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INFLUENCE OF INCUBATION PERIOD ON LARVAL SURVIVAL AND EARLY DEVELOPMENT IN *Clarias gariepinns*

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Abstract

There is a need to provide fish seeds that can grow uniformly fast in large quantities for supply to the farmers. Investigation was carried out to find out the effect of incubation period on survival and growth rate. The phenomenon of differential growth (jumpers' phenomenon) negates fingerling production by cannibalisms. Fry that were hatched from the same spawn were separated based on the hatching time after hatching. These were reared separately to monitor their growth. Result showed that specific growth rate was higher in groups that hatched at mid-point compared to those at the extremes and even the control. Similar pattern was observed in final weights of the fish but there was no significant difference among the weights. We could not attribute these differences to environmental conditions rather to genetic makeup of the eggs.

KEYORDS: *Clariasgariepinns, incubation period,* growth, larvae, survival, fish production, jumpers

1. Introduction

Mostly in the past and often even in the present time, acquisition of fingerlings for culture has been the capture of fingerling from the wild. This practice has a lot of disadvantages. Ogunsina (2010) enumerated the demerits of sourcing seeds from the wild. These include size variation among the fingerlings, vulnerability of wild fingerling to disease contamination, unavailability of seeds when needed; especially in desired quantities, reduced rate of early stages survival due to temperature and other weather condition variation especially during stocking period, which is at the beginning of the rain in the tropical regions. Also, African catfish fingerlings caught from the wild may have in one way or the other experienced stunted growth due to lack of readily available good food. Therefore, there is need for hatchery reared fry so that consistent good food is provided in sufficient quantities at regular intervals for smooth transitional growth. With hypophysation, availability of fingerlings is promising and reliable. This ensures availability of seeds all year round hence ensuring sustainability of aquaculture industry. Instead of depending on natural regime of seeds availability, the hatchery operator rather set the

time table for seeds availability (Machariaet *al.,* 2005).

It is on record that capture fishery is dwindling (Cengiz *et al.*, 2001). Culture fishery is growing to replace part of a proportion of capture fisheries (FAO, 2006; Jim-Siaki *et al.*, 2015; Obasi *et al.*, 2017). Emiaso (2004) noted that there is a concomitant increase in establishment of hatcheries in Nigeria over the years to boost fingerling production. The need for increased fish production has propelled a lot of other researches related to fish seed production (Brzuska, 2003; Akar and Ali 2006; Shourbela *et al.,* 2014).

According to Ada *et al*. (2020) Clarias 'has no equal in Nigerian fish culture because it satisfies the criteria of a fish required for Aquaculture in Nigeria and Ghana more than any other species'. It has been regarded as equivalent of albino rat in terrestrial research environments.

Other admirable features of these catfishes include the fact that they canendure transportation stress more than any fish within the region. But *Heterobranchus*, which is one of the major species of Clarridae, is less hardy and cannot reproduce when induced in captivity. It grows fast and has higher size at age infinity. *Clarias* also grows almost at the same rate and reached the size at infinity much earlier. Farmers that need annual growth cycle in their farms prefer the culture of *Clarias* to *Heterobranchus*. This is coupled with the fact that *Heterobranchus* seeds are more difficult to obtain.

In the continued struggle to improve fish fingerling production, Kristanto*et al.* (1998) studied the effects of incubation technique on hatching rate, hatching kinetic and survival of larvae in the Asian catfish, *Pangasius hypothalamus (Siluriformes: Pangasiidae)*. These workers observed that there were no differences in the rate of egg hatching when different incubation methods were applied. They also concluded that survival depended on intensity of care rather than incubation method. Kristanto *et al.* (1998) reported that the eggs of *Pangasius species*, an Asian cat fish, become sticky on contact with water and ordinary sack as substrate and no substrate led to hatchability of 64.75 % and 20.70 % respectively. On egg density per area, Akomoda and Gabriel (2005) reported that egg density higher than 4.05 g/cm^2 will lead to hatchability of less than 50 %. Akomoda and Gabriel (2005) studied the effects of materials used as kakaban (nesting materials) for incubation on the hatching rate. They concluded that polyvenil chloride (PVC) frame with mesh net (1mm x 1mm) as substrate (kakaban) led to a higher hatching rate of 79.40 % compared to 77.55 % hatchability when ordinary fine net material was used. The present work is an investigation into the influence of incubation period/duration on the survival and early development in African catfish, *Clariasgariepinus*. Quick and Brutton(1984, cited in Ada *et al*. 2020) reported that incubation period is influenced by temperature. Higher temperatures lead to shorter periods of incubation. This is because higher temperatures cause faster developments. These writers also reported that higher temperatures lead to faster developments but cause a production of organisms with smaller final weight and length (size).

