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Research article

Evaluating the effectiveness of SCUBA-based visual searches for an invasive tunicate, *Ciona intestinalis*, in a Prince Edward Island estuary

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Abstract

Visual searches are a common method of detecting invasive species in coastal waters, but the statistical properties of search methods have rarely been evaluated. Understanding the error rate (especially false negatives) and effective detection distance of searches can improve survey design, and quantify the uncertainty in risk assessments used to inform invasive species management efforts. An experiment using artificial tunicates ("decoys") was conducted in Hillsborough Bay, Prince Edward Island, to determine the effectiveness of SCUBA divers conducting underwater visual searches for the vase tunicate, *Ciona intestinalis* (Linnaeus, 1767). Single decoys and clusters of three decoys, constructed from water-filled, ivory-coloured balloons 5-6 cm in length, were placed at a blue mussel, *Mytilus edulis* (Linnaeus, 1758), aquaculture site on buoys, lines and mussel socks. The probability of detecting tunicate decoys on a mussel sock in the experiment is 89.8% (±SD 7.1), known in this paper as a true positive. The probability of not detecting tunicate decoys actually placed on a mussel sock in the experiment is 10.2% (±SD 7.1), known in this paper as a false negative. Divers detected 79.2% (±SD 7.1) of single decoys and 94.0% (±11.4) of clusters. Divers were able to detect single decoys from a measured horizontal distance of 2.7 m (±0.8), and clusters from 2.8 m (±0.9). The typical detection distance for real *C. intestinalis* estimated by divers was, on average, 2.1 m (range 1 - 3 m), and tunicates of lengths ≥ 2.9 cm (range 1-4 cm) could be detected.

Key words: aquatic invasive species, search theory, *Ciona intestinalis*, rapid response, early detection, Prince Edward Island

Introduction

Fouling of surfaces in the marine environment by non-indigenous tunicates (Chordata: Ascidiacea) has become a global problem in the past few decades (e.g., Lambert 2007; Locke and Carman 2009; Locke 2009). Severe fouling by tunicates on suspended blue mussel aquaculture equipment in Prince Edward Island (PEI), Canada, has increased production and processing costs due to competition between tunicates and mussels for food and space, the expense of tunicate control measures, and logistical and personnel requirements related to the increased weight and volume of equipment fouled by tunicates (Thompson and MacNair 2004; Locke et al. 2007). PEI aquaculture supplies 80% of the blue mussels in the North American market (DFO 2006). Mussel aquaculture directly and indirectly generated revenues of more than CAD $36 million in PEI, representing approximately 1% of the gross domestic product of the province and more than 622 person-years of employment in 2004 (DFO 2006).

Since its first reported occurrence in PEI in 2004, the vase tunicate, *Ciona intestinalis* (Linnaeus, 1767), has rapidly increased in abundance to become the nuisance species of greatest concern to bivalve aquaculture. This solitary tunicate, which reached an abundance of 5 individuals/cm^{-2} on settling plates in 2006, is considered a serious threat to the viability of the mussel aquaculture industry in PEI (Ramsay et al. 2008). A quarantine approach was implemented in PEI, prohibiting (1) transfers of mussels from tunicate-infested to uninfested estuaries, and (2) processing of harvested mussels from infested estuaries in fish plants adjacent to uninfested estuaries. This approach successfully contained the occurrence of *C. intestinalis* to three estuaries adjacent to its original point of introduction for three years (Locke et al. 2009).

In 2007, however, *C. intestinalis* was detected for the first time in Hillsborough Bay, an important mussel spat collection area which provides most of the "seed" material for mussel aquaculture throughout PEI. Mussel seed distribution from this estuary was permitted in only the few infested estuaries in 2007 and 2008, which caused shortages of mussel seed in many non-infested estuaries which had formerly been supplied from Hillsborough Bay.

