**ENVIRONMENTAL EVIDENCE**

## SYSTEMATIC REVIEW PROTOCOL

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# How strong is the effect of invasive ecosystem engineers on the distribution patterns of local species, the local and regional biodiversity and ecosystem functions?

Gil Rilov[1*], Rebecca Mant[2], Devin Lyons[3], Fabio Bulleri[4], Lisandro Benedetti-Cecchi[4], Jonne Kotta[5], Ana M Queirós[6], Eva Chatzinikolaou[7], Tasman Crowe[3] and Tamar Guy-Haim[1]

## Abstract

**Background:** One of the most influential forms of biological invasions is that of invasive ecosystem engineers, species that affect other biota via alterations to the abiotic environment. Such species can have wide-reaching consequences because they alter ecosystems and essentially "change the rules of existence" for a broad suite of resident biota. They thus affect resources or stressors that affect other organisms. The objective of this systematic review will be to quantify the positive and negative impacts of invasive ecosystem engineers on ecosystem structure and functioning, and to identify factors that cause their effects to vary.

**Methods:** We will search a number of online databases to gather empirical evidence from the literature on the impacts of invasive ecosystem engineers on: (1) species richness and other univariate and multivariate measures of biodiversity; (2) productivity and abundance of algae, and animals; and (3) biogeochemical cycling and other flows of energy and materials, including trophic interactions. Data from relevant studies will be extracted and used in a random effects meta-analysis in order to estimate the average effect size of invasive ecosystem engineers on each response of interest.

**Keywords:** Biological invasions, Ecosystem engineers, Biodiversity, Ecosystem functioning

## Background

Ecosystem engineers (also termed habitat modifiers or bioconstructors) are defined as organisms that affect other biota via alterations to the abiotic environment [1,2] either directly with their bodies (e.g., add structure) or their activities (e.g., dig a hole) or indirectly through their biotic interactions (e.g., eat the canopy and let more light in the forest). Such species create, destroy, or otherwise modify habitats, and thereby affect resources or stressors (e.g., living space, sediment load, light availability and temperature) that have an impact on other organisms. Invasive species have the potential to play a dominant role as ecosystem engineers and can have

ecosystem-level impacts by modifying their receptive environment, thus inhibiting or facilitating other species, either invasive or indigenous [3].

### Ecosystem engineers

Jones et al (1994) identified two types of ecosystem engineers (that are not mutually exclusive, i.e., a species can be both types) based on the way they alter the ecosystem:

*Autogenic engineers* modify the environment with their own bodies which act as part of the engineered habitat and this engineering is dynamic (when engineers grow they can modify the environment in different ways). For example, as mussels grow, their shells increase the available habitat for other organisms (e.g., by providing settlement space and/or ameliorating environmental stress).

*Allogenic engineers* are species that modify the environment by mechanically changing living and non-living materials (or structures or landscapes) from one physical

* Correspondence: rilovg@ocean.org.il
[1]National Institute of Oceanography, Israel Oceanographic and Limnological Research, Haifa, Israel
Full list of author information is available at the end of the article

state to another. Different types and numbers of other organisms will thrive in the area where an ecosystem engineer modified the environment than would in a non-modified area. The classical examples for such engineers are beavers that alter the flow of rivers by building dams. Mussels can also be considered to be allogenic engineers. By extensively filtering the water in a lake or a bay they can clear it enough to increase light penetration thus affecting benthic macroalgal communities by facilitating growth.

There are numerous examples of animals and plants that function as ecosystem engineers. Trees in the forest are an example of autogenic engineers on land, whereas, coral reefs or kelp forests can be considered as engineers in the sea. Allogenic engineers that shape their environment on land are elephants and termites. Burrowing worms and sea urchins play a similar role in the sea.

## Marine bioinvasions

Invasive species are species that have spread beyond their natural biogeographical range to new regions, usually with human assistance, and have the potential to affect the native ecosystem and its biodiversity [2]. Invasions, in and of themselves, are rated high as a cause of native biodiversity loss and economic damage [4,5]. But invasions also interact with all other factors that compromise the integrity of marine ecosystems, such as habitat destruction, pollution and climate change. Biological invasions are also assumed to induce ecosystem-scale impacts (i.e., significant effects on community biodiversity and/or ecosystem functions), though this has yet to be clearly demonstrated [6].

