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Larval development and dietary ontogeny of a critically endangered galaxiid within a Mediterranean climatic zone of Australia

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SUMMARY

The taxonomy of critically endangered and geographically isolated populations of the Spotted Galaxias (*Galaxias truttaceus*) from either side of the Australian continent has only recently been resolved with those in Mediterranean-climatic Western Australia considered to be an evolutionary significant unit. Ontogenetic changes in gross morphology, pigmentation, fins and fin folds of wild-caught *Galaxias truttaceus* from south-western Australia are described between post-hatch and juvenile life stage. Newly hatched larvae are 5.3 mm in body length (BL), elongate, lightly pigmented, have well-developed eyes and mouth, a large yolk sac and straight gut. A medial unpaired fin fold extends ventro-distally from myosepta 5-8, around the notochord and then dorso-proximally to myosepta 7-12. The commencement and completion of fin development is C, D, A $\rightarrow$ P $\rightarrow$ V, with the full complement of adult fin meristics present by 27 mm BL. The juvenile life stage is reached at *c* 34 mm BL when pigmentation and body shape are consistent with those of adult stages. Larval *G. truttaceus* can be discriminated from other sympatric congenerics on the basis of pigmentation and the position of the dorsal fin origin proximal to that of the anal fin, and from other sympatric species that are less-elongate and/or have fewer myomeres and are more heavily pigmented. A distinct ontogenetic shift in diet from aquatic to terrestrial prey occurs at transition to juvenile life stage, and presumably reflects both a change in habitat (from lentic to lotic) and the attainment of adult morphology.

Keywords: Galaxiidae, ontogeny, south-western Australia

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INTRODUCTION

The Galaxiidae, a family of Southern Hemisphere fishes containing seven genera and approximately 65 species (Eschmeyer & Fong, 2015), are small (< 25 cm), elongate fishes, restricted to Australia's Mediterranean and cool-mild temperate climatic zones, New Caledonia, New Zealand, South Africa and South America (Raadik, 2014). Australia has the greatest diversity of galaxiids (McDowall & Frankenberg, 1981) and boasts five genera and 37 species (Raadik, 2014; Coleman *et al.*, 2015) that are restricted to the Mediterranean climatic and cooler temperate regions of south-western and south-eastern Australia, including Tasmania. Of the five members of the Galaxiidae found in Western Australia's Mediterranean climatic zone (which includes inland waters within the Southwestern Province and the southern Pilbara Province (see Morgan *et al.*, 2014)), three are endemic to the Southwestern Province; *Galaxias occidentalis* Ogilby 1899, *Galaxiella munda* McDowall 1978 and *Galaxiella nigrostriata* (Shipway 1953) (Allen *et al.*, 2002). The Southwestern Province has exceptional endemicity, with over 80% of all freshwater fishes being unique to the region, and further contains a number of endemic cryptic species awaiting description (Morgan *et al.*, 2014). An additional two galaxiids, which also occur in south-eastern Australia, are *Galaxias maculatus* (Jenyns 1842), the most naturally widespread freshwater fish in the Southern Hemisphere (Berra, 1981), and *Galaxias truttaceus* Valenciennes 1846. These two galaxiids have populations that may be diadromous or landlocked (Humphries, 1989; Morgan *et al.*, 2016).

Although a number of other galaxiids are diadromous, including most populations of *G. truttaceus* in south-eastern Australia (McDowall & Frankenberg, 1981; Humphries, 1989), populations of *G. truttaceus* in the Mediterranean Southwestern Province of Australia are landlocked and potamodromous (Morgan, 2003; Morgan *et al.*, 2005, 2016). Unlike most freshwater fishes in this climatic zone, which spawn during the winter and spring when the majority of precipitation occurs and stream flows peak (Pen & Potter, 1990, 1991), the latter populations of *G. truttaceus* migrate upstream within rivers to spawn in mid-late autumn (Morgan, 2003). The early stages then use downstream lacustrine environments as a nursery before migrating back into the river systems as juveniles (Morgan, 2003; Morgan & Beatty, 2006; Close *et al.*, 2014).

Although migratory fishes are a conspicuous component of coastal riverine fish communities of Australia, biological knowledge of early life history stages of most species remains largely undocumented (Miles *et al.*, 2014). In many cases, this has hampered conservation management and consequently many of Australia's diadromous and potamodromous species are under increasing threat from a range of environmental impacts (Miles *et al.*, 2014). Ecological studies of early life history stages of fish require accurate species discrimination. Larval descriptions are available for only five of the 37 Australian members of the Galaxiidae including *Galaxias olidus* Günther 1866 (Close, 1995), *G. maculatus* (Benzie, 1968; Chapman *et al.*, 2009), *Galaxias occidentalis*, *Galaxiella munda* and *Galaxiella nigrostriata* (Gill & Neira, 1994). In addition to those galaxiid species listed above that occur in Western Australia's Mediterranean climatic zone, larval descriptions are also available to discriminate other species of the region including *Nannatherina balstoni* Regan 1906 (Gill & Morgan, 1998), *Lepidogalaxias salamandroides* Mees 1961 (Gill & Morgan, 1999), *Pseudogobius olorum* (Sauvage 1880) (Neira *et al.*, 1998) and *Afurcagobius suppositus* (Sauvage 1880) (Neira *et al.*, 1998). A developmental series of the critically endangered *G. truttaceus* have not been described and this hampers those ecological studies that require the larvae of sympatric galaxiids to be differentiated.

