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Review

Distribution and Diversity of Freshwater Crabs (Decapoda: Brachyura: Potamidae, Gecarcinucidae) in Iranian Inland Waters

Ardavan Farhadi, Muzaffer Mustafa Harlıoğlu

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ABSTRACT

This article reviews the current knowledge of primary freshwater crabs (Decapoda, Brachyura) in Iranian inland waters, with the purpose of classifying the exact number of species, the threat status, and their distribution and diversity. Previous studies have reported that Iranian inland waters have eight freshwater crab species and there was no accurate information on the distribution of freshwater crab species in Iran. This review article describes that an additional six freshwater crab species, *Potamon gedrosianum, P. magnum, P. mesopotamicum, P. ilam, Sodhiana blanfordi, and S. iranica*, are also present in Iran. Therefore, there are 14 freshwater crab species currently known in Iran, which belong to two families (Gecarcinucidae and Potamidae). The genus *Potamon* is represented by 11 species, and the genus *Sodhiana* is represented by 3 species (found in south and south east of Iran). In addition, this review presents a distribution map and the possible threats for each species.

Keywords: Brachyura, decapoda, freshwater crabs, distribution, Iran

INTRODUCTION

Primary freshwater crabs (Yeo et al., 2008, 2012) include more than 1,476 species worldwide and occupy 20 percent of all the brachyurans (Yeo et al., 2008; Cumberlidge et al., 2009). They are composed of five families; Pseudothelphusidae Ortmann, 1893 (Neotropics; Wehrtmann et al., 2010) and Trichodactylidae H. Milne Edwards, 1853 (Mexico, Central and South America; Collins et al., 2006), Potamonautidae Bott, 1970 (Africa and Madagascar; Cumberlidge and Daniels, 2008), and, Potamidae Ortmann, 1896 (North Africa, southern Europe, Asia; Brandis et al., 2000), Gecarcinucidae Rathbun, 1904 (Seychelles, Asia; Shih and Ng, 2011),

Freshwater crabs live in both tropics and subtropics in most regions of the world (Yeo et al., 2008). They can be found in most freshwater ecosystems, from clear, quick-flowing rivers to moderate-flowing rivers, as well as in freshwater swamps, stagnant ponds and rice fields, and even in tree hollows and leaf axils (Yeo et al., 2008; Cumberlidge et al., 2009).

However, some freshwater crab species, such as *Potamon* Savigny, 1816, are only present in freshwater habitats and are not able to live or survive for a long time in saline water, while other genus, such as *Parathelphusa* H. Milne Edwards, 1853, is able to remain alive in saline water for a short time (Yeo et al., 2008). Terrestrial freshwater crab species can live far from continual freshwater sources; they are able to move among the forest floor litter or, sometimes, are even able to climb trees (Ng, 1988; Ng and Tay, 2001; Cumberlidge et al., 2005). Continual immersion in fresh water is not urgent for terrestrial freshwater crab species and they can receive water from food, drinking dew or casual water; they can obtain water by capillary or osmotic uptake from moist substrata (Yeo et al., 2008).

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Freshwater crabs play important ecological roles especially in tropical regions (Dobson et al., 2007a,b). In addition, they are medically important as a threat to human health (i.e. as intermediate hosts of paragonimiasis in Asia, Africa, and the Neotropics) (Maleewong, 2003; Blair et al., 2008) and as a source of medical and pharmaceutical materials (i.e. chitin and chitosan) (Rinaudo, 2006). Freshwater crabs are not used only as a food source, but are also used as food additives and fertilizers, especially crab processing residues are used as feeding additive (Bilgin and Fidanbaş, 2011). Therefore, in addition to the importance of marine crabs, there has also been an increase in the value of freshwater crabs in recent years on a global scale (Yeo et al., 2008).

Two different families of freshwater crabs are present in Iran i.e., Gecarcinucidae and Potamidae (Pretzmann, 1962). Many Potamid species moved to several geographically isolated areas, and adapted to dominant environments (Keikhosravi and Schubart, 2014a). These freshwater crabs in the Eurasian area have extended from the Mediterranean region to the east-Himalaya (Klaus et al., 2011; Gherardi, 2007). The genus *Potamon* in Iran includes three sub-genera; *Pontipotamon* Pretzmann, 1962 (Ashourdan et al., 2014), *Centropotamon* Pretzmann, 1962 (Nasrollahzadeh et al., 2011), and *Orientopotamon* Pretzmann, 1962 (Brandis et al., 2000).

The subgenus *Pontipotamon* is dominant in the south and the south-west Caspian region, the subgenus *Centropotamon* is dominant in the south Caspian Sea region in the center and south of Iran, while the subgenus *Orientopotamon* has been reported from the east Caspian Sea and southeast Iran (Pretzmann, 1962).

Brandis et al. (2000) and Sharifian et al. (2014), examined seven *Potamon* species (*Potamon bilobatum* Brandis, Storch and *Türkay, 2000*, *P. elbursi* Pretzmann, 1976, *P. ibericum* (Bieberstein, 1809), *P. persicum* Pretzmann, 1962, *P. ruttneri* Pretzmann, 1962, *P. strouhali* Pretzmann, 1962, *P. transcaspicum* Pretzmann, 1962) and one gecarcinucid species (*Sodhiana rokitanskyi* (Pretzmann, 1971)) from Iran. Recent active studies bring the number of Iranian species to 14 (Sharifian et al., 2014; Keikhosravi and Schubart, 2014a,b; Keikhosravi et al., 2016; Sharifian et al., 2017). The

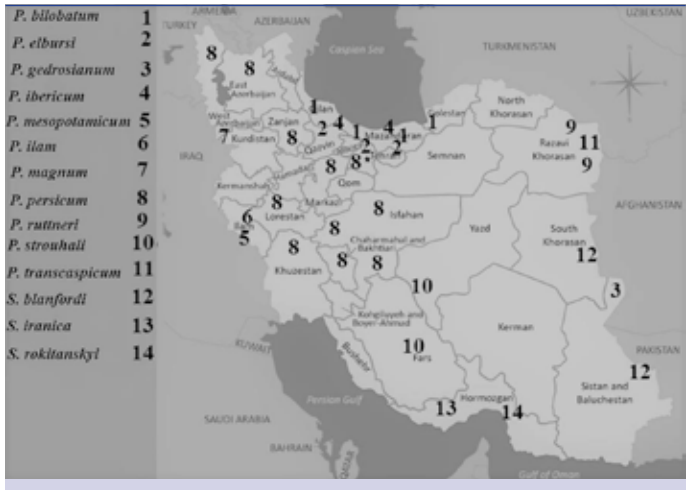

Figure 1. Distribution of freshwater crab species in Iran

genus *Potamon* is represented by 11 species in Iranian inland waters; *P. bilobatum*; *P. elbursi*; *P. gedrosianum* Alcock, 1909; *P. ibericum*; *P. mesopotamicum* Brandis, Storch and Türkay, 1998; *P. ilam* Keikhosravi and Schubart, 2014; *P.* (*Centropotamon*) *magnum* Pretzmann, 1962; *P. persicum*; *P. ruttneri*; *P. strouhali* and *P. transcaspicum*. In addition, three species of the genus *Sodhiana* are found in Iranian inland waters; *Sodhiana blanfordi* (Alcock, 1909); *Sodhiana iranica* Sharifian, Kamrani and Sharifian, 2014 and *S. rokitanskyi*. Figure 1 shows the distribution of freshwater crab species in Iran.

This study is aimed to consolidate and update the distribution and biodiversity of freshwater crabs in Iranian inland waters.

Taxonomy

Family Potamidae Ortmann, 1896

***Potamon bilobatum* Brandis, Storch and Türkay, 2000**

Potamon bilobatum Cumberlidge, 2008a (type locality: Mazandaran and Gilan provinces, Iran); Nasrollahzadeh et al., 2011: 280.

Potamon (Pontipotamon) bilobatum Brandis, Storch and Türkay, 2000: 26-28 (type locality: Mazandaran, Iran).

Distribution in Iran. This species was reported only in the north of Iran from the Elburs Mountains, in Gilan (Rasht, Kelachay), Mazandaran (Chalus, Tonekabon) and Golestan Provinces (Gorgan) (Cumberlidge, 2008a; Nasrollahzadeh et al., 2011).

Remarks. This species is listed as Least Concern in International Union for Conservation of Nature (IUCN) red list of threatened species. Since there is no record showing reduction in the extent and quality of its habitat. This species has been identified in three provinces in Iran (Cumberlidge, 2008a; Nasrollahzadeh et al., 2011). This species was found in Lakan stream when the water temperature, salinity, hardness, and pH was 16-18°C, 330-430 mg/l, 12-16°d, and 7-7.7 respectively (Nasrollahzadeh et al., 2011).

***Potamon elbursi* Pretzmann, 1976**

Potamon elbursi Pretzmann, 1976b: (type locality: northern Iran); Keikhosravi and Schubart, 2014a: (Namak Lake and south Caspian Sea drainages).

Distribution in Iran. Keikhosravi and Schubart (2014a) redescribed *Potamon elbursi*, proving its occurrence as a native species of northern Iran. *P. elbursi* is distributed from the north to the central and southern slopes of the western Alborz Mountains (the north of Tehran and Qazvin), and from the south to the sources of two drainage systems (northeast of Tehran), Namak Lake and south Caspian Sea drainages. Only at one point does the distribution extend northward through the Alborz Mountains (Sefidrud valley), reaching the Caspian Sea (Keikhosravi and Schubart 2014a; Keikhosravi et al., 2015).

Remarks. *P. elbursi* is distinguished from *P. persicum* by the morphology of the first gonopods in males (Keikhosravi and Schubart 2014a). Consistent and marked genetic divergence was also recognized in the mitochondrial 16S rRNA and cytochrome oxidase subunit I genes (Keikhosravi and Schubart 2014a).

***Potamon gedrosianum* Alcock, 1909**

Potamon gedrosianum Alcock, 1909 (type locality: Pishin Valley, Baluchistan, Pakistan); Quddusi, et al., 2005 (Sindh, Punjab and Baluchistan Provinces, Pakistan); Keikhosravi et al. 2016 (Zabol, southeast of Iran).

Potamon gedrosianum waziristanis Pretzmann, 1965: 297 (Kabul, Afghanistan).

Potamon gedrosianum torbenwolffi Bott, 1967 (type locality: west of Afghanistan.).

Distribution in Iran. *Potamon gedrosianum* is found in Afghanistan, northeastern Iran (Keikhosravi et al., 2016), northwestern Pakistan, western India and the western tributaries of the Indus River (Cumberlidge, 2008b). For the first time, in 2016, *P. gedrosianum* has been reported in Iran by Keikhosravi et al. (2016) in Zabol (southeast of Iran).

Remarks. Alcock (1909) reported a new subspecies of *Potamon fluviatile* (i.e. *P. f. gedrosianum*) from Pakistan. Recently, Brandis et al. (2000) synonymized this newer subspecies and ranked it at species level. *P. gedrosianum* and *P. ruttneri* have very similar morphology. Also, gonopod morphology is constant or has very limited variation at intraspecific level in both species (Brandis et al., 2000). However, *P. gedrosianum* and *P. ruttneri* are distinguished by the structure of the first male gonopod (G1) and carapace features (Brandis et al., 2000).

Cumberlidge (2008b) listed *P. gedrosianum* as Least Concern in IUCN red list of threatened species in view of its wide distribution in four countries, tolerance of a degree of habitat modification, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category (Cumberlidge, 2008b).

***Potamon ibericum* (Bieberstein, 1809)**

Cancer ibericum Bieberstein, 1809: 3-5 (type locality: Mazandaran, North of Iran).

Potamon ibericum Scombathy, 1916 (type locality: near Yerevan, Armenia.); Charmantier, 1992 (southern France).

Potamon (Pontipotamon) ibericum Bieberstein, 1808: (Mazandaran, North of Iran).

Potamon (Pontipotamon) ibericum tauricum natio *bithyniensis* Pretzmann, 1983: 281-300 (Turkey)

Potamon (Pontipotamon) ibericum tauricum natio *cappadociensis* Pretzmann 1983 (Cappadocia, central Turkey).

Potamon (Pontipotamon) ibericum tauricum natio *troijensis* Pretzmann (Thassos, Greek island).

Potamon albanicum Starobogatov and Vassilenko, 1979: 1795, (type locality: Cyprus).

Potamon ibericum meandris Pretzmann, 1963 (type locality: Shahpasant, Iran).

Thelphusa fluviatilis taurica Czerniavsky, 1884 (type locality: Chios, Greek islands). Pretzmann, 1976b (Rasht, Iran). Pretzmann, 1963: 376 (Trabzon, Turkey; Samsun, Turkey).

Distribution in Iran. *Potamon ibericum* occurs in a wide and highly fragmented regions. *P. ibericum* present from the Danube River to the Black Sea and the Caspian. *P. ibericum* is found in Bulgaria, Ukraine, Crimea, Georgia, Armenia, and Azerbaijan. *P. ibericum* is a species also present in the region to south of the Caspian Sea region in Turkmenistan (Cumberlidge, 2008c) and Iran. In Iran, *P. ibericum* is present in the north of Iran from the Elburs Mountains, in the Sefidrud opening connected to the Caspian Sea, and in the coastal region of the Caspian Sea, Gilan Province (Langerud, Rudsar, Kelachay) and Mazandaran Provinces (Chalus) (Ashourdan et al., 2014; Parvizi et al., 2017).

Remarks. *P. ibericum* has a wide distribution and the relatively high number of localities and records. *P. ibericum* is assessed here as Near Threatened (NT) in IUCN red list of threatened species. Because it is possible that populations of *P. ibericum* in parts of its range might be in danger of extirpation in the future, especially those on islands or near centers of human population on the mainland (Cumberlidge, 2008c).

***Potamon mesopotamicum* Brandis, Storch and Türkay, 1998**

Potamon mesopotamicum Brandis, Storch and Türkay, 1998 (type locality: Khabur River, Syria); Naser, 2009 (southern regions of Iraq); Keikhosravi and Schubart, 2014b: 119 (Ilam, west of Iran).

Distribution in Iran. *Potamon mesopotamicum* is found at the Turkey-Syria border (Sanliurfa Province), Syria (sources of the Khabur River in Ras al-Ain; Nar al-Khabur) (Brandis et al., 1998; Esser and Cumberlidgea, 2008a) and recently Naser (2009) extended the range of *P. mesopotamicum* to southern regions of Iraq, over 400 km to the southeast. In the Al-Huwaizah marshes, *P. mesopotamicum* lives in areas of still, shallow water, on mud or among submerged aquatic plants (*Ceratophyllum* sp.). In addition, recently *P. mesopotamicum* has been reported in the west of Iran (Ilam) on the Iran-Iraq border (Keikhosravi and Schubart, 2014b).

Remarks. Potential threats for the survival of *P. mesopotamicum* and other aquatic organisms in these marshes include the application of pesticides by fishermen and illegal overfishing (Al-Helfi, 2005).

***Potamon ilam* Keikhosravi and Schubart, 2014**

Potamon ilam Keikhosravi and Schubart, 2014b (type locality: Ilam, south west of Iran).

