Aquaculture 496 (2018) 134–145

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Breeding, larval development, and growth of juveniles of the edible sea urchin *Tripneustes depressus*: A new target species for aquaculture in Ecuador

Jorge I. Sonnenholzner-Varas[a,*], Noelia Touron[b], María Manuela Panchana Orrala[b]

[a] *Escuela Superior Politécnica del Litoral, ESPOL, Centro Nacional de Acuicultura e Investigaciones Marinas, Facultad de Ciencias de La Vida, km. 30.5, Vía Perimetral, Guayaquil, Ecuador*

[b] *Escuela Superior Politécnica del Litoral, ESPOL, Centro Nacional de Acuicultura e Investigaciones Marinas, km. 30.5, Vía Perimetral, Guayaquil, Ecuador*

ARTICLE INFO

Keywords:
Tripneustes depressus
Larviculture
Juveniles
Fast-growing
Sea-ranching

ABSTRACT

Sea urchin fishery has gradually expanded in different countries and territories around the world, including South America, and the sea urchin *Tripneustes depressus* is a feasible candidate for aquaculture. The aim of this study was to prepare a biotechnical protocol for rearing larvae and production of juveniles. Large *T. depressus* were collected at monthly intervals at a subtidal rocky coast off Palmar, Santa Elena, Ecuador from January 2015 to December 2017. *T. depressus* spawns when exposed to thermal shock in low illumination room. Seven spawning events were recorded between March and May and between July and August. On average, females spawned approximately $3.60 \pm 0.83 \times 10^7$ oocytes (mean diameter: $78.4 \pm 2.1\,\mu m$) and males spawned approximately $5.60 \pm 1.10 \times 10^7$ spermatozoa respectively). A cohort of May 13, 2017 was followed for 250 days. Embryo and larval stages were completed within 48 h and 21 days, respectively, at $26.5 \pm 0.5\,^\circ C$. Larvae were fed a mixed diet of *Tysochrisis lutea*, *Chaetoceros gracilis* and *Rhodomonas* sp. at concentrations ranging from 2.5×10^3 to 2.0×10^4 cells /ml as they increased in size. Larvae were measured considering: Total Length (TL), Medium Base Line (MBL) and Base Width (BW). On day 2 after fertilization (AF): $311.7 \pm 2.0\,\mu m$, 158.9 ± 1.0 and 270.7 ± 3.4 ($n = 10$), respectively. On AF$_{16}$: $544.7 \pm 4.0\,\mu m$, 273.6 ± 6.9 and 355 ± 3.5 ($n = 10$). On AF$_{21}$, larval rudiment size was equal in size to that of the stomach and larvae were competent. Their body measurements, and specifically total length at that food concentration, showed no phenotypic plasticity. Thirty two days post spawning (DPS) all juveniles had open mouths and a functional gut for feeding (size: 0.40 ± 0.01 mm DT), and at DPS$_{98}$ three size categories were differentiated. Sea urchin sizes by categories at DPS$_{98}$ and DPS$_{152}$ were, respectively: small (2.20 ± 0.1 mm DT and 23.9 ± 0.45 mm TD), medium (4.90 ± 0.08 mm DT and 33.3 ± 0.3 mm TD); and large (7.20 ± 0.11 mm DT and 43.8 ± 0.5 mm DT) with zero mortality. *T. depressus* reach 4 cm diameter at 188 days after metamorphosis and can reproduce after six months of life, when it reach approximately 4.2 cm diameter), although the gonads are not yet commercial in size. This protocol is an initial perfectible protocol for sea urchin aquaculturing. However, we have made recommendations for consideration that include increasing survivorship and supplying post-larvae for juvenile production.

1. Introduction

Ecuador (in the eastern tropical Pacific, ETP) is a country with a long tradition in coastal-artisanal fishery on rocky-reef ecosystems, for some marine invertebrates, such as lobsters, crabs, mollusks and sea cucumbers (Alava et al., 2015; Henchion et al., 2017).

The high demand for consuming high-quality animal protein of marine origin, consequently has made some natural populations drastically diminish by overfishing (Manabu, 2007; Hearn, 2008; Wolff et al., 2012; Castrejón and Charles, 2013; Martínez-Ortiz et al., 2015). This has triggered concerns about structural and functional changes of shore reefs through trophic cascades (Okey et al., 2004; Glynn, 2008; Sonnenholzner et al., 2011a), and decreasing the income of coastal communities. The fishermen have sought to diversify its fishing by targeting a wider range of marine benthic animals and exploring fishing grounds along the mainland of Ecuador and around the Galapagos Archipelago. But, paradoxically, aquaculture is poorly developed in Ecuador (FAO, 2016; Pullin, 2017).

A comprehensive spatio-temporal dynamics on basic biological and ecological issues (embryonic phase, larvae and juvenile) for potential

---

* Corresponding author at: Escuela Superior Politécnica del Litoral, Campus "Gustavo Galindo" Km. 30.5, Vía Perimetral, Casilla: 09-01-5863, Guayaquil, Ecuador.
E-mail addresses: sonnenhol@espol.edu.ec (J.I. Sonnenholzner-Varas), notouron@espol.edu.ec (N. Touron), mpanchan@espol.edu.ec (M.M.P. Orrala).

https://doi.org/10.1016/j.aquaculture.2018.07.019
Received 1 April 2018; Received in revised form 2 July 2018; Accepted 11 July 2018

new marine resources for fishery and aquaculture is lacking. Due to the decline in some fisheries (*i.e*, sea cucumber, Sonnenholzner et al., 2017), interest has developed in a sustainable and alternative aquaculture production (supported with population restocking) in order to divert fishing pressure away from traditional fisheries. In this sense, sea urchin aquaculture is very pertinent.

Sea urchin fishery has gradually expanded in different countries and territories around the world (including South America, Andrew et al., 2002). At least twenty-one species of sea urchins have been heavily harvested and extensively implemented in the sea urchin trade, especially for some Asian countries (a total production of about 73,000 metric tons, Castilla-Gavilán et al., 2018). This trade represents an estimated at about 200–300 million US$ (Shimabukuro, 1991; Sun and Chiang, 2015; Castilla-Gavilán et al., 2018). > 70% of this production comes from six different areas from the Pacific region (Micael et al., 2009; FAO, 2016). Japan has long been the major lucrative market for global sea urchin production (annual domestic demand of about 50,000 metric tons whole weight) and is the major driver of global sea urchin prices. A top-quality (as fresh, dried, salted or brined sea urchin roe) can sell for over USD $60/kg (Sun and Chiang, 2015).

Roe is part of a special gastronomy part of fish markets and are prized in delicacy cuisine (in sushi or sashimi) for their sensory attributes, in terms of palatability, flavor and color (Keesing and Hall, 1998; AAFC, 2002; Devin, 2002; Muthiga, 2005; Rahman et al., 2012). They also are consumed for the high-quality protein content, polyunsaturated fatty acids; and potential use in medicine because of its rich chemical constituents and biochemical compounds, such as polysaccharides, PUFAs, β-Carotene, xanthophylls and others nutrients (Kaneniwa and Takagi, 1986; Lawrence et al., 1997; Yur'eva et al., 2003; Archana and Babu, 2016). Nonetheless, the supply of domestic sea urchins is limited and the feasibility of this industry is largely dependent on sufficient natural populations. At this juncture, this important industry is kept virtually unstable.

Because several sea urchin populations have diminished as result from ineffective fisheries policies and management (a declined pattern in catches has been noticed in several countries in the Pacific region, as Chile, China, Japan, New Zealand, and in many areas of Southern and Western Australia), interest in sea urchin aquaculture has developed rapidly and has grown steadily over the past few years (Lawrence, 2013; Lawrence and Agatsuma, 2013; Paredes et al., 2015). Sea urchin aquaculture can help alleviate the pressure for overexploitation of wild sea urchins (Barker et al., 1998; Huggett et al., 2006; Liu et al., 2010; Kayaba et al., 2012; Eddy et al., 2012; James et al., 2016).

Tripneustes is a genus of toxopneustid echinoids that recognizes three extant edible species with commercial value, *T. ventricosus* (Lamarck, 1816) from both sides of the Atlantic, *T. gratilla* (Linnaeus, 1758) in the Indo-West Pacific, and *T. depressus* (Agassiz, 1863) in the central eastern Pacific (Fig. 1a–c). A study of the phylogeography of the pantropical sea urchin *Tripneustes* by Lessios et al. (2003) revealed that based on morphology, COI, and bindin data, *T. depressus* from the eastern Pacific is in fact the same species as *T. gratilla* from the western Pacific. In the international market the gonads of *T. ventricosus* and *T. gratilla* are good vis-à-vis quality competitive in the trade with other species (Scheibling and Mladenov, 1987; Rahman et al., 2009; Pena et al., 2010). A number of studies have examined the biology, ecology, fisheries and aquaculture relevant issues of *T. gratilla* (Shimabukuro, 1991; Cyrus et al., 2013; Lawrence and Agatsuma, 2013) and *T. ventricosus* (Keesing and Hall, 1998; Andrew et al., 2002; Robinson, 2004; Wolcott and Messing, 2005), but no detailed information exists for *T. depressus* (Sonnenholzner et al., 2013).

T. depressus is a very desirable species for fishery and is a feasible candidate for aquaculture in Ecuador for its biological attributes, such as: large-size, short time to maturity, high growth rate, high reproductive effort and output, and short life span (Lawrence, 1990; Lawrence and Bazhin, 1998; Lawrence, 2013). They are part of a conspicuous assemblage of coral reef-associated communities that inhabits moderate hydrodynamic leeward rubble and open sloping rocky shores with interspersed coral-sandy rubble pockets (but never on pure sand or muddy sand) on encrusting coralline, calcareous articulated algae on rhodolith beds and on extensive and dominant soft, fleshy brown macroalgae (varieties include *Padina* and *Sargassum* species) that support their grazing activity (Sonnenholzner and Lawrence, 2002; Lawrence and Sonnenholzner, 2004; Foster et al., 2007; Irving and Witman, 2009; Uthicke et al., 2009; Sotelo-Casas et al., 2016) in shallow waters (preferentially at average 12 m depth) to 73 m depth from the Gulf of California, México to Lobos de Afuera islands, Perú, including the Galápagos and Clarion Islands (Maluf, 1988; Idrovo and Sonnenholzner, 1994) (Fig. 1a–c). In Galápagos, *T. depressus* is spread out throughout all five eco-regions with different sea temperatures ranging from 20.3–27.1 °C (Banks, 1999; Brandt and Guarderas, 2002), and bathymetrically between 3 and 40 m depth, it ranges from 17.3–28.5 °C, in the central coast off Ecuador (Datos de la Estación Oceanográfica *El Pelado* del Cenaim-Espol 2013–2017). Traditionally, this species has been locally consumed as food by fishers in Manabí (on the mainland coast of Ecuador) and Galápagos.

Since 2014, considering the importance of *T. depressus*, the Centro Nacional de Acuicultura e Investigaciones Marinas of the Escuela Superior Politécnica del Litoral of Ecuador (CENAIM–ESPOL) is promoting research studies on *T. depressus* diversify aquaculture. These studies will contribute for future management plans (as alternating new fisheries based on aquaculturing), leading to the sustainability of benthic resources in Ecuador. The aim of this study was provide

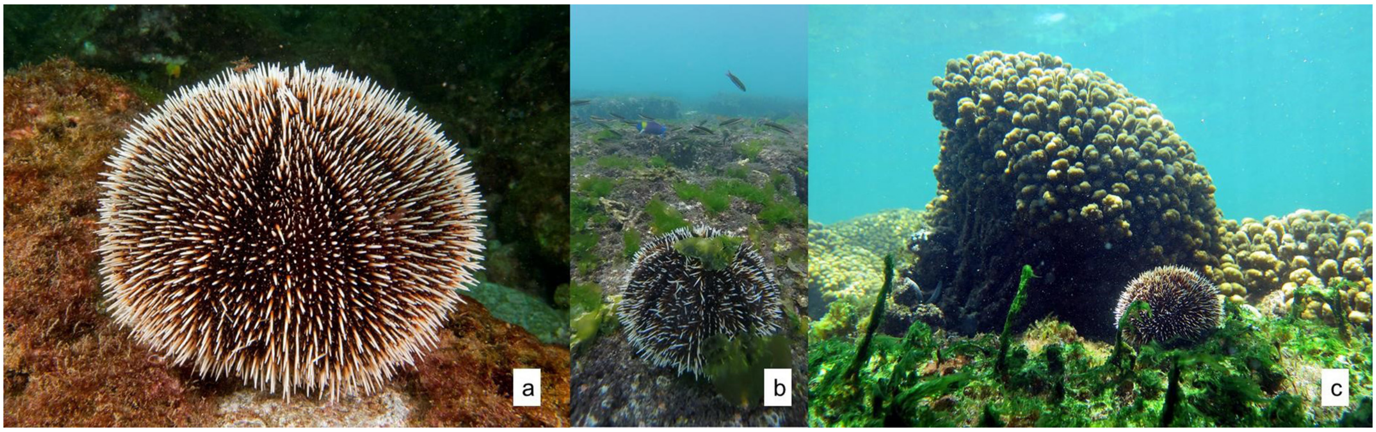

Fig. 1. View of large sea urchins *Tripneustes depressus* in open rocky and coral reef habitats; a) specimen at 12 m depth, *El Pelado* Island, Santa Elena, Ecuador; b) a covered specimen with foliose macroalgae *Ulva lactuca* at 3 m depth, Bahía Santa Elena, Guanacaste, Costa Rica; and c) specimen at 2 m depth associated with a large *Pocillopora verrucosa*, Punta Galeras, Baja California Sur, México.

