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COPARATIVE EVALUATION OF LIVE FEED CONSUMPTION RATES IN LARVAL PERIOD OF SEABREAM (*Sparus aurata*) AND SEABASS (*Dicentrarchus labrax*) AT COMMERCIAL LEVEL

Onur EYÜBOĞLU[1], Murat YIGIT[2]

[1] Department of Aquaculture, School of Graduate Studies, Canakkale Onsekiz Mart University, 17020-Çanakkale, Türkiye
[2] Department of Aquaculture Industry Engineering, Faculty of Marine Science and Technology, Canakkale Onsekiz Mart University, 17020-Canakkale, Türkiye

Onur Eyüboğlu: onurcaneyuboglu@gmail.com, https://orcid.org/0009-0002-3472-8078
Murat Yigit: muratyigit@comu.edu.tr, https://orcid.org/0000-0001-8086-9125

**Corresponding author: Onur EYÜBOĞLU, onurcaneyuboglu@gmail.com, +90-541-5462926*

Abstract
The present study conducted a comparative evaluation of live feed consumption rates in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) during the larval phase. In a commercial scale marine hatchery, four tanks of seabream and four seabass larvae groups were used in the study with rotifer (*Brachionus plicatilis*) and artemia (*Artemia salina*) as live feed during the production period of 2021-2022. The data obtained were evaluated proportionally by comparing the feed consumption amounts in the larval period and the transition period to the postlarva stage. The findings in the present study revealed higher rotifer and artemia consumption rates for seabass compared to the seabream larvae. It has been noted that the seabass larvae consumed 15.44% more rotifers and 37.82% more artemia than the seabream larvae which corresponded to 1.18-fold increment in rotifer consumption and 1.61-fold in artemia consumption levels for the seabass over the seabream larvae under the conditions applied in this study. Despite the higher temperature range in the seabream larvae tanks than the seabass conditions, seabass larvae consumed higher amounts of rotifer and artemia, irrespective of temperature compared to the seabass larvae, which evidenced the species-specific nature of larvae feeding. The results of this study conducted at commercial scale provide useful data for hatchery management in terms of establishment of feeding protocols for seabream and seabass larvae production and estimation of investment and operational costs in marine aquaculture business.
Keywords: Artemia, *Brachionus plicatilis*, *Dicentrarchus labrax*, feed consumption, larvae feeding, marine hatchery, rotifer, *Sparus aurata*

Introduction

Hatchery based aquaculture of seabream and seabass in Türkiye has been intensified since the early 1980's. Initially, a production model was carried out in which fry individuals were caught from the natural environment and fed up to market size in cage systems deployed in coastal areas close to the shore. However, when it was understood that natural stocks were not infinite, it was forbidden to catch juvenile fish from natural resources in order to protect wild populations and to switch to a sustainable production model. Aquaculture production has increased by 1.8% in 2021 compared to the previous year. About 59% of the production share was made up of aquaculture products. Among these, seabream, seabass and trout are the main key players in the Turkish aquaculture industry (TEPGE, 2022). Although remarkable successes have been achieved today, it is well known that feed costs constitute the largest item in production expenses. Considering that a significant portion of the production costs, up to 50-60% (Fernández-Sánchez et al., 2022; 58%), is still constituted by feed costs, it is worse to investigate the estimation of feeding levels that may help business management. Among the feed costs for the first three months, live food constitutes around 50% (Person-Le Ruyet et al., 1993). Especially in the hatchery, the availability of food and digestion efficiency are important factors for providing energy enough for growth and development during the larval stage, which is also important for the best survival rates (Houde & Schekter, 1980; Pedersen, 1997). Due to the fact that weakness and vulnerability during the larval period, many factors among which the size, uncompleted organs and physiological functions, as well as energy stores are low, the survival rates during this growth phase are under great risk that needs special care Fuiman & Cowan, 2003). Due to the characteristics of seabream and seabass larvae, live feed is required for the first feeding phase. Therefore, some of the important cost rates come into play at this stage. Different production protocols and feeding methods are being applied in marine hatcheries. Here, the importance of feeding strategy emerges once again. Compared to the adult stages of fish, the pre-larval and larval stages are very important because mortality rates are very high when nutritional needs are not met. In this respect, the food chain in nature is used as a model for feeding larval forms in aquaculture. Single-celled aquatic organisms can photosynthesize the basis of the food chain in aquatic environments; namely phytoplanktonic ones. Rotifer and artemia are the most important zooplanktonic organisms used in the feeding of many larval forms, especially fish larvae. Further, there are a number of different feeding protocols for seabream and seabass, with a remarkable variation of data for the feed uptake of seabream or seabass larvae (Parra et al., 2000; Rocha et al., 2008; Süzer et al., 2011), however, results of laboratory scale investigations Show remarkable variations among results, that leads farmer to confusion with the arising question regarding the presence of right feeding protocols for seabream or seabass larvae. Moreover, the larval stage is the most vulnerable to fish, the larval stage is important in planning and producing annual production targets in aquaculture management strategies. Inappropriate or sudden changes in feeding management and aquaculture conditions can easily cause mass mortality, while under -or overfeeding has adverse effects on survival rate and best survival rates during the larval stage can be achieved with optimal larval rearing conditions and good management practice.

The present study, conducted for a period of four months, focused on commercial size hatchery rather than laboratory or research-facility-based investigations, which in turn gives hope for the determination of live feed consumption rates in the larval period in commercial enterprises and to create a data source that can be used in production protocols.

Materials and Methods

Research location and marine hatchery

The study was carried out in a commercial marine hatchery located on the Turkish coast of the Aegean Sea. Seabream (*Sparus aurata*) and Seabass (*Dicentrarchus labrax*) larvae were used. The consumption rates of live feed (Rotifer, *Brachionus plicatilis*) and (Artemia, *Artemia sp.*) were comparatively evaluated in terms of consumption rates in a daily and monthly basis during the production season of 2021-2022.

Seabream Larvae Feeding Method and Experimental Conditions

The first 24 hours following the incubation of the larvae was accepted as "Day-1" and the age of the larvae was calculated on the basis of daily values accordingly. Conical shaped tanks with a volume of 20 tons were used in the production of the larvae (Figure 1). The study was carried out with 4 replications in 4 tanks with a volume of 20,000 m^3 each. Larvae were placed in each tank medium with a stock density of 130-150 individuals per liter.

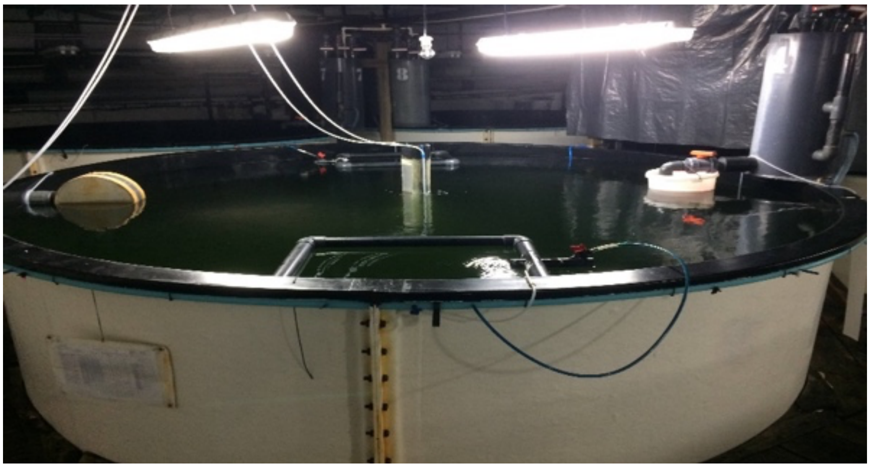


Figure 1. Larvae culture tanks used in the study

The calculation was applied as follows:

For each tank medium with a volume of 20,000 m^3:

$$TLsb = (TV\ x\ \left(\frac{LC}{l}\right))$$

where

TL: total larvae in tank

TV: tank volume in cubic meter (m^3)

LC: larvae count

l: liter

Following this equation,

20,000 l x 130 individuals/l = 2,600,000 individuals of seabream larvae in total

20,000 l x 150 individuals/l = 3,000,000 individuals of seabream larvae in total

The average of these two values were used in the formulation as follows:

$$ALC = (LC1 + LC2)\ /\ 2$$

where

LC1: larvae count 1

LC2: larvae count 2

Average Larvae Count (ALC) = (2,600,000 + 3,000,000) / 2 = 2,800,000 larvae in total. Then, a total of 2,800,000 seabream larvae were placed in each tank with a volume of 20,000 m^3.

Since the mouth size of seabream larvae is quite small (~100 µ) (Alpbaz, 2023), it was decided to use S-type rotifers with a size range of 40-80 µm for larval feeding. Since the size of a single rotifer is at micron level and the estimated amounts of rotifers offered to the larvae is over millions, that means around hundred times rotifers of the 2,800,000 larvae count are to be introduced into the tank with a volume of 20,000 m^3, the exact count may not be always possible. Hence, the number of rotifers in a mL flask has been counted and pooled proportionally for the estimation of rotifer counts in the entire tank. Then the amount of water corresponding to the need of 2.8 million larvae was filtered and transferred to the larval tank. The ratio of rotifer per larvae, that is the number of rotifers given per larvae in seabream tanks was estimated using the equation given below:

$$RLR = ((R\ (prey/ml)\ x\ 1000)\ x\ TV)\ /\ LN)$$

where
RLR is for rotifer : larvae ratio
R is rotifer
TV: tank volume
LN: larvae number

The ratio of artemia per larvae, that is the number artemia supplied per larvae in seabream tanks was estimated using the equation given below:

$$ALR\ (artemia/larvae) = ((A\ (prey/ml)\ x\ 1000)\ x\ TV)\ /\ LN)$$

where
ALR is for artemia: larvae ratio
A is artemia
TV: tank volume
LN: larvae number

In the tank environment, daily water change was set to 5-10% a day, and the water flow rate was gradually increased in direct proportion to the age of the larvae. The water temperature in the experimental tanks of seabream larvae was kept constant between 17-19 °C. However, after the Pre-larvae period, the water temperature followed the normal sea water conditions. The pH and salinity values in the water supplied to the tanks were measured in the range of 6.5-7.5 and ‰ 34-36, respectively. Nitrogen concentrations (NH_3, NH_4, NO_2, NO_3) in the aquatic environment were < 0.01 mg/L and the dissolved oxygen value was measured in the range of 6.5-9.6 mg/l.

