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The Semi-Intensive Seed Production Method for Marine Fishes

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Abstract: Artificial juveniles are crucial for advancing commercial aquaculture in terms of their quality, quantity, and cost. The three key factors for successful aquaculture are egg, feed, and water. Eggs need to be healthy and genetically advanced to exhibit fast growth, superior meat yield, disease resistance, and early maturation. Premium traits were carefully selected and refined through generations (F1 and F2) to stabilize these desirable qualities for commercial applications. Consequently, hatcheries should prioritize the production of premium juveniles to meet growing demands. The aim of this study was to establish a fish seed production method capable of generating premium juveniles at low cost and with ease. The Semi-Intensive Seed Production (SISP) method is tailored for breeders, small investors, and fishermen, who may lack extensive knowledge or access to advanced technology required for intensive microalgae, rotifers, and Artemia cultivation. SISP selects ideal characters by natural selection during the seed production period, and the method can be applied to other species. Furthermore, the method accepted small investments to join aquaculture, which solved one of the bottlenecks of the business, and the method can be applied to other species. The SISP does not require a significant upfront investment or high operational expenses; instead, it requires a sizable tank (20 m^3 and 2 m depth), water supply, agricultural fertilizer, an air blower, and artificial feeds. Rather than relying heavily on cultured rotifers and Artemia, SISP leverages wild-bloomed phytoplankton and zooplankton. Research conducted with various fish species in Chile and Cuba from

2010 to 2024 demonstrated that while SISP exhibited a lower survival rate (2%) compared to Intensive Seed Production (ISP) (70%), it offered cost-efficiency and superior quality advantages. Nonetheless, SISP encountered challenges in meeting the demand for mass production culture owing to its dependence on live feeds, which hinge on phytoplankton.

Keywords: *Oplegnathus insignis*; *Seriola lalandi*; *Engraulis ringens*; *Graus nigra*; *Lutjanus analis*

海水鱼半精养苗种生产方法

摘要：人工幼体对于提高商业水产养殖的质量、数量和成本至关重要。水产养殖成功的三个关键因素是蛋、饲料和水。鸡蛋需要健康且基因先进，才能表现出快速生长、优异的产肉率、抗病性和早熟性。优质性状经过几代（F1和F2）精心挑选和完善，以稳定这些商业应用所需的品质。因此，孵化场应优先生产优质幼鱼，以满足不断增长的需求。本研究的目的是建立一种能够以低成本、轻松地生产优质幼鱼的鱼苗生产方法。半精养种子生产(SISP)方法是为育种者、小投资者和渔民量身定制的，他们可能缺乏微藻、轮虫和丰年虫精养所需的广泛知识或先进技术。SISP在种子生产过程中通过自然选择选择理想的性状，该方法可以应用于其他物种。此外，该方法接受小额投资加入水产养殖，解决了业务瓶颈之一，并且该方法可以应用于其他物种。SISP不需要大量的前期投资或高额的运营费用；相反，它需要一个相当大的水箱（20立方米和2米深）、供水、农业肥料、鼓风机和人工饲料。SISP没有严重依赖养殖轮虫和丰年虫，而是利用野生浮游植物和浮游动物。2010年至2024年在智利和古巴对各种鱼类进行的研究表明，虽然SISP的成活率(2%)低于集约苗种生产(ISP)(70%)，但它具有成本效益和卓越的质量优势。然而，由于依赖浮游植物的活饲料，SISP在满足大规模养殖需求方面遇到了挑战。

关键词：显著鰤;大西洋真鲹;凤尾鱼;黑鸦;红目真鲷

1. Introduction

The stagnation in capture fisheries production over the past two decades, as highlighted by the FAO [1], has had a significant impact on various species, such as snow crabs in Alaska [2, 3], cod in the North Atlantic [4, 5], and anchovy in South America [6, 7]. This decline in wild fish stocks is exacerbated by the threats of climate change and overfishing, putting many species at risk of extinction. However, the demand for fish products continues to increase, driven by concerns about health benefits and global population growth. In response to these challenges, aquaculture has emerged as a crucial industry for meeting the growing demand for seafood. The rise in market prices has incentivized the development of aquaculture, making it a key player in providing sustainable seafood sources for the future [8-10].

Juveniles produced in hatcheries play a crucial role in the aquaculture industry, ensuring a standardized size, healthy growth, disease resistance, and younger maturation [11-14]. The Intensive Seed Production (ISP) method is widely used for producing fish juveniles, involving feeding rotifers and Artemia with enriched materials, culturing up to 100 larvae per liter of water, with a survival rate of 60% until the juvenile stage. However, this method requires significant inputs, such as microfilters, UV light, and concentrated microalgae, under strict sanitary controls and advanced technology to minimize contamination, leading to increased production costs, especially in countries where such equipment must be imported [15-16].

In 2010, the authors produced Pacific beak fish (*Oplegnathus insignis*) juveniles using the ISP method in northern Chile, utilizing cultured microalgae, rotifers, and Artemia. However, the juveniles obtained did not meet the expected standards of quantity and quality because of the lack of available concentrated phytoplankton and enrichment materials. In response to this challenge, the ISP method was modified to leverage local resources using nutritious seawater and intense sunlight available without rain. This approach allows for the multiplication of wild phytoplankton and copepods through the application of aeration and agricultural fertilizers in large outdoor tanks. This modified method, called Semi-Intensive Seed Production (SISP), proved to be effective in facilitating the production of marine juveniles in several areas. This study aims to improve the quality and quantity of juveniles produced using the SISP method while minimizing associated costs.

2. Materials and Methods

The SISP method begins with the selection of premium larvae, development of acclimatization of larvae when released, monitoring of larval survival, availability of live feeds, harvest method, and cost and quality evaluation (Figure 1).

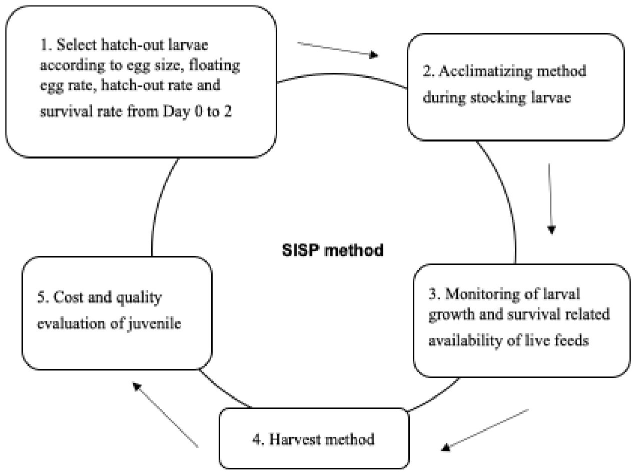

Figure 1. The SISP method flowchart (The authors' elaboration)

The SISP method is based on the use of a large-volume tank with a capacity of over 20 m3, located outdoors and exposed to direct sunlight, equipped with an aeration system and a constant water supply. In the initial stage, the tank is filled with unfiltered seawater and kept under constant aeration, while fertilizers such as potassium nitrate (3 mg/L) and triple superphosphate (0.5 mg/L) are applied. After approximately a week, the water takes on a brown color due to phytoplankton bloom, while nauplii and adult copepods begin to appear, providing food for the larvae. However, it is crucial to maintain strict control over the water pH, as an increase above 8.8 can corrupt phytoplankton. In the case of excessive blooming, sunlight exposure can be reduced or water exchanged to maintain an optimal environment. The larvae, known as dph 2, are ready to begin feeding actively and are released into the tank at a density of 1–2 per liter. They are harvested around dph 60, by which time the juveniles have reached a length of approximately 3 cm and show a survival rate of 2 to 3 %. Through the SISP method, we have successfully produced various fish species, including the Pacific beak fish (*Oplegnathus insignis*) known as "San Pedro", the yellowtail amberjack (*Seriola lalandi*) called "Dorado", the anchovy (*Engraulis ringens*) known as "Anchoveta", the red cusk-eel (*Genypterus chilensis*) (although with unsatisfactory results), the mutton snapper (*Lutjanus analis*) referred to as "Pargo criollo", the common snook (*Centropomus undecimalis*) known as "Róbalo común", and the Peruvian rock seabass (*Paralabrax humeralis)* called "Cabrilla común".