Marine biological invasions are a fast growing environmental concern that is facilitated mostly through the growth of trade as organisms traverse oceans attached to the hulls of ships, carried within ballast water, via aquaculture, as live marine seafood and bait, by the aquarium trade and through canals connecting different bodies of water [2]. Although we have been much slower to realize the extent and impacts of invasions in the sea compared to those on land, our experiences with problematic aquatic invasions continue to mount. For example, the green alga *Caulerpa taxifolia*, a popular aquarium species, now carpets many square kilometers of seafloor in the Mediterranean suffocating other species; *Caulerpa racemosa* increases sedimentation and creates an anoxic sediment layer underneath its roots thus causing the degradation of seagrass (*Posidinia oceanica*) meadows; a comb jelly native to the western Atlantic, caused the collapse of fisheries when introduced into the Black and Caspian Seas; and invasive marsh grasses and mangroves are transforming wetlands around the world.

## Marine invaders as ecosystem engineers

There are quite a few studies, and several reviews among them, that treat marine invaders as habitat modifiers or ecosystem engineers [1,2,7], but to the best of our knowledge a comprehensive systematic review of invasive ecosystem engineers that includes meta-analysis on the size and direction of the impacts has not yet been conducted. This is in contrast, for example, to two (though limited) such analyses on freshwater systems, one on carp and crayfish impacts and one on the impacts of dreissenid mussels [8,9]. Although some marine invasive engineers have been intensely studied in the last decade, it is still unclear at this point if there is enough data published to allow a comprehensive meta-analysis of their impacts on the marine environment. The purpose of this systematic review is to investigate the current state of knowledge on the topic and to scope for existing data thus aiming to perform a meta-analysis in order to achieve evidence-based generalizations on the phenomenon and its impacts on marine ecosystems. In this study, we consider all brackish and transitional waters (e.g., estuaries) as marine ecosystems.

## Objective of the review

This review is aimed to identify the type, direction and strength of effects of invasive species that are recognized as potential ecosystem engineers on the distribution patterns of native species, biodiversity patterns and ecosystem functions in the marine environment. Wherever possible, we will try to identify/separate the engineering effect of the invasive species investigated from other effects (e.g., direct food web effects, such as cases when the invasive species are used as food source by local species or when they affect local species directly as predators or grazers). This is a global review that includes all regions and all species, and studies that will be considered relevant based on the criteria stated below.

### Primary question

How strong is the effect of invasive ecosystem engineers on (a) the distribution patterns of local species, (b) the local and/or regional biodiversity (α and γ diversity) and (c) the ecosystem functions?

Secondary question 1: are there any indigenous species at risk of extirpation or extinction due to the invasion of these ecosystem engineers?
Secondary question 2: is there a difference in the strength of the impact between autogenic and allogenic invasive engineers?

The list of components that will help guide the search and analysis of the data is shown in Table 1. With regard to the primary question, it is important to consider how

**Table 1 Is a list of components that will be investigated for the review questions**

| Population | Exposure | Outcomes (response variables) | Comparators |
|---|---|---|---|
| Marine and brackish (e.g., estuarine) assemblages including both native and other invasive species | Introduction, establishment and spread of exotic ecosystem engineers | Species richness, species evenness, total community cover, total community biomass, single species abundance (cover, count), single species biomass, individual growth of native species, individual size of native species, community productivity, nutrient cycling, metabolism, decomposition, carbon flux, respiration, sediment stabilisation, sediment mixing, resilience, temporal stability, resistance, invasibility | Invaded vs non-invaded plots<br><br>Invaded plots vs Invader removal plots<br><br>Control plots (no Invader) vs Invader transplanted plot |

we should measure change in distribution patterns: presence and absence in a particular area, range shifts, recruitment patterns, size frequencies, etc. The selection depends on the type and quality of the data. Also, engineers can both reduce or increase diversity, therefore they can provide evidence on the direction of the effect. Because some invasive engineers are known to facilitate the establishment of other invaders [a phenomenon referred to by some investigators as an invasional meltdown, sensu 10], this aspect needs also to be considered in the analysis and discussion. In addition, the scale (spatial and temporal) of the effect should be considered.

