
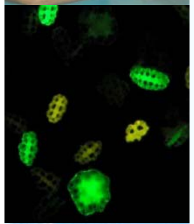


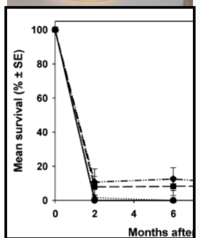

Principles and science of stocking sea cucumbers into the wild

Steven Purcell
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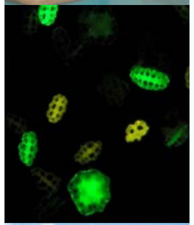
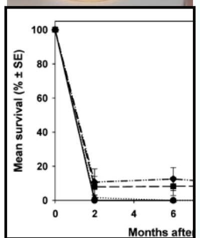
National Marine Science Centre

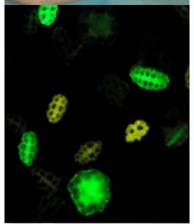
Southern Cross UNIVERSITY
A new way to think

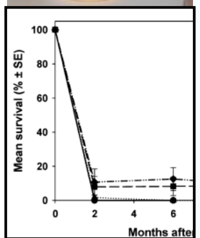




Presentation overview

- Invertebrate stocking: a new era
- Scales of release, different methods and timeframes
- Preserving the integrity of wild stocks
- The dangers of translocation
- Disease screening
- Chemical marking of cultured juveniles
- Use of sea pens
- Surveys to assess the success of sea ranching and restocking
- Likely economic returns ?
- Conclusions

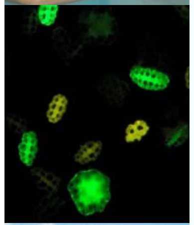



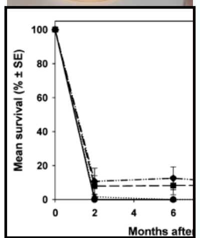
Stocking of invertebrates

- Scallops
- Abalone
- Queen Conch
- Shrimp
- Crayfish and Lobsters
- Sea Urchins
- Giant Clams
- Topshell (Trochus)
- Sea Cucumbers

Jumping in too early

Why did enhancement programs have such poor success?



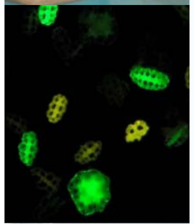


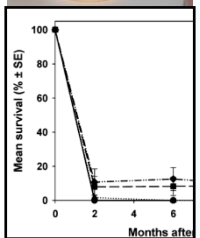
- Restocking and sea ranching are relatively new fields
- Enhancement activities were driven by the ability to produce juveniles

  But…

  1. Goals ?
  2. Cost $$$!!
  3. Predation?, habitat requirements?, post-release movement?
  4. Animals were rarely marked to prove the success of captive-release

➢ Poor success in many case studies gave a bad name to stock enhancement and restocking

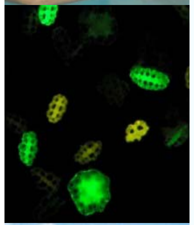



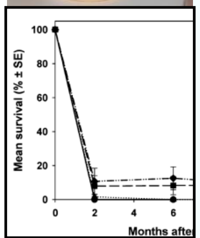



Marine stocking - a 'new era'

Bartley and Bell (2008) – *Rev. Fisheries Sci.*

- Challenges in this 'new era' of stocking include the need to:
  - critically assess costs and benefits,
  - conduct research to work out how to release animals so they survive well, and
  - use science to prove the cost-effectiveness of stocking

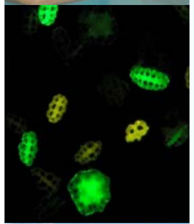


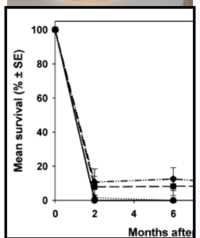


Sea cucumber management and the role of restocking and sea ranching

- Stock Enhancement
  - Increasing stocks and commercial harvests in an existing fishery
- Restocking
  - Restoring breeding populations in a depleted fishery
- Sea Ranching
  - Growing shellfish in an open area defined by a lease or through property rights
- Sea Farming
  - Growing marine organisms in a bounded space – e.g. a fenced off area or in a pond or embayment

Definitions: see Bell et al. (2005, Adv. Mar. Biol.) and Bell et al. (2008, Rev. Fish. Sci.)

Scales of release

Stock Enhancement

- Mass release at low density into broad areas

Restocking

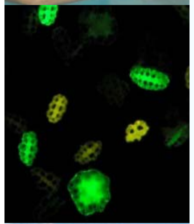
- Moderate-scale release at moderate to high density in relatively small areas

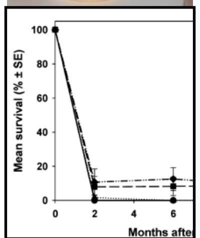
Sea Ranching

- Release at high density into restricted areas

Sea Farming

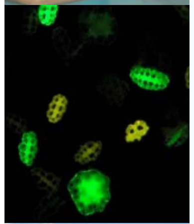
- Release at very high density

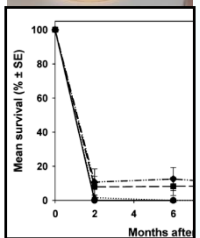




Restocking – 7 golden rules

1. It is a management tool - analogous to 'captive release' programs
2. Applicable when there are so few animals that you can't form effective effective breeding populations by aggregating remnant animals in the fishery
3. Survival will be poor unless the release methods are determined and used
4. The released animals must be protected as a nucleus breeding population
5. The protected area (sanctuary/reserve/NTZ) is big enough so that the majority of animals don't move out of it into grounds where they can be fished
6. At maturity, the animals are in high enough densities to find each other easily to allow successful natural breeding
7. The restocked breeding population is sited such that their offspring can settle in areas within the target fishery

Key steps of restocking

Correct the management system

Nominate existing reserves or create NTZs for restocking sites

Collect broodstock and breed them in a hatchery

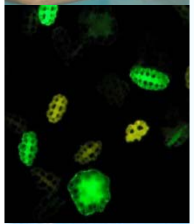
Larval supply from the restocked breeding population rejuvenates recruitment in neighbouring fishing grounds

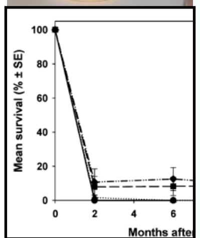
Release juveniles in ways to maximise survival to maturity

