Aquaculture Methods and Early Growth of Juvenile Horseshoe Crabs (*Limulus polyphemus*)

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Abstract Current knowledge of horseshoe crabs (HSC) has been derived, in large part, from field studies. Comprehending the biology and conservation of HSC could be facilitated and augmented by understanding and improving their culture methods. Although many researchers and even lay people are capable of getting animals to the early stages of development, very few are successful in getting them to survive for longer periods of time. The Aquatic Research and Environmental Assessment Center (AREAC) has been successful in rearing HSC in annual cohorts, some for more than 7 years. We have used indoor recirculating aquaculture systems (RAS) containing medium prepared with artificial sea salts. Animals have been given various diets, including both natural and specially formulated feeds. We have traced the earliest developmental stages through juvenile development in animals that were derived from eggs fertilized in the field and laboratory. This chapter will discuss the problems and successes of culturing adult and developing HSC in RAS, methods of fertilization, feed regimes, growth, and survivorship and observations on HSC development from egg to juvenile.

1 Introduction

We have witnessed, in past years, dramatic reductions in the population of horseshoe crabs in most parts of the world where they have existed – some almost to extinction. Although not as severe in the Far East, populations of the Atlantic horseshoe crab, *Limulus polyphemus*, have also declined. There is a concerted effort to address this problem; however, the various stakeholders including pharmaceutical companies, commercial fishermen, shorebird advocates, and environmentalists have largely differed in estimating the significance of the problem and the method of approaching its solution (Berkson and Shuster 1999;

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Odell et al. 2005). It is certain that whichever method is adopted in its conservation, there will continue to be a harvest demand. Sustainable harvests of a species serving such a vital role in ecosystem processes may only be achieved by augmenting wild populations with cultured individuals. The ultimate goal of this form of stock enhancement and restoration must be to produce large numbers of healthy young horseshoe crabs that can be returned to the ecosystem with an optimistic anticipation of their survival. In this chapter we discuss horseshoe crab aquaculture methodologies using land-based, indoor, intensive production of animals in recirculating (water reuse) aquaculture systems (RAS).

Growth in horseshoe crabs is generally slow taking 9–12 years to reach maturity (Shuster and Sekiguchi 2003). Therefore, aquaculture production of mature animals is clearly not feasible. However, production of juveniles to be released into the environment for stock enhancement can be an effective management strategy as observed by its use in salmonid and bivalve fisheries. Botton et al. (2003) estimated the survival rates of trilobites transitioning from the planktonic phase to the epibenthic form to be 2.5%. The authors then estimated a log-fold decrease in the density of juveniles occurring with each molt from the second instar to the fourth instar at the end of their first summer. Although, data describing the survival of fourth instars to maturity are limited, it is believed that increasing the strength of the first-year class through aquaculture stock enhancement may lead to improved viability and stronger adult-year classes.

Horseshoe crabs, including adults and embryos, have been maintained in captivity largely for research purposes. Brown and Clapper (1981) described their methodologies for obtaining gametes and culturing embryos for studying the earliest stages of development. More recently, Smith and Berkson (2005) presented their work on system design, water quality, health issues, and nutritional demands. They described a chronic mortality that occurs in juvenile and adult HSC when they are held in captivity for greater than 6 months. They believe that the mortality is a result of a hypoproteinemic deficiency that is associated with inappropriate diet formulation. Clearly, nutritional requirements must be further studied in order for HSC aquaculture to be successful.

The growth of juvenile horseshoe crabs has been studied in field (Rudloe 1981; Botton et al. 2003) and laboratory experiments (Sekiguchi et al. 1988; Lee and Morton 2005). Typically, growth measurements are expressed in prosomal width. However, the quantity of feed distribution in aquaculture is calculated on a per weight basis. Lee and Morton (2005) fed *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, a mixture of fresh seafood in excess at 50% per wet weight for 5½ months. Both species increased approximately 24 and 71% in wet weight and prosomal width, respectively, after each molt. Similar studies have been lacking for the American horseshoe crab. In addition, further studies need to identify a cost-effective feed type and feeding regime. Lastly, another important consideration is the ability to produce large numbers of healthy juveniles. Therefore, all future studies should be directed toward production on a large scale.