Though *Heterobranchus* has higher length and weight at age infinity, *Clarias* do compete with it for growth within the first one year. As it is easier to obtain *Clarias* seeds than *Heterobanchus, Clarias* is more commonly cultured. Getting seeds in large number and in uniform size is what give *Clarias*an advantage over *Tilapia* (Amoussou 2017).It is said to be highly fecund. Fecundity here is the number of eggs which are found in the ovary of fish. It could also be described as the egg laying capacity of a fish in one season (Nandikeswari, 2014). The relative fecundity of a fish is expressed as the number of eggs per unit weight

of fish measured in mg. Both fecundity and relative fecundities vary with fish species and season as well as age, size and food availability (Murua *et al*., 2003).

Based on the favourable characteristics of Clariids as cultivable fish species (Olaniyi *et a*l., 2013; Olaniyi and Omitogun, 2014; Solomon *et al*., 2019; Kritonto *et al*., 1998; FAO, 2001), said they have made significant contribution over the years in the production of animal protein in many countries of the world The features that make it the most cultured and highly acceptable for consumption in Nigerian and Ghana have been mentioned in the articles of Okey *et al.(*2018) and Amisah*et al.(*2009).

In effort to get the fingerling available all year round, many researchers have recommended hypophysation in preference to other methods of seeds acquisition (Ogunsina, 2010)). To improve hatchability and survival of early stages, so many people have contributed in different areas. For instance, Akomoda and Gabriel (2015) and Machaiia (2005) had worked on hatching success of *Clariasgariepinus* egg using different incubation substrate and varying egg area density. Olaniyi and Omitogun (2014) studied the embryonic and larval developmental stages of African giant catfish *Heterobranchusbidorsalis.* Olaniyi and Omitogun (2013) worked on stages in the early and larval development of the African catfish Clarias*gariepinus*.

Lombardo (2011) worked on killifish and calculated embryo survival as mean of live embryosand hatching rate as the percentage of number of viable larvae after hatching, divided by the number of fertilized eggs.

Santi (2017) noted that high but optimal temperatures are responsible for regulation of gametogenesis and even sex determination in *Clariasgariepinus* as he demonstrated that a temperature of up to 36.5 ^{0}C can result in all male fish populations.

Differential growth rates and survival of juvenile fish, a phenomenon described as jumpers is a concern to fish farmers. This phenomenon is prominent in *Clarias* fingerlings. It has far reaching consequences. Individuals that grow faster become aggressive and cannibalize the stunts. This causes a lot of losses in the number of individual fish and thereby reducing the profit margin of the hatchery operators.

Several workers have investigated the probable causes of this phenomenon of jumpers. These include (Nguenga *et al*., 2001; Uedem-Naa and Nwalili, 2017; Wang*et al*., 2020; Uwuemesi, 2021). No definite conclusion has been reached. One possible factor not yet investigated could be the length of incubation of eggs, having in mind that Quick *et al.* (1984) pointed out that rate of development increases with temperature but result in smaller final sizes. This work will reveal if the length of incubation has anything to do with jumping phenomenon.

2. Materials and methods

The site of the experiment was in Obubra governmental area of Cross River State, Nigeria the area is situated at Latitude 6 - 7^0 N and Longitude 8 - 9^{0}East. It is a tropical rain forest zone with two distinct rain regimes having dry season between December and April; and wet season between May and November. Its average rain fall is between 207.22 – 220.01 mm to mm per year.

Female and male broods were acquired from Shepherd Agricultural Farm in Calabar and Prof Fidelis B. Ada's fish farm in Akamkpa respectively. These two locations are within the same geographical zone with the same rainfall regime and vegetation. Sexes were identifiable using criteria of Yisa and Olufiagba (2004). They were transported to the wet laboratory of The Department of Fisheries and Aquatic Science, University of Cross River State, Nigeria for the experiment. The weight of brood stock was

measured using a kitchen scale (which was a spring balance) to the nearest g.