Protect the released animals in the sanctuary for the term of their lives

Breeding populations in local fishery are rebuilt

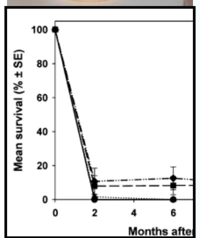
The offspring from restocked animals can be fished





Sea Ranching – 7 precepts

1. It is an extension of aquaculture - “put, grow, and take”
2. Beneficiaries have full property and access rights
3. Like restocking, the release methods are well founded to give good survival
4. Most animals need to remain inside leased/managed area
5. Harvest efficiency will be high – poaching is minimal and animals can be easily harvested
6. Animals are harvested at a size that maximises economic returns per released juvenile
7. Success = the money made from harvesting the animals far outweighs the cost of producing juveniles
   - ☝ Was more money earned from sea ranching than could have been gained from banking the money to produce/buy juveniles and earning the interest? or investing it in other aquaculture/fisheries initiatives?



Key steps of sea ranching

Gain access rights over an area of optimal habitat for releasing and growing the animals

→ Produce juveniles in a hatchery or buy them

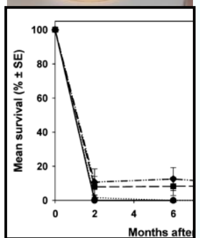
→ Release juveniles in ways to maximise survival to maturity

→ Protect the released animals in the area until they reach an optimum market size

→ Harvest all animals

Improved recruitment to neighbouring fishing grounds is a secondary effect

Preserving the integrity of wild stocks

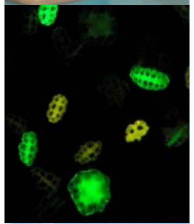


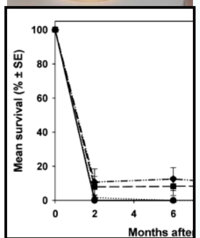
- Wild stocks will be needed in future:
  - Broodstock for producing juveniles in hatcheries
  - Rebuilding healthy wild populations resilient to disease, natural distasters, other natural and human-induced disturbances

- This relies on us to:
  - Ensure the **genetic diversity** and genetic structure of each population is not compromised
  - Ensure we don’t introduce, or increase the prevalence of, **disease** into wild populations

Potential problems from translocation

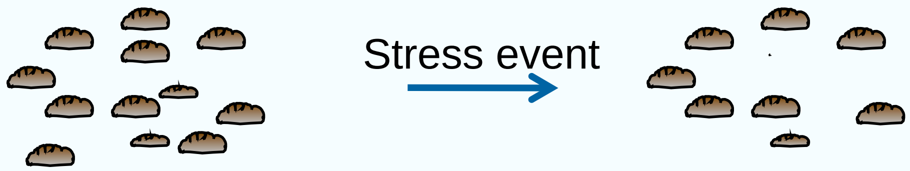
- Can reduce genetic diversity of wild stocks:
  - 'Introgression' - introduction of foreign alleles
    1. Interbreeding of introduced stock with wild stock
    2. Out competing native alleles
    - Introgression reduces the genetic identity of stocks and the unique (and potentially advantageous) genetic composition of native stocks

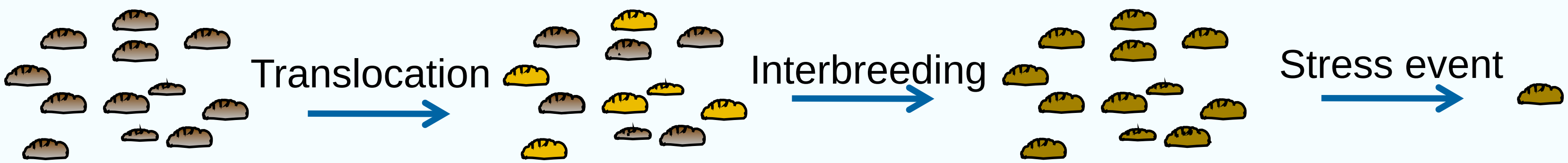




Potential problems from translocation

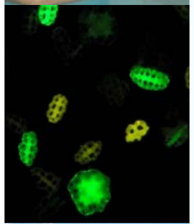
- ‘Outbreeding depression’ can occur from interbreeding of introduced stock with native stock
  - Disturbs adaptations native stock have to local environment

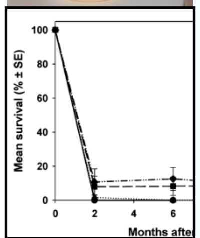




- Interbreeding of translocated stock have been shown to have long-lasting effects, that tend to be disadvantageous in the long term rather than beneficial

- Translocation problems are more of a risk with species that have limited larval dispersal – e.g. *Holothuria scabra*





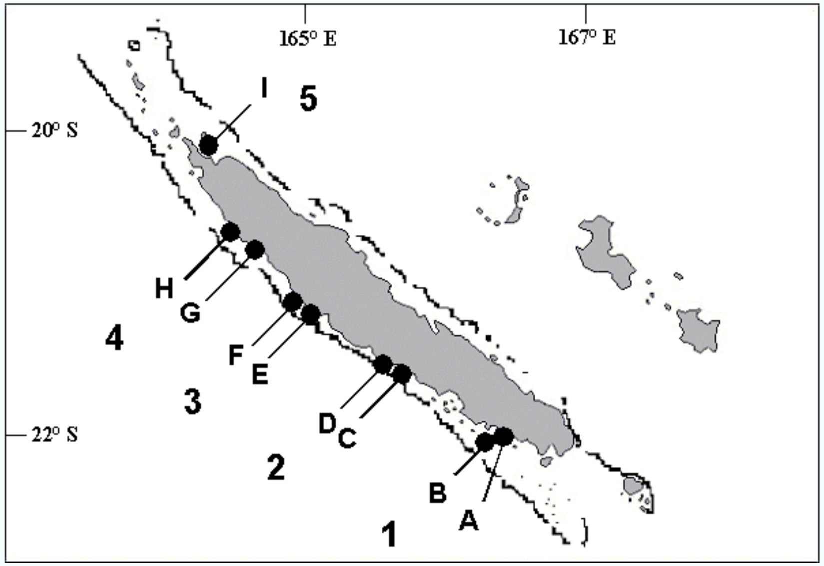
Limited gene flow and larval dispersal of sandfish

