Technical Report: Restoring Angasi oyster reefs – what is the endpoint ecosystem we are aiming for and how do we get there?

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Summary

Three dimensional reefs and beds created predominately by the native Angasi Oyster or Flat Oyster (*Ostrea angasi*) were once common across its range in Australia's southern coastal waters but were lost during the mid-late1800s and early 1900s due to combination of destructive fishing practices, overfishing and changes to estuarine conditions. Despite the continued presence of low densities of individuals and cessation of commercial fishing, reefs structures and their associated communities have shown little signs of natural recovery. Since 2014, a range of reef restoration projects have commenced, guided by international restoration protocols that prioritise the reestablishment of the keystone species (oysters or mussels) on artificial stable substrates placed in locations where reefs have previously existed. These projects invariably need clear and realistic goals and objectives for restoration which are aided by the use of a reference ecosystem as a model or target, for the local ecosystem being restored, yet few reference sites exist. Reefs established Angasi, are absent from much or all of its geographical distribution and the ecosystem has been poorly studied to-date; hence, the application of restoration target ecosystems will largely depend on deriving a model based on historical descriptions and observation of what remains. Here we develop an interim reference model to help set restoration objectives and recommended procedural framework for Angasi reef restoration based on available knowledge of the ecology, biology and aquaculture of the keystone species. We use the Port Phillip Bay oyster reef restoration project as a case study for using the reference model to guide interventions and evaluate the progress of recovery.

Introduction

Oyster reefs or beds, generated predominately by the native Angasi Oyster or Flat Oyster (*Ostrea angasi* Sowerby 1871, hereafter Angasi reefs), were once common features of bays and estuaries throughout its range across southern Australia but have been decimated since European settlement, with fewer than 10% of reefs remaining (Alleway and Connell 2015; Ford and Hamer 2016; Gillies et al. *in press*). The ecosystem is developed as Angasi recruit and affix to each other and similar hard substrates in high densities, creating three-dimensional dense beds and reefs. These biogenic structures are further colonised by other shellfish (including mussels, scallops) mobile epifauna (e.g. echinoderms, molluscs, crustaceans) sessile invertebrates (e.g. ascidians, sponges, hydroids) fish and where light permits, algae, resulting in the development of diverse biological communities. Such reefs and beds can have densities of greater than 50 oysters/m^2 and individual reefs can reach up to 1.5 ha in size (Figure 1).

Several projects to restore Angasi reefs have recently been initiated by non-government organisations (NGOs), governments and community groups, with a number of these coordinated by The Nature Conservancy (http://www.natureaustralia.org.au/our-work/oceans/). The development of these projects has stemmed from growing community interest in the historical loss of shellfish and biogenic reef habitats across Australia and the potential for these reefs and their ecosystem services to be recovered (Fitzsimons et al. 2014; Gillies et al. 2015a). Success in restoring such habitats has been demonstrated in the United States and elsewhere where hundreds of reefs have now been restored with many at system-wide scales (Schulte et al. 2009**;** Schrack et al. 2012; La Peyre et al. 2015; http://www.projects.tnc.org/coastal/;

The success of these early projects relies on two major elements: having clear and realistic goals and objectives for restoration (Ehrenfeld 2000; Baggett. et al. 2014; McDonald et al. 2016) and having reference models for a range of restoration approaches and techniques that could feasibly lead to the recovery of the target ecosystem. Here we develop an interim reference model for Angasi reefs (in the absence of full ecological descriptions of natural reef ecosystems) based on available knowledge of Angasi ecology, biology and aquaculture; and suggest appropriate objectives, approaches and techniques for restoration that show promise for Angasi reef recovery.

[Typesetter to insert Box 1 and Figure 1. somewhere here]

Devising a reference model for Angasi reef restoration

Ecological restoration organisations and oyster restoration practitioners recommend the development of project goals and objectives using a reference ecosystem as a model, or target, for the local native ecosystem being restored (Baggett et al 2014; SER 2004, McDonald et al. 2016a,b). Yet the majority of Angasi reefs were lost between the mid-1800s to mid-1900s (Alleway and Connell 2015, Gillies et al. 2015a, Ford and Hamer 2016). As a result, very few scientific studies have been published describing their structure, ecology or function, impeding the ability of restoration managers to set appropriate objectives for Angasi reef restoration. Furthermore, only a handful of locations comprising natural Angasi reefs are known, none of which can be considered 'pristine', with even the largest and most well-known reef network still subject to commercial oyster fishing (Jones and Gardener 2016). Lastly, known reference sites are not always located in close proximity to restoration sites, such that direct comparison of sites may not be appropriate.