Distribution in Iran. *Potamon ilam* is found in most of the rivers in Ilam (south west Iran) that drain into the Tigris River (except for some rivers in the eastern part), but it is not found in the Tigris River itself, where *P. mesopotamicum*, *P. persicum*, and *P. magnum* occur (Keikhosravi and Schubart, 2014b).

Remarks. Keikhosravi and Schubart (2014b) suggested that *Potamon ilam* is distinguished from *P. persicum* and *P. mesopotamicum* by the shape of the first gonopod and carapace characters. In addition, other differences between these taxa are evi-

dent from nuclear 28S rRNA and mitochondrial 16S rRNA gene sequences (Keikhosravi and Schubart, 2014b).

***Potamon magnum* Pretzmann, 1962**
Potamon (*Centropotamon*) *magnum* Pretzmann, 1962 (type locality: eastern Turkey); Alsalami and Rahma, 2015 (Al-Kufa river, Iraq); Luay and Jawair, 2013 (Greater Zab river Kurdistan Region-Iraq).

Distribution in Iran. *Potamon magnum* occurs mostly in eastern Turkey in the upper reaches of the Euphrates and Tigris Rivers, in northern Syria, northeast of Iraq, in western Armenia and western Iran (Baneh) (Cumberlidge, 2008d; Ali and Latef, 2017).

Remarks. Cumberlidge (2008d) assessed *Potamon magnum* as Least Concern in IUCN red list of threatened species based on its relatively wide distribution (EOO 145,000 km²) and the relatively high number of localities and records in five countries. *Potamon magnum* populations may nevertheless be under threat from rapid anthropogenic changes affecting their habitat, such as water diversion and pollution. No information exists on the population dynamic or density of *P. magnum* (Cumberlidge, 2008d). Alsalami and Rahma (2015) showed that the most important factors that affect presence of *P. magnum* is the water temperature and salinity. The highest density reached (32.89) individual / M^{O} at a temperature of 20°C during April, no crab observed was observed during January and February when the water temperature was 14.5°C and 14.1°C respectively (Alsalami and Rahma, 2015).

***Potamon persicum* Pretzmann, 1962**
Potamon persicum Pretzmann, 1962: 205 (type locality: Isfahan, Iran); Khatami, 2002 (type locality: Jajrood River, Tehran, Iran); Ardalan et al., 2011: 179 (Elburs Mountains); Keikhosravi and Schubart, 2014a: 116 (Kohkiluyeh buyerahmad province, Chaharmahal Bakhtiari province Esfahan, Khuzestan province, Iran). Keikhosravi and Schubart, 2014b: 117 (Khuzestan province Tehran province, Iran).

Potamon (*Centropotamon*) *hueceste armenicum* Pretzmann, 1983 (type locality: Makoom Northwest of Iran).

Potamon (*Centropotamon*) *magnum elbrusi* Pretzmann, 1962 (type locality: Rasht, Iran).

Potamon (*Centropotamon*) *magnum vangoelium* Pretzmann, 1976a (Mazandaran, Iran part).

Potamon (*Centropotamon*) *persicum* Pretzmann, 1962 (type locality: Isfahan, Iran).

Potamon (*Centropotamon*) *persicum kermanshahi* Pretzmann, 1976b (type locality: Kermanshah, West of Iran)

Potamon magnum armenicum Pretzmann, 1962 (type locality: Northwest of Iran); Pretzmann, 1963: 375.

Distribution in Iran. *Potamon persicum* is found in Iran (Azarbayejane Gharbi, Azarbayejane Sharqi, Gilan, Markazi, Hamadan, Bakhtaran, Lorestan, Esfahan Provinces), Turkey, Iraq and in Armenia. Also, this species is found in the Tigris-Euphrates river systems and in the region from Lake Van to the Elburs Mountains and in the region south of Esfahan in Iran (Cumberildge, 2008e; Ardalan et al., 2011; Keikhosravi and Schubart 2014a,b).

Remarks. Cumberildge (2008e) assessed *Potamon persicum* as Least Concern in IUCN red list of threatened species based on its wide distribution (E00 1 million km²) and the high number of localities and records from Iran, Turkey, Iraq and Armenia (Cumberildge, 2008e).

***Potamon ruttneri* Pretzmann, 1962**
Potamon ruttneri Pretzmann, 1962 (type locality: Amirabad, Tabas, Iran); Keikhosravi et al., 2016 (Khorasan Razavi, Iran and Herat, Afghanistan).

Potamon gedrosianum lindbergi Pretzmann, 1966: 297 (type locality: west of Afghanistan)

Potamon gedrosianum linberglundi Bott, 1967 (type locality: northwestern Afghanistan).

Distribution in Iran. *P. ruttneri* is found in northeastern Iran between Mashhad and Birjand. It is also found in northwestern Afghanistan (Herat and Oruzgan Provinces) (Cumberlidge, 2008f; Keikhosravi et al., 2016).

Remarks. *Potamon ruttneri* is listed as Least Concern in IUCN red list of threatened species because of its wide distribution in Iran and Afghanistan (Cumberlidge, 2008f).

***Potamon strouhali* Pretzmann, 1962**
Potamon strouhali Pretzmann, 1962: 205 page (type locality: Yazd, Iran); Farzanpay and Pretzmann, 1974 (type locality: southeast Iran).

Potamon (Orientopotamon) eiselti Pretzmann, 1976a (type locality: Niriz, Iran)

Potamon (Orientopotamon) strouhali Pretzmann, 1962; 205 (type locality: Yazd, Iran): Pretzmann, 1963: 379.

Potamon strouhali shurium Pretzmann, 1976b (type locality: Shiraz, Iran).

Distribution in Iran. *Potamon strouhali* is found in southeast Iran (Yazd and Shiraz) up to the Afghan border region.

Remarks. *P. strouhali* is listed as Least Concern in IUCN red list of threatened species in (Cumberlidge, 2008g).

***Potamon transcaspicum* Pretzmann, 1962**
Potamon transcaspicum Pretzmann, 1962: 205 (type locality: northeast Iran); Keikhosravi et al., 2016 (type locality: Zabul, Afghanistan and Zardkoohi, Sabzevar, Iran).

Potamon (Orientopotamon) transcaspicum Pretzmann, 1962 (Bojnourd, Iran).

Potamon (Orientopotamon) turkmenicum Pretzmann, 1962 (Bojnourd, Iran); Pretzmann, 1976b.

Potamon (Potamon) zarudnyi Starobogatov and Vassilenko, 1979: 1790-1801 (type locality: Cyprus).

Distribution in Iran. *Potamon transcaspicum* is found in Ukraine (Crimea), Turkmenistan and north east of Iran Khorasan Province (Sabzevar) (Cumberlidge, 2008h; Keikhosravi et al., 2016).

Remarks. *P. transcaspicum* is listed as Least Concern in IUCN red list of threatened species because of its wide distribution (Cumberlidge, 2008h).

Family Gecarcinucidae Rathbun, 1904

***Sodhiana blanfordi* (Alcock, 1909)**
Paratelphusa (*Paratelphusa*) *blanfordi* Alcock, 1909: 375 (Type locality: Baluchistan, Pakistan); Pretzmann, 1963: 379.

Sartoriana *blanfordi* Bott 1970 (Type locality: Southern Iran); Cumberlidge, 2008i (Type locality: Baluchistan, Pakistan); Kamrani et al., 2009 (Type locality: Southern Iran).

Sodhiana blanfordi Yeo and Ng, 2012 (Baluchistan, Pakistan); Sharifian et al., 2014 (south east of Iran).

Distribution in Iran. It is found in the south east of Iran, Sistan and Baluchestan Province and Hormozgan Province (Bastak), Pakistan and Afghanistan (Cumberlidge, 2008i; Sharifian et al., 2014).

Remarks. It is listed as Least Concern in IUCN red list of threatened species. *Sodhiana blanfordi* extent of occurrence is in area less than 2,000 km² and all individuals occur in fewer than five localities (Cumberlidge, 2008i).

***Sodhiana iranica* Sharifian, Kamrani and Sharifian, 2014**
Sodhiana iranica Sharifian, Kamrani and Sharifian, 2014 (type locality: Bastak region in the south of Iran).

Distribution in Iran. This species is a gecarcinucid crab from the Bastak region in the south of Iran (Bandar Abbas), only recently reported to be a new species (Sharifian et al., 2014). It inhabits a freshwater spring located in a semi-mountainous area in Iran (Eellod area), covered by dense stands of common reeds and salt cedar trees in the periphery, with algae mats on the bottom (Sharifian et al., 2014).

Remarks. *S. iranica* is easily separated from *S. rokitanskyi* by having the epigastric cristae lower and less pronounced, and coming close to or almost in contact with the postorbital cristae, whereas they are more prominent in *S. rokitanskyi* (Sharifian et al., 2014).

Sharifian et al. (2017) studied population dynamic of *S. iranica*. No significant differences were detected among mean male and female carapace widths and the sex ratio (male:female) was 1:0.7 in *S. iranica*. The maximum life span of *S. iranica* is evaluated to be 1.1- 4.9 years (Sharifian et al., 2017). In addition, Sharifian et al. (2017) suggested that suitable management is essential for conservation of *S. iranica* in the freshwater spring of the Eelood Area (near Bandar Abbas).

***Sodhiana rokitanskyi* (Pretzmann, 1971)**
Liotelphusa (Sartoriana) *rokitanskyi* Pretzmann, 1971 (type locality: Minab, Iran).

Sartoriana rokitanskyi Esser and Cumberlidge, 2008b: (type locality: Minab, Iran); Mirzadeh et al., 2011 (Hormozgan Province, Iran).

Sodhiana rokitanskyi Yeo and Ng, 2012 (south of Iran).

Distribution in Iran. *Sodhiana rokitanskyi* is only found in Iran. The type locality is the Minab River near Minab, east of Bandar Abbas, Geno and Rudan marshes, in Hormozgan Province, southern Iran (Esser and Cumberlidge, 2008b; Mirzadeh et al., 2011; Yeo and Ng, 2012). Kamrani et al. (2009) described the characteristics of the marsh crab, *S. rokitanskyi*. *S. rokitanskyi* belongs to the family Gecarcinucidae, which is related to true crabs.

Remarks. Yeo and Ng (2012) subsequently revised *Sartoriana* Bott, 1969, and transferred three species to a new genus, *Sodhiana*: *S. blanfordi* (Alcock, 1909), *S. afghaniensis* (Pretzmann, 1963), and *S. rokitanskyi* (Pretzmann, 1971).

There is not enough information about this species to make a thorough assessment of *S. rokitanskyi*. Therefore, *S. rokitanskyi* is listed as Data Deficient (Esser and Cumberlidge, 2008b).

CONCLUSION

Studies on freshwater crabs in Iranian inland waters are limited to species reorganization and their diversity. Further studies are required to investigate habitat requirements, population structure, disease, biology and ecology of Iranian freshwater crabs. In addition, further studies are needs to survey fisheries (i.e. evaluate maximum sustainable yield and economy benefits), aquaculture (i.e. assay possibility of successful aquaculture) and industry importance (i.e. chitin and chitosan levels) of freshwater crabs in Iran.

Conservation measures require a complete inventory of distribution and habitat requirements of freshwater crab species, evaluations of population levels and trends, and creation of protected areas. Fortunately, most of the freshwater crabs of Iran are listed as Least Concern in the IUCN Red List of Threatened Species.

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Original Article

Quality Changes of Thermal Pasteurized Mussels (Mytilus galloprovincialis) During Refrigerated Storage at 4±1°C

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ABSTRACT

This study investigated the changes in quality of pasteurized mussels during storage at 4°C±1°C. Mussels (*Mytilus galloprovincialis*) were harvested from the Marmara Sea in March. The mussel flesh was packed with lemon juice, apple vinegar, finely chopped onion, salt, and black pepper. Then, all the packets were pasteurized (at 70°C for 8 min) and stored for 21 days at 4°C±1°C. Results showed that the contents of protein, fat, and ash of pasteurized mussels were significantly higher ($p<0.05$) than those of raw samples. The moisture content of mussels was significantly decreased ($p<0.05$) after heat treatment. The meat yield of pasteurized mussels was 10.42%. Sensory evaluation results indicated that the acceptability of pasteurized mussels at refrigerated storage was limited to 9 days. The TVB-N value of pasteurized samples exceeded the acceptability limit of 22–25 mg/100 g on the 9th day. The TMA-N amount of pasteurized mussels remained lower than the acceptability limit during storage. The initial microbial load of the mussel samples reduced after the pasteurization process. This reduction was observed in the total mesophilic and psychrophilic bacterial count, with the yeast mold counts after pasteurization being 2.44, 2.07, and 2.37 log cfu/g, respectively.

Keywords: Pasteurization, mussel, quality, ready-to-eat

INTRODUCTION

Nowadays, the trend towards ready-to-eat food at high quality makes thermal pasteurization applications important for food. Thermal pasteurization (usually below 100°C) is a classic food preservation method that provides food safety, which reduces undesirable pathogenic vegetative cells, and deteriorates microorganisms, inactivates enzymes, and prolongs the shelf life of foods. Pasteurization used in the food industry does not kill all microorganisms in food. It is used in the food industry targeting pathogens that are important for public health and microorganisms that cause food spoilage. Today, pasteurization is regarded as a technology in which the desired food quality is preserved with minimum loss and pathogenic microorganisms are killed (Skipnes, et al., 2002; Peng et al., 2017).

Mussels are rich in nutrient components; also their economic value is high. They are very popular in many countries in terms of hunting and processing compared to other aquatic products. They are relatively inexpensive, contain high quality protein source, and are usually consumed raw, fresh, frozen and canned. The mussels have low fat and cholesterol content. İt is also very rich in vitamins including, A, B1, B2, B6, B12 and C, free amino acids, trace elements (selenium, calcium, iron, magnesium and phosphorous) and glycogen. At the same time they have high pH. This makes them a suitable substrate for the growth of microorganisms (Goulas, et al., 2005; Ovissipour, et al., 2013). On the other hand shellfish feed by filtering water. They accumulate various chemicals and microorganisms in their bodies (Vernocchi et al., 2007). Especially, raw and partially cooked

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molluscan shellfish (mussels, clams and oysters) are the most common tools of foodborne bacterial and viral diseases (Rippey, 1994).

Depending on all these properties, the mussel is a food that can deteriorate very quickly. Therefore, there is a need for appropriate storage technologies that prolong both shelf life and nutritional and sensory qualities. However, very little information and data are available on the quality changes that have taken place during the storage period, especially after heat treatment.

The purpose of this study, is to investigate the quality changes of mussel pasteurized with lemon juice, apple vinegar, onion, salt and black pepper during cold storage.

MATERIAL AND METHOD

Mussels Supply and Handling

Mussels (*Mytilus galloprovincialis*) were purchased from a wholesale fish market in Istanbul. The mussels were harvested from the Marmara Sea in March. Approximately 550 mussels were transferred to the laboratory in chilled polystyrene boxes within 60 minutes. Dead mussels were discarded after inspection. The average weight and length of the mussels were 23.94±6.80 g and 6.99±0.78 cm. The mussels were washed and shucked by hand using a sterile shucking knife. Raw mussel flesh analysis was done immediately.

