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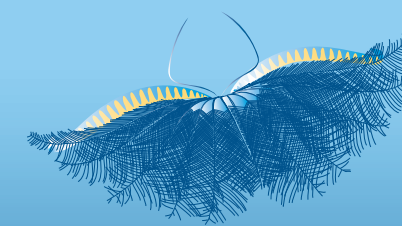
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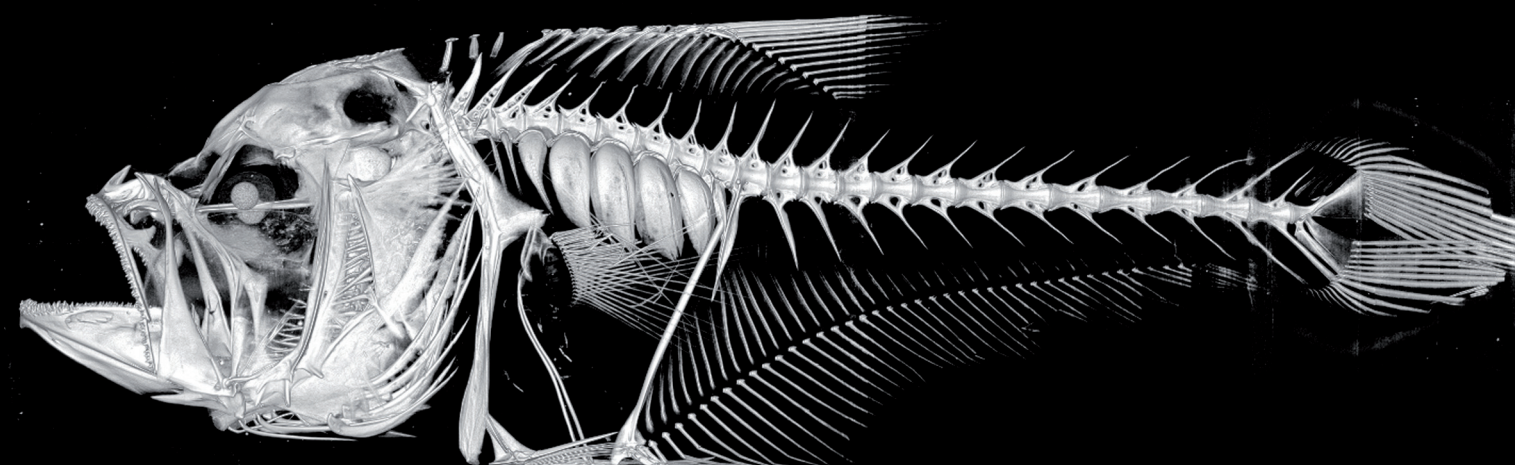
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EVOLUTIONARY, MOLECULAR AND COMPARATIVE ZOOLOGY

Larval nurseryfish, *Kurtus gulliveri* (Perciformes : Kurtidae), in the Adelaide River of the Northern Territory: their season, fellow travellers and unusual rib anatomy

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Abstract. This study was undertaken to determine the spawning season of the nurseryfish, a species with a unique method of parental care found only in northern Australia and southern New Guinea. Monthly samplings with an ichthyoplankton net at the same locality in the Adelaide River over multiple years yielded no larval nurseryfish in February, March, April and May. Larvae first appeared in June and were collected each month until January. From this we conclude that the nurseryfish spawning season in the Adelaide River is June–January. Twelve other larval fish species from 10 families were collected in the plankton tows with nurseryfish. Larval nurseryfish are readily identified and separated from other larval fishes by their peculiar rib anatomy, an important diagnostic character of *Kurtus*. The development of the unusual rib anatomy is examined with three-dimensional micro-computerised tomography scans of 12–21-mm standard length (SL) specimens. The bony protection of the swim bladder formed by the ribs is visible in the smallest postflexion specimens examined and is essentially complete by 19 mm SL. These ecological and anatomical observations add another pixel to the big picture of nurseryfish life history.

