Conservation Forests & Lands
Fisheries Division
Victoria

ARTHUR RYLAH INSTITUTE FOR ENVIRONMENTAL RESEARCH

TECHNICAL REPORT SERIES No. 80

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ISSN 0810-5774

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SUMMARY

Mature, female Macquarie perch, *Macquaria australasica*, were collected from the wild in the Mitta Mitta River and the Mitta Mitta River arm of Lake Dartmouth in north-eastern Victoria during the normal breeding season (Oct.-Dec.) and induced to spawn by either single or incremental, intraperitoneal injections of human chorionic gonadotrophin (HCG) at dosages of 500-1000 IU/kg HCG. Injected fish were held at 18-20^{o}C and either spawned in brood tanks or their eggs and sperm were hand-stripped and mixed together. Courtship behaviour, following injections of HCG, is described. Only ripe fish injected with 750-1000 IU/kg HCG produced eggs that were fertilized. Females which produced eggs which were fertilised, did so within 26 h of the last incremental injections and within 38.8-42.8 h of single injections.

The natural spawning mechanisms of Macquarie perch in Lake Dartmouth are described in an attempt to explain the apparently variable results of HCG-induced ovulation and spawning of this species.

INTRODUCTION

The Macquarie perch, *Macquaria australasica* (Cuvier) (Percichthyidae), is an endemic, Australian, freshwater fish valued for conservation and recreational angling. However, its distribution and abundance within its natural geographic range have declined markedly (Cadwallader, 1981) and, in at least part of this range, its conservation status is now considered vulnerable (Cadwallader *et al.*, 1984). Consequently, attempts have been made to artificially culture Macquarie perch to supplement depleted natural stocks. In the wild, it has been suggested that Macquarie perch spawn annually, during spring or early summer, at water temperatures above 16.5^{O}C, and lay demersal, slightly adhesive eggs in shallow, flowing water over a rock or gravel substrate (Wharton, 1968; Cadwallader and Rogan, 1977).

The technique of hormone-induced spawning (Lam, 1982) has been used to culture several endemic, Australian, freshwater fishes, including Murray cod, *Maccullochella peeli*, golden perch, *Macquaria ambigua* and silver perch, *Bidyanus bidyanus*, e.g. see Wyse (1973), Rowland (1983, 1984, 1985) and Cadwallader and Gooley (1985). Wharton (1973) attempted to induce spawning in *M. australasica* by injecting human chorionic gonadotrophin (HCG) into the musculature of pond-held and wild brood fish, but the fish produced no viable eggs. In the present study, efforts were made to develop a reliable technique for inducing spawning of wild brood Macquarie perch by intraperitoneal injections of HCG. To facilitate this objective, attempts were made to further elucidate the natural spawning mechanisms of Macquarie perch.

MATERIALS AND METHODS

Wild brood Macquarie perch were collected from Lake Dartmouth (147°31'E, 36°35'S) and its tributaries, in north-eastern Victoria, during the 1983, 1984 and 1985 breeding seasons (October to December). Resettable, minimum-maximum thermometers were used to periodically monitor water temperatures in the Mitta Mitta River (the largest inflowing river) and the Mitta Mitta River arm of Lake Dartmouth, where the fish for this study were collected. The fish were taken by angling or were captured in gill nets and drum nets. The captured fish were held in 60 L, thermally insulated, aerated containers and transported to the laboratory daily. Efforts were made to minimise stress on the fish in transit by transporting them in river water containing 5 g/L NaCl and 50 mg/L $CaCl_2$, or containing an anaesthetic solution of 40 mg/L Stresnil (azaperone).

In the laboratory, fish were anaesthetised in a solution of 10 ppm Hypnodil (metomidate hydrochloride), weighed (wet weight, to the nearest 50 g), measured (total length, TL, and/or standard length, SL, to the nearest 5 mm), sexed and tagged (according to sex only). When only standard length was measured, we calculated total length from the equation.

$$TL = (SL - 2.756)/0.8182$$

derived by Cadwallader (1984).