The ontogenetic changes in gross morphology, pigmentation, fin folds and fins of wild-caught *G. truttaceus* are described between post-hatch and juvenile life stage. Preliminary information on ontogenetic shifts in diet is also presented. The construction of a novel developmental series of larvae will allow for diagnostic characters to be identified for application to future research in field-collected samples and provide a basis for further ecological study.

METHODS

Fish collection

Larvae of *G. truttaceus* were collected from the Moates Lake drainage system, situated 30 km east of Albany on the southern coast of Western Australia (Figure 1). The catchment is drained by two small (< 15 m wide) tributaries; the perennial Goodga River (16 km^2) and Black Cat Creek (4 km^2). Specimens were collected opportunistically between May and July 1999 using a fine mesh (250 µm) sweep net, a conical plankton net (80 cm diameter, 500 µm mesh) and a small seine net (3 mm mesh nets, 5 m length, 1.5 m depth). Additional specimens of early-preflexion stage *G. truttaceus*, collected from the Goodga River between 2013 and 2015 using conical drift nets (500 µm; 0.5 m diameter), were also examined to identify the length at hatching. All these latter specimens had enlarged yolk-sacs and were presumed to be immediately post-hatch. All specimens retained for larval description and dietary analysis were euthanised by anesthetic overdose, fixed in 10% buffered formaldehyde and later preserved in 100% ETOH.

Identification of larvae and juveniles

Larvae were identified as belonging to the Galaxiidae by their elongate body (> 50 myomeres; body depth (BD) ~ 6-12% body length (BL), a long gut (~ 70-85% BL) and presence of a single posteriorly placed dorsal fin (Gill & Neira, 1994, 1998). With the exception of *G. maculatus*, all other sympatric species spawn during winter-spring after the distinct April-May spawning period of *G. truttaceus* (Pen & Potter, 1990, 1991; Morgan *et al*., 1995; Morgan, 2003) and thus only larvae of *G. truttaceus* and *G. maculatus* were expected at the time collections were made, although *G. maculatus* has protracted spawning period with larvae present in the Moates Lake system year round (Chapman *et al*., 2006). Larval stages of *G. truttaceus* were distinguished from all other sympatric species using a developmental series (*sensu* Leis & Rennis, 1983) from a positively identified juvenile to the smallest collected larva using comparisons of general morphology, number of myomeres, morphometrics, pigment patterns, and fin development and meristics, available in previous larval description of sympatric species of the Galaxiidae (Gill & Neira, 1994; Chapman, 2003), Perchythyidae (Gill & Morgan, 1998), Gobiidae (Neira *et al*., 1998).

Larval descriptions

Developmental changes in gross morphology, dorsal and ventral unpaired medial fin folds, fins and pigmentation were documented through preflexion, flexion and postflexion (pre-squamation) stages of larval development (Leis & Trnski, 1989). In addition, a transitional stage was included to represent fish that had attained adult fin meristics, but not coloration nor gross morphology (i.e. broadening of the head and body) typical of juvenile and adult life stages (Allen *et al*., 2002; Morgan *et al*., 2011). For the purposes of this study, individuals with adult meristics, morphology and coloration were considered juveniles (~ 34 mm total length (TL) for *G. truttaceus*).