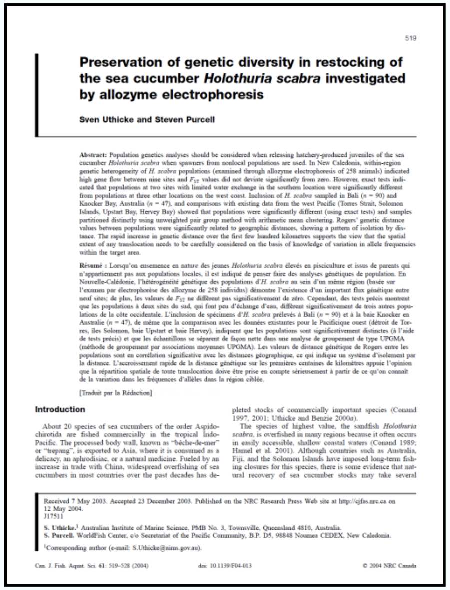
- On regional scale, genetic differences related to geographic distance
- Restricted gene flow in sandfish at scales <100 km

Lesson for restocking and sea ranching

- Juveniles should only be translocated near to the natal sites (where broodstock parents were collected)
- Juveniles cultured from broodstock in one country should NOT be shipped to another country

Uthicke and Purcell (2004) – *Can. J. Fish Aquatic Sci.*





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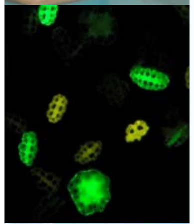
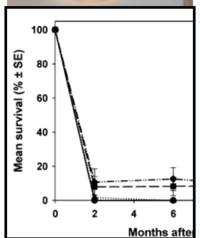
Preservation of genetic diversity in restocking of the sea cucumber *Holothuria scabra* investigated by allozyme electrophoresis

Sven Uthicke and Steven Purcell

Introduction

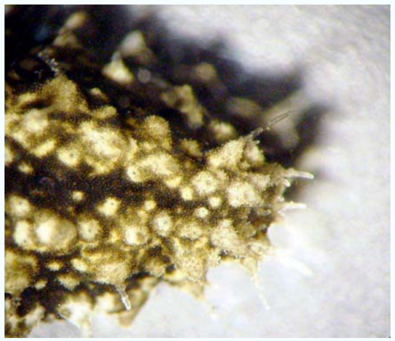



Minimising introduction of diseas

Perform a simple visual disease check in your own hatchery, using established protocols

Mouth

Anus



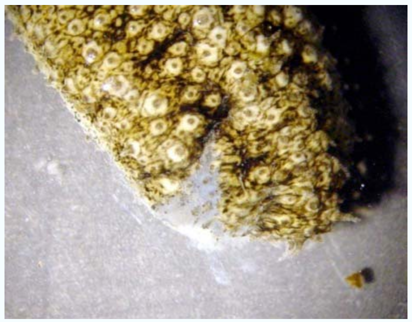
Lesions?

Discoloration?

External parasites?

White spots or fluffy patches?

Excessive mucus?



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An external check for disease and health of hatchery-produced sea cucumbers

Steven Purcell[1] and Igor Eeckhaut[2]

Abstract

Sea cucumber diseases that arise in the hatchery can cripple production and undermine restocking programmes. A rapid protocol for the external examination of juvenile sea cucumbers was developed in order to screen for disease and poor health. After selecting a random sample of juveniles from the entire group, each individual is examined for one minute under low-power microscopy. Six external criteria are checked, including abnormalities of the mouth, anus, papillae, body colour, and signs of excess mucus, unhealed lesions, parasites and macroassociates. If more than 5% of screened animals were "unhealthy" or more than 2% were "diseased", then the whole group from which the sample was derived should be considered unfit for grow-out or restocking. In such cases, the handling and environmental conditions in the hatchery should be improved and the entire group quarantined and treated. The protocol sets a standardised procedure for checking large numbers of juveniles for many infectious diseases, and is a starting point for further development of standardised protocols.

Introduction

Production of hatchery-reared sea cucumbers has gained global interest, and is underway in Australia, China, Ecuador, Kiribati, Madagascar, Malaysia, New Caledonia and Vietnam. Regardless of whether juveniles are to be used for restocking, stock enhancement or land-based grow-out, diseases can arise in the hatchery and cripple production. Identifying diseases in the hatchery is a precondition for their treatment and a prerequisite for releasing juveniles into the wild. Until recently, however, little was known about sea cucumber diseases.

Diseases can be biomolecular (e.g. hereditary), induced by pathogens, or arise from abiotic factors (Kinne 1980). The United Nations-FAO funded workshop in China (October 2003), "Advances in Sea Cucumber Aquaculture and Management", improved the collective knowledge of biotic diseases and parasites affecting sea cucumbers. Wang et al. (2004) reported that various pathogens, including bacteria, fungi and other parasites, could affect *Apostichopus japonicus* larvae and juveniles in culture conditions. Clinical signs of infections include lesions starting around the mouth or anus, whitish ulcerations on the skin or papillae, excessive mucus on the body, skin discolouration, and changes in behaviour and appearance (e.g. the infected animals can become thin, weak and sluggish). Eeckhaut et al. (2004) provided a list of bacteria, protozoans and metazoans (e.g. flatworms, gastropods and crustacean parasites) that can potentially cause disease in sea cucumbers. They also reported a contagious bacterial disease of *Holothuria scabra* that begins as a white lesion close to the anus, followed by a lesion that progresses quickly over the body. Microscopy and biomolecular techniques have proved instrumental in identifying the bacterial species associated with that disease, called "skin ulceration disease" (Becker et al. 2004). Symptoms of many of the diseases reported by Wang et al. (2004) and Eeckhaut et al. (2004) can be seen externally by low-power microscopy so it should be possible to screen juveniles for visible symptoms of diseases prior to transfer to grow-out ponds or into the wild.

Aside from true biotic diseases, some sea cucumber illnesses can be viral, chemical or from poor culture environment. For example, excess aeration or inappropriate temperatures cause illness and death in sea cucumber larvae (Hamel et al. 2001; Wang et al. 2004). In New Caledonia, copper wire placed in tanks was sufficient to kill juveniles within days (S. Purcell, unpubl. data). Illnesses may be subtle with no lesion or malformation. Instead, the animals become unhealthy and the symptoms could include sluggishness or change in body colour. Illness from chemical contamination could reduce feeding in juveniles and make them more prone to predation when they are released into the wild.