We at the Brooklyn College (City University of New York) Aquatic Research and Environmental Assessment Center (AREAC) have instituted a program to study the captive breeding and maintenance of HSC in specially designed systems. It has comprised more than 7 years of intensive field and laboratory studies. What follows is a report of our successes and failures in the aquaculture of HSC and our recommendations for future applications and continuing research.

2 Aquaculture Methods

Our annual production schedule is summarized in Fig. 1. This timetable has been followed, with some modification, for almost 8 years, so that our oldest animals are now more than 7 years old. Our annual activities begin in the months of May and June, a period that is the spawning season for HSC in the New York City region.

Eggs are collected and fertilized in the field or in AREAC. The fertilized eggs are maintained in McDonald jars (Aquatic Eco-Systems Inc.; Fig. 2) and

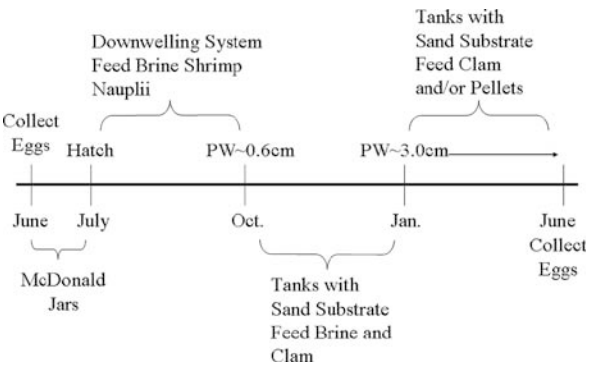

Fig. 1 An annual horseshoe crab aquaculture production schedule at AREAC (PW, prosomal width)

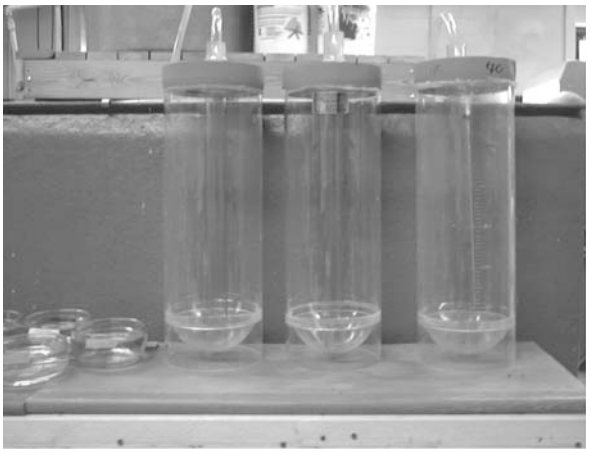

Fig. 2 Egg culture units; finger bowls are on the left and McDonald jars are on the right

transferred to downwelling systems (see below) between July and September. In October animals are transferred to larger recirculating aquaculture systems (RAS) and fed brine shrimp nauplii and finely chopped clam meat. In January, the chopped clams are supplemented with commercial fish food pellets with high protein content (35–40%). The next annual cycle is repeated (as it has in nature for 440 million years!!) in ensuing Mays.

2.1 Egg Collection and Culture

The major source of our animals is derived from Plumb Beach (N45° 35.002′ W073° 55.311′), a sandy beach on the north side of the Jamaica Bay inlet (Brooklyn, NY, USA). Plumb Beach, which generally has a robust spring spawn of HSC, is part of an urban US National Park – Gateway National Recreation Area. Fertilized eggs were derived from field collections in the sediment of spawn sites formed the previous evening. Gametes were also obtained by manual stripping in the field or in animals brought to AREAC. In this procedure slight pressure is applied anterior to the genital operculum (French 1979). We have also utilized a method of electrical stimulation, using a two pronged wire that is connected to a 3–4 V dry cell. The probes are placed below the gonopores and current applied in short intervals to release the gametes (Brown and Clapper 1981). The released eggs are collected from the gonopores by hand and the sperm is collected with a 1 ml pipette. Eggs and sperm are immediately placed together in a fingerbowl with sea water. After 30–60 min the water is changed in the fingerbowl or the eggs are placed in a McDonald jar (Fig. 2). Neither of these methods of artificially stripping HSC will produce sufficient numbers of eggs for large-scale production. Prolonged or repeated stripping will result in lesions below the gonopores and/or damage to the genital operculum. Collection of naturally spawned eggs appears to be a better strategy for aquaculture, at least until methodologies of induction of spawning are identified. We have also been concerned that egg quality may decrease if eggs are manually collected from HSC that have already completed spawning for the season. To examine this, we sampled four individual crabs from the shallow subtidal zone at Plumb Beach in late July and compared egg dry weights ($n = 30$) between the crabs (Fig. 3). There was no significant difference ($P > 0.05$) in egg quality as determined by egg dry weight between the crabs. Furthermore, we have also compared the dry weight of larvae produced from natural spawns ($n = 120$; pooled from four individuals) and artificial spawns ($n = 120$; pooled from four individuals) and found that there was no significant difference ($P > 0.05$) between the two groups (Fig. 4). Therefore, it appears as though methods to induce spawning will not produce inferior eggs and larvae.

