Rates of inbreeding using DNA fingerprinting in aquaculture breeding programs at various broodstock fitness levels — a simulation study

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Abstract. A simulation study was used to examine the potential use of DNA fingerprinting (DNA tagging) as a tool to avoid excessive inbreeding by identifying suitable candidate breeders in genetic selection programs. 'Broodstock fitness' (the ability of broodstock to survive from harvest and reproduce) needs to be considered in designing breeding programs using DNA tagging. In this study, reduced broodstock fitness increased inbreeding exponentially. The level of inbreeding was also dependent on the intraclass correlation (t), selection intensity, number of individuals DNA tagged (N_{DNA}), number of families maintained (N_f) and the number of candidate breeders retained per sex/family at harvest (C). With a broodstock fitness of 0.90, DNA tagging could theoretically achieve a selection intensity, in terms of the total phenotypic variance, of 2.90 standard deviations with 800 000 graded at harvest, while maintaining an inbreeding rate of 1.0% per generation (N_{DNA} = 800, N_f = 30, C = 4, t = 0.3). In practice, the numbers required could be achieved by growing families in individual facilities (e.g. sea cages for barramundi or ponds for prawns). When mechanical grading is not possible, the selection pool may be limited to a level where physical tagging is feasible. In this case, there was no advantage in selection response using DNA tagging compared with physical tags. DNA tagging as a selection tool may be more feasible when broodstock fitness is above 0.6, and may fill a niche where industry infrastructure is not large enough to support separate rearing of families or where physical tagging is not economically viable or suitable. DNA tagging may also be useful as a means of recovering families in backup facilities where families have been pooled to reduce infrastructure costs. Due to the random nature of DNA sampling, not all families may be recovered and a reduction in selection pressure may facilitate family recovery.

Additional keywords: selection, tagging.

Introduction

In theory, high selection intensities can be applied in highly fecund aquaculture species. For example, prawns (*Penaeus* sp.) may yield a million nauplii (Arcos *et al.* 2004; Menasveta *et al.* 1994), and barramundi (*Lates calcarifer*) yield several million eggs per spawn (Davis 1984). The challenge in aquaculture is to implement practical breeding programs to maximise selection response through high intensity selection, while managing inbreeding. The management of inbreeding is essential for commercial selection programs because inbreeding can reduce selection response by more than one third (Bentsen and Olesen 2002), and has been responsible for reduced productivity in aquaculture (Eknath and Doyle 1990; Gjerde *et al.* 1983; Keys *et al.* 2004; Kincaid 1976; Pante *et al.* 2001). A maximum level of inbreeding per generation of between 0.5 and 1.0% is generally regarded as suitable for long-term genetic gain, with less than 1.0% per generation recommended by Goddard (1992), Bentsen and Olesen (2002), Meuwissen and Woolliams (1994) and 0.5% recommended by (Nicholas 1989).

Mass selection and best linear unbiased prediction (BLUP) are generally not feasible in aquaculture breeding programs because high selection intensity often leads to the selection of a limited representation of families. This results in either excessive inbreeding over time (Sonesson *et al.* 2005), or a substantial loss of selection response from the necessity to re-introduce wild families to reduce inbreeding. In practice, selection programs not using tagging to identify families can fail to achieve the predicted response and selection intensity may need to be restricted to avoid deleterious inbreeding (Bentsen and Olesen 2002).

Within-family selection is an efficient strategy to maintain acceptable rates of inbreeding while applying high selection intensities, because the effective population size can be twice the number of parents. The difficulty in applying within-family selection is the necessity to identify family groups, requiring families to be raised in isolation till broodstock stage or communal rearing with some form of family identification. Traditionally, family identification has been achieved in fish by growing larvae to a size large enough to allow tagging using methods such as ink or freeze

branding, fin clipping, external tags (Bentsen and Gjerde 1994) or visual elastomer implant tags (Arce *et al.* 2003). Another method of tagging animals is through DNA fingerprinting (DNA tagging) to assign individuals to parents. Doyle and Herbinger (1994) proposed the use of DNA tagging as a means of achieving high intensity selection with acceptable inbreeding levels, by maximising the identification and recovery of families and minimising between-family environmental variation. One advantage of DNA tagging is that family groups can be mixed at a stage well before physical tagging, eliminating the need for separate family nursery facilities. In Australia, there has been interest in developing and promoting DNA tagging techniques (Jerry *et al.* 2004; Li *et al.* 2003; Wilson *et al.* 2002) for potential use in aquaculture breeding programs.

The simulation study reported in this paper expands on the concept developed by Doyle and Herbinger (1994), and examines the sensitivity of variables affecting rates of inbreeding when using DNA tagging as a tool for family identification. Unlike physical tagging where family pedigrees can be determined immediately, DNA tagging is based on random family sampling of candidate breeders with their pedigree determined from laboratory analysis of a tissue sample. The probabilities of sampling sufficient numbers in different families can easily be assessed through the use of computer modelling to determine the practical application of DNA tagging. This was achieved by predicting the effect on inbreeding under a range of scenarios including levels of survival and fertility of broodstock from harvest to breeding age.

