Aquaculture and Fisheries Management 1993, **24**, 751–760

Development and growth heterogeneity in oriental river prawn, *Macrobrachium nipponense* (De Haan) (Palaemonidea), in onthogenesis

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Abstract. At all onthogenesis stages of the Oriental river prawn, *Macrobrachium nipponense* (De Haan), size heterogeneity has been traced within one egg clutch, at different developmental stages of larvae, young and adult individuals. The problem of predetermining heterogeneity is discussed. Uneven larval development of individuals from a single clutch is shown. Also, it is shown that the first to become adults are the individuals hatched from small eggs. Conclusion is made that in estimating development and growth heterogeneity the genetic factor has a primary nature. Available data accounting is essential for selective culturing of prawns under controlled conditions with the required growth characteristics.

Introduction

Subtropical species, i.e. oriental river prawn, *Macrobrachium nipponense* (De Haan), are prospective candidates for culture in thermoelectric power station cooling reservoirs, which play an important role in productive processes in such reservoirs, being also an object for fishing and aquaculture (Khmelyova, Guiguinyak & Kulesh 1988). The development of world aquaculture made it possible to control the growth and reproduction of prawn populations being reared which determine the most important commercial properties, i.e. growth rate and level. Quantitative parameters of growth and reproduction, in their turn, are directly related to heterogeneity of certain individuals in a population or sample. The heterogeneity effect is closely related to the specific nature of the object under study, i.e. to freshwater prawns in our case. The peculiarity of their biology lies in the fact that in onthogenesis they pass through qualitatively different, substantially lengthy stages (larval, juvenile, mature and definitive) whereas adult individuals are characterized by a complex social structure which is particularly pronounced in the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). It is noteworthy that growth heterogeneity is usually most strongly pronounced in the animals placed under adverse conditions, particularly when their density is high, related to which is the effect of the group considered as one of the population self-regulation mechanisms (Shwartz, Pyastolova, Dobrynskaya & Runkova 1976). Considering these aspects it is worthwhile analysing the data available in the literature, which are not numerous and at times conflicting.

Ra'anan & Cohen (1984), for example, reviewed how group growth or the effect of the group influenced size composition of giant freshwater shrimp populations. According to the authors, as early as 2 weeks after the experiment commences two size classes are formed in juvenile individuals. This results in large dispersion from 0·02 to 0·09 g, in contrast to separately growing individuals, which do not exceed 0·02–0·055 g. The first class included the 'leaders', which grow extremely fast. The second class, 'followers', was observed only when

the 'leaders' were available and its emergence was due to heavy growth repression b leading individuals. The available findings indicate that size dispersion in the populati giant shrimp juvenile individuals is to a great extent the result of behavioural intera rather than genetic differences which determine the growth. Based on the results of studies performed by Kuris, Ra'anan, Sagi & Cohen (1987) and Sagi, Milner & Cohen (1 it is concluded that the principal factor limiting giant shrimp growth and productivity is ma complex social structure — small males, average males with orange claws, and la individuals with blue claws. The interaction between them causes the inhibiting effect. Suc complex social structure may be genetically conditioned. One can change feeding conditio density and abiotic factors but one will still fail to obtain tangible results unless the mal structure is altered. However, purposeful influence of the medium on the social structure giant shrimp males represses the genetic effect which makes the estimate of heredita heterogeneity senseless (Malecha 1977 quoted from Lester 1988). Nonetheless, Lester (198 cites data about heredity influence on the growth of two Penaeus species, *Penaeus stylirostr* Stimpson and *P. vannamei* Boone, at the start of the life-cycle. The author shows that size o the larvae from a single family (or clutch) produced at the early stages by a single femal correlates with the above index at the mysis or post-larval stage. This dependence can b clearly traced for *P. stylirostris*, i.e. we can see that size hereditability is more strongl pronounced in this species. Heterogeneity in the growth of larvae from different families formed by different females was also observed, which is attributable to the combination of maternal heredity and genetic difference between families, the genetic range of growth variations within the family being likely wider than between families, according to Lester (1988).

Thus, based on information derived from the literature the question persists — whether the initial growth heterogeneity or, in other words, genetic leadership of this or that individual within the group is predetermined or this phenomenon is caused by other factors (such as medium influence, the effect of the group, etc.) or whether it is the result of a combination of different factors. In this aspect studies on the animals originating from a single family (clutch) during a prolonged onthogenesis period seem to be prospective.

