Iranian Journal of Fisheries Sciences 21(5) 1156-1165 2022
DOI: 10.22092/ijfs.2022.127711

Research Article

Life stages and seasonal variances of salinity tolerance of *Triplophysa yarkandensis* (Day，1877) in Tarim River

Zhichao W.[1]; Xiaolei W.[2]; Junyuan W.[1*]; Benno K.[3]; Daoquan R.[1]; Cuifang Y.[4*]

Received: November 2017 Accepted: December 2018

Abstract

In order to explore the influence of increased salinization on fish populations in Tarim River, sodium chloride (NaCl) salinity tolerance in *T. yarkandensis* (a native fish species), was measured in laboratory by applying slow acclimation (SA, gradual salinity increase) and direct transfer (DT) methods in spring season. Whereas Probit method was used to calculate the median lethal concentration (LC_{50}). Results of slow acclimation for adult, juvenile and larva fish (LC_{50}) showed 16.51 g/L, 12.94 g/L and 8.13 g/L, respectively. Results of salinity tolerance (LC_{50}) by DT method, were recorded as 16.16 g/L, 11.01 g/L, and 7.37 g/L for adult, juvenile and larva, respectively. The salinity tolerance of the fish showed age-dependent increasing tendency. The experiments have also been conducted in three other seasons to study the relationship between salinity tolerance of adults and seasonal salinity variances in Tarim River. Results showed a significant S relationship between river's salinity and the LC_{50} of adults (data mixed the SA and DT) by using equations $y=e^{2.788-0.121/X}$ (R^2=0.994, P=0.003) for DT, and $y=e^{2.771-0.120/X}$ (R^2=0.927,P=0.037) for SA. These findings suggest that *T. yarkandensis* has a physiological adaptation for osmoregulation in developmental processes and in dealing with the seasonal salinity variances of the Tarim River.

Keywords: *Triplophysa Yarkandensis*, Salinity tolerance, Tarim River, Age, Seasonal variances

1- Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Key Laboratory of Animal Husbandry Science and Technology of Xinjiang Production & Construction Corps，College of Life Science, Tarim University, Alar,843300, China
2- Shandong First Medical University, Tai'an, 271016, China
3-Catholic University Eichstätt-Ingolstadt, Eichstätt, 85072, German
4-College of Information Engineering, Tarim University, Alar, 843300, China
**Corresponding author's Email: 67785053@qq.com, 420084752@qq.com

Introduction

Tarim River is the longest inland river of China flowing its path in North-West of the country. In the past few decades, the increasing salt concentration make its water saline and the salt concentration varies seasonally (Li *et al.*, 2006). Alike to other rivers in arid and semi-arid area, the sources of salt to the Tarim River can be attributed to rock weathering in mountain areas, high evaporation rates and human activities, including land-clearing and irrigation (Williams, 1987; Williams and Williams, 1991;Chen *et al.*, 2008).

Diversity of fish fauna of Tarim River system is poor known only 15 species have been reported till now (Wang *et al.*, 2010), which is well below the world average in comparison with other rivers of similar size (Walker, 1983). Many species, such as the Forehead Swelling Loach (*Triplophysa bombifrons*, Herzenstein), are found exclusively in the Tarim River Basin, and some others such as Tarim Schizothoracin (*Schizothorax biddulphi*, Gunther) and the Big-Head Schizothoracin (*Aspiorhynchus laticeps*, Day) are both endemic and endangered (Le and Chen, 1998).

Most native species have been declining in range and abundance since 1960, due to the introduction of exotic fishes, the massive cultivation of waste lands, construction diversion of water resources for irrigation (Mark and Zhang, 2001) and their continued survival is now of high concern. Human activities within the river basin, causing a gradual increase in river salinity, are a potential threat for fish survival. Since 1970 average salinity of the Tarim River was increasing and have exceeded 1 g/L. Regression analysis of the salinity change from 1960 to 2000 showed a significant exponential relationship, which can be described by the equation $y=2E\text{-}21e^{0.0241X}$ ($R^2=0.9145$) (Chen *et al.*, 2008).

The effects of increased river salinity on the native flora and fauna of Tarim River have not been investigated. There are few or nearly no surveys of salinity tolerance in native species conducted until now. Therefore, the main aim of this study was to improve our knowledge about salinity tolerance of *T. yarkandensis* (the widespread fish species in the Tarim River Basin) at different developmental stages and supply additional information to protect *T. yarkandensis* biodiversity with regard to Tarim River's salinization and its seasonal variances.

