Criteria for release strategies and evaluating the restocking of sea cucumbers

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Abstract

Conservative management should be the key to sustainable sea cucumber fisheries, and the release of hatchery-produced juveniles could speed the recovery of depleted stocks. Advances in methods for culturing sea cucumbers have allowed juveniles to be produced in high numbers for restocking. However, the lack of research on release methods and assessment of stock recovery jeopardizes the success of restocking programs.

A key criterion before releasing juveniles should be genetic similarity of stocks at release sites and sites of broodstock collection. Research is then needed on release methods, including the optimal mode of transportation, release habitat, times of the day and season for release, and the most cost-effective size for release. Acclimation of juveniles to improve survival may include behavioural conditioning at the hatchery or temporary protection from predators upon release. Field experiments using replicate pen enclosures in New Caledonia have shown high initial survival and growth of hatchery produced *Holothuria scabra* juveniles in certain habitat types.

Further to experiments on release methods, cost-benefit analyses at a larger scale will require larger experiments and an accurate evaluation of restocking effects beyond natural recruitment. Until tagging methods for juveniles are developed, such research is likely to utilise experimental designs involving multiple release sites and control sites without released animals. Such "BACI" designs are appropriate for analysis by ANOVA statistics or by Non-Linear Mixed Effects Models using repeated surveys. In order for restocking to gain credibility as a wise investment for resource management, programs will need to rigorously demonstrate that releases of hatchery-produced juveniles contribute substantially to the replenishment of stocks.

Keywords: Restocking, release methods, conditioning, BACI designs, modelling, sea cucumbers, holothurian

海参渔业增殖策略

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摘要

资源保护是发展可持续海参渔业的关键，放流人工苗种有可能加速已衰退资源的恢复。海参养殖技术上的进步可以在高密度下将幼参养到可供放流的规格，然而，在放流技术和资源评估方面的研究不足，影响到资源增殖的成功。

在放流前，一个关键的指标是放流的幼参在遗传性上要与亲参采集地和放流点的海参遗传性相近。在放流方法上，需要研究最佳的运输方法、放流地的生境、放流的季节和时间，以及放流成本与幼参规格之间的关系。为了提高放流的成活率需要研究海参幼体在育苗池中和暂养时防范敌害的行为。在新喀里多尼亚进行的野外网围实验表明，人工育成的糙海参（*Holothuria scabra*）苗在一定的生态环境条件下，最初阶段的成活率和生长都很高

有关放流方法尚待进一步研究，包括大规模放流的经济效益分析和增殖效果的精确评估。在幼参标志放流尚未成功前，海参资源增殖的研究只能在实验方法的设计上予以考虑，如选择多重放流地点和不放流的对照点。这种BACI(Before After Control Impact)设计模式采用统计学上的方差分析或是用非线形混合效果模式进行分析是适合的。为了获得对海参资源增殖放流投资的可信度，尚需要严格地论证人工苗种放流对资源补充的贡献率。

关键词：放流、放流方法、行为、BACI设计，模式、海参

Introduction

Overfishing of sea cucumber stocks worldwide has raised the importance of fisheries management in sustaining this industry because populations do not recover quickly (Uthicke, 2004). Restocking of sea cucumber stocks with hatchery-produced juveniles, as a management measure for remediation of breeding populations, is likely to be employed only when sites are depleted by overfishing. Less frequently, releases of juveniles could be used for stock enhancement (sensu Bell and Nash, 2004), i.e. to improve the productivity at sites with relatively low natural recruitment, or to increase access of species to alienated habitats (Bell, 1992). If the problem is overfishing, then the solution in the first instance is to ensure firm and conservative management to prevent overfishing.

Management of sea cucumber resources should ideally be based on the knowledge of stock size through visual surveys or statistics on catch per unit effort. Several options for management may be applied in combination, using management tools common to other marine invertebrates (see Purcell, in press), with an understanding of the biology and ecology of the species involved. For instance, some species, such as *Holothuria nobilis,* are usually found only at a large and mature size (FAO, 1990; Uthicke and Benzie, 2000), so minimum size limits may be inappropriate for this species. In artisanal fisheries, the strategies should be adopted in participation with fishers and customary owners to promote respect and compliance of regulations. Once these strategies are in place, restocking may be an effective way to rebuild breeding populations for fisheries.

