Update on “tetracotyle” type metacarcariae infection data and implications for sardine movement

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Introduction

Previous work examining infection of sardine by a digenean “tetracotyle” type metacercarian parasite (thought to belong to the genus *Cardiocephaloides*) has shown that locality (west or south coast) is a major contributor to explaining patterns of variability in three indices of infection (Weston *et al*., in. prep). Those authors assessed spatial and temporal variation in infection by examining 1 318 adult fish (>14.0 cm *CL*) collected throughout the year from commercial purse-seine catches taken from the putative western (n = 641) and southern (n = 677) sardine stocks over the period March 2011 to December 2012. Infection prevalence (% of the sample infected), mean infection intensity (number of parasites.infected fish^{-1}) and mean parasite abundance (number of parasites.fish^{-1}) were recorded, and Generalised Linear Models were used to assess the dependency of these three indices on putative *Stock*, *Season*, *Year*, sardine caudal length (*CL*), and their possible interactions. A binomial distribution was assumed for the prevalence data and a negative binomial distribution for infection intensity and parasite abundance, and the most parsimonious model for each index selected based on the Akaike’s Information Criterion (AIC). Significant models were derived for all three parasite indices, with the covariates *Year* and *Stock* explaining the majority of variation in infection prevalence, *Stock* and then *Season* that in infection intensity, and *Stock* and then log-transformed *CL* that in parasite abundance (Table 1).

Sardine from the putative western stock showed higher prevalence, infection intensity and parasite abundance levels than their southern counterparts for most of the year expect spring, when values were typically similar for all three indices (Fig. 1a, d and g). All three indices were higher in 2012 than in 2011 (Fig. 1b, e and h), and all three showed an increase with fish size (Fig. 1c, f and i) for fish from both stocks. However, whereas the increase in infection intensity with size was similar for both stocks this pattern differed markedly for prevalence of infection and parasite abundance. Western fish of 14 cm *CL* showed high (~70%) prevalence of infection and this increased to >90% for fish of 18 cm *CL* and larger. In contrast, less than 10% of small (<15 cm *CL*) southern fish were infected, around 33% of 18 cm *CL* were infected and 70-80% of fish >21 cm showed infection. The 95% confidence levels for the two stocks did not overlap. Small (14 cm *CL*) and medium (19 cm *CL*) sardine from the west had 0.8 and 3.7 parasites each, respectively, whereas southern fish of these sizes had 0.05 and 1.5 parasites each. The 95% confidence limits for the parasite abundance against *CL* plots only overlapped for sardine of 20 cm *CL* and larger. Despite observed seasonal and interannual variation these results clearly demonstrate the dominant spatial signal in parasite loads, and have been taken as supporting the hypothesis hypothesis of discrete western and southern sardine stocks (Weston *et al*., in prep.). Data on infection of around 3 000 sardine collected from >100 localities around the SA coast during research surveys also shows a clear spatial signal, with parasite abundance decreasing from west to east (van der Lingen *et al*., 2014).

Table 1: Outputs from the GLMs for prevalence of infection, infection intensity and parasite abundance of "tetracotyle" type metacercariae in sardine; note that the three factors that explain the highest %s of variation in each index are given in bold (from Weston *et al*. in prep.).

