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Growth of *Penaeus monodon* × *Penaeus esculentus* tiger prawn hybrids relative to the parental species

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Abstract

Interspecies hybrids were produced by artificial insemination of *Penaeus monodon* females with *P. esculentus* males. Successful spawnings (those in which some eggs hatched) were achieved from nine of the 17 *P. monodon* × *P. esculentus* matings attempted (53%). Mean egg numbers produced ranged from 158,000 to 438,000 but hatch rates were low (< 4% in all cases). Cumulative survival of the hybrids and both parental species was similar, and around 30% at 4 weeks. There was no indication of hybrid vigour for growth. However, growth rate of hybrid larvae (0.048 g day^{-1}), was the same as those of pure *P. monodon* (0.047 g day^{-1}) and significantly greater than that of *P. esculentus* larvae (0.033 g day^{-1}). The relative growth rate among parental species and the hybrids was determined accurately by rearing progeny of pure and hybrid matings in parallel under controlled conditions. The colour pattern of the hybrids was intermediate between that of *P. monodon* and *P. esculentus*. Sex ratio was significantly skewed in favour of males in the hybrids (proportion of males was 0.86 compared with 0.56 in the parental species), possibly suggesting females are the heterogametic sex in penaeids. The hybrids had the fast growth rate of *P. monodon*, and some of the attractive colour pattern of *P. esculentus*.

Keywords: Inter-species hybrids; Hybridization; Survival and growth; Penaeid prawns; *P. monodon*; *P. esculentus*

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1. Introduction

Inter-specific hybrids have attracted attention because they can improve productivity through hybrid vigour, combine desirable characteristics found in one species with those of another, or produce animals that are sterile (Chevassus, 1983; Hedgecock, 1987; Longwell, 1987; Menzel, 1987). The development of artificial insemination techniques and in-vitro fertilization for crustaceans has assisted the production of inter-species crosses in this group (Clark et al., 1973; Berg et al., 1986). Several interspecies hybrids of penaeid prawn species have been achieved — *Penaeus setiferus* × *P. stylirostrus* (Lawrence et al., 1984), *P. setiferus* × *P. schmitti* (Bray et al., 1990), reciprocal crosses of *P. monodon* and *P. penicillatus* (Lin et al., 1988), and *P. monodon* × *P. esculentus* (Benzie et al., 1995).

The number and hatch rate of hybrid eggs, and the survival of hybrids to post-larval stages have been low compared with the results of intra-specific matings, and most reports focus on these issues rather than the characteristics of the hybrids (Benzie et al., 1995). Growth rates relative to the parent species have not been assessed in most studies, but Lin et al. (1988) recorded that the reciprocal *P. monodon* × *P. penicillatus* hybrids grew faster than both of the parental species and suggested that hybrid vigour had been demonstrated. Benzie et al. (1995) successfully produced *P. monodon* × *P. esculentus* hybrids in an attempt to create a line having the fast growth rate of *P. monodon* and the attractive banding patterns that enhance the market value of *P. esculentus*. However, these authors noted that the lack of controls in their experiment prevented a critical assessment of the growth rates of the hybrid.

Prawn growth rates are highly variable depending on the precise environmental conditions in which they are reared. No published study of prawn hybrids to date has included the appropriate controls (i.e. the culture of pure larvae from each of the parental species together with the hybrids) to determine the growth rate of the hybrids relative to the parental species.

The aim of the present paper is to report the results of experiments where hybrids were reared together with larvae produced from pure parental species matings to provide a critical assessment of the growth and survival of *P. monodon* × *P. esculentus* hybrids relative to their parental species.

2. Materials and methods

2.1. Larval rearing and experimental design

Broodstock of both species were trawled near Cairns, North Queensland and freighted by air and road to the maturation unit. Males and females from both species were stocked into different 4.5-m diameter and 1-m deep tanks. The animals were acclimatised for 1 week at 28–29°C, on a 14:10 light cycle, a diet of fresh-frozen squid, molluscs and an in-house paté containing ox liver, and a daily exchange of more than 200% filtered seawater. All broodstock were then weighed, measured (total length and carapace length), eye tagged and returned to their respective tanks. Female broodstock

were also moult tagged with 2 mm squares of waterproof paper glued to the carapace (this was to track subsequent moulting events), and one eye was ablated.

