Oviposition and egg mass morphology in barred frogs (Anura: Myobatrachidae: *Mixophyes* Günther, 1864), its phylogenetic significance and implications for conservation management.

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ABSTRACT

Several species of the Australo-Papuan genus *Mixophyes* (barred frogs) have declined markedly and are now considered threatened. During field investigations into the possible causes of declines, we observed oviposition and numerous egg masses (700+) of the four species from southeast Australia. From these and published observations there are two markedly different ovipositional processes within *Mixophyes*. In *M. coggeri*, *M. fasciolatus* and *M. iteratus* eggs are laid in water then deposited terrestrially, propelled from the foot of the floating female onto a near-vertical or overhanging stream bank (Ovipositional Process 1). In *M. balbus* and *M. fleayi* eggs are deposited aquatically in the shallow riffle zones of small streams, either into a rounded nest depression in the substrate (Ovipositional Process 2A), or occasionally directly onto bedrock (Ovipositional Process 2B). These observations of ovipositing and egg masses show that there are three reproductive modes in the genus: mode 2 - eggs and exotrophic tadpoles in lotic water (*M. fleayi*), mode 4 - eggs and early larval stages in constructed basins, exotrophic tadpoles in streams subsequent to flooding - (*M. balbus* and *M. fleayi*), and mode 18 (terrestrial eggs above water, exotrophic hatchlings move to water (*M. carbinensis*, *M. coggeri*, *M. fasciolatus*, *M. iteratus* and *M. schevilli*)). *Mixophyes fasciolatus* was the only species to use both lentic as well as lotic waters for larval development. For the Australian species amplexus was axillary, occasionally shifting during the process of oviposition to inguinal. Amplexus for the New Guinean *M. hihiorlo*, is unknown, as is the ovipositional process and reproductive mode. The phylogenetic significance of our observations is that *Mixophyes* species have non-foamy egg masses, which accords with their recent placement in the family Myobatrachidae. However, no other members of this family show either of the described processes of oviposition, and furthermore we observed amplexus in *Mixophyes* to be axillary whereas all other reports for myobatrachids are of inguinal amplexus. Our findings have consequences for management of the habitat of barred frogs, three of which are considered threatened. The construction of creek crossings (for vehicles, bikes, horse riding, pedestrians) in riffle zones, the trampling of creek banks by cattle, horses, pigs and humans and changes to the hydrology of creeks by damming or regulating flows that alter the connection between hydrology and stream bank structures, are all likely to have a negative impact on reproductive success.

Key words: natural history, threatened, declining, amphibian, breeding, reproduction, management, spawn

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Introduction

Barred frogs (*Mixophyes*) are moderate to large ground-dwelling amphibians of the family Myobatrachidae (sensu Frost *et al.* 2006). Seven species occur in eastern Australia and an eighth species, *M. hihiorlo*, is known only from

the type collection from Namosado in the Southern Highlands of Papua New Guinea (Donnellan *et al.* 1990; Mahony *et al.* 2006; Menzies 2006). The Australian species breed in streams in forests of the coastal plains

and adjacent ranges of the mesic east coast (Gillespie and Hines 1999; Hines, Mahony and McDonald 1999; Meyer, Hines and Hero 2001; Hoskin and Hero 2008) and *M. fasciolatus* also breed in lentic waters (Mahony 1993; Hines, Mahony and McDonald 1999; Lemckert 1999; Meyer, Hines and Hero 2001; Parris 2002). Three of the four south-eastern Australian species have suffered serious population declines and are listed as threatened in the IUCN Redlist (IUCN 2012), and under state and federal legislation (Gillespie and Hines 1999; Hines, Mahony and McDonald 1999; Hero et al. 2007) (Table 1). Basic life history information is important for understanding and ameliorating threats to species. Despite the extensive distribution of Australian *Mixophyes*, and conservation concern for three species, published information on their breeding biology is limited and at times conflicting. Here we describe different oviposition behaviours and resulting form of egg masses among the four *Mixophyes* species from south-eastern Australia and compare this to what is known for other members of the genus. Shared reproductive characters are examined in the context of systematic relationships within *Mixophyes* and these inform debate over the familial placement of this genus.

Until the late 1960s *Mixophyes* was considered a single species, *M. fasciolatus* (e.g. Moore 1961). Straughan (1968) revised the genus, describing *M. balbus* and *M. iteratus*, raising *M. fasciolatus schevilli* to species status and redefining *M. fasciolatus*. The only information we have found on oviposition or egg masses of *Mixophyes* prior to Straughan's (1968) revision, was in Straughan's (1966) unpublished PhD thesis, for *M. fasciolatus (sensu*

stricto Straughan 1968). The site of egg deposition was reported for *M. fleayi* when this species was described in 1987 (Corben and Ingram, 1987). Donnellan, Mahony and Davies (1990) described *M. hihiorlo*, the only known member of the genus from Papua New Guinea, and the *M. schevilli* complex was reviewed by Mahony et al. (2006) who described two new species, *M. carbinensis* and *M. coggeri*. These latter two taxonomic works did not include information on oviposition or egg masses for the respective species. In the following section, we provide a summary of published accounts (roughly in chronological order) of oviposition and the form of egg masses of *Mixophyes*. The unpublished PhD of Straughan (1966 unpubl.) was included as it provided information not presented in Straughan's subsequent (1968) revision of the genus *Mixophyes*.

Early accounts of *Mixophyes* provide scant information on breeding with Fletcher (1889) surmising that they breed in summer, amplexus is axillary and oviposition is "in water in the ordinary manner". The first description of the egg mass of *Mixophyes* is that of Straughan (1966 unpubl., p. 26) and refers to *M. fasciolatus sensu stricto*: "Amplexus is axillary and is initiated out of water at the calling site of males. Only one clasped pair was seen to enter water and this was on disturbance. Egg masses are deposited in loose detritus along banks (plate 5), and contain 1400 to 2000 eggs. Embryo development takes place in the litter and is summarised in fig. 2. When larvae hatch they are washed by rain or flood into the water body where development is completed." Straughan's (1966 unpubl.) plate 5 is a photograph of

Table 1. The list of *Mixophyes* species, their common names, authority, conservation status (IUCN 2012) and legislative status (**Qld** – Queensland Nature Conservation Act 1992, **NSW** – New South Wales Threatened Species Conservation Act 1995, **Vic** – Victorian Flora and Fauna Guarantee Act 1988, **Fed** – Commonwealth Environment Protection and Biodiversity Conservation Act 1999). Codes for conservation and legislative conservation status: **EN** = endangered, **LC** = listed as least concern or not considered threatened, **NA** = not applicable as the species does not occur in that jurisdiction, **VU** = vulnerable.

Scientific name	Common names	Authority	Conservation status	Legislative status			
				Qld	NSW	Vic	Fed
<i>Mixophyes balbus</i>	Southern Barred Frog Silver-eyed Barred Frog Stuttering Frog	Straughan, 1968	VU C1+2a(i)	NA	EN	VU	VU
<i>Mixophyes carbinensis</i>	Carbine Barred Frog	Mahony, Donnellan, Richards & McDonald, 2006	LC	LC	NA	NA	LC
<i>Mixophyes coggeri</i>	Mottled Barred Frog	Mahony, Donnellan, Richards & McDonald, 2006	LC	LC	NA	NA	LC
<i>Mixophyes fasciolatus</i>	Great Barred Frog Great Barred River-frog	Günther, 1864	LC	LC	LC	NA	LC
<i>Mixophyes fleayi</i>	Fleay's Barred Frog	Corben and Ingram, 1987	EN B2ab(ii,iii,iv,v)	EN	EN	NA	EN
<i>Mixophyes hihiorlo</i>		Donnellan, Mahony and Davies 1990	Data deficient	NA	NA	NA	NA
<i>Mixophyes iteratus</i>	Giant Barred Frog Giant Barred River-frog	Straughan, 1968	EN B2ab(ii,iii,iv,v)	EN	EN	NA	EN
<i>Mixophyes schevilli</i>	Northern Barred Frog	Loveridge, 1933	LC	LC	NA	NA	LC

an *M. fasciolatus* egg mass in-situ, sitting upon leaf litter. His figure 2 illustrates embryonic development over time and shows diameter (most likely capsule) increases from 2.1mm at stage 12 to 3.6mm at stage 25 (stages as per Gosner 1960, sample size not stated).

It appears likely that Straughan's observations were the basis for the first published account of the life history of *Mixophyes*, by Martin (1967, p. 179): "...*Mixophyes fasciolatus*, is characterized, at least in some situations by terrestrial oviposition of non-foamy egg-masses and subsequent aquatic larval life. Breeding occurs along fast-flowing streams, and the tadpole is of the mountain-stream type. Male frogs call from depressions in leaf litter up to three metres from the water's edge. Amplexing pairs have been observed in similar sites and also in the water or on rocks near the water. Egg-masses have been collected in litter on the bank of a dry gully which becomes flooded after rain (Straughan, personal communication). No information is available on the length of time for which the eggs can remain viable in such sites prior to flooding."

For *M. balbus* (Point Lookout, NSW), Watson and Martin (1973, p. 37) describe eggs as being pigmented with ovidiameter about 2.8mm, laid in clusters on rocks or gravel near the edge of flowing streams. It is not clear whether the eggs they observed were within the stream or laid terrestrially. However, they later state the life histories of *M. balbus* and *M. fasciolatus* appear to be essentially identical (quoting Martin 1967), inferring that the eggs of *M. balbus* are terrestrial.

The view that *Mixophyes* have terrestrial eggs and aquatic larvae is held by Heyer and Liem (1976), Barker and Grigg (1977), Tyler (1985, 1994), Robinson (1993), Barker, Grigg and Tyler (1995) and Hoser (1989) with the latter adding that *M. balbus* lay their eggs in vegetation adjacent to streams. Corben and Ingram (1987) observed eggs of *M. fleayi* under a rock about 30cm from water and attended by an adult.