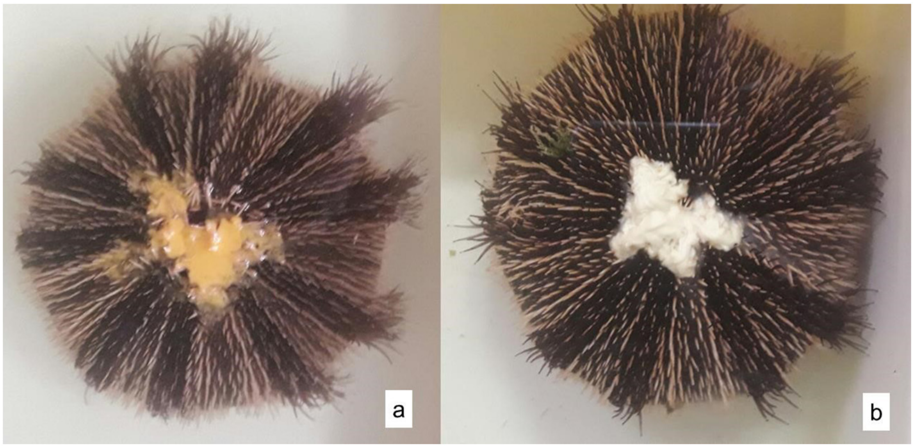

Fig. 2. Aboral view of large *Tripneustes depressus* spawning at laboratory conditions in May 13, 2017; a) female releasing oocytes (14 cm TD) and b) male releasing sperm (13 cm TD).

protocols for rearing competent larvae and producing juveniles of *T. depressus* on a small scale.

2. Materials and methods

2.1. Ethical procedures (in vivo and experiments)

All the procedures realized considered in this study followed the guidelines for ethical and responsible researches using in vivo animals and experiments for echinoderms (Kilkenny et al., 2010; Rubilar and Crespi-Abril, 2017).

2.2. Sea urchin collection

Large sea urchins, *T. depressus* (mean test diameter: 12.5 ± 0.5 cm and wet weight: 759.4 ± 9.1 g; $N = 200$) were collected by diving at high tide, between 10 and 12 m depth, early in the morning (before 0900 h) at monthly intervals along the subtidal rocky coast off Palmar, Santa Elena, Ecuador (15°59′00"S 80°44′00"W) from January 2015 to December 2017 (Fig. 1a).

Specimens were transported individually in double plastic bags with multi-track zipper (29.9 × 26.8 cm, 3.8 l size, Diamond), filled with seawater using styrofoam containers kept at 22 °C to the aquaculture Centre (CENAIM) in San Pedro de Manglaralto, Santa Elena, Ecuador. After collection, sea urchins were gently rinsed with sterile and filtered seawater (SFSW) and kept them individually in flat-bottomed 40 l rectangular tanks. Test diameter and body weight were determined using a caliper (in centimeters) and a spring scale with 0.1-g accuracy, respectively.

2.3. Culture system

2.3.1. Seawater quality and environmental conditions

The seawater used during the spawning, hatching and larval rearing was 1 and 5 μm filtered and UV treated (seawater flow rate used was kept constant 1 l min^{-1}, as sterile and filtered seawater: SFSW), temperature between 22 and 27 °C, salinity 35 ppt, pH of 8.2, dissolved oxygen of 5–6 mg O_2 l^{-1}, and kept in semidarkness condition (0.5 μE m^{-2} s^{-1}).

2.3.2. Maintenance of large and juvenile animals

In this study, we defined juveniles are individuals after metamorphosis, before production of gametes, and also have had opened its mouth with a functional jaw mandible for initiating feed. And we utilized the term large sea urchins, instead adult, since these rapid-growth sea urchins begin producing gametes when they are small size, and they would be adult by definition at 0.4 ± 0.01 mm diameter.

The large sea urchins (from the natural population and after spawning) and the juvenile sea urchins (cultured at CENAIM) were in outdoor conditions. Large sea urchins were kept in 500 l flow-through stock tanks (approximately, 50 l per animal, $N = 10$ per tank), open circuit (water exchange rate in 5 h, 100%), aeration and no direct lighting. Juveniles were separated by three size-classes (by their test diameter): (*i*) 1.1–3.9 mm; (*ii*) 4.1–6.0 mm and (*iii*) 6.1–9.1 mm. During this time, both were fed on the brown algae *Padina durvillaei*. Twice a week the tanks were cleaned removing uneaten food, fecal pellets and debris by siphoning. Seawater temperature and dissolved oxygen were recorded daily by a multi-parameter probe (YSI-550A, USA).

2.3.3. Spawning induction and observation

Laboratory scale observations were firstly made for providing an indirect and rapid assessment in the application of stimuli for spawning in mature sea urchins, as follows: *i*) haphazardly sampled urchins with 1–1.5 ml of 0.5 M KCl injections with a 26 gauge needle into the coelom via the peristomial membrane; *ii*) manual shaking of the animal, and *iii*) thermal shock (water temperature raised 4–5 °C for 10 min) in low illumination room condition.

2.3.4. Spawning and fertilization

Seven cohorts of sea urchins *T. depressus* were produced, but cohort of May 13, 2017 of was reared over a period of 250-days in captivity. Mature oocytes (bright orange; Fig. 2a) were collected through 30 μm Nytex mesh net, and washed with SFSW, then were pooled (approximately 400 to 600 eggs ml^{-1}) and shed into a 4 l jar. Sperm (semen off-white creamy streams; Fig. 2b) was dry-collected. Afterwards, 2 ml of diluted sperm (1 drop of dry semen with 10 ml of SFSW) were added and gently stirred with a plastic paddle for 1 min (pooled sperm: oocyte: 20:1). After three minutes the fertilization membrane was assessed and the spermatozoa removed by three consecutive washes with SFSW (with a 30-μm sieve). Sperm concentration and motility (as rapid sperm) were selected for the pools (three individuals per pool) prior to oocyte fertilization.

2.3.5. Embryonic development, larval rearing, and microalgal diets

Eggs were incubated in SFSW for 48 h at 26 °C until the pluteus stage in 500 l fiberglass cylindroconical tanks in static SFSW without aeration at a density of 0.5 eggs ml^{-1}. Embryo development was scored as: fertilized egg; unequal cleavage; equal to or > 16-cell stage; equal to or > 32-cell stage, hatching blastula, blastula, and gastrula.

Larvae were reared for 20 days until competency in 500 l fiberglass cylindroconical tanks in aerated static SFSW, with three partial (50–75%) water changes per week (larvae were collected with a

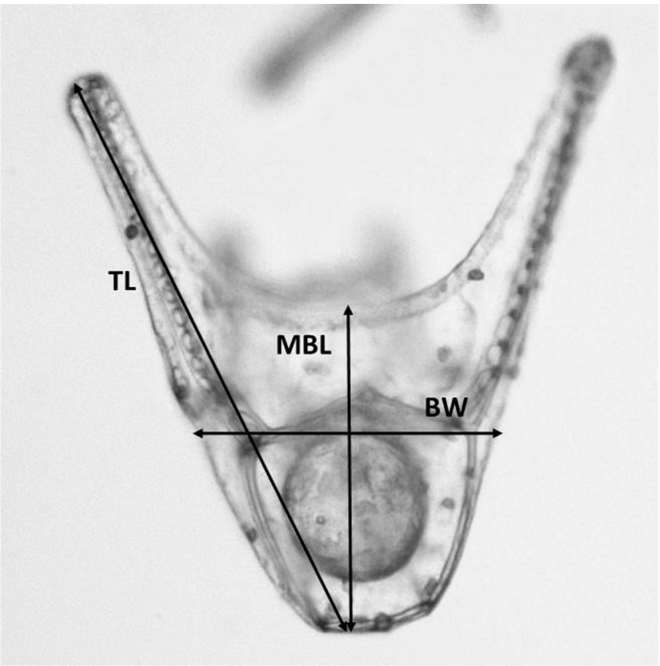

Fig. 3. Digital image of a two-armed *Tripneustes depressus* pluteus larvae depicting the three morphometric characteristics used to compare larval growth performed by TL = total length, MBL = mid-body line length, and WB = width base (40× magnification), as suggested Scholtz et al. (2013).

100–125 μm mesh). A complete water change and thorough cleaning tanks were done once every ten days. Prism larvae were stocked at a density of 1 larvae ml^{-1}. Density was estimated for each batch as the proportion of swimming larvae (up to the eight-armed pluteus stage). Larvae stages were scored as: prism, two-arm, four-arm, six-arm, and eight-arm. Once larvae were staged, the percentage of larvae in each stage were calculated and averaged among the replicate tanks. Larvae were fed (36 h after fertilization, when the functional gut appeared) twice a day with a mixture of three live microalgae species: *Tysochrisis lutea* (=*Isoschrysis galbana*, strain CI/03), *Chaetoceros gracilis* (strain CCH2–2/06) and *Rhodomonas sp* (strain CR16/03) at a concentration ranged from 2.5×10^3 to 2.0×10^4 cells ml^{-1} of seawater, by adjusting the food level (dictated by the daily checking of the gut with a dissecting microscope for the fullness, color pigmentation and presence of algae) every day until attaining metamorphic competence. During larval rearing, information was compiled to measure the larval performance: survival counts assessed volumetrically on days 7, 14 and 21, and at the end of rearing and measurements (maximum dimensions, Fig. 3).

2.3.6. Settlement and metamorphosis

Seven days after reaching competence (indicated by larval morphology and tactile behavior), larvae with a large juvenile rudiment were placed (with a density of 1 larval ml^{-1}) in 10 days pre-conditioned 5000 l fiberglass rectangular flat bottom tanks with an inoculum of the benthic diatom *Navicula* sp. (Strain CN13/03 at $1.3\dot{}10^5$ cell ml^{-1}) forming a biofilm, as a settlement inducer for competent larvae. Seawater was 20% replaced per day and gently aerated. Juveniles were at 32 days post-settlement = DPS, with a size of 0.1 cm diameter). They foraged upon a lush green carpet of the algae *Cladophora* sp., afterwards, semi-dried small pieces of *Padina durvillaei* were introduced.

2.3.7. Counting, morphometric and growth measurements

Sperm and oocytes densities were determined by means of a hemocytometer (Neubauer counting chamber) and a six multi-well plate (area: 9.5 cm^2, diameter: 34.8 mm, Cellgro Corning), respectively, under a compound microscope and a stereoscope microscope using a 4× and 40× objectives, respectively. Measurements of oocytes, zygotes, embryo and larvae were made under a compound microscope and test diameter of juveniles ($DPS_{32–250}$) was made on freshly prepared samples using a profile projector microscope (Mitutoyo). All material photographed was fitted with an image analysis software *i*Works 2.0.