The discovery of *C. intestinalis* in the Hillsborough Bay system led to questions about the effectiveness of search techniques currently used to support tunicate management in PEI. Typically, searches have been conducted by SCUBA divers, often only one pair of divers searching a portion of a large water body over the course of one or two dives. In the event that non-indigenous tunicates are detected, management policy in PEI considers the estuary to be infested until two years pass without further detection of that tunicate (Locke and Hanson 2009). During that time, containment (quarantine) or other management measures may be required to suppress the dispersal of the tunicate to non-infested water bodies through aquaculture transfers or harvests. If tunicates are not detected by a search, the probability that the search could have failed to detect a presumably small population of tunicates in the water body becomes important. Specifically, knowledge of the rate of false negative error results, i.e., the non-detection of a tunicate that was present in the water body, associated with a given search effort, is required in order to allow for well-informed management actions.

Here, we report the results of an experiment to determine the detection rate of SCUBA divers searching for artificial *C. intestinalis* on an aquaculture site in Hillsborough Bay, PEI. The experiment was conducted to estimate detection distances and false negative error rates, which will be used as inputs to a search theory model that is currently under development. This model will inform future searches for invasive tunicates in estuaries in Atlantic Canada by determining the probability of false negative results associated with a given search effort, and conversely the effort required to attain an "acceptable" probability of false negatives as inputs to risk assessments of management options. The acceptable level of uncertainty in the results is a judgement that would have to be made by aquaculture managers and the industry.

Methods

To determine the detection distance and error rates of diver surveys, an experiment was designed using a known number of artificial *Ciona intestinalis* on aquaculture structures in Hillsborough Bay, PEI (49°09.173' N, 63°10.455' W). The experiment would be conducted while divers were conducting a previously scheduled large-scale tunicate survey of Hillsborough Bay. Using real *C. intestinalis* for the experiment in 2008 would have posed an unacceptable risk because it was possible that *C. intestinalis* had not persisted in Hillsborough Bay following the detection of a small number of individuals in 2007.

C. intestinalis is a solitary tunicate, which attaches to hard substrate in subtidal waters, and grows to a maximum length of approximately 10 cm. The appearance of the tunicate varies from almost transparent to a pale orange colour (Figure 1A). Artificial tunicate "decoys" were constructed from ivory-coloured latex balloons filled with water, approximately 5 - 6 cm in length (Figure 1B). The decoys were positioned as single balloons or as clusters of three balloons, fastened to aquaculture structures with plastic ties. These were intended to simulate early stages of establishment, when tunicates would occur as isolated individuals or small clusters. In PEI, all mussels are grown off-bottom, in mesh sleeves or "socks" suspended from an anchored longline system which is kept at or near the surface by styrofoam or pressurized plastic buoys (DFO 2006). Tunicates typically attach to any of these structures; therefore, the decoys were distributed on socks, ropes and buoys. The socks were ~2.5 - 3 m in length with the tops ~1 m below the surface. The divers who placed the decoys were experienced tunicate searchers and positioned the decoys in locations where *C. intestinalis* would naturally occur, such as crevices among the mussels, and on the underside of near-surface structures.

Four decoy sequences were established, each of which included 11 socks and their intervening rope and buoy. Six single decoy tunicates and six cluster tunicates were distributed among the eleven mussel socks. The decoys were deployed on August 19, 2008.

The experiment was conducted August 26, 2008 between 09:00h and 12:00h using nine SCUBA divers who were not involved in the initial placement of the decoys. Before commencing the search, the horizontal detection distance was determined for each diver using single and clustered decoys placed at depths of 0, 1 and 2 m on a sock. Each diver estimated their distance from the sock using a calibrated rope attached to the sock next to each of the decoys (one estimate per diver per depth for each decoy type), and recorded the distance on an underwater tablet. After the dive, all participants completed a questionnaire, specifically regarding their level of experience searching for tunicates, estimates of detection distance and minimum detectable size for real *C. intestinalis* (in the case of experienced tunicate searchers) and a list of factors that they considered to affect detectability. Detection distances of real and decoy tunicates were compared using paired t-tests in order to validate the use of the decoys. Environmental conditions (Secchi depth using a black and white limnological Secchi disk 10 cm in diameter, near-surface (<0.5 m) temperature using a thermometer, and near-surface salinity using a temperature-compensated refractometer) were measured at the start and end of the experiment.