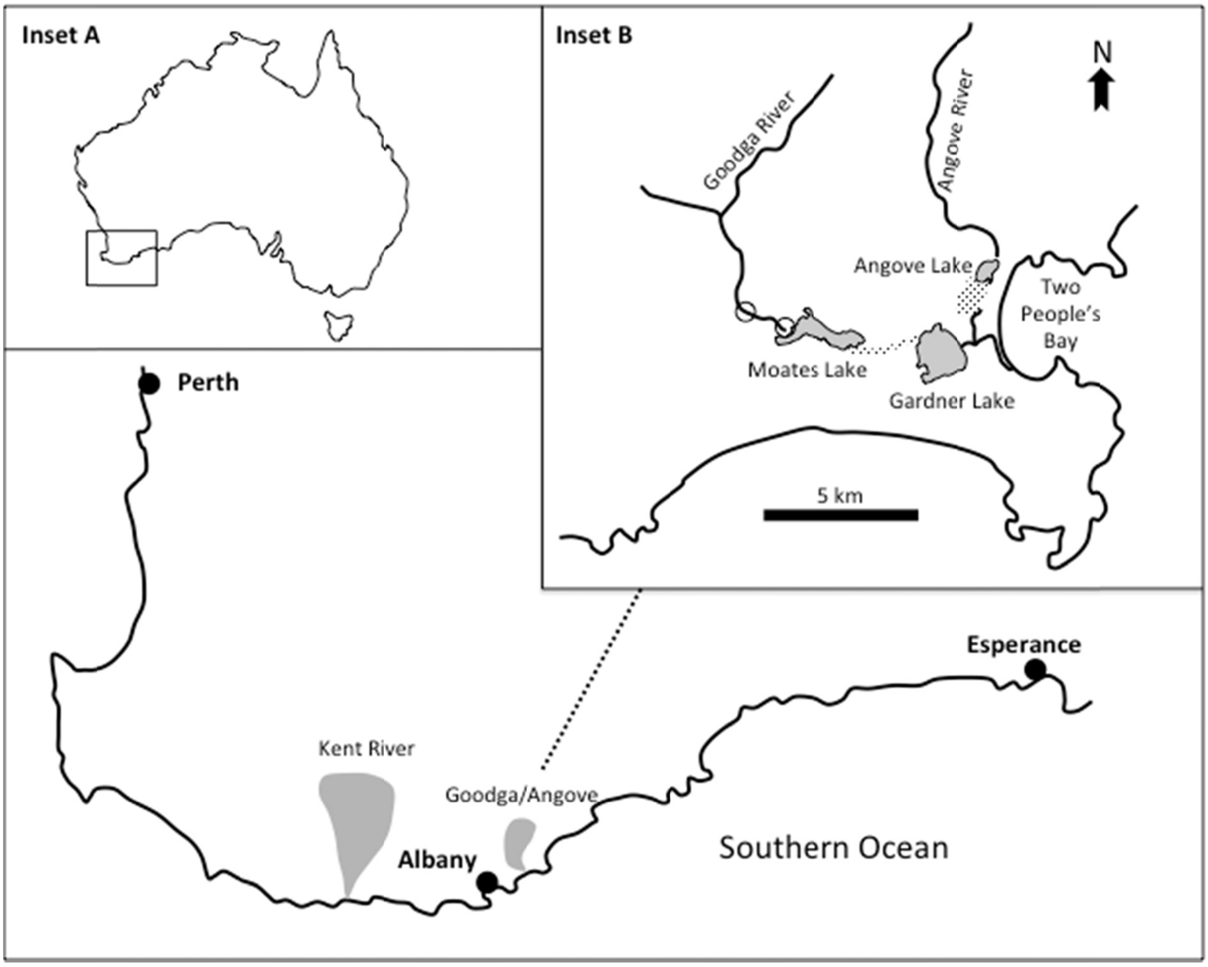

FIGURE 1. Distribution of *Galaxias truttaceus* within the Mediterranean climatic zone of Australia's Southwestern Province (shaded). Inset A shows location within Australia, and Inset B shows those sites (O) within the Moates Lake system from which specimens were sampled for the description of larval development and diet. Stippled area in Inset B depicts seasonally inundated land.

Morphological measurements were made to the nearest 0.01 mm using either a stereomicroscope with a calibrated ocular micrometer for small larvae (< 10 mm) or vernier callipers for specimens > 10 mm BL. BL was measured as the distance between the snout and the tip of the notochord in preflexion and flexion developmental stages, and the total length was measured in postflexion larvae and transitional stages. Where length measurements are given in association with developmental changes, measurements relate to the smallest larva with the described morphology or meristic characters.

Ontogenetic changes in body shape were described using relative dimensions of head length (HL), head depth (HD), body depth (BD), preanal length (PAL), mouth width (MW) and mouth length (ML) expressed as a proportion (%) of BL. Body proportions were defined as: HL, distance from the snout to the posterior margin opercular membrane; HD, distance between the dorsal midline of the supraoccipital crest and the ventral surface of the angular; BD, distance between body margins through the anterior margin of the pectoral fin base; PAL, distance from the snout to the vent; MW, distance between the lateral extremities of the paired maxillae, and; ML, distance between the anterior tip of the premaxilla and the posterior most opening of the mouth.

PLATE 1. (Top) The critically endangered Spotted Galaxias (*Galaxias truttaceus*) (photograph: Mark Allen) from the Mediterranean climatic zone of Australia's Southwestern Province; (middle) the larval nursery habitat in Moates Lake with the Southern Ocean in the distance following a bushfire in 2012 (photograph: Stephen Beatty): (bottom) the western side of Moates Lake and the meandering entrance to the Goodga River (photograph: Stephen Beatty).

