Biology and Reproductive Ecology of the Endangered Cooper Creek Catfish (*Neosiluroides cooperensis*) and Implications for Its Conservation

Kate Burndred[1] and David Sternberg[2]

Abstract

The Cooper Creek catfish (*Neosiluroides cooperensis*) is an endangered species, endemic to the Cooper Creek catchment of the Lake Eyre Basin in Central Australia. The species is considered at risk from a range of significant biological and anthropogenic stressors, including the recent, rapid spread of translocated sleepy cod (*Oxyeleotris lineolata*) throughout its range. Little is known of *N. cooperensis* biology and ecology due to its cryptic nature and restricted distribution within a remote geographical landscape. This study undertook targeted sampling to collect critical biological information, to better evaluate the species' response to current and future threats. Despite a low catch rate, some important biological observations were made. Notably, a ripe female was collected (TL: 409 mm, W: 575.5 g) with eggs ranging in size from 2.48 mm to 3.30 mm, and an estimated fecundity of 4370 eggs. Patterns in reproductive biology indicate the species is likely to be an annual batch spawner, possibly cued by early summer storms. Dietary analysis showed a narrow diet [Levins' standardised niche breadth: 0.33 (B_A)] dominated by gastropods and bivalves. Findings from this study provide significant new information regarding the species' reproductive biology and ecology, in particular life-history similarities and dietary overlap with invasive *O. lineolata*. Our findings validate some of the perceived threats to *N. cooperensis* and will enable future work to accurately assess risks to population viability. Ultimately, these findings will be integral to the development of a conservation plan for Cooper Creek catfish.

Keywords: Lake Eyre Basin, Plotosidae, endemic species, sleepy cod (*Oxyeleotris lineolata*), life history

[1] *Water Services, Department of Regional Development, Manufacturing and Water, Mackay, QLD 4740, Australia* (Kate.Burndred@rdmw.qld.gov.au)
[2] *Water Services, Department of Regional Development, Manufacturing and Water, Townsville, QLD 4810, Australia* (David.Sternberg@rdmw.qld.gov.au)

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Introduction

Neosiluroides cooperensis (Allen & Feinberg, 1998) is endemic to the Cooper Creek system in the Lake Eyre Basin drainage in Central Australia. This monotypic genus belongs to the Plotosidae family and shares an ancient lineage with other catfish species found across the Basin [i.e. *Neosilurus hyrtlii* Steindachner, 1867; *Neosilurus gloveri* Allen & Feinberg, 1998; and *Porochilus argenteus* (Zeitz, 1896)]; however, it remains the sole member of the *Neosiluroides* genus (Wager & Unmack, 2000). The species has important ecological value and cultural significance to local Indigenous groups, the Dieri and the Yandruwandha Yawarrawarrka,

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who traditionally identify *N. cooperensis* as 'Capi' – a customary food source still sought after today (Constable et al., 2015).

Historically, *N. cooperensis* may have been found throughout the wider Lake Eyre Basin waterway network during the wetter climatic phases of the Pleistocene era (Unmack, 2001); however, its present distribution is now restricted to larger, more permanent waterholes of the Cooper Creek system. The species has been recorded from 38 localities within the Cooper catchment, occupying an area of approximately 128 km^2 (Arthington et al., 2019). Contemporary survey data confirm this species is naturally rare across its range, typically representing less than 1% of total catch (DRDMW, 2021). The cryptic nature of *N. cooperensis* and its restricted distribution have resulted in a very limited biological and ecological knowledge base. Sparse catch records (e.g. Balcombe et al., 2007; Kerezsy et al., 2011; DRDMW, 2018) and brief biological observations from captive specimens (e.g. Unmack, 1996) are insufficient to adequately assess the risks to this species from purported threats. Key knowledge gaps remain in relation to reproductive patterns, life-history strategy, trophic status, habitat preferences and movement dynamics.

