A critical review of the statistical analysis in Becker, B H, Press, D T & Allen, S G 2011 Evidence for long-term spatial displacement of breeding and pupping harbour seals by shellfish aquaculture over three decades. *Aquatic Conservation: Marine and Freshwater Ecosystems*

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Overview

The analyses reported in Becker et al (2011) provide convincing evidence that the proportion of harbour seals and pups counted at the three haul-out sites closest to oyster cultivation in Drakes Estero was lower in years of high oyster production.

There is some evidence that the proportion of the Point Reyes harbour seal population using Drakes Estero was also lower in years when oyster production was high. However, I think the effect of natural disturbance caused by the presence of an aggressive elephant seal at a neighbouring colony (Double Point) on this proportion may have been under-estimated. The result may also be sensitive to the exclusion of two data points from the early 1980s that are separated from the rest of the time series by an interval of 13 years. The robustness of the results to these data points and to the way in which natural disturbance at Double Point is modelled should be investigated. In addition, I do not think it is appropriate to consider the proportion of pups counted in Drakes Estero, and the proportion of all seals as separate time series. This is because pups and their mothers (which almost always haul out together at the time of year when counts were conducted) generally make up more than half the total counts. The proportion of all other seals (immature animals, adult males, non-breeding adult females) counted in Drakes Estero does not appear to be related to the level of the oyster harvest.

1. Introduction

This paper uses a discontinuous time series (there are no counts between 1984 and 1996) of harbour seal counts from all colonies in the Point Reyes area, which covers almost three decades, to investigate the effects of mariculture, in this case oyster cultivation, and short-term human disturbance on one colony (Drakes Estero) within that area. Within this time series, the population experienced a number of perturbations that may have affected the number of seals hauled out at the time of the surveys. These included low levels of oyster harvest between 1999 and 2005, three ENSO events, and the presence of an aggressive elephant seal at one colony in 2003. With the exception of two of the ENSO events, it is possible to compare counts before, during and after each perturbation.

The basic statistical analysis relies on linear mixed-effects models, Generalised Linear Mixed Models (GLMMs) and Generalised Estimating Equations (GEEs) using the proportion of seals (either pups or all seals) hauled out in Drakes Estero as the response variable and a range of explanatory variables, including the annual oyster harvest. A subset of the analysis is duplicated using a Bayesian approach.

I think this is an appropriate statistical approach, and I have not attempted to reproduce the R and WinBUGS code used to perform this analysis or to rerun the code that the authors have supplied. I understand that other, better qualified, reviewers have done this. Instead, I focus here on the statistical and biological assumptions that underpin the analysis. In particular, I consider the choice of response variable (and the authors' interpretation of its statistical properties) and the surrogates used for the explanatory variables. I also describe an alternative form of analysis that would take account of the underlying dynamics of the Point Reyes harbour seal population, rather than just considering how the members of the population distribute themselves among the different colonies each year.

The authors examined the response of seals "to mariculture ... at three spatial scales": sub-site, intra-colony, and regional. I now consider their analysis at each of these spatial scales.

2. Sub-site spatial scale

In this analysis the authors investigate the relationship between the maximum number of seals, or the maximum number of seal pups, counted each year between 1997 and 2009 at each of the eight seal haul-out sites within Drakes Estero and the observed daily levels of human disturbance at those haul-out sites. They also use the ratio of pups to adults as an alternative response variable. They conclude that there is no detectable effect of disturbance on any of the response variables, and I see no reason to question this conclusion.

3. Intra-colony spatial scale

The authors analyse the potential effects of the level of oyster harvest level (high or low), human disturbance rate, years since an ENSO event, and the maximum number of seals counted outside Drakes Estero on the proportion of all seals or pups counted in Drakes Estero seen on the three haul-out sites that are closest to mariculture operations. They use a combination of GLMMs (with year as a random effect) and GEEs for this analysis with a binomial link. This seems entirely appropriate - on the day of a count, each seal that hauls out in Drakes Estero undertakes a binomial experiment, because there are only two possible outcomes: it can haul out in the upper estero or it can haul out in the lower estero. The results of this analysis show clear evidence for an effect of oyster harvest and years since an ENSO event on the proportion of all seals and pups hauling out in the upper estero.