2.1. Pacific Beak Fish (*Oplegnathus Insignis*) "San Pedro"

The broodstock was captured in Antofagasta Chile in 2007. SISP was developed by the Private Corporation for the Development of Arturo Prat University (CORDUNAP) in 2010 and 2011(Project INNOVA CORFO 2005 code: 05-CN11PPD-0). Unfiltered water was filled and given gently aerated in a cylindrical fiber tank, 4 m diameter and 2 m height 20 m3, and supplied water 10 to 30 % daily outdoors in March 2010. The water color turned green and then brown after two weeks due to phytoplankton propagation (Figure 2A). Hatched-out larvae were released at 10 larvae per L density, fed millions of unenriched rotifers and Artemia, and fed artificial feeds daily depending on the mouth size of the larva.

2.2. Yellowtail Amberjack (*Seriola Lalandi*) "Dorado"

The SISP was developed by the Universidad Arturo Prat (UNAP) and CORDUNAP in February 2010 to produce yellowtail amberjack juveniles (project FDI-CORFO 00C7PT-09). The water was supplied continually by siphons from a 20 m3 tank with naturally bloomed copepods to a cylindrical conical fiber tank, 0.6 m3 (Figure 2B). Hatched-out larvae were stocked with two larvae per L density and fed thousands of unenriched Artemia and artificial feeds daily, depending on the mouth size of the larva. The system utilizes the copepods of the SISP inside the drain water to produce juveniles additionally.

2.3. Anchovy (*Engraulis Ringens*) "Anchoveta"

SISP was developed by UNAP and CORDUNAP in 2011 to produce anchovy juveniles (Project SUBPESCA, 2011 code: ID 4728-31-LP11, PUCV). Unfiltered seawater was introduced to a 60 m3 concrete rectangular tank on July 11, 2011, at 50% of volume through a 1 mm mesh bag net to eliminate wild fish juveniles and debris. Water was gradually supplied up to 60 m3 within a week, and agricultural fertilizers (3 g of potassium nitrate and 1 g of triple superphosphate per m3) were applied after diluting the water with a blender. This mixture was applied four times to assist the phytoplankton blooms. Water initially turned green in the first week and then shifted to a brown color (Figure 2C). However, the transparency never dropped below 1m.

Several copepod species bloomed from the 7th week and were observed to accumulate at the surface in the morning. The broodstocks, approximately 20 cm TL, were captured near Iquique, Chile, using a special net, and brought to Universidad Arturo Prat Ciencias del Mar Campus Huayquique on July 31, 2011. The fish spawned the same night due to the stress of handling, resulting in an 80% hatch-out rate. On August 4, 16,771 larvae at dph 1 were stocked in the tank. ORIGO-Green and ORIGO-Culture, Skretting brand-dried phytoplankton powder, were fed at 100 g daily for three weeks after stocking the larvae. The rearing water remained clear owing to the lack of phytoplankton growth, and the macroalgae began to grow.

2.4. Red Cusk-Eel (*Genypterus Chilensis*) "Congrio Colorado"

The SISP was developed by CORDUNAP and Quintay Marine Research (CIMARQ) Chile to produce red cusk-eel juveniles in 2011. Unfiltered water was filled into a cylindrical tank, galvanized shells, 8 m diameter and 3 m height 150 m3, with polypropylene liner (Figure 2D), and installed airlift, PVC 32 m/m, 3 m length, and the water exchanged 5 to 10 % daily in December 2011. The water color turned green and then brown after two weeks due to phytoplankton propagation. Hatched-out larvae were released 5 and 0.1 larvae per L density for operations twice.

2.5. Common Snook *(Centropomus Undecimalis)* "Róbalo Común"

SISP was developed by the Japan International Cooperation Agency (JICA) and Fisheries Research Center (CIP) to produce common snook juveniles at Santa Cruz del Sur Cuba in August 2013. Larval siblings were released into four SISP tanks on August 9, 2013. The 4,000 larvae were stocked indoors in a cylindrical fiber tank, 4 m diameter 8 m3, and fed copepods and live and dry phytoplankton. The 100,000 larvae were stocked in a rectangular fiber tank (20 m3) and fed wild copepods that were supplied from the reservoir by siphons with lighting during the night (Figure 2E). The 300,000 larvae stocked in the reservoir, 1,600 m3, were fed artificial feeds for larvae and moist pellets from dph 68. In addition, 10,000 larvae were stored in a floating cage with a 0.5 m/m mesh in the reservoir and continually fed plankton by airlift (Figure 2F).

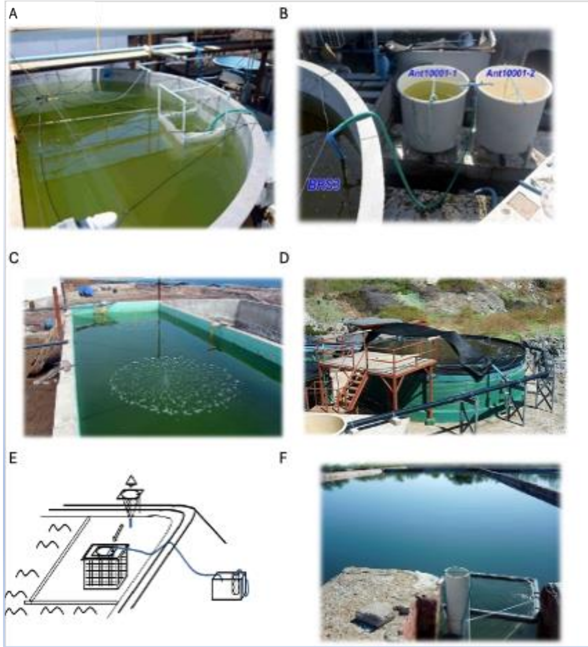

Figure 2. A) Pacific beak fish culture by SISP with phytoplankton blooming; B) Yellowtail amberjack culture by SISP with cylindrical conical fiber tank; C) Anchovy culture by SISP with rectangular concrete tank; D) Feeding copepods by siphon with lighting to snook; E) and F) Snook culture by SISP with reservoir and floating cage (The authors' elaboration)

2.6. Mutton Snapper (*Lutjanus Analis*) "Pargo Criollo"

The SISP was developed by JICA and CIP to produce mutton snappers at Santa Cruz del Sur Cuba in 2013 and 2014. There were three operations with production in October and November 2013. The 137,700 larvae were stocked in a rectangular concrete tank, 3 m depth 40 m^3, and fed artificial feeds, live Artemia, and wild copepods that were supplied from the reservoir by siphons with lighting during the night. A total of 546,600 larvae were stocked in the reservoir (1,600 m3) and fed artificial feed and dry phytoplankton. The 180,000 larvae were stocked in a rectangular concrete tank, 3 m depth 90 m3, and fed dry phytoplankton and artificial feed (Figure 3A).

2.7. Peruvian Rock Seabass (*Paralabrax Humeralis*) "Cabrilla Común"

The SISP was developed by the UNAP project FONDEF-ANID IDEA 2016, code: ID 16I20437, to produce Peruvian rock seabass at Iquique and Rio Seco Chile from 2018 to 2021. The larvae were released into a rectangular fiber tank (20 m3, cylindrical swimming pool (4 m diameter, 1 m depth 35 m3 (Figure 3B), and cylindrical tank, and galvanized shells (6 m diameter, 2 m depth, 60 m^3. Unfiltered water was filled with gentle aeration and fertilizers were applied as per the protocol. The water color turned green and then brown after two weeks due to phytoplankton propagation, and copepods propagated naturally. Supplied water or covered the water surface when the pH rose to more than 8.7 to avoid corruption of phytoplankton.

The larvae were released into the tank at density of 1 to 5 larvae/L after acclimatization to adjusted water temperature less than 1°C. The water supplied and artificial feeds were provided depending on the mouth of the larva.

