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# Effect of salinity, light intensity and tank size on larval survival rate of shoemaker rabbitfish *Siganus sutor* (Valenciennes, 1835)

**Fourooghifard H.[1]*; Matinfar A.[2]; Roohani Ghadikolaee K.[1]; Moezzi M.[1]; Abdolalian E.[1];Zahedi M.R.[1]; Tamadoni Jahromi, S.[1]**

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**Abstract**

The hatching rate and larval survival of shoemaker rabbit fish *Siganus sutor* at different environmental conditions were surveyed in 2014. Adult and sub adult fishes were captured from coastal waters of Lavan Island, Hormozgan Province, Iran and were transported to Persian Gulf and Oman Sea Ecological Research Center, and reared until reproduction season. Induced spawning took place using HCG hormone (500 IU $kg^{-1}$ body weight), during April – May when the environmental temperature was reached around 26-28 $^{o}C$. Eggs were stocked at a density of 25 Eggs $L^{-1}$ in different tank size (40, 300 and 2400 L, tanks) to determine the effects of tank size on eggs, hatching rate and survival of fish larvae. Three salinities (25, 30 and 37 ppt), and three light intensity (10000, 5000, and 2000 lux) were examined in 300L tanks and survival rates of larvae were assessed on days 3, 5, 7, 10, 15 and 20 of culturing period. There was no significant difference between hatching rate of *S. sutor* eggs, in various hatching tanks ($p$>0.05), but survival rates of larvae was significantly higher in 300 L and 2400 L tanks ($p$<0.05), the survival rate of *S. sutor* larvae was significantly higher in 30 ppt salinity than the others ($p$<0.05). There was no any significant difference between survival rate of larvae in salinity 25 and 37 ppt ($p$>0.05). The survival rate of larvae was significantly higher in 2000 lux intensity of light ($p$<0.05), under intense light (5000 and 10000 lux), the mortality of larvae was increased.

**Keywords**: Rabbitfish, Salinity, Light intensity, Survival rate, Hatching rate, *Siganus sutor*, Persian Gulf

1-Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Abbas, Iran,

2-Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran,

*Corresponding author's Email: fourooghifard@yahoo.com

## Introduction

In the recent years developing of mariculture has been regarded as an important contributor to the economies of the southern provinces of Iran. Biotechniques of artificial reproduction and seed production of some species of marine fish such as rabbit fish (*Siganus sutor*) is still in its nascent stages of development (Fourooghifard *et al*., 2009; Fourooghifard *et al*., 2017).

Rabbit fish belongs to the Siganidae family, which are widely distributed in the indo pacific, Indian Ocean, Red sea and Eastern Mediterranean regions and are subtropical, euryhaline, herbivorous fish and some species, are potentially important for aquaculture (EL-Dakar *et al*., 2007; Ignatius, 2009)

Rabbit fish are generally regarded as good food fish in the spite of their relatively small size, some species have been cultured because of their herbivorous food habits, rapid growth and economic value (Randall *et al*., 1997).

In general, the main factors that induce larvae mortality are biotic factors (e.g., starvation, diseases, parasites, and predation) and abiotic factors (e.g., oxygen, pH, salinity, toxic substances and temperature) (Kamler, 2011). More detailed information is needed about the water quality parameters and the environmental conditions (e.g. light conditions, temperature, turbulence, noise, etc.) that affect larval and juvenile performance and their development. The rearing condition in fish hatcheries is very different compare to the natural environment, and our knowledge about how the physical and chemical factors influence the growth and survival of larvae and juvenile of fishes is limited (Helvik *et al*., 2009).

Most of the species belong to Siganidae family are very tolerant to changes in salinity, it has been recommended that the incubation salinity of *S. guttatus* can be within the range of 10–51 ppt, and for yolk-sac larval maintenance, within the range of 14–37 ppt (Young and Dueñas, 1993). A tolerance of extreme fluctuations in salinity (2-55ppt) permits the species, *Siganus vermiculatus* alive in mangrove swamps (Gundermann *et al*., 1983).