A theme arising from restocking programs is the relatively high cost of culturing and releasing animals in the high numbers needed to restore breeding populations (Bell and Nash, 2004). Released juveniles also tend to have high mortality in the initial period following release. Therefore, release of hatchery-produced sea cucumbers may not be cost-effective for a 'put-and-take' mode of stock enhancement. This is because the financial returns per animal surviving to harvest size are likely to be low. In contrast, if sufficient numbers of animals survive and are protected from fishing, the flow-on benefits to restoring larval production of the population and promoting self-recruitment at sites could make restocking cost-effective in the long term. The latter case of restocking requires a fishery closure at the release site for long enough to allow several reproductive seasons of the surviving animals (Bell and Nash, 2004).

In contrast to the volume of literature on culture methods for sea cucumbers, the published information on restocking is limited. The success of restocking marine invertebrates is poor unless the protocols for releasing hatchery-produced juveniles are well determined (e.g. queen conch, Stoner and Glazer, 1998; rock lobster, Mills *et al.,* in press; abalone, Heasman *et al.,* 2003). It can be detrimental to future programs when restocking is conducted without an understanding of these release criteria, because poor success due to inappropriate release methods can be inferred as proof that restocking with that species is not viable. To ensure that restocking reaches its full potential, credible experiments must be conducted to determine where, when and how to release juvenile sea cucumbers so that post-release survival and growth are maximised (Battaglene and Bell, 1999).

Once release criteria are identified by field experiments, research is still needed to show the efficacy of larger-scale restocking (Leber, 1999). The second phase of experiments would need to be longer in duration to allow an assessment of the relative costs per animal surviving to a mature size. Of importance too is knowledge of the number of breeders needed to regenerate the fishery through self-recruitment. The methods used in these two research phases -identifying release criteria and assessing cost-effectiveness of restocking - will differ due to the scale and time frames of the experiments. The focus of this paper is to illustrate some of the considerations for these experiments, using examples from current research from a collaborative program in New Caledonia on the development of restocking methods for the sandfish, *Holothuria scabra.*

Release Strategies

Establishing criteria for release

Experiments on release methods are important in the case of sea cucumbers because hatchery-produced juveniles (Figure 1) are vulnerable to predators (Dance *et al*., 2003) and can undergo stress upon release into the wild or during transport from the hatchery to the release site. The hatchery environment is quite different from natural habitats. Natural behaviours, such as seeking refuge, predator avoidance and feeding, may be poorly developed at the time of release. Research is needed to identify modes of transport that minimise stress, then to determine the best strategies for the locations, habitats, juvenile sizes and times of release (Purcell *et al*., 2002).

Figure 1. *A group of juvenile* Holothuria scabra *(1-5 g) produced in the hatchery in New Caledonia, ready for release in an experiment to test restocking strategies. (Photo: S. Purcell).*

Preserving genetic diversity

A prerequisite for releasing juvenile sea cucumbers, either for experimental purposes or for restocking, is to minimise the risk of modifying the genetic diversity of stocks at release sites. This is an issue when juveniles are translocated to non-native sites, i.e. away from sites where the parental stock was collected. This issue goes beyond theoretical concepts of genetic contamination, as empirical examples from stocking of fishes show that the genetic effects of translocations tend to be detrimental to wild stocks, and that these effects can be irreversible (Hindar *et al.,* 1991; Waples, 1995; Utter, 1998).

In the case of sea cucumbers, some species, such as *Holothuria scabra,* can have restricted gene flow between populations over distances of less than 100 km (Uthicke and Purcell, in press), whereas gene flow can be relatively broad for other species, like *Holothuria nobilis* (Uthicke and Benzie, 2000). If the juveniles are to be translocated away from native sites, genetic studies should be conducted to determine if stocks (albeit remnant) at release sites are different from parental stocks. In New Caledonia, two southern sites were excluded for release experiments because populations there were found to be significantly different from populations at other release sites and from populations where broodstock were collected. Efforts should also be made to use as many spawning broodstock as possible to produce a group of juveniles with a range of alleles reflecting the breadth of genetic variation within the parental stock.