Each of the nine divers searched along the line of 11 mussel socks in each sequence for sets of decoy tunicates randomly hidden in the sequence. Each diver was given an underwater tablet to record how many tunicates they viewed in each sequence. Each diver recorded two numbers for each sequence (a count for single tunicates and a count for clusters of tunicates), totaling 72 reports.

Statistical analysis of the count data involved the determination of Type I and II error rates. In this case, the Type I error is caused by mistakenly identifying a specimen as a tunicate or counting a single tunicate more than once (false positive). In a real search for an invasive species, however, this is not an issue because field misidentification of collected specimens should be correctable following further examination of specimens at the surface or laboratory, and double-counting a real tunicate when surveying an area for presence/absence management purposes is much less important. We note here that it is not our intention to trivialize the difficulty of correct identifications: incorrectly identifying a species may have important consequences such as the inappropriate destruction of native species. We do, however, suggest that the error rates of identification in the laboratory versus those in the field ought to be quantified separately, and our experiment relates only to field determinations. Type II error is the probability of not detecting a tunicate that is indeed present (false negative). This is a much more serious error in the context of tunicate management as it could lead to the incorrect conclusion that a species is absent from an area, particularly when the number of individuals in the area is small or a relatively small proportion of the total area is surveyed (Taylor 1997).

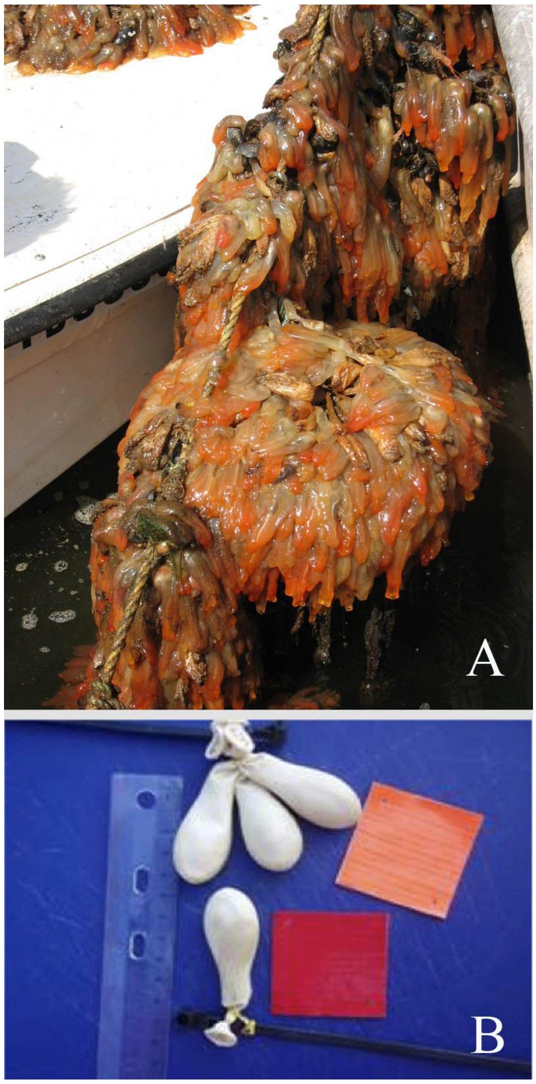

Figure 1. A) *Ciona intestinalis* showing the range of colours present in PEI populations (photo credit: J.L. Martin); B) Decoys of *C. intestinalis* used in the experiment.

Both false and true decoy tunicate sightings were considered to determine the appropriate distribution for the model because there were at least seven reports of values exceeding the number of decoys actually hidden. A combination of the binomial and Poisson distributions are used for the model. The binomial distribution, $P_b(i)$, is used to model the number of correctly reported tunicates. The binomial formula (Finkelstein and Levin 2001) is as follows:

$$P_b(i) = \frac{n!\,p^i(1-p)^{(n-i)}}{i!(n-i)!} \qquad \text{(Equation 1)}$$

where n is the number of decoy tunicates hidden in a sequence (in this case n = 6), p is the probability of correctly reporting a decoy tunicate, and i is the number of positive detections reported ($0 \le i \le n$ and i is an integer). A Poisson distribution, with rate λ (Finkelstein and Levin 2001), is used to model the number of falsely reported tunicates. $Pp(j)$ is the probability of diver falsely detecting j ($0 \le j$ and j is an integer) tunicates while searching through one experiment sequence. The formula for this probability is

