Inbreeding among cultivated Atlantic cod (*Gadus morhua*) and its effects on offspring

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Background Information

- Atlantic cod farming in Eastern Canada is a developing industry and differs in several ways from salmon farming- ex. unlike farmed salmon, cod spawn in sea cages, releasing eggs into wild
- Due to broodstock selection process, often thousands of related fish in each cage ► inbreeding likely to occur, could have negative affects on offspring
- Cod in cages may not be local to cage site, and potentially unsuited to life outside the cage ► interbreeding with local conspecifics could result in outbreeding depression in hybrid offspring
- If effects of inbreeding are severe enough, may be used as a tool to mitigate effects of 'escapes through spawning' on wild cod populations

Tank Spawning Experiment

Question

- Do cultivated Atlantic cod inbreed in captivity?

Hypothesis

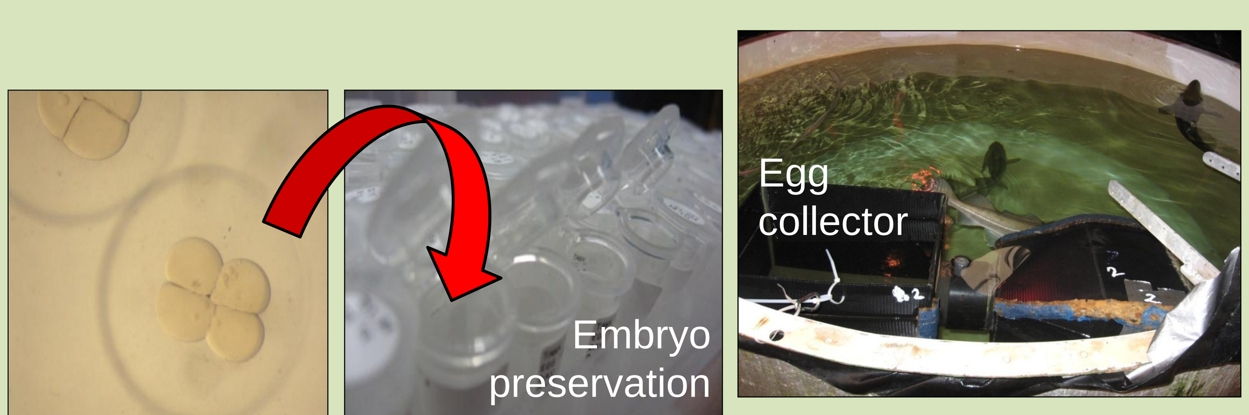
- Females will not discriminate against related males as partners when spawning. Inbreeding will occur.

Predictions

- Will see egg batches fertilized by brothers & unrelated males across the tanks

Methods

- Trios of 4-5 yr old cultivated Atlantic cod placed in 10 tanks
  - ►Sister, brother, unrelated male
- Matched by L, W, & condition factor
- Feb. – Mar. 2010 spawning season
- Tanks checked daily for egg batches
- Viable eggs collected using floating egg collector at top of water column.



↳ Minimum of 20 embryos from each batch preserved in ethanol for genetic analysis.

- 5 of the 10 tanks spawned
- Total of 19 batches (see Table 1)

Parentage Assignment

- DNA microsatellite analysis to determine embryo sire
- Four primers used: Gmo8, Gmo19, Gmo35, and Gmo37