Materials and methods

The model assumed a normal distribution between and within families. Selecting animals at 1 tail of a distribution (e.g. growth) will often lead to a limited number of families represented with unacceptable rates of inbreeding. Variables thought to influence rates of inbreeding were included in the model such as; proportion selected (P), phenotypic intraclass correlation (t), number of individuals DNA tagged (N_{DNA}), number of animals per family/sex retained as candidates for breeding (C) and the number of families in the breeding population (N_f) were considered by this simulation.

Nomenclature

The notation used is: t, phenotypic intraclass correlation — a measure of the between-family group variance over the total variance (Falconer 1972); r, the correlation of breeding values between members of families; h^2, heritability of individual values; c^2, proportion of variance contributing to family means; H, total harvest size at selection; P, proportion selected at harvest (e.g. right-hand side of distribution); C, maximum number of animals per sex/family retained as candidates for mating, with culling implemented once DNA fingerprinting results known to reduce costs of maintaining broodstock; N_{DNA}, number of DNA tagged animals ($N_{DNA}<HP$); N_m, number of male broodstock desired in the breeding population; N_f, number of female broodstock desired in the breeding population (equal to number of families in a monogamous population); X_y, phenotypic mean of family y; A_{ym*} (A_{yf*}), number of male (female) sibs identified using DNA tagging in family y; A_{ym} (A_{yf}), number of male (female) sibs retained as candidates for potential breeding at harvest which is a subset of A_{ym*}

(A_{yf*}) culled to C; T_{ym*} (T_{yf*}), number of reproductively fit male (female) sibs surviving to breeding age in family y which is a subset of A_{ym} (A_{yf}); T_{ym} (T_{yf}), number of male (female) sibs from family y contributing to the next generation which is a subset of T_{ym*} (T_{yf*}); R_m (R_f), total number of males (females) contributing to next generation over all families which is the sum of T_{ym} (T_{yf}); σ_m^2 (σ_f^2), variance of male (female) sib numbers per family contributing to next generation; $\sigma_{m,f}$, covariance of male and female sib numbers from each family contributing to next generation; σ_B^2 (σ_W^2) between-family (within-family) variance component; i_{Bs} (i_{Ws}) standardised selection intensity in terms of total phenotypic variance for between-family (within-family) selection.

The parameters A_{ym*}, A_{ym}, T_{ym*} are intermediate steps used in the simulation to determine the number of males from family y which pass on genes to the next generation (T_{ym}), the sum of which is the total number of males contributing to the next generation (R_m).

Family sampling

The model assumes discrete generations with monogamous males and females with sampling of families achieved assuming equal family stocking and survival across all families. Phenotypic means X_y, of each family y were sampled from the between-family variance component (σ_B^2) as $X_y \sim N(0,\sigma_B^2)$, where $\sigma_B^2 = t \times V_P$ with t being the intraclass correlation and V_P the total phenotypic variance (Falconer 1972). The deviate X_y was scaled to standard deviation units of the within-family variance component (σ_w^2), as X_y/σ_w where $\sigma_w^2 = (1 - t)V_P$. The desired proportion selected in the population P, was equal to the average proportion selected from all N_f families determined from:

$$P = \sum_{y=1}^{Nf} \left[1 - \Phi(x - X_y/\sigma_w)\right]/N_f \qquad (1)$$

where $\Phi()$ is the cumulative distribution function of the standard normal distribution and x is the common truncation point on phenotype across all families solved by iteration to give the desired P. From the total harvest size H, the number of animals available for DNA sampling was HP. The number of N_{DNA} tagged animals was randomly chosen from a subset of HP as not all animals selected were DNA tagged. The initial numbers available in each family, $HP.[1 - \Phi(X_y + x)]$ were adjusted to emulate sampling without replacement for each DNA sample taken to give the total number available A_{y*} within each family. The number of males were determined from random sampling of the binomial distribution using $A_{ym*} \sim binomial(A_{y*}, 0.5)$ which assumes an equal sex ratio. The number of females were then determined as $A_{yf*} = A_{y*} - A_{ym*}$. The number of males ($A_{ym*}$) and females ($A_{yf*}$) retrieved within each family using DNA tagging were truncated to a maximum number of candidates per sex/family (C) to give the number of males (A_{ym}) and females (A_{yf}) retained for potential breeding in family y.

