



Technical Memorandum 49

Reproduction in the freshwater
mussel *Velesunio angasi* in
response to the release of water
from Ranger Uranium Mine to
Magela Creek

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Summary

Biological studies of the population of freshwater mussel, *Velesunio angasi*, inhabiting the braided, sandy channel of the Magela Creek about the Ranger outflow pipe, were undertaken during the 1984/85 Wet season (March–May 1985). In particular, reproductive activity of female mussels was monitored to determine whether or not activity was altered by the release of water from Retention Pond No. 4 (RP4) that occurred between 12–23 March 1985. For assessment of reproductive activity, female mussels from the channel receiving RP4 waters were sampled above and below the outflow pipe prior to, during and after release. Assessment was based on: (1) examination of mussels for the presence and stages of development of the glochidial larvae that are nurtured in marsupial portions of the inner gills; and (2) determination of the amount or quantity of larvae present in the marsupia.

Prior to the RP4 release, reproductive activity of mussels was similar upstream and downstream of the outflow pipe. During the release (five days after commencement) however, an effect was observed in mussels sampled from four sites between the point of release and 120 m downstream. Over this distance, a decrease in both the proportion of females bearing embryos and larvae and in the quantity of larvae present in the marsupia was found in mussels sampled at increasing proximity to the source of discharge waters. These observations were in contrast to the higher reproductive activity of mussels sampled simultaneously, upstream of the release pipe. In particular, fewer larvae were present in the marsupia of females sampled from all downstream sites in contrast to those found in the marsupia of females sampled upstream. These patterns at downstream sites were evidence of suppression of gonadal activity and larval production during the RP4 release, whilst an unnatural occurrence of few glochidia in the marsupia suggested either abortion or unusually protracted release of larvae.

In mussels sampled after the RP4 release, the stage of larval development at the two downstream sites closest to the pipe (within 50 m), though similar among mussels from the same site, differed between the two sites, and both differed from all of the other sites sampled. Such observations, together with those made during and after release, indicate that marsupia of females from these two sites were empty for a total period of about 12 days, including a period of 8–9 days after RP4 release. Recovery was slowest in mussels from the site closest (< 20 m) to the release point.

The decrease in reproductive activity of mussels found in downstream sites, at closer proximity to the source of RP4 release, was correlated with increasing electrical conductivity. Elevated water temperatures (up to 0.5°C) were found at the first two sites sampled downstream of the release pipe. Warmer waters, however, should have the effect of *increasing* the intensity of larval production.

The constituent(s) in RP4 waters responsible for suppression of reproductive activity in *V. angasi* was not identified. Early indications are that the same reduced reproductive activity was also present in the mussel population resident in RP4. The digestive glands of mussels native to RP4 and of creek mussels sampled within 50 m downstream of the release point during the RP4 release, were coloured a dark red. This colouration and an orange-red pigment present in fluids secreted by the gland were unique to these mussels, not having been observed among mussels from other waterbodies of Magela Creek.

It is argued that any suppression of reproductive activity *per se* caused by RP4 (or other mine waste-water) releases is unlikely to result to any significant degree in diminishing stocks of mussels downstream of the point source of release. However, such releases could adversely affect newly-metamorphosed juvenile mussels that are recruited downstream of this point

during the Wet season should this life-stage also prove to be sensitive to Ranger mine waters. The suitability of reproductive activity in *V. angasi* as a biological monitor of possible adverse effects upon the biota in future releases of Ranger waste waters is advocated.

Associated studies of the population of *V. angasi* in the Magela Creek channel during the 1984/85 Wet season included estimation of mussel density and determination of environmental influences of larval production. A mean density estimate of 7.1 mussels (> 0-year old)/m of creek length (all braids included) was derived for a 50 m reach of creek sampled intensively downstream of the release pipe. It is likely that the shell-free dry weight of *V. angasi* in the Magela Creek channel exceeds that of all other benthic macroinvertebrates. Larval production, as measured by gravidity (ie % of all females sampled at any one time bearing embryos and larvae in the marsupia), was found to be significantly correlated with water temperature.

Reproduction in the freshwater mussel *Velesunio angasi* in response to the release of water from Ranger Uranium Mine to Magela Creek

1 Background and aims

The freshwater mussel *Velesunio angasi* is abundant in many of the permanent billabongs of Magela Creek (Humphrey & Simpson 1985). A resident population is also common (see below) in the ephemeral, braided creek channel upstream of Mudginberri Billabong. Mussels occurring in the sandy creek channel are obliged to aestivate at the cessation of creek flow and during the ensuing Dry season, by burrowing into the sandy and loamy banks and amongst roots of *Pandanus aquaticus*.

The sexes of *V. angasi* are separate and the mussel larvae, termed glochidia, are harboured and nurtured in marsupial portions of the inner gills of mature females. In Magela Creek, larval development is rapid and may be completed in less than 10 days. Upon maturity, the glochidia are released into the water by the mother. In order for further development of the larvae to proceed, a short and obligate parasitic phase upon the gills and fins of fish is necessary. Upon metamorphosis (between 3 and 14 days depending upon water temperature) the juvenile falls to the sediments to begin the free-living benthic stage (Humphrey & Simpson 1985).

Spawning and breeding of *V. angasi* in Magela Creek are repetitive and occur year round. Superimposed upon this repetitive reproductive cycle, the relative activity of which is only marginally slowed to any degree by low water temperatures, major interruptions to gonadal activity and larval production are found. These are associated with seasonal lulls in dissolved oxygen (DO) concentrations and with seasonally high turbidities (Humphrey & Simpson 1985). Allison and Simpson (1989) also observed suppressed larval production in mussels transplanted to Ranger's Retention Ponds 2 and 4. Because of the ephemeral nature of the environment, spawning and breeding by mussels inhabiting the braided, sandy creek channel are confined to the Wet season (~ Dec–May). DO concentrations are high and turbidities sufficiently low that the intensity of larval production by these 'creek mussels' is generally dependent upon temperature alone (see below).

Because reproductive activity of *V. angasi* in the Magela Creek can be so described and quantified in relation to environmental factors, this information can provide useful data upon which to monitor the effects of environmental disturbance such as potential pollutants. To this end, the reproductive activity of female creek mussels in the vicinity of the Ranger outflow pipe was monitored to determine whether or not activity was altered by the release of Retention Pond No. 4 (RP4) water that occurred between 12–23 March (see table 5 for details).