The species was listed as Endangered as part of a review by the International Union for Conservation of Nature (IUCN) in 2019, due to significant conservation threats (Arthington et al., 2019). The recent introduction of the invasive sleepy cod [*Oxyeleotris lineolata* (Steindachner, 1867)], which has colonised most of the Cooper catchment within a decade of introduction, represents the greatest potential threat to *N. cooperensis* through both predation and competition (Sternberg & Cockayne, 2018). Furthermore, under a conservative climate scenario, waterhole persistence is predicted to decrease over time (Cockayne, 2021), which will reduce available habitat for *N. cooperensis* and likely compound competition with *O. lineolata* (Morrongiello et al., 2011; Arthington et al., 2019).

This paper aims to fill key biological and ecological knowledge gaps for *N. cooperensis* using data obtained from wild specimens. Findings from this study of wild specimens will supplement the recent review of threats to *N. cooperensis* and its listing as Endangered on the IUCN Red List of Threatened Species (Arthington et al., 2019), and this paper will discuss findings in the context of the species' biology, ecology and interspecific relationships with translocated *O. lineolata*. Outcomes from this project will help guide future targeted monitoring activities and assist in the development of long-term recovery plans for *N. cooperensis* in the Lake Eyre Basin.

Materials and Methods

Field Collection

Specimens of *N. cooperensis* were obtained via community and citizen group fishing events, and by routine and targeted fish sampling associated with the Lake Eyre Basin Rivers Assessment (LEBRA) (Table 1). LEBRA is a monitoring program designed to assess the condition of watercourses and catchments in the Lake Eyre Basin, their related natural resources, and those factors likely to affect them such as water resource development and land use change. The main collection method for community events was daytime hook-and-line angling, while LEBRA fish sampling employs two large double-winged fyke nets (10 m wings, 12 mm mesh, 5 m funnels, 1.2 m high; T & L Netmaking, Melbourne, Victoria, Australia) and six small single-winged fykes (3 m wing, 4 mm mesh, 3 m funnel, 0.6 m high; T & L Netmaking) set overnight (<19 hrs set time) (see Sternberg and Cockayne, 2018).

Monitoring sites were selected from rivers across the Cooper Creek catchment, with an emphasis on permanent waterholes representative of the surrounding landscape, hydrology, water chemistry and geomorphic diversity (Thoms et al., 2009). Specimens obtained from LEBRA sampling were collected from fyke nets set near semi-submerged woody debris, at depths of less than 2 m in waterholes with previous records of *N. cooperensis*. Specimens obtained from community events were all angled from within the main channel and littoral zones of waterholes. Stream flow and rainfall records representative of conditions preceding 2019–2020 sampling periods are provided in Figure 1. During the weeks preceding the targeted sampling in November 2019, late spring and early summer storms were occurring across the district – producing small, scattered rainfall events (<10 mm mean hourly total).

Table 1. Location and number of *N. cooperensis* collected between 2019 and 2020 (LEBRA: Lake Eyre Basin Rivers Assessment). All specimens were frozen on site for later analysis, except LEBRA targeted samplings which were processed *in situ* and subsequently frozen.