Larvae were placed in a flat focal plane for measuring them on total length (TL), base width (BW), and medium base line (MBL) (Scholtz et al., 2013, Fig. 3). Measurements were made every day by image analysis of fresh samples of 10 larvae. Competence was considered as achieved when at least 80% of the observed larvae showed that the rudiment was at least equal or larger in size than the stomach. The following calculation (the midpoint method) was made to determine the percentage change for growth uniform differences over time:

$$\%\Delta = [(S_1 - S_0)/(S_1 + S_0/2)] \times 100$$

where S_0 represents the initial size and S_1 is the total final size of larvae in each measurement by category. All values are expressed in millimeters. The denominator is the average of the starting and ending values.

At DPS_{95} of culture, juvenile sea urchins were separated into three size-categories: (*i*) small: 1.1–3.9 mm; (*ii*) medium: 4.1–6.0 mm and (*iii*) large: 6.1–9.1 mm and maintained in independent tanks for avoiding the intraspecific competition between large and small individuals (as suggested Grosjean et al., 2003). Size is expressed by the ambital test diameter (TD) the external diameter of the test at the ambitus excluding the spines. TD was measured with a manual sliding caliper at the nearest 0.1 mm. Specific growth rate (SGR) was calculated, as follows:

$$SGR\ (\%day^{-1}) = [(\ln\ TD_2 - \ln\ TD_1)/\Delta t] \times 100$$

where ln is natural logarithm, TD_1 and TD_2 are the average test diameters of the sea urchins (in millimeters) at time 1 and 2 in days, respectively, and Δt is the growth interval in days ($t_2 - t_1$). SGR is expressed as a percentage of sea urchin size increase per day.

2.4. Statistical analysis

Significant differences in the growth of juvenile sea urchins were tested using one-way ANOVA and post hoc Tukey HSD test. Assumptions of normality and homogeneity of variance were confirmed prior to the analysis using Levene test. Differences were regarded as significant at $P < 0.05$. All results are given as mean ± SE. Statistical analyses were carried out using Statistica 10.0 software.

3. Results

3.1. Spawning

Of the three methods tested to induce spawning in *T. depressus*, increasing seawater temperature 4 to 5 °C in in the dark was the most efficient and non-invasive method for obtaining gametes 20 min after animals arrived in the laboratory (see Subsection 2.2., Fig. 2a, b). Seven spawning events mostly occurred between March and May and between July and August (2015–2017). The spawning by females and males was continued intermittently for approximately 30 min. No spawning occurred after intracoelomic injection of 1 ml of KCl 0.5 M and manual shacking. Several sea urchins died after potassium chloride injection.

The mean diameter of unfertilized oocytes was 78.4 ± 2.1 μm (*N* = 110, 6 females, 50 oocytes of each, Fig. 4a). The mean of the spermatozoa head and tail are 15.0 ± 0.1 μm and 45.0 ± 0.2 μm, respectively. In average oocyte and spermatozoa number spawned by females and males were $3.60 \pm 0.83 \times 10^7$ oocytes and $5.60 \pm 1.10 \times 10^7$ spermatozoa, respectively.

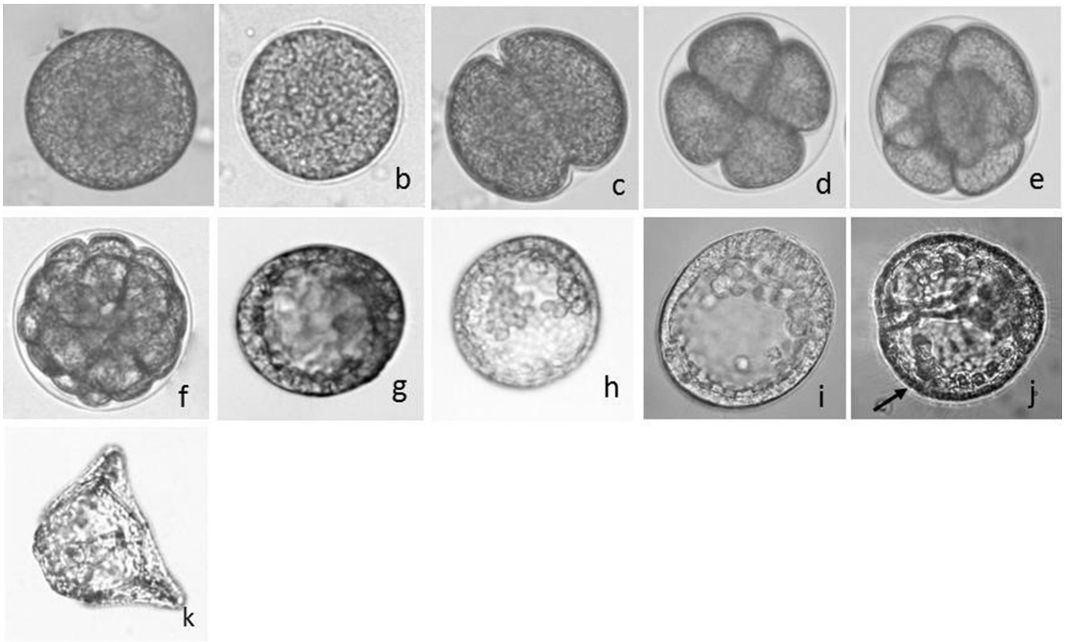

Fig. 4. Early cleavages of *Tripneustes depressus* under light microscopy; a) Oocyte; b) egg; c) embryo with two cells before the second cleavage; hialine layer is visible between cells; d) animal pole view of an embryo with 4 cells; e) embryo with 16 cells; f) arrangement of micromeres and macromeres on the vegetal pole of a 16 cell embryo; g) vegetal pole of an embryo with 56 cells; h) lateral view of an embryo with 108 cells; i) embryo during blastula formation; j) embryo during gastrulation, as post-hatching swimming blastula with thickened vegetal pole epithelium and final stage of archenteron invagination touching the anterior pole; k) prism larvae. Note: black arrow indicates ciliated band of gastrula.

3.2. Fertilization and embryonic development

Dynamics of *T. depressus* cell divisions is shown in Table 1. The embryo stages were completed within 48 h at 26.1 ± 0.7 °C (Fig. 4b–j). The fertilization envelope was observed with the release of polar bodies (Fig. 4b). The eggs exhibited holoblastic cleavages (until 32-cell stages) at approximately 27–60 min after fertilization (AF) (Fig. 4c–e, Table 1). The morula (128-cell stage) occurred 2 h AF (Fig. f–g, Table 1). The embryo hatched and developed into the late blastula and an early ciliated gastrula stage 7 h AF (Fig. 4h, i, Table 1). An early full-size spherical gastrula occurred 20 h AF (Fig. 4j, Table 1) and a bilateral pyramidal prism larva with a complete digestive tract occurred 48 h AF (Fig. 4k, Table 1).

Table 1
Development of *Tripneustes depressus* from fertilization to 40 mm diameter juvenile. Time is in hours after fertilization.

Stages	Description	Time	Size (μm)
Embryo	Fertilized eggs with complete fertilization membrane and expulsion of polar bodies	3–12 min	85.5
	Fist division (2cell stage)	16 min	100.0
	Second division (4-cell stage)	34 min	107.0
	Third division (8-cell stage)	55 min	109.0
	Fourth division (16-cell stage)	1 h 18 min	115.0
	Fifth division (32-cell stage)	1 h 45 min	120.0
	Morula	2 h 18 min	125.0
	Hatching blastula	4 h 56 min	128.0
	Blastula	7 h 07 min	130.0
	Gastrula	7 h 40 min	150.0
	Gastrula with archenteron	20 h	150.0
Larvae	Prism	48 h	201.0
	2-arm pluteus	2 d	312.0
	4-arm pluteus	6 d	455.0
	6-arm pluteus	9 d	485.0
	8-arm pluteus	11 d	497.0
	Pre-competent larva, ciliated ring and growing rudiment	16 d	545.0
	Competent larva, complete rudiment	21 d	477.0
Juvenile		30 d	630.0
	Juvenile (day-1 with open mouth)	54 d	850.0
	Juvenile (day-40)		1000
	Juvenile (day-61)		4000
	Juvenile (day-83)		5000
	Juvenile (day-111)		10,000
	Juvenile (day-145)		20,000
	Juvenile (day-188)		40,000

3.3. Larval development and growth

The echinopluteus larval stages were completed within 11 days AF (Fig. 5a–j, Table 1) at 26.0 ± 0.5 °C. On day-2 AF, the size of the early feeding larvae of two-armed stage (Fig. 5a) was TL: 311.7 ± 2.0 μm, MBL: 158.9 ± 1.0 μm and BW: 270.7 ± 3.4 μm, $N = 10$ (Fig. 6). These larvae showed fenestrated rods and the esophagus, sphincter, digestive tract and anus were clearly visible (Fig. 5a–c). On day-9 AF, the size of the six-armed stage (Fig. 5d) was TL: 504.5 ± 3.7 μm; MBL: 265.1 ± 3.5 μm; BW: 270.7 ± 3.5 μm; N = 10 (Fig. 6) with the highest growth increment variations at 47%, 50% and 49%, respectively (Fig. 6). On day 11 AF, the size of the eight-armed stage (Fig. 5f) was TL: 524.1 ± 4.3 μm; MBL: 258.4 ± 5.1 μm; BW: 322.8 ± 3.5 μm; N = 10 (Fig. 6). Their growth increment variation decreased at 4%, 3% and 18%, respectively (Fig. 6). On day 16 AF, the size of the larvae was TL: 544.7 ± 4.0 μm; MBL: 273.6 ± 6.9 μm; BW: 355.0 ± 3.5 μm; N = 10 (Fig. 6). The antero-lateral arms were reduced in length, the lateral larval body extensions were bent towards the previous end, and its body shape was rounded (Fig. 5f) with very low increment variations at 4%, 6% and 9%, respectively (Fig. 6). The rudiment grew and attained approximately one third the length of the stomach (Fig. 5f, Table 1). On day 20 AF, the larval rudiment reached a size-equal to that of the stomach and occupied a large part of the larva body, constricting the stomach to the left side. The larva developed cilia over its whole body (Fig. 5g,h, Table 1). On day 21 PF, the movement of the first pedicellariae, which were emerging from the larval rudiment, was observed (Fig. 5i). This indicated that larvae were ready for the settlement and metamorphosis (Fig. 5j, Table 1).