During the study, the brightness in the facility was kept under control and the lights were turned on as soon as the larvae started to take feed from day-3 onward, right after consumption of the food sac, and adjusted to remain at the same level for 24 hours throughout the pre-larval period.

The "Green Water Technique" was used during the study, where algae (*Spirulina sp.*) were added to the medium, in order to provide nutrient availability for the rotifers, to support the maintenance of the pH balance of the medium, and to create a background that would facilitate the larvae to follow the rotifer in the water.

With the opening of the mouth (day-3), rotifers were offered to the larvae. After approximately 2 weeks, artemia was gradually introduced to the medium on day-16. With the addition of artemia, the amount of artemia was gradually increased by decreasing the amount of rotifer supplied. According to daily consumption, increases and decreases were observed, and feeding levels were recorded. During the gradual transition from rotifer to artemia, the meal arrangement was adjusted to 5 rotifer -and 4 artemia meals a day, and the meal times were arranged as given in Figure 2.

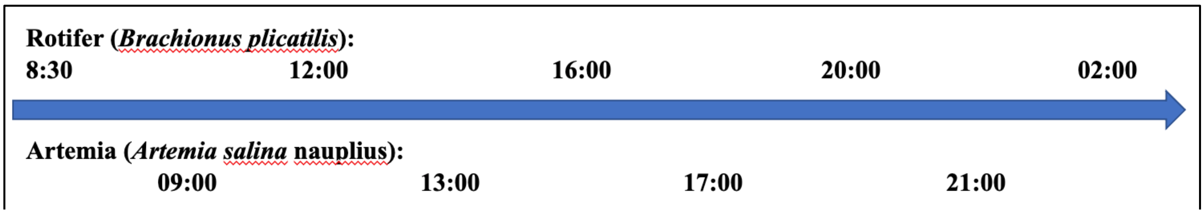


Figure 2. Meal arrangement and feeding times during the transition from rotifer to artemia in seabream larvae

Seabass Larvae Feeding Method and Experimental Conditions
Same as with seabream, the first 24 hours following incubation for seabass larvae was accepted as "Day-1" and the larval age was calculated on the basis of daily values accordingly. Conical shaped tanks with a volume of 14 tons were used in the production of seabass larvae. The study was carried out in 4 tanks of 14.000 m^3 with 4 replications. Larvae were placed in each tank medium with a stock density of 120-150 individuals per liter.

The calculation was applied as follows:
For each tank medium with a volume of 14,000 m^3:

$$TL = (TV\ x\ \left(\frac{LC}{l}\right))$$

where,
TL: amount of total larvae in tank
TV: tank volume in cubic meter (m^3)
LC: larvae count
l: liter

Following this equation,
14,000 l x 120 individuals/l = 1,680,000 individuals of seabass larvae in total
14,000 l x 150 individuals/l = 2,100,000 individuals of seabass larvae in total

The average of these two values were used in the formulation as follows:

$$ALC = (LC1 + LC2)\ /\ 2$$

where
LC1: larvae count 1
LC2: larvae count 2

Average Larvae Count (ALC) = (1,680,000 + 2,100,000) / 2 = 1,890,000 larvae in total. Then, a total of 1,850,000 seabass larvae were placed in each tank with a volume of 14,000 m^3.

Since the mouth size of seabass larvae (~400-420 µ) is larger than that of seabream larvae (~100 µ), it can be noted that it is possible to offer artemia directly instead of starting with rotifers in larval feeding, because the size of artemia (~165-175 µ) is quite suitable for the mouth size of seabass larvae. Therefore, in hatcheries with seabass production only, it may not be necessary to offer rotifer, and instead direct feeding with artemia could be initiated. However, in hatcheries where both seabass and seabream are produced, and rotifer production units already exist, first start of feeding with rotifers and then gradually switch to artemia might further improve larval development. In such hatcheries, the start with rotifers is a common practice also in seabass production. Hence, in this study, a commercial hatchery production with seabream and seabass was followed. Further, this provided a possible comparison for both seabream and seabass when both received the same live feed of rotifers and artemia.

Again, as mentioned in the section on feeding seabream larvae, the size of the rotifer is at micron level and the amounts of rotifers offered to the larvae is over millions, that means around hundred times rotifers of the 1,890,000 larvae count is to be introduced into the tank with a volume of 14,000 m^3, the exact count may not be always possible. Hence, the number of rotifers in a mL flask has been counted and pooled proportionally for the estimation of rotifer counts in the entire tank. Then the amount of water corresponding to the daily rotifer supply for 1.89 million larvae was filtered and transferred to the larvae tank.

The ratio of rotifer per larvae, that is the number rotifers given per larvae in seabass tanks was estimated using the equation given below:

$$RLR = ((R\ (prey/ml)\ x\ 1000)\ x\ TV)\ /\ LN)$$

where
RLR is for rotifer : larvae ratio
R is rotifer
TV: tank volume
LN: larvae number

The ratio of artemia per larvae, that is the number artemia supplied per larvae in seabass tanks was estimated using the equation given below:

$$ALR\ (artemia/larvae) = ((A\ (prey/ml)\ x\ 1000)\ x\ TV)\ /\ LN)$$

where
ALR is for artemia: larvae ratio
A is artemia
TV: tank volume
LN: larvae number

Daily water change in the experimental tanks was set at 5-10% a day, and the water flow rate gradually increased in direct proportion to the larvae age. The water temperature in the tanks was kept constant between 14-18 °C at the pre-larvae stage but then followed the natural course of the seawater supply. The pH and salinity values were measured in the range of 6.5-7.5 and ‰ 34-36, respectively. Nitrogen (NH_3, NH_4, NO_2, NO_3) in the water body was <0.01 mg/l and the dissolved oxygen was measured in the range of 6.5-9.6 mg/l. During the study, the brightness of the environment was kept under control and the lights were turned on as soon as the larvae started to take feed by day-3 when they consumed the food sac, and the light was adjusted to remain "turned on" for 24 hours and throughout the pre-larval period.

Again, the "Green Water Technique" was used during the study, where algae (*Spirulina sp.*) were added to the medium, in order to provide nutrient availability for the rotifers, to support the maintenance of the pH balance of the medium, and to create a background that would facilitate the larvae to follow the rotifer in the water.

Rotifer was introduced to the seabass larvae when the mouth opened by day-6. On day-12, enriched artemia was added to the medium incrementally. With the addition of artemia, the amount of artemia was gradually increased by gradually decreasing the amount of rotifer. According to daily consumption, increases and decreases were observed in feeding. During the gradual transition from rotifer to artemia, the meal arrangement was adjusted to 5 rotifer and 4 artemia meals a day, and the feeding meal times were arranged as presented in Figure 3.

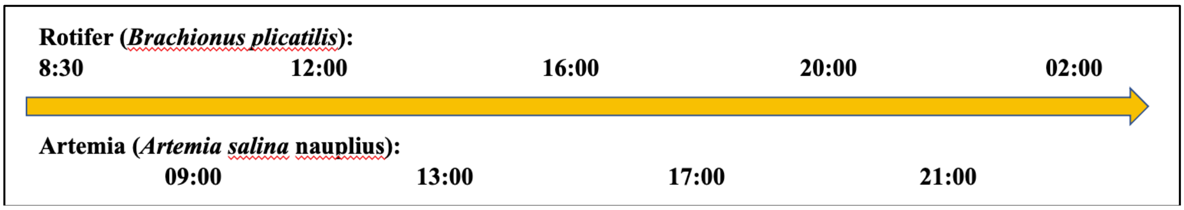


Figure 3. Meal arrangement and feeding times during the transition from rotifer to artemia in seabass larvae

Results

The data obtained are compared with the feed consumption amounts in the pre-larval period and the transition period to the postlarval stage, and the proportional values are below.

Table 1. General protocol of water conditions, physicochemical parameters applied in the hatchery for seabream larvae

Fish species		**Seabream** *(Sparus aurata)*	**Seabass** *(Dicentrarchus labrax)*
Larvae age		**Day-1***	**Day-1***
Parameter	**Unit**		
Amount	Individuals	2,600,000 – 3,000,000	1,600,000-2,000,000
Stocking density	individuals per liter (larvae/L)	130-150	120-150
Tank Volume	Liter (L)	20,000	14,000
Temperature	°C	17-19	14-18
pH	-	6,5-7,5	6,5-7,5
Salinity	‰	34-36	34-36
Nitrogen (NH_3-NH_4-NO_2-NO_3)	mg/L	< 0.01	< 0.01
Dissolved oxygen	mg/L	6.5-9.6	6.5-9.6
Photoperiod	Hour dark:light	with mouth opening 24:0	with mouth opening 24:0
Algae	-	-	-
Rotifer (*B. plicatilis*)	meals/day	5	5
Artemia (*Artemia sp.*)	meals/day	4	4

*Day-1: 24 hours after larvae incubation

Based on the findings in this study, feeding protocols were presented in tables and figures according to the feed uptakes in seabream and seabass larvae during the experiment. The rotifer supply for larvae started when the mouth showed function (mouth opening) that corresponded to day-3 in seabream, and day-6 in seabass larvae. It was determined that the mouth size suitable for artemia corresponds to the day-16 in seabream larvae, which was the day when artemia was introduced to the larvae tank. For the seabass larvae, artemia was introduced by day-12. Feeding protocols were prepared based on daily feed amounts, and the feeding protocol for seabream larvae has been given in Table 2.