Family matings

Not all animals will survive from harvest to breeding age nor will they all be fertile, the product yielding the probability of 'broodstock fitness' (B). The number of fertile males (T_{ym*}) and fertile females (T_{yf*}) available at breeding age for each family y were determined as $T_{ys*} \sim binomial(A_{ys}, B)$ of sex s (m or f). The level of broodstock fitness was assumed constant across all families for each level of broodstock fitness simulated.

Matings were managed by attempting to equalise the total number of sibs represented by each family in order to reduce inbreeding. The preference was to sample 2 sibs from each family to replace their parents. By chance both sexes were not always available within each family and numbers were supplemented from the opposite sex or other families where available.

With poor broodstock fitness, the total number of fertile males available at breeding age from all families may be less than the number

of desired males N_m. It may also be possible that there are more males than females resulting in surplus males to discard. Males finally retained for breeding were chosen from those families which had the least number of sibs represented to give the number of male broodstock used for breeding T_{ym} (with similar procedures for females to give T_{yf}). The total number of viable male (female) broodstock used as breeding replacements R_m (R_f) was determined from the sum of T_{ym} (T_{yf}). Vectors T_{ym} and T_{yf} had variances σ_m^2 and σ_f^2 respectively with covariance $\sigma_{m,f} = \sigma_{f,m}$.

Effective population number and inbreeding

The effective population size N_e was based on a drift variance model by Hill (1979), equation 9:

$$\frac{1}{N_e} = \frac{1}{16R_m}\left[2 + \sigma_{mm}^2 + 2\left(\frac{R_m}{R_f}\right)\sigma_{mm,mf} + \left(\frac{R_m}{R_f}\right)^2 \sigma_{mf}^2\right]$$
$$+ \frac{1}{16R_f}\left[2 + \sigma_{ff}^2 + 2\left(\frac{R_f}{R_m}\right)\sigma_{fm,ff} + \left(\frac{R_f}{R_m}\right)^2 \sigma_{fm}^2\right] \quad (2)$$

where σ_{sm}^2 (σ_{sf}^2) is the variance in the number of viable males (females) used as broodstock from parents of sex s (m or f) and $\sigma_{sm,sf}$ is the covariance of the number of male and female offspring from parents of sex s.

In this simulation, the animals used as breeder replacements (R_m and R_f) are without parental pedigree information. We know that male and female contributions to the next generation will have sires represented in more than one family if R_m is less than N_m (similarly for dams). By assuming parents are sampled for breeding in a similar way to their offspring, the ratio of desired:recovered male (female) broodstock retrieved from progeny N_m/R_m (N_f/R_f) was used to infer the genetic contribution of sires (dams) to produce female and male progeny with variance components:

$$\sigma_{mm}^2 = \sigma_m^2\left(\frac{N_m}{R_m}\right)^2, \ \sigma_{ff}^2 = \sigma_f^2\left(\frac{N_f}{R_f}\right)^2, \ \sigma_{mf}^2 = \sigma_f^2\left(\frac{N_m}{R_m}\right)^2$$

$$\text{and } \sigma_{fm}^2 = \sigma_m^2\left(\frac{N_f}{R_f}\right)^2$$

and covariances

$$\sigma_{mm,mf} = \sigma_{fm,ff} = \frac{\sigma_{m,f} N_m N_f}{R_m R_f}$$

which leads to

$$\frac{1}{N_e} = \frac{1}{16R_m}\left[2 + \sigma_m^2\left(\frac{N_m}{R_m}\right)^2 + \frac{2\sigma_{m,f}N_mN_f}{R_f^2} + \sigma_f^2\left(\frac{N_m}{R_m}\right)^2\right]$$
$$+ \frac{1}{16R_f}\left[2 + \sigma_f^2\left(\frac{N_f}{R_f}\right)^2 + \frac{2\sigma_{f,m}N_fN_m}{R_m^2} + \sigma_m^2\left(\frac{N_f}{R_m}\right)^2\right] \quad (3)$$

The rate of inbreeding per generation ΔF, was calculated using the classical formulae: $\Delta F = 1/(2N_e)$ (Falconer 1972) using the harmonic mean of N_e determined from 50 iterations.

Selection intensity

The average within-family selection intensity (i_W) was determined by weighting contributions from each family and sex (T_{ym} and T_{yf}) to the next generation from 3 stages of selection, (i) at harvest where *HP* animals are selected from *H* available, (ii) after DNA tagging where animals are truncated to the desired number of breeding candidates (from A_{ys*} to A_{ys}), and (iii) during mating where animals surplus to reproductive requirements are discarded (from T_{ym*} to T_{ym}). All 3 stages of selection were pooled assuming a genetic correlation between them of 1 (e.g. were selected on the same trait such as initial weight at harvest). Between-family selection intensity (i_B) was

measured as an average deviation of each family from the population mean weighted by the contributions from each family (T_{ym} and T_{yf}).