2.8. Sea Chub (*Graus Nigra*) "Mulata"

The project was developed by the UNAP project FONDEF-ANID IDEA 2016, code ID 20I10242, to produce sea chub juveniles in 2022 and 2023. The project reared 13 captured wild broodstocks, consisting of four females and nine males with a biomass of 13.1 Kg, with the water supply flowing through three to five times a day the fish were fed mussel meat 3 to 5 % of biomass 5 days a week. The Spawning occurred between 16 and 17°C frequently when the temperature decreased, and the egg diameter was 1.148 mm. The fish spawned 16 days naturally, collected 0.43 x106 eggs, and showed a floating egg rate as fertilized eggs and hatch-out rate were 52.9% and 50.8%, respectively.

The SISP will operate from October to December 2023. The unfiltered water was filled and agricultural fertilizers were applied as per the protocol, with gentle aeration in a cylindrical fiber tank, 4 m diameter, 2 m depth, and 20 m^3. The 10,000 larvae dph 2 released in the tank after acclimatization in 5 days culture (Figure 3C).

2.9. SISPL (SISP with Lighting) Indoor

LED light enhances phytoplankton growth [17, 18] and SISPL (SISP with lighting) is an operation with continuous indoor lighting. The system enhances phytoplankton and zooplankton propagation and minimizes the temperature rise during summer. However, SISPL incurs the cost of housing, lights, and energy. We must compare the product cost to the SISP outdoors [19].

The SISPL230202 experiment was conducted for 30 days from February 2 to March 1, 2023. Fill up unfiltered water continuously for three days, consequently filling up a full cylindrical fiberglass 12.5 m3 tank. Fertilizers, potassium nitrate 3 g and 1 g/m3 triple superphosphate 1 g/m^3, were soaked in water in a hand mixer and applied to the whole surface of the tank from Day-1 to D1, D6, and D22 when there was less phytoplankton. Four units of airlift, a PVC pipe of 32 m/m, and a length of 2 m were installed diagonally to mix water at the surface and bottom. In addition, eight units of 200 W LED light were installed on the surface. They did not supply water until day 10, then supplied unfiltered water 50% per day continuously until the end of the experiment. Water temperature, pH, DO and Photosynthetic Photon Flux Density (PPFD) were measured using a smartphone (Tent Buddy) in the morning and afternoon at 09:00 and 14:00. Phytoplankton was observed under a microscope, and live feeds were counted as nauplius and adult copepods, nauplius of barnacles, larva of polychaetes, and native rotifers in one litter of water samples collected from the whole surface of the water and filtered through a 62 µm mesh. Small barnacles (600 g) collected at the shoreline were installed inside a plastic cage and hung in water (Figure 3E).

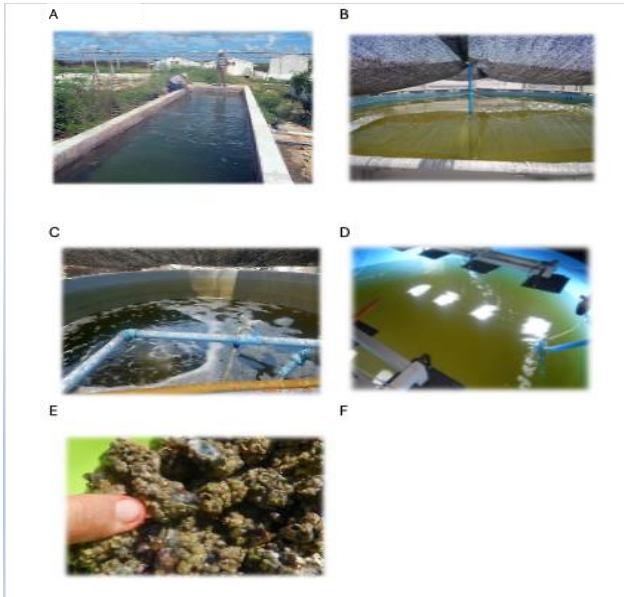

Figure 3. A) Snapper culture by SISP with concrete tank; B) Peruvian rock seabass culture by SISP with cylindrical swimming pool; C) Sea chum culture by SISP with cylindrical fiber tank (The authors' elaboration)

The experiment, SISPL230204, was conducted for 30 days from February 4 to March 5, 2023. The unfiltered water was filled continuously for three days until it was filled into a full cylindrical fiberglass 0.5 m^3 tank. Fertilizers were applied as usual from day -1 to D1, D4, D10, D20, and D27 when few phytoplankton became clear. One unit of airlift, a PVC pipe of 32 m/m and 0.3 m long, for mix water at the surface and bottom. Two units of LED light (35 W) were installed 10 cm from the surface. The unfiltered water was supplied on D6 and D8 at 150 % per day. Water temperature, pH, DO, and PPFD were measured. Phytoplankton and live feed were counted as usual. Small barnacles (100 g) were collected and installed as usual on D9 and D17.

3. Results

3.1. Peruvian Rock Seabass (Paralabrax Humeralis) "Cabrilla Común"

SISP100314 and SISP100326 were harvested at dph

47 and dph 59, and survival rates were 0.4 % and 2.7 % in May 2010. water temperature fluctuated between 16 and 22 °C and consumed water 13 and 19 m3 respectively. Rotifers and copepods grew 1,000 to 3,000 per L and fed 265 thousand of rotifer and 69 thousand of Artemia per juvenile to SISP100326. The Juvenile production was 671, survival 0.4 %, at 47 dph and 6,774, 2.7 %, at dph 59 respectively (Tables 1 and 2, Figure 4).

Table 1. Summary of semi-intensive seed batch production of: Pacific beakfish (*Oplegnathus insignis*) "San pedro" in 2010, Yellowtail amberjack (*Seriola lalandi*) "Dorado" in 2010, Anchovy (*Engraulis ringens*) "Anchoveta" in 2011, Red cusk-eel (*Genypterus chilensis*) "Congrio Colorado" in 2011, common snook (*Centropomus undecimalis*) "Robalo común" in Cuba 2013, mutton snapper (*Lutjanus analis*) "Pargo criollo" at Cuba SCSC in 2013, and Peruvian rock seabass (*Paralabrax humeralis*) "Cabrilla común" except no production operation from 2018 to 2023 (The authors' elaboration)