Table 2. Tank-1, Seabream Larvae Feeding Protocol

Day	**Temperature (°C)**	**Salinity (‰)**	**Water exchange (%/hour)**	**Light intensity (Lux)**	**Photoperiod Light:dark hours**	**Feeding**	
						Rotifer R, individual/ml	**Artemia EG,** individual/ml
1.	17-18	34-36	10	0	0:0	NF*	NF
2.	17-18	34-36	10	0	0:0	NF	NF
3.	17-18	34-36	3-4	1000	24:0	9	-
4.	17-18	34-36	3-4	1000	24:0	6	-
5.	17-18	34-36	3-4	1000	24:0	12	-
6.	17-18	34-36	5	1000	24:0	20	-
7.	17-18	34-36	5	1000	24:0	24.5	-
8.	17-18	34-36	5	1000	24:0	29	-
9.	17-18	34-36	5	1000	24:0	31	-
10.	17-18	34-36	5	1000	24:0	39	-
11.	17-18	34-36	6-7	1000	24:0	45	-
12.	17-18	34-36	6-7	1000	24:0	47	-
13.	17-18	34-36	6-7	1000	24:0	53	-
14.	17-18	34-36	6-7	1000	18:6	55.5	-
15.	18-19	34-36	8	1000	18:6	56.5	-
16.	18-19	34-36	8	1000	15:9	49	2.1
17.	18-19	34-36	8	1000	15:9	48	3.0
18.	18-19	34-36	8	1000	15:9	32	3.6
19.	18-19	34-36	8	1000	15:9	39	5.2
20.	18-19	34-36	10	1000	15:9	40	6.8
21.	18-19	34-36	10	1000	15:9	39	7.7
22.	18-19	34-36	10	1000	15:9	39	8.5
23.	18-19	34-36	10	1000	15:9	16	9.3
24.	18-19	34-36	10	1000	15:9	16	10.4

25.	18-19	34-36	10	1000	15:9	12	12.2
26.	18-19	34-36	10	1000	15:9	8	13.8
27.	18-19	34-36	10	1000	15:9	4	15.3

*NF: no feeding
R: rotifer
EG: enriched artemia

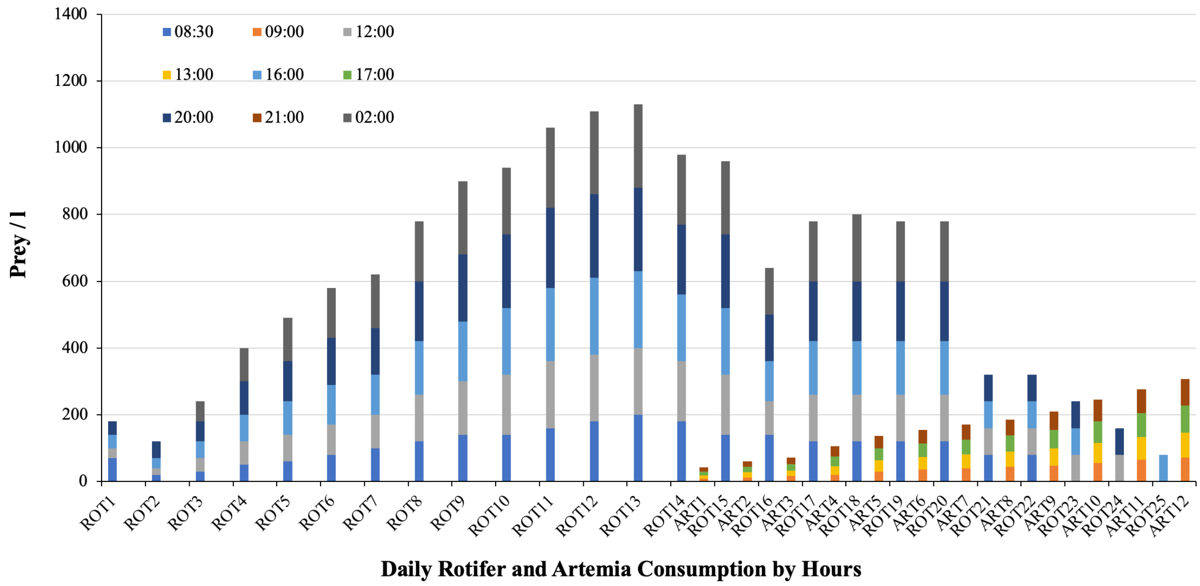


Figure 4. Tank-1, Seabream larvae feeding by hours on daily basis

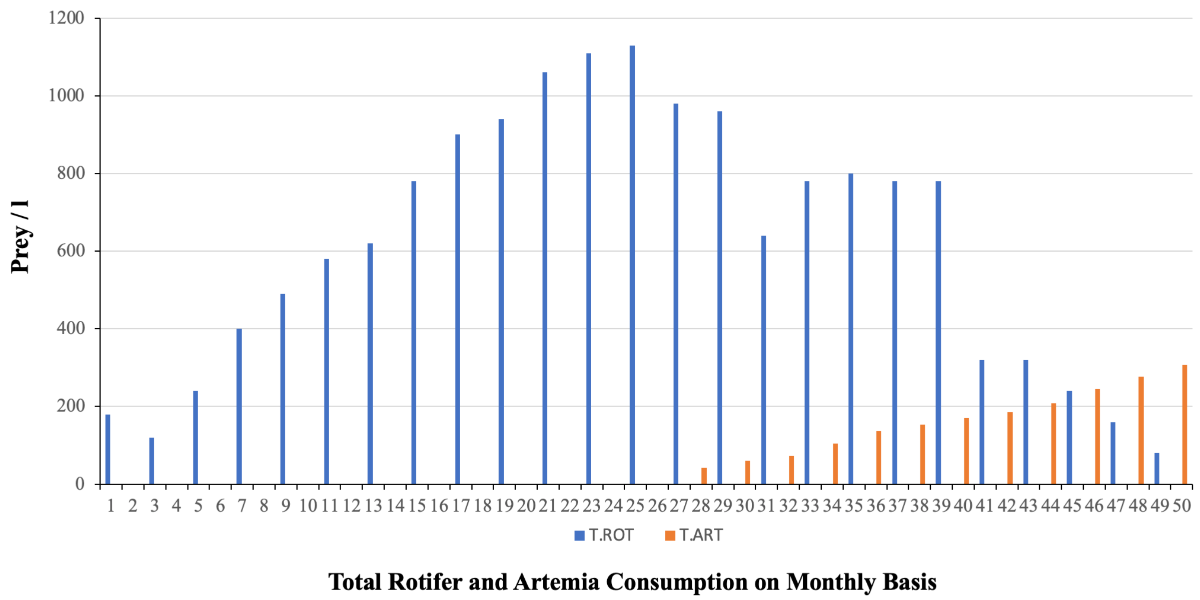
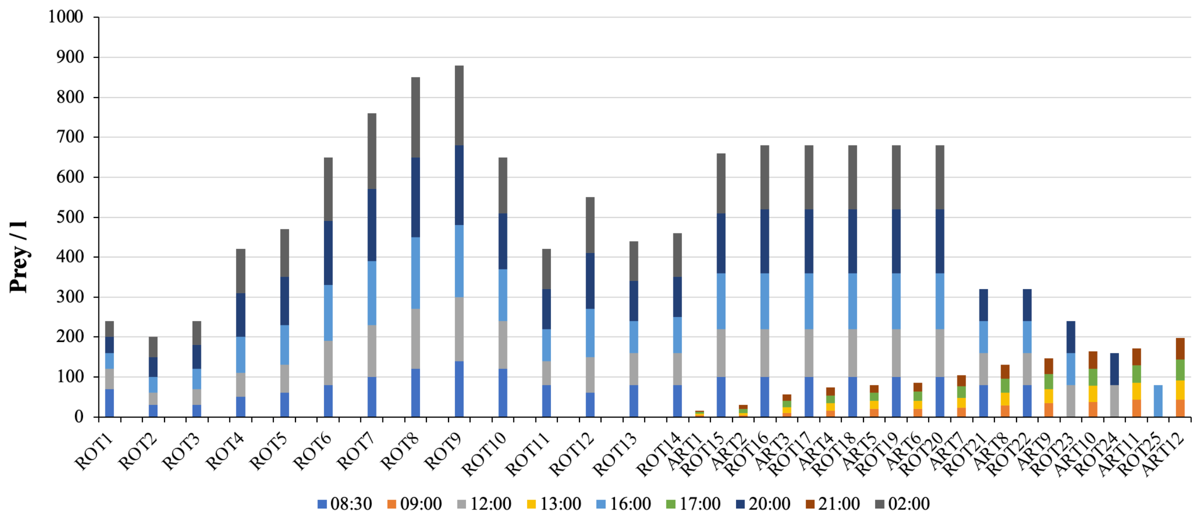


Figure 5. Tank-1, Seabream larvae feeding by days on monthly basis

Table 3. Tank-2, Seabream Larvae Feeding Protocol

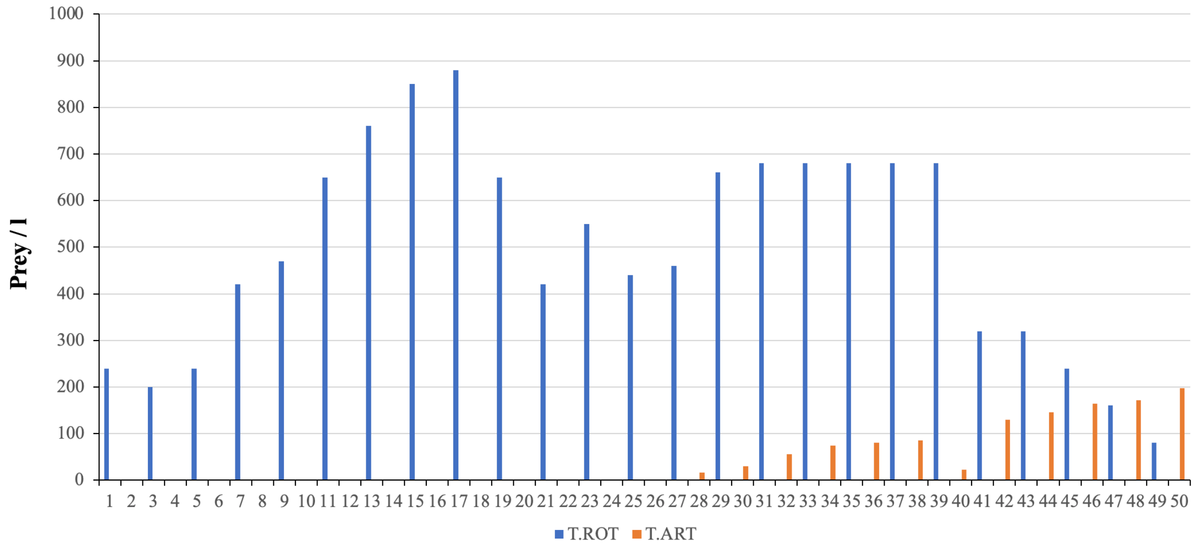
Day	Temperature (°C)	Salinity (‰)	Water exchange (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	Artemia EG prey/ml
1.	17-18	34-36	10	0	0:0	NF	NF
2.	17-18	34-36	10	0	0:0	NF	NF
3.	17-18	34-36	3-4	1000	24:0	R=12	-
4.	17-18	34-36	3-4	1000	24:0	R=10	-
5.	17-18	34-36	3-4	1000	24:0	R=12	-
6.	17-18	34-36	5	1000	24:0	R=21	-
7.	17-18	34-36	5	1000	24:0	R=23.5	-
8.	17-18	34-36	5	1000	24:0	R=32.5	-
9.	17-18	34-36	5	1000	24:0	R=38	-
10.	17-18	34-36	5	1000	24:0	R=42.5	-
11.	17-18	34-36	6-7	1000	24:0	R=44	-
12.	17-18	34-36	6-7	1000	24:0	R=32.5	-
13.	17-18	34-36	6-7	1000	24:0	R=21	-
14.	17-18	34-36	6-7	1000	18:6	R=27.5	-
15.	18-19	34-36	8	1000	18:6	R=22	-
16.	18-19	34-36	8	1000	15:9	R=23	EG=0.8
17.	18-19	34-36	8	1000	15:9	R=33	EG=1.5
18.	18-19	34-36	8	1000	15:9	R=34	EG=2.8
19.	18-19	34-36	8	1000	15:9	R=34	EG=3.7
20.	18-19	34-36	10	1000	15:9	R=34	EG=4
21.	18-19	34-36	10	1000	15:9	R=34	EG=4.3
22.	18-19	34-36	10	1000	15:9	R=34	EG=5.2
23.	18-19	34-36	10	1000	15:9	R=16	EG=6.5
24.	18-19	34-36	10	1000	15:9	R=16	EG=7.3
25.	18-19	34-36	10	1000	15:9	R=12	EG=8.2
26.	18-19	34-36	10	1000	15:9	R=8	EG=8.6
27.	18-19	34-36	10	1000	15:9	R=4	EG=9.8