The authors calculate an "effect size" by multiplying the change in the proportion of seals or pups using the upper estero in years of high oyster harvest by the mean count in Drakes Estero over the time series. I am not convinced of the validity of this calculation, because the total number of seals counted in Drakes Estero in the years of low oyster harvest is higher than in years of high harvest (mean adults = 919 vs 666, mean pups = 405 vs 250 for 2000-2004 as the years of low harvest). Since this analysis provides no evidence that the reduced number of seals in Drakes Estero in years of high oyster harvest was associated with that harvest, use of the overall mean counts overestimates the impact of the high harvest.

4. Regional spatial scale

This analysis uses essentially the same set of covariates as the intra-colony analysis (oyster harvest level (high or low, or actual harvest level), human disturbance rate, years since an ENSO event, maximum count outside Drakes Estero). However, year is treated as a fixed effect, presumably because only one count is used for each year, so GLMs rather than GLMMs are used. Two additional covariates (maximum count at Double Point, the nearest colony, and proportion of Drakes Estero seals using haul-out site A, which became exposed to predators in 2004) were included. The Double Point counts were included to take account of the presence of an aggressive elephant seal in 2003; the proportion of seals at haul-out site A was included to take account of its reduced suitability for pupping in recent years.

In this analysis, the response variable is the proportion of pups or of the total number of seals in the Point Reyes population that were counted in Drakes Estero. I think this proportion is calculated as the maximum count in Drakes Estero divided by the sum of the maximum counts for all of the colonies on Point Reyes. As with the intra-colony analysis, this proportion is treated as a binomial or quasi-binomial variable. That would be appropriate if the counts at colonies other than Drakes Estero were made on the same day as the maximum count at Drakes Estero. However, this does not seem to be the case. If, as I suspect, the proportion is based on the maximum count (or possibly a single count) at each of the other colonies, then I am not sure what it represents. It is certainly not the outcome of a binomial experiment in which each seal chooses either to haul out in Drakes Estero or elsewhere, and I don't know what its statistical properties should be.

The implications of this for the validity of the analysis are probably not too severe. I suspect that the authors are treating this proportion as an estimate of the outcome of a true binomial experiment, and this is captured in the Bayesian analysis in which it is assumed that the distribution of the response variable is over-dispersed, because it includes an unestimated variance component. The Bayesian analysis, which ironically doesn't make use of any prior information, yields effectively the same results (at least for the pup counts) as the GLM analysis, which is reassuring. However, the calculated proportion is probably an over-estimate of the true proportion, because it appears that many more counts were made in Drakes Estero than at the other colonies. In general, I would expect the value of the maximum count at a particular colony to be directly related to the number of counts on which it is based. Again, I am not sure of the implications of this for the analysis. The simplest solution to this problem is to use the sub-set of counts from all colonies that are closest together in time in each year, rather than the maximum counts from each colony, to calculate the proportion of seals using Drakes Estero.

I think the use of total seals and pups as independent response variables is potentially misleading. Not only do the total counts include pups, but these pups are effectively counted twice, because mothers and pups almost always haul out together during the peak pupping period. Figure 1 shows the changes in the number of seals counted at all colonies and at Drakes Estero with the counts of pups and their presumed mothers removed (i.e. total seals - 2xpups). These are effectively counts of immature animals, adult males, and non-breeding females. The number of these animals counted in all the Point Reyes colonies doubled between 1999 and 2004, and then falls sharply. However, the proportion of these animals counted in Drakes Estero (Figure 2) does not show the clear peak during the period

of low oyster production that is evident in the proportion of pups counted (Figure 2) or in the count of total seals. I therefore suspect that the significant relationship between the proportion of all seals counted in Drakes Estero and the level of the oyster harvest is largely, if not entirely, a consequence of changes in the proportion of mothers and pups.