Packaging and Pasteurization Process

Approximately, twenty five (approx. 150g) mussel flesh was placed in a thermostable polyethylene-polyamide pouch (O_2 permeability of 160 cm^3/m^2 per 24 h at 23C, 0% RH and water vapor permeability of 8.5 g/m^2 per 24 h at 38C, 90% RH.) with lemon juice (10 mL), apple vinegar (10 mL), fine-chopped onion (10 g), salt (0.5 g) and black pepper (0.5 g). The packages were heat sealed under atmospheric air. Then, all packed samples were pasteurized (70°C for 8 min) by using autoclave. A total 18 packages were prepared. Pasteurized samples were stored refrigerated (4±1°C) conditions. After 0, 6, 9, 12, 15, 18 and 21 days randomly chosen two packages were removed for analysis.

Yield Determination

After thermal pasteurization, mussel meat was subsequently drained by placing on filter paper and then covered with another filter paper for five minutes. Consenquently the yield was compared with that from freshly unopened mussels. Mussels (25 pieces) yield are calculated by the following equation:

Yield (%): [cooked mussel meat weight (g)/unopened mussel weight (g)] X 100 (Cruz-Romero et al., 2007; Bongiorno et al., 2015).

Proximate Analyses

The mussel samples were analyzed in triplicate for determination of the proximate composition: the lipid content of mussels was determined by the soxhelet method of the AOAC (1998a), the moisture content by the method of Mattissek et al. (1992), the ash content by the AOAC (1998b) method and total crude protein by the Kjeldhal method (AOAC, 1998c).

Sensory Analysis

The sensory attributes of pasteurized mussels were evaluated on each sampling day by ten trained panelists. Sensory analysis was performed in individual booths under controlled conditions of air circulation, light, temperature and humidity. Pasteurized mussels were evaluated on the basis of appearance, odor, texture and taste characteristics. The scale points were: 10-9=excellent; 8.9-8=very good; 7.9-6=good; 5.9-4=sufficient; < 4=unacceptable (Karl et al., 2001). The overall quality score was calculated as the average value of the score of the each attributes evaluated.

Microbiological Analyses

All microbiological analyses were performed in duplicate. Twenty five grams of mussel flesh for analysis was transferred aseptically to a sterile stomacher bag containing 225 mL of peptone from meat (Merck, 1.0214) (0.1%) and homogenized in a stomacher (IUL Instrument, Spain) for 60 second. Appropriate serial dilutions were prepared (1:10 diluent, 0.1% peptone water). Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) were determined using Plate Count Agar (PCA, Merck, 1.05463) after incubation for 24-48 hours at 37°C and for 10 days at 7°C, respectively (Baumgard, 1986). Violet Red Bile Agar (Merck, 1.01406) and Violet Red Bile-Mug Agar (Merck, 1.04030) were used for the enumeration of total coliform bacteria and *Escherichia coli* after incubation at 30°C 18-24 hours and 35°C 18-24 hours, respectively (Feng et al., 2002). Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466) was used for the enumeration of yeast and mold (YM) after incubation at 25°C for 5 days (Tournas et al., 2001). *Salmonella* spp. determination was performed according to Andrews et al., (2007). Twenty five grams of mussel flesh was added to the 225 mL Lactose broth (Merck, 1.07661), and incubated at 37°C for 24-48 hours. After the incubation, 0.1 mL homogenate was transferred into the 10 mL of Rappaport-Vassiliadis Broth (Merck 1.07700.500) and then incubated at 42°C for 24 hours for the selective enrichment. At the same time 1 mL homogenate was transferred in to 10 mL Tetrathionate Broth (Merck 1.05285) and then incubated for 24 hours at 43°C. After the incubation, a loopful of broth cultures were streaked onto XLT4 (Merck 1.13919) and Bismut Sulfite Agar Base (Merck 1.05418). Plates were incubated at 35°C for 48 h. After that, identification tests have been performed on suspected salmonella colonies. Mussel samples were analyzed for the presence of *Vibrio* spp. using FDA Bacteriological Analytical Manual (Kyasner and Depaola, 2004). Twenty five grams of mussel flesh was added to the 225 mL alkaline peptone water (1% NaCl+1% peptone from meat), after, a loopful of homogenate from alkaline peptone water was streaked on Thiosulphate Citrate Bile Salt Agar Base (TCBS, Merck 1.10263). Plates were incubated at 35°C for 18-24 h and identification tests have been performed on suspected colonies. Three agar plates per dilution were made in each medium.

Chemical Analysis

Chemical analyses for performed triplicate. For the pH analysis mussel samples were homogenized and diluted with distilled water at 1:1 (w/v) ratio. After that, the pH value of the mussel samples was measured with pH meter (Hanna pH 211 Microprocessor pH meter, Ann Arbor, MI) (Olafsdottir et al., 1997).

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) analyses were carried according to the methods described by Schormüller (1968). Mussel samples were boiled with MgO (Merck, 1.05862) and vapor components held with 0.1N HCl (Merck, 1.00317). The amount of TVB-N was calculated after the titration with 0.1N NaOH (Merck, 1.06498). The results were expressed as milligrams of TVB-N per 100 grams of mussel flesh. For the estimation of TMA-N content of mussel flesh, 10 g samples were homogenized with 10% trichloracetic acid solution (90 mL)(Merck, 100807) and homogenized samples were filtrated by Whatman No: 1 filter paper. After filtration, 4 mL filtrate was shaken with 10 mL toluene (Balmumcu Ltd.), 1 mL 20% formaldehyde (Merck, 1.04002) and 3 mL 50% potassium hydroxide (Carlo Erba, 362257). Following this, upper layer (5 mL) was separated and mixed with 0.02% picric acid (5 mL). Absorbance was measured by a spectrophotometer (UV/VIS T80, PG Instruments Ltd) at 410 nm. The TMA values were calculated from the standard curve and expressed as mg/100g samples.

Statistical Analysis

The average of the results from experimental studies was used for statistical analysis. The data were analyzed using IBM Statistical Package for the Social Sciences 21 software (SPSS Inc.; Chicago, IL, USA). Differences between means were analyzed by one way analysis of variance (ANOVA) followed by Tukey and Games-Howell tests. T-test was used to compare the results of the proximate composition. Differences at ($p<0.05$) were considered significant.

RESULT AND DISCUSSION

In our study, pasteurized mussels had approximately 10.42% yield. Cruz-Romero et al., (2007), calculated the yield of traditional pasteurized oyster (at 75°C for 8 min) range 1.5-15.5%. Bongiorna et al., (2015), observed that, for *Mytilus galloprovincialis* maximum value of meat yield (cooked) in May (26.3%) and minimum value of yields in January (12.5%). In another study, Vernocchi et al., (2007), reported that meat yield (cooked) for *Mytilus galloprovincialis* ranged from 13.4% (July) and 25.2% (January). Cavalheiro et al., (2013) reported the global weight loss of the blue mussels as 17.20% after vapor cooking. Meat yield of bivalve species may vary depending on gametogenic cycle of animals, water temperature, salinity, pH, food availability and catching season (Orban et al., 2002; Yildiz et al., 2011).

Table 1. Proximate composition (%) of raw and pasteurized mussels.

	Raw Mussel	Pasteurized Mussel
Protein	10.61±0.09[a]	20.00±0.41[b]
Fat	2.11±0.10[a]	3.82±0.09[b]
Moisture	86.19±0.11[a]	73.46±0.20[b]
Ash	1.02±0.01[a]	2.18±0.03[b]

a-b: Different letters show significant differences ($p<0.05$)

Proximate composition of raw and pasteurized mussel is shown in Table 1. The raw mussel meat had a moisture content of 86.19%, protein 10.61%, fat 2.11% and ash 1.02%. Our results are similar to the results of many studies with *Mytilus galloprovincialis* (Khan et al, 2005; Fuentes et al., 2009; Özden et al., 2010; Ulusoy and Özden, 2011; Bongiorno et al., 2015). It is known that catching season, size, age, food availability, sex and reproductive cycle may influence the meat yield and nutritional composition of mussels (Orban et al, 2002; Stratev et al., 2017; Merdzhanova et al., 2018). The moisture, protein fat and ash contents of pasteurized mussel meat were 73.46, 20.00, 3.82 and 2.18% respectively. The protein, fat, and ash contents of pasteurized mussels were significant higher ($p<0.05$) than raw samples. Shelf life of stuffed mussels at 4°C in modified atmosphere packaging was studied by Ulusoy and Özden (2011). In their study, the protein, fat, and ash contents of stuffed mussels was found statistically significant higher after cooking as observed in our study. In this study, the moisture content of mussels significantly decreased ($p<0.05$) after heat treatment, in agreement with data reported by Cruz-Romero et al. (2007), Ulusoy and Özden (2011) and Lekjing et al., (2017).

The pH value of pasteurized mussels during cold storage are shown in Table 2. Manousaridis et al., 2005, recommended the following pH scale points as a basis for evaluating the freshness of mollusks (oysters); 6.2-5.9=good; 5.8=off, 5.7-5.5= musty and 5.2 and below sour or putrid. In our study, pH value measured in raw mussel was 6.20±0.00. Approximate pH values (6.30, 6.20 and 6.20) were reported by Manousaridis et al., 2005, Masniyom and Benjama, (2007) and Bongiorno et al., (2018), respectively. In our study, initial pH of the sauce (lemon juice, apple vinegar, fine-chopped onion, salt and black pepper) was 2.55±0.03 which is acidic. The pH value of samples decreased after treatment with lemon juice and vinegar, similar to results reported by others (Sallam et al., 2007; Cosansu et al., 2011; Cosansu et al., 2013). Bhunia et al., (2017), observed that a decrease in initial pH of blue mussels after treatment with red sauce (red tomato sauce, salt, and paprika).

The scores of sensory evaluation of pasteurized mussels are presented in Table 2. During the first 6 days, mussel samples were rated at 8.9-6 scores. This range of points is defined as "very good" and "good". Acceptability scores for sensory quality of pasteurized mussel samples stored at 4±1°C decreased with time of refrigerated storage. The limit of acceptability of sensory quality was reached after 9th day of storage. Pasteurized mussel samples were determined as unacceptable (2.20±0.93) at the 12th day of storage. In a similar study, Ulusoy and Özden, 2011, reported that the shelf life of stuffed mussel with air packed was 11th day. In another study, sensory quality of cooked and chilled mussels without vacuum conditions unacceptable at 7th day (Bongiorno et al., 2018).

In this study, initial TVB-N content of raw mussels were 17.52±1.94 mg/100g. This value slightly higher than reported by previous studies (Masniyom and Benjama, 2007; Bongiorno et al., 2018). During refrigerated storage, TVB-N content increased in pasteurized mussels until end of storage (21 days), reached value of 29.24±0.38 mg/100 g (Table 2). The TVB-N content is often

Table 2. Changes in sensory, pH, TVB-N and TMA-N values of raw and pasteurized mussels stored at 4±1°C.

Days	Sensory score	pH	TVB-N (mg/100g)	TMA-N (mg/100g)
0 (Raw Material)	7.66±0.27	6.20±0.00	17.52±1.94	1.65±0.13
0 (Pasteurized samples)	8.62±0.38^{a}	4.65±0.05^{a}	7.90±0.69^{a}	3.39±0.00^{a}
7	7.02±0.62^{b}	4.57±0.03^{ac}	21.92±0.98^{b}	3.34±0.19^{ab}
9	4.60±0.55^{c}	4.65±0.07^{a}	22.58±0.15^{b}	3.37±0.17^{a}
12	2.20±0.93^{cd}	4.84±0.07^{b}	22.24±0.67^{b}	3.54±0.28^{ab}
15	1.85±0.73^{d}	4.43±0.06^{c}	23.21±0.47^{b}	3.40±0.00^{a}
18	1.40±0.35^{d}	4.45±0.00^{c}	24.35±0.29^{c}	2.75±0.25^{ab}
21	1.17±0.20^{d}	4.54±0.04^{ac}	29.24±0.38^{d}	2.49±0.05^{b}

a-d: Different letters in the same column show significant differences ($p<0.05$)
TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen

Table 3. Changes in microbial counts of raw and pasteurized mussels stored at 4±1°C.

Days	TAMB (logcfu/g)	TAPB (logcfu/g)	YM (logcfu/g)
0 (Raw Material)	5.59±0.02	6.18±0.11	6.30±0.02
0 (Pasteurized mussel)	3.15±0.10^{a}	4.11±0.01^{a}	3.93±0.02^{a}
7	3.35±0.10^{ab}	2.20±0.17^{b}	3.35±0.31^{ab}
9	3.66±0.05^{b}	2.10±0.17^{b}	3.10±0.17^{b}
12	3.56±0.07^{ab}	2.00±0.00^{b}	3.10±0.17^{b}
15	3.20±0.17^{ab}	2.00±0.00^{b}	2.10±0.17^{c}
18	3.41±0.10^{ab}	1.20±0.17^{bc}	<100
21	3.47±0.00^{ab}	1.10±0.17^{c}	<100

a-c: Different letters in the same column show significant differences ($p<0.05$)
TAMB: total aerobic mesophilic bacteria; TAPB: total aerobic psychrophilic bacteria; YM: yeast and mold

used as an index to evaluate the freshness and quality of seafood (Masniyom and Benjama, 2007). There are different views on acceptable limits in various literature. Kietzman et al., (1969) stated the TVB-N limit values in fish and fish products as follows; 25.00 mg/100g or lower TVB-N values; "very good", 30.00 mg/100g TVB-N values; "good", 35.00 mg/100g TVB-N values; "marketable" and over 35.00 mg/100g TVB-N values; "unacceptable". Sikorski et al., (1990) cited TVB-N acceptability limit value for fatty fish is 20 mg/100g and 17 mg/100g for oyster. The limit of acceptability is suggested as 22-25 mg/100g by Goulas et al., (2005), while it is 15 mg/100g according to Erkan (2005). Ulusoy and Özden (2011), reported the TVB-N value of cooked stuffed mussels reached to 21.90 mg/100g and exceeded the acceptable limit after nine days of storage. In our study, for pasteurized mussels the sensory score was approximately 4.60 on the 9th day when the TVB-N value reached 22.58±0.15 mg/100g. Panelists who performed sensory analysis evaluated mussel samples as "unacceptable" on the 12th day. At the 12th day of storage TVB-N content of mussel samples was 22.24±0.67 mg/100g. Our results were in accordance with that of Masniyom and Benjema, (2007) who reported a TVB-N value of 20 mg/100g for green mussel samples after 9 days of refrigerated storage. In our study, it would be more realistic to use the 22-25 mg/100g TVB-N unacceptable limit value recommended by Goulas et al., (2005) for the mussel as compared with the value of 35.00 mg/100g proposed for fish (Kietzman et al., 1969).