$$P_p(j) = \frac{e^{-\lambda}\lambda^j}{j!} \qquad \text{(Equation 2)}$$

where λ is the average rate of finding false positives. The binomial and Poisson distributions were combined to determine a probability distribution for the reported number of real and false tunicates. The probability of reporting k tunicates is the sum over the combinations of real and false positives giving a reported total of k:

$$P(k) = \sum_{i+j=k} P_b(i)P_p(j) \qquad \text{(Equation 3)}$$

Each of the nine divers reported four values (one value for each sequence searched), $k_1,\ldots,k_4$. The probability of a diver reporting a given set of four values is modeled as the product:

$$L = P(k_1)P(k_2)P(k_3)P(k_4) \qquad \text{(Equation 4)}$$

This is what we refer to as the likelihood function, a function of the parameters p and λ. An estimate for p and λ that best fit the sequence $k_1,\ldots,k_4$ is found by maximizing L. This is commonly referred to as a maximum likelihood estimate (Devore 2000). Thus, two sets of estimates (one for single decoy and one for cluster decoy) of p and λ are determined for each of the 9 divers. Expected values calculated from this model were compared to the observed counts, using a chi-squared goodness of fit analysis in Maple (version 11.01, Maplesoft, Waterloo Maple Inc).

Results

Environmental conditions during the experiment were an overcast sky with 50% cloud cover, Secchi depth of 2.75 m, water temperature of 19°C, salinity of 28 PSU, and a weakly ebbing tide approaching slack tide at the end of the experiment.

Overall, divers detected 79.2% (±7.0) of single decoys and 94.0% (± 11.4) of clusters. Divers were able to detect single decoy *Ciona intestinalis* from a measured horizontal distance of 2.7 m (± 0.8), and decoy clusters of *C. intestinalis* from 2.8 m (± 0.9). There was no effect of depth. For real *C. intestinalis*, the typical detection distance (not specified as horizontal or vertical distance) estimated by divers was, on average, 2.1 m (range 1 - 3 m), and tunicates of lengths above 2.9 cm (range 1 - 4 cm) could be detected. A two-sample paired t-test verifies there was no difference in detectability of single versus cluster decoy *C. intestinalis* (degrees of freedom = 8 and a statistical p-value = 0.31).

The mean maximum likelihood estimate of p, the probability of a true positive result, is 89.7% (±12.9) and the estimate of λ, the rate of reporting a false positive, is 0.159 (±0.261). The standard deviation of p and λ reflects the effects of diver experience on detection rates as well as an element of chance (e.g., events such as a small deviation in the depth at which the diver swims, or the occurrence of a distraction causing the diver to look away from the substrate being searched, could cause the diver to miss detecting a tunicate). Figure 2 shows the expected values calculated from the model for the estimates of λ and p along with the observed values. A contingency table analysis (chi-squared goodness of fit test) indicated that the model was a good fit to the observed data (χ^2 = 6.96, statistical p-value = 0.64).

The true positive detection probability (p) is the probability of finding a tunicate given that it is present; therefore, considering p = 89.7% (±12.9), the probability of a false negative error (1-p) is 10.2% (±12.9).

Discussion

Visual surveys are included in several widely implemented invasive species monitoring protocols (Campbell et al. 2007), and can be an important tool to inform invasive species management, but quantifying uncertainty in the survey data is essential to risk analysis (Vose 2000). In most invasive species surveys, records of presence are statistically robust, but records of absence may have limited value depending on the survey design (Campbell et al. 2007).