More detailed accounts of oviposition and or egg masses appeared from the mid-1990s. Marantelli (1995) describes the egg mass from captive *M. fasciolatus*. A pair in a small terrarium comprising two terrestrial areas separated by water up to 18 cm deep laid 1100 eggs scattered all over the tank. Some eggs were in water, others above water attached to rocks or on the sides of the pond, through leaf litter and on the walls of the tank up to a height of 40 cm. Oviposition was not observed but he postulated that fertilised eggs are thrown at the surfaces to which they wish them to adhere. Many eggs became desiccated, with only those in water and to about 50 mm above water remaining viable, hatching after 11–18 days (temperature 16–21°C). Hatching tadpoles were observed to wriggle violently, seemingly to rupture the capsule, and to assist them in reaching the water.

In February 1998 one of the current authors (HH) gave a presentation at the annual general meeting of the Australian Society of Herpetologists at Yungaburra in Queensland, on oviposition in the four species of *Mixophyes* species from south-eastern Australia (Knowles *et al.* 1998 unpubl.). This presentation described two modes of oviposition among these species. *Mixophyes balbus* and

M. fleayi constructed a 'nest' in the shallow running water, with eggs deposited in a shallow excavation in the stream bed or pasted directly onto bed rock. *Mixophyes fasciolatus* and *M. iteratus* deposited their eggs out of water, under overhanging banks or on steep banks, of larger pools. Subsequently, information from this presentation is cited in the literature as "Knowles *et al.* (1998)", "Knowles *et al.* (in prep.)", or as personal communications from one or more of the current authors. The data and observations underlying these sources of information are detailed in the results section below.

Lewis (2000) provides the first published account of egg laying in *M. balbus*. A pair was observed to create eight nest depressions in the gravel and detritus substrate in shallow water (up to 4 cm deep) of an upland second order stream. Eggs were laid in four main clumps (and a few small scattered clumps), with an estimated total of 250 eggs. Nest depressions averaged 10 cm in diameter. Nests were evenly spaced over an 80 x 50 cm area, averaging 15–20 cm distance between each depression. Amplexus was observed between 02:20 hours and 09:10 hours with the eggs most likely laid during daylight hours. A colour photograph of the pair in amplexus is provided, but the egg mass is not illustrated.

A photograph of the breeding habitat of *M. fleayi* at Lamington Plateau and of an egg mass of *M. fasciolatus* on an undercut bank in the D'Aguilar Range, both southeast Qld is provided by Meyer, Hines and Hero (2001).

For the south-eastern Australia species of *Mixophyes*, Anstis (2002, 2013) describes the breeding sites, embryos and oviposition based largely on the observations of Knowles *et al.* (1998 unpubl.) and Lewis (2000). In addition she presents egg mass counts of three female *M. balbus* held captive from the onset of amplexus: 647 and 642 (Olney State Forest) and 1019 eggs (Point Lookout). She shows photographs of *M. balbus* eggs (labelled Gloucester Tops but from Sharpes Ck, Barrington Tops NP, NENSW, Table 2), *M. fasciolatus* ovipositing and egg masses (Bat Cave Ck, Nightcap NP, NENSW, Table 2, Figure 2), a pair of *M. fleayi* in a nest depression with eggs (Gap Ck West, Main Range NP, SEQ, Table 2), and eggs (Yabba Ck, Yabba NP, NENSW, Table 2) and eggs of *M. iteratus* (Desert Ck, Washpool NP, NENSW, Table 2, Figure 11).

Banks *et al.* (2003) report on spawning of *M. fasciolatus* in captivity over the period 1998–2000. During this time they observed oviposition once and egg masses on 13 occasions. Eggs were deposited on rocks and branches/bark at the water's edge. More specific details are provided for the first three egg masses observed. On 13 Apr 1998 approximately 500 eggs were laid in two clumps on a flat water-soaked piece of timber overhanging the water, approximately 10 cm above the surface of the water. A small number of eggs was within a narrow cavity on wet rocks under the timber and some were in water. Eggs hatched after eight days with embryos dropping directly into the water. The eggs laid in water failed to hatch. Subsequent egg masses, laid on 24 Sep 1998 and 26 Oct 1998 were similar but with a smaller (approximately 300) number of eggs. During the third laying, oviposition was

Table 2. Locations where oviposition or egg masses of *Mixophyes* species were observed. **Latitude** and **Longitude** are rounded to the nearest minute (datum is GDA94). **Alt** is the mean altitude (m) of the stream section (n.b. some stream sections surveyed were in excess of 1000 m long). Abbreviations used in **Locality** are: **Ck** = creek, **trib.** = tributary, **R.** = river, **MENSW** = mid-east New South Wales, **NENSW** = northeast New South Wales, **SEQ** = southeast Queensland, **NP** = National Park, **SF** = State Forest. Species abbreviations used in **Oviposition** and **Egg masses** are **Mba** = *M. balbus*, **Mfa** = *M. fasciolatus*, **Mfl** = *M. fleayi*, **Mit** = *M. iteratus*. Atypical egg masses (i.e. those with very low numbers of eggs or with eggs in scattered or diffuse clumps or obviously contained multiple clutches of eggs) are excluded.

Latitude & Longitude	Stream order	Alt	Locality	Oviposition	Egg masses
24° 22'S 151° 00'E	1	840	Kroombit Ck north branch, Kroombit Tops NP, SEQ		Mfa x 2
24° 23'S 151° 01'E	2	800	Kroombit Ck south branch, Kroombit Tops NP, SEQ		Mfa x 1
26° 43'S 152° 34'E	2	715	East Kilcoy Ck, Conondale NP, SEQ		Mfl x 1
26° 43'S 152° 35'E	2	680	North Booloumba Ck, Conondale NP, SEQ		Mfl x 16
26° 52'S 151° 35'E	2	970	Barker Ck, Bunya Mountains NP, SEQ		Mfa x 12
26° 52'S 151° 36'E	2	930	Upper Tim Shea Ck, Bunya Mountains NP, SEQ		Mfa x 2
26° 52'S 151° 37'E	3	685	Lower Tim Shea Ck, Bunya Mountains NP, SEQ		Mfa x 1
26° 53'S 151° 36'E	2	955	Saddletree Ck, Bunya Mountains NP, SEQ		Mfa x 4
27° 09'S 152° 43'E	3	205	Jacky Ck, D'Aguiar NP, SEQ		Mfa x 3
27° 24'S 152° 48'E		520	Bullocky's Knob pond, D'Aguiar NP, SEQ		Mfa x 7
27° 55'S 153° 10'E	2	425	Sandy Ck, Tamborine NP, SEQ		Mfa x 2
27° 59'S 152° 21'E	3	730	Dalrymple Ck south branch, Main Range NP, SEQ		Mfa x 12; Mfl x 83
27° 58'S 152° 20'E	4	665	Dalrymple Ck, near Main Range NP, SEQ		Mfa x 3
28° 03'S 152° 22'E	3	680	Gap Ck West, Main Range NP, SEQ	Mfl x 6	Mfa x 2; Mfl x 413
28° 15'S 152° 28'E	3	750	Condamine R., near Main Range NP, SEQ		Mfl x 5
28° 17'S 152° 23'E	3	895	Unnamed trib. Condamine R., Main Range NP, SEQ		Mfl x 2
28° 11'S 153° 07'E	3	635	Cainbale Ck, Lamington NP, SEQ	Mfl x 1	Mfa x 5; Mfl x 5
28° 11'S 153° 08'E	1	690	Unnamed trib. West Canungra Ck, Lamington NP, SEQ		Mfa x 2
28° 12'S 153° 06'E		690	Dam, upper Duck Ck, near Lamington NP, SEQ		Mfa x 11
28° 13'S 153° 07'E	3	775	Stockyard Ck, Lamington NP, SEQ		Mfa x 1; Mfl x 7
28° 13'S 153° 08'E	2	525	Bundoomba Ck, Lamington NP, SEQ		Mfl x 41
28° 13'S 153° 08'E	2	585	Darraboola Ck, Lamington NP, SEQ		Mfl x 3
28° 14'S 153° 08'E	3	780	Morans Ck, Lamington NP, SEQ		Mfl x 1
28° 14'S 153° 19'E	2	230	Tallebudgera Ck, near Springbrook NP, SEQ		Mfl x 1
28° 15'S 153° 17'E	2	200	Couchy Ck, Numinbah, NENSW		Mfl x 6
28° 19'S 152° 52'E	2	670	Long Ck, Border Ranges NP, NENSW		Mfl x 3
28° 22'S 153° 04'E	2	760	Unnamed trib. Brindle Ck, Border Ranges NP, NENSW		Mfl x 2
28° 22'S 153° 04'E	3	760	Brindle Ck, Border Ranges NP, NENSW	Mfl x 2	Mfl x 5
28° 33'S 153° 15'E	2	230	Beech Ck, Nightcap NP, NENSW		Mfl x 2
28° 33'S 153° 17'E	2	460	Tunttable Ck, Nightcap NP, NENSW	Mfa x 1	Mfa x 1; Mfl x 29
28° 36'S 153° 18'E	2	210	Unnamed trib. Bat Cave Ck, Nightcap NP, NENSW	Mfa x 4	Mfa x 4

Latitude & Longitude	Stream order	Alt	Locality	Oviposition	Egg masses
28° 38'S 152° 29'E	2	500	Yabbara Ck, Yabbara NP, NENSW	Mfl x 2	Mfa x 4; Mfl x 2
29° 16'S 152° 26'E	3	200	Desert Ck, Washpool NP, NENSW	Mit x 1	Mit x 6
29° 26'S 152° 10'E	3	800	Rockadooie Ck, Washpool NP, NENSW		Mba x 1
30° 15'S 153° 05'E	1	130	Bucca Bucca Ck, Orara East SF, NENSW	Mit x 1	Mit x 10
32° 03'S 151° 40'E	2	370	Sharpes Ck, Barrington Tops NP, NENSW	Mba x 1	Mba x 6
32° 14'S 151° 46'E	2	280	Frying Pan Ck, Chichester SF, NENSW		Mba x 1
33° 12'S 151° 20'E	2	60	Little Jilliby Jilliby Ck, near Gosford, MENSWS	Mit x 1	Mit x 1
33° 01'S 151° 26'E	1	200	Gap Ck, Watagans NP, MENSWS	Mba x 2	Mba x 2
TOTAL				Mba x 3; Mfa x 5; Mfl x 11; Mit x3	Mba x 10; Mfa x 79; Mfl x 627; Mit x 17

observed. While in amplexus, the female gathered the eggs with her back feet, whereupon she rolled to one side and with a quick flick of the foot, kicked the eggs onto a nearby wet vertical rock surface. It was assumed that the eggs were fertilised by the male while being held in the female's foot just prior to being kicked on to the rock.