2 Methods

2.1 Census of mussels in the Magela Creek channel

As part of any assessment of the full impact of release contaminants on mussels in the Magela Creek channel, reliable information on distribution and abundance is necessary. These data are available for billabong populations (Humphrey & Simpson 1985) but density estimates had not previously been made for the creek population.

Density estimates of mussels in the Magela Creek channel were derived from sampling undertaken in April 1985. A 50 m reach of the creek (all braids included) located approximately 200 m downstream of the release pipe was sampled. This site was situated downstream and outside the reach where reproductive activity in mussels was affected by RP4 release (section 3.2, Response over the study period). The section of the creek censused was as typical as could be possible for the sandy channels—the total area was sufficiently large and contained every type of creek habitat, ie a number of braids, shrub-lined and shrubless banks, and small to large clumps of *Pandanus aquaticus* rooted in midstream of the braids.

Collections of mussels were made by hand, diving with or without mask and snorkel. Following Humphrey and Simpson (1985, section 3.2.1) the sampling method used was 'Type 1'. By this method a specified habitat (eg along a bank) is searched systematically and repeatedly until no more mussels are recovered. Because mussels were sampled by hand in this fashion, some 40% of small mussels between 20 and 27 mm in length may have been missed; most mussels below 19 mm are assumed to have escaped detection (Humphrey & Simpson 1985, section 3.2.2). The vast majority of mussels that were missed by hand collecting were of the size that corresponds to the juvenile, young-of-year class (Humphrey & Simpson 1985, section 6.4.2).

After a particular habitat was denuded of mussels, they were counted and then returned to the sediments from where they came. Counts from all likely and potential habitats sampled in this manner were tallied until a grand mussel total for the 50 m section of the creek was reached. A mean density estimate of mussels per metre of creek length was then derived.

2.2 Assessment of reproductive activity over the study period

Adult mussels from the channel receiving RP4 water were sampled above (25–150 m) and below the outflow pipe over three periods: two days prior to RP4 release (9 March); five days after initial release (17 March); and twelve days after the last day of release (5 April). These sampling periods are termed pre-, during, and post-release respectively. During release, mussels were also sampled from an additional control site, parallel to the outflow pipe, but in the eastern channel (a braid receiving different water from that flowing past the pipe). During and post release, mussels below the outflow pipe were sampled at four sites: site 1, 13–20 m; site 2, 32–57 m; site 3, 91–96 m; and site 4, 110–115 m downstream. During release, an additional site was sampled 700 m below the pipe. Reproductive activity of female mussels was assessed in the laboratory in the following manner:

- 1 the stage and development of embryos and larvae present in the marsupia were determined microscopically;
- 2 the marsupia were scored for fullness of embryos and larvae (arbitrary scale 0–6); and
- 3 the inner gills of all females were excised and weighed separately and together with the visceral mass. An 'inner gill index' was then calculated as the proportion of the total visceral mass comprising the inner gills (expressed as a percentage).

(For part 1 details see Humphrey & Simpson 1985.)

The measurements of water temperature and electrical conductivity used in the present study were made using a Hydrolab meter. The source of other limnological data used in the study is acknowledged in the appropriate text or table.

3 Results and discussion

3.1 Creek census

The total number of mussels (> 0-year old) sampled from the 50 m reach of creek amounted to 355. From this figure a mean density estimate of 7.1 mussels/m creek length was reached.

Billabong and creek densities of mussels are not directly comparable as much of the creek environment is shifting and unstable, and is uninhabitable for macroinvertebrates. For habitats that are available to mussels nevertheless, it appears that densities of creek mussels are comparable with, if not higher than, most billabong populations.

No information is presently available on density and biomass of the other macroinvertebrates occurring in the Magela Creek channel. However, if the sum total biomass of macroinvertebrates other than *V. angasi* is a similar figure to those derived for billabong populations (Outridge 1988), then the shell-free dry weight of *V. angasi* would comprise a biomass far exceeding that of the other benthic macroinvertebrate fauna. The implications of the effect of potential pollutants upon these relative contributions of macroinvertebrate biomasses are considered below.

3.2 Reproductive activity

Environmental influences

Of the known factors that may affect larval production of *V. angasi* in Magela Creek (see above), DO concentrations are never low enough nor turbidities high enough in the creek channel during the Wet season to suppress production of larvae (Humphrey & Simpson 1985, section 7.10.1). In the clean flowing waters of the sandy channel, the mussel population is exposed to the highest DO concentrations and amongst the lowest turbidities of any population in Magela Creek. This is similar to the situation in Mudginberri Billabong where, essentially, water chemistry of Wet season quality prevails throughout the year (Walker & Tyler 1982, 1983; Walker et al 1982, 1984); in particular, DO concentrations are rarely low enough nor is turbidity high enough to significantly reduce the production of larvae. In Mudginberri Billabong, water temperature was found to be the only significant environmental determinant of monthly larval production of mussels measured over a 27-month period. At this site, water temperature was positively correlated with larval production ($P < 0.001$), accounting for 48% of the variation in monthly percentages of females bearing embryos or larvae in the marsupia (Humphrey & Simpson 1985).

To determine whether a similar water temperature/larval production relationship prevails in mussels from the Magela Creek channel, data pertaining to water temperature and monthly larval production were gathered for regression analysis. Records of monthly larval production (ie percent of all females sampled at any one time that were gravid) were available for the 1979/80, 1980/81 and 1981/82 Wet seasons (Humphrey & Simpson 1985) and for the months February–April inclusive of the 1984/85 Wet season (present study). Monthly water temperature records for stations on the Magela Creek channel were available from NT Water Division (NT Department of Transport and Works). These data are extensive and it was possible therefore to obtain temperature records from a date very close to that (and occasionally on the date) when mussels were sampled. Water temperature was measured by the NT Water Division, generally between the hours of 0800 and 1400. For analysis in the present study, readings were standardised to a set time (0800) by extrapolating from a 24 hour temperature cycle that was compiled from data collected at 2–6 hourly intervals between 22–23 March 1985 (table 1). The temperature range and heating cycle recorded over the 24-hour period in March 1985 is at this stage assumed to be reasonably representative of the range that could be expected in the Magela

Creek channel during the Wet season. Certainly, the extrapolated data agree well with ranges calculated from miscellaneous NT Water Division data that were occasionally collected twice daily.

