Short Communication © CSIRO 1999 *Mar. Freshwater Res.*, 1999, **50**, 467–8

Detection of predation on Australian native fishes by *Gambusia holbrooki*

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Abstract. Clearing and staining of *Gambusia holbrooki* facilitated identification of juvenile fishes among the gut contents, and a feeding trial with captive *Gambusia* allowed assessment of gut transit time and degradation of melanotaeniid larvae. Regurgitated fishes and eggs in fixative solutions were also investigated. These techniques, extending to 12 h the post-feeding interval in which fishes may be found and differentiated among *Gambusia* gut contents, were tested on 631 wild-caught *Gambusia* collected in eastern Australia; an ingested native fish could be identified in the gut of 18 *Gambusia*, and a cannibalized fish in the gut of three.

Extra keywords: gut contents, melanotaeniid

Introduction

Gambusia spp. (Cyprinodontiformes : Poeciliidae) have been introduced to many countries, primarily for mosquito control. Twenty-one Australian populations were identified as a single species (Lloyd and Tomasov 1985) usually referred to as *G. holbrooki* Girard (Wooten *et al.* 1988). In the present paper, *'Gambusia'* refers to *G. holbrooki* and the closely related *G. affinis*.

Gambusia is reputed to have reduced endemic fish populations by aggressive interactions, including predation on juveniles (Schoenherr 1981). Experiments in aquaria and field enclosures have shown that *Gambusia* can extirpate *Poeciliopsis occidentalis* (Poeciliidae) (Meffe 1985) and *Heterandria formosa* (Poeciliidae) (Belk and Lydeard 1992). The principle form of interaction with *H. formosa* was predation (Schaefer *et al.* 1994). Following extirpation, enduring re-establishment of *H. formosa* was dependent on complete eradication of *Gambusia* (Lydeard and Belk 1993).

In Australia, it was speculated that *Gambusia* preyed on the eggs and larvae of melanotaeniids (Arthington and Lloyd 1989; Arthington 1991). However, the authors are not aware of any report detailing the identification of fishes other than cannibalized juveniles among the gut contents of wild-caught *Gambusia*. This report describes the use of simple techniques to confirm the above speculation.

Materials and methods

The study area was a 10 km stretch of the upper Orara River near Karangi, New South Wales (30°15′S,153°00′E). Here, *Gambusia* was sympatric with juvenile native fishes during the period of the study (October 1997 to January 1998).

To facilitate identification of ingested fishes, juvenile fishes were collected with a tea-strainer (0.6 mm pore size) and preserved in 4% formaldehyde. Some specimens were bleached in caustic peroxide, cleared in boracic trypsin solution, stained in caustic alizarin red solution, and examined under a stereo microscope.

To assess the gut transit time and degradation of melanotaeniid larvae, a captive feeding trial was undertaken. Seventy-four post-juvenile *Gambusia* were collected with a hand-net and transferred to uncovered 4 L aquaria (eight or nine fish per aquarium) containing broad-leafed aquatic foliage (*Valisneria* sp.). The aquaria were placed outdoors in shade. Three weeks after capture, *Gambusia* were acclimated for three days to a regime of once-daily feeding (0800 hours) with a mixture of commercial flake food and live wild-harvested melanotaeniid larvae. On the final day of the trial (5 November 1997; ambient air temperature 17–23°C), ~130 larvae (size range 4 mm total length (TL) to 9 mm standard length (SL)) were divided into nine portions and offered to the *Gambusia* (one portion per aquarium). Eight *Gambusia* were selected (one fish from each aquarium) at intervals between 0.5 and 12 h after feeding, and fixed in 4% formaldehyde solution. Four fishes from each sample were cleared and stained prior to excision of the gut *en bloc* (pharynx to rectum) for examination of contents *in situ*.

To assess predation on native fishes, post-juvenile *Gambusia* were collected over 1–2 h (13 occasions) at various times of the day during the study period. Fishes were fixed in formaldehyde solution, then removed for clearing and staining. Fixative solutions were strained and the residue was examined.

Results

Juveniles of six species of fishes (*Gambusia*, *Retropinna semoni* (Salmoniformes), *Melanotaenia duboulayi* (Melanotaeniidae), *Rhadinocentrus ornatus* (Melanotaeniidae), *Pseudomugil signifer* (Pseudomugilidae) and *Hypseleotris galii* (Eleotridae)) could be differentiated on the basis of pigmentation, size of the mouth, body length and flexion status (Table 1). Following clearing and staining, additional characters were the number of vertebrae and morphology of the caudal skeleton.

