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Response to bidirectional selection for naupliar length in *Artemia franciscana*

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Abstract

Bidirectional mass selection for naupliar length was practised in *Artemia franciscana* from Great Salt Lake, UT, USA, with the aim of developing two divergent lines, namely small naupliar size (SNS) and bigger naupliar size (BNS). A random-bred control line was also maintained to quantify the environmental effects. The mean naupliar length in the base population was 486.99 ± 2.11 μm in males and 490.58 ± 1.82 μm in females. Six generations of selection for smaller naupliar size in the SNS line resulted in a phenotypic response of –45.32 μm and –37.52 μm decreases in naupliar size in males and females respectively. In the BNS line, responses (increase in size) from five generations of selection for bigger size were 8.59 μm and 35.80 μm respectively. The cumulative genetic gain in males and females of the SNS were –41.72 μm and –38.76 μm compared with 12.64 μm and 39.48 μm, respectively, in the BNS line.

Keywords: bidirectional, *Artemia*, selection response, individual selection, phenotypic response, realized response

Introduction

A major problem encountered by aquaculturists is the availability of appropriate food, especially live food, for rearing larvae and juveniles of finfish and shellfish in controlled systems. Ingestibility of food by larval or juvenile fish or shellfish is determined to a great extent by the size of the food particle in relation to the mouth size of the predator (Vanhaecke & Sorgeloos 1980). *Artemia* cysts are the most extensively used live food. Vanhaecke & Sorgeloos (1980) observed wide variations among the different strains of *Artemia* with respect to the size of cysts, nauplii and adults. The implications of these findings are far reaching and call for genetic manipulation to develop different lines/strains of *Artemia* of different size specifications to suit the requirements of various species of cultured aquatic animals. Selective breeding, as used in farm animals and plants, is the time-tested genetic manipulation technique that can play a major role in developing these lines. However, before resorting to large-scale breeding programmes, it is essential to carry out selective breeding experiments and to evaluate the response to selection. Selective breeding in *Artemia* remains a grey area with no reported work to date, although Leger, Bengtson, Simpson & Sorgeloos (1986) suggested that high heritability and wide variations in *Artemia* cysts could be exploited through selective breeding techniques. This paper reports the phenotypic and realized genetic response to selection for smaller and larger naupliar size in *Artemia franciscana*.

Materials and methods

Artemia franciscana (Kellogg 1906) from Great Salt Lake, UT, USA, was used in the present investigation. The base population was raised by hatching the cysts of San Francisco Bay brand (Inve Aquaculture, batch no. 425 G, 06345).

The method of selection followed for picking the desirable individuals to become the parents of the next generation was by mass with the trait under selection consisting of naupliar size (length in μm). Bidirectional mass selection was practised with the aim of developing two divergent stocks.

For this purpose, the base population was divided into three equal parts to be designated the small naupliar size (SNS) line, the big naupliar size (BNS) line and the control line. The criteria of selection were smaller naupliar size in the SNS line, larger naupliar size in the BNS line and no selection in the control line. The mean naupliar lengths in the base population were 486.99 ± 2.11 μm in males and 490.58 ± 1.82 μm in females. Heritability estimates of naupliar size of the base population estimated from parent–offspring regression analysis were 0.5851 ± 0.2153 and 0.3766 ± 0.1893 in males and females respectively (Shirdhankar 1999).

Schematic representation of the mass selection programme is depicted in Fig. 1. The selection programme in the SNS line was initiated by selecting pairs from the base population of the SNS group and keeping each pair in separate bottles. Ten nauplii from each of the pairs were reared individually in separate bottles constituting the S_0 generation of the SNS line. On the ninth day, when sexual dimorphism was clear, all individuals selected on the basis of smaller naupliar size from the S_0 generation were kept as pairs in separate bottles to facilitate mating and breeding. Ten progeny (reared individually in separate bottles) from each of these pairs constituted the first selected generation (S_1) of the SNS line. Selection of individuals was made on the basis of smaller naupliar size from the S_1 generation to facilitate mating and breeding to produce nauplii of the S_2 generation. This process of selection and breeding was repeated until the sixth selected generation (S_6) of SNS was produced. Throughout the selection process, due care was taken to avoid inbreeding. Recording of naupliar length was carried out individually from each animal in every generation. The selection and breeding in the second group (BNS) was carried out as outlined above, but with the difference of selecting bigger nauplii in place of smaller nauplii, and the selection was practised until the fifth selected generation (S_5 of BNS) was available. Numbers of individuals measured according to sex, line and generation are given in Table 1. The selection intensities for each generation are presented sex-wise in Table 2.