Trio	Batch	Brother	Unrelated	G value, P value	G_H (df), P value
1	Feb 27	2	**44**	47.32, <0.001	na
2	*Pooled*	*66*	***310***	*171.91, <0.001*	*86.93 (9), <0.001*
2	Feb 15	1	16	15.96, <0.001	
2	Feb 16	0	36	49.91, <0.001	
2	Feb 17	1	13	12.2, <0.001	
2	Feb 19	0	24	33.27, <0.001	
2	Feb 23	3	38	35.37, <0.001	
2	Feb 25	1	28	31.50, <0.001	
2	Mar 2	2	46	49.91, <0.001	
2	Mar 9	12	53	27.97, <0.001	
2	Mar 11	25	23	0.08, 0.773	
2	Mar 15	21	33	2.69, 0.101	
3	*Pooled*	*1*	***101***	*130.16, <0.001*	*1.79 (2), 0.409*
3	Feb 11	0	21	29.11, <0.001	
3	Feb 12	0	39	54.07, <0.001	
3	Feb 20	1	41	48.77, <0.001	
4	*Pooled*	***27***	*4*	*19.13, <0.001*	*0.12 (1), 0.734*
4	Feb 7	18	3	11.89, <0.001	
4	Feb 28	9	1	7.36, <0.01	
5	*Pooled*	***70***	*27*	*19.74, <0.001*	*47.82 (2), <0.001*
5	Feb 10	0	15	20.79, <0.001	
5	Feb 11	36	4	29.45, <0.001	
5	Feb 17	34	8	17.32, <0.001	

Table 1. Batches produced & number of embryos each male sired (per batch & pooled for trio). Significant *G* value indicates one male fertilized significantly more eggs than other .

The heterogeneity *G*-test (G_H) was used to determine whether one male was dominant over all batches in the trio, where more than one batch was spawned. This was the case in trios three & four.

Results

- Inbreeding occurred in all tanks
- No clear difference in female mate choice

The Big Picture

- Cultivated, captive Atlantic cod do inbreed
- Could have escaped inbred embryos from cage spawnings
- May face slightly lower hatching success & smaller body depth, but overall, inbreeding not likely to ruin out survival entirely
  - ► potential for farmed-wild cod interbreeding.
- Farmed-wild hybrid offspring could face decreased fitness & outbreeding depression, damaging chances at survival.

