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Research Article

Experimental cultures of giant lion's paw *Nodipecten subnodosus* in equatorial waters of the eastern Pacific: progress in larval development and suspended culture

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ABSTRACT. The bivalve *Nodipecten subnodosus* is one of the largest scallop species and has been selected as an appropriate species for mariculture, adapting the culture technology for production in equatorial waters of the eastern Pacific Ocean. A study of its larval development was performed, comparing standard technology with different treatments in terms of larval density (two and four larvae mL^{-1}), temperature (24, 27, and 29ºC), photoperiod limitation (darkness) and antibiotic treatment (Florfenicol 1.2 mg L^{-1}). The juveniles obtained (11 mm) were transplanted to suspended culture in Ayangue Bay, Santa Elena Province, Ecuador. Results show suboptimal larval cultures, probably due to water quality. The only treatment that produced competent larvae for metamorphosis (>50% larvae with eyespot) was the darkness treatment after 18 days when the larvae reached 193.4 ± 15.69 µm in length with 33±1.48% survival. However, growth rate (6.2 µm d^{-1}) was also lower than that reported for the *N. subnodosus*. Juveniles in intermediate culture showed the highest growth rates so far reported for the species (9.3 mm $month^{-1}$) and reached 64 mm in eight months. The rapid growth in suspended culture conditions with the estimation of >55% survival, suggest *N. subnodosus* as an emerging species for the diversification of aquaculture in Ecuador.

Keywords: bivalve; scallop; tropical aquaculture; growth; survival

INTRODUCTION

The bivalve Giant Lion's Paw *Nodipecten subnodosus* (G.B. Sowerby I, 1835) is one of the largest pectinid species since some organisms can measure up to 220 mm in anteroposterior length and weigh almost 2 kg. It inhabits lagoons, bays, and channels of more than 6 m deep with strong currents. Natural banks generally have a low density of organisms, <2 ind m^{-2} (Ponce-Díaz *et al*., 2011), and there is great interest to increase their production. In this regard, several studies have generated aquaculture technology, particularly in Mexico (Maeda-Martínez & Lodeiros, 2011). However, although the species is distributed in the tropical and subtropical eastern Pacific, from the coasts of Peru in Paita, Piura, 4.7°S, to Isla Cedros in Baja California Sur, Mexico, 28.2°N (Coan & Valentich-Scott, 2012), no research on this species has been reported for tropical waters.

Studies on the relationship of environmental patterns and their influences on the growth of some marine bivalves, have allowed to establish their ecological importance within natural systems (Bayne & Worral, 1980; Griffiths, 1980b; Suchanek, 1985; Jørgensen, 1990); as well as in their culture potential (Grant, 1996; Saxby, 2002; Lander *et al.*, 2012), and even leading nowadays to the use of these close relationships for recover ecosystem services (Bergström & Lindegarth, 2016; Petersen *et al*., 2019). In the particular case of pectinids, Ponce-Díaz *et al*. (2011) mention that studies in "long-life cycle" show that the growth of individuals in natural conditions is affected by environmental aspects of the area (temperature and food availability). These hypotheses have been of great importance within the genus *Nodipecten*, to help understand the behavior of organisms in their early stages of life from larvae to juveniles (Rupp *et al*., 2011; García-Pánames *et al*.,

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2011), and promoted aquaculture programs in different regions (Freites-Valbuena *et al*., 2011; Mazón-Suástegui *et al*., 2011; Rupp *et al*., 2011).

In Ecuador, research is being carried out in order to diversify the aquaculture production, which until now has been mainly dominated by shrimp farming (*Penaeus vannamei*). The bivalve *N. subnodosus* has been selected as a promising species for mariculture, by adapting the culture technology and optimizing it for massive production (Alvarez *et al*., 2008).

In the present study, information is generated to establish the culture of *N. subnodosus* in Ecuador, by optimizing its larval development, concerning the effect of larval density, temperature and photoperiod, and testing the feasibility of spat growth in suspended culture in the sea.