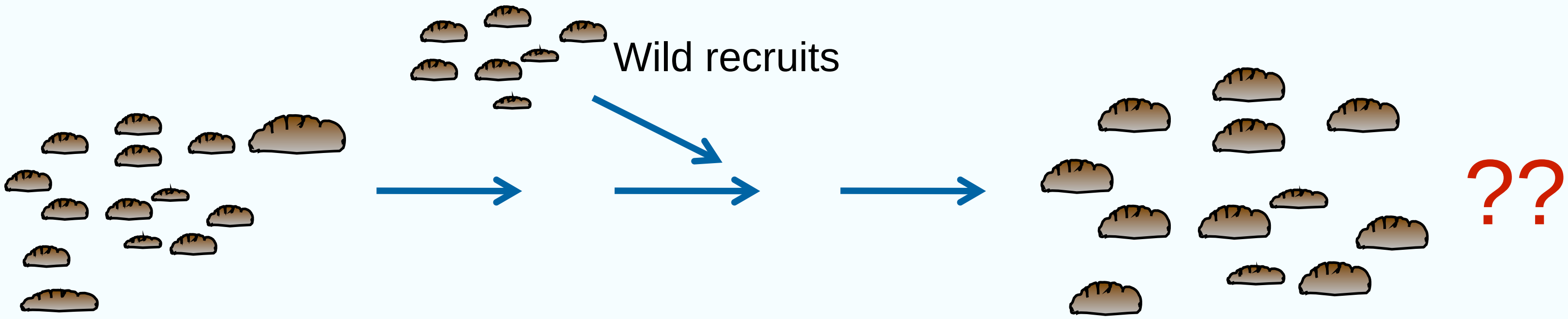
The impacts of releasing diseased or unhealthy juveniles into the wild can be indirect or direct. First, contagious diseases from hatchery-reared sea cucumbers can spread to native stocks, competitors or predators. For example, some bacterial strains can be infectious to other invertebrates

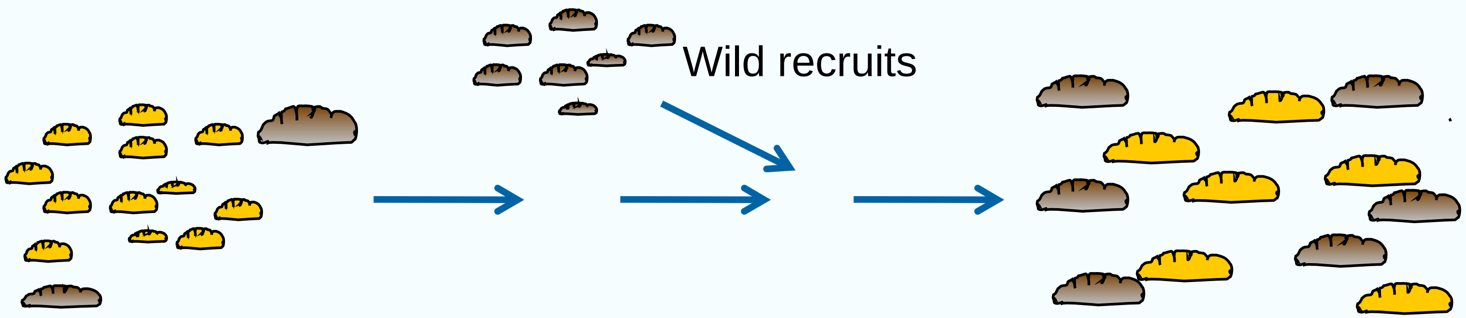
1. The WorldFish Center, c/o Secretariat of the Pacific Community, BP D5, 98848 Noumea Cedex, New Caledonia. Email: s.purcell@cgiar.org
2. Laboratoire de Biologie Marine, Université de Mons-Hainaut, 6 ave. du Champs de Mars, B-7000 Mons, Belgium

Why should we need to mark or tag cultured juveniles?

1. To be able to assess the survival rate of released juveniles ⇨⇨ cost effectiveness?
2. To show that the animals we later harvest came from the animals we released

   ➢ Provides proof of stock ownership





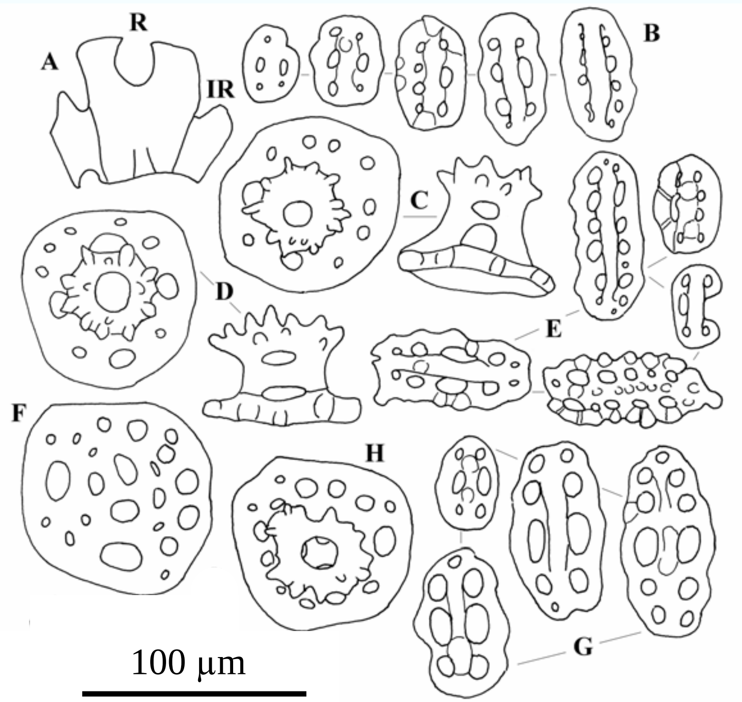
Chemical marking fluorescently colours sea cucumber spicules

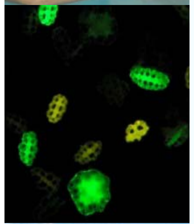
Spicules (‘ossicles’)

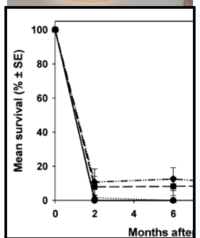
- In the outer body wall – mainly in the dermis
- Calcareous
- Very small (less than 1/10th of a mm!)
- Produced as the animal grows

