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Original Article

Effect of density on some physiological responses to transportation stress in *Mesopotamichthys sharpeyi* (Günther 1874) fingerlings

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Abstract: In the present study, the effect of density on transportation stress in *Mesopotamichthys sharpeyi* fingerlings was evaluated. For this purpose, four different densities, including 40, 80, 120 and 160 g/l were used as treatments each with 3 replicates. Simulation of transport procedure was carried out for 4 hrs. The blood samples were collected from the fish prior to loading from the stocking tank (control), after 4 hrs of transportation and from released fish into recovery glass tanks at 6, 12, 24, 48 and 96 hrs after transportation during recovery period. For blood sampling, fish immediately anesthetized by adding 2% 2-phenoxy ethanol and the blood samples were prepared. The cortisol, glucose and lactate value of plasma were measured. The results showed a significant increase in cortisol and glucose levels (in highest density) after transportation in all treatments ($P < 0.05$). Lactate did not show a significant difference in experimental groups ($P > 0.05$). The results showed that only cortisol level was significantly different with basal level at 96 hrs. Our findings showed that this species can be transported at higher densities up to 120 g/l.

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Introduction

In aquaculture, fish is exposed to stressful stimuli during transport, handling, netting and stocking at high densities (Dobsikova et al., 2009). Transporting fish is inevitable in aquaculture (Gbore et al., 2006), therefore, reduction of harmful effects of caused stress is a fundamental goal for its successful growth and production (Ashly, 2007). Hence, rearing programs of fishes in aquaculture industry usually involve subjecting fish to a variety of practices such as weighing, grading, transporting or increasing rearing density, all of which can be stressful to the fish.

Fish transportation is considered a traumatic procedure that exposes fish to a series of adverse stimuli responsible for several physiological responses. These stimuli include capture in ponds, handling, transportation, inappropriate stocking densities, physical handling, unfavorable water

quality and introducing fish to a new environment (Urbinate et al., 2004; Harmon, 2009). Stocking density in fish transportation can be led mechanical abrasion due to their contact (Urbinati et al., 2004). In addition, during transporting, fish is encountered a decrease in dissolved oxygen and an increase in carbon dioxide levels. Excretion of nitrogenous wastes also increase the level of ammonia in the transport medium. The increase of CO₂ concentration decrease water pH (Dobsikova et al., 2006). The poor water quality parameters are caused physiological stress affecting fish health (Kayali et al., 2011).

Mesopotamichthys sharpeyi (Günther 1874) is a commercially valuable cyprinid species of Karoun river drainage of Tigris basin, which has been considered as an important candidate for the aquaculture industry of Iran in recent years. Due to the low number of the hatcheries to produce

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Table 1. Composition of basal diet used in this study.

| Ingredients diets | (g/100g) |
|-----------------------|----------|
| Fish meal | 39 |
| Soybean meal | 23 |
| Casein | 15 |
| Wheat flour | 7.5 |
| Rice flour | 7.5 |
| Fish oil ^b | 6 |
| Vitamin premix | 1 |
| Mineral premix | 1 |

M. sharpeyi larvae, distribution and transport of produced larvae to rearing farms at long intervals is necessary. Since suitable methods and conditions to reduce fish stress is favorable in aquaculture, therefore, this study was conducted to find a suitable density for the transportation of this species to rearing farms and characterize physiological responses of *M. sharpeyi* during this process.

Materials and Methods

A total of 180 *M. sharpeyi* (with a mean length and weight of 10 ± 1 g and 9.8 ± 1 cm, respectively) were obtained from Shahid Maleki farm (Ahwaz, Khuzestan Province, Iran) and transported to the laboratory of marine natural resources department, (Khorramshahr University). The fish were acclimatized for one week in well-aerated 300 liters polyethylene tanks. During this period, they were fed twice a day with commercial pellet at 3% body weight. Diet composition is presented in Table 1. Water temperature ($25.6 \pm 0.84^\circ\text{C}$), dissolved oxygen (7.11 ± 0.36 mg/L) and pH (7.42 ± 0.18) were recorded daily during the experimental period. Eighty g/l is a routine density that used by practioniors for fish transportation, hence, densities of 40, 80, 120 and 160 g/l were considered as treatments in this study (each with 3 replicates). Simulation of transportation procedure was performed for 4 hrs (Urbinat et al., 2004), which represents the average transportation time. After examination, each group was introduced into 100 L glass aquarium for recovery and fed once a day with commercial fish pellet as 3% body weight, but they were not consumed the food. Fish were sampled prior to loading from the stocking

