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Selective Breeding Approach to Study the Nauplii Size Variations in Artemia

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Abstract

Aquaculture plays a significant role in providing healthy protein and meeting food security for the marginalized communities. Aquaculture in most cases relies on hatchery-produced fish seeds. Hatchery seed production success depends on the larval feeding, and presently most of the seed production commences with live feeds. Artemia is considered the universal live feed in aquaculture for its universal availability, easy storage, and nutritional advantages. The objective of the present study is to reduce the nauplii size by following the selective breeding approach using the cysts collected from the hypersaline habitats of Kelambakam (CKF), Southeast India. Nauplii length reduction was evident with a selection gain of -2.40 μm in G1, -9.86 μm in G2, and -10.70 μm in G4. The study reduced the nauplii length of Artemia by 14.9 percent from the 15-generation selective breeding approach using the Artemia strain collected from India. The study reports that the genetic effect on the nauplii length in response to the selection is significant. Cumulative selection gain of the mass selection was evident from the variance values. Non-additive genetic factors also contributed substantially to the total genetic variance and are indicated by the higher h2 values. The increase in hatching percentage (10% increase) may be due to the cumulative effect of the selection. Live feed quality standards are defined regarding nutrient content, size, hatching percentage, etc. The selective breeding approach seems an ideal option for developing superior strains to replace the imported strains.

Keywords: Selective breeding, artemia nauplii, aquaculture, smaller nauplii, heritability, algae

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Introduction

Fisheries and aquaculture sectors have been the leading contributors to global food security and nutrition in the past two decades. The total capture fisheries and culture production reached a record of 214 million tonnes in 2020, comprising 178 million tons of aquatic animals and 36 million tonnes of algae, three a slight increase (3 percent) from the previous 2018 record (213 million tonnes) (FAO, 2022). Boosts in aquaculture production is evident; it contributed 37%, which is significantly high compared to 12 percent of the total output in the late 1980s. The total aquaculture contribution was 122.6 million tonnes; 87.5 million tonnes were contributed by aquatic animals and 35.1 million tonnes by algae. 87% of the total production was utilized for human consumption, which is higher when compared to the 1960s when it was 67% only.

FAO states that Blue Transformation is a vision for sustainably transforming aquatic food systems, a recognized solution for food and nutrition security and environmental and social well-being, by preserving aquatic ecosystem health, reducing pollution, protecting biodiversity, and promoting social equality. The United Nations General Assembly declared the International Year of Artisanal Fisheries and Aquaculture 2022 to enhance global awareness and understanding of small-scale artisanal fisheries and aquaculture; foster action to support its contribution to sustainable development; and promote dialogue and collaboration between and among actors and partners, engaging critical public and private stakeholders to address challenges and opportunities for small-scale fisheries and aquaculture to achieve the Sustainable Development Goals (SDGs) (FAO, 2022). Aquaculture production entirely relies on the seed production industry which is the main bottleneck in this industry. Several factors contribute to the growth of this sector, including research and vital inputs like quality

water and feed. Artificial feeds play a crucial role in post-larval rearing, especially starting one month after hatching, while live feeds remain essential for survival before that period. Since live feeds are considered a necessary part of all the fish/shrimp hatcheries, a nutrient-rich optimum starter diet is crucial for hatcheries and breeders. Even though there are methods for enrichment of the live feeds, the innate nutrient quality of live feeds is often not achieved. The primary focus is on finding optimal live feed species to replace traditional ones in hatcheries. However, no perfect replacement has been found for Rotifers or Artemia. Rotifers are valued for their size, while Artemia stands out as an ideal starter diet due to its other qualities and is more commonly sought after than Rotifers as a live feed. The only limitations for Artemia as live feed is its bigger nauplii size (>450 micron) and lack of some crucial nutrients such as EPA and DHA. At hatcheries, through enrichment, the Nutrient deficiency issues are balanced. However, the larger size is still an issue that needs to be addressed. The live feed industry needs to catch up in selective breeding programs, and attempts in this regard are less. Pioneering works were done in pair-based scale breeding programs to improve the desired traits in leading aquaculture species such as salmonids in the 1970s pair (Gjedrem, 1985), for Nile tilapia in 1988 (Eknath et al., 1991) and for marine shrimp *P. vannamei* in 1993 (Fjalestad et al., 1997). In this context, we conducted a study to validate the effect of selective breeding in Artemia for reducing nauplii size. Falconer (1960) defined *heritability* as the regression of breeding value on phenotypic value (h2 = BGP), which is equivalent to the square of the correlation between breeding values and phenotypic values (h2= r2GP). Selective breeding in *Artemia franciscana* for reduction of the naupliar length by Shirdhankar & Thomas (2003) has shown that it is amenable to selection as the genetic gain realized was substantial. The objective of the present study is to study the reduction in nauplii size using selective breeding as a tool.

