

Hybridization and Larval Rearing of *Sparidentex hasta* x *Acanthopagrus latus* and their Reciprocals

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Abstract: *Sparidentex hasta* is superior over *Acanthopagrus latus* in market price and growth rate while *A. latus* is superior over *S. hasta* in number of eggs produced, hatching rate, larval survival and eventually number of fry produced. Thus, the possibility of obtaining a hybrid with close resemblance in appearance and growth rate to *S. hasta* and being superior in fry production resembling *A. latus* would be an encouraging hybrid to seek as being the aim of this study. The broodstocks used for this study, *S. hasta* and *A. latus*, were injected with human chorionic gonadotropin hormone to initiate spawning. The eggs were collected and manually fertilized successfully to produce the following types: *A. latus* (♀) x *A. latus* (♂), *A. latus* (♀) x *S. hasta* (♂) (Hybrid I), *S. hasta* (♀) x *S. hasta* (♂), and *S. hasta* (♀) x *A. latus* (♂) (Hybrid II). The larval survival rate at 54 day after hatching of *A. latus* and Hybrid II was significantly the highest ($p < 0.05$) followed by Hybrid I and *S. hasta*. The mean fry weight of Hybrid II was significantly higher ($p < 0.05$) than both parent's fry at age of 54-d, and not significantly different ($p > 0.05$) from Hybrid I fry. The resemblance of hybrid II to *S. hasta* in body appearance will fetch a better market price while being similar to *A. latus* in larval survival will imply superiority in fry production. Thus hybrid II would be selected and recommended for further work to evaluate its growth rate to market size fish.

Keywords: *Sparidentex hasta*, *Acanthopagrus latus*, hybrids, egg fertilization, hatching, survival.

INTRODUCTION

Over the last twenty years, an increasing literature on hybridization of the family *Sparidae* has started to appear, as in the 1980s in Japan [1] and in the early 1990s in Europe [2]. Skakelja *et al.*, [3] compared the karyological characteristics in four genera of the family *Sparidae* and concluded that it has high level of homogeneity which implies the high possibility of successful intergeneric hybridization. More than eleven species in seven genera with more than fifteen different crosses were cited in the literature; *Pagrus major* x *Eyynniss japonica* [1], *Sparus aurata* x *Diplodus puntazzo* and *Sparus aurata* x *Diplodus vulgaris* [2], *Diplodus sargus* x *Dentex dentex* [4], *Pagrus major* x *Acanthopagrus schlegeli* and *Pagrus major* x *Sparus sarba* [5], *Sparus auratus* x *Acanthopagrus bifasciatus* [6] *Dentex dentex* x *Pagrus major* and their reciprocals [7], *Sparus auratus* x *Pagrus major* [8], *Pagrus pagrus* x *Pagrus auriga* [9], *Pagrus major* x *Acanthopagrus schlegeli* [10], *Sparus auratus* x *Pagrus pagrus* [11].

Preliminary work in Kuwait on the hybridization of *A. latus* x *S. hasta* [12] indicated the possibility of a good candidate for aquaculture. The study concluded similar growth between *S. hasta* and the hybrid produced (*A. latus* female with *S. hasta* male). The reciprocal cross was not produced in that study. There were no previous publications available on the study of hybrids between *A. latus* x *S. hasta*.

The research on hybridization of the two sea breams *A. latus* x *S. hasta* was first reported in 2000 [12] and secondly in 2008 [18], since then no further scientific work was published.

A. latus and *S. hasta* are classified under the same order Perciformes and family *Sparidae*, but different subfamilies; *Sparinae* and *Denticinae*, respectively [13]. Both of *S. hasta* and *A. latus* is protandrous hermaphrodites, changing sex to female starting from the second year of age. The two species are considered of high commercial values in Kuwait and the Arabian Gulf region fetching a price of around \$US 10-15 per kg. The preferred marketable size of *S. latus* is around 300 to 700 g while for *S. hasta* is around 500 to 2000 g. The two species were produced commercially in floating cages in Kuwait and the Gulf region exceeding 300 tons per year [14].