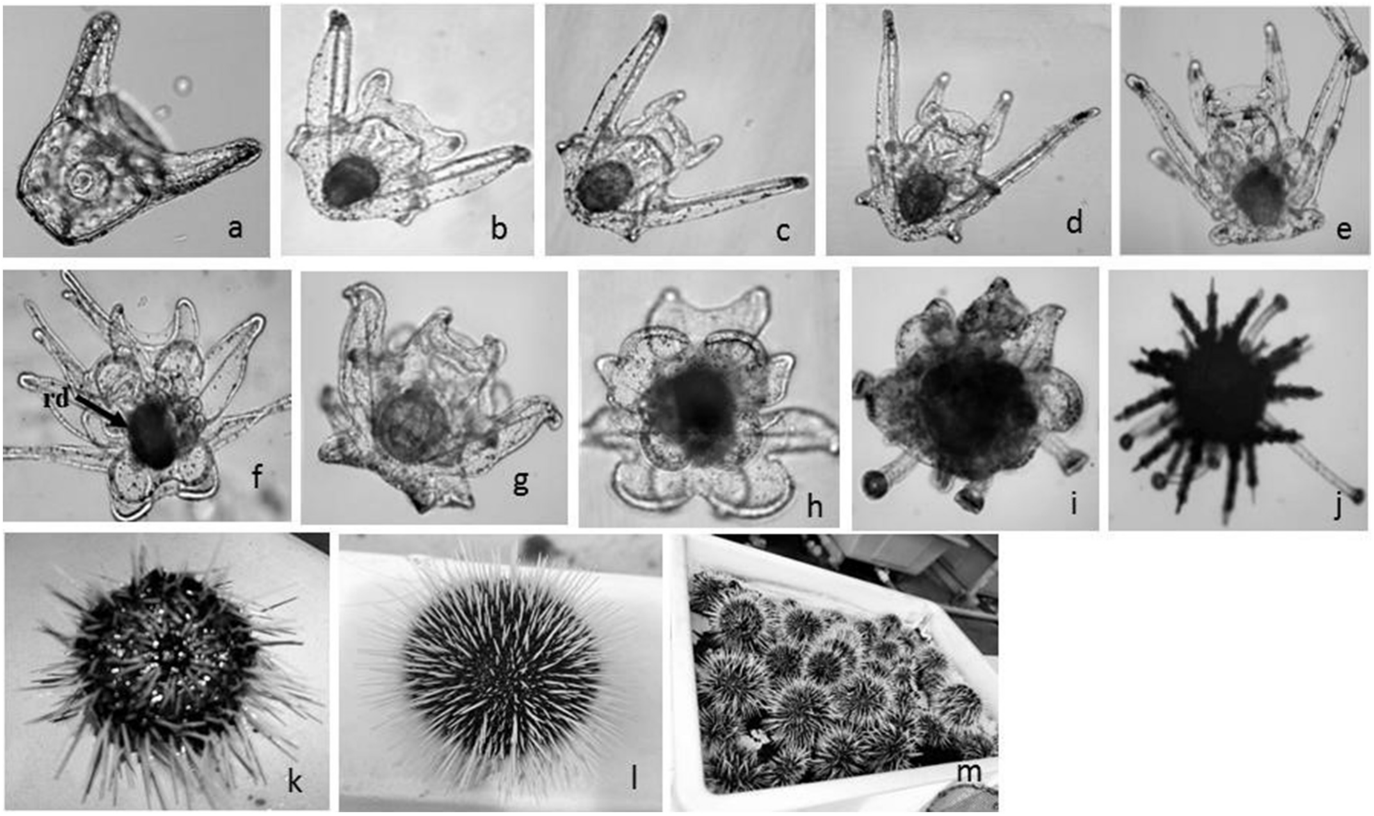

Fig. 5. Larval development and juvenile of *Tripneustes depressus*. a) early two-armed pluteus; b) two-armed; c) four-armed; d) six-armed; e) eight-armed; f) a competent eight-armed pluteus larva with juvenile rudiment occupied most of the body and displaced the larval gut; g) pre-metamorphic larvae; h) pre-metamorphic larvae with pedicels; i) larval tissue regression of the pre-metamorphic larvae with ambulacral feet; j) aboral view of a recently metamorphosed sea urchin showing podia and spines; k-m) oral and aboral views of juveniles at laboratory. Arrow indicates a well-developed rudiment (rd).

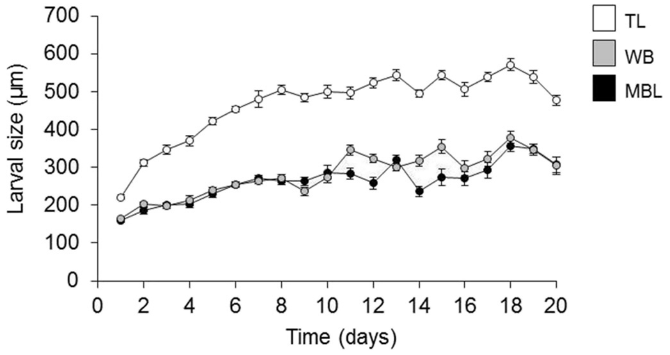

Fig. 6. Larval morphometrics of *Tripneustes depressus*. TL = total length, MBL = mid-body line length, and WB = width base. Error bars denote s.e.

3.4. Size variability and growth of juveniles

Size variability and growth performance for juveniles are given in the Figs. 5k–m, and 7 to 9. At DPS_{20} juveniles fed *Navicula* sp. (Figs. 5j,k; 7a). At DPS_{35}, sea urchins without opened mouth passed to juvenile stage with an average size of 0.40 ± 0.01 mm TD (Figs. 5l; 8a,b; Table 1). At this size, animals opened their mouth with a movable jaw structures, as Aristotle's lantern, and developed a functional gut. They initially fed on turfs of *Enteromorpha* sp. and passed to blades of *Padina durvilaei* (Figs. 5m; 7b,c). Two remarkable rapid-growth episodes occurred (SGR ranged from of 0.8 to 4.2% d^{-1}, Figs. 8a,b, 9) and were intercalated by a long slow-growth episode between 53 and 82-day of culture (SGR ranged from 0.1 and 0.3% d^{-1}, Figs. 8a,b, 9). At $DPS_{82–95}$, growth rates of juveniles were well differentiated into three size groups at ($F_{[2197]}$ = 961.63, *P* = .0000, Fig. 9).

At 98-days of culture, all juveniles were separated in three size-categories: *i*) small: 2.20 ± 0.07 mm TD; *ii*) medium: 4.90 ± 0.08 mm TD and *iii*) large: 7.20 ± 0.11 mm TD, and maintained in independent tanks (Fig. 8c,d). After this (until 242-days of culture), the growth of *T. depressus* was synchronous and alternated (as rapid and slow-moderate growth episodes) for all three sea urchin size-categories (Fig. 8c,d). During the slow-moderate growth episodes, small individuals grew faster (0.6 ± 0.06% d^{-1}) than large individuals ($F_{[2,27]}$ = 3,7644, *P* = .036; Fig. 9), but during the rapid growth episodes, all individuals grew fast and there were no differences between categories ($F_{[2,23]}$ = 0.2282, *P* = .798). The higher amounts of growth were recorded at one event synchronically for all size-categories of sea urchins, between days 160 and 180, but medium and large individuals showed a high-frequency of size increments (Figs. 8c,d). The final sizes for small, medium and large sea urchins were 23.9 ± 0.45 mm TD, 33.3 ± 0.3 mm TD, and 43.8 ± 0.533.3 ± 0.3 mm TD, respectively (Figs. 8c,d, 9).

3.5. Survival

Based on all our records of the culturing of *T. depressus* between 2015 and 2017, during the first day post-hatching (from fertilization to the prism larvae stage) survivorship was between 91 and 95% (Fig. 10). Before metamorphosis, from the prism to the eight-arm larvae stages, survivorships were around 75 and 80% (Fig. 10). We reported high mortalities between 80 and 93% after metamorphosis and settlement process (Fig. 10). Overall in the cohort of 13 of May 2017, approximately 280,000 larvae were seed in the culture tank, and of them, 18,000 reached the juvenile stage. At DPS_{95} of culture, the data for juveniles in Fig. 10 indicates survival was ca., 10%.

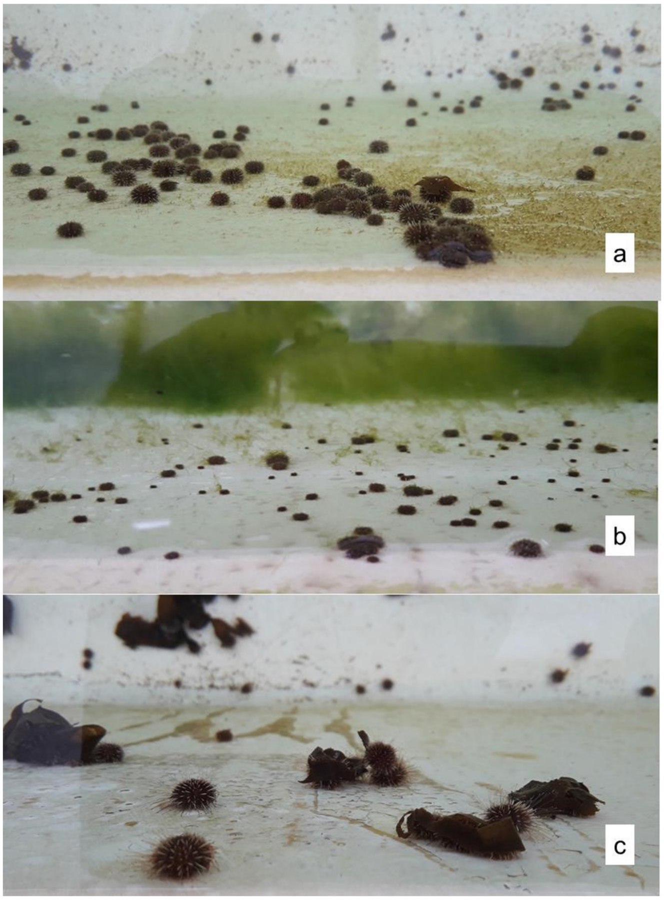

Fig. 7. Culturing of juveniles of *Tripneustes depressus* in outdoor tanks. a) a sea urchin barren ($^{<}$4 mm TD, DPS$_{20}$) feeding *Navicula* sp. film with other sessile diatoms; b) small juveniles (2.20 ± 0.07 mm TD, DPS$_{98}$) feeding the brown macroalgae *Padina durvilaei* and c) exhibiting cryptic covering behavior. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The results of this study promise an advance for production of juveniles (as seed) of the edible and native sea urchin *Tripneustes depressus* in Ecuador. Our protocol is based on larval and juvenile culture over three years (January 2015 to November 2017) It provides basic information and timing schedules on hatching and development of the larvae and juveniles, diets for promotion of growth increasing their survivorship, and consideration of systematic broodstock selection for effective spawning under laboratory scale conditions. Our results will be useful for culturing *T. depressus*, but it must be calibrated for the different regions of the ETP.

4.1. Spawning and reproduction of large sea urchins

Although KCl is an effective chemical used to induce spawning in mature echinoids during the breeding season (Giudice, 1973; Thompson, 1983; Levitan et al., 1992); it was not for *T. depressus*. Animals were not stimulated for spawn and can die. A similar unfavorable case was reported in for Giudice (1973) who noted that *Arbacia* can release a fertilization inhibitor from the epidermis or from the perivisceral fluid when they were stimulated with KCl, which needs to be carefully washed away from the eggs to prevent unfertilization. Based on our laboratory scale observations, *T. depressus* is very sensitive to temperature stress (4–5 °C) and light change as stimuli (see Fig. 1b) to induce spawning. This reaction to light was reported for *T. esculentus* (= *T. ventricosus*) where Lewis (1958) determined that it shows responses to change in light intensity. Therefore, we decided to apply a non-invasive and non-destructive inducer instead of the classical intraoral injection of 0.5 M KCl method. For this reason, large sea urchins were transported to the laboratory in a thermostatic bath in the dark to maintain their pre-spawning state until their arrival to the laboratory (see sessions 2.1, 2.2).

T. depressus does not show a restricted spawning period with variable intensities. We observed seven spawning events between March–May and July–August, corresponding to warm/humid and cold/dry periods, respectively, from January 2014 to November 2017. This suggests that *T. depressus* has a continuous, non-discrete and synchronic gametogenic activity throughout the year, with two peaks of gonadal development for reproduction, where the mean monthly seawater temperature fluctuated from 19 to 30 °C, and rainfall was < 150 mm per year (information based on the meteorological station at CENAIM–ESPOL). Similar patterns of an extended, nearly continuous period of reproduction with peaks (as a seasonal cycle of gonadal growth in the winter and the summer) have been reported for *T. gratilla* in Madagascar, Kenya, and Philippines (Muthiga, 2005; Toha et al., 2017), and *T. ventricosus* in coasts off Florida (McPherson, 1965). Nonetheless, both species there seems to be a tendency for semiannual reproduction into any broad geographical pattern or any one seasonal temperature regime (Pearse, 1974). Based on histological examination of gonads (data unpublished), *T. depressus* may reach their sexual maturity in less than one year, particularly the males at sixth months-old, and females one month after. This is particularly similar to *T. gratilla* reared in hatchery in the Philippines at 27 °C, which reached sexual maturity in six to seven months (Creswell, 2011).

Although, the factors that control the reproduction of *T. depressus* for producing successful larval supply through the year were not studied here, we consider that some conditions required for *T. gratilla* in the western tropical Pacific may apply for *T. depressus* in Ecuador. Pearse (1974) concluded that reproductive effort in *T. gratilla* does not correlate with temperature. Contrary to this Muthiga and Jaccarini (2005), Muthiga (2005) and Chen and Chang (2012) determined that the reproductive cycle, gonad development and spawning events of the Kenyan and Indonesian populations of the sea urchin *T. gratilla* are affected by annual and intra-daily environmental conditions, as sea temperature, daily light intensity, phases of moon, and benthic productivity (as food available offer). It's well known that both direct illumination and changes in day length are correlated with sea temperature in tropical shallow-waters, and both factors may affect sexual reproduction in marine invertebrates (Coma et al., 2000; Shpigel et al., 2004; Farhadian et al., 2014). Lodeiros et al. (2018) reported a good environmental data for mariculture of the oyster *Pteria sterna* at sites where large *T. depressus* were mostly collected in this study (between Palmar and Ayangue sites). They determined that temperature, salinity, concentration of seston and chlorophyll *a* were highly variable all year long, and suggested that high temperatures during the reproduction period of *P. sterna* can be the most negative influential trait, since the daily temperature range was > 6 °C (mid-March 2015). Higher intraday temperature variation was observed during Jan-Feb (4 °C), Feb-Apr (up to 6 °C), and Jun-Jul (4.3 °C). Variation during the rest of year was < 1 °C. Although, it is not clear whether the environment variables mentioned here may contribute to the reproductive pattern in *T. depressus*, a high variation in sea temperature and non-uniform light conditions by turbidity occur. In addition, this variation is often exacerbated by season.