On the final day of the feeding trial, 70 adult *Gambusia* remained (four unexplained disappearances), and juvenile *Gambusia* were present in three aquaria. Some *Gambusia* placed in fixative solutions at 0.5 and 1.0 h after feeding regurgitated gut contents, including ingested juveniles.

In non-cleared and stained *Gambusia* specimens, small melanotaeniid larvae (<5 mm TL) were found up to 2 h after feeding. Larger larvae were identifiable up to 3 h after feeding. At 4 h after feeding, some larger larvae could be identified from otoliths and the melanophore pattern of semi-digested skin. Larvae could not be identified 6 h after feeding in non-cleared and stained *Gambusia*.

10.1071/MF98106 1323-1650/99/050467

Table 1. Known characters of juvenile fishes considered during analyses of gut contents of *Gambusia*

	Gambusia	*R. semoni*	*M. duboulayi*	*R. ornatus*	*P. signifer*	*H. galii*
Size at hatching	≥4.7 mm, post-flexion	≥4.6 mm, pre-flexion	≥3.7 mm, pre-flexion	≥4.1 mm, pre-flexion	≥4.4 mm, post-flexion	≥2.8 mm, pre-flexion
Vertebrae	31–32	48–51	34–36	34–36	29–31	30–31
Hypurals	caudal fan	5 hypurals	5 hypurals	4 hypurals	2 hypurals	two hypurals
Pigmentation	moderate	translucent	heavy	heavy	maculi	light
Mouth size	intermediate	small	small	small	small	large

In cleared and stained *Gambusia*, recently hatched (non-calcified) melanotaeniid larvae could not be identified. Larger larvae were readily discernible up to 6 h after feeding: the vertebral column remained articulated, and some cranial bones remained aggregated about the otoliths. At 8 h after feeding, the otoliths were discernible, the column was disarticulated, and individual vertebrae stained poorly among faecal matter. At 12 h after feeding, one female (23 mm SL) was observed with a rectal faecal pellet containing a few centrae. At 3 h and 6 h after feeding, the gut of a male (19 mm SL) and a female (26 mm SL) each contained an ingested fish (6 mm and 8 mm SL respectively) identified as cannibalized *Gambusia*.

Some larvae (≤4.5 mm) found in the regurgitate were strongly pigmented, and were identified as melanotaeniids. Differentiation of larger fishes in the regurgitate or gut contents was generally straightforward, whereas smaller, well-masticated fishes among the gut contents were not easily identified. The acidic environment of the predator's gut caused some dissolution of ingesta, as suggested by poor staining of skeletal material 4 h after experimental feeding.

Regurgitated larvae among the residue of solutions used to fix 631 wild-caught *Gambusia* included ten melanotaeniids, one *Gambusia* and one *H. galii*. Regurgitated larvae, displaying wounds consistent with 'mastication', were distinguishable from juvenile *Gambusia* (without external trauma) delivered in these solutions. Twenty-one *Gambusia* were found with an ingested fish: 18 (2.8%) with an ingested melanotaeniid or pseudomugilid larva, and three with a cannibalized fish.

Discussion

In a survey of cannibalism, fishes were recognizable among *Gambusia* gut contents for less than 4 h after feeding (Meffe 1985). Thus, the proportion of rapidly degraded dietary items (including ingested fishes) may be underestimated (unlike lignified plant debris and arthropod body-parts) in routine gut content analysis (Arthington and Lloyd 1989; Arthington 1991).

Examination of the fixative solution for regurgitated items has not been reported in other studies of piscivory (Dionne 1985; Meffe 1985; Nesbit and Meffe 1993).

The significance of predation by *Gambusia* on native fishes cannot be ascertained from this isolated study, since newly hatched fishes may be an important dietary resource for species other than *Gambusia*, and analysis of gut contents of endemic fishes was beyond the scope of this study. However, these techniques (differentiation between sympatric juvenile fishes, examination of regurgitate, and clearing and staining *Gambusia*) extend to 12 h the post-feeding interval in which fishes may be found and differentiated among *Gambusia* gut contents.

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Manuscript received 20 July 1998; revised 27 January 1999; accepted 29 March 1999