## Methods
### Search strategy
We will search for relevant studies using Web of Science and Scopus databases, with the terms listed in Table 2. The general terms and the terms for ecosystem structure, biogeochemical cycling, and productivity will be combined within brackets and separated using the Boolean operator 'OR'. They will then be combined with the terms for invasive species using the Boolean operator 'AND'.

An asterisk (*) indicates a 'wildcard', which allows the database or search engine to look for multiple words that have different endings, e.g. estuar* captures [estuary OR estuaries OR estuarine]. Quotation marks ("") around two words restricts the search to instances where that phrase occurs.

When reading the full-text papers we will look for further relevant material (cited papers) that may include useful data for this systematic review that might have been missed by the search. In the case of papers reporting incomplete information (e.g., missing standard deviations or errors, or number of replicates), we intend to contact the authors in order to seek for the retrieval of missing information.

### Study inclusion criteria
Studies will be evaluated for inclusion at three successive levels: First we will evaluate them by their title to remove citations spuriously returned by our search.

Next, we will evaluate the remaining citations based on their abstracts. Several reviewers will assess a subset of the studies (~5-10 % depending on the total number that came up in the search), and a Kappa statistic relating to the assessments will be calculated. If the statistic indicates that reviewers are inconsistent in their assessment, discrepancies will be discussed and the inclusion criteria will be clarified or modified. Finally, the remaining studies will be evaluated as full text. When it is not clear if a study meets our inclusion criteria at one of the initial levels of screening, they will be re-evaluated at the next level of the systematic review.

Evaluations will be based on whether their populations, exposures, comparators, outcomes, and study types are considered relevant according to the following criteria:

### Relevant populations
Any ecosystems or ecosystem components affected by marine or brackish water invasive species that can be considered ecosystem engineers.

### Relevant exposures
The presence of non-indigenous species that can be considered an ecosystem engineer. We will consider all effects of these species but will make an effort to separate the engineering effect from other types of effects.

### Relevant comparators
(1) Experiments comparing "treated" (engineer present) and "control" (engineer absent) conditions; (2) Experimental and observational time series where abundance of engineer species varies; (3) Observational studies comparing areas with and without engineers, or the effect of the same invasive engineer in different geographical areas (a geographic context).

### Relevant outcomes
We will begin by searching for studies examining a broad range of outcomes. Due to limited resources, we may later decide to exclude studies examining some of these outcomes. This decision will be based on the number of studies found for different outcomes, not the

