Effects of stocking density on the survival, growth, size variation and condition index of juvenile rabbitfish *Siganus rivulatus*

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Abstract Spinefoot rabbitfish, Siganus rivulatus, is an economically important species of herbivorous fish that is relatively easy to rear and thus considered to be suitable for aquaculture. Juveniles are generally reared in nursery systems before being stocked into growout cages or ponds. We report here our evaluation of the effects of stocking density on the survival, growth, feed efficiency and condition index of S. rivulatus juveniles in nursery tanks. The experiment was conducted in a recirculating system of twelve 52-1 aquaria connected to a biological filter and a sand filter. Juvenile fish (average weight 6.5 g) were stocked into aquaria at four stocking densities (10, 20, 30, and 40 fish/aquarium) with three replicate aquaria per treatment. Diet was provided at 3% body weight daily divided into two feedings. Fish were weighed weekly for 8 weeks and the diet increased accordingly. Survival was greater than 95% in all treatments, with no significant differences observed among treatments. There were also no differences in specific growth rate (SGR 2.12-2.27) of the fish among treatments. Growth rate was linear during the 8 weeks in all treatments, and harvested biomass increased proportionally to stocking density (198, 401, 600 and 785 g per increasing stocking density, respectively). Feed efficiency (FE 0.67–0.71) of the fish did not vary significantly among treatments. The coefficient of variation was high (35-41%) among the harvested fish, but it also did not differ significantly among treatments. The final condition indices of the fish in all treatments were similar to each other but significantly greater than the initial values (P < 0.05). The results suggest that there is no apparent effect of stocking density at the levels tested on the survival and growth of juvenile rabbitfish.

Keywords Rabbitfish · Siganus rivulatus · Stocking density

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Introduction

Siganids are a relatively small family of herbivorous fishes widely distributed in the Indo-West Pacific Region (Woodland 1983). The opening of the Suez Canal in 1869 linked the Red Sea to the Mediterranean and resulted in the invasion of the eastern Mediterranean Sea by two siganid species, one of which is *Siganus rivulatus* (Por 1978; Boudouresque 1999; Fishelson 2000; Galil 2000; Quignard and Tomasini 2000). *Siganus rivulatus* was first recorded in the Levant basin in 1927 (Tortonese 1970) and has subsequently established large populations in its new environment. As such, it can be considered amongst the most successful of Lessepsian fishes (George 1972; Ben-Tuvia 1985; Papaconstantinou 1990). Additionally, rabbitfish have a high market value in Eastern Mediterranean countries (Stephanou and Georgiou 2000).

Siganids are economically important and relatively easy to rear and, as such, are considered suitable for aquaculture (Lam 1974; Bryan and Madraisau 1977; Juario et al. 1985; Hara et al. 1986). Various researchers have studied the feasibility of culturing the rabbitfish S. rivulatus in the Mediterranean region (Ben-Tuvia et al. 1973; Popper et al. 1973, 1979; Popper and Gundermann 1975; Stephanou and Georgiou 2000), yet to date little data are available on the growth of juveniles in nursery tanks prior to stocking in ponds or cages. One of the most important factors that has yet to be determined is how many juveniles can be reared in nursery tanks. Stocking density may or may not cause detrimental effects on fish survival and growth (Barton and Iwama 1991), depending on the species of fish being reared. In many fishes, an inverse correlation exists between growth rate and stocking density (Carr and Aldrich 1982; Mackintosh and De Silva 1984; Papoutsoglu et al. 1990; Lambert and Dutil 2001; El-Sayed 2002), while in others growth ameliorates with stocking density (Jørgensen et al. 1993; Papoutsoglou et al. 1998; Wallace et al. 1988). Alternatively, some species of fish, such as Atlantic salmon Salmo salar reared in tanks and tambaqui Colossoma macropomum reared in cages, have shown no difference in growth with changes in stocking densities (Kjartnsson et al. 1988; Gomes et al. 2006).

Since *S. rivulatus* is a schooling fish in nature (Popper and Gunderman 1975), one would expect little competitive behavior among individuals reared at high densities. In the study reported here, juvenile rabbitfish were reared at four densities, and the effects on survival, growth, feed efficiency and condition index were evaluated.