Interim reference model and restoration planning and evaluation framework

The reference model proposed in Table 1 is structured as a framework for the planning and tracking progress of an Angasi reef restoration project. It is based on a database prepared by the authors (Gillies et al. *in press*) drawing information from papers describing the composition, structure and function of Angasi reefs. This review included descriptions of quantifiable ecological attributes (e.g. oyster density, patch size, reef biodiversity) and ecosystem services (finfish productivity, filtration and denitrification rates) typically used for setting restoration goals and objectives (Baggett et al. 2014; zu Ermgassen et al. 2016, McDonald et al. 2016). The published literature on Angasi largely focuses on attributes most relevant to aquaculture and commercial harvesting, with only a few publications describing their natural ecology and function (Gillies et al. *in press*). Where data were unavailable or limited, we used information available in the grey literature, proxy information from related *Ostrea* species or from established shellfish reef restoration guides (e.g. Baggett et al. 2014, zu Ermgassen et. al. 2016a).

Deriving objectives to measure success

In this interim reference model we provide a set of objectives and metrics which can be used to measure the success of restoration methods and gauge the development of oyster habitat against predefined ecological objectives (Table 1). We advocate programs should use a Before-After-Control-Impact (BACI) design for community structure and function attributes when assessing restoration performance as recommended by Brumbaugh et al. (2006) and Baggett et al. (2014) with the use of multiple control sites to assess variation in space amongst control and impacts sites preferable i.e. *beyond BACI designs* (Underwood 1992).

[Typesetter to insert Table 1 somewhere here]

Identifying restoration approaches

The majority of Angasi reefs were lost or severely degraded through commercial dredge fishing by removing, burying or breaking up the reef complex. Changes to water flow, salinity, sedimentation rates, including increased re-suspension, and water pollution have further negatively altered physical conditions required for reef formation, with disease, predation and introduction of exotic species also likely to have contributed to ecosystem collapse and/or inhibited natural recovery (Alleway and Connell 2015; Ford and Hamer 2016; Gillies et al. in press). Despite closure of most oyster fisheries by the early 1900s and more recent improvements to water flow, sedimentation and water quality in some estuaries, evidence for natural recovery of reefs is limited. Information on the existence of extant reefs, and even those that are degraded, is compounded by the lack of any systematic searches for reef ecosystems or distribution maps (Gillies et al. *in press*).

Technical approaches to restoration can range from natural regeneration, through assisted regeneration to reconstruction approaches; all of which are preceded by the removal or mitigation of causal factors or threats (SER 2004, McDonald et al. 2016a,b). Like most oyster reef restoration projects, the major threats that need to be overcome before natural recovery of Angasi reefs can occur are: 1) lack of suitable substrate, 2) depleted supply of larvae and 3) sedimentation. Approaches for ameliorating disease and predation, particularly during early stages of growth and reef development, should also be considered (Box 2). Considering the widespread removal of reefs, sedimentation of suitable substrates and the fact that Australian estuaries now contain few larvae for recolonization, the process of restoring reefs through *natural regeneration* is unlikely to be a common restoration method in the short-term. More active intervention is therefore likely to be required in most cases to overcome the above-listed barriers and to initiate an ongoing recovery processes.

Where natural spat supply does not occur but other conditions are intact, *reintroduction* of larval supply may conceivably be the only intervention required. In theory, for example, it may be possible for Angasi larvae to be 'seeded' into suitable habitats. Although no trials of this approach have been carried out in the modern era for restoration purposes in Australia, the release of other oyster species such as Rock Oyster (*Saccostrea glomerata*) or non-native species such as Pacific Oyster (*Crassostrea gigas*) either through aquaculture or direct introduction, has led to increased adult oyster biomass when conditions are suitable at both the site level and estuary scales (Mitchell et al. 2010; Bishop et al. 2010) and the reintroduction of spawning adults was a common practice during the early years of modern aquaculture in Australia (1890-1920) (Roughley 1922; Neil 2001). Where natural spat supply still occurs but barriers such as availability of substrate for oysters to attach to or predation are preventing natural recovery, the removal of these barriers could be considered an *assisted regeneration* approach. An example of such an approach would be the installation of shell cultch or limestone to an estuary, such as has been used extensively in reef restoration projects in the United States (Brumbaugh and Coen 2009; La Peyre et al. 2014). The addition of new substrate provides several benefits, including: increasing surface area for oyster settlement, reducing predation (by increasing foraging effort of predators and/or increasing physical protection of oysters through creation of interstitial spaces) and reducing the effect of smothering of oysters from suspended or mobile bottom sediments by providing elevation and different surface orientations. A yet higher *reconstruction* approach would require removal or management of the full range of barriers including substrate, predation, disease and larval supply.