Materials and Methods

To carry out the selective breeding experiment, the Artemia cysts from the hypersaline habitats of Kelambakam (CKF), Southeast India (12047′ N - 800 13′ E) were used. The Artemia cysts (1gm Lr-1) hatched to develop the generation following the established procedure of Sorgeloos, (1986). Nauplii were maintained in 35 ppt seawater (10Lr) with adequate aeration and fed daily with microalgae *Isochrysis galbana*.

The selective breeding experiment was by following the standard method described by Falconer, (1981), called mass selection. In the selection process, we selected Artemia nauplii according to the size, carried out the selection using customized filtering units of various mesh-sizes (500, 480, 450, and 400μm), and selected 10% of the base population (1.5 lakhs nauplii) for the experiment. About fifteen thousand nauplii, were reared separately as per the standard procedure for 15 days, until adulthood and till they formed pairs and started copulation. First-formed pairs of 1000 units were collected using hand nets and incubated in another breeding tank for hatching. On the third day, the young ones started to appear in the breeding tanks, which were collected, and carefully transferred into nauplii rearing tanks and reared following the standard procedure. These were denoted as generation G1, and this protocol was repeated and the selected nauplii were denoted from G2 to G15 respectively.

Generation-wise nauplii length, the diameter of the cyst, percentage hatching of the selectively bred Artemia, and the reference samples collected from various parts of India, namely (Vedaranyam (VDA), Tuticorin (TTJ), Marakanam (TMM), Tamaraikulam (TNM) and Gujarat (GMJ) were measured, and heritability estimated following the protocol mentioned below.

The variance component analysis was used to estimate the component of variance, and Heritability was estimated from it. The linear statistical model used was

$$Yik = \mu + P + eik$$

Where,

Yik = Observation of the kth progeny of the ith sire

μ = Overall mean

Pi = Effect of ith sire, where i= 1, 2, 3…..P

eik = Random error attributed to individuals, assumed normally and independently distributed with mean zero and variance σ2e.

The degree of freedom (D.F.), the sum of squares (S.S.), the mean sum of squares (M.S.), and the expected sum of squares (EMS) used for the estimation of heritability are given below:

Analysis of variance

Source of variation	D.F.	SS	MS	EMS
Between pairs	P-1	SS_P	MS_P	$\sigma^2_W + K_1 \sigma^2 P$
Between progeny within pairs	n-P	SS_W	MS_W	σ^2_W

Where,

P = Total number of pairs

N = Total number of progenies

K_1 = Average number of progenies per sire

σ^2 = Pair component of variance

σ^2_W = Error variance component

Computational Formula

Sources of variation	Sum of squares	Mean squares
Correction terms (C.T.)	$\frac{Y\ldots^2}{n_i}$	---------
Between pairs	$\Sigma \frac{Y\ldots^2}{n_i}$ –C.T.	$MS_P = SS_P / S\text{-}1$
Progeny within pair	$\Sigma_i \Sigma_j Y_{ij}^2 - \Sigma \frac{Yi}{n_i}$	$MS_W = SS_W / n. - P$

Estimation of variance and heritability

$$\sigma^2_W = MS_W$$

$$\sigma^2_P = \frac{MS_P - MS_W}{K1}$$

$$h^2_P = \frac{2\sigma^2_P}{\sigma^2_P + \acute{o}^2_W}$$

The value of K_1 was calculated from the following formula:

$$K_1 = \frac{1}{P\text{-}1} \left[n. - \frac{n_i^2}{n.} \right]$$

Standard error of heritability was calculated as per Swiger et al., (1964) using the following formula.

$$\text{S.E. } h^2 = 2\sqrt{\frac{2(n\text{-}1)\ (1\text{-}t)^2\ [1+K_1\ \text{-}1)\ t\ ]^2}{K_1^2\ (n.P)\ (P\text{-}1)}}$$

Where,

't' is interclass correlation

$$t = \frac{\sigma^2_P}{\sigma^2_P + \sigma^2_w}$$

Heritability pooled over generations, as detailed by Enfield *et al.* (1966).

$$\text{Pooled } h^2 = \frac{\frac{h_0^2}{V_0} + \frac{h_1^2}{V_1} + \frac{h_2^2}{V_2} + \frac{h_3^2}{V_3} + \ldots\ldots \frac{h_n^2}{V_n}}{\frac{1}{V_0} + \frac{1}{V_1} + \frac{1}{V_2} + \frac{1}{V_3} + \ldots\ldots \frac{1}{V_n}}$$

$$\text{S.E. of Pooled } h^2 = \frac{1}{\frac{1}{V_0} + \frac{1}{V_0} + \frac{1}{V_0} + \frac{1}{V_0} + \ldots\ldots \frac{1}{V_0}}$$

Where,

h_0^2, h_1^2, h_2^2, h_3^2, h_4^2, …… h_n^2 are the heritability of character in the corresponding generation G_0, G_1, G_2, G_3, G_4, …… G_n.