A very significant linear regression equation ($P < 0.01$) was found to describe the relationship between monthly larval production (per cent of gravid females, arcsine transformed) and water temperature. The relationship and derived regression equation are shown in figure 1. The significant relationship between larval production and water temperature could be expected; the environment prevailing in the Magela Creek channel during the Wet is equitable and temperate, with recorded DO concentrations being particularly high and turbidities low. As found in the Mudginberri Billabong population, therefore, the breeding pattern of the creek population represents similarly a background cycle primarily dependent upon water temperature.

It is important to note that the intensity of oogenesis in *V. angasi* is immediately reflected in the intensity of larval production (Humphrey & Simpson 1985). The measure of response therefore of larval production to any environmental factor, such as temperature, is in turn a measure of the response of gonadal activity.

Response over the study period

Larval development and/or production in adult females collected from the various creek sites and at the particular sampling dates is represented:

- 1 by histograms of the marsupial appearance of females,
- 2 as mean marsupial fullness scores, and
- 3 as mean inner gill indices

in figures 2, 3 and 4 respectively. The following sections describe the reproductive activity of mussels at the various sampling locations pre-release, during release and post-release. Reproductive activity is discussed in terms of both larval development (ie appearance of marsupia, fig 2) and production. Larval production is represented by:

- 1 'per cent gravidity' ie percentage of all females sampled that bore embryos and larvae – hereafter abbreviated to 'gravidity' (fig 2); and
- 2 'content' ie the relative contribution of larvae by fullness or weight, as measured by mean marsupial fullness scores (fig 3) and mean inner gill indices (fig 4) respectively.

A comparison of figures 3 and 4 shows that both measures of 'content' are virtually identical, so further references to content are based on the calculations of inner gill indices only (fig 4).

Table 1 Water temperature readings (°C, ~1 m depth) measured at intervals over a 24 hour period during the RP4 release (22–23 March 1985), for sampling sites in the Magela Creek channel

Distance from release pipe (m)	Time of day (date)						Mean	(SD)
	1000 (22/3/85)	1140	1423	1840	2245	0743 (23/3/85)		
-10	29.6	30.4	31.5	31.3	30.2	28.6	30.3	(1.08)
0	–	–	–	32.3	31.3	30.2	–	–
13	29.9	30.9	31.9	31.5	30.5	29.2	30.7	(1.00)
20	30.3	30.7	31.8	31.4	30.4	29.1	30.6	(0.95)
32	30.3	30.6	31.8	31.4	30.4	29.0	30.6	(0.98)
57	30.2	30.5	31.7	31.4	30.3	28.9	30.5	(0.99)
75	–	–	31.6	31.3	30.3	28.8	–	–
91–96	30.0	30.4	31.5	31.3	30.2	28.6	30.3	(1.04)
110–115	29.9	30.4	31.5	31.3	30.2	28.7	30.3	(1.02)

Over the entire study period (9 March–5 April 1985) over 80% of females from all sites, except those downstream of the outlet pipe during the RP4 release, bore embryos or larvae in the marsupia. This intensity in gravidity is high and reflects the response by mussels to the warm water temperature that prevailed over the period (early morning readings in excess of 25°C).

Pre-release

Prior to RP4 release, both larval development (fig 2) and production (gravidity and content, figs 2 and 4 respectively) were similar upstream and downstream of the release pipe.

Spawning and breeding are generally asynchronous among mussels at any given time and location in Magela Creek (Humphrey & Simpson 1985), so that it is usual to observe amongst the females, marsupia with all stages of larval development present. The appearance of the marsupia of pre-release females was unusual, therefore, in that a synchronised spawning had occurred; early and developing larvae only, and no mature glochidia, were present in the marsupia of females sampled (fig 2). It is possible that such occasions of synchronised spawning and breeding are the result of sudden discharge fluxes; these may have a major disruptive effect upon breeding cycles through changes in water temperature and other water quality variables.

During release

Reproductive activity During RP4 release, larval development (fig 2) and production (figs 2 and 4) in mussels sampled upstream, in the eastern channel and at the site 700 m downstream of the release pipe, were similar. Approximately 50% of females bore glochidia in the marsupia that had matured from the very early developmental stages of eight days prior (ie pre-release). The remaining 40–50% of females bore earlier development stages that were the result of spawning after very recent glochidial maturation and release (fig 2). Larval content during release was highest in mussels sampled upstream of the release pipe and in the eastern channel (fig 4).

Downstream of the pipe at sites 1–4 (see above, section 2.2), similar proportions of females bore mature glochidia during release (~ 40–50%). However, progressively fewer females bore early and developing larvae in the marsupia at increasing proximity to the release pipe. Further, mean larval content declined similarly in females sampled at increasing proximity to the pipe. The low mean larval contents were caused by: (a) the presence of empty marsupia amongst the females sampled (fig 2); and (b) trace or small amounts of glochidia that occurred in many of the females that did bear mature larvae (see fullness scores in fig 5).

Thus, development of the larvae that were present in the marsupia of females sampled from the four sites downstream of the release pipe at the time of RP4 release was *not* subsequently interrupted by RP4 release. This is indicated because glochidia present in the marsupia of approximately 50% of the females sampled, had matured from the very early developmental stages that were present eight days prior to release. The presence, however, of progressively fewer females bearing early and developing larvae in the marsupia at increasing proximity to the release pipe indicated that gonadal development (section 3.2, Environmental factors) and production of subsequent broods of larvae were suppressed. This suppression was most intense at the site closest to the pipe, where no early and developing larvae were found amongst the females sampled.

Normally in creek mussels at larval maturity, discharge of glochidia from the mother occurs quickly and over a very short period. This is indicated by the observation that glochidia are most often present in the marsupia of females in large quantities and only rarely in small or trace amounts that would indicate protracted release (fig 5). The marsupial appearance of females sampled downstream of the release pipe during release was unusual, therefore, in that small or trace amounts of larvae were often present (fig 5). Thus, glochidial release in these mussels was abnormal, although it is not clear at this stage whether the low fullness scores represent premature or unusually protracted release.

The observations of reduced gravidity and content, and marsupial appearance of glochidial-bearing females from downstream sites were in contrast to the higher reproductive activity and marsupial appearance of mussels sampled simultaneously, upstream of the release pipe. In particular, the larval content of females sampled from all downstream sites was significantly lower than the content found in females sampled upstream (fig 4).