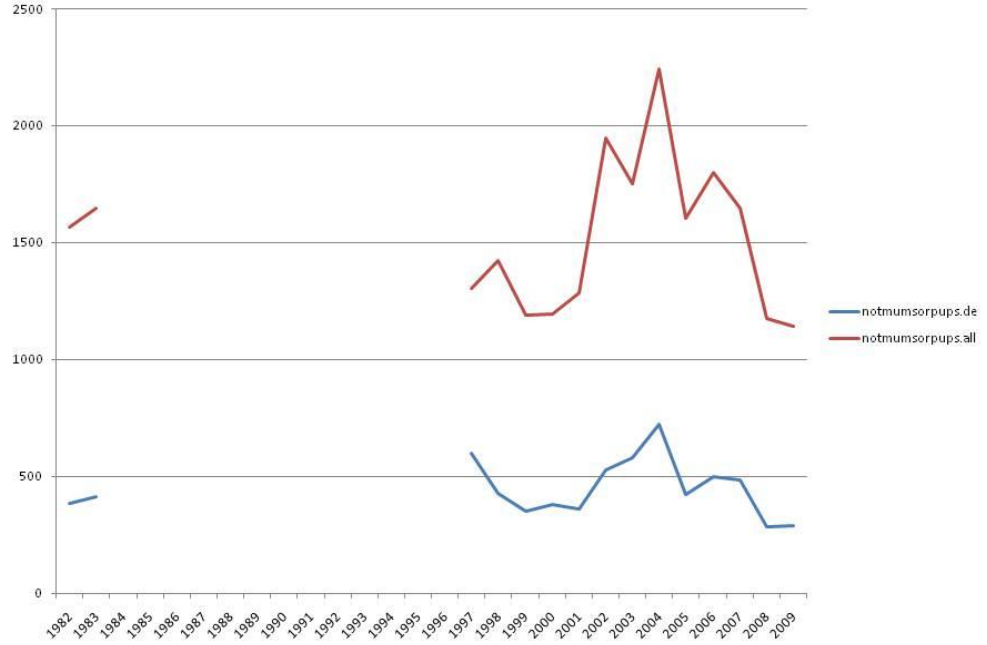

Figure 1. Changes over time in the numbers of immature, adult male and non-breeding female harbour seals counted at Drakes Estero (blue line labelled "notmumsorpups.de") and at all colonies on Point Reyes (red line labelled "notmumsorpups.all")