Activity	Location	Sampling dates	Collection method	Number captured
Community events				
Windorah Yellowbelly Fishing Competition	Cooper Creek at Windorah Bridge (vicinity) (25.3701°S, 142.7429°E)	May 2019	Angling	0
Longreach Sleepy Claw Bust	Thomson River at Apex Park (vicinity) (23.4084°S, 144.2298°E)	May 2019	Angling	0
Isisford Fishing Competition	Barcoo River at Oma Waterhole (24.2875°S, 144.3125°E)	July 2019	Angling	0
Longreach Yellowbelly Fishing Classic	Thomson River at Apex Park (vicinity) (23.4084°S, 144.2298°E)	August 2019	Angling	7
LEBRA targeted sampling	Thomson River at Stonehenge (24.4481°S, 143.3543°E)	November–December 2019	Fyke and seine nets	0
	Thomson River at Longreach weir (23.3645°S, 144.2945°E)			1
	Thomson River at Apex Park (vicinity) (23.4084°S, 144.2298°E)			0
	Thomson River at Camoola (22.9884°S, 144.5078°E)			0
LEBRA annual monitoring	Thomson River at Ag College waterhole (23.3512°S, 144.3292°E)	May–June 2019; August–September 2020	Fyke and seine nets	0
	Towerhill Creek at Lamermoor (21.3431°S, 144.6475°E)			0
	Cornish Creek at Bucksleas (22.4701°S, 144.8745°E)			0
	Barcoo River at Avington Road (24.3078°S, 145.2889°E)			0
	Barcoo River at Killman Waterhole (24.2758°S, 144.3661°E)			0
	Barcoo River at Retreat (25.1855°S, 143.2814°E)			0
	Kyabra Creek at One Mile (25.8482°S, 143.0517°E)			0
	Vergemont Creek at Noonbah (24.0844°S, 143.1285°E)			0
	Darr River at Darr (23.216°S, 144.0817°E)			0
	Thomson River at Stonehenge (24.4481°S, 143.3543°E)			0
	Cooper Creek at Windorah Bridge (25.3701°S, 142.7429°E)			1
	Wilson River at Noccundra (27.8244°S, 142.5873°E)			0
	Cooper Creek at Durham Downs (27.0524°S, 141.9037°E)			1

A post-winter flow event occurred in the upper and mid reaches of Cooper Creek in early November and lasted for approximately two weeks, before the channels returned to zero flow.

All *N. cooperensis* specimens were euthanised in an ice slurry at the point of capture and transferred frozen to the laboratory for entire processing, except for specimens collected during targeted monitoring which were processed immediately to assess reproductive stage and then frozen for later analysis. All non-target fish species were removed from nets upon collection and returned to the water immediately.

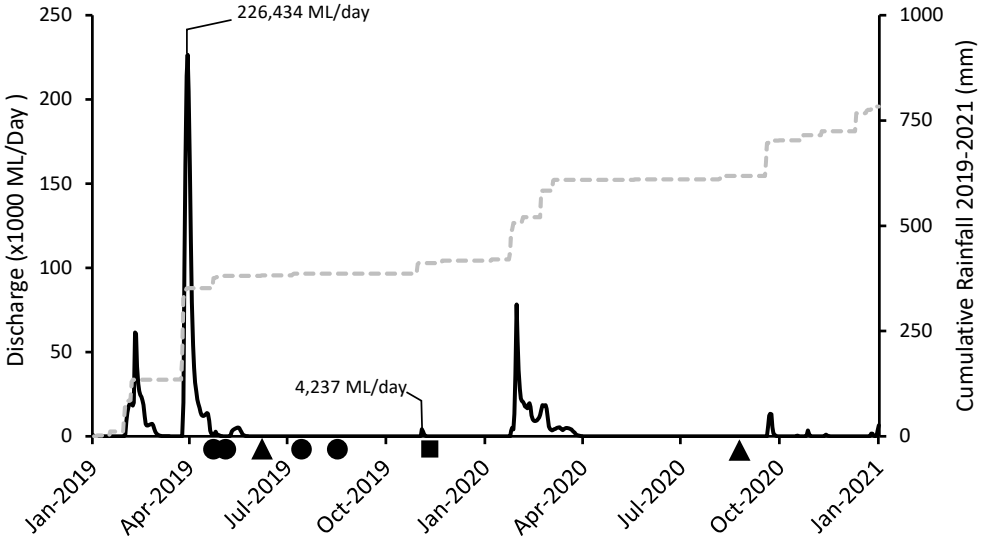

FIGURE 1. Discharge (ML/day: black line) and rainfall (mm/day: grey dashed line) representative of conditions recorded in Cooper Creek throughout sampling activities (GS 003203A; Thomson River at Stonehenge). Symbols below axis: Circle = Community sampling events; Square = LEBRA targeted sampling; Triangle = LEBRA annual monitoring. See Table 1 for details of sampling locations.