Materials and methods

Specimens of *T. yarkandensis* were collected from the drainages or the upper reaches of the Tarim River, near Alar city (40°32'23.50" N, 81°17'41.61" E) from September 2010 to May 2012. Captured fishes for the experiments were sorted into three groups (adult, juvenile and larva stages) based on the morphology and the total length (Zeng and Tang, 2010). The adult group samples are 10-12 cm in total length, juvenile samples are about 2 cm, and larva group samples are about 0.6 cm. Fishes were separately maintained in 3 groups for one week in 100L aquaria at

20 ℃ prior to experiment. During this time and then throughout experimental period, adult fishes were fed with pellet diet and pupa of honeybee (best baits for the fishing in the trunk of the Tarim River), while the larva and juvenile fishes were fed with *Artemia* nauplii.

Round glass aquaria (H 90 cm×D 50 cm) were used for experiments. Washed river sand was added as substrate and sodium chloride was used for preparation of different salinity concentrations. Tanks were initially filled with 14 L of distilled water mixed with sodium chloride at concentration of 0.30 g/L and they were fitted with internal power filters. For adult group, the water re-circulated with a speed of 60 $L\cdot h^{-1}$. All groups were maintained at 20°C in temperature controlled room, applying a natural light/dark cycle.

Two tanks (one experimental, one control) were used for different groups but without replication. 20 adult, 16 juvenile and 16 larva fishes were randomly selected for each tank. Samples were allowed to acclimatize for two days before experiment begun. Salinities were increased in the speed of 2 g/L·day in the experimental tanks by adding sodium chloride. Mortality was measured 24 hours after salt addition and counted against current salinity. Whether fishes were in obvious distress was also recorded. Dead fishes were removed and water lost by evaporation replaced with distilled water.

Tanks used for direct transfer experiments were set up in the same way as those for slow acclimation experiments. Preliminary experimental salinities were 5, 10, 15, 20, 25 and 30 g/L, then, according to the preliminary results, experimental salinities were adjusted to 6.20, 7.07，9.99，11.90，14.14 g/L with ratio of equality of 2^{-2}. Ten adult, juvenile, larva fishes were placed directly into each tank. Mortalities were counted after 4, 8, 12, 24, 48, 72 and 96 hours. LC_{50} values were calculated for each exposure time as described by Green (1965).

The salinity of Tarim River was monitored with a portable field multiparameter water quality monitoring system (YSI650 MDS, Doppler America) from 2010 to 2012. Relationship between seasonal salinity variances of Tarim River and salinity tolerance of *T. yarkandensis* was examed using nonlinear regression analysis by SPSS software (Version 13.0 for windows, SPSS Inc.).

Results

Salinity tolerance of T. yarkandensis in Spring at different life stages

The results of slow acclimation experiments are shown in Table 1. Deaths of adult fishes occurred between 14 and 20 g/L salinity. The salt concentration and fish mortality interdependency curve (Fig. 1) indicated an LC_{50} of 16.51 g/L. Only one death occurred in the control tank. Individuals began to show signs of distress at salinities higher than 14 g/L. Most specimens swam in an uncoordinated fashion and were unable to maintain balance properly when salinity reached to 18 g/L and higher.

The death of juvenile appeared at the concentration of 10 g/L and all others died at 18 g/L . Inter-dependency curve of salinity and survival, as shown in Figure 2, pointed out an LC_{50} of 12.94 g/L. No death occurred in the control tank. Individuals began to show signs of distress at salinities higher than 14 g/L.

Table 1: Results of slow acclimation experiments in Spring.

Group	Probit =a +bx	LC_{50} (g/L)
Larva	Probit=-2.58130+3.12646X	8.13
Juvenile	Probit=-6.31844+5.6816X	12.94
Adult	Probit =-9.70859+7.97218X	16.51