T. depressus (as short-living sea urchins) that inhabits shallow rocky shores off Palmar, and typically lives in a group with patchy distribution pattern between 5 and 12 m deep (Idrovo and Sonnenholzner, 1994; Sonnenholzner and Lawrence, 2002; Lawrence and Sonnenholzner, 2004). This distribution pattern reported to the long-lived *Strongylocentrotus franciscanus* for increasing their fertilization success (Leahy et al., 1981; Levitan, 2004; Mercier and Hamel, 2009).

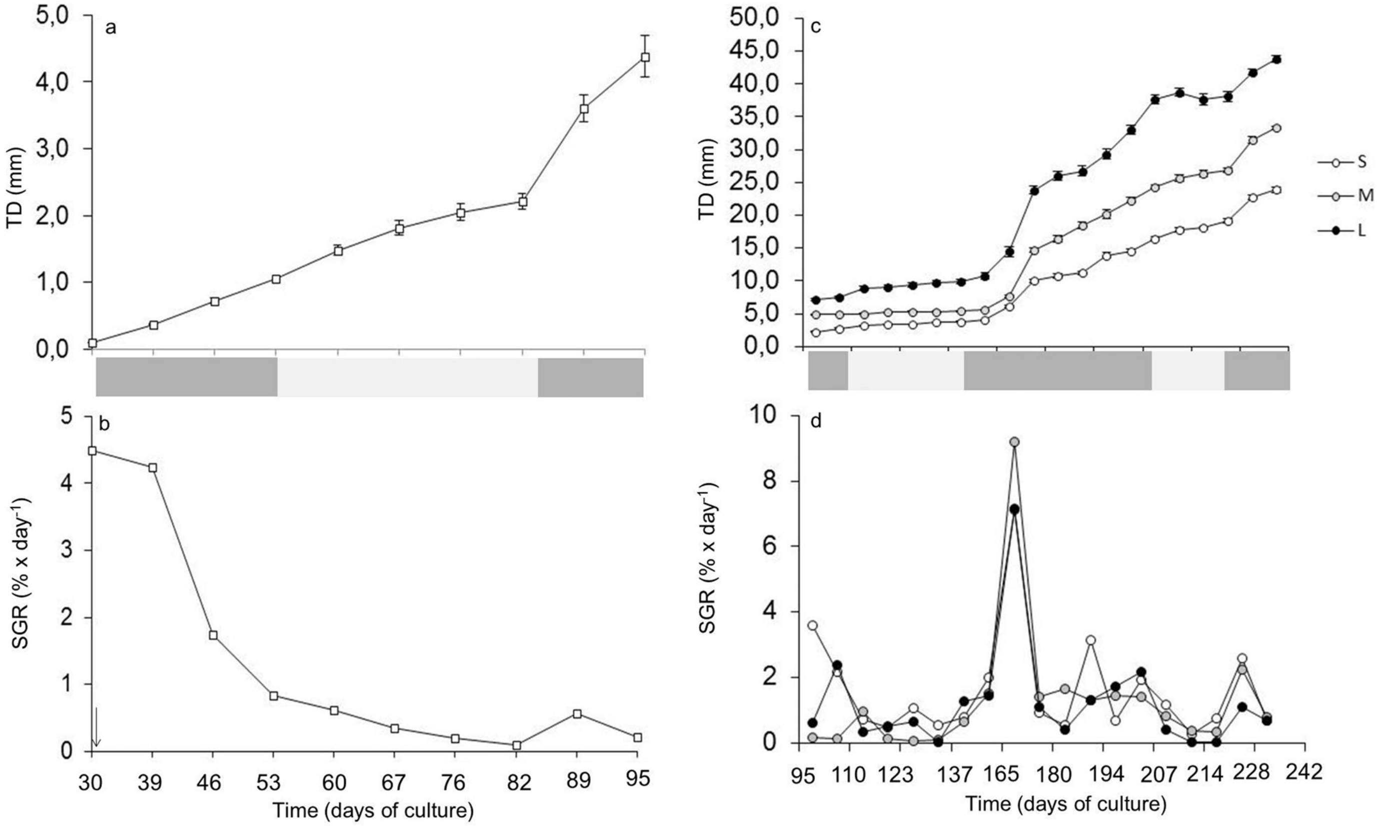

Fig. 8. Growth increments in size (test diameter, TD) and specific growth rates in juveniles of the sea urchin *Tripneustes depressus*, respectively; a,b) DPS_{95}, and c,d) DPS_{250}. Dark and light grey bars in the x axis mean rapid- and slow-growth phases. Data are expressed as mean ± s.e. ($N = 50$).

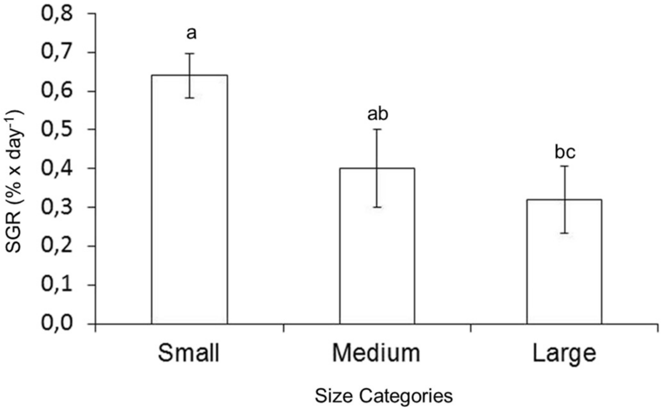

Fig. 9. Specific growth rates (SGR) during slow-growth episodes by size categories in juveniles of the sea urchin *Tripneustes depressus*. Error bars denote s.e.

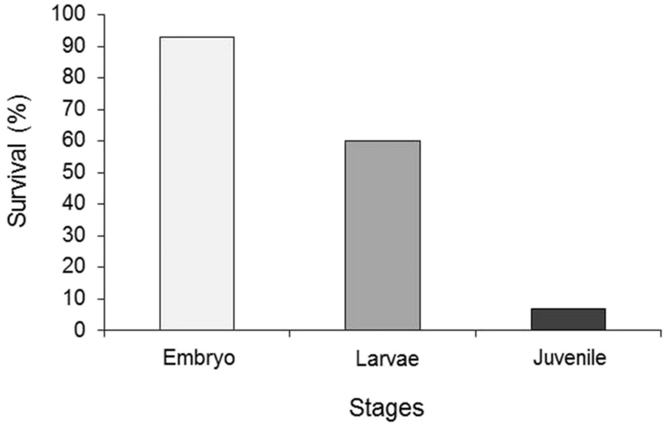

Fig. 10. Survivorship of the sea urchin *Tripneustes depressus* by stages as embryo, larvae and juvenile.

In the case of *T. depressus*, female's location in the reef and her proximity to males in high environmental variable conditions are crucial for the survival and production of new recruits. Nojima and Mukai (1985) and Shimabukuro (1991)*fide* Toha et al. (2017) determined that some *T. gratilla* have a tendency to live in couples, while populations that showed no individual-to-individual body contact were observed, even with high density. These issues are very important, since another interesting finding was that, at the end of this study, we were challenged to collect sea urchins in the field to evaluate their gonad production. The capture effort increased and the number of animals collected progressively decreased throughout three last years (between 2014 and 2017). This explains to us that their densities apparently have a high variability at very, short time periods at intervals of perhaps between four and five years until the next recruitment event in 2018. This study coincided with a similar natural event noticed in the Galápagos Archipelago, during 2016–2017. Reported densities of *T. depressus* in Ecuador are approximately $^{<}$0.1 to 5.0 individuals per m^2 and vary considerably from year to year (Brandt and Guarderas, 2002; Lawrence and Agatsuma, 2013). Based on our results it is well known that population densities of *T. depressus* in Ecuador can rapidly diminish or completely disappear at monthly intervals.

4.2. Larviculture

Comparatively, the mean egg size of the tripneustids echinoids, *T. depressus* from Ecuador (78.4 ± 2.1 μm), *T. ventricosus* from the Caribbean and Panamanian coasts (80.0 ± 1.1 μm) and *T. gratilla* from the Indo-Pacific (85.2 ± 1.3 μm) seems different in size, but their planktotrophic larvae are competent to feed at 2.5, 2.0 and 3.5 day post-fertilization, respectively (Lewis, 1958; Lessios, 1987; Byrne et al., 2008). Lessios (1987) studied temporal variation in mean egg size of *T. ventricosus* from different females collected from the same locality on the same day. He determined monthly and inter-annual differences, and suggested that size of mature eggs is not determined by reproductive state of the parental population. In a later study, Byrne et al. (2008) demonstrated that the growth dynamic larvae of *T. gratilla* larvae is not divergent under unfed and fed conditions (with no evidence of phenotypical plasticity in growth). They suggested that larvae maintain a nutrient storage buffer against starvation, which may exceed 8 days, as a facultative feeding period, considerably longer than that of other echinoids with similarly sized eggs, emphasizing that egg quality may be more important than egg size.

Our findings are important to understand the life history strategy of *T. depressus* and determine their resistance to starvation, risks of mortality, successful metamorphosis, and an optimal quality production of juveniles, considering two factors, high sea temperature and low food conditions; which are characteristic at tropical waters (Reitzel et al., 2005). In our laboratory, we pre-evaluated the feeding of *T. depressus* larvae (at a density of 1 larva per milliliter) with low concentration of microalgae (with five different species with < 500 cell ml^{-1}). Larvae died in less than five days (data unpublished). This suggests that larval development *T. depressus* (with small egg) proceeds immediately after the third day post-fertilization and the larvae cannot survive more than five days in very low food conditions. They do not have the ability to use maternal reserves (as endogenous energetic lipid reserves, particularly triglycerides) as was reported for *T. gratilla* (Byrne et al., 2008). In this context, the tri-algal diet used here (*Tysochrisis lutea = Isoschrysis galbana*, *Chaetoceros gracilis* and *Rhodomonas* sp) at concentrations ranging from 2.5×10^3 to 2.0×10^4 cells ml^{-1} were adjusted by feeding rations to ensure apparent satiation for the non-facultative *T. depressus* larvae. This combined diet positively contributed to meet balanced nutritional needs in some of the essential polyunsaturated fatty acids (PUFAs and DHA) for achieving the competence with a continuous growth performance, non-delayed metamorphosis, and no abnormalities, with high survival rates of larvae. Therefore, although the final output in term of larval survival was high after rearing, but it cannot be affirmed that this combined diet leads to success in settlement. Further research is needed for this stage. With supporting data from larval morphometric, the larvae did not exhibit phenotypic plasticity. Indeed, the phenotypic plasticity of the post-oral arm size (as Total Length) makes it a good indicator of the morphological development of larvae in response of the food type and availability (Strathmann et al., 1992; Soars et al., 2009). Gallager et al. (1986) suggested that larvae fed with *Rhodomonas* sp. produce the highest lipid content, as PUFA content (22:6n-3 and 20:5n-3), and high levels of carbohydrates, which is a good physiological condition indicator and the capacity for successful metamorphosis. Therefore, the specific growth of *T. depressus* larvae reported here could be explained by the carbohydrates ingested (Brown et al., 1998). Other authors have confirmed that *Rhodomonas* sp. alone may provide the critical nutrients or energy (for their higher digestibility) for echinoderm larval development (Castilla-Gavilán et al., 2018).

The larva of *T. depressus* is intermediate temperature tolerant, with a wide temperature range, between 23 and 29 °C, and has a relatively short larval stage lasting between 25 and 30 days at 26 °C. Our results agree with those of Rahman et al. (2009), who experimentally studied the early development to *T. gratilla* at Okinawa Island, Japan. Our results indicate that the optimum range of temperature for *T. depressus* was between 25 and 27 °C for normal development.