Transfer of disease

Before releasing progeny from the hatchery, strict checks for disease and parasites should be carried out to avoid introduction of harmful organisms to the wild stock. Diseases and parasites for sea cucumbers are not well documented but this is an area of recent research (Eeckhaut *et al.,* 2004; Wang *et al.,* 2004). Diseases can arise from bacterial, fungal and viral infections and are more associated with intensive culture conditions in the hatchery. Limits should be set *a priori* on the acceptable level of disease, parasites and infections of juveniles to be released from the hatchery. The entire group of juveniles should be quarantined and not used for restocking if an unacceptable proportion is unfit as this may indicate that a high number are disease carriers. Further research is needed to understand the frequency and range of diseases occurring naturally in wild juveniles so comparisons with the situations in hatcheries can be made.

Transportation methods

Transport and handling from the hatchery to the wild are stressful and juveniles can die or become vulnerable to predators if transportation methods are crude. It is necessary to know the densities and duration of transport that can be comfortably sustained by the animals without producing adverse effects, particularly for restocking to distant sites. Juvenile sandfish produced in the hatchery have proven to be very hardy for transport at high density over 24 h (Purcell *et al.,* in prep).

Reducing the temperature for transport may be useful in reducing the activity of juveniles and promote lower rates of oxygen consumption and excretion of wastes. However, caution is needed when modifying transport temperature because juveniles may be more prone to temperature shock once released into warmer water in the wild. Some effort should be made to determine the best transport media, which may not be water. For instance, substrata such as damp cloth or vegetation have been shown to be better transport media than water for many other marine invertebrates used in stock enhancement, such as trochus (Dobson, 2001) and abalone (Heasman *et al.,* 2003).

Habitats for release

The best habitats for releasing juveniles may not be the same as the habitats where adults are found. Recruitment of larvae may occur in certain habitats, with juveniles migrating to 'adult habitats' later in life. Settlement of sandfish can occur in shallow seagrass beds (Mercier *et al.,* 2000) and distributional patterns suggest that they move to deeper areas later in life at some sites (Hamel *et al.,* 2001). Adult habitats may offer better foods but a higher risk to predators, or may not have suitable substrata for larval settlement. Moreover, juveniles may survive and grow better in certain micro-habitats within the general habitat in which they occur with adults.

A study in New Caledonia showed that micro-habitat features can account for a majority of the variation in the survival of hatchery-produced sandfish released in the wild (Purcell and Blockmans, unpublished data). In optimal habitats, survival of juveniles released into enclosures (non-covered) over one week was consistently greater than 90 %, whereas survival in other habitats was much lower and highly variable. Identifying the right habitat features to release juveniles is thus critical for restocking.

Experiments should examine a broad range of biotic and abiotic variables, rather than testing habitat types (e.g. "shallow seagrass") that are too broad and can vary in specific features from one location to another. In New Caledonia, the percentage survival of juvenile sandfish released into enclosures was often highly variable at the scale of tens of metres, with standard deviations averaging 32 % of the mean *(n* = 12 experimental treatment combinations). A high level of replication is therefore needed at local (e.g. within-site) scales, in addition to broader (e.g. site or location) scales, in field experiments on restocking strategies.

Size and density for release

The time and space required to culture sea cucumber juveniles to a large size is a primary impediment to land-based culture. Large-scale enhancement trials with other marine taxa indicate higher survival of hatchery-produced juveniles when released at a larger size (Zhao *et al.,* 1991; Bell, 1992; Munro and Bell, 1997). Releases of hatchery-produced juvenile *Apostichopus japonicus* into ponds in China indicate that survival is positively related to size (Chang *et al.,* 2004). Clearly, the germane issue is the trade-off in costs to produce large juveniles compared with the increased post-release survival. It could be that producing and releasing many more small juveniles at low cost is more economical for restocking, despite lower survival (e.g. Zhao *et al.,* 1991). A sound knowledge of the most cost-effective size for release is thus vital to the economic viability of a restocking program. Evaluating the optimal size-at-release requires knowledge of rates of survival and production costs for juveniles of various sizes, which is part of a program in New Caledonia on developing methods for restocking of sandfish (Purcell *et al.,* 2002).