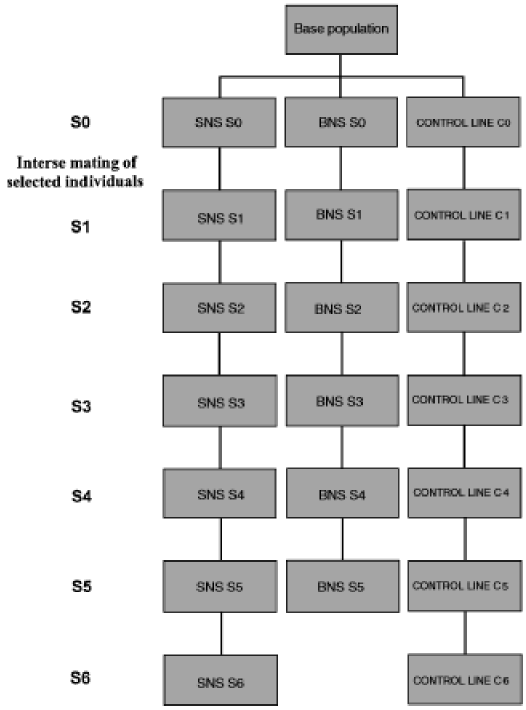

Figure 1 Schematic representation of mass selection programme.

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

Line	Sex	Generations S_0	S_1	S_2	S_3	S_4	S_5	S_6
SNS	Male	239	223	246	226	102	95	116
	Female	255	195	275	202	154	190	118
	Total	494	418	521	428	256	285	234
BNS	Male	239	187	215	162	65	78	–
	Female	255	171	186	153	91	168	–
	Total	494	358	401	315	156	246	–
Control	Male	239	98	75	70	51	63	45
	Female	255	112	91	88	59	81	58
	Total	494	210	166	158	110	144	103

Table 2 Generation-wise selection intensities

Line	Parents	Selection intensity		
		Male	Female	Average (im + if)/2
SNS	S_1	–0.6923	–1.1942	–0.9433
	S_2	–0.3179	–0.3797	–0.3487
	S_3	–0.7982	–0.9649	–0.8816
	S_4	–0.8011	–0.7225	–0.7618
	S_5	–0.4921	–0.7138	–0.6030
	S_6	–0.5255	–0.9790	–0.7523
Average		–0.6045	–0.8257	–0.7151
BNS	S_1	0.7435	0.5807	0.6621
	S_2	0.2461	0.2164	0.2312
	S_3	0.7513	0.7687	0.7600
	S_4	0.6889	0.5516	0.6203
	S_5	0.4195	0.7483	0.5839
Average		0.5699	0.5731	0.5715

The control line, which originated from the same base population, was also regenerated along with each of the selected generations but without any selection. Breeders were picked at random. The individuals in the control line were maintained similar to those of the selected lines.

Response to selection was calculated at both genetic and phenotypic levels. Realized phenotypic response per generation (mean phenotypic response) for the selected trait (naupliar size) was estimated within sex for each line separately from the regression of generation mean on generation number.

Realized genetic gain (ΔG) per generation was estimated in a similar manner, but using generation means of selected lines corrected for control deviation. Estimation of generation mean, corrected for control deviation, was done using the formula:

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

where S and C represent the selected and control lines, respectively, while subscripts represent the generation. Environmental effects between generations were corrected by taking the mean difference between selected and control lines and assuming that the environment had similar effects on them. The regression of generation means on generation number was calculated.

Naupliar length was measured by ocular micrometer under a compound microscope. The ocular micrometer was standardized using the stage micrometer of 0.01 mm division. The factor obtained after standardization of the ocular micrometer was used to convert the ocular readings into naupliar lengths.

Wherever necessary, the data was subjected to ANOVA test to compare difference in the means recorded. The regression coefficients were also tested for significance. Significant results are mentioned as $P < 0.05$. Statistical analysis was carried out according to the methods given by Snedecor & Cochran 1967). Required programmmes were prepared in Fox base for data analysis.

