Saudi Journal of Biological Sciences 27 (2020) 1717–1721

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Review

Molecular identification of *Neoechinorhynchus rutili* parasite diagnosed in some fish species caught in Menzelet dam lake in Kahramanmaras province (Turkey)

Mikail Özcan *, Neslihan Bozdoğan

Kahramanmaraş Sütçü Imam University, Faculty of Agriculture, Department of Fisheries, Kahramanmaras 38000, Turkey

ARTICLE INFO

Article history:
Received 1 December 2019
Revised 27 April 2020
Accepted 27 April 2020
Available online 7 May 2020

Keywords:
Fish
Parasite
Neoechinorhynchus rutili
PCR

ABSTRACT

This study details the molecular identification of the parasite *Neoechinorhynchus rutili* diagnosed in fish (*Capoeta barroisi, Cyprinus carpio, Barbus rajanorum*) caught in Menzelet Dam Lake in Kahramanmaras Province, Turkey. Parasite samples were obtained from the intestines of fish caught from January to June of 2013. The collected parasites were stored in sample vials containing 70% alcohol. Using staining methods and based on morphology, 120 *N. rutili* specimens were identified. DNA isolation of *N. rutili* was accomplished using special tissue sets for parasites. Specific primers were utilized in the molecular identification of *N. rutili* using polymerase chain reaction (PCR) and it was possible to verify that all of the parasites contained *N. rutili* molecules. In conclusion, using multiple methods we successfully identified and confirmed the presence of *N. rutili* parasites in the fish caught in Kahramanmaras Province. The process of identification of *N. rutili* using morphology and staining methods is time-consuming; however, PCR was successfully performed in a short time to accomplish the same results. The success of this study may lead to more original and extensive work aimed at the efficient molecular identification of parasitic agents found in fish.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Contents

* Corresponding author.
E-mail address: mikailozcan@ksu.edu.tr (M. Özcan).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Aquaculture has been drawing increasing significant worldwide attention on a daily basis. Fish diseases that adversely affect reproduction, growth, and nutrition lead to enormous economic losses;

https://doi.org/10.1016/j.sjbs.2020.04.047
1319-562X/© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

therefore, it is extremely important to identify the diseases that challenge fish farming and search for ways to eradicate them (Dal, 2006).

Aquaculture can be exercised in almost any place where water is available. Fishing, pisciculture, and aquaculture are performed in both saltwater and freshwater. In recent years, fishing and pisciculture have continued to experience worldwide growth. At the same time, technological advancements have played a major role in the decreasing availability of stock as more and more fish are caught in the seas and lakes. Turkey is home to seas of varied properties, with an increasing number of species, temperature, and salinity and decreasing populations from north to south. Inland, Turkey has approximately 200 natural lakes, over 300 dam lakes, 33 major rivers, and roughly 750 ponds with economic significance in terms of aquaculture. Given this potential, it is of great importance to utilize the areas that have been in use in the aquaculture sector efficiently (ZMO, 2017).

Fish represent the healthiest source of protein for humans in many parts of the world and particularly in developing countries. The world population has been consuming water products of animal origin to meet a large part of its protein requirements. The current protein deficit, as well as its growth over time, will be impacted by aquaculture (Dick and Choudhury, 1995).

Although there is a high volume of fishing in Turkey compared to other countries, the region has a very low quantity of fish. While other areas are involved in fishing, the Black Sea region is the leading fish producing area in Turkey. According to statistics for the year 2017, of the 354,318 metric tons of fish caught, 322,173 were saltwater fish and 32,145 were freshwater fish (TÜİK, 2018).

Fishing has been analyzed in detail in Ataturk, Keban, Karakaya, and Seyhan dams, which are among the major dams of Turkey. Information has been provided concerning the species of fish caught and the means of fishing in use (Duman and Çelik, 2001; Pala, 1996; Anonim, 1995; Avşar and Özyurt, 1999).