This last-listed approach has been applied in a number of recent cases. For example, the addition of hard substrate such as old shell or limestone is a common method used in the United States (Brumbaugh et al. 2006). Hatcheries are used to restock oysters in a number of restoration projects in Chesapeake Bay, MD where environmental conditions make recruitment less reliable and natural reproductive capacity is considerably reduced from historical numbers (https://chesapeakebay.noaa.gov/images/stories/pdf/2016marylandoysterimplementationupdate.pdf). A similar approach has been trialled for Angasi in Port Phillip Bay, Victoria (see case study below) with other projects located in Albany, Western Australia and Gulf St Vincent, South Australia also proposing to use similar reconstruction techniques.

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Case study: Application of the reference model to Port Phillip Bay and its use for tracking recovery over time

Port Phillip Bay prior to European settlement in 1836 had extensive reefs and beds of Angasi and Blue Mussel (*Mytilus edulis galloprovincialis*) which may have covered up to 50% of the seafloor (Ford and Hamer 2016). These reefs were destroyed largely through commercial dredge fishing for oysters throughout the late 1800s and early 1900s, and then for mussels and scallops from the 1960s until the mid-1990s, when the Victorian Government banned the practice of using dredges for shellfish harvest in Port Phillip Bay (Ford and Hamer 2016). In 2014, a local recreational fishing club (The Albert Park Yachting and Angling Club), Victorian Government (through Fisheries Victoria) and The Nature Conservancy formed a partnership to trial the restoration of Angasi reefs and blue mussel beds at three locations within Port Phillip Bay (http://www.natureaustralia.org.au/our-work/oceans/restoring-shellfish-reefs/port-phillip-bay/).

Process of restoration

Prior to the establishment of the partnership, a desktop feasibility study was undertaken by Fisheries Victoria and the Albert Park Yachting and Angling Club (Hamer et al. 2013) which considered many of the ecosystem's attributes identified in Table 1. These included: identification of previous threatening processes (*Attribute 1, threats* e.g. commercial harvest, water quality, predators), assessment of the logistical viability of undertaking restoration (*Attribute 2, logistical requirements* e.g. access to hatcheries, vessels and plant equipment and management and approval processes) and site selection, which was based, in part, on historical reef locations and environmental suitability (*Attribute 3, suitability of physical conditions*). This study provided a recommendation to establish in-water trials and helped inform the methods undertaken in first stage of restoration.

The first stage of the project (2014-2016) was a small-scale experimental trial at two locations with the primary objectives to: 1) assess and compare growth and survival rates of Angasi spat and small juveniles set onto scallop shell (cultch) and deployed to the seabed, with and without a base of hard substrate (limestone rubble) (*Attribute 1*), and 2) test feasibly, costs and logistics of hatchery and field grow-out procedures, within a restoration context (*Attribute 2*). Importantly, the first stage experiment was not a trial of restoration but aimed to test approaches to inform design of larger scale restoration attempts that would follow. This stage was necessarily conducted at a 'small scale' involving 1m^2 replicate plots of the various treatments and controls. Sampling of the replicate plots involved removal of the cultch for measurement in the laboratory. Port Phillip Bay has well oxygenated vertically mixed waters with salinity similar to oceanic levels and temperatures rarely exceeding 24°C or dropping below 10°C, physical parameters which are ideal for Angasi (*Attribute 3*). The suitability of the physical characteristics of Port Phillip Bay are further confirmed through the presence of live Angasi throughout the Bay and the evidence of historical reefs at both experimental areas.