4.3. Post-settlement and growth of juveniles

In our study, the high mortality in the transition between larvae and juveniles is remarkable, which would be a major problem in the production seeds, and this represents a limitation of our study. Therefore, our high rate of mortality will be discussed based on the positive findings of Rials et al. (2018) who determined a good inducer for settlement, and high growth and survival rates.

Rials et al. (2018) experimentally evaluated the suitability of different cues (treatments as unialgal films by *Tetraselmis marina*, *Nitzschia sp*, *Cylindrotheca closterium*, and mixed) as inducers of settlement and diet after metamorphosis for promoting the growth as increase in test diameter and survival of juveniles of the slow-growth sea urchin *Paracentrotus lividus*. They monitored metamorphosed individuals for 32 days on these treatments and found one treatment (*C. closterium*) that showed higher survival rates (confidence intervals: 74–100%), high settlement and growth rates ($DPS_{21–26}$: 73.4 μm /day). We used a direct film of the pennate diatom *Navicula* sp. for coating the tank walls as the substrate, as inducer and diet for post-settlement of *T. depressus*. At $DPS_{32,}$ survival was low ca., 10%, but the calculated daily growth rates was 60.0 μm /day.

Rials et al. (2018) determined that the released dissolved organic matter, mainly free amino acids, with higher protein content, as lipid and fatty acids (that represent concentrations at 14:0, 16:0, 16:1(n-7), 18:1 and 20:5(n-3)) and nitrates of the pennate diatom *C. closterium* (as unialgal biofilms) in *P. lividus*, is nutritionally very important due its high digestibility, contributing on the development of the life stages from the larvae to adults for different species of sea urchins (Salas-Garza et al., 2010; Lawrence et al., 2013; Watts et al., 2013). Taken this into account, we recommend an experimental test with *C. closterium* for improving general performance of all these phases as an inducer film and as the primary food for post-settlement juveniles of *T. depressus* to decide the proper moment (probably occurs for individuals between 3 and 6 mm TD, according to Onitsuka et al., 2015) to start a macroalgal diet (Rials et al., 2018).

There are very few studies on fisheries ecology of fast growing echinoids (Lawrence, 2013). Therefore, our study contributes to the understanding of growth patterns for ruderal echinoids, which has been associated to a “bet-hedging” strategy; where large-size, high growth rate, short life span, short time to maturity, a high rate of adult survival, and high annual reproductive effort and output are adaptations to compensate for the low and highly variable survival rate of first-year juveniles (Stearns, 1976; Roff, 1992). Lawrence and Bazhin (1998) pointed out that ruderal species with economic interest, such as *T. depressus* are more susceptible to stress. Therefore, we must consider more care for the management of this species in the wild *T. depressus,* because of its biological characteristics, must be included as an interesting species for the global urchin roe industry, but based on aquaculture and restocking operations, and not as a direct fishery.

In this study, early stage (as post-metamorphic juveniles) *T. depressus* (0.1 mm TD) opens its mouth with functional gut at 5 wk. of age after post-settlement (0.4 mm TD). They continue developing their podia, spines, and pedicellariae as long as the thermal (26–28 °C), salinity (31–35 ppt), pH (8.2), dissolved oxygen (5–6 mg O_2 l^{-1}) and food (*Padina durvillaei*) regimes are appropriate. In this study, we used selected dry cuts and leaves of the brown macroalgae *P. durvillaei*, since at a laboratory scale experiment it showed that it dominantly consumes *P. durvallaei* than *Sargassum ecuadoreanum*, *Gracilaria lemaneiformis*, *Ulva lactuca*, and *Codium fragile* (unpublished data). It is well known that some species can grow rapidly to a total diameter of 1–2 cm in the first year (Moore, 1966) but *T. depressus* can reach 1 cm at 152 days after post-settlement. Many sea urchin species can reproduce in their second year of life, but *T. depressus* can reproduce after six months of life. These results are in agreement with other observations in the Philippines that *T. gratilla* may spawn throughout the year (Toha et al., 2017).

Our results contrast with the study of Miller and Emlet (1999) who studied environmental effects on early development of two slow-growing sea urchin species (*Strongylocentrotus franciscanus* and *S.*

purpuratus), focusing on their larval feeding condition (based on food ration regimes) and different temperatures. They determined that larval food-rations significantly affect the development of traits in spines, podia, and pedicellariae in juveniles up to and after the onset of feeding, which begins 9 days after metamorphosis at 14 °C. Juveniles from the high-ration larval culture were significantly larger after metamorphosis and grew faster than ones from the low-ration culture. Other differences relate to time of development of jaws and pedicellariae. Juveniles from the high-food larval cultures were comparable in size to juveniles collected from the field at 14 days of age, but ones from the low-food larval cultures were much smaller. Here, we report growth rates of juveniles of *T. depressus* which seems that the nutritional requirement for somatic growth was satisfactory with a high survival rate (90%). Nonetheless, the growth rate of juveniles at 95 days post-settlement was affected by an emergent and divergent separation into three size categories (large, medium and small individuals). Where, the access of smaller sea urchins to food was mostly limited by large ones generating a chronic growth inhibition. Ebert (1975) determined a strong positive correlation between growth rate and mortality in natural populations of fast growing sea urchins that may have higher rates of natural mortality. Here, given this, at 95 days after post-settlement of *T. depressus* (juvenile culture of the same cohort of May, 13 May 2017) we reported three subpopulations of new recruits with differentiated size structure (as small, medium and large individuals), which was mostly composed of small inhibited-growth individuals (60%) and with an evident retarded growth. Cannibalism was mostly by medium-size congeners to small-size individuals. Therefore, sub-population of small individuals can be drastically reduced. Sonnenholzner et al. (2011a),b) reported recurrent cannibalism events by conspecifics in small sea urchins of *Strongylocentrotus purpuratus* under starving conditions.

T. depressus is an appropriated species for aquaculture, because here we showed that when animals are early isolated (before 95-days after post-settlement) into different tanks by size-classes, intra-size cannibalism does not occur, and then mortality is reduced to zero. In this sense, *T. depressus* has a ruderal strategy for allocating more energy to production (for growth and reproduction) than to protection and maintenance, as consequence, they grow rapidly and have great roe production at an early age (Lawrence, 1990; Lawrence and Bazhin, 1998). Therefore, they would have to have a predictable recruitment to persist (Ebert, 1975, 1982; Rowley, 1990). This pattern is contrary to those commercial species with unpredictable recruitment, which must live longer to persist, and may invest more resources in body construction - growing heavier skeletons, more slowly, while still allocating resources to reproduction, such as the sea urchins from temperate ecosystems, e.g., the family Strongylocentrotidae (Ebert et al., 2018). Our results indicate that the changes in growth rate (as an alternated biphasic growth pattern, such as, rapid and slow-moderate growths (as was shown in Figs. 8 and 9) in all three sea urchin size groups are not size dependent and this suggest that the changes are based on a culturing system change.

Finally, for the aquaculture of juveniles of *T. depressus*, the brown macroalgae *P. durvillei* can be applied as functional food (Vadas et al., 1982) that promote health condition for growing and protection of their beneficial intestinal microbial flora (Fenchel and Harrison, 1976; Lawrence and Lane, 1982). *P. durvillei* dominates inter- and subtidal flora, especially at sites where large sea urchins were collected. This alga has a high content of ashes (relatively high concentrations of SO_4^{-2}, calcium carbonate as extracellular calcium precipitated in the form of aragonite and organic matter (34.4%), carbohydrates (primarily mannitol as the major carbohydrate of low molecular weight, 44.2%) and total dietary fiber (7.6%), but low in proteins (5.24 ± 0.1%) and lipids (essential fatty acids like omega-3, omega-6 and PUFAs, with 0.69%) as usual for these macroalgae (Goecke et al., 2012). Therefore, our seed production protocol provided a good environment to cultivate juveniles for commercialization and stock enhancement. But further research on natural and formulated diets for juvenile sea urchins *T. depressus* is needed.

4.4. Perspectives

Due to the decline in sea cucumber and spiny lobster fisheries, interest has developed in Ecuador for new fisheries (Wolff et al., 2012; Castrejón and Charles, 2013; Alava et al., 2015), in order to divert fishing pressure away from traditional fisheries. For example, in 1998, local fishermen of Galápagos requested the Autoridad Interinstitucional de Manejo of the Galápagos Marine Reserve (AIM-GMR, Resolución No. 003–2002) for permission to open the fishery of *T. depressus*. The AIM-GMR suggested realizing biological and ecological surveys of *T. depressus* to synthetize information of their population state in the Archipelago prior for establishment a new non-traditional fishery in Galápagos. Therefore, this initiative continues to be pertinent based on this newly reported information. *T. depressus* is occasionally caught for local consumption by fishermen on the Ecuadorian mainland coast and Galápagos (Luna and Darwin, 2000; Sonnenholzner et al., 2013). So, there is interest in consumption and aquaculture of this native marine invertebrate species in Ecuador.

Aware of these conditions, the Ecuadorian Government developed a number of laws to regulate this sector. In 2012 the Acuerdo Ministerial # 458 was published for the regulation and control of aquaculture leases, which could be awarded in areas with rocky bottoms where the cultivation of marine invertebrates may be technically feasible for reproduction and restocking natural populations (López-Alvarado et al., 2016). Beside of that *T. depressus* has a high importance as human food. Recently, Salas-Rojas et al. (2014) reported that *T. depressus* has importance for human health. This species produces antimicrobial peptides with a direct and stable activity against Suid herpesvirus type 1 and/or rabies virus. They have been characterized as new molecules with antiviral activity for designing new drugs from the coelomic fluid of this commercial and edible sea urchin species.

Consequently, this study has developed a perfectible new culture technology for supplying juvenile seed of the native sea urchin *T. depressus* for sea urchin aquaculture purposes in Ecuador. In other parts of the world, there are producers who grow species of slow-growing sea urchins. For this reason they have to face high investments and require waiting approximately three years to achieve sea urchins of 55 mm DT (such as *Paracentrotus lividus* in Europe). In contrast with this, *T. depressus* is the opposite. It is a species of sea urchin of rapid growth, and therefore, the investment of money for its cultivation will be much smaller. Additionally in its first year of cultivation the animals will be larger in size with larger gonads.

Acknowledgements

This work was supported by the Secretaría Nacional de Educación, Ciencia, Tecnología e Innovación of Ecuador (SENESCYT) for providing funds for the project titled: “Desarrollo de protocolos de domesticación para el uso sostenible de nuevas especies marinas para consumo de alimentos y repoblación de bancos naturales” (grant number: PIC-2014-CENAIM-002). We are grateful to John Lawrence and John Chamberlain for their comments and criticisms. We also thank to Juan Carlos Navarro, Daniel González and Manuel González for contributing photographic material to Fig. 1.

References

AAFC (Canadian Agriculture and Food International Program of Agriculture and Agri-Food Canada), 2002. Sea urchin fishery profiles: A background document produced by Explorations Unlimited Inc. pp. 83.

Alava, J.J., Lindop, A., Jacquet, J., 2015. Marine fisheries catch reconstructions for continental Ecuador: 1950-2010. Working paper #2015-34. Fisheries Centre, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada, pp. 25.

Andrew, N.L., Agatsuma, Y., Ballesteros, E., Bazhin, A.G., Creaser, E.P., Barnes, D.K.A., Botsford, L.W., Bradbury, A., Campbell, A., Dixon, J.D., Einarsson, S., Gerring, P.K., Herbert, K., Hunter, M., Hur, S.B., Johnson, C.R., Juinio-Meñez, M.A., Kalvass, P., Miller, R.J., Moreno, C.A., Palleiro, J.S., Rivas, D., Robinson, S.M.L., Schroeter, S.C., Steneck, R.C., Vadas, R.L., Woodby, D.A., Xiaoqi, Z., 2002. Status and management of world sea urchin fisheries. Oceanogr. Mar. Biol. Ann. Rev. 40, 343–425.

Archana, A., Babu, K.R., 2016. Nutrient composition and antioxidant activity of gonads of sea urchin *Stomopneustes variolaris*. Food Chem. 197 (PtA), 597–602.