The practice of commercial fishing is done in Menzelet, Sir, and Aslantas Dam Lakes in Kahramanmaras Province. There is no research available, except for the records kept by the Provincial Directorate of Agriculture, on important information such as the status of fishing in these areas, the fishing equipment in use, and the annual fishing amounts. For optimal lake management and stock identification it is very important to know the amount of fishing occurring in dam lakes. Fishing in Kahramanmaras is performed using primitive equipment, and it is not possible to make the best use of the stock. Among the species involved in fishing in Menzelet Dam Lake are *Silurus glanis, Cyprinus carpio, Barbus rajanorum, Capoeta capoeta, Capoeta barroisi,* and *Leuciscus cephalus* (Alp et al., 2003).

Fish encounter many disease agents, including parasites, in their environments. Parasites are mechanically and functionally harmful for living things and have preying exploitative effects as well, feeding on the host's nutrients, and impairing respiration. These parasites both reduce the demand in the market and cause major economic losses, weakening and even killing the fish by taking advantage of their food (Ekingen, 1983).

Certain undesirable natural or human-related incidents in the aquatic environment can upset the host-parasite balance, leading in turn to the growth of one or more parasite types and major losses of fish (Hoffman, 1967). Thus, natural and cultivated fish can suffer major losses due to parasitic diseases.

Diagnosing the parasites in fish is very important both scientifically and in terms of human health. Because fish are an important nutritional source, consuming fish that accommodates any kind of parasites may be harmful to human health; therefore, it is important for the health of humans to have fish in the market in a healthy condition. The availability of healthy high-quality products increases the demand for such products, leading in turn to increased earnings (Hoffman, 1967; Grabda, 1991).

The aquatic environment is very favorable for the development and continuation of the parasitic life cycle. Although parasitic infections are commonly found in fish in their natural environment, pathologies caused by parasites are very rarely encountered in fish (Barber and Poulin, 2002).

Acanthocephalans, also known as spiny-headed worms, have proboscis with chitin hooks. Mature parasites lay their eggs in the intestines of fish. Some copepod crustaceans pick them up when they come out. The first larval stage called acanthor becomes acanthella in the second larval stage. Fish encounter the agent upon eating crustaceans. Sometimes the agents use the fish as a second intermediate host, and when high in number they can seriously harm the intestines. Due to their simple biological structure, increased efforts in fish cultivation may increase their significance (Karabulut, 2009).

Neoechinorhynchus rutili, an acanthocephalan worm, lives in the small intestine of freshwater fish (Tınar, 2006).

The distribution of *N. rutili*, redefined by is described in Canada, Northwestern lands, Alaska and Washington coasts, the polar circle, Sweden, Finland, Russia, Central Europe, and continuously along the North Holarctic region (Sheridon and Pratt, 1964).

Adverse effects as severe as mortality can be seen in fish having intense *N. rutili*. Their proboscis being short, the parasites attach low on the intestinal mucosa and cause various degrees of inflammation. Young fish may exhibit worse manifestations (Arda et al., 2005; Tınar, 2006).

Morphologically indistinguishable species can have absolute genetic differences. Therefore, molecular techniques are useful for identifying the species that are hard to distinguish otherwise (Aksakal and Erdoğan, 2007).

Polymerase Chain Reaction (PCR) is a method of in vitro DNA synthesis. It is a simple but very successful technique used for reproducing the specific zones in the genomic DNA of an organism. PCR can reproduce millions and even billions of copies of DNA molecules in a short time. This method thus ensures identification with high precision (Saiki et al., 1988; Mullis, 1990; Bej et al., 1991; Taylor, 1993; Marx, 1988; Coote, 1990; Quirke, 1992; Arda, 1995; Arı, 1999).

There is no comprehensive research in Turkey involving molecular identification of *N. rutili* which is one of the significant agents of parasitic diseases among fish. This study was intended to use PCR for molecular identification of the *N. rutili* parasite diagnosed by morphology and staining methods in the fish species caught in Menzelet Dam Lake in Kahramanmaras, Turkey.