Only running-ripe males with motile sperm were selected for experiments; most were given a single intraperitoneal injection of HCG solution (200 IU/kg HCG) to maximise sperm production while in captivity. Putative, mature females, distinguished by their characteristic, enlarged abdomen and swollen genital papilla, were given either a single intraperitoneal injection of HCG solution or a series of three consecutive, daily, incremental, intraperitoneal injections. The incremental dosages were 10%, 10% and 80% respectively of the total dosage. The total volume of each injection was standardised to 0.1 ml/100 g body weight. During the 1983 breeding season, both single (at dosages of 750-1000 IU/kg HCG) and incremental injections (at total dosages of 250-1000 IU/kg HCG) were used. As a control group, some fish were also injected with distilled water. It was subsequently decided that all fish used in spawning trials during the 1984 breeding season would be injected incrementally with a total dosage of 1000 IU/kg HCG, and during the 1985 breeding season all fish would be injected with a single dosage of 1000 IU/kg HCG.

Injected fish were placed in 60-120 L tanks at temperatures of 18-20°C. Fish were held either individually or in various combinations of one or more females with one or more males, at ratios ranging from one female to one to three males. Any courtship behaviour was noted. Fish were left to spawn in the tanks or, alternatively, both males and females were anaesthetised and removed (after the females were thought to have ovulated) and the eggs and sperm hand-stripped. Before being stripped, the fish were rinsed in fresh water to remove all traces of anaesthetic. Sperm motility of the donor males was checked microscopically (x 400). Only males with active sperm were used. Eggs were stripped into a dry, calibrated bowl; the sperm was either stripped directly onto the eggs or collected in a calibrated, plastic syringe and then dispersed onto the eggs at a rate of 5 mL sperm per 50-150 mL eggs. For each injected female, the latency time between the last injection and spawning or stripping was recorded to the nearest 0.1 h. When the time was

not known, e.g. for fish left to spawn in the brood tanks, the latency period was estimated.

Fish collected during the 1984 breeding season were also examined for gonadal development and external, morphological changes associated with late-stage ovarian maturation, both under natural conditions and after the injection of HCG administered incrementally at a total dosage of 1000 IU/kg HCG. These fish were sacrificed, either at the time of capture (no HCG) or at various intervals after the last HCG injection. The ovaries were removed and the oocytes were examined under a microscope (x 20).

RESULTS

Hormone-induced spawning

Initial trials indicated that intraperitoneal injections of HCG would induce ovulation in sexually mature Macquarie perch. Trials carried out during the 1983 breeding season indicated that either single or incremental injections of HCG, at total dosages ranging from 500 to 1000 IU/kg HCG, would induce spawning, although fertilisation did not always occur (Table 1). Most fertilised eggs were obtained by incrementally injecting females with HCG at daily intervals (total dosage of 1000 IU/kg HCG). However, during the 1984 trials, only 9% of females which received incremental injections produced eggs that were fertilised (Table 2). The latency period (from the last injection) for viable eggs was less than 26 h. During 1985, 22% of females injected with a single dosage of 1000 IU/kg HCG produced eggs that were fertilised after a latency period of 38.8-42.8 h (Table 3).

Oocytes sampled from sexually mature fish collected during the 1984 breeding season exhibited little variation in appearance or structure and, based on the appearance of pre-injection oocytes alone, it was not possible to predict the likelihood of ovulation and spawning in fish injected with HCG. Although HCG-induced oocyte development appeared to be consistent in all sampled fish, not all fish ovulated or produced eggs that were fertilised. Also, external, morphological characters varied little between brood fish prior to being injected with HCG, although the genital papilla was markedly distended in females which had ovulated or were about to ovulate, with or without being injected with HCG. Prior to injection with HCG the oocytes were pale-yellow in colour, generally opaque and oil particles were small and not readily apparent when examined. After the fish had been injected with HCG, and immediately before ovulation, the oocytes turned honey-coloured, more translucent and the oil particles coalesced to form several larger, more obvious oil globules. Oocyte diameter correspondingly increased from 1.45 to 1.60 mm at this time. After ovulation, and before spawning, some eggs were normally visible in the ovarian lumen through the translucent, distended genital papilla. It was often necessary when stripping fish to facilitate passage of the eggs by physically rupturing the thin membrane of the genital papilla. The diameter of recently fertilised eggs ranged from 1.75 mm to 2.00 mm. Males were generally running ripe throughout the spawning season.