*NF: no feeding
R: rotifer
EG: enriched artemia



Daily Rotifer and Artemia Consumption by Hours

Figure 6. Tank-2, Seabream larvae feeding by hours on daily basis



Total Rotifer and Artemia Consumption on Monthly Basis

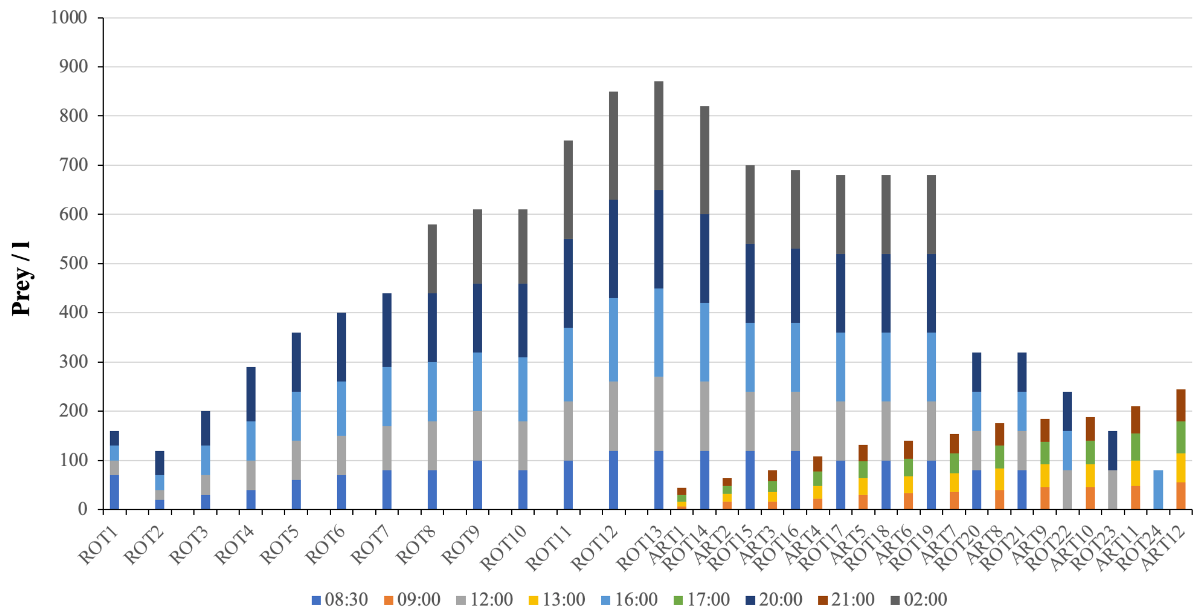
Figure 7. Tank-2, Seabream larvae feeding by days on monthly basis

Table 4. Tank-3, Seabream Larvae Feeding Protocol

Day	Temperature (°C)	Salinity (‰)	Water exchange (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	Artemia EG prey/ml
1.	17-18	34-36	10	0	0	NF	NF
2.	17-18	34-36	10	0	0	NF	NF
3.	17-18	34-36	3-4	1000	24	R=8	-
4.	17-18	34-36	3-4	1000	24	R=6	-
5.	17-18	34-36	3-4	1000	24	R=10	-
6.	17-18	34-36	5	1000	24	R=14,5	-
7.	17-18	34-36	5	1000	24	R=18	-
8.	17-18	34-36	5	1000	24	R=20	-

9.	17-18	34-36	5	1000	24	R=22	-
10.	17-18	34-36	5	1000	24	R=29	-
11.	17-18	34-36	6-7	1000	24	R=30,5	-
12.	17-18	34-36	6-7	1000	24	R=32,5	-
13.	17-18	34-36	6-7	1000	24	R=34,5	-
14.	17-18	34-36	6-7	1000	18	R=37,5	-
15.	18-19	34-36	8	1000	18	R=42,5	-
16.	18-19	34-36	8	1000	15	R=43,5	EG=1,5
17.	18-19	34-36	8	1000	15	R=41	EG=3,2
18.	18-19	34-36	8	1000	15	R=35	EG=4
19.	18-19	34-36	8	1000	15	R=34,5	EG=5,4
20.	18-19	34-36	10	1000	15	R=34	EG=6,6
21.	18-19	34-36	10	1000	15	R=34	EG=7
22.	18-19	34-36	10	1000	15	R=34	EG=7,7
23.	18-19	34-36	10	1000	15	R=16	EG=6,8
24.	18-19	34-36	10	1000	15	R=16	EG=9,2
25.	18-19	34-36	10	1000	15	R=12	EG=9,4
26.	18-19	34-36	10	1000	15	R=8	EG=10,5
27.	18-19	34-36	10	1000	15	R=4	EG=12,2

*NF: no feeding
R: rotifer
EG: enriched artemia



Daily Rotifer and Artemia Consumption by Hours

Figure 8. Tank-3, Seabream larvae feeding by hours on daily basis

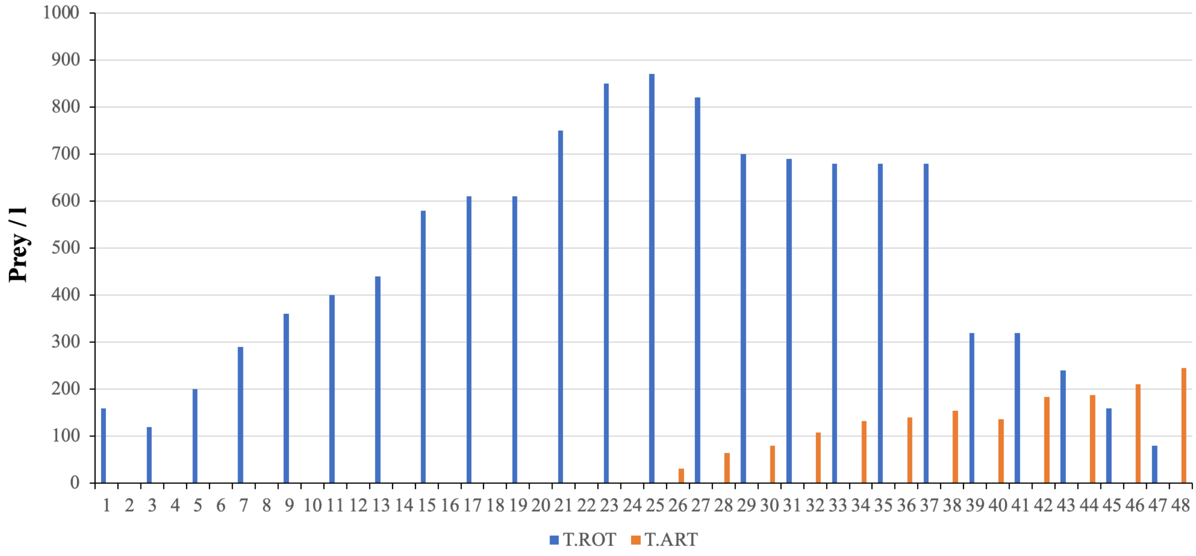


Figure 9. Tank-3, Seabream larvae feeding by days on monthly basis

Table 5. Tank-4, Seabream Larvae Feeding Protocol

Day	Temperature (°C)	Salinity (‰)	Water exchange (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	Artemia EG prey/ml
1.	17-18	34-36	10	0	0	NF	NF
2.	17-18	34-36	10	0	0	NF	NF
3.	17-18	34-36	3-4	1000	24	R=12	-
4.	17-18	34-36	3-4	1000	24	R=10	-
5.	17-18	34-36	3-4	1000	24	R=12	-
6.	17-18	34-36	5	1000	24	R=23	-
7.	17-18	34-36	5	1000	24	R=25,5	-
8.	17-18	34-36	5	1000	24	R=32,5	-
9.	17-18	34-36	5	1000	24	R=38	-
10.	17-18	34-36	5	1000	24	R=42,5	-
11.	17-18	34-36	6-7	1000	24	R=44	-
12.	17-18	34-36	6-7	1000	24	R=37,5	-
13.	17-18	34-36	6-7	1000	24	R=28	-
14.	17-18	34-36	6-7	1000	18	R=25,5	-

15.	18-19	34-36	8	1000	18	R=28	-
16.	18-19	34-36	8	1000	15	R=25,5	EG=1
17.	18-19	34-36	8	1000	15	R=30	EG=2
18.	18-19	34-36	8	1000	15	R=38	EG=3,7
19.	18-19	34-36	8	1000	15	R=39	EG=4,1
20.	18-19	34-36	10	1000	15	R=40	EG=4,4
21.	18-19	34-36	10	1000	15	R=39	EG=4,6
22.	18-19	34-36	10	1000	15	R=39	EG=5,5
23.	18-19	34-36	10	1000	15	R=39	EG=6,9
24.	18-19	34-36	10	1000	15	R=16	EG=7,7
25.	18-19	34-36	10	1000	15	R=16	EG=8,3
26.	18-19	34-36	10	1000	15	R=12	EG=8,6
27.	18-19	34-36	10	1000	15	R=8	EG=9,8

