Update on the processing for "tetracotyle" type metacercariae of sardine from commercial catch samples, 2011-2014, and preliminary analyses thereof

C.D. van der Lingen[1,2], C.C. Reed[3], M. Hendricks[1], I. Nunkoo[3] and H. Winker[4]

[1] Resources Research, DAFF; [2] Marine Research Institute, UCT; [3] Department of Biological Sciences, UCT; [4] SANBI

Introduction

Generalised Linear Model (GLM) analyses of data on infection of sardine by a digenean "tetracotyle" type metacercarian parasite from 1 318 sardine collected in 2011 and 2012 were published by Weston *et al.* (2015), who reported that putative stock (western or southern) was the most significant contributor in explaining deviance seen in infection intensity (# parasites.infected fish^{-1}) and parasite abundance (# parasites.fish^{-1}), and the 2nd-most important (after the covariate log(CL)) in explaining deviance in infection prevalence. Western fish had significantly higher parasite loads compared to southern fish and these results were taken as support for the hypothesis of multiple sardine stocks off South Africa. GLM analyses of parasite data for 2 319 sardine collected over the period 2011-2013 (which includes the data used by Weston *et al*., 2015) were reported by van der Lingen *et al.* (2014), who found that putative stock was now the most important contributor for all three indices of infection (prevalence, infection intensity and parasite abundance) and that western sardine again showed higher indices of infection than did southern sardine. Sardine from both western and southern stocks showed an increase in the prevalence of infection with increasing fish size (van der Lingen and Winker, 2014), and an analysis that focused on parasite prevalence in larger fish (dominated by fish of ages of 2 and older) concluded that prevalence continued to show a significant increase over these size classes (Ross-Gillespie and Butterworth 2014), indicating that there must be some movement of older (>1 year old) sardine from the western to the southern stock.

The collection and processing of sardine from commercial catch samples to examine levels of infection by a digenean "tetracotyle" type metacercarian parasite continued in 2014 following recommendations by the Review Panel at the 2013 International Stock Assessment Workshop that data on the presence of parasites by length should be included in sardine assessments (Smith *et al*., 2013), a recommendation endorsed by the Review Panel at the 2014

International Stock Assessment Workshop (Dunn *et al*., 2014). This document presents an update of sardine samples processed in 2014 and initial results from GLM analyses of prevalence for the 4-year dataset.

Methods and materials

A total of 2 008 commercially-caught sardine were collected and processed in 2014 with large sample sizes collected from both western and southern stocks (Table 1), although poor catches of sardine off Port Elizabeth in 2014 meant that most southern sardine examined were from catches made in the Mossel Bay area. A total of 4 327 sardine from 131 samples collected over the period 2011-2014 have now been examined (Table 1). Three indices of infection were used, namely infection prevalence (% of the sample infected), mean infection intensity (number of parasites.infected fish^{-1}) and mean parasite abundance (number of parasites.fish^{-1}).

Table 1: Number of fish (and number of samples) from commercial catch samples examined for “tetracotyle” type metacercarian parasites by putative stock and in total, 2011-2014.

Year	Western	Southern	Total
2011	269 (12)	169 (7)	438 (19)
2012	373 (16)	509 (20)	881 (36)
2013	358 (11)	641 (23)	999 (34)
2014	1 206 (25)	802 (17)	2 008 (42)
Total	**2 206 (64)**	**2 121 (67)**	**4 327 (131)**

Results

Fish of a similar size range were sampled from both stocks in 2014, as in previous years, and plots of infection prevalence and mean parasite abundance per 0.5 cm length class show a clear increase in these parameters with increasing fish size for sardine from both stocks (Fig. 1). Prevalence-at-length was appreciably higher for western compared to southern sardine whilst mean parasite abundance did not appear to be markedly different. Outputs from the updated GLM for prevalence are shown in Table 2. That model explained 20% of observed variance in tetracotyle prevalence (compared to 21% for n=2 years and 27% for n=3 years), with *Stock* the most important contributor followed by *Year* and then *Length*. The most important interaction term was the *Year:Stock* term, indicating that differences between the two stocks are not the same in every year.