tank (control) in 3 replicates, immediately after transport (transportation effect) and 6, 12, 24, 48 and 96 hrs after transport (during the recovery period) based on Urbinat et al. (2004). For prevention of handling stress, fish were anesthetized by 0.2% 2-phenoxy ethanol to the glass aquarium and then blood samples were taken from the caudal vasculature using a heparinized syringe. Survival was recorded in an experimental unit at the end of transportation and recovery. Mortality rate determined by dividing the number of dead fish by number transported fish, multiplied by 100 (Gbore et al., 2006).

Because of the small size of fish, each blood sample resulted from a pool of 3-4 fish (Urbinat et al., 2004). The extracted blood was centrifuged at 3000 rpm for 10 min. The collected serum in eppendorf tubes was stored at -80°C for further assays (Acerete et al., 2004). Cortisol was measured in duplicate, using RIA kit (Immunotech, Czech Republic) with a sensitivity of 10 nM (Ghaedi et al., 2013). Glucose and lactate were determined by enzymatic colorimetric and by enzymatic method using commercial kits (Pars Axmoon, Iran), respectively (Salati et al., 2010).

Water temperature, pH, total ammonia and dissolved oxygen were monitored in the tank (before loading), immediately after the fish transportation and 6, 12, 24, 48 and 96 hrs after transport (Table 2). Water temperature, pH and dissolved oxygen were measured using HATCH multimeter and total ammonia was determined by Nessler method according to standard method (Standard method, 1992).

The data was analysed by SPSS 11.5 software. Data are presented as mean \pm SE. One way ANOVA and Tukey's post hoc were used to compare the parameters between treatments at each sampling time and different time at each density. $P < 0.05$ was accepted for statistical significance.

Results

Blood cortisol was increased significantly in all treatments compared to the basal value after 4 hrs of

Table 2. Water quality parameters before and after transportation of *Mesopotamichthys sharpeyi* and during recovery period.

| Parameters | density | Before transport | After transport | Empty aquarium | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 96 hrs |
|--|----------------------------------|------------------|-----------------|----------------|-------|--------|--------|--------|--------|
| PH | 40 g ^l ⁻¹ | 7.16 | 7.31 | 7.16 | 7.20 | 7.30 | 7.25 | 7.25 | 7.24 |
| | 80 g ^l ⁻¹ | 7.16 | 7.11 | 7.16 | 7.16 | 7.17 | 7.16 | 7.85 | 7.36 |
| | 120 g ^l ⁻¹ | 7.16 | 6.80 | 7.60 | 7.83 | 7.64 | 7.37 | 7.78 | 7.36 |
| | 160 g ^l ⁻¹ | 7.16 | 7.34 | 7.30 | 7.27 | 7.21 | 7.19 | 7.85 | 8.50 |
| DO (mg ^l ⁻¹) | 40 g ^l ⁻¹ | 8.60 | 0.83 | 8.70 | 8.20 | 7.90 | 7.80 | 7.48 | 7.36 |
| | 80 g ^l ⁻¹ | 8.60 | 0.78 | 8.60 | 8.08 | 8.01 | 8.10 | 7.64 | 7.83 |
| | 120 g ^l ⁻¹ | 8.60 | 0.76 | 8.70 | 8.40 | 8.30 | 8.50 | 8.20 | 7.90 |
| | 160 g ^l ⁻¹ | 8.60 | 0.85 | 8.40 | 8.37 | 8.33 | 8.11 | 8.06 | 7.81 |
| NH ₃ (μg ^l ⁻¹) | 40 g ^l ⁻¹ | 13.30 | 36.70 | 8.4 | 19.8 | 15.3 | 21.6 | 11.4 | 26.2 |
| | 80 g ^l ⁻¹ | 13.30 | 52.70 | 7.2 | 8.0 | 7.4 | 12.9 | 13.2 | 14. |
| | 120 g ^l ⁻¹ | 13.30 | 63.40 | 10.7 | 23.5 | 19.2 | 15.5 | 16.5 | 24.2 |
| | 160 g ^l ⁻¹ | 13.30 | 64.90 | 11.3 | 16.2 | 17.0 | 13.6 | 19.2 | 16.6 |

