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Original article

A survey on osmoregulatory potential of Bream, *Abramis brama* (Berg, 1949) fry for restocking management programs

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ABSTRACT

Bream (*Abramis brama*, Berg, 1949; Family: Cyprinidae) is commercially valuable fish in the Caspian Sea fishing industry. Iranian Fisheries Organization annually produces and release up to 19 million Bream fries size for recruiting of this species. Its fries are mostly released into the Anzali wetland with 4 ppt salinity. Meanwhile, they sometimes are released into Sefidrood River (0.5 ppt), Sefidrood River estuary (8 ppt) and directly into the Caspian Sea (12 ppt). To determine whether these alternative locations are suitable for release, sixty fingerling size Bream (0.5±0.13 g b.w.) were exposed to four salinity levels of these locations *i.e.* 0.5, 4, 8 and 12 ppt, for 120 hrs. The results displayed that accumulated mortality rate was significantly lower in exposed fish to 4 ppt (P<0.05) in compare to others. No differences in size of gill chloride cells were observed among treatments; however, chloride cell number was significantly higher in all treatments except for 4 ppt treatment (P<0.05). Hind-gut epithelium thickness significantly decreased in specimens at 8 and 12 ppt treatments after 120 hrs (P<0.05). The results do not suggest releasing Bream fries directly into the Caspian Sea and also, the Anzali wetland cwas confirmed as the suitable releasing site.

1. Introduction

Bream (*Abramis brama*, Berg, 1949; Family: Cyprinidae) is distributed in Europe and Asia: east from the Pyrenees and north from the Alps and Middle Asia, in the basins of the North, Baltic, Black, Caspian and Aral seas. They are benthopelagic and potamodromous fishes (Riede, 2004). Adults occur usually in still and slow-running waters where they inhabit in large shoals (Vostradovsky, 1973). This species is commercially valuable fish in the Caspian Sea fishing, but its populations have experienced a decline since 1940s. Overfishing, environmental pollutions and destruction of spawning sites are the most likely reasons for this stock decrease.

For recruiting purpose of this species valuable fish, the Iranian Fisheries Organization (Shilat) annually produces up to 19 million fries (~0.5 g) at the Shahid Ansari Hatchery Center (Guilan Province, Iran) (SAHC), which are released into the Caspian Sea. Most of these fries are released into the Anzali wetland which has salinity of 4 ppt and according to Shilat report, it is supposed to be successful. In addition, a number are released at other locations with different salinity profiles including the Sefidrood River (0.5 ppt), Sefidrood River estuary (8 ppt) and directly into the Caspian Sea (12 ppt) as occasional target areas for releasing. The success of these juvenile bream at other sites is unknown, as the osmoregulatory capabilities of Bream fries has not been experimentally described.

Fish challenged with an altered environmental osmolality must maintain their body osmolality and ionic balance by changing behavior, such as drinking rate (Tytler and Blaxter, 1988; Ura *et al*., 1996; Miyazaki *et al*., 1998), stress hormone levels (Brown *et al*., 2001) and functions of the osmoregulatory surfaces (Hwang and Hirano, 1985; Hwang *et al*., 1989; Arai *et al*., 1997; Perry, 1998; Kelly and Woo, 1999). In teleosts, gills, kidneys and intestine are the most premier organs responsible for osmoregulatory homeostasis (Evans, 1993). The gills and specifically, chloride cells (CC) located on the gill epithelium are generally considered to be the principal route to regulate of monovalent ions in a high or low concentration gradient by changing its size and number (Karnaky, 1986). In metamorphosed fish, it is well established that branchial chloride cells, or "mitochondria- rich cells", are the primary site for discharge of excess Na^+ and Cl^- in an hyperosmotic environment (Zadunaisky 1984; Alderdice, 1988) and uptake of $Ca2^+$ and Cl^- in a hypoosmotic environment (Greco *et al*., 1995; Perry, 1998; Rombough, 1999).