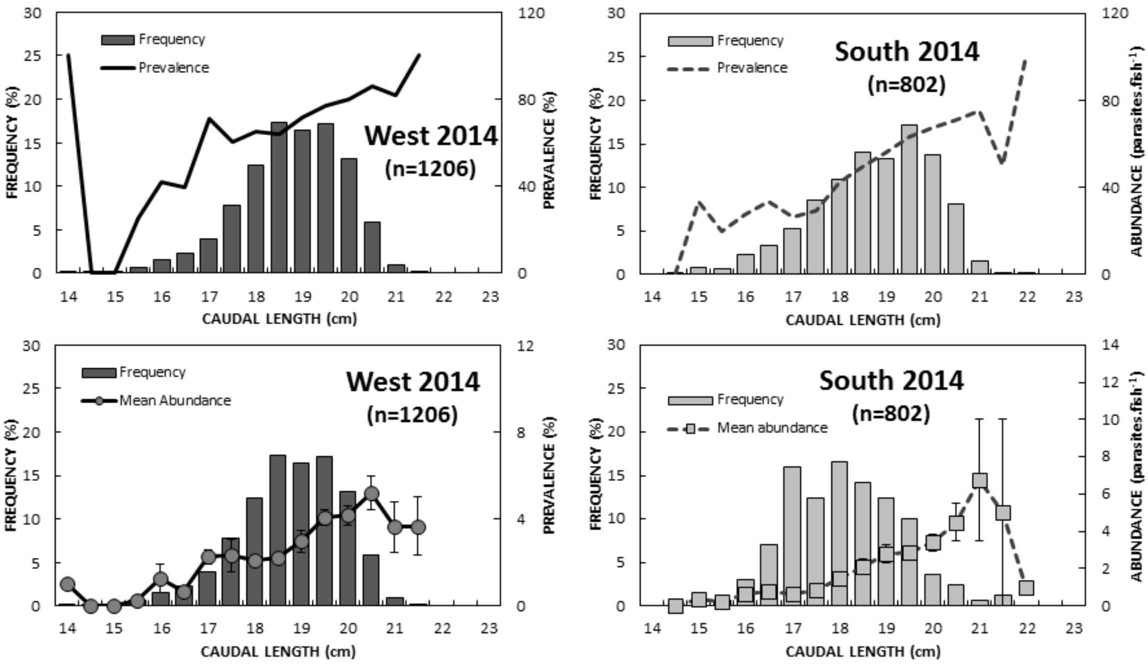

Figure 1: Length frequency distributions (histograms; left axis), and infection prevalence (upper plots) and mean parasite abundance (lower plots; one standard error is shown) by length class for sardine from commercial catches taken to the west ("West") and east ("South") of Cape Agulhas in 2014.

Table 2: Analysis of deviance for the binomial GLM (total variation explained = 20%) fitted to parasite prevalence data 2011-2014. The table summarises the AIC score, residual deviance (Res. Dev.), changes in the residual deviance (Δ Dev), the percentage of the total reduction in deviance explained by each factor (% explained), and corresponding p-values when a χ^2-test was applied to test for significance. The three most important terms in explaining variance are shown in bold.

Model Structure	AIC	Res. Dev	Δ Dev	% explained	$p(\chi^2)$
NULL	5998.6	5996.6			
+ Stock	**5594.1**	**5590.1**	**-406.5**	**31.45**	**< 0.001**
+ Year	**5246.3**	**5236.3**	**-353.8**	**27.37**	**< 0.001**
+ Season	5209.0	5193	-43.3	3.35	< 0.001
+ Length	**4870.7**	**4852.7**	**-340.3**	**26.33**	**< 0.001**
+ Stock:Season	4820.2	4796.2	-56.5	4.37	< 0.001
+ Year:Stock	4747.668	4717.7	-78.5	6.07	< 0.001
+ Year:Length	4740.0	4704	-13.7	1.06	< 0.01

Plots of predicted prevalence of infection by length class for each stock in each year are shown in Figure 2 (note that data from samples in Spring were not included due to evidence of high mixing in that season; see van der Lingen *et al*., 2014 and Weston *et al*., 2015), together with the observed mean prevalence and their associated approximate 95% confidence intervals.