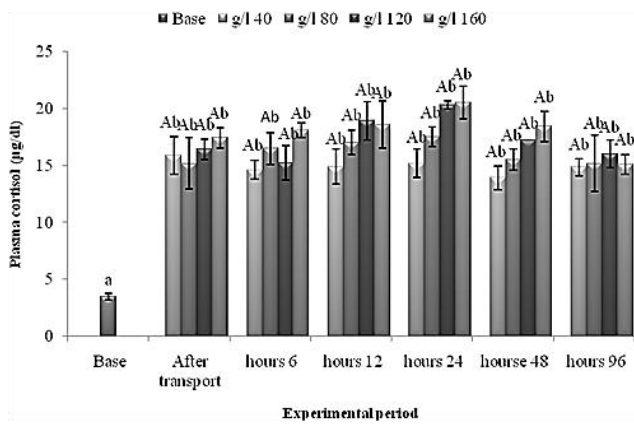


Figure 1. Plasma Cortisol (μg/dl) levels of *Mesopotamichthys sharpeyi* in response to transportation stress in different densities. Same capital letters indicates no differences among treatments within sampling time and lower case letters among same treatment in different sampling ($P<0.05$).

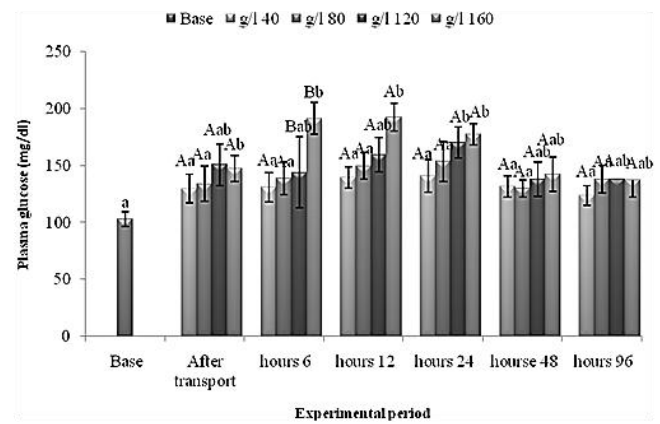


Figure 2. Plasma glucose (mg/dl) level of *Mesopotamichthys sharpeyi* in response to transportation stress in different densities. Same capital letters indicate no differences among treatments within sampling time and lower case letters among same treatment in different sampling ($P<0.05$).

the transportation ($P<0.05$) (Fig. 1). After 4 hrs transportation period, the blood cortisol levels of fish submitted to the highest loading density were higher than fish at lowest loading density, but their differences were not significant ($P>0.05$).

A little increase in cortisol levels were observed in 6, 12 and 24 hrs compared to 0 hrs after transportation, but it was not significantly different. The reduction in cortisol levels continued after 48 hrs recovery. In the present experiment even after 96 hrs, the cortisol levels was remained elevated.

After transportation, an increase in glucose was recorded compared to the base value, but these changes were only significant in 160 g/l treatment ($P<0.05$) (Fig. 2). Fish transported in the highest densities showed higher values of glucose, but no

significant differences were verified in values of blood glucose level in all densities ($P>0.05$). In the recovery aquarium, the glucose value showed a little increase at 6 and 12 hrs after transportation compared to 0 hrs but was not significant. The glucose levels in all densities with the exception of lower density at 48 and 96 hrs after transport, returned to near baseline levels at the end of the recovery period.