The number, size and location of branchial chloride cells are known to alter with changes in environmental salinity; however the degree of change in cells can be influenced by the range of change in salinity as well as the species and age of fish (Hwang and Hirano, 1985; Kelly and Woo, 1999; Kelly *et al*., 1999; Caberoy and Quinitio, 2000). Transfer of euryhaline fish from freshwater to saltwater generally causes a proliferation of branchial chloride cells and an increase in branchial Na^+, K^{+-}ATPase activity (Sasai *et al*., 1998; Martinez-Alvarez *et al*., 2005) and exposure of freshwater fish to high salinities has been shown to induce and increase in the total number of chloride cells in some fishes (Hwang *et al*., 1989). Changes in morphology of chloride cells following transfer to saltwater and freshwater suggest that filament and lamellar chloride cells are important in saltwater and freshwater osmoregulation, respectively.

It is well known that fishes living in fresh water drink little water, but those in the salt water must drink salt water permanently to prevent dehydration. Hence, this is led to decrease of hind-gut epithelium thickness in order to faster and easier transfer of ions for increasing blood osmotic pressure and prevention body dehydration. Therefore, in hyperosmotic environments, the gastrointestinal tract plays an important role in intestinal salt and water uptake as osmotically-obliged water loss must constantly be compensated (Wilson *et al*., 1996).

This study was conducted to survey the salinity tolerance of Bream to different salinity levels relevant to its realising points in re-stocking program. Hence, based on above mentioned releasing points (Anzali wetland, 4 ppt; Sefidrood River, 0.5 ppt; Sefidrood River estuary, 8 ppt and the Caspian Sea, 12 ppt), Bream was exposed to this four different salinity levels. The salinity preparedness was assessed by indicating the mortality rate, and indices of both histological ion regulatory capacity (*i.e.*, number and size of gill chloride cells and hind-gut epithelium thickness). The findings of this research will provide insight into which locations would be most suitable for reintroduction of fingerling size Bream.

2. Materials and methods

2.1. Fish and experimental conditions

Sixty fries of Bream, *Abramis brama*, (0.5±0.13 g) were obtained from artificially propagated Bream from SAHC (Guilan, Iran). They were reared up to 0.5 g in ponds with 2 ppt salinity. Specimens after weight screen, were introduced into 40-l tanks with same salinity for 3 days as adaptation period. Then, fries were randomly divided to four 40-l experimental treatments (0.5, 4, 8, and 12 ppt) with three replicates for 120 hrs. To prepare Salinities, water from Caspian Sea (12 ppt), Anzali wetland (4 ppt) and Sefidrood River (0.5 ppt) were transferred to SAHC. Furthermore, saline water of Caspian SAHCOF Sea was diluted to prepare 8 ppt salinity concentration with fresh water. All tanks were aerated at the same rate with a central aeration system. Cumulative mortality was recorded in tanks after 3, 6, 9, 12, 24, 48, 72 and 120 hrs following the experiment, and dead fish were removed at the defines times.

2.2. Tissue collection and histological study

Four anesthetized fries by MS222 from each treatment were removed at 3, 6, 9, 12, 24, 48, 72 and 120 hrs and fixed into buffered 4% formalin. Then, second gill filament and hind-gut were dissected out, and prepared for histological study based on Hewitson and Darby (2010). The sections were stained using Hematoxylin-Eosin (Druy and Wallington, 1980). The chloride cells (CC) which are usually located at the lower and base of filaments and lamella were counted in part of 5 filaments of gill histological sections from each sample. CC cells were easily determined with a conventional light microscope (Zice, Germany) as they were bigger with a faint color in H&E stained sections. Mean length and width of the cells was used to calculate size of CCs. Hind-gut epithelium thickness was evaluated in sections using a micrometer mounted in the optic lens of a light microscope (Zice, Germany).

2.3. Water quality measurements

Water pH (Orion, 410A) ranged from 7.23 to 7.27 and water temperature (YSI, 57) from 24 to 26˚C in all salinities during the experimental period.

2.4. Statistical analysis

Treated group means were compared within each time point using one-way analysis of variance (ANOVA). Assumptions of equal variance and normal distribution were met in all cases. Data were analyzed using Minitab 14 and statistical significance determined at $P<0.05$.

3. Results

3.1. Mortality rate

Effect of salinity on cumulative mortality of Bream between times and treatments were shown in Fig. 1. Little mortality was observed under all conditions during early 9 hrs of exposure. Afterward, it was significantly increased in treatments with 12, 8 and 0.5 ppt salinity. Total mortality rate was lowest in 4 ppt (8.1%) treatment. After 24 hrs exposure, mortality rate was relatively constant in 0.5, 4 and 8 ppt but it was increased significantly in 12 ppt up to 80% at the end of the experiment. Therefore, fish exposed to 12 ppt had significantly higher mortality than three others (Fig. 1).

