustose algae), S (sessile invertebrates: Bivalvia, Cirripedia and Cnidaria), H1 (invertebrate herbivores with rhipidoglossan, taenoglossan and docoglossan radula), H2 (invertebrate herbivores Polyplacophora and Echinoidea), C (carnivores Asteroidea). See Table 2 for a complete list of species within each TG.

As a proxy of changes in community structure that were driven by pollution in this zone, we assessed statistical differences (Mann–Whitney U-test) in the abundance of each TG between reference and polluted sites considering the 2 years cycle. This was carried out using all data collected for each TGs (i.e., the sum of its component species) on each transect and sampling date together. Significant differences ($p < 0.05$, Mann–Whitney U-test) in TG abundances observed at the polluted site with respect to the reference site were encapsulated in a vector $\mathbf{O}$ (see Results), whose elements $o_i = 1$ and $o_i = -1$ indicate increased and decreased abundance of TG i respectively; $o_i = 0$ indicates no significant difference between sites in the abundance of TG i.

From the recorded TGs and the ecological interactions among them (see Table 2), we built the qualitative community matrix $\mathbf{A}$ (see "Results"), whose elements a_{ij} are -1, 1 and 0 for negative, positive and null direct effects of TG j on TG i respectively. All TGs were assumed to be self-regulated (i.e., all diagonal elements of $\mathbf{A}$ were negative), given the current evidence supporting that direct density dependence is the rule across every taxon (Brook and Bradshaw 2006). Matrix $\mathbf{A}$ includes the basic structure of interactions among TGs (off-diagonal elements) in both reference and polluted sites, which were considered to be two system states. Each system state was characterized by the abundance of the component TGs, here referred to collectively as "community structure". Vector $\mathbf{O}$ summarizes the observed change in community structure but only considers the direction of changes observed in each TG and not their magnitude. In this study we developed a method for finding the set of sustained perturbations ("press perturbations" sensu Bender et al. 1984) on TGs that best explain the observed changes in community structure depicted by $\mathbf{O}$. For brevity, hereafter we will refer to a sustained perturbation in the growth rate of a species (via altering its birth or death rate by a small and constant value over time) as an "input".

Our retrospective analysis of changes in community structure included four steps: (1) development of an equation that permits predicting the change in TG equilibrium abundances in response to multiple inputs. (2) Using this equation to calculate the predicted changes in TG abundances for each of the possible input sets, denoted by input vectors $\mathbf{K}$. Each prediction considers the direction of change in TG abundance (vectors $\mathbf{P_K}$) as well as the probability $\mathbf{Q_K}$ of correctly predicting each change in abundance. (3) Calculation of the similarity Ω between each predicted change in community structure and the observed change $\mathbf{O}$. (4) Finding the TG that, being impacted by the stressor, best explains the observed change in community structure (shift in system state). This was carried out by determining the input set $\mathbf{K^*}$ that yields the prediction of changes in TG abundance which best matches $\mathbf{O}$. The details of these procedures are explained in the next two subsections.

Once the input vector corresponding to the best prediction was selected, plausible hypotheses were formulated about the inputs that caused the observed changes. The hypotheses were composed of the following elements:

(a) direct negative impacts on the rate of change of TGs, and (b) positive or negative impacts on the interaction strengths between TGs. Reversing the rationale of Dambacher and Ramos-Jiliberto (2007), we inferred the impact on interactions from impacts on the involved TGs.

All analyses we performed using our own Matlab routines (version 2010a for OS X) run in a Mac Pro desktop computer.

Qualitative predictions to single and multiple perturbations

Qualitative predictions represent the direction of change in equilibrium population density of species i (or equivalently, trophic group i) in response to a sustained positive input on the rate of change of species j (Bender et al. 1984; Puccia and Levins 1985). For single inputs (i.e., when only one species is perturbed), the matrix of qualitative predictions $\mathbf{P}$ is obtained from the sign of the inverse of $-\mathbf{A}$, or equivalently for stable systems $\mathbf{P} = \mathrm{sgn}(\mathrm{adj}(-\mathbf{A}))$. Dambacher et al. (2003) went a step further in the study of community interactions by developing a measure of certainty $\mathbf{W}$ that each element of $\mathbf{P}$ gives the correct prediction, which is calculated as

$$w_{ij} = \frac{\mathrm{adj}(-\mathbf{A})_{ij}}{t_{ij}} \tag{1}$$

where $t_{ij} = \mathrm{permanent}\left(\min \mathrm{or}|\mathbf{A}_{ij}|\right)^{\mathrm{T}}$ is the total feedback associated with p_{ij}. Nevertheless, environmental perturbations exerted by a pollutant or any other stressor rarely affect only one species, but rather a set of species at one or more trophic levels. Indirectly, a stressor can also affect more than one species through modifying the strength of an interspecific interaction.

