Principles and science of stocking marine areas with sea cucumbers

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Abstract

Clearly stating the goals of stocking builds an essential platform for success. The scales, methodologies, management and time frames of the interventions can then be matched to the original goals. Stock enhancement, restocking and sea ranching will involve different stocking strategies. The genetic risks to wild stocks must be minimised by preventing translocation of juvenile sea cucumbers to different locations than those where broodstock were collected, unless studies show broad genetic homogeneity of the stock. Cultured juveniles are easily marked by immersion in a fluorochrome solution (e.g. tetracycline or calcein), which provides a long-term, unequivocal means of distinguishing hatchery-produced animals from wild conspecifics. Use of open sea pens is an experimental tool that provides better estimates of early stocking success. Juvenile density can be assessed by searching through sand and mud in quadrats by hand, whereas sub-adults and adults can be surveyed visually in transects with a stratified arrangement. Proponents of sea cucumber stocking in the wild should be conservative and realistic about the expected returns; 1 in 5–10 (10–20%) of released juvenile sea cucumbers surviving to market size is a benchmark. Clear goals, use of existing technology, and realistic expectations in sea ranching and restocking of sea cucumbers will provide the foundation for success.

Background

Stocking of marine invertebrates

While fish have been stocked into the sea since long ago, stocking of cultured marine invertebrates is mostly fairly recent (Bell et al. 2005). Notable invertebrates used in marine stocking include scallops and other bivalves, sea urchins, abalone, lobsters, Queen conch, giant clams and trochus. In the past, most stocking programs were unsuccessful in biological and economical terms (Leber et al. 2005; Bell et al. 2006). Poor survival of the released juveniles can be attributed, to a large extent, to inept knowledge about how, when and where to release the animals so that they may survive in high numbers (Liao et al. 2003; Purcell 2004; Lorenzen et al. 2010). Consequently, stocking programs started releasing cultured juveniles before the technology was developed to know how they should be released. This is unfortunate because stocking was thus criticised as a questionable management intervention even before the technology for many species was given the chance to be developed and proven (Hilborn 1998; Molony et al. 2003).

In recent times, criticism about stocking success has fostered a new era for programs to both develop release strategies through research before large-scale releases and conduct stocking in a responsible way (Blankenship and Leber 1995; Lorenzen et al. 2010). Key elements to responsible stocking are: (1) a requirement to demonstrate stocking success using marking of juveniles, (2) precautions to avoid disease transfer from hatchery stocks to the wild and (3) making efforts in the hatchery to produce juvenile cohorts with a wide genetic pool that closely matches the genetic make-up of the wild stocks among which the juveniles are released. As a consequence, greater scientific rigour in stocking programs is now giving back confidence in restocking, sea ranching and stock enhancement as potentially cost-effective management tools (Bell et al. 2006, 2008).

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Stocking of sea cucumbers

Stocking marine areas with sea cucumbers is a relatively nascent intervention (Battaglene and Bell 2004; Bell et al. 2005). Small-scale trials of stocking cultured sandfish (*Holothuria scabra*) in the sea appear to have commenced in the early 1990s in India (James 2004) and the late 1990s in Solomon Islands (Dance et al. 2003).

The Australian Centre for International Agricultural Research (ACIAR) embarked on a long-term program to assess the best tropical candidate species for restocking, develop hatchery technology for producing juveniles en masse, develop optimal release strategies, and apply the technology on a larger scale to test whether tropical sea cucumbers could be restocked or grown economically for village-based sea ranching. The first component, in Solomon Islands, determined that sandfish (*Holothuria scabra*) was the best species for tropical stocking, developed enough hatchery technology to produce them reliably for small-scale releases (Battaglene 1999; Battaglene et al. 1999) and studied the juvenile ecology (Mercier et al. 1999, 2000). The second component, in New Caledonia, adapted the larval culture and grow-out methods (Agudo 2006), developed methods to transport the juveniles (Purcell et al. 2006a) and technology for mark–recapture research (Purcell et al. 2006b; Purcell and Blockmans 2009), assessed release density and size-at-release in long-term release experiments (Purcell and Simutoga 2008), and evaluated restocking design (Purcell and Kirby 2006). The third component, being conducted in the Philippines and the Northern Territory, Australia, aims to determine whether the benefits of stocking sandfish for village-based sea ranching outweigh the costs of stocking (Juinio-Meñez 2012; Fleming 2012).

