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Wayne Knibb, Jane Quinn, Rob Lamont, Paul Whatmore, Nguyen Hong Nguyen, Courtney Remilton

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REPRODUCTIVE BEHAVIOUR OF CAPTIVE *Fenneropenaeus merguiensis*: EVIDENCE FOR MONOGAMY AND HIGH BETWEEN FAMILY VARIANCES FOR OFFSPRING NUMBER.

Wayne Knibb[a,c], Jane Quinn[a], Rob Lamont[a], Paul Whatmore[a], Nguyen Hong Nguyen[a], Courtney Remilton[b].

[a]The University of the Sunshine Coast, Maroochydore, Queensland 4558, Australia.

[b]. Seafarm, Bruce Hwy, Cardwell, QLD 4849, Australia.

[c] Corresponding author

wknibb@usc.edu.au

Faculty of Science Health and Education

University of the Sunshine Coast

Locked Bag 4

MAROOCHYDORE DC QLD 4558

AUSTRALIA

Tel: +61 7 5430 2831

Abstract

The purpose of this paper was to determine aspects of the reproductive biology of captively bred *Fenneropenaeus merguiensis* under commercial broodstock production conditions that relate to the design and implementation of genetic improvement programs for this species.

First, we tested whether there is evidence for polygamy vs monogamy by genotyping females, the material found in their thelycums, and material that leaches out of the thelycum using DNA microsatellite loci. All genotypes in all animals and tissues tested could be accounted for using a monogamy model.

Second we compared the accuracy of pedigrees formed under assumptions of monogamy vs polygamy. Pedigrees were formed using microsatellite genotypes from 73 dams and 400 offspring. Sibship groups and dam-offspring groups from pedigrees developed assuming monogamy almost always had the same mtDNA haplotypes, suggesting a high accuracy of the pedigrees, but those formed under the assumption of polygamy were less accurate, and together these results also support the monogamy model.

Third, we assessed the between family variance in offspring family numbers from two sets of mass spawnings of about 40 inseminated females per spawn. About half of the offspring originated from just a few percent of the dams, i.e. many dams contributed few offspring. These data can help predict optimal sample sizes required for accurate future estimates of genetic parameters.

Keywords

Banana, prawns, shrimp, monogamy, polygamy, variable, fecundity.

Introduction

Across aquaculture, new species are being domesticated and new genetic selection programs developed, including one for the banana shrimp, *Fenneropenaeus merguiensis*. A sound understanding of the reproductive biology of shrimp in captivity, whether the species mating behaviour is polygamous or monogamous and the between family variances in offspring number can assist with the planning and operation of genetic improvement programs and the collection of data concerning genetic parameter estimates.

The relevance of monogamy vs polygamy under conditions of mass matings in captivity is that monogamy precludes the creation of half sib groups and can limit the estimation of genetic parameters since maternal effects cannot be partitioned from the additive effects (at least in shallow pedigrees). If so, dedicated artificial insemination (AI) experiments may be required for estimation of genetic parameters. Further, in some cases especially where few marker loci are available, or when only DNA from one parent

(the mothers) is available, accurate pedigree assignment using DNA markers can rely of which assumption - monogamy or polygamy - is used.

In view of the utility of this information for genetics, there are surprisingly few data whether mating in prawn species or indeed among the decapod crustaceans, is monogamous or polygamous. The lack of data is further surprising given that mating systems are a fundamental aspect of reproductive biology in this group. Those few data available for wild crustacean suggest that mating behaviour is linked to life history traits, territoriality, and habitat type. For example, temporary monogamy was inferred by behavioural and morphometric observations on the symbiotic shrimp *Pinna carnea* (Baeza et al., 2011), and thought to be linked to the commensal behaviour of this shrimp species. The snapping shrimp (genus *Alpheus*) appear to be monogamous, a behaviour thought to the linked to the territorial nature of this species (Mathews, 2002). Both serial monogamy and serial polygamy were observed from behaviour of the American *lobster, Homarus americanu*s (Atema, 1986; Cowan and Atema, 1990). Specifically for shrimp, overnight video recordings of *Penaeus monodon* did not reveal polygamous mating behaviour for females (Marsden et al., 2013). These reports have several things in common, namely, the use of behavioural observations during a given time interval as the primary data, that they leave open the possibility of serial polygamy and last, the conclusions were not confirmed using DNA markers.