Two days after moulting, each female was artificially inseminated using one complete spermatophore and returned to the tank. Female *P. monodon* were inseminated using spermatophores from either a *P. monodon* or a *P. esculentus* male, and *P. esculentus* females were inseminated using only *P. esculentus* males. Most matings attempted were *P. esculentus* × *P. monodon* crosses to maximise the number of hybrids. Space and numbers of prawns were limited, so only six pure *P. esculentus* matings and two pure *P. monodon* matings were made.

The gonadal index of each ablated and inseminated female was monitored daily until stage IV development (ripe ovaries). Stage IV females were placed in separate 150 l spawning tubs with 28°C recirculated water. After spawning, the entire egg batch was harvested into a 4-l beaker and five 2-ml samples were taken to estimate total egg number. Hatch rates for each batch were estimated in a similar fashion, after harvesting nauplii larvae from the hatching upwellers. Naupliar condition was estimated 1 day after hatching by microscopically examining sub-samples from each batch. Up to three replicate batches of 4000 larvae from each of the *P. monodon* and *P. esculentus* families (i.e. the controls) were stocked into separate 50-l tubs for larval rearing. As a result of low numbers of hybrid larvae, three families were stocked at lower densities (two at 3000 and one at 1000).

Larvae were reared on a mixed diet of resuspended *Chaetoceros muerilli* paste, dried *Spirulina*, Frippak micro-capsules, *Artemia*, and Frippak post-larval pellets. Water temperature was maintained at 28°C throughout the experiment. Filtered, preheated seawater was used to exchange 20% of the total volume in each tub from mysis stage one (M1) up to post-larval stage one (PL1). Water exchange was then increased to 40% until post-larval stage six (PL6), when it was increased to 50% until the end of the hatchery phase (i.e. at PL15). At PL15, each batch was harvested and 20 individuals, selected at random, were weighed and measured.

After the hatchery phase, up to five replicates of 100 animals were selected at random from each of the pure parental and hybrid families. Each set of 100 animals was stocked into an $80 \times 30 \times 30$ cm plankton mesh cage. Up to 20 cages were suspended in each 4.5-m diameter by 1-m deep nursery tank. Larvae were fed ad libitum on a formulated pellet diet supplemented with a daily ration of minced squid. Larvae were exposed to a natural light cycle and water quality was maintained using 100% exchange of filtered seawater daily. At 4, 7, and 12 weeks after hatching, 20 individuals from each replicate were selected at random, measured for total length, weighed and replaced. At 7 weeks, the density of larvae in each cage was reduced to 50 individuals. Ten of the discarded larvae from each family were frozen for genetic analysis.

At 12 weeks, all animals were harvested and pooled into three groups: pure *P. monodon*, pure *P. esculentus*, and *P. monodon* × *P. esculentus* hybrids. A total of 98 individuals was taken at random from each of these pooled populations. Each individual was measured for total length, weighed, and tagged with a numbered juvenile streamer tag. Forty-nine were stocked in each of two tanks, giving equal stocking densities of each group, and a total stocking density of 9.2 prawns m^{-2} in each tank. The prawns were fed formulated pellets ad libitum, supplemented with a daily ration of minced

squid. Mortalities were estimated by recording floating streamer tags each day. After 3 months, the remaining prawns (now 7 months old) were harvested, measured for total length, weighed, and sacrificed.

2.2. Laboratory analysis

Samples of abdominal muscle from the parents and individual hybrid progeny were snap-frozen in liquid nitrogen and stored at −80°C until analysis. Five enzymes showing fixed gene differences between *P. monodon* and *P. esculentus* [glucosephosphate isomerase (GPI) EC 5.3.1.8, lactate dehydrogenase (LDH) EC 1.1.1.27, malate dehydrogenase (MDH) EC 1.1.1.37, 6-phosphogluconate dehydrogenase (PGD) EC 1.1.1.44, and phosphoglucomutase (PGM) 5.4.2.2] were screened following Benzie et al. (1995) [details in Ballment et al. (1993)].

2.3. Statistical analysis

Statistical analysis was carried out using programs in Statview (SAS Institute, 1998). Differences between survival curves were tested using Mantel–Cox statistics, and ANOVA was used to test the significance of differences in length or weight between species and hybrids using the means of the replicates within each group as base data.