The gained experience over the past thirty years in breeding and fry production of *A. latus* and *S. hasta* at Aquaculture, Fisheries and Marine Environmental Department (AFMED), Kuwait Institute for Scientific Research (KISR), will rank *A. latus* as superior over *S. hasta* in hatching rate, larval survival and eventually number of fry produced [12]. The mass fry production of *A. latus* is known to be unproblematic compared to *S. hasta*. The cannibalistic behavior of *S. hasta* starting from age of the third week onwards explains the difficulty of *S. hasta* mass fry production [15]. Probably, the non cannibalistic character of *A. latus* could be the reason for this superiority over *S. hasta* in mass fry production.

S. hasta juveniles showed faster growth rate than *A. latus* which has an effect on growth duration to market size. Thus, the shorter duration of the grow-out operation will have positive effect on the economics of the farm [12].

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Therefore, obtaining a hybrid similar to *A. latus* in hatchery performance with close resemblance in appearance and similar or higher growth than *S. hasta* would be the aim of this study.

This manuscript covers all the activities carried on the broodstock maintenance, selection and sexing of *A. latus* and *S. hasta* breeders. It also covers hybridization success, fertilization and egg hatching; larval rearing procedures; larval survival and growth performance for the following crosses; *A. latus* (♀) x *A. latus* (♂), *A. latus* (♀) x *S. hasta* (♂) (Hybrid I), *S. hasta* (♀) x *S. hasta* (♂), and *S. hasta* (♀) x *A. latus* (♂) (Hybrid II).

MATERIALS AND METHODS

Maintenance, Selection and Sexing of *A. latus* and *S. hasta* Breeders

The broodstock were kept in a flow through system. Filtered ambient seawater (40 g l⁻¹) was pumped continuously to the broodstock tanks to achieve at least 8 to 10 changes day⁻¹. Water quality was monitored daily for temperature and dissolved oxygen, and weekly for pH and salinity. The incoming ambient and borehole seawater were controlled and mixed to achieve 28 to 30 °C in summer and 22 to 24 °C in winter (spawning temperature). The tanks bottom were siphoned and cleaned every other day.

The broodstock used were bred at AFMED, KISR as the third filial generation (F3). The selection made during the first two filial generations was based on faster growth character. *A. latus* male will reach maturity during first year while the female during the second year. Similarly, *S. hasta* male will reach maturity at end of the first year while the female at the end of the third year. The production of the selected F3 breeders of *A. latus* and *S. hasta* required 6 and 9 yr, respectively [12].

Feed Preparation and Feeding

The broodstock were fed with highly nutritious feed containing the essential nutrients, such as adequate levels of highly unsaturated fatty acids (HUFA), vitamins and minerals [15]. The breeders' feed consisted of two components; namely, fresh or frozen small fish (sardine, mullet, shrimp, squid) mixed with additives (vitamins, minerals and marine oil), and a semi dry pellets for breeders by INVE, Greece. The breeders were fed until satiation.

Sex Ratio and Biomass Manipulation for Spontaneous Spawning

A. latus and *S. hasta* mature breeders were stocked in two 30 m³ fiberglass circular tanks with a sloping bottom. The yearly thermal cycle of the water temperature is ranging between 14 to 34 °C. The gradual increase of the winter water temperature from 14 to 20 °C (February) for *S. hasta* and to 24 °C (March) for *A. latus* will trigger the final gonadal maturation and ovulation. This event coincides with the increasing of the photoperiod from 10 hr and 20 min to 11 hr and 20 min. The initiation of spermiation in both species will start six weeks before spawning. Off-season, the functional breeders were kept at normal stocking densities of 10 to 15 kg m⁻³. Three to four weeks before spawning, the breeders were sexed by gently pressing their abdominal area toward the genital opening. The males were identified by

milting while females were sampled by a catheter to determine the egg developmental stage under the microscope which was predicted previously from several trails, when the examined eggs is expected to be ovulated. Thus, the approximate date of spawning could be estimated. The sex ratio was corrected to be 1 (♀): 1 (♂) for both species to ensure successful spontaneous spawning. This correction was mandatory due to the fact that both *S. hasta* and *A. latus* are protandrous hermaphrodite and the nature of group spawning behavior of both species. At the same time, the fish biomass was adjusted to ensure optimal fish density. The comfort biomass for spawning differs from one fish species to another, but it can be generalized to 5 and 7 kg m⁻³ for *S. hasta* and *A. latus*, respectively. The mean body weight of the males and the females of *S. hasta* were 1.0-2.0 and 3.1-5.8 kg, respectively. However, the mean body weight of the males and the females of *A. latus* were 0.15-0.35 and 0.55-1.8 kg, respectively.