Another aspect of shrimp reproduction important for genetic planning is the relative contributions of different dams to the offspring in the next generation after mass matings since this variation can impact the efficiency and accuracy of the estimation of genetic parameters. Great variation will limit the numbers of parents and resulting families in the ensuing pedigrees and AI and or a period of separate rearing may be required for efficient experimental designs.

We have assessed using nine newly developed DNA microsatellite markers whether females carry DNA from one or more males in their thelycum. We have also compared the accuracy of pedigrees constructed using DNA markers under assumption of either monogamy versus polygamy by checking the pedigrees using mtDNA haplotypes, assuming that real dam-offspring and full sib groups will have the same mtDNA

haplotypes. Also, using DNA markers we have estimated the variable contributions to offspring numbers made from mass spawning dams in two different spawning events under commercial conditions.

Methods

Shrimp samples

All shrimp were from the Seafarm farm site at Cardwell, Australia (latitude 18° 16' 0S, longitude 146° 1' 60E, altitude 0 m). The daily average temperature in Cardwell is between 14 and 32ºC, with the minimum average of 19ºC and the maximum average of 29ºC over the last 103 years (Australian Bureau of Meteorology, 2012). The water temperature in cultured ponds varies between 25ºC and 32ºC. The annual rainfall is 2129 mm, occurring mainly from December to April with a peak in January, February and March.

For the tests of DNA from males in females, thirty-five thelycum were dissected from sexually mature and potentially inseminated female broodstock at the Seafarm site and shipped from Seafarm to the University of the Sunshine Coast. One eyestalk from each female was also dissected, placed in ethanol along with the dissected thelycum and shipped to USC for analysis. Upon arrival at USC, 16 samples were examined under a dissecting microscope with 8 being identified as containing a spermatophore inside or outside of the thelycum. For all 16 samples, DNA was extracted from the eyestalk plus a section of muscle tissue removed from the underside of the dissected thelycum. Additionally, each tube was centrifuged at 2000 rpm for 5 minutes and the resulting pellet was extracted for DNA. For the 8 samples visually assessed as having spermatophore present, DNA was extracted from the spermatophore following removal from the thelycum. To compare pedigrees formed assuming polygamy vs monogamy, two pedigrees, A and B, were considered. Pedigree A had 37 known dams and 200 sampled offspring, and pedigree B had 36 dams and 200 sampled offspring. For these pedigrees, whole animals were shipped to USC frozen below -60ºC. All DNA extractions were performed using a DNeasy Blood & Tissue kit (QIAGEN) according to the manufacturer's instructions. DNA was PCR amplified and subsequently

genotyped using nine microsatellite primer pairs developed from 454 Next Generation sequencing as follows.

Development of microsatellite loci

Approximately 5 µg of a pooled DNA sample from 20 *F. merguiensis* individuals was submitted to the Australian Genome Research Facility (AGRF; Brisbane, Australia; http://agrf.org.au/) and used to construct a random library that was sequenced on a 454 shot-gun GS-FLX platform (Roche Applied Science; Mannheim, Germany). Sequences were trimmed for length and quality using CLC Genomics Workbench v6 software (CLC Bio, Aarhus, Denmark). We obtained 65 129 reads with an average length of 367 bp and searched for microsatellite loci having a minimum of six repeats for di-nucleotides, and four repeats for tri- and tetra-nucleotides, using the QDDv2b pipeline (Meglécz et al., 2010) and PRIMER 3 (Rozen and Skaletsky, 1999). Primers were designed for 84 microsatellite-containing sequences with suitable flanking regions.

Microsatellite loci were initially screened for successful amplification and variation using eight wild and eight farmed animals and amplified individually in 12.5 µL reactions containing approximately 20 ng of genomic DNA, 1 × reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/mL gelatin), 200 µM of each dNTP, 250 ng BSA (Roche), 2.0 mM $MgCl_2$, 0.4 µM of each primer, and 0.5 U of *Taq* F1 DNA polymerase (Fisher Biotec; Wembley, Australia). PCR was performed using a MaxyGene® thermocycler (Axygen; Tewksbury, USA) with the following cycling conditions: initial denaturation step at 95ºC for 3 min; 35 cycles at 94ºC for 30 s, 52ºC for 30 s, 72ºC for 45 s; with a final extension at 72ºC for 10 min. PCR products were visualised on 3.0% agarose 0.6 × TBE gels (140V; ~110 min) stained with EtBr to look for evidence of polymorphism, prior to labelling the forward primer of each of 16 potentially useful microsatellite loci with FAM, VIC, NED or PET fluorescent dyes (Applied Biosystems, Melbourne, Australia).