In this connection the purpose of this paper is to study size indices and development heterogeneity in oriental freshwater prawn individuals throughout the onthogenesis period: stage 'A' — non-segmentable eggs and embryo eye forming (or pigmentation) stage; 'zoea' plankton larval stage; from the 'post-larval' stage until the first egg-carrying females appear and, based on heterogeneity, to single out different size and weight groups of prawn in early onthogenesis, determining their functional role in the growth process.

Methods

Experiments were conducted under conditions optimal for rearing the oriental river prawn, i.e. temperature 27–28°C, water oxygen concentration never dropped below 6–8 mg O_2/l, pH 7·5–8·5. Egg-carrying females were kept in fresh water. Freshwater prawn eggs are known to be ellipsoidal. Measurement of egg length and width was taken using binoculars. Once larvae hatched, the entire clutch (family) was transferred from the aquarium to a fishing crib, which

was then lowered into a recirculation water management system tank. Salinity of artificially prepared water in such a system was 6%. Larval density in the fishing crib was 200 specimens/l and 300 specimens/l. To determine moulting frequency and zoea stage duration, larvae were kept separately in 50-ml experimental vessels. Water was changed once every 2 days. To perform experiments for investigating size of larvae from a single clutch, the first 'early individual' group was segregated from the larvae which were first to metamorphose for 19–24 days, which corresponded to 1–6 days from the time the first individual metamorphosed to post-larva (see Fig. 2). The second, 'mean individual' group was made up from individuals metamorphosing for 25–30 days, or 7–12 days after the first individual metamorphosed to post-larva, and the third, 'late individual' group — from those metamorphosing for 31–36 days, or 13–18 days respectively (see Fig. 2). To investigate post-larval growth, each group was placed under identical conditions: temperature 27–28°C, salinity 6%. The original larval density (with each group in a separate tank) was 55 specimens/m^2. All experiments to investigate larval differential growth were carried out six times. Survival ability at the end of the experiments constituted 70–76%. Oriental river prawn larvae were fed on brackish rotifers, artemia cyst, rasped fish, whereas post-larvae were fed on fresh-frozen fish and mixed fodder.

Results and discussion

Out of the aggregate characteristics determining prawn quantity variation the essential factor is the number of eggs in a clutch while egg size is a critical determinant for survival and growth of newborn individuals, particularly among aquatic animals whose parents do not nourish their young (Fowler 1972; Gall 1974; Ware 1975). Table 1 presents fertility and size characteristic of eggs and larvae in females of different age. The unit of heterogeneity is proposed to be the 'heterogeneity coefficient' ('HC'), i.e. maximum-to-minimum size ratio for a given sample. Table 1 shows that eggs (stage 'A') are heterogeneous in size, the heterogeneity coefficient varies with females of different size, ranging from 1·6 in smaller individuals to 1·30 and 1·40 in larger females. Prevailing in the clutch at the same stage are 0·65 and 0·70mm eggs, which account for 30% and 27% of the total amount of eggs respectively (Fig. 1a). At the eye pigmentation stage 'HC' does not vary in females of different size and equals to 1·4. This can possibly be attributed to the fact that at this embryogenesis stage the egg size range is somewhat narrower and, in contrast to stage 'A', is characterized by the availability of one 0·65 mm size class accounting for 64% of the entire clutch (Fig. 1b). At both stages egg size does not actually undergo any change, though, according to Mashiko (1983), during embryonic development towards eye pigmentation stage egg volume increases by 20%.

Larval hatching always begins in the dark and lasts from several hours to 2–3 days. Should the hatching continue for two days, approximately half of zoea is released during the first night, then during the daytime the above process is discontinued and is completed the following night (Kulesh & Alekhnovich 1982). Mashiko (1987) has observed that embryogenesis duration in freshwater prawns at steady temperatures is directly proportional to egg volume, i.e. the larger the egg volume, the longer females carry their eggs. Thus, it would be proper to assume that the smallest larvae are the first to hatch whereas large individuals are the last to hatch from the largest eggs.