Fluorochromes

- **Tetracycline (and oxytetracycline)**
- **Calcein**
- **Calcein blue**
- **Xylenol orange**



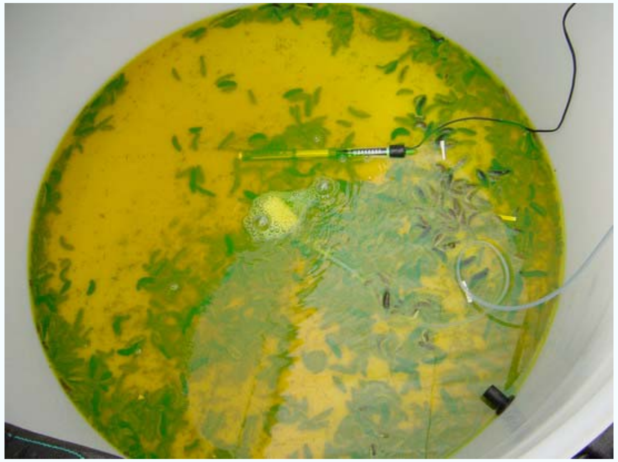



Chemical marking with flurochromes

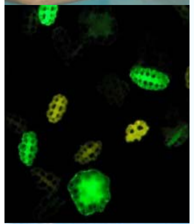
- The juveniles must be in a period of active growth before immersion marking

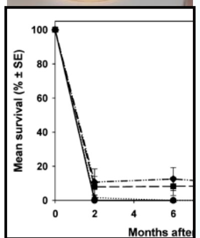
Keeping juveniles in a bare tank overnight

Fluorochrome stock solution added to tank with aerated seawater and heater



Tanks shaded and animals left in immersion solution for 24 h



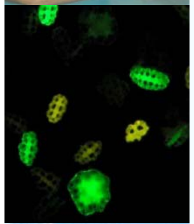


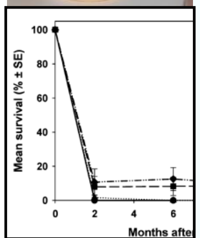
Marker verification - sampling

Taking a sample of outer body wall and bring back to the lab

Digest soft tissue in bleach to get cleaned spicules

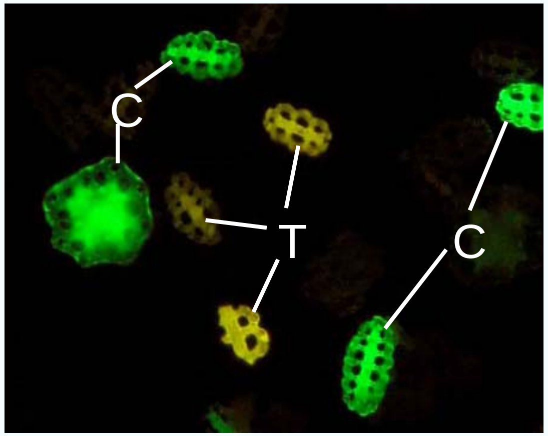
Check spicules to see if any are marked with a fluorochrome