The commencement of formation of the caudal (C), dorsal (D) and anal (A) fins corresponds to the first appearance of anlagen, the pectoral fin (P) to the first appearance of incipient fin rays and the ventral (pelvic) fin (V) to the first appearance of fin buds (Neira *et al*., 1998). Measurements and counts of myomeres and rays of the paired fins were made on the left side of the body. Pigment refers to melanin.

Illustrations were prepared with the aid of a camera lucida. Care was taken to ensure that specimens used for measurements and illustrations were not unduly distorted. Those specimens illustrated were chosen on the basis of having morphometric and meristic characteristics typical of the developmental stage. Given that some individual variation in development exists, differences between the level of development of individual fish and that for the entire sample for each stage do occur.

Material examined

Galaxias truttaceus larval descriptions: 16, 6.9-10.9 mm total length (TL), Moates Lake Western Australia, D. Morgan; 20, 16.4-20.9 mm TL, Moates Lake Western Australia, D. Morgan; 18, 17.1-22.7 mm TL, Moates Lake Western Australia, D. Morgan; 24, 27.7-34.1 mm TL, Moates Lake/Goodga River Western Australia, D. Morgan; 10, 31.2-38.7 mm TL, Goodga River Western Australia, D. Morgan; 15, 11-100 mm TL, Moates Lake/Goodga River Western Australia, D. Morgan, cleared and stained. All collected between 25/5/99-23/7/99. Additional 20, 5.3-8.7 mm BL, Goodga River Western Australia, 2013-2015, P. Close and J. Berkelaar.

Dietary analysis

All specimens used for dietary analysis were measured for length and categorised by developmental stage as described above. Stomachs were removed and their contents examined under a dissecting microscope. For larval stage specimens that lacked a distinct stomach, the anterior portion of the intestinal tract was removed for analysis. For all specimens examined, each prey item was identified to the lowest possible taxon and allocated to broad taxonomic groups and 'fish eggs'. All terrestrial fauna (i.e. spiders, ants and winged insects) were grouped together as they are predominantly found on the water surface and indicate surface feeding (Gill & Morgan, 2003).

Stomach content of each specimen was described in terms of i) the percentage frequency of occurrence of each ingested prey type (%F), and ii) relative contribution by volume (%V) of each prey type to total stomach content (Hynes 1950). For determination of %V, the fullness of each stomach was estimated and assigned a value between zero (empty) and 10 (fully distended stomach). The percentage contribution of each item to the total stomach volume was then estimated (see Hyslop, 1980; Schafer *et al*., 2002; Gill & Morgan, 2003). Individuals with empty stomachs or completely unidentifiable stomach contents were removed from subsequent analysis.

RESULTS

Size range of developmental stages

A total of 98 wild caught specimens of *G. truttaceus* including larval, transitional and juvenile stages of development were used to describe ontogenetic changes in morphology, meristics and pigmentation (Table 1). Sixteen preflexion larvae collected during 1999 were examined and ranged in BL from 6.9 to 10.9 mm (Table 1). Additionally, early-preflexion stage larvae, collected between 2013 and 2015, indicated length at hatching was 5.3 mm BL. Twenty flexion stage larvae were examined and ranged in BL from 16.4 to 21.9 mm. Based on the degree of notochord flexion, all flexion stage larvae examined were considered to be mid-late stage flexion and thus the BL for larvae at early stages of notochord flexion are likely to be considerably less than that reported here (i.e. < 16.4 mm BL). The range in BL for postflexion stage larvae (n=18) and 'transitional' stage specimens (n=24) was 17.1-22.7 mm and 27.7-34.1 mm, respectively. The smallest juvenile specimen examined was 31.2 mm BL.

Gross morphology

Larvae are very elongate in body plan throughout larval development (Figure 2) with a total myomere count between 54 and 59. Mean PAL is between 71 and 88% BL throughout larval, transitional and juvenile stages and mean BD increases from 3.7% BL in preflexion larvae to 13% BL in juvenile stages (Table 1). The anus is located below myomeres 40 to 42 in all stages. All other body proportions increased progressively in size from preflexion larvae through to juveniles (Table 1). The smallest larva collected was 5.3 mm BL (2015 sample). Preflexion larvae had a functional mouth (MW = 2.3% BL; ML = 1.7% BL) with small caniniform teeth in both upper and lower jaws (prominent by late-flexion), well developed eyes (ED =2.5% BL), a yolk sac (Figure 2) and a small, anteriorly-located gas bladder (not shown in Figure 2). In preflexion larvae, the gut is simple and straight. A distinct anterior stomach develops during flexion. Adult gut morphology was apparent in the smallest juvenile examined (31.2 mm BL).