3. Results

All larvae were vigorous and had no obvious abnormalities. At each locus tested (GPI*, LDH*, MDH-2*, PGD* and PGM*), all the hybrids were heterozygous for the alleles found in the alternative homozygous states in the respective parents and their pure progeny. These data established that all hybrid families were inter-specific hybrids.

Spawnings were observed in 14 of the 17 inter-specific matings achieved by artificial insemination (82%). This figure was comparable to those for the pure parental matings (Table 1). Of the successful hybrid spawnings, 64% hatched. This figure was compara-

Table 1
Summary of matings, spawning success, and hatchings from pure matings of *P. monodon* and *P. esculentus*, and crosses of *P. monodon* females with *P. esculentus* males

Mating (female × male)	Matings number	Spawnings		Spawnings which hatched		
		Number	% of matings	Number	% of spawns	% of matings
P. monodon × *P. monodon*	2	1	50	1	50	50
P. monodon × *P. esculentus*	17	14	82	9	64	53
P. esculentus × *P. esculentus*	6	6	100	6	100	100

Two individuals produced repeat spawns from the same mating, but these individuals were counted as having one spawning. In these cases, the total egg number (i.e. the sum of those in the partial spawnings) was used for calculations in this table (there was no obvious difference between the hatch rates in the repeat spawns from one individual).

Table 2
Mean ± S.D. (with range in parentheses) of egg number and hatch rates in successful spawns, and survival to early- and mid-juvenile stages, for pure matings and hybrid crosses of *P. monodon* females and *P. esculentus* males

Mating (female × male)	Number of eggs in thousands	Hatch rate [(%) hatching]	Survival (%) to PL15	Survival (%) to 6 months
P. monodon ×	120.0 ± 0	24.4 ± 0	30.1 ± 0	2.1 ± 0
P. monodon	(120–120)	(24.4–24.4)	(30.1–30.1)	(2.1–2.1)
P. monodon ×	314.8 ± 29.6	1.3 ± 0.4	28.5 ± 27.1	0 ± 0
P. esculentus	(157.6–438.4)	(0.0–3.8)	(1.8–75.2)	(0)
P. esculentus ×	138.3 ± 17.8	28.5 ± 7.6	27.0 ± 25.5	2.3 ± 2.8
P. esculentus	(64.0–189.2)	(3.1–54.2)	(1.8–75.2)	(0–5.9)

ble to that of the pure *P. monodon* spawnings. Although only two matings could be included in this experiment, it is usual for about 50% of artificially inseminated *P. monodon* females to spawn in any given experiment. The number of eggs produced per spawning by the hybrids (157,000–438,000) was greater than the pure *P. monodon* spawn (120,000) or the pure *P. esculentus* spawns (64,000–189,000) (Table 2). This may have been the result of the greater size of the *P. monodon* females mated with the *P. esculentus* males (Table 2). However, hatch rates were an order of magnitude lower in the hybrids (1–2% compared with 25–30%) (Table 2). The hatch rate for the single *P. monodon* spawning in the present experiment (24.4%) was at the low end of the range usually obtained in our system for artificially inseminated females. From a group of 38 *P. monodon* females in another, unrelated experiment, the mean hatching rate was 42 ± 2.1% (s.e.). Survival was highly variable among replicates, but the average performance of the hybrids to PL15 and at 28 weeks was almost identical to that of the pure *P. esculentus* larvae and the only *P. monodon* family stocked (Table 2). The