Genotyping

Loci with consistent PCR amplification, clear allelic variation, and clarity of electrophoretic signatures were selected for genotyping, nine were used for the thelycum and related samples, ten to determine the pedigrees. Primer pairs were analysed for complementarity and possible interactions using Multiplex Manager software (Holleley and Geerts, 2009) and subsequently grouped into 2 pools (pool 1 with 4 primers and pool 2 with 5 primers) for amplification using Qiagen Multiplex PCR Plus Kits (Qiagen, Germany). Final volumes were optimized (25 µL) to reduce costs. PCR reactions contained ultra-pure water, Multiplex PCR Master Mix (2x), Q-Solution (5x), primer premix (10x) and DNA (10 ng/µL). Cycling conditions were: an initial denaturation at 95ºC for 15 min, followed by 35 cycles of 95ºC for 30 s, 57ºC for 90 s, and 72ºC for 30 s; with a final extension at 68ºC for 30 min. PCR products were separated by capillary electrophoresis on an AB 3500 Genetic Analyser (Applied Biosystems) and fragment sizes were determined relative to an internal lane standard (GS-600 LIZ; Applied Biosystems) using GENEMARKER v1.95 software (SoftGenetics; State College, USA) and double-checked manually. Individuals with low or missing peaks were amplified and genotyped a second time. MICRO-CHECKER v2.2.3 (van Oosterhout et al., 2004) was used to look for evidence of large allele dropout, extreme stuttering and null alleles, based on 1000 bootstraps and a 95% confidence interval. Tests for HWE at each locus and genotypic linkage equilibrium among pairs of loci were conducted in FSTAT v2.9.3.2 (Goudet, 2001). Numbers of alleles, observed and expected heterozygosities, and the fixation index (F_{IS}) as a measure of past inbreeding (Wright, 1965) were determined using GENALEX v6.41 (Peakall and Smouse, 2006). Polymorphic information content (*PIC*) was computed in CERVUS v3.0 (Kalinowski et al., 2007).

Mitochondrial DNA sequencing and analysis

The mtDNA control region (D-Loop, 310bp) was amplified and sequenced using the primers DLoopF (5'-TCCTCTTGTTTTCCCCCTTT-3') and DLoopR (5'-GGATTCAATATAGGCATTTAT-3') (Wilson et al., 2000). Reaction volumes of 25 µL contained approximately 10 ng of genomic DNA, 1 x reaction buffer (67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2 SO_4$, 0.45% Triton X-100, 0.2 mg/mL gelatin), 200 µM of each dNTP, 250 ng BSA (Roche), 1.5 mM $MgCl_2$, 0.4 µM of each primer, and 0.25 U of *Taq* F1 DNA

polymerase (Fisher Biotec; Wembley, Australia). PCR was performed using a MaxyGene® Gradient Thermal Cycler (Axygen Scientific) with the following cycling conditions: initial denaturation step at 95ºC for 3 min; 35 cycles at 94ºC for 30 s, annealing temperature of 55ºC for 30 s, 72ºC for 45 s; final extension at 72ºC for 10 min. A sample of each PCR product was run on 1.5% agarose 0.6xTBE gels to check both amplification quality and quantity with subsequent generation of single stranded forward sequences using DLoopF (9.6 pmol/reaction) as the sequencing primer on an ABI 3750XL Genetic Analyser (Applied Biosystems). Sequence chromatographs were aligned and edited where necessary using SEQUENCHER v4.9 (Gene Codes Corp.).

Results

Monogamy / Polygamy testing

A maximum of two alleles at a given loci, for each of nine loci, was detected in the 16 dam eye stalks, and for some female / loci combinations, only one allele, presumably a homozygote, was evident (Table 1, full data given in Supplementary Table 1). A maximum of two alleles per loci were detected in hundreds of other prawn samples of pleiopods and eyestalks, data not tabulated.