Laboratory Processing

Once in the laboratory, frozen fish samples were defrosted and excess water removed. The sex of fish was determined by examining the urinogenital papilla (Unmack, 1996). Morphometric characters were measured to the nearest millimetre using vernier callipers, according to Pusey et al. (2004). Wet weight was determined to the nearest 0.01 g. A length–weight relationship was obtained by applying the exponential regression equation: $W = a(SL)^b$, where W = Weight (g), SL = Standard Length (mm), a = the intercept and b = the slope of the log-transformed linear regression (Sternberg & Cockayne, 2015).

To remove the gonads, an incision was made from the urinogenital pore through the pelvic girdle towards the head, exposing the abdominal cavity. Gonads were then dissected, weighed, and staged according to Pusey et al. (2004). Mature gonads were inspected, and where possible, egg diameter was measured to the nearest 0.1 mm and egg weight was determined to the nearest 0.01 g. The morphology of eggs was closely inspected under a compound microscope (ZEISS Axiolab5, Carl Zeiss Pty Ltd, Germany) and described in detail. Gonadosomatic index (GSI) is a metric that represents the gonad mass as a proportion of the total body mass and was calculated as: GSI = (Weight[gonad] ÷ Weight[wet]) × 100 for mature males and females (see Pusey et al., 2004). An increased GSI value indicates further development of the gonads. GSI of eviscerated individuals was also calculated using the formula: GSI[eviscerated] = (Weight[gonad] ÷ Weight[eviscerated]) × 100 for ripe males and females. Summary statistics (mean, standard error and range) were generated for all reproductive traits

for Stage I, II, III, IV and V males and females. Fecundity was estimated using the gravimetric method: after determining the weight of the ovary, three small samples of 0.1 g each were taken from the anterior, middle and posterior of the ovary. The number of ova in each sample was counted, and total number of ova calculated using the formula: Fecundity = (average number ova from sample) × (total ovary weight) ÷ 0.1.

Stomach contents were removed by dissecting the digestive tract between the œsophagus and the intestine, and eviscerating prey items into a bag. Contents were then weighed 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 fishes, macrocrustaceans, microcrustaceans, other aquatic invertebrates, terrestrial invertebrates, terrestrial vertebrates, plants, 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. Eviscerated fish weight was calculated as: Weight[eviscerated] = Weight[wet] – (Weight[gonad] + Weight[prey]), which includes the stomach tissue but not the stomach contents (i.e. prey items).

Results

Sample Size

Ten specimens (3 males, 7 females) were sampled from four sites across three sampling events (June 2019, August 2019, November 2019). Total length ranged from 196 mm to 409 mm (mean 328.5 mm), and weight ranged from 51.5 g to 575.50 g (mean 319.15 g). The standard length–weight relationship was derived from the log-transformed linear regression: W = 3.3473(SL) – 5.8828, and best described as: $W = 1{\times}10^{-5.8828}(SL)^{3.3473}$, $R^2 = 0.97$, $P < 0.001$, $n = 10$.

Reproductive Biology

Sexual dimorphism was restricted to differences in urinogenital papilla shape: females having a smooth, rounded triangular shape; males having a longer, tapered, cylindrical shape.

Two *N. cooperensis* collected in June 2019 were both relatively small males (L_T = 196 mm, W_W = 51.5 g and L_T = 302 mm, W_W = 142.5 g) in immature (GSI_E = 0.20%) and early-developing (GSI_E = 0.14%) stages, respectively. One immature male (L_T = 223 mm, W_W = 78 g, GSI_E = 0.13%) and six females ranging from early to late developing stage (GSI_E = 0.56%–3.41%) were collected in August 2019 (Table 2). For females where developing ova were measurable, the standard length–fecundity relationship was derived from the log-transformed linear regression: F = 5.638(SL) – 10.771, and best described as: $F = 1{\times}10^{-10.7707}(SL)^{5.6368}$, $R^2 = 0.73$, $P < 0.05$, $n = 6$.