Trimethylamine is a non-protein nitrogenous compound, and is responsible for further fish degradation. TMA is formed by the reduction of Trimethylamine oxide (TMAO) caused by microbial action and possibly through the activity of endogenous enzymes. At the same time, it contributes to the characteristic ammonia-like off-odor in fish spoilage (Sikorski et al., 1990; Gram and Huss, 1996; Goulas et al., 2005). TMA-N limit values for fish and other seafood were determined as follows. 4.00 mg/100g TMA-N content; "good", 10.00 mg/100g TMA-N content; "marketable" and 12.00 mg/100g TMA-N content; "unacceptable" (Connell, 1980). Sikorski et al., (1990), suggested 5 to 10 mg TMA-N per 100g as the rejection limit. In our study, initial value of TMA-N in raw mussels was found 1.65±0.13 mg/100g. Approximate TMA-N values were reported by Kaba and Erkoyuncu, (2005) and Erkan, (2005). TMA-N value of pasteurized mussels did not exceed the limit value during storage period (Table 2).

Total viable count is an important criterion in assessing the quality of fresh and refrigerated seafood (Chouhan, et al., 2015). Changes in the microbiological count during cold storage were shown in Table 3. In our study, the initial total aer-

obic mesophilic bacteria count in raw mussel was 5.59±0.02 log cfu/g. Linton et al., 2003 also reported that total aerobic count for mussel samples was 5.00 log cfu/g. Total aerobic mesophilic bacteria count decreased (3.15±0.10 log cfu/g) after pasteurization. Total bacterial count reached maximum load (3.66±0.05 log cfu/g) at the 9th day of storage. In this study, total aerobic psychrophilic count in raw mussel was 6.18±0.11 log cfu/g. Similar, psychrotrophic count (>6 log cfu/g) was found by Linton et al., (2003) for mussel. After pasteurization, the total aerobic psychrophilic load was reduced (4.11±0.01 log cfu/g). Total psychrophilic count of pasteurized mussels decreased steadily throughout the storage period (Table 3). Many studies have shown that various heat treatment application in aquatic products are effective in reducing total bacterial load (González-Fandos et al., 2004; Martínez-Alvarez, et al., 2009; Mol et al., 2012; Cosansu et al., 2011, Cosansu et al., 2013, De Lima et al., 2017 ; Doğruyol and Mol, 2017 ; Bongiorno et al., 2018). The results of our study are consistent with the findings of other researchers. The raw material had initial yeast-mold counts of 6.30±0.02 log cfu/g. Yeast-mold load of the raw material decreased (3.93±0.02 log cfu/g) after pasteurization process (Table 3). The counts of yeast-mold decreased throughout storage. The yeast and mold were not detected in the later days of storage. Velammal et al., (2017) did not detect fungal colonies throughout the storage period in cooked meat of brown mussel. Kilinc and Cakli (2005a,b) did not detect mold and yeast in pasteurized sardines during the cold storage. *Vibrio* spp. and *Salmonella* spp. were not detected in raw and pasteurized mussels in our study. Total coliform bacteria and *Escherichia coli* were found 5.28±0.07 and 2.23±0.33 log cfu/g in raw mussel, respectively. In our study, the pasteurization process eliminated total coliform bacteria and *Escherichia coli* in mussels. Thermal processing and refrigeration are important means of controlling these bacteria. Cosansu et al., (2011) and (2013) reported no growth of these bacteria in sous-vide fish.

CONCLUSION

In our study, the pasteurization process reduced the microbial load of the mussels and stabilized during the storage. Pasteurization was very effective on microbial flora. According to the TVB-N and sensory analysis results obtained in the study, the pasteurized mussel can be safely consumed for nine days storage at 4°C.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

Behavior and Stereotypies of Nile Tilapia (*Oreochromis niloticus*) in Response to Experimental Infection with *Aeromonas hydrophila*

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ABSTRACT

Motile *Aeromonas* septicemia (MAS) is a common bacterial fish disease that may cause severe economic losses. This novel study was conducted to investigate behavioral changes of Nile tilapia (*Oreochromis niloticus*) in response to experimental induction of MAS. *Aeromonas hydrophila*, which is the causative agent of MAS, was isolated from diseased *O. niloticus* and used for the experimental infection of *O. niloticus* by intraperitoneal and intramuscular injections for inducing MAS. Each injection route had its control group. Fish behavior was recorded daily using a digital video camera for 7 consecutive days post injection in intraperitoneally and intramuscularly injected fish. On day 14 post injection, the behavior of intramuscularly injected fish was recorded again. Experimentally infected *O. niloticus* showed various clinical signs such as exophthalmia, ocular hemorrhage, congested gills, and skin and fin hemorrhages. The behavior of apparently healthy and experimentally infected *O. niloticus* was recorded and analyzed by scan observation. The experimentally infected fish exhibited cessation of normal behavior. Buccal-opercular movement and signs of aggression increased, whereas foraging, schooling, and shoaling frequencies decreased. Two abnormal behavioral patterns (stereotypies) of apparently healthy *O. niloticus* following injections were recorded. Post injection, intraperitoneally injected fish performed circular swimming on day 3 and 7, whereas intramuscularly injected fish exhibited vertical movement on the days 4, 5, 7, and 14. The frequency of circular swimming increased over time; however, vertical swimming frequency decreased by day 14. These results revealed that MAS had a severe effect on the behaviors of apparently healthy fish. Therefore, monitoring the behavior of *O. niloticus* may provide a useful and noninvasive tool for assessing fish health and diagnosing MAS early.

Keywords: Fish behavior, stereotypies, motile *Aeromonas septicemia*, Nile tilapia

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INTRODUCTION

There is an increasing demand on fish meat world-wide due to its high quality and healthy protein content and hence aquaculture industry showed a significant development in the last decades. However, bacterial diseases may threaten this industry due to serious impacts such as retarded growth or mortalities which cause economic losses. Motile Aeromonads septicemia (MAS) is one of the most virulent bacterial fish disease in cultured fishes, especially Nile tilapia *Oreochromis niloticus* (Pavanelli et al., 2008; Pridgeon and Klesius 2011 and 2012; El-Araby et al., 2016).

Fish behavior reflects the biochemical and physiological changes induced by stress and/or disease conditions. Therefore, it can be used as an early indicator to the health status of fish (Martins et al., 2012). In addition, it is targeted to predict economic impacts of diseases and stress on the aquaculture. Moreover, behavior is fast and easy to observe (Martins et al. 2012). Thus, it provides a useful tool for assessment of fish health and early diagnosis of diseases in cultured fish.

Ventilatory activity, achieved with bucco-opercular movements, is measured by the flow of ventilated water over gills per unit time (Martins et al., 2012). This renewal of oxygenated water is well regulated in healthy fish (Martins et al., 2012). Foraging behavior is the search for and exploitation of food resources (Danchin et al., 2008). Common types of social behavior in fish are, shoaling, schooling and aggression (Pavlov and Kasumyan, 2000). Shoaling refers to fish that swim together in an unstructured pattern, whereas schooling implies synchronized and polarized swimming (Faucher et al., 2010). In addition, the swimming of three fish together is described as shoaling and four and more fish swimming together is called schooling (Khalil et al., 2013). Impairment of foraging frequency and social interactions of these behavioral patterns may threaten the survival of fish in an ecosystem.

Stereotypic behavior is defined as a behavioral pattern that is repetitive invariant, and are frequently indicative of an sub-optimal environment (Mason, 1991). Furthermore, stereotypic behavior is believed to be an adaptation to stress (Vestergaard, 1981; Anonymous, 1989). Common stereotypical behaviors in fishes are circular swimming ; fish swimming in fixed circular pattern for more than 20 sec (Almaza´n-Rueda et al., 2004) and vertical swimming ; fish swimming vertically with head broke the water surface) (Kristiansen and Fernö, 2007).Thus, observation of these two stereotypies are performed by fish is an indication of stress.

Experimentally induced infections of MAS by intramuscular and/or intraperitoneal injection were studied in several fish species including Nile tilapia; *O. nilticus* (Banu and Yılmaz, 2011), catfish; Clarias gariepinus (Korni, 2015; Korni et al., 2016), Pangasius; Pangasianodon hypophthalmus (Naha et al., 2016), causing a mortality of up to 100% in infected species. These studies mainly investigated hematological and histopathological alterations; however there were no reports about behavioral alterations induced by MAS. Thus, the aim of this study was to evaluate behavioral changes in cultured *O. niloticus* following experimental infection of *Aeromonas hydrophila*as as an indicator of fish health.

MATERIAL AND METHOD

Fish Samples

A total of 140 *O. niloticus* individuals with average body weight (96±5 g) were provided alive from a fish hatchery, Beni-Suef, Egypt, in late spring season at water temperature 23±2°C. They were brought back to the wet laboratory of Fish Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. They were acclimatized for 15 days in two fiberglass tanks (800 L each) capacity supplied with chlorine-free tap water and continuous aeration. The fish were fed with standard commercial fish feed with a ratio of 3% body weight once a day during the acclimatization and experimental study. .

Bacterial Isolate

Aeromonas hydrophila strain was previously isolated from diseased *O. niloticus* and identified using API 20E and PCR technique in Department of Fish Disease and Management, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef , Egypt and published by (Korni et al., 2017) The hemolysin gene forward primer was 5´-GCCGAGCGCCCAGAAGGTGAGTT-3´ and the reverse primer was 5´-GAGCGGCTGGATGCGGTTGT-3´. The PCR mixture was 20 µL reaction. Volumes of each (10 µL) PCR product were subjected to electrophoresis in a 1.5% (w/v) agarose gel (Casiano et al., 2010).

Determination of Median Lethal Dose (LD_{50}) of *Aeromonas hydrophila* Isolate in Healthy *O. niloticus*

A total of 60 *O. niloticus* (96±5 g) were equally divided into 6 groups each of 10 fish An overnight culture of the isolate was adjusted to densities of 1.5×10^8 (group 1), 1.5×10^7 (group 21),, 1.5×10^6 (group 3), 1.5×10^5 (group 4), and 1.5×10^4 (group 5), (Korni et al., 2017). Each dilution was injected intraperitoneally (IP) to a fish in each group at a dose of 0.4 ml/fish and the fish of the sixth group was used as control and injected with 0.4 mL of sterile saline. All fish groups were closely observed for two weeks. Mortality rates were recorded daily, and the organs were aseptically streaked on brain heart infusion agar for re-isolation and re- identification.

Experimental Design of Pathogenicity and Behavior Studies

A total of120 clinically healthy *O. niloticus* with average body weight (92±3 g) were used for monitoring the pathogenicity of *A. hydrophila.* The identified isolate was inoculated onto BHIA at 28°C for 18 hours. Pure bacterial culture was suspended into a sterile saline and adjusted to $1.5x10^8$ CFU/mL. Fifteen days after acclimation; the fish were randomly divided into four equal groups (30 fish/group). Each group was sub-divided into 3 replicates (10 for each). First and second groups were kept for controls and injected IP and IM respectively with 0.4 ml of sterile saline. The third and fourth groups were given IP and IM injections with 0.4 mL bacterial suspension of 1.5×10^8 CFU/mL. All fish groups were kept under daily observation for two weeks. Behavior of the fish, clinical signs and mortalities were recorded. All freshly dead fish were submitted to bacteriological isolation and identification of the disease causative agent to verify the specificity of mortality. The present study was approved by Beni-Suef University's Institutional Animal Care and Use Committee (BSU-IACUC).

Behavioral Measurement

Behavior of injected fish (IP and IM groups) was recorded during the seven consecutive days post injection. The behavior of survived fish in the IM group was observed on fourteenth post injection. Behavior was captured for 15 min session once a day using digital video camera (SONY, Japan). Three videos for each group (one video for each replicate) were recorded. Time of recording was fixed daily (8:00-8:15). Behavior of experimentally infected and apparently healthy fish was analyzed by scan observation (Barlow et al., 2009). Number of opercular movements min^{-1} of four fish in each aquarium was counted without moving them from aquarium Foraging as shown in Figure 1a and social behavior including aggression (fish bit another; Figure 1b), schooling (at least 4 fish swimming together; Figure 1c), and shoaling (3 fish swimming together; Figure 1d) were analyzed. Abnormal behaviors (stereotypies) were also recorded. Frequencies of all normal and abnormal behaviors were calculated among the recorded videos period (Figure 1e, f).

Statistical Analysis

Statistical analyses were performed using Advanced Models 16.0 software Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA). Behavior data was analyzed using independent t-test. A significant difference between the injected groups and corresponding control is considered if $p<0.05$.

RESULT AND DISCUSSION

Pathogenicity Test

Experimentally infected *O. niloticus* in IP and IM injected fish showed signs of septicemia as congested gills, exophthalmia, ocular hemorrhage, disintegration of tail fin (Figure 2 a, b) and skin and fin hemorrhages (Figure 2 c, d). The post mortem findings of experimentally infected fish reveled congested kidney, liver and spleen. The IP injected fish showed more severe clinical signs and higher mortality rate than IM injected ones (80%). In the control group, fish showed neither clinical signs nor mortalities (Table 1).

Behavioral Alterations

Fish showed clinical signs of experimentally induced MAS infection. Fish exhibited cessation of all normal behavioral patterns, aggressed by apparently healthy fish, and gathered around the source of aeration. Moreover, normal behavioral patterns of apparently healthy fish in injected groups (IP and IM) were altered significantly compared to control group. These alterations were in a fluctuating manner among the days of observation post injection.