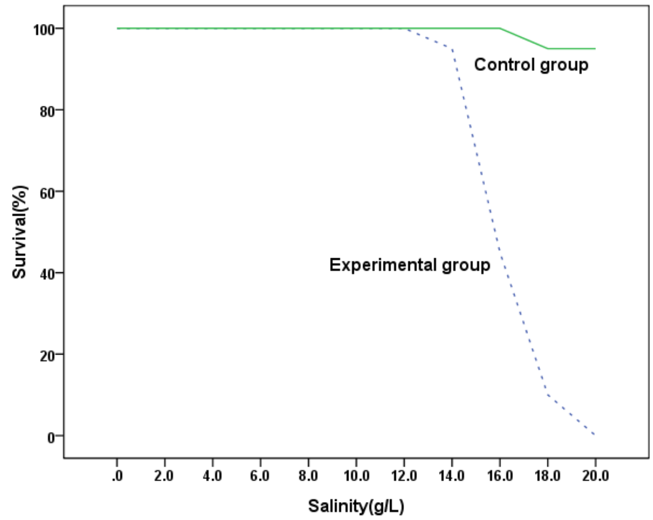

Figure 1: Interdependency curve of salinity and survival for adults in Slow Acclimation experiments.

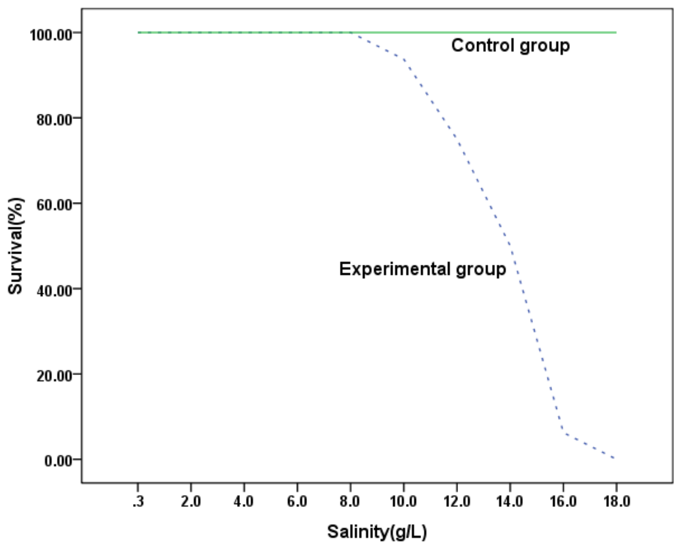

Figure 2: Interdependency curve of salinity and survival for juveniles in Slow Acclimation experiments.

The mortality of larva samples have been recorded between 2 to 18 g/L.The salt concentration and fish mortality interdependency curve (Fig. 3) indicated an LC_{50} of 8.13 g/L. There was no death record in control treatment. Individuals began to show signs of distress at salinities higher than 10 g/L.

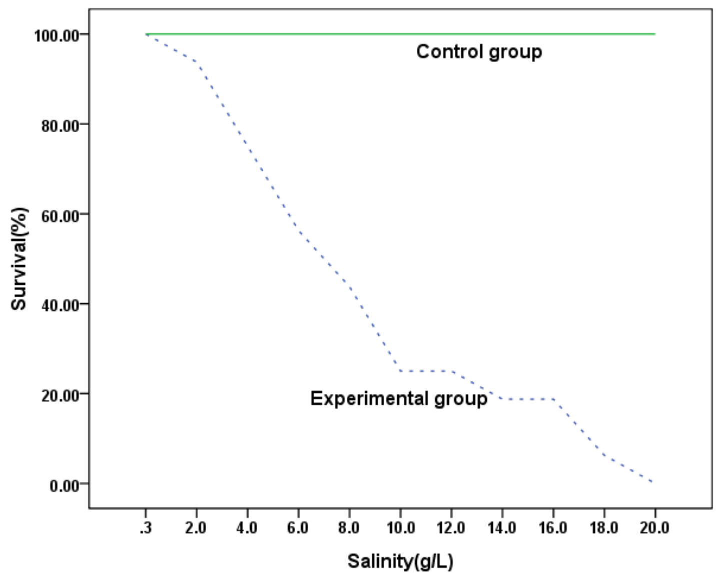

Figure 3: Interdependency curve of salinity and survival for larvae in Slow Acclimation experiments.

The value of LC_{50} was calculated for direct transfer experiments at 4, 8, 12, 24, 48, 72 and 96 hours after the start of the experiment. Transformed data to LC_{50} and exposure $time^{-1}$ interdependency can be described by linear relationship, y=16.162+17.249X (R^2=0.708, P=0.018) for adult fishes. Here the y represents intercept, viz. the LC_{50} at infinite exposure time, equal to 16.16 g/L. For juvenile fishes the linear relationship is equal to y =11.545X+11.007 (R^2=0.863, P=0.002), where the LC_{50} at infinite exposure time is 11.01 g/L. For larva group the linear relationship is described by y=7.365+30.200X (R^2=0.599, P=0.041) and the LC_{50} at infinite exposure time is 7.37 g/L. With regard to the results of direct transfer and slow increasing salinity, the salinity tolerance in all three fish groups showed increasing tendency for both experiments.