Correlate(s) of suppressed reproductive activity Neither the DO concentrations nor the turbidity of released RP4 water could account for suppressed reproductive activity in mussels sampled downstream of the release pipe during the RP4 release. Measurements made over the release period showed that DO concentrations were actually higher in RP4 release water than those prevailing naturally in the creek (tables 2 & 3), while concentrations of suspended solids were similar if not lower in release water than in creek water (table 2). Released RP4 water was warmer than that prevailing naturally in the creek. Consequently, elevated water temperatures (up to 0.5°C) were found at the first two sites sampled downstream of the release pipe (tables 3 & 4). Warmer waters should have the effect of *increasing* the intensity of larval production in creek mussels, particularly gravidity (section 3.2 'Environmental influences'). The observed patterns in larval production, however, were contrary to this expectation.

Electrical conductivity appeared to be the only variable measured over the study period that could account for suppressed reproductive activity of creek mussels during RP4 release; the decrease in reproductive activity of mussels found in downstream sites, at closer proximity to the source of RP4 release, correlated with increasing conductivity.

Table 2 Measurements of suspended solids and dissolved oxygen made during the 1985 discharge from RP4. Gauging station 821009 on the Magela Creek channel is several kilometres downstream of the Ranger release pipe. (Source: Ranger Uranium Mines Pty Ltd)

Site	Date	Constituent concentration (mgL ⁻¹)	
		Suspended solids	Dissolved oxygen
RP4 pump discharge	12/03/85	13	—
	13/03/85	5	—
	14/03/85	10	—
	15/03/85	6	—
	16/03/85	<5	—
	17/03/85	8	—
	18/03/85	<5	—
	19/03/85	10	7.0 (RP4)
	20/03/85	14	—
	22/03/85	7	—
Magela Creek (GS821009)	12/03/85	21	—
	13/03/85	7	6.1
	14/03/85	<5	6.3
	15/03/85	<5	6.5
	16/03/85	<5	6.4
	17/03/85	8	5.9
	18/03/85	<5	6.4
	19/03/85	10	—
	20/03/85	14	6.3
	22/03/85	7	7.3

Conductivities measured during the release period were between 4–30 μScm^{-1} higher than natural creek values at the four sites downstream of the release pipe (13–120 m, tables 3 and 4). (Measurements were made at the dilution range of 95–104:1, Magela Creek: RP4 water release water, that prevailed at the end of the mixing zone; see table 5.) The chemical constituent(s) in RP4 waters, metallic or otherwise, responsible for suppression of reproductive activity in *V. angasi* is (are) not known.

Post release

By 5 April 1985, thirteen days after the cessation of the RP4 release, a recovery in reproductive activity was evident in mussels sampled downstream of the release pipe. In particular, larval production was similar among mussels from both upstream and downstream sites; gravidity was high (exceeding 85%, fig 2) and content had returned to pre-release values with similar values in mean content found among mussels from all sites (fig 4).

The stages of larval development among mussels from upstream and downstream sites, however, differed (fig 2). In particular, the stage of larval development at the two downstream sites closest to the pipe outlet (within 50 m), though similar among mussels from the same site, differed between the two sites, and both differed from all of the other sites sampled (fig 2). The similar reproductive patterns observed in mussels within each of the two sites indicated a synchrony in the recommencement of spawning and breeding following a breeding cessation during release. Recommencement of breeding activity in mussels at the two sites immediately downstream of the waste-pipe outlet lagged several days behind activity in mussels sampled from further downstream (30–60 m) (ie the developmental time between 'early and developing larvae' and 'mature glochidia'). It is most likely that at this site (30–60 m) most mussels had recommenced breeding some 8–9 days prior to sampling (because the reproductive cycle occurs over a 9–10 day period and mussels were sampled with mostly glochidia in the marsupia), or 5–6 days after the last day of RP4 release. Thus, it is likely that reproductive activity in mussels from the downstream site closest (< 20 m) to the discharge point had only recommenced a few days prior to sampling, or 8–9 days after RP4 release.

Because the marsupial appearance of post-release females from sites 3 and 4 downstream of the release pipe resembled the upstream (undisturbed) condition (fig 2), it would seem that reproductive activity of these mussels was not seriously impaired over the release period. Hence, some developing and mature larvae were present in the marsupia of these mussels at all stages of RP4 release, and no complete curtailment of activity occurred that would cause a synchronised spawning and brooding at recovery (such as observed for mussels from sites 1 and 2).

Table 3 Water quality data collected at various times during the RP4 release from sites in the Magela Creek channel (12–19 March 1985). Site 1 is 15 m upstream; site 2 is 10 m downstream; and site 3 is 700 m downstream of the Ranger release pipe. (ERISS unpublished data)

Physico-chemical parameter	Site 1			Site 2			Site 3		
	n	mean	SD	n	mean	SD	n	mean	SD
Conductivity (μScm^{-1})	14	15.6	1.5	15	27.3	2.6	15	17.4	1.4
Dissolved oxygen (mgL^{-1})	13	6.1	1.5	13	6.4	1.7	8	6.6	1.5
Temperature ($^{\circ}\text{C}$)	13	29.9	2.0	15	30.4	2.5	10	29.6	2.0
pH	13	5.5	0.2	12	6.0	0.2	8	5.8	0.2

Table 4 Water temperature and conductivity data collected during the RP4 release at sampling sites in the Magela Creek channel. Values are means of six measurements made at intervals over a 24 hour period (22–23 March 1985). Additional data (*) provided by ERIS Chemistry section.

Distance from release pipe	Conductivity (μScm^{-1})			Temperature ($^{\circ}\text{C}$)	
	mean	(SD)	Chemistry section *	mean	(SD)
0–10 ¹	17.0	(0.0)	12.4	30.3	(1.1)
13–20	45.4	(9.9)	42.4	30.6	(0.9)
32–57	38.6	(3.0)	31.5	30.5	(0.9)
91–96	22.0	(1.1)	22.0	30.3	(1.0)
110–115	21.4	(1.0)	21.5	30.3	(1.0)

* spot readings only, taken on 14 March 1985.