MATERIALS AND METHODS

Larval bioassays

Organisms from the Manabí province coast (Salango), Ecuador (1°35'09"S, 80°51'55"W), were collected by means of autonomous diving and transferred to tanks at CENAIM-ESPOL laboratory, where they were conditioned until maturation induced to spawnand larval culture, following Mazón-Suástegui *et al*. (2011). Fifteen mature scallops were selected for spawning by thermal "shock", obtaining a percentage of success ≥70%, Approximately, 20 million embryos were incubated in 1-ton tanks (in duplicate) filled with microfiltered (0.45 µm) and UV light treated seawater (FSW). Mixotrophic larvae were placed in conical tanks of 500 L, at a density of 4 larvae mL, in triplicate, and were cultured with FSW for 6 days with total exchange every 24 h.

Larval development was performed under conditions of FSW at a density of 2 larvae mL^{-1} in 50-L conical dark tanks (100 000 larvae per tank in triplicate), at temperatures of 27 ± 0.3°C, salinity 34 ± 0.2 and continuous aeration. Larvae were treated with antibiotics (Florfenicol 1.2 mg L^{-1}) and fed with a diet of *Isochrysis galbana* (T-iso clone) and *Chaetoceros gracilis* in a 3:1 ratio during the first larval stages (1st to 8th day), 1:1 in the intermediary stages (9th-14th day) and 1:3 in the finals stages (15-18th day), using daily rations from 10 000 to 50 000 cells mL^{-1}. This treatment was called standard because these conditions have been reported in other studies of the *N. subnodosus* (Mazón-Suástegui *et al*., 2011). The other treatments consisted of variants to the standard, as for larval density (4 larvae mL^{-1}), temperature (24 ± 0.2 and 29 ± 0.3°C), culture in darkness by limiting the natural light intensity, using a mesh of dark shade (>85%) and treatment without antibiotics. All treatments started with 6-day veliger larvae after fertilization and were performed in triplicate.

Larval developments under each treatment were evaluated during the water exchange periods (every two days) when the larvae of each treatment were concentrated by sieves and poured into 1 L containers. Then, three subsamples of each replicate were counted using an Olympus CX31 compound microscope at 40X to estimate density and survival. The larvae were also photographed at 100X with a digital camera (Lanoptik MDX 501) adapted to the microscope, and 25 randomly selected larvae were measured in their anterior-posterior axis, using the iWork 2.0 program. Special attention was taken for the observation of larvae with the ocular spot at the end of the experiment (18th day) in order to determine the metamorphic competitive stage. The anteroposterior length and survival data, after verification of their normal distribution and homogeneous variances, were tested at day 16th for all treatments, and day 18th for the treatments that had surviving larvae (those with limited light intensity and at 29°C) one-way ANOVA and a Tuckey-Kramer or Duncan *a posteriori* test were performed. The statistical tests significance level was 0.05 (Zar, 2010).

Juvenile culture

Competent larvae close to metamorphosis (double-ring/active foot), were transferred to tanks (500-L), that contained spat collectors (shade mesh 75%). The culture was fed a diet of *I. galbana* and *Ch. gracilis* 3:1 at daily rations of 90 000 cells mL^{-1}, until they reached 11.0 ± 2.20 mm in anteroposterior length. Then, they were arranged in baskets (pearl nets) at a base occupancy density of 25% (370 juvenile specimens per replica in triplicate), and suspended from a long line located in Ayangue Bay, Santa Elena Province (1°59'1.59"S, 80°45'35.15"W) following the methodology of Ventilla (1982) for scallops' culture. Juveniles confined in four experimental replicates (pearl nets) were sampled, usually every two months, when the pearl nets were changed. Scallops were counted to estimate survival and measured at their maximum anteroposterior axis with an electronic caliper (0.1 mm accuracy) to determine the growth.

RESULTS

Larval bioassays

Both, standard (2 larvae mL^{-1}) and larval density treatments (4 larvae mL^{-1}), showed a similar growth curve, reaching statistically same length sizes (149.2 ± 5.09 and 154.2 ± 10.02 µm, respectively; Fig. 1a). At

day 16th, survival was also statistically same (12.0 ±1.07 and 13.3 ± 2.28%, respectively), with a total larval mortality at the end of the experiment (day 18th). No competent larvae for metamorphosis were obtained from these treatments.