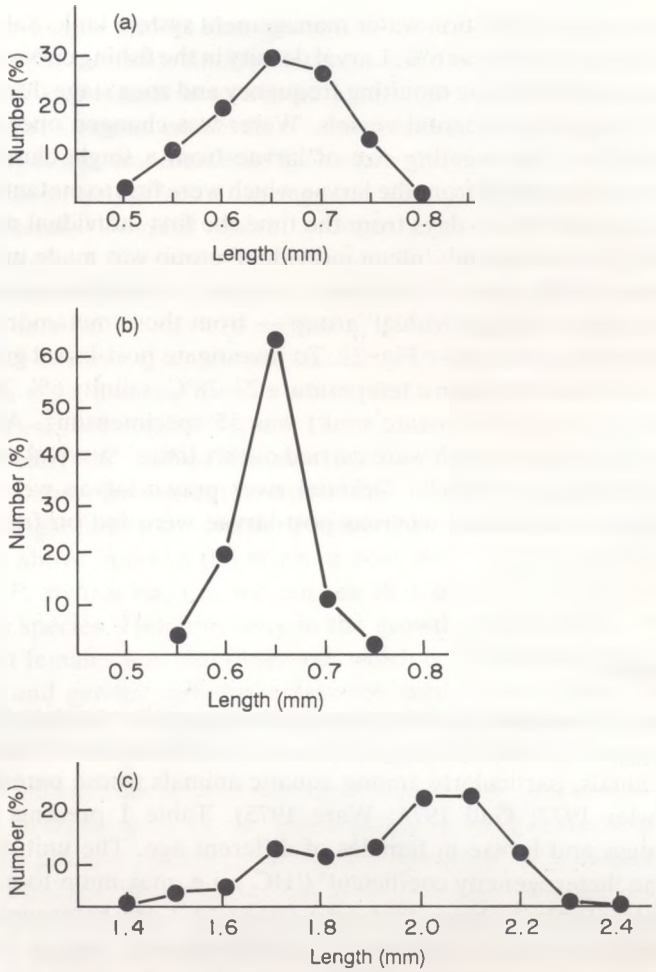


Figure 1. Size characteristic (minimal length) of eggs and larvae of oriental river prawn derived from a single clutch: (a) at stage 'A' (n = 370); (b) at 'eye pigmentation' stage (n = 300); (c) larvae at 'zoea stage I' (n = 639).

On the whole, one-day-old larvae (zoea stage I) are characterized by a rather wide size range — from 1·4 to 2·4 mm. In Fig. 1c, it can be seen that no strongly pronounced leading class is observed at this stage of onthogenesis. As to their quantitative composition, larvae fall into four size groups, i.e. 1·7 mm; 1·8 mm; 1·9 mm and 2·2 mm, each constituting 10–12% of the total clutch strength. Variation and heterogeneity coefficients for larvae hatched by females of different size also vary in wider ranges (Table 1).

Should the larval stage proceed under strictly standard conditions, its duration for each individual from a single egg clutch varies significantly and lasts from 15 to 35 days and more at 27–28°C and with 6% salinity. Oriental river prawn larvae are known to pass through nine zoeal stages in their development, each subsequent stage being separated by a moulting (Kwon & Uno 1969). However, as our investigation of different versions of larval development of individuals from a single clutch (family) suggests, this conformity to natural laws is not always observed. As can be seen from Table 2, the entire spectrum of larval

levelopment from a single clutch is characterized by mandatory zoea initial stages which are ıpproximately equal in duration and are separated from one another by moultings. Of particular interest for identifying larval development physiology, in our view, is zoea stage IV, which is the longest stage, and even for shortened larval development in version I it constitutes 5 days. At this zoea stage two or three moultings occur. Attention is drawn to the fact that it is this larval development period (6–12 days), which is characterized by the highest mortality rate, at times up to 50%. Visual observation indicates that it is at zoea stage IV that reserve nutrients are consumed completely (pellet-shape fat inclusions located in the vicinity of the digestive tract can be clearly seen through the binoculars) and the larva commences exogenous food intake. In the shortened versions of larval development (I and II) larvae pass through six zoea stages only, characterized by seven or eight moultings only. It is noteworthy that in the first version larvae metamorphose from stage V to VII to become post-larvae immediately after the next moulting. In the second version zoea stages V, VIII and IX are missing. Missing in the third version is stage IX only while the characteristic of version 4 is the availability of all nine zoea stages and the noticeable increase in the duration of the larval development period up to 26 days. On the whole, based on Table 2, it can be concluded that shortened larval development in a clutch can be observed in the smallest individuals, i.e. in larvae which are the first to hatch, and, on the contrary, larger individuals are characterized by an extended metamorphosis period when they pass through all the zoea stages. There is a whole range of intermediate versions between them when this or that zoea stage is missing or its duration varies which is related to the hereditary factors of both parents.