While few invasive species surveys have explicitly determined the statistical properties of the data obtained by their protocols (except see Hewitt and Martin 1996, 2001), at least one study has examined the detection probability of visual surveys for tunicates at the bay-wide level (McFadden et al. 2007). McFadden et al. (2007) conducted experiments to develop estimates of detection probability for *Styela clava* Herdman, 1881 at different levels of water clarity, for different infestation scenarios and for two methodologies (underwater SCUBA searches and searches from the hulls of moored boats). There was a low probability of detecting small numbers of *S. clava* at most of the 24 locations studied. In a scenario with one *S. clava* present in a water body, the detection probability of out-of-water searchers was 2 - 54%; in another scenario where 200 m of the water body's perimeter was affected, the detection probability by divers was 8 - 72% (McFadden et al. 2007). These high levels of variability suggest a large element of chance in the detection of invasive species during early stages of an incursion with low population densities, regardless of visual search method.

In our Hillsborough Bay experimental setup, the mean probability of correctly detecting a *Ciona intestinalis* decoy that is present in the area surveyed is 89.7% (±12.9) but this is not a bay-wide estimate. Typically, only a portion of each bay would be surveyed for tunicates in PEI. Surveys have focused on aquaculture sites, navigational buoys and docks because these were the only habitats where *C. intestinalis* had been found in PEI during the first four years following its initial detection (Locke et al. 2007). Observations of *C. intestinalis* attached to eelgrass in two other PEI estuaries in the summer of 2008 (Locke, pers. obs.) caused us to expand our search to eelgrass habitats in Hillsborough Bay in 2008 and 2009. In Hillsborough Bay,

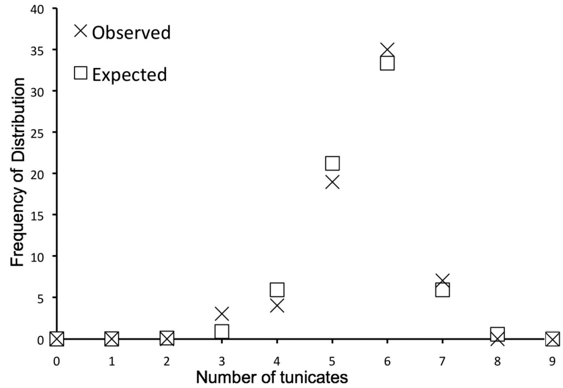

Figure 2. Comparison of expected counts of diver counts of *Ciona intestinalis* decoys, estimated from a combined Poisson-binomial distribution mathematical model, to the observed counts in the experiment. The graph represents a visual display of the goodness of fit of observed *C. intestinalis* count data and model data calculated using λ = 0.159 and p = 0.897 for the Poisson distribution and binomial distribution, respectively.

these searches did not detect any *C. intestinalis*. Determination of detection probability of *C. intestinalis* taking into account the proportion of the bay surveyed, or of the proportion of appropriate habitat surveyed, will follow in a later paper. Relatively few invasive species surveys have had the resources to examine 100% of the potential habitat in a water body. Exceptions include two post-eradication survey protocols: (1) *Caulerpa taxifolia* (Vahl) C. Ag. in two California lagoons, where more than 100 ha of benthic habitat was completely covered by divers swimming 1 m apart (Mooney et al. 2005), and (2) *Mytilopsis sallei* (Recluz, 1849) in Darwin Harbour, with follow-up surveys that have continued for more than a decade (Willan et al. 2000).

False negative error is the most important error, in terms of making effective management decisions, that could result from a tunicate survey because it may result in a failure to undertake necessary management action. False negative errors can be minimized through the use of an appropriate statistical model in survey design and the determination of adequate sample sizes and distribution of sampling effort (Campbell et al. 2007). The probability that a visual survey will detect the presence of a target species depends on the number of individuals present, the distribution of those individuals, the area or volume of the water body, the detection distance, the surveyed area or volume, and the survey design (Hayes et al. 2005).

According to the divers, conditions for a tunicate survey were good during our experiment. Several environmental factors that they claimed could reduce their ability to detect tunicates included water temperature (with detection rate declining as divers become either too cold or too warm), poor lighting conditions, and high sediment load in the water column. Divers also stated their detection abilities deteriorated as they became tired. The experiment was conducted during the first 30 minutes the divers were in the water, so the error rate might have been higher if they had been tested later in the day. We assume that level of experience would also affect the results, although we were unable to test this experimentally as virtually our entire team of divers was experienced in tunicate searches.