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Introduction

Nurseryfish are unique among fishes in that the males carry the egg mass on a supraoccipital hook (Berra *et al.* 2004), a system known as forehead brooding (Balon 1975). Field work on the life history of *Kurtus gulliveri* was begun in 2001. Prior to this, the most recent papers on the biology of this species date back to Weber (1913: pl. 12–14) and de Beaufort (1914). The species is euryhaline (Crook *et al.* 2015) and occurs in rivers in northern Australia and southern New Guinea (Berra 2003). A second species, *Kurtus indicus*, occurs in southern Asia, but its reproductive behaviour is unknown. *K. gulliveri* is a predatory species, with a diet consisting of insects, prawns and fishes (Berra and Wedd 2001). Longevity is known to be up to four years (Berra and Aday 2004) and maximum size recorded is 590 mm standard length (SL) (Weber 1913) but is more commonly seen up to 333 mm SL (Berra 2003).

The histology of the supraoccipital hook on which the males carry the eggs was discussed by Berra and Humphrey (2002). They showed that the bony hook contains a cavity and suggested

that the egg mass is held in place by engorgement of the fleshy tissue around the hook with blood supplied from within the cavity. Crypts within the tissue under the hook grip the chorionic filaments of the egg mass and hold it in place. He *et al.* (2016) illustrated how the male's hook forms by resorption and growth of various components of the supraoccipital bone. The unusual rib and swim bladder anatomy of adult specimens was described by Carpenter *et al.* (2004).

Early life history of nurseryfish was reviewed by Berra and Neira (2003), and drawings of stages from fertilised egg to yolk-sac fry to miniature adults were presented. Berra *et al.* (2007) recorded preliminary data on spawning seasonality, but their report did not include all months of the year, thus necessitating the current paper. The purposes of this paper are to define the spawning season based on plankton tows in every month of the year, to provide a list of other fish species found in the plankton with nurseryfish, and to show the rib development, which is a diagnostic taxonomic feature, at a larval size in a three-dimensional micro-computerised tomography (CT) scan.

Materials and methods

Ichthyoplankton tows utilising a 500- μm mesh net with a 50-cm² mouth and a length of 2 m were made in the Adelaide River from the Arnhem Highway bridge upstream in the direction of the mouth of Marrakai Creek (12°40.95'S, 131°20.030'E), a distance of ~2.0 km. Tows were made just below the surface at a boat speed of ~2 km h⁻¹ during daylight from 0900 until 1500 hours. Each tow lasted 20 min. A river map is given in Berra (2003).

A total of 22 tows, representing all months, was made from 2001 to 2013. To the best of our ability we maintained sampling effort, location, and duration of tows constant but variations

between samples could be due to a variety of factors such as tidal movements, spring versus neap tides, discharge rates related to wet/dry seasons, etc. The larval fish were preserved in 10% formalin and later identified using Carpenter and Liem (1998), measured and deposited in the Northern Territory Museum. As an interesting aside, it should be noted that on at least two occasions the moving plankton net attracted the attention of the large saltwater crocodiles, *Crocodylus porosus*, that thrive in the Adelaide River, home to the 'Jumping Crocodile' tours. A 5-m crocodile charged the net, pushing a bow wave ahead of its snout. We had to abandon such tows and wait until the crocodile moved on.