The spawning season of both species of fish used in this study was in February and March, 2007. Since *A. latus* spawns after *S. hasta* by 3 to 4 wk, the water temperature was manipulated in *A. latus* tank by gradual increase from 19.0 to 22.5 °C by the end of January to synchronize gonad development of the two species to ensure hybridization. The standard procedures adopted for broodstock maintenance and husbandry were given by [15].

Testing the Hybridization Success, Fertilization, Egg Floating and Egg Hatching

The hybridization was carried out manually by stripping the ripe eggs from female *A. latus* and adding the milt of male *S. hasta* [7]. This process was standardized to produce all the needed fertilized eggs of the reciprocal cross and the original parent fishes over 10 days period. Each cross was the result of mixing one female eggs with 3 males for each trail. Sometimes more females were used (2 to 3) in order to obtain the required number of fertilized eggs. The number of manually striped trials for *A. latus* x *A. latus*, hybrid I, *S. hasta* x *S. hasta* and hybrid II was as follows: 9, 16, 13 and 21, respectively.

The standard procedure used for the preparation of fish stripping and eggs collection was started by selecting runny males and big, soft bellied females of *A. latus* and *S. hasta*. The selected females were transferred to two new tanks of 30 m³ fiberglass circular tanks. The males of both parental species (50 each) were stocked in two separate 30 m³ fiberglass circular tanks. Twenty two of *S. hasta* (♀) and thirty eight *A. latus* (♀) were injected with primary and secondary doses separated by 24 hr. The ovulating hormone used was the chorionic gonadotropin HGC manufactured by Pregnyl, Holland. The amount of hormone injected ranged from 250 to 1000 IU kg⁻¹ (International Unit per kilo gram of fish, based on belly size and softness). The body weight ranges were 3.1 – 5.8 kg and 0.55 – 1.8 kg of *S. hasta* and *A. latus* (♀), respectively. The ovaries in the selected soft-belly females were sampled by using the catheter to obtain eggs, which were examined under the microscope to determine their size and development stage. The ova size determines whether or not to inject the ovulation hormone. Fish with ova larger than 500 microns in diameter were injected. The method used to inject the hormone was intramuscular via the dorsal muscle. The success of manually induced eggs from

Table 1. The Total Amount of Floated, Sunken Eggs and their Floating, Hatching and Fertilization Percentages Based on Several Trials

Fish Type	Floating (ml)	Sunken (ml)	Floating (%) [*]	Fertilization (%)	Hatching (%)
<i>A. latus</i> (♀) x <i>A. latus</i> (♂)	140	180	43.8 ^a	64.5 ^a	76.6 ^a
<i>A. latus</i> (♀) x <i>S. hasta</i> (♂)	395	590	40.1 ^a	65.3 ^a	63.8 ^b
<i>S. hasta</i> (♀) x <i>S. hasta</i> (♂)	350	940	27.1 ^b	53.3 ^a	34.2 ^c
<i>S. hasta</i> (♀) x <i>A. latus</i> (♂)	716	990	42.0 ^a	74.6 ^a	57.5 ^b

* Values with different superscript in each column are significantly different at $p < 0.05$ according to Duncan's multiple-range tests using SPSS for Windows.

the ovulated females was 80% of the injected females. The number of males used for the production of the four crosses was 50 each, no hormone injection was used.

The only soft belly female that gave transparent eggs when applying a gentle pressure on its belly was considered to be ready for fertilization. Usually the duration between injection and egg collection was about 598 (one injection) to 1196 (two injections) degree hours at 23 °C. The soft belly female was gently hand striped during the morning hours into a dry plastic bowl. When adequate volume (>200 ml) of viscous and transparent eggs of *S. hasta* eggs were obtained, 2 or 3 *A. latus* (♂) were milted (>2 ml) immediately onto the collected eggs. The eggs and milt were gently mixed and kept for 2 to 3 min then hydrated by adding seawater and kept for 5 to 10 min. The hydrated eggs were then washed by passing gently through a 200- μ m mesh to remove all excess sperms. The eggs were separated into floating and sunken by a settling method using a 1000-ml graduated cylinder. The same procedures were repeated to get the fertilized eggs for the four types of crosses. Examinations of the floating eggs to check fertilization percentage, embryo development stage were carried to each of the four types using a powerful dissecting microscope.