An investigation on the effects of various salinity levels among (0‰, 5‰, 10‰, 15‰, and 20‰) on survival rate of common carp (*Cyprinus carpio*) larvae, showed that survival rate of larvae significantly reduced in salinities 15‰, and 20‰ (Malik *et al*., 2018).

Light is another factor which is essential for life of most plants and animals, even if a few species are able to do without it, as it is in the case of the deep sea aphotic zone or in caverns (Boeuf and Le Bail, 1999). Light intensity, quality and photoperiod are extremely variable and can change over a huge range, fish move within their environment and often their environment moves around them and can affect the light that the fish receives (Sumpter, 1992). Photoperiod and light intensity can affect the behavior, survival and growth rate of fishes as shown in *Clarias gariepinus* (Appelbaum and McGeer, 1998; Almazán-Rueda *et al*., 2004; Adewolu *et al*., 2008).

Tank size and shape can also affect the survival rate of fish larvae, for example, *Epinephelus coioides* larvae cultured in 3 $m^3$ tanks demonstrated higher survival rate (19.8%) at 24 day after hatching (DAH), compared with only 7.4% for those in 0.5 m3 tanks at 21 DAH (Duray *et al*., 1997). Survival rate of seven-band grouper *E. septemfasciatus* and devil stinger *Inimicus japonicus* larvae was higher in deeper tanks compare to the others (Ruttanapornvareesakul *et al*., 2007). Feeding rate of sergeant major, *Abudefduf saxatilis* larvae was significantly lower in 60 L rearing tanks and growth rates were significantly higher in 120 L rearing tanks, no larvae survived beyond 10 DAH in 60 L rearing tanks (Wittenrich *et al*., 2012).

The present study summarizes observations on the effect of salinity, light intensity and tank size on hatching and survival rate of Shoemaker rabbit fish *S. sutor* larvae.

## Materials and methods

This study was conducted at the Persian Gulf and Oman Sea Ecological Research Center (PGOSERI), Bandar Abbas, Iran, 2013. About 150 individuals of *S. sutor* (200-350 g bodyweight), were captured from the northern reefs of Lavan Island, Hormozgan, Iran (during October to November, 2012) and were transported to PGOSERI, aquaculture department. The fish were stocked in 5 $m^3$ round fiber glass tanks filled with filtered sea water at a density of 10 fish $m^{-3}$ (3-4 kg $m^{-3}$) and reared until reproduction season (Duray and Juario, 1988). The salinity was maintained at 37-38 ppt and temperature ranged between 18 – 28$^o$C. The tank water was exchanged 20% on every two or three days with filtered seawater and aerated with constant aeration. Feeding was done twice a day with commercial shrimp pellet ( 40% protein, 5% fat) and crab meat at a rate of 3% body weight (Fourooghifard, 2003).

Induced spawning of fish (350-450 g body weight) took place using injection of HCG hormone at a dosage of 2 IU $g^{-1}$ body weight (Ayson, 1991). Injections were administered twice (by 24 hour intervals) to each female, intramuscularly under the dorsal fin, at 8.00-9.00 a.m., during April–May when the environmental temperature was about 26-28$^o$C. Males were injected using $LHRH_{a2}$ hormone at a dosage of 20 µg per kg body weight under the dorsal fin at the same time with the second injections of females (Garcia, 1993). Injected fish were introduced to the spawning tanks at a ratio of 1 female to 2 males, after second injections (Hara *et al*., 1986). Spawning always took place 2 days after hormone injection.

Eggs of *S. sutor* like other siganids, are demersal and adhesive, so some eggs collectors consisting of corrugated fiberglass sheets were placed on the bottom of spawning tanks, prior to spawning (Nelson *et al*., 1992). The egg collectors were pull out from the spawning tank and were washed using a gentle water flow and fertilized eggs were collected in a plastic basin.

The diameter of eggs and length of larvae were measured using a stereomicroscope equipped with a microcomputer. Three different salinities (25, 30 and 37 ppt) were examined, in 300 L polyethylene tanks containing 250 L water, to determine the effect of salinity on survival rate of *S. sutor* larvae,. Eggs were stocked at a density of 25 Eggs $L^{-1}$**.** Effects of tank size (40 L , 300 L and 2400 L tanks) on hatching rate of eggs and survival rate of *S. sutor* larvae were assessed on days, 3, 5, 10, 15 and 20 after hatching (DAH).