While most of the surveys for tunicates in PEI have been conducted using divers, it may be worthwhile to investigate other visual methods such as the use of underwater cameras. Grey (2009), for example, found similar patterns of presence-absence of tunicates were obtained by an observer in the water investigating the undersides of floating docks or by a camera-based survey of the sides of the same docks. While a camera-based method may also be affected by the fatigue or comfort level of the operator, the results may be less dependent on these factors than diver surveys. Disadvantages of camera methods include the challenges of navigating either a remotely operated or tethered camera among the complex physical structures of mussel aquaculture sites, and the need to collect specimens (Locke, pers. obs.). Obtaining the actual specimen(s) for confirmation of identification and vouchering is essential, especially in the case of an initial infestation of an area, or for easily misidentified species, and can be quite difficult in the absence of a dive team (Locke, pers. obs).

Visibility was moderate (Secchi depth of 2.75 m) during our experiment, and probably determined the maximum detection distance of 2.7 - 2.8 m for the decoys. The estimated maximum detection distance of ~2.1 m (range 1 - 4 m) for real *C. intestinalis* was recalled by divers from previous experiences under a range of lighting conditions. Divers normally would not conduct a tunicate survey under conditions when visibility was much less than 2 m. The divers' regular routine of swimming within arms length of the socks is closer than the detection distance that was measured for the experiment. The protocol followed by divers searching for tunicates on mussel sites in PEI typically involves divers swimming at a horizontal distance of ~0.7 to 1 m from the mussel socks. A pair of divers travel along each line of socks parallel to and on either side of the longline. One diver swims near the top of the sock (usually ~ 0.5 - 1.5 m in depth), surveying the underside of ropes and buoys as well as the upper portion of the socks, while the second diver swims at approximately mid-depth of the socks (~ 1.5 - 2 m).

Survey efficacy as tested with synthetic *Caulerpa taxifolia* also highlighted the importance of visibility to diver-based searches (Mooney et al. 2005). Overall, a greater proportion (72% vs. 58%) of the *C. taxifolia* was detected in a lagoon with water clarity > 1 m than in one where only specimens located directly within line of sight could be seen. The presence of eelgrass reduced detectability relative to unvegetated habitat, which is consistent with visual interference by eelgrass. Size mattered; only 42-55% of single-frond *C. taxifolia* patches were detected, but the detection rate of 1 metre patches approached 100%, i.e., double that of single fronds (Mooney et al. 2005). Our results comparing single versus clusters of three *C. intestinalis* decoys indicated that mean detection rate increased only 19% for a three-fold increase in size. In our experiment, however, a two-sample paired t-test verifies there was no difference in detectability of single versus cluster decoy *C. intestinalis* (degrees of freedom = 8 and a statistical p-value = 0.31).

We have assumed that false positives, i.e. when a species is mistakenly identified as non-indigenous, have little relevance in understanding the statistical properties of visual searches as taxonomic errors should be corrected in the laboratory. However, as Campbell et al. (2007) correctly point out, sightings with no specimen collection, or lack of taxonomic expertise, may result in such errors. For example, McFadden et al. (2007) found that 56% of the sightings of *Styela clava* reported to them by the public (including aquaculture farmers and processors, boating associations and marinas, and dive clubs) were false positives, based on specimens they requested for 75 reports. However, errors of identification are by no means confined to the public. McFadden et al. (2007) also received two reports of historical infestations from researchers who had collected but misidentified *Styela clava* in two New

Zealand harbours in 2002, three years before its presence was correctly reported. We are similarly aware of an instance in Atlantic Canada where a non-indigenous amphipod was misidentified as a native species during several years of study by a university researcher, and several instances when native species were misidentified as European green crab, *Carcinus maenas* (Linnaeus, 1758) by biology professionals.

In our experiment, we have started to obtain the necessary field data to develop a survey design that could be used either to minimize false negative error associated with diver-based tunicate surveys in PEI, or at least to quantify the error rate associated with different levels of effort. Further research is underway to develop a model of tunicate population growth and distribution that can be used in conjunction with the field data to develop geospatial aspects of the survey design; as well as an analysis to compare the efficacy and cost-effectiveness of diver-based versus other survey methods.

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