*NF: no feeding
R: rotifer
EG: enriched artemia

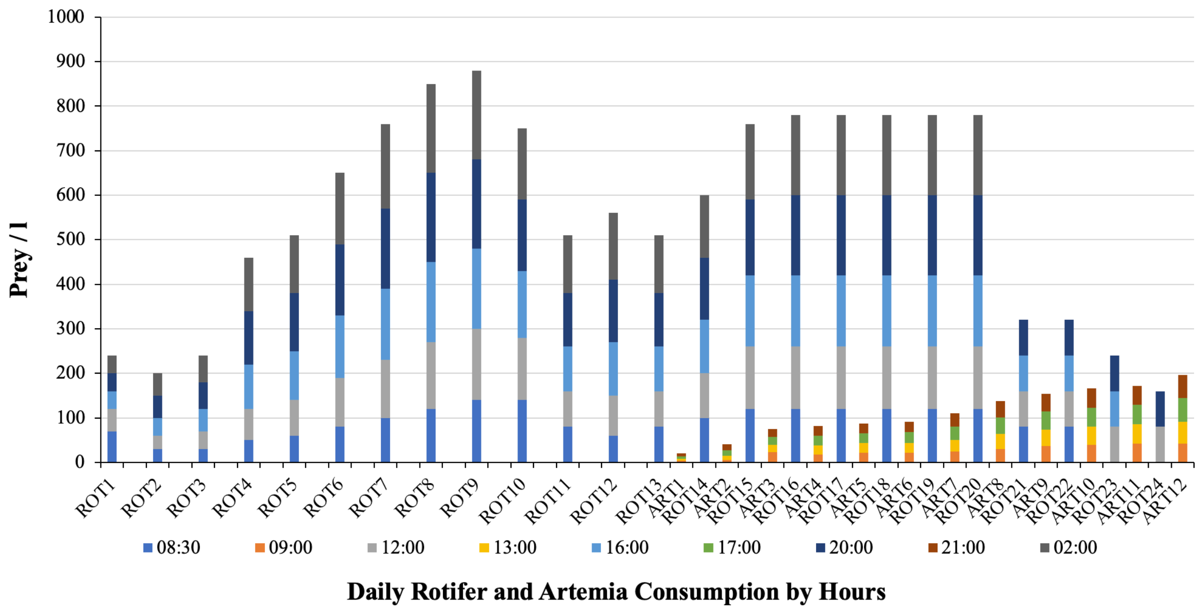


Figure 10. Tank-4, Seabream larvae feeding by hours on daily basis

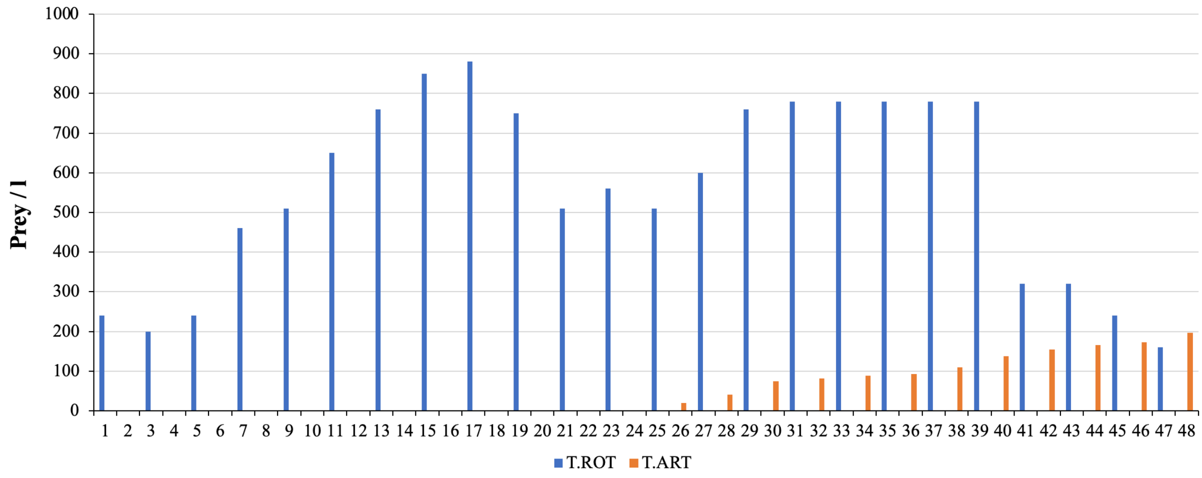


Figure 11. Tank-4, Seabream larvae feeding by days on monthly basis

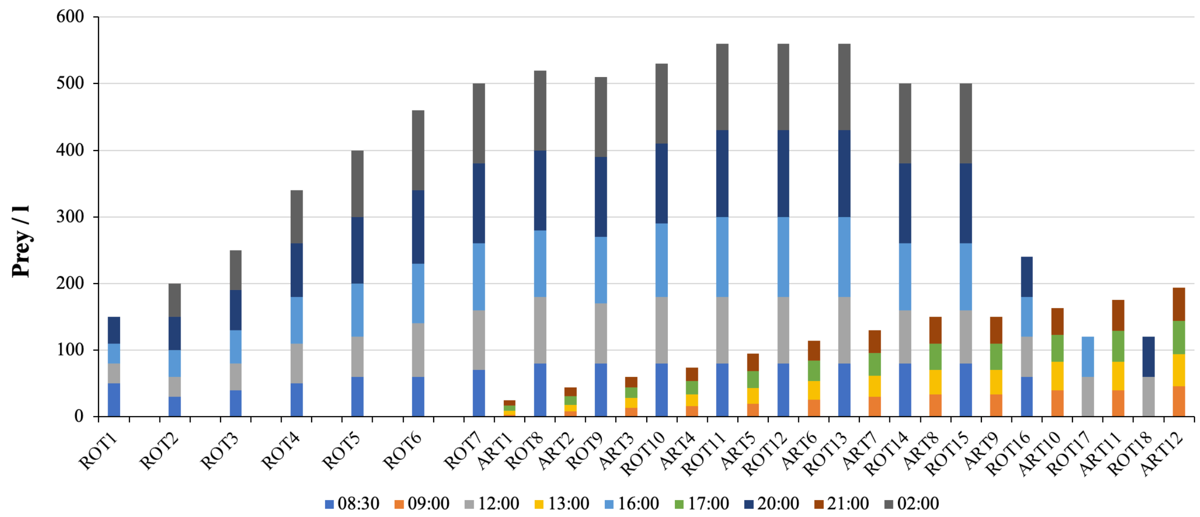
The feeding protocols for seabass larvae have been given in Table 6.

Table 6. Tank-1, Seabass Larvae Feeding Protocol

Day	Temperature (°C)	Salinity (‰)	Water exchange rate (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	Artemia EG prey/ml
1.	14-15	34-36	10	0	0:0	NF	NF
2.	14-15	34-36	10	0	0:0	NF	NF
3.	14-15	34-36	10	0	0:0	NF	NF
4.	14-15	34-36	10	0	0:0	NF	NF
5.	14-15	34-36	10	0	0:0	NF	NF
6.	15-16	34-36	5-6	1000	24:0	R=10.7	-
7.	15-16	34-36	5-6	1000	24:0	R=14.3	-
8.	15-16	34-36	5-6	1000	24:0	R=17.9	-
9.	15-16	34-36	5-6	1000	24:0	R=24.3	-
10.	15-16	34-36	5-6	1000	24:0	R=28.6	-
11.	16-17	34-36	8	1000	24:0	R=32.9	-
12.	16-17	34-36	8	1000	24:0	R=35.7	EG=1.8
13.	16-17	34-36	8	1000	24:0	R=37.2	EG=3.1
14.	16-17	34-36	8	1000	18:6	R=36.4	EG=4.3
15.	16-17	34-36	8	1000	18:6	R=37.9	EG=5.3
16.	17-18	34-36	10	1000	15:9	R=40	EG=6.8
17.	17-18	34-36	10	1000	15:9	R=40	EG=8.1
18.	17-18	34-36	10	1000	15:9	R=40	EG=9.3
19.	17-18	34-36	10	1000	15:9	R=35.7	EG=10.7

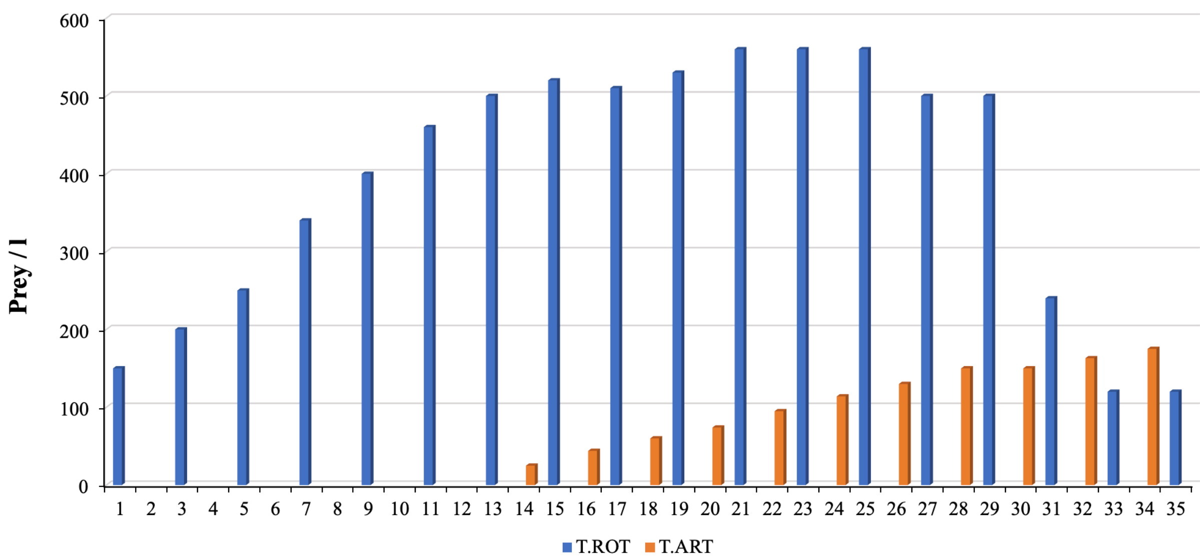
20.	17-18	34-36	10	1000	15:9	R=35.7	EG=10.7
21.	17-18	34-36	10	1000	15:9	R=17.1	EG=11.6
22.	17-18	34-36	10	1000	15:9	R=8.6	EG=12.5
23.	17-18	34-36	10	1000	15:9	R=8.6	EG=13.9