The second stage of the project (2016-2018) has involved the deployment of two reefs comprised of a base of larger limestone rock that has had scallop cultch with juvenile Angasi layered on top (each reef is approximately 300 m^2 in size). An assessment and monitoring program has been implemented to measure the success and cost-effectiveness of restoration at more relevant restoration scales, along with collection of additional data on fish and other biodiversity. This first true restoration trial includes testing logistics of large-scale substrate deployment, oyster growth, survival and deployment density (*Attribute 4, primary ecosystem former*) as well as describing species composition that develop on the reefs including fish usage (*Attribute 4, community composition*). Methods used to assess Attributes 1 and 4 include oyster growth and survival (using quadrat-based measurements e.g. Baggett et al. 2014), Reef Life Survey visual observations (including predator observations, http://reeflifesurvey.com/) and Baited Remote Underwater Videos (BRUVS) deployed by local angling groups (Citizen Science). Oysters will also be analysed for *Bonamia* sp. 12 months post-deployment (from February 2017).

The third stage of the project (2018-2021) aims to reconstruct up to 20 ha of Angasi reef across the two locations, with the final reef design (i.e. footprint, height, substrate materials), oyster metrics (age and density at deployment, deployment times) and reef management (e.g. predator exclusion, disease mitigation, long-term management overlays) to be formulated, in part, from the results and learnings from the second stage restoration trial and the other Angasi restoration projects occurring elsewhere. Restoration objectives for the third stage include an overall adult oyster density of minimum 50 oysters/m^2, a diverse assemblage of reef-associated epifauna and the production of recreationally important fish species. Ecological targets for reef biodiversity and ecosystem services (Attribute 5) have yet to be determined pending more detailed ecological assessments of natural Angasi reefs (see Knowledge Gaps below).

Collaboration with local shellfish growers and recreational fishers to fill gaps in knowledge

Importantly, the initial feasibility study (Hamer et al. 2013) included a one day workshop with recreational fishers and ecologists which helped confirm the location of previous reef areas and which fostered community support from the onset of the project. Further input from commercial shellfish fishermen through an oral history and participatory GIS study (Crawford 2015) helped verify the location of old oyster reefs and mussel beds. Equally important has been the involvement of shellfish hatchery staff and local mussel growers who are regularly consulted during each stage of the restoration project and who have been employed at various times to assist with oyster deployment and husbandry. Furthermore, project staff regularly consult Angasi growers and hatchery staff from other states through informal discussions and formal networks (e.g. Australian Shellfish Reef Restoration Network, State Oyster Association Conferences) to increase knowledge in animal husbandry, disease management and reproduction.

Results to date

The data on survival rates of spat and small juveniles over 6 months post-deployment, suggest survival rate can be from 30-50% with a rubble base. However, the trial experiment also showed that the small plots and small sized rubble (40 – 70 mm aggregate, 10-15 cm elevation) used were vulnerable to loss of cultch, disturbance and sedimentation both from wave disturbance and bioturbation. This has likely had a significant impact on the survival and growth observed over longer time periods. In mobile sediment environments larger size rubble and larger size plots, with greater elevation, are likely required to improve cultch retention, oyster survival and growth, and overall success.

The results of the first stage experimental trial demonstrated that requirements for success will likely vary among sites, particularly depending on exposure to physical disturbance and sedimentation. The addition of a hard substrate base (in this case limestone rubble) was most important at the more exposed site, with growth and survival of oysters set on scallop cultch higher on substrate than when deployed directly on sandy/muddy bottoms (P. Hamer, Victorian Fisheries Authority, 2016, *unpublished data*). At the less exposed site survival and growth over the first 6 months post-deployment for small spat was higher with a rubble base, and for the larger juveniles growth was higher with a rubble base, but survival was similar to the sediment treatment. Overall, the results so far suggest clear advantages from providing a hard raised substrate base for growth and survival of spat and small juveniles. Finally, deploying the oysters to the seabed as larger sized juveniles after a period of grow out on mid-water long-lines, appears to provide gains in survival and final size, over deploying them as smaller spat.

Major predators observed to affect juvenile oyster survival include flatworms, and boring snails. While larger predators such as seastars, most notably the native 11 arm seastar (*Coscinasterias muricata*), were observed occasionally in plots, observation of drill holes in dead oysters and flat worms inside others, suggested that the flatworms and predatory snails caused most of the predation on the small spat and juvenile oysters. Further experiments are planned to test predation resistance of larger oysters to larger predators such as seastars.

Logistics and labour cost associated with preparation and field husbandry for temporary grow-out of oysters on mid-water long lines are not trivial and will limit the extent of application of this practice. However, it may be required in the early stages of restorations to expedite the growth and establishment of a base level of reproductive adults. In any case more cost effective methods, including using of biodegradable materials, will need to be developed for mid-water grow out to be applied at larger scale for restoration purposes. Hatchery production of larvae is now well established but capacity is limited, and improved methods are required to increase production, larval survival and settlement rates.