Artificial Fertilization Experiment

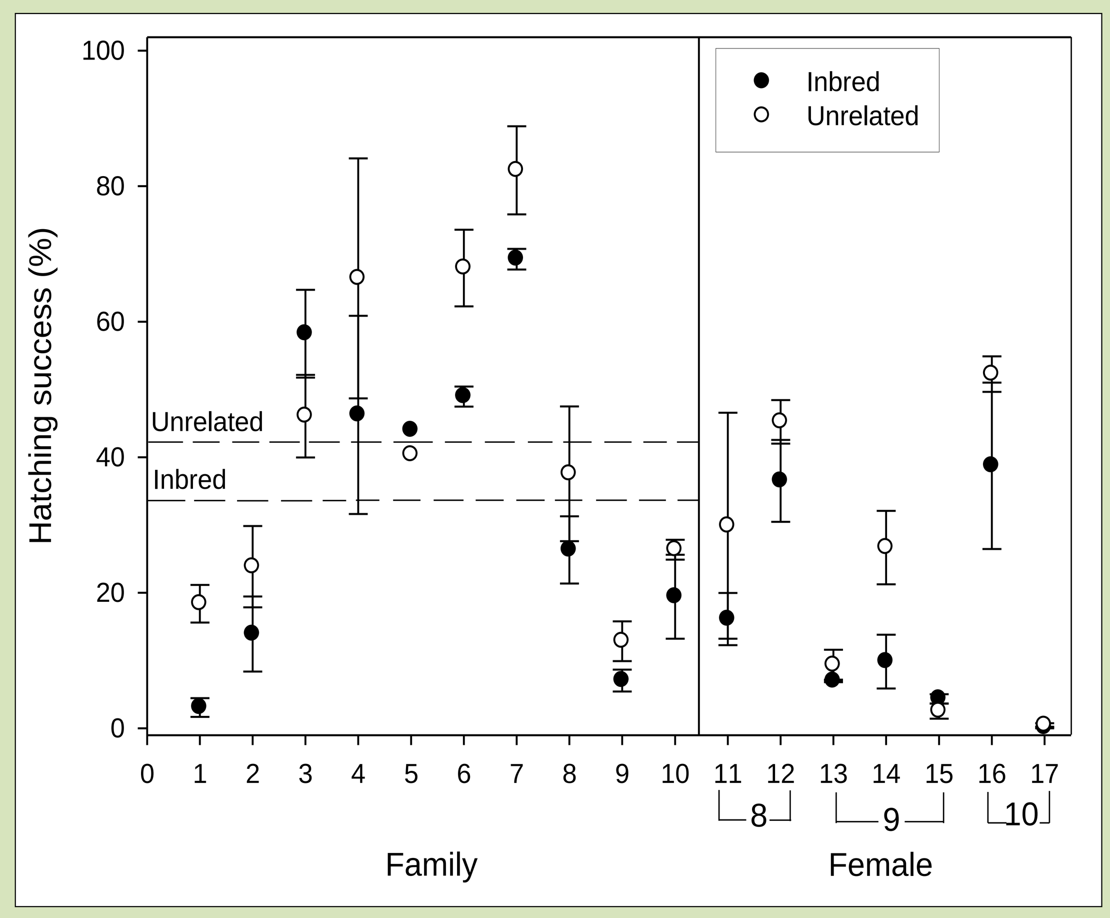


Figure 5. Mean hatching success (%) (standard error) of inbred (●) & unrelated (○) crosses. Dashed lines indicate mean value for cross type as labelled. Families with multiple females are broken down by female in right panel.