Broods were fed with industrial pelleted floating feeds (Top feeds)of pellets size 9 mm. The fish were fed 6 % of their body weight divided into two rations fed at 900 -1000 hours and 1700 – 1800 hours (Mills, 1986). Feeding of larvae was carried out by using powder feeds (trout starter with 45 % protein) produced by Finstar Foder in Vasa Finland. Appelbaum and Kamler (2000) found that this feed is not inferior to Artemianauplea in terms of protein and energy. Water change took place every two days in brood tanks.

Length of fish was measured with the help of a measuring board. Total lengths, which is a distance from the most anterior part of the snout the most posterior part of the tail fin were recorded in mm. However, some scientist argued that standard length should be used for fear of caudal fin mutilation. Our broods were apparently healthy with complete fin rays.

2.1 Hormone administration

The hormone used was ovaprim. This was done at the rate of 0.5 ml/kg of fish Injection was given intramuscularly. The syringe was pointed towards the head of the fish above the lateral line with the needle at 45 degrees to body of the fish. After the injection, the fish (brood) was checked to monitor the fishes' readiness for ovulation. The first check was six hours after and followed by check frequency of 1 hour intervals. The fish was ready for stripping when the blue green and translucent eggs were observed coming out from the vent of the fish after 12 hours. The weight of eggs too were weighed with the kitchen weighing balance model SH150 made in China to the nearest gram. Total egg mass from a gravid female was weighed One gram of eggs were weighed in triplicates and counted. The weight of one egg was calculated from the formula

$$W = \frac{\text{weight of I g of eggs}}{\text{number of eggs in 1 g}}$$

To stripe the eggs, the female brood was moped dry. A clean towel was used to hold the head rejoin firmly while a gentle pressure was applied on the abdomen to expel eggs. These were collected in a clean dry plastic bowl for fertilization. Size of eggs was obtained by measuring a random sample of 10 eggs using a microscope with ocular micrometer to the nearest 0.01 mm.

Eggs were fertilized by mixing with milt obtained from macerated testes of a slaughtered male (Shourbela *et al.,* 2014). The milt was prevented from sticking together by adding saline solution prepared by adding 9 g of table salt to 1 L of water. Milt was obtained from a male measuring 510 g after sacrificing the specimen. It was dissected by cutting transversely through anterior position of the abdomen followed by a ventro-lateral cut to expose the viscera. The bi-lubed testis was removed and mobbed dry with a tissue paper.

2.2 Incubation

The fertilized eggs were spread into nesting materials (the kacaban) inside the tanks containing water from a stream. The water was not changed for the period of incubation so as not to disturb the eggs or larvae. The eggs were monitored for several parameters. These include percentage of fertilized eggs. After 12 hours' fertilization, a sample of eggs was obtained by taking a portion of eggs from the central part of the kacaban in triplicates. They were placed in a Petri dish and examine under a binocular microscope at X3. Eggs that were not fertilized looked dull/whitish and were separated from the fertilized eggs that looked transparent/and greenish. These two types of eggs were counted (fertilized and unfertilized). Fertilization rate was calculated from

$$\frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

Hatching rate was also calculated from:

Number of hatched eggs (larvae)x 100
Total number of fertilized egg

2.3 Design

Fry rearing tanks of uniform size 45 by 30 cm^2 with the inner water depth of 20 cm were used for fry rearing. Each tank was fitted with inlet of 1.5 cm diameter and an outlet of 0.5 cm diameter PVC pipes. The tanks were exposed at ambient weather conditions. The treatments were made of different lengths of time the eggs incubated before hatching within the same environmental conditions. Treatment I was given as the time from zero hours when the eggs were fertilized to a period of 50hours (control). Treatment 2 is the period between 20 hours to 26hours. The fish eggs were incubated in the first tank (treatment 1) in triplicates from the point of fertilization to the time the eggs started to hatch within 20 hours to 26 hours. Treatment 3 is the period 26 hours to 32hrs. Treatment 4 is the time between 32 hours to 38hrs. Treatment 5 is the time between 38hours to 44 hours while treat treatment 6 is period between 44 to 50 hours after fertilization. The nest containing the eggs was removed with a gentle shake to cause the already hatched larvae to fall into tank 2. The nest was immersed in tank 3 which is treatment 3. All larvae hatched in each tank were reared in the respective tanks. For easy computations, larvae population were made uniform (200 each).