Banks, S.A., 1999. The Use of AVHRR Data in Determining Sea Surface Temperature Zonation and Variability across the Galápagos Marine Reserve. M Sci Thesis. Southampton Oceanographic Centre, UK, pp. 46.

Barker, M.F., Keogh, J.A., Lawrence, J.M., Lawrence, A.L., 1998. Feeding rate, absorption efficiencies, growth, and enhancement of gonad production in the New Zealand sea urchin *Evechinus chloroticus* Valenciennes (Echinoidea: Echinometridae) fed prepared and natural diets. J. Shell. Res. 17, 1583–1590.

Brandt, M., Guarderas, P., 2002. Erizos de Mar. In: Danulat, E., Edgar, G.J. (Eds.), Reserva Marina de Galápagos. Línea Base de la Biodiversidad. Fundación Charles Darwin/ Servicio Parque Nacional Galápagos, Santa Cruz, Galápagos, Ecuador, pp. 396–418.

Brown, M.R., McCausland, M.A., Kowalski, K., 1998. The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. Aquaculture 165, 281–293.

Byrne, M., Sewell, M.A., Prows, T.A.A., 2008. Nutritional ecology of sea urchin larvae: influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla*. Funct. Ecol. 22, 643–648.

Castilla-Gavilán, M., Buzin, F., Cognie, B., Dumay, J., Turpin, V., Decottignies, P., 2018. Optimising microalgae diets in the sea urchin *Paracentrotus lividus* larviculture to promote aquaculture diversification. Aquaculture 490, 251–259.

Castrejón, M., Charles, A., 2013. Improving fisheries co-management through ecosystem-based spatial management: the Galápagos Marine Reserve. Mar. Policy 38, 235–245.

Chen, C.P., Chang, K.-H., 2012. Reproductive periodicity of the sea urchin, *Tripneustes gratilla* in Taiwan compared with other regions. Intl. J. Invert. Reprod. 3, 309–319.

Coma, R., Ribes, M., Gili, J.-M., Zabala, M., 2000. Seasonality in coastal benthic ecosystems. Trees 15, 448–453.

Creswell, R.L., 2011. Developing echinoderm culture for consumption and stock enhancement in the Caribbean. In: Lovatelli, A., Sarkis, S. (Eds.), A Regional Shellfish Hatchery for the Wider Caribbean: Assessing its Feasibility and Sustainability. FAO Regional Technical Workshop. 18–21 October 2010, Kingston, Jamaica. FAO Fisheries and Aquaculture Proceedings. No. 19 FAO, Rome, pp. 141–145.

Cyrus, M.D., Bolton, J.J., De Wet, L., Mace, B.M., 2013. The development of a formulated feed containing *Ulva* (Chlorophyta) to promote rapid growth and enhanced production of high quality roe in the sea urchin *Tripneustes gratilla* (Linnaeus). Aquac. Res. 45, 159–176.

Devin, M.G., 2002. Land-based echinoculture: a novel system to culture adult sea urchins. In: Yokota, Y., Matranga, V., Smolenicka, Z. (Eds.), The Sea Urchin: From Basic Biology to Aquaculture. AA. Ballema Publishers Swets & Zeitlinger, Lisse, pp. 145–159.

Ebert, T.A., 1975. Growth and mortality of post-larval echinoids. Am. Zool. 15, 755–775.

Ebert, T.A., 1982. Longevity and life history and relative body wall size in sea urchins. Ecol. Monogr. 52, 353–394.

Ebert, T.A., Barr, L.M., Bodkin, J.L., Burcham, D., Bureau, D., Carson, H.S., Caruso, N.L., Caselle, J.E., Claisse, J.T., Clemente, S., Davis, K., Detwiler, P.M., Dixon, J.D., Duggins, D.O., Engle, J.M., Estes, J.A., Groth, S.D., Grupe, B.M., Halmay, P., Hebert, K.P., Hernández, J.C., Jurgens, L.J., Kalvass, P.E., Kenner, M.C., Konar, B., Kushner, D.J., Lee, L.C., Leighton, D.L., Montaño-Moctezuma, G., Munk, J.E., Olguin, I., Palleiro, J.S., Parker, D.O., Pearse, J.S., Pondella, D.J., Rogers-Bennett, L., Schroeter, S.C., Shelton, A.O., Sonnenholzner, J.I., Taniguchi, I.K., VanBlaricom, G.R., Watson, J.C., Weitzman, B.P., Williams, J.P., Yakimishyn, J., Zhang, Z., 2018. Size, growth, and density data for shallow-water sea urchins from México to the Aleutian Islands, Alaska, 1956–2016. Ecology 99, 761. https://doi.org/10.1002/ecy.2123.

Eddy, S.D., Brown, N.P., Kling, A.L., Watts, S.A., Lawrence, A., 2012. Growth of juvenile green sea urchins, *Strongylocentrotus droebachiensis*, fed formulated feeds with varying protein levels compared with a macroalgal diet and a commercial abalone feed. J. World Aquacult. Soc. 43, 159–173.

FAO, 2016. FAO yearbook. In: Fishery and Aquaculture Statistics. 2014. Rome, Italy.

Farhadian, O., Yussoff, Md, Arshad, A., 2014. Effects of salinity, temperature, light intensity and light regimes on production, growth and reproductive parameters of *Apocyclops dengizicus*. Iran. J. Fish. Sci. 13, 30–46.

Fenchel, T., Harrison, P., 1976. The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In: Anderson, J.M., MacFadyen, A. (Eds.), The Role of Terrestrial and Aquatic Organisms in Decomposition Processes. Blackwell, Oxford.

Foster, M.S., McConnico, L.M., Lundsten, L., Wadsworth, T., Kimball, T., Brooks, L.B., Medina-López, M., Riosmena-Rodríguez, R., Hernández-Carmona, G., Vázquez-Elizondo, R.M., Johnson, S., Steller, D.L., 2007. Diversidad e historia natural de una comunidad de *Lithothamnion muelleri* y *Sargassum horridum* en el Golfo de California. Cienc. Mar. 33, 367–384.

Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as an index of growth and viability in three species of bivalve larvae. Aquaculture 56, 81–103.

Giudice, G., 1973. Developmental Biology of the Sea Urchin Embryo. Academic Press, Inc.

Glynn, P.W., 2008. Food-Web Structure and Dynamics of Eastern Tropical Pacific Coral Reefs: Panamá and Galápagos Islands. In: McClanahan, T.R., Branch, G.M. (Eds.), Food Webs and the Dynamics of Marine Reefs. Oxford University Press, pp. 185–208.

Goecke, F., Escobar, M., Collantes, G., 2012. Chemical composition of *Padina fernandeziana* (Phaeophyceae, Dictyotales) from Juan Fernández Archipelago. Chile. Rev. Latinoam. Biotecnol. Amb. Algal 3, 95–104.

Grosjean, Ph., Spirlet, Ch., Jangoux, M., 2003. A functional growth model with intraspecific competition applied to a sea urchin *Paracentrotus lividus*. Can. J. Fish. Aquat. Sci. 60, 237–246.

Hearn, A., 2008. The rocky path to sustainable fisheries management and conservation in the Galápagos Marine Reserve. Ocean Coast. Manage. 51, 567–574.

Henchion, M., Hayes, M., Mullen, A.M., Fenelon, M., Tiwar, B., 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods 6, 1–21.

Huggett, M.J., Williamson, J.E., de Nys, R., Kjelleberg, S., Steinberg, P.D., 2006. Larval settlement of the common Australian sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. Oecologia 149, 609–619.

Idrovo, F., Sonnenholzner, J., 1994. Distribución y abundancia de *Tripneustes depressus* A. Agassiz, 1863 y *Diadema mexicanum* A. Agassiz 1863 (Echinodermata: Echinoidea) en Punta Los Frailes, Parque Nacional Machalilla, Manabí, Ecuador. Reporte técnico de investigación No. 72. Facultad de Ciencias Naturales, Escuela de Biología, Universidad de Guayaquil, pp. 20.

Irving, A.D., Witman, J.D., 2009. Positive effects of damselfish override negative effects of urchins to prevent an algal habitat switch. J. Ecol. 98, 337–347.

James, P., Noble, C., Hannon, C., Stefansson, G., Þórarinsdóttir, G., Sloane, R., Ziemer, N., Lochead, J., 2016. Sea urchin fisheries, management and policy review (Activity A4.2.1 of the URCHIN project), Nofima Report 18/2016. pp. 18.

Kaneniwa, M., Takagi, T., 1986. Fatty acids in the lipid of food products from sea urchin. Bull. Jpn. Soc. Sci. Fish. 52, 1681–1685.

Kayaba, T., Tsuji, K., Hoshikawa, H., Kikuchi, Y., Kawabata, K., Otaki, I., Watanabe, T., 2012. Effect of low temperature rearing, using deep-sea water, on gonadal maturation of the short-spined sea urchin, *Strongylocentrotus intermedius*, in Rausu, Hokkaido. Fish. Sci. 78, 1263–1272.

Keesing, J.K., Hall, K.C., 1998. Review of harvests and status of world sea urchin fisheries points to opportunities for aquaculture. J. Shellfish Res. 17, 1597–1604.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 8, 1–5.

Lawrence, J.M., 1990. The effect of stress and disturbance on Echinoderms. Zool. Sci. 7, 17–28.

Lawrence, J.M., 2013. Chapter 2: sea urchin life history strategies. In: Lawrence, J.M. (Ed.), Sea Urchins: Biology and Ecology. Elsevier B.V., pp. 15–23.

Lawrence, J.M., Agatsuma, Y., 2013. Chapter 32: *Tripneustes*. In: Lawrence, J.M. (Ed.), Sea urchins: Biology and Ecology. Elsevier B.V., pp. 491–507.

Lawrence, J.M., Bazhin, A., 1998. Life-history strategies and the potential of sea urchins for aquaculture. J. Shell. Res. 17, 1515–1522.

Lawrence, J.M., Lane, J.M., 1982. The utilization of nutrients by post-metamorphic echinoderms. In: Jangoux, M., Lawrence, J.M. (Eds.), Echinoderm Nutrition. A. A. Balkema, Rotterdam, the Netherlands, pp. 331–371.

Lawrence, J.M., Sonnenholzner, J., 2004. Distribution and abundance of asteroids, echinoids, and holothuroids in Galápagos. In: Heinzeiller, T., Nebelsick, J.H. (Eds.), Echinoderms, München. Proc. 11th Int. Echinoderm Conf. Taylor and Francis Group, London, pp. 239–244.

Lawrence, J.M., Olave, S., Otaiza, R., Lawrence, A.L., Bustos, E., 1997. Enhancement of gonad production in the sea urchin *Loxechinus albus* in Chile fed extruded feeds. J. World Aquacult. Soc. 28, 91–96.

Lawrence, J.M., Lawrence, A.L., Watts, S.A., 2013. Chapter 9 - feeding, digestion and digestibility of sea urchins. In: John, M.L. (Ed.), Developments in Aquaculture and Fisheries Science. Elsevier, pp. 135–154.

Leahy, P.S., Hough-Evans, B.R., Britten, R.J., Davidson, E.H., 1981. Synchrony of oogenesis in laboratory-maintained and wild populations of the purple sea urchin (*Strongylocentrotus purpuratus*). J. Exp. Zool. 215, 7–22.

Lessios, H.A., 1987. Temporal and spatial variation in egg size of 13 Panamanian echinoids. J. Exp. Biol. Ecol. 114, 217–239.

Lessios, H.A., Kane, J., Robertson, D.R., 2003. Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. Evolution 57, 2026–2036.

Levitan, D.R., 2004. Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation for sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. Am. Nat. 164, 298–309.

Levitan, D.R., Sewell, M.A., Chia, F.S., 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentortus franciscanus*. Ecology 73, 248–254.

Lewis, J.B., 1958. The biology of the tropical sea urchin *Tripneustes esculentus* Leske in Barbados, British West Indies. Can. J. Zool. 36, 607–621.

Liu, H., Zhu, J., Kelly, M.S., 2010. Recent advances in sea-urchin aquaculture and enhancement in China. Bull. Aquacult. Ass. Can. 108, 30–37.

Lodeiros, C., Rodríguez-Pesantes, D., Márquez, A., Revilla, J., Freites, L., Lodeiros-Chacón, C., Sonnenholzner, S., 2018. Growth and survival of the winged pearl oyster *Pteria sterna* (Gould, 1851) in suspended culture in the tropical Eastern Pacific: influence of environmental factors. Aquac. Res. 49, 832–838.