GLM (and pseudo-R^2 value)	**Factor**	**Residual DF**	**Residual Deviance**	**Δ Deviance**	**p-value**	**% Deviance explained**
Prevalence of infection (0.17)						
	Null	1315	1821.6			
	Stock	1314	1741.9	79.7	<0.001	**25.89**
	Season	1311	1697.5	44.5	<0.001	14.46
	Year	1310	1608.6	88.9	<0.001	**28.89**
	Log(CL)	1309	1559.6	48.9	<0.001	**15.91**
	*Stock*Season*	1306	1516.9	42.7	<0.001	13.87
	*Stock*Log(CL)*	1305	1513.9	3.0	0.0820	0.98
Infection intensity (0.29)						
	Null	687	907.3			
	Stock	686	809.3	97.9	<0.001	**37.21**
	Season	683	754.8	54.5	<0.001	**20.70**
	Year	682	714.5	40.3	<0.001	**15.29**
	Log(CL)	681	682.3	32.2	<0.001	12.24
	*Stock*Season*	678	648.2	34.1	<0.001	12.96
	*Stock*Log(CL)*	677	644.0	4.2	<0.05	1.59
Parasite abundance (0.30)						
	Null	1315	1810.1			
	Stock	1314	1609.5	200.6	<0.001	**36.3**
	Season	1311	1519.7	89.8	<0.001	16.3
	Year	1310	1422.3	97.4	<0.001	**17.6**
	Log(CL)	1309	1324.7	97.6	<0.001	**17.7**
	*Stock*Season*	1306	1277.8	46.9	<0.001	8.5
	*Stock*Log(CL)*	1304	1257.6	20.2	<0.001	3.7