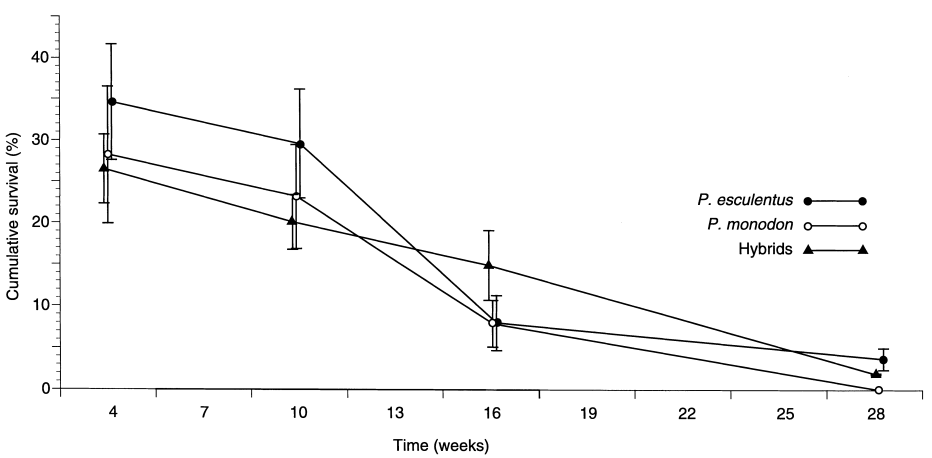

Fig. 1. Cumulative survival (% ± s.e.) of pure *P. monodon* larvae, pure *P. esculentus* larvae, and *P. monodon* × *P. esculentus* hybrids over 28 weeks.

Table 3
Growth data using weight and total length (mean ± S.D. with sample size in parentheses) for pure *P. monodon*, pure *P. esculentus* and hybrid larvae, together with the probability that the values differ between groups

Age (weeks from hatching)	*P. esculentus*	Hybrids	*P. monodon*	Probability that means differ		
				esc/mon	esc/hyb	mon/hyb
	Weight (g)					
4	0.006 ± 0.003 (8)	0.006 ± 0.002 (5)	0.004 ± 0.001 (3)	0.31	0.75	0.24
7	0.112 ± 0.025 (8)	0.149 ± 0.025 (5)	0.133 ± 0.019 (3)	0.23	0.02 *	0.39
10	0.450 ± 0.050 (8)	0.704 ± 0.084 (5)	0.776 ± 0.067 (3)	< 0.001 * * *	< 0.001 * * *	0.17
16	2.742 ± 1.054 (6)	4.052 ± 0.493 (5)	3.973 ± 0.184 (3)	0.03 *	0.01 *	0.93
28	15.003 ± 0.090 (4)	No data	18.773 ± 1.365 (3)	0.04 *	–	–
	Length (mm)					
4	11.64 ± 1.37 (8)	12.00 ± 0.77 (5)	11.76 ± 0.21 (3)	0.81	0.94	0.77
7	25.94 ± 2.50 (8)	31.56 ± 6.07 (5)	29.13 ± 1.51 (3)	0.34	0.06	0.45
10	41.48 ± 1.62 (8)	47.68 ± 2.04 (5)	50.07 ± 1.08 (3)	< 0.001 * * *	< 0.0002 * * *	0.09
16	75.10 ± 8.43 (6)	84.72 ± 3.08 (5)	82.63 ± 0.60 (3)	0.010	0.02 *	0.64
28	132.43 ± 1.21 (4)	No data	140.23 ± 4.58 (3)	0.04 *	–	–

* $P < 0.05$.
* * $P < 0.01$.
* * * $P < 0.001$.

cumulative survival curves for both species and their hybrids were not significantly different from each other over the 6-month period for which they were held, and by which time all the hybrids had died, a few of the pure parental crosses remained (Fig. 1).

The average total length and weight of larvae at PL15 showed no relationship to the initial larval stocking density (data not shown). However, there was considerable variation among replicates in the survival rate, leading to marked differences in prawn density that might have affected growth rates. Graphs of the average length and weight of each replicate at each time period demonstrated no relationship between these variables and density, except at prawn densities less than 200 per tank at PL15 (data not shown). Subsequent analyses excluded those replicates with densities less than 200 per tank at PL15. A total of nine replicates was excluded, five from the hybrids and four from *P. esculentus*.