V_0, V_1, V_2, V_3, V_4, V_n are the squares of the standard error of corresponding heritabilities.

Artemia nauplii Selection data: Before selecting the parents, the selection differentials are calculated as the difference between the mean of the selected individuals who have parented the next generation and the mean of the population (Falconer, 1960) and the Standardized selection differential (Falconer, 1981).

$$\text{Standardized selection differential (i)} = \frac{\text{Selection differential}}{\text{Phenotypic standard deviation}}$$

Predicted response and selection gain in each generation were estimated from the full-sib data following the method described by Falconer, (1981).

Predicted genetic response (R) = i $\sigma^2 p$ h^2

R = Average predicted response per generation

i = Standardized selection differential

$\sigma^2 p$ = Phenotypic standard deviation of the trait under selection

h^2 = Pooled heritability of selected trait

Pooled heritability was used for the prediction of response since it is supposed to be more accurate than individual generation estimates (Kinney & Shoffner 1967).

Data details: Analysis of variance (ANOVA) and post-hoc tests were run using SPSS program 13.0 (SPSS Inc, Chicago, USA).

Results and Discussion

Morphological observations revealed the length of the freshly hatched Artemia nauplii within the base generation (G0) ranged from 400.0 μm to 570.0 μm with a mean value of 517.0±39.8μm (Table 1.).

Table 1. Generation-wise mean nauplii length (μm) in selectively bred Artemia

Generation	Mean nauplii length with SD* (μm)
G0	517.0±39.8^{a}
G1	514.6±20.5^{ab}
G2	504.7±38.5^{abc}
G3	501.7±20.3^{c}
G4	491.0±38.7^{cd}
G5	490.1±19.4^{cd}
G6	482.5±23.1^{de}
G7	477.1±27.1^{ef}
G8	471.4±27.1^{efg}
G9	464.1±30.1^{fgh}
G10	463.4±24.8^{fgh}
G11	459.4±21.4^{gh}
G12	454.5±29.1^{hj}
G13	452.2±25.0^{hjk}
G14	444.4±31.8^{jk}
G15	439.3±27.0^{k}

* SD= Standard Deviation
Values with the same superscript are not significantly different at (P>0.01).

The heritability estimates of Artemia nauplii (first-day length) is presented in Table 4.2. Heritability estimates of the selected Artemia showed generation-to-generation variations. Heritability was 0.99±0.36 in the base generation while it varied between 0.36 and 1.64 in other generations. Though, the heritability estimates and the standard errors associated with individual generations varied widely, the pooled heritability and standard error of the selected trait was 0.96±0.01 (Table 2).

Table 2. Heritability estimate and standard error of the Artemia nauplii (first-day length)

Generation	Heritability	Standard error
G0	0.99	0.36
G1	1.10	0.30
G2	0.73	0.39
G3	1.27	0.24
G4	0.47	0.18
G5	0.98	0.35
G6	1.09	0.28
G7	0.31	0.23
G8	0.36	0.31
G9	1.36	0.24
G10	1.21	0.29
G11	1.15	0.22
G12	1.64	0.21
G13	0.42	0.28
G14	0.53	0.30
G15	1.46	0.19

The phenotypic investigation reveals a distinctive trend in the base nauplii size (517.0±39.8 μm), showcasing a notable reduction under the aegis of the selective breeding program. The study reports a heritability estimate of 0.99±0.36 for the first-day length of the base generation, with subsequent generations displaying values ranging from 0.36 to 1.64. The pooled Heritability with SE is reported at 0.96±0.01. These estimates gain significance when contextualized against the backdrop of economically essential traits in fish and shellfish, coupled with the high fecundity and short generation intervals (1–4 years) prevalent in most aquatic species. The implications of these findings align with the insights provided by Gjedrem (2012), where high heritability values contribute to substantial genetic gains in aquaculture breeding programs. Gjedrem & Thodesen (2005) reinforce this notion with 21 estimates of selection response for growth rate in various aquatic species, averaging 14% per generation.

A deeper look into the selective breeding dynamics reveals a -25.97-selection differential in the base generation, a range observed from -33.17 to -8.66 μm (Table 3). The standard selection differential at the

base generation is -0.65, spanning from -0.95 to -0.27. The phenotypic standard deviation values of 39.84 μm, 38.75 μm for G4, and 38.59 μm for G2 intricately detail the complexity of the genetic alterations across generations.