Studies have shown that restocking of temperate abalone would be most effective at low density, because survival of released progeny is positively density-dependent (e.g. Heasman, 2003). The effect of the density of released juvenile sea cucumbers on their survival in restocking should therefore be investigated.

Times for release

The time of release of sea cucumbers into the wild could be important because they have diurnal behaviours, which may make them less susceptible to predators at certain times. For example, juvenile sandfish have a daily burrowing cycle in which they hide in mud during the day, and would be less vulnerable to visual predators during daytime (Mercier *et al.* 1999, 2000). Dance *et al.* (2003) hypothesised that releases would be better at night when predators are less abundant. However, juvenile sandfish released in New Caledonia survived equally at different release times, indicating that this was not a critical release criterion at that site (Purcell, unpubl. data). In that experiment, the juveniles took some hours to burrow and were presumably vulnerable immediately upon release. Thus, the time of release may be important only at sites where predators are active at certain times.

Figure 2. *Juvenile sandfish (*Holothuria scabra*) produced at a hatchery in New Caledonia. The sizes range from about 2 g to 15 g. Knowing which size is most cost-effective for release is pivotal to restocking (Photo: S. Purcell).*

Seasons for release

Similarly, the release season is likely to have an important bearing on survival and growth of juveniles, mainly in sub-tropical/high-latitude sites. The natural spawning period for sandfish in New Caledonia is November to February (Conand, 1993) and the pentactula larvae would settle on seagrass blades after a couple weeks in the plankton (Mercier *et al.,* 2000). Natural recruitment to the benthic population living on and in the sediments would presumably be at least a month or more after the juveniles had gained size from feeding epiphytically on the seagrass.

Two release experiments using juvenile sandfish (of approximately the same size and from the same culture batch) were conducted in New Caledonia at the same site using similar methodology during two months, April and July. Juveniles of this size would probably occur predominantly from February to April, in view of spawning in November to January (Conand, 1993). Although this comparison is limited to just two periods, the results suggest that releases of juveniles are best in the natural season for recruitment. In July (winter), survival of juveniles was lower and the growth was slightly negative, compared with high survival and positive growth in April (Figure 3).