Effect of light intensity (10000, 5000, and 2000 lx) on larvae growth was surveyed by adjusting the distance of light source from the water surface. Light intensity at the surface of water was measured using a light meter, Lutron, LX-1108 (Puvanendran and Brown, 1998) (Fig. 1).

**Figure 1: Adjusting of light intensity by changing the distance of light source from the water surface.**

All experiments were performed with 3 replications. Hatching rates were obtained by counting the larvae after 1 DAH and using the following formula (Molla *et al*., 2008).

$$\text{Hatching rate} = \frac{\text{Number of larvae}}{\text{Total eggs}} \times 100$$

Survival rates were obtained by counting the number of alive larvae per liter. Larvae were fed *Nannochloropsis oculata* and sieved rotifer (45- 80 µm) *Brachionus plicatilis* at a density of 500,000 cells $ml^{-1}$ and 15-20 ind. $ml^{-1}$, respectively.

Data were analyzed using SPSS 22.0 software through the application of parametric tests. Data were treated statistically by one-way analysis of variance (ANOVA), differences between the treatments means were compared by Duncan test. Differences were considered significant at $p<0.05$.

## Results

The fertilized eggs of *S. sutor* were adhesive, round and about 625.05±6.15 µm (n=30) in diameter (Fig. 2).The eggs of *S. sutors* hatched in temperatures higher than 28°C. Average length of newly-hatched larvae (1 day larvae) was about 2.97±0.68 mm with un-pigmented eyes. (Fig. 3) They started exogenous feeding 2 or 3 days after hatching, when their total length was about 2.8- 2.9 mm.

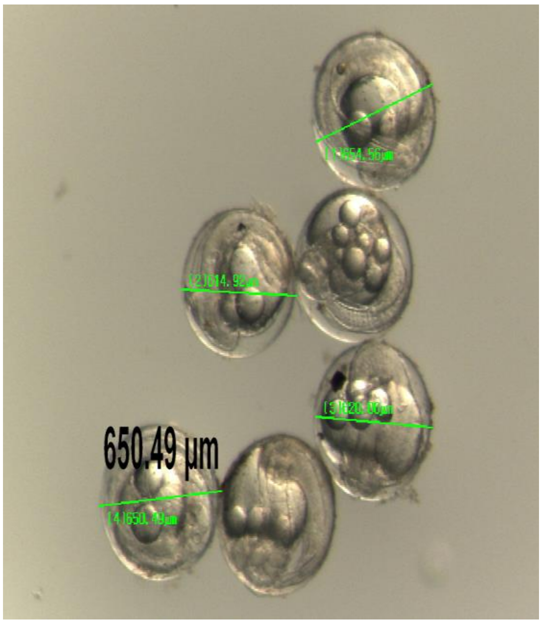


**Figure 2: fertilized eggs of *Siganus sutor* (16 hours after spawning).**

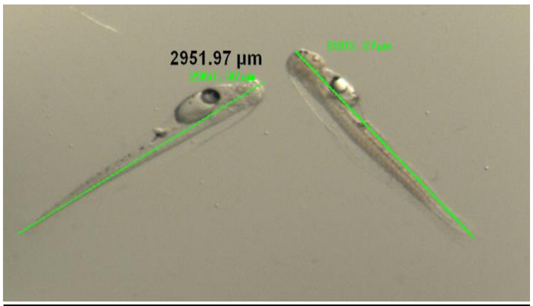


**Figure 3: newly hatched larvae of *Siganus sutor* (16 hours after hatching).**

Result of hatching rate of *S. sutor* eggs in 40 L aquarium was a little, lower than the other tanks, however, the difference between them was not significant ($p$>0.05) (Fig. 4).