Standardised selection intensity of between-family (i_{Bs}) and within-family (i_{Ws}) selection expressed in terms of total phenotypic variance were determined as $i_{Bs} = i_B \times \sigma_B \times r/t$ and $i_{Ws} = i_W \times \sigma_W \times (1 - r)/(1 - t)$ given the phenotypic variance $V_P = 1.0$, and the correlation of breeding values between members of full sib families $r = 0.5$. The value ($i_{Bs} + i_{Ws}$) multiplied by the heritability (h^2) is equivalent to the total response to selection in standard deviation units.

Results

The results indicated that inbreeding was highly sensitive to broodstock fitness levels, intraclass correlation coefficients, number of breeding candidates selected, selection intensity, number of individuals DNA tagged and the number of families in the selected population. The rate of inbreeding per generation was a function of all combined parameters. The effect of each parameter is illustrated below while fixing others constant.

Effect of intraclass correlation (t) and broodstock fitness on inbreeding

The intraclass correlation had a large effect on the rate of inbreeding as shown in Figure 1 with 400 DNA samples. With an intraclass correlation of 0.30, a broodstock fitness of 0.5 gave a rate of inbreeding less than 1% per generation. Intraclass correlations of 0.4 and above achieved inbreeding rates above 1% at all broodstock fitness levels.

Effect of DNA sample size (N_{DNA}) and broodstock fitness on inbreeding

Increasing the number of individuals DNA tagged reduced the rate of inbreeding (Fig. 2). With 30 families and an intraclass correlation of 0.25, the rate of inbreeding was less than 1% with 200 individuals DNA tagged when broodstock fitness was greater than 0.75.
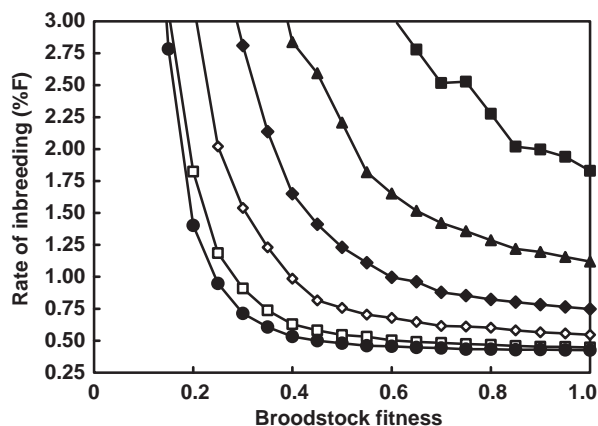

Figure 1. Inbreeding per generation at different levels of broodstock fitness and intraclass correlation $t = 0.05$ (●), $t = 0.10$ (□), $t = 0.20$ (◇), $t = 0.30$ (◆), $t = 0.40$ (▲), $t = 0.50$ (■), ($N_m = N_f = 30$, $N_{DNA} = 400$, $P = 1\%$, $H = 40000$, $C = 4$).
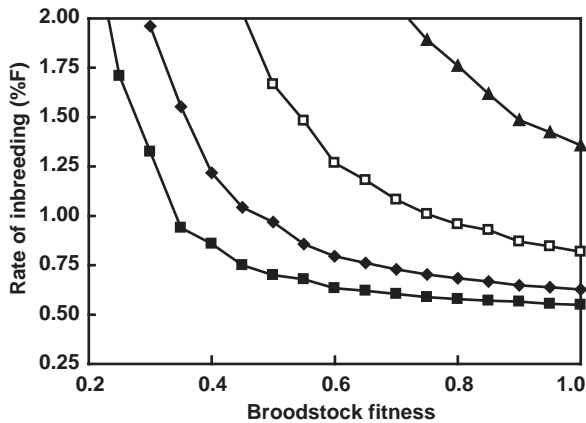
Figure 2. Inbreeding per generation at different levels of broodstock fitness and number of individuals DNA tagged 100 (▲), 200, (□), 400 (◆) and 800 (■), ($N_m = N_f = 3\,0$, $t = 0.25$, $P = 1\%$, $H = N_{DNA}/P$, $C = 4$).

Effect of candidate numbers (C) and broodstock fitness on inbreeding

Preferably 2 animals from each family will pass on their genes to the next generation but with poor broodstock fitness this goal may not be achieved as candidates may be infertile or die before breeding age. Increasing the number of candidates from 1 to 2 per sex and family, had a large impact on the rate of inbreeding at low broodstock fitness levels (Fig. 3). There was a diminished effect of reducing the rate of inbreeding with an increase in the maximum number of breeding candidates retained, with little difference from 8 to 16 candidates (Fig. 3).