All the alleles evident for the eyestalks, but no additional alleles, were found again in the muscle (behind the thelycums) (Table 1, full data given in Supplementary Table 2), notwithstanding one missing case, which is consistent with the repeatability of the DNA markers and also suggests that contamination of this tissue by sperm, if it occurs, is infrequent.

In half of the samples spermatophores could be identified. In these samples, the number of alleles recorded ranged from two to four (Table 1, full data given in Supplementary Table 3). That is, only DNA from one male and one female are needed to account for the allele numbers and there is no evidence for two males and one female. After accounting for the alleles found in the eyestalk, the residual number of alleles in the spermatophores ranged from zero to two only.

The number of alleles at a given locus in the 16 pellet samples ranged from 1 to 4 (again, indicating only one male), and from 0 to 2 after accounting for alleles in the eyestalk (Table 1, full data given in Supplementary Table 4).

For a given tissue and genotype, it is possible male polygamy exists but was not detected because the hypothetical second male shared alleles with either the female or the first male. Assuming, in the first instance, that in every case there is a second male contributing equal amounts of detectable DNA as the first, then the probabilities of failing to detect it can be estimated.

For example, in the case of locus one, sample one, in the pellet sample (Supplementary Table 4) the genotype detected was 119,128, 134,152. There are 13 alleles detected at this loci in the eyestalks with frequencies ranging from 0.031 to 0.188, which we assume are typical for the population (Table 2).

The chance the hypothetical second male has a detectable allele for its first allele is the sum of frequencies of alleles 113, 116, 125, 131, 137, 143, 146, 149, 155 which is 0.594, so the chance of not detecting the hypothetical second male considering the first allele is 1-0.594 or 0.406. The chance of failing to detect at the first *and* the second allele is 0.406^2 or 0.165. The calculation can be made in a different way, using all the expected genotype frequencies for loci one, and the results are the same.

For loci 1, eight of the 16 pellet samples had four alleles (Table 1) and following the above argument, the chance of failing to detect the second male is the product of all the individual probabilities of failing to detect, which is 1.15468×10^{-07}. Certainly considering more loci and even cases with fewer than four alleles would further diminish the calculated probability. On the other hand, if polygamy is less than 100%, then these probabilities would be increased. If the second male contributes less or undetectable amounts of DNA, then no evidence for polygamy would be adduced.

Comparing the accuracy of pedigrees assuming polygamy or monogamy

First, we considered data just from the 73 dams. Sib (sister) groups for the 73 dams were formed by analysis of the dam genotypes with the COLONY software first under the assumption of monogamous mating for both dams and sires. There were 37 potential dams from mating group A and 36 potential dams from mating group B, which each group representing different lineages. 121 pairs of dam sibs (sisters) were identified assuming mongamy at P>0.95 based on DNA microsatellites (Table 3; note, a given animal can be present in more than one pair or dyad) and no sibs were identified between mating groups.

Seventy-two of the 73 dams were haplotyped, and of these, 24, 2, 28, 11, 8 and 1 had haplotypes 1, 2, 3, 4, 5 and 6 respectively. Haplotypes 1, 2, 4 and 6 occurred exclusively in group A animals (for both dams and offspring), and haplotypes 3 and 5 occurred exclusively in group B (dams and offspring).

Of the 121 pairs of full sibs identified above, there were mtDNA sequence data for 119 pairs of sibs. Each of the 119 sib pairs had the same mtDNA sequence. That is, there was no evidence under the assumption of monogamy, that incorrect sib groups were being formed.

These analyses for dams were repeated using COLONY and assuming polygamy for dams and monogamy for sires. Detectible polygamy was considered unlikely for sires, since of the 37 or 36 dams per mating group are taken from a communal tank of hundreds of same age mixed sex animals, so the chance biologically for finding polygamy for males would be quite low on the grounds that if a given male mates e.g. twice, the second female is unlikely to be sampled in the group of 37 or 36. Assuming polygamy for females, 110 pairs of full sibs and 73 pairs of half sibs were identified at P>0.95 (Table 3). Of the 108 full sib pairs, there were eight pairs of sibs that did not have the same mtDNA haplotypes, although all identified sib pairs were from the same mating group (A or B). Of the 73 half sib pairs, there were 28 pairs that did not have the same mtDNA haplotypes, although most of these (24) were from the same mating group (A or B).

