CHAPTER 7

REPRODUCTION

7.1 Introduction

Reproduction in freshwater mussels has been extensively investigated and is reasonably well understood for the unionaceans of northern temperate climes. The North American fauna in particular, has received considerable attention and in general two phases can be recognised in the progress of studies in this region: (a) commercial interest in freshwater mussels in the early 1900's led to a spate of broad, general studies concerned primarily with discovering means of rearing mussels or restocking heavily exploited and depleted populations (Lefevre and Curtis, 1910, 1912; Surber, 1912; Coker et al., 1921; Howard, 1922); (b) more intensive and specific investigation has been carried out only in comparatively recent times, part of the impetus for this work being the threat made to species whose ranges have been significantly reduced by anthropogenic changes to their environments. Indeed attention in regard to the latter has not only been directed at North American unionids (e.g. Trdan, 1981; Trdan and Hoeh, 1982; Zale and Neves, 1982, a,b,c) but at species equally endangered in parts of Europe, e.g. Margaritifera margaritifera (Bauer, 1979; Young and Williams, 1984 a, b).

By comparison, reproduction in freshwater mussels from the tropics and southern latitudes has received scant attention. Although the broad anatomical and life stage differences that distinguish the southern mutelaceans and hyriid unionaceans from northern forms have been

described (e.g. Fryer, 1961; Parodiz and Bonetto, 1963), breeding patterns and life cycles for the vast majority of mussels are unknown.

Among tropical freshwater mussels, documentation of the sexuality and breeding seasons (Bloomer, 1931; Lomte and Nagabhushanam, 1969; Ghosh and Ghose, 1972; Nagabhushanam and Lohgaonker, 1978) and parasitic stages (Seshaiya, 1941, 1969) of the Indian unionids Lamellidens and Parreysia, is the most extensive. More comprehensive, ecological studies however, are few and comparatively recent. Noteworthy are Kenmuir's (1980, 1981 a, b) investigations of the reproductive biology of two mutelids, Aspatharia wahlbergi and Mutela dubia, and the unionid, Caelatura mossambicensis, in Lake Kariba and Lake McIlwaine, Zimbabwe. Fryer's (1961) thorough study was the first description of the parasitism and development of a mutelid haustorium (Mutela bourguignati of Uganda) upon a host fish. Otherwise, the only other study of significance is a reasonably complete account of reproduction in Anodonta woodiana in Plover Cove, Hong Kong carried out by Dudgeon and Morton (1983).

Very few studies have investigated the reproductive biology of the hyriids of the Australasian region, though the situation has improved in temperate Australia somewhat in recent times. Glochidia have been described and fish hosts found for Hyridella menziesi (Percival, 1931), Velesunio ambiguus (Hiscock, 1951; McMichael and Hiscock, 1958; Walker, 1981b), H. drapeta (Atkins, 1979), and Alathyria jacksoni (Walker, 1981b). Published reports include glochidial descriptions of an additional five species - A. p. pertexta, A. profuga, H. australis (McMichael and Hiscock, 1958), H. australis, H. depressa and

Cucumerunio novaehollandiae (Jones and Simpson, in prep.); while there are unpublished records of at least a further three - V. angasi, V. moretonicus and Westralunio carteri (Walker, pers. comm.). Thus the glochidia of eleven of the seventeen Australian hyriids have been described. From observations made of the appearance of glochidia (Percival, 1931; McMichael and Hiscock, 1958; Atkins, 1979) and of brooding females (Walker, 1981b; Jones and Simpson, in prep.), the probable breeding seasons of a number of species from temperate Australasia (section A4.3) have been inferred. Collection of material for the studies by Walker (1981b) and Jones and Simpson (in prep.) was regular and seasonal and thus the breeding seasons of the species involved are more or less confirmed. Among the species studied by Jones and Simpson, gonadal and larval development of C. novaehollandiae were also described.

The above review accentuates the paucity of knowledge regarding the reproduction of both tropical and Australian freshwater mussels. No Australian or South American hyriid, has been thoroughly investigated in all aspects of its reproductive biology. The study of reproducton in Velesunio angasi therefore provides an important contribution to the knowledge relating particularly to the hyriids, and also to freshwater mussels of the tropics. Important aspects of the reproduction of V. angasi studied included gonadal development, structure of the breeding population, larval production, glochidial release and parasitism. Worldwide information of this completeness for populations from specific locations is available for only a number of unionacean species, namely: Elliptio complanata (USA) (Matteson, 1948); Pleurobema cordatum (USA) (Yokley, 1972); Anodonta cygnea

(Italy) (Giusti et al., 1975); four (sympatric) lampsilines (USA) (Zale and Neves, 1982 a, b); and A. woodiana (Dudgeon and Morton, 1983), while collectively, the reproductive biology of Margaritifera margaritifera throughout its holarctic range is well known (Murphy, 1942; Bjork, 1962; Roscoe and Redelings, 1964; Smith, 1976, 1979; Bauer, 1979; Young and Williams, 1984 a, b).

Literature reviews appropriate to the following sections on gonadal development and structure of the breeding population, larval production, and glochidial release and parasitism, appear in Appendix 4, sections A4.1 to A4.3 respectively.

A. GONADAL DEVELOPMENT

7.2 Materials and methods

The material for histological study was taken from sexually mature mussels, randomly selected from the collections made monthly in Georgetown, the Magela Creek channel, Mudginberri and Nankeen billabongs (section 3.3). Collectively, a diversity of billabong types was represented, each varying considerably in limnological character. Nevertheless, rationalising the large amount of material to be processed, the annual gametogenic cycle of Velesunio angasi was followed more closely in Mudginberri billabong than in the other waterbodies. In this environment, regular seasonal patterns were apparent in larval production but seasonal changes in water quality were least discernible in comparison to the other waterbodies. The pattern of larval production in the reasonably equitable environment

of Mudginberri, apparently represented a baseline breeding cycle. In other waterbodies, interruptions to this pattern caused by regular and seasonal fluctuations in oxygen concentration (Nankeen), turbidity (Georgetown and Nankeen) and exposure and inundation (Magela Creek channel) might occur. Description of this background breeding cycle assumed priority in the studies undertaken here.

Individuals collected between June 1980 and May 1981 were used for study. Monthly samples from Mudginberri billabong were processed over this period, while quarterly samples taken in June, September, December 1980, and March 1981 from Georgetown and Nankeen, were used. Aestivating mussels collected during November and December 1980, and submerged mussels taken in January 1981 from the Magela Creek channel, were processed. Each monthly sample comprised five individuals of each sex. Specimens were fixed in Bouin's solution and preserved in 70% ethanol. Sections were taken transversely through the central visceral mass at 6 μm and stained with Mayer's haematoxylin and alcoholic eosin.

No broad stages of gonadal maturation could be assigned to individuals as superficially the sections of testes and ovaries bore a resemblance between all individuals, sites and collection dates. The gonads were mature and sperm and primary oocytes present year round. The more subtle changes in gonadal condition, however, were quantified in the following manner:

For the testes of each individual, the proportion of the different spermatogenic stages was determined in 5 seminiferous tubules. The

tubules chosen were spaced evenly across the section dorsally to ventrally (i.e. at predetermined intervals), and were selected using an eyepiece graticule. Camera lucida drawings of each tubule in relation to the different stages were made upon graph paper, and the relative areas of each spermatogenic stage determined by counts of the squares filled with the respective cell type. Sperm in the lumen of the tubules were present in more or less varying densities. To account for this variation, the areal presence of sperm was ranked according to an arbitrary scale, 1-5, of density. The relative areas of sperm was then adjusted accordingly. The values of the relative areas of each spermatogenic stage for the 5 tubules were averaged and a percentage composition by cell type was determined for the testes of each individual. 50 cells of each spermatogenic stage were measured under an oil immersion lens, using an eyepiece graticule.

Measurements and counts of the free primary oocytes were used to assess the female gonadal cycle. Oocyte counts were made on each of 25 follicles. Five fields of view were selected and located in the same manner as described for the testes. Within the field of view, the numbers of oocytes within each of 5 adjacent follicles were counted. A mean number of oocytes per follicle averaged over the 25 counts, was calculated for each individual. Simultaneously, at each field of view selected in the ovaries, the diameters across both axes of all oocytes through which the nucleus had been sectioned, were measured using an eyepiece graticule. For each individual, data were accumulated on at least 20 oocytes. After averaging the values of the two measurements made of each oocyte, a mean oocyte diameter was calculated for each individual.

7.3 Sex determination

7.3.1 Sexual dimorphism in shell size and shape

In the Magela Creek waterbodies, there is a tendency for female Velesunio angasi to grow slightly faster than males; in billabongs where females were underrepresented in the older age classes, however, differences in growth rates were least apparent (section 6.4.2). In only 2 of the 12 populations sampled, were significant growth disparities (as determined by the confidence intervals about the parameter, L_{∞} , of the von Bertalanffy growth equation) observed between the sexes (Table 5.20).

With respect to shape, there were no tendencies observed between relative height (i.e. in relation to total length) and sex (section 4.3.1). Of the populations sampled both males and female tended to have higher or lower shells equally. In only one billabong, moreover, did the slope of the length-height regression differ significantly between the sexes. In 9 of the 12 waterbodies, females tended to be more inflated (wider) than males (section 4.4), yet in only one billabong did the slope of the length-width regression differ significantly between the sexes.

The conclusion reached is that while obesity is the only morphometric index that consistently portrays a sexual dimorphism, nevertheless differences between the sexes in either growth rate, relative height or obesity are trivial and none of the indices has a broader application to sex determination. External shell characters in most

instances therefore, could not be used to confidently predict sex of V. angasi in the Magela Creek.

7.3.2 Internal anatomy

For routine work, sex was determined by both morphology of the inner gills and nature of the gonads ('visceral sex', Heard, 1975).

The inner gills of female V. angasi are modified as marsupia and are easily distinguishable from either the male or non-marsupial condition. While McMichael and Hiscock (1958) have described the anatomy and structure of the marsupial and non-marsupial demibranchs of V. angasi, for practical recognition their appearance in mussel populations from the Magela Creek is as follows. The inner, marsupial gills of females are notably thickened and the striation of the modified water tubes appears uniformly regular and even over the entire gill. Generally the gills are pigmented a light tan and this coloration is similarly uniform throughout the gill. The non-marsupial condition comprises both pairs of gills in males and the outer pair in females. These gills are conspicuously thinner, and the structure of the water tubes gives the appearance of a very irregular striation and reticulation throughout. Coloration is similarly variable both across the gill and between individuals where in the latter instance, the range in colour may vary from light tan to a deep, crimson red. Although the gill may be uniform in coloration, in most cases an irregular, yellow-orange pigmentation is prominent at its base, losing intensity over the remainder of the gill. Only in female mussels from the Magela Creek channel, was the marsupial

appearance restricted to a portion of the inner gills. In these mussels, the marsupium occupied between one and two thirds of the gills, the remainder having the non-marsupial appearance.

Determination of the visceral sex in V. angasi was easily accomplished as the gonads were in a mature condition year round. By piercing the visceral mass with a probe, the ripe, yolky oocytes of females exuded and were clearly visible as minute, white specks amongst the body fluids. The body fluids similarly exuding from males, however, mainly comprised a white, milky fluid containing spermatogenic products (of which sperm predominated, section 7.4.2). For mussels from Georgetown, Mudginberri, the Magela Creek channel and Nankeen billabong, the body fluids containing the gonadal products were in all instances smeared upon microscopic slides and examined under high power magnification to confirm the nature of the gonadal tissues. The gravid condition of many of the females throughout the year in most populations, greatly assisted in determining sex.

For individuals from all populations, sex was determined both by the presence or absence of marsupial, inner gills and by puncturing the visceral mass to observe the exuded gonadal products. With few exceptions (section 7.5), there was always a consistent correlation between the visceral sex and sex as determined by inner gill morphology.

7.4 Gonad histology

Velesunio angasi is dioecious and only very occasional individuals are

hermaphroditic (section 7.5). The gonads of both sexes ramify the visceral mass and within the follicles and tubules the various gametogenic stages develop.

7.4.1 Stages of gametogenesis

Stages of sperm maturation

The follicles or seminiferous tubules of the testes, each contain discrete cell aggregations or 'nests' of the various spermatogenic stages arranged in successive layers so that cells in the more advanced stages occur more or less regularly in succession towards the centre of the tubule (Fig. 7.1 B). In the spermatogenesis of *V. angasi*, six distinct stages were observed.

1. Spermatogonia - These cells have a mean nuclear diameter of 4.1 µm and are slightly angular in appearance. No cytoplasm is observed and the nucleus is compact and basophilic. Spermatogonia always occurred in nests adjacent to the tubule walls (Figs 7.1 C and D).