TABLE 1. Range (mean ± 1 SE) of body length* and proportions of larval, transitional and juvenile stage *Galaxias truttaceus***. All measures are in mm.

Measurement	Preflexion (n=16)	Flexion (n=20)	Postflexion (n=18)	Transitional (n=24)	Juvenile (n=10)
Body length	6.9 – 10.9 (8.6 ± 0.3)	16.4 – 21.9 (18.8 ± 0.3)	17.1 – 22.7 (20.6 ± 0.6)	27.7 – 34.1 (30.8 ± 0.4)	31.2 – 38.7 (35.1 ± 0.6)
Body depth	2.6 – 5.0 (3.7 ± 0.3)	4.9 – 6.8 (5.8 ± 0.1)	5.5 – 8.2 (7.1 ± 0.2)	8.2 – 10.9 (9.4 ± 0.1)	11.9 – 13.9 (13.2 ± 0.2)
Head length	6.0 – 7.6 (6.9 ± 0.1)	11.9 – 15.7 (14.0 ± 0.2)	12.6 – 18.2 (14.6 ± 0.4)	16.1 – 19.9 (18.4 ± 0.2)	19.5 – 24.3 (22.2 ± 0.5)
Head depth	4.2 – 6.0 (5.0 ± 0.2)	4.6 – 8.5 (7.2 ± 0.2)	6.7 – 11.1 (8.5 ± 0.2)	8.1 – 10.7 (9.0 ± 0.1)	11.1 – 12.7 (12.2 ± 0.2)
Snout length	0.9 – 1.7 (1.2 ± 0.1)	1.7 – 4.3 (3.1 ± 0.1)	2.5 – 4.6 (3.4 ± 0.1)	3.5 – 5.3 (4.5 ± 0.1)	4.5 – 5.9 (5.2 ± 0.2)
Mouth width	2.3 – 3.4 (2.8 ± 0.1)	4.12 – 5.7 (4.7 ± 0.1)	4.6 – 6.5 (5.2 ± 0.14)	4.3 – 5.6 (4.7 ± 0.1)	6.7 – 8.0 (7.2 ± 0.1)
Mouth length	1.7 – 3.1 (2.4 ± 0.1)	3.2 – 5.4 (4.3 ± 0.1)	3.8 – 6.4 (5.1 ± 0.2)	4.7 – 6.5 (5.5 ± 0.1)	7.4 – 8.3 (7.6 ± 0.2)
Preanal length	71.6 – 87.5 (75.6 ± 1.0)	71.0 – 83.6 (80.4 ± 0.8)	76.1 – 86.2 (80.5 ± 0.7)	69.3 – 78.2 (74.4 ± 0.5)	71.6 – 78.2 (75.5 ± 0.8)
Eye diameter	2.5 – 3.2 (2.9 ± 0.1)	3.8 – 4.7 (4.3 ± 0.1)	4.3 – 5.3 (5.1 ± 0.2)	4.7 – 6.0 (5.1 ± 0.1)	5.5 – 6.4 (6.0 ± 0.1)

*, NB some variation in body proportions of preflexion-flexion and postflexion-juvenile stages attributable to change in body length measurements; BL measured as notochord length for preflexion and flexion stages, and as total length for postflexion, transitional and juvenile stages;

**, Data do not include additional early-preflexion specimens collected between 2013 and 2015.

Development of fins

The start and completion of fin development in *G. truttaceus* follows the pattern: C; D; A → P → V. In preflexion larvae a dorsal medial fin fold extends distally from approximately myosepta 5-8, around the notochord, then ventro-proximally to myosepta 7-12. Rays of caudal, dorsal and anal fins are developing by 16.4 mm BL (flexion stage). As all examined flexion stage larvae were considered to be mid-late stage, the BL at which anlagen for these fins first appears is unknown. Pterygiophores and associated fin rays of the dorsal and anal fin are visible by ~ 17 mm BL. Adult meristics for caudal (C 16-17), dorsal (D 8-12) and anal (A 11-14) are present by 22 mm BL after notochord flexion is complete. Pectoral fin buds are present at hatching (not shown Fig. 2). Pectoral fin rays were visible in all flexion stage larvae (i.e. > 16.4 mm BL) with adult meristics (P 7-13) present by approximately 27 mm BL. Ventral fin rays appear by 27 mm BL and develop sequentially from dorsal to ventral with adult meristics (V 5-8) present in juvenile specimens greater than 31 mm TL.