Marked spicules under fluorescent microscope

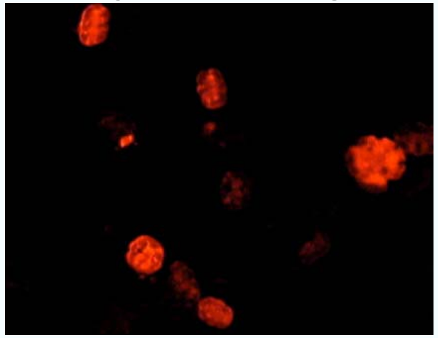
White light only

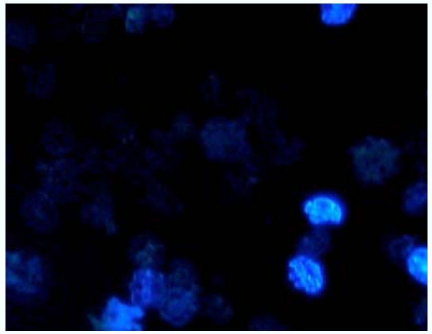


Spicules marked with tetracycline (T) and calcein (C) sequentially

UV light only

Xylenol orange

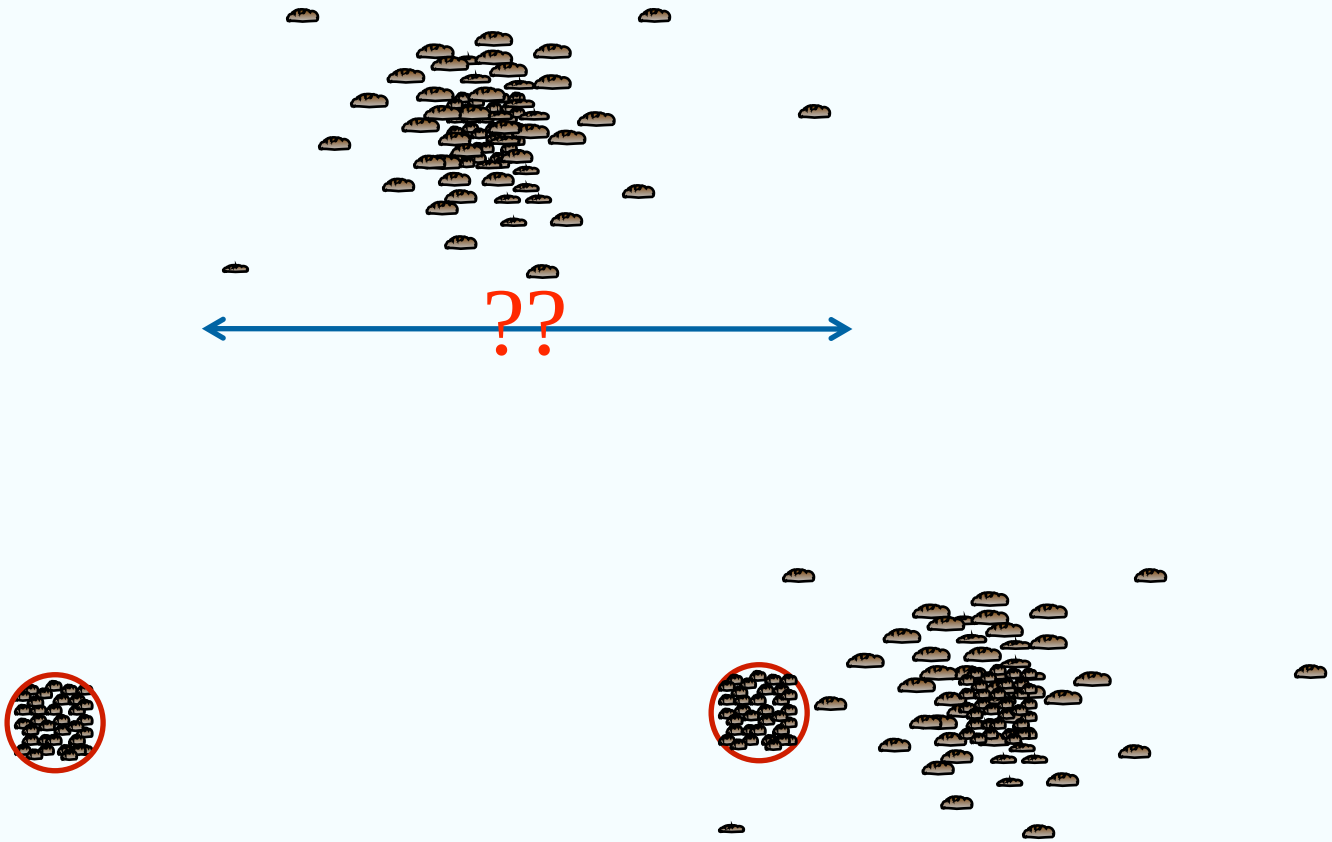


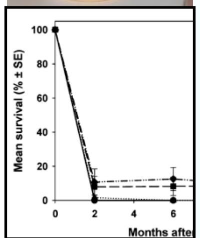


Calcein blue

Sea pens – an experimental tool

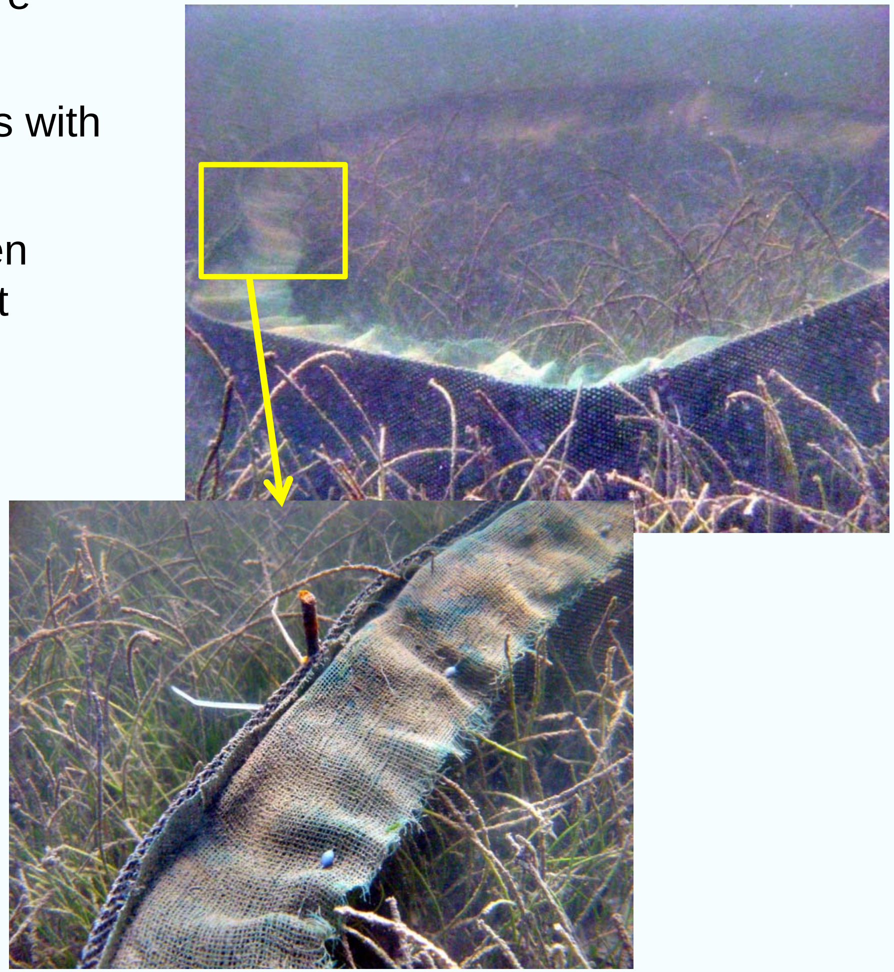
Gauging the post-release survival of juvenile sea cucumbers: A dilemma

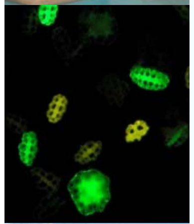


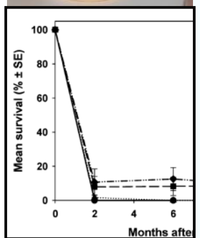


Small sea pens

- Small sea pens (1 m^2 to 10 m^2)are great for short-term work
- Chance of escapement increases with smaller pens
- A netting skirt at the top of the pen significantly reduces escapement

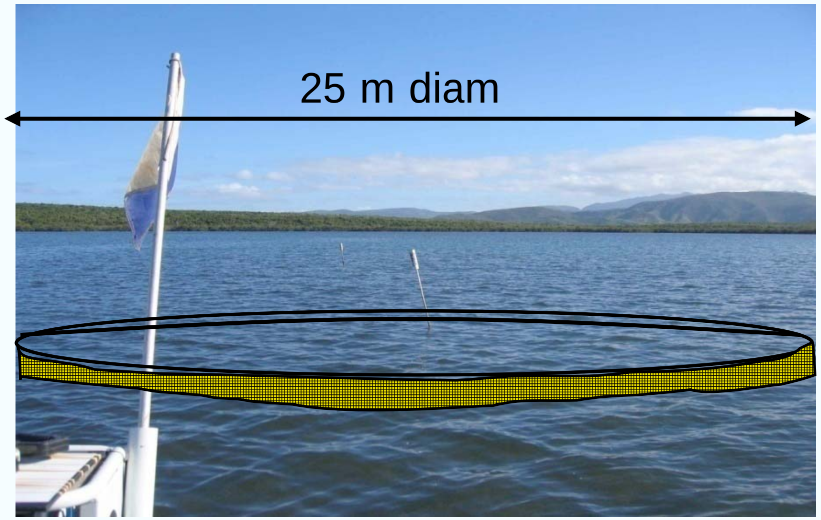


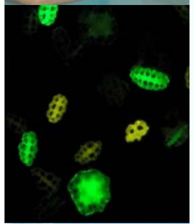


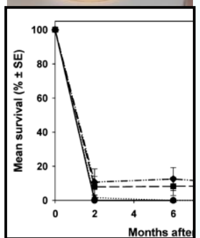


Large sea pens

- Pens need to be open to replicate the natural conditions – so that we can assume similar mortality in and out of the pens
- A strip of antifouling on the mesh should reduce escapement
- Big pens (100-1000 m^2) are harder to install
- 500 m^2 pen – 2 days to install 3 pens
- Pens are difficult to set up in firm sand – muddy sand is best for sandfish