We have experimented with the culturing of eggs and have compared hatch successes of eggs placed in small (12.5 cm in diameter) finger bowls and

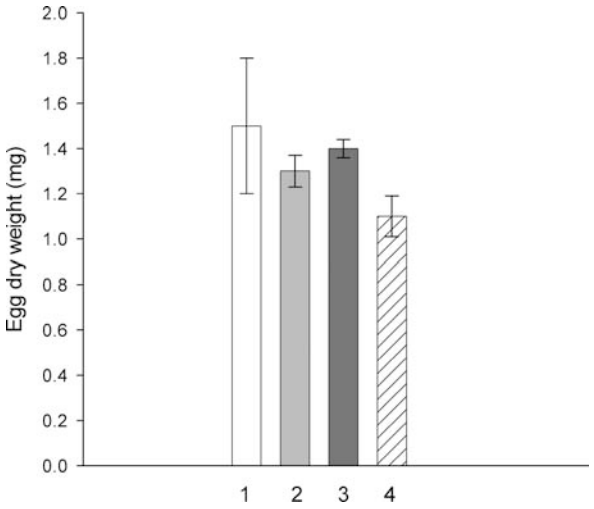

Fig. 3 The egg dry weights (n = 30) of four artificially spawned horseshoe crabs collected in July (error bars; ±SE)

McDonald jars. The eggs in the finger bowls are not agitated except during water changes which are typically done once per week. The greater hatch success (>75%) occurs when we use the McDonald jars where the eggs are constantly agitated, while our results with the finger bowls are far more variable. Typically, we will keep 300–500 eggs in a McDonald jar with a flow of 40 l min^{-1}. This density and flow rate are adequate for the number of crabs we have been producing. These values would have to be increased for large-scale production to be efficient, as these jars are 6 l in capacity and can hold 100,000 trout eggs. The use of static finger bowls is also adequate for culturing eggs. However, in our experience the eggs are more prone to fungal infection. Regardless of egg culture methodology, fertilized eggs generally hatch within 1 month.

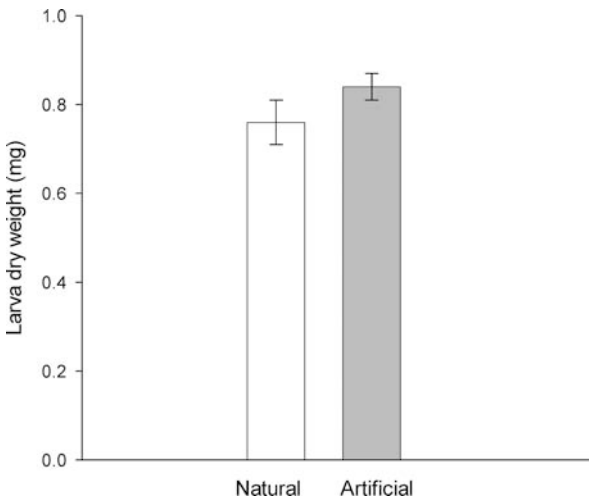

Fig. 4 The mean dry weight (±SE) of larva (n=120) from natural and artificially spawned horseshoe crabs

2.2 Larvae and Juvenile Culture

In all phases of animal rearing, recirculating aquaculture systems (RAS) are employed. The capacities of these systems range from 6 l (McDonald jars) to 2,000 l (our larger RAS are used to house adult HSC). The saltwater we utilize is constituted from artificial sea salts (Instant Ocean®) and filtered New York City tap water. Table 1 summarizes the water quality parameters that we have determined to be effective. For the most part, they reflect the characteristics of the water in the animal's natural habitat (Jamaica Bay, NY). These conditions are maintained throughout the year. Water quality is measured thrice weekly.