For the case of multiple inputs, we extend Eq. 1 to assess the effects on species i of simultaneous inputs on more than one species. The formula is

$$w_{i\mathbf{K}} = \frac{\sum_j \mathrm{adj}(-\mathbf{A})_{ij} k_j}{\sum_j t_{ij}|k_j|} \tag{2}$$

where k_j are elements of the column vector $\mathbf{K}$ containing elements 1, -1 and 0, which represent positive, negative and null inputs to species j, respectively. We assume that the effects on species i of multiple inputs to the set of species $\mathbf{K}$ are additive. Thus the sign of $w_{i\mathbf{K}}$ represents the direction of change in equilibrium population density of species i as a product of the inputs specified in $\mathbf{K}$. These qualitative predictions are contained in a vector $\mathbf{P_K}$, with elements 1, -1, and 0 for $w_{i\mathbf{K}} > 0$, $w_{i\mathbf{K}} < 0$, and $w_{i\mathbf{K}} = 0$, respectively. In the same way that $\mathbf{P}$ and $\mathbf{W}$ give qualitative predictions and certainty of those predictions for the case of single inputs, $\mathbf{P_K}$ is associated with the certainty measure $\mathbf{W_K}$ in the more general scenario of either single or multiple inputs.

In order to test which combinations of inputs provide the best explanation to the observed changes in population densities between the reference and perturbed systems, we constructed a list with all possible combinations of positive, negative, and null inputs to all species in the network. For our network composed of 7 TGs, this yields 2,187 input vectors $\mathbf{K}$. For each of the input vectors $\mathbf{K}$ we calculated the corresponding predictions $\mathbf{P_K}$ and $\mathbf{W_K}$.

Retrospective analysis

To contrast observation and predictions, we measured the distances between qualitative changes in population density observed in the field (vector $\mathbf{O}$), and each of the qualitative prediction vectors $\mathbf{P_K}$ derived from the set of input vectors $\mathbf{K}$. The distance metric developed here considers the number of predictions matching the sign of the corresponding observations (i.e., how many species are correctly predicted to change their equilibrium size), but also considers as a weighting factor the probability $q_{i\mathbf{K}}$ that a given qualitative prediction matches the predictions based on parameterized quantitative models. Thus, the probability $q_{i\mathbf{K}}$ of correctly predicting the direction of change in population density from the element $p_{i\mathbf{K}}$, is calculated according to Hosack et al. (2008) as an asymptotic (sigmoid) function of certainty $w_{i\mathbf{K}}$ and total feedback t_{ij}:

$$q_{i\mathbf{k}} = \frac{\exp(\beta_w w_{i\mathbf{k}} + \beta_{wt} w_{i\mathbf{k}} t_{i\mathbf{k}})}{1 + \exp(\beta_w w_{i\mathbf{k}} + \beta_{wt} w_{i\mathbf{k}} t_{i\mathbf{k}})} \tag{3}$$

A set of values for shape parameters β_w and β_{wt} (Table 3) were obtained from Hosack et al. (2008). The probabilities for the entire set of species in the community were arranged into the corresponding vector $\mathbf{Q_K}$.