Results

Phenotypic response

The generation-wise mean values of naupliar length of both SNS and BNS lines are presented sex-wise in Table 3. The mean naupliar length of females was always greater than that of males in both the selected and control lines, although the differences were not statistically significant except in the first, third and sixth generations of SNS, and base, fourth and fifth generations of the BNS line ($P > 0.05$).

The total phenotypic response in the SNS line, i.e. the cumulative decrease in naupliar size from six generations of selection for small naupliar size was –45.3177 μm in males and –37.5220 μm in females. The corresponding values in the BNS line were 8.5923 μm and 38.7966 μm for males and females respectively. In the SNS line, the average phenotypic response per generation calculated from regression of generation means on generation numbers was

Table 3 Mean, standard error and coefficient of variation of (%) naupliar length in SNS and BNS lines

		Male		Female	
Line	Generation	Mean (μm ± SE)	CV (%)	Mean (μm ± SE)†	CV (%)
SNS	0	486.9912 ± 2.1136	6.71	490.5754 ± 1.8157	5.92
	1	467.5543 ± 1.6068	5.13	472.8036 ± 1.7946++	5.31
	2	467.8579 ± 1.5877	5.33	471.2891 ± 1.4797	5.21
	3	453.1580 ± 1.8442	6.12	459.7709 ± 2.2112++	6.85
	4	466.6716 ± 2.1132	4.57	469.4052 ± 1.8077	4.79
	5	455.5490 ± 3.0104	6.47	460.3879 ± 2.0097	6.02
	6	441.6735 ± 2.3345	5.72	453.0534 ± 3.6845++	8.83
	b ± SE§	–5.7554 ± 1.4947**	–	–4.9743 ± 1.1997*	–
BNS	0	486.9912 ± 2.1136	6.71	490.5754 ± 1.8157+	5.92
	1	504.5246 ± 1.9710	5.34	506.1070 ± 2.0245	5.25
	2	491.7750 ± 1.6087	4.81	494.7871 ± 2.006	5.53
	3	492.7043 ± 2.2332	5.79	496.2098 ± 2.5047	6.24
	4	494.4246 ± 3.2401	5.28	505.3978 ± 3.3415++	6.34
	5	495.5835 ± 2.8021	5.03	529.3720 ± 2.7009++	6.61
	b ± SE§	0.3883 ± 1.5395	–	5.5222 ± 2.1979*	–

+ and ++ indicates that female naupliar length is significantly different from male naupliar length at $P < 0.05$ and $P < 0.01$ respectively.

‡'b' values are significant at *$P < 0.05$ and **$P < 0.01$ respectively.

§Regression of generation means on generation number.

–5.7554 ± 1.4947 μm and –4.9743 ± 1.1997 μm in male and females respectively. The corresponding values in the BNS line were 0.3883 ± 1.5395 μm and 5.5222 ± 2. 1979 μm. The mean phenotypic responses were statistically significant ($P < 0.05$) except for that of BNS males.

Realized genetic gain

The observed phenotypic response is the combined effect of both genetic and environmental factors. As the environment rarely remains the same over the period of selection, separating these effects becomes rather difficult. One of the most commonly used methods for removing the environmental effect from the phenotypic gains and for determining genetic gain is the use of an unselected control population, preferably from the same stock as that of the selected population. Such a control line was used in the present study.

Although the mean naupliar length varied from generation to generation, the regression of the control mean on the generation number was not significant ($P > 0.05$). This fact suggests that fluctuations in the control mean resulted from random changes in the environment. Consequently, the genetic gains in the selected generations were calculated after correction of the generation mean of the selected lines to control means. The cumulative genetic gains realized in each generation of the two lines, estimated as control deviations, are presented in Table 4 and Fig. 2a–d.

In the SNS line, the total genetic gains, i.e. decrease in naupliar length realized from six generations of individual selection for smaller naupliar length, was –41.7244 μm in males and –38.7585 μm in females. However, in the BNS line, the total genetic gains from five generations of selection were 12.6427 μm and 39.4836 μm in males and females respectively.

The realized mean genetic gain per generation, estimated from the regression of control-corrected generation means on generation numbers, was –5.2585 ± 1.2517 μm in males and –5.2289 ± 0.9683 μm in females of the SNS line, and 0.9338 ± 0.9338 μm in males and 5.3493 ± 2.5384 μm in females of the BNS line. The mean genetic gains were statistically significant except for that of BNS males ($P > 0.05$).