Gaps in Knowledge

No published studies currently exist which describe reef assemblages or ecosystem function of Angasi reefs, whilst the extent to which diseases such as *Bonamiosis* may prevent or inhibit natural recovery is still unclear. In Port Phillip Bay, remanent Angasi reefs exist close to one of the restoration sites (Wilsons Spit), although these remnant reefs have yet to be assessed for their biological attributes, due to their recent discovery during the pilot project. Large, old Angasi from these reefs have tested positive for Bonamia, but have otherwise appeared in healthy condition. The level of genetic resistance to mortality from Bonamia is unclear, and requires further study. Current hatchery practice is to source larvae from brooding adults collected from Bonamia free aquaculture sites. This mode of selection maybe selecting against the Bonamia resistant traits of the old large wild stock surviving in areas that do have Bonamia.

Because of the lack of available data on reef composition and function, we recommend practitioners use available data on rocky reef habitats if they exist in the vicinity (e.g. Reef Life Survey, http://reeflifesurvey.com/) to assist in identifying the local species pool available for colonization.

Similarly, along with biodiversity assessments, we suggest future surveys of natural reefs should measure functional attributes such as filtration, fish production and denitrification, in addition to structural parameters such as reef aerial dimension and height, oyster density, size frequency, growth and survival. The lack of diversity and biomass information on natural reefs considerably inhibits the development of longer-term objectives based on community assemblage and ecosystem services. Consequently we recommend that an adaptive management process is adopted to enable the development of more detailed restoration objectives as more information becomes available from restoration projects, Angasi aquaculture, traditional knowledge and from future scientific studies of natural reefs.

Conclusion

The reference model and framework for Angasi reef restoration proposed in this paper, as demonstrated by its application to the Port Phillip Bay restoration project, highlights key restoration attributes which can be considered for Angasi restoration projects, but could also be adapted for other *Ostrea* species. We hope that our reference model and progress tracking system will be trialled by restoration practitioners and the community to set objectives for oyster habitat restoration based on current knowledge, and to assess habitat suitability for future projects (e.g. Pollack et al. 2012).

Paying consideration to factors during site selection such as: presence of existing Angasi, spat density or evidence of recruitment, sedimentation rates, prevalence of *Bonamia* and location of historic reefs will help guide restoration practitioners to sites which have the required attributes to support Angasi reef restoration. We expect as more information comes to hand from ecological surveys of existing reefs, and with new reefs likely to be discovered, our reference system will be updated to better reflect Angasi-specific reef function and composition. Key knowledge gaps which would assist in setting restoration targets and in communicating the benefits of restoration include: biodiversity and community assemblages associated with reefs, ecosystem function and ecosystem services. We suggest community assemblages, biomass, reef function and ecosystem services should be given priority for future research to help inform restoration goals, monitoring programs and develop the case for further restoration. Lastly, project managers should adopt an adaptive management system when designing and managing Angasi reef restoration projects to incorporate new information as it becomes available so that project objectives and monitoring protocols can be adjusted accordingly.

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Box 1. *Restoration ecology of Angasi oyster reefs*

The ecosystem's dominant species, *Ostrea angasi,* belongs to the family *Ostredidae* and is closely related in appearance and genetically to the European Flat Oyster (*Ostrea edulis*) in Europe and the Bluff Oyster (*O. chiliensis*) from New Zealand and South America*.* Although they occur intertidally (Author's pers. obs.), Angasi predominately occupy the sublittoral zone where they settle on hard surfaces, such as other oysters, rocky outcrops and pylons, down to a depth of 40 m (Edgar 2000). As adults they can be broken from these hard structures or overgrow small fragments of settlement substrate, surviving as solitary individuals and creating areas of mixed beds and reef comprising both lose and cemented oysters which are then further colonised by other species (Figure 1). Their distribution covers southern Australia, from northern New South Wales to southern Western Australia including Tasmania. Across the total geographic range of Angasi, there is very little genetic divergence among sites, suggesting little or no population structure (Heasman et al. 2004).

Angasi oysters are hermaphrodites and can change sex multiple times within a spawning season. They are brooders, holding their eggs within the female mantle cavity where they are fertilized. Larvae are released after about 7-10 days incubation with a pelagic phase of 14-21 days. They produce fewer eggs than *Crassostrea* species, typically between 1-3 million larvae, with the main brooding period from October to March, although spawning can occur year round in warmer waters (Hickman and O'Meley 1988). Adults reach reproductive maturity from about 2 years and growth rates vary from 8-40 mm per year with adults obtaining sizes as large as 18 cm and can weigh up to 400 g (Mitchell et al. 2000; Jones and Gardener 2016).