2. Sperm morulae - These are large multinucleate structures, each drupel of which resembles in appearance the spermatogonium. Collectively they have a mean diameter of 11.0 µm . Sperm morulae are often associated with spermatogonia and occur more or less singly scattered along the periphery of the tubule walls (Fig. 7.1 C).

3. Primary spermatocytes - Division of the spermatogonia produces primary spermatocytes which have a mean nuclear diameter of 5.3 µm. The nuclei of these rounded cells are slightly granular in appearance as dense chromatin material is abundant. Primary spermatocytes occur in nests more or less centripetal to the spermatogonia (Fig. 7.1 D).

4. Secondary spermatocytes - These cells, produced by divisions of the primary spermatocytes were rarely observed. This is in accordance with the observation of Tranter (1958); apparently division is very rapid at this stage. The nuclei are characteristically granular in appearance and measure 3.5 μm in mean diameter. Secondary spermatocytes when present, occur in nests adjacent to the primary spermatocytes (Fig. 7.1 D).

5. Spermatids - The secondary spermatocytes divide to produce spermatids. The nuclei of these spherical cells have a mean nuclear diameter of 2.7 μm, and resemble the spermatogonia in staining quality. Invariably, the spermatids occurred in large nests outside the central body of spermatozoa (Fig. 7.1 D).

6. Spermatozoa - The transformation of the spermatids into spermatozoa occurs in the centre of the tubule lumen. The nuclei of these small cells stain intensely and homogeneously and are bullet-shaped in appearance, having mean dimensions of 3.8 x 1.4 μm (Figs 7.1 C and D).

Oocyte maturation

The classification of oocyte maturation stages was based on the general scheme used by Tranter (1958) for describing the histological changes in the ovaries of the Australian pearl oyster, Pinctada albina.

Oogonia and early previtellogenic oocytes (oocytes 1 and 2 of Tranter, 1958) stain heavily as little observable cytoplasm is present in these cells. They are found imbedded in the follicle walls and were

infrequently observed in the sectioned material.

At the onset of vitellogenesis, the oocyte becomes attached to the follicle wall amongst the nutritive granules, by a flat broad base of attachment (Fig. 7.2 C). The cytoplasm of the semi-oval cell stains less heavily than the nucleus (oocyte 3).

The oocyte increases in size and the cytoplasm stains more heavily as yolk accumulates (Fig. 7.2 C). The base of attachment constricts and the stalked oocyte becomes almost spherical in appearance (oocyte 4).

By this stage, the oocyte has grown towards the centre of the lumen; final detachment from the follicle wall occurs and the ripe oocyte lies free in the lumen (Fig. 7.2 C). After detachment, the cell is termed the free or primary oocyte.

Amongst the sectioned material, no atretic oocytes were ever observed.

7.4.2 Seasonal histological changes in the gonads

7.4.2.1 The testes

Examination of the proportions of spermatogenic stages calculated for each individual from the one time and location, revealed little variation in the relative proportions between the 5 individual testes. Because of this similar appearance, the proportions were averaged over the 5 individuals to provide a combined composition by cell type for each month and location. The relative proportions of spermatogenic

stages observed for each month and waterbody are shown in Figure 7.3.

Having calculated the relative proportions of spermatogenic stages, it is apparent that spermatogenesis occurred throughout the year as indicated by the presence of most of the early stages in the testes at any one time. The relative equitability of the environment in Mudginberri in particular, is reflected in the similar constancy at which spermatogenesis takes place in males throughout the year in this billabong (Fig. 7.3). However, elsewhere periods of inactivity were noted. Inactive phases were recognised by relative absence of the stages of typical spermatogenesis (spermatocytes and spermatids). In accordance with the findings of others (Ropes and Stickney, 1965; Heard, 1975; Jones and Simpson, in prep.), most of the spermatogenic activity is atypical during these phases, activity being directed towards production of sperm morulae. The characteristic appearance of the testes at these times is shown in Figures 7.1A and C. Sperm morulae, spermatogonia and spermatozoa (residual?) are chiefly observed. Atypical and typical spermatogenesis are nevertheless not mutually exclusive events, and sperm morulae are often found amongst the typical spermatogenic units (e.g. Magela Creek channel, January; Mudginberri, June, July, September; Nankeen, September - Fig. 7.3). Inactive phases where found in the testes of males in Georgetown during the early-mid Dry (June and September), in the testes of aestivating Creek mussels (November and December) and early Dry season testes in Nankeen (June).

Other than the periods stated above, the testes were otherwise active, and spermatozoa were dominant. Activity in Mudginberri mussels was

discernible throughout the year. The appearance of the testes during active phases is shown in Figures 7.1 B and D. Spermatozoa and the stages characteristic of typical spermatogenesis dominate the tubules.

Active gonads with the presence of sperm (and ripe oocytes) throughout much (if not all) of the year, is concomitant with the notion that spawning in V. angasi is repetitive. This could be expected in equitable, warm-water and tropical environments, and in relation to the testes, implies that sperm is continuously produced and released throughout the year. The actual presence and quantity of sperm in the seminiferous tubules therefore, give no indication of the intensity of spermiogenesis and resultant spawning, as the sperm is constantly in a state of flux. Rather, under these circumstances, the presence and abundance of the stages of typical spermatogenesis - spermatocytes and spermatids (but especially the latter) may be expected to reflect spermiogenic activity and subsequent release of sperm, i.e. spawning intensity.

Spermiogenic activity of V. angasi in the present study was found to respond to the same factors that influence oocyte and larval production in the female cycle. To demonstrate this, the following regression analyses were performed. In the reproductive cycle of the female, larval production (% of females brooding embryos and larvae in the marsupia at any one time and location) was extensively studied and environmental correlates that might influence it, determined. (Larval production nevertheless, directly and immediately reflects the simultaneous production of oocytes occurring in the ovaries, section 7.4.2.2.) Larval production for the same period and locality was

regressed against each of the spermatogenic stages occurring in the testes. Both variables were arcsine transformed, and the results of the linear regressions for each spermatogenic stage and location are shown in Table 7.1. As a whole year's data were analysed from Mudginberri billabong, more confidence is stored in the conclusions drawn from these results. From the results in general, the following conclusions and remarks can be made:

1. The abundance of spermatids correlated positively with larval production. In Mudginberri billabong this correlation was high and a very significant regression relationship was found ($P < 0.01$). This same relationship was the strongest found in Nankeen billabong. These results indicate that spermiogenesis and spawning in males occur with the same intensity as larval production (and thus oocyte production and spawning) in females.

2. In the three billabongs, all stages of typical spermatogenesis correlated positively with larval production.

3. The relative compactness of sperm in the tubules (the 'empty' category of Table 7.1) correlated negatively with larval production, indicating that spermiogenesis and larval production proceed with the same intensity. In Mudginberri, this relationship was significant ($P < 0.05$), even though sperm abundance declined over the same gradient. The latter suggests that spawning of males in Mudginberri billabong is exceedingly intense and that sperm release is a continuous event, peaking with larval production.

4. The presence of sperm morulae (and thus atypical spermatogenesis) in the tubules correlated negatively with larval production (and also spermiogenic activity).

5. Spermatogonia appeared to play a passive role in cycles of

spermatogenesis as they were constantly present in the tubules (Fig. 7.3). Their omnipresence indicates that they act as a reservoir of germ cells for all spermatogenic redevelopment.

Of the factors that promote larval production (and therefore oocyte production) in the billabongs, increasing water temperature (Mudginberri and Georgetown) and oxygen concentration (Nankeen), and decreasing turbidity (Georgetown) are strongly implicated (section 7.10.1). According to the relationships found in point 1. above therefore, spermiogenic activity responds to these same factors. Thus, it appears likely that the intensity of spawning in both sexes of V. angasi is simultaneous.

Of final interest is the observation that spermatogenesis proceeded apparently uninterrupted in mussels from the Magela Creek channel, aestivating during November and December. Normal physiological processes such as gametogenesis in V. angasi therefore, are not suspended during dormancy. Increasing ambient temperatures were presumably responsible for the increase in spermatogenic activity observed in Creek mussels over the period encompassing aestivation and subsequent inundation (January).

7.4.2.2 The ovaries

Superficially it was assumed that owing to the presence of primary oocytes in the ovaries year round, in association with the gravid condition of the marsupia for much of the year, oogenesis and spawning in female V. angasi occurred throughout the year. However, the exact

nature, intensity and timing of the events could not be shown without investigating the seasonal activity and development of oocyte maturation in the ovaries. The oogenic cycle in the ovaries was followed by monitoring seasonal changes in the size and numbers of primary oocytes. In the ovaries of both temperate (e.g. Zale and Neves, 1982a; Jones and Simpson, in prep.) and tropical (Dudgeon and Morton, 1983) unionaceans, either the absence of primary oocytes or a sharp decline in oocyte diameter immediately after a period of reproductive maturity has been assumed to indicate that spawning had occurred.

For each location and sampling period, a mean oocyte number per follicle and mean oocyte diameter were calculated from the individual observations averaged for the 5 sectioned ovaries. The values and 95% confidence intervals about the means, are shown in Table 7.2. From microscopic inspection of the ovaries, changes and patterns in oocyte sizes between individuals, localities and sampling periods were not visually discernible. However, periods of inactivity just as in the male gametogenic cycle, or a partially spawned appearance in the ovary, were readily observed by visual assessment of oocyte numbers in the follicles. In a relatively inactive or partially spawned ovary, primary oocytes were noticeably fewer (Fig. 7.2 A) than in the active ovary (Fig. 7.2 B).

Mean oocyte number

Both oogenic activity in terms of mean numbers of primary oocytes per ovarian follicle, and the percentage of gravid females observed over

all individuals examined from the same monthly collections, are plotted with respect to billabong and against time in Figure 7.4. Clearly, the ovaries and marsupia are in close communication with each other and there is an immediacy of response to the intensity and activity of oogenesis by spawning and subsequent production of larvae. The breeding pattern is apparently a repetitive one for unlike distinct, seasonal breeding cycles observed elsewhere, spawning in V. angasi does not result in an immediate fall in oocyte numbers in the follicles. Rather oocyte and subsequent larval production occur simultaneously, and the only indication of a spawning stress was observed in Mudginberri billabong (for which the seasonal cycle is complete) when between July and November, the intensity of larval production was apparently so high that a lag in oogenic activity was observed (Fig. 7.4).

For the 12 monthly observations in Mudginberri billabong, analysis of variance (AOV) testing was performed to discern whether the mean oocyte numbers of the 5 observations differed when they were partitioned according to the different stages of gravidity observed in the respective marsupia. Although considered in detail in a later section (section 7.9), the marsupial condition for the purposes of this analysis, was classified: 'empty'; with 'developing larvae'; and 'mature larvae'. The analysis revealed no significant difference ($P > 0.05$) in oocyte numbers among the different marsupial states. The mean oocyte numbers determined were: 4.50 (empty); 4.15 (developing larvae); and 5.30 (mature larvae).

When the partitioned data were replotted with respect to season,

however, particular patterns emerged (Fig. 7.5). In the active phases of the oogenic and larval cycles (from the peaks in activity observed in Fig. 7.4), least oocytes were observed in the gonads of females simultaneously brooding developing larvae (Fig. 7.5). This is to be expected as presumably, individuals either (1) with empty marsupia that have recently discharged their larvae and whose marsupia are ready to receive a new batch of eggs or (2) with fully developed glochidia, have had a longer period since the previous spawning for the gonads to have recovered. During relatively inactive phases of the oogenic and larval cycles (Fig. 7.4), however, least oocytes were observed in the ovaries of females with empty marsupia (Fig. 7.5). Various environmental factors at these times inhibit or retard gonadal and larval development (section 7.10.1), and apparently females with empty marsupia represent a condition in which oogenesis is slowed to such an extent that not enough eggs are availble for spawning and subsequent brooding.

Mean oocyte diameter

Oogenic activity in terms of mean oocyte diameters, and the percentage of gravid females observed from the same monthly collections, are plotted with respect to billabong and against time in Figure 7.6. Seasonal patterns in oocyte diameters are less obvious than the patterns involving oocyte numbers. Only the diameters of free, primary oocytes were measured and presumably a relatively advanced, developmental threshold is required before the oocytes break away from the follicle wall. Tranter (1958) in fact, thought it doubtful whether there would be any further growth of primary oocytes after

they had broken free into the lumina of the ovarian follicles in Pinctada albina. However, assuming eggs discharged into the marsupia are of a constant size, then the differences in sizes of free, primary oocytes observed in the lumina of the ovarian follicles of V. angasi between sampling periods and locations (Table 7.2), are themselves evidence that either vitellogenic growth, or expansion of the eggs must occur here. (It was noted for V. angasi nevertheless, that larger eggs were produced in higher trophic environments - Georgetown, Mudginberri, Nankeen billabongs - in that order, Fig. 7.6.)