Fig. 1. Menzelet Dam with fish hunting.

2. Materials and methods

2.1. Materials

Menzelet Dam is located on the River Ceyhan. This dam is approximately 27 km from Kahramanmaras city center in the northwestern direction. A map of Menzelet Dam from where the fish samples used in this study were collected is provided in Fig. 1.

This study was performed on 3 fish species commonly caught in Menzelet Dam Lake in January, February, March, April, May, and June of 2013 with the help of fishermen using gill nets. The fish species reviewed were *Capoeta barroisi, Cyprinus carpio,* and *Barbus rajanorum*. In the study, a total of 270 fish were evaluated, including 267 *Capoeta barroisi*, 1 *Cyprinus carpio,* and 2 *Barbus rajanorum*.

2.2. Methods

The 270 fish caught in Menzelet Dam Lake were transferred live in plastic buckets to the Fish Diseases Laboratory of Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Aquaculture, where they were anesthetized with 2-phenoxyethanol. The fish species were identified using the method of Geldiay and Balık (1996). With their species identified, the fish were autopsied according to information by Arda et al. (2005). The fish were cut ventrally along the abdomen starting from the anus using a pair of small and thin pin scissors. The digestive tract was placed into a dark-waxed Petri box, longitudinally cut from end-to-end using the same pair of scissors, and stretched on a wax base utilizing pins. Prepared as such, the digestive tract was examined under a binocular stereo microscope using fine needles and brushes. After counting the parasites and recording their host organ, the parasites were removed using the brushes and fine needles and moved to Petri boxes that contain 0.9% physiologic saltwater. The parasites were then taken out of the solution, examined under a light microscope, identified, and eventually stored in Eppendorf tubes containing 70% ethyl alcohol to be used in further research.

After being carefully cleaned by brushes, the acanthocephalans were placed directly in Alcohol, Formalin, Acetic acid (AFA) solution for identification. They were kept in this solution for 3–7 days, then, placed in 70% ethyl alcohol for long-term storage. Following the fixation procedure, parasite samples were placed in Semichon's acetocarmine staining media. At the end of the staining procedure, when the parasite bodies absorbed different densities of stain, their anatomic structures were observed in detail. The staining procedure was followed by dehydration using an alcohol series to dehydrate the parasites and crystallization for a better view of the internal tissue structures using xylol and lactophenol. A glass slide was thoroughly cleaned, and a drop of Canada balsam was placed in the center. The prepared material in the crystallization agent was picked up using a brush or quill and placed into the closing media, followed by a cover glass which was slid from one corner of the closing media, being careful not to leave air bubbles behind. The cover glass was pressed using a clamp so that the material would be evenly fixed and left at room temperature to dry. The type and stock number of the parasite were noted on one corner, and the locality and species of the host organism, the organ hosting the parasite, and the examination date were noted on the other corner of the dried preparation (Merdivenci, 1984; Williams and Jones, 1994).

The parasites were identified using the methods of Bauer (1987) and Ekingen (1983).

Parasite samples were taken from 70% alcohol and placed in Eppendorf tubes containing sterilized distilled water. The parasite remained in sterilized distilled water for 24 h to allow the alcohol to evaporate. Each one of the parasite samples was cut into pieces of 0.2 gr using sterile scissors. The parasite pieces were crushed in Eppendorf tubes using a sterile crushing apparatus and, after adding 2 ml of sterile distilled water, were centrifuged at 5000 rpm for 10 min. Following centrifugation, the water remaining on top of the Eppendorf tube was discarded and 20 μl of proteinase K and 180 μl of lysine buffer were added to the pellet remaining at the bottom of the Eppendorf tube, and all were mixed. These samples were incubated for 1 h at 56 °C in a shaking water bath, mixed for 15 s in a vortex, and 200 μl of lysine buffer was added and mixed in the vortex. After adding 200 μl of ethyl alcohol, the sample was vortexed again. The mixture was transferred to the spin column tube inside a mixture collection tube and centrifuged at 10000 rpm for 1 min. The lower tube containing the liquid was emptied and replaced under the spin column where 500 μl of wash buffer was added and centrifuged at 8000 rpm for 1 min. The liquid accumulating under the spin column was removed and 500 μl of wash buffer was added in the spin column and centrifuged at 12000 rpm for 3 min. The tube of liquid beneath the spin column was replaced with a new Eppendorf tube after which 200 μl of elution buffer was added into the spin column and incubated for 2 min at room temperature then centrifuged at 8000 rpm for 1 min. After placing in the spin column, the Eppendorf tube was stored at −20 °C.