3.2. Histology

Chloride cell density in the gill of 12 ppt treatment was increased significantly at the beginning of the experiment. In this treatment, chloride cell density was relatively constant during 9 to 24 hrs and then it was increased significantly again until the end of experiment. Specimens of 0.5 and 8 ppt treatments showed sudden increments in chloride cell density from 12 to 24 hrs and relatively constant density afterward. A significant increase of chloride cell density was not observed during experiment in 4 ppt treatment (Fig. 2). Despite of the increase in the density of chloride cells, their size remained constant and no significant differences were seen in all treatments during experiment. A significant decrease in hind gut epithelium thickness was observed throughout the experiment in the fish exposed to higher salinities (8 and 12 ppt). No significant changes occurred at either 0.5 or 4 ppt, although during first 9 hrs hind gut epithelium thickness showed an insignificant increase in 4 ppt and decrease in 0.5 ppt treatments (Fig. 3).

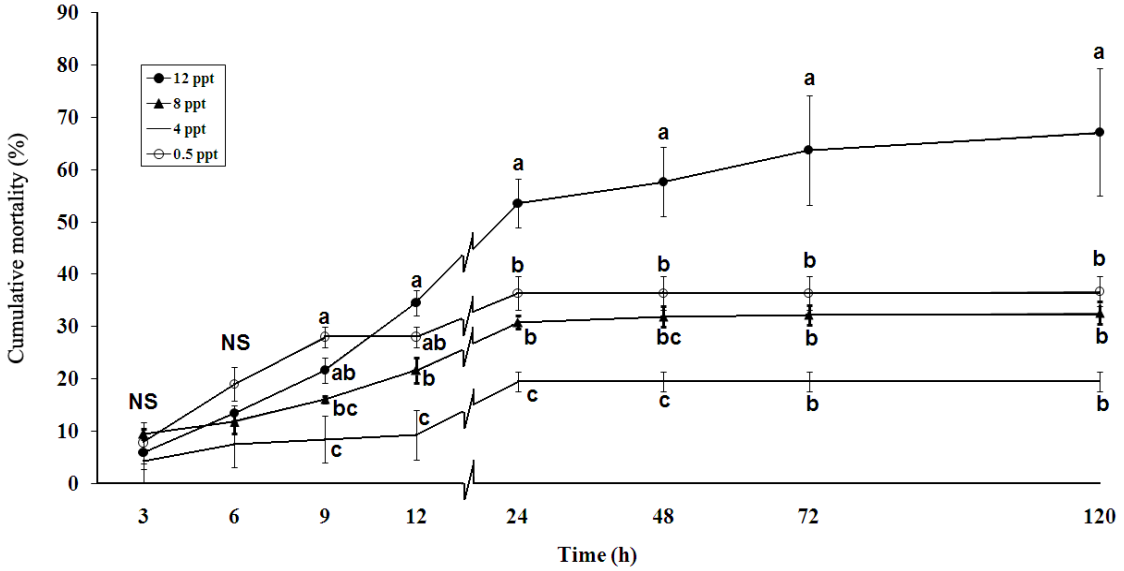

Fig. 1. Effect of salinity on cumulative mortality in Bream. Values are means ± SEM. Different letters over values indicate significantly different accumulated mortality between salinity exposures at that time point (P<0.05).

