

Efficacy of selection in sexually breeding *Artemia* (*Artemia franciscana*, Kellogg, 1906)

Mangesh M Shirdhankar¹, Palhani C Thomas² & Satish K Barve¹

¹College of Fisheries, Shirgaon Ratnagiri, Maharashtra, India

²Central Marine Fisheries Research Institute, Ernakulam, Kerala, India

Correspondence: M M Shirdhankar, College of Fisheries, Shirgaon Ratnagiri, Maharashtra 415 629, India. E-mail: mangeshshirdhankar@yahoo.com

Abstract

Bi-directional selection for smaller naupliar size (SNS) and bigger naupliar size (BNS) was practiced to develop two divergent lines. The efficacy of bi-directional mass selection in *Artemia franciscana* was evaluated by comparing the predicted genetic gains with the realized genetic gains. Two sets of predictions were made using two heritability estimates, e.g., the heritability estimate from full-sib analysis (h^2) and the estimate from regression of offspring on mid parent (b_{op}). Predictions with the full-sib heritabilities were of very high magnitude as compared with predictions with b_{op} heritabilities. The predictions based on b_{op} were more or less in agreement with realized genetic gain, while the predictions with heritability estimates based on full-sib analysis were much higher than the realized gains. Ratios of realized gain to predicted gain based on full-sib heritabilities were 0.2302 and 0.2152, respectively, for males and females of the SNS line, and 0.0471 and 0.2248, respectively, for males and females of the BNS line. Ratios of realized gain to predicted gain based on b_{op} were 1.5348 and 0.6069 for males and females of the SNS line and 0.1028 and 0.9503 for males and females of the BNS line. Ratios of realized gain to predicted gain based on full-sib heritability were of low magnitude in all the cases as the heritability estimates based on full-sib analysis were inflated by non-additive genetic variance. The ratios of realized gain to predicted gain based on b_{op} were high in both sexes of SNS and females of BNS, indicating high efficacy of selection as b_{op} includes only additive genetic variance. However, it was of low magnitude in BNS males. Thus, the heritability estimates based on regression of offspring on mid parent (b_{op}) are more

reliable than that of heritability estimates based on full-sib analysis (h^2) for predicting the selection response in *Artemia*.

Keywords: realized genetic gain, predicted genetic gain, *Artemia*, heritability

Introduction

Ingestion of *Artemia* nauplii by the larval or the juvenile fish or shellfish is determined to a great extent by its size as a food particle in relation to the mouth size of the predator (Dugan, Hagood & Frakes 1975; Sick & Beaty 1975; Smith 1976; Purdom & Preston 1977). *Artemia* in different stages of the life cycle is the most extensively used live food and there is a great need for developing *Artemia* nauplii of different size to suit the requirements of various species of fish in aquaculture industry. Selective breeding has played a significant role in modifying the performance of farm animals and plants. On similar lines, genetically modified lines of *Artemia* can be developed to meet the required size of *Artemia* nauplii and different life stages of *Artemia* to the aquaculture industry. The genetic studies hitherto carried out are limited to the fields of biochemical genetics, cytogenetics and molecular genetics. Heritability values for certain quantitative traits of *Artemia* were estimated by Shirdhankar and Thomas (2003a) and Shirdhankar, Thomas and Barve (2004), whereas the response to bi-directional selection for naupliar length of *Artemia franciscana* was studied by Shirdhankar and Thomas (2003b). Although Leger, Bengtson, Simpson and Sorgeloos (1986) suggested that high heritability and wide

variation in cyst could be exploited through selective breeding techniques, the efficacy of selection in sexually breeding *Artemia* has not been tested. Thus, an attempt has been made to test the efficacy of selection for size in *Artemia* by comparing the predicted genetic gain with the realized genetic gain.

Materials and methods

The base population was divided into three groups as follows: (1) smaller naupliar size (SNS), (2) bigger naupliar size (BNS) and (3) control lines. These groups were maintained after hatching *A. franciscana* cysts (San Francisco Bay Brand, Inve Aquaculture, batch no. 425 G, 06345).