The PCR mixture was prepared with a total volume of 50 μl containing 25 μl Master mix, 1 μl Forward Primer, 1 μl Reverse Primer, 1 μl DNA, and 22 μl dH_2O. A specific pair of primers for *N. rutili* was used from which 50 pmol and 1 μl template DNA were added from each of the pair of primers (BD1 GTCGTAACAAGGTTTCCGTA; BD2 TATGCTTAAATTCAGCGGGT) (Near et al., 1998; Král'ová-Hromadová et al., 2003; Goméz et al., 2002).

In PCR amplification, the pre-denaturation stage for 10 min at 94 °C was followed by a total of 35 PCR cycles, denaturation at 94 °C for 30 s, hybridization at 56 °C for 45 s, 1 min of DNA synthesis at 72 °C, and final elongation at 72 °C for 12 min. A total of 7 μl was collected from the DNA products amplified in PCR, mixed with 3 μl of blue-orange loading solution, and placed in previously prepared wells over 1.5% agarose gels with the last well saved for the DNA marker. After the gel was subjected to electrophoresis using Tris/Borate/EDTA buffer in 1.5% agarose gel at 80 V for 1.5 h, it was stained for 30 min with ethidium bromide. It was photographed with a polaroid camera system in a dark room using an ultraviolet trans-illuminator looking at the specific DNA bandwidth for *N. rutili*.

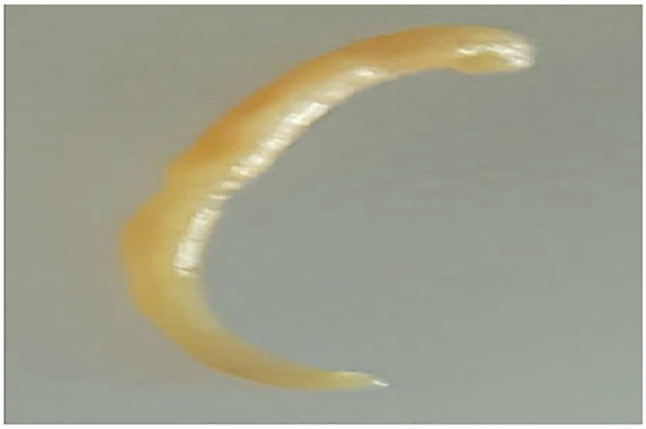

Fig. 2. Neoechinorhynchus rutile microscope view.

Fig. 3. Microscopic after staining N. rutile.

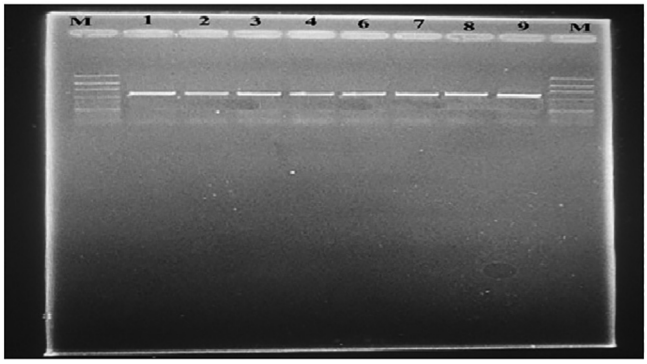

Fig. 4. Analysis of in PCR using primer pair BD1-BD2 of DNA sa.