An interpretation of the data of Figure 7.6 is as follows. During periods of gonadal activity (as previously discerned by mean oocyte numbers in the follicles) larger eggs are closely associated with increased spawning and intensity of larval production (Mudginberri between July and November; Nankeen in September). Only after a period of peak ovarian activity and larval development does the effect of repetitive spawning finally outpace oocyte development, reflecting in smaller egg sizes (Georgetown in March, Mudginberri in December and April). During inactive phases in the ovary, the fewer oocytes present (Fig. 7.4) are nevertheless fully matured and developed. Their larger size (Fig. 7.6) suggests that they have accrued and developed for some period of time in readiness for the next spawning (Georgetown between September and December; Mudginberri between January and March; Nankeen in June and March). The small egg sizes observed in Mudginberri during July, the coolest month, are best explained by a retardation of oogenesis caused by low water temperatures.

Exceptions to the above patterns (e.g. Nankeen in December) may also be explained after partitioning individual observations according to different stages of gravidity. AOV testing in Mudginberri billabong revealed a significant ($P < 0.05$) difference in oocyte size among the different marsupial states (as classified earlier). Multiple range testing (least significant different test: Zar, 1974, p. 151) showed that while the oocyte means of individuals with empty marsupia (47.9 μm) and ones bearing mature larvae (48.9 μm) were compatible, the oocyte means in individuals bearing developing larvae (43.2 μm) were significantly lower than either ($P < 0.05$).

When the partitioned data were replotted with respect to season (Fig. 7.7), the same general patterns emerged as were discovered when mean oocyte number was partitioned according to the different stages of gravidity. Active ovarian development resulted in larger eggs in females both brooding mature larvae and with empty marsupia. (The same interpretation of this phenomenon can be given to oocyte diameter, as was previously given in relation to oocyte number.) During inactive phases, however, some females with empty marsupia bore larger eggs in the ovaries than those with developing larvae in the marsupia (Georgetown in September, Mudginberri in June, Nankeen in December). As mentioned above, this might reflect additional development available for the eggs owing to their retention in the ovaries for relatively prolonged periods.

Overall

Oogenic activity in terms of production of primary oocytes is best represented by mean numbers of oocytes per ovarian follicle. That the pattern of larval production may be directly superimposed upon that of oogenic activity reflects the close communication of the ovaries and marsupia: the intensity of oogenesis is immediately reflected in the intensity of larval production. A series of repetitive breeding cycles is concomitant with this description. (Very strong evidence for the latter is given in section 7.15.2.) Knowledge of primary oocyte size adds supplementary information concerning recognition of peaks in spawning intensity (marked by declines in oocyte diameter) and periods of ovarian recovery (indicated by increases in oocyte diameter when oocyte numbers are low).

Evidence from the previous section (section 7.4.2.1) suggests that the timing of both spermiogenic at least, and oogenic activity (and therefore spawning of both sexes) is the same, and both cycles are presumably therefore, influenced by the same environmental factors (described in section 7.10.1).

Finally, just as spermatogenesis proceeded uninterrupted in aestivating mussels from the Magela Creek channel between November and December (1981), so too oogenic activity proceeded uninterrupted (Table 7.2). Both mean primary oocyte numbers and diameters increased over the period, presumably in response to increasing ambient temperatures. Inundation during January resulted in decreases in oocyte numbers and sizes presumably as a result of spawning.

B. STRUCTURE OF THE BREEDING POPULATION

7.5 Hermaphroditism and sexual integrity

Among the mussels from Georgetown, Mudginberri and Nankeen billabongs, and the Magela Creek channel sexed by inspection of gonadal smears, individuals were occasionally found in which both ripe eggs and sperm were present. Heard (1975) classified occasional hermaphrodites amongst normally dioecious unionids as male or female hermaphrodite, according to the predominant gonad prevailing. Smears, however, are known to be a less reliable technique both for determining the presence of hermaphroditism and for assessing the comparative abundance of ovarian and testicular tissue (Heard, 1975). Having determined by smears that the gonads were of an hermaphroditic nature, the assignment into male or female hermaphroditic categories was made according to inner gill morphology: male hermaphrodites possessed non-marsupial inner gills while the inner gills of female hermaphrodites were marsupial in appearance.

The incidence of hermaphroditism according to different age classes of mussels from the populations investigated, is shown in Table 7.3, while seasonal incidence of hermaphroditism amongst male and female hermaphrodites is displayed in Table 7.4. Among the individuals histologically sectioned, occurred 6 hermaphrodites; 3 of these had not previously been discovered from gonadal smears. Thus the incidence of hermaphroditism among the populations studied, as shown in Table 7.3 needs to be corrected for cases where the condition is undetected by smears. Assuming that the incidences are only half

represented, nevertheless hermaphroditism in V. angasi is uncommon and is confined to less than 2% of each of the populations investigated (Table 7.3).

The nature of the gonads of sectioned hermaphrodites is given in Table 7.5. Testicular tissue predominated (5 out of 6 cases), and the discrete ovarian follicles or testicular tubules were mostly confined to distinct regions of the gonad (5 out of 6 cases) (Fig. 7.2 D). In contrast, the majority of known North American hermaphroditic unionids either bear gonads with monoecious acini (in which both eggs and sperm are produced) or gonads with intermingled zones of male and female acini (Heard, 1975). Only one hermaphrodite sectioned in the present study displayed the latter condition (Table 7.5).

Summarising known information on North American unionids, Kat (1983d) states that hermaphrodites reproducing chiefly as males are either very uncommon or when present in appreciable numbers contain less than 5% female tissue. In V. angasi the predominant visceral sex of hermaphrodites corresponded with the morphology of the inner gills in 5 out of 6 cases (Table 7.5). If the same relationship is applicable to all the hermaphrodites examined, then hermaphrodites of V. angasi in the Magela Creek reproduce both as males and females more or less equally. Twelve male and 14 female hermaphrodites were detected by gonadal smears (Table 7.4). Moreover, the ovarian follicles in male hermaphrodites occupied more than 5% of the gonadal tissue (Table 7.5). Thus the nature of hermaphroditism in the hyriid V. angasi differs from that found in unionids. According to Kat (1983d), unionids reproducing chiefly as females are common and are highly

variable with respect to the amount of male tissue found in the gonad. While male and female hermaphrodites were equally common, nevertheless the gonads of female hermaphrodites of V. angasi are apparently equally variable. Two individuals with female inner gills contained testicular tissue varying from less than 40% to more than 90% (Table 7.5). This observation accords with Kat's (1983d) hypothesis that disruption of hormonal levels determining sex by developmental error should result in considerable variability in male:female gonadal ratios among females as the sex of females is hypothesised to be determined by high hormone levels.

Hermaphroditism has been previously implicated as providing evidence of sex reversal in unionids (e.g. Bloomer, 1934, 1935, 1939). The possibility of consecutive, rhythmical consecutive, and alternative sexuality (Coe, 1943) was investigated in V. angasi.

Consecutive sexuality is sex reversal in which there is a single change in the functional sexuality of the individual, usually from male to female (Coe, 1943). Protandry is one such example. Chi-square analyses were performed on the data of Table 7.3 to discern whether occasional hermaphrodites were distributed evenly amongst the age classes of the various populations studied. No evidence against the hypothesis of equal distribution ($P > 0.05$) was found, although the chi-square value of the Mudginberri data was high ($\chi^2 = 39.4$ on 31 DF). The incidence of hermaphroditism amongst the large numbers of young of year mussels examined in this billabong is disproportionately high (Table 7.3).

Inspection of Table 7.6 reveals that the sex ratios of young of year mussels are often strongly biased in favour of males, particularly in populations where smaller individuals are well represented (Georgetown and Mudginberri billabongs). Sexual maturity in V. angasi is size dependent (section 7.7) and from Table 7.7 it is apparent that the gonads of small individuals function initially as males. (This observation is best exemplified in populations in which larger sample sizes are available.) The apparent protandry is not the result of an earlier maturation of males; once mussels exceed 30 mm in length, few immature and indeterminate gonads are found (Table 7.7) and yet the proportion of females still remains relatively low.

Presumably amongst juveniles, protandry is accompanied by hermaphroditism in which the dominant gonad does not necessarily correlate with inner gill morphology. No histology was undertaken to investigate this, however. Further, an intermediacy should be noted in the inner gills between the marsupial and non-marsupial condition of at least some individuals. This was not observed. Presumably the change in morphology occurs very rapidly. In any case the distinction between marsupial and non-marsupial gills in mussels so small is not always so clear.

In section 6.7.1, significant declines were noted in the proportions of females, with increasing age in some mussel populations of the Magela Creek. Long term mark-recapture studies may be needed to determine whether consecutive sexuality is the cause of these declines. However, there is very little evidence to suggest that this form of sex reversal did occur in the populations in question as: 1)

incidence of hermaphroditism, that may suggest a sex change, is overall very low; 2) in Mudginberri billabong where a rapid decline in the proporton of females was observed over the oldest age classes (Fig. 6.34), no noticeable increase in the incidence of hermaphroditism was noted (Table 7.3); and 3) significantly, visceral sex and the dominant gonad of hermaphrodites, are consistently correlated with morphology of the inner gills of mature mussels regardless of their age. For these populations, differential mortality between the sexes may be the best explanation available for the observed change in sex ratio with age (section 6.7.1).

Rhythmical consecutive sexuality is observed where the initial phase is male, followed by a series of alternating female and male phases throughout life (Coe, 1943). For the reasons given in points 1) and 3) above, it is very doubtful that this type of sexuality characterises populations of V. angasi in the Magela Creek.

Alternative sexuality is a sex reversal in which adults function seasonally as separate sexes. No patterns were observed in the seasonal incidence of hermaphrodites amongst the different populations (Table 7.4) that were suggestive of a sex change confined to a definite period of the year at least. Figure 7.8 shows the seasonal fluctuations observed over the study period in the sex ratios of mussels between the different populations of the Magela Creek. The decline in the proportion of females observed during the Wet-Dry interchange (April and May, 1981) in Jabiluka billabong suggested a strategy whereby females changed sex in response to the anoxic environment that prevailed at the time. During January of the

following Wet season (1982), known female mussels from the respective billabongs (sexed by observing individuals aborting larvae in the laboratory) were marked and subsequently released in Jabiluka and Mudginberri. Upon recollection at the end of May (1982), all individuals were found to be female, showing that no sex change had occurred over the period.

Nevertheless while no more than random sampling error is assumed to account for the seasonal fluctuations observed in the sex ratios in most populations, definite patterns were discernible in both Georgetown billabong and the Magela Creek channel (Fig. 7.8). Both patterns are almost in phase with one another, and two cycles are apparent each year. The proportion of females is lowest at the end of the Dry and again at the end of the Wet. Peaks in proportions of females are observed in between these periods.

Although the sexes tend to occupy different habitats in different seasons in Georgetown billabong (section 6.8.1), the nature of sampling in this billabong using transects, was such that no sampling biases were likely (section 3.1). In Georgetown as in all the billabongs, visceral sex consistently correlated with morphology of the inner gills of mature mussels, regardless of season. Moreover, hermaphrodites were least common in this billabong (Table 7.3), both factors arguing against alternative sexuality. No satisfactory explanation is available to explain the seasonal patterns in sex ratios observed in Georgetown billabong. (The only apparent environmental factor that resembles the diphasic pattern is water temperature but the relationship between temperature and sex ratio in

V. angasi is unknown.)

During 1981, a peak in female proportions was observed in Creek mussels at the onset of aestivation (July), and declined over the entire period of dormancy (up to December). It is tempting to suggest that the rigours of dormancy promote a strategy whereby it is energetically more expedient somehow, for these mussels to aestivate as males. The highest proportion of hermaphrodites was observed in this mussel population (Table 7.3), and of further note is that the inner gills of females are not entirely marsupial in appearance (section 7.3). A sex reversal is therefore not inconceivable for mussels from the Magela Creek channel, particularly in consideration of the rigours of their environment.

In conclusion, it is highly unlikely apart from protandry, that sex reversal occurs seasonally or throughout the adult life of billabong populations of V. angasi (and in all likelihood that of the Magela Creek channel). Hermaphroditism is only occasional, and by all appearances sexual integrity in V. angasi is high. Further long-term investigations are required to determine the causes of the observed fluctuations in the sex proportions of mussels in Georgetown billabong and the Magela Creek channel. Sampling biases may yet explain the patterns.