Relationship between seasonal salinity variances of Tarim River and salinity tolerance of T. yarkandensis

In the direct transfer experiments, linear relationship between LC_{50} values and exposure $time^{-1}$ in spring can be described by y=16.162+17.249X (R^2=0.708, P=0.018), in summer, the correlation can be described by y=12.474+23.917X (R^2=0.838, P=0.004), as well as y=15.422+17.556X (R^2=0.730, P=0.014) and y=15.686+20.252X(R^2=0.752, P=0.011) for autumn and winter respectively. Here the y represents intercept, viz. the LC_{50} at infinite exposure time, equal to 16.16 g/L (spring), 12.47g/L (summer), 15.42g/L (autumn) and 15.69g/L in winter. In the slow acclimation experiments, the Probit equation of different seasons was showed in Table 2. Table 3 showed the results of LC_{50} of the adult for both slow acclimation and direct transfer experiments in different seasons, furthermore the salinity of the Tarim River has been presented in experimental years (2010-2012) .There is significantly S relation between Tarim River's salinity and the LC_{50} (date mixed the SA and DT), the equation is $y=e^{2.788-0.121/X}$ (R^2=0.994, P=0.003) for DT, and $y=e^{2.771-0.120/X}$(R^2=0.927, P=0.037) for SA.

Table 2: Probit equation of slow acclimation experiments in Different Seasons.

Season	Probit =a +bx	LC_{50} (g/L)
Spring(May)	Probit =-9.70859+7.97218X	16.51
Summer(August)	Probit=-14.10859+12.90854X	12.94
Autumn(October)	Probit =-14.28950+12.2559X	14.65
Winter(January)	Probit=-14.35362+12.12296X	15.38

Table 3: Results of LC_{50} and Tarim River's Salinity in Different Seasons.

Specimens collection month	Tarim River's salinity (g/L)	LC_{50} (g/L)	
		SA	DT
Spring (May)	11.04	16.51	16.16
Summer (August)	0.46	12.39	12.47
Autumn (October)	2.03	14.65	15.42
Winter (January)	5.43	15.28	15.69

Discussion

Salinity tolerance is an important parameter in the fish biology, especially in marine culture. It affects the survival, metabolism, disease resistance, osmoregulation etc. during the fish development(Varsamos *et al.*, 2005). Investigations on the salinity tolerance of freshwater species also have shared a common ecological motivation. Investigations by Bringolf *et al.* (2005), Schofield *et al.* (2006), and Susanne *et al.* (2012) suggested the low salinity tolerances as barriers to invasion for the flathead catfish, goldfish and Eurasian round goby, respectively. While the main aim of the current study is to evaluate the future of *T. yarkandensis* in Tarim River, the salinity tolerance study should provide valuable information about the species' ability to deal with osmoregulatory stressors and could further be extrapolated for evaluating its population change with increase of salinity in the Tarim River.

According to the Brett (1979) ecological categorization standards, the fish of *T. yarkandensis* in the Tarim River belongs to euryhaline fresh water fish. With regards to the results of LC_{50} for larva, juvenile and adult fishes, the salinity tolerance tends to increase throughout its life developmental stages. The increase in salinity tolerance in other teleosts is either age-dependent, as in *Alosa sapidissima* (Zydlewski and

McCormick, 1997 a, b), Cobia (Cynthia and Holt, 2006), Atlantic sturgeon (Edwin and David, 2009) or size-dependent as in salmonids (Parry, 1958; Farmer *et al.*, 1978; McCormick and Naiman, 1984), *Acipenser transmontanus* (Mojazi *et al.*, 2009), *Oreochromis aureus* and O. *niloticus* (Watanabe *et al.*, 1985). Our results of *T. yarkandensis* seem similar with the above fishes as its salinity tolerance increased with the developmental stages.