Other species in the ecosystem

No published account currently exists detailing the biodiversity associated with Angasi reefs. Visual observations by the authors, however, on intact reefs in Tasmania and degraded reefs in Port Phillip Bay indicate species diversity and trophic structure is likely to be high, with reefs having representatives from all major functional groups. Common species observed on reefs include: Autotrophs (several species of red, green and brown algae including *Undaria pinnatifida, Sargassum* sp. and *Ulva* sp.); suspension feeders (*Mytilus galloprovincialis*, *Mimachlamys asperrimus*, Veneridae bivalves and various species of barnacles, zooanthids, sponges, ascidians and hydroids) mobile grazers (*Heliocidaris erthrogramma*), generalists (e.g. *Guinusia chabrus, Mitra glabra, Palaemon serenus, Pagurixus handrecki),* predators (e.g *Hapalochlaena maculosa*, *Coscinasterias muricata*, *Anthothoe albocincta* nudibranchs) and fishes (e.g. *Meuschenia freycineti*. *Pseudolabrus rubicundus)*. Many of these species can also be observed on rocky reefs or other hard surfaces suggesting that Angasi reefs may have similar levels of diversity to nearby rocky reefs.

Box 2. Bonamiosis – parasitic disease of oysters

The largest disease risk affecting Angasi is Bonamiosis, a protozoan parasite (likely *Bonamia exitiosa*) that primarily infects blood cells (haemocyts) and which can kill adult oysters, yet is less acute than *Bonamia ostreae* which infects *O. edulis* in Europe (Corbeil et al. 2009). Whilst Bonamiosis has decimated aquaculture populations in the past (Crawford 2016); the extent to which the disease may prevent natural recovery or inhibit future restoration is still unclear. Only limited testing of natural populations has occurred and the incidence of *Bonamia* sp. in natural populations has been found to be variable across southern Australia (Crawford 2016), with up to 20-30% in New South Wales estuaries (Heasman et al. 2004). The persistence of populations in the presence of the *Bonamia* sp. parasite suggests some resistance or tolerance to the disease yet the ability to develop resistant oysters using selective breeding is still to be assessed and should be prioritised. Other parasites include mud worms (*Polydora* complex) and flatworms, while predators such as sea stars, gastropods, fish and rays are also known to affect wild oyster populations.

Considering current limited knowledge on the distribution of *Bonamia* and the possible resistance or tolerance of Angasi in some populations, restoration practitioners should ensure they adhere to state guidelines on the movement and transport of oysters. Restoration can consider using oysters from areas where *Bonamia* is known to exist but should not transfer oysters to areas where it is uncertain if Bonamia is present. The culture of Angasi has occurred in hatcheries across Australia since the 1970s and commercial oyster growers are continually improving husbandry practices to combat disease and optimise feeding and growth rates (Crawford 2016). Partnering with the aquaculture sector to source oyster spat, identify local threats and manage disease risk should be a key consideration for restoration projects.