**Table 2 The general search terms that will be used for the review**

| Search terms | Population | Outcome |
|---|---|---|
| **Exposure** | | |
| ("alien species" OR "alien organism*" OR "invasive species" OR "invasive organism*" OR "species invasion" OR "introduced species" OR "introduced organism*" OR "species introduced" OR "species introduction" OR "allochthonous species" OR "nonindigenous species" OR "non-indigenous species" OR "nonindigenous organism*" OR "non-indigenous organism*" OR "non native species" OR "non-native species" OR "non-native organism*" OR "exotic species" OR bioinvaion OR "bioinvasive species" OR "bioinvasive organism*") | (marine OR brackish OR estuar* OR coastal OR shallow OR sea OR seas OR maritime OR lagoon* OR pelagic OR benth* OR demersal OR shore* OR intertidal OR subtidal OR ocean* OR bay OR cove) | *Terms for ecosystem structure*<br><br>("species richness" OR diversity OR "community structure" OR evenness OR biodiversity OR bio-diversity OR "biological diversity" OR "Shannon-Weaver" OR "Shannon-Weiner" OR "Shannon index" OR "Simpson Index" OR "abundance-biomass curve*" OR "species abundance distribution*" OR "community similarity" OR "community dissimilarity")<br><br>*Terms for biogeochemical cycling, flows of energy and materials*<br><br>("Energy flow*" OR "Energy flux*"OR "Flow* of energy" OR "Flux* of energy" OR biogeochemical OR "Nutrient cycl*" OR "cycling of nutrient*" OR "Nutrient dynamics" OR "nutrient flux*" OR "Nutrient flow*" OR "Flow* of nutrient*" OR "Flux* of nutrient*" OR "cycling of carbon" OR "carbon cycl*" OR "carbon stor*" OR "carbon flow*" OR "carbon flux*" OR "flow* of carbon" OR "flux* of carbon" OR "cycling of sul*ur" OR "Sul*ur cycl*" OR "Flow* of sul*ur" OR "flux* of sul*ur" OR "sul*ur flux*" OR"sul*ur flow*" OR "Hydrogen sul*ide" OR "cycling of nitrogen" OR "nitrogen cycl*" OR "Flow* of nitrogen" OR "flux* of nitrogen" OR "nitrogen flux*" OR "nitrogen flow*" OR denitrification OR "cycling of phosphorus" OR " phosphorus cycl*" OR "Flow* of phosphorus" OR "flux* of phosphorus" OR "phosphorus flux*" OR "phosphorus flow*" OR "cycling of oxygen" OR "oxygen cycl*" OR "Flow* of oxygen" OR "flux* of oxygen" OR "oxygen flux*" OR "oxygen flow*" OR anoxi* OR hypoxi* OR bioturbation OR grazing OR foraging OR herbivory OR predation OR scavengers OR scavenging OR respiration OR ecosystem metabolism OR "sediment stabilisation" OR "sediment mixing" )<br><br>*Terms for productivity*<br><br>(primary product* OR "secondary product*" OR "carbon fixation" OR "community respiration" OR "ecosystem respiration" OR "community metabolism" OR "ecosystem metabolism" OR "abundance of benthic" OR "productivity of benthic"OR "benthic biomass" OR "biomass of benthic" OR "benthic metabolism" OR "benthic respiration" OR ((abundance* OR biomass* OR productivity OR mortalit* OR survival OR growth OR cover OR densit*) AND (fauna* OR animal ORinfauna* OR epifauna* OR fish OR macroinvertebrate OR invertebrate OR macrofauna* OR mesofauna* OR meiofauna* OR epibenthic OR seagrass OR eelgrass OR cymodocea OR zostera OR posidonia OR seaweed OR macroalgal OR macroalgae OR fish* OR bird* OR seabird OR shorebird))) |

apparent magnitude or direction of effects. Initially we will search for studies on:

(1) Change in the structure and diversity of communities or community components at two levels: (a) Changes in structure measured by univariate diversity (richness) or evenness indices (Shannon's, Simpson's, Pielou's, etc), or by multivariate indices such as assemblage similarity patterns that are derived by the similarity matrices; (b) Changes in community components measured as abundance, biomass, density or cover of individual species or by parameters or statistics describing abundance-biomass curves, species-abundance distributions, or similar.

(2) Ecosystem functions considered here are of two types: (a) Productivity of ecosystem or ecosystem components, measured as carbon fixation, respiration or other rate measurements; (b) Flows of energy and material between ecosystems or ecosystem components. Biogeochemical cycling of nitrogen, phosphorus, carbon, oxygen, and sulphur, including both static and dynamic measures (e.g., static- nitrogen pool, dynamic- denitrification rate).

### Relevant types of study design
Empirical studies conducted in the field or in the laboratory.

### Potential effect modifiers and reasons for heterogeneity
It is likely that we will find different sources of heterogeneity among studies also depending on the: (1) study type (lab, field, observational, experimental); (2) scale of the study (spatial, temporal); (3) design (replication, controls, confounding effects, BACI design); (4) execution (independence of samples, independence of treatments, randomization, sampling techniques and protocols); (5) biogeographic region of study.

### Study quality assessment
We will extract all relevant details from each full-text article selected, in order to categorize each selected article as relevant through the use of the attributes summarized in Table 3. We will extract the exact information and raw data, not just the category into which the paper falls.