¹ away from effluent plume

Table 5 Details of Ranger's RP4 release during the 1985 late Wet season (Source: Ranger Uranium Mines Pty Ltd)

Release	Date	Time	Magela flow m^3s^{-1}	Pump rate m^3s^{-1}	Dilution at end of mixing zone (Magela: pump)
1	12/03/85	0927 (start)	37	0	37:0
		0930	~37	0.166	229:1
		1147	37	0.166	229:1
		1726	37	0.216	172:1
1	13/03/85	0810	~13	0.175	74.3:1
		1119	13	0.216	60.2:1
		1430	13	0.175	74.3:1
1	14/03/85	0845	13	0.133	97.7:1
		1301	13	0.125	104:1
		1600	13	~0.125	104:1
1	15/03/85	0848	9.5	0.133	71.4:1
		1506	9.5	~0.133	71.4:1
		1609	~9.5	0.100	95.0:1
1	16/03/85	0850	15	0.100	150:1
1	17/03/85	1350	13.5	0.142	95.1:1
1	18/03/85	0820	15	0.158	94.9:1
		1546	13.5	0.142	95.1:1
1	19/03/85	0810	19	0.117	162:1
		0939	19	0	19:0
		(off)			
2	22/03/85	0823 (start)	9.5	0.100	95:1
2	23/03/85	0800	9.5	0.100	95:1
		0820	~9.5	0	9.5:0
		(off)			

3.3 Other observations relating to effects of RP4 waters

The finding of suppressed reproductive activity in creek mussels exposed to various dilutions of RP4 water is consistent with similar observations made by Allison and Simpson (1989). They found that billabong mussels transplanted to, and present in, RP4 for a period during 1980 and 1981, failed to produce larvae. (In other transplants between billabongs, the data of Allison and Simpson show that transplanted mussels normally assume a reproductive condition similar to mussels native to the particular billabong.)

A resident population of *V. angasi* occurs in reasonably high densities in RP4. There are early indications that reduced reproductive activity is also the rule for these mussels. On two sampling occasions in 1985 (April & May), recorded gravidities were only 19 and 41% respectively (table 6). From the observations of section 3.2 (Response over the study period: during release), it would appear that neither DO concentration, turbidity nor temperature of RP4 water could account for such low activity in larval production. Values of these physico-chemical variables in RP4 water over the release period were within ranges that would normally result in high larval production. Although collection of mussels from RP4 occurred some weeks after these limnological parameters were measured, it is unlikely that water quality in the pond would have changed to any significant degree. (In relation to DO in particular, by virtue of the shallow nature of the pond and the absence, to a great degree, of macrophytic growth, concentrations should be high year round, especially in comparison with concentrations recorded in the billabong waters.) Again, the constituent(s) in RP4 responsible for a suppressed activity in reproduction of both natives resident in, and billabong mussels transplanted to, RP4 is not known.

A final effect observed of RP4 water upon *V. angasi* was that the digestive glands of both mussels resident in RP4 and creek mussels sampled within 50 m downstream of the release point (ie sites 1 & 2) during RP4 release, were coloured a dark red. This colouration and an orange-red pigment in fluids secreted by the gland are apparently unique among mussels from waterbodies of Magela Creek. Other than that the pigmented fluids are the result (presumably) of filtration and ingestion of particles from RP4 water, no further explanation of their presence and function can be forwarded.

Table 6 Details of larval development (marsupial appearance) and production (gravidity) of female mussels sampled from RP4 during April and May 1985

Sampling occasion	Marsupial appearance			Gravidity (%)
	Empty	Early and developing larvae	Glochidia	
25/04/85	25	6	0	19
07/05/85	13	8	1	41

4 Conclusion

In relation to adverse effects of RP4 discharge (present and future) into the Magela Creek channel on the resident population of *V. angasi*, suppression of reproductive activity *per se* is unlikely to result to any significant degree in diminishing stocks of mussels. This is because the contribution of larval production made by female mussels immediately downstream of the release point to recruitment in the 'affected' reach, is only one of three potential sources. Other sources are displacement of new recruits by currents from upstream portions and importantly, the extensive movements of glochidial-bearing fish that occur in the creek channel during the Wet season.

It was suggested above (section 3.1) that the biomass of the *V. angasi* population is likely to exceed that of all other benthic macroinvertebrates in the sandy channels of Magela Creek. On this

assumption, therefore, if any further waste-water releases were to adversely affect the distribution and abundance of benthic invertebrates downstream of the Ranger release pipe, a relatively large biomass at least would be lost were they to directly affect the population of freshwater mussels. However, since 1991 RP4 water has become essentially non-toxic to aquatic organisms tested at the Environmental Research Institute of the Supervising Scientist (ARRRI 1992).¹

Finally, in any further releases of Ranger waste waters, reproductive activity in the population of *V. angasi* resident in the sandy channels of Magela Creek should provide a useful response in the monitoring of adverse biological effects if the previously recorded toxicological effects reappeared. The large population, the large size of individuals (and consequent ease at which larval content may be recognised and determined) and ease at which mussels may be collected, all contribute to the suitability of the population in this role (see ARRRI 1987).

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¹ In 1994 the Alligator Rivers Region Research Institute (ARRRI) merged with the Commonwealth's Environment Protection Agency, and is now called the Environmental Research Institute of the Supervising Scientist (ERISS).