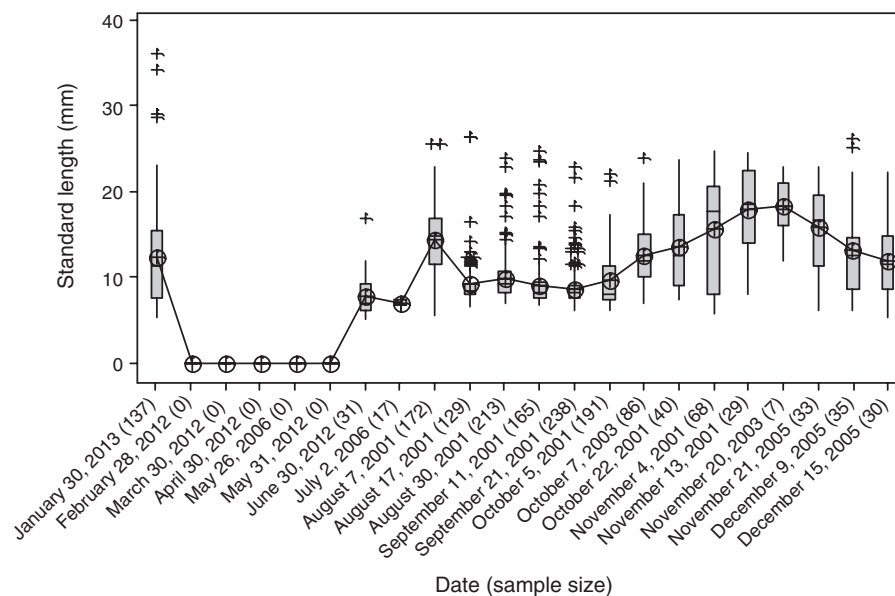


Fig. 1. Standard length (mm) of larval nurseryfish and sample size collected on various dates over a twelve-year period. The box in the boxplot represents the middle 50% of lengths with 25% above and 25% below it. Means are indicated by circles. Medians are shown by horizontal lines and ranges are indicated by vertical lines. Outliers are denoted by plus signs. Outliers are defined to be standard lengths more than 1.5 box-lengths beyond the edges of the box.

Table 1. Larval fish species collected from the Adelaide River during 2001–13 in 20-min plankton tows with a 500- μm mesh, 2-m-long net with a 50-cm² mouth
n, no. of specimens caught

Family	Species	Common name	<i>n</i>	Length (mm SL)
Engraulidae	<i>Thryssa brevicauda</i> Roberts	Short-tail thryssa	30	15–26
	<i>Thryssa</i> sp.		120	7–25
Ariidae	<i>Nemapteryx armiger</i> (De Vis)	Copper catfish	1	50
Mugilidae	<i>Ellochelon vaigiensis</i> (Quoy & Gaimard)	Diamond-scale mullet	1	4
Ambassidae	<i>Ambassis interruptus</i> Bleeker	Longspined glassfish perchlet	3	18–32
Leiognathidae	<i>Leiognathus equulus</i> ^A (Forsskål)	Common ponyfish	1	9
Sciaenidae	<i>Johnius novaeguineae</i> (Nichols)	Paperhead croaker	6	15–41
Toxotidae	<i>Toxotes chatareus</i> (Hamilton-Buchanan)	Seven-spot archerfish	4	8–14
Gobiidae	<i>Caragobius rubristriatus</i> (Saville-Kent)	Red eelgoby	1	76
	<i>Gobiopterus</i> spp.		26	6–8
	<i>Periophthalmus darwini</i> Larson & Takita	Darwin's mudskipper	1	8
Kurtidae	<i>Kurtus gulliveri</i> Castelnau	Nurseryfish	1621	5–36
Soleidae	<i>Leptachirus darwinensis</i> Randall	Tailed sole	3	4–4

^AIdentification based on adults reported by Berra *et al.* (2004).

The specimens used for CT examination were obtained from the Northern Territory Museum in Darwin (NTM S.14644–005 12, 15, and 21 mm SL, Wildman River, Northern Territory, May 1998). Synchrotron radiography and microtomography were done at the Shanghai Synchrotron Radiation Facility utilising 14.0 keV monochromatic X photons. The synchrotron microtomography was performed according to previously published protocol (He *et al.* 2013).

Results and discussion

Fig. 1 plots the standard length of larval nurseryfish and number collected each month over a twelve-year period (2001–13). No nurseryfish were taken in the plankton tows during February through May. They first appear in June and persist through January. The inherent variability of northern Australian rivers with variation in the timing of rainfall and the length of wet/dry