In order to calculate the distance between observation and each qualitative prediction, we first transformed each of the elements of $\mathbf{O}$ and $\mathbf{P_K}$ into Cartesian coordinates within a three dimensional space. Axes x, y, and z represent changes in equilibrium density of a species pointing towards $+$, $-$, and 0 respectively. Hence, positive, negative, and zero elements in $\mathbf{O}$ will be assigned coordinates (1, 0, 0), (0, 1, 0), and (0, 0, 0.5) respectively. For nonzero elements in $\mathbf{P_K}$, the coordinates are $(q_{i\mathbf{K}}, 0, 0)$ and $(0, q_{i\mathbf{K}}, 0)$ respectively. The coordinates for zero elements in $\mathbf{P_K}$ will be (0, 0, 0.5) if the corresponding element in $\mathbf{W_K}$ is 1, and (0, 0, 0) if the corresponding element in $\mathbf{W_K}$ is 0. No other values of $w_{i\mathbf{K}}$ are allowed for $p_{i\mathbf{K}} = 0$. The rationale behind shortening the z-axis is that the distance between predictions and observations of opposite sign should be greater than the distance between predicting zero change and observing non-zero change or vice versa.

Table 3 Sensitivity of input vector selection to changes in values of shape parameters β_w and β_{wt} of Eq. 3

Parameter set		Overall similarity	$q_{i\mathbf{K}}$ per trophic group						
β_w	β_{wt}	Ω	A1	A2	A3	S	H2	H3	C
3.460	0.034	0.69	0.83	0.45	0.47	**0.92**	0.54	0.21	0.96
3.443	0.033	0.68	0.71	0.43	0.51	**0.76**	0.49	0.91	0.87
3.514	0.040	0.70	0.92	0.51	0.21	**0.78**	0.54	0.75	0.84
3.518	0.042	0.70	0.89	0.47	0.90	**0.54**	0.22	0.75	0.87
4.042	0.026	0.70	0.89	0.51	0.74	**0.50**	0.93	0.81	0.47
3.779	0.022	0.68	0.25	0.21	0.75	**0.54**	0.78	0.76	1.00
6.965	0.062	0.82	0.21	0.91	0.53	**0.23**	0.79	0.81	0.98
8.138	0.079	0.84	0.25	0.74	0.50	**0.92**	0.51	0.40	0.98

For all tested parameter values, the same input vector $\mathbf{K}^* = [0, -1, 0, -1, 1, 0, -1]$ was selected as the one rendering predictions that best fitted (i.e., highest Ω) the observed changes in abundance. The probability of correctly predicting a group's abundance change $q_{i\mathbf{K}}$ after an input $\mathbf{K}$ is shown. The abundance change in group S (in bold) was erroneously predicted

Finally, for each pair of observed and predicted points, which represent changes in equilibrium density of a species i, the Euclidean distance was calculated between them as $d_i = \sqrt{\Delta x_i^2 + \Delta y_i^2 + \Delta z_i^2}$. The similarity Ω between observation and prediction vectors will then be:

$$\Omega = 1 - \frac{1}{n\sqrt{2}}\sum_i d_i, \tag{4}$$

where $n\sqrt{2}$ is the largest possible distance between an observed and a predicted vector with n elements (i.e., species) each. Ω is then considered as a measure of the goodness of fit between $\mathbf{O}$ and $\mathbf{P_K}$, taking values between zero and one, with larger values representing a better fit between observation and prediction.

Results

Qualitative community matrix and changes in population abundances

The qualitative community matrix obtained for the Chañaral intertidal system was:

$$A = \begin{bmatrix} -1 & 0 & 0 & 0 & -1 & -1 & 0 \\ 0 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & -1 & 0 & -1 & -1 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & -1 \\ 1 & 0 & 1 & 0 & -1 & 0 & -1 \\ 1 & 1 & 1 & 0 & 0 & -1 & -1 \\ 0 & 0 & 0 & 1 & 1 & 1 & -1 \end{bmatrix},$$

where the component TGs were sorted in the matrix as: A1, A2, A3, S, H1, H2 and C.

With the exception of S, the abundance of all TGs varied significantly between reference and polluted sites. H1 was the only TG showing a significantly higher abundance in

Table 4 Mean and standard deviation of abundance of the trophic groups (TGs) at reference and polluted sites

Trophic group	Reference (Zenteno)		Polluted (Palito)	
	Mean	SD	Mean	SD
A1*	24.43	11.74	6.79	8.06
A2*	15.19	8.66	6.49	4.48
A3*	24.16	9.77	2.50	3.26
S	3.56	2.97	3.71	3.12
H1*	33.85	43.71	624.94	802.54
H2*	0.15	0.37	0	0
C*	0.52	0.45	0	0

Significant differences (U-test, $p < 0.05$) indicated by asterisks

the polluted system. On the other hand, H2 and C were not recorded in the polluted site, where they were considered to occur with very low abundance. From the changes in abundance observed in the reference and the polluted sites, a vector of observed qualitative changes in density of TGs was obtained, $\mathbf{O} = [-1, -1, -1, 0, 1, -1, -1]$ for A1, A2, A3, S, H1, H2, and C, respectively (see Table 4 for abundances in reference and polluted sites).