After transportation, no significant difference was found in the blood lactate between treatments ($P>0.05$) (Fig. 3). In the present experiment, 24 hrs after transportation the blood lactate showed an increase and its concentration was decreased in 48 and 96 hrs. This alterations were not significant from that of the base value ($P>0.05$).

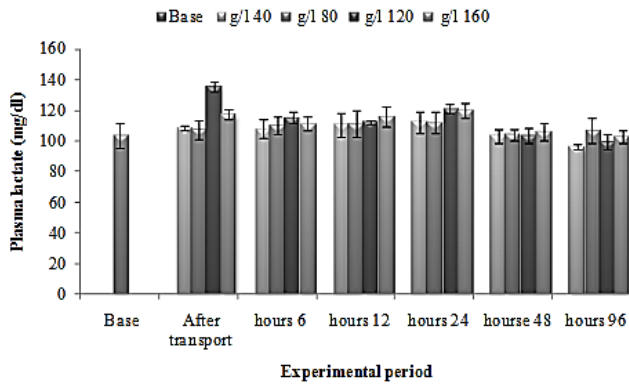


Figure 3. Plasma lactate (mg/dl) level of *Mesopotamichthys sharpeyi* in response to transportation stress in different densities. Same capital letters indicate no differences among treatments within sampling time and lower case letters among same treatment in different sampling ($P < 0.05$).

The results showed increasing the mortality in plastic bags and aquariums in higher densities (Table 3).

Discussion

In the present study, the blood cortisol increased in all treatments compared to the basal values after 4 hrs of the transportation. Furthermore, the blood cortisol levels of those fish submitted to the highest loading density were higher than those of the lowest loading density, but these differences were not significant. In response to stressful events, the hypothalamic portion of the brain stimulates the release of adrenocorticotrophic hormone (ACTH). ACTH is circulated into the anterior kidney, where it stimulates the interrenal cells to produce cortisol and other corticosteroid hormones (Kubilay and Ulukoy, 2002). Similar findings have been reported in other fish species. Carneiro et al. (2009) in Jundiã (*Rhoadia quelen*), Abreu et al. (2008) in Matrinxã (*Brycon amazonicus*), Adamante et al. (2008) in *Salminus brasiliensis* fingerlings, Acerete et al. (2004) in *Perca fluviatilis* L., Bolasina (2011) in juvenile *Paralichthys orbignyanus*, Hur et al. (2009) in *Paralichthys olivaceus*, Urbinati et al. (2004) in Matrinxã (*B. cephalus*), Dobsikova et al. (2009) in *Cyprinus carpio* and Grutter and Pankurest (2000) in *Hemigymnus melapterus* reported an increase in plasma cortisol level in response to transportation stress. Whereas, Dobsikova et al. (2009) and Svobodova et al. (1999) reported a decrease in

Table 3. Mortality rate (%) during the study.

| | 40 g ^l ⁻¹ | 80 g ^l ⁻¹ | 120 g ^l ⁻¹ | 160 g ^l ⁻¹ |
|-------------------------|---------------------------------|---------------------------------|----------------------------------|----------------------------------|
| Polyethylene bag | 0.79 | 5.55 | 6.34 | 11.90 |
| Aquarium | 0.00 | 4.76 | 3.17 | 3.17 |
| Total | 0.79 | 10.31 | 13.48 | 15.07 |