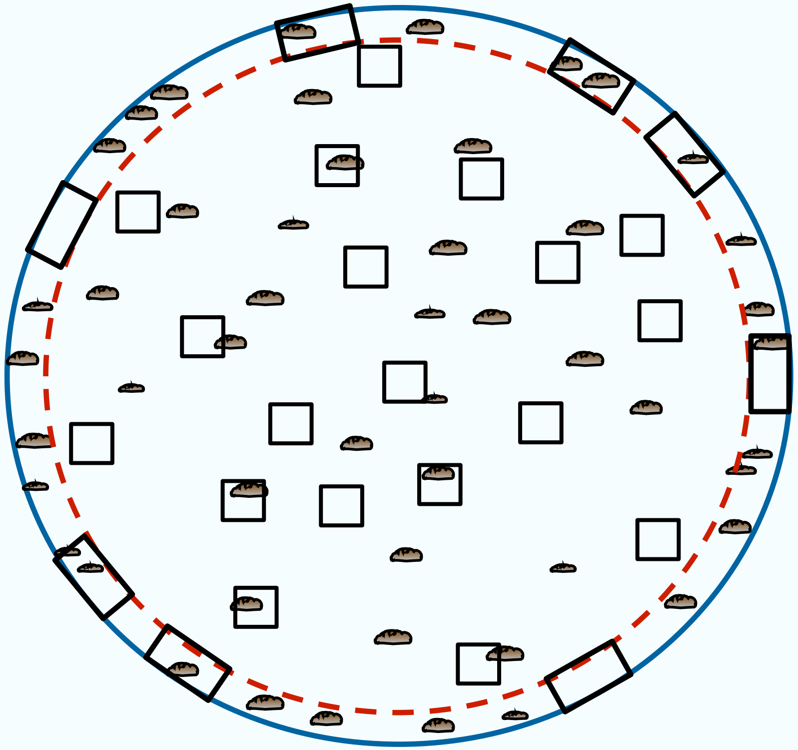



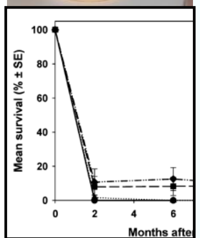



Field surveys to assess the success of stocking

QUADRAT SURVEYS IN SEA PENS

- Quadrats need to be set randomly – in pre-determined positions
- Need to assess border separately
  - This requires stratified sampling





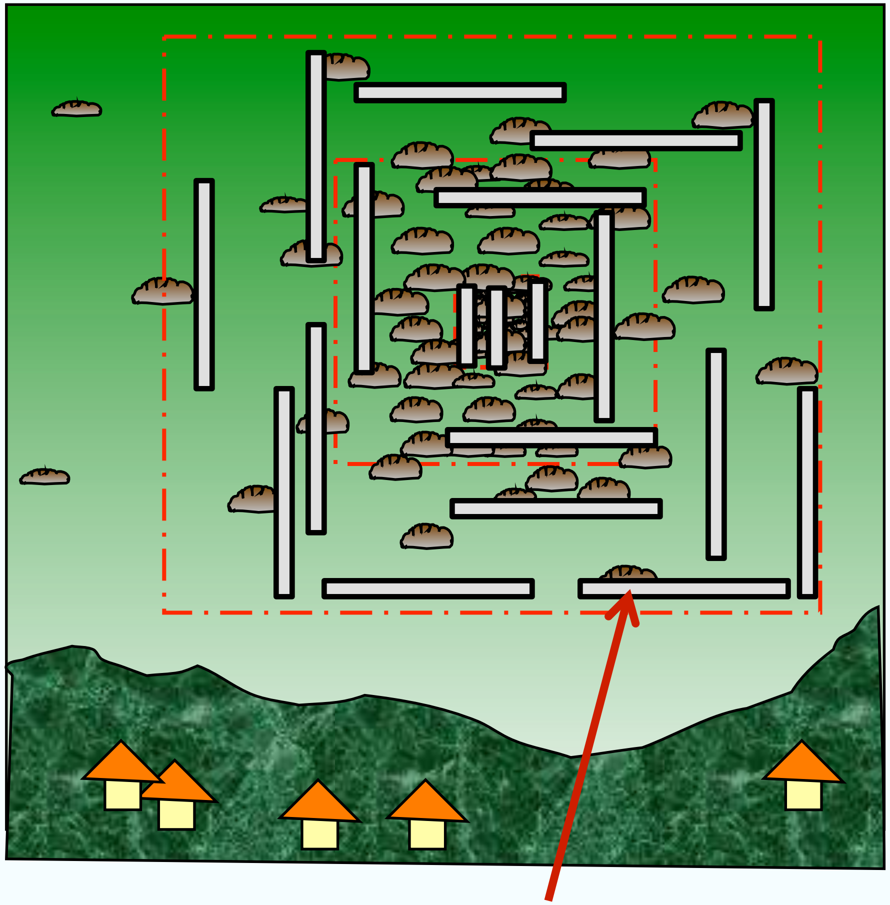
Assessing the success of sea ranching

- Release the marked juveniles into a core area in a natural habitat

- Allow 1 year for them to attain a size at which they can be seen in surveys

- Assess survival of larger sandfish by using transect surveys within dispersion zones and verifying the origin of the animals using analysis of their spicules