Among various North American unionids studied by Kat (1983d), occasional hermaphroditism among populations of predominately dioecious species was found to be associated with the presence of digenean trematodes within the gonads. Cercarial infections were also

noted in the gonads of V. angasi. In severe cases gametes were entirely absent and mussels were rendered functionally sterile by the sporocysts (Fig. 7.1 E). No doubt all heavily infected individuals were detected by gonadal smears. Histology, however, showed that 2 out of 5 infections found were so light that they were not previously detected by smears.

The seasonal occurrence of cercarial parasitism as determined by gonadal smears is shown for the different waterbodies in Table 7.8. Corrected infection rates from histological observations are also given in this table. The infection rates overall are low, but during the Dry season in Georgetown billabong up to 10% of the population was infected. Infections in mussels from the Magela Creek channel and Mudginberri and Nankeen billabongs invariably occurred only during the Wet season. In Georgetown, however, peak infections were observed during the mid Dry and were associated with a period of low physiological condition (body weights) of mussels (section 8.7.1). Because of the generally low incidence of infections observed, the reproductive potential of populations of V. angasi was presumably not affected to any significant degree. Female mussels, however, are apparently more susceptible to infection than males (Table 7.8).

There was no correspondence between incidence of cercarial infection and occasional hermaphroditism in the Magela Creek waterbodies. In fact the Georgetown billabong population had the highest incidence of cercarial infection but the lowest incidence of hermaphroditism, whilst the reverse situation applied to the population from the Magela Creek channel. Presumably very high incidences of parasitism (up to

80% in Kat's (1983d) populations) are required in order for trematodes to cause hermaphroditism via disruption to hormonal levels. Errors in developmental processes are likely to be responsible for observed hermaphroditism amongst populations of V. angasi in the Magela Creek. That the incidence of hermaphroditism is highest in the Magela Creek channel, where normal metabolic processes such as filtering and reproduction are periodically brought to an abrupt halt at the onset of dormancy each Dry season, is supportive to this hypothesis.

7.6 Sex ratio

The sex ratio of mussel populations in the Magela Creek varied with age. In most waterbodies (and probably in all populations) the primary gonad is apparently male (section 7.5) and within several billabongs a decline in the proportion of females with age was observed (Fig. 6.34). In one or two populations moreover, seasonal fluctuations in sex ratios were discerned (section 7.5, Fig. 7.8).

Averaged over seasons and age classes, however, the proportion of females in the waterbodies invariably falls below a 1:1, male:female sex ratio (Table 7.6). Tests of departure from a 1:1 sex ratio were performed on the proportions, using the normal approximation to the binomial test (Zar, 1974, p. 289). Significant departure from equal proportions of the two sexes (H: $p = 0.5$) resulted for populations from Jabiluka ($P < 0.05$) and Buffalo and Leichhardt ($P < 0.01$) billabongs.

The low proportions of females observed may partly be explained in

terms of longevity, and dissolved oxygen concentration in the billabongs. The longest-lived populatios are found in Mudginberri and Buffalo billabongs and in Mudginberri at least, males live significantly longer than females (section 6.7.1). It is notable, however, that both environments in which females are most underrepresented - Jabiluka and Leichhardt billabongs (Table 7.6), have the lowest mean concentrations of dissolved oxygen, averaged over the seasons, of the populations sampled (Table 2.7). This finding adds further strength to the claim that low dissolved oxygen concentrations are most stressful to females, and the decline observed in the proportions of females with increasing age are very likely the result of this stress (section 6.7.1). Low values of dissolved oxygen that are at least periodic in most of the billabongs, may partly explain why the proportions of females are invariably always lower than those of males.

7.7 Age and size at sexual maturity

Gonadal maturation in V. angasi was determined by examination of smears for the presence of spermatozoa or primary oocytes. From Table 7.7 it is apparent that gonadal maturity is size dependent, and the mature gonad is first distinguishable from the undifferentiated gonad somewhere between the size ranges 25.0-29.9 and 30.0-34.9 mm. These size classes generally lie well within the growth attained by mussels in their first year in all study populations (Tables 6.9-6.19).

Gonadal maturity was followed in more detail in juvenile mussels from Mudginberri billabong. Gonadal smears were made of 79 young of year

mussels at various times during 1981. All mussels greater than 30 mm in length possessed mature gonads. Details of the gonadal appearance of all mussels below this size are shown in Table 7.9. Below 30 mm, the sex of most individuals cannot be reliably determined. Age at gonadal maturation can be determined from the data of Table 7.10. 50% of mussels reach maturity at approximately 0.5 years of age, while all mussels are mature by an age of 0.8 years. Mature gonads may be found in individuals as young as 0.2 years.

The mean sizes of female young of year mussels in which brooding larvae were found, were used to determine the age and size at which mussels first spawned and brooded young. The results for a number of populations are shown in Table 7.11. (Details of the developmental stages of the larvae are given in section 7.9). The broader range in size at first gravidity, as compared to the size range at gonadal maturation, is more apparent than real, partly because of low sample size, and because larval production is very sensitive to various limnological factors such as temperature, dissolved oxygen and turbidity (section 7.10.1). The greater size recorded of females brooding young in Leichhardt billabong for example, resulted from anoxic conditions during the early Dry seasons that prevented brooding at an early age (section 7.10.1). The minimum mean sizes at which females were first observed brooding young (Table 7.11) are therefore assumed to be the sizes at which females in most populations may potentially spawn and become gravid. Thus first gravidity like gonadal maturity is size dependent and occurs at a size of approximately 40 mm.

Again age and size at which females first brooded young was studied in greater detail in Mudginberri billabong. The marsupial condition of all females collected during September, October and November 1981 is shown in Table 7.12. No brooding young were found in mussels below a size of 36.0 mm, and most females greater than 38 mm in length were gravid. The mean age at first spawning and brooding in Mudginberri billabong, extrapolating to the population as a whole, is reached at approximately 0.8 years (Table 7.10).

Assuming a size at first spawning and brooding of 40 mm, and extrapolating from the growth data of Tables 6.9-6.19, the mean age at first spawning and brooding lies within an age span of from 0.6 to 1.5 years (Table 7.11) depending upon the growth rates of mussels within the various waterbodies. In all populations, however, sufficient growth is reached by a more or less significant proportion of individuals, that some spawning and brooding by young of year mussels occur.

7.8 Senescence

The percent of gravid females with respect to age is plotted in Figures 7.9 and 7.10, for each of the Magela Creek waterbodies. In 7 out of the 10 populations, gravidity was observed to decline significantly with age. The populations in which significant declines were noted and the respective regression equations describing the relationships are shown in Table 7.13. Both linear and quadratic weighted regression equations were fitted to the data (the dependent variable being arcsine transformed), but in only two populations

(Leichhardt and Jabiluka billabongs) was the quadratic model appropriate (Table 7.13).

There was a decline in gravidity with age in most of the populations of V. angasi in the Magela Creek. This decline appears to operate over much of the life-spans of mussels in the various populations (Figs 7.9 and 7.10). Larval production correlates directly with the mean number of primary oocytes in the ovarian follicles of V. angasi (Fig. 7.4). Thus with increasing age, oogenesis and oocyte production decline in activity. In the largest of the females histologically sectioned (85 mm from Nankeen billabong), the ovarian follicles were entirely devoid of primary oocytes - an unusual occurrence in all of the mussels sexed and sectioned. No attention was paid to the likelihood of the same decline in reproductive activity in male V. angasi. Superficially, no discernible changes were noted in the testes from the many gonadal smears made, and from the sections examined. In both sexes, however, condition (relative body weight) was noted to decline with age (section 8.7.2). Declining reproductive activity is compatible with this observation.

The data of Mudginberri, Buffalo, Leichhardt and Jabiluka billabongs clearly indicate a peak in reproductive potential reached in the fourth, fifth, third and sixth years of life respectively (Figs 7.9 and 7.10) after which a long and gradual senile or post-reproductive phase occurs. In other waterbodies, the full reproductive potential is reached in the very first year or so of life (Figs 7.9 and 7.10).

In all probability the long and gradual senile phase of declining

reproductive activity V. angasi is limited to or most prominent at least in females. Again the protracted nature of the decline in reproductive potential, may be suggestive of a physiological weakening of females by Dry season stresses (e.g. turbidity and anoxia) to which they are particularly sensitive to by virtue of the brooding, feeding and respiratory function of the gills. Repetitive spawning (section 7.11) might further exacerbate the decline. A post-reproductive and senile phase in females of V. angasi provides further supportive evidence that females are shorter-lived than males amongst several populations at least in the Magela Creek (section 6.7.1).

C. LARVAL PRODUCTION

7.9 Stages and rate of larval development

Stages of larval development

The development of brooding young of V. angasi was observed and divided into three stages. Such a classification was originally thought useful for determining the times of spawning, incubation period, the period of glochidial release and the number of broods of larvae produced per year. All developmental stages were studied under low power microscopy, using living material. Photomicrographs of the stages are shown in Figure 7.11. The large amounts of yolk material present in the early embryological stages precluded any attempts at studying the cleavage processes. By all accounts nevertheless, the appearance of the developmental stages of larvae of V. angasi apparently does not differ greatly from the records of Lillie (1895)

and Wood (1974a) for unionids.

Although a continuum of development is present in embryogenesis (Fig. 7.11), and therefore the classifications are rather arbitrary, certain morphological features were very characteristic of each stage. Including the non-gravid condition, the larval classifications are as follows:

'Empty' - No developing embryos or larvae were present in the marsupia.

'Early larvae' - The developmental continuum of early larvae is shown in Figure 7.11 A-C. Early larvae included developmental stages from zygote through all cleavage divisions to at least gastrulation. The embryonic appearance ranged from an early spheroid (Fig. 7.11 A) to a more advanced elliptical (Fig. 7.11 B-C) mass of cells enclosed within a vitelline membrane. Apparently the larger dorsal end is the rudimentary, ectodermal shell gland. No other morphological features, however, characterise the embryos. (The stages represented in Fig. 7.11 A-C are relatively advanced embryos.)

'Developing larvae' - Other than minor (largely unrecognisable) cellular developments, the most discernible feature of the developing larvae following gastrulation, is the appearance and development of the larval adductor muscle. Developing larvae are shown in Figure 7.11 D-F, and the adductor muscle is apparent as a cross band of striated tissue within the centres of the larvae. In the latter stages of this developmental phase, slight invagination of the larval

mantle is apparent (seen in the right-most larvae in Fig. 7.11 F).

'Glochidia' - The chief distinguishing feature of the transformation phase of young larvae into glochidia is the continuing invagination of the larval mantle to affect the bifid condition of the mature larva. Accompanying this movement is the formation of the larval shell. As yolk is absorbed, the shell becomes progressively thinner until the typical translucent appearance of the mature glochidium is reached. The various glochidial stages are shown in Figure 7.11 G-I. Early glochidia (Fig. 7.11 G) are enclosed in the vitelline membrane. The shell margins are not necessarily partitioned fully and remain untoothed. Mature glochidia are invariably free of the vitelline membrane, and possess well developed teeth (Fig. 7.11 H) and a sticky, larval thread (Fig. 7.11 I).

Accompanying each of the developmental stages is a corresponding change in colour of the larvae when observed in the intact marsupia, brought about by yolk absorption and shell formation. Early larvae appeared white to pale yellow; developing larvae, yellow to light brown; and glochidia, a light to a darker shade of tan. Development in fact could be followed on colour changes alone. However, the embryos and larvae of all gravid females dissected were sorted into stages microscopically. No unfertilised eggs were encountered, and without exception developmental stages were highly synchronised, indicating that the young mature and are released from the mother at the one time. Finally, in eutrophic waters (e.g. Island, JaJa, Jabiluka and Leichhardt billabongs) the larval masses were noted to fill the entire inner gill of females. In other populations, the

portion of the gill used to incubate young was generally restricted to the inner two thirds.

Rate of larval development

Because 1) larval development was not synchronised between individuals collected at the same time and location, 2) females brooded young continuously for most of the year, and 3) the developmental time was very short in relation to the monthly interval between samples, the staging of larval development performed routinely on gravid females did little towards assisting the determination of the rate at which young develop in the marsupia. Faced with the same problems, Kenmuir (1981b) monitored the developmental rate of larvae in tropical Lake Kariba by periodically prising open the valves of marked female mussels from the lake, probing the marsupial gills with sharp-pointed forceps and staging the larvae so sampled. The results achieved by these methods, however, must be viewed with some suspicion. Disturbance of brooding females commonly results in abortion (noted by Kenmuir (1980) himself, amongst many other authors - see section A4.2), and the resulting patterns observed may merely reflect the attempts of the disturbed mussels at re-establishing the marsupial brood with a subsequent alteration in the release date.