The floating eggs were incubated in a suspended open top cylindrical cloth mesh with 400 micron at the bottom and with 100 micron of side walls. Four of these nets were placed in a rectangular incubation trough with running seawater. A single small air stone was placed in the center of each hatching net. The floating eggs for each of the four types of crosses collected as four separate treatments were incubated to calculate the mean hatching percentages. Egg hatching started after 32 hrs from fertilization at a temperature of 21.0 °C. The volume of floating eggs collected, fertilization and hatching percentages for each of the four types of crosses carried out were considered as one treatment with several unequal replicates.

Compare Growth and Survival Performances of *A. latus* and *S. hasta* Larvae and their Hybrids

The newly hatched larvae from the four different types were collected from the egg hatching nets and their total length was measured using a high power dissecting microscope. The four types of the larvae produced namely; *A. latus* (♀) x *A. latus* (♂), *A. latus* (♀) x *S. hasta* (♂) (Hybrid I), *S. hasta* (♀) x *S. hasta* (♂), and *S. hasta* (♀) x *A. latus* (♂) (Hybrid II), were stocked randomly in twelve

round fiberglass 1-m³ tanks as four treatments with three replicated each. Each hatching net was divided into the three replicates of each cross until the required number of larvae was fulfilled. The initial larval stocking density for each of the four types was 30 larvae l⁻¹. The larval rearing procedures and live food production (algae, rotifers and *Artemia*) and enrichments were followed according to [16, 17 and 18, respectively]. Two inert encapsulated larval diets (Proton 2/3, 200 to 300 μ m in size) and commercially available (Proton 3/5, 300 to 500 μ m in size) larval diets from INVE Aquaculture Nutrition in Greece, were used for weaning. The duration of the experiment was 54 days.

This study focuses on the following features: The total 208 larval length (n=30) was measured at 0, 5, 10, 15, 39 and 54 DAH (Day After Hatching); survival at 10 and 54 DAH; fry weight at 54 DAH; and cannibalistic behavior at 20 to 40 DAH. All the larval rearing factors were standardized to all of the four treatments unless of the type of fish.

Water Quality

Water-quality parameters, in the larval rearing tanks such as daily water temperature, dissolved oxygen (DO), and weekly pH and ammonia were monitored and recorded.

Data Analysis

The raw data of total floating eggs, fertilization and hatching percentages, together with the larval survival, total length, fry weight, ammonia contents, DO and pH were analyzed using one way analysis of variance (ANOVA) with unequal replicates. Data from the different larval rearing tanks for the four different types of fry produced were tabulated and prepared for statistical analysis. ANOVA was conducted using SPSS for Windows (13.0, SPSS Inc., Chicago, USA). If significant differences between means were determined, the Duncan's multiple-range test was applied. In all analyses, the statistical significance level was at $p < 0.05$ [19].

RESULTS

Fertilization, Egg Floating and Egg Hatching

The total amount of eggs obtained for the four types of fish according to their buoyancy (floating and sunken) and their percentages of floating, hatching and fertilization were measured (Table 1). *S. hasta* showed significantly lower floating percentage (27.1%, $p < 0.05$) compared to the other three fish types which showed no significant difference