Figure and Table captions

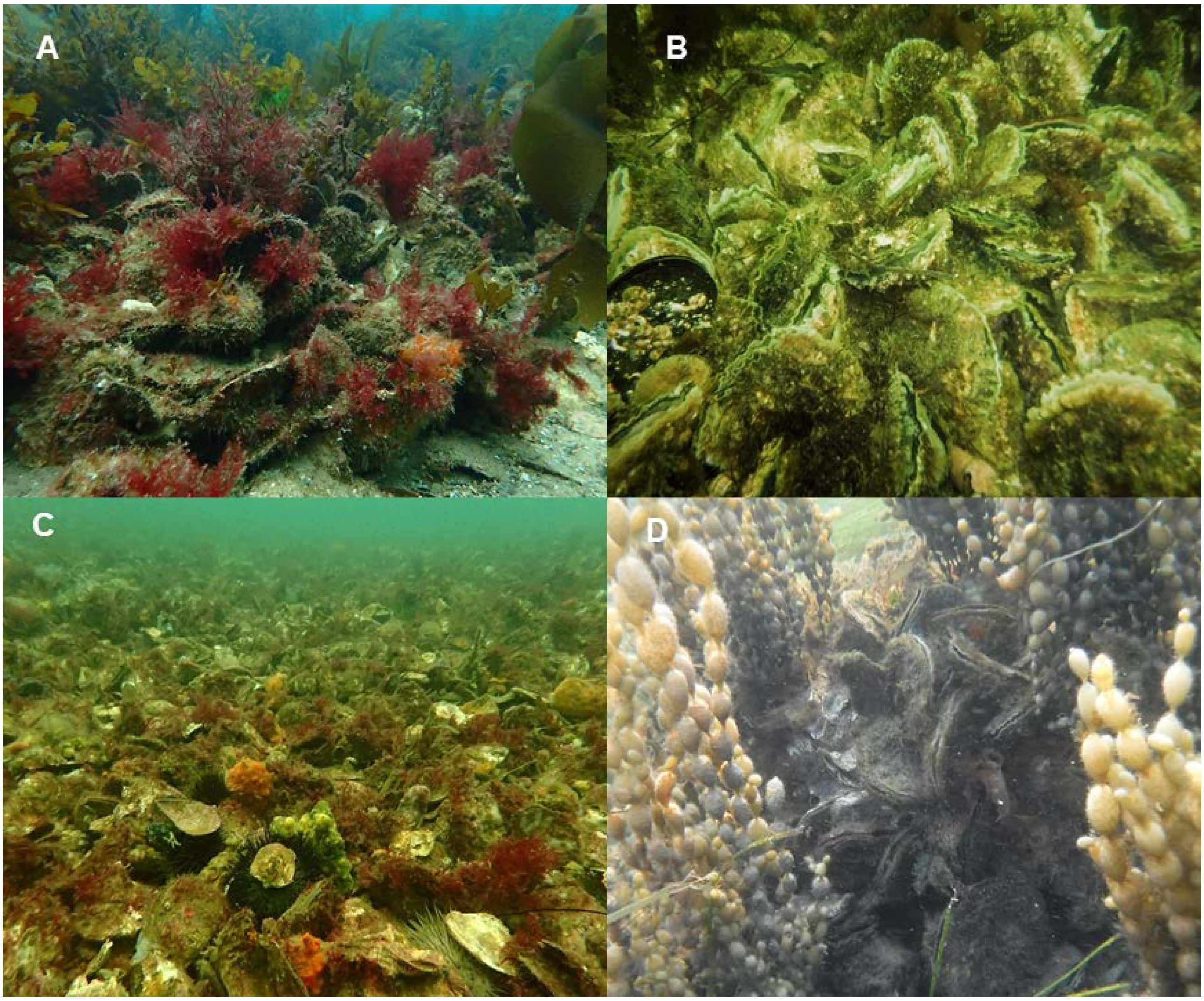

Figure 1. Natural Angasi oyster reefs in Georges Bay, Tasmania displaying different vertical profiles and reef structure (Reef height in Figure 1A and 1D approx. 0.3 m). © C Gillies. The Nature Conservancy

Table 1. Recommended reference system and restoration targets for Angasi reef restoration.

Attribute (or metric)	Recommended objective	Measurable indicators	Interim reference condition	Minimum detection period	Notes and further references
1. Absence of threats	Key threats reduced or eliminated sufficient to allow oyster growth and survival	Disease prevalence (*Bonamia* sp.) Sedimentation Predation	Bonamia prevalence assessed, restored reefs with similar levels of Bonamia as local populations. Biosecurity protocols followed. Use of local oysters as broodstock Density, survival and growth targets maintained above mortality (see *Primary ecosystem former* below)	Ongoing	Heasman et al. 2004; Crawford 2016 Proestou et al. 2016 *State aquaculture biosecurity protocols* Recognise that removal of *all* threats is unlikely to be feasible even in the long-term. Consider designs which *reduce the impact* of threats such as increasing surface complexity and/or more vertical angles to provide refuge from sedimentation and predation. Assess the need for protective caging of juvenile oysters from predators during early stages. Consider position and shape of reef in relation to water flow to reduce sedimentation, and to aid natural recruitment.

