A study of tagging methods for the sea cucumber *Cucumaria frondosa* in the waters off Maine

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The sea cucumber fishery in waters off Maine is developing and has recently experienced great increases in landings, corresponding to expanding export markets. Between 1994 and 1996, reported landings ranged from one to three million pounds (Fig. 1). In 1999, reported landings were over eight million pounds and rose to over nine million in 2000 (Feindel[1]). Like other developing fisheries, we have little information about the biology and ecology of the sea cucumber off Maine, limited data on the fishery, and little knowledge about the key life history processes that characterize its population dynamics. Therefore, we have a limited understanding of the current status of the resource and the impacts the fishery may have on the stock.

Most research done so far has been done on the red sea cucumber (*Parastichopus californicus*) in the northwestern United States and British Columbia (e.g., Bradbury et al., 1996; Zhou and Shirley, 1996; Phillips and Boutillier, 1998; Perry et al., 1999; Cripps and Campbell, 2000). Limited research has been done along the eastern coast of North America, although some studies have added to our knowledge of *Cucumaria frondosa*. A study of the ecology and behavior of *C. frondosa* was conducted in Maine by Jordan (1972). More recent studies have focused on fertilization success and feeding behavior as it relates to aquaculture (Hamel and Mercier, 1997; Medeiros-Bergen and Miles, 1997; Singh et al., 1998). These studies have improved our knowledge of *C. frondosa* but still do not provide enough information on growth, one of the life history processes most important for understanding the population dynamics of sea cucumbers in waters off Maine.

Because of the lack of hard tissue in sea cucumbers to lay down growth increments, tagging is the method undertaken to determine age. Tagging studies of sea cucumbers are difficult because external tags are frequently lost and internal tags can be shed through the body wall. Sea cucumbers have been tagged *in situ* with limited success (Shelley, 1981; Conand[2]) by using a small T-bar tag that is inserted through the body wall with a tagging gun (Harriott, 1980). Previous studies of manual tags in *P. californicus* in Washington indicated that the presence of these tags had no significant effect on the behavior of the sea cucumbers and did not affect their ability to react to shifts in the salinity of the experimental medium (Fankboner[3]). Typically, necrosis of the tissue surrounding the tag occurs and tags fall out within a few months (Morgan, 2000). Morgan suggested that this method may be adequate for tagging broodstock in captivity over short periods of time and stated that tag loss may be minimized by ensuring that the T-bar is pushed right through the dermis and attempting to make a puncture wound that is as small and clean as possible. Schroeter et al. (2001) used tags to conduct a growth study of *P. parvimensis* in California by following individuals through repeated surveys at two sites. Only 17.6 % of 1224 tagged animals were recaptured over one year. These studies indicate that manual tags may not be an effective means for studying growth because of the high frequency of shedding tags by sea cucumbers.

Previous researchers have also used fluorescent dyes to stain the calcareous plates surrounding the buccal cavity of sea cucumbers but with varied results. Successful staining has depended on the timing of the injection of the dye and the deposition of calcium for growth of the mouthparts (Conand[2]). Other work has suggested that fluorescent marking by dyeing the ossicle of the juveniles with tetracycline may be useful (Tanaka[4]). However, injec-

[1] Feindel, S. 2002. Status of the Maine sea cucumber *(Cucumaria frondosa)* fishery. Report to the standing legislative committee on marine resources, 35 p. Department Marine Resources, West Boothbay Harbor, ME 04575.

[2] Conand, C. 1989. Aspidochirote holothurians of New Caledonia lagoon: biology, ecology and exploitation. Studies and thesis, 393 p. ORSTOM, Paris.

[3] Fankboner, P. V. 2002. Seasonal visceral atrophy and response to salinity by *Parastichopus californicus* (Stimpson): osmoregulation? SPC Beche-de-mer Information Bulletin no. 17, 5 p. Université de La Réunion, Laboratoire de biologie marine, 97715 Saint-Denis Cedex, La Réunion, France.

[4] Tanaka, M. 2000. Diminution of sea cucumber *Stichopus japonicus* juveniles released on artificial reefs. Bulletin Ishikawa Prefecture Fish Research Centre 2:19–29.

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tions are time-intensive and marks often become difficult to discern after several months. Also, sea cucumbers could not be located soon after release in some follow-up investigations.

Yanagisawa (1995) successfully used a hot wire to brand the sea cucumber *Stichopus japonicus*. Although most of the marked juveniles could be distinguished three months later, it seemed to be difficult to discern their marks after four months.