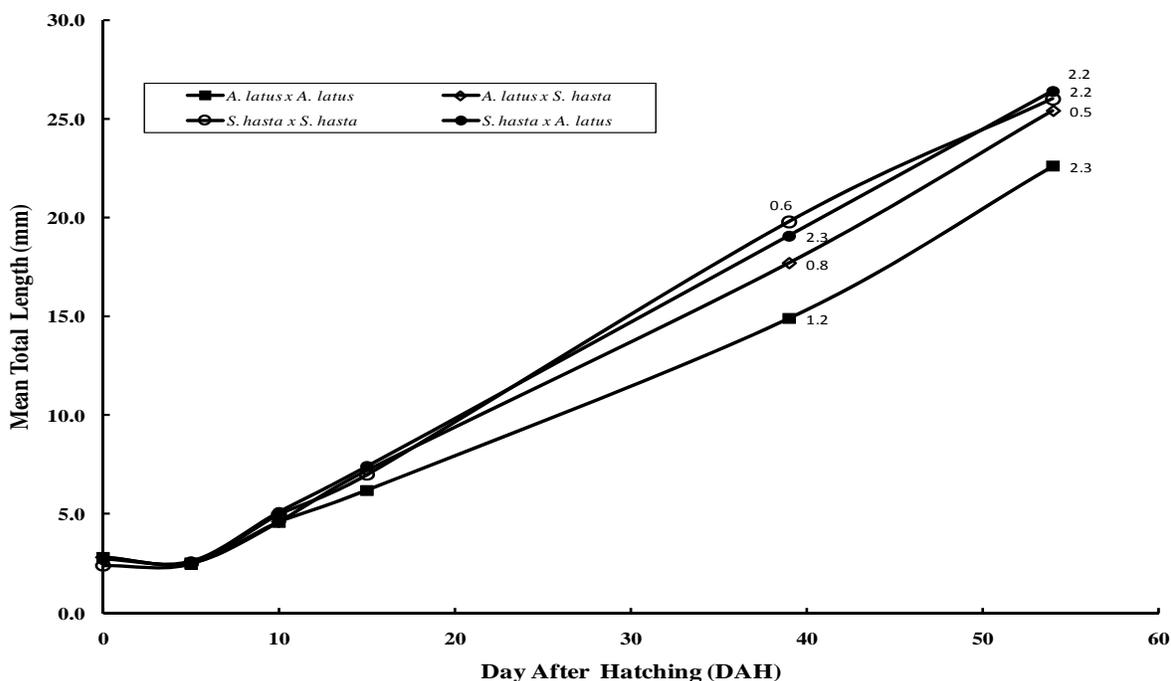


Fig. (1). Mean total length of the *A. latus* x *S. hasta* (Hybrid I), *S. hasta* x *A. latus* (Hybrid II) and their parents fry up to 54 DAH. Standard deviation at age 0, 5, 10 and 15 cannot be presented in the figure due to low variations between treatment means. (Values shown above each bar represent the Standard Deviation).

Table 2. Survival of Produced Fry on 10 and 54 DAH

Fry Type	10 DAH (%) Mean ± SD	54 DAH (%) Mean ± SD
<i>A. latus</i> x <i>A. latus</i>	28.9 ^a ± 3.2	6.4 ^a ± 1.0
<i>A. latus</i> x <i>S. hasta</i>	4.5 ^c ± 0.9	3.1 ^b ± 0.2
<i>S. hasta</i> x <i>S. hasta</i>	6.1 ^c ± 1.9	2.5 ^b ± 1.5
<i>S. hasta</i> x <i>A. latus</i>	13.1 ^b ± 0.9	5.9 ^a ± 0.6

Each value is the mean of three replicates.

SD = Standard deviation.

Values with different superscript in each column are significantly different at p<0.05.

among them (p>0.05). On the other hand, *A. latus* showed significantly higher hatching percentage (76.6%, p<0.05) compared to the other three fish types. Moreover, the hatching percentage of Hybrid I and Hybrid II are significantly higher (63.8%, 57.5%, respectively, p<0.05) than *S. hasta* (34.2%). No significant differences in the fertilization (%) were found (p>0.05) among the four types of fish examined.

Hatched Larval Count

The total numbers of hatched larvae obtained, from several manual fertilization trials over 10-days period, for the four types of fish were estimated. The total number of larvae obtained from Hybrid II cross was 599,480, followed by Hybrid I with a total of 567,500 larvae. The total number of hatched larvae for *A. latus* was 320,675 and 198,600 larvae for *S. hasta*.