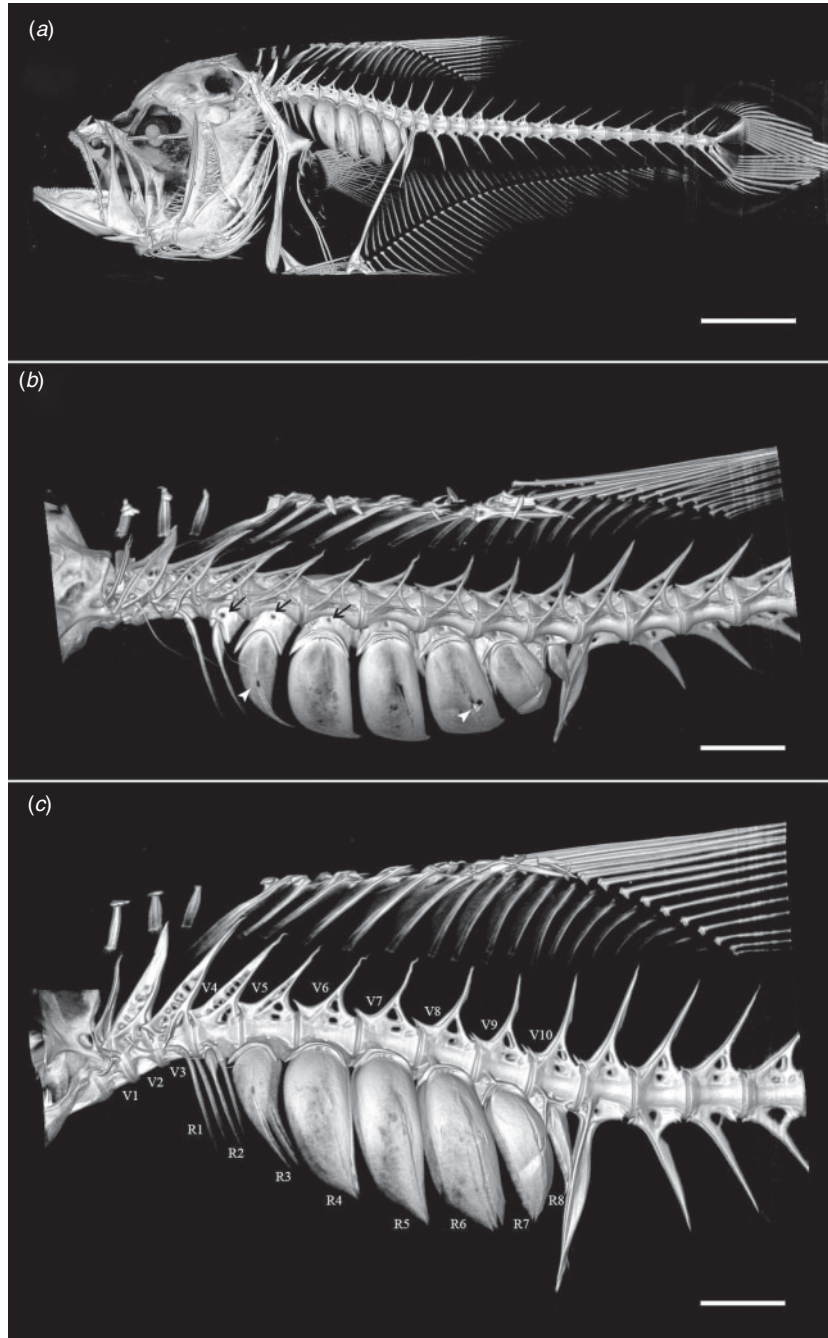


Fig. 2. Three-dimensional CT rendering of late-stage postflexion larva (21 mm SL). Scale bar: 2 mm in (a); 0.75 mm in (b, c). Black arrows indicate foramina of parapophyses and white arrowheads indicate foramina of ribs in (b). Vertebrae and ribs are numbered in (c).

seasons may affect ecological patterns from year to year. The number of larvae collected may also be impacted by neap versus spring tides or incoming versus outgoing tides. We also do not know the duration of egg carrying. With those qualifications in mind, Fig. 1 strikingly demonstrates that the spawning season of *K. gulliveri* falls between June and January based on the data collected. It is reasonable to assume that other Northern Territory river systems may have a similar pattern, give or take a month to account for the local conditions, but seasonal data on larval fish in Northern Territory rivers are lacking. Pusey *et al.* (2015) provided the best analysis to date of the presence of small fishes and their environmental conditions in these rivers.