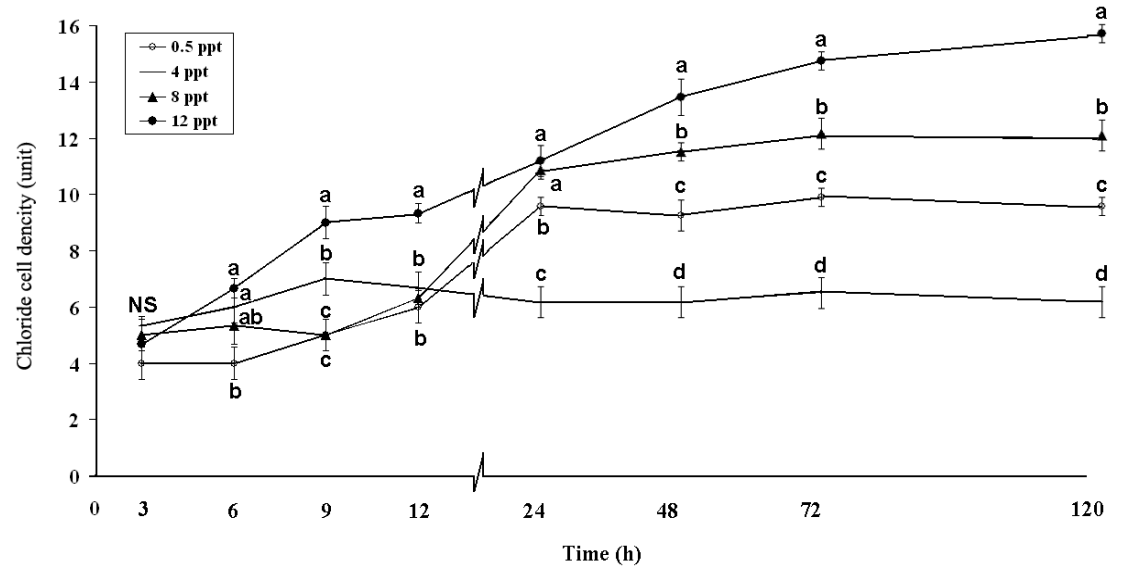

Fig. 2. Effect of salinity on chloride cells density in gills of Bream. Values presented as means ± SEM. Different letters over values indicate significantly different accumulated mortality between salinity treatment groups at that time point (P<0.05).

4. Discussion

Result of this study revealed that freshwater-adapted juvenile bream could not tolerate direct transfer to salinities of 12 ppt. Hence, its osmoregulatory mechanisms maybe insufficiently developed to adapt this salinity or unable to respond quickly enough to prevent massive osmoregulatory disturbance. After the initial 24 hrs exposure time, little mortality was observed in treatments but 12 ppt treatment, suggesting Bream fry can tolerate those salinities. Chervinski (1984) states that there are two types of freshwater fish – the so-called primary freshwater fishes (migrating completely in freshwater) and secondary freshwater fishes (experience saline habitats during a part of their life history). The primary freshwater fishes such as Clarids and Cyprinids are only able tolerate low

salinity levels below 9.75 g/L, whereas others such as Cichlids, Cyprinodontids and Poecilids are able to survive in higher salinity levels through gradual adaptation due to a more developed osmoregulatory capability. The upper tolerance limit of fry Bream in this study was identical to the tolerant limit of 8 ppt in the primary freshwater fish described by Chervinski (1984). This also suggested that fry Bream are true-freshwater species and will not grow if reared in salinities higher than 8 ppt.