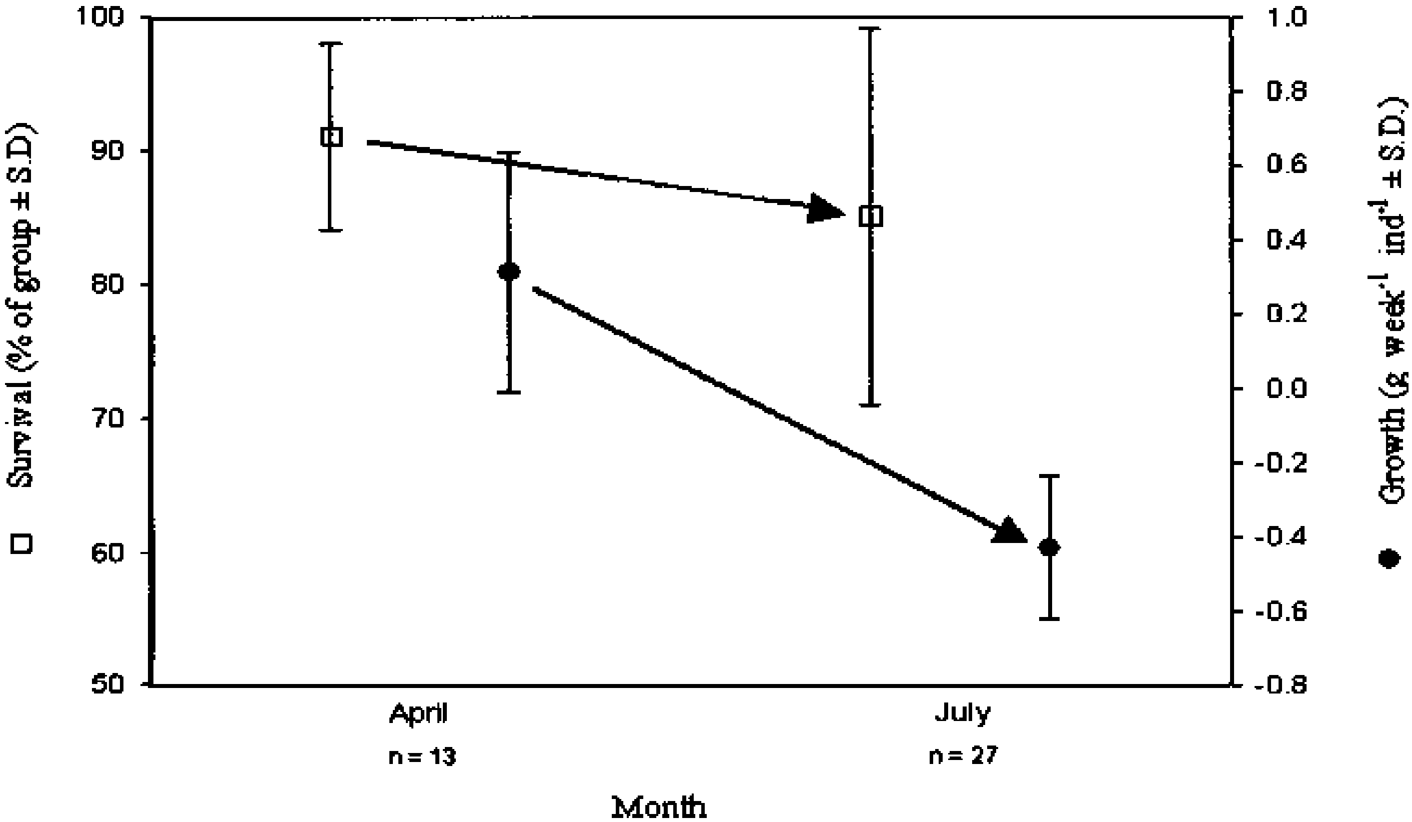

Figure 3. *Mean estimates for survival and growth of hatchery-produced juvenile sandfish (1-10 g) released in two experiments at Ouano, New Caledonia. To compare seasonal differences, the data for the April experiment are displayed only for enclosures in the same habitat used in the July experiment. Arrows indicate trends in the seasonal changes in survival and growth. The numbers of enclosures (n), which each received 25 juveniles, are indicated below the graph.*

Behavioural conditioning of juveniles

The juveniles of many sea cucumber species are rarely found in the wild. Very small juvenile sandfish of less than 10 mm in length were found by Mercier *et al*. (2000) on seagrass blades when still in the initial phase of feeding on hard surfaces. They also found that, once off seagrass, wild sandfish juveniles (10-100 mm) predominantly burrow in sediments during the morning and early afternoon, and only 5-15 % of individuals can be found on the surface at these times. In contrast, Mercier *et al*. (1999) showed that a greater proportion (>20 %) of hatchery-produced juveniles are on the surface in the morning and early afternoon. Likewise, results from the field releases in New Caledonia show that around 15-75 % of hatchery-produced juveniles are on the surface (Figure 4) during morning and early afternoon at the end of three separate experiments (Figure 5). Hatchery-produced juveniles therefore appear to be less cryptic than wild juveniles of the same size range.

Figure 4. *A hatchery-produced juvenile sandfish (approx. 3 g), in the centre of the photo, partially buried in sediments at a field site in New Caledonia. (Photo: S. Purcell).*

A common problem with restocking of marine invertebrates is the naïve behaviour of cultured juveniles compared to wild ones, particularly regarding behaviour in seeking refuge (e.g. abalone, Schiel and Welden, 1987; queen conch, Stoner and Glazer, 1998). If the burrowing of sandfish during the day is a natural behaviour to avoid predation, as suggested by Mercier *et al*. (1999), the findings suggest a benefit in releasing juveniles from the hatchery earlier and at a smaller size to avoid the development of naïve behaviours from long-term culture. It may also be advantageous to employ methods for behavioural conditioning at the hatchery to encourage a higher frequency of burrowing of sea cucumber juveniles like sandfish, or cryptic behaviour in reef species.