Hoskin and Hero (2008) describe the egg laying behaviour of *M. coggeri* as "pair in amplexus flicks eggs out of water to stick on rock or earth bank overhanging water" presumably based on a detailed account published subsequently by Hoskin (2010). Hoskin's is the only published account of oviposition for *M. coggeri*. Eggs were deposited on the roof and walls of the overhanging rock and adjacent earth bank of a stream, by the pair sitting or floating in shallow water and the female flicking eggs and water upwards using her hind legs. Hoskin likened the egg flicking behaviour to that shown in a photograph of *M. fasciolatus* in Anstis (2002, p. 218). Most eggs (approximately 300) were stuck to the roof of the overhang in an area 60 x 30 cm about 20 cm above the water. Other eggs (approximately 150) were scattered on the adjacent sloping earth bank and rocks 10 to 30 cm from the water, with a small number of eggs in the pool.

Further information on the egg masses of the north-eastern Australian species (*M. carbinensis*, *M. coggeri*, and *M. schevilli*) is presented in Anstis (2013), based largely on the unpublished observations and photographs of Tim Hawkes. Each of these species deposits eggs on moist near-vertical or sloping bank or rock above a stream pool, with oviposition behaviour assumed similar to that observed in *M. fasciolatus* (Knowles *et al.* 1998 unpubl.) and *M. coggeri* (Hoskin 2010). A count of the number of eggs within a single egg mass of *M. schevilli* was 74 and of four *M. carbinensis* egg masses averaged 196 (range 144–311).

In summary, existing published information on oviposition and egg mass morphology in *Mixophyes* is limited and at times contradictory. Oviposition is described from observations of a pair of *M. balbus* (Lewis 2000) and *M. coggeri* (Hoskin 2010) in the wild, and from a pair of *M. fasciolatus* in captivity (Banks *et al.* 2003). In the following sections of this paper we publish details of the observations of Knowles *et al.* (1998 unpubl.), with additional observations up to early 2012. We have observed oviposition in wild *M. balbus* (three times),

M. fasciolatus (five times), *M. fleayi* (11 times) and *M. iteratus* (three times) and have observed numerous egg masses of these species (summarised with locality details in Table 2). These observations greatly increase knowledge of the breeding biology of *Mixophyes* species, and clarify some previously published observations and assumptions on oviposition and egg masses in the genus.

Methods

The observations presented here were made during survey and monitoring activities targeting *Mixophyes* species in southeast Queensland and northeast New South Wales (e.g. Newell, Goldingay and Brooks 2013). These activities were typically at night, along stream transects, using spotlights or head torches to locate active animals. Where time and conditions permitted we observed pairs of *Mixophyes* in amplexus for extended periods, to document oviposition. In these cases we noted one or more of the following: time and location, prevailing weather conditions, method of oviposition and length of the oviposition sequence. We photographed the mode and site of oviposition, and the resulting egg masses.

In addition we observed many more *Mixophyes* egg masses during surveys and monitoring or through targeted searches. At times these targeted surveys covered hundreds of metres of stream, focusing on areas with breeding choruses and, for some species, large sample sizes were gathered from across their distributional range. Notes were made of the deposition site and the shape and dimensions of egg masses. For a subset of these we counted or estimated the number of eggs, described the development stage as per Gosner (1960) and measured a small series of eggs (capsule diameter)

Results

Ovipositional process

We observed two distinct ovipositional processes. *Mixophyes fasciolatus* and *M. iteratus* were observed (five and three times respectively) to deposit eggs terrestrially above water by kicking them onto an overhanging bank or log (referred to as Ovipositional Process 1) whilst *M. balbus* and *M. fleayi* deposited eggs aquatically in a constructed depression or 'nest' in the stream bed (referred to as Ovipositional

Figure 1. A pair of *Mixophyes fasciolatus* in amplexus, during the process of spawning. Note that the pair is in the water, with the female using her forelimbs to brace against the stream bank. A number of eggs, already laid, are visible on the rock above the frogs. Unnamed tributary of Bat Cave Ck, Nightcap NP, NENSW, 24 Sep 1995. Photo, R. Knowles.

Figure 2. A photograph capturing the moment when a female *Mixophyes fasciolatus*, in amplexus, propels fertilised eggs out of the water and on to the embankment and overhanging rock. Arrows indicate eggs stuck on rock above water. Unnamed tributary of Bat Cave Ck, Nightcap NP, NENSW, 24 Sep 1995. Photo, R. Knowles.

Process 2). Ovipositional Process 2 was observed three times for *M. balbus* and 11 times for *M. fleayi*.

Ovipositional Process 1: observed in *M. fasciolatus* and *M. iteratus*

The following is a description of the ovipositional process of *M. fasciolatus*, at an unnamed tributary of Bat Cave Creek, Nightcap NP, NENSW (Table 2), 24 Sep 1995 (five amplectant pairs observed between 00:00 and 02:20hrs).

1. Males were calling from terrestrial positions, typically within 5 m of the stream, and facing the stream. Females were attracted to the calling males.
2. Amplexus occurred terrestrially. The female, with the male on her back, moved to the stream where she moved into the water and along the bank. Amplexus was initially axillary, but shifted at times during oviposition to be pectoral or inguinal.
3. The amplexing pair floated in the water facing the chosen egg deposition site (a more or less vertical surface of an overhanging rock, which formed part of the bank), see Figure 1. The female spread her forearms out in front of her body under the water: this appeared to brace the pair.
4. Just prior to laying a batch of eggs, the female moved the pair sideways, either to the right or to the left, less than one centimetre.
5. The male then moved his vent down into contact with the female and squeezed his hind legs inwards.
6. As the male moved his vent down eggs were expelled from the female's cloaca. They were presumably fertilised by the male during his downward movement observed in 5.
7. The female moved either her right or left hind limb rapidly through the water with an upward flicking motion towards the oviposition site, creating a clearly audible splash of water. Eggs were propelled with the water. The movement of the eggs from the female's cloaca to the position where they were propelled out of the water by the webbing of the foot was not observed. The movement of the female's leg in propelling the eggs was rapid and it was difficult to determine exactly what was occurring with the naked eye. Photographs show however, that a splayed webbed foot is likely to be responsible for propelling the eggs from the water onto the deposition site (Figure 2).
8. The eggs were propelled approximately 10–15 cm towards the deposition site (the overhanging rock) where the eggs adhered (Figures 1, 2 and 3).
9. The female's motion, in propelling eggs towards the deposition site, was so vigorous that her body swung around to a position almost parallel to the bank, before she returned to a position facing the bank. As a result of this movement, the male's body was partially displaced from the female (Figure 2), before he returned to the normal amplexing position (Figure 1).

For one pair, oviposition took place between about 00:00 and 00:41hrs with egg laying bouts (steps 4–9 above) at intervals of 15–55 seconds with a mean of 31 seconds ($n =$

9). In *M. iteratus* the interval between bouts of egg laying ranged between 34 seconds and 3 minutes 36 seconds, with a mean interval of 1 minute and 24 seconds ($n = 11$).

Ovipositional Process 2: observed in *M. balbus* and *M. fleayi*

The following is a description of the ovipositional process of two pairs of *M. fleayi*, Yabba Creek, NENSW (Table 2), 07 Feb 2000.

1. Males were calling from terrestrial positions, within the stream bed or within 5 m of the stream, and facing the stream. Females were attracted to the calling male.
2. Amplexus occurred terrestrially and was axillary. The female, with the male on her back, moved down to the stream.
3. The pair moved along the creek to a riffle where the stream bed comprised small stones under approximately 2 cm of gently flowing water. The pair made several rotations in the one spot, with the female shuffling her feet. This resulted in a more or less circular depression or 'nest' amongst the stones. No eggs were laid during this nest construction phase.
4. Just prior to laying a batch of eggs, the amplexing pair made a small downward movement into the nest area. The female rapidly ventriflexed so that her dorsum arched downwards, with her head held up. Simultaneously the male quickly placed his feet on the female's groin or upper legs (Figures 4 and 5), before both frogs dropped back down together again. During this process the female extruded a small clump of eggs (possibly 20–30 eggs) (Figure 4) presumably fertilised by the male as extruded.
5. The pair rotated, usually 45–90°, in an anti-clockwise direction, with the female shuffling her feet backwards into the walls of the nest, presumably pushing the eggs into the substrate.
6. The pair then pushed downwards into the nest area again, with their bodies lower than a normal sitting position (Figure 5). After a pause of up to several minutes the pair repeated steps 4 and 5, always rotating in an anticlockwise direction after laying a batch of eggs.