plasma cortisol in *C. carpio* during transportation. A little increase in cortisol levels were observed in 6, 12 and 24 hrs after transportation. Furthermore, after 96 hrs, the cortisol levels was remained elevated that may be due to new environment and starvation stress during recovery period. New environment is a stressor that can alter the recovery procedure. Many other factors including genetic, developmental, and environmental (e.g. temperature, nutrition and water quality) factors influence stress responses in fishes (Barton, 2002). Animals respond to challenges in their environment through several interacting mechanisms, including behavioral, hematochemical, physiological and neuro-hormonal parameters (Fazio and Ferlazzo, 2003). Barcellose et al. (2001) observed similar result in *R. quelen* after transference to growing tank, which could explain the fast effect (Adamante et al., 2008). The cortisol levels showed reducing after 48 hrs recovery. Similar results were observed by Carmichael (1983) who subjected largemouth bass (*Micropterus salmonides*) to long distance transport. Carneiro et al. (2009) in Jundiã (*R. quelen*), Abreu et al. (2008) in Matrinxã (*B. amazonicus*), Hur et al. (2009) in *P. olivaceus* and Urbinati et al. (2004) in Matrinxã (*B. cephalus*) reported that the blood cortisol values returned to normal value during the first 24 hrs after transportation. Such rapid decreases in plasma cortisol levels could be related to the low intensity of stress (Urbinati et al., 2004). Adamante et al. (2008) in fingerlings of *S. brasiliensis* and Sulikowski et al. (2006) in flounder, *Pseudopleuronectes americanus* were reported that cortisol levels of lower densities, returned rapidly to near baseline levels.

After transportation, an increase in glucose was recorded compared to base value, but this change was significant only in 160 g/l treatment. The high glucose level is characteristic of the secondary

response which is caused by an increase in catecholamines and cortisol levels and provides energy for the fish to respond to the demand generated by the behavioral response to stress stimuli (Carneiro et al., 2009). Urbinati et al. (2004) in Matrinxã (*B. cephalus*), Abreu et al. (2008) in Matrinxã (*B. amazonicus*), Carneiro et al. (2009) in Jundiã (*R. quelen*), Dobsikova et al. (2009) in (*C. carpio*), Nomura et al. (2009) in (*Salmo salar*) and Hur et al. (2009) in (*P. olivaceus*) reported an increases in blood glucose level following encountered stress. In contradict, Acerete et al. (2004) in *P. fluviatilis* did not record any change in blood glucose level.

The low magnitude of glucose responses can indicate low activation of the brain-sympathetic-chromaffin cell axis, and hence a low release of catecholamines, which seems to occur at a higher extent of severe stresses (Abreu et al., 2009). Elevated cortisol secretion under stress increases the activation of plasma glucose by activity of the gluconeogenesis enzyme (Hur et al., 2009). In the recovery aquarium, the glucose concentration showed a little increase at 6 and 12 hrs after transportation compared to that of 0 hrs but was not significant. This increase may be due to the new environment and demanding energy for behavioral responses to stress.

In the present study, fish transported in high densities showed higher values of the glucose, but no significant differences were verified in values of blood glucose level in all densities. Similar to our findings, Abreu et al. (2008) in Matrinxã (*B. amazonicus*) and Carneiro et al. (2009) in Jundiã (*R. quelen*) reported that glucose level is directly related to fish density. The glucose level was returned to near baseline levels at the end of the recovery. Decrease in the glucose level appears to be a high glucose demand to supply the energy (Martinez-Alvarez et al., 2002). Also, it may be as result of appetite decrease and starvation due to consumption of plasmatic glucose. Glucose levels in all densities with the exception of lower density at 48 and 96 hrs after transport, returned to near baseline levels at the end of the recovery, which may be due

to intensity of stress in different experimental groups. This difference can be related to low demand for breakdown of energy resource.

After transportation no significant difference was verified in values of blood lactate in all densities. Hur et al. (2009) in *P. olivaceus*, Dobsikova et al. (2009) in *C. carpio*, Inversen et al. (2005) in *S. salar*, Nomura et al. (2009) in *S. salar* and Inversen et al. (1998) in *S. salar* were reported lactate increased after transportation. Blood lactate is considered as a secondary stress response that is released from white muscle following vigorous exercise (Nomura et al., 2009). In the present study, 24 hrs after transportation, the lactate showed an increase and it was decreased in 48 and 96 hrs, which was not significantly different from base value. The lactate level in lower density showed a rapid decreases that could be related to low hypoxia. No significant difference in lactate levels were observed in this study that could be related to low severity of transportation stress for *M. sharpeyi*.