Table 1 Water quality parameters maintained in recirculating aquaculture systems for horseshoe crab culture

Temperature	20–25°C
Salinity	27–33 ppt
Dissolved oxygen	>5.0 mg l^{-1}
pH	7.5–8.8
Ammonia	<1.0 ppm
Nitrite	<0.6 ppm

When the HSC eggs hatch and the larvae emerge, they are moved to downwellers where our best survival rates have been achieved. These downwellers are typically used in our bivalve aquaculture programs. Generally, our downwelling systems are composed of a 320 l rectangular fiberglass trough (Fig. 5) with several PVC cylinders approximately 30 cm in diameter covered at one end with a 180 μm Nitex® screen. Each downweller is created by adding an airlift, which enters the top of the cylinder and forces seawater from the trough into the cylinder. A flow of 1.5–2.0 l min^{-1} is maintained into the cylinder. This flow rate helps minimize

Fig. 5 An example of the downwelling systems used in the culture of horseshoe crabs through the second or third instars

the occurrence of external epiphytes and fungus. The use of the downwelling system does not conflict with our annual bivalve production cycles since the young bivalves are moved to field grow-out sites prior to HSC egg hatching. Therefore, this provides an additional use for these systems and could be used by bivalve aquaculturists interested in producing HSC for stock enhancement.

We compared the percentage of total lipid and carbohydrates occurring in eggs (24 h post-fertilization) and larvae (24 h post-hatch) to observe changes in these energy substrates. There was no significant difference between the lipid content of eggs and larvae. However, we did observe a significant reduction in carbohydrates from egg to larvae (Fig. 6). The larvae do not have to be fed until after the first post-hatch molt (Brown and Clapper 1981). Following the first post-hatch molt the HSC are fed brine shrimp nauplii (~450 μm). The 180 μm screen at the bottom of the downwellers serves to retain the brine shrimp for HSC consumption. The downwellers are removed from the system three times per week and gently sprayed with seawater to remove the organic material that has accumulated on the mesh screen, as well as on the HSC. The downwellers are sprayed with freshwater and scrubbed, after removing HSC, on an irregular basis. The HSC are cultured in the downwelling system through September and have at that time a mean prosomal width (PW) of 0.6 cm.

By October of the first year (see production schedule, Fig. 1), animals are moved to RAS tanks of various dimensions (see Schreibman and Zarnoch (2005) for details on RAS). In all cases a layer of sand is placed at the bottom of the tank. Larger juveniles (>1.0 cm PW) utilize a fine substrate for burrowing which also minimizes epiphyte accumulation.

These HSC are fed chopped clam meat thrice each week at approximately 1.5% of their body weight. They will also accept, and are offered periodically,

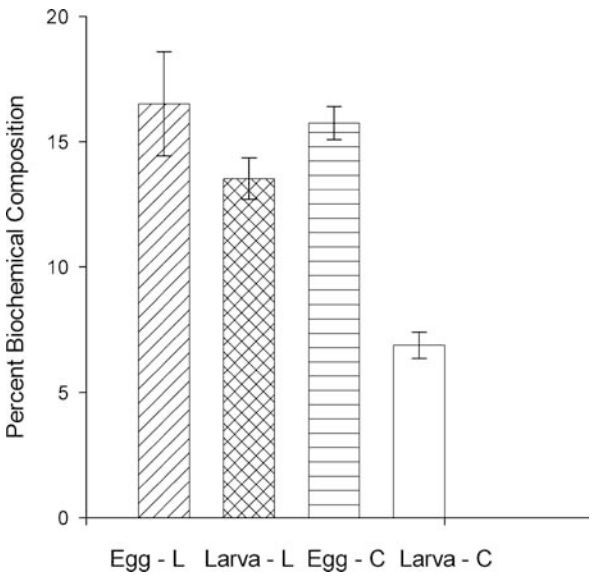

Fig. 6 The change in total lipid and carbohydrate content in eggs (n = 30) to newly hatched trilobites (n = 30). Units are given as a percentage of the biochemical constituent per unit dry weight (DW) of the tissue (L, lipids; C, carbohydrates)