A mass selection programme was practiced to develop SNS by selecting smaller-size nauplii while the BNS line was modified by selecting larger-size nauplii. Selection was practiced for six generations in the SNS line and for five generations in the BNS line. The control line was also regenerated and maintained but without selection. Each pair of selected male and female of both lines was maintained in a 200 mL plastic bottle containing 90 g L⁻¹ seawater (Browne 1980). The pairs were maintained in these bottles till they released nauplii. Ten nauplii drawn randomly from all maintained pairs in each generation were reared individually in 50 mL bottles till secondary sexual characters were developed. The *Artemia* in all stages of life cycle were fed using the third diet of Maeda-Martinez, Obregon-Barboza and Dumont (1995) with little modification (20 g baker's yeast + 0.5 g Spirulina powder + 1.8 mL cod liver oil). The naupliar length was recorded in micrometres with the help of a standardized oculometer, but is expressed as an average with four decimal places. The number of pairs bred to obtain the next generation and the number of nauplii reared in each generation for recording naupliar length are given in Table 1.

The heritability values of the selected trait (naupliar size) were estimated from full-sib data (h^2) and from regression of offspring on mid parent (b_{op}) as per the procedure given by Becker (1975). Heritability for each line was initially estimated within sex and within generation. These estimates were then pooled over generations, within sex and line, to provide mean estimates.

Selection differentials

The expected selection differential was calculated as the difference between the mean of the selected indi-

Table 1 Number of pairs mated and individuals measured according to sex, line and generation

Line	Generations	Number of pairs	Sex		Total
			Male	Female	
SNS	S0	61	239	255	494
	S1	65	223	195	418
	S2	63	246	275	521
	S3	50	226	202	428
	S4	39	102	154	256
	S5	39	95	190	285
BNS	S6	30	116	285	234
	S0	61	239	255	494
	S1	43	187	171	358
	S2	48	215	186	401
	S3	37	162	153	315
	S4	26	65	91	156
Control	S5	33	78	168	246
	S0	61	239	255	494
	S1	65	98	112	210
	S2	78	75	91	166
	S3	79	70	88	158
	S4	54	51	59	110
	S5	71	63	81	144
	S6	51	45	58	103

SNS, smaller naupliar size; BNS, bigger naupliar size.

viduals as parents for the next generation and the mean of the population before selection of parents. The effective selection differential was calculated by weighing each parent by the number of its offspring.

The effect of natural selection was calculated from the ratio of effective selection differential to expected selection differential (Falconer 1981).

Standardized selection differential (Intensity of selection)

$$i = \frac{\text{Effective selection differential}}{\text{Phenotypic standard deviation}}$$

As selection was applied in both the sexes, the value of intensity of selection (i) was estimated as the means of the two sexes i.e. $i = 1/2 (im + if)$ (Falconer 1981).

Predicted genetic response

The predicted genetic response per generation was calculated for each line separately within sex following the procedure described by (Falconer 1981). The following formula was used to predict the response:

$$\text{Response(R)} = i\sigma_p^2 h^2$$

where R is the average predicted response per generation; i the average intensity of selection; σ_p^2 the phenotypic standard deviation of trait under selection; and h^2 the pooled heritability of selected trait.

Pooled heritability estimates from full-sib data and offspring on mid parent were used to predict the response as it is supposed to be more accurate than individual generation estimates (Kinney & Shoffner 1967).

Realized genetic gain

Realized genetic gain (ΔG) per generation was estimated using generation means of selected lines (corrected for control deviation). Generation mean, corrected for control deviation, was estimated using the formulae (Falconer 1981).

$$\Delta G = (S_n - C_n) - (S_0 - C_0)$$

S and C represent the selected and control lines, while the subscripts represent the generation number. The regression coefficient (b) of generation mean (Y) on generation number (X) was calculated along with the standard error. The regression coefficient was tested for the significance, and significant difference is expressed as $P < 0.05$ at the 5% level of significance or $P < 0.01$ at the 1% level of significance (Snedecor & Cochran 1967).