Table 1. Egg and larva heterogeneity in oriental river prawn

Female length (mm)	Number of eggs in sample	Minimal size of egg larva (mm)	Maximal size of egg larva (mm)	Heterogeneity coefficient ('HC')	Variation coefficient (c.v.) (%)	Modal class (Mo) (mm)	Maximum frequency of the encountered modal class (%)
Eggs at stage 'A'							
43	369	0·50	0·80	1·60	9·7	0·65	30·6
47	115	0·50	0·70	1·40	7·9	0·66	39·1
57	56	0·50	0·60	1·30	8·8	0·61	33·9
62	54	0·50	0·70	1·40	6·7	0·68	40·7
				1·43	8·3		
Eggs at 'eye pigmentation' stage							
41	100	0·56	0·75	1·40	5·4	0·64	67·6
42	111	0·56	0·75	1·40	5·4	0·66	61·0
65	100	0·50	0·70	1·40	5·9	0·64	53·0
				1·40	5·6		
Larvae at 'zoea stage I'							
42	166	1·30	2·30	1·77	11·0	2·13	25·3
47	160	1·50	2·30	1·53	7·8	2·03	24·2
50	230	1·50	2·30	1·53	10·3	1·90	31·2
54	153	1·60	2·40	1·50	8·8	2·00	35·6
62	90	1·70	2·50	1·47	7·1	2·09	23·0
				1·56	9·0		

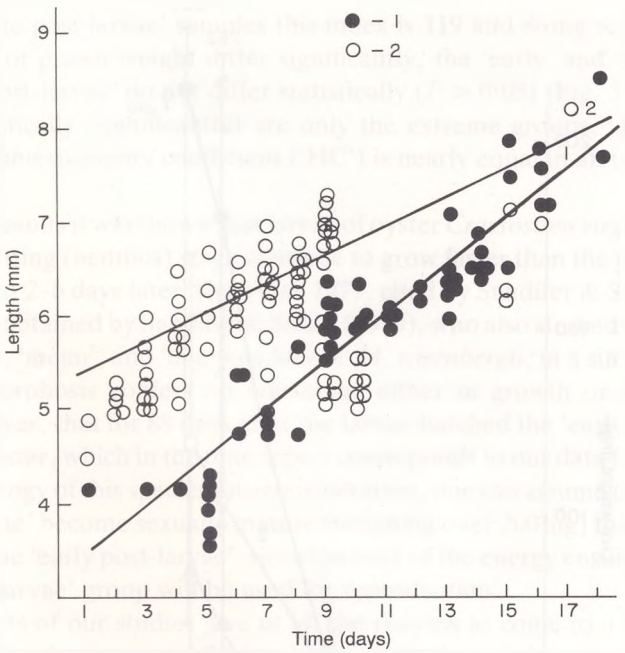
Relationship between metamorphosis duration and the number of larval stages as their dependence on parental polulation is shown for *Palaemonetes pugio* prav particularly markedly for *P. vulgaris* (Sandifer & Smith 1979). Thus, five to twelv stages correspond to 14–25 days (unfortunately, the authors failed to indicate the dura each stage), though the bulk of the individuals pass through seven to nine stages or 14–19 days and minimum and maximum values are intrinsic to solitary specimens only authors conclude that, in addition to the influence of environmental factors, the tre certain larvae to pass through a given number of stages may be hereditary. On the other duration of larval development is not determined by hereditability directly, though it h on the number of stages.

Considering the above, larval development can be divided into two periods of time. I until the moment the first larva passes through all zoea stages, metamorphosing to post-l and second, until all the individuals from a single clutch or sample complete their l development. Both metamorphosis stages are approximately of the same duration. Thu the first clutch, with larval density 300 specimens/l, the first stage lasted for 15 days survival ability by the end of the larval development was 23%. In the second clutch, larval density 200 specimens/l, the first stage lasted for 19 days and the second for 18 d while survival ability amounted to 38%. Of particular importance for studying heterogen

