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Supplementary material

The ongoing invasion of translocated sleepy cod (*Oxyeleotris lineolata*) in the Lake Eyre Basin, central Australia

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SUPPLEMENTARY APPENDIX 1.

Methods for the collection of *O. lineolata* biological information

Laboratory

Between September 2014 and May 2015, a total of 245 *O. lineolata* were humanely destroyed, frozen on site and retained for laboratory for analysis. Once in the laboratory, frozen fish samples were defrosted and patted dry to remove excess water. The total length ($\text{Length}_{\text{TOTAL}}$) was measured to the nearest millimetre using Vernier callipers, and the wet weight ($\text{Weight}_{\text{WET}}$) determined to the nearest 1/100 of a gram.

To remove the gonads, an incision was made from the urogenital pore through the pelvic girdle towards the head, exposing the abdominal cavity. Gonads were then dissected out, weighed ($\text{Weight}_{\text{GONAD}}$), bagged and staged according to Pusey *et al.* (2004).

Sagittal otoliths were removed from defrosted specimens in the laboratory, according to McDougall (2004). Briefly, two sagittae were extracted from the otic bulla, washed, dried, weighed individually, mounted in clear casting resin, and polished to expose the primordial region. Annual increments of the right otolith where then counted at a magnification of x 10 to x 40 under reflected light using a stereomicroscope according to (Russell *et al.* 2010). A 25% random otolith re-count was undertaken and Coefficient of Variation (CV) used as a measure of ageing precision. Age estimates with a CV below ≈5% are considered to be reliable (D. Lou, *pers comm*).

Stomach contents were removed by dissecting the digestive tract between the oesophagus and the intestine, and eviscerating prey items into a bag. Contents were then weighed ($\text{Weight}_{\text{PREY}}$) and pressed and pressed to an even thickness of 2 mm, visually scored over graded graph paper with the relative volumetric contribution of prey items to the total gut content measured in the number of graph squares covered (Hyslop 1980; Balcombe *et al.* 2005). Prey categories were derived from Pusey *et al.* (2004) and included fish, macrocrustaceans, microcrustaceans, other aquatic invertebrates, terrestrial invertebrates, terrestrial vertebrates, plant, algae, detritus, and unidentified. Allotted squares were summed for each diet category and expressed as the percentage of total dietary contribution. Unidentified food items were omitted from all diet analyses. Average stomach fullness (fyke derived fish) was 24.2% (± 3.6%); median (10%); with 22% of fish found to have empty stomachs. This suggests that feeding within nets by captured individuals was relatively limited, however fish found to have a stomach fullness >80% were removed from all analysis. Dry season dietary composition for a further eight Lake Eyre Basin fish species was drawn from Balcombe *et al.* (2005) and summarised according to Pusey *et al.* (2004).

Eviscerated fish weight was calculated as: $\text{Weight}_{\text{EVISCERATED}} = \text{Weight}_{\text{WET}} - (\text{Weight}_{\text{GONDAD}} + \text{Weight}_{\text{PREY}})$ which includes the stomach tissue, but not the stomach contents (i.e. prey items).

Data Analysis

All analyses were performed in R v 3.2.0 (R Core Team, 2015).

Length–weight relationships for *O. lineolata* were obtained by applying the exponential regression equation: $= aL_T{}^b$, where W = $\text{Weight}_{\text{WET}}$ (g), L_T = $\text{Length}_{\text{TOTAL}}$ (cm), a = the intercept and b = the slope of the log-transformed linear regression (Dulčić *et al.*, 2009). This was also performed with W = $\text{Weight}_{\text{EVISCERATED}}$.

Gonadosomatic index (GSI) was calculated as: GSI = ($\text{Weight}_{\text{GONAD}}$ / $\text{Weight}_{\text{WET}}$] x 100 for stage III males and femals (see Pusey *et al.* 2004). We also calculated the GSI of eviscerated indivuals using the formula: $\text{GSI}_{\text{EVISCERATED}}$ = ($\text{Weight}_{\text{GONAD}}$ / $\text{Weight}_{\text{EVISCERATED}}$) x 100 for stage III males and females. Summary statistics (mean, standard error, and range) were generated for all reproductive traits for stage II and III, males and females.

The population age structure of Sleepy cod was estimated by generating a von Bertalanffy growth equation: $l_T = l_\infty\left(1 - e^{-K(t-t_0)}\right)$, where l_T = $\text{Length}_{\text{TOTAL}}$ (mm); l_∞ = asymptotic length at which growth is zero; K = growth rate; t = age (years); t_0 = age at which length equals zero, using annual age estimates for 242 individuals between 40 – 302 mm TL. Initial growth parameters were obtained by using the function 'vbStarts' in the package FSA. The function 'nls' was used to estimate final parameters for the growth equation. The growth equation was then used to convert length data into age estimates for a total of 668 fish collected from the Cooper Creek catchment. Separate growth equations for males and females were not possible due to persistent fitting errors within 500 iterations.

The trophic niche breadth of each species (O. *lineolata* from this study, and the species reported in Balcombe *et al.*, 2005) was calculated using the Levin's Standardized Index: $B_i = \frac{1}{(n-1)}\left[\frac{1}{(\sum_j {p_{ij}}^2)} - 1\right]$, where B_i = standardized index of niche breadth, p_{ij} = proportion of diet of predator i on prey j, and n = total number of prey items (resources). B_i values vary from 0 to 1, where lower values indicate prey specificity and higher values indicate equal proportions of prey consumption (generalist prey consumption) (Krebs 1989).