The tenth specimen collected in November 2019 was a gravid female (L_T = 409 mm, W_W = 575.5 g, GSI_E = 8.91%) (Figure 2; Table 2). No eggs were exuded from the gravid specimen when firm pressure was applied, and no eggs were present in the oviduct upon dissection. The paired ovaries were turgid, well vascularised, approximately equal in size, weight and shape, and uniform in texture. *In situ* ova were evenly distributed from the posterior to the anterior of the ovary, and their size and shape were relatively consistent; little atresia was observed (<1.0 %). The eggs were spherical, translucent, bright amber in colour, and slightly adhesive with no oil droplets or observable surface structures. Mature eggs ranged in diameter from 2.48 mm to 3.30 mm (mean egg diameter = 2.96 mm), and weight from 0.0129 g to 0.0164 g (mean egg weight = 0.0147 g). Total fecundity was estimated to be 4370 eggs.

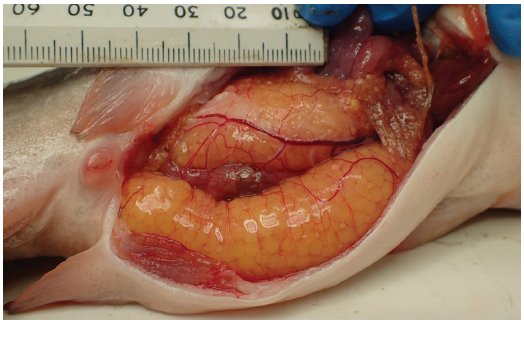

FIGURE 2. Peritoneal cavity of Stage V, gravid female *N. cooperensis* (LT = 409 mm) showing posterior urinogenital papilla (left of image), paired ovaries with vascular network (centre), and visceral organs with mesentery containing larval parasitic nematode (unknown species) (right). Scale in millimetres.

Table 2. Reproductive parameters derived from female *N. cooperensis* specimens (n = 7). Data presented are minimum–maximum (mean ± standard error). Total length (L_T); wet weight (W_W); eviscerated gonadosomatic index (GSI_E); maturity stages follow Pusey et al. (2004).

Parameter	Stage I	Stage II	Stage III	Stage IV	Stage V
Count (*n*)	1	2	2	1	1
L_T (mm)	371	336–358 (347 ± 11)	378–391 (384.5 ± 6.5)	321	409
W_W (g)	461	297–376 (337 ± 39)	453–483.5 (468.3 ± 15.25)	273	575.5
GSI_E (%)	0.563	0.80–0.96 (0.88 ± 0.08)	1.73–2.04 (1.89 ± 1.56)	3.41	8.91
Egg weight (g)	—	(0.0008 ± 0.00005)	(0.0011 ± 0.0001)	(0.0072 ± 0.0005)	(0.0145 ± 0.0004)
Egg diameter (mm)	<0.1–0.8	0.4–1.4 (0.87 ± 0.05)	0.6–1.5 (1.10 ± 0.06)	1.8–2.6 (2.36 ± 0.09)	2.48–3.30 (2.96 ± 0.09)
Total fecundity	—	1826–3972 (2899 ± 1073)	4013–6177 (5094 ± 1082)	1235	4370

Targeted sampling between November and December 2019 found individuals of the catfishes *N. hyrtlii* and *P. argenteus* to be gravid and running ripe, gauged by distended bellies with some exuding spat and spawn when slight pressure was applied. This was confirmed by dissecting a sample of individuals. Some specimens of *O. lineolata* were also observed to be gravid, running ripe and spent; also gauged by the swollen appearance of bellies and confirmed by dissecting a sample of individuals.

Dietary Analysis

All *N. cooperensis* specimens had dietary matter in their stomachs, and fullness ranged from 10% to 70% (Table 3). Gut fullness was not influenced by fish size (W_W: F = 1.55, p >0.05, R^2 = 0.163; L_T: F = 1.78, p >0.05, R^2 = 0.161). Levins' standardised niche breadth was low (B_A = 0.33). *Neosiluroides cooperensis* diet was dominated by molluscs (*Velesunio* spp., *Notopala* spp.) and macrocrustaceans (*Macrobrachium australiense*) in most individuals (Table 3). No evidence of piscivory or consumption of terrestrial food sources was observed.

