**Nested frequency analysis as an alternative processing method for settlement plates**

**Brett R. Howard1\*, Katie P. Gale2, Calley L. Wasser1, Fiona F. Francis3, Thomas W. Therriault1**

**1 Fisheries & Oceans Canada, Pacific Biological Station, Nanaimo, BC V9T 6N7, Canada**

**2 Fisheries & Oceans Canada,**

**3 Fisheries & Oceans Canada, IOS**

\*Author for correspondence: [brett.howard@dfo-mpo.gc.ca](mailto:brett.howard@dfo-mpo.gc.ca)

(ORCID 0000-0003-4103-1324)

**Abstract**

**Introduction**

The value of long term ecological data from field surveys is well-understood in the practice of ecology. The absence of ‘baseline’ historical data as a significant challenge for conservation and resource management. Selecting a method for ecological field surveys is a complex problem involving trade-offs between accuracy, efficiency, and the purpose of the survey. However, there is an additional component of time – that is the time since the initiation of the study or survey. The availability of assets at the beginning of a project will change over time. This may mean changing funding, changes in staff size or competency, or technological and scientific advancements. However, there is a trend in long-term data collection in ecology to resist change. Thus, although the context of a long-term study or survey change, the method in which the survey is conducted will remain fixed over time, often to the detriment of the data set and its ability to be analyzed or mined for valuable insight.

A well-known example of this is the failure of NOAA to change their reef survey method from X to Y in the WHEN. NOAA’s reef survey began in the WHEN and the method at the time was novel…. Because?? However, by XXXX, the proliferation of NGOs working in the area of coral reef restoration and conservation meant that new and improved survey methods were abundant, and by XXXX most workers in this field had adopted REEF as a standard survey method. NOAA however did not change to this method until XXX. As such there is a X year-long period in which the data collected by NOAA, possibly the largest agency collecting this sort of data, could not be easily compared to other data sources.

The resistance to change in survey methodology is understandable. First, there is a fear that a substantial change in survey method will invalidate past data collection or restrict the ability to merge datasets across organizations. At best, changing survey methods means that any analysis of longterm data will require a caveat about how the methods changed over time. Second, new methods are rightfully subject to scrutiny as they may not be any better at collecting the same information, and in fact could be worse. Third, there is the consideration of barriers to access, as a new method may require access to expensive technology or expertise. For example, the cost of DNA-based monitoring methods or remotely operated technology (e.g., ROVs, drones, etc.) is a significant barrier to transitioning to these new methods for underfunded research programs. A study by so and so showed that the use of ROVs for fisheries management by governments is XX times greater in North American and European countries. (also cite Favaro here?)

Long-term surveys, by accident or design, are often especially valuable for detecting and tracking rare species in an environment, as the rarer the species the more search effort required. In the field of invasion ecology, the difference between detecting a non-native species when it is still rare, through intensive and regular surveys, and detecting a non-native species by accident once it becomes abundant enough to be captured in random or one-time surveys can be the difference between a low-cost, manageable problem and a very expensive, potentially intractable one. Surveys for non-native species have been responsible for heading off many potentially disastrous invasions. For example…. ?

Since 2007 (?) the Department of Fisheries and Oceans Canada has maintained an annual survey of biofouling communities in marinas, docks, and wharves. The program began as part of a short-term study, but interest in biofouling and non-native fouling tunicates (e.g. *Botrylloides violaceus*, *Botryllus schlosseri*) from aquaculture operators and port authorities was sufficient to justify a long-term monitoring project. The methods used were based off of the Smithsonian and aligned with similar ‘settlement plate’ surveys conducted by the DFO on the Atlantic Coast and WHERE ELSE. By 2014 the basic method had become largely standardized. However, changes in staffing, funding, and priorities have gradually caused a misalignment between the needs of the survey and the sampling design being used. In recognizing this, we decided to consider re-designing the survey using a sampling method more common in rangeland ecology – nested frequency analysis. This method gained some attraction in the XXXXs, but it was typically too labour intensive to use for long-term applications and never achieved widespread popularity, despite several studies identifying it as the most consistently accurate method for rangeland community surveys (cite). Fortunately, NFA has been revitalized with the development of SampleFreq, a free photo analysis program designed to perform nested frequency analysis in fast, repeatable way. In this paper we compare several methods of surveying biofouling communities, with a focus on understanding the spread and abundance of non-native species, using several common methods applied in this field, and the novel application of NFA using SampleFreq.

**Methods & Analysis**

Since 2014, the standard method used by the Pacific Region of DFO to survey biofouling communities has been to deploy ~14 cm2 PVC plates, roughened on one side and weighted with a small brick, 1 m into the water column by hanging them off of floating structures (i.e. docks). The roughened side, facing downwards, becomes a settlement surface for fouling species, which are allowed to grow naturally from mid-June to late September. Every year, ten settlement plates are deployed in this manner, at upwards of 40 sites (e.g., marinas, docks, wharves, etc.) per year. Variation in the deployment methodology occurred, usually due to delays in getting the plates in the water and the spacing of plates at a site. Upon retrieval, plates were analysed in two ways: rapid visual scans for non-native species of concern (presence/absence) and a detailed survey of both native and non-native taxa under a compound microscope using a point-intercept method (abundance). A stringed grid would be laid over the plate and all organisms identified to the lowest possible taxonomic level at each point. Due to the labour-intensive nature of this method, five of the ten plates were randomly chosen for this process. Additionally, photographs were always taken of the plates, but these were not analysed and were only intended as a historical record if needed.