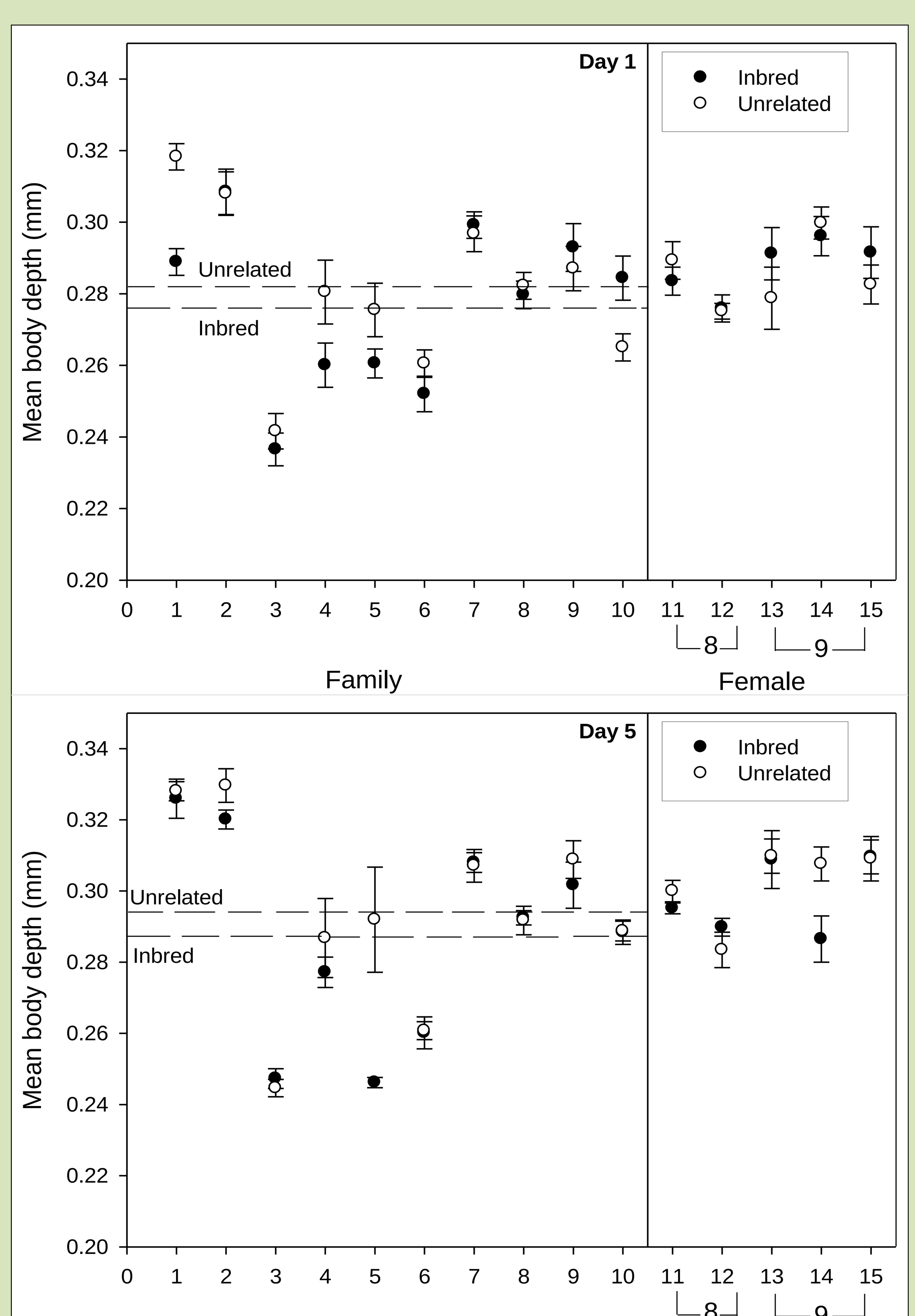


Figure 6. Mean body depth (mm) of inbred (●) & unrelated (○) crosses on days 1 & 5 (standard error). Dashed lines indicate mean value for cross type as labelled. Families with multiple females are broken down by female in right panel.