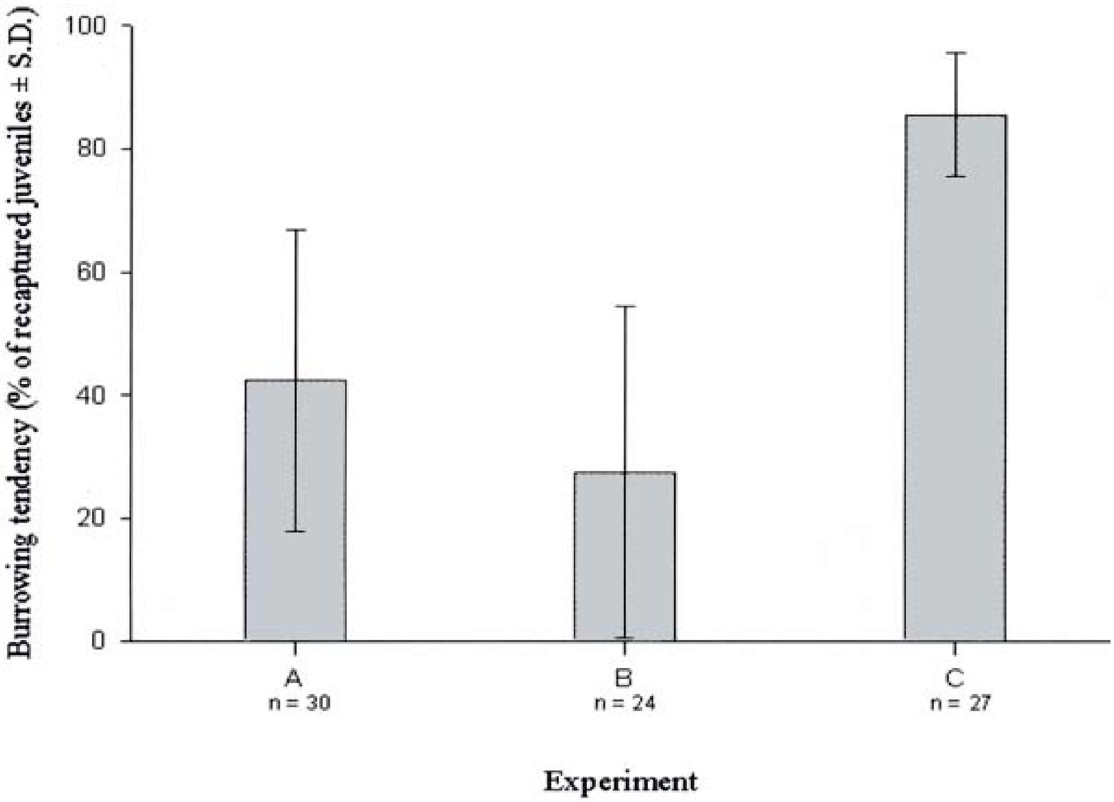

Figure 5. *Bar graph of mean percentage of hatchery-produced juveniles buried in sediments in the wild at the end of three release experiments in New Caledonia.* ***A****: experiment using 2-10 g juveniles, 8-10 d duration, 25 juveniles per enclosure, n = 30 enclosures.* ***B****: experiment using 2-10 g juveniles, 5 weeks duration, 25 juveniles per enclosure, n = 24 enclosures.* ***C****: experiment using 1-5 gjuveniles, 5-7 d, 25 juveniles per enclosure, n = 27 enclosures.*

Acclimation of juveniles

Acclimation of juveniles in the wild before releasing them to the risks of predation is a further approach that could increase survival rates for restocking programs. Enclosures or cages for temporarily holding juveniles could be placed at sites prior to release, acting as an intermediate 'half-way-home' to acclimate juveniles during the first days or weeks of release. Predation can be intense in the early stages of releasing sea cucumbers, particularly from fishes (Dance *et al.,* 2003). An experiment in New Caledonia indicated that survival of sandfish could be increased twofold in the first month of release by containing the juveniles in enclosures to exclude large predators (Purcell and Blockmans, unpubl. data).

Protecting juveniles until they recover from the stress of translocation, or until they reach a larger size, could increase the success of restocking (Dance *et al.,* 2003) and could overcome to some extent the need for pre-release behavioural conditioning. But containing juveniles for long periods of grow-out is impractical when considering high numbers, such as hundreds of thousands, as this would need large areas of enclosures.