Morphology

All *N. cooperensis* specimens were in excellent condition, with no external signs of disease or injury at time of capture. Upon dissection, the eggs and larvae of parasitic nematodes (unknown species) were observed throughout the mesentery of the abdominal cavity of eight specimens (Figure 2).

Table 3. Diet breadth and composition of 10 wild-caught *N. cooperensis* from the Cooper Creek catchment. Data are minimum–maximum (mean ± standard error).

Parameter (unit)	
Count (*n*)	10
Total length (mm)	196–409 (328.5 ± 21.2)
Weight (g)	51.50–575.50 (319.15 ± 54.5)
Gut fullness (%)	10–70 (34 ± 6.4)
Gut weight (g)	1.90–18.30 (9.10 ± 1.7)
Diet category (% contribution)	
Molluscs	0–80 (30 ± 9.4)
Macrocrustaceans	0–80 (25 ± 8.9)
Microcrustaceans	0–10 (1 ± 0.9)
Other aquatic invertebrates	0–10 (1 ± 0.9)
Vegetation	0–20 (7 ± 1.7)
Algae	0–5 (1 ± 0.5)
Detritus	0–15 (5 ± 1.6)
Unidentified	0–100 (30 ± 9.8)
Levins' standardised niche breadth (B_A)	0.33

Observation of the buccal cavity showed two discrete patches of villiform maxillary teeth under the fleshy upper lip, and a circular vomerine tooth patch consisting of clustered smooth molariform

teeth (Figure 3 and Figure 4). The lower jaw contained a narrow band of villiform mandibular teeth behind the inferior lip, leading into a wider patch of irregularly arranged smooth molariform teeth. Two large, elliptical patches of coarse pharyngeal teeth border the œsophageal opening.

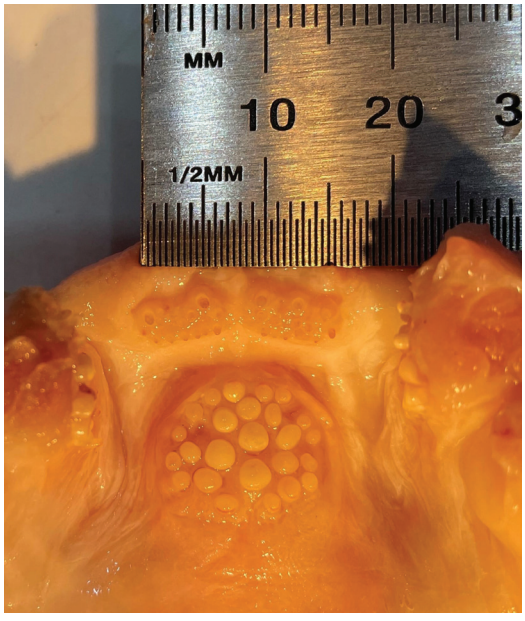

FIGURE 3. Dissected upper jaw of adult *N. cooperensis*, demonstrating the arrangement of the maxillary and vomerine tooth patches.

FIGURE 4. Dissected upper jaw of adult *N. cooperensis*, showing sharp villiform teeth which sit under the fleshy upper lip.

Discussion

This study has provided the first confirmed evidence of a wild, gravid female *N. cooperensis* and offers novel biological and life-history information relating to the trophic and reproductive ecology of this species. Considering the recent declaration by the IUCN that *N. cooperensis* is Endangered, this information is valuable for quantifying the potential interspecific threats from *O. lineolata* in the Lake Eyre Basin.