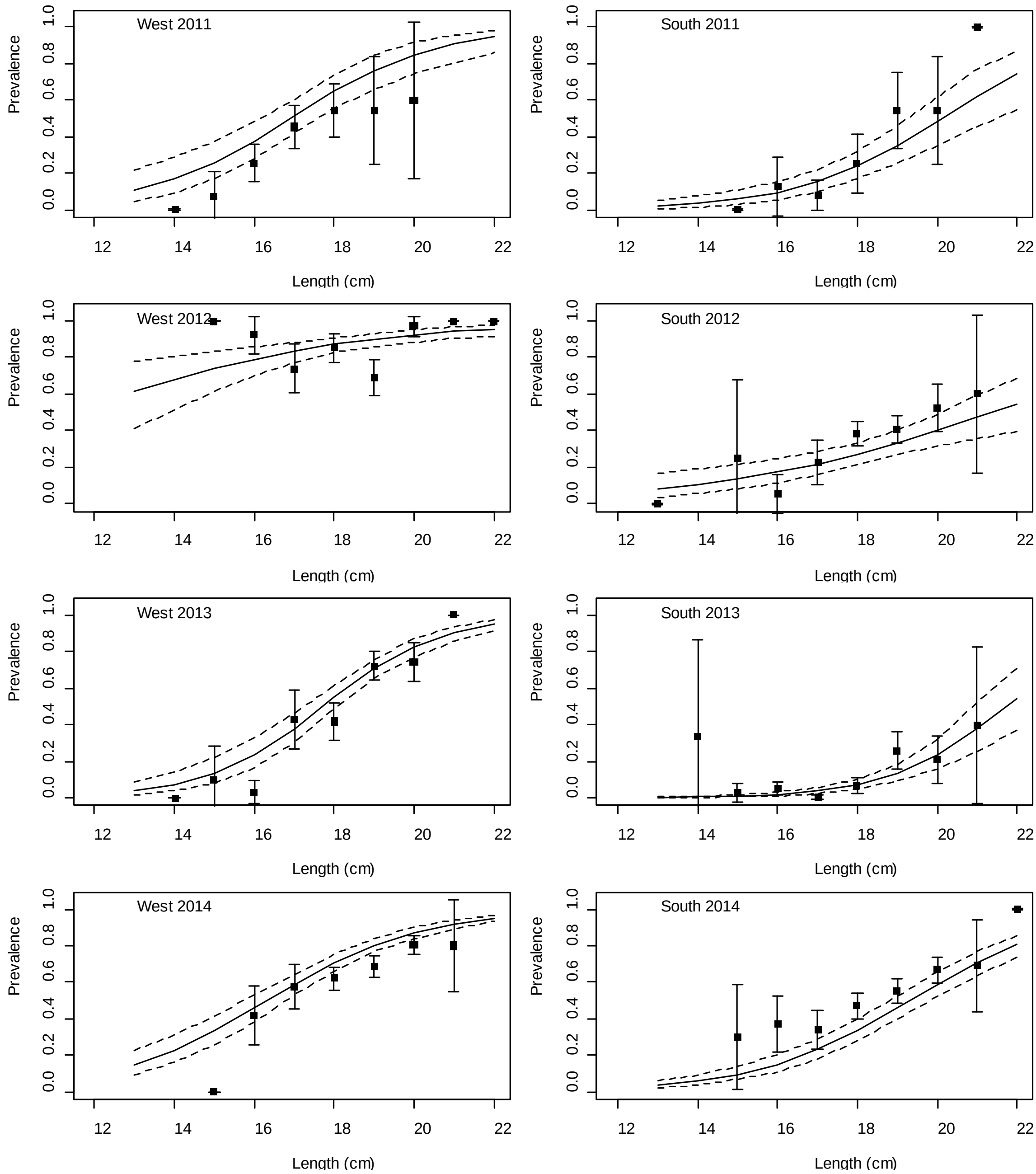

Figure 2: Predicted prevalence (± 95% CI, dashed lines) of "tetracotyle" type metacercariae infection in *Sardinops sagax* by year for fish from both the putative western and southern sardine stocks (values have been standardized to Winter). Solid squares denote observed mean prevalence by length bin and year (excluding Spring). Error bars approximate 95% CI, calculated as $CI_{95\%} = \hat{p}_L \pm z\sqrt{\hat{p}_L(1-\hat{p}_L)/n_L}$.

Predicted prevalence of infection by year and season for three size classes of fish (16, 18 and 20 cm CL) are shown in Figure 3. That infection prevalence differs between years is evident, with 2012 and 2014 showing higher prevalence of infection and 2011 and 2013 showing lower prevalence. In addition, the differences between stocks in infection prevalence also differs between years, with larger differences observed in 2012 and 2013 compared to 2011 and 2014, hence the significance of the (*Year:Stock*) interaction term which was not significant in previous analyses.