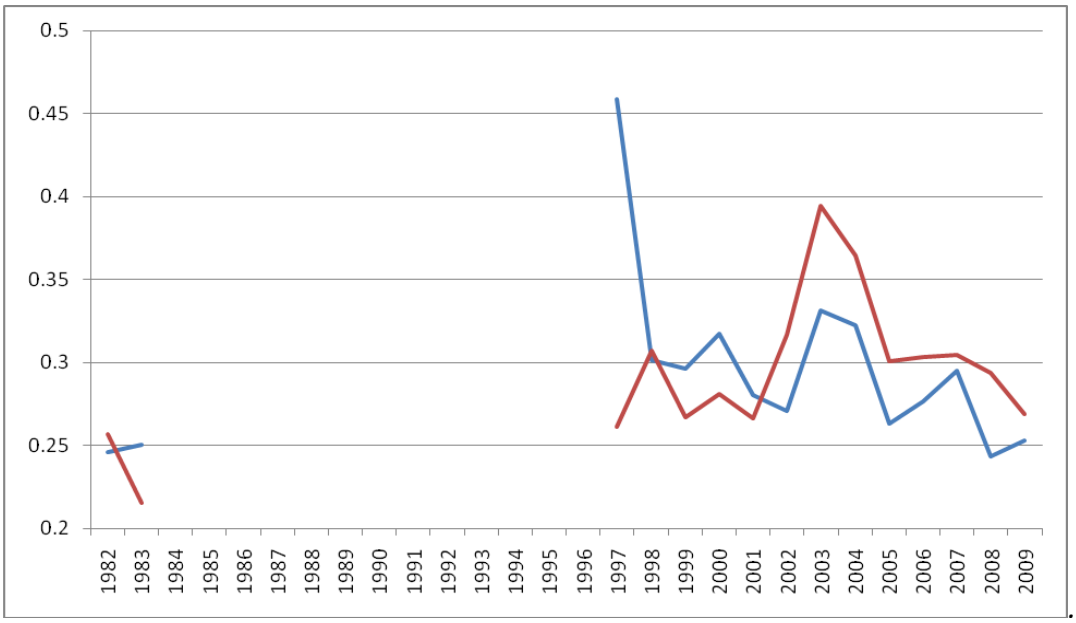

Figure 2. Changes over time in the proportion of immature, adult male and non-breeding female harbour seals counted at Drakes Estero (blue line) and the proportion of pups (red line) counted at the same colony.

The authors applied a weight of 0.3 to the proportion of all seals using Drakes Estero in 1997 because they believe it is an outlier. The explanation for this decision is somewhat mystifying ("a "die-off of mostly adult females ... during the breeding season ... that did not appear to affect pups" - I do not see how this can happen, given the high pregnancy rate usually observed in harbour seal populations), and no reason is given for why the ratio of pups to adults in 1997 is used as the basis for the weighting. I think the authors should test the robustness of their conclusions by rerunning the analysis with the same weight applied to all the data points.

The authors find that the best fitting model is one that includes high or low oyster harvest, number of seals at Double Point, and proportion of seals at site A. They conclude that the proportion of pups hauling out in Drakes Estero was 7% lower in years of high oyster harvest. Again, I have some concerns about the way in which the effect size has been calculated, because I don't think the maximum count at Double Point provides a satisfactory surrogate for the hypothesised effect of the aggressive elephant seal. The number of seals and the number of pups counted at Double Point tracks the numbers in Drakes Estero closely until 2003 , when it diverges sharply (see Figure 4, below), pup numbers then converge over a period of 2-5 years. I think it would be more appropriate to model this disturbance in exactly the same way as the 1998 ENSO event: a fixed effect (whose size is estimated by the appropriate coefficient in the GLM) in 2003, whose influence declines exponentially over time.

I suspect that an analysis using this covariate will still show a significant relationship between the proportion of pups counted in Drakes Estero and the level of oyster harvest, because of the low proportion of seals hauling out in Drakes Estero in 1982 and 1983, which are years of high oyster production but when there was no disturbance at Double Point. However, the inclusion of these two data points in the analysis needs to be justified, because they are separated from the rest of the time series by an interval of 13 years, as long as the remainder of the time series. In addition, Drakes Estero seems to have been less important as a breeding location for harbour seals in these two years than it was after 1997 (see Figure 3 below). The authors should test the robustness of their results by rerunning the analysis without the counts from these two years.

5. Accounting for population dynamics

The number of harbour seals counted at the different colonies on Point Reyes varied between 2481 and 4769, a factor of two, during the period 1997-2009. These are large fluctuations for a harbour seal population in the absence of major die-offs, none of which was recorded in the period. These fluctuations make it difficult to analyse the raw count data using linear models. I can therefore entirely understand why Becker et al. chose to use the proportion of seals counted in Drakes Estero as their main response variable. However, the fluctuations are also likely to have affected the proportion of seals counted in Drakes Estero in ways that are not captured by the simple linear relationship with the number of seals counted at colonies outside Drakes Estero tested by Becker et al.

I assume that the main conservation concern behind the analysis in Becker et al is whether the level of the oyster harvest in Drakes Estero influences the number of seals in Drakes

Estero and in the entire Point Reyes population. Modelling the proportion of seals at one colony cannot provide this information. The obvious way to do this is to construct a state-space model of the dynamics of the Point Reyes population (see, for example, Buckland, S. T., K. B. Newman, L. Thomas, and N. B. Koesters. 2004. State-space modelling for the dynamics of wild animal populations. Ecological Modelling **171:** 157–175, which contains an example of such a model for seals). This model could take account of the fact that individual seals are likely to show some fidelity to one colony - the analysis in Becker et al assumes that where a seal hauls out in a particular year is entirely independent of its behaviour in previous years. It could also account of variations in haul-out behaviour among age and sex classes, because these can affect the number of seals counted at each colony. The following paragraphs illustrate why I think it is important to take account of these issues in order to understand the changes that have been observed at Drakes Estero.

