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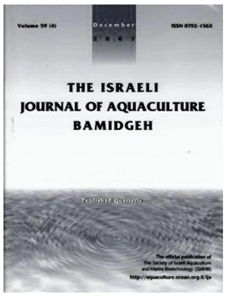

Rearing White Grouper (*Epinephelus aeneus*) in Low Salinity Water: Effects of Dietary Salt Supplementation

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Abstract

The white grouper (*Epinephelus aeneus*) is native to the Mediterranean Sea and the eastern Atlantic Ocean. It is an important species in fisheries of this region, having high market value. Our project of white grouper domestication in brackish water systems aims at developing culture methods for local conditions. The study evaluates the potential of culturing white grouper in low salinity water and the effect of an NaCl-enriched diet on this species. The results of a three-month culture period, conducted at a commercial farm, demonstrate that white grouper can grow in 3 ppt salinity and that 3% dietary salt supplementation can significantly improve its growth. However, the overall growth performance in low salinity was below expected for this species and further research is needed to develop culture practices for white grouper.

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Introduction

The white grouper (*Epinephelus aeneus*) is a perciform fish (family Serranidae) native to the Mediterranean Sea and the eastern Atlantic Ocean. It is an important species in the fisheries of this region since it is very popular among customers and possesses high market value. A white grouper domestication project was initiated over a decade ago and some progress has been achieved in reproduction and nutrition (Hassin et al., 1997; Lupatsch and Kissil, 2005; Gorshkov, 2010). However, the focus of these efforts was to develop culture practices in sea water. Grouper species (e.g., *E. akaara*, *E. coioides*, *E. malabaricus*, *E. polyphekadion*, *E. tauvina*) are cultured in Southeast Asia, mostly in the sea or high salinity water (Liao and Leano, 2008). The white grouper and other local grouper species are not commercially cultured in the Mediterranean region.

The native habitat of groupers is coral reefs, and they do not tend to occupy estuaries or rivers. Some grouper species, however, are euryhaline and can tolerate a wide range of salinities (Woo and Wu, 1982; Caberoy and Quiniti, 2000). Fresh water is hypo-osmotic for groupers, causing diffusive ion loss and osmotic water gain. The passive flux of ions (mainly Na^+ and Cl^-) from the fish to the external medium must be overcome by active ion uptake. While specialized cells in the gills recover some of the secreted ions, ingested food may substantially contribute to ion uptake (Marshall and Grosell, 2006). The addition of salt to the diet can result in better feed efficiency and greater weight gain in marine and freshwater species reared in fresh water (Salman and Eddy, 1988; Gatlin III et al., 1992; Nandeesha et al., 2000; Harpaz et al., 2005; Arockiaraj and Appelbaum, 2010; Cnaani et al., 2010).

In this study, we evaluated the ability of white grouper to tolerate low salinity water and examined whether dietary salt supplementation can improve its adaptation, as an initial evaluation of the potential for culturing white grouper in inland waters.

Materials and Methods

Fish. White grouper juveniles (3.1 g) were obtained from the commercial nursery of Kibbutz Maagan Michael, Israel. The fish were maintained in the nursery in water salinity of 20 ppt and transferred with nursery water to a commercial growout facility at Kibbutz Kfar Ruppin, Israel. The fish were stocked in six 250-l net cages (200 fish per cage) placed in a 20-m^3 growout system. The water in the system recirculated through a biofilter. Local well water (salinity 3 ppt) was added gradually over three weeks until all the nursery water was replaced and a salinity of 3 ppt was reached (Table 1).

Table 1. Chemical composition of water in the nursery (Maagan Michael) and growout facilities (Kfar Ruppin).

Location	*Nursery*	*Growout*
Salinity (ppt)	20	3
Conductivity (mS/cm)	29.1	4.47
Cl (mg/l)	5,700	1,281
Na (mg/l)	4,813	178
Ca (mg/l)	256	273
K (mg/l)	198	16
Mg (mg/l)	574	147

Diets. A base diet and a salt-enriched diet were prepared by Zemach Feedmill (Zemach, Israel) based on the formula developed by Lupatsch and Kissil (2005). The feed was in the form of sinking extruded pellets, 2 mm diameter. The diets were identical except that NaCl was added to the salt-enriched pellets at 3% of the food weight, resulting in a proportional reduction of the ratio of the ingredients replaced by the salt (Table 2). Fish were fed throughout the day using conveyor belt feeders. The daily feeding rate was 3% of the fish biomass and feed amounts were adjusted following periodic weighing of the fish.