Effect of family numbers (N_f) on inbreeding and broodstock fitness

When the intraclass correlation (*t*) was 0.25, increasing family numbers had its largest effect when broodstock
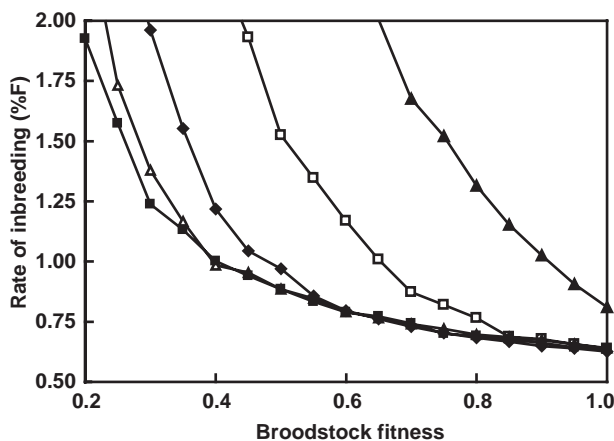

Figure 3. Inbreeding per generation at different levels of broodstock fitness and number of candidate broodstock retained for breeding 1 (▲), 2 (□), 4 (◆), 8 (△) and 16 (■), ($N_m = N_f = 30$, $N_{DNA} = 400$, $t = 0.25$, $P = 1\%$, $H = 40000$).

fitness was high (Fig. 4) and tended to converge as broodstock fitness was reduced. As before, an increase in the intraclass correlation gave an increase in the rate of inbreeding per generation.

Effect of subdividing families between different ponds on inbreeding and broodstock fitness

The effect on the rate of inbreeding by subdividing 30 different families into 1, 3 and 10 ponds (cohorts) while keeping the total number of individuals DNA tagged constant at 400 is shown in Figure 5. With 10 ponds there were 3 families represented and only 40 DNA samples in each pond. There was no advantage by subdividing families in different cohorts to reduce inbreeding without an increase in the total number of individuals DNA tagged across all ponds.

Use of DNA tagging and physical tagging with harvest size restricted to 10000

With up to 4 individual animals selected as candidates for breeding per family and sex (*C* = 4), and harvest numbers (*H*) limited to 10000, physical tagging and DNA tagging systems were compared at broodstock fitness levels from 0.3 to 0.8. Family numbers were adjusted using physical tagging to achieve an inbreeding rate of 1.0% per generation, while the number of animals DNA tagged were adjusted to achieve an inbreeding rate of 1.0% while maintaining 30 families (Table 1). There was no significant benefit through increasing the number of families as both between and within-family selection intensities competed for a limited number of animals performance tested. As expected, as broodstock fitness increased, selection intensity also increased. Fewer families were required using physical tagging to achieve the same inbreeding level as DNA tagging although little between-family selection was possible. With the same number of families as DNA tagging, physical tagging achieved the same selection intensities (selection response) as DNA tagging.
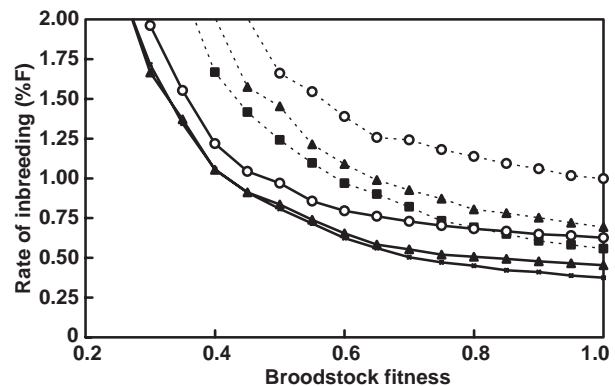

Figure 4. Inbreeding per generation at different levels of broodstock fitness with 30 (○), 50 (▲) and 75 (■) families and intraclass correlations of 0.35 (dotted line) and 0.25 (solid line), ($N_{DNA} = 400$, $P = 1\%$, $H = 40000$, $C = 4$).
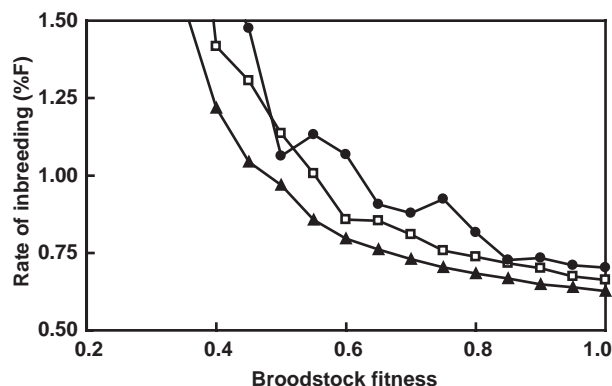
Figure 5. Inbreeding per generation at different levels of broodstock fitness with N_f families equally partitioned into either 1 pond (▲), 3 ponds (□) or 10 ponds (●). Total number of animals DNA tagged (N_{DNA}) was divided equally among X ponds with harvest size of each pond equal to $N_{DNA}/(X.P)$, ($N_m = N_f = 30$, $N_{DNA} = 400$, $t = 0.25$, $P = 1\%$, $C = 4$).