The rate of bucco-opercular movement min^{-1} was significantly increased (hyperventilatory activity) in IP injected fish among all

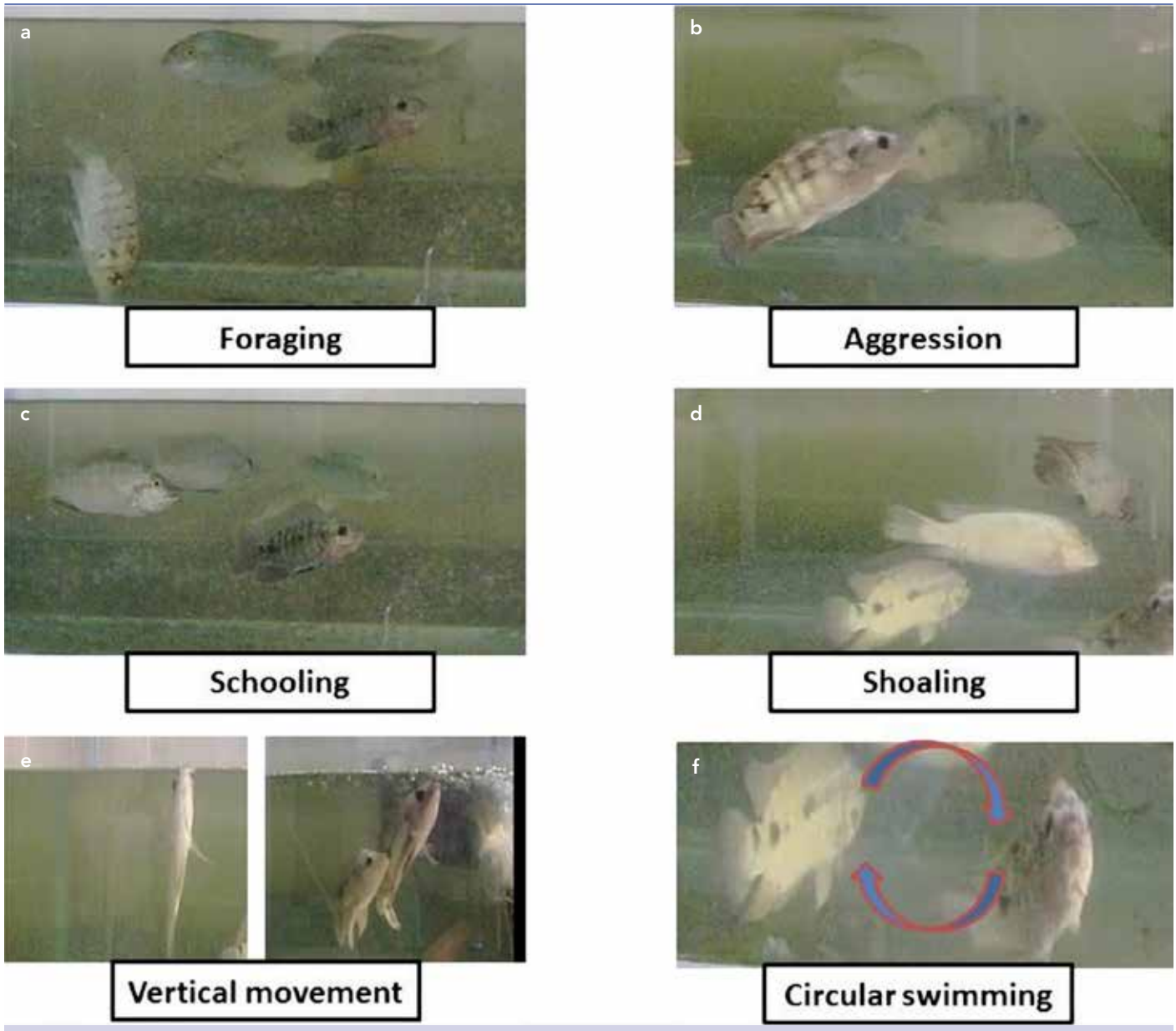

Figure 1. a-f. Normal behaviors (a-d) and stereotypies [abnormal behavior (e, f)] of *O. niloticus*

days of observation post injection (Figure 3a). In addition, IM injected group exhibited significant hyperventilatory activity on all days of observation except the first fourth and fourteenth days. Furthermore, the IP injected group showed significant decrease in foraging frequency in relation to control fish. Similar results were observed in IM group except on the fourth, fifth, and sixth days post injection (Figure 3b).

Social behavior of IP injected fish was disturbed. Aggression frequency was significantly increased during all the days of observation (Figure 3c), however, shoaling and schooling frequencies significantly decreased on the third, fourth, fifth, sixth, and seventh days compared with the control group (Figure 3d, e). Moreover, IM group showed impaired social behavior and significantly increased aggression among the observation period except on the second and third days post injection (Figure 3b). Shoaling frequency decreased on day fourteen only (Figure 3e), whereas schooling behavior significantly decreased on the third, seventh, and fourteenth days.

Table 2 showed two stereotypies (i.e., circular swimming and vertical movement) of apparently healthy *O. niloticus*. IP injected fish performed circular swimming with frequency of 1.6±2.3 and 7.6±5.5 on the third and seventh days respectively. IM injected fish exhibited vertical movement with frequency of 2.6±2, 4.6±1.5, 13±7.6 and 0.3±0.5 on the fourth, fifth, seventh and fourteenth days post injection, respectively.

Aeromonads are commonly found in all types of freshwater environments and various motile aeromonas species such as *A. hydrophila, A. caviae, A. sobria, A. veronii and A. schubertii* are pathogens of cultured freshwater fishes, however *A. hydrophila,* is the main cause of MAS (Akayli et al., 2011; Öztürk and Altınok 2014).

Figure 2. a-d. Clinical signs of motile *Aeromonas* septicemia in experimentally infected *O. niloticus*. (a). ocular hemorrhages, hemorrhage at pectoral fin base and disintegration of caudal fin (Scale bar 0.75 cm). (b). exophthalmia (Scale bar 1 cm). (c). severe hemorrhages on the skin (Scale bar 0.5 cm). (d). at dorsal fin (Scale bar 1 cm)

Table 1. Mortality % of *O. niloticus* caused by experimentally induced MAS.

	Mortality%		
Day post injection	Control	Ip injected group	Im injected group
1st	0	25	0
2nd	0	26	0
3rd	0	29	0
4th	0	37,5	20
5th	0	40	18,7
6th	1	33	153
7th	3	50	9
14th	3	100	0

Table 2. Stereotypies of *O. niloticus* caused by; control group (injected with 0.4 mL saline), IP (injected 0.4 mL of bacterial suspension of 1.5×10^8 CFU/mL) and IM (injected with 0.4 mL of bacterial suspension of 1.5×10^8 CFU/mL)

Group	Stereotypies	Day of onset post injection	Frequency
Ip	Circular swimming	3rd	1.6±2.3
		7th	7.6±5.5
Im	Vertical movement	4th	2.6±2
		5th	4.6±1.5
		7th	13±7.6
		14th	0.3±0.5

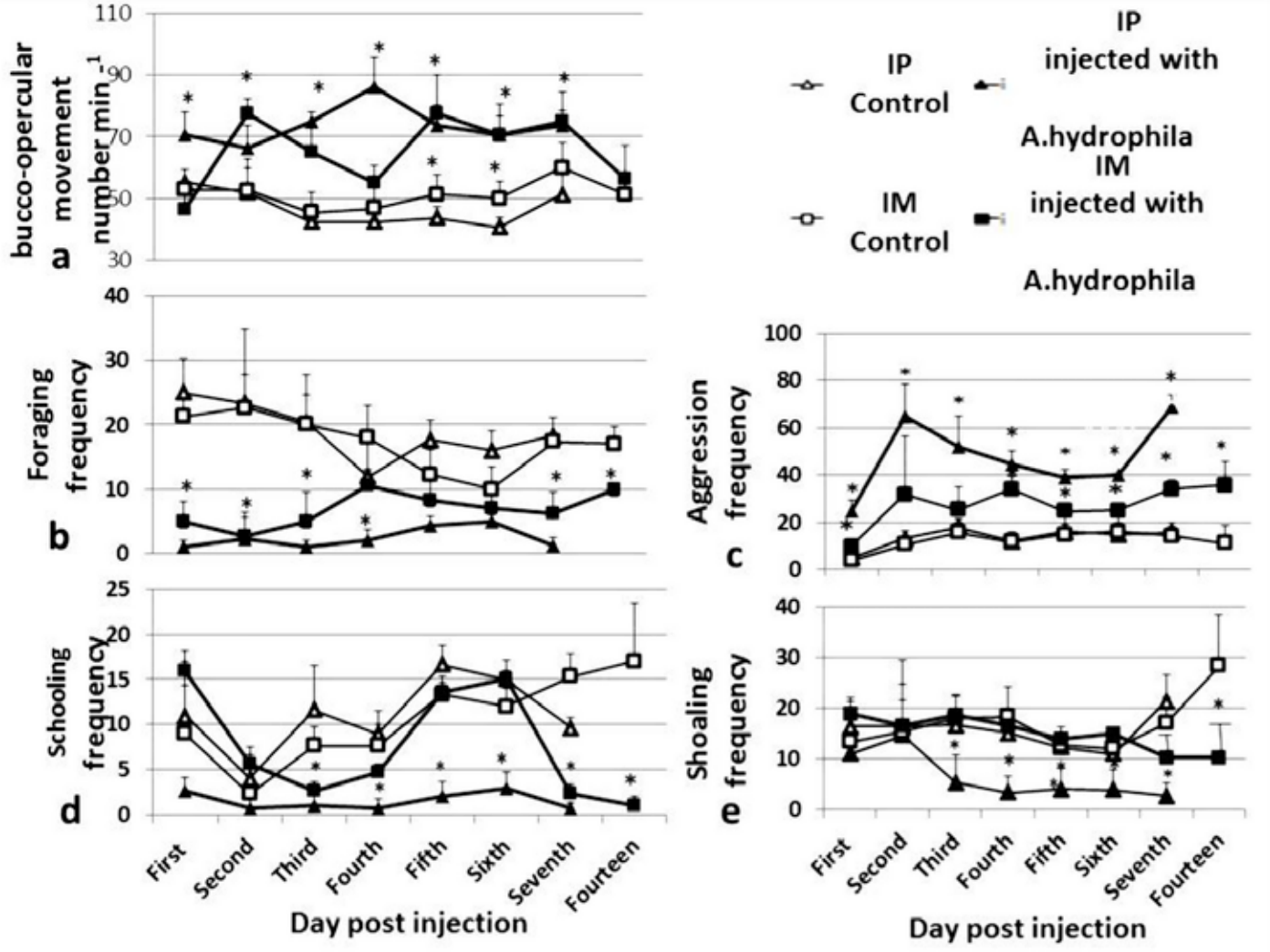

Figure 3. a-e. Effect of experimentally induced MAS (*A. hydrophila*) on behavior of min -1 *O. niloticus*, Control group (injected with 0.4 mL saline), IP (injected 0.4 mL of 1.5×10^8 CFU mL) and IM (injected with 0.4 mL of 1.5×10^8 CFU/mL). All values are the mean ± SD (n=3). The asterisk (*) indicates a significant difference between the injected groups and corresponding control (white blot) according to independent t test at $p<0.05$

In this study, extracellular products secreted by the causative agent of MAS disease (*A. hydrophila)* were responsible for pathogenicity. In addition, the reported signs of septicemia are attributed to the presence of hemolysis virulence gene in the isolate. Kristiansen and Fernö (2007) recorded higher mortality in IP injection. Moreover, Sarker (2009) recorded mortalities reached to 100% in IP and IM injected carp and perch.

Concerning the behavioral data, experimentally infected fish showing the clinical signs of MAS stopped performance of all normal behavioral patterns. Cessation of such behaviors is a common indicator of fish sickness (Martins et al., 2012). In addition, diseased fish stayed near aeration source due to hypoxia caused by gills inflammation (Noga 2011). Infected fish with bacteria showed different abnormal swimming patterns such as erratic swimming, whirling on the surface, and C-shaped body curvature at the surface in Red tilapia and mullet infected with streptococci (Evans et al., 2002; Zamri-Saad et al., 2010).

Normal behavioral patterns of apparently healthy fish in injected groups (IP and IM) were altered significantly compared to control. These alterations were in a fluctuating manner among the days of observation post injection. The reported behavior of apparently healthy fish in IP injected group revealed an acute form of the behavioral response to pathogens and stressors (Martins et al., 2012). The observed hyperventilatory activity may be attributed to the gill congestion or low hematocrit (White et al. 2008). This is an attempt from the fish's internal environment (homoeostasis) to maintain oxygen (O_2) status, blood and tissue acid–base balance (pH) (Martins et al., 2012). The observed hyperventilatory activity was supported the findings of Noga (2011) and Khalil et al. (2017). Hyperventilatory activity may result from many biotic and abiotic stressors (Martins et al., 2012; Khalil et al., 2017). Therefore, rate of bucco-opercular movement could be linked with other welfare indicators in the fish ponds such as water quality, blood lactate, glucose, and hematocrit (Martins et al., 2012; Noga, 2011, Khalil et al., 2017) as well as other behavioral changes to achieve an accurate interpretation of hyper/hypoventilatory activity.

Our data revealed decrease of foraging behavior of apparently healthy fish. This is may be due to stress posed by infection and impairment of fish social behavior (Krause and Ruxton, 2002; Martins et al., 2012; Kujur and Parganiha, 2013). These data were similar to that observed on *O. niloticus* suffered from a piscirickettsiosis-like condition (Mauel et al., 2007) and in Atlantic salmon suffered from vibriosis (Danchin et al., 2008). Decrease of feeding behavior is a sensitive indicator to health status of fish that leads to reduction of growth and reproduction (Volkoff et al., 2010).

The results of this study showed that, the impairment effect of MAS disease on social behavior of fish expressed in an increase of aggression bouts and a decrease of group swimming (schooling and shoaling) frequency, reduction of schooling and shoaling might be attributed to the decrease of individual movement induced by parasitic infestation as reported in Atlantic salmon (Wagner et al., 2003), Sockeye salmon *Oncorhynchus nerka* (Tierney and Farrell, 2004*)* and Bull trout *Salvelinus confluentus* (Jones and Moffitt, 2000). Fish aggregation is controlled by visual clues (Ruhl and McRobert, 2005), hence the observed decrease shoaling and schooling may be attributed to eye lesions caused by aeromonous hydrophilla.

Although increased aggression is an indicator of fish health and welfare, it must be accompanied with scars and lesions on the bitten fish (Martins et al., 2012). Social behavior enhance foraging, reproducion, and protection against predators (Krause and Ruxton, 2002; Kujur and Parganiha, 2013). Thus, social behavior alterations were usually used for assessment of fish health and welfare.

Circular swimming and frequencies increased over time, however, frequency of vertical swimming was decreased on the fourteenth day. On the fourth, fifth and sixth day post injection, mortalities were 37.5%, 40% and, 33% while, foraging, schooling, and shoaling showed no significant alterations in relation to the control fish. This indicated that, all fish that had exhausted resistance to infection during these three days died while fish that were still resisting infection performed the recorded normal behavior. Thus, behavior reflects immunity competence of fish.

The observed circular swimming (stereotypes) in IP injected fish was similar to that performed by African catfish as an indicator to stress (Almaza´n-Rueda et al., 2004*).* This behavioral pattern was almost followed by a high mortality on the third (27%) and seventh (50%) day. Furthermore, vertical swimming was recorded in the IM injected group. This behavior might be exhibited by fish due to hypoxia caused by gills inflammation (El-Araby et al., 2016), though no clinical signs of disease were observed. We hypothesize that, the recorded time-dependent increase of the recorded stereotypes frequency may indicate an increase of fish attempts to resist pathogen until exhaustion/failure that ended by mortality. The observed behavioral changes of apparently healthy fish may be a nonspecific response to MAS, however, it may be used as a useful, fast and non-invasive tool to assess and predict the resulted economic losses of the disease before occurrence of mortalities. Retardation of growth and reproduction is highly expected due to impairment of critical behaviors such as swimming, foraging, and schooling.

CONCLUSION

These results revealed that, MAS had a serious effect on the behavior of apparently healthy fish. Consequently, severe economic losses are predicted, such as retarded fish growth and decreased reproduction. Moreover, data suggests behavior measurement of Nile tilapia as a useful and fast and non-invasive tool for early diagnosis of the bacterial disease (specially MAS) in aquaculture.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Beni-Suef University's (Institutional Animal Care and Use Committee; BSU-IACUC).