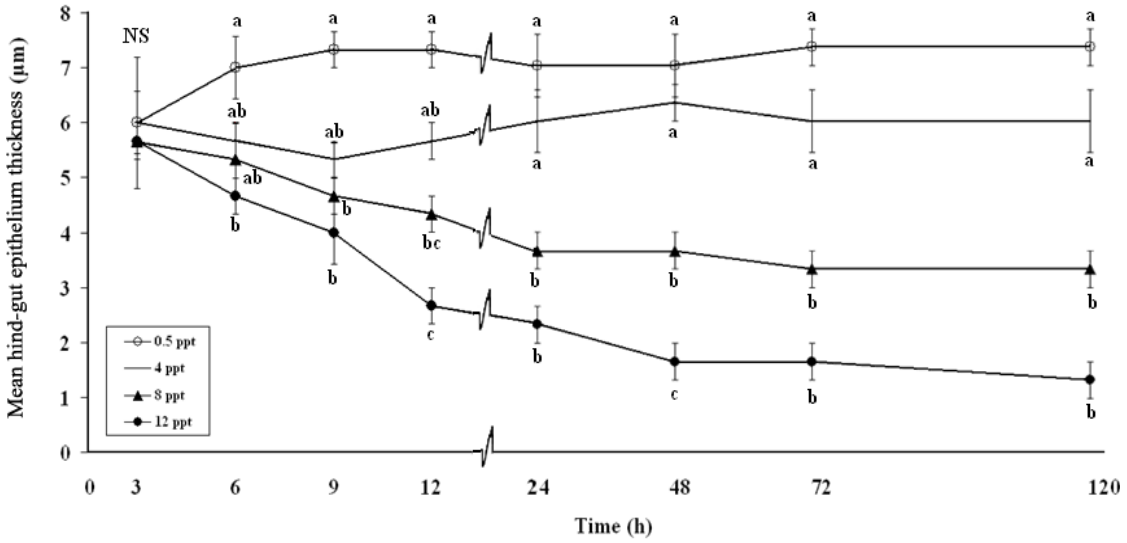

Fig. 3. Effect of salinity on hind-gut epithelium thickness in Bream. Bars represent means ± SEM. Different letters over bars indicate significant difference between salinities at that time point (P<0.05).

In this experiment, Bream fry chloride cells density increased after transfer to all treatments except 4 ppt. Bream fries could not acclimate themselves to 12 ppt and this probably as result of low number of chloride cell production to overcome this salinity level. Whereas, chloride cells seem enough at 0.5 and 8 ppt treatments after 24 hrs exposure time. For some years now, a seawater challenge (direct transfer to seawater for 24 hrs) has been used to estimate preparedness for entry into a seawater environment in a number of teleost fishes (e.g., salmonids, Brauner *et al*., 1992). The magnitude of haematological and iono-regulatory disturbance and gill CC differentiation associated with this challenge has been experimentally demonstrated to be a good predictor of long-term salinity tolerance in these fishes as indicated by survival, ion homoeostasis, and swimming performance (Brauner *et al*., 1992). Several studies have documented increases in chloride cell density and size in confrontation with a saline water (e.g., Foskett *et al*., 1983; Hoar 1988; Laurent and Hebebi 1989; Greco *et al*., 1996; Altinok *et al*., 1998; Sardella *et al*., 2004). However, Laiz-Carrio *et al.* (2005) reported that the number and size of CC were significantly increased when sea bream fish were exposed to salinity extremes (5% and 60%), and had fewer and smaller cells in intermediate salinities (15% and 25%).

There was no difference in chloride cell size during experimental period and treatment levels. This could be related to low age of fry and immature chloride cells (Thomson and Sargent, 1977). Contrary to our study, the number of branchial chloride cells in mudskipper, *Gillichthys mirabilis*, did not change, but chloride cells became larger when fish were transferred from 30 % to 60 % (Yoshikawa *et al*., 1993). Also, chloride cells of tilapia acclimated to a hyperosmotic (60 %) environment was two-fold and four-fold larger than those of fish reared in seawater and freshwater, respectively (Uchida *et al*., 2000). In addition, chloride cells of tilapia bear well-developed tubular networks and an increase in the size was associated with a major elevation in Na^+, K^{+-}ATPase activity (Uchida *et al*., 2000). On the other hand, Cioni *et al*. (1991) reported that neither chloride cells number nor their size increased in tilapia exposed to different salinity levels. Also, King *et al*. (1989) reported no increase in the number and size of chloride cells in cyprinodontids because of their ability to high salinity tolerance.

In this study, thickness of hid-gut epithelium layer decreased significantly when fish exposed to high salinity levels (8 and 12 ppt), while no significant difference was observed in low salinity of 0.5 and 4 ppt. This result is in agreements with the reports of Sundell *et al.* (2003); Ando and Nagashima (1996) and Grady and Wolters (1990),

but the result of Pickering and Morris (1973) on lamprey shows a contrary one. Basically, the increase in salinity level is caused to increase of hind-gut epithelium thickness (Grady and Wolters, 1990; Ando and Nagashima, 1996; Sundell *et al.,* 2003. Therefore, no significant change in epithelium layer thickness of 0.5 ppt treatment can confirm increasing of hind-gut epithelium in confrontation with saline habitats.

5. Conclusion

Although Bream fry could tolerate salinities between 0.5 to 8 ppt, but 4 ppt treatments shows best results and 12 ppt treatments was worse one. Therefore Anzali wetland with 4 ppt salinity is suggested as best releasing site and the Caspian Sea is strongly not suggested for this size of bream. Future study on other releasing sizes for bream restocking is suggested.

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