Table 3. Selection differential of length (μm), Phenotypic standard deviation (μm), and Standardized Selection differential of the fifteen generations of selected Artemia

Generation	Selection differential (μm)	Phenotypic standard deviation (μm)	Standardized Selection differential
G0	-25.97	39.84	-0.65
G1	-33.17	35.52	-0.93
G2	-29.98	38.59	-0.78
G3	-32.54	34.38	-0.95
G4	-24.17	38.75	-0.62
G5	-25.78	27.48	-0.94
G6	-21.26	23.15	-0.92
G7	-16.52	27.19	-0.61
G8	-13.73	27.10	-0.51
G9	-10.19	30.13	-0.34
G10	-11.43	24.86	-0.46
G11	-11.82	21.44	-0.55
G12	-14.19	29.19	-0.49
G13	-14.02	25.09	-0.56
G14	-8.66	31.84	-0.27
G15	NA	27.09	NA

The culmination of this genetic interplay manifests in the artemia nauplii length after 15 generations, settling at 439.3±27.0 μm, a substantial reduction from the base generation's 517.0±39.8 μm. The selection gain of -2.40 μm in G1, -9.86 μm in G2, and -10.70 μm in G4 elucidates the gradual nature of the genetic modifications, with the lowest recorded in G10 (-0.76 μm). The cumulative selection gain of -5.13 μm attests to the profound impact of the 15-generation selective breeding initiative (Table 4).

A parallel investigation into cyst size dynamics reveals a reduction from 224.83±14.81μm to 212.5±9.4μm. This reduction, while seemingly modest, assumes significance when benchmarked against reference strains (VVC, SFB, ASL, TBS) and other indigenous strains (236.4 to 219.6 μm). Duncan's multiple range test substantiates the homogeneity of

Table 4. Predicted, realized, and cumulative gain (μm) in nauplii length of Artemia from fifteen generations of selective breeding

Generation	Predicted gain (μm)	Realized gain (μm)	Cumulative gain (μm)
G0	-24.93	NA	NA
G1	-31.84	-2.40	-2.40
G2	-28.78	-9.86	-12.26
G3	-31.24	-2.97	-15.23
G4	-23.20	-10.70	-25.93
G5	-24.75	-0.95	-26.89
G6	-20.41	-7.61	-34.49
G7	-15.85	-5.33	-39.82
G8	-13.18	-5.78	-45.60
G9	-9.79	-7.21	-52.81
G10	-10.97	-0.76	-53.57
G11	-11.35	-3.94	-57.51
G12	-13.63	-4.96	-62.47
G13	-13.46	-2.31	-64.78
G14	-8.31	-7.76	-72.54
G15	NA	-5.13	-77.67

the selected Artemia, further validated by the remarkable increase in hatching percentage from 54.4% in the base generation to 64.58% in the selectively bred strain.

The consequential reduction in nauplii length by 14.9% from the 15-generation selective breeding approach, employing an Artemia strain sourced from India, is a standout outcome. This Artemia, identified as belonging to the species *Artemia franciscana* (Vikas et al., 2012), showcases a reduction not only in nauplii size but also in cyst dimensions, by 5%. This dual reduction is accompanied by an increase in hatching percentage, an interconnected response to the selection process.

The significant reduction in nauplii length to 439.3±27.0 μm from 517.0±39.8 μm underscores the pivotal outcome of the study. This selectively bred strain, when juxtaposed against the commercial strain's length of 502.6±97.13 μm, positions itself as an important contributor to hatchery starter diets. Comparative analyses with global strains reveal the smaller size of the present Artemia, underscoring the efficiency of the selective breeding approach. Changes in gene frequencies at specific locations, as explained by Falconer (1981) and Liang et al. (2010),

help us understand how genetics influence the observed responses.

Studying heritability estimates from the full sibs produced from pair mating helps uncover complex genetic differences. The pooled h2 estimate of 0.96 ± 0.01, while slightly lower than the estimates for other *A. franciscana* strains, delineates the profound genetic influence on nauplii length variations. The variations in heritability estimate values, acknowledged by Briski et al. (2008), McLaren & Corkett (1978), and Durborow et al. (1985), underscore the multifaceted nature of dominance deviation, epistatic interaction, and maternal effects.

Conclusion

In essence, this study explores selective breeding within the live feed sector of aquaculture, uncovering the intricate genetic foundation of observable traits. From the reductions in nauplii and cyst dimensions to the increase in hatching percentage, each outcome contributes to an understanding of the impact of selective breeding on key traits. As the aquacultural landscape continues its evolution, studies on selective breeding can play a pivotal role for enhanced productivity and sustainability.

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