For one pair, 14 bouts of egg laying (steps 4–6 above) occurred at intervals of between 2 and 6 minutes (mean = 3.8 minutes), and the mean interval for 17 bouts in a second pair was 4.2 minutes. In *M. balbus* (Sharpes Ck, Barrington Tops NP, NENSW, on 11 Feb 2000 between 22:10 and 23:28 hours), 25 bouts of egg laying took place at intervals between 1.5 and 5 minutes (mean = 3.08 minutes).

Oviposition sites and egg masses

The different ovipositional processes and depositional sites produced distinctive forms of egg masses. These observations enabled targeted searches of potential breeding sites, in particular in areas in proximity to choruses of calling males. Furthermore, as eggs take many days to hatch, egg masses were present well after breeding events. Egg masses are also locatable during daylight hours which enabled increased and more efficient search effort.

Figure 3. A stylised illustration of Ovipositional Process 1 (e.g. *Mixophyes fasciolatus*). The amplexant pair of frogs is floating in water, braced against a partially undercut/overhanging stream bank. Artwork, G. Hancox.

Figure 4. A pair of *Mixophyes fleayi* in amplexus, during the process of spawning. Gap Creek West, Main Range National Park, SEQ, 15 Sep 1996. Note that the pair has created a shallow depression in the stream bed amongst the gravel substrate, in shallow running water. A small clump of eggs can be seen leaving the female's cloaca (oviposition step 4). Photo, R. Knowles.

As a result, the number of egg masses detected (over 700) was substantially higher than the number of occasions on which we observed oviposition (22 occasions, see Table 2). The morphology of egg masses resulting from the two ovipositional processes are described below with quantitative data summarised in Tables 3 and 4.

Table 3. Dimensions of *Mixophyes* egg masses from Ovipositional Process 1. Refer to Table 2 for additional locality details. Atypical egg masses (i.e. those with very low numbers of eggs or with eggs in scattered or diffuse clumps or obviously contained multiple clutches of eggs) are excluded. SE = standard error of the mean. Species abbreviations as per Table 2. All measurements are in millimetres.

Species	Location	Shortest dimension				Longest dimension				Lowest egg above water				Highest egg above water			
		Mean	SE	Range	n	Mean	SE	Range	n	Mean	SE	Range	n	Mean	SE	Range	n
Mfa	Kroombit Ck north branch, Kroombit Tops NP, SEQ					600			1								
Mfa	Barker Ck, Bunya Mountains NP, SEQ					478	54.4	160 -850	4	50	0.0	50 -50	2	143	27.5	115 -170	2
Mfa	Bullocky's Knob pond, D'Aguilar NP, SEQ	65			1	183	44.4	100 -300	4	90			1	178	13.0	155 -200	3
Mfa	Sandy Ck, Tamborine NP, SEQ					275	25.0	250 -300	2					150			1
Mfa	Dalrymple Ck south branch, Main Range NP, SEQ	158	33.8	100 -230	4	223	38.3	120 -500	9	32	14.7	0 -100	7	157	13.8	115 -220	9
Mfa	Gap Ck West, Main Range NP, SEQ	64			1	163			1	27			1	96			1
Mfa	Dam, upper Duck Ck, near Lamington NP, SEQ					183	49.1	100 -270	3					95	23.6	60 -160	4
Mfa	Yabbra Ck, Yabbra NP, NENSW	227	62.3	150 -350	3	320	20.8	280 -350	3	50	28.9	0 -100	3	223	29.6	180 -280	3
Mfa	Unnamed trib. Bat Cave Ck, Nightcap NP, NENSW	125	26.3	80 -200	4	245	24.7	180 -300	4								
Mfa	All sites (n=9)	149	22.7	64 -350	13	273	29.6	100 -850	31	42	9.8	0 -100	14	154	11.1	60- 280	23
Mit	Desert Ck, Washpool NP, NENSW	157	56.7	100 -270	3	387	109.1	240 -600	3	67	27.3	30 -120	3	137	18.6	100 -160	3
Mit	Bucca Bucca Ck, Orara East SF, NENSW	198	20.9	120 -300	9	347	25.2	250 -500	9	45	5.0	40 -50	2	149	31.0	108 -210	3
Mit	All sites (n=2)	188	20.3	100 -300	12	357	30.3	240 -600	12	58	15.9	30 -120	5	143	16.4	100 -210	6

Eggs deposited out of water

We observed 79 egg masses of *M. fasciolatus* from 19 sites in southeast Queensland and northeast NSW and 17 of *M. iteratus* from three sites in northeast and mid-east NSW (Table 2). Dimensions of egg masses of these species are provided in Table 3.

The egg masses of *M. fasciolatus* were large (Table 5) with a mean of 1461 eggs (range 740–2800, $n = 8$) and generally rectangular-shaped, extending, on average, along 273 mm of stream bank, $n = 13$, and averaging 149 mm in vertical

extent, $n = 31$ egg masses). The mean depth of water beneath the egg masses of *M. fasciolatus* was 231 mm ($n = 12$, range 40–450 mm) and eggs were positioned between 0 and 280 mm above the water line (Table 3).

The eggs of *M. fasciolatus* are propelled out of the water onto an adjacent bank or overhanging structure (Figures 6–10). When selecting oviposition sites within streams, *M. fasciolatus* preferred pools. Oviposition sites were on steeply sloping, partially or completely overhanging banks (Figures 6, 7, 9 and 10) or tree trunks, beneath

Table 4. Dimensions of *Mixophyes* egg masses from Ovipositional Process 2. Refer to Table 2 for additional locality details. Atypical egg masses (i.e. those with very low numbers of eggs or with eggs in scattered or diffuse clumps) are excluded. SE = standard error of the mean. Species abbreviations as per Table 2.

Species	Locality	Shortest dimension (mm)			Longest dimension (mm)			n
		Mean	SE	Range	Mean	SE	Range	
Mba	Rockadooie Ck, Washpool NP, NENSW	70			80			1
Mba	Sharpes Ck, Barrington Tops NP, NENSW	95	5.0	70-100	110	7.3	90-140	6
Mba	Frying Pan Ck, Chichester SF, NENSW	120			120			1
Mba	Gap Ck, Watagans NP, MENS	65			70			1
Mba	All sites (n=4)	92	6.2	65-120	103	7.3	70-140	9
Mfl	East Kilcoy Ck, Conondale NP, SEQ	100			100			1
Mfl	Dalrymple Ck south branch, Main Range NP, SEQ	123	4.4	80-190	149	6.9	80-260	36
Mfl	Gap Ck West, Main Range NP, SEQ	104	2.6	55-180	125	4.4	55-250	79
Mfl	Condamine R., near Main Range NP, SEQ	120	10.0	110-130	140	10.0	130-150	2
Mfl	Cainbale Ck, Lamington NP, SEQ	120			155			1
Mfl	Darraboola Ck, Lamington NP, SEQ	103	2.5	100-105	103	2.5	100-105	2
Mfl	Bundoomba Ck, Lamington NP, SEQ	116	9.1	80-150	124	10.7	80-150	8
Mfl	Long Ck, Border Ranges NP, NENSW	58	2.5	55-60	63	2.5	60-65	2
Mfl	Yabba Ck, Yabba NP, NENSW	100			160			1
Mfl	All sites (n=9)	109	2.2	55-190	130	3.5	55-260	132

washed out stumps (Figure 8), or on fallen logs above water. Most egg masses were observed on steeply sloping (i.e. near vertical) surfaces, but on a few occasions (e.g. at least four egg masses at Dalrymple Ck south branch, Main Range NP, SEQ) they were also observed on the ceiling of undercut banks (i.e. deposited on a more or less horizontal surface above the water). Often a feature of oviposition sites on steeply sloping banks was the presence of overhanging vegetation (see Figures 7 and 9) which presumably provided protection of eggs from direct sunlight and or desiccation, particularly at sites in open forest (e.g. Dalrymple Ck south branch, Main Range NP, SEQ). Some oviposition sites contained clutches of eggs from numerous pairs (Figures 9 and 10) and some oviposition sites were used on multiple occasions. Several times we also observed egg masses with very low numbers of eggs or with eggs in scattered or diffuse clumps, possibly as a result of the pair choosing an unsuitable oviposition site and subsequently moving, harassment by other males, and / or disturbance by nocturnal predators such as rough-scaled snake *Tropidechis carinatus* which frequented oviposition sites. Often eggs were several layers deep. They were typically firmly attached to the substrate and each other, such that they were not easy to dislodge. On several occasions hatching tadpoles were seen wriggling free of eggs and dropping or wriggling down to the water below.

Unlike other *Mixophyes*, *M. fasciolatus* often calls around and breeds in lentic (pond) as well as lotic (stream) habitats. Egg masses were observed at two ponds in southeast Queensland. The first site at Bullocky's Knob, D'Aguilar NP (Table 2) is a steep sided pond probably formed by quarrying rock. At this site *M. fasciolatus* egg masses were deposited in similar situations and

Figure 5. A pair of spawning *Mixophyes fleayi*, Gap Creek West, Main Range National Park, SEQ 05 Dec 2001. The pair has created a shallow depression in the stream bed amongst the gravel substrate, in shallow running water (oviposition step 3). The upper image shows the pair during oviposition step 4, at the instance when the female expelled a small clump of eggs (see Figure 4). The lower photo shows the pair resting within the nest hollow between bouts of egg laying (oviposition step 6). Photos, H.B. Hines.