Since many animals share the same mtDNA haplotype, it is possible the number of actual pedigree errors under the assumption of polygamy was substantially underestimated.

Secondly, we considered data both the 400 genotyped offspring and the 73 genotyped dams in the COLONY analysis. Three hundred and fifty-five of the 400 offsrping were also haplotyped, and of these, 113, 16, 152, 51, 22 and 1 had haplotypes 1, 2, 3, 4, 5 and 6 respectively. Haplotypes 1, 2, 4 and 6 occurred exclusively in group A animals, and haplotypes 3 and 5 occurred exclusively in group B.

Assuming monogamy, 1792 full sib dyad offspring pairs were found with a P > .95 (from COLONY full sib dyad output) of which 1409 pairs were available where both members of the pair had mtDNA data (Table 3). Of these 1409, no pairs had different mtDNA sequences.

Under the assumption of polygamy, 1562 and 553 full and half sibs respectively were identified with a high probability (P>.95 in full or half sib dyad output). Of these, 1207 and 427 pairs were identified where both members of the pairs had mtDNA data. There were 3 full sib pairs that had different mtDNA haplotypes but 63 half sib pairs that had different mtDNA haplotypes.

Last, we considered offspring-dam assignments assuming either monogamy or polygamy for dams and monogamy for sires (Table 3). Under the assumption of monogamy, 380 dam-offspring pairs were identified at P > 0.95 and for 267 of these, both pairs were haplotyped. Of the 267, there were 2 pairs with dissimilar haplotypes. Assuming polygamy for the dams, very similar results were evident (Table 3).

Contribution of females to offspring under mass spawning

There were 37 dams eye-stalk ablated and spawned to produce the A group, of which 200 offspring were sampled. Assuming monogamy, and considering only maternity assignments with P>.95 from the

COLONY “maternity” output, 29 of the 37 dams or 78.4% had progeny detected by the DNA genotyping and 192 of the progeny could be assigned (Figure 1). Similarly, for group B, 27 out of 36 or 75.0% of potential dams were detected, and 188 of the 200 progeny were assigned (Figure 1).

Considering only those females with offspring, the mean count of pond A dam offspring groups was 6.62±0.76 (std. error) and was not statistically different from the average count for pond B which was 6.90±1.08. Neither (square root transformed) counts nor average proportions (after arc sine square root transformations) per dam varied between the two ponds (assessed using ANOVA). The variance of counts was not different between pond A and pond B (Levene statistic); similar results were found for proportions.

Discussion

This report, specifically the absence of evidence of DNA from a second male in a given female, does not refute the hypothesis that female banana shrimp carry sperm from only a single male at a given time, and consequently, females probably tend to be monogamous within a given moulting cycle or spawning event. This is the first time DNA markers have been used to test hypotheses of monogamy vs polygamy in crustaceans. The lack of evidence for contemporaneous polygamy is consistent with a variety of behavioural studies in crustaceans (Cowan and Atema, 1990; Mathews, 2002; Baeza et al., 2011; Marsden et al., 2013). The present study did not test the possibility of sequential polygamy for females over time, for example in different moult cycles. Sequential polygamy would almost certainly be the case if the females were to have multiple spawns over various moult cycles. We did not specifically investigate polygamy in males.

Following on from the results that no DNA from a second male was evident, our expectation was that pedigrees built assuming contemporaneous monogamy for females should be more accurate than those assuming polygamy. Accuracy of the pedigree was tested using the criterion whether sib groups, or dam-