Table 2. Chemical composition (%) of diets (proximate analysis).

	Base diet	*Salt enriched*
Protein	50	50
Fat	13	13
Fiber	2.5	2.3
Ash	10.5	13
Calcium	1.98	1.98
Phosphorus	1.60	1.59
Sodium	0.49	1.43
Vitamin premix	1	1

Analyses. Fish were reared for 92 days. At the end of the experiment, each fish was individually weighed and the survival rate was calculated for each cage. The specific growth rate (SGR) was calculated as 100($\ln_{\text{final body wt}} - \ln_{\text{initial body wt}}$)/days of growth. The feed conversion ratio (FCR) was calculated as wt of given food/wt gain. Statistical analyses were conducted separately for each parameter using *t* test, with the two diets as classes. For preparation of histological sections, fish were anesthetized using clove-oil and a piece from the first gill arch was removed and kept in buffered neutralized formalin for later analysis. Samples were dehydrated in a series of increasing concentrations of ethanol, and embedded in methacrylate. The samples were sectioned at 3 µm and stained with hematoxylin and eosin.

Results

The white grouper were able to survive and grow during three months in low salinity water. There were significant differences (p<0.01) between diets in both growth and survival. Fish fed the salt-enriched diet were 45% heavier and their SGR and FCR were better by 25% and 33%, respectively (Table 3). In comparison to histological sections from fish kept in 20 ppt nursery water, sections from fish kept three months in 3 ppt water showed hyperplasia of epithelial cells in the gill lamella and filaments, as well as an increase in the number of chloride cells (Fig. 1). These effects were more pronounced in fish that received the supplemental salt diet than in fish that received the basal diet.

Table 3. Mean±standard deviations of weight, specific growth rate (SGR), feed conversion ratio (FCR), and survival of white grouper fed a base diet or a diet with 3% supplemental salt, after being kept 92 days in low salinity (3 ppt) water.

	Base diet	*Salt enriched*	*F-ratio*	*Probability*
Final wt (g)	13.2±1.2	19.2±0.5	68.8	*P* = 0.001
SGR	1.58±0.09	1.98±0.02	48.4	*P* = 0.002
FCR	2.56±0.29	1.71±0.13	21.3	*P* = 0.009
Survival (%)	60.7±2.5	77.8±3.9	41.3	*P* = 0.003