Species	Batch	Tank		Stocking			Production				Feeding per juvenile ($n/10^3$, g)		
		No.	m^3	Date	Batch	n	dph	n	g	%	Rotifer	Artemia	Artificial feeds
Pacific beakfish	SISP100314	BRS1	23	04-Apr-10	SANANT100404	172,020	47	671	152	0.4			
(*Oplegnathus insignis*)	SISP100326	BRS3	23	06-Apr-10	SANANT100406	248,000	59	6,774	3,319	2.7	265	69	1.1
"San pedro"													
Yellowtail kingfish	SISP100217	CON07	0.6	17-Feb-10	DORANT100213	1,231	58	17	27	1.4	0	366	8.6
(*Seriola lalandi*) "Dorado"													
Anchovy	SP110711	CN1	62.0	04-Aug-11	ANCIQQ110803	17,771	52	258	80	1.5	5	5	0.0
(*Engraulis ringens*) "Anchoveta"													
Red cusk-eel	SISP111114	DIV	145	23-Nov-11	COCQUI111123	704,150	7	0	0	0.0	0	0	0.0
(*Genypterus chilensis*)	SISP111204	DIV	145	29-Dec-11	COCQUI111223	10,225	16	0	0	0.0	0	0	915.0
"Congrio colorado"													
Common snook	SISPC130722T4	T4	8	9-Aug-13	ROBPTB130807	4,000	5	0		0.0	Feeding dry microalga		
(*Centropomus*	SISPC130808G1	G1	20	9-Aug-13	ROBPTB130807	100,000	5	0		0.0	Feeding copepods		
undecimaris)	SISPC130809FCR	FCR	1	9-Aug-13	ROBPTB130807	10,000	5	0		0.0	Floating cage inside REV3		
"Robalo"	SISPC130809REV3	REV3	1,600	9-Aug-13	ROBPTB130807	300,000	71	3,017	6,212	1.0	35	21	7.6
Mutton snapper	SISPC131011C1	C1	40	12-Oct-13	PARPCE131010	137,700	55	308		0.2	42	88	11.4
(Lutjanus analis)	SISPC131026R3	REV3	1,600	1-Nov-13	PARPCE131029	546,600	93	17,891	66,197	3.3			2.0
"Pargo"	SISPC131102C2	C2	90	5-Nov-13	PARPCE131104	180,000	71	2,022		1.1			3.3
Peruvian rock seabass (Paralabrax humeralis) "Cabrilla común"	SISP190211	SISP1	20.0	25-Feb-19	CABCAV190225	589,997	44	3		0.0			
	SISP190310	SISP3	35.0	31-Mar-19	CABCAV190331	70,000	66	45	0	0.1			0.2
	SISP190402	SISP1	20.0	22-Apr-19	CABCAV190422	90,000	60	800	17	0.9			0.3
	SISP191029	SISP3	32.0	7-Jan-20	CABCAV191212	189,200	71	115	324	0.1			3.9
	SISP191121	SISP1	20.0	15-Dec-19	CABCAV191214	91,100	68	45		0.0			
	SISP191128	NAR3	2.0	9-Dec-19	CABCAV191214	29,600	44	6		0.0			
	SISP200226	SISP3	32.0	16-Mar-20	CABCAV200317	139,500	57	416	92	0.3			3.3
	SISP200228	SISP1	20.0	12-Mar-20	CABCAV200310	77,000	56	135	125	0.2			13.0
	SISP200910	SISP3	35.0	15-Oct-20	CABCAV201006	338,300	46	138	42	0.0			
	SISP210103	SISP4	5.0	24-Dec-20	CABCAV210105	140,000	51	42	44	0.0			
	SISP201202	SISP3	35.0	24-Jan-21	CABCAV210105	40,000	57	257	378	0.6			1.1
	SISP210311	SISP3	35.0	14-Apr-21	CABCAV210412	119,900	85	108	157	0.1			10.7
	SISP210525	SISP6	60.0	10-Jul-21	CABCAV210710	48,000	60	265	326	0.6			
	SISP230809	E4	23.0	29-Sep-23	CABCAV230929	21,800	61	376	981	1.7			0.9
	SISP240118	E3	23.0	25-Jan-24	CABCAV240123	29,800	48	276	187	0.9			1.5
	Total					2,014,197		3,027					
Sea chub	SISP231017	E3	23.0	22-Oct-23	MULANF231020	10,000	60	22	1	0.2			15.5
(Graus nigra) "Mulata"													

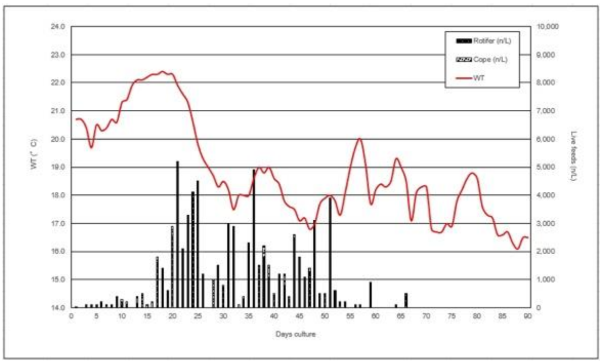

Figure 4. Water temperature and live feeds of SISP100326 with Pacific beak fish (*Oplegnathus insignis*) at E3 from March 26 to June 24, 2010 (The authors' elaboration)

The project applied pole sampling, inserted PVC pipe 40 m/m into the tank and closed pipe mouth, and took water samples with larvae during the night to estimate the number of larvae on April 15, 2010 (Figure 5). The Sampling of SISP100314 in dph 11 and SISP100326 in dph 9 showed the larvae survived 16 and 25 %, respectively. The method is reliable for estimating larvae in the tank until they develop a caudal fin to swim quickly.

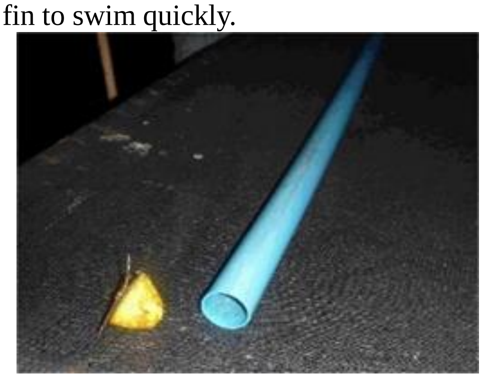

Figure 5. Sampling tube (The authors' elaboration)

3.2. Yellowtail Amberjack (*Seriola Lalandi*) "Dorado"

SISP100217 was harvested on dph 58, and the survival rate was 1.4 % in April 2010. Water temperature fluctuated between 18 and 24°C and consumed water 79.9 m^3 (Tables 1 and 2). Rotifers and copepods grew 1,000 to 3,000 per L and fed 366 thousand Artemia per juvenile to SISP100217 (Figure 6). Yellowtail amberjack juveniles showed rapid growth and strong cannibalism. Thus, it is recommended to harvest in dph 30–45 to minimize carnivorism and increase production.

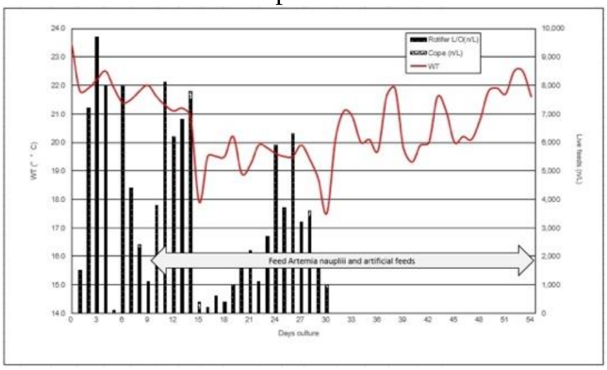

Figure 1. Water temperature and live feeds of SISP100217 with Yellowtail amberjack (*Seriola lalandi*) "Dorado" at CON7 from February 12 to April 12, 2010 (The authors' elaboration)

3.3. Anchovy (*Engraulis Ringens*) "Anchoveta"

Juveniles were harvested on dph 52 in September. 24, 2011. The average water temperature was 16.8°C between 15 and 19°C and consumed 505 m^3 water during the culture period and 75 days (Tables 1 and 2). ORIGO Green and Culture were fed 5 g per juvenile. We collected 258 juveniles, TL 41.7 mm, SL 35.4 mm, BW 0.31 g. and survival rate showed 1.5 % (Figure 7). Interestingly, the anchovy grew to 4 cm on dph 52.

Table 2. Summary de las environmental conditions of semi-intensive seed batch production of: Pacific beakfish (*Oplegnathus insignis*) in 2010, Yellowtail amberjack (*Seriola lalandi*), Anchovy (*Engraulis ringens*) in 2011, Red cusk-eel (*Genypterus chilensis*) in 2011, common snook (*Centropomus undecimalis*) in Cuba 2013, mutton snapper (*Lutjanus analis*) at Cuba SCSC in 2013, and Peruvian rock seabass (*Paralabrax humeralis*) except no production operation from 2018 to 2023 (The authors' elaboration)