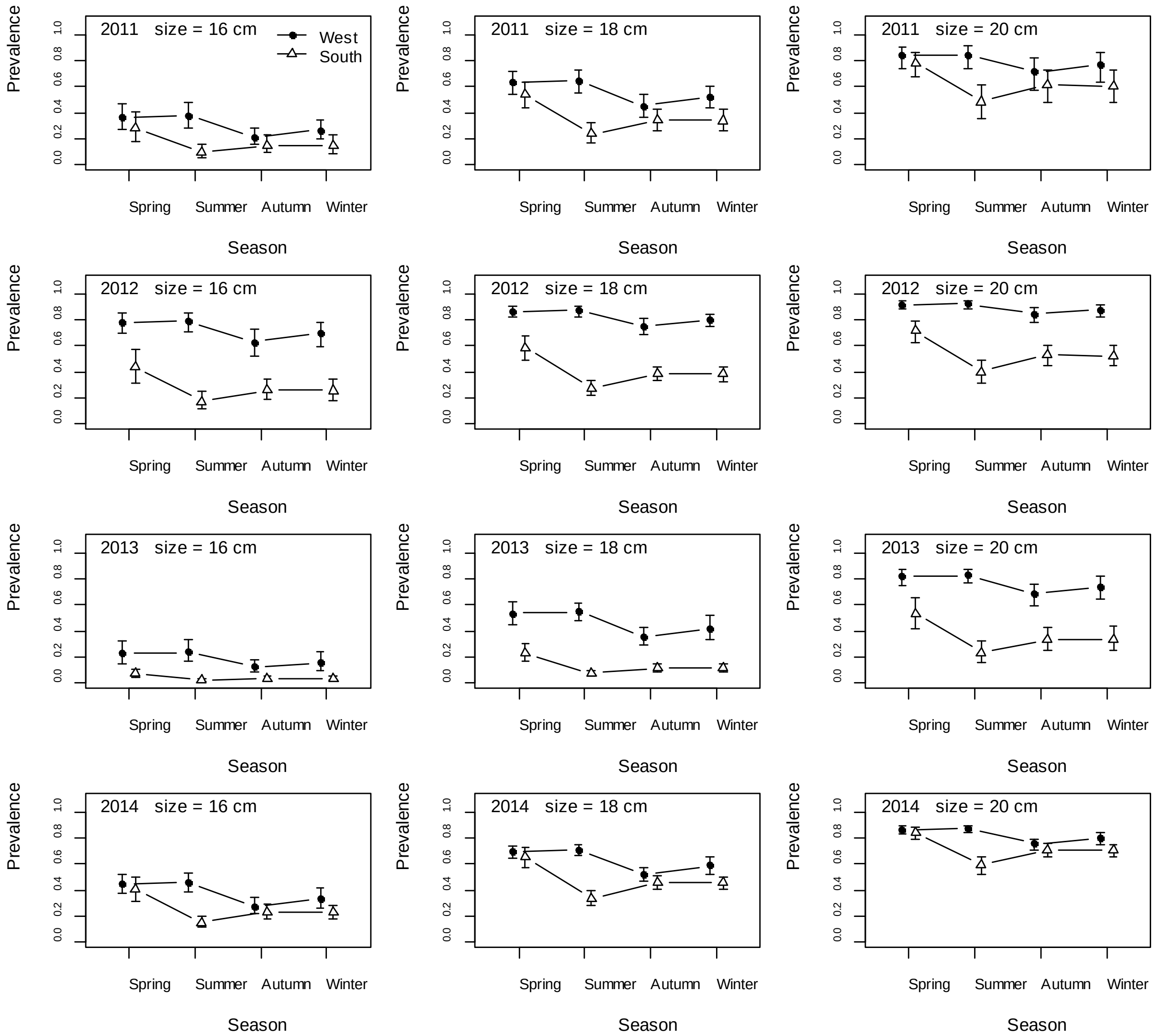

Figure 3: Predicted prevalence (± 95% CI) of infection in *Sardinops sagax* by "tetracotyle" type metacercaria by year (2011-2014), season (Spring to Winter) and size class (16, 18 and 20 cm CL) for fish from the putative western (filled circles) and southern (empty triangles) sardine stocks.

Discussion

The addition of new data from >2 000 sardine collected and processed in 2014 has further corroborated previous results that *Stock* explains the majority of variation in prevalence of infection by the "tetracotyle" type digenean, followed by *Year* and then *Length*. These were also the three most important factors (and in the same order) for the 3-year dataset, but not for the 2-year dataset, where *Length*, *Stock* and then *Year* were dominant. These new results add support to the hypothesis of multiple sardine stocks, and although GLMs for infection intensity and parasite abundance have yet to be derived they will be conducted soon and presented to the SWG-PEL. The parasite data on prevalence and infection intensity of sardine from commercial catch samples collected over the period 2011-2014 is now available for inclusion into stock assessment models.

Samples of sardine have been collected for parasite analysis from pelagic recruit and spawner biomass surveys since 2010, in addition to opportunistically-collected samples from South Africa, including during the KZN sardine run, and also from Namibia. Results from preliminary analyses of these data up to 2012 were presented by van der Lingen *et al.* (2015), who plotted distribution maps of mean parasite abundance for three size classes of fish (<12.5cm, 12.5-16.5 cm, and >16.5 cm CL) and reported strong gradients in this index around the SA coast. Mean abundance was highest off the west coast and western part of the south coast, low to moderate off the remainder of the south coast, and close to zero for fish off the east coast, and no infected sardine from Namibia (n = 174 fish) were observed. Samples collected during 2013 and 2014 are presently being processed and parasite data collected and collated. Because of the inclusion of juvenile fish in that dataset it may provide new insights into infection of sardine by this parasite, and possible sardine movement patterns, not obvious from analyses of commercial catch samples where the smallest sardine processed was 14 cm CL. It is hoped that analysis of those data will be completed by mid-2015, despite present logistical constraints.

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