Purposes of stocking

The goals of stocking interventions will govern the management regulations needed and the spatial context of the releases. It is easy for agencies to develop a keen interest in culturing and stocking sea cucumbers in the wild without a clear description of the ultimate goals of the intervention. Such ambiguity can lead to false expectations of the likely outcomes, ownership or access issues, and the scale of releases and companion measures needed to achieve success. The path to failure in stocking programs is therefore often paved with uncertainty about the ultimate goals.

Stocking is a general term used here to mean the release of sea cucumbers into the sea with the expectation that they will then grow to larger sizes. Bell et al. (2005, 2008) and Bartley and Bell (2008) defined different types of stocking interventions, which are paraphrased, respectively, below.

- *Sea ranching*: *the release of cultured juveniles into open (non-bounded) habitats in the sea for harvesting once they reach market size*. This is a 'put, grow, and take' strategy relying on sole access rights (e.g. via lease of an area) to the proponents, without a main objective of increasing the yield of the overall fishery.
- *Restocking*: *the release of cultured juveniles into natural habitats to build nucleus breeding populations that will subsequently breed and replenish recruitment to repopulate the broader fishery*. This modality is predicated on protection of the released animals from fishing, ideally for their life span.
- *Stock enhancement*: *the release of cultured juveniles into the broader fishery to grow and later improve yields to fishers granted access to fishing grounds.* This modality does not have a main objective of rebuilding egg supply for generational stock rebuilding, and does not rely on sole access to stocked areas within the fishery.

Sea farming is another type of stocking, which is done into impoundments and artificial habitats (e.g. earthen ponds) supplied with sea water, but it is not examined in this paper.

The pathways to impact in restocking interventions are rather long compared with sea ranching (Figure 1). The main reason is because restocking relies not only on the survival of released animals to maturity, but also that they breed in the wild and that their offspring repopulate fishing grounds and survive to maturity (also see Molony et al. (2003)). The success of this latter, vital step of restocking is most difficult to demonstrate scientifically (Battaglene and Bell 2004; Purcell 2004). In contrast, sea ranching requires only that the stocked animals survive in high numbers to a market size.

Proponents should be explicit about whether the aim is to release animals that will be harvested by a particular group of people, or to rebuild depleted wild populations, or to enhance fishery yields for all fishers.

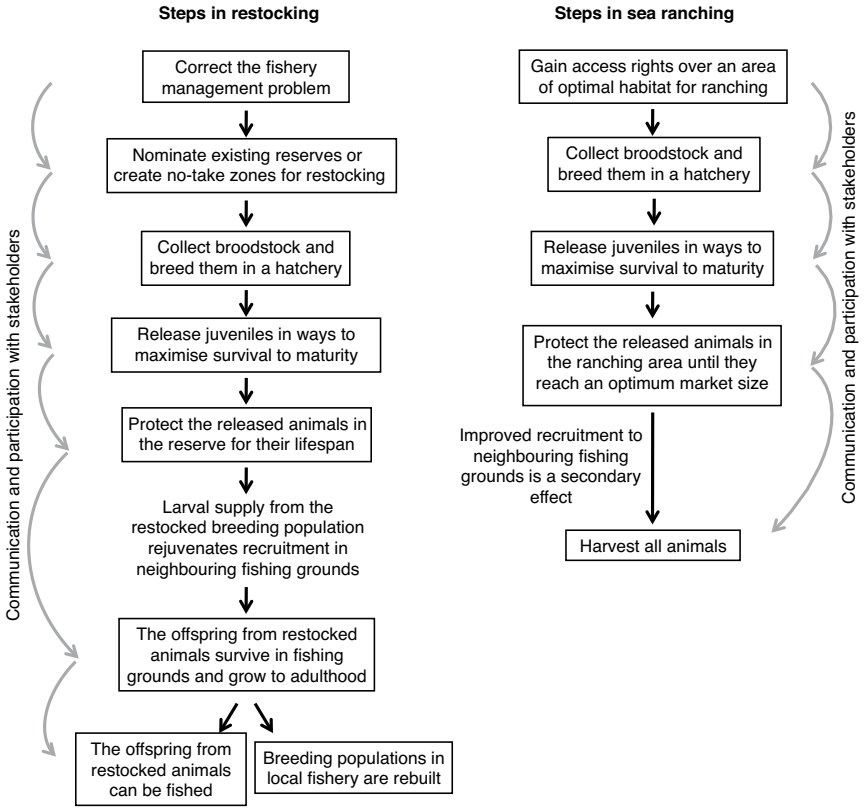

Figure 1. Important steps in restocking and sea ranching. Restocking relies on survival of the restocked animals to maturity and survival of their offspring to maturity. Also important through the steps of both interventions is frequent communication and participation of stakeholders.