Results

Selection differentials and selection intensities

Sex-wise expected and effective selection differentials along with ratios of expected to effective selection dif-

ferentials are presented in Table 2 for the SNS and BNS lines. Both expected and effective selection differentials were of almost similar magnitude in both lines, except in the second generation of the BNS line. Both expected and effective selection differentials averaged over generations were slightly higher in females. The mean values for expected and effective selection differentials were -16.6780 and $-16.3966 \mu\text{m}$, respectively, in SNS males while these estimates were -19.9266 and $-22.3101 \mu\text{m}$ in SNS females. The expected and effective selection differential values in the BNS line were 16.2308 and $15.8700 \mu\text{m}$, respectively, for males while similar estimates for females were 17.1180 and $17.0019 \mu\text{m}$. The phenotypic standard deviations and intensity of selection are presented in Table 3.

Predicted genetic gain

The predicted mean genetic gains from six generations of mass selection for naupliar length in the SNS and BNS lines were estimated separately for both sexes using pooled estimates of heritability, mean phenotypic standard deviations of naupliar length of the generations from which parents were selected and selection intensities averaged over the generations. Two sets of predictions were made: (1) using pooled heritability estimates from full-sib analysis (h^2) and (2) using heritability estimates from b_{op} . Values of both the predictions are compared in Table 4, along with realized genetic gain. Predictions made with full-sib heritability were much higher than realized gain, whereas predictions made with heritability

Table 2 Expected and effective selection differentials of selected trait (naupliar length) in mass selection

Line	Parents	Selection differential						Expected/ effective
		Male (Sm)		Female (Sf)		Average (Sm + Sf)/2		
		Expected	Effective	Expected	Effective	Expected	Effective	
SNS	S1	-22.3572	-22.6214	-29.0732	-34.6934	-25.7152	-28.6574	0.8973
	S2	-7.5564	-07.6271	-09.7647	-09.5395	-08.6606	-08.5833	1.0090
	S3	-22.5564	-19.9178	-20.9441	-23.6779	-21.7502	-21.7978	0.9978
	S4	-21.0624	-22.2104	-22.6878	-22.7640	-21.8751	-22.4872	0.9727
	S5	-11.4735	-10.5024	-14.6694	-16.0655	-13.0714	-13.2839	0.9840
	S6	-15.0626	-15.5006	-22.4208	-27.1208	-18.7417	-21.3107	0.8794
BNS	S1	24.3535	24.2932	16.5644	16.8711	20.4090	20.5822	0.9916
	S2	12.3317	06.6932	10.5133	05.7445	11.4225	06.1883	1.8458
	S3	14.9593	17.7644	18.5833	21.0320	16.7713	19.3982	0.8646
	S4	19.9610	19.6422	19.1587	17.0895	19.5599	18.3659	1.0650
	S5	09.6486	10.9570	20.7704	23.9843	15.2095	17.4707	0.8706

SNS, smaller naupliar size; BNS, bigger naupliar size.

Table 3 Phenotypic standard deviations and selection intensities of selected trait (naupliar length) in mass selection

Line	Parents	Phenotypic standard deviation (σ_p)		Selection intensity (i)		Average($im + if$)/2
		Male	Female	Male	Female	
SNS	S1	32.6751	29.0519	-0.6923	-1.1942	-0.9433
	S2	23.9953	25.1242	-0.3179	-0.3797	-0.3487
	S3	24.9532	24.5385	-0.7982	-0.9649	-0.8816
	S4	27.7239	31.5053	-0.8011	-0.7225	-0.7618
	S5	21.3425	22.5058	-0.4921	-0.7138	-0.6030
	S6	29.4958	27.7016	-0.5255	-0.9790	-0.7523
Average		26.6976	26.7379	-0.6045	-0.8257	-0.7151
BNS	S1	32.6751	29.0519	0.7435	0.5807	0.6621
	S2	26.9532	26.5514	0.2461	0.2164	0.2312
	S3	23.6426	27.3393	0.7513	0.7687	0.7600
	S4	28.5118	30.9818	0.6889	0.5516	0.6203
	S5	26.1223	32.0504	0.4195	0.7483	0.5839
Average		27.5810	29.1950	0.5699	0.5731	0.5715

SNS, smaller naupliar size; BNS, bigger naupliar size.