In the temperature treatments, the highest growth and survival were observed at 29°C (Fig. 1b), reaching a mean size of 178.9 ± 0.50 µm in length and survival of 33.3 ± 1.08%, whereas larval lengths in the standard culture (27°C) and 24°C treatments were statistically same at day 16th (148.1 ± 0.87 and 149.2 ± 5.09 µm, respectively). At day 16th, mortality at the 24°C treatment was slightly smaller than the standard treatment (survival of 20 ± 4.98 *vs*. 18.0 ± 1.84%; $P < 0.05$), but larval mortality was total at the end of the experiment. No metamorphosed competent larvae were observed in any of these treatments.

Culture larvae without antibiotic showed a similar behavior, both in length (larvae did not exceed 160 µm at day 16th), and survival (total mortality at day 18th) in comparison with the standard culture (Fig. 1c); while, the treatment with limited light intensity (darkness) showed the highest growth, reaching a mean size of 193.4 ± 15.69 µm at the end of the experiment, when there was a survival of 33.0 ± 3.48%. In this treatment, competent larvae for metamorphosis were observed (71.5 ± 18.15% larvae with eye spot).

In the treatments that promoted greater performance (darkness, Fig. 1c and temperature 29°C, Fig. 1b), survival did not show significant differences (33.0% *vs*. 33.3%, respectively, $P > 0.05$). However, the larval length was significantly greater in darkness, with respect to the treatment at 29°C (193.4 ± 15.69 *vs* 178.9 ± 0.44 µm, respectively).

Juvenile culture

The juveniles (produced under darkness condition) showed an accelerated growth from the beginning of the experiment (mid-January 2016) to June 2016 (Fig. 2a), reaching a size of 52.1 ± 6.09 mm. After this period, the juveniles showed a decrease in the growth rate, achieving a final size of 63.9 ± 2.73 mm, after 8.5 months of rearing (September 2016). Although the growth was accelerated during the beginning of culture, in this phase, survival decreased drastically, reaching values of 58.9 ± 5.76%, after 6 months. From this moment, survival remained without significant variations until the end of the experiment ($P > 0.05$), with an accumulated value of 55.5 ± 2.18% (Fig. 2b).

DISCUSSION

Except for the treatments in darkness and at a temperature of 29°C, the larval development of *N. subnodosus* in the different treatments was similar to the standard treatment, not reaching sizes greater than 160 µm with total mortality at the end of the experiment (day 18th). These results suggest that the culture conditions were suboptimal. The high sensitivity of *Nodipecten* larvae to bacteria can explain this situation since often *Vibrio* produce epizootics in bivalve mollusks larval cultures (Luna-González *et al*., 2002; Bem *et al*., 2011). Bacteria, like-*Vibrio,* were found in high density in the culture systems, even after using antibiotics (>200 bacteria L^{-1}, J. Revilla *unpubl. data*). This argument could also be the explanation for the similar results observed in the treatment without antibiotic, both in growth and survival, compared with the standard treatment (total mortality at day 18th). In this sense, microbiological control using Florfenicol at the concentration used (1.2 mg L^{-1}) may not be sufficiently effective in quantity and/or specificity, to attack the bacteria associated with a decrease in larval survival. The dose of Florfenicol used was below those recommended for *Nodipecten nodosus* (3 mg L^{-1}; Bem *et al*., 2011), which provided a significant increase in larval survival during the first 9 days in this species. In any case, a pathological study is recommended accompanied by antibiograms with antibiotics authorized in aquaculture activities, probiotics and other alternatives (management, homeopathic, natural substances) that could improve the larval culture.

Larvae at the 29°C treatment reached mean sizes close to 180 µm, with survival > 30% at the end of the experiment. However, the mean size at which eyespot could be detected was slightly above that described in *N. subnodosus* (≈174-177 µm; Mazón-Suástegui *et al*., 2011). Eyespot larvae were not observed, and in the subsequent days the mortality was total (data not shown). By the contrary, larvae in darkness (at 27°C) had a survival >30%, reached a size >190 µm on day 18th and >70% presented eye spots, indicating its competence to metamorphosis. It is hypothesized that some interaction must exist in the improvement of larval culture by keeping them in the dark, which may be associated with the low bacterial load or physiological effects related to photoperiod in this species.