Preserving the integrity of wild stocks

Risks of translocation

The ability to produce juveniles in the hatchery often spurs the desire to release them at various sites for various purposes. However, the genetic identity of local stocks, even those suppressed to low levels by fishing, should be maintained (Hindar et al. 1991; Utter and Epifanio 2002; Lorenzen et al. 2010). Some sea cucumbers such as black teatfish (*Holothuria whitmaei*) have high gene flow among populations, suggesting that larvae travel long distances and maintain genetic mixing among populations (Uthicke and Benzie 2000, 2003). In contrast, species such as the sandfish (*Holothuria scabra*) have restricted gene flow, causing certain populations to be relatively isolated from others, even within a country, and leading to unique genetic differences between populations at scales of less than 100 km (Uthicke and Benzie 2001; Uthicke and Purcell 2004). Native stocks may have particular genes that predispose them to cope much better with local environmental stresses that may occur periodically (Templeton 1986; Waples 1995).

Stock translocation may lead to reduced fitness of resident populations through *outbreeding depression* and *introgression* (Utter 1998; Uthicke and Purcell 2004). That is, introduced stock can outcompete with local stock (both ecologically and reproductively) or can interbreed with local stocks, leading to a loss in the genetic differentiation between populations. It is possible that introgression of foreign stocks could reduce the fitness of the population to deal

with occasional environmental stresses (Figure 2). Such effects are not just theoretical; studies show that translocation of fish can negatively affect local populations, and the introduction of foreign genes can lead to long-lasting effects that are usually irreversible (Hindar et al. 1991; Waples 1995; Utter 1998).

Are there some instances when translocation of foreign stock could be responsible? In some cases, populations have been depleted to extinction such that teams of divers could not find even a small number to serve as hatchery broodstock for restocking, and years have passed without successful natural recruitment (Bell et al. 2005). If proponents can produce rigorous data to convincingly show this to be the case, foreign translocation of new stock may be the only practical solution to restoring populations, but such interventions should not be swayed by private economic interests. Additionally, responsible restocking in such cases would use broodstock of the closest populations from which broodstock can be collected.

Population viability relies on genetic variability among individuals (Waples 1995). Using a large number of spawning animals in each spawning event in the hatchery, and taking care with using different sperm from different males to fertilise different groups of eggs (to avoid sperm dominance), will help to produce genetically diverse juveniles for stocking in the wild (see Utter 1998).

Technology for stocking

Use of markers

In a 'responsible approach' to stocking (Blankenship and Leber 1995; Lorenzen et al. 2010), cultured animals stocked in the wild are first tagged or marked. Marking the juveniles allows them to be distinguished from wild conspecifics, and provides a means to evaluate the effectiveness of the intervention (Figure 3). The ability of sea cucumbers to shed tags inserted in their body wall or coelomic cavity prevents the retention of most tags used in fisheries biology, including streamer tags, T-bar tags, coded-wire tags, visible implant elastomer tags and passive induced transponders (Conand 1990; Kirshenbaum et al. 2006; Purcell et al. 2006b, 2008).

Genetic 'fingerprinting' of individual sea cucumbers provides an accurate marking method (Uthicke and Benzie 2002; Uthicke et al. 2004), but the method is relatively costly. This method has not been applied yet to cultured sea cucumbers. Alternatively, sea cucumbers can be marked with fluorochromes, which fluorescently marks the ossicles (spicules) in the outer body wall of the animals. This procedure can be as cheap as 2 cents (US) to mark a 5-g juvenile (Purcell et al. 2006b). Fluorochromes such as tetracycline and calcein have been shown to be