Interruptions to larval development in mussels from the Magela Creek populations occurred often, and resulted from adverse environmental conditions (section 7.10.1). During these periods, it was common for all females to have aborted brooding young or to be inhibited from further production of larvae. Use was made of this phenomenon in

ascertaining the rate of larval development in V. angasi in the Magela Creek. Having sampled mussels in a synchronised condition of empty marsupia, the subsequent sampling date at which mature larvae reappeared in the marsupia indicated the rate of larval development.

From Figures 7.12-7.14 it is seen that recovery, after such periods of non-gravidity, to the stage of mature larvae present in the marsupia can occur by the time of the proceeding monthly collection. Glochidia appeared after 40 days in Island (Jun./Jul., 1981), 41 days in Corndorl (Jan./Feb., 1982), 42 days in Leichhardt billabong (Jun./Jul., 1981) and 44 days in the Magela Creek channel (Dec./Jan., 1981). All of these periods encompass both winter and summer months, and therefore even for the slowest rates in winter, larvae had developed within a 40-42 day period. Sampling at shorter intervals was carried out in Corndorl billabong (Nov./Dec., 1981) and in the Magela Creek channel (Nov./Dec., 1981), for which recovery after a period of cessation in larval production was noticed (Figs 7.12 and 7.13). Glochidia appeared after 15 days in Corndorl and after 12 days in the Magela Creek channel. Of interest is the observation that the latter population had been aestivating 12 days prior to collection in December, 1981; subsequent inundation resulted in immediate spawning and very rapid development of active, mature glochidia, free of the vitelline membrane. Larvae were probably mature even for some days prior to collection.

Development rates are presumably affected by temperature, and larvae may require a longer period to develop during the winter than the minimum rate of 12 days recorded during the summer. However, the

winter rate does not exceed 40 days and is probably much closer to the maximum of 12 days noted during summer.

7.10 Seasonal pattern of larval development

7.10.1 Between waterbodies

Seasonal breeding activity of *V. angasi* in relation to larval production was determined by compiling the monthly occurrences of the gravid stages recorded over the entire period of investigation, for each of the study populations. Larval production in each of the populations, represented by histograms of the monthly marsupial appearance of females, is shown in Figures 7.12-7.14. From these figures, it is apparent that at any one time and location, all developmental stages of larvae may be present in different individuals comprising the population. The asynchronised nature of larval development within the populations and the observation that larval production can occur over the entire year were superficially suggestive of repetitive breeding in *V. angasi* (substantiated in section 7.11).

Seasonal patterns of larval production were discernible within most of the waterbodies, but rarely was the same pattern common between any two locations. Major interruptions to larval production in particular were a feature of several populations, especially those from the floodplain billabongs (Figs 7.12-7.14). Thus the seasonal appearance of larval production varied quite considerably amongst the waterbodies.

While no one obvious environmental variable was the cause of the marked variations observed both within and between the billabongs, several factors - of climatic and limnological nature, seasonal in occurrence, and in combination - were strongly implicated to affect the patterns of larval production. A largely aseasonal breeding cycle was evident for V. angasi populations in the Magela Creek. Within this general perspective, however, a background breeding pattern dependent upon temperature was apparent while superimposed upon this, the cycle was shown to be interrupted by adverse environmental conditions, especially the effects of dissolved oxygen and turbidity. The effect of temperature upon larval production was clearly evident in Mudginberri billabong as shown in Figure 7.15, while the significance of turbidity in Georgetown (Fig. 7.15), and dissolved oxygen in Nankeen (Fig. 7.15) and the other floodplain billabongs (Fig. 7.16) could also clearly be demonstrated. (It is worth noting that larval production in V. angasi merely mirrors the intensity of oogenesis (section 7.4.2.2). Therefore the measure of the response of larval production to various environmental factors is in turn a measure of the response of gonadal activity.)

Having delineated several environmental variables of importance to larval production, attempts to model the breeding cycle over all billabongs and within billabongs, were made using a multiple regression approach. Independent variables chosen were temperature, dissolved oxygen, turbidity, chlorophyll and time. The inclusion of chlorophyll and time of year, was made on the assumption that the former is a measure of food availability, of conceivable importance to

larval production, while the latter was included as a measure of other (unmeasured) seasonal effects. Such effects may include aspects of the physiology of the mussel itself, e.g. relative condition and previous spawning history.

While the choice of temperature, dissolved oxygen, turbidity, chlorophyll and time was fixed, stepwise multiple regressions were performed on the untransformed data, various transformations of the data and on various multiplicative combinations of the data from each billabong, to determine whether or not transformations were required and whether consistent synergistic or antagonistic effects among the variables were present. The results indicated that a log transformation of dissolved oxygen only was required for the regression analyses (also indicated from inspection of the residuals of appropriate regressions). No other significant factors resulted that were suggestive of consistent synergism or antagonism amongst the variables. Multiple regression analyses were performed on the five independent variables against monthly larval production (percent of gravid females, arcsine transformed) of each billabong. An equation was also derived using the data of the billabongs combined.

The resulting multiple regression equations are shown in Table 7.14. An AOV of the regression coefficients over the billabongs showed very strong evidence against the assumption that all 8 regression equations estimated the same population regression ($P < 0.001$). Even when the billabongs were grouped according to the hydrological classifications of backflow, channel and floodplain, the equations within the groups still differed in each case ($P < 0.001$). Nevertheless, within the

broad billabong types, some environmental influences were strong and common in effect. Three environmental factors at least have a very significant bearing upon the patterns of larval production observed amongst the different billabongs:

1) Temperature - The major determinant of larval production in Mudginberri billabong is apparently water temperature (Table 7.14), and increasing water temperature is conducive to production of young (as might be expected). The relationship between monthly larval production and temperature for Mudginberri billabong is shown in Figure 7.15, and a highly significant linear regression equation ($P < 0.001$) was found to describe it (Table 7.15). The environment in Mudginberri billabong is the most equitable and temperate of all the Magela Creek waterbodies, and dissolved oxygen is generally adequate and turbidity very low year round. The breeding pattern in this billabong therefore, apparently represents a background cycle, primarly dependent on water temperature. Except for Nankeen, the sign of the partial regression coefficient for temperature amongst the billabongs was consistently positive and in two other billabongs (Georgetown and Island) the variable was significant ($P < 0.05$) in the regression equation (Table 7.14).

2) Turbidity - At high concentrations, suspended solids (as measured by turbidity) had a mostly negative effect upon larval production. In Georgetown, the 'dirtiest' of the Magela Creek billabongs, the relationship is clear (Fig. 7.15), and a highly significant linear regression equation ($P < 0.001$) was found to describe the relationship between monthly larval production and turbidity (Table 7.15). The significant, but positive sign of the partial regression coefficient

for turbidity in Nankeen billabong is ambiguous. No correlation was found between larval production and turbidity when the variables were regressed on their own, and the significance of the parameter in the multiple regression equation is suggestive of intercorrelation between the other independent variables. Similarly, in the non-turbid and marginally turbid billabongs, the positive sign of the coefficient in the regression equations (Table 7.14) may merely reflect increasing algal production that turbidity partially measures during the Dry season. Thus, at high loads only, suspended solids apparently inhibit larval production, very likely through an effect of interference to normal gill functioning of brooding females.

3) Dissolved oxygen - In all but one billabong, the partial correlation coefficients of dissolved oxygen in the multiple regression equations are positive. It is particularly noteworthy that the coefficients in three of the five floodplain billabongs are significant (Table 7.14), and it is clear that dissolved oxygen has a marked influence upon larval production in these billabongs (Fig. 7.16). In four of the five floodplain billabongs, significant linear regression equations were derived to describe the relationships between dissolved oxygen and larval production (Table 7.15).

From Figure 7.16, larval production is suppressed at each Wet-Dry season interchange (April-June), apparently in response to the same seasonal lulls observed in dissolved oxygen concentration. Later in the Dry, however, the same correspondence is not so clear. At these times algal populations have increased and the resultant respiratory lull measured in early morning dissolved oxygen concentration may be followed by a supersaturated reading in the afternoon as a result of

photosynthesis (section 2.3.2.2). At the Wet-Dry interchange, however, algal populations are low and the effects of the relative anoxia resulting from macrophytic decomposition are sustained, in all likelihood, throughout much of the day. Thus, the response of larval production to dissolved oxygen concentration may be an integrated measure of the sustained effect of low dissolved oxygen. Spot monthly readings of dissolved oxygen are probably of little value in this regard. Nankeen is limnologically the least productive of the floodplain billabongs and presumably dissolved oxygen concentrations are less dependent upon algal blooms. The close correspondence of larval production to dissolved oxygen in this billabong therefore (Fig. 7.15), may indicate that the early morning readings of dissolved oxygen are an effective measure of the daily concentration. In spite of an apparent relationship between dissolved oxygen and chlorophyll, however, no multiplicative combination of the two variables resulted in any consistent and significant patterns from the stepwise regressions.

At low sustained concentrations, dissolved oxygen must either inhibit further larval production (via reduced oogenic activity, section 7.4.2.2) or induce abortion in brooding females. In the former, relative anoxia is assumed to directly interfere with oogenesis at least, while in the latter the brooding larvae may impose a serious respiratory burden upon the mother or conceivably, themselves asphyxiate if their development occurs more or less independently of the parent.

4) Overall - Because larval production responds differently to the

same environmental conditions between billabongs no overall model can accurately predict larval production given a limited set of variables as used in these analyses. In any case, on statistical grounds, an equation derived from the data of all the billabongs for predictive purposes is invalidated. Thus within billabongs larval production may respond in a predictable fashion to the immediate environment; yet responses averaged over all billabongs differ sufficiently to suggest that control of breeding is also influenced by more complicated physiological mechanisms. Other unmeasured, environmental influences and synergistic and antagonistic effects may also be influential to breeding.

Nevertheless, the combined equation as derived in Table 7.14 whilst not providing significant predictive value (having a low coefficient of determination of 39%), indicates by way of the sign and significance levels of the coefficients a measure of the relative importance of the environmental parameters studied, to larval production. According to the multiple regression equation, the strongest influence of the environmental factors studied is dissolved oxygen. Above a threshold value, the influence of dissolved oxygen is not particularly marked, but at low sustained concentrations breeding of mussels is suppressed. Increasing water temperatures also significantly enhance breeding, while suspended solids at high concentrations inhibit it. Algal abundance (as measured by chlorophyll) as a source of food available to mussels is apparently unimportant in determining larval production.

In the Magela Creek channel, the availability of water appears to be

the only requirement for breeding as mussels were observed in a gravid condition for the entire duration of the Wet season (Fig. 7.12). Breeding commences almost immediately after the Creek begins to flow in about December, continues through the Wet season and ceases at the cessation of flow prior to aestivation in about May. Continuous breeding over the Wet season is no doubt enhanced by the high oxygen concentration of the flowing waters and generally low loads of suspended solids.

7.10.2 Within billabongs

The percentages of gravid females observed within each depth interval over the study period, were estimated and plotted according to depth and/or sampling location in Georgetown, Mudginberri and Buffalo (Fig. 7.17) and Leichhardt and Nankeen (Fig. 7.18) billabongs.

Given that larval production generally declines with mussel age (section 7.8), and that age is related to depth in the billabongs according to the relationships described in section 6.5.3.1 and shown in Figures 6.25-6.27, the relationships drawn here are merely the inverted images of Figures 6.25-6.27. Thus larval production decreases with depth in Georgetown, Mudginberri and Buffalo billabongs (Fig. 7.17) in response to increasing age over the same gradient. (The significance of the encircled point in Buffalo billabong shown in Figure 7.17, is considered in section 6.5.3.1). In Leichhardt and Nankeen billabongs, larval production is least at intermediate depths (Fig. 7.18), corresponding to the sites where the oldest mussels are found. The relationship found in Nankeen billabong, however, is not

exactly an inversion of the quadratic relationship between age and depth as shown in Figure 6.27. Larval production at the shallowest and greatest depths (Fig. 7.18) was less than might be expected, as the youngest age classes are heavily represented here. The effects of anoxia may be assumed to be most pronounced at depth, possibly explaining the suppressed breeding at the deepest station. At the shallowest depth, wave-induced resuspension of the silty sediments during the Dry season (see section 6.4.3.1) may interfere with larval brooding, again perhaps explaining the slightly reduced breeding activity here.