In the present study, the mortality was observed in fish exposed to transport stress for 4 hrs. Despite providing proper condition in the applied closed systems, the method can become a limiting factor, as well as an important stress factor due to the accumulation of metabolites in water such as carbon dioxide and total ammonia, and also due to the decreasing of dissolved oxygen (DO) and changing PH (Adamante et al., 2008).

In our study, mortality in plastic bags and aquariums were increased in higher densities. The higher stocking densities overload with produced metabolites is led stress (Conte, 2004). In addition, fish transported at high densities are submitted to mechanical abrasion due to contact between them, an important stress factor which can cause loss of fish scales and mucus facilitating the infection process and diseases (Urbinati et al., 2004). Transportation consists of several traumatic events (stressors), including capture, loading, transport, unloading and stocking (Inversen et al., 2005). Pavlidis et al. (2003) in Red porgy (*Pagrus pagrus*), without water renewal, reported an increased mortality with

increasing stocking density. A high density associated with a very long time of transportation can stress fish, impair transportation efficiency, and cause mortality, as well as cause negative effects on animals performance. Hence, an acute stress during transportation can predispose fish to pathologies after the stock-term due to immunosuppression caused by stress (Adamante et al., 2008). Abreu et al. (2008) in Matrinxã (*B. amazonicus*) no mortality was registered through the week following the transport. Matrinxã demonstrated to be a crowding tolerant-species in transport. Also Urbinati et al. (2004) in Matrinxã (*B. cephalus*) no mortality was observed among the fish during the whole experimental period.

Based on little changes in physiological indicators of stress after transportation and low mortality rate at different densities in the present study, it can be concluded that *M. sharpeyi* fingerlings could be transported at higher densities up to 120 g/l without significant changes in physiologic status.

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چکیده فارسی

اثر تراکم بر روی برخی پاسخ‌های فیزیولوژیک به استرس انتقال در ماهیان انگشت قد بنی (*Mesopotamoichthys sharpeyi* (Günther, 1874

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چکیده:

در مطالعه حاضر اثر تراکم بر استرس انتقال در ماهیان انگشت قد بنی *Mesopotamichthys sharpeyi* مورد بررسی قرار گرفت. برای این منظور چهار تراکم ۴۰، ۸۰، ۱۲۰ و ۱۶۰ گرم بر لیتر در سه تکرار در این مطالعه مورد استفاده قرار گرفت. شبیه سازی فرایند انتقال به مدت ۴ ساعت انجام شد. نمونه خون ماهیان قبل از بارگیری در تانک های ذخیره سازی (کنترل)، پس از ۴ ساعت انتقال و از ماهیان در تانک‌های ریکاوری توزیع شده در زمان‌های ۶، ۱۲، ۲۴، ۴۸ و ۹۶ ساعت پس انتقال جمع‌آوری شد. برای نمونه‌برداری از خون، ماهیان بلافاصله پس از صید با ماده بیهوشی ۲- فنوکسی اتانول ۰.۲٪ بیهوش شدند و نمونه خون از آن‌ها اخذ شد. پس از جداسازی پلاسما میزان کورتیزول، گلوکز و لاکتات در آن‌ها اندازه‌گیری شد. نتایج افزایش معنی‌داری در میزان کورتیزول و گلوکز پس از انتقال در همه تراکم‌ها نشان داد ($P > 0.05$). لاکتات افزایش معنی‌داری در گروه‌های آزمایشی نشان نداد ($P < 0.05$). فقط سطح کورتیزول در زمان ۹۶ ساعت پس از انتقال با سطح پایه اختلاف نشان داد. یافته‌های ما نشان می‌دهد این گونه می‌تواند در تراکم‌های بالا تا ۱۲۰ گرم بر لیتر جابه‌جا شود.

کلمات کلیدی: استرس، کورتیزول، لاکتات، گلوکز، *Mesopotamichthys sharpeyi*.