high protein (35–40%) commercial fish pellets. We have instituted a program of nutritional studies in an attempt to improve and accelerate growth rates. In a recently completed study (Tzafrir-Prag et al., in prep.) juvenile HSC were fed increasing levels of energy and protein (0, 1, 2, 3% BW^{-1}) through pelleted feeds composed of fish meal or clam meat. This resulted in a linear gain in protein and energy. However, the partial efficiency of utilization, which accounts for losses (i.e., heat) in protein and energy when food energy is transformed into usable forms, was low (0.13 for protein and 0.16 for energy) indicating that there was poor assimilation of the feed. This is probably related to molting dynamics and should be further investigated. For example, Barthel (1974) described a quiescent period of 5–6 days prior to molt for instar 3 HSC and 1 day of inactivity after the molt.

2.3 Molting and Growth

Molting and concomitant growth in HSC is an exciting phenomenon and has been a major interest of our laboratory. Shuster and Sekiguchi (2003) have eloquently reviewed the current knowledge of molting and growth. Here we describe our observations in an aquaculture setting. Several molts occur in the first year while only two or three occur in the ensuing 18 months (Fig. 7);

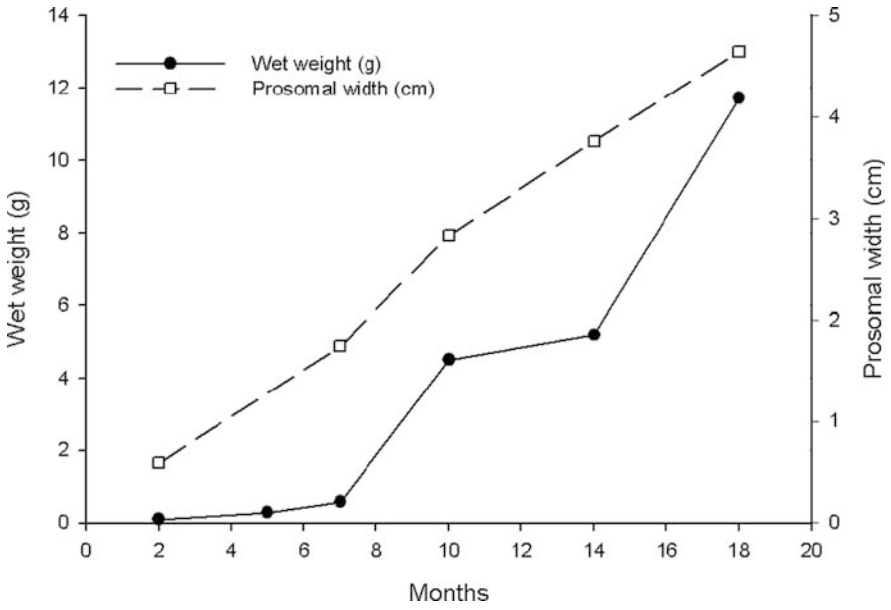

Fig. 7 The mean growth of successive cohorts of horseshoe crabs cultured from trilobites in recirculating aquaculture systems at the Aquatic Research and Environmental Assessment Center, Brooklyn College, NY, USA