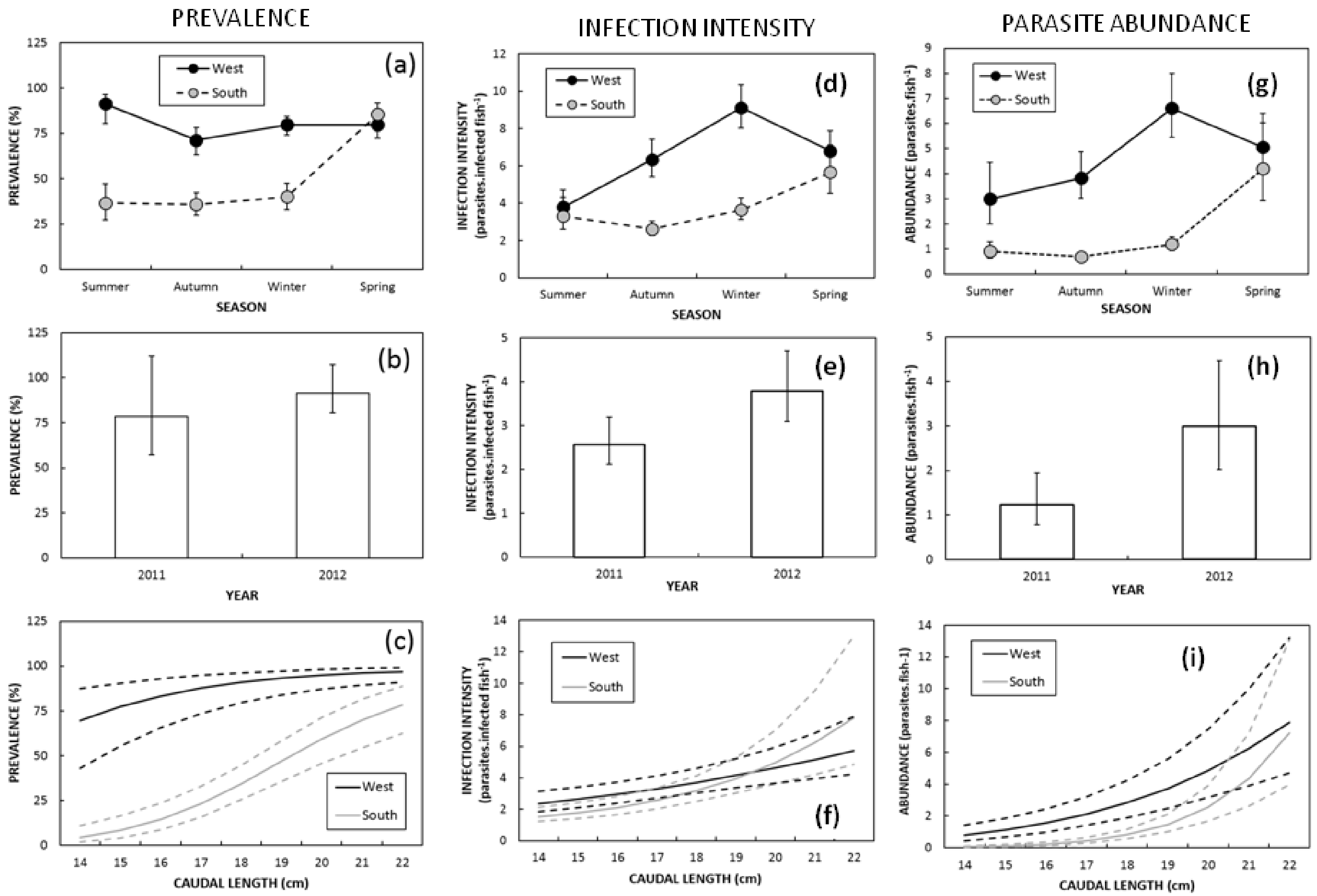

Figure 1: Outputs from generalized linear models (GLMs) of infection of sardine by a digenean "tetracotyle" type metacarian parasite (with putative *Stock* [West and South], *Season*, *Year* [2011 and 2012] and fish size [*CL*] as input parameters) showing predicted prevalence (%; a, b and c), predicted average infection intensity (number parasites.infected fish^{-1}; d, e and f) and predicted average parasite abundance (number parasites.fish^{-1}; g, h and i) for *Season* by *Stock* (upper panel; normalized for a fish of 18.1 cm *CL*), *Year* (centre panel; normalized for a fish of 18.1 cm *CL*) and fish size (*CL*; lower panel). The 95% confidence limits are shown (from Weston *et al*., in prep.).

New data

Preliminary, raw data (i.e. not analysed by GLM or other models) on infection by the "tetracotyle" type metacercariae parasite of sardine collected from commercial catches in 2013 are presented and compared to raw data for 2011 and 2012. A further 999 fish from 34 samples, mostly from the south coast, have been examined (Table 2).

Table 2: Number of fish examined (and number of samples) for "tetracotyle" type metacercariae parasites by putative stock, 2011-2013.

Year	Western	Southern
2011	268 (12)	169 (7)
2012	373 (16)	508 (20)
2013	358 (11)	641 (23)

Sardine collected in 2013 had a broadly similar length distribution to those collected previously, and prevalence of infection again increased with *CL* for fish from both stocks (Fig. 2).