Figure 6. Oviposition site and egg mass of *Mixophyes fasciolatus*, Stockyard Ck, Lamington NP, SEQ, 11 Dec 2001. Top – oviposition site on bank of stream (circled in white). Centre – close-up of egg mass with some overhanging debris removed, the opaque gelatinous material on the right are the remnants of capsules following hatching of tadpoles and some unviable eggs. Bottom – close-up of eggs, showing well-developed strongly pigmented tadpoles (stage 23) just prior to hatching. Photos, H.B. Hines.

resembled those typical of stream habitats (seven egg masses, all deposited above water on a more or less vertical bank). The second pond was a small, shallow farm dam in upper Duck Ck, near Lamington NP (Table 2). Most of the perimeter of this dam was gently sloping, such that typical oviposition sites of *M. fasciolatus* were limited. Of the 11 egg masses observed here, six were on a more or less vertical low earthen bank and four were

Figure 7. Oviposition site and egg mass of *Mixophyes fasciolatus*, Dalrymple Ck south branch, Main Range NP, SEQ, 17 Oct 2011. Top – oviposition site on the stream bank (circled in white), protected from sunlight by dense growth of *Polia crispata*. Bottom – close-up of the eggs (stage 9-10), attached to rocks and earth of the more or less vertical stream bank. Photos, H.B. Hines.

deposited out of water onto the sides of a fallen log that sloped from the bank into the water. Another large egg mass however, was deposited on very gently sloping mud about 60 cm from the water's edge.

Whilst the development of egg masses was not monitored, rotting eggs were noted in at least eight *M. fasciolatus* egg masses from six sites (e.g. Figure 10), of which most or all eggs were affected in four egg masses. A small proportion of desiccated eggs were also observed at several sites. At the dam in upper Duck Ck, near Lamington NP, SEQ (Table 2) one of the egg masses was almost entirely desiccated: this dam is in open forest and the egg masses were laid on a fallen log above water, with no overhanging vegetation providing protection from sunlight.

The egg masses of *M. iteratus* were large (Table 5) with a mean of 1988 eggs (range 1660–2200 eggs, $n = 4$) and roughly rectangular-shaped with mean dimensions of 188 x 357 mm ($n = 12$, Table 3). Egg masses were positioned over water (Figure 11), 30–280 mm deep (mean depth 120 mm, $n = 13$), and the eggs were placed between 30 and 210 mm ($n = 6$) above the water line (Table 3). All of the egg masses of *M. iteratus* were deposited on the ceiling of undercut banks of the stream pools (i.e. deposited on a more or less horizontal surface above the water).

Figure 8. Oviposition site and egg mass of *Mixophyes fasciolatus*, Dalrymple Ck south branch, Main Range NP, SEQ, 17 Oct 2011. Top – oviposition site, underneath a partially washed out tree stump (circled in white). Bottom – close-up of eggs (stage 8–9) attached to rocks and earth on the sides and ceiling of the undercut bank. Photos, H.B. Hines.

A feature of pools of streams in eastern Australia is the occurrence of undercut banks. These are commonly associated with the roots of trees and shrubs that grow adjacent to the stream, which bind the soil and stones of the banks, resulting in a more or less stable undercut created by the long-term erosional effects of stream flow. Undercut banks vary in size, but in streams where *Mixophyes* occur they are typically 20–30 cm deep and with a ceiling 5–10 cm above the water, extending along a bank for many metres. Undercut banks do not occur along riffle zones. From our limited observations *M. iteratus* appears to rely on the ceilings of these undercut banks for oviposition. As it is difficult to survey beneath undercut banks it is possible that these sites are also used by other *Mixophyes* species with Ovipositional Process 1, as shown for *M. coggeri* by Hoskin (2010) and our observations of four egg masses of *M. fasciolatus* on the ceilings of undercut banks at Dalrymple Ck south branch, Main Range NP, SEQ.

Eggs laid in shallow, gently flowing water

We observed 10 egg masses of *M. balbus* from four sites in northeast and mid-east NSW and 627 egg masses of *M. fleayi* from 19 sites across the full extent of its geographical range, which extends from NENSW to SEQ (Table 2). These two species shared similar oviposition

Figure 9. Oviposition site and egg masses of *Mixophyes fasciolatus*, Dalrymple Ck south branch, Main Range NP, SEQ, 17 Oct 2011. Top – oviposition site of two egg masses on a more or less vertical stream bank (circled in white), partially obscured by overhanging vegetation. Centre – closer view of the oviposition site and two egg masses. Bottom – close-up of the eggs (stage 4–8) of the left hand egg mass, showing the density of eggs in some situations. Photos, H.B. Hines.

sites with similar egg mass shape and dimensions (Table 4) (Figures 12–16). Typically, eggs were placed in gently flowing water of riffle zones within a rounded nest constructed in the substrate of sand, gravel and or leaf litter (~100 mm diameter, 30–40 mm deep). The mean number of eggs per egg mass for *M. fleayi* was 576 eggs (range 120–1750, $n = 30$), while for *M. balbus* there was a mean of 448 eggs per egg mass (range 130–824, $n = 8$; one-tailed t -test, $t = 0.26$, n.s.).

Table 5. The number of eggs observed in egg masses of *Mixophyes* species. Refer to Table 2 for additional locality details. Species abbreviations as per Table 2. Atypical egg masses (i.e. those with very low numbers of eggs or with eggs in scattered or diffuse clumps) are excluded.

Species	Location	Date	Number of eggs	Count or estimate
Mba	Rockadooie Ck, Washpool NP, NENSW	Feb-98	550	Estimate
Mba	Sharpes Ck, Barrington Tops NP, NENSW	Oct-96	500	Count
Mba	Sharpes Ck, Barrington Tops NP, NENSW	Oct-96	550	Count
Mba	Sharpes Ck, Barrington Tops NP, NENSW	Feb-00	130	Estimate
Mba	Sharpes Ck, Barrington Tops NP, NENSW	Feb-00	250	Estimate
Mba	Sharpes Ck, Barrington Tops NP, NENSW	Feb-00	550	Estimate
Mba	Frying Pan Ck, Chichester SF, NENSW	Nov-01	824	Count
Mba	Gap Ck, Watagans NP, MENSU	Dec-95	230	Count
Mfa	Dalrymple Ck south branch, Main Range NP, SEQ	Nov-96	1331	Count
Mfa	Yabbara Ck, Yabbara NP, NENSW	Jan-00	2005	Count
Mfa	Yabbara Ck, Yabbara NP, NENSW	Jan-00	1400	Estimate
Mfa	Yabbara Ck, Yabbara NP, NENSW	Jan-00	1210	Estimate
Mfa	Unnamed trib. Bat Cave Ck, Nightcap NP, NENSW	Sep-95	1200	Count
Mfa	Unnamed trib. Bat Cave Ck, Nightcap NP, NENSW	Sep-95	740	Count
Mfa	Unnamed trib. Bat Cave Ck, Nightcap NP, NENSW	Sep-95	2800	Count
Mfa	Unnamed trib. Bat Cave Ck, Nightcap NP, NENSW	Sep-95	1000	Count
Mfl	Dalrymple Ck south branch, Main Range NP, SEQ	Feb-97	1750	Estimate
Mfl	Dalrymple Ck south branch, Main Range NP, SEQ	Dec-97	900	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Sep-96	966	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Sep-96	1250	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Sep-96	941	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Oct-96	587	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Dec-96	709	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Mar-97	874	Count
Mfl	Gap Ck West, Main Range NP, SEQ	Jan-01	798	Count
Mfl	Bundoomba Ck, Lamington NP, SEQ	Oct-96	800	Estimate
Mfl	Bundoomba Ck, Lamington NP, SEQ	Oct-96	200	Estimate
Mfl	Bundoomba Ck, Lamington NP, SEQ	Oct-96	500	Estimate
Mfl	Bundoomba Ck, Lamington NP, SEQ	Oct-96	300	Estimate
Mfl	Darraboola Ck, Lamington NP, SEQ	Oct-96	590	Count
Mfl	Tuntable Ck, Nightcap NP, NENSW	Mar-01	480	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Mar-01	360	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Apr-03	660	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Aug-03	265	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Aug-03	740	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Sep-05	200	Estimate

Species	Location	Date	Number of eggs	Count or estimate
Mfl	Yabbra Ck, Yabbra NP, NENSW	Feb-00	500	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Sep-05	345	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Sep-05	150	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Sep-05	200	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Feb-06	850	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Feb-06	505	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Aug-03	250	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Aug-03	120	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Sep-02	175	Estimate
Mfl	Tuntable Ck, Nightcap NP, NENSW	Apr-03	310	Estimate
Mit	Desert Ck, Washpool NP, NENSW	Nov-95	2200	Count
Mit	Bucca Bucca Ck, Orara East SF, NENSW	Jan-95	1660	Count
Mit	Bucca Bucca Ck, Orara East SF, NENSW	Jan-00	1890	Count
Mit	Bucca Bucca Ck, Orara East SF, NENSW	Feb-00	2200	Count

Figure 10. Mass spawning of *Mixophyes fasciolatus*, Jacky Ck, D'Aguilar NP, SEQ, 7 Jan 1999. Top – oviposition site, showing multiple clutches, totalling many thousands of eggs, on a more or less vertical earthen stream bank. The paler area in the centre of the egg mass is rotting eggs. Bottom – close-up of the late stage eggs (stage 23-4) showing heavily pigmented tadpoles and highly vascularised yolk sacs. Photos, H.B. Hines.

Figure 11. Oviposition site and egg mass of *Mixophyes iteratus*, Desert Ck, Washpool NP, NENSW, Dec 1995. Top – oviposition site, eggs were laid onto the ceiling of the undercut stream bank immediately below the compass (indicated by the white arrow). Bottom – close-up of the eggs (stage 19) on the ceiling of the undercut stream bank. Photos, R. Knowles.