2.4 Growth measurements of fry

Growth changes considered in this experiment were the length and weight of the fish.,Weights were measured in batches using a sensitive spring balance (model: ……) to the nearest 0,01 g/ the length was measured using a method applied by Agadjihouede *et al.,* (2011). A transparent water proof paper was used to cover a graduated paper on which the larvae were placed for length measurement.

2.5 Weight and length gains

This was derived from the formula weight gain (WG) = final mean weight (FW)-initial,weight (IW). (Ayinla and Akande, 1988;,Popoola and Fammagun, 2017). Length gain (LG) was calculated using the formula LG = FL – IL

Where FL = final length and IL = initial length

2.6 Percentage weight gain

Percentage weight gain was obtained by substituting the values in the formula

PWG = (final weight – initial weight / initial weight) x 100

(Popoola and Famuagun, 2017)

2.7 Specific growth rate

This was obtained using this formula

SGR= log final weight – log initial weight x 100 over time (in days) between two measurements

(Ada *et al.* 2020; Popoola and Famuagun, 2017)

2.8 Survivor rate (SR)

SR = (Survivors/ Total number of fish stocked) × 100

(Akinwole *et al*., 2006;Omodu *et al.*, 2017; Popoola and Famuagun, 2017)

This was calculated in interval of seven days and plotted in survival curves as shown in 1.

2.9 Water quality parameters measurements

Water quality could be anything in the water, be it physical, chemical or biological, which affects the normal physiological functions of fish. These parameters were measured to monitor a relatively stress free environment that may meets the physical, chemical and biological standards for the fishes' normal health and normal physiology.

The temperature of the water in which the fish were contained was measured using a mercury in

glass thermometer and electronically by a Dissolved oxygen meter BLE-9100to the nearest degree Celsus.

Dissolved Oxygen was measured using electronic, method. The Dissolved oxygen meter BLE-9100was used for oxygen determination.

pH was measured using electronic method. The pH meter model WTW PH 90was used for pH determination.

The Conductivity was measured using Aqua-probe 2000 (AP2000) conductivity meter manufactured by AQUAREAD COOPERATION. The red and black leads of the meter were plugged into the positive and negative ports respectively. The digital meter was turned on. The measurement dial was switched to the resistance mode (Omega which is the same as ohm). The temperature of the water used in the experiment was at the same temperature as room temperature. The electrodes were dipped into the sample water. Before each measurement, the electrodes were washed and dried. Chi square (χ^2) was used to test the homogeneity of survivors of larvae between groups while growth was analysed using one way analysis of variance

3. Results

Fertilization rate in the different replicates of the experiment showed that replicate 1, 2 and 3 to be 84 $\pm$ 2,29 %, 84 00 + 3.00 % and 84.00 $\pm$0.5 % respectively. Survival was taking at the end of seven days after hatching. This was recorded for different replicates as 54.60 %,53.96 % and 54.08%; this gave a mean survival for this experiment as 54.21 $\pm$ 0.25 %. Survival rates were all the same in all the groups. Though there was a peak in the middle, x2 analysis did not reveal the heterogeneity among treatments ($p < 0.5$). Specific growth rate was statistically different among groups ($p < 0.5$) with group 32 – 38 hours being the highest, followed by group 26 – 32 hours' group. Percentage weight gain and mean weight gain followed similar pattern as specific weight gain as seen in Table 2. However, analysis of the length at day 7did not show any difference between groups. Extending the experimental period could bring the difference out.