Table 4 Predicted and realized mean genetic gain in naupliar size (selected trait) of SNS and BNS lines

Line	Sex	Genetic gain			Ratio of realized gain to predicted gain based on	
		Predicted based on		Realized	h^2	b_{op}
		h^2	b_{op}			
SNS	Male	-21.3934	-3.4263	-5.2585 ± 1.2517**	0.2302	1.5348
	Female	-24.2940	-8.5773	-5.2289 ± 0.9683**	0.2152	0.6096
	Mean	-22.8437	-6.0018	-5.2437	0.2296	0.8737
BNS	Male	19.7738	9.0805	0.9338 ± 1.5409 (NS)	0.0472	0.1028
	Female	23.7973	5.6292	5.3493 ± 2.5384*	0.2248	0.9503
	Mean	21.7855	7.3591	3.1416	0.1442	0.4269

* $P < 0.05$.

** $P < 0.01$.

NS, not significant; SNS, smaller naupliar size; BNS, bigger naupliar size; h^2 , full-sib analysis; b_{op} , offspring on mid parent.

estimate from regression of offspring on mid parent (b_{op}) were comparable to the realized gains. The ratios of the realized gains to the expected gains, which give an indication of the efficacy of selection and effect of natural selection on artificial selection, are also presented in Table 4.

Realized genetic gain

The phenotypic response is a combined effect of both the genotype and the environment. The cumulative genetic gains realized in each generation of two lines were calculated as the deviation from the control. The mean genetic gain per generation along with standard errors, estimated from the regression of control-corrected generation means on generation

numbers, are presented in Table 4. The realized mean genetic gains were statistically significant for SNS males and females ($P < 0.01$) as well as for BNS females ($P < 0.05$) but were not significant for BNS males ($P > 0.05$).

Discussion

Comparison of the predicted and realized genetic gain is used to evaluate the efficacy of selection. In the present study, predictions based on b_{op} were in agreement with realized genetic gain. Predictions using heritability estimates of full sibs were much higher than the realized gain. Gjerde (1986) concluded that predicted responses to selection usually prove to be overestimates because most sources of

bias cause an overestimation of heritability values. Results from the present study showed a similar pattern. Predictions of response using heritability from full-sib data were different from the realized response, whereas the predictions based on the b_{op} heritability values were comparable to realized response. This suggests that heritability estimated from full-sib data was indeed inflated by non-additive genetic variances, environmental co-variances and maternal effects, unlike the heritability from b_{op} , which contains only additive genetic variance. Therefore, it is advisable to use only heritability estimates from parent–offspring regression for prediction of selection response in *Artemia*.

The ratios of realized gains to predicted gains b_{op} were high in both sexes of SNS and females of BNS, indicating high efficacy of selection. Comparison of these ratios revealed that the values were highest in SNS males selected for small naupliar size, but very low in BNS males selected for large naupliar size. It is a well-known fact that when this ratio approaches unity, as observed in SNS males of the present study, it indicates that natural selection is also favouring artificial selection Falconer (1981). In contrast, when the ratio approaches zero, it indicates natural selection acting against artificial selection. Thus, it can be presumed that nature prefers smaller males and larger females. As larger males beyond the optimum size may not be preferable for the buoyancy during copulation, larger females may be ideal for reproduction and maternal requirement.

Selection of males for SNS line resulted in a better genetic response than the selection for BNS line. Moav and Wohlfarth (1976) have reported differential responses to bi-directional selection for the same trait in common carps (*Cyprinus carpio* L.). They did not obtain any response from five generations of selection for high growth rate; however, a relatively strong response was realized from three generations of selection for a slow growth rate. They suggested that selection for high growth had reached a plateau, involving overdominance, which maintains genetic variation. In the present study, selection in both directions responded positively, although the rate of response was relatively of a lower magnitude in the line selected for larger naupliar size. There are no reports on the predicted and realized genetic gains in *Artemia*, to compare with the findings of this study.