*NF: no feeding
R: rotifer
EG: enriched artemia



Daily Rotifer and Artemia Consumption by Hours

Figure 12. Tank-1, Seabass larvae feeding by hours on daily basis



Total Rotifer and Artemia Consumption on Monthly Basis

Figure 13. Tank-1, Seabass larvae feeding by days on monthly basis

Table 7. Tank-2, Seabass Larvae Feeding Protocol

Day	Temperature (°C)	Salinity (‰)	Water exchange rate (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	Artemia EG prey/ml
1.	14-15	34-36	10	0	0:0	NF	NF
2.	14-15	34-36	10	0	0:0	NF	NF
3.	14-15	34-36	10	0	0:0	NF	NF
4.	14-15	34-36	10	0	0:0	NF	NF
5.	14-15	34-36	10	0	0:0	NF	NF
6.	15-16	34-36	5-6	1000	24:0	R=10.7	-
7.	15-16	34-36	5-6	1000	24:0	R=21.4	-
8.	15-16	34-36	5-6	1000	24:0	R=30.7	-
9.	15-16	34-36	5-6	1000	24:0	R=33.6	-
10.	15-16	34-36	5-6	1000	24:0	R=35	-
11.	16-17	34-36	8	1000	24:0	R=41.4	-
12.	16-17	34-36	8	1000	24:0	R=42.8	EG=2.7
13.	16-17	34-36	8	1000	24:0	R=42.8	EG=3.4
14.	16-17	34-36	8	1000	18:6	R=45	EG=4.1
15.	16-17	34-36	8	1000	18:6	R=47.1	EG=4.4
16.	17-18	34-36	10	1000	15:9	R=47.1	EG=7.9
17.	17-18	34-36	10	1000	15:9	R=47.1	EG=9.2
18.	17-18	34-36	10	1000	15:9	R=47.1	EG=11.1
19.	17-18	34-36	10	1000	15:9	R=45.7	EG=13
20.	17-18	34-36	10	1000	15:9	R=17.1	EG=14.7
21.	17-18	34-36	10	1000	15:9	R=12.8	EG=

*NF: no feeding
R: rotifer
EG: enriched artemia

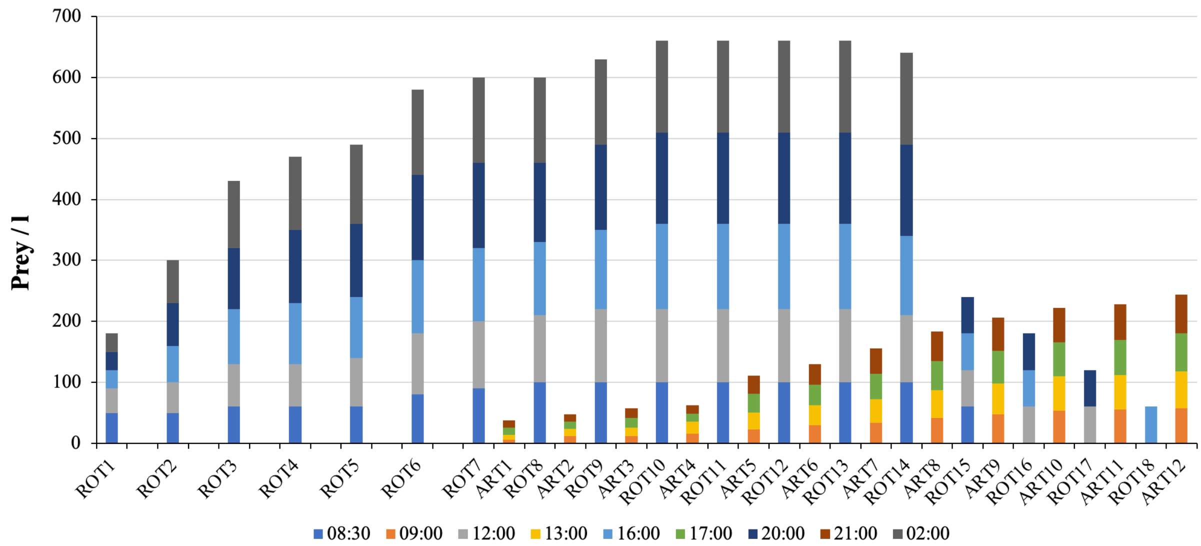
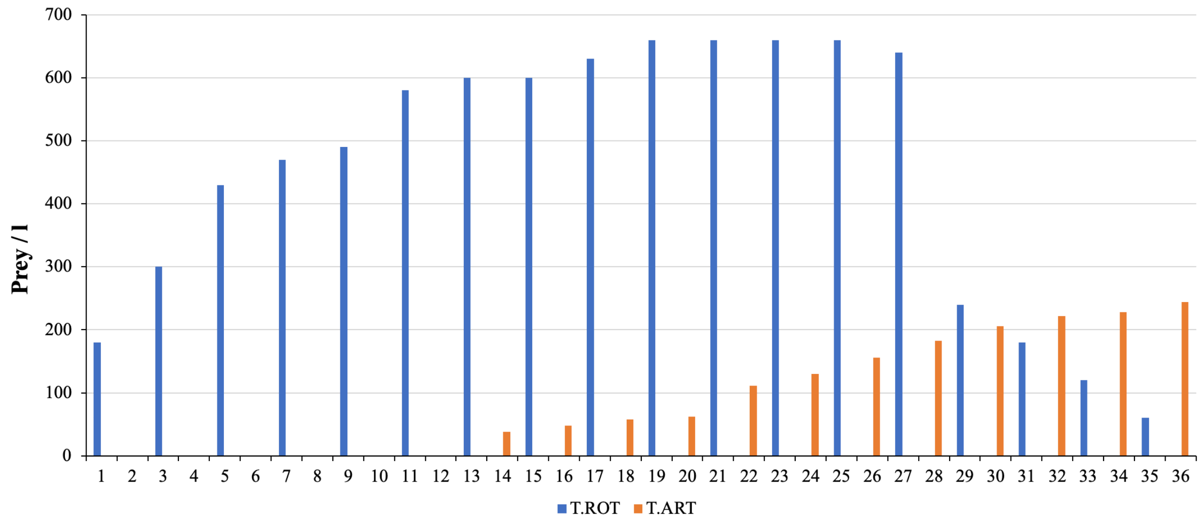


Figure 14. Tank-2, Seabass larvae feeding by hours on daily basis



Total Rotifer and Artemia Consumption on Monthly Basis

Figure 15. Tank-2, Seabass larvae feeding by days on monthly basis

Table 8. Tank-3, Seabass Larvae Feeding Protocol

Day	Temperature (°C)	Salinity (‰)	Water exchange rate (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	**Artemia EG** prey/ml
1.	14-15	34-36	10	0	0:0	NF	NF
2.	14-15	34-36	10	0	0:0	NF	NF
3.	14-15	34-36	10	0	0:0	NF	NF
4.	14-15	34-36	10	0	0:0	NF	NF
5.	14-15	34-36	10	0	0:0	NF	NF
6.	15-16	34-36	5-6	1000	24:0	R=22.1	-
7.	15-16	34-36	5-6	1000	24:0	R=22.1	-
8.	15-16	34-36	5-6	1000	24:0	R=23.6	-
9.	15-16	34-36	5-6	1000	24:0	R=35	-
10.	15-16	34-36	5-6	1000	24:0	R=40	-
11.	16-17	34-36	8	1000	24:0	R=42.9	-
12.	16-17	34-36	8	1000	24:0	R=45.7	EG=2.4
13.	16-17	34-36	8	1000	24:0	R=45.7	EG=3.9
14.	16-17	34-36	8	1000	18:6	R=45.7	EG=5.9
15.	16-17	34-36	8	1000	18:6	R=45.7	EG=7.8
16.	17-18	34-36	10	1000	15:9	R=47.1	EG=9.7
17.	17-18	34-36	10	1000	15:9	R=47.1	EG=11.6
18.	17-18	34-36	10	1000	15:9	R=45.7	EG=13
19.	17-18	34-36	10	1000	15:9	R=47.1	EG=14.1
20.	17-18	34-36	10	1000	15:9	R=17.1	EG=15.2

21.	17-18	34-36	10	1000	15:9	R=12.9	EG=16.6
22.	17-18	34-36	10	1000	15:9	R=8.6	EG=18
23.	17-18	34-36	10	1000	15:9	R=4.3	EG=20.4