Using the Student's t-test (Sokal and Rohlf, 1969), no significant differences were found between the length or weight of fish which produced eggs that were fertilised and that of fish which produced no eggs or eggs that were not fertilised during each spawning season (TL - 1983, $P > 0.2$; 1984, $0.2 > P > 0.1$; 1985, $P > 0.2$; weight - 1983, $P > 0.2$; 1984, $P > 0.2$; 1985, $0.2 > P > 0.1$). However, a comparison of the length and weight of females injected in 1983 and 1984 with that of females injected in 1985 indicated that the latter were significantly larger (TL, $P < 0.001$; weight, $P < 0.001$).

During the three spawning seasons, water temperatures in the Mitta Mitta River ranged from daily minima of 7-18^{o}C to daily maxima of 7-21^{o}C. Females which produced eggs which became fertilised were collected in mid to late spring, when temperatures in the river were increasing and ranged from daily minima of 9-17^{o}C to daily maxima of 13-21^{o}C (Fig. 1). Of the females which produced eggs that were fertilised, 78% were collected at minimum water

Table 1. Details of trials for HCG-induced spawning of Macquarie perch (total length range 370-420 mm; weight range 1000-1500 g) collected during the 1983 breeding season (9 November - 15 December) at Lake Dartmouth; IU/kg HCG, total HCG dosage of single and incremental injections; n, number of fish injected; Eggs produced, number of injected fish (n) producing non-fertilised eggs and eggs that were fertilised (in parentheses); Latency time, time between last or only injection and egg laying or stripping.

IU/kg HCG	n	Eggs produced	Latency time (h)	
			Eggs laid	Eggs stripped
Single				
0	2	0		
750	9	7(1)	49.6-61.9	
			52.2	
			52.3-61.8	
			61.8-62.0*	
			62.8	
			64.2	
			64.4-64.9	
			69.3-69.7	
1000	1	1	52.2-68.8	
Incremental				
0	2	0		
250	1	0		
500	2	2	25.8-38.3	47.6
750	3	3	17.0-19.3	31.9
			24.0-41.8	
1000	7	5(2)	17.3-18.5	17.3*
			19.8-22.9	18.0
			19.9	20.3
			19.9-23.3*	

* time for those fish which produced eggs that were fertilised

Table 2. Details of trials for HCG-induced spawning of Macquarie perch (total length range 350-430 mm; weight range 900-1550 g) collected during the 1984 breeding season at Lake Dartmouth and given incremental injections of a total dosage of 1000 IU/kg HCG; n, number of fish injected; Eggs produced, number of fish injected; Eggs produced, number of injected fish (n) producing non-fertilised eggs and eggs that were fertilised (in parentheses); Latency time, time between last injection and egg laying or stripping.

Date (1984)	n	Eggs produced	Latency time (h)	
			Eggs laid	Eggs stripped
25 Oct.	4	2	25.4-37.7	
			25.4-37.9	
2 Nov.	1	1	26.7-40.1	
5 Nov.	4	1	20.3-20.9	
6 Nov.	2	(1)		25.5*
7 Nov.	2	1		18.7
9 Nov.	1	1	24.0-38.8	
12 Nov.	6	3(1)	0.6-15.1*	21.0
			41.3-45.3	21.5
				24.4
13 Nov.	2	1		21.5
14 Nov.	2	0		
16 Nov.	5	1(1)	1.2-16.1*	
			25.2-38.6	
20 Nov.	2	1	1.9-14.9	
21 Nov.	4	2	1.8-14.9	
			2.3-15.3	
23 Nov.	2	1	0.4-14.8	
27 Nov.	2	2	14.7-19.3	
			23.1-38.9	
28 Nov.	5	3(1)	2.2*	
			4.2-17.8	
			20.6-26.0	
			26.5-43.0	
29 Nov.	1	1	3.3-18.9	
4 Dec.	1	1	22.4	

* time for those fish which produced eggs that were fertilised

Table 3. Details of trials for HCG-induced spawning of Macquarie perch (total lenght range 375-435 mm; weight range 900-1800 g) collected during the 1985 breeding season at Lake Dartmouth and given a single injection of 1000 IU/kg HCG; n, number of fish injected; Eggs produced, number of injected fish (n) producing non-fertilised eggs and eggs that were fertilised (in parentheses); Latency time, time between injection and egg laying or stripping.