The constant presence of small larvae in each month from June through January demonstrates that spawning is occurring in those months. The increase in mean size of larvae as the months progress beyond the dry season of June–September shows that the larvae that hatched at the beginning of the spawning season continue to increase in size each month until growth slows down in December and January as the wet season begins and/or until the larvae reach a size that is no longer collected by the plankton tows. Pusey *et al.* (2015), using a much larger beam trawl with a minimum mesh of 6 mm in the South Alligator River of the Northern Territory caught 4415 individuals of 81 taxa including 72 *K. gulliveri*, the juveniles of which were present only in the dry season. This is in keeping with our results.

Because of the complexity of the Adelaide River system, it is not possible at this time to say what initiates or halts spawning by nurseryfish. Larval sampling throughout the river from mouth to headwaters is currently underway to identify the salinity range of spawning for this species.

Table 1 lists 13 species from 11 families taken in plankton tows in the Adelaide River. Berra *et al.* (2004) reported 31 fish species from 19 families taken in gill-nets and by electrofishing during the nurseryfish field work. Nurseryfish larvae of all sizes are easily distinguished from other larval fishes in the Adelaide River by their compressed, hatchet-shaped body with a large mouth and head, a very narrow caudal peduncle, and the presence of the ‘rib window’. Nurseryfish are more or less miniature adults by the time they are ~25 mm SL. At this size they are capable of swimming well enough to elude the plankton net, as shown by their relative scarcity as outliers in Fig. 1, or they may also begin to inhabit waters deeper than the surface plankton tows when they reach that size. After nurseryfish, unidentified larval engraulid species were the most frequently collected larvae in the plankton net. In the study by Pusey *et al.* (2015) with a much bigger net in the South Alligator River, sciaenids and engraulids dominated the species richness and abundance.

Some systematised information is available on Australian larval fishes of coral reefs (Leis and Rennis 1983), shore lines (Leis and Trnski 1989), and temperate regions (Neira *et al.* 1998), but knowledge of the larval fish fauna of the Adelaide River and other rivers of the Northern Territory is sorely lacking.

Fig. 2 includes three-dimensional renderings of a later stage postflexion (21 mm SL) larval nurseryfish with emphasis on the ribs. The ‘rib window’ (expanded ribs covering the swim bladder) is a diagnostic feature of the Kurtidae (Berra 2003; Carpenter *et al.* 2004). The peculiar rib configuration of this species is apparent from ~9 mm SL. The osteological basis of the ‘rib window’ is clearly visualised by both radiography and CT

scans. Fraser (2013) compared the skeleton of the two species of *Kurtus*. Both species have the unusually wide ribs protecting the swim bladder. His descriptions, based on radiographs and cleared and stained material, are readily appreciated when examining our Fig. 2.

In adult *K. gulliveri*, there are 10 trunk (precaudal) vertebrae and eight pairs of ribs. The anterior two vertebrae are without pleural ribs, and Vertebrae 3–10 bear ribs. The third to eighth pair of ribs are expanded and protect the swim bladder, whose lobes nest within the concavity of the expanded ribs (de Beaufort 1914; Carpenter *et al.* 2004; Fraser 2013). There are no parapophyses on the first to third vertebral centra, so the first pair of ribs directly articulate with the third vertebral centrum. The fourth to ninth vertebrae bear wing-like parapophyses. The second pair of ribs are expanded slightly at the proximal end, and articulate with the anterior edge of the fourth vertebral parapophysis. The fifth to ninth vertebral parapophyses are well developed and with a central notch. The third pair of ribs are expanded at the proximal end and taper distally, whereas the fourth to seventh ribs are greatly expanded. The third to seventh pair of ribs articulate laterally to the wing-like fifth to ninth vertebral parapophyses. The tenth vertebral parapophysis is not visible, and bears the eighth pair of ribs, which are less expanded than the third to seventh pairs (Fig. 2).

A foramen exists in each parapophysis (Fig. 2b), as described by Fraser (2013; p. 36). It is further noteworthy that foramina also exist in the third to seventh expanded ribs (Fig. 2). The foramen in the anterior expanded ribs are also observed in our micro-CT scanning of adult specimens (He, personal observation). The ossified supraneurals can be seen in Fig. 2.

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