Selection limits of DNA tagging

The limits of DNA tagging were tested with an intraclass correlation of 0.30, DNA sample size of 800 and a breeding population of 30 families with up to 4 candidates per sex per family retained. Selecting the best 0.1 and 0.01% at harvest resulted in 800 000 and 8 million being graded. An inbreeding rate less than 1.0% per generation was achievable with a broodstock fitness of 0.9 or greater when selecting the best 0.1%, which in theory, achieved standardised selection intensity in terms of total phenotypic variance for within-family ($i_{Ws} = 2.33$) and between-family ($i_{Bs} = 0.57$) which was equivalent to the selection intensity in terms of within-family variance ($i_W = 3.89$) and between-family variance ($i_B = 0.62$). With a higher selection intensity of 0.01%, a broodstock fitness of 1.0 resulted in an inbreeding rate of 1.2% per generation with $i_{Ws} = 2.65$ and $i_{Bs} = 0.69$.

Using DNA tagging as a means of family recovery

The mean variance of sib numbers per family contributing to the next generation σ_s^2 of sex s (m or f), rose with reduced broodstock fitness and then declined as fewer sibs per family

Table 1. Standardised between-family (i_{Bs}) and within-family (i_{Ws}) selection intensity with system requirements using physical and DNA tagging to maintain an inbreeding rate of 1% per generation, with 10000 ranked at harvest with up to four candidate breeders for each sex within each family

Broodstock fitness	Tags N_{DNA}	Intraclass correlation (t)	Families N_f	Selection intensity		
				i_{Bs}	i_{Ws}	$i_{Bs}+i_{Ws}$
0.3	590	0.25	30	0.30	1.32	1.62[A]
0.4	350	0.25	30	0.49	1.37	1.85
0.5	270	0.25	30	0.55	1.41	1.96
0.8	170	0.25	30	0.67	1.51	2.18
0.3	905	0.35	30	0.26	1.40	1.67
0.4	505	0.35	30	0.41	1.47	1.88
0.5	390	0.35	30	0.49	1.51	2.00
0.8	255	0.35	30	0.57	1.62	2.19
0.3	>1000[B]	0.45	30	0.31	1.53	1.83
0.4	660	0.45	30	0.40	1.58	1.98
0.5	500	0.45	30	0.41	1.77	2.18
0.8	385	0.45	30	0.50	1.75	2.25
0.3	720	0.35	40	0.39	1.34	1.73
0.3	630	0.35	50	0.47	1.29	1.76
0.3	565	0.35	100	0.65	1.13	1.77
0.3	570	0.35	150	0.73	1.04	1.76
0.5	360	0.35	40	0.60	1.43	2.03
0.5	340	0.35	50	0.66	1.39	2.05
0.5	335	0.35	100	0.82	1.25	2.06
0.5	345	0.35	150	0.88	1.16	2.04
0.3	Physical	0.35[C]	19	0.02[D]	1.51	1.53
0.4	Physical	0.35	16	0.07	1.64	1.71
0.5	Physical	0.35	15	0.06	1.72	1.78
0.8	Physical	0.35	14	0.05	1.85	1.90

[A]Physical tagging will achieve these levels of selection intensity utilising both between and within-family selection.
[B]Rate of inbreeding achieved with 1000 DNA tags was 1.2%.
[C]Intraclass correlation used to estimate i_{Ws} and i_{Bs}.
[D]Limited between-family selection due to recovery of family losses.

of each sex were retrieved (Fig. 6). Highest values of σ_s^2 occurred with a high selection pressure at harvest ($P = 1\%$). Lowest values of σ_s^2 occurred with random sampling, which was effectively achieved when there was no selection pressure at harvest ($P = 100\%$). When broodstock fitness was greater than 0.80, random sampling resulted in the variance of sib numbers close to zero, with a mean of 2 sib numbers per family. An increase in σ_s^2 occurred in these unselected populations with a decrease in broodstock fitness as family losses were filled by representatives from surplus animals from other families, and then declined with fewer surplus replacements being available. When the intraclass correlation (t) was increased from 0.25 to 0.35, similar trends were observed with an increased sib variance.

Using the same parameters as Figure 6 ($N_m = N_f = 30$, $N_{DNA} = 400$, $t = 0.25$, $C = 4$), and a broodstock fitness of 0.4, the proportion selected at harvest (P) of 1, 5, 10, 20, 40 and 100% yielded standardised selection intensities ($i_{Ws} + i_{Bs}$) of 2.20, 1.72, 1.48, 1.21, 0.90 and 0.54, respectively, which included selection at both harvest (when $P<100\%$) and at postharvest when surplus animals were culled. Respective inbreeding at these P-values were 1.22, 0.82, 0.70, 0.62, 0.56 and 0.54%.