The results of previous worldwide sea cucumber tagging studies vary in success by species, investigator, and location. It is also possible that the technique varied significantly for studies involving the same styles of tagging. Therefore, the conclusions of these previous tagging studies may not be applied to the sea cucumber population off Maine. Factors such as dermal thickness and seasonal fluctuations likely alter the capacity of the sea cumcumber to retain tags.

The objective of this study was to ascertain whether a reliable means of tagging the Maine sea cucumber could be identified. Tagged animals would provide information on growth and movement for later studies. This important biological information would be critical for sea cucumber stock assessment and management.

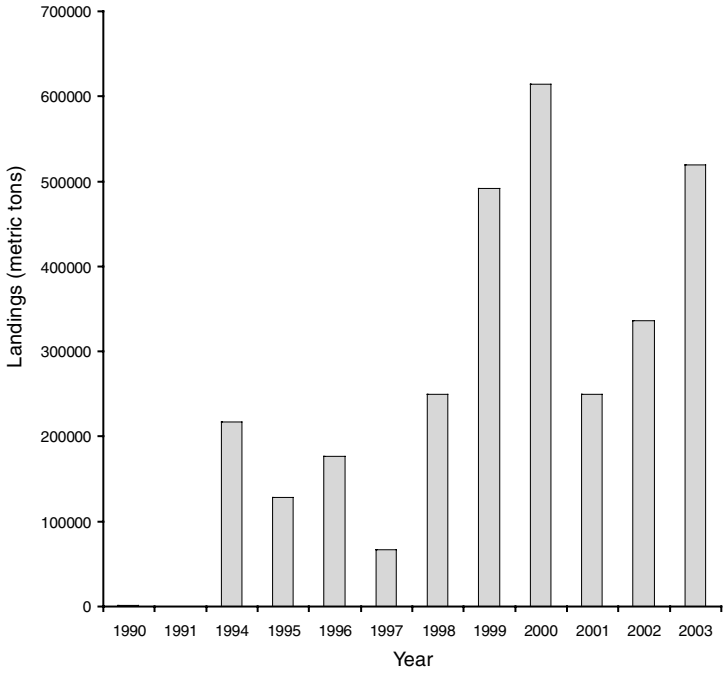

Figure 1

Historical landings (metric tons) for the sea cucumber *(Cucumaria frondosa)* fishery in Maine.

Materials and methods

The study was carried out between April and September 2003 to determine the effectiveness of various tags. Initially, animals were tested by using 1) long and short double T-bar tags inserted with a tagging gun; 2) dart tags; 3) streamer tags; and 4) cinch-up tags. A control group was also included in which animals were punctured with the tagging gun to observe any mortality from the piercing. It was evident in the first minute that dart tags, streamer tags, and cinch-up tags would not stay within the cucumber body wall and these methods were not considered further.

Next, 5) a rope gun was tested as a means of scarring (branding) animals. Rope guns are normally used to burn through rope by applying the hot tip to the surface of a rope. For tagging purposes, the rope gun tip was applied to the dermis of *C. frondosas* to produce a recognizable scar; the process is similar to that used in branding cattle. Although the rope gun created a noticeable mark, many animals already possessed significant scarring from being dragged during harvesting or from interactions with fishing gear and other marine organisms, and the new scar would not stand out to an observer. The branding method was therefore also discarded.

Finally, 6) liquid orange and yellow fluorescent dyes composed of visible implant elastomer (VIE) were injected into the animals. VIE is a silicone-based material that is mixed immediately before use and quickly becomes a pliable, biocompatible solid. VIE tags were injected internally beneath the epidermal tissue within tube feet, along the body wall, or near the oral cavity. The dye did not spread and all marks were small pin points visible only under ultraviolet light.

Short and long double T-bar tags and fluorescent dyes served as the remaining tagging methods studied during the course of the investigation. Seventy tagged sea cucumbers from the Gulf of Maine were observed over time (Table 1). Ten animals were injected with orange and yellow fluorescent dyes and 45 animals were punctured with long or short T-bar tags. Long tags had a distance of 45 mm between T-bar ends. Short tags had a distance of 19 mm between T-bar ends. Twenty-one tags punctured the body wall just under the muscle so that one end remained inside the animal (single-anchor tags). The remaining 24 passed completely through the body wall twice so that both ends of the T-bar tags were exposed (double-pass tags). Finally, 15 control animals were punctured with the tagging gun, 9 with double punctures and 6 with single punctures. Sea cucumbers were kept in tanks in the laboratories at the Maine Department of Marine Resources in West Boothbay Harbor, Maine. The presence and absence of tags was noted every day from April to September 2003 for each sample. Notation was made when tags were partially in place or no longer observable at all.