Pigmentation

Pigmentation is typically sparse in preflexion larvae and becomes more prominent during 'transitional' and juvenile life stages (Figure 2). External pigmentation in preflexion larvae is confined to a paired row of stellate melanophores on either side of ventral midline (17-24 melanophores) and a single row on the dorsal mid-line (4-33; generally >16). Stellate melanophores are also present on the ventral midline at, or just posterior to, the anal vent (0-4) and along the ventral midline of the caudal peduncle (0-6; generally >2). The head and nape are largely unpigmented during preflexion, although a few (up to five) large stellate melanophores may be present in some specimens. Approximately 50% of examined specimens possessed a single, internal stellate melanophore within the otic capsule. Between 5 and 11 stellate melanophores are present on the lateral and ventral surface of yolk sac. During flexion, melanophores on the head increase in size and number, and a line of irregularly spaced melanophores develops along the lateral line. Pigment along the dorsal and ventral midlines remains largely unchanged. This pigment becomes more dense (melanophores coalesced) in the region surrounding anlagen of the dorsal and anal fin (Figure 2). In postflexion and transitional stages, stellate and punctate melanophores become more numerous and develop on either side of the lateral line, and on and around the pectoral base, operculum and jaw. Adult pigmentation is present in juveniles at approximately 45 mm BL; heavily pigmented with overall brownish red coloration, dark circular pigment spots surrounded by silvery white halos dorsolaterally, fins orange-reddish in colour.

Diet

Over half of all fish examined contained prey in their gut. For those with prey, all postflexion and transitional stage *G. truttaceus* contained dipteran larvae, which contributed ~ 98% to stomach content volume (Table 2). One fish also consumed ostracods. Early juvenile stages (i.e. 35-49 mm BL), consumed eight distinct dietary categories (Table 2), although terrestrial fauna strongly dominated both in terms of their contribution to stomach volume (81%) and frequency of occurrence in individual fish (89%). Of the aquatic diet, amphipods contributed most (10%) to stomach volume and amphipods, dipteran larvae and fish eggs contributed most (> 10% each) to frequency of occurrence.

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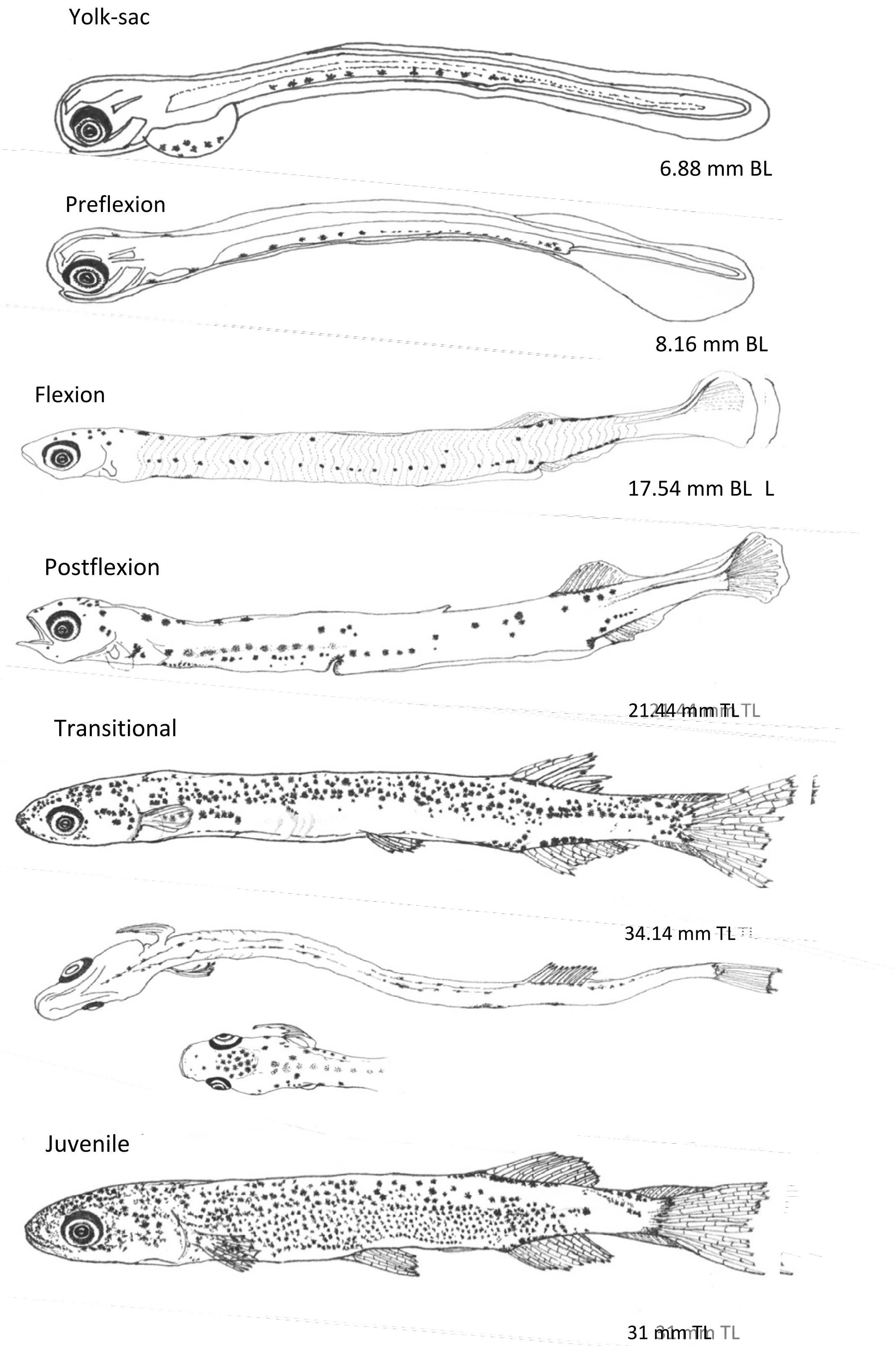