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Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

Effects of Live Food Feeding on Growth Performance and Some Histological Parameters of *Herotilapia multispinosa* and *Amatitlania nigrofasciata*

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ABSTRACT

Aquarium fish feeding is an expensive hobby. Live foods such as *Artemia* are highly expensive and widely used. Therefore, aquarists look for alternative live food sources to feed their fish. Nematodes and annelids are good sources of protein and are easy to produce. In this study, we evaluated the effects of nematode and annelid feeding on the on growth performance and some histological parameters of *Herotilapia multispinosa* and *Amatitlania nigrofasciata* compared with commercial feeds. *A. nigrofasciata* fish groups fed with live and commercial food showed the highest survival rates of 91% and 100%, respectively. However, *H. multispinosa* fish groups fed with live food showed inhibited growth. Histologic examination of the liver and intestines revealed negative effects on these aquarium fish. Thus, right feeding regime practices should be established for using nematodes and annelids as fish food sources.

Keywords: Annelids, nematodes, aquarium fish, *Herotilapia multispinosa*, *Amatitlania nigrofasciata*

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INTRODUCTION

Aquariums are generally accepted as a hobby all around the world. Yet, the aquarium fish industry is a very big and important industry in terms of fish production. The aquarium fish trade costs about 659 million US dollars in the world. Supporting industries like fish feeds, filters, water chemicals and other equipment totals up to a 30 billion US dollars industry. Over 4000 freshwater and 1400 marine species of fish are in circulation for aquarium trade and an estimated 1 billion fish is sold every year (Whittington and Chong 2007, Tolon and Emiroğlu, 2014). It is evident that, aquarium fish are very cost effective compared to other fisheries products (Galib and Mohsin 2010, Saxena 2003). Growing aquarium industry increased the demand for ornamental fish which increased the need for aquarium fish culture establishments. Presently, freshwater species used in aquariums are 90% cultured and 10% wild captured whereas marine species are only 5% cultured. Yet marine fish are much more profitable and sought for by the hobbyists (Hekimoğlu 2005; Gümüş et al. 2014; Türkmen and Aktuğ, 2011).

Cichlid species are the most common species used for aquariums as well as the most cultured aquarium species. Most of them are preferred by the middle class and low income consumers for their affordable prices. One of the most important problems for these consumers is the cost of feeding. Quality feeds are important for both fish producers and home consumers for fish health, breeding and growth performance and visual quality (Naylor et al., 2009). High quality feeds are available as commercial products with high prices. Especially, larval feeding products like artemia and granulated feeds are very high priced feeds which are commonly used. The most used live food for larval feeding is Artemia sp. Which is

collected from the salt lakes around the world like Utah, Great salt Lake, Iran, Urmia Lake, İzmir, Çamaltı Saltworks. These sources are limited and dependent on natural stocks and growing need for artemia by aquaculture hatcheries increases the prices every year (Korkut et al., 2003; Tosun et al., 2015). This high prices results in the need for alternative live foods with low production costs.

Figure 1. Nematod culture media and climbing nematodes on the walls

Nematodes and Annelids are important alternatives for fish feeding with their low production costs and nutritional qualities. *Panagrellus redivivus* is a free living non-parasitic nematode which can be suitable for larval feeding. *P. redivivus* is the preferred nematode species for most of the aquarists with their ease of production and nutritional composition (Bruggemann, 2012). *Enchytraeus sp.* are annelid species used in aquarium fish feeding. Easy and economic growth of these live foods gets the attention of both fish producers and scientists for further research and production techniques (Tosun et al., 2015; Şahin et al., 2017). Starter culture costs for these live feeds are low and they are easily produced with low cost raw materials like water and oatmeal. One of the important aspect of producing annelids and nematodes is the ease of nutritional composition enhancement. Inclusion of fish meal and oil results in culture mediums results in high quality nutritional quality both for nematodes and annelids (Delbare and Dhert, 1996; Ricci, 2001; Santiago et al., 2003; Reyes et al., 2011; Schlechtriem et al., 2004; Kumlu et al., 1998; Santiago et al., 2004; Ercan et al., 2018).

In this study, we evaluated the effects of annelid and nematode feeding on *Amatitlania nigrofasciata* and *Herotilapia multispinosa* growth performances and some histologic parameters in aquariums.

MATERIAL AND METHOD

Nematodes

Panagrellus redivivus cultures used in this study were obtained from the Live Feed Laboratory of Aquaculture Department, Faculty of Aquatic Sciences, İstanbul University. *P. redivivus* cultures were inoculated in 1l plastic cases (Figure 1). The culture medium consisted of oat meal, water and yeast (baker's yeast) 200 L mixture (Tosun et al., 2015). Culture media was kept at 24°C. 10 plastic cases were used for nematode production and all received 2 ml starter cultures. Plastic cases were kept in Live Feed Culture Laboratory, Faculty of Aquatic Sciences. Microworms climbing the walls were harvested with a spatula to feed fish (Figure 1).

Annelids

Enchytraeus albidus was obtained from the Live Feed Laboratory of Aquaculture Department, Faculty of Aquatic Sciences, İstanbul University. *E. albidus* starter culture was inoculated in two 6.8 l (33 cm x 23 cm x 9 cm) plastic cases. Coconut turf was used as growth media with weekly additions of baby formula, milk, yoghurt and kefir (Memiş et al., 2004). Climbing worms were easily harvested and used as food (Figure 2).

Control Feed

Commercial aquarium flake feeds (AHM – Tropical mix flake) were used for control groups. Proximate composition of the feed is given in Table 1.

Fish

Herotilapia multispinosa (rainbow cichlid) *and Amatitlania nigrofasciata* (convict cichlid) were obtained from the Aquarium Fish Laboratory of Aquaculture Department, Faculty of Aquatic Sciences, İstanbul University.

Forty-eight convict cichlids with 0.54±0.27g mean weight (SD±) and 2.8±0.53 cm mean length were placed in four glass aquaria (A1, A2, B1, B2, each stocked with12 fish). 44 Rainbow cichlids with 0.14±0.09 g mean weight (SD±) and 1.4±0.36 cm mean length were placed in four glass aquaria (A3, A4, B3, B4, each stocked with 11 fish).

Aquariums

A total of eight aquariums were used during the experiment. 52.5 l (L70 cm x W30 cm x H30 cm) glass to glass aquariums which were part of a recirculating system with mechanic and biologic filtration were used. Water depth was 25 cm and water temperature was 22±2°C during the experiment. Aquariums were labeled as A1, A2, A3, A4, B1, B2, B3, B4. This study has been designed as 2 parallel for every group.

Table 1. Proximate composition of the commercial flake feed.

Commercial feed proximate composition	
Moisture	8% max
Crude Protein	47% min
Crude Lipids	4% min
Ash	7% max

Table 2. Feeding regime and fish distribution.

Species	Groups	09:00	12:30	16:00
Amatitlania nigrofasciata	A1 – A2	Nematod + Annelid	Flake feed	Nematod + Annelid
Amatitlania nigrofasciata	B1 – B2	Flake feed	Flake feed	Flake feed
Herotilapia multispinosa	A3 – A4	Nematod + Annelid	Flake feed	Nematod + Annelid
Herotilapia multispinosa	B3 – B4	Flake feed	Flake feed	Flake feed

Figure 2. Annelid culture media

Feeding Regime

A1-A2 and B1-B2 aquariums were stocked with convict cichlids, A3-A4 and B3-B4 aquariums were stocked with rainbow cichlids. A group aquariums were fed with live food twice a day (ad-libitum) at 09.00 and 16.00 and with flake feeds at 12:30. B group aquariums were fed only flake feeds at the same hours (Table 2).

Measurements

The experiment lasted for 3 months (90 days). Fish were measured for length by a ruler (mm) and weight (Digital scale, 0.01g) for every 30 day intervals. Data was analyzed using Student's T-test (Excel 2013, Microsoft).

Histological Examination

Randomly selected fish (3 from each group) were examined histologically. Histologic changes in liver and intestines were targeted. Fish were anaesthetized with 2-phenoxiethanol. After ventral incision, all fish was fixated in 10% formalin solution. Processed samples were placed in paraffin and cut into 4-5 µm cross-sections. Cross-sections were stained by Hematoxylin & Eosin method and examined under microscope (Culling, 1972).

RESULT AND DISCUSSION

Weight and Length Measurements

Weight Measurements

Mean weight gain results for live food fed groups are given in Figure 3.

Convict cichlids received live foods better than rainbow cichlids. Rainbow cichlids showed weight loss in the last period during the experiment.

Mean weight gain results for commercial flake feed fed groups are given in Figure 4.

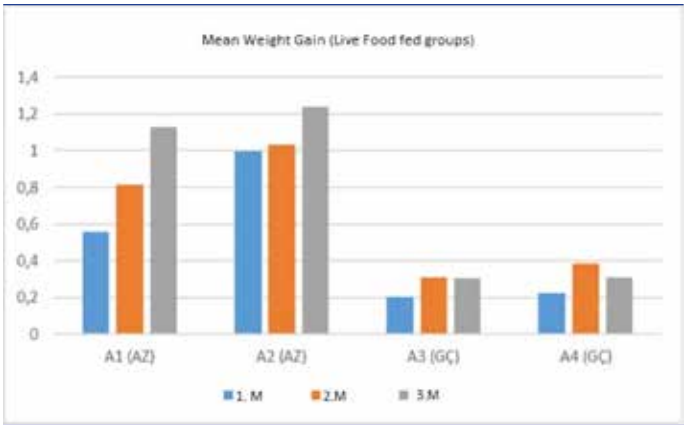


Figure 3. Mean weight gain comparison between live feed fed groups

Both fish species fed with commercial feeds had continuous growth in weight till the end of the experiment. Although A1-A2 groups have better results in terms of mean weight gain, live food feeding did not have statistically important differences with B1-B2 groups (p>0.05). Yet, when compared with A2-A3, B3-B4 groups have statistically better mean weight gain. Hoyland (2015) achieved continuous growth in *labrus bergylta* larvae with live feeds which was similar for convict cichlids response to live feeds in our study. We can reach to the conclusion that rainbow cichlids weight gain is inhibited with live food feeding. Mohseni et al. (2012) reported that *Huso huso* larvae, fed with live foods showed less growth in comparison to granulated pellets which is a similar result to our results with rainbow cichlids.

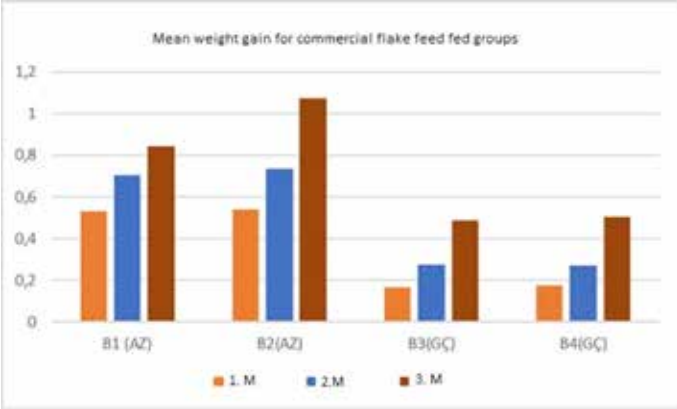


Figure 4. Mean weight gain results for commercial flake feed fed groups

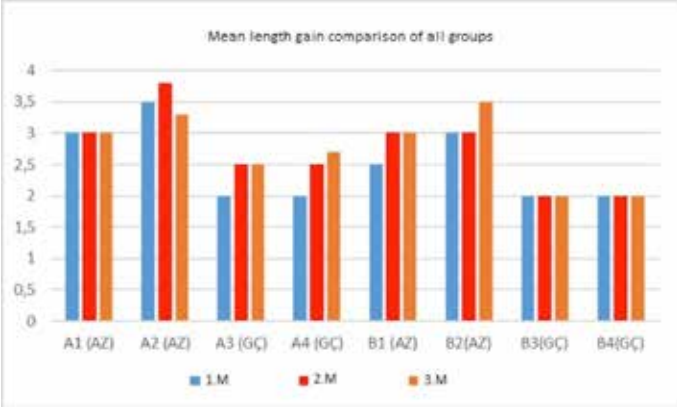


Figure 5. Mean length comparison of all groups
AZ: Convict cichlid; GÇ: Rainbow cichlid; M: month

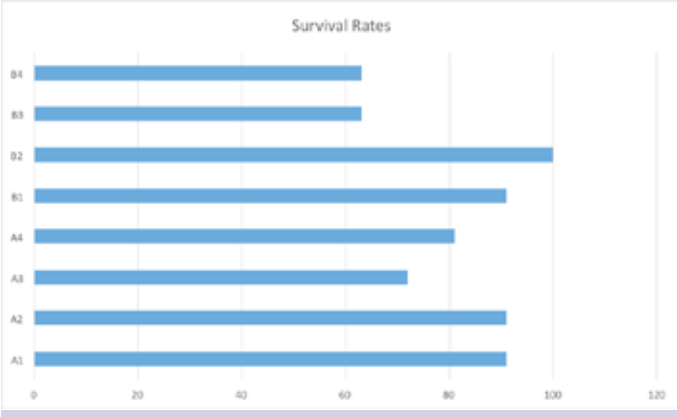


Figure 6. Survival rates (%) for the groups at the end of the trial

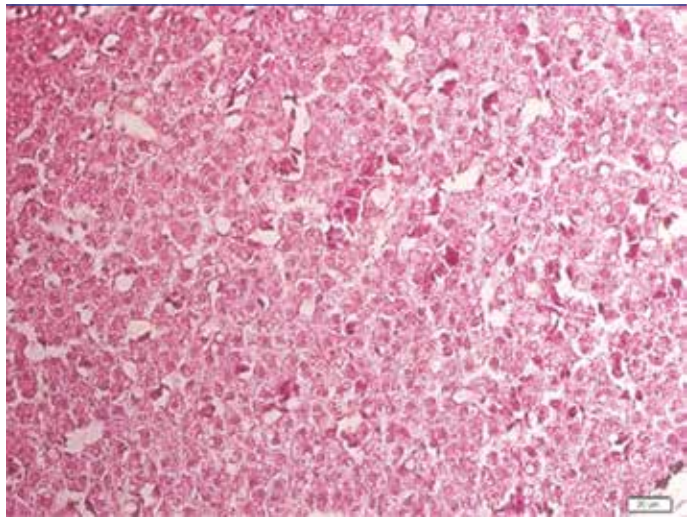
Length Measurements

Mean length measurements for all groups are given in Figure 5.

As it is evident in the given table, all groups except B3-B4 showed growth in length during the trial period. Commercial feed fed groups did not have significant differences in terms of growth in length compared to nematode and annelid fed groups.

Survival Rate

Calculated survival rates are given in Figure 6. Highest survival rates were calculated for A1, A2 (91%) and B1, B2 (91% and 100%) groups. Lowest survival rates were calculated for 63% for B3 and B4 (Figure 6).