Materials and methods

The present work was conducted at the marine laboratory of the American University of Beirut (AUB). Juvenile rabbitfish were caught in traps in Batroun Bay and transported to AUB where they were held for 1 week in a 1-m^3 round tank and trained to accept a commercial dry feed. The research setup consisted of a recirculating system of twelve 52-1 ($58 \times 30 \times 30 \text{ cm}$, L × W × H) glass aquaria connected to a biological filter, a water pump and a sand filter, which was filled with filtered seawater. The flow rate into each aquarium was 55 l/h. Water used in the experiment was pumped from the sea off the Batroun beach ata depth of 15 m, stored in large closed tanks, chlorinated for 48 h, dechlorinated and EDTA added to remove possible heavy metal contamination. The water level in all aquaria was maintained using an internal standpipe, and each aquarium was equipped with a submersible air diffuser connected to a regenerative blower. The faces and tops of all aquaria were covered with Styrofoam sheets in order to reduce mortality due to

jumping and stress related to people feeding the fish and maintaining the system. Water temperature was maintained at 28°C, salinity at 35 ppt, oxygen remained above 5.5 mg/l, pH was held between 7.9 and 8.2, ammonia was below 0.02 mg/l and nitrite was below detection limits. The light regime was set at 12:12 h (light:dark).

At the start of the experiment, fish were hand sorted to similar sizes. Sorted fish $(6.5 \pm 0.06 \text{ g})$ were divided into groups often and placed in 30 buckets. An additional 20 fish were individually weighed and their length measured to estimate the condition index. Fish in each bucket were group weighed and stocked into the aquaria, resulting in four stocking densities (10, 20, 30 and 40 fish per aquarium) with three replicates per treatment. All fish in each aquarium were group weighed every 2 weeks. They were offered a 50% crude protein, 20% lipid trout commercial diet (Golden Extruded, Chile) at 3% body weight divided into two daily feedings during the week following weighing. The ration was then increased by 20% and offered to the fish for 1 week until they were weighed again. Eight weeks post-stocking, all fish were harvested, each group was weighed and counted, and then each fish was individually weighed and its length measured.

Weight gain and survival for each treatment were determined. The average weight of fish and the coefficient of variation for each tank were also calculated. Feed efficiency (FE = weight gain × 100/diet fed) was estimated based on feed inputs. Length and weight measurements were used to calculate Fulton's condition index: $K = 100 \times W/L^3$, where W is the weight in grams and L is the total length in centimeters. The specific growth rate (SGR) was calculated as: SGR = $100 \times [(LnW_f - LnW_I)/t]$, where Ln is the natural log, W_f is final weight of fish, W_I is the initial weight and t is time in days. A growth curve (weight vs. time) for each replicate was drawn using all biweekly weight measurements for each tank, and the slopes were compared among treatments. All results were compared using one-way ANOVA and the Student–Newman–Keuls multiple comparison test (Steel and Torrie 1980). All statistical analyses were conducted using SPSS (V.8 for Windows; SPSS, Chicago, IL, USA).

Results

There were no apparent effects of stocking density (10, 20, 30, 40 fish per 52-1 aquarium) on the survival and growth of rabbitfish at the levels tested. Survival was greater than 95%, and growth during the 8-week experimental period was greater than 300% in all treatments (Table 1). Similarly, no differences in SGR and FE were observed among treatments. The final condition indices of the fish in all treatments were similar to each other but significantly greater than the initial condition index. Harvested biomass increased proportionally to stocking density (Table 1), and there were no differences in the coefficient of variation among treatments. Growth was linear over the 8 weeks in all treatments, with no differences apparent in slope (Table 2).

Discussion

The rabbitfish *Siganus rivulatus* is a schooling fish in nature, and this behavior is apparent in the results of this study reported here. There was no influence of fish number (10, 20, 30, 40) per unit volume of container (52 l) on survival and growth. Similar results were observed by Gomes et al. (2006) working with tambaqui. These researchers stocked fish at 20, 30, 40 and 50 fish/m³ and found no differences in survival or growth among treatments.