López-Alvarado, J., Ruíz, W., Moncayo, E., 2016. Offshore aquaculture development in Ecuador. Int. J. Res. Educ. 1, 1–6.

Luna, S., Darwin, E.C.C., 2000. Distribución poblacional y ciclo reproductivo del erizo de mar blanco *Tripneustes depressus* (Echinodermata: Echinoidea) en las Islas Galápagos. Licenciatura Tesis. Universidad San Francisco de Quito, Quito, Ecuador.

Maluf, L.Y., 1988. Composition and Distribution of the Central Eastern Pacific Echinoderms. Technical Reports, Number 2. Natural History Museum of Los Angeles County.

Manabu, E., 2007. Report of Study of Fisheries Products Distribution and Consumption State in Santa Cruz Island. JICA/Fisheries and Aquaculture International Co., Ltd, pp. 40.

Martínez-Ortiz, J., Aires-Da-Silva, A.M., Lennert-Cody, C.E., Maunder, M.N., 2015. The Ecuadorian artisanal fishery for large pelagics: species composition and spatio-temporal dynamics. PLoS One 10, e0135136.

McPherson, B.F., 1965. Contributions to the biology of the sea urchin *Tripneustes ventricosus*. Bull. Mar. Sci. 15, 228–244.

Mercier, A., Hamel, J.F., 2009. Chapter 2: Gametogenesis. In: Mercier, A., Hamel, J.F. (Eds.), Endogenous and Exogenous Control of Gametogenesis and Spawning in Echinoderms. Adv. Mar. Biol Vol. 55. pp. 7–72.

Micael, J., Alves, M.J., Costa, A.C., Jones, M.B., 2009. Exploitation and conservation of echinoderms. Oceanogr. Mar. Biol. An. Rev. 47, 191–208.

Miller, B.A., Emlet, R.B., 1999. Development of newly metamorphosed juvenile sea urchins (*S. fransiscanus* and *S. purpuratus*): morphology, the effects of temperature and larval food ration, and a method for determining age. J. Exp. Mar. Biol. Ecol. 235, 67–90.

Moore, G.P., 1966. The use of trabecular bands as growth indicators in spines of the sea urchin *HeLiocidaris erythrogramma* Aust. J. Sci. 29, 52–54.

Muthiga, N.A., 2005. Testing for the effects of seasonal and lunar periodicity on the reproduction of the edible sea urchin *Tripneustes gratilla* (L) in Kenyan coral reef lagoons. Hydrobiologia 549, 57–64.

Muthiga, N.A., Jaccarini, V., 2005. Effects of seasonality and population density on the reproduction of the Indo-Pacific echinoid *Echinometra mathaei* in Kenyan coral reef lagoons. Mar. Biol. 146, 445–453.

Okey, T.A., Banks, S., Born, A.B., Bustamante, R.H., Calvopiña, M., Edgar, G.J., Espinoza, E., Fariña, J.M., Garske, L.E., Reck, G.K., Salazar, S., Shepherd, S., Toral-Granda, V., Wallem, P., 2004. A trophic model of a Galápagos subtidal rocky reef for evaluating fisheries and conservation strategies. Ecol. Model. 172, 383–401.

Onitsuka, T., Niwa, K., Unuma, T., Umezu, Y., 2015. Dietary shifts in the juvenile sea urchin *Strongylocentrotus intermedius* associated with the development of digestive enzymes. Mar. Biol. 162, 869–880.

Paredes, E., Bellas, J., Costas, D., 2015. Sea urchin (*Paracentrotus lividus*) larval rearing – culture from cryopreserved embryos. Aquaculture 437, 366–369.

Pearse, J.S., 1974. Reproductive patterns of tropical reef animals: three species of sea urchins. Proc. Second Int. Coral Reefs Symp. 1, 235–240.

Pena, M.H., Oxenford, H.A., Parker, C., Johnson, A., 2010. Biology and fishery management of the white sea urchin, *Tripneustes ventricosus*, in the eastern Caribbean. FAO Fisheries and Aquaculture Circular, No. 1056. Rome, FAO, pp. 43.

Pullin, R.S.V., 2017. Diversification in aquaculture: species, farmed types and culture systems. In: Harvey, B., Soto, D., Carolsfeld, J., Beveridge, M., Bartley, D.M. (Eds.), Planning for Aquaculture Diversification: The Importance of Climate Change and Other Drivers. FAO Technical Workshop, 23-25 June 2016, Rome Italy, pp. 15–36.

Rahman, S., Tsuchiya, M., Uehara, T., 2009. Effects of temperature on hatching rate, embryonic development and early larval survival of the edible sea urchin, *Tripneustes gratilla*. Biologia 64, 768–775.

Rahman, M.A., Yusoff, F. Md, Arshad, A., Shamsudin, M.N., Amin, S.M.N., 2012. Embryonic, larval, and early juvenile development of the tropical sea urchin, *Salmacis sphaeroides* (Echinodermata: Echinoidea). Sci. World J. 1–9. https://doi.org/10.1100/2012/938482.

Reitzel, A.M., Miles, C.M., Heyland, A., Cowart, J.D., McEdward, L.R., 2005. The contribution of the facultative feeding period to echinoid larval development and size at metamorphosis: a comparative approach. J. Exp. Mar. Biol. Ecol. 317, 189–201.

Rials, D., Rial, P., Casal, A., Costoya, N., Costas, D., 2018. Induction of settlement, growth and survival of juveniles of *Paracentrotus lividus*. Aquaculture 483, 16–20.

Robinson, S., 2004. The evolving role of aquaculture in the global production of sea urchins. In: Lawrence, J.M., Guzman, O. (Eds.), Sea Urchin Fisheries and Ecology. Destech Publications, Lancaster, Pa, USA, pp. 343–357.

Roff, D.A., 1992. The Evolution of the Life Histories. Theories and Analysis. Chapman and Hall, London, New York, pp. 1–535.

Rowley, R.J., 1990. Newly settled sea urchins in a kelp bed and urchin barren ground: a comparison of growth and mortality. Mar. Ecol. Progr. Ser. 62, 229–240.

Rubilar, T., Crespi-Abril, A., 2017. Does echinoderm research deserve an ethical consideration? Rev. Biol. Trop. 65, S11–S22.

Salas-Garza, A., Parés-Sierra, G., Ponce-Márquez, M.A., 2010. Culture of benthic diatoms and effect of dissolved organic carbon on metamorphosis of the white sea urchin *Lytechinus pictus* (Verrill). Aquac. Res. 41, 535–544.

Salas-Rojas, M., Galvez-Romero, G., Antón-Palma, B., Acevedo, R., Blanco-Favela, F., Aguilar-Setién, A., 2014. The coelomic fluid of the sea urchin *Tripneustes depressus* shows antiviral activity against Suid herpesvirus type 1 (SHV-1) and rabies virus (RV). Fish Shellfish Immun. 36, 158–163.

Scheibling, R.E., Mladenov, P.V., 1987. The decline of the sea urchin, *Tripneustes ventricosus* fishery of Barbados: a survey of fishermen and consumers. Mar. Fish. Rev. 49, 62–69.

Scholtz, R., Blton, J.J., Macey, B.M., 2013. Effects of different microalgal feeds and their influence on larval development in the white-spined sea urchin *Tripneustes gratilla*. Afr. J. Mar. Sci. 35, 25–34.

Shimabukuro, S., 1991. *Tripneustes gratilla* (sea urchin). In: Shokita, S., Kakazu, K., Tomomi, A., Toma, T., Yamaguchi, M. (Eds.), Aquaculture in Tropical Areas. Midori Shobo, Tokyo, Japan, pp. 313–328.

Shpigel, M., McBride, S.C., Marciano, S., Lupatsch, I., 2004. The effect of photoperiod and temperature on the reproduction of European sea urchin *Paracentrotus lividus*. Aquaculture 232, 343–355.

Soars, N.A., Prowse, T.A.A., Byrne, M., 2009. Overview of phenotypic plasticity in echinoid larvae, *Echinopluteus transversus* type vs. typical echinoplutei. Mar. Ecol. Progr. Ser. 383, 113–125.

Sonnenholzner, J., Lawrence, J.M., 2002. A brief survey of the echinoderms communities of the central and southern marine-coastal wetlands of the continental coast off Ecuador. B. Ecotropica. 36, 23–35.

Sonnenholzner, J., Lafferty, K., Ladah, L.B., 2011a. Food webs and fishing effect parasitism of the sea urchin *Eucidaris galapagensis* in the Galapagos Islands. Ecology 92, 2276–2284.

Sonnenholzner, J., Montaño-Moctezuma, G., Searcy-Bernal, R., 2011b. Effect of macrophyte diet and initial size on the survival and somatic growth of sub-adult *Strongylocentrotus purpuratus*: a laboratory experimental approach. J. Appl. Phycol. 23, 505–513.

Sonnenholzner, J., Brandt, M., Francisco, V., Hearn, A., Luzuriaga, M., Guarderas, P., Navarro, J.C., 2013. Chapter 6: Echinoderms of Ecuador. In: Alvarado, J.J., Solís-Marín, F.A. (Eds.), Echinoderm Research and Diversity in Latin America. Springer-Verlag, Berlin, Heidelberg, pp. 183–233.

Sonnenholzner, J.I., Searcy-Bernal, R., Panchana, M., 2017. The potential for propagation of the commercial sea cucumber *Isostichopus fuscus* (Ludwig, 1875) by induced transverse fission. Reg. Stud. Mar. Sci. 9, 35–42.

Sotelo-Casas, R.C., Cupul-Magaña, A.L., Rodríguez-Zaragoza, F.A., Solís-Marín, F.A., Rodríguez-Troncoso, A.P., 2016. Structural and environmental effects on an assemblage of echinoderms associated with a coral community. Mar. Biodivers. https://doi.org/10.1007/s12526-016-0622-y.

Stearns, S.C., 1976. Life-history tactics: a review of ideas. Q. Rev. Biol. 51, 3–47.

Strathmann, R.R., Fenaux, L., Strathmann, M.F., 1992. Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. Evolution 46, 972–986.

Sun, J., Chiang, F.S., 2015. Chapter 2: Use and exploitation of sea urchins. In: Brown, N.P., Eddy, S.D. (Eds.), Echinoderm Aquaculture. J. Wiley & Sons, Inc., pp. 25–45 Published.

Thompson, R.J., 1983. The relation between food ration and reproductive effort in the green sea urchin, *Strongylocentrotus droebachiensis*. Oecologia 56, 50–57.

Toha, A.H.A., Sumitro, S.B., Hakim, L., Widodo, N., Binur, R., Suhaemi, Anggoro, A.W., 2017. Review: biology of the commercially used sea urchin Tripneustes gratilla (Linnaeus, 1758) (Echinoidea: Echinodermata). Ocean Life 1, 1–10.

Uthicke, S., Schaffelke, B., Byrne, M., 2009. A boom-burst phylum? Ecological and evolutionary consequences of density variations in echinoderms. Ecol. Monogr. 79, 3–24.

Vadas, R.L., Fenchel, T., Ogden, J.C., 1982. Ecological studies on the sea urchin, *Lytechinus variegatus* and the algal seagrass communities of the Miskito Vays. Nicaragua. Aquat. Bot. 14, 109–125.

Watts, S.A., Lawrence, A.L., Lawrence, J.M., 2013. Chapter 10 - nutrition. In: John, M.L. (Ed.), Developments in Aquaculture and Fisheries Science. Elsevier, pp. 155–169.

Wolcott, R., Messing, C.G., 2005. A comparison of diets and water agitation methods for larval culture of the edible sea urchin, *Tripneustes ventricosus* (Echinodermata: Echinoidea). Bull. Mar. Sci. 77, 177–190.

Wolff, M., Schubauer, A., Castrejón, M., 2012. A revised strategy for the monitoring and management of the Galapagos sea cucumber. Int. J. Trop. Biol. 60, 539–551.

Yur'eva, M.I., Lisakovskaya, O.V., Akulin, V.N., Kropotov, A.V., 2003. Gonads of sea urchins as the source of medication stimulating sexual behavior. Russ. J. Mar. Biol. 29, 189–193.