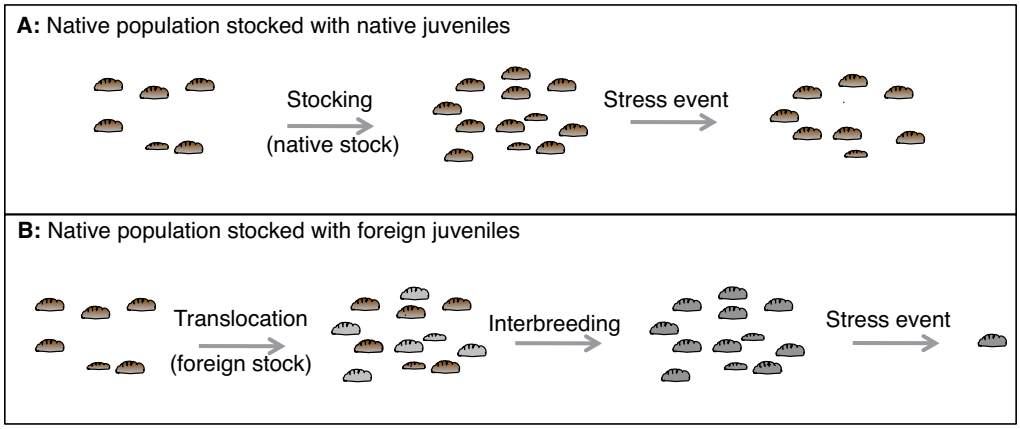

Figure 2. Illustration of one risk of translocation of foreign stock. **A**: Hatchery-produced juveniles from local (native) broodstock are stocked into the local population, the genetic identity of the stock is preserved, and the population is able to cope well with a stress event. **B**: Hatchery-produced juveniles from foreign broodstock (from a genetically different population) are translocated into the local population, the genetic identity of the stock is greatly reduced through introgression, and the interbred population no longer has the previous tolerance to cope with certain stress events.

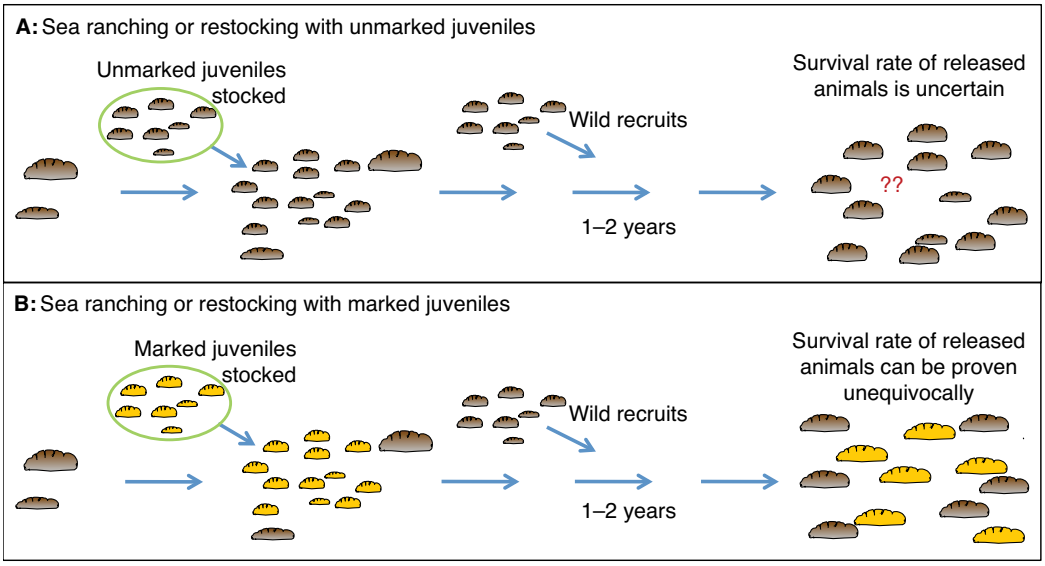

Figure 3. Diagrammatic illustration of pitfalls in releasing unmarked sea cucumbers in the wild. **A**: Unmarked cultured juveniles are released into an area that receives some natural recruitment of wild juveniles—it is impossible to validate how many, or what percentage, of the cultured animals survived over time. **B**: Marked cultured juveniles are released into an area that receives some natural recruitment of wild juveniles—the markers allow the cultured animals to be later distinguished from wild conspecifics mixed in the population to validate how many, or what percentage, survived over time.

suitable for up to about 2 years (Purcell and Simutoga 2008) (Figure 4), and 2-month trials with calcein blue and xylenol orange have shown long-term promise (Purcell and Blockmans 2009).

Cultured juveniles can be immersed in a marker solution in mass numbers in the hatchery within completely shaded flat-bottom tanks (Figure 5). The animals must be in a growth phase for the ossicles in their body wall to take up the fluorochromes (Purcell and Blockmans 2009). Fluorochromes are combined into the carbonate structure of ossicles during the process of calcification, and only that portion (e.g. 10–50%) of their ossicles being developed will be marked (Purcell et al. 2006b; Purcell and Blockmans 2009). Some juveniles may be slightly yellowish for a short time after immersion, but afterwards they are