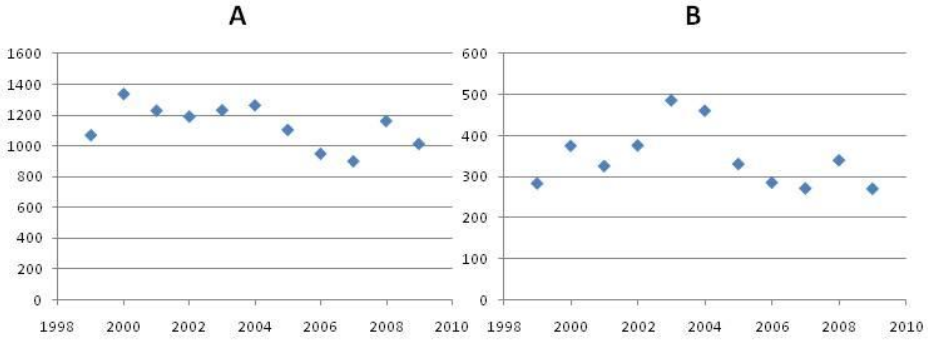

Figure 3. Changes over time in the numbers of pups counted at all colonies on Point Reyes (A) and at Drakes Estero (B) since the 1998 ENSO event.

Figure 3 indicates that, since the 1998 ENSO event, the number of pups counted each year at all of the Point Reyes colonies, and at Drakes Estero alone, show no consistent trend. The number of pups counted at Drakes Estero was, however, particularly high in 2003 and 2004. The same peak is evident at a number of other colonies (Figure 4), and it coincides with a sharp reduction in the number of pups counted at Double Point in 2003. Up until 2003 the number of pups counted at Drakes Estero and Double Point follow remarkably similar trajectories, and these trajectories gradually converge again after 2004 (Figure 4).

The most likely explanation of these changes is that a large number of mothers and pups deserted Double Point in 2003, presumably because of the presence of the aggressive elephant seal, for other colonies, particularly Drakes Estero. These animals gradually returned to Double Point from 2004 onwards. It is also noticeable that the changes in the numbers of pups counted at Drakes Estero after 2004 are very similar to those observed at all of the other colonies except Double Point.

Figure 4. Changes over time in the numbers of pups counted at Double Point (blue line labelled "pups.dp"), Drakes Estero (red line labelled "pups.de") and all other colonies on Point Reyes (green line lablled "pups.notde&dp").

Figure 1 shows that the number of immature, adult male and non-breeding adult females counted in all the Point Reyes colonies doubled between 1999 and 2004, and then fell sharply. Given that the number of pups born each year was effectively constant during this period and that no major die-offs were reported, I can think of only three explanations for these changes: a dramatic increase in juvenile survival between 1999 and 2004, followed by an even more dramatic decline in the survival of all age and sex classes after 2004; a dramatic and temporary increase in the proportion of time that non-breeding animals spend hauled out during the period 2002-2004; or a temporary immigration of approximately 1000 animals. A state space model should be able to distinguish between these hypotheses.

Unfortunately, these changes in the structure and distribution of the Point Reyes population occurred at exactly the same time as the sharp decline in the size of the oyster harvest. It is very difficult to disentangle the effect of these different factors using simple covariates (such as the number of seals counted at Double Point, or the total number of seals at other colonies) in a linear model. I think their putative effects have to be modelled explicitly in a state space model that could be fitted to all of the count and telemetry data that is available

on this population using Bayesian techniques. However, I can entirely understand why Becker et al did not take on this rather daunting task.