Determining whether replication has been carried out appropriately might be somewhat subjective at times. Therefore, the following criteria will be considered: (1) do the controls appear to be spatially/temporally independent of the affected areas? (2) do the replicates appear to be independent of one another in space and time (are they interspersed geographically, spread sufficiently in time, etc.)? (3) are the controls and affected areas sufficiently similar (considering, for example, habitat and substrate type, degree of exposure, salinity, proximity to human activities)?

If clear evidence is available in the paper that all of these criteria are met, then the controls should be considered valid, if there is evidence of failure to meet any one of these criteria, the controls should be considered invalid and if it is not possible to make a proper assessment based on the information provided, then the study should be classified as 'unclear' in this regard.

If the authors refer to the replicates being assigned randomly, or make reference to use of a random number table, they will be classified as being randomly assigned. If the authors refer to the allocation as haphazard, or make reference to a procedure such as throwing a quadrat over their shoulder, allocation will be classified as 'haphazard'. All other methods will be classified as 'other', and they will be described so that their susceptibility to bias can be assessed.

We will record the presence of factors that may have caused the observed changes other than the presence of invasive species. These factors might include pulse or chronic sources of disturbance. For example, the occurrence of accidents due to human activities (e.g. oil spill, nuclear wastes, organic wastes (e.g. aquaculture)) or natural extreme events (e.g. storms; run-off due to heavy rains) during the execution of the study could mask the effects of invasive species. Details of these events, if given, will be taken into account to evaluate whether a study will have to be retained or rejected. This, although clearly reported, will be somewhat subjective.

Details of the confounding variables will be given for each paper when present. Confounding variables will be formally accounted for if possible; otherwise, studies will have to be rejected.

In the case of observational (i.e. not experimental) studies, we will carry out sensitivity tests according to the following categories used to score studies:

Category of study
CI
1 Control - 1 Impacted
≥ 2 Control - ≥ 1 Impacted
BA
1 Before - 1 After
≥ 2 Before - ≥ 2 Impacted
BACI
1 Control - 1 Impacted : 1 Before – 1After
≥ 2 Control - ≥ 1 Impacted: 1 Before – 1After
1 Control - 1 Impacted : ≥ 2 Before - ≥ 2 Impacted
Beyond BACI

The meta-analysis will include all studies, and will be repeated separately for each category of study to check how the category of the study can influence the results of the analysis. Of course, these analyses will be carried out only for those categories for which a sufficient

**Table 3 Attributes considered for categorizing full-text articles**

| Study type | Comparison between invaded and non-invaded plots | Comparison between invaded and invader removal plots | Comparison between non-invaded and invader transplanted plots | |
|---|---|---|---|---|
| *Study location* | | | | |
| *Latitude* | | | | |
| *Longitude* | | | | |
| *Distance from major urban or industrial centers* | | | | |
| *response variable(s)* | | | | |
| **Study type** | lab* | field | | |
| **Study settings** | observational | experimental | | |
| **Spatial scale - extent** | <1 km$^2$ | 1-100 km$^2$ | spanning area >100 km$^2$ | |
| **Spatial scale – grain (*size of plots*)** | plots <1 m | plots >1 m | individual | sub-individual (i.e. portions of an individual) |
| *Description of the hierarchical design - space* | | | | |
| **# of spatial scales included** | 1 | 2 | >2 | |
| **Temporal scale - extent** | | | | |
| **Temporal scale - grain** | | | | |
| *Description of the hierarchical design - time* | | | | |
| ***Number of temporal scales included*** | 1 | 2 | >2 | |
| **Design - replication (plot)** | Non-replicated | Poorly replicated (n = 2) | sufficiently replicated (2 < n < 4) | Well replicated (n > 4) |
| *if well replicated: how many replicates?* | | | | |
| **Design - controls/reference sites** | None | Procedural controls | Unmanipulated controls | Both |
| **Design - CI** | | | | |
| *How many Control sites?* | | | | |
| *How many Impacted sites?* | | | | |
| **Design - BA** | | | | |
| *How many times Before?* | | | | |
| *How many times After?* | | | | |
| **Design - BACI** | | | | |
| *if Beyond BACI: how many Control sites?* | | | | |
| *if Beyond BACI: how many Impacted sites?* | | | | |
| *if Beyond BACI: how many 'Before' times?* | | | | |
| *if Beyond BACI: how many 'After' times?* | | | | |
| **Design - confounding** | Confounded | Not confounded | | |
| *If High probability of confounding, list confounders* | | | | |
| *Execution - specify the sampling method* | | | | |
| **Execution - sample independence** | Not independent | Probably independent | Independent | |
| **Execution - treatment independence** | Not independent [11] | Independent [11] | | |
| **Execution - randomisation (allocation of sampling units)** | Neither | Haphazard | Random | |
| **Confounding variables** | Certainly present | Likely present | Not present | |