Date (1985)	n	Eggs produced	Latency time (h)	
			Eggs laid	Eggs stripped
22 Oct.	5	1	42.5	
23 Oct.	6	5		33.3
				35.4
				45.3
				47.1
				47.5
25 Oct.	2	1		40.9
30 Oct.	2	0		
31 Oct.	2	1	45.9	
6 Nov.	1	0		
7 Nov.	5	3	38.8	47.3
				47.5
9 Nov.	7	5(2)	40.3	40.4*
				40.5
				40.9
				41.3*
				49.3
				49.3
10 Nov.	3	1		46.0
11 Nov.	6	1(5)	39.0	38.8*
				39.0*
				39.5*
				40.9*
				41.0*
12 Nov.	1	(1)	42.0*	
13 Nov.	5	1(2)	41.4*	42.4
			42.8*	
				41.8*
14 Nov.	5	1(1)		42.2

* time for those fish which produced eggs that were fertilised

temperatures ranging from 13 to 16°C, and 89% were collected at maximum temperatures ranging from 14 to 18°C. During the 1985 spawning season, a running-ripe female was collected from the river at the same time as those fish used in the spawning trials which, after being injected with HCG, produced eggs which were fertilised. Spent females were subsequently collected from the same location during the next few days.

Courtship behaviour

Courtship behaviour consisted of one or both fish mouthing the caudal peduncle and fin, nudging the vent and rubbing against the lateral line of the other fish, often while swimming vigourously throughout the brood tank. Males typically initiated courtship immediately after the two sexes were placed together. Females often displayed similar, reciprocal courtship and, on occasions, also initiated courtship with unresponsive males. Males tended to court more vigourously after being kept isolated from females, either individually or with other males, for several days before being added to the brood tank. Courtship behaviour diminished markedly after 20-30 h if one of the pair was unresponsive. Injected males were no more inclined to court females than were non-injected males.