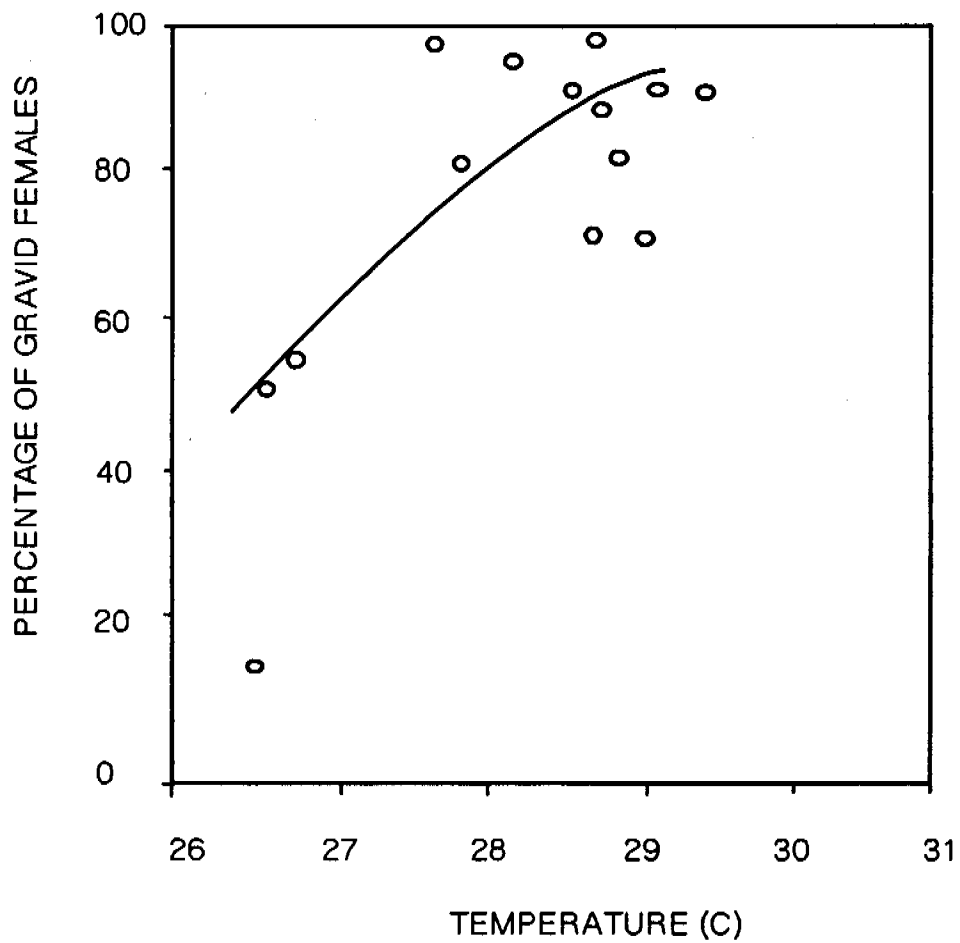


Figure 1 Relationship between the percentage of gravid female mussels found among all females examined at any one time and water temperature, in the Magela Creek channel.

Fitted regression equation is

$$Y = -4.29 + 0.191X \quad (P < 0.01, r^2 = 0.519)$$

where Y = gravidity observed at any one time ($\arcsin\sqrt{(Y \text{ in percent}/100)}$, radians), and x = water temperature (°C).

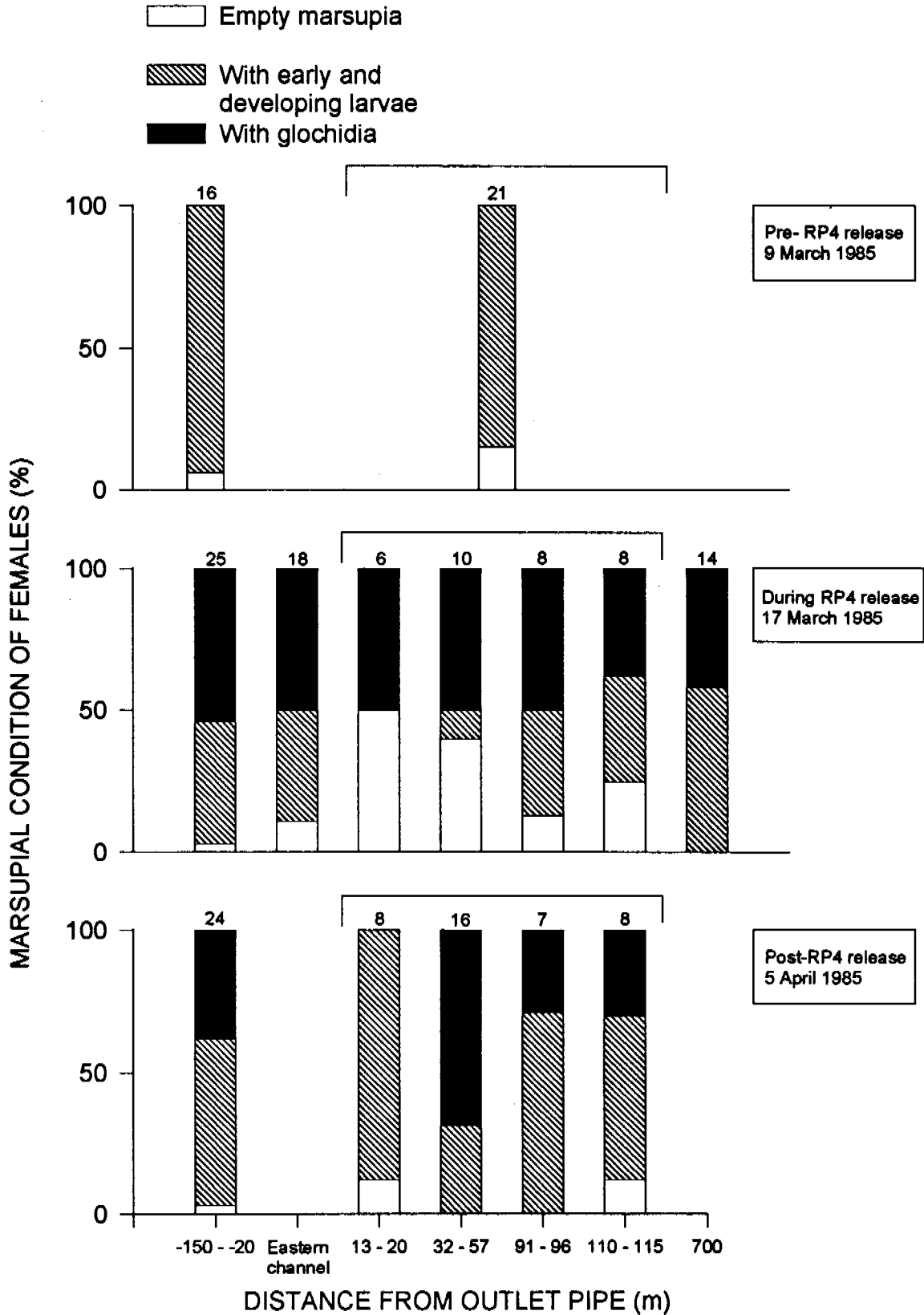


Figure 2 Relative proportions of the various marsupial appearances found among female mussels from sites of the Magela Creek channel during the study period. Numbers of females sampled indicated above histograms while parentheses enclose sites where reproductive effects were observed during RP4 release. Pre-release mussels from the downstream site were sampled over the range 13–115 m from the outlet pipe