The detailed observations from a ripe specimen help augment some of the existing hypotheses regarding the species' spawning strategies and question current theories pertaining to their breeding biology. The eggs from the wild specimen were smaller than reported for a captive specimen of similar size: 2.48 mm to 3.30 mm (mean = 2.96 mm), c.f. 2.75 mm–4 mm (Unmack, 1996); and not significantly larger than the eggs of other Australian plotosids (Pusey et al., 2004; Wager & Unmack, 2000). The eggs were strongly demersal, lacking oil constituents, and although they were slightly adhesive, possessed no adhesive structures suggesting they are adapted to settle onto substrate. The uniform distribution of mature eggs throughout the ovary indicates that *N. cooperensis* is indeed likely to spawn annually (Kereszy, 2010), in one episode or in successive batches over a short time frame (i.e. days to weeks).

The theory that *N. cooperensis* spawns in summer, at water temperatures around 26°C (Unmack, 1996), is supported by previous observations of female *N. cooperensis* with distended bellies consistent with egg production, recorded during early summer (Kerezsy, 2010; DRDMW, unpublished data). Furthermore, a single male displaying a swollen, highly vascularised urinogenital papilla has been recorded in November (DRDMW, unpublished data). During targeted sampling, abundant, medium-bodied, non-target species were observed ripe and running ripe (*P. argenteus*, *N. hyrtlii* and *O. linoleata*), suggesting local conditions had triggered gonad development and fish were preparing to spawn. Sampling was undertaken in the weeks following several small rainfall events; water levels were slowly receding, daily mean water temperatures ranged from 27.3°C to 34.0°C, and occasional storms were occurring throughout the Cooper catchment daily. Preparation for a synchronous

spawning event associated with these 'optimal' conditions would ensure maximum exploitation of invertebrate resources following within-channel flow pulses, and greater chances of juvenile recruitment (Humphries et al., 1999; King, 2004). It therefore seems most likely that *N. cooperensis* spawns on an annual cycle in the warmer summer months, adapted to take advantage of early or intermittent summer flows that drive increased productivity within waterholes (Welcomme et al., 2006; Kerezsy, 2010; Kerezsy et al., 2011). Unmack (1996) observed that in comparison to other Australian plotosids, Cooper Creek catfish have a much lower fecundity with significantly larger eggs and may be capable of mouthbrooding. The present study recorded smaller egg size and higher fecundity in a mature wild-caught female; therefore, it seems that mouthbrooding is less plausible. The theory should not be discredited, though, until ripe and/or spent male specimens are closely inspected.

In comparison to other plotosids, the morphology of *N. cooperensis* eggs and ovaries is remarkably similar to *Tandanus tandanus* (Mitchell, 1838), which are nest-building, annual batch spawners that provide a high level of parental care (Burndred et al., 2017). Relatively stable flow conditions are required during the early development phase of *T. tandanus* larvae, when males closely guard and protect their brood, a strategy that seems unlikely to apply to *N. cooperensis* due to their opportunistic spawning associated with unpredictable hydrology. In contrast, the co-occurring *N. hyrtlii* is a widely recognised annual flood spawner (e.g. Kerezsy et al., 2011), broadcasting a comparatively high number of smaller, strongly demersal eggs, which develop in substrate without parental care (Orr & Milward, 1984). The way in which male and female *N. cooperensis* coalesce in highly turbid conditions, the mechanisms of spawning and fertilisation, the duration of egg and larval development, and the mode of parental care (if any) remain largely unknown.