number of studies is available. Categories will be reported in the summary Excel spreadsheet.

Papers suffering from one or more of the following major flaws could be rejected: (1) Design – confounding: confounded; (2) Execution – sample independence: not independent; (3) Execution – treatment independence: not independent; (4) Randomization: no randomization in the allocation of experimental units to different treatments or, in the case of observational studies, of control sites. Specific details of the reasons why a study has been deemed as suffering by one or more of the above-mentioned flaws will be provided.

### Data extraction strategy

We will extract the response variables listed within the column Outcomes in Table 1 and 2. These will include response variables describing the response at the level of native assemblages, populations and individuals, in plots or sites where the invasive species was present or absent. In addition to the presence, we will also record the density or cover at which the invader was found.

In addition to being categorized in terms of aspects of study quality, studies that meet the inclusion criteria will be described in terms of their: (1) region (from NOAA large marine ecosystem list); (2) geographic coordinates (field studies only); (3) dates (start and end dates, sampling dates); (4) habitat type (e.g. rocky subtidal, beach, open coast, lagoon); (5) aims/focus; (6) study design (beyond those aspects covered in quality assessment); (7) response variables measured.

Data on all of the outcome measures listed above will be extracted from the relevant papers. Means and measures of variation (standard deviation, standard error, confidence intervals) will be extracted from tables and graphs, using image analysis software (such as IMAGEJ or DATATHIEF) when necessary. In case that the required data will not directly be extractable from papers, authors will be approached and asked to provide either raw data or relevant information (e.g. means, standard deviation/variance, sample size). If only raw data are provided rather than summary statistics, these will be extracted and the summary statistics will be calculated. Data on potential confounding variables or effect modifiers will also be extracted. Date and location data may be used to obtain estimates of additional effect-modifying variables from other data sources (e.g. data or digital maps of sea surface temperature, primary productivity, potential solar radiation). If the required data cannot be extracted from the paper, the authors will be asked to provide it. Several reviewers will independently extract data from different papers, but a subset of papers will be processed by all reviewers to verify that data extraction is repeatable.

### Data synthesis and presentation

The review will firstly present the number and type of studies that cover the impact of invasive ecosystem engineers on each of the different outcome measures of interest (i.e. diversity, species richness, productivity of different parts of the ecosystem and nutrient cycling of different nutrients). Where sufficient studies present data on the same outcome measure, a meta-analysis will be conducted. Where different measures of the same outcome can be meaningfully combined in a single effect, we will use standardised response measures (e.g. log response ratios, Hedged' *d*). Initially, information from all studies of a given outcome will be combined, and a random-effects meta-analysis will be used to estimate effect sizes. Where possible, subgroup analysis or meta-regression will be conducted to assess the impact of study quality categories, different outcome measurements and other potential effect modifiers. Results of the meta-analysis will be reported graphically using standard approaches. Forest plots will be used to represent effect sizes and funnel plots and normal q-q plots to assess whether there a publication bias exists.

### Author details

[1]National Institute of Oceanography, Israel Oceanographic and Limnological Research, Haifa, Israel. [2]Bangor University, Bangor, UK. [3]University College Dublin, Dublin, Ireland. [4]University of Pisa, Pisa, Italy. [5]Marine Biology Department, Estonian Marine Institute, Tallinn, Estonia. [6]Plymouth Marine Laboratory, Plymouth, UK. [7]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Heraklion - Crete, Greece.

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