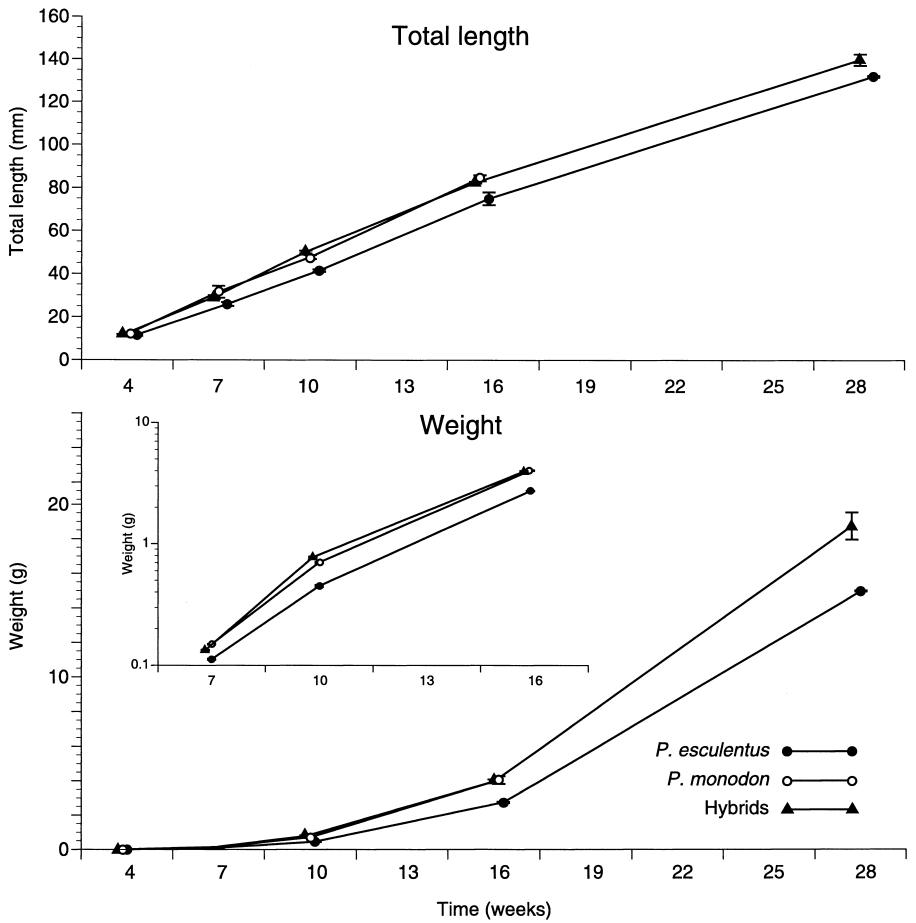

Fig. 2. Total length, mm (±s.e.) and wet weight, g (±s.e.), of pure *P. monodon* larvae, pure *P. esculentus* larvae, and *P. monodon* × *P. esculentus* hybrids over 28 weeks. The insert graph for weight illustrates growth from 10 to 16 weeks on a log scale to more clearly demonstrate the difference in growth of *P. esculentus* from *P. monodon* and the hybrids.

The total length and weight of the hybrids were not statistically different from *P. monodon* or *P. esculentus* larvae at PL15, that is, 4 weeks from hatching (Table 3; Fig. 2). At 7 weeks, the hybrids were significantly heavier, but not significantly longer than *P. esculentus* larvae. Thereafter, the hybrids were consistently and significantly heavier and longer than *P. esculentus* larvae, but not significantly different from *P. monodon* larvae. From 4 to 16 weeks, weight gain of the hybrids (0.048 g day^{-1}) was similar to that of *P. monodon* larvae (0.047 g day^{-1}) and significantly greater than that of *P. esculentus* larvae (0.033 g day^{-1}). All hybrids had died prior to the 28-week measurements where growth rates from PL15 for *P. monodon* were 0.112 g day^{-1}, and 0.089 g day^{-1} for *P. esculentus*. It should be stressed that the progeny for *P. monodon* in this experiment were derived from only one mating, and represent a limited sample for the species as a whole. However, growth rates were comparable to those observed for several *P. monodon* families in other experiments at the same facility (0.12–0.13 g day^{-1}; Benzie et al., 1995), suggesting the control was reasonably representative.

The proportion of males at 4 months was 0.56 for both *P. monodon* and *P. esculentus*, but was 0.86 for the hybrids, indicating a considerable bias in sex ratio towards males in the hybrids. There is marked sexual dimorphism in tiger prawns, with females growing to a larger size than males. Divergence in size is usually observed at about 6–7 months old, although individuals can be accurately sexed at 4 months old. There was no significant difference in weight or total length between males and females of *P. monodon*, or *P.esculentus* or their hybrids at 4 months (the last time period for which there were data for the hybrids) (Table 4). Differences in growth rate among the two parental species and their hybrid were not therefore influenced by the differences in sex ratio among the groups.

