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INFLUENCE OF BRACKISH WATER ON SURVIVAL AND GROWTH OF THE JUVENILE WHITE GROUPER, *EPINEPHELUS AENEUS*

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Key words: brackish water, cortisol, geothermal water, grouper, osmoregulation, salinity

Abstract

The natural habitat of the white grouper, *Epinephelus aeneus,* is the Mediterranean Sea, which has a salinity of approximately 35 ppt. As fish species vary in their tolerance of environmental conditions, potential production in specific conditions must be determined empirically. The growth, survival and cortisol level of 1.7 g grouper juveniles grown in diluted sea water (4 ppt) or brackish water (4 ppt) from the Tsofar well in the Arava (southeastern Negev, Israel) was compared to the growth of similar fish in sea water (43 ppt). Survival in all treatments was 100%. During the first ten days, the fish grown in brackish water grew significantly less ($p<0.01$) than the fish grown in full-strength or diluted sea water. There were no significant differences between the treatments during the second growth period or in the final weight (approximately 9 g). Total cortisol concentrations ranged 2.7-4.5 ng/l and did not significantly differ between treatments. Results indicate that the white grouper can flourish in water with salinity as low as 4 ppt and that there are no detrimental chemical factors in the brackish water from the Tsofar well.

Introduction

The use of marginal lands and water resources for food production is becoming necessary since high quality water resources are becoming increasingly scarce. The need to efficiently use water resources has led to the development of intensive fish farming with recirculated water and/or integration with traditional agriculture (Koren, 1992). The chemical compositions of the water used in such projects vary according to the source, but

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invariably differs from the chemical composition of sea water. Presently, the major crop in these farms is the hardy red tilapia hybrid (Koren, 1992*)*, a fish with a low market price. As farmers, naturally, are interested in high-revenue crops, the growth potential of the white grouper in brackish water is of interest in this area.

The natural habitat of the white grouper, *Epinephelus aeneus,* is the Mediterranean Sea, which has a salinity of approximately 35 ppt. As fish species vary in their tolerance to environmental conditions, production potential must be determined empirically for each species. Since the red groupers, *Epinephelus akaara* (Woo and Wu, 1982) and *Epinephelus coioides* (Caberoy and Quiniti, 2000), are extremely euryhaline, we decided to test the potential production of *Epinephelus aeneus* in brackish water. Chua and Teng (1980) obtained optimum growth rates in *Epinephelus salmoides* grown in salinities below the salinity of the species' natural habitat. Bœuf and Payan **(**2001) found that only one of 21 marine fish species they tested and/or reviewed reached its optimal growth rate in seawater salinity; all others grew best in brackish water at salinities lower than those in their natural habitats. In tilapia, *Oreochromis mossambicus,* Ron et al. (1995) found that salinity influences food intake and, consequentially, the growth rate.

The mineral contents and quality of brackish water from different wells vary, making experiments for each water source essential. Comparing the growth of the white grouper in diluted sea water to growth in full-strength sea water may help distinguish between osmoregulatory effects and effects of chemicals in the brackish water.

The plasma cortisol level is widely used as a general indicator of stress in vertebrates, particularly in fish (Pickering and Pottinger, 1989). An elevated plasma cortisol level can be indicative of a stress-inducing chemical component in the brackish water. As chronically high cortisol levels in the plasma increase susceptibility to disease in fish (Pickering and Pottinger, 1985) and suppress somatic growth (Pickering, 1993), any water resource causing stress would definitely be considered detrimental to fish production.

This experiment was performed to compare the growth potential and survival of grouper juveniles in diluted sea water, brackish water from the Tsofar well, and full-strength sea water.

Materials and Methods

Animals. Epinephelus aeneus juveniles (1.67 g) were obtained from the nursery of the National Center of Mariculture (NCM) in Eilat, Israel. The fish were spawned by NCM broodstock and grown in NCM facilities, in a free-flow system with sea water of a salinity of 43 ppt.