Table 6. Diameter and development stage of *Mixophyes* eggs. Refer to Table 2 for additional locality details. Development stage as per Gosner (1960). SE = standard error of the mean. Species abbreviations as per Table 2.

It is our experience that measurements of egg diameters are affected by a number of factors such as the degree of hydration, method of measurement, as well in changes in the shape of the egg in the latter stages of development. These are field measurements taken using calipers, with the eggs on a solid substrate and are the diameter of the capsule (see Anstis 2002, p. 70).

Species	Locality	Development stage	Mean	SE	Range	n
Mfa	Kroombit Ck north branch, Kroombit Tops NP, SEQ	Late, >20	4.6	0.07	4.1 - 5.0	13
Mfa	Bullocky's Knob pond, D'Aguilar NP, SEQ	Late, >20	5.7	0.13	5.4 - 6.1	6
Mfa	Bullocky's Knob pond, D'Aguilar NP, SEQ	Early, <5	3.2	0.04	3.1 - 3.3	6
Mfa	Dalrymple Ck south branch, Main Range NP, SEQ	Early, <5	2.6	0.06	2.2 - 3.0	16
Mfa	Gap Ck West, Main Range NP, SEQ	Late, >20	4.7	0.10	4.3 - 4.9	6
Mfl	Gap Ck West, Main Range NP, SEQ	Mid, <12	2.8	0.04	2.5 - 3.0	10
Mfl	Gap Ck West, Main Range NP, SEQ	Early, <5	3.0	0.04	2.7 - 3.3	17
Mfl	Bundoomba Ck, Lamington NP, SEQ	Late, >20	3.2	0.05	3.0 - 3.6	11
Mfl	Bundoomba Ck, Lamington NP, SEQ	Mid, 17-19	3.3	0.06	3.0 - 3.6	9
Mfl	Brindle Ck, Border Ranges NP, NENSW	Early, <5	4.2	0.03	3.7 - 4.5	50

Figure 12. Nest and eggs of *Mixophyes fleayi*, Gap Ck West, Main Range NP, SEQ, 26 Oct 2004. Top – nest constructed in gravel in shallow, gently flowing water. Most eggs are packed into the gravel around the perimeter of the nest depression. Note the larger stone on the upstream side of the egg mass which probably provides some protection from higher stream flows. Bottom – close-up of eggs, (~stage 9). Photos, H.B. Hines.

Egg masses of *M. fleayi* were typically laid in shallow, gently flowing water of glides and riffles of higher order streams (mostly stream order two and three – Table 2). Egg masses tended to be laid in the centre of the stream (see Figure 18) and were only very rarely seen towards or at the stream margin. This bias in nest site preference resulted in a very patchy distribution of egg masses along streams with some streams having very limited suitable oviposition microhabitat. The form of the egg mass varied depending upon the substrate and stream morphology. The vast majority of egg masses were laid in a nest depression made in the stream bed by the female whilst in amplexus – formed by her shuffling her hind limbs amongst the substrate, whilst spinning around (step three in Ovipositional Process 2 described above). The resultant nest was more or less circular where the substrate was fine and homogenous, such as sand or gravel (Figure 12). More irregular substrates (leaf litter, coarse gravel) or less homogeneous substrates resulted in less symmetrical nests (Figure 13). For recently laid (i.e. Gosner stage <10) *M. fleayi* egg masses at Gap Ck West, Main Range NP, SEQ, the mean depth of water in the centre of the nest depression was 34 mm (n = 37, SE = 1.5, range = 15–45 mm). In larger streams or steeper sections of streams, egg masses were often laid immediately downstream of a larger stone, presumably providing some protection from higher flows (Figure 12).

Some streams that lacked sandy or gravelly riffles or glides had suitable oviposition sites in sections where shallow water flowed gently over wide areas of exposed bedrock in the stream bed. Oviposition was not observed but at least 10 egg masses were found in these situations, at three sites. Eggs were laid directly onto the bed rock as a single layer, or up to a few layers deep (Figure 15). In these cases the eggs adhered strongly to the bedrock such that they would be capable of withstanding substantially increased flows of water. We refer to this as Ovipositional Process 2B to distinguish it from the more frequently encountered process described above (which we refer

Figure 13. Nest and eggs of *Mixophyes fleayi*, Gap Ck West, Main Range NP, SEQ, 15 Mar 2007. Top – nest constructed in leaf litter and gravel in shallow, gently flowing water. Most eggs are packed into the litter and gravel around the perimeter of the nest depression. Bottom – close-up of eggs (~stage 18). Photos, H.B. Hines.

Figure 14. Egg masses of *Mixophyes fleayi*, following falls in water level of the stream, Gap Ck West, Main Range NP, SEQ, top – 15 Mar 2007, bottom – 22 Oct 1998. In both cases the eggs remained moist and viable. Photos, H.B. Hines.

to as Ovipositional Process 2A). At some breeding sites Process 2B formed the majority of egg masses observed (e.g. parts of Bundoomba Ck, Lamington NP, SEQ). However even at streams where there were substantial areas of gravelly riffles and glides, occasional egg masses were observed on bedrock (e.g. one of 413 egg masses at Gap Ck West, Main Range NP, SEQ). These observations show that Ovipositional Processes 2A and 2B are not particular to certain populations of *M. fleayi* but are likely to be flexible responses within populations and potentially individuals, presumably to exploit available substrate with suitable hydrology.

In addition to the *M. fleayi* egg masses listed in Tables 2 and 4 we occasionally observed egg masses with very low numbers of eggs (e.g. 10–15 eggs) or scattered over larger areas and not of the form of typical egg masses. As with *M. fasciolatus* this may have been a result of the amplexant pair choosing an unsuitable oviposition site and subsequently moving, harassment by other males, and/or disturbance by nocturnal predators. At times at Gap Ck West, Main Range NP, SEQ large numbers of male *M. fleayi* congregated on the gravel banks used for oviposition which resulted in many physical interactions between calling males and between males and amplexant pairs

(see Figures 7 and 9 of Stratford *et al.* 2010). Amplexant males were observed on at least two occasions to fend off other males with their hind limbs. Rough-scaled snakes *Tropidechis carinatus* were also frequently observed at oviposition sites and one was observed attempting to ingest a female *M. fleayi* (Figure 17).

Calling activity (O'Reilly and Hines, 2002, Stratford *et al.* 2010) and oviposition (Hines unpublished data) in *M. fleayi* (and presumably *M. balbus*) are strongly linked to stream flow and season. Peak breeding activity occurs as the stream falls back to basal flow following a period of higher flow, from spring through summer to early autumn. Depending upon the temperature of the water, egg development can take up to several weeks (Hines unpublished data). During the period of development the stream level sometimes dropped causing the nest depression to drain. Eggs appeared to remain viable as long as the substrate was moist (even if no surface water was visible) (Figure 14). On a number of occasions recently hatched tadpoles were found alive, sheltering within the moist substrate, with only their tails sticking out of the gravel. However, on several occasions desiccated eggs and/or tadpoles were observed when the substrate dried out. At other times the stream level rose

following oviposition and often in these circumstances large conspecific tadpoles or tadpoles of *M. fasciolatus* were observed foraging on the eggs (this was observed at eight sites). Large *Mixophyes* tadpoles usually inhabited much deeper water (pools) than that used by *M. fleayi* for oviposition (glides and riffles). If the stream remained near basal flow hatchling tadpoles were observed within the nest depression (Figure 16), where they appeared to eat the remains of egg capsules, before moving out to the surrounding stream.

Many egg masses of *M. fleayi* contained a small proportion of rotting eggs, presumably unfertilised or unviable eggs that succumbed to aquatic fungi. Occasionally entire egg masses were affected, for example on 05 Feb 2002 at Gap Ck West, Main Range NP, SEQ (Table 2) five egg masses were rotten, most likely due to desiccation followed by rewetting from rainfall.

Potential egg predators

Most streams where *M. fleayi* occur support a low species richness of fish. East of the Great Dividing Range eels (southern shortfin eel *Anguilla australis* and or longfin eel *A. reinhardtii*) are present at low density at all altitudes. At lower altitudes (e.g. Tallebudgera Ck, near Springbrook NP, SEQ – Table 2) additional species, such as Cox gudgeon *Gobiomorphus coxii*, are present. Eels are absent from streams running west of the Great Dividing Range, but mountain galaxias *Galaxias olidus*, are often abundant, and the freshwater catfish *Tandanus tandanus*, was also present at one site (Condamine R., near Main Range NP, SEQ – Table 2). In contrast to our observations of tadpole predation, we never saw fish attacking egg masses of *M. fleayi*. On the morning of 08 Mar 1997 at Gap Ck West, Main Range NP, SEQ (Table 2) some *M. fleayi* eggs were taken from an egg mass and offered to *G. olidus* in a nearby pool. The fish immediately attacked the small clumps of eggs, with larger fish appearing capable of ingesting several eggs at a time. Small fish had problems ingesting single eggs but rapidly ate smaller egg fragments created by the bigger fish. This was repeated with similar results for a second egg mass about 800 m further upstream.

In contrast the lower order streams that often form the habitat for *M. fasciolatus* and in particular *M. iteratus* (e.g. Mary, Stanley and Coomera Rivers, SEQ) are rich in fish species, including large predatory species such as gudgeons (*Gobiomorphus* spp., *Mogurnda adspersa*), eels (*Anguilla* spp.), catfish (*T. tandanus*), Australian bass (*Macquaria novemaculeata*) and cod (*Maccullochella* spp.) (Pusey, Kennard and Arthington 2004), likely to find *Mixophyes* eggs highly palatable. While we have not tested this assumption widely, on 18 Nov 1996 a small number of *M. fasciolatus* eggs were offered to *G. olidus* that were living in the pool immediately below an oviposition site, at Dalrymple Ck south branch, Main Range NP, SEQ (Table 2). The eggs were immediately attacked and consumed by the fish.