Species	Batch	WT (°C)			pH			DO (mg/L)			Water (m^3) consumptio
		AVG	Max	Mini	AVG	Max	Mini	AVG	Max	Mini	
Pacific beakfish	SISP100314	19.3	21.7	16.6	8.08	8.66	7.76				23.4
(*Oplegnathus insignis*)	SISP100326	18.6	22.4	16.1	8.02	8.67	7.66				28.7
"San pedro"											
Yellowtail kingfish	SISP100217	20.6	23.5	17.5	8.27	8.56	7.77				79.9
(*Seriola lalandi*) "Dorado"											
Anchovy	SP110711	17.1	18.2	15.3				8.60	11.54	5.93	505.4
(*Engraulis ringens*) "Anchoveta"											
Red cusk-eel	SISP111114	20.1	22.6	18.0	8.96	9.56	8.33	13.99	18.60	11.40	130.5
(*Genypterus chilensis*)	SISP111204	20.7	23.7	18.3	8.68	8.90	8.47	9.29	14.43	5.59	443.7
"Congrio colorado"											
Common snook	SISPC130722T4	27.7	29.0	26.3	7.7	8.0	7.3	5.60	6.40	3.60	8.0
(*Centropomus*	SISPC130808G1	28.3	29.7	26.7	8.4	8.6	8.2	6.70	7.50	5.70	30.0
undecimaris)	SISPC130809FCR	31.0	32.0	30.0	8.2	833.0	8.0	6.20	7.30	5.70	1.0
"Robalo"	SISPC130809REV3	31.2	33.6	28.8	8.2	8.9	7.3	6.20	10.10	4.20	940.0
Mutton snapper	SISPC131011C1	28.9	32.3	25.2	8.4	8.9	7.7	7.00	11.30	4.50	8.0
(Lutjanus analis)	SISPC131026R3	27.3	31.6	22.0	8.6	9.2	8.1	7.40	13.40	3.50	2560.0
"Pargo"	SISPC131102C2	27.9	30.9	25.5	8.3	8.9	7.7	6.40	11.60	4.10	144.0
Peruvian rock seabass (Paralabrax humeralis) "Cabrilla común"	SISP190211	22.2	23.6	20.4	8.4	8.8	7.9	7.75	12.16	6.06	115.0
	SISP190310	20.1	22.3	17.4	8.4	8.6	7.9	8.40	12.60	6.37	173.3
	SISP190402	18.5	20.9	16.2	8.2	8.5	7.6	8.35	11.10	6.53	141.0
	SISP191029	21.6	25.0	17.7	8.4	8.7	8.0	7.91	14.81	4.82	679.0
	SISP191121	21.6	23.7	20.2	8.4	8.6	8.0	6.98	8.75	5.17	234.0
	SISP191128	21.3	24.4	18.8	8.3	8.7	8.1	7.00	7.95	5.24	0.0
	SISP200226	20.6	23.0	17.8	8.3	8.7	7.8	7.56	11.71	5.22	626.5
	SISP200228	20.6	22.0	18.1	8.1	8.6	7.7	6.14	7.81	4.29	192.0
	SISP200910	18.7	20.5	16.8	8.2	8.6	7.8	5.86	8.37	4.59	311.5
	SISP210103	20.4	21.8	19.0	8.4	8.6	8.7	6.09	7.80	4.61	17.0
	SISP201202	20.6	21.9	18.7	8.3	8.6	7.5	6.45	8.51	4.99	550.4
	SISP210311	17.6	20.1	15.4	8.3	8.6	7.3	7.40	10.00	5.10	732.8
	SISP210525	18.6	21.3	16.1	8.3	8.8	7.9				64.0
	SISP230809	19.2	21.9	17.8	8.4	8.8	8.0	7.91	9.58	6.86	50.6
	SISP240118	22.5	24.4	20.4	8.1	8.8	8.1	7.52	8.48	6.21	62.1
Sea chub	SISP231017	19.7	22.6	17.3	8.5	8.9	8.0	7.88	9.75	6.85	52.9
(Graus nigra) "Mulata"											

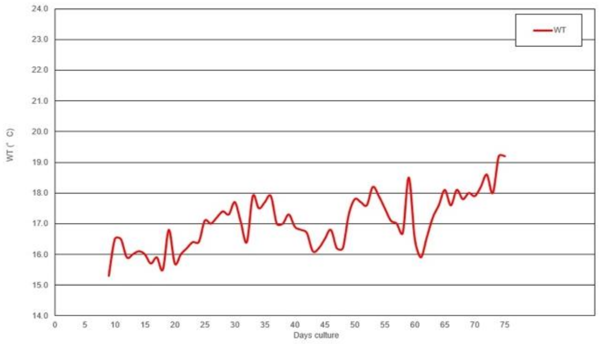

Figure 7. Water temperature of SISP110711 with Anchovy (*Engraulis ringens*) "Anchoveta" at CN1 from July 11 to September 24, 2011 (The authors' elaboration)

3.4. Red Cusk-Eel (*Genypterus Chilensis*) "Congrio Colorado"

SISP111114 and SISP111204 started on November 14 and December 4, 2011, respectively. The average water temperature showed 20.1 and 20.7 °C between 18 to 24 °C and consumed 131 and 444 m3 water during the culture period respectively (Tables 1 and 2). The ORIGO Culture was fed 915 g of SISP111114. Copepods and larvae were not observed within a week after the larvae were stocked. It is possible that the larvae died because of the lack of copepods and high pH. The microalgae did not grow well because the SISP tanks were located at the back of the cliff and sunlight passed in a short time (Figure 8). Therefore, it is necessary to add light to improve phytoplankton growth.

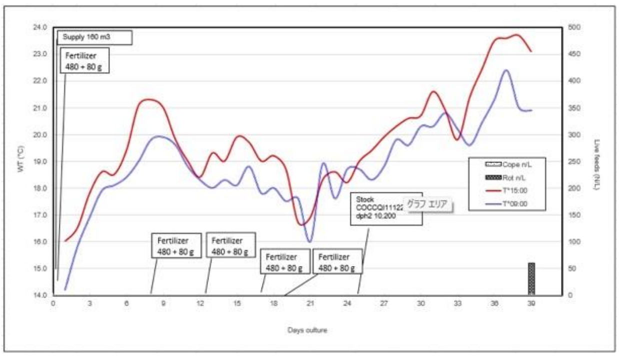

Figure 8. Water temperature and live feeds of SISP111204 with red cusk-eel (*Genypterus chilensis*) "Congrio Colorado" COCCQI111223 at DIV from December 4, 2011, to January 14, 2012 (The authors' elaboration)

3.5. Common Snook (*Centropomus Undecimalis*) "Róbalo Común"

The larvae of SISP130722 at T4 disappeared until dph 5, even though the larvae showed stomach contents, not copepods, due to the lack of copepod nauplii (Tables 1 and 2). The larvae of SISP130808 at G1 were eliminated until dph 5 lacked feeds, which showed that the larvae took air bubbles inside the intestine. The larvae of SISP130809 in the floating cage were eliminated until dph 5, and feed was not taken. The mesh of the cage was stocked by phytoplankton. These three batches showed a shortage of live feed, and the larvae died.

The SISP130809 at REV3 harvested 3,017 juveniles at dph 71 on October 17, 2013. The average water temperature was 31.2°C between 31 and 34°C during the culture period. The juveniles showed TL 58.9 mm, SL 46.7 mm, BW 2.06 g., and a survival rate of 1.0 %, even though the sampling showed a few copepods (Figure 9). The project fed rotifers, Artemia, and artificial feeds of 25 and 21 thousand, and 7.6 g per juvenile, respectively. The large volume of water provided them a chance to meet live feeds or even smaller siblings to survive juveniles. The size of the juveniles varied greatly owing to strong carnivorism. This suggests that harvest days should be shorter than dph 60 to improve survival.

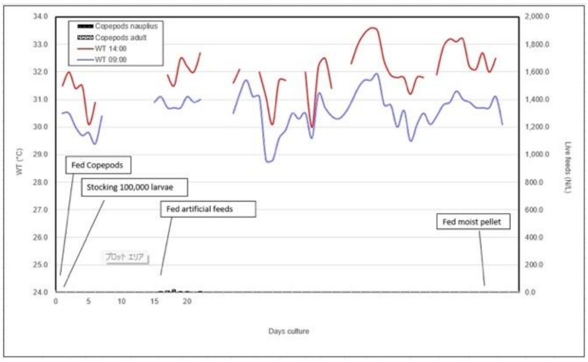

Figure 9. Water temperature and live feeds of SISP130809 with Common snook (*Centropomus undecimalis*) "Róbalo común" at REV3 from August 9 to October 17, 2013 (The authors' elaboration)

The growth curve of common snook juvenile is shown in Figure 10.