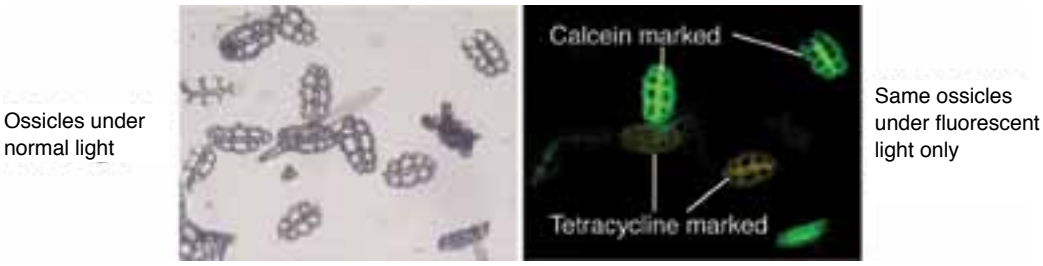

Figure 4. Ossicles (spicules) of *Holothuria scabra* individuals that had previously been marked sequentially by tetracycline then calcein (2 weeks later). Left: a field of view of ossicles under the microscope with normal light; right: the same field of view of the same ossicles under fluorescent light in an epifluorescence microscope. Note that about half of the ossicles have been marked—some that were fully formed were not marked during the immersion treatment.

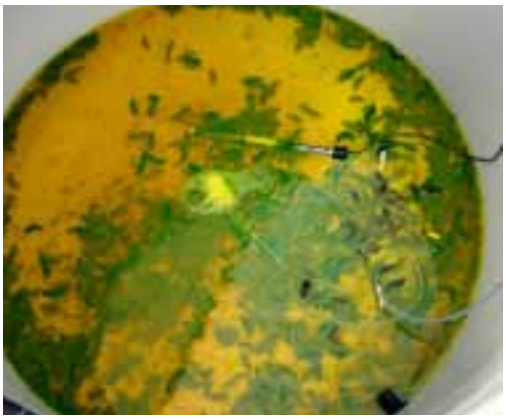

Figure 5. Fluorochrome stock solution is added to a large tank with aerated sea water and a heater to maintain water conditions. The animals are left for 12–24 hours in the solution to enable effective marking of the ossicles within their outer body wall.

indistinguishable in outer appearance from unmarked animals. If ossicles are unmarked, or weakly marked, after an immersion treatment, it may be that (1) the animals were not growing well before the treatment (i.e. they were 'stunted'), so their ossicles were not being developed; (2) the conditions, such as the temperature of the immersion solution, were not well maintained; or (3) the fluorochrome chemicals were inactive—e.g. tetracycline can be damaged by light and heat.

The materials needed for verification of fluorochrome markers are surprisingly basic, and the methods are cheap and simple (Figure 6). Small tissue samples can be taken from the ventral surface of the sea cucumbers in the field. Most ossicles are about 50–100 µm long and there are thousands of ossicles in each cubic millimetre sample of outer body wall of sandfish (Purcell et al. 2006b). Once in the laboratory, the samples are simply soaked for 30 minutes in household bleach to digest the soft tissue, which leaves the ossicles in the sample container. The ossicles are rinsed with fresh water to remove the bleach, then dried and observed under an epifluorescence microscope.

Use of sea pens

In some situations, sea pens may be used for farming sea cucumbers to market size. For instance, it may be important to separate sea cucumbers from other animals or to keep them from moving into other areas where they can be fished (e.g. Robinson and Pascal 2009, 2012). However, sea pens can be costly (materials and set-up), require regular maintenance and do not allow sufficient space for large numbers of animals unless the pens are very large. Sea ranching of large numbers of sea cucumbers would involve an area (e.g. a sheltered bay) of good habitat in which the ranching proponents have exclusive access to the animals, and where the animals could be released into that area without sea pens. So long as the habitat is optimal or good for the species, the sea cucumbers will not be likely to move far in the years before they are harvested (Mercier et al. 2000; Purcell and Kirby 2006). Sea pens are, therefore, mostly advantageous as experimental tools to help the researcher better estimate survival and growth of released sea cucumbers.

Up to a size of about 50–100 g, juvenile sandfish can crawl up the walls of sea pens made of plastic mesh. Escape then causes an underestimation in survival rates. We conducted short trials in a hatchery tank with sand to test different designs of small (0.1 m^2) prototype sea pens in an attempt to find a design that would prevent 2–10-g juveniles from escaping. Copper wire sewn to the upper edge of the mesh deterred animals from moving over it and escaping, but was toxic. In a weakly replicated ($n = 2$) test over 24 hours, fewer juveniles escaped (climbed over) pens with mesh skirting (mean = 25% escape) compared with pens with the upper edges folded inwards (mean = 60% escape) or pens with simple straight edges (mean = 70% escape). Juveniles were observed to crawl up the mesh wall, but fell back into pens when they crawled to the edge of the net skirts. We therefore used small sea pens of 1 m^2 with mesh skirts for small experiments on release strategies (Figure 7). Later, we tested escape rates of similar sized juveniles from prototype pens in the hatchery that had a strip of antifouling painted on the upper edge, and found that escape rates over 24 hours were comparable with those using the mesh skirts. As mesh skirts were difficult to make, we used an antifouling strip on the upper 10-cm surface of the pen walls for large pens (e.g. 500 m^2). Note that the risk of escape is much higher with smaller pens because the animals are in close proximity to the pen walls; hence, escape rates from small pens are not indicative of those from large pens.