Assessing restocking success

There is much recent interest worldwide in restocking of sea cucumbers in the wild using hatchery-produced juveniles (Battaglene and Bell, 1999). However, no study to date has evaluated this approach, by estimating the survival of juveniles released in the wild, to know if it is viable for rebuilding breeding populations.

Once release methods are developed, studies should be conducted to estimate the rates of post-release survival (Bell and Nash, 2004), at least over the short-term. In this regard, marking juveniles with a long-lasting tag would be an effective way to test the success of restocking. Tagging studies could permit an evaluation of temporal changes in mortality rates, sightability in surveys and movement of released animals, which would allow a forecasting of the likely survival and time frames for replenishment of breeding populations. Some visual and chemical tags have been successful for relatively short periods (Harriott, 1980; FAO, 1990), but are not applicable for marking juveniles that need to be identified 2-3 years later. Some genetic tagging methods are available (Shaklee and Bentzen, 1988), but these rely on selecting broodstock with rare alleles and it is unclear that this approach would not contaminate the genetic diversity of remnant stocks at release sites (even when supposedly selectively-neutral markers are used). The development of a simple chemical or visual tag for juveniles is needed, as noted by Hamel *et al.* (2001). Until tagging methods for juveniles are developed, such research is likely to utilise experimental designs involving multiple release sites and control sites without released animals (discussed below).

Two possible designs that could be used to assess the effects of restocking, for example two years after juveniles are released and expected to be at a mature size, are illustrated in Figure 6. In both cases, there is a residual population of the species of sea cucumber at the time of release, and the release sites and control sites are both protected from poaching or fishing. These two designs are explained in more detail below and anticipate some natural recovery of the populations at control sites, due to natural recruitment, but increased recovery at release sites due to the release of juveniles.

Figure 6A illustrates a BACI (Before-After-Control-Impact) design (after Green, 1979) in which surveys are conducted at a release site and a control site, both before and after the restocking effect (i.e. after 2 years). Due to variability among replicate surveys at the restocked site, the power of this design to detect a restocking effect is relatively weak and the estimate of population increase would have a large error.

In contrast, Figure 6B illustrates a simplified version of the "Beyond BACI" design (Underwood, 1991, 1992), in which multiple control sites are used and the surveys are conducted at multiple times before and after the restocking effect. The design is more powerful for detect restocking effects and should not necessarily be restricted to one restocked site. If abundances of sea cucumbers vary seasonally, for example due to migration or shifts in cryptic behaviour, then Non-Linear Mixed Effects models are more appropriate, and rely on repeated surveys through time.