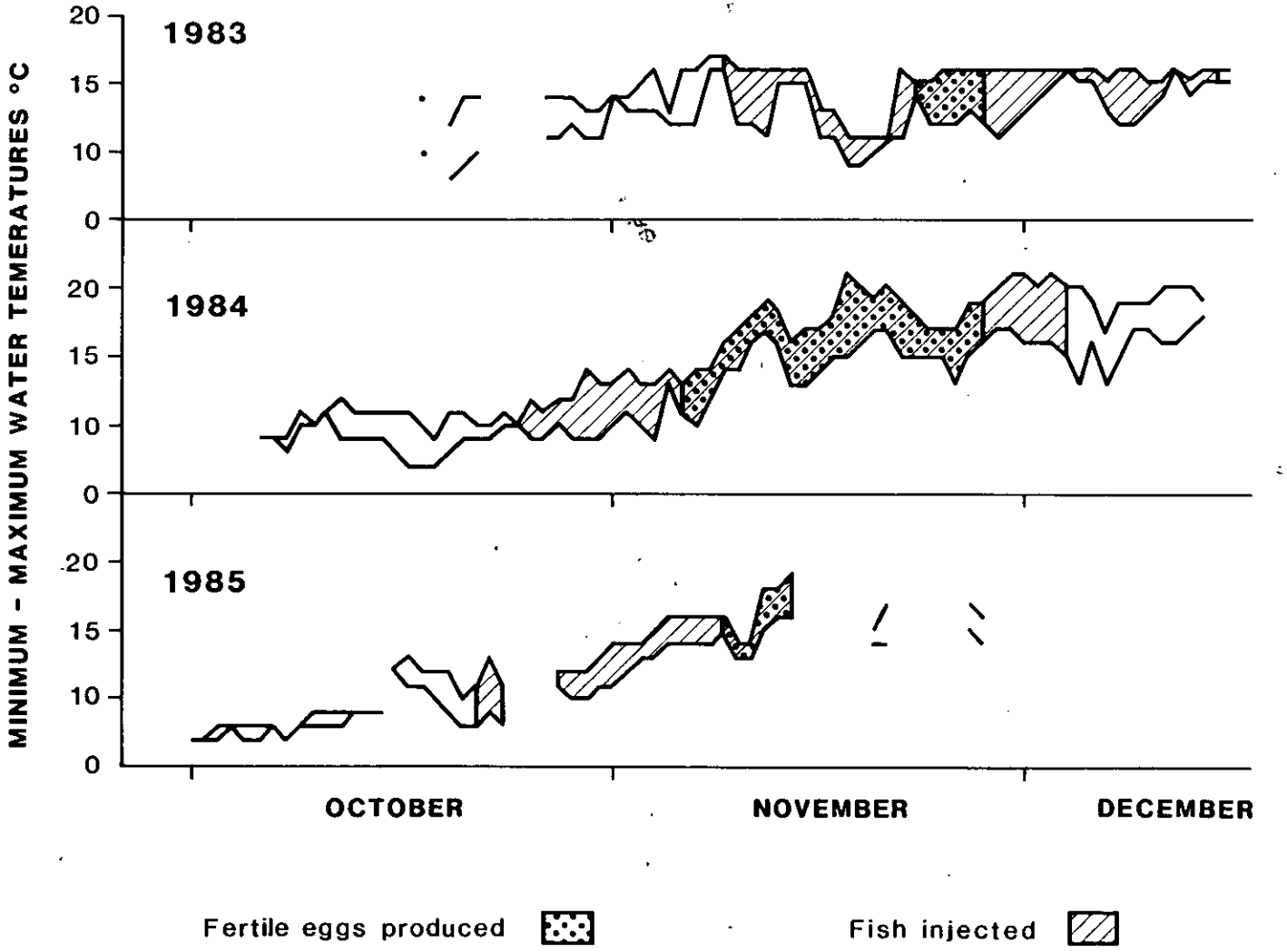

Figure 1: Maximum and minimum water temperatures recorded in the Mitta Mitta River, above Lake Dartmouth, between October and December, 1983, 1984 and 1985. The temperatures at which female Macquarie perch were collected and injected, and at which these fish produced eggs that were fertilised, are marked.

DISCUSSION

Intraperitoneal injections of HCG at dosages of 500-1000 IU/kg HCG induced ovulation and spawning in sexually mature Macquarie perch collected from the wild in the normal breeding season. Only fish injected singly or incrementally with dosages of 750-1000 IU/kg HCG produced eggs that were fertilised. Apparently, HCG injected at 750-1000 IU/kg HCG does not induce gross ovarian maturation, but simply triggers ovulation, together with associated late-stage oocyte development, and spawning in sexually ripe fish only. Golden perch, silver perch and Murray cod, held in earthen ponds, ovulated and spawned following single intraperitoneal injections of 500-2000, 200-2000 and 800-2000 IU/kg HCG respectively (Rowland, 1983, 1984, 1985). In each case fish were removed from the ponds during the natural breeding season, and so were presumably at advanced stages of gonadal development.

During the spawning trials, the response of female Macquarie perch to HCG injections at the same dosage frequently differed. It was difficult to accurately identify ripe fish on the basis of morphological and oocyte appearance, the reproductive condition of brood fish being only subjectively assessed at capture. Selected, putative, mature females were subsequently shown (after dissection) to exhibit considerable variation in ovarian development and, therefore, females collected from Dartmouth at any one time during the breeding season and used in the spawning trials were not likely to be all at the same stage of development, hence the often varied response to the HCG injections. It should also be noted that the significantly larger size of brood fish injected during 1985, compared with 1983 and 1984 (when fish were injected with a range of dosages including 0-750 IU/kg HCG), indicates that many fish injected in 1985 may have been older, with a consequent higher proportion of sexually mature individuals. This would perhaps partly explain the increased success of HCG-induced spawnings in 1985.