Experimental procedures. Three randomly divided groups were acclimated for approximately three hours to one of the following conditions: Tsofar well-water (4 ppt), sea water diluted to 4 ppt, and sea water from the original environment of the fish (43 ppt). For each treatment, there were four replicates, i.e., 15 fish were weighed and stocked into each of four aquaria containing 27 l of the water. After ten days, the fish from each replicate were weighed and counted to determine survival and growth rate, expressed as Specific Growth Rate (SGR). SGR was calculated as follows: SGR =100 x $(Lnw_f - Lnw_i)\backslash d_f - d_l$, where w_i and w_f represent the initial weight and weight on the day of measurement, respectively; d_f-d_l represents the number of days between initial stocking and the day of measurement. Two fish from each replicate were analyzed for total cortisol concentration. The prolonged effects of the treatments and acclimation were appraised by restocking 12 graded and weighed fish into each of two aquaria per treatment. The fish were weighed after an additional two weeks and at 39 days after the initial stocking.

Analytic procedures. Fish samples were removed from the aquaria quickly so that the total sampling time required less than 4 min. The fish were stored at -70°C prior to the total cortisol extraction procedure and determination of the cortisol concentration by radioimmunoassay.

Experimental conditions. Full-strength seawater was provided in a free-flow system at an exchange rate of 6.5 l/h. In the brackish and diluted seawater treatments, water circulated through a closed system at a rate of 27 l/h and was replaced at a rate of 6% of the water volume per day. Aeration kept dissolved oxygen levels above 4.0 mg/l. Water temperatures were stable at 23.5±0.4°C. The pH in the full-strength seawater treatment was above 8.0 and was kept at 7.6, or higher, in the two closed systems by daily addition of NaOH. Alkalinity levels did not drop below 1.0 meq/l in the closed systems or below 2.0 meq/l in the seawater treatment. The ammonia level did not exceed 0.5 ppm in the closed systems and was negligible in the seawater. Light intensities ranging from 1000 to 1400 lux were provided by 9 W light fixtures placed on both sides of each aquarium, according to a schedule of 19 light:5 dark hours.

Water quality. The water supplied to the two closed systems was stored in separate 2500 l tanks. Tsofar well water was aerated for four days prior to use, according to routine procedures in commercial production facilities utilizing this water. Water in the storage tank had a pH of 6.3 and an alkalinity of 0.2 meq/l. Table 1 shows the chemical analysis of the well and sea water.

Feed. Commercial feed (Coppens International, The Netherlands), containing at least 56% protein and 12% lipids, was dispensed by electric conveyor belt. The daily feed ration was 8% of the initial fish biomass with a 7% daily increase to account for expected growth during the first ten days and 7% of the initial biomass with a 6% daily increase for the following two weeks. During the last two weeks, the fish were fed an NCM-prepared diet consisting of 50% protein and 12% lipids at a rate of 4% of the fish biomass at the previous measurement. These rations provided *ad libitum* amounts of food in all treatments.

Statistical analysis. Means and standard deviations were calculated for each parameter. The results were analyzed by Anova and Duncan multiple range tests using software SPSS for Windows' Student Version 6.1.3 9 (SPSS Inc., 1985-1995).

Results

Fish survival was 100% throughout the experiment in all treatments. The fish grown in Tsofar water grew significantly less ($p<0.01$) than the fish grown in full-strength or diluted sea water during the first ten days of growth (Table 2). However, during the second period there were no significant differences between treatments either in growth rate or in final weight. There were no significant differences in total cortisol concentrations, which ranged 2.7-4.5 ng/l (Table 3).

Discussion

The major aim of the experiment was to test the potential of growing white grouper in brackish water from a specific source in a recirculating water system. These conditions were tested in fish of the smallest size that are commonly transferred to commercial grow-out facilities. The fast growth rate and higher sensitivity to environmental conditions of smaller fish can be used to study, within a short time and with limited water, potential problems that may be caused by water quality. Using such small fish enabled us to compare fish survival and growth in various treatments until the fish weighed at least 500% of their initial weight.