Discussion

Generation-wise phenotypic response in naupliar length realized from bidirectional selection for

Table 4 Cumulative genetic gain in naupliar length of SNS and BNS lines

		SNS			BNS		
Sex	Generation	Selected (Sn)	Control (Cn)	Genetic gain (Sn–Cn)	Selected (Sn)	Control (Cn)	Genetic gain (Sn–Cn)
Male	0	486.9912	486.9912	0.0000	486.9912	486.9912	0.0000
	1	467.5543	486.6831	–19.1285	504.5246	486.6831	17.8415
	2	467.8579	483.3373	–15.4794	491.7750	483.3373	8.4377
	3	453.1580	481.474	–28.3160	492.7043	481.474	11.2303
	4	466.6716	487.6906	–21.0190	494.4246	487.6906	6.7340
	5	455.5490	482.9408	–27.3918	495.5835	482.9408	12.6427
	6	441.6735	483.3979	–41.7244	–	–	–
	b±SE			–5.2585** (±1.2517)			0.9338 NS (±1.5409)
Female	0	490.5754	490.5754	0.0000	490.5754	490.5754	0.0000
	1	472.8036	490.9359	–18.1323	506.1070	490.9359	15.1711
	2	471.2891	489.0627	–17.7736	494.7871	489.0627	5.7244
	3	459.7709	487.6298	–27.8589	496.2098	487.6298	8.5800
	4	469.4052	494.5766	–25.1714	505.3978	494.5766	10.8212
	5	460.3879	489.8884	–29.5005	529.3720	489.8884	39.4836
	6	453.0534	491.8119	–38.7585	–	–	–
	b±SE	–	–	–5.2289** (±0.9683)			5.3493* (±2.5384)

'b' values are significant at $^{*}P<0.05$ and $^{**}P<0.01$.
NS, not significant.

reducing naupliar length in the SNS line and for increasing naupliar length in the BNS line showed that the response in both lines was in the desired direction. The total cumulative decrease in naupliar length from six generations of selection for smaller naupliar size, namely –45.3177 μm and –37.5220 μm in males and females, respectively, was to 9.31% and 7.65% of the naupliar size of the base population. Similarly, the cumulative increase of 8.5923 μm and 38.7966 μm in males and females of the BNS line from five generations of selection for larger naupliar size was to 1.76% and 7.91% of the naupliar size of the base population. The mean decrease in the naupliar length per generation in the SNS line was –5.7554 μm and –4.9743 μm for males and females respectively. The corresponding average increase per generation in the BNS line was 0.3833 μm and 5.5222 μm respectively. It can thus be seen that, although both sexes readily responded to selection for decrease in naupliar size in the SNS line (Fig. 2a and b), there was a differential response to selection for the larger size in the BNS line (Fig. 2c and d). In the BNS line, the females showed a 14.5 times higher response than males, whereas both sexes showed a comparable response in the SNS line. It is rather difficult to explain whether this low response in males resulted from the attainment of the genetically preset maximum size for that sex or any other reasons. There are no reports on response to selection in *Artemia*. But Moav & Wohlfarth (1976) also reported a positive response to selecting the smaller individuals and a lack of response to selecting larger individuals of common carp. The results of the present study have also shown similar trends.

The point to be noted in this context is that the male nauplii were always smaller than females in both SNS and BNS lines as well as in the base population. The smaller size of males compared with females may be associated with the need for males to clasp the female and to maintain buoyancy during copulation. Males might have reached the size limit set by nature and, hence, exhibited a lower response when larger sized nauplii were selected.

The realized response calculated by subtracting the mean control values of each generation from the corresponding selected generation mean is free from environmental effects and therefore gives the true genetic gains from selection. Compari-