Discussion

The need to assign individual broodstock to parental families using DNA tagging, physical tagging, or separate family grow-out facilities, is an essential requirement of sustainable breeding programs with high selection intensity. In assigning individuals to parental families each method has a different set of risks and costs that need to be evaluated. Even when DNA tagging is possible, it does not mean that it is the most cost effective. There is no unique answer to all
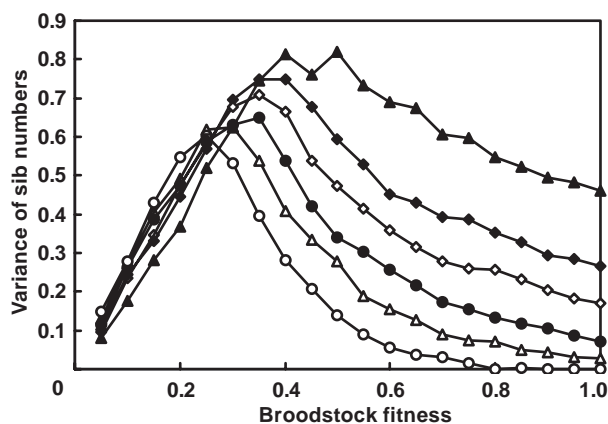

Figure 6. Variance of sib numbers within each sex contributing to the next generation, at different levels of broodstock fitness and at various harvest sizes (H) and proportions selected at harvest (P); $H = 40000$, $P = 1\%$ (▲); $H = 8000$, $P = 5\%$ (◆); $H = 4000$, $P = 10\%$ (◇); $H = 2000$, $P = 20\%$ (●); $H = 1000$, $P = 40\%$ (△); and $H = 400$, $P = 100\%$ (○), ($N_m = N_f = 30$, $N_{DNA} = 400$, $t = 0.25$, $C = 4$).

aquaculture species as many species have unique biological differences, however, it appears that DNA tagging may be more feasible as a selection tool when broodstock fitness is above 60%.

Inbreeding

Although there have been general recommendations that inbreeding per generation should not exceed 1% (Bentsen and Olesen 2002; Goddard 1992; Meuwissen and Woolliams 1994; Nicholas 1989), there is no single inbreeding rate that is suitable for all selective breeding programs. Other recommendations have been based on the lifespan of the breeding program, with cumulative inbreeding rates as low as 5–10% over that period (Tave 1999). If the expected lifespan of an aquaculture breeding program is 50 years, species with short generation lengths of about 1 year would require an effective population size of 250–500, which corresponds to inbreeding rates per generation of 0.4 and 0.2%, respectively. At these inbreeding rates it becomes important to manage broodstock fitness as a means of reducing overall infrastructure costs. With a 1% selection intensity and a broodstock fitness of 100%, a 0.38% inbreeding rate was just achieved in this study with 75 families using 400 DNA samples with a population having a low intraclass correlation of 0.25 (Fig. 4).

Reducing DNA costs

Optimising the number of families in a selection program can be one way of minimising DNA tagging requirements (Table 1). With too few families it becomes more important to retrieve most families to maintain inbreeding and this requires more DNA tags. With too many families more DNA tags will be required to limit inbreeding caused by increased between-family selection.

Mitochondrial DNA tagging and nuclear DNA tagging may have different cost structures of which the cheapest technology could be used. Figure 6 revealed that the variance of sib numbers contributing to the next generation may be greater than zero, meaning that female lines will be lost with substitutes from other families required to maintain the desired population size. These lost female lines will mean that not all families could be distinguished using mitochondrial DNA, which limits its long-term application in breeding programs.

Another possible way to reduce DNA costs was examined by splitting families into separate groups while maintaining the same number of DNA tags. The results from Figure 5 indicate that this does not result in a decrease in inbreeding. It appears that lower inbreeding will occur by increasing DNA tags in one pond, rather than splitting families into separate ponds and using the same total number of DNA tags.

Practical application to finfish breeding

In northern Australia, barramundi (*Lates calcarifer*) is the dominant aquaculture species, and in southern Australia

members of the Salmonidae family are dominant. Both of which are grown in sea cages. Each sea cage may have 30 000–40 000 animals that may be graded by mechanical means. The size of the sea cages provides a limit to achieving ultra-high selection differentials using DNA tagging. Therefore, it is unlikely that the ultra-high selection differentials simulated in this paper would be achieved using DNA tagging. In this situation very high selection intensities could be achieved with multiple cages randomly allocated per family without the need to rear communal families. Random environmental cage effects could be modelled with BLUP to assist between-family selection.