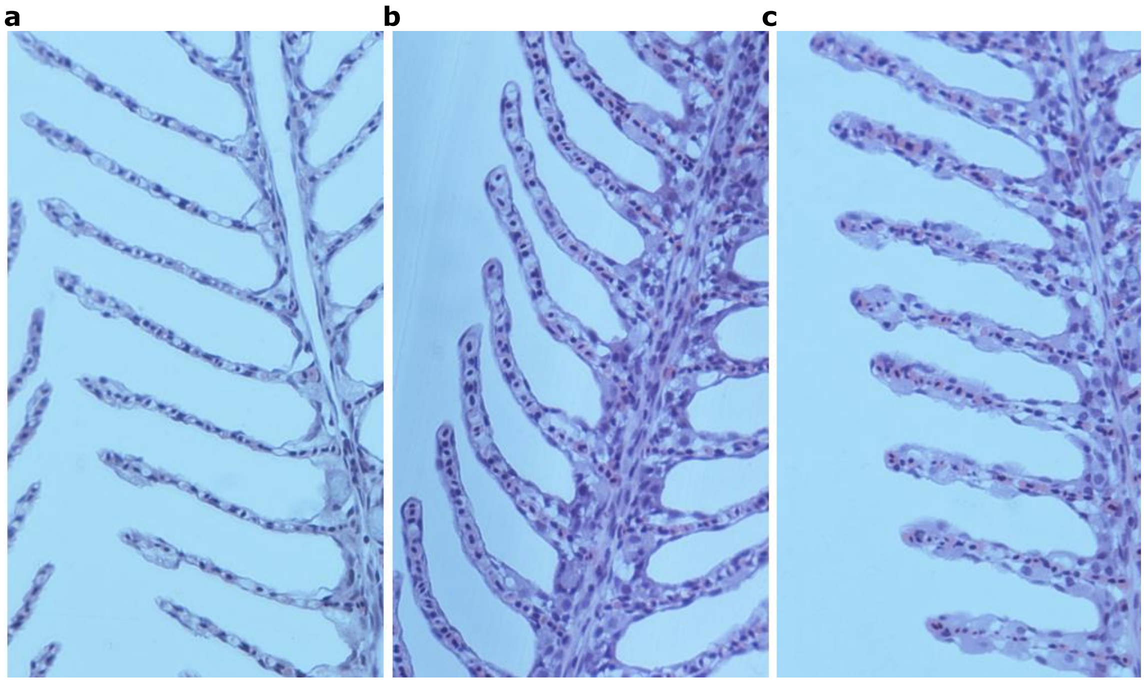

Fig. 1. Histological sections of the lamella of white grouper gills from (a) fish from the nursery raised in 20 ppt salinity, (b) fish receiving the base diet after being kept three months in 3 ppt salinity, and (c) fish receiving a diet supplemented with 3% NaCl after being kept three months in 3 ppt salinity.

Discussion

To some extent, white grouper is a euryhaline fish (Peduel and Ron, 2003). The current study demonstrates that white grouper juveniles can live and grow in salinity levels as low as 3 ppt, less than a tenth of seawater salinity. Based on the results of this study, this salinity seems to be below the normal range for this species and a significant environmental stressor for the fish.

Supplemental dietary salt can have a positive effect on the growth of salmonid species in fresh or low salinity water (MacLeod, 1978; Smith et al., 1987; Salman and Eddy, 1988). Addition of NaCl to the diets of the marine euryhaline red drum (*Sciaenops ocellatus*) and Asian sea bass (*Lates calcarifer*) resulted in better feed efficiency and greater weight gain when reared in fresh water, but not in brackish or sea water (Gatlin III et al., 1992; Harpaz et al., 2005). Freshwater species such as carps (*Cyprinus carpio* and *Cirrhinus mrigala*) and tilapia (*Oreochromis* spp. hybrid) also attain better feed efficiency and weight gain with dietary NaCl supplementation (Nandeesha et al., 2000; Cnaani et al., 2010). The growth improvement in this study was much higher than observed for other species, possibly due to the conditions in which the white grouper juveniles were reared, i.e., well below the natural range of salinities to which this species is accustomed and thus presenting a heavy burden on their ion regulation systems.

The large differences in SGR and FCR between treatments that differed only in NaCl content exemplify the importance of finely calibrating nutritional components in white grouper diets. However, even the better growth obtained with the salt-enriched diet was not as good as expected for this species. Maricultured groupers of similar size can have an FCR close to 1.0 (Lupatsch and Kissil, 2005; Williams, 2009), much better than the 1.7 obtained in this study. The SGR of fish of similar size in Lupatsch and Kissil (2005) was 25% better than that of fish receiving the supplemented salt diet and 50% better than that of fish receiving the control diet in our study. Calibration of dietary nutritional components, in addition to changes in mineral contents, might improve the relatively low growth performance of this study.

Physiological effects of supplemented dietary salt can lead to growth differences. As seen by the notable hyperplasia of epithelial cells and increased number of chloride cells in the histological sections, the gill structure seems to be highly influenced by environmental salinity. Such a profound effect of dietary minerals on tissues and organs, together with the considerable change in water salinity, indicates that the nutritional requirements of white grouper should be thoroughly investigated and diets should be developed that take culture conditions into consideration. Since these effects are accelerated in faster-growing fish, it is possible that the higher oxygen demand due to growth-related metabolic pathways leads to increased blood flow to the gills, which in turn leads to increased ion loss from the blood to the hypo-osmotic environment.

The main conclusion of this study is that white grouper have the ability to live and grow in low salinity water such as that found in springs and underground wells. Such water cannot always be used for irrigation, and using it for aquaculture of fish with high market value may provide additional income in arid regions. Further research is required to understand the physiological responses of white grouper to low salinity and nutritional effects on these responses as a step towards developing and optimizing culture protocols for white grouper.

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