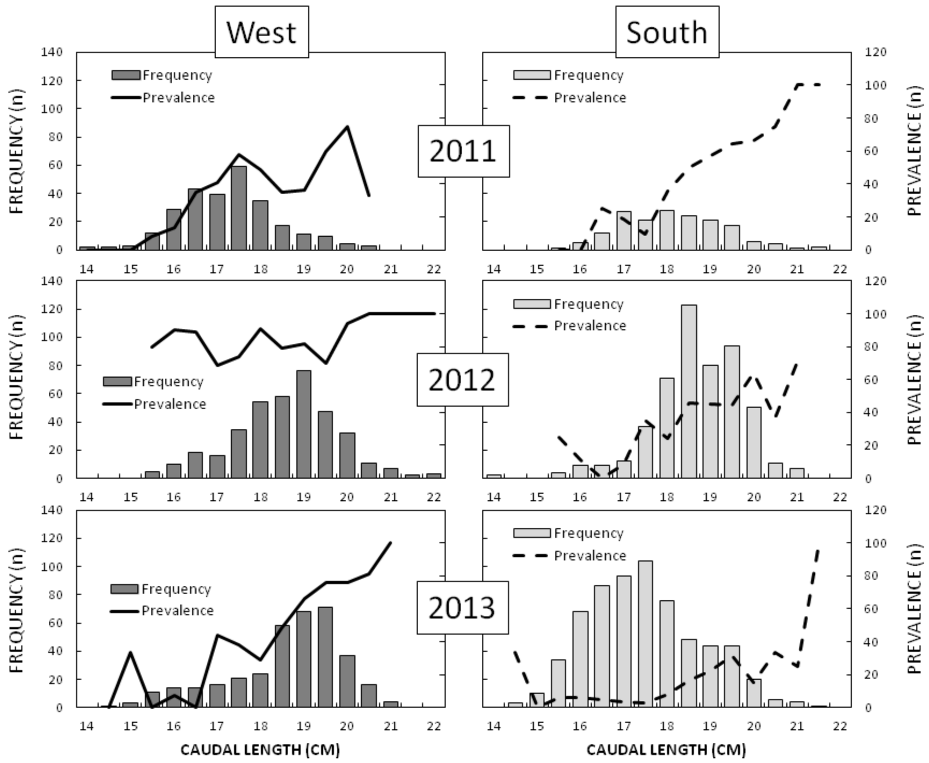

Figure 2: Length frequency distributions (histograms; left y-axis) and prevalence by length class (lines; right y-axis) for sardine from commercial catches taken to the west of Cape Agulhas (dark histograms and solid lines) and those taken to the east of Cape Agulhas (light histograms and dashed lines) by year, 2011-2013 (note that data from 2011 and 2012 were used in the GLM analysis by Weston *et al.,* in prep.).

Direct comparisons of prevalence of infection by length class and parasite abundance by length class for sardine from the western and southern stocks each year individually are presented in Figure 3. In 2011 neither prevalence of infection by length nor parasite abundance by length differed markedly for sardine from the western and southern stocks, and whilst the highest prevalence and abundance values were in fact for large fish from the south these came from small samples of <5 fish. Differences were marked in 2012, with both prevalence and parasite abundance higher for western than southern sardine. The 2013 data also show a marked inter-stock difference, particularly for prevalence of infection and parasite abundance levels in sardine of 18 cm *CL* and larger. Infection levels in 2013 appeared to be more similar to those observed in 2011 than in 2012.

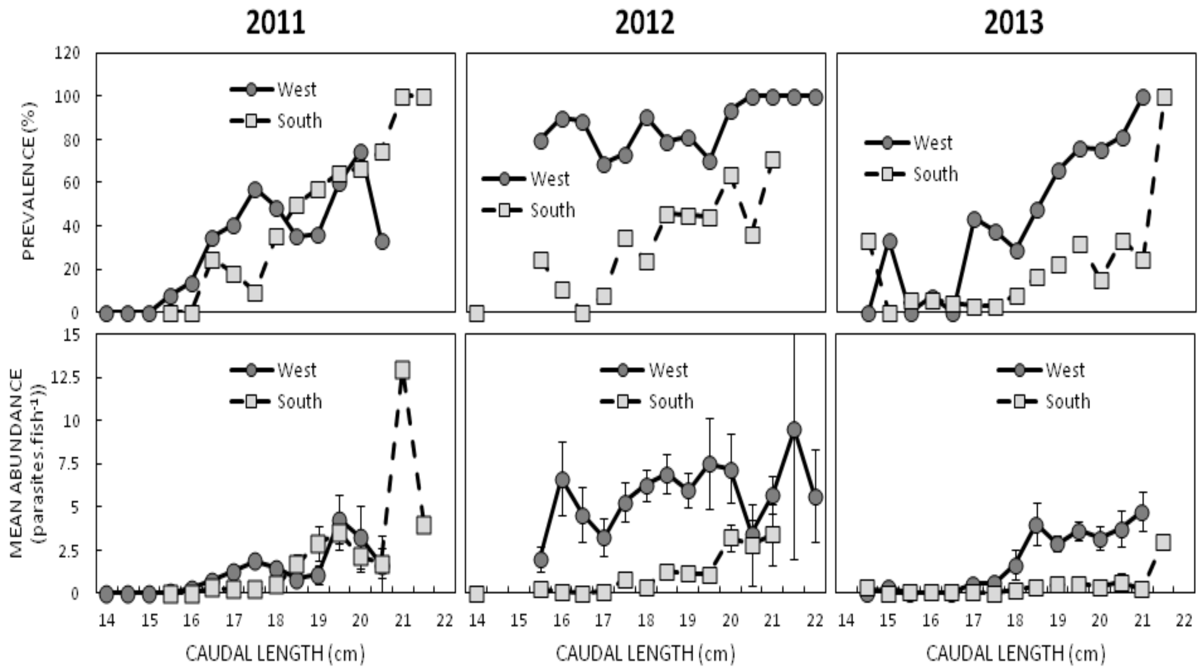

Figure 3: Prevalence (%; upper panel) and average parasite abundance (number parasites.fish^{-1}; lower panel; standard erros are shown) by length class of sardine from commercial catches taken to the west of Cape Agulhas (dark circles and solid lines) and those taken to the east of Cape Agulhas (light squares and dashed lines) by year, 2011-2013 (note that data from 2011 and 2012 were used in the GLM analysis by Weston *et al*., in prep.).