In practice, there are many biological issues that need to be considered when evaluating the suitability of DNA tagging for different species. Barramundi, which is a cannibalistic species with larger fish preying on smaller fish, provides a good example of some of the biological issues that may occur (Macbeth *et al.* 2002). Age differences as small as 24 h could give older fish a size advantage they never relinquish (Tave 1995) with small differences in fingerling weight in barramundi maintaining a distinct advantage (Rodgers and Bloomfield 1993). This may mean a reasonably high intraclass correlation (t). A value of $t = 0.58$ was used in a fish simulation by Doyle and Herbinger (1994) although it was unclear if this value was based on experimental data. With $t = 0.50$, 30 families would be insufficient to maintain inbreeding rate of 1.0% per generation even with a broodstock fitness of 1.0 (Fig. 1). Synchronising spawning of large numbers of families in barramundi is also a very difficult task. Even with progeny from a single spawn, a series of grading and mixing of fingerlings is required to reduce variation and associated cannibalism before communal rearing, a process that may result in some unfavourable selection or family loss. A detailed understanding of these biological issues is essential in the overall evaluation of DNA tagging as it may not be appropriate technology for all finfish species.

Practical application to prawn breeding

Partial harvest of prawn ponds is not recommended as it can suspend noxious sulphate compounds causing mortalities. This means that ponds are better harvested and sorted in one day. Unfortunately there is no mechanical means of grading live prawns as they are prone to handling stress, have appendages that inhibit the use of mechanical grading and are sexually dimorphic in size (Kenway *et al.* 2005). This necessitates the need for hand grading with 250–500 per person-day being achievable. This may mean a maximum harvest size of about 10 000, a number that is also possible to physically tag using elastomer tags (Arce *et al.* 2003). The rate of genetic response to selection using physical tags should be at least as good as DNA tagging. Physical tagging leads to easy measurement of family data with means and variances determined with the potential for

BLUP based between-family selection and more accurate assessment of genetic correlations with other traits such as survival or reproductive traits. From this perspective physical tagging may be more attractive than DNA tagging.

Effective management of inbreeding may be the difference between success and failure of a prawn breeding program, because once prawns are harvested from ponds and relocated into smaller tanks for breeding they can loose their condition over a period of a few weeks. Prawns should be mated and spawned as soon as possible making timely family identifications a necessity. Unfortunately female prawns cannot be mated until they moult which usually occurs around the new moon. Not all prawns will moult on the first new moon, so maintaining some family lines starts to become problematic. Once prawns moult they also need to mate and undergo natural or induced gonad maturation. Not all females will reach this stage, nor at the same time and the ones that do spawn may be infertile or spawn with insufficient nauplii to stock larval rearing facilities. These problems have made some species of prawns (and other newly domesticated aquaculture species) difficult to breed in captivity with broodstock fitness possibly below 0.20. At these low levels of broodstock fitness some margin for error may be required in designing a breeding program. Particularly since reproductive biology may also interact with seasonal variability, and inbreeding tends to increase exponentially with a reduction in broodstock fitness (Figs 1–4). As physical tagging may require fewer families to manage inbreeding (Table 1), it provides added opportunity to place selection pressure on reproductive traits to improve broodstock fitness using the same reproductive infrastructure required for DNA tagging.

Physical tagging does have disadvantages in prawns. Families will need to be grown to about 1–2 g before tagging can be easily achieved. During this period there may be some genotype by environment interactions between tank and pond grow-out of post-larvae to tagging at 1–2 g. However, these interactions are expected to be small and may be more than offset by some selection for growth at tagging age, as there is an estimated genetic correlation of 0.70 between 1 and 20 g harvest weight (M. Kenway, M. Macbeth, M. Salmon, C. McPhee, J. Benzie, K. Wilson, W. Knibb unpublished data). Another disadvantage of physical tagging is the logistical need for family sorting before grading. Perhaps individual ponds per family may be the ultimate alternative with no family tagging required, which in practice, may have the benefit of achieving much higher selection differentials than which could be achieved in a one pond system using DNA tags or physical tags as handling stress and mortalities may be reduced.

Model limitations

The model used in this study could be fine tuned in a number of ways. Most importantly, the model did not simulate

inbreeding from cumulative family losses over multiple generations and consequently the current model may have underestimated inbreeding particularly at lower survival rates. The model did not include the reduced effects of inbreeding through mutation (Wei *et al.* 1996). The model also assumed that there is no correlation in fertility, and also survival, between parents and offspring, which could potentially increase inbreeding. The model also assumed all families are stocked at equal densities with equal survival and departures from this assumption would also increase inbreeding.

Conclusions

Inbreeding was highly sensitive to broodstock fitness, the intraclass correlation, selection intensity, number of individuals DNA tagged, number of families maintained and the number of candidate breeders retained per family at harvest. DNA tagging should be technically feasible for high intensity selective breeding when broodstock fitness levels are above 60%, but may not be suitable in aquaculture species having broodstock fitness levels below 30–40% where physical tagging may be more appropriate.

Acknowledgments

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