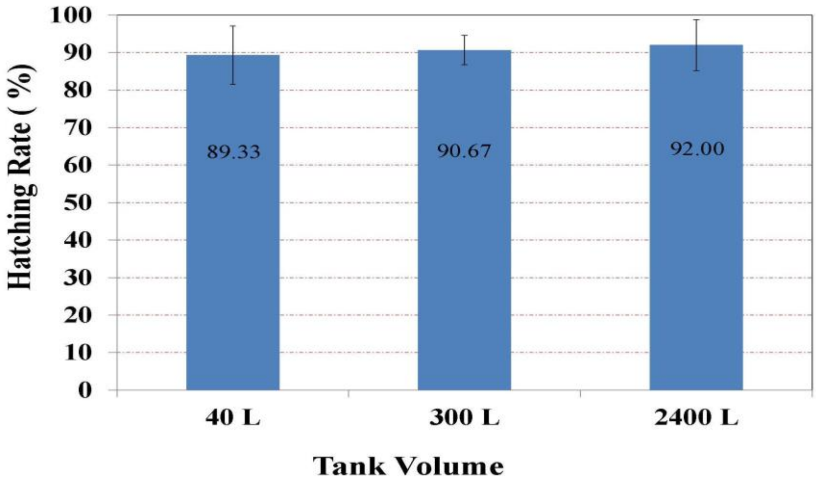


**Figure 4: hatching rate of *Siganus sutor* eggs in various tanks.**

Survival rates of *S. sutor* larvae during the first 20 days after hatching were affected by the tank size and ranged from 0.0, 2.53 and 3.67% for 40, 300 and 2400L tanks respectively, on day 20 of culturing period. Survival rate of *S. sutor* larvae in 2400 L tank was significantly higher than the other tanks ($p$<0.05). All of the *S. sutor* larvae reared in 40 L aquariums died during 10 days after hatching (Fig. 5).

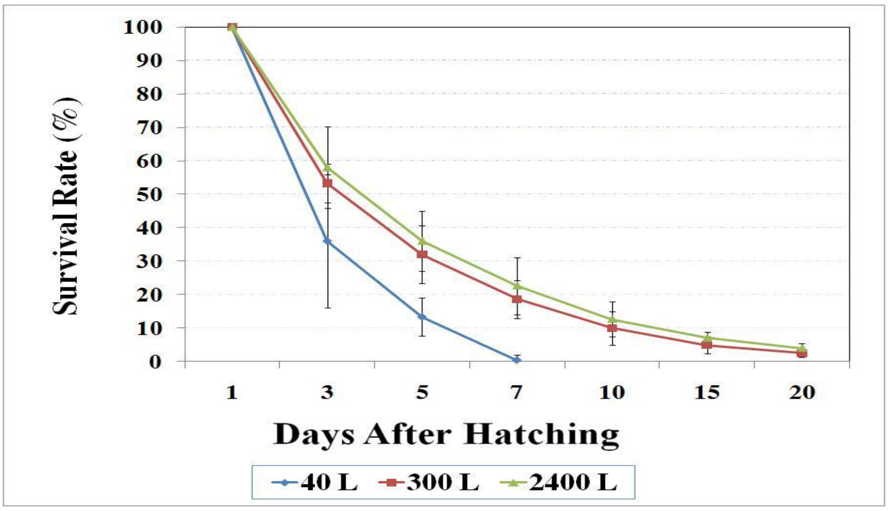


**Figure 5: survival rate of *Siganus sutor* larvae in various tanks.**

Light intensity affected the survival rate of *S. sutor* larvae. The survival rate of *S. sutor* larvae ranged from 2.55, 1.87 and 0.0% for 2000, 5000 and 1000 Lux of light intensity respectively which was significantly higher in 2000 lux intensity of light on day 20 of experimental period ($p<0.05$). By increasing of light intensity of light, mortality of larvae increased (Fig. 6).