Surveys

Surveys for juvenile sandfish <100 g need to be done by hand because the animals often bury in the sediment during part of the day at this small size. This makes large transects impractical for surveying

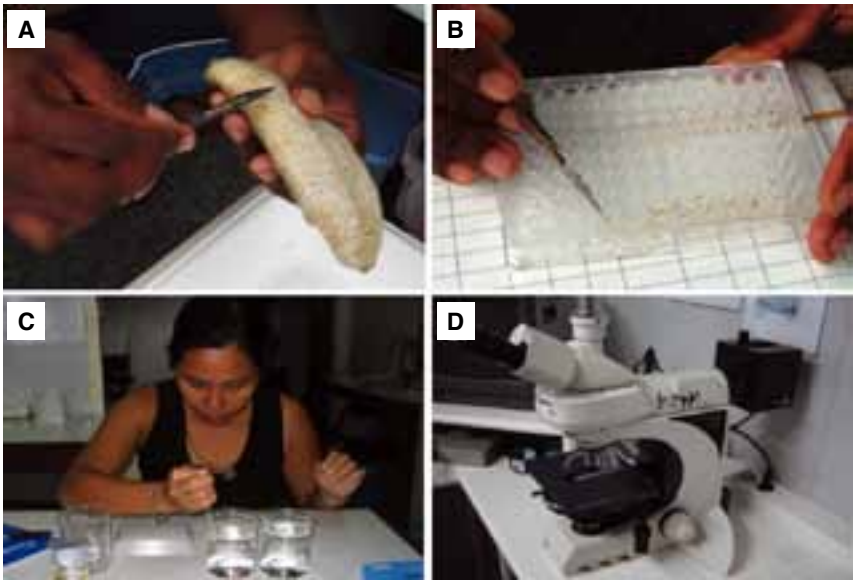

Figure 6. Steps in collecting and processing tissue samples of sea cucumbers to distinguish marked animals from unmarked (wild) ones. **A**: A tiny sample (a few mm^2) of the outer body wall is taken from the ventral surface of the animal, which is returned to the sea. **B**: The tissue sample is placed into a cell of a tray and buffered alcohol is added to preserve it. **C**: The alcohol is removed, bleach is added for 30 minutes to digest the soft tissue, then the bleach is removed and the ossicles are rinsed five times with freshwater. **D**: Once dry, the tray is placed under an epifluorescence microscope to look for fluorescently marked ossicles.

Figure 7. A small sea pen of 1 m^2 set into a seagrass bed. A mesh skirt on the upper edge of the pen mesh helps to prevent juveniles from escaping by climbing over the sea pen wall.

juvenile sandfish. The solution is to assess densities of juveniles within randomly placed quadrats of 1–2 m^2 by laying the quadrat and manually searching through the upper 5 cm of sediment by hand.

It is useful to estimate the survival rate in the initial months after release, when the animals are still juveniles. Within sea pens, quadrat surveys for sandfish should be stratified—some should be placed against the inner wall of the sea pen and some in the centre area of the pen (Figure 8). This is necessary because sea cucumbers will tend to gather near the edge of the sea pen through random movements (Jeanson et al. 2003), so this zone should be surveyed separately (Purcell and Simutoga 2008).

Once the animals in a sea pen are large enough to count reliably using visual census, the sea pen may be removed to allow the animals to displace over a larger area and avoid crowding. Conversely, the sea-ranching program may have simply released animals into the open and waited 6–12 months before doing visual surveys. In most sea-ranching situations, the animals