*NF: no feeding
R: rotifer
EG: enriched artemia

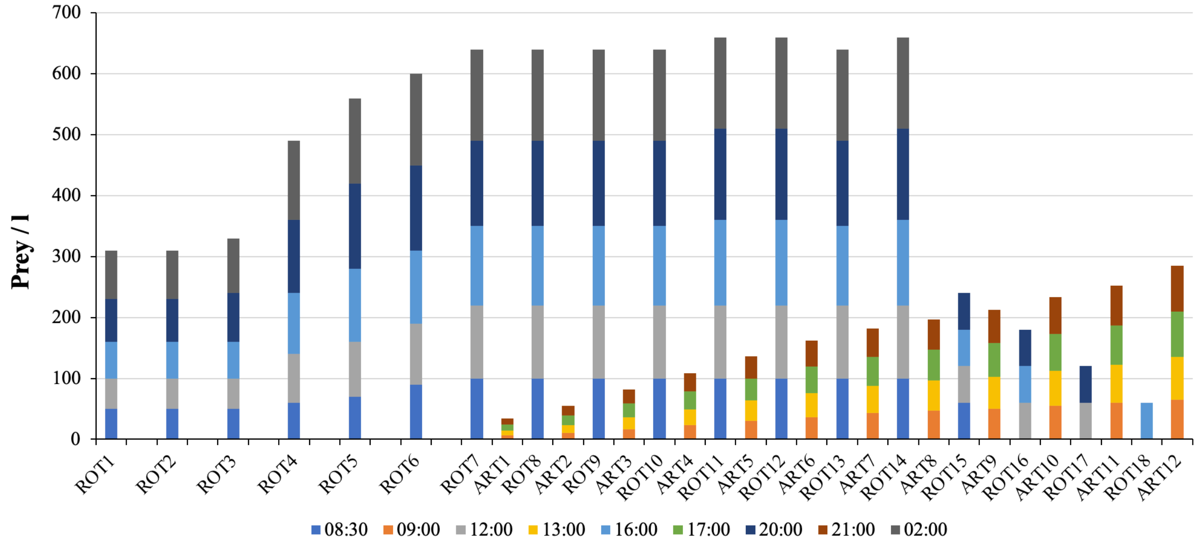


Figure 16. Tank-3, Seabass larvae feeding by hours on daily basis

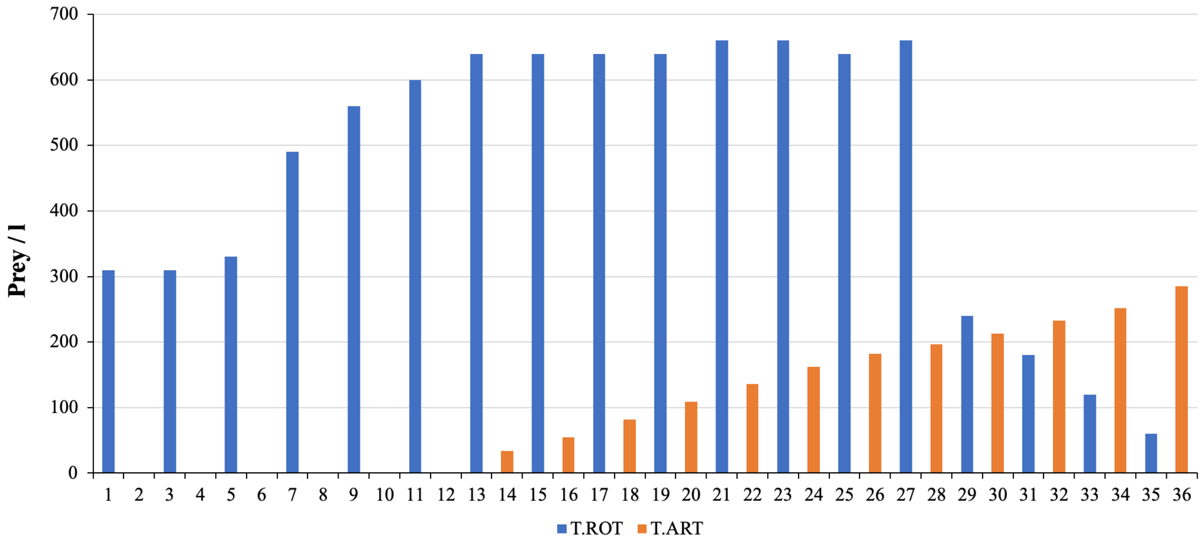
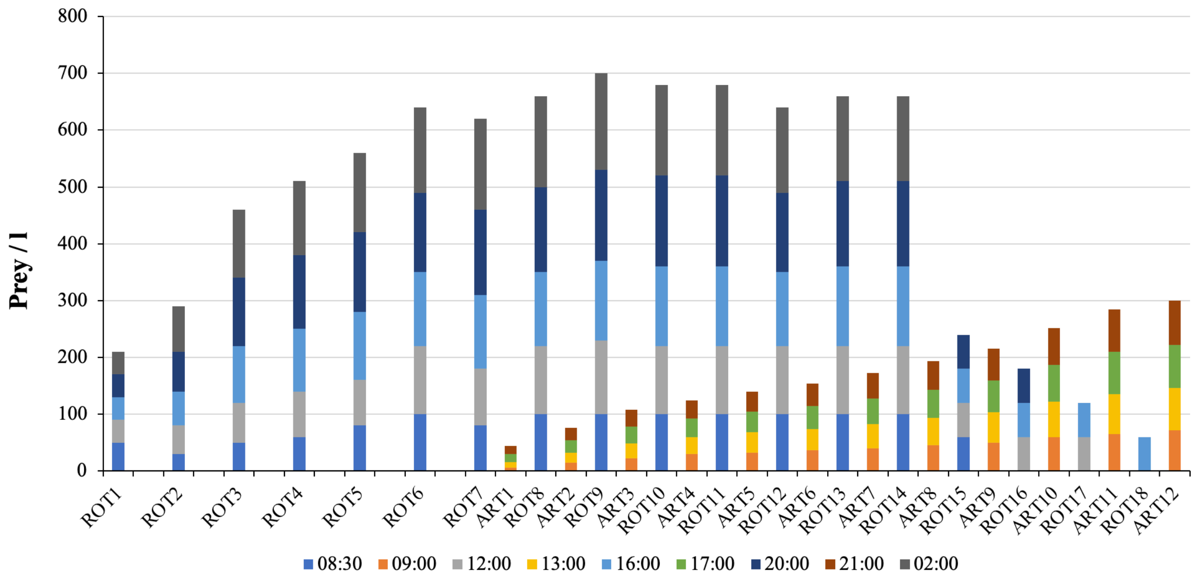


Figure 17. Tank-3, Seabass larvae feeding by days on monthly basis

Table 9. Tank-4, Seabass Larvae Feeding Protocol

Day	Temperature (°C)	Salinity (‰)	Water exchange rate (%/hour)	Light intensity (Lux)	Photoperiod Dark:light (hour)	Feeding	
						Rotifer R prey/ml	Artemia EG prey/ml
1.	14-15	34-36	10	0	0:0	NF	NF
2.	14-15	34-36	10	0	0:0	NF	NF
3.	14-15	34-36	10	0	0:0	NF	NF
4.	14-15	34-36	10	0	0:0	NF	NF
5.	14-15	34-36	10	0	0:0	NF	NF
6.	15-16	34-36	5-6	1000	24:0	R=15	-
7.	15-16	34-36	5-6	1000	24:0	R=20.7	-
8.	15-16	34-36	5-6	1000	24:0	R=32.9	-
9.	15-16	34-36	5-6	1000	24:0	R=36.4	-
10.	15-16	34-36	5-6	1000	24:0	R=40	-
11.	16-17	34-36	8	1000	24:0	R=45.7	-
12.	16-17	34-36	8	1000	24:0	R=44.3	EG=3.1
13.	16-17	34-36	8	1000	24:0	R=47.1	EG=5.4
14.	16-17	34-36	8	1000	18:6	R=50	EG=7.7
15.	16-17	34-36	8	1000	18:6	R=48.6	EG=8.9
16.	17-18	34-36	10	1000	15:9	R=48.6	EG=10
17.	17-18	34-36	10	1000	15:9	R=45.7	EG=11
18.	17-18	34-36	10	1000	15:9	R=47.1	EG=12.4
19.	17-18	34-36	10	1000	15:9	R=47.1	EG=13.8
20.	17-18	34-36	10	1000	15:9	R=17.1	EG=15.4
21.	17-18	34-36	10	1000	15:9	R=12.9	EG=18
22.	17-18	34-36	10	1000	15:9	R=8.6	EG=20.4
23.	17-18	34-36	10	1000	15:9	R=4.3	EG=21.4

*NF: no feeding
R: rotifer
EG: enriched artemia



Daily Rotifer and Artemia Consumption by Hours

Figure 18. Tank-4, Seabass larvae feeding by hours on daily basis

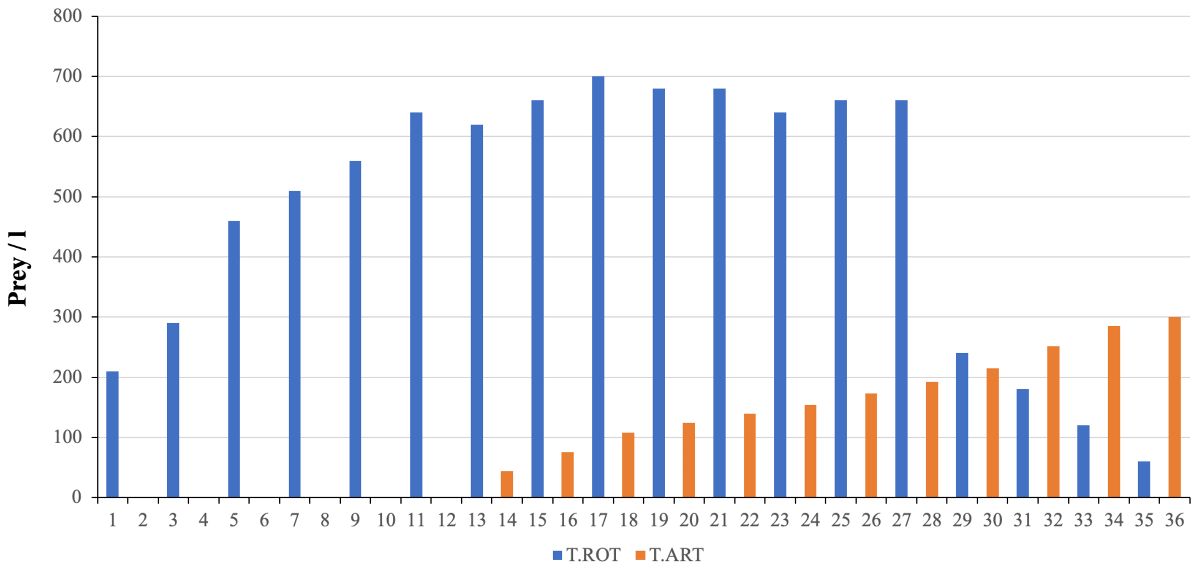


Figure 19. Tank-4, Seabass larvae feeding by days on monthly basis

Live eggs were accepted as age "day-1" after incubation. By day-5, the larvae were transferred to culture tanks, and by the end of day-6 the larvae were examined under the microscope and it was observed that the mouth was functional (open mouth formation), and feeding with Rotifer was initiated. By day-12, Artemia nauplii was gradually introduced. According to the findings, the daily amounts of live feed used for seabream and seabream larvae have been presented in Table 10.

Table 10. The mean and standard deviation values with the amounts of rotifer and artemia given to the experimental groups of seabream and seabass larvae

Fish species	**Seabream** *Sparus aurata*		**Seabass** *Dicentrarchus labrax*	
Live feed	**Rotifer** prey / ml	**Artemia** prey / ml	**Rotifer** prey / ml	**Artemia** prey / ml
Tank 1	30.78	8.15	27.86	9.81
Tank 2	24.82	5.22	32.37	10.03
Tank 3	24.18	6.95	34.00	12.29
Tank 4	26.84	6.45	31.86	10.92
Mean ± SD	**26.66 ± 2.75**	**6.45 ±1.12**	**31.52 ± 2.41**	**10.76 ± 1.04**

Based on the findings in the present study on feeding seabream larvae with four replicated, it was determined that a total of 26.66±2.75 prey/ml Rotifer and 6.45±1.12 prey/ml Artemia were used. For the seabream larvae experimentations, again conducted in four replicates, this was recorded as 31.52±2.41 prey/ml for rotifer and 10.76±1.04 prey/ml for artemia.