FIGURE 2. Stages in the development of early life history of *Galaxias truttaceus* between post-hatch and juvenile life stage (illustrations by Fiona Rowland). Developmental stages are defined by Neira *et al*. (1998) as: Yolk-sac - presence of a yolk sac ventrally in the gut region; Preflexion - the stage between complete absorption of the yolk and the start of upward bending of the notochord; Flexion - the stage from the commencement of notochord flexion to the time when the hypural plates

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FIGURE 2 (CONTINUED). assume a vertical position; Postflexion – the stage between completion of notochord flexion and the transition to juvenile morphology; Transitional–the period of metamorphosis of morphological features from those of the larval stage to those typical of the juvenile stage and where the ventral pigmentation distinguishes it from all other sympatric species; Juvenile – the stage from attainment of the full complement of external meristic characters (fin rays and scales) to first sexual maturity.

TABLE 2. Percentage volumetric contribution (%V) and percentage occurrence (%F) (in parentheses) of prey items to the stomach contents of postflexion and 'transitional' stage larvae and juveniles of *Galaxias truttaceus*.

Prey type	Postflexion and 'transitional' (20-34 mm BL; n = 7)	Juvenile (35-49 mm BL; n = 28)
Ostracoda	1.8 (14.3)	1.1 (7.1)
Amphipoda	-	10.6 (14.3)
Collembola	-	0.8 (3.6)
Diptera larvae	98.2 (100)	1.2 (14.3)
Trichoptera larvae	-	0.4 (3.6)
Coleoptera larvae	-	1.9 (3.6)
Terrestrial fauna	-	81.3 (89.3)
Fish eggs	-	2.4 (10.7)
Mean gut fullness (± SE)	3.1 (0.3)	2.8 (0.4)

DISCUSSION

Mediterranean climatic populations of *G. truttaceus* on the west coast of Australia exhibit morphological differences to populations on the opposite side of continental Australia (McDowall & Frankenberg, 1981) and sub-structure at nuclear and matrilineal genetic markers suggest discrete western and eastern Australian sub-populations with limited contemporary gene flow (Morgan *et al.*, 2016). Many eastern Australian populations are diadromous, whereas all populations on Australia's south-west coast are potamodromous and landlocked (Humphries, 1989; Morgan, 2003, Morgan *et al.*, 2016). Morphological features, such as a reduced number of fin rays and vertebrae in land-locked populations compared to diadromous populations may have evolved as a response to the need to have a greater swimming ability in environments with higher flow. The following discussion of the key diagnostic characters that distinguish *G. truttaceus* from other species is restricted to those sympatric species within the Western Australian distribution. In Western Australia, the species is known from only three catchments located on the south coast (Morgan, 2003; Colman, 2010; Close *et al.*, 2014; Morgan *et al.*, 2016) and occurs sympatrically with teleost fishes belonging to the Galaxiidae, the Percichthyidae and the Gobiidae (Morgan *et al.*, 1998; 2011).

While larval *G. truttaceus* and *G. maculatus* are sympatric, all other species with which these co-occur as adults spawn during winter-spring (June- November), after the distinct April-May spawning period of *G. truttaceus* (Morgan, 2003) and thus only larvae of *G. truttaceus* and *G. maculatus* can be expected in samples collected in autumn – early winter. Additionally, both these galaxiid species (as well as *G. occidentalis*) possess in excess of 50 myomeres, whereas all other sympatric species possess less myomeres; Gobiidae, 24-34 (Neira *et al.*, 1998), Perchythyidae, 31-35 (e.g. *N. balstoni*, Gill & Morgan, (1998)) and *Galaxiella* species, 38-44 (Gill & Neira, 1994; Neira *et al.*, 1998) and tend to be more heavily pigmented with melanophores (see Gill & Neira, 1994; Gill & Morgan, 1998; Neira *et al.*, 1998).