Implications for sardine movement

The increase in all three indices of infection by the 'tetracotyle" type parasite with increasing fish size indicates cumulative infection and that this parasite is long-lived in sardine, the latter considered by some to be by far the most important criterion for an effective parasite biotag (Lester, 1990; Lester and MacKenzie, 2009). The longer fish spend in the parasite endemic area, where transmission of the parasite from the first intermediate host to sardine occurs, the higher the number of parasites each fish will likely contain. Because parasite distributions (and those of their hosts at different life history stages) are strongly driven by temperature-salinity profiles and specific water masses (Reed *et al.,* 2012) and because the South African west and south coast differ markedly in terms of these characteristics, our working assumption at present is that the endemic area of the 'tetracotyle" type parasite is to the west of Cape Agulhas. The pattern of increasing prevalence, infection intensity and parasite abundance with increasing fish size for sardine from the putative western stock fits this assumption. However, such increases with increasing fish size for sardine from the southern stock does not, at least not under the assumption that sardine movement occurs only from west to south in November as recruits age to 1-year olds, presently used in sardine stock assessment models (de Moor and Butterworth, 2013).

Increases in infection indices with increasing fish size for sardine from the southern stock could possibly be explained by one or more of several reasons:

1. The hypothesis that the parasite endemic area occurs only to the west of Cape Agulhas is incorrect;
2. Direct (fish-to-fish) transmission of the parasite occurs on the south coast, with infected 1–year old fish from the western stock that move to the south coast infecting southern fish;
3. Some southern fish move to the west coast for some part of the year during which they become infected, before returning to the south coast; and
4. Some of the fish moving from the west to the south coast do so at a later stage of their life; i.e. not only as 1-year old fish.

The identity of the first intermediate host (thought likely to be an inter-tidal or sub-tidal gastropod based on work on *Cardiocephaloides* elsewhere) remains undetermined at present, and investigation into this issue is only planned once the parasite has been definitively identified through genetic sequencing of metacercariae and comparison with the genetic sequence of adult *Cardiocephaloides physalis* collected from African penguins. Until the parasite itself and its first intermediate host have been definitively identified, the location and extent of the parasite endemic area cannot be determined. Based on the life cycle of Strigeid digenean parasites elsewhere, direct transmission of the 'tetracotyle' type parasite from one sardine to another is considered to be highly unlikely if not impossible, as the metacercariae are infective to the final avian host only (K. MacKenzie, pers. comm.). Alongshore movement of three ("cool", "temperate" and "warm") sardine stocks off California and Mexico, corresponding to changes in the California Current strength and associated environmental conditions, has been postulated (Felix-Uraga *et al.,* 2004). The KZN sardine run is itself a seasonal movement (van der Lingen *et al.,* 2010), hence it seems plausible that seasonal sardine movement could occur off other parts of South Africa, and some evidence for seasonal along- and across-shore movement of sardine as inferred from seasonal changes in the centre of gravity (CoG) of commercial catches is given in van der Lingen (2014). In particular, a westward movement from summer to spring of the CoGs of sardine catches off Mossel Bay is seen, which could indicate the movement of some southern fish onto the west coast, although the constraints and limitations of using catch data to infer fish movement weaken such inference. Larger fish generally have a higher mobility compared to smaller fish, hence the suggestion that sardine >1-year old move from the west to the south coast is also plausible. Unfortunately, however, the parasite data cannot distinguish between these two movement hypotheses and new approaches will have to be utilized to better understand intra-annual sardine movement.

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