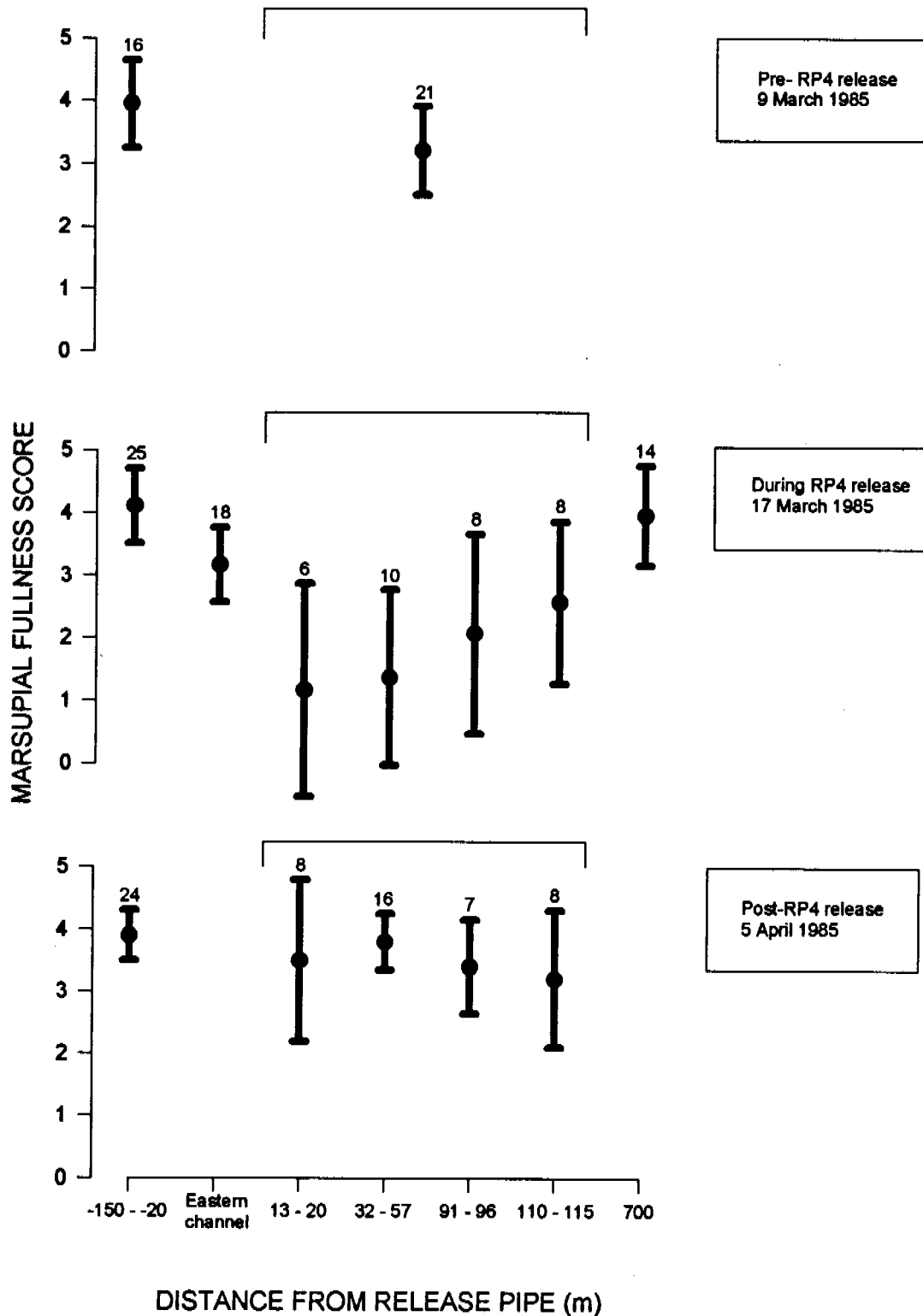


Figure 3 Marsupial fullness scores of female mussels sampled from sites of the Magela Creek channel during the study period. Mean scores shown, with 95% confidence limits indicated by bars and the numbers of females sampled indicated above the bars. Solid parentheses enclose sites where reproductive effects were observed during RP4 release.