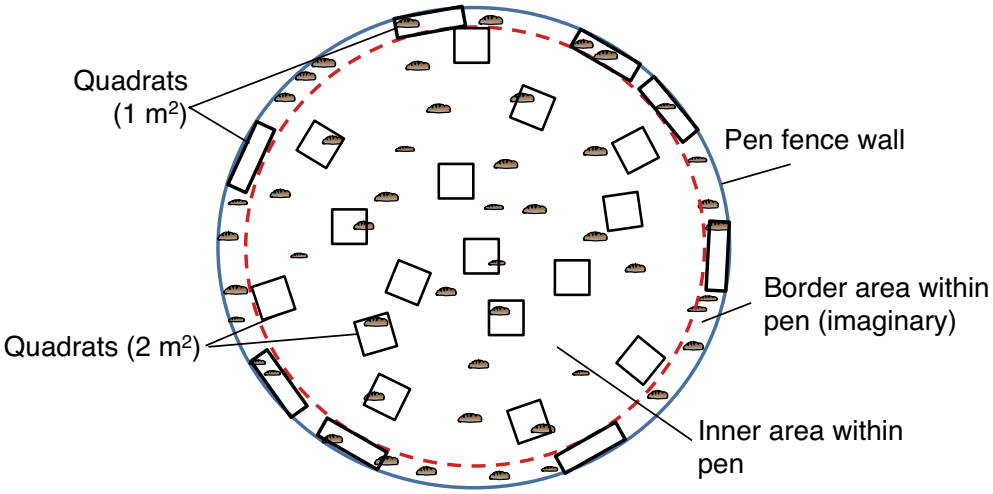

Figure 8. Potential placement of random quadrats within experimental sea pens. The number of animals within the border area—50 cm inside the inner wall of the pen mesh—can be sampled with rectangular 1-m² quadrats (2 × 0.5 m). Animals within the inner area can be sampled with square 2-m² quadrats (1.41 × 1.41 m).

would be released near the middle of the managed area at moderate density (e.g. 1/m²). Through random displacement over long time intervals (see Purcell and Kirby 2006), some of the animals will move relatively large distances from the release area (e.g. 100 m), many would move short distances from the release area (e.g. up to 50 m) and many would stay in the release area. The uneven density of released animals calls for a stratified survey design (Figure 9). Zones can be marked out—for example, using buoys at the corners—to delineate the release zone (central zone), a middle zone and the outer zone. Transects can then be laid randomly in each zone, increasing replication in zones successively further from the release area to account for greater variability in counts within the replicate transects from increasing patchiness and sparseness of sea cucumbers.

Where to release?

Nature should be a useful guide to choosing good sites for stocking sea cucumbers. For example, sandfish (*Holothuria scabra*) larvae appear to settle on seagrass blades, and juveniles are known to inhabit shallow seagrass beds (Mercier et al. 2000). Sites with a current or previous history of hosting the species should be a sensible start. It may be that some sites never really were home to the species of sea cucumber but could serve as good stocking sites; however, this will generally be rare. Avoid sites with widely varying environmental conditions; for example, areas periodically subject to freshwater deluges. Likewise, avoid areas that may be vulnerable to pollutants (see Purcell and Simutoga 2008).

In an experiment in New Caledonia, we set up 30 replicate 1-m² experimental sea pens with net skirts 15 cm into sediments within various locations in a bay such that each pen had a different undisturbed habitat composition (S.W. Purcell, unpublished data). A group of 25 juveniles (2–10 g) was weighed and released into each sea pen. After 1 week, the juveniles surviving in each enclosure were collected by hand and an air-uplift suction device, and re-weighed. Preliminary analyses suggest that microhabitats for optimal growth and survival of the juveniles would have the following traits to ensure their protection from predators and allow them to bury easily:

- shallow—0.2 m to about 2–3 m depth
- a low proportion of coral or coral rubble in the sediments
- moderate penetrability of sediments; muddy-sand appeared best and should allow a hand to be forced easily to a few centimetres depth
- moderately high seagrass coverage, preferably in the genera *Cymodocea*, *Thalassia* and *Syringodium*.