Galaxias maculatus is known to undertake protracted spawning with larvae present in coastal lakes year-round (Chapman *et al.*, 2006). Larvae and juveniles of *G. maculatus* and *G. truttaceus* are sympatric in the Moates Lake drainage system, and probably in coastal lakes associated with the two other rivers in which these species co-occur as adults. Taxonomic discrimination between these two species is possible using pigment patterns: *G. truttaceus* possesses paired stellate melanophores on the ventral midline, not present in *G. maculatus; G. maculatus* has a single line of melanophores on the upper lateral surface of the gut, not present in *G. truttaceus* (Chapman *et al.*, 2009). Larger larvae and juveniles of *G. truttaceus* can be distinguished by anterior origin of dorsal fin, which is proximal to that of the anal fin in *G. truttaceus* and adjacent or behind the anal fin in *G. maculatus*. Compared to *G. maculatus*, late-larval stages of *G. truttaceus* are more heavily pigmented, particularly on the latero-dorsal surface, by larger stellate and punctate melanophores (*c.f.* Chapman *et al.*, 2009). While *G. occidentalis* spawns later (June-September) than *G. truttaceus*, early-larval stages of these species can be distinguished by pigment (2-29 melanophores) along the dorsal and ventral midlines of the caudal peduncle; a characteristic diagnostic for *G. occidentalis* (Neira *et al.*, 1998) and lacking in larval *G. truttaceus*. *Galaxias occidentalis* also possess melanophores along the isthmus and around the cleithral symphysis during larval stages (Neira *et al.*, 1998) not present in *G. truttaceus*. In flexion larvae, coalesced melanophores are located on the ventral and dorsal midline around anlagen of the dorsal and anal fins in *G. truttaceus* but not *G. occidentalis* (*c.f.* Neira *et al.*, 1998). Larger larvae and juveniles of *G. truttaceus* can be distinguished by the anterior origin of dorsal fin, which is proximal to that of the anal fin in *G. truttaceus* and adjacent to the anal fin in *G. occidentalis*.

Previous examination of the diet of Western Australian populations of *G. truttaceus* identified that preflexion stages consumed exclusively copepods and that terrestrial prey, of which 98% was insect, dominated (65-96%) the diet of adult fish (Morgan, 2003). We identified that dipteran larvae dominated the diet during postflexion larval stages and that the dominance of terrestrial prey starts as early as the juvenile stage. This dramatic ontogenetic shift in diet coincides with a distinct shift from lentic to lotic habitats that occurs when juvenile fish undertake an upstream recruitment migration to adult riverine habitat (Morgan & Beatty, 2006; Close *et al.*, 2014), but may also be related to seasonality of prey availability, noting that Morgan (2003) found terrestrial fauna (insects) in the stomachs to be lowest in winter (65%) and spring (76%).

Similar ontogenetic shifts in diet have been described for other freshwater fishes of Mediterranean south-western Australia. *Nannatherina balstoni* also preys on small aquatic fauna (predominantly cladocerans, ostracods, copepods and dipteran larvae) during larval stages, after which terrestrial fauna contribute increasingly to the diets during juvenile and adult life stages (Gill & Morgan, 1998). Gill and Morgan (2003) found a similar shift in diet for larval *G. nigrostriata* and *L. salamandroides* from cladocerans, copepods, ostracods and dipterans during larval stages to terrestrial fauna and larger benthic prey in juvenile stages. The pronounced shift from small aquatic prey to terrestrial prey items in both studies was thought to be related to ontogeny, rather than seasonality in prey abundance.

This study has provided the first detailed morphological description of larvae of the critically endangered *G. truttaceus*. Key diagnostic characters that distinguish Australian populations of *G. truttaceus* from other sympatric *Galaxias* species include the distribution and density of pigmentation and the position of the dorsal fin origin relative to that of the anal fin. Larvae of *G. truttaceus* may be easily distinguished from other sympatric species belonging to Gobiidae, Perchythyidae and *Galaxiella* that have significantly fewer myomeres and/or are less elongate and tend to be more heavily pigmented with melanophores. Accurate discrimination of *G. truttaceus* from other sympatric species provides a basis for further ecological investigations on the ecology of early life history stages of this species in Mediterranean Western Australia. Because *G. truttaceus* exhibits morphological differences among populations in western and eastern Australia, the transferability of the key diagnostic features described here to eastern Australian populations remains unknown.

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AUTHOR CONTRIBUTIONS

FR contributed to the writing and illustrated the larvae; PC collected the samples 2013-2015 and contributed to the writing and interpretation; SB and MA contributed to the collection of recent samples and earlier drafts of the manuscript; HG assisted in the illustrations and descriptions of larvae; JB contributed to larval collections in 2013-2015 and earlier drafts of the manuscript; DM conceived the study, collected all early larval samples and contributed to the writing and interpretation.

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