$Y = 0.0102X^2 + 0.0693X + 4.2183$, $R^2 = 0.9758$, Y: Total length (mm), X: Days post hatch-out

$Y = 0.0009X^2 – 0.0426X + 0.2866$, $R^2 = 0.9758$, Y: Body weight (g), X: Days post hatch-out

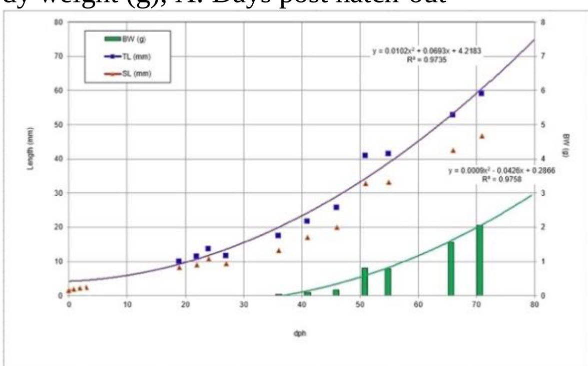

Figure 10. The growth of common snook

(*Centropomus undecimalis*) “Róbalo común” ROBPTB130807 until dph 71 at REV3 od SCSC from August 9 to October 17, 2013 (The authors’ elaboration)

3.6. Mutton Snapper (*Lutjanus Analis*) “Pargo Criollo”

The average water temperature of SISP131011 at C1 was 28.9°C between 25 and 32°C. The juveniles harvested 308 in dph 55, survival rate 0.2 % on December 4, 2013. The average water temperature of SISP131102 C2 was 27.9°C between 26 and 31°C. The juveniles harvested 2,022 in dph 55, survival rate 1.1 % on January 14, 2014. The average water temperature of SISP131026 REV3 was 27.3°C between 22 and 32°C. The Juveniles harvested 17,891 in dph 93, survival rate of 3.3 % on January 30, 2014. The juveniles consumed 35,220 g of artificial feed until harvest and consumed 2.0 g per juvenile (Tables 1 and 2, Figure 11). The snapper showed better survival than the snook because of its omnivorous or carnivorous habits.

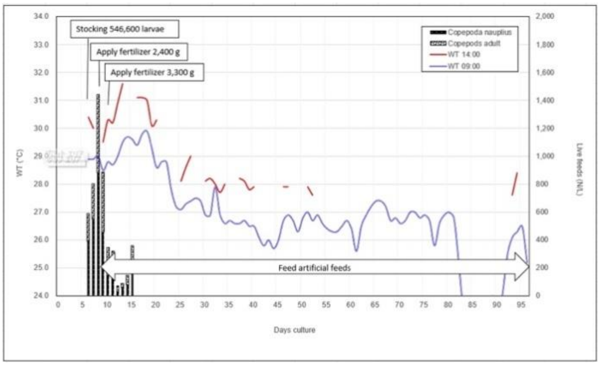

Figure 11. Water temperature and live feeds of SISP131026 at REV3 with mutton snapper (*Lutjanus analis*) “Pargo criollo” F1 in Cuba from October26, 2013 to January 30, 2014 (The authors’ elaboration)

The growth curve of mutton snapper juvenile, PARSAS120707 showed (Figure 12).

Y = 10.913e0.0231x, R2 = 0.953, Y: Total length (mm), X: Days post-hatching.

Y = 0.1128e0.0458x, R2 = 0.9325; Y: Body weight (g); X: Days post-hatching

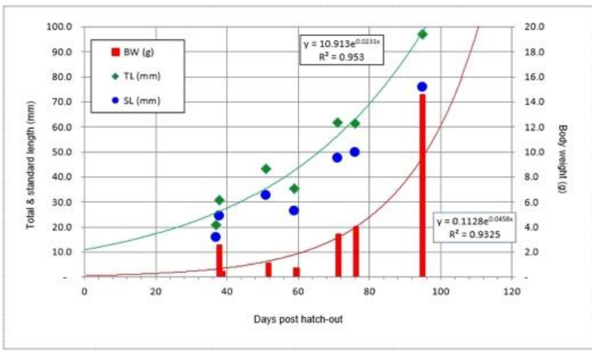

Figure 12. The growth of mutton snapper (*Lutjanus analis)* “Pargo criollo” PARSAS120707 at Cuba SCSC in 2013 (The authors’ elaboration)

3.7. Peruvian Rock Seabass (*Paralabrax Humeralis*) “Cabrilla Común”

Fourteen operations produce juveniles 2,751 out of 21 operations (Tables 1 and 2). The best survival rate was SISP230809, and the average water temperature was 19.2°C between 18 and 22 °C. The juveniles harvested 376 in dph 60, with a survival rate of 1.7 % on November 29, 2023 (Figure 13). Even when live feeds grew to 190 per L in 55 days of culture, the phytoplankton were corrupted, the water color became clear, and then fed artificial feeds mainly. The feeds consumed 0.9 g at a harvested.

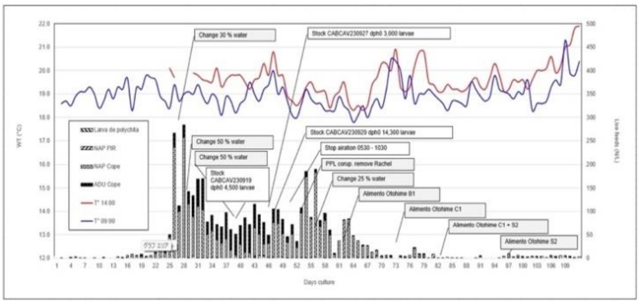

Figure 13. Water temperature and live feeds of SISP230809 with Peruvian rock seabass (*Paralabrax humeralis)* “Cabrilla común” F2 from August 9 to November 28, 2023 (The authors’ elaboration)

Another sample of SISP, SISP210525, was operated with a 60 m3 cylindrical tank at a fisherman village from May 25, 2021. The Copepods adult and nauplius grew to 91 L after 15 days of culture. A total of 19,900 larvae, CABCAV210614 dph 2, were released on Jun 16, and 28,100 larvae, CABCAV210710 dph 3, were added to the tank on July 13, 2021. The operation had no feeding nether supply water nor aeration until harvest, 106 d of culture. Even so, 326 juveniles were harvested at dph 86, TL 30 mm, body weight 0.5 g, and survival rate was 0.6 % on September 8, 2021. The juveniles were produced without water supply, aeration, or feeding until harvest. Juveniles consume copepods and maggots. These phenomena suggest that large volumes of water take advantage of juveniles that can survive easily.

3.8. Sea Chub (*Graus Nigra*) “Mulata”

The SISP showed average water temperature of 19.7°C between 17 and 23°C and water supplied three times with volume 53 m3. The 22 juveniles were harvested at dph 60, survival rate 0.2 % on December 19, 2013 (Table 1). Live feeds, adult copepods, and nauplii grew at a maximum density of 224 per L on dph 36. Artificial feeds, Otohime B1 and C1, were fed from dph 13, consuming 341 g until harvest, and consumed 15.5 g per juvenile. The bubbles covered the water surface from dph 41 to 43 due to changing water quality, and the bubbles reduced sunlight inside the water and propagation of phytoplankton (Figure 14). The tank bottom showed many sediments that died of plankton and algae, and it feeds on the benthos copepods Harpacticoide.