Retrospective analysis

For each of the 2,187 input vectors $\mathbf{K}$ and their associated probabilities $\mathbf{Q_K}$ of correctly predicting the direction of change in TG density, their similarity Ω with vector $\mathbf{O}$ was calculated. No significant relationship between the number of non-zero entries in $\mathbf{K}$ and the values of Ω was found. The input vector with the largest Ω corresponds to the best hypothesis about the set of perturbed TGs to which the observed changes in community structure are attributable. The selected input vector was $\mathbf{K}^* = [0, -1, 0, -1, 1, 0, -1]$, representing negative inputs to TGs A2, S and C, and

positive input to H1. This input vector renders the following predictions of qualitative change in TG abundance: $P_{K*} = [-1, -1, -1, -1, 1, -1, -1]$. Note that although all three TGs of algae were predicted to be depleted by copper pollution, one of the herbivore TGs (H1) was predicted to decrease and the other (H2) to increase in abundance, thus escaping the fundamental correlation based on direct predator–prey effects alone and showing the importance of indirect effect in determining community structure. A comparison between P_{K*} and O indicates that the selected input vector is expected to drive changes in TG abundance that match all the observed changes, with the exception of S. This TG was predicted to decrease in abundance, but the field data for this group were not significantly different between sites (Table 4).

The selection of the vector $K*$ is based on qualitative predictions P_K and probabilities Q_K. Although P_K is invariant for each input set K, there are different forms of calculating Q_K, which could alter our results. Nevertheless, the selected $K*$ was robust to the different ways (i.e., chosen values of β_w and β_{wt}) of calculating Q_K (Table 3). On the other hand, the change in the carnivores C was predicted with the highest associated probability. This means that this prediction is particularly robust to the value of elements in the community matrix.

The selected input vector $K*$ could result from a direct impact on the growth rate of the species, from impacts on their interactions (Dambacher and Ramos-Jiliberto 2007), or from a combination of both. This rationale allowed identification of a single hypothesis about the network components, i.e., TGs and interactions among them, whose perturbation caused the observed changes in community structure. This hypothesis is depicted in Fig. 1, and states that the stressor led to the observed community changes through directly decreasing the growth rate (e.g., via increased mortality or diminished reproduction) of TGs A2 and S, and suppressing the interaction between C and H1 (Fig. 1). Note that a negative input to any TG can also be translated into an enhancement of its self-limitation, but no further attention was paid here to this interpretation.

Discussion

In this study we assessed the pathway by which a pollutant entered into an ecological network and drove a shift in community structure via effects that were spread through the web of interactions among species. This was achieved by developing an extension of the qualitative analysis tools that allow predicting changes in equilibrium abundances of interacting populations, in response to a sustained perturbation affecting the growth rate of multiple species simultaneously. Thus, we tested all possible combinations
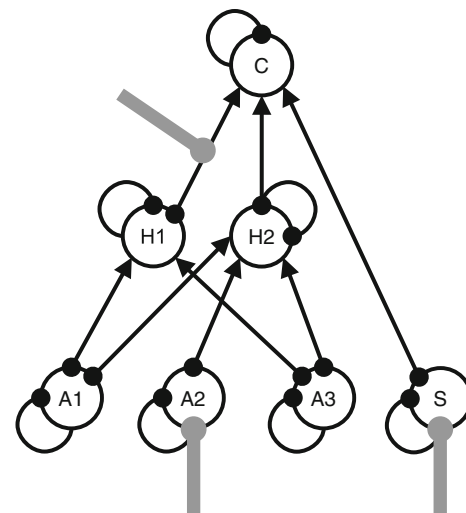