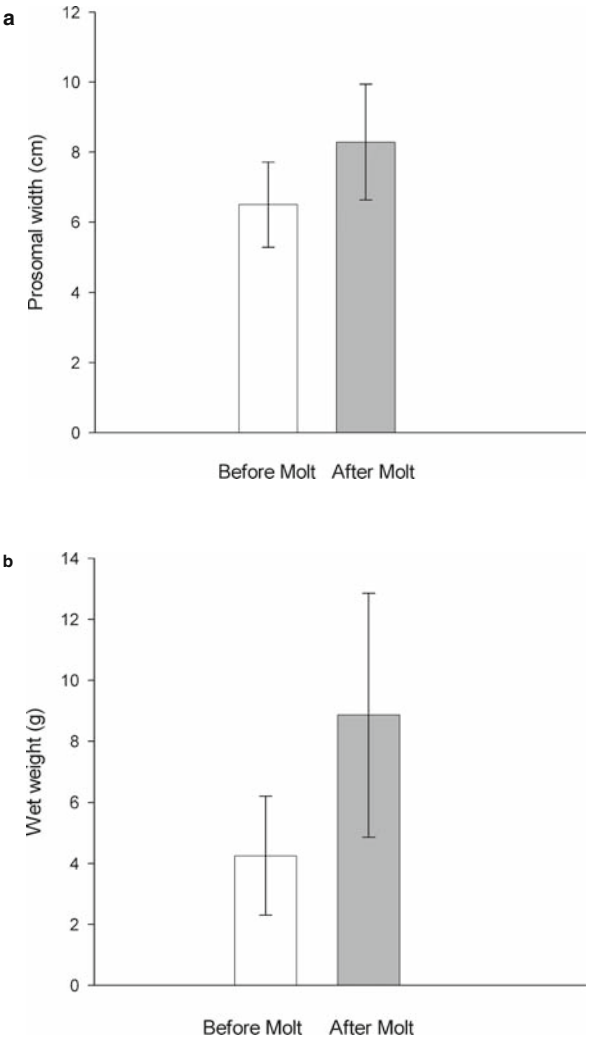

Fig. 8 **a** (*Top*) The increase in the prosomal width of a juvenile horseshoe crab after molting. **b** (*Bottom*). The increase in wet weight of a juvenile horseshoe crab after molting

this is similar to the molting schedule described by Shuster and Sekiguchi (2003). In each molt there is a major increase in the wet weight and prosomal width of the emerging animal. As depicted in Fig. 8a, b, there was a 15–20% increase in prosomal width and a 100% increase in weight as it emerged from the molt. An examination of the growth supports the notion of stepwise growth (Sekiguchi et al. 1988) with each molt. We have also determined (Fig. 9) that the percentage of HSC that molt increases with increasing light duration (20% in 0 h of light, 70% with 12 h of light, and 80% with 24 h light). However, 10% mortality occurred during the molting process in the

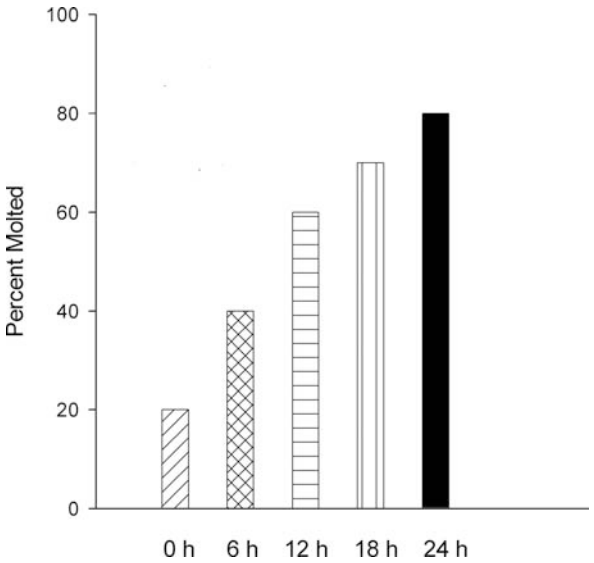

Fig. 9 The influence of light cycle on the molting of juvenile horseshoe crabs

groups held at 18 and 24 h of light, while no mortality occurred in the 0 and 12 h treatments. Therefore, we have adopted a 12 h/12 h light–dark cycle at AREAC.

3 Conclusions

We have developed or modified methods that permit the successful culture of HSC from fertilization to 7-year (and counting)-old juveniles. The growth observed in our studies seems to follow what has been described from field observations (Shuster and Sekiguchi 2003). This work should provide a basis for further studies which examine large-scale production of HSC for stock enhancement/restoration purposes. However, this is only the beginning of a new and exciting field of study in aquaculture and much more research is needed for large-scale production.

As described, AREAC utilizes RAS systems that permit long-term experiments in which environmental variables can be controlled year round. However, future research efforts may consider utilizing land-based systems that are nearshore allowing for the use of natural seawater (with its organic material) which may provide an additional feed type, particularly for the first and second instars. There is also a definite need for additional studies on induction of spawning including the influence of environmental factors, diet, social interactions, and physiological mechanisms. Furthermore, research should address the influence of molting hormones (i.e., 20-hydroxecdysone) on the acceleration of growth rates.

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