Dietary analysis confirms that *N. cooperensis* is an invertivore favouring mussels, macroinvertebrates and snails (Unmack, 1996; Wager & Unmack, 2000). The positioning of the underslung mouth, lined with bands of villiform teeth, is well suited for striking benthic prey, and the form and arrangement of vomerine teeth in the upper buccal cavity are adapted for crushing hard shell and chitin. Importantly, the dietary habits of *N. cooperensis* reveal a relatively high trophic niche overlap with *O. lineolata*. Both species show a dietary preference for macrocrustaceans and other aquatic invertebrates (Pusey et al., 2004); however, in the Lake Eyre Basin, fishes also dominate the diet of *O. lineolata* (Sternberg & Cockayne, 2018). This translocated species was also found to prey on juvenile and small plotosid catfish (*P. argenteus*) (Sternberg & Cockayne, 2018), suggesting that *N. cooperensis* is likely to be impacted by *O. lineolata* through both competition for food and direct predation. These interactions are predicted to intensify during dry periods, when waterholes become isolated and contract to a series of pool habitats. In the Lake Eyre Basin, waterhole persistence is predicted to reduce as much as 30% by 2070 based on current global warming trends (Cockayne, 2021), suggesting the two species will most likely incur increased interactions as available dry season habitat declines throughout the Cooper Creek catchment. Furthermore, the strength of this interspecific competition is likely to become more intense due to shifting population structures and changing reliability of shared food resources, as seen in other catchments (Olden et al., 2008; Morrongiello et al., 2011). Fish assemblage shifts caused by the incursion and integration of *O. lineolata* have been postulated by Kerezsy et al. (2014) and Sternberg & Cockayne (2018).

Although several other medium-bodied species were collected from nets during targeted monitoring, *O. lineolata* dominated catch at all sites. This was despite high sampling effort in waterholes with relatively consistent *N. cooperensis* catch rates, at a time when the species was most likely to be active (DRDMW, unpublished data). It is accepted that *O. lineolata* has colonised most of the Cooper catchment over the last decade (Sternberg & Cockayne, 2018), and although the unexpectedly low *N. cooperensis* abundance recorded in this study may simply be due to chance, it may represent a tangible shift in fish population structure, particularly in major waterholes.

The presence of mature, running ripe and spent *O. lineolata* in all targeted waterholes is of notable concern. This study provides further evidence of niche overlap with *N. cooperensis*, where mature adults may be competing for habitat, specifically

during spawning, and developing larvae and juveniles may compete for resources following flood events. *Oxyeleotris lineolata* are highly fecund serial spawners (Herbert & Graham, 2004), which are likely to spawn over a prolonged period in the Cooper Creek catchment, particularly when storms are prevalent. Larvae develop rapidly and are capable of exogenous feeding at hatching (Herbert & Graham, 2004), so the likelihood of larval and juvenile *O. lineolata* overlapping with larval and juvenile *N. cooperensis* is very high. Interspecific competition that persists across multiple life stages is likely to place substantial pressure on native fish species, particularly in times of prolonged drought and environmental stress when resources are limited. Furthermore, it is unclear to what extent other factors such as meso- and microhabitat segregation may influence the strength of interspecific niche overlap throughout the species' life stages. Further detailed research into the early life history of *N. cooperensis* will help to identify critical requirements for their successful recruitment, and therefore enable measurement of the intensity and impacts of competition with young-of-year *O. lineolata*.

Identifying the factors that give introduced species competitive advantages or disadvantages over native species is a key requirement for determining and managing the threat of alien fish species, particularly under future climatic scenarios. Given their predominantly benthic habitat, and confirmed dietary and reproductive overlaps, *O. lineolata* and *N. cooperensis* are highly likely to interact frequently, particularly during times of waterhole drying. Under these conditions, *O. lineolata* would be expected to maintain a competitive advantage over *N. cooperensis* in the short term, due to its dietary adaptability and more aggressive nature. However, further reproductive information is required for both species to quantify the long-term population viability of *N. cooperensis* in the Lake Eyre Basin.

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Author Profiles

Kate Burndred is an Aquatic Ecologist at the Department of Regional Development, Manufacturing and Water in Mackay, Australia. She has studied the early life history of freshwater fishes for over 20 years across Australia's inland waterways and is interested in the links between fish reproductive biology, recruitment and the environment.

David Sternberg is an Aquatic Ecologist at the Department of Regional Development, Manufacturing and Water in Townsville, Australia. His main research interests are associated with the persistence of freshwater fishes in arid and semi-arid regions, fish life-history diversity and its functional relationship with gradients of environmental variation.