Many species of crayfish co-occur with *Mixophyes* species, in particular from the genus *Euastacus*. The diet of Australian crayfish is poorly known but they are considered polytrophic (Coughran and Furse in press). *Euastacus* in the laboratory feed on a range of foods, including meat, and in the field they are readily attracted to protein-based baits (e.g. Furse 2010). *Euastacus sulcatus* is a large species that forages widely along streams and adjoining forests, by day and by night (Coughran 2013) and is common at many *M. fleayi* breeding sites. For example it is abundant at Gap Ck West, Main Range NP, SEQ (Table 2) where we have observed many egg masses in the stream bed (413) over a range of years, seasons, times of day and climatic conditions. On several occasions we observed *Euastacus* in close proximity to *M. fleayi* egg masses but surprisingly we did not see evidence of egg predation.

Discussion

Oviposition has now been observed in the wild for *M. balbus*, *M. fleayi*, *M. fasciolatus* and *M. iteratus* (our observations; Lewis 2000) and *M. coggeri* (Hoskin 2010). The natural egg deposition sites and the form of egg masses for all Australian species of *Mixophyes* are now known. Within the genus there are two markedly different ovipositional processes and resultant egg mass structures.

Figure 15. Egg mass (~stage 19) of *Mixophyes fleayi*, laid directly onto bed rock (Oviposition Process 2B), under very shallow gently flowing water, Bundoomba Ck, Lamington NP, SEQ, 13 Dec 1999. Photo, H.B. Hines.

Figure 16. Hatchling *Mixophyes fleayi* tadpoles (stage 25) in the water filled nest depression, Gap Ck West, Main Range NP, SEQ, 12 Nov 1998. Photo, H.B. Hines.

Ovipositional Process 1 involves a floating amplexant pair propelling eggs out of the water, resulting in an egg mass on the sides of the stream bank or on the ceiling of an undercut stream bank, and is now known from *M. fasciolatus* and *M. iteratus* (observations presented here), *M. coggeri* (Hoskin 2010, Anstis 2013) and *M. carbinensis* and *M. schevilli* (Anstis 2013). Ovipositional Process 2 involves an amplexant pair laying eggs in shallow running water, either in a nest depression constructed within sand, gravel or detritus, or pasted directly onto bedrock, both within the stream bed, and is now known from *M. fleayi* and *M. balbus* (observations presented here; Lewis 2000). Oviposition and spawn of *M. hihiorlo*, a species endemic to Papua New Guinea, remains unknown.

Our use of the term egg mass is equivalent to the concept of a 'group' of eggs *sensu* Altig and McDiarmid (2007), such that a group can comprise eggs from one or more bouts of egg laying and that one or more groups may make up the clutch. Altig and McDiarmid (2007) define a clutch as the total number of eggs deposited per ovulation event. The ovipositional mode (Altig and McDiarmid 2007) of each group of *Mixophyes* eggs is an adherent clump, i.e. a multilayered stack of eggs that lack a common, surrounding surface or matrix, with interstices among eggs and with adjacent eggs adherent. A clutch of *Mixophyes* eggs is therefore one or more adherent clumps, either deposited aquatically or terrestrially. Consequently the egg masses we observed may at times have been part of a clutch, the entire clutch or include eggs from multiple clutches (different pairs of frogs depositing eggs within a short time-span). This is likely to explain the wide ranges in observed counts of eggs in egg masses in the field (Table 5) and our observations of scattered eggs or egg masses with very low counts. This is further supported by Lewis' (2000) observation of a pair of *M. balbus* that constructed ten nest depressions, of which eight contained eggs. It may also explain discrepancies with high gonadal counts of eggs, for example 4184 eggs for a museum specimen of *M. iteratus* (Hero and Fickling 1996) compared with the four egg masses we counted for this species (range 1660–2200).

The observations of ovipositing, depositional sites and egg masses show that there are three reproductive modes (*sensu* Haddad and Prado 2005) in the genus: mode 2 – eggs and exotrophic tadpoles in lotic water (Ovipositional Process 2B of *M. fleayi*), mode 4 – eggs and early larval stages in constructed basins, subsequent to flooding exotrophic tadpoles in streams (Ovipositional Process 2A of *M. balbus* and *M. fleayi*), and mode 18 – terrestrial eggs above water, exotrophic tadpoles move to water (Ovipositional Process 1 of *M. carbinensis*, *M. coggeri*, *M. fasciolatus*, *M. iteratus* and *M. schevilli*). Furthermore, reproductive mode 18 within *Mixophyes*, was mostly in stream habitats although *M. fasciolatus* also used ponds.

These modes of reproduction are likely to be adaptations to avoid loss of eggs to aquatic predators such as odonates, beetles, fish and tadpoles, whilst maintaining egg moisture and oxygen supply (Magnusson and Hero 1991; Duellman and Trueb 1994; Resetarits 1996; Haddad and Prado 2005; Malone 2004, Altig and McDiarmid 2007). Our

Figure 17. Female *Mixophyes fleayi* being ingested by a rough-scaled snake *Tropidechis carinatus*, Gap Ck West, Main Range NP, SEQ, 06 Mar 2002. Photo, H.B. Hines.

Figure 18. Distance of *Mixophyes fleayi* egg masses from the nearest stream margin ($n = 107$), from four sites in Main Range and Lamington National Parks, SEQ. The distance was the right angle distance from the egg mass (laid in the stream bed) to the nearest wetted margin of the stream.

observations, of tadpoles feeding on egg masses of *M. fleayi* and of sympatric native fish consuming *M. fasciolatus* and *M. fleayi* eggs offered to them, support this hypothesis. Depositing eggs out of water or in shallow water is not without risks as both are prone to desiccation and terrestrial predators (e.g. Richards 1993). Typically the sites used in mode 18 (i.e. eggs laid out of water) are sheltered from direct sunlight, either as a consequence of being deposited behind overhanging vegetation (Figures 7–9), under a dense forest canopy (e.g. rainforest) or under an embankment, rock or other structure (Figures 8 and 11). Also the eggs are within a short distance of the water and in situations where the humidity is high. In *M. balbus* and *M. fleayi* (modes 2 and 4) a drop in stream flow can result in egg masses and/or hatchling tadpoles being stranded

without free water. Our experience with *M. fleayi* suggests that eggs or hatchling tadpoles can survive as long as the substrate remains moist (Figure 14) and the oviposition site receives little or no direct sunlight. Furthermore, we have observed hatchling tadpoles of *M. fleayi* seeking refuge by burrowing into the moist substrate at the oviposition site.

Reproductive mode 4 is only known from a few hylids from central and southern America, e.g. *Smilisca sordida* and the *Hypsiboas semilineatus* species group (Faivovich et al. 2005), four Asian ranids and in the Australian hylid *Litoria jungguy* (Richards, 1993; using the name *L. lesueuri*, see Donnellan and Mahony 2004) (Malone 2004 and references therein). Richards (1993) proposed that the evolution of nest construction in *L. jungguy* was in response to a fine, mobile substrate on which egg deposition was problematic without a depression to protect the egg mass from the stream current. This scenario is unlikely in *Mixophyes* as the substrate is usually much more heterogeneous, coarser and stable and the eggs are mixed into the substrate and bind firmly to it and each other, rather than the gelatinous egg mass of *L. jungguy* which is not incorporated into the substrate. A third hypothesis for the functional significance of nest construction is that it results in elevated temperatures and hence reduced developmental times for eggs within the depression (Richards 1993 and references therein; Malone 2006). Again this is unlikely in *Mixophyes* as nests typically have stream water very gently percolating through them. However additional studies are required to test these hypotheses.

Foamy egg masses are a feature of several limnodynastid genera where eggs are placed in water or in burrows, and Seymour, Mahony and Knowles (1995) argue that the function of the foam is to facilitate gas exchange. Gas exchange would not appear to be a problem for the eggs of *Mixophyes* species laid out of water because they are typically exposed to the air on at least part of their surface (Figures 6–11), and the sites have high humidity which is essential to keep the egg capsule moist so that oxygen can diffuse into the egg. For the species using modes 2 and 4, eggs are placed in cool, shallow, gently flowing water, where oxygenation is likely to be high. If the stream level drops, eggs of these species are exposed to the air (Figure 14) but while they remain moist oxygen diffusion presumably will be high.

Previous accounts of oviposition and egg masses were based on either very low numbers of observations in the field or from captive animals, and sometimes lacked sufficient detail or clarity, or assumed similar ovipositional processes for all *Mixophyes* species. Our observations fill a number of knowledge gaps, thus enabling some clarification of contradictory or potentially misleading statements in the literature, particularly in regard to oviposition site. In the following section we address some of these major discrepancies, supported by our observations.

Straughan (1966 unpubl.) and Robinson (1993) imply that *M. fasciolatus* eggs are laid by the amplexant pair sitting out of water. The six amplexant pairs we observed laid eggs whilst floating in water. In addition the vast majority of the 79 egg masses we observed of this species were in

situations where it would have been impossible for the amplexant pair to lay eggs unless they were floating in water (i.e. laid on vertical banks or beneath undercut banks/overhangs). Furthermore, Straughan's observations (and later cited by Martin 1967) of *M. fasciolatus* eggs being laid in loose detritus along banks appears atypical, as most of our observations were of eggs on near vertical surfaces or beneath overhangs. Our data do not concur with statements by Straughan (1966 unpubl.), Barker and Grigg (1977) or Barker, Tyler and Grigg (1995) that when larvae hatch they are washed by rain or flood into the water body. Most egg masses we saw were protected from rain or run-off, except in extreme conditions, and eggs were firmly attached to the substrate and/or each other. We observed tadpoles hatching by wriggling strongly and rupturing the egg capsule and dropping or wriggling down to the water below, as documented in captivity by Marantelli (1995).