2. Logistical and policy requirements[1]	Projects be situated in appropriate areas which consider long-term protection and management of reefs. Access to shore-based infrastructure (e.g. barge loading areas, hatcheries) during construction and monitoring is cost effective.	Permits obtained Cost effective construction Management overlay obtained Risk assessments complete	n/a	n/a	Shellfish reef restoration can be resource intensive, requiring use of large plant equipment, hatcheries and shoreline access. Oysters can be legally harvested (recreationally) in all states and so projects should give consideration to protecting oysters from future harvests and protection against damage (e.g. anchor, dredge). Reefs should not pose a risk to vessel navigation.
3. Physical conditions	Physical conditions (salinity, temperature, dissolved oxygen) of site(s) within *O. angasi* tolerance levels	Salinity Temperature Dissolved O_2	Salinity: 25-35 ppt 10-29°C (temperatures above 20°C required for spawning) Dissolved O_2: No prolonged periods of hypoxia	Ongoing	O'Sullivan 1980 O'Connor et al. 2015
4. Species composition	*Primary ecosystem former (O. angasi)*				
	Individuals survive to reproductive age	Density Growth	Density > 50 individuals / m^2.	3-4 reproductive cycles (8-10 years)	*O. angasi*: 64-73 oysters/m^2 (Jones and Gardener 2016)

[1] Note, this attribute is additional to those identified in McDonald et al. (2016). We include it here as policy and logistical requirements for oyster restoration should be given equal consideration alongside biological attributes in determining the suitability of restoration sites, particularly in regards to cost effectiveness and long term protection.

		Age classes	Four or more age classes (= approx. two reproductive cohorts)		*O. chilensis (NZ):* 3-125m^2 (Mode 35.5) (Cranfield 1968)
	Viable spawning population Increased recruitment/density	% of population 2+ year class or older % of gravid oysters in population Increase in oyster spat settlement	>5% of population 2+ year class or older 20-30% of females ripe during spawning season Increase in no. of oyster recruits / m^2		O'Sullivan (1980) Hickman and O'Meley 1988. Baggett et al. 2014.
	Reef accretes in size and biomass over time	Reef areal extent Reef height	Positive or neutral change in reef aerial extent and height from original structure	1 reproductive cycle (2 years)	Recorded reef sizes for Angasi range from 4,000-15,000 m^2 (Tarbath and Gardner 2013) Projects should take into consideration *shell budget* where the rate of accretion must exceed shell loss for a reef to persist and also rates of spreading, sedimentation, subsidence and shell degradation (see Powell et al. 2006).
	Community composition				
	Short term- trend of increasing abundance and diversity of native reef-related species	Visual census of invertebrates, fish and algae assemblages e.g. Reef Life Survey methodology http://reeflifesurvey.com/	No empirical data available. Locally derived rocky reef habitat data can assist with determining species pool	2-3 reproductive cycles (4-6 years)	Local species assemblages associated with structured habitat should be identified from local species pool. Data from Reef Life Surveys (http://reeflifesurvey.com/) and local site

	Long term- comparable species richness to nearby rocky reef assemblages/lack of undesirable species				assessments could be used to identify macro assemblages.
5. External exchanges	Increase in Angasi recruitment detected outside of restoration site Community assemblage includes species from local species pool	Settlement plates/spat collectors Visual census of invertebrates, fish and algae assemblages Observed movement of mobile species across habitats e.g. fish-based acoustic arrays	Locally derived habitat data can assist with determining species pool	2-3 reproductive cycles (4-6 years)	Projects should consider establishing a spat monitoring program using a BACI design to detect changes in reproductive biomass before and after reef seeding. Consider placing reefs adjacent to other habitats such as seagrass, saltmarsh and mangrove habitats to enhance recruitment and gene flow between habitats Grabowski et al. 2005; Baggett et al. 2014
6. Ecosystem functionality/ Ecosystem services	Quantifiable change in fish biomass compared to pre-restoration and alternate habitats Trend of decreasing seston and/or chlorophyll a concentrations	Enhanced fish biomass/m^2 Light penetration or seston/chlorophyll a concentrations (in areas of eutrophication)	No empirical data available for ecosystem functionality. Mean fish enhancement in SE Australian seagrasses = 80 g m^{-2} y^{-1} (Blandon and zu Ermgassen 2014) Mean fish enhancement on *C. virginica* reefs in	3-4 reproductive cycles (6-8 years)	See zu Ermgassen et al. 2016a for setting ecosystem service objectives for oyster reef restoration.

	Denitrification enhanced above surrounding substrate	Denitrification rates/nitrogen removal	Atlantic and Gulf of Mexico, USA = 283- 528 g m^{-2} y^{-1} (zu Ermgassen et al. 2016b). See Kellogg et al. (2013, 2014) and Humphries et al 2016 for seston removal and denitrification rates on *C. virginica* reefs in the USA.		