Measurements of Newly Hatched Larvae

Total length measurements of newly hatched larvae (0 DAH) showed that the *S. hasta* larvae had significantly (p<0.05) smaller total length which varied from 2.3 to 2.5 mm with a mean ± SD of 2.4 ± 0.07 mm. The total length

ranges and mean ± SD obtained for *A. latus*, Hybrid II, and Hybrid I were 2.6 to 2.8 mm with 2.7 ± 0.10 mm, 2.6 to 3.0 mm with 2.8 ± 0.13 mm and 2.6 to 2.9 mm with 2.8 ± 0.09 mm, respectively. There was no significant difference (p>0.05) in the total length among these three types of larvae. The mean total length measurements were monitored for the four types of larvae from newly hatched larvae until the end of the experiment, i.e., 0, 5, 15, 39 and 54 DAH as shown in Fig. (1). These results indicated that since 39 DAH, the mean larval total lengths for the three types of fry, i.e., *S. hasta*, Hybrids I and II fry, were significantly higher (p<0.05) than those of *A. latus* fry (Fig. 1).

Larval survival

The mean larval survival for the four different tested larvae at 10 and 54 DAH was shown in Table 2. At day 10, the mean survival rate of *A. latus* (28.9%, 8670 ± 960 larvae) was significantly the highest (p<0.05) followed by Hybrid II (13.1%, 3930 ± 270 larvae). The lowest survival rate was found with hybrid I (6.1%, 1830 ± 570 larvae) and *S. hasta* (4.5%, 1350 ± 270 larvae). At 54 DAH, the mean survival rate of *A. latus* (6.4%, 1920 ± 300 larvae) and Hybrid II (5.9%, 1770 ± 180 larvae) was significantly the highest

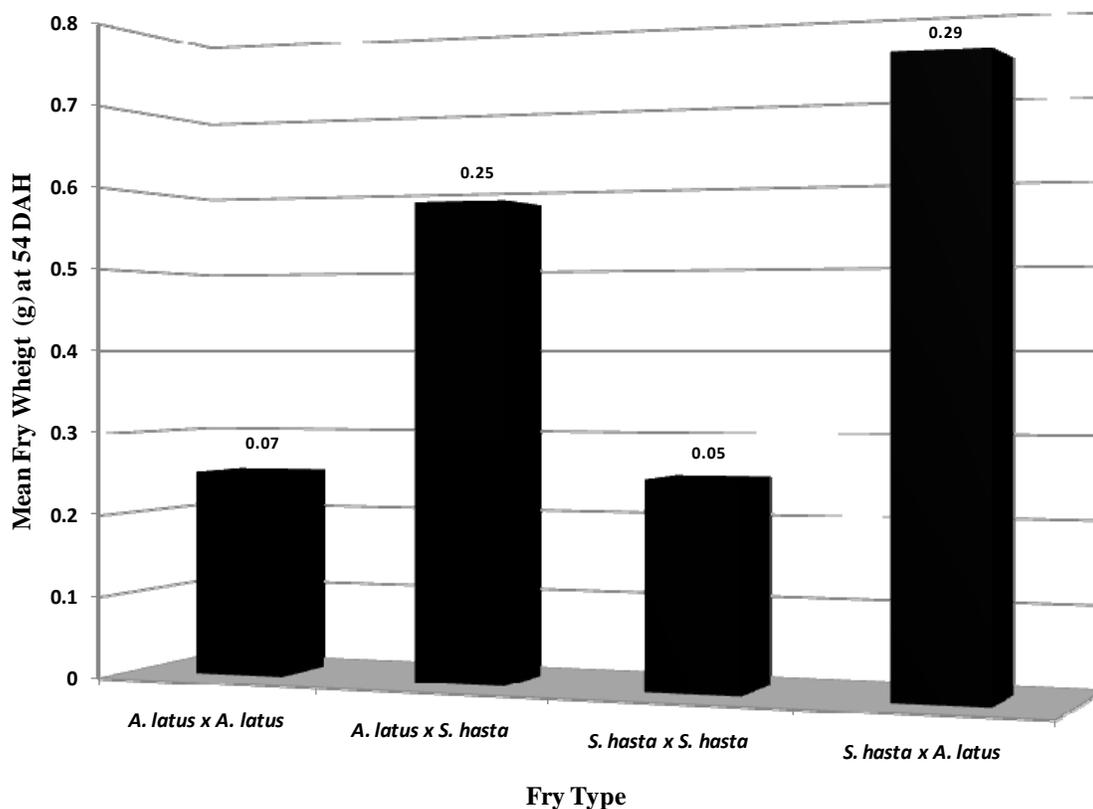


Fig. (2). Mean fry weight of the *A. latus* x *S. hasta* (Hybrid I), *S. hasta* x *A. latus* (Hybrid II) and their parents fry up to 54 DAH. (Values above each bar represent the Standard Deviation).