offspring groups, determined by their genotypes, shared the same mitochondrial haplotype. This method of testing can miss some real errors, because there were only a total of 6 haplotypes shared amongst 73 dams and 400 offspring, so some non sibs could have the same haplotypes. Even so, it was evident that there were more “errors”, or cases where sib or dam-offspring groups failed to share a common haplotype, in pedigrees built assuming polygamy, with “errors” evident for both full and half sibs. To some degree, the “errors” detected for the half sibs may also relate to the power of the genotyping using 10 loci, so perhaps the “fairest” comparison is that for the full sibs. Overall, these results for the accuracy of pedigrees support the conclusions from previous investigations of the female thelycum samples, i.e. that banana shrimp females are monogamous at a given time. There is also the possibility that scoring errors of the genotypes, and that of haplotyping, confounded to some extent the results. However the set of nine DNA microsatellite loci were selected from many candidates on the basis of their quality of scoring (e.g. consistent and stable peaks) and all alleles detected in the eyestalk were redetected in the tissue around the thelycum, suggesting a very high repeatability of the DNA markers. This repeatability was confirmed by re-genotyping known pedigree samples, including four samples that were repeated at least seven times, for all nine primers, where genotypes were consistently scored each time (Knibb unpublished).

There are various implications arising from this study for the future application of genetic programs for this species, which are probably general for shrimps and crustaceans. First, under the present mating arrangements (mass communal matings, spawning of selected females over one or several nights), the genetic structure of the pedigree would be all full sibs due to the monogamous nature of females at a given spawning. Genetic analysis of full sib pedigrees, at least shallow ones, has some limitations, in that maternal effects and common environment effects cannot be separated from genetic effects. Artificial insemination and the formation of half sib families could overcome these limitations. Second, some DNA information of the sire is recoverable from the dam, and, with many loci, this leaves open the possibility of adducing the sire’s genotype which would assist in the development of pedigrees. Third, about half of the offspring in the pedigrees originate from only 3% or so of the dams, so there are substantial unequal contributions of dams to the next generation. Many dams contributed very few offspring to the pedigree. These patterns were consistent in the two different cohorts, and so could be a general feature. Selection

of broodstock from the cohorts over generations for selective breeding, could result in loss of families, because the cohorts derive from few families. Development of full pedigrees, where pedigree information is available for each animal, could avoid the loss of families. Using these data, we can also predict that in order to secure about 30 families each with a minimum of 20 offspring per family for estimation of genetic parameters, we would need to sample at least 2,000 offspring per cohort.

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Table 1. Alleles in different dam tissues for each DNA microsatellite locus.

		DNA microsatellite Loci								
Tissue Type	Number of alleles observed	1	4	5	11	14	47	52	56	57
Dam eye stalk (n=16)	1 allele	0	2	3	7	6	8	10	4	11
	2 alleles	16	14	13	9	10	8	6	12	5
Dam, muscle behind the thelycums (n=16)	1 allele	0	2	3	7	6	8	10	4	11
	2 alleles	16	13	13	9	10	8	6	12	5
Spermatophores (n=8).	1 allele	0	2	0	2	4	3	2	1	6
	2 alleles	2	3	3	5	3	4	6	5	1
	3 alleles	1	1	2	0	0	1	0	1	1
	4 alleles	4	1	1	1	0	0	0	1	0
Spermatophores (n=8) after alleles found in the eyestalk are removed.	0 alleles	2	4	1	5	6	6	5	4	7
	1 allele	1	2	2	2	1	2	3	3	1
	2 alleles	4	1	3	1	0	0	0	1	0
Pellet (n=16)	1 allele	0	0	2	4	4	4	5	1	9
	2 alleles	1	12	6	11	6	8	11	7	6
	3 alleles	7	2	3	0	6	3	0	3	1
	4 alleles	8	2	3	1	0	1	0	5	0

Pellet (n=16) after alleles found in the eyestalk are subtracted	0 alleles	1	8	4	12	8	8	11	5	13
	1 alleles	7	6	6	3	8	7	5	6	3
	2 alleles	8	2	4	1	0	1	0	5	0

Table 2. Allele frequencies for locus FMG001 from 16 eye stalks.