However, considering the number of larvae in each tank environments of 20 m3 volume for seabream and 14 m3 volume for Seabass, the rates of rotifer and artemia nauplii per larvae are presented in Table 11.

Tank 11. The ratios of rotifer or artemia per larvae in both seabream and Seabass tanks

Fish species	**Seabream** *Sparus aurata*		**Seabass** *Dicentrarchus labrax*	
prey / larvae	**RLR** rotifer / larvae	**ALR** artemia / larvae	**RLR** rotifer / larvae	**ALR** artemia / larvae
Tank 1	219.86	60.37	199.00	72.67
Tank 2	177.29	38.67	231.21	74.30
Tank 3	172.71	51.48	242.86	91.04
Tank 4	191.71	47.78	227.57	80.89
Mean ± SD	**190.39 ± 19.67**	**49.57 ± 8.32**	**225.16 ± 17.24**	**79.72 ± 7.72**

RLR = artemia : larvae ratio
ALR = artemia : larvae ratio

At the end of the study conducted for a period of four months, total consumption rates and mean ± SD values obtained during larval feeding processes in both seabream and seabass tanks are presented in Figure 19, and live feed consumption rates for seabream and seabass larvae are given in Table 12.

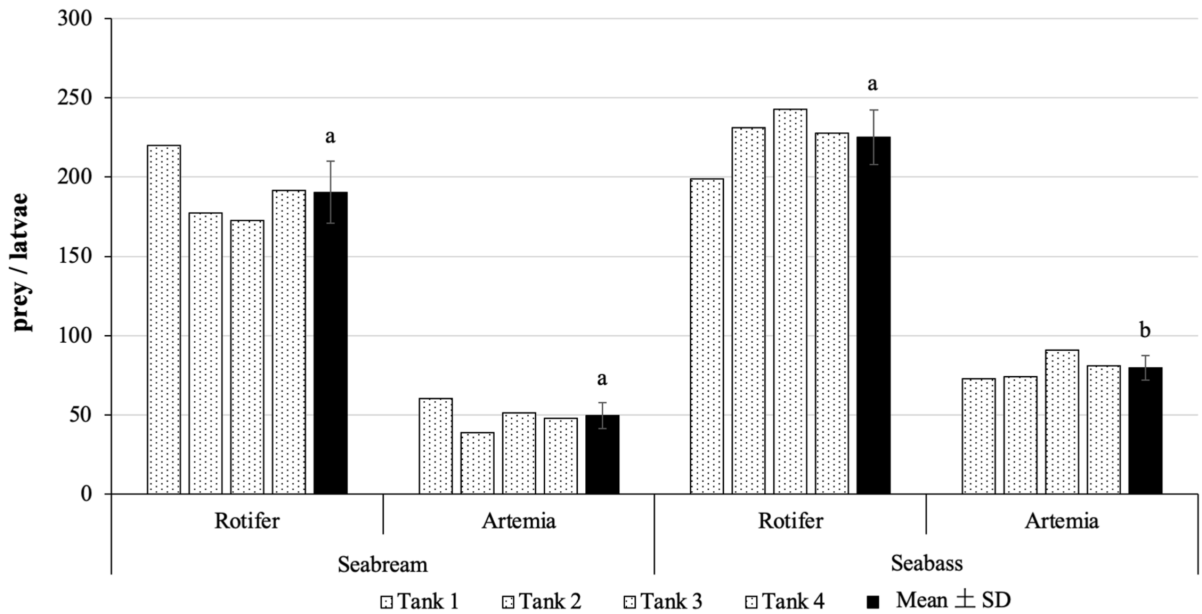


Figure 19. Rotifer and Artemia Consumption Rates in Seabream and Seabass Larvae

Table 12. Consumption rates (prey/larvae, mean±SD) and percent difference of prey feeding levels between seabream and seabass larvae

Larvae	Rotifer consumption		Artemia Consumption	
	prey / larvae	difference (%)	prey / larvae	difference (%)
Seabream	190.4 ± 19.7	15.44	49.6 ± 8.32	37.82
Seabass	225.2 ± 17.2		79.7 ± 7.72	

According to the findings in the present study in regards to rotifer and artemia consumption rates, higher consumption values have been noted in the seabass larvae group compared to the seabream larvae group. Seabass larvae consumed 15.44% more rotifers and 37.82% more artemia than the seabream larvae. This corresponds to around 1.18-fold increase for rotifer and 1.61-fold increment for artemia consumption in seabass larvae compared to the seabream once.

Discussion

In commercial marine hatcheries, broodstock and egg quality, along with optimum feeding protocols are highly important for the successful survival of fish larvae. Therefore, the right amount and the right strategy in feeding may increase the survival rate during the larval period. Eggs of sea bream are 0.9-1 mm in diameter and transparent. The chorion is transparent and thin, and the micropylar hole is approximately 14 microns in size. Lifeless or unfertilized eggs turn opaque after a few hours and sink to the bottom of the incubation tank (Alpbaz, 1990). Live eggs were accepted as age day-1 after incubation. At the end of the day-3, the larvae were examined under the microscope and it was observed that the mouth became functional (open mouth formation), and feeding with Rotifer was then initiated. By day-16, the artemia was introduced to the system and larvae started feeding with Artemia nauplii. In a study regarding prey size selection of gilthead seabream, *S. aurata* larvae with a total length of less than 4 mm preferred feed particles with a diameter of 25-50 µm, and larvae with 4-5 mm length preferred 51-100 µm diameter feed particles, whereas the larvae with the length of over 5 mm showed 101-150 µm particle size (Fernández-Diaz et al., 1994), which is in close agreement with the rotifer size (40-80 µm) used as a first live feed for larvae in the present study. The size of rotifer used as prey in this study was suitable for the mouth size of seabream larvae, that was reported as around 100 µm by Alpbaz (2023), who further indicated that seabass larvae can accept larger sized life feed such as artemia (740-780 µm in length and 225-240 µm in width) compared to the seabream larvae.

The success of larvae feeding may depend on various factors such as light intensities and temperature (Ribeiro et al., 2022). Earlier studies reported that the success in larval feeding is enhanced by larvae growth, and the response for feeding with the development of their biological functional systems such as the skeletal formation, swimming speed, digestive system, feed intake) (Pittman et al., 2013; Herbing, 2001). Light intensity and temperature range has been reported as significantly important for the success of larval feeding with influence on regulating preying ability in larvae (Blaxter, 1988). Moratti et al. (1999) underlined that light intensities varying between 1000 to 3000 lux at water surface are suggested during larvae growth period for both seabream and seabass until the end of day 25 after the hatch, which is advised to decrease to 500-1000 lux afterwards. The photoperiod regime (1000 lux) applied during the course of the present study was in line with the earlier report of Moretti et al. (1999). The temperature of the water body in the culture environment is in great importance for fish, a poikilothermic organism, for metabolic features which regulate the growth that is closely linked to a variety of activities such as digestion, swimming, catabolism effects etc. (Blaxter et al., 1992). The temperature range applied during the course of larval feeding for seabream larvae in the present study (17-19 °C), was in the range of earlier reports of Polo et al. (1991), who found over 90% hatching success for gilthead seabream at 16-26 °C temperatures, and the best temperature range for the mouth opening of fish larvae was underlined as 16-24 °C. However, the temperature range used in the seabass larvae tanks during the course of the present study were slightly lower (14-18 °C). The successful results for the development of larvae in both seabream and seabass larvae in the present study with lower temperature treatments might be explained by the temperature tolerance range of 2 to 4 °C as earlier reported by Polo et al. (1991). Also, Ribeiro et al. (2022) evidenced that seabream larvae treated with either 17 or 19 °C, demonstrated similar feeding activities, underlining similar physiological influences at these temperature ranges, which was also in agreement with Jordaan et al. (2002) who reported similar findings in cod (*Gadus morhua*) larvae.

The tolerance of larvae to temperature variations has been reported as species-specific and there are optimal temperature ranges for best growth performance and physiological activities in different species (Rombough, 1997). Ribeiro et al. (2022) reported higher feed intake levels in seabream larvae when the temperature increased to 25 °C compared to lower temperatures of 17 and 19 °C, which can be explained by the increasing energy needs of fish with the increase of temperature with acceptable limits as was underlined by Brett and Groves (1979) in their study on pacific sockeye salmon (*Oncorhynchus nerka*). In the present study, the temperature range for the seabream larvae tanks was 2 °C higher than those with the seabass larvae. However, the “prey to larvae” ratio in seabass feeding was around 15% and 38% higher than the seabream larvae in terms of rotifer and artemia consumption, respectively, despite the lower temperature range for seabass larvae than the seabream. This highlights the evidence for the species-specific nature of the tolerance to temperature and its effects on fish physiological functions in larvae.

Conclusions

The findings of this study showed differences among the two fish species compared for rotifer and artemia consumption during the larval stage. Understandably, feeding and preying success in larval feeding is closely linked to a variety of conditions as well as the interaction of these factors, and the preying ability might differ among variations of conditions in the culture environment. It was observed that under the conditions applied in this study, seabass larvae consumed 15.44% more rotifers and 37.82% more artemia than seabream larvae, which corresponds to 1.18 times the consumption of rotifer compared to seabream larvae and 1.61 times the consumption of artemia in seabass larvae. While artemia production is limited by the availability of natural resources such as fish meal and fish oil, rotifer production continues through continuous culture in live feed production units. Considering the increasing food demand and decreasing natural resources, seabream farming is important in terms of sustainable and good aquaculture practice. Therefore, in order to reduce the increasing feed costs in aquaculture facilities, breeding and feeding strategies should be developed, optimized and updated with the development of knowledge coupled with new technologies. In this study, although the temperature range for seabream larval tanks was higher than for the seabass larvae conditions, it was found that seabass larvae consume more rotifers and artemia, irrespective to temperature compared to seabream larvae. This finding evidences the species-specific nature of larvae, and underlining that seabream and seabass larval feeding protocols should be applied separately. Therefore, the findings of the present study, conducted at commercial scale provide useful data for farm managers in the establishment of feeding protocols for seabream and seabass production, as well as estimations on investment and operational costs in marine aquaculture enterprises.

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Ethical approval
No ethical approval needed for this study since data from commercial marine hatchery facilities were used.

Informed consent
Informed consent has been obtained from all individual participants involved in the study.

Data availability statement
The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest
There is no conflict of interests for publishing this study. A written approval has been obtained from the commercial hatchery for the use of data in this study.

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