($p < 0.05$) followed by *S. hasta* (3.1%, 930 ± 60 larvae) and hybrid I (2.5%, 750 ± 450 larvae). The survival obtained in this study was rather low and could be probably due to induced spawning and the use of HGC hormone and manually stripping.

Fry Weight

The mean \pm SD of fry weight at 54 DAH of Hybrid II (0.76 ± 0.29 g) was significantly higher ($p < 0.05$) than those of *A. latus* (0.25 ± 0.07 g) and *S. hasta* (0.25 ± 0.05 g) fry, and not significantly different ($p > 0.05$) from that of Hybrid I (0.58 ± 0.25 g) as shown in Fig. (2).

Cannibalism and Jumping Behavior

The experiment was finished at 54 DAH due to high variation in fry size, leading to higher cannibalistic and jumping behavior, which was observed in the following decreasing order: *S. hasta*, followed by hybrid I then hybrid II, while the lowest was observed in *A. latus* fry. These observations were based on visual sighting of number of attacks in the larval rearing tanks and fish jumping incidences of different crosses during the experiment duration.

Appearance at 54 DAH

The observed body shape of both hybrids I and II during the final measurements (54 DAH, $n=30$) at the termination of the experiment were found more closely resemble *S. hasta* in body elongation and depth. A randomly selected fish specimen of the two produced hybrids and their parents was photographed to illustrate the body shape elongation and body depth as shown in Plate 1.

Water quality analysis

The total amount of ammonia measured in each of the rearing tank during this experiment ranged between 0.04 to 0.06 mg l⁻¹. There was no significant difference ($p > 0.05$) in the ammonia contents among the experimental tanks throughout the experiment's duration. The DO and pH readings were found to be within the normal ranges (DO range; 4.9 - 5.2 mg l⁻¹ and pH range; 7.7 - 8.3) with no significant difference ($p > 0.05$) among different treatments. In the larval rearing tanks the initial water temperature for the larval rearing tanks ranged between 19.0 to 20.0 °C (static) and the final water temperature at 54 DAH was 24.0 - 25.0 °C (flow through). There was no significant difference ($p > 0.05$) in the larval rearing temperature among the experimental tanks throughout the experiment's duration.

DISCUSSION

This study has documented a successful cross between *A. latus* (♀) x *S. hasta* (♂) (hybrid I) and *S. hasta* (♀) x *A. latus* (♂) (hybrid II), which can be added to the hybrids produced within the *Sparidae* family. Hybridization among different marine seabream species was studied in European and South East Asia hatcheries [2, 4, 5, 7, 8, 10, 11 and 20].

The insignificant difference in fertilization percentages ($p > 0.05$) between the hybrids and parent fish in this study agree with other findings as in [2 and 4]. The results of this study also agree with the findings of [10] who reported that the mean fertilization rate of the Hybrids is similar to those of the parental cross between female red seabream and male black seabream.