Although the darkness treatment was the one with the highest yield and survival with a growth rate of 6.2 µm d^{-1} at the end of the experiment (evidencing the conditions for fixation and metamorphosis), this growth rate is lower than that reported for other scallops of tropical and subtropical regions, as 8.1 µm d^{-1} for *Argopecten purpuratus*, 6.8 µm d^{-1} for *A. ventricosus* and *N. nodosus* (Uriarte *et al*., 1996; Monsalvo-Spenser, 1998; Mazón-Suástegui *et al*., 2011). It was also much lower than those reported by Villegas-

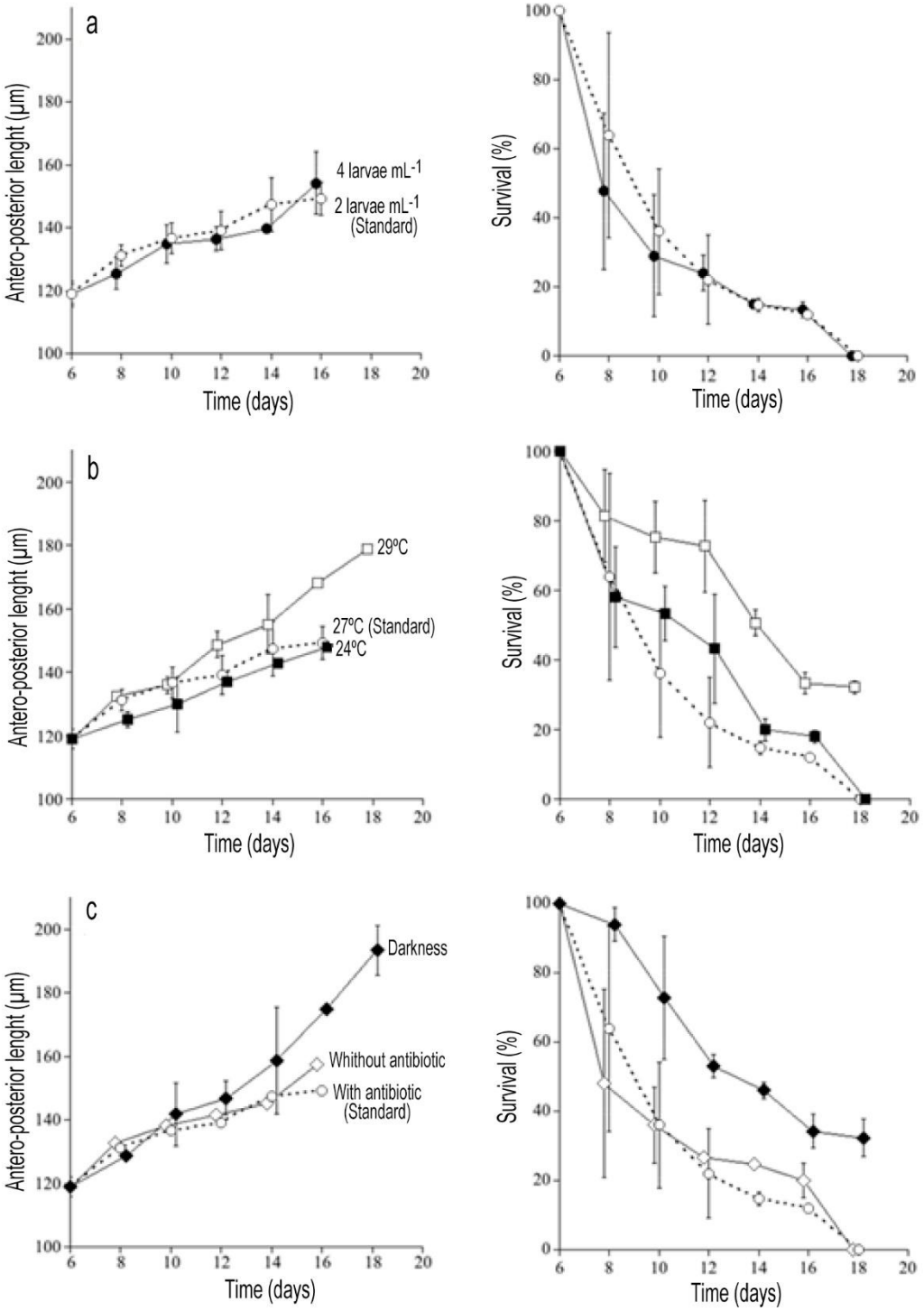

Figure 1. Larval developments (starting with veliger larvae of 6 days post-fertilization) in several treatments: a) at different larval densities, b) different temperatures, c) without antibiotics and in darkness, compared with the standard larval culture proposed for *Nodipecten subnodosus* (2 larvae mL^{-1}, temperature of 27 ± 0.3°C, salinity of 34 ± 0.2, with antibiotic treatment). The vertical lines indicate 95% confidence intervals.

Carrasco (2004) and Mazón-Suástegui *et al*. (2011) in the larval development of *N. subnodusus* from the Pacific subtropical coast of Northamerica (7.3 to 13.3 µm d^{-1}). The latter is considered as one of the highest larval growth rates in pectinid cultures.

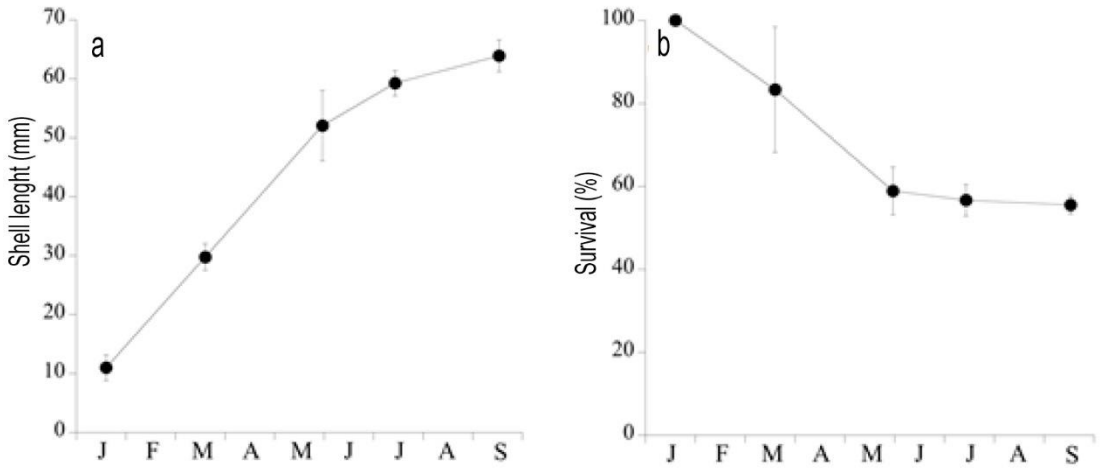

Figure 2. a) Growth in anteroposterior length and b) survival of *Nodipecten subnodosus* spats in suspended culture in Ayangue Bay, Santa Elena Province. Vertical lines indicate 95% confidence intervals.