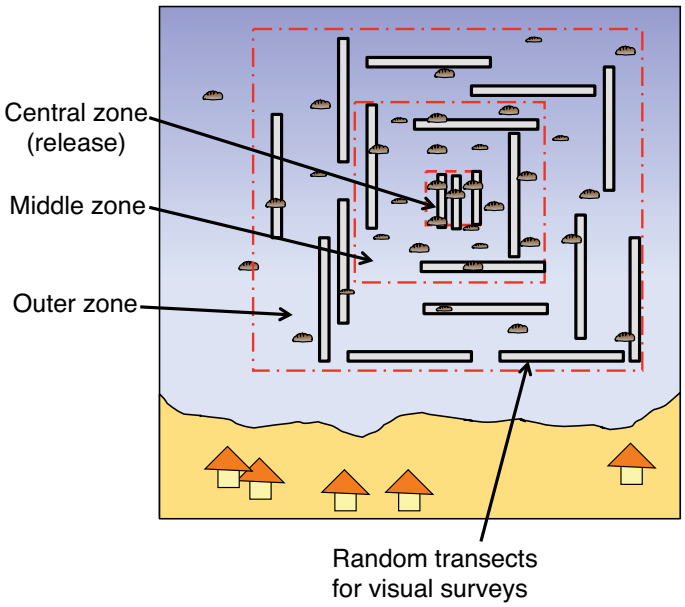

Figure 9. Potential design for transect surveys within a coastal seagrass bed in which cultured sea cucumbers (small oval figures) have been previously released for sea ranching, and have moved out of the central release zone. Bars are belt transects (e.g. 2 × 50 m) laid randomly within three zones (red dashed lines), which are defined at the site by buoys or other permanent markers at the corners of each zone. Stratified sampling is used; that is, the number of transects increases from the central zone to the outer zone because the sea cucumbers are expected to become sparser.

Expected returns

Unfortunately, but quite predictably, most of the small juvenile sea cucumbers released in the wild will not survive. Predation is the biggest hurdle in stocking a wide variety of invertebrates, and most of the released juveniles die or are eaten by predators shortly after being released (Bell et al. 2005). Many different animals eat sea cucumbers, particularly when they are young. Known predators of tropical sea cucumbers include a wide variety of crabs, predatory gastropods, sea stars, sea birds, and fishes including pufferfishes (Tetraodontidae), emperor fishes (Lethrinidae), triggerfishes (Balistidae) and wrasses (Labridae) (Dance et al. 2003; Francour 1997). Personal experience with various release experiments in New Caledonia suggests that invertebrate predators, especially crabs and sea stars, are especially voracious predators of juvenile sea cucumbers. These observations correspond closely with reports of crab predation in Madagascar (Lavitra et al. 2009; Robinson and Pascal 2012).

Modelling of survival rates of 5-g released juveniles showed that 7–20% of sandfish released in New Caledonia could be expected to survive to a good market size of 700 g 2.6 years after being released for sea ranching (Purcell and Simutoga 2008). Therefore, a conservative estimate of survival to this size would be about 1 in 10 (Figure 10). A survival rate of around 20% to this size over roughly 3 years could be achieved if conditions were favourable over the ranching period. This notion corresponds closely with shorter durations of some other recent studies. In Fiji, Hair (2011) determined survival rates of 23–41% for sandfish in sea pens over just 6 months, and the animals had not reached a market size. Similarly, in the Philippines, a survival rate of 39% was reported by Juinio-Menez et al. (2012) for sandfish in a sea ranch;

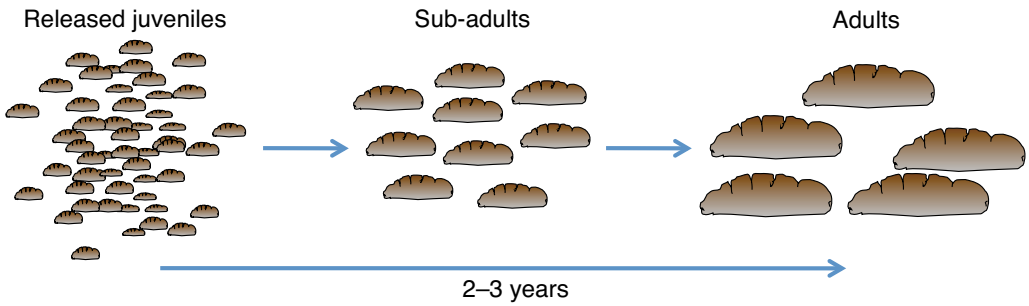

Figure 10. Stylised diagram of changes in size and numbers of sea cucumbers, due to death and predation, from the time of release in the wild to the time at which they reach a good marketable size

however, these comprised juveniles from batches released at various occasions over a 19-month period, and many had been recently released.

Overly optimistic predictions of economic returns from sea ranching will give expectations to villagers that will be difficult to meet, and proponents may benefit more from conservative expectations and praise at exceeding them. Estimates of economic viability of sea ranching can then be made by back-calculating revenue from the harvested animals to the maximum cost of producing juveniles in hatcheries to make a profit (see Leber et al. 2005; Purcell and Simutoga 2008). Two additional points should be considered:

1. As with other mariculture programs, some patience and investment is needed early on because it often takes years to reduce the costs of producing juveniles and to perfect release methods.
2. Benefits to communities extend beyond the economic (Lorenzen et al. 2010); sea ranching and restocking can build technical capacity and foster awareness for better stewardship of the resource by communities, which should be considered a valuable outcome for fishery managers.

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