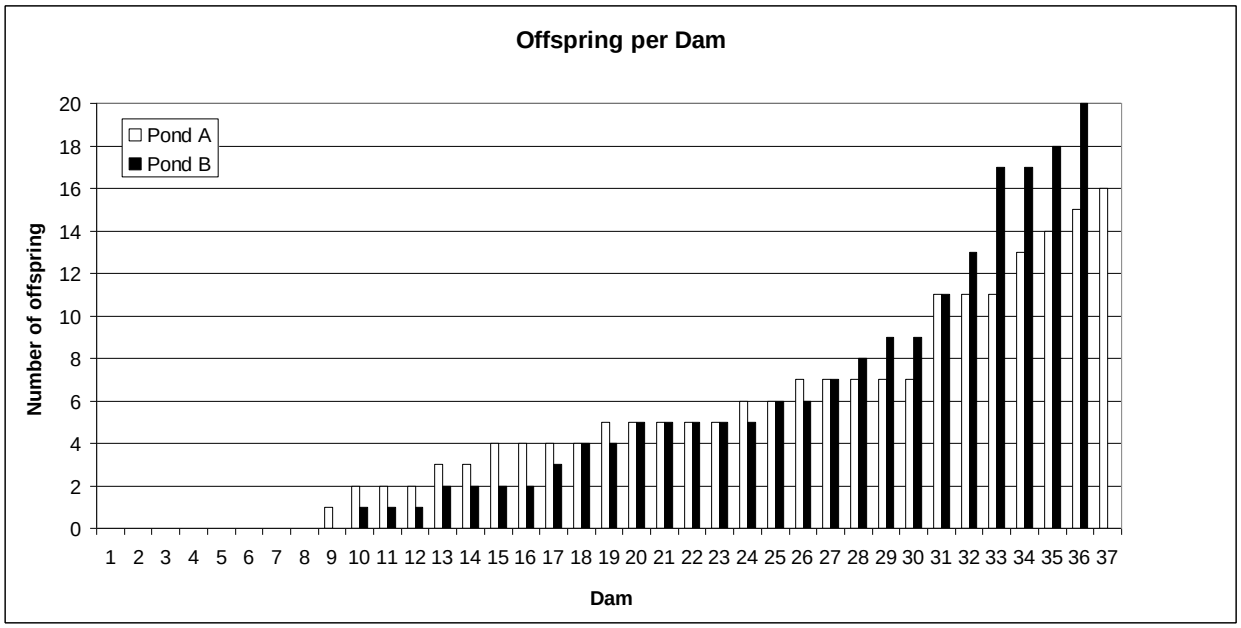
Allele	Frequency
113	0.031
116	0.031
119	0.031
125	0.031
128	0.125
131	0.063
134	0.063
137	0.125
143	0.063
146	0.063
149	0.063
152	0.188
155	0.125

Table 3. Accuracy of pedigrees formed assuming monogamy or polygamy.

	Assuming	Sib type	Number of sister†, sib††, or parent offspring pairs††† identified with P>.95	Number of assignments where both have mtDNA haplotypes	Number of pedigree errors detected by conflicts of mtDNA haplotypes
Sisters among the dams	monogamy	full sib	121	119	0
	polygamy	full sub	110	108	8
	polygamy	half sib	73	73	28
Offspring sibship pairs estimated using both offspring and parental dams	monogamy	full sibs	1792	1409	0
	polygamy	full sibs	1562	1207	3
	polygamy	half sibs	553	427	63
Parent-offspring assignments (using COLONY "maternity" assignments)	monogamy		380	267	2
	polygamy		377	260	4

†for sisters among the dams, †† for offspring sibship pairs, ††† for parent-offspring assignments

Figure 1. Note there were 37 dams in pond A and 36 different dams in pond B, so a given number in the graph refers to two different females.



-novel data: first data testing polygamy vs monogamy in shrimp, and first using molecular tools to resolve this question in crustaceans.

-significance: knowledge of monogamy or polygamy critical to interpreting pedigrees and developing breeding programs

-solves a biological problem important to aquaculture: the between family fecundity estimates permit efficient design of breeding program and estimation of genetic parameters

-of general significance for the biology of crustaceans and has practical outcomes for aquaculture

-major findings and implications: For a given moult cycle, female banana shrimp appear to monogamous, i. e. only have one mating. Thus, progeny from mass matings at a given time will form full sib groups, and as such, some non genetic effects may be included with genetic effects when trying to estimate genetic parameters from this type of pedigree structure. Also, pedigrees formed using COLONY software were more accurate when monogamy was assumed than when polygamy was assumed. Last, about 3% of the dams gave rise to about half of the offspring, suggesting that without pedigree management, the diversity of families and alleles could rapidly be lost, and that very large sample sizes are required to estimate genetic parameters with accuracy.