Fig. 1 Result of qualitative retrospective analysis, showing the proposed hypothesis of perturbations (*thick grey lines ending in circles*) affecting the identified initiator species H1, A2, S and C. Proposed negative inputs to growth rate of species A2 and S, as well as to C–H1 interaction, predict changes in community structure that best matched the observed shifts in species abundance driven by copper pollution in Chañaral bay. Nodes are TGs; *thin lines ending in arrowheads* and *circles* represent positive and negative effects of increasing abundances on per capita growth rate of species. Abbreviations are: A1 = Filamentous and foliose algae, A2 = Corticated and articulated calcareous algae, A3 = Leathery and crustose algae, S = Sessile invertebrates (Bivalvia, Cirripedia, and Cnidaria), H1 = Invertebrate herbivores with Rhipidoglossan, Taenoglossan and Docoglossan radula, H2 = Invertebrate herbivores (Polyplacophora and Echinoida), C = Carnivores (Asteroidea)

of the defined perturbation inputs and contrasted the predicted changes in abundances derived from each of them against the observed differences between an unperturbed and a perturbed community. Then we selected the input set that gave the closest match between prediction and observation. By doing this we identified the potential *initiators*, i.e., the subset of defined TGs whose alterations in growth rate driven by the stressor are hypothesized to trigger the direct and indirect effects that caused the observed changes in community structure. In other words, perturbing initiators might be sufficient to obtain the density changes observed in every TG within the community. In addition, we derived a single set of potential sustained perturbations on TGs and their interactions, capable of explaining the presumed alteration of the growth rate of initiators attributable to copper pollution.

Our data indicate that the structure of Chañaral intertidal community was altered by copper pollution, affecting significantly the abundance of six out of seven TGs. The analyses performed allowed identification of a unique set of four initiator TGs, which were hypothesized to be the primary drivers of the observed changes in community structure. These four initiator TGs were two basal nodes

consisting of a group of algae (A2) and sessile invertebrates S, along with herbivores H1 and the top predator C. Initiators share two essential properties: (a) they are sensitive to the perturbation under consideration, in this case copper pollution, and (b) they occupy a topologically important position within the interaction network for the propagation of effects over the community. This makes initiators idiosyncratic; that is to say, a variable that is an initiator in a given community will not necessarily be an initiator in other communities.

The proximate causes of reduced growth of the initiator TGs may be split into two main categories: those that directly affect some vital rate such as survival, development or reproduction, and those that modify the strength of direct interactions of the focal (initiator) species. On this basis we identified one hypothesis of how the perturbation by copper pollution reduced the growth rate of initiators (Fig. 1). This hypothesis assumes decreasing the growth rate of two species and the suppression of one trophic interaction.

Although there is scarce information about effects of copper on ecological interactions, there is considerable support for harmful direct effects on organisms of many taxa. Several studies have assessed the effect of copper on algae and have demonstrated an adverse effect on growth, development, reproduction and photosynthesis at elevated concentrations (Gledhill et al. 1997; Bidwell et al. 1998; Coelho et al. 2000; Collén et al. 2003; Nielsen et al. 2003). This helps to explain the inferred sensitivity of algae to copper pollution. Nevertheless copper effects on species inhabiting our study site may be partially attenuated by different physiological defences leading to a reduction of oxidative stress (Ratkevicius et al. 2003; Contreras et al. 2007, 2009; González et al. 2010; Ritter et al. 2010; Tominaga et al. 2010). On the other hand, the direct effect of copper on the growth rate of sessile invertebrates (S) could be supported by the high sensitivity, especially of early stages, reported for different species belonging to this group (Jacobson et al. 1993; Royo-Gelabert and Yulea 1994; Qiu et al. 2005; Kennedy et al. 2006). In addition, a decrease in the predation pressure exerted by top predators C on H1 appeared to be important in explaining the community alteration via down-cascade indirect effects, in agreement with earlier empirical research (Correa et al. 2000; Espoz and Castilla 2000). However, our results identified the effect on the interaction strength and not on the growth rate of C as an important force shaping the community structure. Further analyses (results not shown) including plausible non-trophic interactions among TGs, such as whiplash effect from leathery algae and competitive interaction among sessile groups, did not improve the match between prediction and observation.

This study allows us to infer the relationships and interactions implied in the generation of changes in community structure observed at Chañaral bay. Nevertheless, the reliability of our results rests on the quality of the available data, which dictates the resolution and therefore the structure of the assumed food webs. This will be the principal limitation of this tool in any application. In order to deal with this drawback, the greatest effort should be allocated towards designing and executing the best possible sampling procedures, based on substantial developments attained in field ecology (Underwood 1994).