Juveniles obtained exclusively from the darkness treatment showed rapid growth in anteroposterior length under suspended culture conditions, particularly during the first months of the assay. During the experimental period the organisms grew in two phases, at first with a high growth rate (9.4 mm month^{-1}) when they reach about 52 mm in 4.5 month^{-1} and then the growth rate decreased (3.1 mm month^{-1}) until reaching about 64 mm the remaining 3.5 month^{-1}. Considering the cultivation stages indicated by Freites *et al*. (2011) for *Nodipecten* spp. (pre-culture: 3-15 mm, intermediate culture: 15-40 mm, final culture grow-out: >40 mm), the current experiment could cover the intermediate growing stage, starting at 10 mm in shell length, and part of the final grow-out phase, starting at 50 mm. When analyzing the *N. subnodosus* shell length growth studies at the intermediate culture stage (Table 1), the growth rate obtained in the current study (9.4 mm month^{-1}) was higher than the maximum reported in experimental cultures in the coasts of Baja California-Mexico (8.7 mm month^{-1}). The rates reached in part of the final fattening culture stage (5.6 mm month^{-1}) are similar to the maximum rates reported for the species (5.4-6.3 mm month^{-1}, Freites-Valbuena *et al*., 2011). The results related to the growth of *N. subnodosus* juveniles at sea, increase the viability of the lion's paw scallop production with commercial size in less time in equatorial waters as compared to other Pacific Ocean coasts.