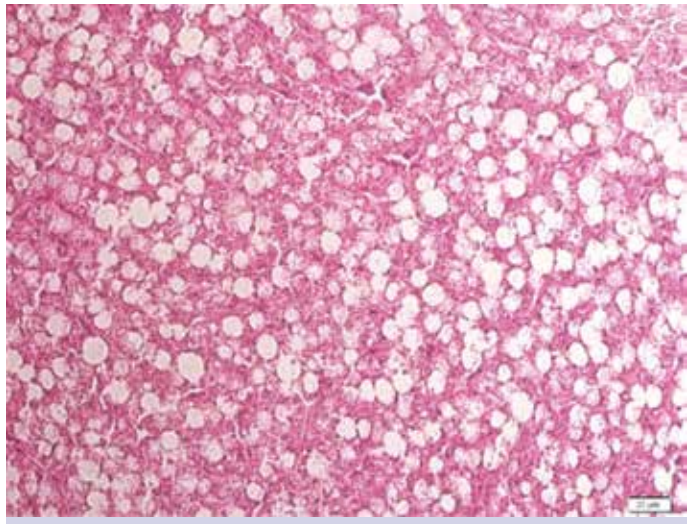
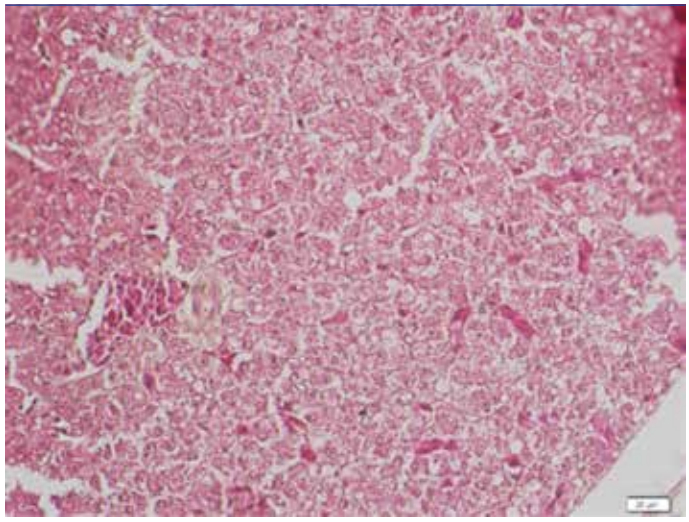


Figure 7. Lipid vacuoles in hepatocyte cells in cross sections of livers from live food fed groups Left; convict cichlid; right; rainbow cichlid



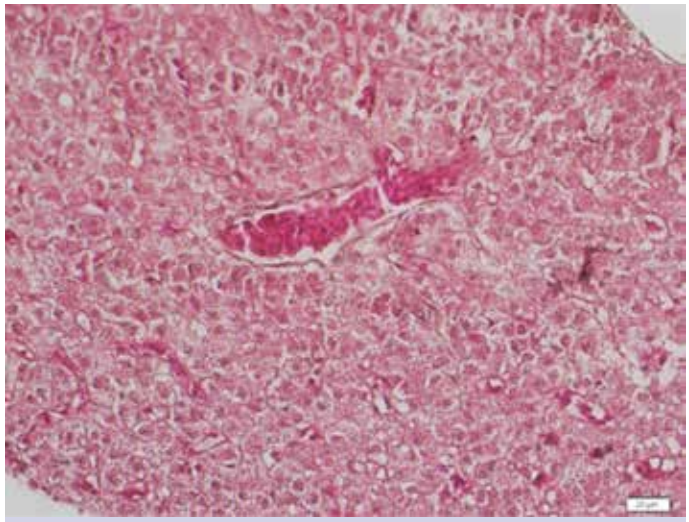
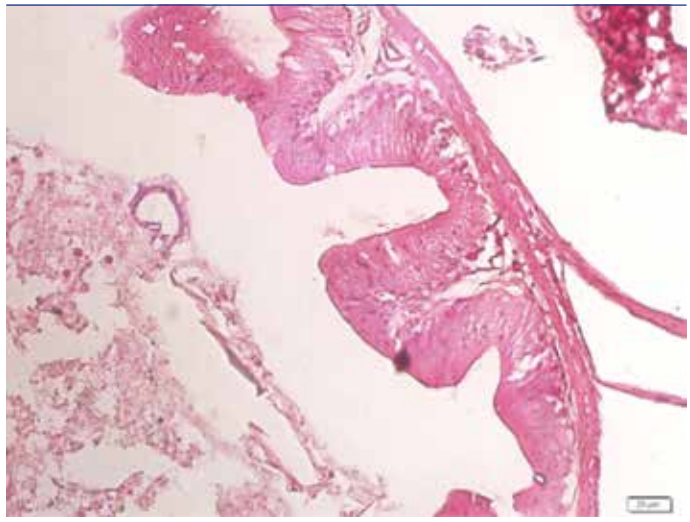


Figure 8. Healthy liver cross sections from commercial food fed groups without lipid accumulation
Left; convict cichlid; right; rainbow cichlid



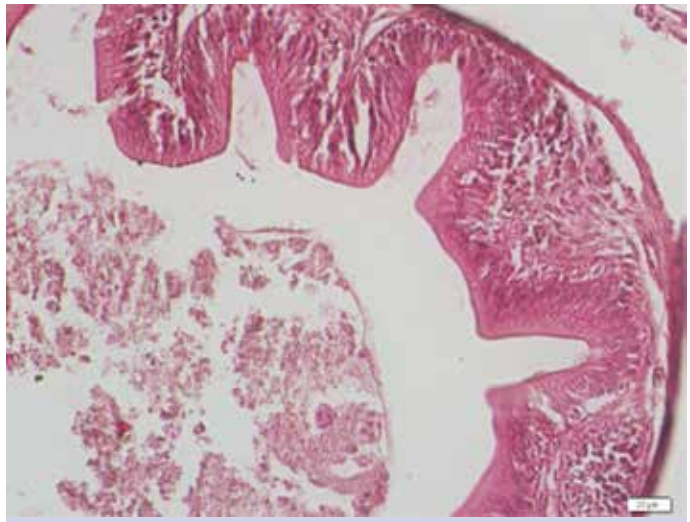


Figure 9. Cross sections of intestines from live food fed groups
Left; convict cichlid; right; rainbow cichlid

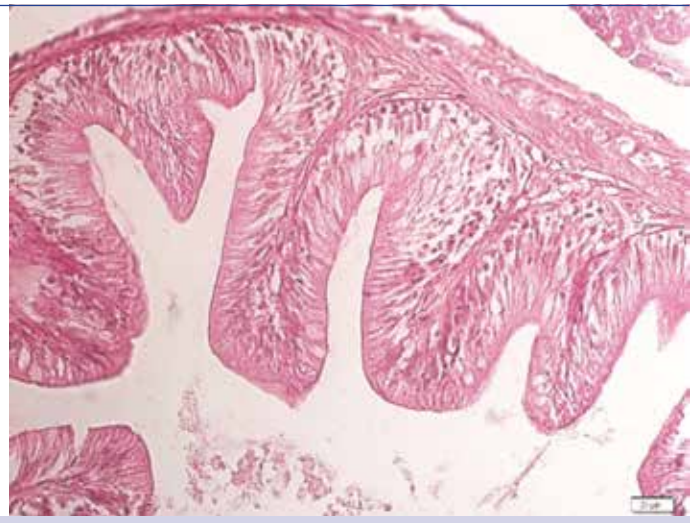


Figure 10. Healthy intestinal cross sections from commercial food fed groups
Left; convict cichlid; right; rainbow cichlid

Histological examination

A1, A2, A3 and A4 liver cross sections showed lipid vacuoles in hepatocyte cells. This showed that live food feeding resulted in lipids accumulation in liver cells for both convict cichlids and rainbow cichlids (Figure 7).

Contrary to the live food fed groups, commercial food fed groups (B1, B2, B3 and B4) had healthy liver cross sections (Figure 8).

Intestinal Examination

Cross sections from the intestines showed that live food fed groups (A1, A2, A3 and A4) had damaging effect. Villus were shortened with absorptive vacuoles on the tips, lamina propria were widened, and ruptures in serosa were found (Figure 9).

In cross sections from commercial food fed groups, intestines showed much healthier characteristics. Villus were longer, enterocytes had centered nucleus, lamina propria were not widened and goblet cells were present (Figure 10).

CONCLUSION

Live foods like nematodes and annelids which are widely used in aquarium fish maintenance and production are important alternatives with their ease of production and low costs. Alternative protein sources are gaining importance with increasing aquaculture production all around the world. *P. redivivus* is mainly important with its fast growth, easy mass production and ease of use as food. In addition, nutritional enhancement is possible. Brüggeman (2012), mentions that small size productions are easily adopted to industrial size production systems. Wilkenfeld (1984), points out that, production of these live foods can be more economical in comparison with artemia which presently is a very high priced commodity. As we demonstrated in our study, growth performances and survival rates of the fish fed with alternative live foods are not different compared with commercial feeds. This shows that, cheaper alternatives can be used for aquarium fish instead of expensive commercial products.

Our histological findings show that, amount of live feed used is important for healthy individuals. Raised amount of live feeds may result in internal damages to fish. Enhancing the live feeds or balancing the amount in the feeding regime should be evaluated in future studies. Culture mediums to produce suitable nematodes or annelids has to be formulated for better nutritional quality and healthy aquarium fish.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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Review

An Overview of the Factors Affecting the Migration of Sturgeons in Yeşilırmak

Serap Ustaoğlu Tırıl[1], Devrim Memiş[2]

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ABSTRACT

The Yeşilırmak is one of the rivers in Turkey where sturgeons migrate for spawning. It originates in the Kösedağ Mountains in Sivas and flows into the Black Sea at the plain of Çarşamba. The Yeşilırmak plays a major role in agricultural production and irrigation, and it also supplies energy due to the hydroelectric power plant (HPP). Serious structural changes and significant habitat loss for sturgeons and other fish species have occurred due to the dams and HPPs in the Yeşilırmak, especially since the 1970s. It is also a known fact that fish passages in these constructions are not suitable for the sturgeons to pass through. Unfortunately, coastal regulations along the river have had a negative effect on the habitats. Although the Yeşilırmak has lost quite a lot of its natural structure and its waterway gradually shortened due to the HPPs, sturgeons can still migrate upstream into it. Therefore, related sectors and stakeholders should consider the importance of the Yeşilırmak in their planned activities for the future of sturgeons in Turkish waters. In this study, the major factors affecting the sturgeon migration in the Yeşilırmak will be evaluated.

Keywords: Yeşilırmak, sturgeon, dam, hydroelectric power plant

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INTRODUCTION

Many fish species in the world are facing the danger of extinction due to various activities in their living and breeding ground which lead to habitat loss and pollution, in addition to man-made causes such as illegal and/or overfishing which are also responsible for their extinction. Due to these reasons, almost all species of sturgeon, which are of great importance due to the black caviar, now considered as "a symbol of luxury and high status" worldwide, are categorized by the IUCN (International Union for Conservation of Nature) in the red list of threatened animals as CR (critically endangered): species facing an extremely high risk of extinction in the wild (Anonymous, 2018a).

Sturgeons have existed for about 200 million years and are referred to as "living fossils" (Kirschbaum and Gessner, 2001). It is understood from the historical records that sturgeons were captured in Dniester in the 2500s BC, served with decorations in the great celebrations and printed money bore pictures of them. It is also reported that sturgeon caviar is of great political interest and wars were caused due to caviar (Reichle, 1997). From the 20th century, sturgeon began to be overfished worldwide. However, from the 1960s, due to the construction of dams, HPP (Hydroelectric Power Plant) and flood control sets on the spawning rivers began to lose spawning areas. At the same time, the destruction of spawning grounds, eggs and larvae due to the removal of sand and gravel from the rivers has also played a role in the decline of stocks. Agrochemicals, domestic and industrial wastes contaminated rivers which increased the destruction of spawning areas (Hochleithner, 1996). Since the same adverse conditions are still present despite some measures such as sturgeon fishing ban and stock enhancement, there is no improve-

ment in the critical situation of sturgeons. Five sturgeon species (*Acipenser gueldenstaedtii, Acipenser nudiventris, Acipenser stellatus, Acipenser sturio and Huso huso*) are naturally found in the Turkish waters and these species migrate into the large rivers (e.g. Sakarya, Yeşilımak, Kızılırmak) to spawn. In addition, until the 1970s sturgeon fishing was economically important in Turkish fishery and was known to have an important share in fishing activities especially in Çarşamba, Bafra, Karasu and Istanbul (Anonymous, 2015b). In 1989, FAO (Food and Agriculture Organization of the United Nations) experts reported that *A. sturio, H. huso, A. gueldenstaedtii, A. stellatus* and *A. nudiventris* were found in the fishing ports of the Turkish coast and the most common species of sturgeons were *A. sturio* and *H. huso* (Edwards and Doroshov, 1989). But in recent years the situation has completely changed and sturgeons are at the brink of extinction in Turkish waters. It is known that over-fishing, construction of dams and HPPs, flood control sets and pollution in rivers played a very significant role in this situation (Anonymous, 2015b).

Figure 1. Yeşilırmak

The Importance of Yeşilırmak for Sturgeons and the Effects of Hydroelectric Power Plants

Yeşilırmak is 519 km long and is derived from Kösedağ located within the boundaries of Sivas Province and flow into the Black Sea in Samsun-Çarşamba (Figure 1). While passing through Tokat, Amasya and Samsun, it connects with Çekerek, Tersakan and Kelkit Streams.

Yeşilırmak, streams and brooks located in the west and east of the Yeşilırmak spilled into the sea individually, formed Yeşilırmak river basin which is one of the 25 river basins of Turkey (Figure 2). The Yeşilırmak Basin (Number 14) contains all and/or parts of Tokat, Samsun, Amasya, Çorum, Sivas, Yozgat, Gümüşhane, Giresun, Erzincan, Ordu and Bayburt. Yeşilırmak River Basin, covering approximately 5% of the surface area of Turkey, which is the third largest precipitation area covers an area of 39628 km^2 of the country. Silts carried by Yeşilırmak and its branches forms the Çarşamba Plain (Anonymous, 2015a).

Yeşilırmak is a meandering river and forms the fertile Çarşamba Plain. Çarşamba Plain is a delta plain in the Central Black Sea Region. There is Çaltı Cape on the eastern end and Cıva Cape on the western end of the plain. There are shallow lakes, marshes and lagoons in the north side of the plain. Yeşilırmak, which flows from the Cıva Cape to the Black Sea, plays an important role in the formation of Çarşamba Plain, has an important role in the agricultural potential of the region (Kadıoğlu, 2016).

Moreover, along with the contribution in the agricultural production of Turkey, Yeşilırmak also plays an important role in the electricity production. There are 21 dams and/or HPPs for irrigation and electricity production on Yeşilırmak, which made an irregular flow regime of the river. HPPs in Yeşilırmak has a total capacity of 887 MW, which meets approximately 3.4% of the total electricity produced from HPPs in Turkey (Table 1) (Anonymous, 2018b). Hasan Ugurlu and Suat Ugurlu dams, Kumköy HPP and Çarşamba HPP are the closest to the mouth of the Yeşilırmak in Samsun.