7.11 Duration of incubation and frequency of brood production

The correlation between the percent of brooding females and the incidence and intensity of parasitism of the host fishes by the glochidia over the seasons (section 7.15.2), clearly indicates that larvae are released from the mother as soon as they have matured. From the data of section 7.9 therefore, larvae are brooded and released within at most 12 days during summer and within at most 40 days (probably much shorter) during winter. Unfortunately, it is not known over what time span the larvae are released to ascertain more precisely the duration of an entire reproductive cycle, from one brood to the next. Possibly the period of release of glochidia is no longer than a week. A very conservative estimate would place the period at two weeks. In all likelihood therefore, an entire reproductive cycle is completed within a period of two weeks and one month.

When reproductive activity is most intense in the waterbodies, few

females were found in a non-gravid condition, for often prolonged periods (e.g. Sept.-Dec., 1980 in Mudginberri billabong, Fig. 7.12). This indicates that a rest period between broods is extremely short during these phases of activity, and females are therefore breeding repetitively. Based upon an estimate of one month for the duration of an entire reproductive cycle, and considering that in most billabongs, there are active phases in which one cycle is immediately followed by another, it is likely that as many as 9 broods are produced each year. If cycles are of fortnightly duration, it is conceivable that as many as 15 broods may be produced each year. The duration of a reproductive cycle, however, needs to be properly ascertained.

D. GLOCHIDIAL RELEASE AND PARASITISM

7.12 Materials and methods

Natural infections

Fish collections from several billabongs of the Magela Creek, were made at various times of the year to investigate glochidial infections of *V. angasi*. Wherever possible (several instances) advantage was taken of the catches of other investigators involved in fish autecological studies in the Region. Only catches made in billabongs and sites known to harbour mussel beds, however, were utilised. Fish were collected by seining the shallows on or adjacent to mussel beds, while gill netting was performed in some deeper sites mainly for capture of the larger fish species. The sampling dates and sites of collection are shown in Table 7.16. All fish from a catch were fixed

in 10% formalin and subsequently transferred to 70% ethanol for later inspection of glochidial infections. When individuals of a fish species were particularly numerous, subsamples only were taken and preserved.

Having ascertained early in the study that Glossogobius giurus, the flathead goby, was a host for the glochidia of V. angasi, regular monthly collections were made of the fish from Mudginberri billabong during 1981 and 1982, to monitor the seasonal incidence of parasitism. G. giurus is benthic in habit and fish were collected by diving over sites where mussels occurred, using small dip nets for their individual capture. An effort was made each month to capture at least 20 fish. However, during the late Dry season of 1981 (Sept.-Dec.), water clarity deteriorated to such an extent that fish were difficult to locate. Sample numbers were therefore low during these months (Table 7.17).

In the laboratory, fish were inspected under a dissecting microscope for the presence of encysted glochidia. These were conspicuous as small, semi-opaque tubercles on the host's tissues. Rationalising the time required to inspect the fishes for presence of glochidia, inspections were made of the fins only unless otherwise indicated (section 7.1).

Laboratory induced infections

The duration of the parasitic period of the glochidia of V. angasi upon a host fish, G. giurus, was determined in the following manner:

Fish were collected from Mudginberri billabong two weeks prior to experimentation to allow naturally occurring infections to be voided. Individuals were kept in billabong water (from Mudginberri) throughout the study and, during the experiments, water was changed daily. Prior to experimentation fish were fed, but were starved during the experiments.

Each trial comprised artificial exposure of the fish to glochidia, at a specific water temperature. Two trials were performed, at 22° C during the Dry season of 1981, and at 30° C during the Wet season of 1982. Six fish were used in each trial, and each fish was held in a 1 litre glass beaker individually aerated. All beakers were held in a waterbath where water temperature was thermostatically controlled to within 1° C. A barrier of nylon gauze mesh was placed a few centimetres above the bottom of the beaker through which newly metamorphosed larvae could pass, to prevent them being fed upon by fish.

Mature glochidia from 5 or 6 mussels collected from Mudginberri billabong, were used to infect fish. Glochidia were excised from the marsupial gills of mussels, placed in a water-filled petri dish, agitated and mixed, and then pipetted into the beakers holding the fish. Only glochidia free of the vitelline membrane and with active, snapping movements were used in the infections. Fish were exposed to the glochidia for several minutes, after which they were transferred to fresh beakers and water to begin the parasitic phase.

After initial infection, fish were removed from the beakers every 24 hours and the bottom debris individually siphoned and carefully collected into water-filled petri dishes for microscopic inspection. (Fish were again transferred to fresh beakers and water.) Newly metamorphosed larvae were readily discernible from sloughed glochidial shells by their opaque shells (indicative of the new internal structures), and by occasional and conspicuous movements of the feet. At each individual inspection, the number of metamorphosed juveniles was recorded. The bottom debris of each beaker was inspected for at least 3 days after the last juveniles were found.

A number of other fish species were exposed to artifical infections to determine whether or not they were host for the glochidia of V. angasi. In these cases, the procedures were followed according to the previous descriptions of infections of G. giurus, except that the trials were run at 22° C only, and for Leiopotherapon unicolor and Lates calcarifer, 3 and 1 individuals respectively were tested. The single individual of L. calcarifer was placed in a 40 l perspex aquarium for the duration of the trial. The presence of metamorphosed juveniles in the bottom debris was the criterion that a fish species was host to the glochidia of V. angasi.

7.13 Site of attachment of the glochidia upon the host fish

The body surfaces of individuals of 4 species of fish only, naturally infected with glochidia of V. angasi, were thoroughly examined to determine the sites of attachment. These species were Ambassis (spp. complex), Glossogobius giurus, Oxyeleotris lineolatus and Tandanus

ater. Internal surfaces examined were mouth, opercula and gills, while external surfaces comprised the fins and body surface other than the fins. The mean numbers of encysted glochidia found on each of the fish tissues in relation to fish species, number of fish examined, and sampling location, are shown in Table 7.18.

For each species examined, encysted glochidia were proportionately more common on the internal body surfaces than on the external surfaces. For individuals of each fish species, the number of glochidia recorded were combined for both the internal and external body surfaces. From these data, a mean number of glochidia per individual was estimated for both internal and external body surfaces, for the 4 naturally infected fish species. A chi-square test was performed over the data of the 4 species to determine whether or not there were disparities between the species in the relative proportions of glochidia found on the internal and external body surfaces. No evidence was found against the hypothesis that the relative proportions of glochidia on either body surfaces were the same between gall 4 fish species (χ^2 = 2.92, 3 D.F.). In all of the data a tendency was found for the benthic feeding G. glurus and T. ater to harbour proportionately more gill infections, as might be expected. (Ambassis spp. are more commonly observed in mid-waters, while O. lineolatus frequents the benthos, but is less dependent upon it for food - Bishop et al., 1981).

To discern whether total body infections were correlated with those observed only on the external body surfaces, the mean number of infections with respect to internal (Y) and external (X) body surfaces

were subjected to regression analysis over the four species. The resulting linear regression equation ($Y = 0.72 + 2.36X$) proved non-significant (2 degrees of freedom only, $P < 0.20$), but correlation between the infections observed on each of the body regions was relatively high ($r^2 = 69\%$). Similar regression analysis was performed between internal and external (fins only) infections over the individual data for G. giurus. As this species was used as a monitor of glochidial release over the seasons (section 7.15.2), and as only fin infections were scrutinised, it was important to demonstrate that fin and internal body infections were correlated. Using the data that comprised the observations in Table 7.18, a very significant linear regression equation was found to describe the relationship between internal infections (Y) and infections on the fins (X) of G. giurus. The regression equation is:

$$Y = 7.573 + 2.17X \qquad (P < 0.01,\ r^2 = 0.344).$$

Further observations are required over a wider range of fish species with a broad range of habits, to confirm whether or not attachment of glochidia to the fish body is proportionately higher on the internal surfaces (chiefly the gills) than the external surfaces (mainly fins). Although the chi-square test used here showed that there were no disparities, between the species examined, in the relative proportions of glochidia found on the different body surfaces, 3 of the 4 fish species are chiefly bottom-dwelling. Gill infections therefore could have been expected to be higher than fin infections in the 3 bottom-dwelling species. Nevertheless, considering that many of the fish species from the Magela Creek feed from or on the bottom (apparent in the data of Bishop et al., 1981), gill infections generally, may prove to be higher than fin infections. This is

contrary to the general observation, that hooked or toothed glochidia tend to parasitise the external surfaces of their host fish (see section A4.3).

7.14 The host fish species and host specificity

Observations on natural infections of glochidia of V. angasi, upon the fins of fish species from various Magela Creek billabongs, are summarised in Table 7.19. The sampling periods that comprise the total number of fish examined for each billabong are given in Table 7.16. The percent of gravid female mussels recorded at each sampling period (Table 7.16), and a mean percent of gravid females observed overall are given in Tables 7.16 and 7.19 respectively.

The order at which species appear in Table 7.19 is based more or less upon highest to lowest incidence (% of fish infected) and intensity (mean number of glochidia per infected fish) of infection when the data from all billabongs were averaged. While the order may change slightly as gill infections are scrutinised more, early indications are that differences between fish species in the site of attachment of glochidia are slight (section 7.13, above). Therefore the results of Table 7.19 while not providing total data on incidence and intensity of infection, nevertheless provide an adequate measure of the relative degree of infection between the different fish species. It should be noted, however, that particularly in view of the observation that gill infections outnumber fin infections (section 7.13), some low infections were possibly missed. The list of Table 7.19 therefore, may possibly be an underestimate of the total number of known hosts

occurring from the observations on natural infections.

Trdan and Hoeh (1982) emphasised that the designation of a fish species serving as a suitable host required a combination of observations on naturally encysted glochidia and artificial laboratory infections. The artificial infections performed on the six fish species tested in the present study (Table 7.20), resulted in successful metamorphosis of glochidia. A diversity of families, sizes and life habits were represented in these species, suggesting that host fish specificity of V. angasi is low if existing at all. The natural infections observed on the fish species shown in Table 7.19 therefore, are likely to indicate that glochidia have the potential to parasitise and successfully metamorphose from the respective species.

The 19 known fish hosts of the glochidia of V. angasi are shown in Table 7.20. The ranking is based upon an order thought to represent most to least important host to V. angasi. By 'importance' is meant the number of glochidia that would eventually metamorphose from a fish species under field conditions, and is fairly subjectively scaled according to information on overall abundance of a fish species throughout the Magela Creek (Table 2.8), and its incidence and intensity of infection of glochidia (Table 7.19).

From Table 7.19 both incidence and intensity of infection, are low in fish species of the Magela Creek, even considering that gill infections are not represented. Thus the highest intensity of infection is observed on G. giurus which bears upon the fins a mean number of 4.1 glochidia per infected fish or 16.5 glochidia upon all

the body surfaces (using the regression equation from section 7.13). Seventy-three percent of G. giurus were found infected, and the next highest incidence of infection was for Amniataba percoides where only 40% of the individuals were found infected. These results are somewhat surprising given that densities of mussels from all the sampling sites were high, and that high proportions of gravid female mussels were present in the populations at any one time (Table 7.16).

As mentioned above, no evidence was found of host fish specificity of V. angasi and in relation to natural infections specifically, no one fish family stood out as having a consistently higher incidence or intensity of infection than another. Infections are apparently more influenced by fish behaviour than any other factor. The feeding guilds of fish species identified as hosts to V. angasi are shown in Table 7.20. Generally speaking, bottom dwelling and feeding fishes observed highest incidence and intensity of infection.

All species listed in Table 7.20 are known to feed in both benthic and mid-water habitats (Bishop et al., 1981), although Toxotes chatareus and Melanotaenia splendida tend to inhabit and feed in mid- and surface waters. G. giurus feeds exclusively on the benthos explaining no doubt the high infections observed on this species (Table 7.19). Ambassis spp., Glossamia aprion and Oxyelectris lineolatus from direct observations, were notably inactive in the water column. The latter two species probably capture prey in an ambush fashion, observing long periods of inactivity between predatory movements. This relative inactivity may provide more opportunities for infection. Scleropages jardini, Lates calcarifer, Megalops cyprinoides, Strongylura kreffti

and Leiopotherapon unicolor are no doubt more active in their predatory habits and in all likelihood, may not often frequent the benthos. This may account for the generally low infections observed on these species. The fins of the eeltailed catfishes Tandanus ater and Porochilus rendahli are probably not particularly suitable for glochidial infections, as the membranes between the fin rays may be too coarse and slimy for attachment. Similarly the fins of larger individuals of S. jardini, L. calcarifer and O. lineolatus may prove too coarse for glochidial attachment. In the eeltailed catfishes at least, however, the gills have been shown to bear relatively large infections (section 7.13). Not enough samples were collected of many species, however (Table 7.10), to speculate upon the relative degree of infection.