The results of the experiment indicate that the production of white grouper in brackish water has potential. The significantly lower growth in Tsofar well water during the first period than during the second, indicating that the fish needed a period of acclimation to the experimental conditions. The significantly lower growth in the first period was the result of the negligible feed intake of the fish during the first two days after stocking. The growth compensation measured in the second growth period shows that the fish had acclimated by the second growth period and the high growth potential of the grouper in brackish water

Conditions in a recirculating water system can deteriorate when technical problems arise. With this in mind, it is interesting to note the results of a six-day preliminary experiment where the pH of the Tsofar water ranged 6.32-7.49 and the alkalinity 0.4-0.7 meq/l (unpublished results). The 20 fish in the 27-liter aquarium did not eat, therefore they did not

Table 1. Chemical analysis* of Tsofar well water compared to sea water.

Parameter	*Tsofar water (ppm)*	*Diluted water (ppm)*	*Sea water (ppm)*
Dissolved matter	7,850	NM	NM
Hardness ($CaCO_3$)	3,308	NM	NM
pH	6.7	NM	8.5
Flouride (F)	1.1	NM	NM
Bicarbonate (HCO_3)	155	NM	171
Sulfate (SO_4)	836	381	3,671
Nitrite (NO_2)	<0.01	NM	0
Nitrate (NO_3)	1.5	NM	0
Ammonia (NH_4)	1.29	NM	0.027
Sodium (Na)	1,170	NM	15,755
Potassium (K)	78	NM	1,251
Calcium (Ca)	1,000	70	483
Magnesium (Mg)	194	162	1,690
Total iron (Fe)	4.6	NM	0.1
Hydrogen sulfide (H_2S)	0		NM

NM = not measured
* The chemical analysis of the well water was carried out by FRL Engineering Ltd. The sea water was analyzed by the Hevel Eilot Research Station Laboratories.

grow, but their survival was 97.5±2.9%. The fish in the diluted sea water were exposed to a pH of 7.16-7.89 and alkalinity of 1.2-1.6 meq/l (the recommended alkalinity range is 2-3 meq/l according to Wedemeyer, 1996). In spite of the low alkalinity, survival was 100% and weight increased from 1 to 2.1 g. This weight gain was the same as that of the control fish, grown in free-flowing sea water with an alkalinity of 2.5 meq/l and a pH of 8.0. The insensitivity to extreme environmental conditions exhibited by the grouper is an important advantage for fish grown in a recirculating culture system.

Several aspects of cortisol activity can be influenced by experimental conditions. Cortisol is an accepted indicator of stress because the cortisol plasma level usually rises during acute stress. Pickering and Pottinger (1989) showed that plasma cortisol levels remained high for at least four weeks when rainbow trout, *Salmo gairdneri* Richardson, were subjected to chronically stressful conditions. On the other hand, Lemarie et al. (2000) grew seabass, *Dicentrarchus labrax*, for 63 days in such extreme levels of pH and CO_2 that the fish were lethargic, discolored and lost reflexes, yet the plasma cortisol level remained in the normal range for seabass in ambient conditions. Flodmark et al. (2002) showed that acute stress might lead to a rise in plasma

Table 2. Growth rates and survival of juvenile white groupers grown in full-strength or diluted sea water or brackish well water.

Treatment	Nov. 24 - Dec. 3, 2002			Dec. 3, 2002 - Jan. 1, 2003			Survival
	Weight gain (g)	SGR (%)	Final weight (g)	Weight gain (g)	SGR (%)	Final weight (g)	%
Tsofar well water (4 ppt)	1.09±0.25*	5.6±1.2*	2.78±0.21*	7.2±1.2	4.2±0.4	10.2±1.1	100
Diluted sea water (4 ppt)	1.73±0.08	8.0±0.4	3.37±0.0	5.0±1.2	3.0±0.4	8.6±1.3	100
Sea water (43 ppt)	1.62±0.03	7.5±0.18	3.30±0.0	6.2±0.2	3.5±0.0	9.6±0.4	100

* significantly different ($p<0.01$) from other treatments

cortisol levels in brown trout, *Salmo trutta,* that return to normal levels when the stressful situation continues for at least four days. Apparently there can be habituation to or compensation for prolonged stress by physiological or behavioral changes.