The hybrid larvae were green–brown in colour initially, but in time developed a relatively consistent banding and colour pattern that was intermediate to that of the pure *P. monodon* and pure *P. esculentus* juveniles. The hybrids had blue and yellow pleopods characteristic of *P. monodon* juveniles and sub-adults, rather than the red–brown and white pleopods characteristic of *P. esculentus*. *P. esculentus* has finer brown bands on the uropods and body than *P. monodon* and the hybrids had bands more like *P. esculentus*, but these were occasionally broader, reminiscent of *P. monodon*, particularly on the carapace.

Table 4
Weight and total length (mean ± S.D. with sample size in parentheses) of each sex for pure *P. monodon*, pure *P. esculentus* and hybrid larvae, at 16 weeks old

Sex	*P. esculentus*	Hybrids	*P. monodon*
	Weight (g)		
Male	2.29 ± 0.68 (51)	4.04 ± 1.22 (434)	3.99 ± 1.37 (55)
Female	2.15 ± 0.56 (40)	3.43 ± 1.17 (70)	4.00 ± 1.38 (43)
	Length (mm)		
Male	70.82 ± 7.26 (51)	84.70 ± 8.47 (434)	82.75 ± 10.26 (55)
Female	72.48 ± 6.62 (40)	80.14 ± 8.15 (70)	82.42 ± 9.50 (43)

4. Discussion

The proportion of *P. monodon* females inseminated by *P. esculentus* males, which spawned and whose eggs hatched (53%), was high relative to the first report of 15% by Benzie et al. (1995). However, hatch rates of hybrid eggs were similar ($<4\%$) to previous reports, and an order of magnitude less than that in the parental species. Benzie et al. (1995) noted that the survival rate of the *P. monodon* × *P. esculentus* hybrids was high relative to other penaeid hybrids. Despite the low hatch rate relative to other inter-species penaeids, this meant that the proportion of larvae surviving to PL20 from a given number of eggs was similar to other penaeid hybrids (about 1%). The present experiment demonstrated clearly that the survival rate of hybrid larvae was almost identical to that of pure *P. monodon* or *P. esculentus* larvae when reared under the same conditions. This suggests the main obstacle to hybrid production lies in the successful fertilization and hatching of eggs, rather than in the viability of the larvae that do hatch.

When the data from the present study and those from Benzie et al. (1995) are combined, the proportion of *P. monodon* × *P. esculentus* matings that produced larvae is 37%. This figure is similar to that recorded for matings between *P. penicillatus* and *P. monodon* (10–30%) (Lin et al., 1988), *P. setiferus* × *P. schmitti* (30–40%) (Bray et al., 1990), and reciprocal crosses of *P. setiferus* and *P. stylirostris* (30–40%) (Lawrence et al., 1984). Hatch rates of the *P. monodon* × *P. esculentus* eggs were 1.3%, similar to previous reports of 2.4% by Benzie et al. (1995). They are an order of magnitude more than the hatch rates of 0.2% recorded for *P. setiferus* × *P. schmitti* crosses (Bray et al., 1990), and an order of magnitude less than the hatch rates of 30% recorded for *P. monodon* × *P. penicillatus* crosses (Lin et al., 1988).

Growth of the hybrids was demonstrated to be significantly faster than pure *P. esculentus* larvae, but not significantly different from *P. monodon*. There was no evidence of hybrid vigour in the sense that hybrids outperform both parental species. From 4 to 16 weeks, the weight gain of the hybrids (0.048 g day^{-1}) was similar to that of *P. monodon* larvae (0.047 g day^{-1}) and significantly greater than that of *P. esculentus* larvae (0.033 g day^{-1}). The comparison with *P. monodon* has to be qualified in that progeny from only one family was available for specific comparison in the experiment. However, growth rates for *P. monodon* up to 28 weeks of 0.112 g day^{-1} in this experiment compare well with results for growth rates of pure *P. monodon* (0.12–0.13 g day^{-1}) reared successfully on other occasions in the same tank system over similar periods of time (Benzie et al., 1995). These data suggest that the growth in the larvae from the one mating available for *P. monodon* in the present experiment reflected the average performance of that species in the tank system and were not skewed, so providing an adequate control.