Since the salinity tolerance in any kind of fish is always limited, therefore, they can only live in environment with salinity concentration lower than their limits. Chen *et al.* (2008) made an exponential model of the Tarim River salinity variance based on the hydrological data of the period of 1960 to 2000, and with the prediction in the year 2015, the average salinity of Tarim River might reach to 3.01 g/L, while in the year 2020 it might be even 3.40 g/L.These predictions were based on scenarios of different supply of water from the glacier and various evaporation rates in different seasons. It has to be pointed out that the salinity of Tarim River always showed two crests and two hollows every year *i.e.* in April or May with the highest salinity, which is 4-5 times of the average salinity of the year (Chen *et al*., 2008). So while looking to the predictions based on later data of salt concentration in Tarim River, the salinity can reach to 13.6-17 g/L in April or May 2020. This exceeds the tolerance of *T. yarkandensis*.

Kefford *et al.* (2004) found that laboratory salinity tolerances (slow acclimation LC_{50}) of freshwater adult fishes had better correspondences with their maximum field salinities (field distribution). In this situation, the fish *T. yarkandensis* in the Tarim River will be under threat or in dangerous in the year 2020. Moreover, if considering the overfishing and the numerous water diversions and dams along the river, the population of the *T. yarkandensis* in Tarim River probably may face more danger. But relative to our results, *T. yarkandensis* doesn't show same pattern of salinity tolerance in different seasons. The salinity tolerances were in positive correlation with the seasonal salinity variances of Tarim River. It probably means that with half century's accompany with the seasonal salinity variances of Tarim River, *T. yarkandensis* has adaption to the seasonal environmental changes, and their physiological functions accommodate to the environmental fluctuation. This physiological adaption already reported in notothenioid teleost, marine copepod *etc.* (Vargas-Chacoff *et al.*, 2016; Karlsson *et al.*, 2018). Interestingly, there is a paradoxical result for larva, the salinity of Tarim River in spring is higher than the salinity tolerance of *T. yarkandensis* larva (Table 3), as the most larva hatch in this season. In our opinion, one reason probably the larvae were hatched in early spring, when the salinity of Tarim River is less than 11.7 g/L. The other reason probably lies on the farmland drainages and channels (salinity always fluctuated in 4-5 all the year), which directly connect with the Tarim River. They supply the refuge habitats to the larva, and in our field investigations we indeed found schools of larvae swimming in these drainages in spring seasons.

Acknowledgements

This work was supported by the Project SuMaRiO - Sustainable Management of River Oases along the Tarim River (wp4.1).

References

Brett, J.R., 1979. Environmental factors and growth. In: W.S. Hoar, D.J.Randall, and J.R.,Brett(ed.). Fish Physilolgy, volume 8, Bioenergetics and Growth. USA: Academic Press lnc., 599-675.

Bringolf, R.B., Kwak, T.J., Cope, W.G. and Larimore, M.S., 2005. Salinity tolerance of the Flathead Catfish: implications for dispersal of introduced populations. *Transactions of the American Fisheries Society*, 134, 927–936. http://dx.doi.org/10.1577/T04-195.1

Chen, X.B., Yang, J.S., Yang, Z.H., Hu, S.J., Liu, G.M. and Wang, Y.L., 2008. Influence of wasteland development on Tarim River quality and primary countermeasure—A case study from Alar hydrometric station. *Journal of Agro-Environment Science*, 27(**1**),0327- 0332.

Cynthia, K. F. and Holt, G.J., 2006. Responses of cobia *Rachycentron canadum* larvae to abrupt or gradual changes in salinity. Aquaculture, 254(**1–4**), 275-283. http://dx.doi.org/10.1016/j.aquaculture.2005.10.046

Edwin, J.N. and David, H.S., 2009. Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I. Laboratory results. *Journal of Experimental Marine Biology and Ecology*, 381, Supplement, S150-S160. http://dx.doi.org/10.1016/j.jembe.2009.07.018

Farmer, G., Ritter, J. and Ashfield, D., 1978. Seawater adaptation and parr-smolt transformat on of juvenile Atlantic salmon, *Salmo salar*. *Journal of the Fisheries Board of Canada*, 35, 93–100.

Green, R.H., 1965. Estimation of tolerance over an indefinite time period. Ecology, 46, 887.

Karlsson, K., Puiac, S. and Winder, M., 2018. Life-history responses to changing temperature and salinity of the Baltic Sea copepod *Eurytemora affinis*. *Marine Biology*, 165(**2**), 30. https://doi.org/10.1007/s00227-017-3279-6

Kefford, B.J., Papas, P.J., Metzeling, L. and Nugegoda, D., 2004. Do laboratory salinity tolerances of freshwater animals correspond with their field salinity? *Environmental Pollution*, 129, 355-362. https://doi.org/10.1016/j.envpol.2003.12.005

Le, P.Q. and Chen Y.Y., 1998. China red data book of endangered animals: pisces. USA: Science Press.