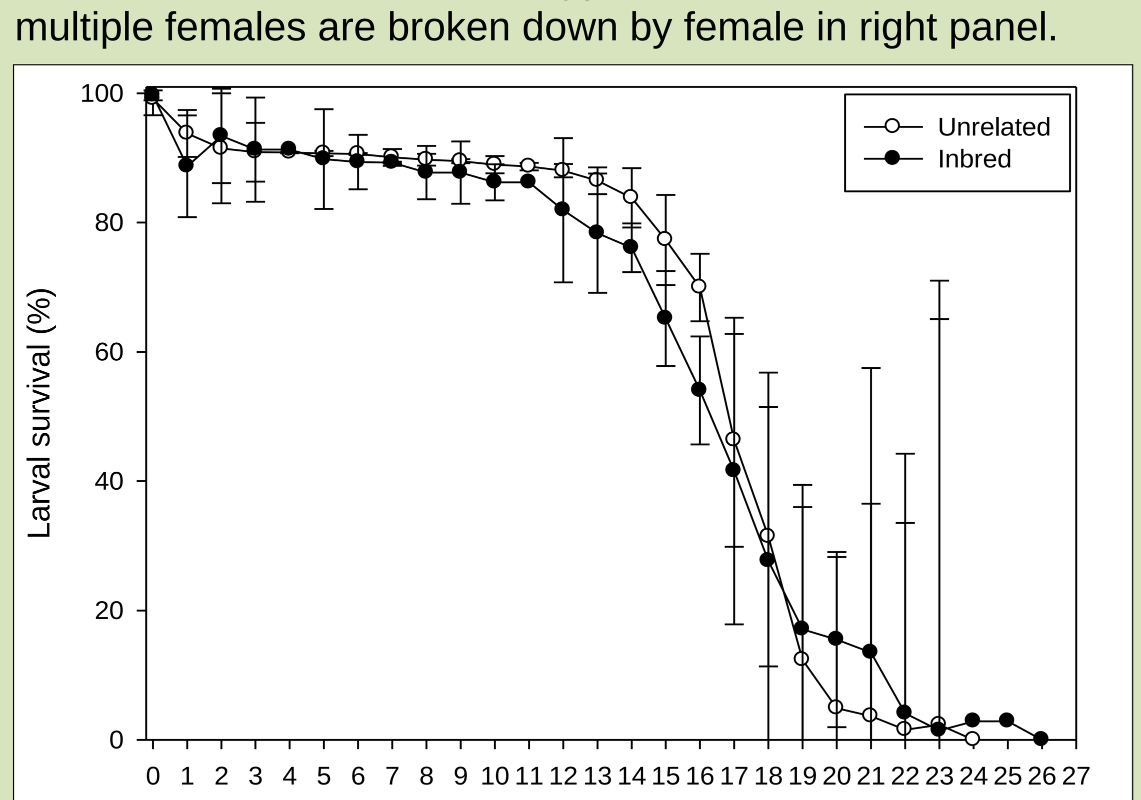


Figure 7. Mean cumulative larval survival (%) of offspring from inbred (●) & unrelated (○) crosses (standard deviation). Data was pooled from each cross until 100% mortality was reached.

Question

- What is the effect of inbreeding on Atlantic cod offspring?

Hypothesis

- Inbreeding causes reduced survival & quality of offspring.

Predictions

Compared to unrelated counterparts...

- Inbred embryos → lower hatching success
- Inbred larvae → higher mortality rate
- Inbred embryos and larvae → higher incidence of deformities

Methods

- Gametes manually stripped from cod of known genetic background (supplied by Huntsman Marine Science Centre, St. Andrews, NB, Canada)
- Artificial fertilization to make inbred & non-inbred offspring from each female (Figure 1)
- 14 females, 10 families represented

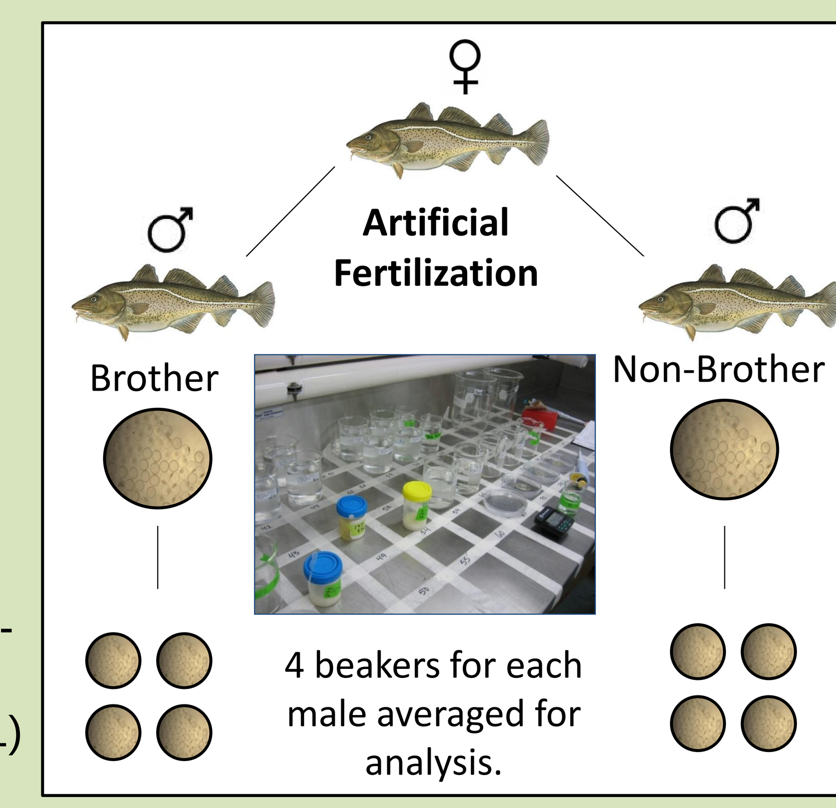
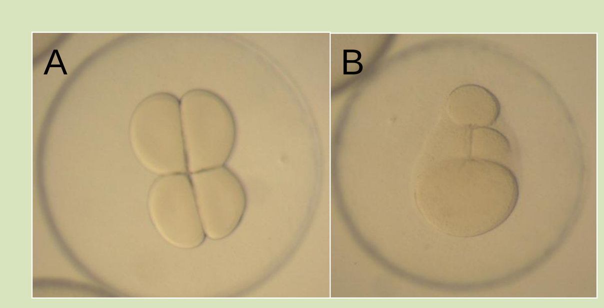


Figure 1. Experimental setup: female egg batch split, each half fertilized with sperm of brother or unrelated male, incubated, subdivided into 4 beakers of ~250 embryos.