7.15 Glossogobius giurus as a monitor of glochidial release

7.15.1 Duration of the parasitic period

The mean numbers of newly metamorphosed juveniles of V. angasi per infected G. giurus are plotted at daily intervals subsequent to initial infection, and with respect to temperature in Figure 7.19. At lower temperatures (22° C) the duration of the parasitic period was protracted, and the period of larval metamophosis spanned 3-15 days after initial infection. Recovery of juveniles was greatest on the 11th day. At higher temperatures (30° C), however, the duration of parasitism was very short (Fig. 7.19) and the period of metamorphosis spanned a relatively short period of 9 days, i.e. 2-10 days after infection. Greatest recovery of juveniles was on the 6th day.

Thus, there was a relationship between the duration of the period of metamorphosis of glochidia of V. angasi, and water temperature. Metamorphosed juveniles were recovered earlier from the host fish at 30° C than 22° C. Liberation from the host tissues was more protacted at 22° C, but at either temperature, metamorphosed juveniles left the fish during the middle of the period of parasitism. It was noted during the trials that those glochidia encysted after 2 days generally continued development through to metamorphosis. Very few sloughed off glochidia were found in the bottom debris after 2 days. At 22° C at least, the duration of the parasitic period was observed to be similar in all 6 species artificially infected (Table 7.20). Thus, the duration of attachment by glochidia of V. angasi does not appear to be influenced by host species to any extent.

7.15.2 Seasonal incidence of parasitism

The mean number (per fish) of encysted glochidia of V. angasi (and standard deviation) parasitising G. glurus from Mudginberri billabong during 1981 and 1982 are shown in Table 7.17. The monthly means are plotted with monthly percentages of gravid female mussels from the billabong observed over the same period, in Figure 7.20. All of the fish collected by diving, exceeded 60 mm. Only infections upon the fins of these fish were observed and counted. However, infections on the fins were significantly correlated with infections recorded on other body regions of G. glurus during March 1981 (section 7.13). There is no reason to suspect that the same correlation would not hold at other times of the year, as the same habits of G. glurus and

habitat, were observed year round. Therefore the infections observed on the fins, although only relative, nevertheless directly reflect the actual monthly intensities of infection.

From Figure 7.20, monthly intensity of infection of G. giurus clearly correlates with the monthly percentage of gravid female mussels. Thus, glochidia are released from the marsupia after a very short developmental time. Release of glochidia from brooding mussels, as monitored by the intensity of infection of G. giurus, is in direct proportion to their seasonal production. Given that water temperatures in Mudginberri billabong always exceed 25° C and average 28.2° C over the seasons (from the data of Fig. 2.20), recovery of metamorphosed juveniles from the host fishes would span a period no longer than two weeks; most recovery averaged over the seasons would be accomplished only 8 days after infection (extrapolating from the data of section 7.15.1 above). Thus the seasonal intensity of recruitment (with only a slight lag phase) is in direct proportion to glochidial production. (This same relationship is assumed to apply to the other Magela Creek waterbodies as well.) From Figure 7.20 therefore, recruitment would appear to be greatest during the latter period of the Dry season (Aug.-Dec.) in Mudginberri. From the data of section 6.6.1, however, recruitment of V. angasi in billabongs of the Magela Creek is clearly seasonal and occurs during the Wet and early Dry seasons in association with periods of highest dissolved oxygen content. Thus, there is a marked seasonal disparity between the intensity of larval production and subsequent metamorphosis of larvae (Figs. 7.12-7.14) and actual recruitment of juveniles observed in the sediments of the Magela Creek waterbodies. This apparent anomaly in

aseasonal breeding but seasonal recruitment of V. angasi is discussed below.

E. DISCUSSION

Gonadal development

But for very occasional hermaphrodites (see below), the sexes of V. angasi are separate, in accordance with the general condition prevailing in freshwater mussels. As in the majority of species, sex can be determined by both gonadal appearance and by morphology of the marsupial gills. Sex in V. angasi is readily determined by gonadal smears, as ripe eggs and sperm in sexually mature mussels are present year round. Nevertheless, for all practical purposes sex is most easily and reliably determined by gill morphology alone, as consistent correlation is found between sex as determined by identification of gametes and sex as determined by inner gill morphology.

In unionaceans from temperate regions, gametogenesis generally continues throughout the year, but is most intense during the warmer months when more or less synchronised spawning occurs (Matteson, 1948; van der Schalie and van der Schalie, 1963; Stein, 1969; Yokley, 1972; Giusti et al., 1975; Heard, 1975; Smith, 1979; Zale and Neves, 1982a; Jones and Simpson, in prep.). Even in tropical climates oogenesis is reportedly slowed in the cooler seasons; spermatogenesis nevertheless, may continue unabated throughout the year, and spawning may be protracted over the warmer months (Lomte and Nagabhushanam, 1969; Ghosh and Ghose, 1972; Nagabhushanam and Lohgaonker, 1978; Dudgeon and

Morton, 1983). Thus in unionaceans from all climates, seasonal gonadal activity is influenced to a lesser or greater degree by water temperature. The presence of spermatozoa and primary oocytes in the respective gonads of the sexes of V. angasi throughout the year, suggested that gametogenesis was a continuous event. Nevertheless, while gonadal activity was found to be synchronised between males and females, relatively inactive phases, as identified by presence of stages of atypical spermatogenesis in the testes and by fewer numbers of primary oocytes in the ovaries, could be recognised. As in all other unionaceans studied, gonadal activity in V. angasi could also be shown to be responsive to water temperature. However, superimposed upon an apparent repetitive reproductive cycle (the relative activity of which is only marginally slowed to any degree by low seasonal water temperatures), major interruptions to gonadal activity were observed that were associated with seasonal lulls in dissolved oxygen concentrations and with seasonally high turbidities in the billabongs. These factors are further considered below.

That the period of spermatogenic activity invariably overlaps the same period of oogenic activity in unionaceans, has been interpreted as meaning that sperm are released over a timespan that overlaps ovulation in females, thus ensuring successful fertilisation (e.g. Matteson, 1948; Dudgeon and Morton, 1983). The timing of spermiogenic and oogenic activity of V. angasi is the same, and both sexes therefore are assumed to spawn both simultaneously and at a similar intensity. As sperm are present in the testes at all times of the year, however, it is probable that some sperm are released even during inactive phases of spermatogenesis. Absence of spawning in females

moreover, is most conspicuous when for brief periods the marsupia in some populations may be entirely devoid of brooding larvae.

Further consideration of the breeding patterns observed in populations of V. angasi from the Magela Creek is given below.

Structure of the breeding population

Hermaphroditism in V. angasi is very occasional and in the Magela Creek was confined to less than 2% of each of the populations investigated. In North American unionaceans similarly, when hermaphroditism is encountered amongst predominately dioecious species, incidence is reportedly low (Kat, 1983d). The incidence of hermaphroditism in V. angasi is the first reported for any hyriid. The nature of hermaphroditism in V. angasi differs from other unionaceans, but whether these differences are representative for the hyriids as a whole of course, is not known. Unlike North American unionaceans with monoecious acini or gonads with intermingled zones of male and female acini (Heard, 1975), the discrete follicles or tubules of hermaphroditic V. angasi were mostly confined to discrete regions of the gonad. Further, hermaphroditic unionids of North America reproduce chiefly as females and if male hermaphrodites are present, generally less than 5% of the gonadal tissue is female (Kat, 1983d). In V. angasi, however, hermaphrodites reproduced both as males and females more or less equally. Moreover, the ovarian follicles in male hermaphrodites occupied more than 5% (between 10 and 40%) of the gonadal tissue.

Kat (1983d) found an association between presence of cercarial trematodes within the gonads and occasional hermaphroditism among populations of predominately dioecious species of unionids. No such association was observed in populations of V. angasi, however, where incidence of parasitism was generally low (up to 10% infected at any one time), and seasonal in nature. Because of the low incidences of infection, the reproductive potential of mussel populations in the Magela Creek is assumed not to have been affected to any significant degree by cercarial parasitism. High incidences of cercarial infections observed in some unionids, however, have been shown to have severely affected the reproductive potential of respective populations (e.g. Zale and Neves, 1982a; Kat, 1983d).

Both evidence for (Bloomer, 1934, 1935, 1939; Tudorancea, 1969, 1972) and against (Heard, 1975; Dudgeon and Morton, 1983; Young and Williams, 1984a) sex reversal in unionaceans have been presented. From limited mark-recapture studies on known female mussels and from the close correlation observed between sex as determined by the gonad and sex as determined by inner gill morphology in animals from all seasons and of all ages, sex reversal is assumed to be absent from adult populations of V. angasi in the Magela Creek. Heard (1975) reached a similar conclusion in relation to the sexuality of various North American anodontines. Declines in the proportions of females with age observed in a number of populations in the Magela Creek can best be explained by differential and age selective mortality between the sexes. Tudorancea (1969, 1972) observed this phenomenon in some European unionids. Seasonal patterns were observed in the sex ratios of mussels from both Georgetown billabong and the Magela Creek

channel. While sampling biases may affect the patterns observed in Georgetown, both long and short term mark-recapture studies would be needed to resolve the question of sex reversal in V. angasi for the respective populations.

Hermaphroditism in adult populations of V. angasi is apparently the result of none other than developmental error in sexual determination. Such cause has also been implicated for hermaphroditism in other normally dioecious bivalves including unionaceans (e.g. Coe, 1943; van der Schalie, 1970; Kat, 1983d). However, the relatively high incidence of hermaphroditism observed in young of year mussels in Mudginberri billabong, did provide evidence suggestive of consecutive sexuality in the form of protandry. In fact the gonads of the smallest individuals of V. angasi from most populations function initially as males. Protandry observed at about the time of sexual maturity may prove to be the general rule for unionaceans (Bloomer, 1939; Tudorancea, 1969, 1972; Kat, 1983d).

In relation to the question of sexuality in V. angasi in the Magela Creek, it is concluded that apart from early protandry, sex reversal appears to be absent. Hermaphroditism occurs at a low incidence in all populations investigated and otherwise the dioecious condition is a stable one and sexual integrity is high.

The sexes of V. angasi in the Magela Creek populations are biased in favour of males. Apart from age specific differences in sex ratios males live longer than females in many of the waterbodies, which presumably accounts for the observed disparities in proportions of the

sexes. Repetitive spawning and periods of anoxia in particular waterbodies may be causes of selective mortality of females. Elsewhere, the literature shows that there may be more or less predominance of either sex in freshwater mussel populations (section A4.1). However, no statistical evidence has been presented to show that sex ratios vary from 1:1 in any population studied, nor has speculation been offered as to possible reasons for observed disparities from a sex ratio of unity.

Gonadal maturity in V. angasi is size dependent, and is initiated between the size ranges 25.0-29.9 and 30.0-34.9 mm. This length is reached by most mussels during their first year. First gravidity is similarly size dependent, occurring at a size of approximately 40 mm. This mean length is reached within an age span of from 0.6 to 1.5 years depending upon the waterbody. Such early maturity may be the general rule amongst species of freshwater mussels from the tropics. Kenmuir (1980) observed gravid females of 2 out of 3 species studied in Lake Kariba between the ages of 1 and 2, while Dudgeon and Morton (1983) observed that Anodonta woodiana was mature by the end of the first year in Hong Kong. Other fast growing temperate species, also mature at a relatively early age (Table A4.1), but generally never in the first year. Warm, equitable water temperatures, presumably enhance rapid gonadal maturation.

After a peak is reached in reproductive potential relatively early in life, for female mussels at least, in most populations from the Magela Creek a long and gradual decline in reproductivity with increasing age occurs. The same phenomenon has previously been noted only for two

European unionids (Haranghy et al., 1964), while a post-reproductive phase (a decline with age) is reportedly absent in many other species of freshwater mussel (Scruggs, 1960; Stansbery, 1967; Heard, 1975; Kenmuir, 1980). The decline in reproductive effort of female V. angasi in the Magela Creek with age is suggestive of a physiological weakening of mussels by certain environmental stresses to which they, because of the brooding habit, may be particularly vulnerable - e.g. turbidity and anoxia. Repetitive breeding is also, presumably, taxing to the reproductive physiology of female mussels. Both sexes nevertheless, showed a decline in relative body weight in the oldest age classes of most of the Magela Creek populations, corroborative evidence (section 8.7.2) of general senescence in V. angasi.