Results and discussion

Table 1

Tagging study design for the sea cucumber *Cucumaria frondosa* off Maine. Total numbers sampled for each method are indicated as total animals in the right column. Parentheses identify the number of animals sampled.

Tagging method					Total animals
Fluorescent dyes					10
Double T-bar tags					
Double-pass		Single-anchor		45	
Long tag	(9)	Long tag	(6)		
Short tag	(15)	Short tag	(15)		
Control					15
Tagging gun puncture		Double-puncture	(9)	Single-puncture (6)	
Total					70

The tagging study was successful in providing information on retention rates for the different tagging methods observed during the 140 days of the investigation (Fig. 2). Fluorescent dye injections and single-anchor long T-bar tags were the most successful means identified in tagging sea cucumber in the waters off Maine. Eighty percent with dye continued to fluoresce under the UV light and 65% of single-anchor long T-bar tags remained embedded in the sea cucumbers at the end of the investigation on day 140. The T-bar method may be the most practical for short-term research on this species considering the time and expense of fluorescent dye injection. Another advantage over injections is that the tagging gun technique is much more easily repeatable among subjects, whereas the site of injection varied among animals depending on the location of soft tissue.

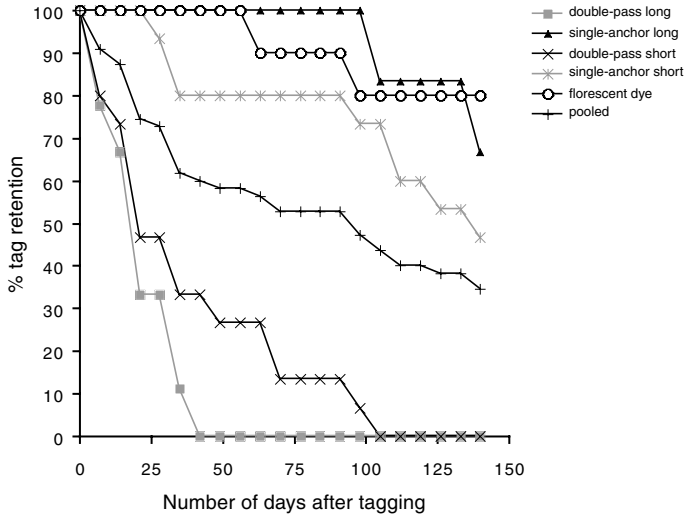

Figure 2

Tagging retention in the sea cucumber *Cucumaria frondosa* for individual-style tags over 140 days of observation in the waters off Maine. Note that florescent dye and single-anchor long T-bar tags were the most successful methods identified.

Double-pass T-bar tags were expelled faster than those in the single-anchor condition. With the double-pass tag, it appeared that the long portion of the tag under the animal's epidermis gradually moved upward until it was expelled outside of the skin without scarring the animal. The longer bars were pushed out the fastest and all were shed before day 50. Shorter T-bar tags took almost twice as long to reach 0% retention as the longer T-bars in the double-pass condition, perhaps because the shorter bars pinched the dermis tightly between tag bars, whereas animals with the longer bars were able to relax their entire body wall. After tags were shed in all T-bar conditions, it was not possible to tell which experimental condition each individual had experienced. No mortality was observed from the tags or from the control punctures.

Five T-bar tagged animals experienced a punctured respiratory tree caused by the tagging gun. The respiratory tree is an internal organ used for breathing by pumping seawater in and out. After the initial puncture, no trace of the injury could be detected and no differences in appearance or behavior were noted throughout the investigation. Although it was not possible to track which individuals had a partially punctured respiratory tree, injured individuals may have shed tags at a different rate than others in their cohorts. Internal injuries may also have affected the results of later studies of growth and movement with the use of manual tags. With the use of a largest possible sample size and careful placement of the tagging gun, this potential bias can be minimized. Additionally, it is important to consider that tag retention rate in the field may vary more than results observed in the laboratory.

We believe that the fluorescent dye injections and single-anchor long-T-bar tagging methods in our investigation will likely prove effective for a longer-term study with additional preliminary tagging analysis. However, a more effective way to study the growth of sea cucumbers may be the combination of caging and tagging, where tagged individuals of similar size are put in the same cage to monitor a change in size.

Future investigations will test different locations for, and methods of, injecting fluorescent dye and single-anchor long T-bar tags and will help to further identify the best tagging method. When the most successful method is determined, a long-term study will allow us to understand how individual animals grow, move, and change both seasonally and annually.

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