Table 2. Development of oriental river prawn larvae from a single clutch

Duration of larval development (days)	I		II		III		IV	
	A*	B*	A	B	A	B	A	B
1	I	1·8	I	2·0	I	2·1	I	2·
2	II	+2·0	II	+2·2	II	+2·2	II	+2·
3	II	–	II	–	II	–	II	
4	III	+2·4	III	+2·6	III	+2·8	III	+2·5
5	III	–	III	–	III	–	III	–
6	IV	+2·9	IV	+3·2	IV	+3·2	III	–
7	IV	–	IV	–	IV	–	IV	+2·7
8	IV	+3·1	IV	+3·4	IV	+3·4	IV	–
9	IV	–	IV	–	IV	–	IV	–
10	IV	–	IV	+3·8	V	+3·7	IV	+2·9
11	V	+3·3	IV	–	V	–	IV	–
12	V	–	VI	+4·0	V	–	IV	–
13	V	–	VI	–	VI	+4·2	V	+3·5
14	VII	+3·5	VI	–	VI	–	V	–
15	VII	–	VII	+4·4	VII	+4·6	V	–
16	VII	–	VII	–	VII	–	VI	+3·7
17	VII	–	VII	–	VII	–	VI	–
18	p/1*	+3·7	VII	–	VIII	+4·9	VII	+4·0
19			p/1*	+4·5	VIII	–	VII	–
20					p/1	+5·3	VIII	+4·4
21							VIII	–
22							IX	+4·8
23							IX	–
24							IX	–
25							IX	–
26							p/1	+5·8

A* — zoea stage; B* — +-moulting, larva length (mm); p/1* — post-larva.



Relationship between the length of a post-larva from a single clutch and larval development duration. a length — from the beginning of rostrum to the end of telson). 1 — Larval density 300 specimens/l. phosing to the post-larval stage in 15 days; 2 — Larval density 200 specimens/l. Metamorphosing to the al stage in 18 days; 3 — Straight line according to equation (1), 2 — according to equation (2).

and generative processes is the second stage shown in Fig. 2. As can be seen from this relationship between size of post-larval and larval period duration is directly rtional in both versions. Numerically, it can be represented as follows:

$$Y=3{\cdot}144+0{\cdot}262\ X \qquad (1)$$

e Y = length of postlarva, mm; X = time from the moment larva starts metamorphosing stlarva (beginning of the second larval development stage),

=0·894

$$Y=5{\cdot}265+0{\cdot}145\ X \qquad (2)$$

=0·969

ccordance with the above equations, two straight lines are drawn in Fig. 2.

After all the individuals from a single clutch passed through the larval development stage, 17–18 days we obtained the entire spectrum of different size post-larvae (Fig. 2), which de it possible for us to trace their growth with differentiation. Figure 3 shows growth ves for three size groups of post-larvae. The first group, 'early post-larvae', included the lividuals which were the first to metamorphose. They were characterized by the last size d weight indices which on average were 5·6 mm and 1·9 mg respectively. The second group, ıean post-larvae', was made up of the individuals which were 5·9 mm long and 2·5 mg in eight. The third group were 'late post-larvae' and measured 6·6 mm in length and 3·3 mg in eight. These groups accounted for 17·4%, 50·7%, 31·9% respectively of the total number of