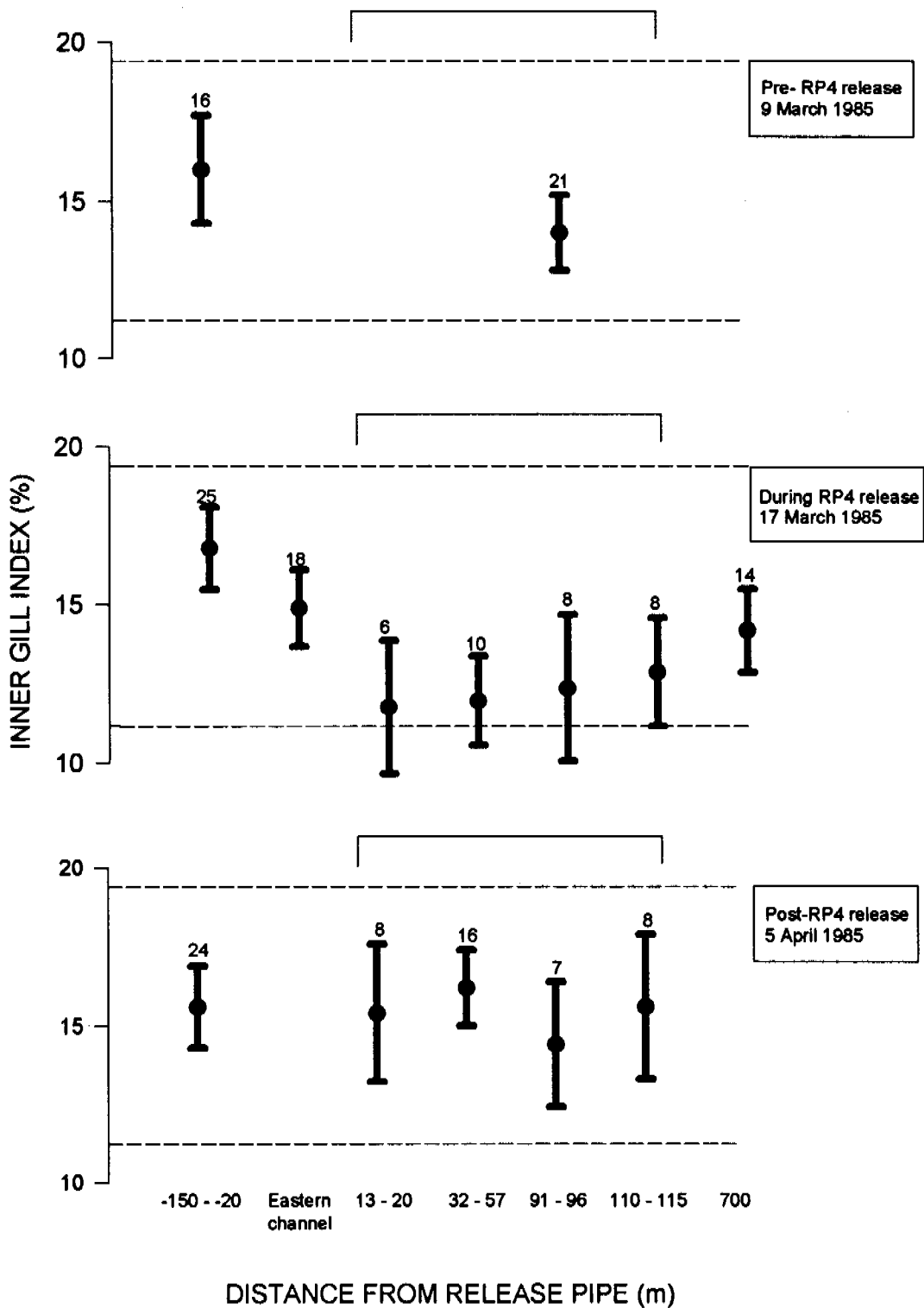


Figure 4 Inner gill indices of female mussels sampled from sites of the Magela Creek channel during the study period. Mean indices shown, with 95% confidence limits indicated by bars and the numbers of females sampled indicated above the bars. Solid parentheses enclose sites where reproductive effects were observed during RP4 release. Upper broken lines indicate expected index if all marsupia were packed with mature larvae (19.4%); lower broken lines indicate expected index if all marsupia were empty (11.1%).

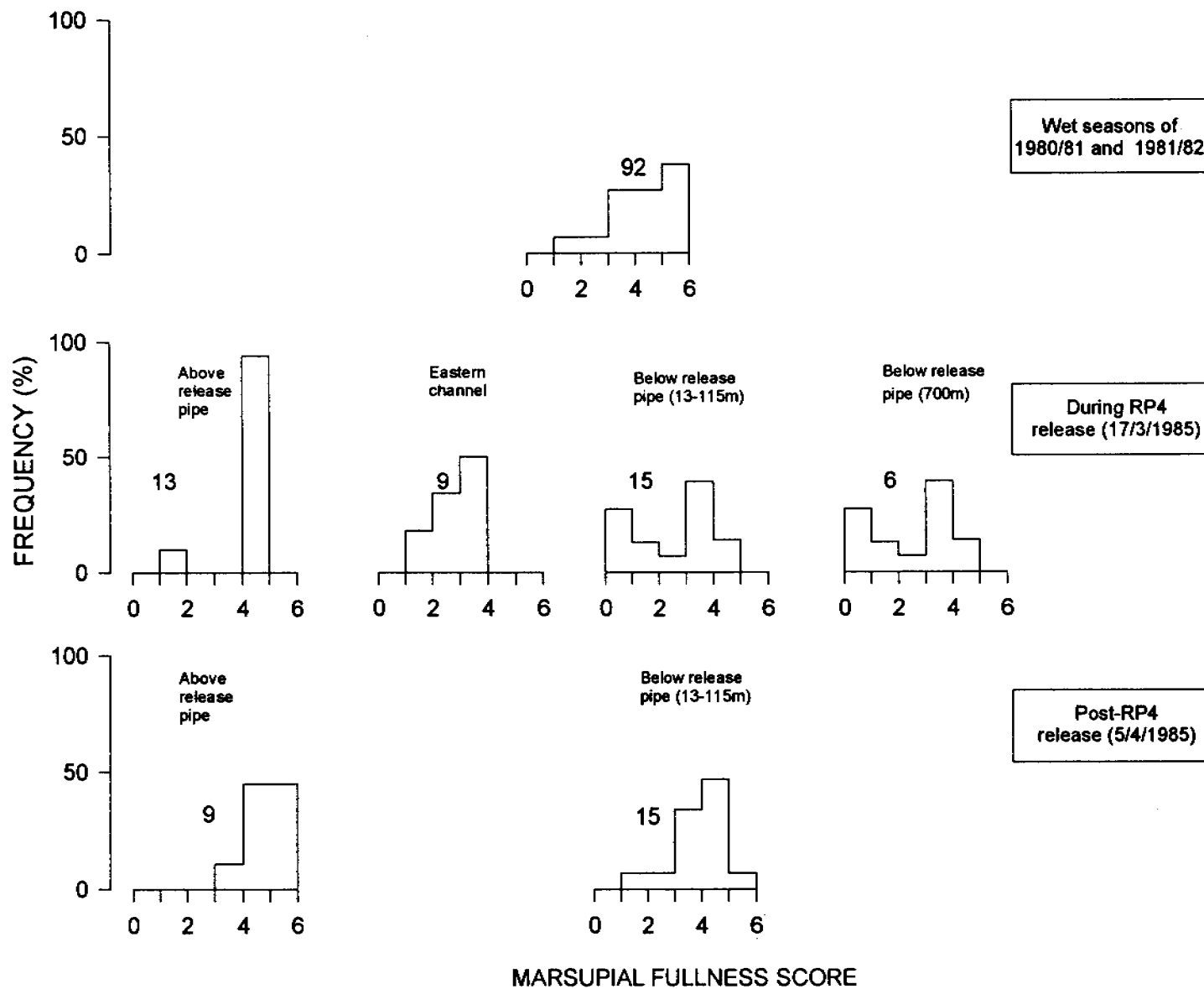


Figure 5 Percentage proportion of marsupial fullness scores of glochidial-bearing female mussels sampled from the Magela Creek channel. Numbers above histograms refer to numbers of females sampled bearing glochidia.

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