Table 1; statistics of broods used in breeding

Brood sex	Weight (g)	Total length (cm)	Weight of reproductive materials	Specific fecundity(eggs/g of fish)	GSI
Female	1,658	83	135.2	64	0.0815
Male	725	71	5.1	-	0.0718

Table 2; biological parameters of *Clariasgariepinus*fry hatched at different durations of latencies (hours after fertilization)

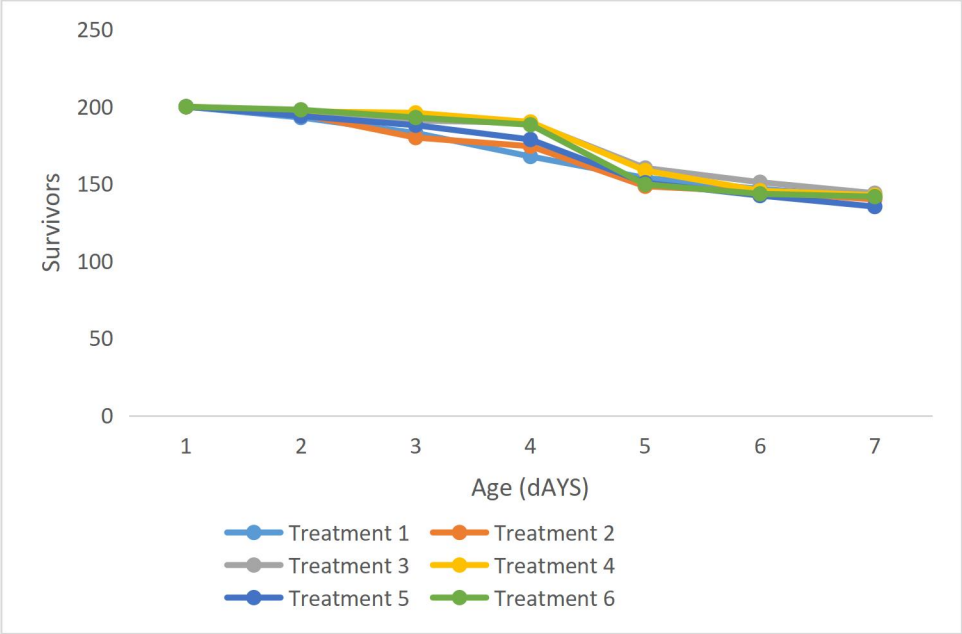

Figure 1; survivor curves for fries from different treatments (1 - 6)

Treatment, (intervals in hours)	0.0 – 50 (control)	20 – 26	26 – 32	32 – 38	38 – 44	44 – 50
Survival, up to 7 days after hatching (%)	70.67 ± 2.08	71.00 ± 2.65	72.00 ±1.00	71.33 ± 2.18	67.67 ±2.51	70.89 ± 2.47
Specific growth rate (mg)	30.44 $\pm 0.52^{a}$	30.75 $\pm 0.74^{ab}$	32.10 $\pm 0.72^{ab}$	31.07 $\pm 0.93^{b}$	31.06 $\pm 0.81^{ab}$	29.86 $\pm 0.70^{a}$
Percentage weightgain (%)	742.31 $\pm 1.52^{b}$	760.77 $\pm 9.23^{c}$	848.46 $\pm 1.49^{e}$	916.92 $\pm 1.58^{f}$	789.00 $\pm 1.76^{d}$	700.77 $\pm 2.13^{a}$
Mean weight (mg) at day 7	10.95 $\pm 0.23^{ab}$	11.19 $\pm 0.69^{ab}$	12.33 $\pm 1.54^{ab}$	13.09 $\pm 1.11^{b}$	11.44 $\pm 0.63^{ab}$	10.51 $\pm 0.56^{a}$
Mean length (mm) at day 7	11,95 ±0.63	12.61 ±0.90	12.72 ±0.48	12.48 ±0.53	12.06 ±0.66	12.37 ±0.54

Table 3; physicochemical properties of water within the incubation period of two days

Treatment, (interval) (hours)	Temperature	pH	TDSx10^{-3} ($msec^{-s}$)	Conductivity	Dissolved oxygen
Before, 0.0	26.45 ± 1.55	7.30 ± 0.22	3.00 ± 0.0	6.00 ± 0.0	6.22± 0.06
0.0 – 50 (control)	26.25 ± 1.44	7.22 ± 0.5	3.85 ± 0.5	7.60 ± 1.0	5.12 ±0.15

20 – 26	26.00 ± 1.56	7.14 ± 0.0	3.00 ± 0.0	6.00 ± 0.0	5.28 ± 0.36
26 – 32	26.12 ± 0.23	7.24 ± 0.32	4.00 ± 0.0	8.00 ± 0.0	5.58 ± 0.08
32 – 38	26.72 ± 1.45	7.11 ± 0.14	3.25 ± 0.75	6.50 ± 1.5	5.20 ± 0.02
38 – 44	26.50 ± 1.35	7.14 ± 0.28	3.75 ± 0.75	7.50 ± 1.25	5.18 ± 0.16
44 – 50	26.46 ± 1.44	7.04 ± 0.60	4.30 ± 0.9	8.60 ± 1.8	5.02 ± 0.14