High cortisol concentration can be the result of the osmoregulatory activity of cortisol. A higher cortisol concentration is expected in fish grown in sea water than in fresh water due to the osmoregulatory activity of cortisol (McCormick, 2001). Ron et al. (1995) found the cortisol concentration in tilapia (*Oreochromis mossambicus*) acclimated to sea water to be 2.5 times the concentration in tilapia grown in fresh water. The higher growth rate and lower respiratory rate of the fish grown in sea water show that the high cortisol level was not a reaction to stress.

The present experimental results reflect the expectation that the cortisol level would be higher in full-strength sea water than in diluted sea water. The high cortisol level in the fish grown in the Tsofar water might have been induced by osmotic disturbance caused by higher levels of some water component(s), possibly metal(s), than those found in the diluted sea water. The proliferation of chloride cells, stimulated by cortisol, can help restore the ionic balance (Jezierska and Witeska, 2001).

Experiments dealing with cortisol as an indication of stress usually measure plasma cortisol levels (Tort et al., 2001; Flodmark et al., 2002). Because of the small size of the fish at the end of the experiment, it was more feasible to measure the total cortisol content. While the plasma level of a hormone is an indication of activity, total cortisol includes produced and stored cortisol and may not necessarily indicate activity at a given time. As very little information is available concerning total cortisol levels or stress reactions in such small fish, it is difficult to discern the meaning of the comparatively low levels of cortisol found in all the treatments.

Generally, the total cortisol concentrations in the groupers (2.7-4.5 ng/l) were considerably lower than concentrations found in gilthead seabream *(Sparus aurata,* Sparidae) of

Table 3. Total cortisol concentrations in juvenile white grouper after 39 days of growth.

Treatment	*Cortisol (ng/ml)*	*No. of Samples*
Tsofar well water (4 ppt)	4.45±1.42	7
Diluted sea water (4 ppt)	2.7±1.6	7
Sea water (43 ppt)	3.41±1.15	6

a similar size (21-26 ng/l). As with the grouper, there were no significant differences in cortisol concentration between the seabream, whether they were grown in brackish water or in sea water (Ron et al., 2002). These findings are not surprising when compared with the results of Woo and Wu (1982) who compared osmoregulatory changes in red groupers *(Epinephelus akaara)* and another member of the Sparidae family, Acantopagrus schlegeli (formerly identified as *Mylio macrocephalus)* after transfer to varying salinities. They found changes in levels of serum osmolality, glucose, Na^+, Ca^+, K^+ and hematocrit, and muscle water to be transient and concluded "that physiological stress is unlikely to occur within the salinity regime of 7 to 30 ppt in red groupers and black sea bream."

In *Sparus aurata* (Tort et al., 2001) and several salmonids (Ferolden et al., 1991; Pottinger et al., 1992) it has been shown that some fish are consistently high or low cortisol responders to acute stress, whereas others may be inconsistent responders. There is no information on this subject in literature concerning groupers. Such innate differences in reaction among fish can influence the significance of the results, particularly when few fish are tested in each treatment.

The multiple functions of cortisol and the problems discussed above make the experimental results regarding cortisol level, inconclusive. It would be interesting to compare our results regarding cortisol levels with other groupers of similar size, challenged by a different kind of stressor, or with larger fish whose blood can be sampled.

The results indicate that the white grouper can flourish in water with salinity as low as 4 ppt. The fact that the fish grew more than five times their initial weight, the low cortisol level, and the 100% survival rate, are good indications that there were no detrimental influences of the chemical content of the Tsofar water on the fish. More conclusive results would be obtained by long-term monitoring of fish growth in these conditions. It is important to ascertain the healthy development of internal organs and the reproductive system in future studies.

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