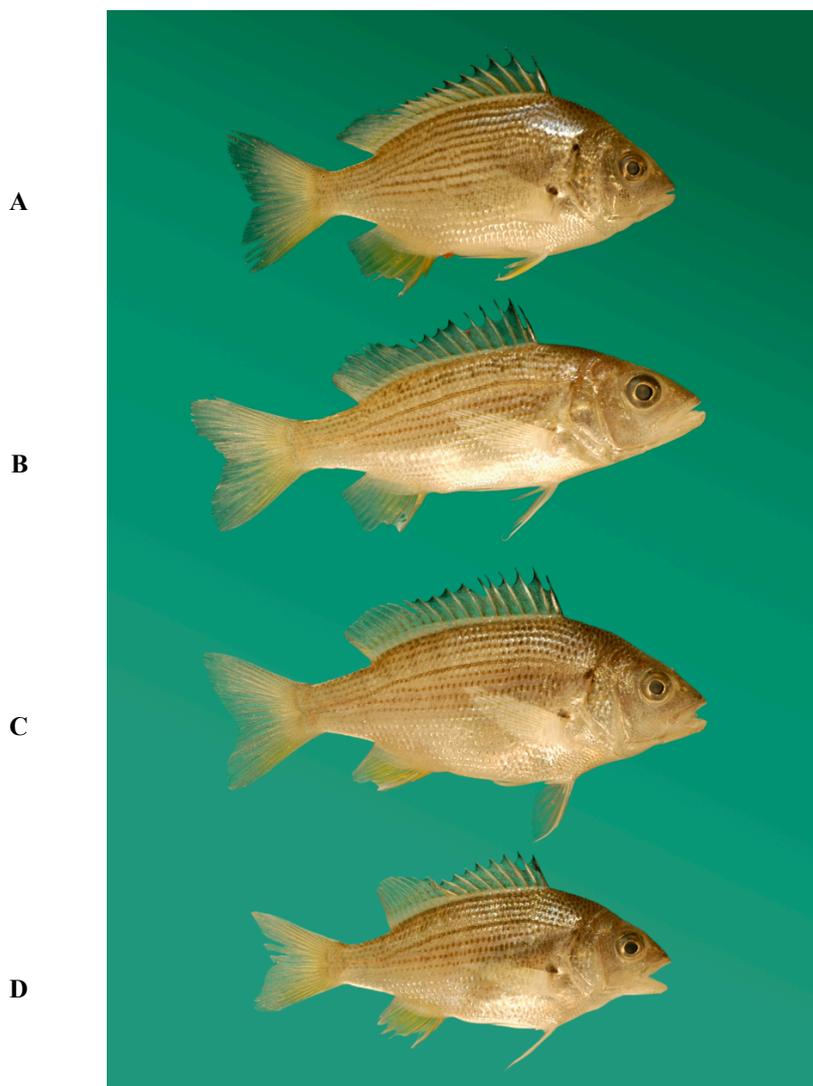


Plate 1: Sample of produced fry 54 DAH.

Note: (A: *A. latus* x *A. latus*, B: *S. hasta* x *S. hasta*, C: *A. latus* x *S. hasta* (Hybrid I), D: *S. hasta* x *A. latus* (Hybrid II)).

Results showed that although the total length of the newly hatched larvae (0 DAH) of *S. hasta* was significantly lower ($p < 0.05$) compared to the other three types of larvae, this difference disappeared starting from 39 DAH indicating that the growth during the larval phase is not critical to fry production.

The low larval survival of *S. hasta* and the relatively high survival of *A. latus* were expected according to previous experience. The superior survival of Hybrid II over Hybrid I is an advantage in fry production in addition to its lower cannibalism.

The mean fry weight of the produced hybrids (I and II) indicated superiority in growth rate over both parents. This statement should be confirmed by a further study on growth rates of the four types of fish.

From the data obtained in the present study and the similarities and differences observed among the four types of fish tested regarding larval survival percentage, jumping and cannibalistic behavior of the four crosses, two groups can be distinguished. The first one includes *A. latus* and the cross *S. hasta* (♀) x *A. latus* (♂) (Hybrid II) having higher larval

survival and very low jumping and cannibalistic behavior, while the second group includes *S. hasta* and the cross *A. latus* (♀) x *S. hasta* (♂) (Hybrid I) having lower larval survival and high jumping and cannibalistic behavior. The resemblance of hybrid II to *S. hasta* in body appearance will fetch a better market price due to its body elongation and depth while being similar to *A. latus* in higher larval survival which will have better economics of the hatchery due to higher fry production.

CONCLUSION

Overall, under the conditions of this study, it was possible to manually fertilize both *A. latus* and *S. hasta* and obtain fertilized eggs of the two hybrids. The newly hatched larvae of the two hybrids *A. latus* (♀) x *S. hasta* (♂) (Hybrid I) and *S. hasta* (♀) x *A. latus* (♂) (Hybrid II) and of both parent were successfully obtained. All the four types of newly-hatched larvae were reared up to fry stage (54 DAH). The larval survival of Hybrid II was found to be close to that of *A. latus* which was the highest at day 10 and 54 DAH. Even though the fry growth performance of the two hybrids was significantly higher ($p < 0.05$) than the fry of both

parents, it is not conclusive at this growth period. Thus, hybrid II would be the choice for further work recommendation to evaluate its growth rate to market size fish. Further studies are required to grow these four types of fry produced to market size in order to evaluate growth performance and survival and to judge their suitability for aquaculture.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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