To evaluate dietary overlap among O. *lineolata* and Lake Eyre Basin species for which dietary data was available, Schoener's Index was used calculated as: $T_{x,y} = 1 - 0.5\left(\Sigma\left|P_{x,i} - P_{y,i}\right|\right)$, where $T_{x,y}$ = Schoener's Index of Dietary Overlap between species x and y, $P_{x,i}$ and $P_{y,i}$ the total proportion of the prey item i in the diet of species x and y, respectively. T varies between 0 and 1 where 0 indicates no overlap and 1 indicates all prey items are found in equal proportions (Schoener, 1970). Values of $T \geq$ 0.60 are considered to represent significant biological overlap (Pusey and Bradshaw 1996).

Dietary overlap was also calculated using Pianka's Index using the equation: $O_{x,y} = \sum P_{i,x}P_{i,y} \Big/ \sqrt{\left(\sum P_{i,x}^2 \times \sum P_{i,y}^2\right)}$, where $O_{x,y}$ = Pianka's index of niche overlap between species x and y, $P_{i,x}$ = the proportion of the i^{th} resource in the diet of species x, and $P_{i,y}$ = the proportion of the i^{th} resource in the diet of species y. Pianka's index follows the same boundaries as the Levin's Standardized Index and Schoeners index above, where 0 indicates total separation and 1 equals total overlap (Pianka 1973).

A basic assumption of these dietary indices is that all prey items were equally accessible to all species in both our dietary analysis of *O. lineolata*, and that of Balcombe *et al.* (2005).

Table 1. Length-weight equations used to calculate fish weight (g) from total length (mm) in order to calculate standardised fish biomass for each species per waterhole, per sampling occasion

See main text for methods. L_T, total length (mm); W, weight (g); ^, Exotic species; #, Hypseleotris species complex identified to genus. *Hypseleotris klunzingeri* used for length-weight equation

Family *Species*	**Length-Weight Equation**	**Reference**
Clupeidae		
Nematalosa erebi (Günther, 1864)	$W = (0.0193/10^{2.798})\ L_T^{\ 2.798}$	Sternberg and Cockayne (2015)
Retropinnidae		
Retropinna semoni (Weber, 1895)	$W = 0.5x10^{-5}\ (0.9414(L_T) - 1.2888)^{3.227}$	Pusey *et al.* (2004); Sternberg *et al.* 2014
Melanotaeniidae		
Melanotaenia splendida tatei (Zietz, 1896)	$W = 2.01x10^{-5}\ (0.8002(L_T) + 2.5490)^{3.020}$	Pusey *et al.* (2004); Sternberg and Cockayne (2015)
Percichthyidae		
Macquaria sp.	$W = (0.0117/10^{3.077})\ L_T^{\ 3.077}$	Sternberg and Cockayne (2015)
Ambassidae		
Ambassis muelleri Kulnzinger 1879	$W = 2x10^{-5}\ (0.8697(L_T) + 0.4577)^{3.119}$	Pusey *et al.* (2004); Sternberg *et al.* 2014
Plotosidae		
Neosilurus hyrtlii Steindachner, 1867	$W = (0.0045/10^{3.179})\ L_T^{\ 3.179}$	Sternberg and Cockayne (2015)
Porochilus argenteus (Zeitz, 1896)	$W = (0.0071/10^{2.881})\ L_T^{\ 2.881}$	Sternberg and Cockayne (2015)
Neosiluroides cooperensis Allen & Feinberg, 1998	$W = 2.78x10^{-6}\ (0.9739(L_T) - 5.0780)^{3.221}$	Sternberg *et al.* (2014)
Terapontidae		
Amniataba percoides (Günther, 1864)	$W = (0.0189/10^{2.937)}\ L_T^{\ 2.937}$	Sternberg and Cockayne (2015)
Bidyanus welchi (McCulloch & Waite, 1917)	$W = (0.0064/10^{3.336)}\ L_T^{\ 3.336}$	Sternberg and Cockayne (2015)
Leiopotherapon unicolor (Günther, 1864)	$W = (0.0107/10^{3.149)}\ L_T^{\ 3.149}$	Sternberg and Cockayne (2015)
Scortum barcoo (McCulloch & Waite, 1917)	$W = (0.0093/10^{3.233)}\ L_T^{\ 3.233}$	Sternberg and Cockayne (2015)
Eloeotridae		
Hypseleotris spp.#	$W = 6x10^{-6}\ (0.7951(L_T) + 1.5102)^{3.318}$	Pusey *et al.* (2004); Sternberg *et al.* (2014)
Oxyeleotris lineolata [Steindachner, 1867]	$W = (0.0146/10^{3.106})\ L_T^{\ 3.106}$	This study.
Cyprinidae		
Carassius auratus (Linnaeus, 1758)^	$W = (0.0318/10^{2.834})\ L_T^{\ 2.834}$	Sternberg and Cockayne (2015)
Poeciliidae		
Gambusia holbrooki Girard, 1859^	$W = 3.98x10^{-6}\ L_T^{\ 3.33}$	Froese and Pauly (2017)

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