Hoser's (1989) statement that *M. balbus* lays eggs on vegetation adjacent to streams is contrary to our observations of 10 *M. balbus* egg masses from four sites, and the observations of Lewis (2000) at another site. We have only observed *M. balbus* and *M. fleayi* using Ovipositional Process 2, where eggs are laid in shallow running water in the stream bed ($n = 627$ for the latter). Occasionally we saw *M. fasciolatus* eggs attached to vegetation overhanging an oviposition site, but in these cases most eggs were attached to the substrate of the bank (e.g. soil, rock, root mass).

Ovipositional Process 2 has only been recognised in *Mixophyes* since the late 1990s (Knowles et al. 1998 unpubl.; Lewis 2000). Consequently much of the previous literature on oviposition and spawn of *M. balbus* and *M. fleayi* has largely been incorrect, assuming egg laying to be the same as for *M. fasciolatus* or based on atypical or poorly documented observations (Watson and Martin 1973, Barker and Grigg 1977, Robinson 1993, Tyler 1994, Barker, Tyler and Grigg 1995). Most intriguing is the account of eggs presumed to be those of *M. fleayi* by Corben and Ingram (1987) as being "found under a rock about 30 cm from water and attended by an adult frog". We have observed oviposition 11 times in *M. fleayi* (always in shallow running water in the stream bed) and have observed in excess of 600 egg masses from 19 sites across the geographical and altitudinal distribution of this species and have never seen eggs in such a situation. The only occasions adult frogs were in association with eggs was during amplexus, or rarely a female sitting in the nest depression during daylight hours, presumably following oviposition. However, males often called from positions in the stream bed, on top or near egg masses but with no indication of being "in attendance" of the egg mass. Corben and Ingram (1987) also described the eggs as resembling *Pseudophryne*, but the capsules were much larger. While capsule diameter varies with stage of development and degree of hydration, our measurements of eggs of varying developmental stage from five *M. fleayi* egg masses from three sites (Table 6), showed capsule diameter ranging from 2.5 to 4.5 mm. The only *Pseudophryne* species that we have observed in the catchments of sites occupied by *M. fleayi* is *P. coriacea*

which has a capsule diameter ranging up to 5.17mm when hydrated (Anstis 2002), somewhat larger than we observed in *M. fleayi* and most other *Mixophyes* eggs (Table 6). Our observations suggest that it is more likely that an adult *M. fleayi* was sheltering under or beside a rock which coincidentally was the oviposition site of a *Pseudophryne* species.

Phylogenetic significance

The phylogenetic relationships and familial placement of *Mixophyes* within Myobatrachoidea (Myobatrachidae + Limnodynastidae) remain unresolved. Older, mainly morphology based studies, placed this genus within Limnodynastidae (e.g. Lynch 1973; Heyer and Liem 1976; Ford and Cannatella 1993; Davies 2003a). Limnodynastidae are united by fusion of the first two vertebrae (atlas and axis) whereas these vertebrae are free in all Myobatrachidae, including *Mixophyes* and *Rheobatrachus* (Lynch 1973; Heyer and Liem 1976; Davies 2003a). Horton (1982) and Donnellan, Mahony and Davies (1990) reported that the relatively simple arrangement of the genioglossus muscle of *Mixophyes* was distinct both from Limnodynastidae, and other Myobatrachidae, with the exception of *Rheobatrachus* (which differs in its fused tongue). By contrast, Ford and Cannatella (1993) suggested that connection between the m. submentalis and m. intermandibularis was a morphological synapomorphy of the Limnodynastidae, including *Mixophyes*. More recent DNA based analyses provide equivocal support, either for an association with Limnodynastidae (Roelants *et al.* 2007) or with Myobatrachidae (e.g.; Frost *et al.* 2006; Pyron and Wiens 2011). There is general agreement that *Mixophyes* is a highly differentiated lineage (Heyer and Liem 1976), with time-calibrated molecular phylogenies estimating divergence from other living relatives in the late Cretaceous, around 85 million years ago (mya), compared with a maximum of 70 mya among other Myobatrachidae and 55 mya among Limnodynastidae, excluding the similarly problematic *Rheobatrachus*, with estimated divergence around 90 mya from other Myobatrachoidea (Pyron 2014; Roelants *et al.* 2007).

The reproductive behaviour, egg mass and larval morphology in *Mixophyes* add to this picture of distinctness. All genera of Limnodynastidae (*sensu* Frost *et al.* 2006) have foamy egg masses, except for the desert-adapted *Notaden* and *Neobatrachus*, whereas Myobatrachidae (*sensu* Frost *et al.* 2006), including *Mixophyes* and *Rheobatrachus*, do not have foamy egg masses. Anstis (2013), however, states that the embryos and larvae of *Mixophyes* share close similarities with the family Limnodynastidae, with embryos having external gills and tadpoles with very similar oral disc and body form. External gills are absent in all other Myobatrachidae (*sensu* Frost *et al.* 2006) except *Rheobatrachus* (Anstis 2013). The position of amplexus in frogs is an evolutionarily conservative character, with shifts among related genera generally associated with changes in oviposition behaviour and sexual dimorphism (Wells 2007). *Mixophyes* has axillary amplexus, unlike other Myobatrachidae genera,

which have inguinal amplexus (Anstis 2013). The functional significance of amplexus position is unclear, but Wells (2007) suggests that axillary amplexus allows fertilization of larger batches of eggs and a reduced oviposition period. There are few comparative data, however, on oviposition period in Myobatrachoids and the form of amplexus is not known from several genera (e.g., *Arenophryne*, *Metacrinia*, *Myobatrachus*, *Rheobatrachus*) (Anstis 2013). Surprisingly, considering the diversity of reproductive biology, there is little differentiation in sperm morphology between *Mixophyes* and limnodynastid frogs (Lee and Jamieson 1992).

The two ovipositional processes in *Mixophyes* reveal that different adaptive strategies have evolved within the genus despite similarities in morphology. Molecular phylogenetic analyses indicate that *M. balbus* and *M. fleayi* are sister taxa (Donnellan *et al.* 1990) and it is likely therefore, that Ovipositional Process 2, with egg masses in shallow running water, is a shared character. Similarly, Ovipositional Process 1, with eggs deposited out of water, unites the other members of the genus which form a sister clade (Donnellan *et al.* 1990). The only member of the genus for which oviposition is unknown is the enigmatic *M. hihiorlo* from Papua New Guinea which is a sister lineage to the Australian species in molecular phylogenetic studies (Donnellan *et al.* 1990). Without outgroup analysis, it is not possible to determine which of the ovipositional processes observed may represent the ancestral and which the derived state, and this may be assisted with observation of the mode in *M. hihiorlo*. Alternatively, it is possible that both processes are derived, as reproductive strategies are highly diverse within Myobatrachidae (*sensu* Frost *et al.* 2006) (Davies 2003b). There are no other members of the family Myobatrachidae that show either of these ovipositional processes. It is likely that, as with other aspects of reproductive strategy, ovipositional process is a character that is open to ecological selection and is not particularly useful for phylogenetic reconstructions beyond the genus level.

Implications for conservation management

Several management issues arise from the observations of oviposition in the barred frogs. The four species of *Mixophyes* which use Oviposition Process 1, *M. carbinensis*, *M. coggeri*, *M. fasciolatus*, *M. iteratus* and *M. scheyvilli* require undercut, overhanging or near vertical stream banks for egg deposition. The formation and retention of these structures is dependent upon or strongly influenced by the root masses of riparian vegetation (e.g. Beschta and Platts 1986; Cummins 1986; Treadwell, Koehn, and Bunn 1999; Pusey and Arthington 2003). Consequently, loss of this vegetation through clearing, logging, grazing, trampling (by stock and or humans) or rooting by feral pigs has the potential to reduce availability of oviposition sites. The presence of vegetation overhanging the stream bank/oviposition site is likely to protect eggs from sunlight and desiccation. Erosion resulting from trampling of stream banks by cattle and horses or loss of riparian vegetation as well as altered flow regimes from impoundments, infrastructure development (e.g. bridge

pylons) or changes in catchment hydrology (e.g. broad scale clearing) will reduce availability of oviposition sites.

Mixophyes balbus and *M. fleayi*, which use Oviposition Process 2, are species of conservation concern (Table 1). These species usually place egg masses in gently flowing, shallow riffles. As riffles have lower banks than the surrounding stream sections stock tend to use them to access or cross the stream. Due to the presence of shallow water, riffles are often used for road, bike, horse-riding and walking track crossings. We observed trampling of *Mixophyes fleayi* egg masses by stock and or humans at several sites, as well as fouling and damage to potential oviposition sites by cattle. Where there are populations of *M. balbus* or *M. fleayi*, stream crossings (for people, stock and vehicles) should avoid riffles. Road crossings, forestry activities, grazing and land clearing can increase

sediment in streams (Parris and Norton 1997; Gillespie and Hines 1999). Higher sediment loads may reduce the availability or quality of oviposition sites through filling of interstitial spaces in the stream bed and blanketing substrates, resulting in increased mortality of eggs from predation, desiccation or flooding (Gillespie and Hines 1999). The weed mistflower *Ageratina riparia* is now well established across the range of *M. fleayi*, particularly at riffles with more open canopy cover. This herbaceous weed forms dense stands within the stream bed and its abundant roots bind the substrate tightly, possibly reducing oviposition site availability or quality. Further research on the potential impacts of this weed on *Mixophyes* species is required. Control activities in riffles should avoid the use of chemicals and be done in winter to avoid the extended breeding season of these two species.

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