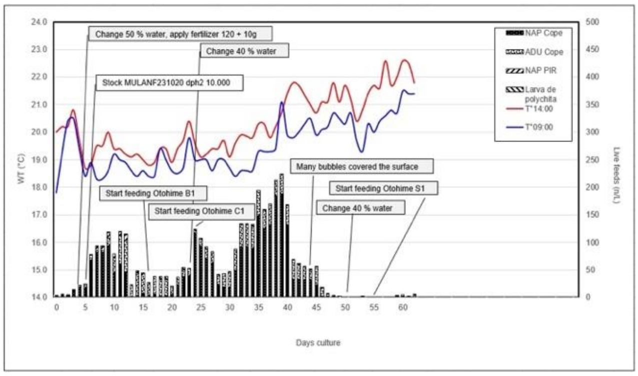

Figure 14. Water temperature and live feeds of SISP231017 with sea chub (*Graus nigra*) "Mulata" at E3 from October 19 to December 19, 2023 (The authors' elaboration)

3.9. SISPL (SISP with Lighting) in Indoor

The water temperature of SISPL230202 fluctuated between 17.8 to 21.5 and the average was 19.3°C. pH was 7.20 to 8.56 and the average was 8.02. DO showed between 6.52 to 13.34 and the average was 8.85 mg/L from February 2 to March 7, 2023 (Table 3). The PPFD fluctuated between 350 900 and with an average of 560 μmol/m2s. The water color was green when Asterionellopsis grew until D4 and suddenly corrupted the water and became transparent in D5. The water color turned brown with a bloom of Skeletonema, Thalassiosira, and diatoms, then when Chaetocerous grew well. Copepod nauplii and adults grew six and one individual per L of water, respectively. Barnacle nauplius showed an average of 21 and a maximum of 222 per L on D27. Polychita and rotifers were 1 and 0 per L on average. In summary, the average live feed of SISPL was 33.2 per L of water per day. Green seaweed grew on the surface of the wall after 3 weeks (Figure 15). It was necessary for 3 weeks to grow live feeds at a density of more than 100 per L for the care of fish larvae.

Table 3. Summary of Experiment SISPL230202 with 12.3 m3 tank from February 2 to March 3, 2023 (The authors' elaboration)

Initial;	2-Feb-23			End;	3-Mar-23		Duration;	30 days		
Fertilizer; 0,4,11,19		Water supply; 50 % / Day			Lighting; LED 200W x 8			PPL; D21-28, ZPL; D21-28		
Barnacle 600g D19	WT (°C)	pH	DO (mg/L)	PPFD μmol/m²s	Copepod		Barnacle nauplius	Polychita larvae	Rotifer (n/L)	Live feeds (n/L)
					Nau	Adu				
Average	19.3	8.02	8.85	560	6	1	21	1	0	33.2
Maximum	21.5	8.56	13.34	900	27	6	222	4	1	254
Minimum	17.8	7.20	6.52	350	0	0	0	0	0	2

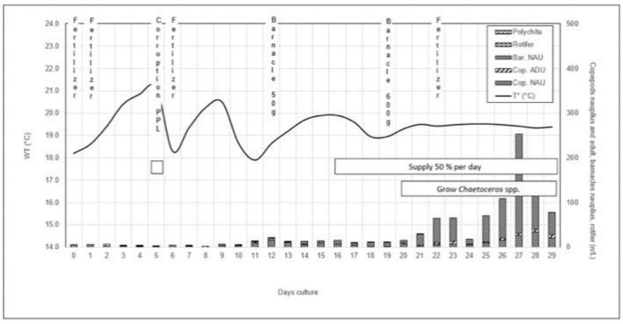

Figure 15. SISPL230202; water temperature and live feeds with 12.3 m^3 tank under LED lights (200 W x 8) from February 2 to March 2, 2023 (The authors' elaboration)

The water temperature of SISPL230204 fluctuated between 17.7 to 22.2 and the average was 20.5°C. pH was 7.57 to 8.50 and the average was 8.08. The DO showed from 6.51 to 14.20, with an average of 7.66. The PPFD fluctuated between 250 720 and with an average of 214 μmol/m^{2}s from February 4 to March 10, 2023 (Table 4). The water color was brown until D2 and suddenly corrupted the phytoplankton and became transparent on D3. Water was supply of water at 70 to 200 % per day from D3 to D8 after applying fertilizers. Skeletonema and diatoms bloomed from D4 to D17, and the water was clear until D29. Copepod nauplii and adults showed 41 and 5 individuals per litter, respectively, on average per day. The Barnacle nauplius showed an average of 22 days. There were very few polychita and rotifers. The live feeds grew to 265 on D29, and the average was 68.5/L of water. The water temperature fluctuated more than that of SISPL230202 because of the smaller volume of water (Figure 16). It was necessary for 3 weeks to grow to live feeds at a density of more than 100 per L, as for feeds of fish larvae. Even so, the productivity of live feed was double that of the large tank SISPL230202.

Table 4. Summary of SISPL230204 with 0.5 m3 tank under LED lights (35 W x 2) from February 4 to March 5, 2023 (The authors' elaboration)

Initial;	4-Feb-23			End;	5-Mar-23		Duration;	30 days		
Fertilizer,0,4,9,20,27		Water supply; 100 % / Day			Lighting; LED 35Wx2			PPL; D4 - 16, ZPL; D19 - 29		
Barnacle 100g D17	WT (°C)	pH	DO (mg/L)	PPFD μmol/m²s	Copepod		Barnacle nauplius	Polychita larvae	Rotifer (n/L)	Live feeds (n/L)
					Nau	Adu				
Average	20.5	8.08	7.66	398	41	5	22	0	0	68.5
Maximum	22.2	8.50	14.20	720	236	22	124	1	0	265
Minimum	17.7	7.57	6.51	250	0	0	0	0	0	0

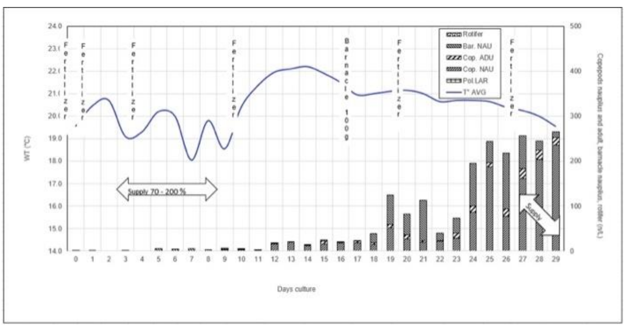

Figure 16. SISPL230204; water temperatures and

live feeds with 0.5 m^3 tank from February 4 to March 5, 2023 (The authors' elaboration)

4. Discussion

The authors suggest that in the field of aquaculture, three items are essential for a successful operation. The items included eggs, feed, and water. The eggs should be healthy and genetically advanced as they grow fast, have more meat, and resist disease. For example, the fertilization rate, hatching rate, and survival duration of unfed larvae post-hatch were weakly correlated with each other ($-0.23 < r < -0.08$), revealing the occurrence of distinct and independent components of egg quality. In addition, studies on the basis of natural resistance of fish to infections to help genetic selection are under study. Livestock history is selected as premium genes and generated continuously as F1, F2, and so on, and fix the characters for merchandise. In reality, in several large-scale experiments and in breeding programs, 10–15% genetic change has been obtained per generation, which is much higher than that reported for other farm animals. Therefore, hatchery producers should produce premium juveniles for their growers [17]. At the point of nutrition for larval culture, the value of fatty acid content and the culture method of rotifers are significant [18, 20]. In addition, CRISPR/Cas9-based genome editing is a tool that allows for the efficient and targeted improvement of important aquaculture traits, for example, acquiring disease resistance and stress tolerance in fish, and is likely to be the solution to many of the current problems in the aquaculture industry [21].

The SISP design for breeders, small investors, and fishermen who have no knowledge and sophisticated technology as an intensive live feed culture and do not require large initial investment and operation costs. The SISP does not require rotifers and Artemia, which use many tanks, microfilters, UV lights, large pumps, workers, chemicals, enriched materials, and large buildings with strict sanitation control. SISP uses wild-bloomed Phyto and zooplankton instead of cultured rotifers and Artemia. The advantages of SISP, comparison of SISP and ISP show that survival rates are 2 % and 70 %, premium quality and low-quality, low-cost, and high cost accordingly. However, SISP cannot meet the demands of mass production culture because of the limited availability of live feeds that depend on the quantity of phytoplankton. We suggest the following suggestions to improve productivity.