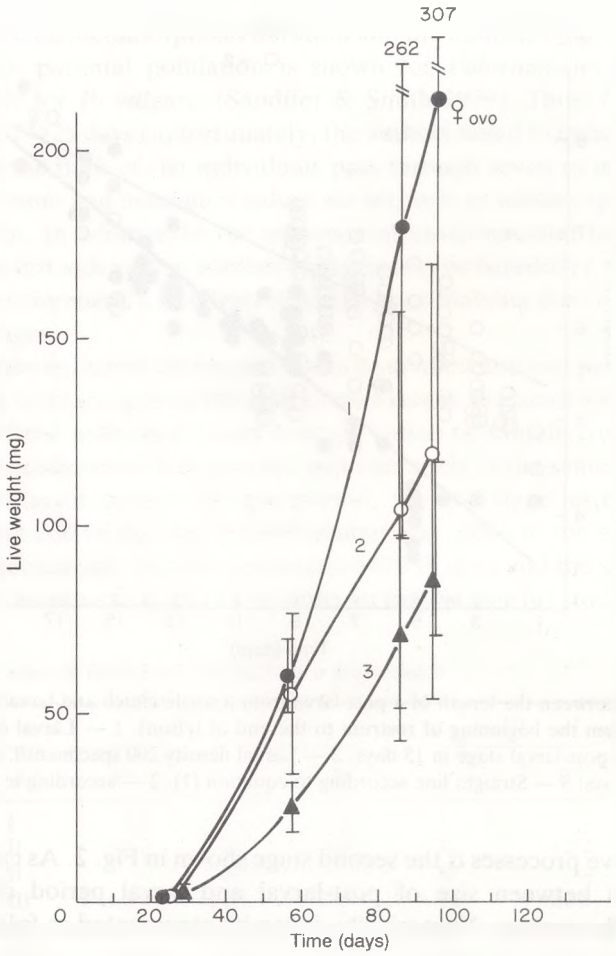


Figure 3. Growth of postlarvae hatched from a single clutch: 1 — 'early post-larvae' group, initial mean w 1·9 mg; 2 — 'mean post-larvae' group, initial mean weight 2·5 mg; 3 — 'late post-larvae' group, initial mean w 3·3 mg.

post-larvae derived from a single clutch. It is noteworthy that about 1% of individuals a characterized by a very prolonged larval development stage — up to 40 days and mo During this period post-larvae grow up to 8 mm in size and 5–6 mg in weight. Similar resu were earlier noted (Sandifer & Smith 1979) for the giant freshwater prawn, *Macrobrachiu rosenbergii*. Despite the fact that, unlike oriental river prawn, larval development of *M rosenbergii* comprises 11 zoea stages and lasts 1·5 times longer, the groups of 'early', 'mea and 'late post-larvae' accounted for 19·4%, 45·7%, 31·2% respectively and with delaye metamorphosis, 1·9%.

As is shown in Fig. 3, the initial weight indices in each group differed statistically ($P <$ 0·05). However, though the initial weight of the 'early post-larvae' was minimal, the firs egg-carrying (8–12%) females were available 94 days after hatching from eggs, i.e. prawn belonging to this group left the rest behind in becoming sexually mature. The mean weight o the individuals coming from the 'early post-larvae' was 212 mg, while for those coming from

e 'mean' and 'late post-larvae' samples this index is 119 and 86 mg respectively. Though nal mean values of prawn weight differ significantly, the 'early' and 'mean post-larvae', nean' and 'late post-larvae' do not differ statistically ($P > 0{\cdot}05$) (Fig. 3).

Differing statistically (significantly) are only the extreme groups, i.e. 'early' and 'late ost-larvae'. The heterogeneity coefficient ('HC') is nearly equal in all three groups — 1·6, 5, 1·7.

Similar to our results it was shown that larvae of oyster *Crassostrea virginica* 'which are the rst to reach the sitting (benthos) stage continue to grow faster than the young whose larvae ew slower and sat 2–6 days later' (Newkirk 1977, cited by Sandifer & Smith 1979). On the ontrary, the data obtained by Sandifer & Smith (1979), who also studied the growth of these roups, i.e. 'early', 'mean', and 'late post-larvae' *M. rosenbergii*, in a similar aspect indicate nat early metamorphosis confers no advantage either in growth or development. It is oteworthy, however, that for 88 days after the larvae hatched the 'early post-larvae' group dividuals grew faster, which in the time aspect corresponds to our data for the oriental river rawn. Taking biology of this species into consideration, one can assume that once the 'mean' nd 'late post-larvae' become sexually mature (weighing over 200 mg) their growth intensity ill equal that of the 'early post-larvae', since the bulk of the energy ensuring somatic growth f the 'early post-larvae' group will be used for reproduction.

Thus, the results of our studies give us all the reasons to come to a determination that hanges in larval development and fluctuations in growth are laid down as early as the egg tage and are conditioned above all by the genetic factor rather than environmental influence. However, this would need proper heritability trials using some form of known breeding tructure probably based on realized heritabilities.

The practical conclusion is as follows:

The first egg-carrying females appear among the 'early post-larvae' hatching from small eggs. Priority of the genetic factor which determines growth heterogeneity is supported by he data produced by Sandifer & Smith (1975), who investigated the impact of density on the degree of body size fluctuations in the giant freshwater prawn. Similar conclusions were made by Golubev (1990) when he studied how temperature affected growth heterogeneity in polymorphous families of the gastropod *Physella integra*. However, great heterogeneity in size and weight of the body, in reaching a specific onthogenesis stage, cannot occur due only to genetic differences. It is also due to the stimulation of growth related to various group effects, such as density, metabolites, physiological influence within the group, environmental factors, increasing or reducing genetical heterogeneity. In this connection, considering influence of the effect of the group and environmental factors, a carefully devised programme or selective breeding should significantly improve growth characteristics in marketable species of prawns.

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