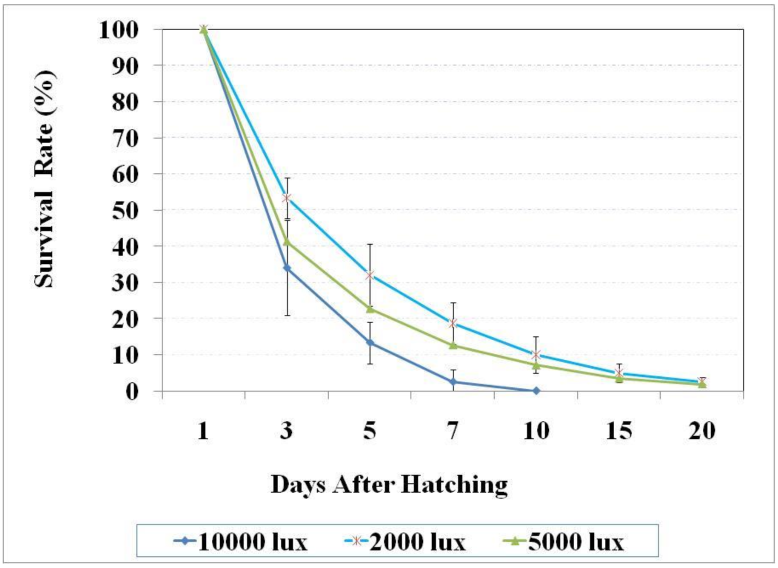


**Figure 6: survival rate of *Siganus sutor* larvae under various intensity of light.**

The survival rates of *S. sutor* larvae were 0.93, 2.53 and 1.33 for salinities 25, 30 and 37 ppt respectively which was significantly higher in salinity of 30 ppt than the others ($p<0.05$). There was no any significant difference between survival rate of larvae in 25 ppt and 37 ppt salinity ($p>0.05$) (Fig. 7).

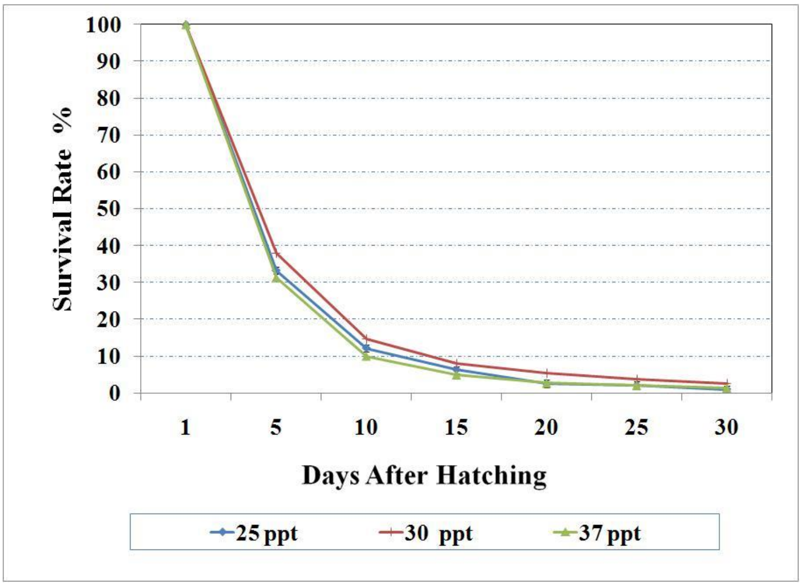


**Figure 7: survival rate of *Siganus sutor* larvae under various salinities.**

## Discussion

Results revealed that fertilized eggs of *S. sutor* were about 625.05±6.15 µm in diameter and average total length of newly-hatched larvae (1 day larvae) was about 2.97±0.68 mm. It can be said that Siganids have one of the tiniest eggs among the marine fishes. It seems that there is no direct relationship between fish eggs diameter and larvae length. According to the available reports, napoleon wrasse (*Cheilinus undulatus*) has a fertilized egg with diameter of 620-670 µm and larvae with total length of 1.5–1.7 mm (Slamet and Hutapea, 2005), egg diameter and larval length of cobia (*Rachycentron canadum*) ranged from 1.14-1.24 and 2.8-3.2 mm respectively (Yousif *et al*., 2011), the egg diameter and larval length of *S. rivulatus* were 630 µm and 1.80 mm respectively (Stephanou and Georgiou, 2000). The shoemaker rabbit fish *S. sutor* has relatively lanky larvae (2.97±0.68 mm), although their fertilized eggs are very tiny (625.05±6.15 µm), so they are very sensitive and fragile.