Table 1 Initial w (FE), coefficient c	eight (W ₁), initial of variation of harv	condition in vested weig	Table 1 Initial weight (W_I), initial condition index (K_I), harvested biomass (B), final average individual weight (W_F), survival (S), final condition index (K_F), feed efficiency (FE), coefficient of variation of harvested weights (CV) and specific growth rate (SGR) of rabbitfish stocked at 10, 20, 30 and 40 fish per 52-l aquarium	biomass (B), final a fic growth rate (SG	verage individua R) of rabbitfish s	weight (W _F), sur tocked at 10, 20, 3	vival (S), final con- 30 and 40 fish per	dition index (K _F), 52-1 aquarium	feed efficiency
Fish/tank (n) W ₁ (g)	$W_{I}(g)$	K_I B $(g)^a$	$B (g)^{a}$	$W_{\rm F}$	$S \left(\% \right) \qquad \qquad K_{F}$	\mathbf{K}_{F}	FE	CV	SGR
10	6.50(0.09)	1.09	198 (6.2) a	21.20 (0.29)	96.7 (3.3)	1.31 (0.01)	0.67 (0.03)	0.35 (0.03)	2.12 (0.05)
20	6.50 (0.06)	1.09	401 (17.9) b	23.20 (0.95)	98.3 (1.7)	1.31 (0.01)	0.70 (0.03)	0.37 (0.01)	2.27 (0.06)
30	6.30 (0.03)	1.09	600 (3.3) c	21.70 (0.59)	98.9 (1.1)	1.31 (0.01)	0.71 (0.02)	0.41 (0.02)	2.21 (0.04)
40	6.50 (0.06)	1.09	785 (30.7) d	22.10 (0.76)	95.8 (2.2)	1.33 (0.01)	0.67 (0.04)	0.37 (0.01)	2.18 (0.06)

10	6.50(0.09)	1.09	198 (6.2) a	21.20 (0.29)	96.7 (3.3)	1.31 (0.01)	0.67 (0.03)	0.35 (0.03)
20	6.50(0.06)	1.09	401 (17.9) b	23.20 (0.95)	98.3 (1.7)	1.31 (0.01)	0.70(0.03)	0.37 (0.01)
30	6.30 (0.03)	1.09	600 (3.3) c	21.70 (0.59)	98.9 (1.1)	1.31 (0.01)	0.71 (0.02)	0.41 (0.02)
40	6.50(0.06)	1.09	785 (30.7) d	22.10 (0.76)	95.8 (2.2)	1.33 (0.01)	0.67 (0.04)	0.37 (0.01)
PSE^b	0.06	I	18.2	0.69	2.24	0.008	0.027	0.018
Values in parenth	Values in parenthesis are the standard error of mean	rd error of	mean					

0.053

^a Values followed by different letters are significantly different from each other at P < 0.05

^b PSE, Pooled standard error

Fish/tank (n)	Slope (a)	Intercept (b)	R^2
10	1.829 (0.012)	5.93	0.99
20	1.963 (0.076)	5.65	0.98
30	1.858 (0.063)	5.47	0.97
40	1.881 (0.075)	5.80	0.98
PSE	0.06	0.07	-

Table 2 Slope, intercept and R^2 of growth linear model (y = ax + b) of rabbitfish stocked at 10, 20, 30 and 40 fish per 52-1 tank

Values in parenthesis are the standard error of mean

Other researchers have reported results where fish such as Arctic charr or European sea bass grew faster at high stocking densities (Wallace et al. 1988; Jørgensen et al. 1993; Papoutsoglou et al. 1998). Conversely, most growth studies report a negative correlation between stocking density and growth rate (Papoutsoglou et al. 1987; Holm et al. 1990; Lambert and Dutil 2001; El-Sayed 2002). However, it is difficult to compare the results of the various authors since some studies were performed with larval fish while others used juveniles and yet others used adults. Since some fishes are cannibalistic as larvae (Hecht and Pienaar 1993) but might or might not exhibit other agonistic behavior as adults, while other fish are non-aggressive as juveniles but become territorial with age (Goldan et al. 1997), it is impossible to compare results among fishes of different age groups (Kjartansson et al. 1988). Furthermore, some researchers report densities as biomass per unit volume, again making comparisons difficult. The results of our study show that rabbitfish juveniles (4–8 months old) can be maintained at densities as high as 40 fish per 52-1 tank (770 fish per m³) with no negative effect on survival and growth, assuming water quality remains within the tolerance levels for the species.