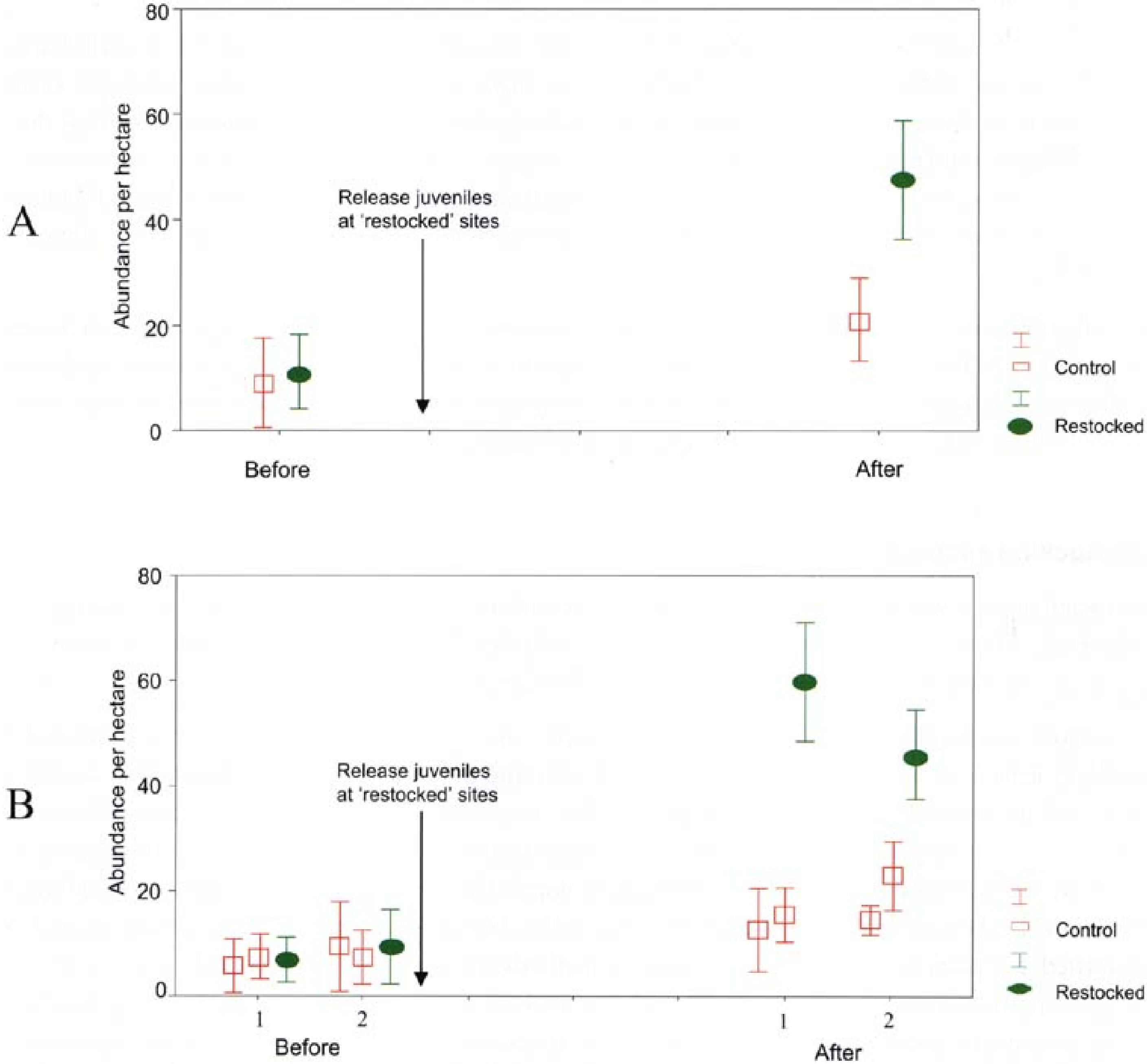

Figure 6. *Plots of mean abundance with error bars at sites in a hypothetical experiment to test restocking of sea cucumbers.* ***A*** *= BACI design;* ***B*** *= Beyond BACI design. The "After" surveys could be, for example, 2 years post-release when released animals would be mature.*

Further to experiments on release methods, cost-benefit analyses at a scale relevant for fishery replenishment will require larger experiments and an accurate evaluation of restocking effects beyond natural recruitment. It is therefore essential to define the explicit indicators of restocking success, in terms of contribution to fishery production, and how these indicators will be measured (Leber, 1999). In order to assess the potential of restocking, the time frames to detect effects should be long enough to allow the hatchery-produced juveniles to reach maturity and contribute reproductively to rebuilding stocks (Heppell and Crowder, 1998). It is likely to be safer to restock at multiple release sites because larval dispersal can be restricted (Uthicke and Purcell, in press) and therefore would result mainly in localised replenishment of neighbouring grounds, and because the survival of juveniles at some sites may be unexpectedly poor.

Conclusions

To enable the release of hatchery-produced juveniles to restore depleted stocks, management plans must protect the sea cucumbers from fishing. The surviving animals can then contribute to egg production and natural rebuilding of the stock on a broader scale. For restocking programs to be cost-effective, criteria for release will need to be determined well. Experiments in New Caledonia have confirmed that survival and growth can be significantly improved if we can understand how, where and when to release the juveniles. Tagging studies would be useful for understanding the post-release survival rates of juveniles overtime, but long-term tags for juvenile sea cucumbers need to be developed. Researchers should then take care in designing experiments to test large-scale restocking so that natural variability in time and space are accounted for, otherwise, these factors may well reduce the power of large-scale (and expensive) experiments to detect and estimate restocking effects.

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