In 2021, plates were deployed using the standard method outlined above at XX sites. However, an additional nine sites were deployed specifically for this methods comparison study. Three sites were deployed as normal, with only ten plates. Another three sites had 20 plates each, and finally three sites were deployed with 30 plates. The number of plates was an important consideration for two reasons. First, there is some doubt as to how well ten plates captures the variation at a site, and how this scales with the size of the site. Second, the nature of any frequency analysis method, where the abundance of a species is represented as a proportion, means that the precision of the measurement is uniformly tied to the number of samples taken per site. As such by deploying only 10 plates, it is only possible to track changes in abundance ±10%, whereas setting 20 plates allows for ±5% precision, and so on. One of the goals of this study was to determine the best number of plates to deploy to capture the most variation with a reasonable amount of precision.

TABLE OF METHODS – PROS / CONS

Current method:

* Plate photo
  + Meant to be done in the field, on **ALL** plates
  + Visual record to go back to
  + Often not great (lighting issues, canopy species hide everything anyways, not truly nadir)
* Simple visual scan for well-known AIS
  + Meant to be done in the field on **ALL** plates
  + Non-destructive
  + Some of the species on the scan sheet make no sense (not actually species, and can’t be ID’d in the field anyways)
  + Presence data
* Point-intercept count
  + 49 gridded points (+1 random), everything at point identified including cover/canopy and order recorded (primary, secondary, etc).
  + Done on random 50% of plates
  + Will detect AIS not observed on scan
  + “abundance” data
  + Destructive

Proposed method:

* Plate photo
  + Done in the lab with proper lighting, true nadir
  + Canopy removed (usually hydroids), noted on species ‘list’ below
  + **ALL** plates
* Nested frequency analysis of photo (using SampleFreq)
  + Prior to destruction of plate when possible
  + Requires a predetermined species list (up to 60 sp./taxa)
  + “abundance” data
  + **ALL** plates
* Species list (maybe?)
  + Pick apart the plate and note all species/taxa seen and VOUCHER anything tricky
  + Presence data
  + **ALL** plates???

DON’T need to demonstrate the validity of Nested Frequency Analysis in general, just its applicability to plates (the point-intercept method is widely used).

**Table 1: pluses and minuses of all methods**

2021 season we need to do a bit of both the current and proposed methods so we can compare:

* Data/accuracy gained
* Data/accuracy lost
* Effort (more or less) NEED AN EFFICIENT WAY TO LOG TIME TAKEN PER TASK

**Question 1: Variation**

One big problem is that, to be really useful, NFA has a recommended minimum of 20 plates, per site, to capture variation in frequencies. But we only put out 10 usually (sometimes fewer on a small dock).

*Frequency precision = 100%/n, and thus the method requires large sample sizes, on the order of 20–50 plots, to achieve useful detection precision. For example, 20 plots yields a detection precision of 100%/ 20 = 5%, but changes in frequency < 5% will be undetectable. For ± 1% precision, 100 plots are required.*

* + Currently 4 sites with 20 plates; change two or all of those to 30?
  + Really can’t do much to test effect of plate # over time this year, can only assess usefulness for within-year questions
  + Makes sense if research question is about differences across ‘regions’ (e.g. HG vs Salish Sea, or PRPA vs Port of Van.)
  + Can you pool across sites? By treating sites (e.g. marinas) as replicates within ‘region’ are we violating important assumptions of a nested design

*Thus, within the constraints of a balanced, nested design, there are 3 design features that can be adjusted when collecting frequency data: the number of randomly located sites (n), the number of frequency plots per site (m), and the size of each plot (as measured by the mean number of plants per plot, d).*

m = # of sites (e.g. marinas per ‘region’) – not actually random?

n = # of plates

d = quadrat size (always optimized using nesting)

Compare variability of point counts to variability of NFA (over time? Between sites? Relative to variability of native species?)

**If you’re putting 30 plates in how much do we standardize inter-plate distance (some docks smaller than others). Or is that part of the question?**

**Question 2: Diversity**

Do you lose significant amount of information on biodiversity (NFA vs Point Count)?

**Question 3: Abundance**

Do point counts and frequencies agree on most abundant species?

**Question 3: AIS Detection/Rarity**

In theory,NFA is better for detecting rare species, which is esp. important with AIS (compare all four methods)

**Application 1: Within-year spatial comparison of frequency AND percent cover (compare) of AIS between PRPA and Port of Vancouver**

Do actual analysis

**Application 2: Spatial and temporal trends over multiple seasons**

Use existing past photos (although not great) at one or two sites to make some figures of freq. over time

* Use some sort of bootstrapping to increase old sample sizes?
* Would come with a lot of caveats

Ideas:

* Depth of plate (help determine if difference between what’s caught on camera vs missed species) or scrape and weigh (may not work with preserved plates)
* Take off all the mussels!!
* Take our usual shitty photos this year too to see how important the fancy set up matters