Li, X., Pan, X.L., He, B.Y. and Wang, X.P., 2006. Water quality assessment and ecological security research in main stream of the Tarim River. *Arid Land Geography*, 29(**5**), 653-657. https://doi.org/10.13826/j.cnki.cn65-1103/x.2006.05.006.

Mark, B.B. and Zhang, S.M., 2001. Threatened fishes of the world: *Aspiorhynchus laticeps* (Day, 1877) (Cyprinidae). *Environmental Biology of Fish*, 61, 380.

McCormick, S.D. and Naiman, R.J., 1984. Osmoregulation in the brook trout, *Salvenilus fontinalis*. II: effects of size, age and photoperiod on seawater survival and ionic regulation. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, 79, 17–28.

Mojazi, A.B., Baker, D.W., Morgan, J.D. and Brauner, C.J., 2009. Size dependent early salinity tolerance in two sizes of juvenile white sturgeon, *Acipenser transmontanus*. *Aquaculture*, 286,121-126. https://doi.org/10.1016/j.aquaculture.2008.08.037

Parry, G.,1958. Size and osmoregulation in salmonid fishes. *Nature*, 181, 1218–1219.

Schofield, P.J., Brown, M.E. and Fuller, P.L., 2006. Salinity tolerance of goldfish *Carassius auratus* L., a non-native fish in the United States. *Florida Scientist*, 69(**4**), 258–268.

Susanne, I.K., Lindsey, R.P., Joshua, E.B. and Carol, A.S., 2012. Salinity tolerance of the invasive round goby: Experimental implications for seawater ballast exchange and spread to North American estuaries. *Journal of Great Lakes Research*, 38(**1**), 121-128. https://doi.org/10.1016/j.jglr.2011.12.010

Vargas-Chacoff, L., Moneva, F., Oyarzún, R., Martínez, D., Saavedra, E., Ruiz-Jarabo, I., Muñoz, J.L.P., Bertrán, C. and Mancera, J.M., 2016. Metabolic responses to salinity changes in the subantarctic notothenioid teleost *Eleginops maclovinus*. *Polar Biology*, 39(**7**), 1297-1308. https://doi.org/10.1007/s00300-015-1854-1

Varsamos, S., Nebel, C. and Charmantier, G., 2005. Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, 141, 401– 429. https://doi.org/10.1016/j.cbpb.2005.01.013

Walker, K.F., 1983. Fish and fisheries of the Murray-Darling river system. FAO/IPFC Workshop on Inland Fisheries, Philippines.

Wang, Z.C., Jiang, Z.F., Zhang, C.Z., Han, C.X., Ba, X. and Wang, N.Z., 2010. Checklist literature study and compilation of bracketed keys for indigenous fishes Tarim River basin. *Progress in Fishery Sciences*, 31(**1**), 22-25.

Watanabe, W.O., Kuo, C.M. and Huang, M.C., 1985. The ontogeny of salinity tolerance in the tilapias *Oreochromis aureus*, *O. niloticus*, and an *O. mossambicus* × *O. niloticus* hybrid, spawned and reared in freshwater. *Aquaculture*, 47, 353–367.

Williams, W.D., 1987. Salinization of rivers and streams: an important environmental hazard. *Ambio*, 16, 180–185.

Williams, M.D. and Williams, W.D., 1991. Salinity tolerances of four species of fish from the Murray-Darling River system. *Hydrobiologia*, 210, 145-160.

Zeng, L. and Tang, W.Q., 2010. Age, body growth and reproductive characteristics of *Triplophysa yarkandensis*. *Chinese Journal of Zoology*, 45(**5**), 29-38. https://doi.org/10.13859/j.cjz.2010.05.003.

Zydlewski, J. and McCormick, S.D., 1997a. The loss of hyperosmoregulatory ability in migrating juvenile American shad, *Alosa sapidissima*. C*anadian Journal of Fisheries and Aquatic Sciences*, 54, 2377–2387.

Zydlewski, J. and McCormick, S.D., 1997b. The ontogeny of salinity tolerance in the American shad, *Alosa sapidissima*. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 182–189.