- Photographs taken of embryos at 4-cell stage to measure deformities (Figure 2) ► Used pre-existing criteria

Figure 2. Examples of Atlantic cod embryo deformities: (A) Normal embryo, (B) Unequal cell size, marginal, incomplete intercell adhesion, cell margins poorly defined.

- Larvae photographed at hatch (day 1) to measure deformities (day 1 only) ► bent tail, bent midsection, curved spine, deformed yolk (Figure 3)

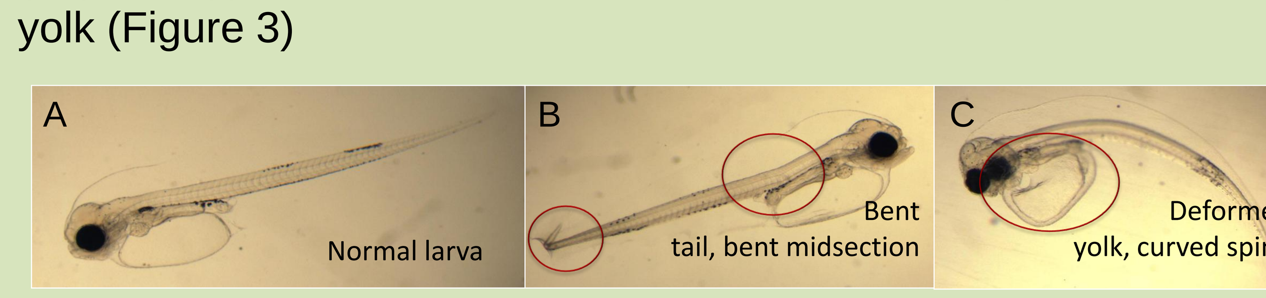


Figure 3. Example of Atlantic cod larval deformities: (A) Normal larva, (B) Bent tail & minor bend in midsection, (C) Deformed yolk sac & curved spine.

- Larvae photographed at hatch (day 1) & day 5 to measure size
  - ► Body length, body depth, eye diameter, yolk area, jaw length (day 5 only) (Figure 4)

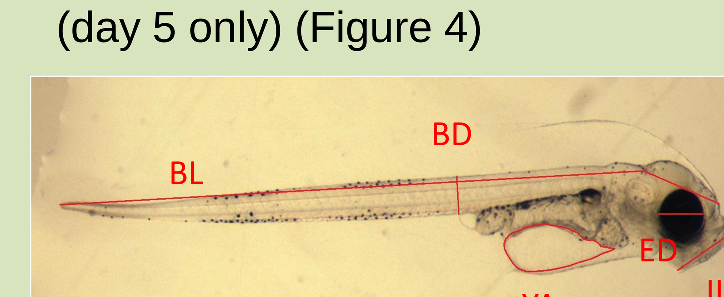


Figure 4. Location of larval size measurements (BL- body length, BD- body depth, YA- yolk area, ED- eye diameter, JL- jaw length)

- Time to starvation used as measure of larval survival

Results

- Offspring from unrelated parents had significantly higher percent hatch (Figure 5) & body depth (Figure 6) than inbred
- No significant differences in percent deformed, all other size measurements, & larval survival (Figure 7)

Future Work

- More interesting results may be found with larger sample size & rearing larvae to look for other possible effects on...
  - ► juvenile growth, feeding conversion efficiency, occurrence of deformities later in larval stage, etc.
- Look at broodstock- maintained for years from few families
  - ► high likelihood of inbreeding
- Extent & effect of inbreeding may be more pronounced