Clayton, Morris and Robertson (1957) stated that both predicted and realized responses to selection would be in close agreement, only at higher intensities of selection. The realized response at lower inten-

sities is usually below expectations. The genetic gain realized in the present study is in accordance with the predicted gains (based on b_{op}) even though the selection intensities were of moderate magnitude, indicating that there is ample scope to improve the response by intensifying the selection intensities.

Conclusion

Predicted gains using heritability estimates from regression of offspring on parent (b_{op}) were close to the realized genetic gain, unlike that of predictions based on full-sib heritability, indicating that heritability estimates from full sibs were inflated by non-additive genetic variance. Therefore, use of heritability estimates from regression of offspring on parent (b_{op}) estimates is more reliable for predicting the selection response in *Artemia*. The ratios of realized gains to predicted gains based on heritability estimates from regression of offspring on parent (b_{op}) were high in both the sexes of the line selected for SNS and females of the line selected for BNS, indicating high efficacy of selection.

References

- Becker W.A. (1975) *Manual of Quantitative Genetics*. Washington State University, Pullman, WA, USA, 170pp.
- Browne R.A. (1980) Reproductive pattern and mode in brine shrimp. *Ecology* **61**, 466–470.
- Clayton G.A., Morris J.A. & Robertson A. (1957) An experimental check on quantitative genetics theory I. Short term response to selection. *Journal of Genetics* **55**, 131–151.
- Dugan C.C., Hagood R.W. & Frakes J.A. (1975) Development of spawning and mass larval rearing techniques for brackish–freshwater shrimps of genus *Macrobrachium* (Decapoda, Palaemonidae), Publication No. 12. Florida Marine Research Laboratory, Florida Department National Research Station Petersburg, 28pp.
- Falconer D.S. (1981) *Introduction to Quantitative Genetics*, 2nd edn. Longman, New York, 340pp.
- Gjerde B. (1986) Growth and reproduction in fish and shellfish. *Aquaculture* **57**, 37–55.
- Kellogg V.L. (1906) A new *Artemia* and its life conditions. *Science* **24**, 594.
- Kinney T.B. Jr. & Shoffner R.N. (1967) Phenotypic and genetic responses to selection in meat type poultry population. *Poultry Science* **45**, 900–910.
- Leger P.H., Bengtson D.A., Simpson K.L. & Sorgeloos P. (1986) The use and nutritional value of *Artemia* as food source. In: *Oceanography and Marine Biology, An Annual Review*, Vol. 24 (ed. by H. Barnes & M. Barnes), pp. 521–623. Aberdeen University Press, Aberdeen, UK.

- Maeda-Martinez A.M., Obregon-Barboza H. & Dumount H.J. (1995) Laboratory culture of fairy shrimp using baker's yeast as basic food in a flow through system. *Hydrobiologia* **298**, 141–157.
- Moav R. & Wohlfarth G. (1976) Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics* **82**, 83–101.
- Purdom C.E. & Preston A. (1977) A fishy business. *Nature* **266**, 396–397.
- Shirdhankar M.M. & Thomas P.C. (2003a) Heritability estimates of naupliar length in *Artemia franciscana* using different methods. *Asian Fisheries Science* **16**, 69–76.
- Shirdhankar M.M. & Thomas P.C. (2003b) Response to bidirectional selection for naupliar length in *Artemia franciscana*. *Aquaculture Research* **34**, 535–541.
- Shirdhankar M.M., Thomas P.C. & Barve S.K. (2004) Phenotypic estimates and heritability values of *Artemia franciscana*. *Aquaculture Research* **35**, 35–39.
- Sick L.V. & Beaty H. (1975) Development of formulated foods designed for *Macrobrachium rosenbergii* larval and juvenile shrimp. In: *Proceedings of 6th Annual Meeting of the World Mariculture society* (ed. by J.W. Avault Jr. & R. Miller), Louisiana State University, Baton Rouge, FL, USA, 557 pp.
- Smith W.E. (1976) Larval feeding and rapid maturation of bluegills in the laboratory. *The Progressive Fish-culturist* **38**, 95–97.
- Snedecor G.W. & Cochran W.G. (1967) *Statistical methods*, 6th edn. The Iowa State University Press, Ames, IA, USA pp. 260–264.