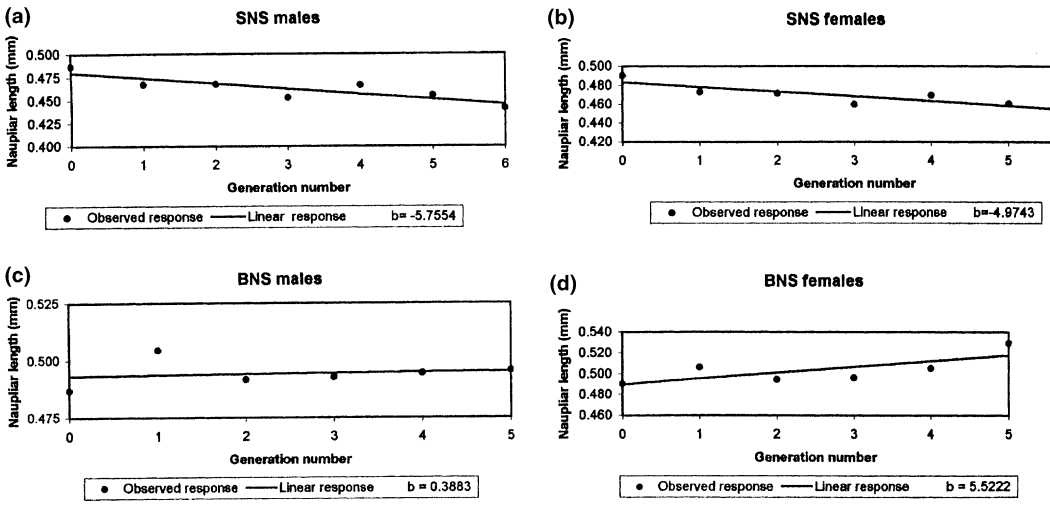

Figure 2 Linear trend of phenotypic response in naupliar length.

son of the genetic and phenotypic gains realized in this study points towards the fact that, although the environment played a role in deviating the phenotypic response from the genetic response, its effect was comparatively small and the genetic gain was substantial. Most of the documented selection studies in aquatic species have reported the response to selection without considering the environmental effects and, therefore, represent only the phenotypic response and not the genetic response.

In the present study, response to bidirectional selection was in the expected direction, although the rate of response was of a higher magnitude in the line selected for reduction in naupliar size. Very few bidirectional selection studies, with reference to growth, have been reported in aquatic animals. Although Moav & Wohlfarth (1976) observed no response from five generations of selection for high growth rate in common carp *Cyprinus carpio*, there was a strong response to selection for slow growth rate. In channel catfish *Ictalurus punctatus*, Bondari (1983) reported response to selection for body weight and length in both upward and downward directions. Huang & Liao (1990) reported little response to mass selection for high body weight as well as for low body weight in tilapia *Oreochromis niloticus*. Behrends, Kingsly & Price (1987) in tilapia *Oreochromis* spp. and Rocchetta, Vanelli & Pancaldi (1996) in guppy *Poecilia reticulata* could not observe any response to selection for growth, because of the prolonged domestication process in these fish. No comparable results from *Artemia franciscana* are available.

Conclusion

Substantial genetic gains were realized from selection for naupliar length in *A. franciscana*. The study indicates the usefulness of selective breeding for development of lines/strains of *Artemia* with different naupliar size specifications to meet aquaculture requirements.

References

Behrends L.L., Kingsly J.B. & Price A.H. III (1987) Bi-directional – backcross selection for body weight in a red strain of tilapia (*Oreochromis*. spp.). *Paper presented at the Second International Symposium on Tilapia in Aquaculture*, Bangkok, Thailand, 16–20 March 1987.

Bondari K. (1983) Response to bi-directional selection for body weight in channel catfish. *Aquaculture* **33**, 73–81.

Huang C.-M. & Liao I.-C. (1990) Response to mass selection for growth rate in *Oreochromis niloticus*. *Aquaculture* **85**, 199–205.

Kellogg V.L. (1906) A new *Artemia* and its life conditions. *Science* **24**, 594.

Leger Ph., 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.

Moav R. & Wohlfarth G. (1976) Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics* **82**, 83–101.

Rocchetta G., Vanelli M.L. & Pancaldi C. (1996) Effects of artificial selection on body size and its reaction norm in guppy fish. *Aquaculture* **137**, 271–284.

Shirdhankar M.M. (1999) *Studies on genetic architecture and predicted response in biometrical traits on sexually breeding* Artemia. PhD Thesis, Central Institute of Fisheries Education (Deemed University), Mumbai, India, 133 pp.

Snedecor G.W. & Cochran W.G. (1967) *Statistical Methods*. Oxford & IBH Publishing, New Delhi. 593 pp.

Vanhaecke P. & Sorgeloos P. (1980) International study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. In: *The Brine Shrimp Artemia* Vol. 3 (ed. by G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers), pp. 393–405. Universal Press, Wetteren, Belgium.