The period of juvenile high growth rates coincides with the moment of high water temperatures in the zone and the lower growth rates with the season of lower water temperature. This positive correlation between temperature and growth rates suggest a main modulation by this factor, due to the fact the food in the study area seems not to be a limitation for bivalve mollusks, since the phytoplankton biomass, estimated by concentration of chlorophyll-*a*, maintained values above 2 μg L^{-1} throughout the year (Lodeiros *et al*., 2018).

An alternative hypothesis is that the decline in growth rate observed was due to the high energetics of the gametogenesis process, which could limit the energy devoted to somatic growth. The first size of sexual maturity of *N. subnodosus* (50-55 mm), under culture conditions reported by Arellano-Martínez *et al*. (2011), coincides with the beginning of the decrease in shell growth. Visual observations of gonad formation for the first time in experimental scallops during April-June 2016 confirm the onset of reproduction among scallops with sizes near 50 mm.

The results showed suboptimal larval cultures of *N. subnodosus*, probably due to deficient water quality. Improvement of larval cultures was obtained from maintaining the larvae in darkness, reason why it is recommended that more studies should be performed including this factor on pathologies control or its influence on the larval microbiota.

The growth of *N. subnodosus* in the intermediate culture size category in equatorial waters of the Province of Santa Elena showed the highest rates so far reported for the species. There is possibly an influence of environmental factors on growth, particularly temperature. Hence, a study is recommended on the influence of environmental factors on the growth and survival under cultivation conditions, given the confluence of oceanographic phenomena in the area, to elucidate theories regarding the environmental modulation in invertebrates and to generate cultivation strategies.

Table 1. Growth rate (mm month^{-1}) reported for *Nodipecten subnodosus* during the intermediary culture in different sites of the eastern Pacific Ocean.

Culture site	Time (days)	Length ± SD (mm)		Growth (mm month^{-1})	Reference
		Initial	Final		
Guerrero Negro Lagoon (B.C., México)	113	22 ± 0.1	52 ± 6.1	8.1	Osuna-García (2006), Mazón-Suástegui & Osuna-García (2004)
Ojo de Liebre Lagoon (B.C.S., México)	150	16	50	6.9	Morales-Hernández & Cáceres-Martínez (1996)
El Coyote estuary (B.C.S., México)	105	24 ± 0.2	52 ± 4.9	8.1	Mazón-Suástegui *et al*. (2003)
Magdalena Island (B.C.S., México)	112	21 ± 0.2	51 ± 8.8	8.1	Mazón-Suástegui *et al*. (2002)
Rancho Bueno estuary (B.C.S., México)	120	20 ± 2.3	55 ± 2.9	8.7	Racotta *et al*. (2003)
La Paz Bay (B.C.S., México)	144	20	60	8.4	Morales-Hernández & Cáceres-Martínez (1996)
El Colorado Lagoon (Sinaloa, México)	163	10.0 ± 3.7	43.4 ± 0.4	6.2	Diarte-Plata (2007)
Ayangue Bay (Santa Elena, Ecuador)	131	10.0 ± 2.2	52.1 ± 6.1	9.4	Present study

The rapid growth in suspended culture conditions with the estimation of high survival (>55%), together with the high economic value, suggest that *N. subnodosus* should be considered as an emerging species for the diversification of aquaculture in Ecuador. It is urged to continue the studies for implementation of the technological package of this species, to optimize spat production in the laboratory and development of the grow-out phase in the sea.

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