☞ Juveniles are expected to move further if the habitat is sub-optimal

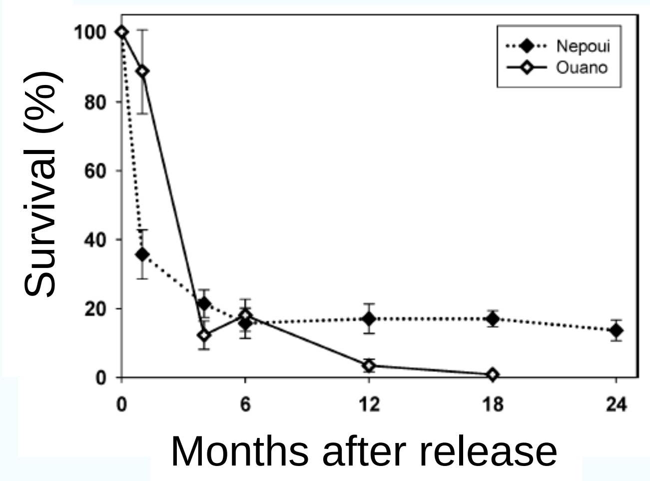


Random transects for visual surveys

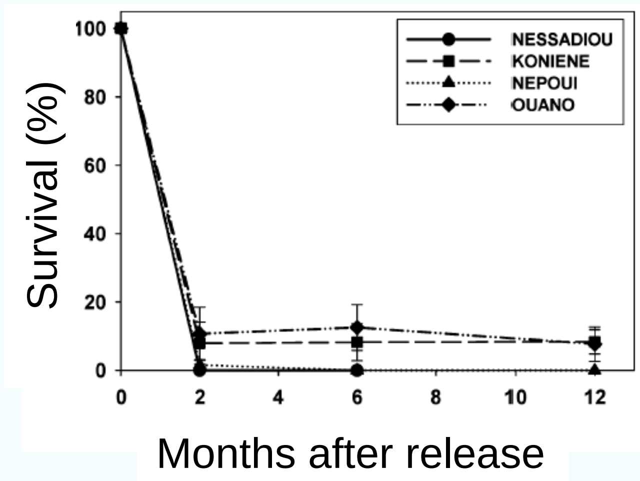
Survival of released juveniles?

- Most of the mortality occurred in the first couple months after release
- At some sites, none of the released animals survived to market size

Experiment 1

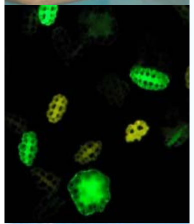


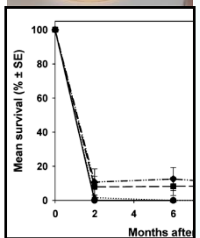
Experiment 2



Purcell & Simutoga (2008) – Rev. Fisheries Sci.

- If animals are released in the correct habitats, at initial sizes of >3 to 10 g, **survival to market size** (700 g) was predicted to be **7-20%**
- Survival may be higher at other localities where predators are removed or where the animals can grow to adulthood faster

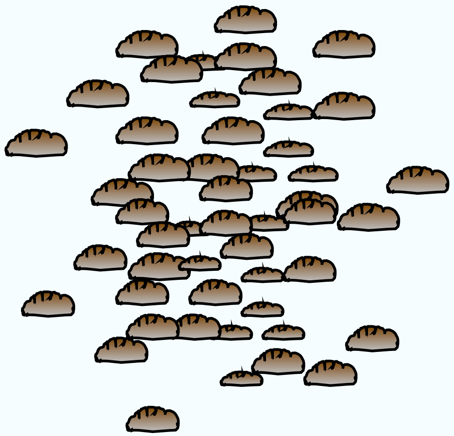


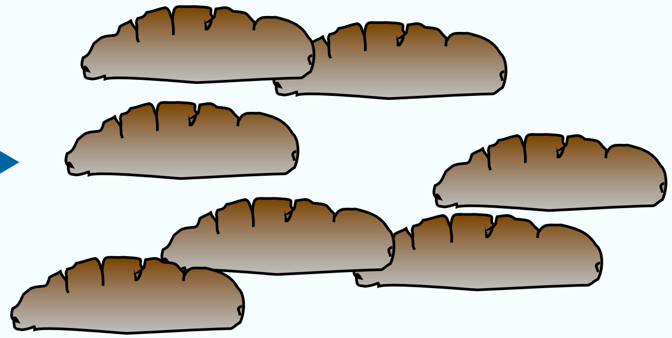


Likely economic returns from sea ranching?

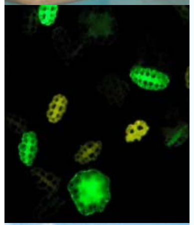
A quick calculation:

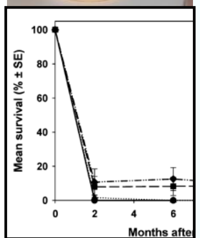
- Conservatively, one-in-ten animals will survive to market size





Total cost per juvenile (3-10 g) of production, release and harvest must be US 24 cents or cheaper, just to break even ← Maybe you only find 80% of those that survived ← 1 adult of 700 g = about US$3

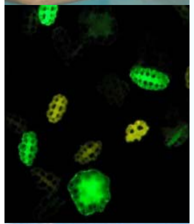



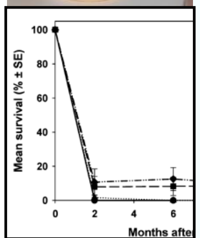
Lessons for sea ranching and restocking

Unpredictability

- Factors beyond those controllable by optimal release strategies can dictate success (e.g., cyclones, predator invasion, flooding)
- Success on <u>one occasion</u> doesn't guarantee success at that site on <u>another occasion</u>
- Success from <u>short-term</u> experiments is no guarantee for success of large-scale releases over <u>longer</u> time frames – some key causes of mortality occur <u>infrequently</u>

➢ <u>Plan</u> on some sites failing – use multiple release sites and multiple times

Lessons for sea ranching and restocking

1. Firstly determine the optimal habitats, sizes and densities to release juveniles..
2. Risks to wild populations (genetic, disease) are real, and should be minimised.
3. Chemical marking of juveniles is cheap and simple. Restocking and sea ranching programs should mark all cultured juveniles to prove cost-effectiveness.
4. A majority of the juveniles you release in the wild will die or be eaten by predators before they reach market size.
5. Sea pens can help assess survival, and visual surveys can be used once the animals are adults.
6. Juveniles need to be produced cheaply for cost effectiveness.
7. Weigh up the costs and expected benefits, and be aware of the timeframes.
8. Sociological issues and constraints will be just as important as biological ones!