In *P. monodon*, the daily growth rate, measured from 3 to 28 weeks in the present experiment (0.112 g day^{-1}), is 2.4 times greater than that measured from 3 to 16 weeks (0.048 g day^{-1}). In *P. esculentus*, it is 2.7 times greater. Comparisons with the growth rates of hybrids reported by Benzie et al. (1995) must take into account the different time periods over which growth was estimated. Benzie et al. (1995) reported the growth rate of the *P. monodon* × *P. esculentus* hybrids from PL20 to 130 days as 0.09 g day^{-1}. Multiplying the 4 to 16 weeks estimate for the hybrids in the present experiment (0.048

g day^{-1}) by a conservative 1.8 times (2.4 less one quarter to correct in part for a comparison with 130 as opposed to 168 days) gives a growth rate of 0.086 g day^{-1}. The estimates of hybrid growth rate from the two experiments are, therefore, reasonably close.

Lin et al. (1988) have been the only authors to specifically address the occurrence of hybrid vigour in penaeid prawns. They stated that they had observed hybrid vigour in reciprocal crosses of *P. monodon* and *P. penicillatus* as the hybrids grew faster than pure larvae of either of the parent species. However, no control lines of pure parental larvae were reared and the average weights of the hybrids were far less than those given by Dall et al. (1990) for *P. monodon* juveniles at 150 days (Benzie et al., 1995). The large effect of environment on growth in penaeids and the lack of controls make it difficult to assess whether or not hybrid vigour occurred in the experiment of Lin et al. (1988). The improved experimental design in the present study of *P. monodon* × *P. esculentus* hybrids, where parental larvae were reared in parallel with the hybrids, has produced the first controlled data on hybrid growth in penaeid prawns.

In contrast to the wide range in body colour and pattern in the *P. monodon* × *P. esculentus* hybrids reported by Benzie et al. (1995), the hybrids in the present experiment exhibited a relatively consistent pattern intermediate between that of the parental species. The combined data from both experiments suggest control of colour pattern by several genes, but that many crosses appear to produce a consistent intermediate type.

Sex ratios were also biased in favour of males, a finding also made by Benzie et al. (1995) where all hybrids surviving at the end of their experiment were male. Benzie et al. (1995) suggested that this result might have been the result of differential resistance of the sexes to disease, as they observed a rapid and catastrophic loss of animals to a viral disease. There was no such rapid and catastrophic death in the present experiment. However, there was a consistent low mortality throughout the present experiment leading to very small numbers of prawns of both species, and no hybrids remaining at 6 months (when the experiment was terminated). It is likely that there was a low level of infection by viruses affecting all groups (detected later in other stocks held at that time), since *P. monodon* can be kept successfully in the system throughout their life cycle. Survival curves for both parental species and the hybrids were not significantly different though, and there was no evidence that the hybrids were particularly susceptible (very low numbers of both the parental species (< 10) remained at the end of the experiment).

In the present experiment, the fact that only hybrids showed the bias towards males, and no sex bias was observed in the parental species, suggests differential survival of the sexes to a disease agent was unlikely, and that the bias was an example of the Haldane effect. There is a strong empirical observation first noted by Haldane (1922) that when one sex is absent or rare in the hybrid progeny, that sex is likely to be the heterogametic sex. The sex determination mechanism in penaeid prawns is not known (Benzie, 1998), but the data from the *P. monodon* × *P. esculentus* hybrids suggest that females are the heterogametic sex.

The original goal in attempting to produce *P. monodon* × *P. esculentus* hybrids was to create a line having the fast growth rate of *P. monodon* and the attractive banding patterns that enhance the market value of *P. esculentus*. The first controlled experiment comparing growth of the hybrids to pure parental species larvae (albeit to only one

family of *P. monodon*) has demonstrated that the hybrids do have the fast growth rate of *P. monodon*. They also have a colour pattern that is largely an intermediate of the colour patterns of *P. esculentus* and *P. monodon*, but for which there appears to be segregation, and therefore, the possibility of matching different aspects of colour and pattern.

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