Larval production

Larval development in V. angasi, from initial spawning to glochidial maturation, is exceedingly rapid and may be completed during the Wet season months at least, in well under 12 days. This time span is the shortest recorded of any species of freshwater mussel, although some North American Quadrula spp. may complete development in 2 weeks (Lefevre and Curtis, 1912). A longer period for larval development has been found in other tropical freshwater mussels: 4 weeks in Anodonta woodiana (Dudgeon and Morton, 1983) and 3-5 weeks for species from Lake Kariba (Kenmuir, 1981b).

The ubiquity of mature primary oocytes and sperm, the presence of gravid females throughout much of the year in many populations, and the knowledge that larval development is exceedingly rapid and that

mature glochidia are released in direct proportion to the intensity that they are produced, provided clear evidence that spawning and breeding of V. angasi in the Magela Creek are repetitive. Elsewhere, such intensity of larval production is apparently matched only in the mutelids Mutela bourguignati (Fryer, 1961) and M. dubia, and the unionid, Caelatura mossambicensis (Kenmuir, 1981a) from tropical Africa. Other tropical species are distinctly seasonal in their breeding - e.g. Anodonta woodiana (Dudgeon and Morton, 1983), although protracted and/or repetitive breeding during the warmer months have been observed for Indian unionids (Seshaiya, 1969; Lomte and Nagabhushanam, 1969; Ghosh and Ghose, 1972; Nagabhushanam and Loghaonker, 1978) and for the mutelid, Aspatharia wahlbergi in Lake Kariba (Kenmuir, 1981a).

Amongst temperate tachytictic breeders from northern climes, multiple broods and repetitive spawning during the warmer months have been observed (e.g. Yokley, 1972; Wood, 1974a) and even some bradytictic forms have been shown to reproduce repetitively over the summer (Heard, 1975). Data from the temperate Australian hyriids also indicates that many of the species studied to date may have the potential to produce a number of broods during the warmer months (Hiscock, 1951; McMichael and Hiscock, 1958; Atkins, 1979; Walker, 1981b; Jones and Simpson, in prep.). Different climatic regions across latitudes appear to be largely influential in determining the reproductive patterns of the respective freshwater mussel groups. Providing warm temperatures are sustained for a long enough period of time, multiple broods may be the norm, suggesting that the breeding of freshwater mussels may be largely opportunistic.

Both wide temporal and spatial variations were found in the breeding patterns of V. angasi between the different populations of the Magela Creek. The superficial appearance observed was one of a number of different populations each breeding in a pattern peculiar to the respective waterbody. Intraspecific variation in the timing and frequency of breeding over a broad geographical range (Giusti et al., 1975; Kenmuir, 1981a,b; Dudgeon and Morton, 1983), or on a more regional scale between neighbouring lakes (Kenmuir, 1981b) or sites of the same river (Walker, 1981b; Young and Williams, 1984a; Jones and Simpson, in prep.) have been attributed to climatic and temperature variations between the various localities. Otherwise both interpopulation (Bjork, 1962; Heard, 1975; Haukioja and Hakala, 1978b; Young and Williams, 1984a) and even intrapopulation variations (Porter and Horn, 1980) in the timing and frequency of reproduction of intraspecifics have been left unexplained.

Interpopulation variation in breeding patterns of unionids recorded at the specific level was attributed by Dudgeon and Morton (1983) to adaptations to conditions peculiar to local environments, whilst Heard (1975) concluded that breeding cycles were apparently not influenced entirely by conspicuous environmental factors such as water temperature and presence or absence of current. Reproductive timing amongst unionaceans has been linked to the habits and activities of the host fishes (section A4.3) although no evidence has been provided to indicate that the activities of the host fishes may influence the timing and frequency of breeding cycles between specific populations of freshwater mussels.

Seasonality in other environmental factors has largely been ignored in terms of its influence upon reproduction in freshwater mussels. Numerous authors, nevertheless, have drawn attention to the sensitivities of gravid female mussels to stressful environmental conditions such as high water temperatures and oxygen deficiences under laboratory conditions (section A4.2). Under field conditions, however, only Matteson (1955) has observed abortion in gravid mussels, in response to unseasonally warm, shallow waters. While breeding of V. angasi in the Magela Creek in equitable and stress-free environments conforms to a cycle apparently temperature dependent, the breeding patterns in other billabongs are clearly interrupted by seasonal deficiences in dissolved oxygen concentrations and seasonally high turbidities.

In V. angasi from the Magela Creek, spawning and larval production are immediate responses to the intensity of gametogensis. Although a background reproductive cycle dependent upon temperature, and which may be interrupted by the aforementioned seasonal stresses, is suggested for mussel populations of the Magela Creek, no overall model could be derived to show this. Prediction of breeding patterns within billabongs may be good, but responses between billabongs to the same environmental parameters differ sufficiently to suggest that control of breeding is also influenced by more complicated physiological mechanisms. Physiological condition of the mussels themselves (e.g. body weights, previous spawning history) in addition to other unmeasured, environmental influences are also presumably, influential to reproductive patterns.

Nevertheless, this study is the first to attempt an elucidation of the factors that may influence breeding patterns, in a species of freshwater mussel that breeds largely aseasonally. The results clearly implicated two other environmental variables apart from water temperature that are influential to breeding in freshwater mussels - namely dissolved oxygen and turbidity. It would be of interest to investigate the extent to which environmental parameters other than water temperature and implicated host fish habits and activities, influence reproductive cycles in other species of freshwater mussel.

Glochidial release and parasitism

From both field and laboratory observations, a total of 19 fish hosts are known for the glochidia of V. angasi in the Magela Creek. In accordance with the observations for unionaceans in general (Kat, 1984) including the Australian hyriids (Atkins, 1979; Walker, 1981b) therefore, it appears that V. angasi also has little host specificity. Considering the comparative richness of the fish community of the Region (section 2.3.3), it is unlikely that a record of at least 19 fish hosts observed for V. angasi will ever be surpassed by another Australian hyriid confined to a specific catchment. Further hosts of V. angasi are likely to be discovered moreover, as gill infections are more closely scrutinised. Only two North American anodontines are known to parasitise a larger number of fish hosts (over 30) than V. angasi (Trdan and Hoeh, 1982), though these reports pertain to known hosts over the entire ranges of the species concerned. As more tropical species are examined, however, even these records are likely

to be surpassed.

Both incidence and intensity of infection by glochidia of V. angasi are low overall in fish species of the Magela Creek. This finding accords with other natural infections recorded in unionaceans elsewhere (section A4.3). The result might be expected for V. angasi as Trdan (1981) observed a negative correlation between the number of species serving as hosts for the glochidia of particular unionids and the infection rate (percent infected and intensity).

While both host specificity and infection rates are low in V. angasi, nevertheless, some species were observed to serve disproportionately as hosts. Bottom feeding and dwelling fishes in the Magela Creek and fishes notably inactive in the water column (e.g. Glossogobius giurus, Ambassis spp., Glossamia aprion, Amniataba percoides) had higher infections than species inhabiting the mid- and surface waters (Toxotes chatareus, Melanotaenia splendida) or species with active predatory habits (e.g. Leiopotherapon unicolor, Lates calcarifer). Elsewhere, hosts occurring in sympatry with mussels may have heavy infections of glochidia (Surber, 1912; Lefevre and Curtis, 1912; Percival, 1931; Fuller, 1974; Giusti et al., 1975; Kenmuir, 1980; Zale and Neves, 1982 a,b; Kat, 1984).

For four species of fish whose body surfaces were thoroughly examined for glochidial infections, infections were observed to be higher on the gills than the fins. This is contrary to the general observation, that hooked or toothed glochidia tend to parasitise the external surfaces of their hosts (section A4.3). More species of fish from all

habitat types need to be examined, however, before conclusions concerning the distribution of glochidia of V. angasi on the fish hosts can be drawn. Nevertheless, there are many recorded exceptions to the general rules concerning the site of attachment of glochidia upon the host tissues (section A4.3). The glochidia of Anodonta cygnea for example are of the hooked variety, but Giusti et al. (1975) found large burdens of infections upon the gills of bottom feeding, host fishes than upon predatory fishes. Upon further examination, this may also prove to be case for infections of the glochidia of V. angasi. Dartnall and Walkey (1979) concluded that such factors as fish size and feeding behaviour of fishes might interact to determine the distribution of the glochidia on fish hosts. Among the few other Australian hyriids (larvae of the toothed variety) whose host fishes are known, the glochidia of Hyridella drapeta apparently attach exclusively to the gills of host fishes (Atkins, 1979). Other velesunionines, however, chiefly parasitise the fins and general body surface of their hosts (Hiscock, 1951; Walker, 1981b).

As for other unionaceans studied, temperature appeared to be the most important factor determining the duration of the parasitic period of glochidia of V. angasi. Metamorphosed juveniles were recovered from host fish on average after 5 days at a water temperature of 30° C, and after 10 days at 22° C. Recovery of metamorphosed juveniles occurred as shortly as within 48 hours after infection at 30° C, and within 96 hours at 22° C. Thus larval metamorphosis in V. angasi is exceedingly rapid, and is matched only by some Indian unionids which may complete the parasitic period in 3 days (Seshaiya, 1969 - Table A4.2). The glochidia of some temperate unionids also have a rapid metamorphosis -

Anodonta grandis and A. imbecilis were observed to complete the parasitic phase on average 6 and 8 days respectively after infection at summer temperatures (Trdan and Hoeh, 1982). Seshaiya (1969) thought that the rapid metamorphosis observed for the larvae of Lamellidens spp. might be an adaptation to the environmental conditions of the tropics. No reference was made, however, as to what these conditions might be. Larval metamorphosis in V. angasi though, is probably no shorter than that which might be expected for any unionacean under the same warm environmental conditions prevailing in the Magela Creek.

By monitoring the seasonal incidence of parasitism of glochidia upon a host fish species, Glossogobius giurus, it was shown that V. angasi releases glochidia throughout the year in direct proportion to their seasonal production. Thus metamorphosed glochidia drop from the fishes onto the sediments throughout the year - again, in direct proportion to their seasonal production. However, recruitment of V. angasi in the Magela Creek is seasonal and occurs during the Wet and early Dry seasons in association with periods of highest dissolved oxygen content (section 6.6.1). It would seem therefore, that much of the energy expended in producing larvae during the Dry season is wasted as little recruitment occurs during these times. A better strategy would seem to be for mussels to accrue reserves and to breed only during the Wet season when conditions are ideal for recruitment.

However, the fact that some Dry season recruitment is observed at all, may be the clue to understanding the aseasonal and continuous breeding pattern of V. angasi. Populations of V. angasi have a very high

reproductive potential, chiefly as a result of early maturation, repetitive spawning, and high longevity. In the deeper billabongs, overall densities are mainly affected by Wet season flow regimes (section 5.4.2). For all the considerable reproductive effort expended by individuals nevertheless, minor (and presumably constant) recruitment has been observed in all of the billabongs during the Dry. In billabongs where low densities of mussels are observed such as in Jabiluka, it is conceivable that a significant proportion of the population are the result of Dry season recruitment, as anoxia at the Wet-Dry interchange may kill many of the Wet season recruits.

Thus environments may be envisaged where breeding during lentic phases results in significant recruitment, and thereby is a successful strategy ensuring the continuation of the population. Over the entire geographical range of V. angasi, a monsoonal climate occurs. However, annual precipitation and stream discharges over this range may be considerably lower than those observed in the Alligator Rivers Region. Low dissolved oxygen concentrations in part may limit Dry season recruitment in the Magela Creek populations. Nevertheless it is likely that in drier regions of its range, there are lentic environments where dissolved oxygen concentrations are sufficiently high to allow major recruitment of V. angasi throughout the year.

In the broader context of the recent climatic history of the Australian environment, the year round breeding strategy of V. angasi may be better appreciated. From the scenario presented by Galloway and Kemp (1981), warmer and considerably wetter phases characterised Australian climates during the Early Tertiary. In the perennial

streams of the time, year round breeding for freshwater mussels was presumably the norm. From the Middle Miocene onwards, there has been a general trend to dryness over the continent. Fluctuating climates characterised the Lower and Middle Pleistocene and the trend to dryness culminated in relative aridity between 30,000-17,000 years B.P. With this increasing aridity, streams presumably became intermittent. Also, wet seasons in monsoonal belts at the height of this dry period, were (presumably) of less intensity than those experienced today. Thus, the reproduction of the freshwater mussels was probably modified under the fluctuating and relatively arid environments to one of opportunistic breeding superimposed on a year-round potential.

Past climates have been drier and more variable than those observed today, and recruitment of V. angasi during periods of low and fluctuating discharges, and under lentic conditions has presumably, ensured the continuation of the species. The opportunistic breeding pattern of V. angasi thus, may have been retained as a guarantee against fluctuating climates and environments.