On the other hand the analytic approach presented here, focused on identifying initiators, provides a valuable approach for better understanding mechanisms behind community disruption driven by human activities. Studies focused on addressing community-level changes driven by environmental stressors often present patterns of changes without detecting their causes. Notable exceptions are the studies of Johnston and Keough (2002, 2003) and Perrett et al. (2006) who were able to detect experimentally direct and indirect effects of pollutants on communities. Their results showed both that indirect effects are important in explaining changes in community structure, and that the strength of ecological interactions (e.g., competition) determines the outcome of perturbing multispecies assemblages. In addition, Rochet et al. (2010), based on previous developments (Trenkel et al. 2007; Dambacher et al. 2009; Trenkel and Rochet 2010), were able to track plausible environmental pressures exerted on a groundfish shelf community that drove observed shifts in community structure. Nevertheless, and contrary to our approach, their study only considered single inputs, which probably explained why their model failed to detect mechanisms of community shifts in several cases.

A necessary next step for applying our technique in future studies is the experimental validation of model assumptions and outcomes. This may be achievable through the use of mesocosm experiments (e.g., Medina et al. 2004), which may allow manipulating multispecies systems at a workable scale.

In addition to generating theoretical insights, our approach has practical implications. First, if impacts on initiators generate community-level alterations, then it is conceivable that recovering the abundance and function of the same initiators may facilitate community restoration. Thus the tools presented in this study could be useful in the design of plans for ecosystem restoration as a guide for obtaining a prioritisation of species. On the other hand, the response of initiators in single-species bioassays could be a good indicator of ecological risk for the exposed community. Standard methodologies for ecological risk assessment and evaluation of environmental impacts exerted by toxicants commonly consider the use of single-species

bioassays (USEPA 1998). These species are selected based on several criteria, commonly including geographical distribution, accumulated biological information, life-cycle length, ease of culture, and ecological relevance. The last criterion, however, is normally based on general ecological knowledge of the species, without considering system-specific properties of the community which determine the set of indirect effects spreading through species interactions. The approach presented here could be used for assessing ecological relevance in the selection procedure for single-species bioassays, promoting an advance towards linking single-species tests with effects at the community level, which has remained as one of the most important challenges in ecotoxicology (Newman and Clements 2008).

Nevertheless, our approach includes a number of limitations that further work should address. Ideally, this methodology should be applied to high quality data on community changes obtained from surveys of community structure carried out before and after the occurrence of a perturbation event. Alternatively, and as was the case in this study, a robust comparison between spatially distinct sampling sites, representing non-disturbed and disturbed stages, may be a useful means to infer causes of shifts in community structure. This approach is commonly used to assess the ecological state of natural areas for conservation or management purposes. On the other hand, in this study it was necessary to lump species into trophic groups because we did not have reliable information about interactions at the level of species. If detailed information is available, our method can be applied without further changes to detect initiator species instead of TGs. Other limitations of our methodology are imposed by the demand of computer power that increases noticeably with network size, and by the fact that the relative importance of initiator species cannot be discerned. The first restriction should be relaxed due to the sustained increase in processing speed attained by the new computers. The second lies at the core assumptions of the qualitative analysis because it deals only with the direction of changes and not their magnitudes. Nevertheless, it is this feature of the technique which gives our approach its main strength; i.e., simplicity and independence from parameter values and shape of functional relationships for analysis. Finally, it must be emphasized that for this type of analysis it is assumed that the perturbation is sustained through time and the system is close to a stable equilibrium. Fortunately, there is evidence that the methods of qualitative analysis are still useful when those assumptions are not strictly satisfied (Hulot et al. 2000; Dambacher and Ramos-Jiliberto 2007). Another strong assumption is that multiple inputs act additively in defining the direction of change of species equilibrium densities. How relaxing this assumption will affect model outputs is a matter of further research.

A desirable next step within this research agenda would be to uncover the role played by non-initiator groups as propagators of indirect effects. In this way it would be possible to gain further insight about the relationship between community structure and its response to pollution and other sources of environmental perturbation.

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