This study showed that the SISP method is feasible to produce fish seeds, such as: *Oplegnathus insignis, Seriola lalandi, Engraulis ringens, Graus nigra, Genypterus chilensis, Lutjanus analis, Centropomus undecimalis, Paralabrax humeralis*, at minimum cost and easily.

4.1. Survival of Larvae from Hatch-Out to Releasing, dph 0 to 2

For example, the larvae of Peruvian rockfish and South Pacific bonito develop their digestive system after hatching (dph) 2 while consuming the yolk sac [22-24]. According to the author´s observations, newly hatched larvae have a survival rate of 40% until dph 2 in the incubation tank, depending on the larval quality (Figure 17). High-quality eggs were selected for the SISP. The batch of spawning eggs was chosen based on fertility, floating eggs divided by total eggs, and hatching rate, hatch-out larvae divided by floating eggs, which are over 90%), and the survival rate of larvae from hatching to dph 2 shows over 40%). Some species of larvae rely on the yolk sac until dph 2 and begin feeding on copepod nauplii the following days.

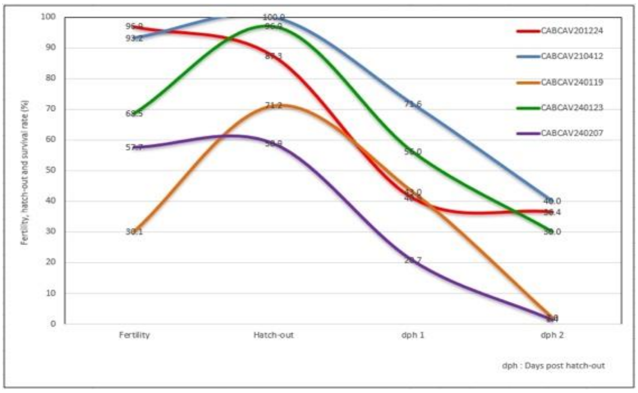

Figure 17. Fertility, hatch-out, and survival rate until dph 2 of Peruvian rock seabass (*Paralabrax humeralis*) larvae (The authors' elaboration)

4.2. SISP Tank Design

Generally, we predict that intensive fish farming will lead to the evolution of more virulent pathogens [25]. We should select the correct material and location of the SISP tank because the cost of the tank is high and sufficient sunlight is required to grow phytoplankton. For example, the cost of a cylindrical 20 m3 fiber tank was 11,800 USD, and a polyethylene liner with galvanized shells was approximately 6,000 USD with 10 years of durability. To economize the cost, the tank consists of a polyethylene liner with a still mesh and post or install a polyethylene liner half-underground at a slope to eliminate shells. In addition, low-cost vinyl swimming pools have been used for the last two years for durability.

4.3. Barnacle Nauplius

Wild zooplankton are an ideal live feed for fish larvae [22]. However, live feed cannot always be substituted because of the biochemical and behavioral constraints of fish larvae [26]. Barnacle nauplii is the ideal live feed for fish larvae instead of copepod adults and nauplii. The nauplius can be cultured by *Chaetoceros spp.*, which are abundant during SISP culture [27]. Many barnacles are available on the coast and can be collected easily, and the nauplius grows to approximately 1 mm within a week. We set the barnacle inside the cage, hung it in the SISP tank, and spawned the nauplius within days.

It is desert weather, almost no rain and strong sunlight, 30°C in a day, and 15°C in the night at the North of Chile. The sunlight is too strong and heats up colored water with phytoplankton (Figure 18). According to recent competition theory, the population dynamics of phytoplankton species in monocultures can be used to make a priori predictions of the dynamics and outcome of competition for light [28-29]. Thus, water surface should be covered by shade, Rachel 80 % reduction) to control the sun light.

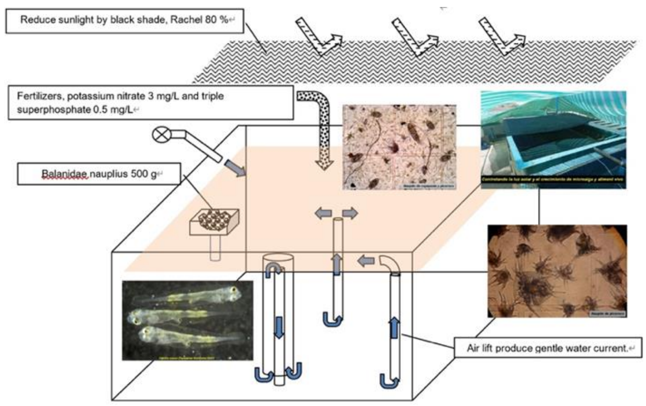

Figure 18. Total design of SISP (The authors' elaboration)

4.4. Mixing Herbivorous or Omnivorous and Carnivorous Juvenile with Large Scale Pond

Some fish have strong carnivores, attack smaller fish, eat or kill, such as yellowtail amberjack, and reduce survival. It is recommended to mix herbivorous or omnivorous larvae with minimizer carnivores.

4.5. Phytoplankton Growth Enhance by LED Lights

Copepods are ideal feeds for fish larvae and suggest that R. salina should be cultivated in a range of irradiance 60–100 μmol/m^{2}s and nutrient excess to obtain algae with high production and a balanced biochemical composition as feed for copepods [30]. LED light is purchased easily in the market at a low cost compared to the growing light that is designed to culture plants indoors. It is effective in growing phytoplankton and producing copepod adults and nauplii with a PPFD of 800 μmol/m^{2}s. Light operates during the night using a timer with a mesh to protect insects and enhance plankton growth. The heat of light must be controlled to avoid increasing the water temperature.

4.6. SISP until dph 45

According to the data, dph 60 juveniles consumed artificial feed (1 g per fish), and carnivorous fish consumed 8 g per fish. This means that nauplius and adult copepods are eaten by post-larva, dph 45, and then juveniles eat artificial feeds until dph 60; copepods are possibly not necessary after the larval stage. Thus, SISP operates until dph 45 and harvests juveniles then rearing juveniles intensively in a nursery tank, 2 m^3, Therefore, the juvenile survival and productivity of the tank will increase.

5. Conclusion

The primary findings indicated that the Semi-Intensive Seed Production (SISP) method can successfully produce juveniles with minimal resources, limited skills, and reduced production costs. In a comparison between SISP and the Mesocosm method, it was noted that SISP necessitates effective water management to sustain phytoplankton and requires feeding to produce juveniles.

This study aimed to efficiently produce high-quality juveniles at a low cost, and our results demonstrate that this goal is achievable. The findings provide new opportunities for small, unskilled investors in rural areas to improve their livelihoods. However, a significant challenge remains regarding production quantity, as survival rates are low, ranging from only 3% to 8%. Future research should focus on developing technologies that can maintain high phytoplankton densities, which are essential for live feed production, such as copepods, in large tanks.

It is recommended that numerous small investors, including fisher unions, implement the SISP method along coastal regions to increase juvenile production for intensive grow-out culture or release into open waters. Furthermore, efforts should be made to genetically enhance juvenile quality through selection techniques from the F1 to F2 generations and beyond.

The future of Semi-Intensive Seed Production (SISP) for marine fish is anticipated to concentrate on improving production efficiency through technological advancements and enhanced management practices as well as optimizing breeding protocols for various species. There has also been an increase in diversification in marine seed production and the adoption of emerging technologies, such as biotechnology and genomics, to improve seed health and disease resistance. All these efforts will be underpinned by a growing commitment to sustainability, emphasizing practices designed to minimize environmental impacts and bolster resilience against climate change.

Declarations

Author Contributions

Conceptualization, M.F. and J.P.D.-V.;

methodology, M.F. and G.B.; software, R.P.-V.; validation, M.F., G.B. and J.P.D.-V.; formal analysis, J.P.D.-V.; investigation, M.F., G.B., R.P.-V. and J.P.D.-V.; resources, M.F.; data curation, J.P.D.-V.; writing—original draft preparation, R. P.-V.; writing—review and editing, J.P.D.-V.; visualization, G.B. and R.P.-V.; supervision, J.P.D.-V.; project administration, M.F. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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