The condition index of the juvenile rabbitfish used in our study improved during the specified growth period. This can probably be attributed to the fact that the feed offered to the fish was very high in protein and lipid, while the diet they consume in nature tends to be low-energy algae (Lundberg and Lipkin 1979; Lundberg and Golani 1995). Weight-length relationships, such as the Fulton condition index used in the present experiment, are used for various reasons, such as comparing a population's 'well-being' among the same fish species in nature (Anderson and Neumann 1996) or evaluating the physiological health of fish. For example, Lambert and Dutil (2001) found a negative effect of an increased stocking density of cod Gadus morhus on the condition index and postulated decreased food intake as the cause. A decreased food intake is often associated with increased stress. Interestingly, however, Lambert and Dutil (2001) observed a significant increase in the condition index in some of their experiments with cod but attributed this result to the fact that they might have started with post-spawning fish. Jørgensen et al. (1993) found that the condition of Arctic charr reared for 9 weeks at high stocking densities improved with time, while the condition of the same fish stocked at low densities for the same time did not. In both of the cited experiments, the condition index changed in parallel with growth. However, K is not always indicative of the direction of growth. When K was backcalculated from data given by Papoutsoglou et al. (1998) for European sea bass, we found no increase in the condition of the fish during the growth period nor differences among treatments, although all fish had grown during the experiment and those at high densities had grown more than those at low densities.

The FE of the fish in our study did not vary with stocking density. These results might be due to the fact that the fish were underfed at 3% body weight and thus were more efficient at converting feed to body weight. There is no published information on feeding levels for *S. rivulatus*. Parazo (1990) offered *S. guttatus* a feed containing 45% protein and 7.9% lipid at 5% body weight daily and obtained rapid growth. Since the food used in the present experiment contained 50% protein and 20% lipid, we decided to offer the fish less feed daily. Accordingly, the present results might not indicate optimal growth rates for the fish, but that was not the purpose of the experiment. The feed efficiency results of our study suggest that an increase in stocking density did not affect fish metabolism or food acquisition; this is in contrast to results described by Koebele (1985) for *Tilapia zilli*. Feed efficiency results are important when making an economic analysis of fish production, but they are not indicative of fish growth potential. Papoutsoglou et al. (1998) and Gomes et al. (2006) found that the FE ameliorated in faster growing fish, while El-Sayed (2002) reported a negative correlation between growth and feed efficiency in Nile tilapia stocked at various densities.

The coefficient of variation (CV) was high (35-41%) among the harvested fish. Jobling and Baardvik (1994) state that CV values above 10% are indicative of non-homogeneity within a group of fish. In aquaculture, as in any kind of animal husbandry, it is desirable to have a homogeneous animal size since that would facilitate feeding, harvesting, marketing and processing. The fish used in our study were sorted before stocking, but the variability in initial size was not estimated and, therefore, we cannot draw any conclusions about the effects of initial size variability on CV of harvested fish. However, since the variability was similar in all treatments, we can conclude that size depensation was due to genetic variation among individuals. As rabbitfish aquaculture develops and cultured populations become more domesticated, size depensation can be expected to decrease. In the meantime, fish from a nursery can be sorted once again before being stocked into growout facilities, which may improve growth and decrease size variability at harvest (Saoud et al. 2005). Further research on size grading before stocking is needed to evaluate the growth depensation of rabbitfish. Average growth rate, however, was similar at all densities. Growth was linear in all treatments throughout the 8 weeks of culture, making it possible to compare SGR values between the initial and final weights of the fish after 8 weeks of culture.

Water quality in the present experiment remained good throughout the 8-week trial. However, as the fish grew larger and feed input increased, the biological filter certainly became more loaded. Although the increase in biomass per tank was directly proportional to the stocking density at the levels tested, total biomass in the system did not reach levels stressful to the filter. An increase in number of fish per culture unit is desirable since high stocking densities generally reduce production costs per unit of fish (Huguenin 1997). However, as biomass per tank increases, so does the quantity of feed offered, resulting in potential water quality and oxygen concentration problems. Therefore, experiments on tolerance to various water quality parameters should be investigated separately from density experiments, and then total biomass possible per unit volume calculated could be based on the metabolic parameters and feed requirements of the fish. However, as stated by Fréchette (2005), stocking density experiments are more relevant when reported as number of fish per volume of water rather than as biomass per volume. Biomass affects water quality, which is a technical and engineering issue that can be addressed, while fish number involves an effect of the fish on each other and, as such, is an effect over which we have no control.

To conclude, the results of our study suggest that *Siganus rivulatus* is a good candidate for intensive aquaculture. Juveniles are relatively easy to rear at high densities in tanks as long as good water quality is maintained, and the fish can tolerate handling when being harvested to be sorted and stocked into growout facilities.

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