Figure 2. River Basin Map of Turkey (National Watershed Management Strategy (2014-2023) - (07/04/2014 date and 29050 numbered Official Newspaper)

Besides its role in the supply of agricultural product and demand of electricity, Yeşilırmak has vital importance for sturgeons, which is very valuable for the biodiversity of the world as well as Turkey. Considering the history of sturgeons in the Turkish waters, sturgeon used to spawning in Samsun, the meeting point of two rivers (Yeşilırmak and Kızılırmak Rivers) with the Black Sea. Until the 1970s, sturgeons were caught in Kızılırmak, Yeşilırmak and Sakarya Rivers and their estuary, and about 8 tons of caviar were produced annually. Caviar industry which was seen as an important source of income for the economy of Turkey, was reported in the "Second Industrial Plan of the Republic of Turkey-1936" and considered worthy for support as being an important branch of industry that had to be improved. In the industrial plan, it was reported that caviar was mostly obtained from *H. huso* in the 1930's, and sturgeons were captured mostly in Sakarya, Kızılırmak and Yeşilırmak, especially around Bafra district in Samsun province. In this plan, it was also reported that more than 2000 sturgeons per year were caught by hook (in Turkish: karmak) in the river estuaries, half of them were mature enough to produce caviar and caviar production reached 4 tons per year. According to the FAO report, prepared by Edward and Doroshov (1989), caviar production was about 8 tons/year between 1940-1970 around Samsun (Ustaoğlu Tırıl and Memiş, 2015).

Besides the Second Industrial Plan of the Republic of Turkey-1939, the above-mentioned FAO report and a few research articles, there are many newspaper articles and other materials e.g. photos and videos which can be considered as important evidences of the presence of sturgeons and their status in the Turkish rivers. The newspaper article in Figure 3 ("This fish is another fish") is one of the most striking examples on this issue. According to this article, at the end of the 1960's, beluga (*H. huso*), ship sturgeon (*A. nudiventris*) and other species (*A. gueldenstaedtii*, *A. sturio* or *A. stellatus*) were caught in the mouths of Kızılırmak and Yeşilırmak and caviar trade was a major source of income for many families in Bafra and Çarşamba distritcts (Hayat Magazine, 1969).

Similar information was found in the Second Industrial Plan of the Republic of Turkey (1939) where it was reported that sturgeon catch and caviar production did not develop enough in those years. It was also reported that the main reason was that the mature sturgeons could not enter the river to spawn due to the logs in the mouth of the rivers (Sakarya, Kızılırmak and Yeşilırmak). It is also understood that the obstacles in sturgeon spawning habitats were not HPPs in those years, on the contrary, the drifted and accumulated logs in the river mouths. It was also suggested in this Plan that these natural obstacles must be cleaned before the spawning season and this should be done regularly. Nowadays, it is known that sturgeons can still enter the rivers, but could not pass through the dam or HPP and was caught under the dam or HPP. (Ustaoğlu Tırıl and Memiş, 2013, Ustaoğlu Tırıl and Memiş, 2015).

Table 1. Dams and HPPs on Yeşilırmak (Anonymous, 2018b).

Dam/HPP Name	Province/District	Installed capacity (MW)
Hasan Uğurlu Dam and HPP	Samsun, Ayvacık	500
Suat Uğurlu Dam and HPP	Samsun, Çarşamba	69
Kumköy HPP	Samsun, Çarşamba	17
Çarşamba HPP	Samsun, Çarşamba	11
Almus Dam and HPP	Tokat, Almus	27
Ataköy Dam and HPP	Tokat	6
Çilehane HPP	Tokat, Almus ve Reşadiye	7
Köklüce HPP	Tokat, Niksar	90
Yeşilırmak 1 HPP	Tokat, Reşadiye	14
Yeşilırmak 2 Regulator and HPP	Tokat, Reşadiye	6
Omala HPP (under construction)	Tokat, Merkez	17
Karakeçili 1 HPP (under construction)	Tokat	7
Çarıklı HPP	Amasya, Merkez	9
Amasya Kale HPP	Amasya, Merkez ve Taşova	29
Midilli HPP	Amasya, Merkez ve Taşova	33
Yavuz HPP	Amasya, Taşova	23
Umutlu HPP	Amasya, Taşova	20
Osmancık HPP	Amasya	9
Taşova Yenidereköy HPP	Amasya, Taşova	2
Karayel HPP (with production license)	Amasya, Taşova	22
Yeşil HPP	Sivas	14

HPP: hydroelectric power plant

Although the habitat structure and flow regime of Yeşilırmak are quite different from its natural structure, it is reported that the mature sturgeons, especially *H. huso*, migrate to Yeşilırmak (Zengin and Ustaoğlu Tırıl, 2012). In this regard, the latest data in Yeşilırmak was reported on 11 March 2012. A *Huso huso*, weighed 150 kg, was caught by the local people in a puddle of the river bed in Yeşilırmak, nearly 20 km far from the Yeşilırmak river mouth. It was a female fish and 20 kg of caviar was obtained from it. This data demonstrates that the sturgeons still migrate to Yeşilırmak (Ustaoğlu Tırıl and Memiş, 2013). Therefore, the part from the Yeşilırmak river mouth to the first HPP (Çarşamba HPP) is very important for the spawning migration of sturgeons.

The habitat of sturgeons in Yeşilırmak was evaluated in one of the studies which was done as a project entitled "Recovery of Sturgeon Population in Turkey: Habitat Assessment and Restocking" supported by the Food and Agriculture Organization of the United Nations (FAO) and coordinated by the Ministry of Agriculture and Rural Affairs of Turkey (MARA). In this study, the area from Suat Uğurlu Dam to the river mouth of Yeşilırmak was evaluated and determined that the construction of Kumköy HPP and Çarşamba HPP caused significant loss of spawning and feeding habitat for sturgeon. In the National Action Plan prepared at the end of this project was highlighted to the critical status of sturgeons in Yeşilırmak in terms of habitat loss. In this Plan, it was also reported that serious rehabilitation studies are urgently needed in Yeşilırmak (Anonymous, 2015b). There are successful studies (Hayes and Caroffino, 2012; Hall et al., 2012; Gessner et al., 2014) for the rehabilitation and restoration of sturgeon habitats in different countries such as Germany, Romania, USA and Canada. It should be urgently assessed whether similar rehabilitation and restoration studies can be carried out in Turkey.


21 bin liralık havyar çıkarılacak olan balık işte bu...

Bu balık başka balık!...

SAMSUN: Bafra ve Çarşamba'da yüzlerce ailenin geçimini sağlayan havyarcılık, bu yıl kendine ümit bağlayanların yüzünü güldürmüştür.

Kızılırmak ve Yeşilırmak nehirlerinin ağızlarında avlanan Şip, Mersin ve Morina balıklarından elde edilen havyarın kilosu toptan 750 - 1000 liraya satılmaktadır.

Havyarcıların verdiği bilgiye göre, geçen hafta Avni Natipoğlu adlı vatandaş, bir balıktan 19 bin liralık havyar çıkartmıştır. Kemal Bayramoğlu adındaki balıkçı da Kızılırmak ağzında yakaladığı dev bir Morinadan 21 bin 500 liralık havyar elde etmiştir. Balıkçılar, yıllardır bu derece yüklü mal yapmadıklarını söylüyorlar.

Figure 3. Newspaper article in Hayat Magazine (29.05.1969)

Threats to Sturgeons in Yeşilırmak

Fisheries on the River Mouth

Estuaries are among the most productive natural habitats in the world in terms of biological productivity due to the rich nutrients transported from the river and accumulated on the river bed. Throughout history, estuaries are important for human settlement and transportation, but today the estuaries are mostly under threat from human activities such as domestic and industrial wastes, shipping and fisheries activities. Despite all the negativity, estuaries give a fascinating insight in the natural habitat, where energy is transformed from sunlight into plant material and then through the steps of a food chain is converted into a rich food supply for fish and birds. Estuaries are important nursery and feeding areas for many fish species because of their high biological productivity. At the same time, these are the important areas for the migration routes of anadromous and catadromous fish species (McLusky and Elliot, 2011).

As in many estuaries in Turkey, the commercial fisheries on the mouth of Yeşilırmak is one of the most important economic income sources for the local people. It is known that during these fishing activities in this region, sturgeons are also caught occasionally together with another fish species. Therefore, commercial fisheries are a significant threat to the sturgeons in this region and special protection strategies (e.g. strictly control or complete fishing ban) should be determined in order to prevent illegal fishing in Yeşilırmak estuaries. Decreasing the mortality resulting from fishing is a very important factor in the rehabilitation of the stocks efficiently. An effective program to decrease the incidental capture of the fish to avoid future losses of the few remaining adult fish and juveniles should be established (Anonymous, 2015b). Moreover, a conservation status should be determined for Yeşilırmak estuaries for the long-term preservation of sturgeons.

Fishing Vessel Traffic and Illegal Settlement Problems

Yeşilırmak estuary is used as relatively low density area for human settlement compared to many other estuaries in Turkey. However, since there is no fishing port around the river mouth, the inner part of the river mouth is used as a fishing port. The increasing use of river mouth as fishing ports and illegal settlement problems are serious threat to Yeşilırmak (Figure 4). Monitoring and control of this situation by authorized institutions and taking necessary precautions are important both in terms of the protection of environment and the future of sturgeons in Yeşilırmak.

Bridges

A bridge is a structure constructed from wood, stone, steel or reinforced concrete, which is built over obstacles such as a stream, valley, rail or other roadway for the purpose of providing passage

over the obstacle. Unfortunately, during the design and construction of bridges over rivers, its impacts on river ecosystem, hydrology, geomorphology, sediment transport, fish and wildlife passage/movement and wildlife habitats are usually not considered. Bridges may directly result in loss or fragmentation of habitat and increase the disruption of ecosystem processes. Changes of hydrology as a result of bridged crossing can cause alteration in size and section of river geomorphology. Negative impacts on habitat may also occur as a result of bridge maintenance. These impacts are most acute and have long-term effects on fish and wildlife movements (Anonymous, 2008).

Figure 4. A view from the Yeşilırmak river mouth (Photo: Google Earth, 2018)

Figure 5. The location of Hürriyet-Kumtepe Bridge on Yeşilırmak (Photo: Google Earth)

There are still 7 reinforced concrete bridges on the section from the river mouth to the first HPP on Yeşilırmak and one bridge is under construction. The bridge, which is the closest to the river mouth (about 4 km) was completed in December 2017 and this bridge is 310 meters long (Figure 5, 6).

Bridge abutments and embankments between them like a dike may affect the movement and passage of sturgeons negatively when water is stored behind the dam or HPP. Therefore, during the design and construction of the bridge the natural life in the river should be considered. The bridges which fully span the watercourse channel provide the best opportunities for maintaining river habitats (Anonymous, 2012). Nevertheless, in the bridges on Yeşilırmak, the natural life in the river has not been considered and critical habitat loss has occurred.

Industrial, Agricultural and Domestic Pollution

The main sources of pollution in rivers are a discharge of untreated industrial and domestic wastewaters and agricultural activities. Industries and cities have generally been located along rivers because the rivers have been a convenient place to discharge wastes. Besides, agricultural activities have also tended to be concentrated near rivers, because the soil around the rivers is highly fertile.

An intensive agricultural production is carried out in the fertile agricultural lands in the Lower Yeşilırmak Basin and vegetables, fruits and rice are mostly cultivated in Çarşamba Plain. Inorganic fertilizers and pesticides are used intensively for agricultural purposes in the plain. In addition, industrial wastewater (for example from

Figure 6. A view of Hürriyet-Kumtepe Bridge on Yeşilırmak (Photo: https://www.youtube.com/watch?v=LB2Xsnu GjZU)

Çarşamba Sugar Factory) and untreated municipal wastewater also contribute to the pollution in Yeşilırmak. Currently, only 15 of 194 settlements (municipalities and villages) treat their domestic wastewaters in treatment plants in Yeşilırmak Basin (Anonymous, 2015b).

Migratory fish (*such as sturgeons and salmonids*) have been able to exploit all physically accessible river habitats. Their eggs generally adhere to stones, gravels or weeds. Therefore, fish are sensitive to modification of the river regime (velocity, erosion, etc.) as well as to the input of toxic substances. Consequently, highly specific physical and chemical conditions are necessary in a river for successful breeding. Migratory fish which return from the sea to spawning habitats in upstream stretches of rivers can be prevented from reaching their spawning areas by physical or chemical barriers along their migratory route. Chemical barriers are stretches of highly toxic or anoxic water in the river. The release into rivers of untreated domestic or industrial wastes high in organic matter results in a decline in oxygen concentration (resulting in anoxia) and a release of ammonia and nitrite downstream of the effluent input (Meybeck et al., 1996). All the below mentioned industrial, agricultural and domestic effluents reach the Yeşilırmak but the effects of these inputs on the spawning of sturgeons in the river are unknown. Therefore, the level of pollution impact and the main criteria which are adversely impacted must be identified and the persistence of pollution in the river is to be assessed (Anonymous, 2015b).

Figure 7. Spawning and feeding areas of sturgeons in Yeşilırmak (Anonymous, 2015b)

CONCLUSION

Considerable habitat degradation and loss have occurred through dams, HPPs, bridges and river bed modifications in Yeşilırmak. However, it is known, that sturgeons can still migrate upstream into Yeşilırmak and are accidentally caught sometimes in the river mouth or in the river. The information in this regard was reported in the National Action Plan for the Conservation and Restoration of the Sturgeons of Turkey, which prepared within the scope of the project entitled "Recovery of Sturgeon Population in Turkey: Habitat Assessment and Restocking" supported between 2008-2011 by the Food and Agriculture Organization of the United Nations (FAO) and coordinated by the Ministry of Agriculture and Rural Affairs of Turkey (MARA). Moreover, the spawning and feeding areas are identified in the habitat evaluation studies during this project which was given in this National Action Plan (Figure 7).

According to the Action Plan, very limited spawning and feeding areas for sturgeons have remained in Yeşilırmak between the last HPP (Çarşamba HPP) and the river mouth (Figure 7). It is of utmost importance to protect these last remaining areas and to avoid practices that would destroy their habitat. In other words; "last minute" measures are needed urgently. Also it is vital to develop communication tools to increase the awareness of local communities about the conservation value and its role for future rehabilitation measures. It would also be very useful to develop a guarding system in cases where potential poaching might affect the population during migration or reproduction (Anonymous, 2015b).

In conclusion, by evaluating the available historical and current data, it is understood that Yeşilırmak is very important for the future existence of sturgeons in Turkish waters. The still existing/under construction or planned obstacles in Yeşilırmak (dams, HPPs, bridges, flood control sets, recreational modifications etc.) must be evaluated in detail by the related institutions taking into consideration the National Action Plan.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Table 1. Limitations for each manuscript type

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Original Article	≤20	250	40
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- Authored Book: Rogers, T. T., & McClelland, J. L. (2004). Semantic cognition: A parallel distributed processing approach. Cambridge, MA: MIT Press.
- Chapter in an Edited Book: Gill, M. J., & Sypher, B. D. (2009). Workplace incivility and organizational trust. In P. Lutgen-Sandvik & B. D. Sypher (Eds.), Destructive organizational communication: Processes, consequences, and constructive ways of organizing (pp. 53-73). New York, NY: Taylor & Francis.

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