During each breeding season, all male Macquarie perch were running ripe and, when given the opportunity, courted females throughout the season, irrespective of whether they were injected with HCG or not. It appeared that males injected with HCG produced a greater quantity of milt than non-injected males, suggesting that dosages as low as 200 IU/kg HCG promote spermiation in Macquarie perch. Despite active courtship being exhibited by both sexes after injection of HCG, many males failed to fertilise apparently viable eggs released by females in the brood tanks. Wharton (1973) also noted that some eggs produced by Macquarie perch injected with HCG were not fertilised even though running-ripe males were present and courtship behaviour occurred before the eggs were shed.

According to Woynarovich and Horvath (1980), incremental hormone injections are more suitable than single injections in promoting final gonadal maturation and that single injections are successful only if the female is fully ripe (as for fish collected in their spawning migration). In the present study, HCG appeared to have a limited effect on gonadal maturation in Macquarie perch and, therefore, incremental injections were no more effective than single injections at the tested dosages. Although the latency period following the last of three incremental injections was at least 12 h shorter than that following a single injection, the overall time in which brood fish were handled was much greater. The extra handling necessitated by incremental injections may have imposed additional stress on the fish and, in many cases, resulted in atresia and yolk resorption, premature ovulation and shedding of eggs, or death. Wharton (1973) reported that treatment with HCG did not induce spawning in

Macquarie perch in which yolk resorption had commenced. Rowland (1985) suggested that handling Murray cod brood fish up to 3 months prior to the breeding season caused atresia and ovarian yolk resorption in most females and that this prevented HCG-induced ovulation. The failure of HCG-injected, golden perch females to spawn has, in some cases, been attributed to the onset of atresia and ovarian yolk resorption (Rowland, 1983).

Wharton (1968) claimed that a water temperature of 16.5^{o}C stimulated lake-dwelling Macquarie perch to migrate into inflowing tributaries, for a distance of about 1.5 km upstream, spawn and then return to the lake. Several of these 'spawning runs' were observed during each breeding season. From observations made in the present study, it appears that increasing photoperiod and water temperature induce gonadal development in Macquarie perch. During spring, large numbers of mature fish congregate in the upper reaches of the Mitta Mitta River arm of Lake Dartmouth, where the relatively low temperature of the inflowing Mitta Mitta River probably creates a thermal barrier to further upstream movement. During periods of warm weather the water temperature of the river and lake assimilate. The rapidly increasing water temperature, ranging from 13 to 18^{o}C, probably stimulates ripe fish to make a short, upstream spawning migration of up to several hundred metres. The time taken for fish to move into the river, undergo courtship, spawn and return to the lake appears to depend primarily on the water temperature in the river. This would normally take 1-2 days because there does not appear to be any parental care displayed for the eggs and, as indicated by the behaviour of fish in the HCG-induced spawning trials, courtship rarely lasted for longer than 20-30 h. The latency times for fish injected with HCG which produced fertile eggs in 1985 suggest that the post-ovulatory stage is relatively short and that spawning may occur up to only 4 h after ovulation. This perhaps explains the difficulty in collecting running-ripe females, although spent females were often caught. During the present study, the breeding season, i.e. the time from the earliest congregations of fish in the upper reaches of the Mitta Mitta River arm to the completion of spawning in the river, lasted about 6 weeks, from mid spring to early summer. Because of the variable thermal regime in the river and the inherent variation in gonadal development of mature fish, spawning may occur either en masse, over a prolonged period, or intermittently, with several small groups of fish migrating upstream on separate occasions. Most HCG-injected fish which produced eggs that were fertilised were collected during their upstream migration, immediately prior to ovulation and spawning.

ACKNOWLEDGEMENTS

The authors wish to thank G. Bell (dec.), R. Donald, J. Douglas, J. Power, R. Price and E. Savage for their valuable assistance both in the laboratory and in the field during the course of this study. We are also grateful to Drs P.L. Cadwallader, D. Evans and S.J. Rowland for their constructive comments on the manuscript and to Mrs M. May for typing the manuscript.

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