4. Discussion

It is earlier indicated in literature that higher temperatures lead to higher metabolic rate (*Teugels, 1982;* Quickand Brutton, 1984). This drives fish to reach their recruitment in a shorter time (Wang *et al*., 2020) or even the fertilized eggs to hatch earlier (Robert, 2007). In this work, the fertilized eggs were exposed to the same ambient temperature of 26 to 27 ^{0}C. This temperature range falls within the range (25 to 32 ^{0}C) acclaimed to be suitable for the development of *Clariasgariepinus* (Afzal *et al*., 2007).

Many workers have found in their works that the size, survival and growth rate of fry depend on the size of eggs. The size of an overall egg also has large yolk on which, the fry depends on for feeding before they begin to feed independently. It is as well-known that the size of the egg depends on the age and size of brood (Umnah, 2020; Bichi *et al*., 2014; Roff, 1992; Marsh, 1986; Miller *et al.* 1988). It is worth asking then, what is responsible for the variation in growth OF eggs that are all sourced from a single brood. One therefore has to look in the direction of genetypic and environmental contributors (Umnah, 2020). It is equally known that there exist intra specimen variations in egg size. It is worth mentioning that although eggs in a particular spawn did not start developing at exactly the same time. And if the eggs which hatched at a particular interval have a certain attribute, it become logical to argue that the hatching time and that attribute(s) which, encourage food intake are linked. Attribute in our case are fast growth and aggression. Individual having these characters are described as jumpers.

Ability of an organism to grow is dependent on ability to ingest, absorb and assimilate (IAA) food substances. The rate of growth is therefore dependent on the quantity and quality of food substances available for the cells to assimilate. It therefore means fish that grow fast have large quantities of desired quality of food substances inundating their cells.

Fertilization rate of 84 per cent of eggs was high indicating that the reproductive materials as well as the environmental conditions were conducive. The work of Olaniyi and Omitogun (2013) produced a hatching rate of 85 per cent in the same fish. Bichi *et al*. (2014) observed fertilization rate of 21 to 65 per cent which was decreasing with size of broods due to immaturity of smaller broods. This equally calls for investiation into the effect of an egg amongst the spawn on the growth of the resultant fish

Work on Pangasiushypophthalmus (Siluriformes, Pangasidae) by Kritanto et al. (1998) showed that hatching period did not influence the survival of fryup to four days after hatching. They discovered that it takes this fish a period of six to eight hours for all the eggs to hatch. Observation in Clariasgariepinus in this experiment shows that the hatching interval ranges from 24 to 48 hours. That means that by the time the last group of eggs are hatching, the first hatched of larvae are 24 hours old. The specific growth is the speed of increment of size of fish per day. It was seen to be significantly different among groups hatching at different times with those hatching early and the late grow slower or have less specific growth among rate than those at the midpoint (see table 2). However, at age 7days, the resultant growth among the fish only produce with significant difference in size at extreme incubation period (44-50hrs)

Giving that extremes shows individuals with smaller sizes, the late hatchers which are

contained in the general pool (control) could have resulted in the low specific growth rate as well as resultant size of members of the hatching batch. It is possible that if the assessment was continued for a longer time, those with higher specific growth rate should produce larger size fishes (Hopkins).

It is still however observed that individuals that were pooled, (treatment 1 that allowed hatching period from beginning to about 50 hours and the late hatching individuals were having individuals with smaller average size. Those that hatched between 26 hours to 44hours had larger sizes.

Mortality was high on day four. This was possibly due to the transition from yolk feeding to exogenous feeding. This was the period of weaning, and for individuals that are not capable of moving from endogenous feeding to exogenous feeding could not survive. After this day (period of weaning), mortality become low and steady

5. Recommendation for further study

For a particular spawn, it is composed of eggs that must have started development at different times. That mean the eggs of a spawn finish and contains eggs of different ages and sizes. It is suggested that should be done to trace the rate of growth to the relative age of the egg in the spawn.

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