The survival rates of *S. sutor* larvae were, 4.00±1.35, 2.53±1.15, and 0.00% in 2400, 300 and 40L tanks respectively, this results revealed that increasing the tank size improves the survival rate of *S. sutor* larvae (Fig. 6). Tank size and shape have been found to affect the survival of fish larvae, for example, *E. coioides* larvae, cultured in 3 $m^3$ tanks demonstrated higher survival rate (19.8%) compared with only 7.4% for those in 0.5 $m^3$ tanks at 21 DAH (Duray *et al*., 1997). The same study on larval rearing of orange-spotted grouper, *E. coioides* indicated the survival rate of 16±9.94 and 10.67% for 2400 and the 300 L tanks respectively (Fourooghifard *et al*., 2017).

In contrast, a study on effect of tanks volume on survival rate of gilthead seabream (*S. aurata*) indicated that mean survival rates in 5 tons tank (12.56±0.47 %) was significantly higher than that in 15 tons tank

(9.83±0.76%) ($p$<0.05), mean total length and width of the larvae on day 38 after hatching were similar in both tanks (Başaran *et al*., 2004).

Light intensity affects the larval survival rate of *S. sutor*. Survival rate of *S. sutor* larvae was significantly higher in 2000 lux intensity of light with a rate of 2.53±1.15 % in comparison with 1.87±0.89% and 0.0% for 5000 lux and 10000 lux respectively ($p$< 0.05).

It has been reported that light intensity had a profound influence on larval growth and survival rate in cod (*Gadus rnorhua*), results obtained from rearing of cod larvae under four light intensities (300, 600, 1200 and 2400 lx) indicated that cod larvae grew and survived better in higher light intensity (2400 lux) (Puvanendran and Brown, 2002). The best growth rate for Chinese long snout catfish (*Leiocassis longirostris*) juveniles was observed under light intensity about 312 lux, the growth rate was significantly reduced at lower or higher intensities while light intensity did not affect the survival rate (Han *et al*., 2005).

Rabbit fish, *Siganus guttatus* larvae reared at continuous light have higher mean survival (31%) and bigger larval size at first feeding than those reared under natural daylight (17%) (Duray, 1998).

Studies have shown that most marine fish larvae are visual feeders and their feeding incidence increases with light intensity (Hunter, 1981; MacKenzie *et al*., 1999; Sawada *et al*., 2000). Lower swimming activity under continuous dim light may result in metabolic savings and increased body mass (Trippel and Neil, 2003). Cod larvae reared in higher light intensity captured prey more efficiently than larvae reared in low light (Puvanendran and Brown, 2002). Light intensity effects are not so clear and depend on the species, it is probably not an important factor for growth stimulation, most species need light to detect and catch feed, often optimal light for growth is not the same as for survival, too much light intensity can be stressful or even lethal (Boeuf and Le Bail, 1999).

Some studies have also suggested that light can act as a stressor in catfish which are known to be nocturnal fish and growth rate would be reduced as day length increase (Appelbaum and McGeer, 1998; Appelbaum and Kamler, 2000)

As result revealed, survival rate of *S. sutor* larvae was significantly higher in salinity of 30 ppt than the other salinities (Fig. 7). A comparative study on the effects of salinity on *S. guttatus* egg development and hatching rate, showed the highest total hatching and percentage of viable larvae at salinity of 24 ppt and the lowest at 8 ppt, larvae that hatched at lower salinities (8, 16 ppt) were relatively longer than those at salinity of 32 ppt and 40 ppt (Duray *et al*., 1986).

Successful rearing of fish larvae needs to understand the roles of various environmental factors which influence larval growth and survival rate. The survival rate of shoemaker rabbit fish, *S. sutor* larvae can be improved using larger tanks with carefully controlled environmental factors such as salinity and light intensity, further study about light intensity, photoperiod and live feed size, must be done to improve the survival rate of this valuable species.

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