3. Result and discussion

Menzelet Dam Lake is geographically positioned on the migration route of some birds.

In the parasitologic examination of fish caught in the area, 267 *Capoeta barroisi,* 1 *Cyprinus carpio*, and 2 *Barbus rajanorum* were found to be infested.

A total of 3 fish species were examined in this study, and 12,533 pieces of *N. rutili* were identified using morphology and staining methods (Figs. 2 and 3).

N. rutili was diagnosed in fish obtained from Menzelet Dam Lake. Isolates were used for DNA isolation and amplified using PCR. The resulting products were subjected to electrophoresis in 1.5% agarose gel. The gel was stained with ethidium bromide, and the results were analyzed with an ultraviolet transilluminator. All of the 120 samples exhibited BD1-BD2 primers specific to *N. rutili* species and 614–644 bp long bands (Fig. 4). Therefore, it was proven that all of the 120 isolates were *N. rutili.*

This research was primarily focused on diagnosing *N. rutili* using classic morphology and staining methods in the fish species caught in Menzelet Dam Lake in Kahramanmaras Province, Turkey. The suspected *N. rutili* samples were successfully confirmed using PCR.

A total of 267 *Capoeta barroisi,* 1 *Cyprinus carpio*, and 2 *Barbus rajanorum* caught in Menzelet Dam Lake were examined, and the *N. rutili* parasite was identified in the fish intestines. Diagnosis of *N. rutili* species was made according to methods of Hoffman (1967), Bykhovskaya-Pavlovskaya et al. (1964), Ekingen (1983), and Kennedy (1974).

In studies performed with freshwater fish, Türkmen (1990); Topçu (1993); Dörücü and İspir (2005); Sağlam and Sarıeyyüpoğlu (2002); Kır and Tekin Özan (2005); Dal (2006); Tekin Özan et al. (2006); Uzunay and Soylu (2006); Karabulut (2009); Karaman (2011); Barata (2012); Mujakić, (2014) reported that they identified the parasite *N. rutili.* The identification of *N. rutili* in this study also supports these previous studies with regard to the identification of the parasite in freshwater fish.

It is stated that the PCR technique is more advantageous than cultures and serological tests because of the ability to identify a few numbers of microorganisms in pure or mixed cultures in as short a period as one day (Lin and Tsen, 1996). Sarabeev et al. (2020), reported molecular identification of species *N. personatus* and *N. yamagutii* in *Gray mullets,* fish hunted in the Atlantic and Pacific Oceans. In their study, they used primers belonging to the genus Neoechinorhynchus and universal eukaryotic primers. Sequences were made for molecular identification based on *N. personatus* and *N. yamagutii* species. The diagnosis was facilitated by the use of a specific primer in PCR amplification. In this research, the use of a specific primer (BD1 GTCGTAACAAGGTTTCCGTA; BD2 TATGCTTAAATTCAGCGGGT) pair for *N. rutili* species prevented any wrong positive reactions of the parasite (Near et al., 1998; Král'ová-Hromadová et al., 2003; Goméz et al., 2002). The PCR technique was successfully applied with negative control at all stages to eliminate the possibility of contamination.

Mujakić (2014) made use of PCR in his research to identify Acanthocephala. Accordingly, molecular diagnosis has been made for *Echinorhynchus salmonis* and *N. rutili* species in the Gacka and Matica rivers, *Acanthocephalus lucii, Acanthocephalus anguillae, Pomphorhynchu laevis,* and *Pomphorhynchus tereticollis* in the Novantica River, and *Dentitruncu truttae* in the Una River. In this study, molecular identification was successfully performed using primers specific for *N rutili.*

While the life cycles of fish, the propagation of parasites, and the resulting economic losses have been reported years ago, there is still insufficient information on fish parasites in Turkey where mass deaths of fish related to acute parasitic diseases continue to occur (Güralp, 1981). Fish with chronic parasitic diseases also suffer excessive weakening and growth, leading to significant problems in marketing. The situation is further exacerbated by the money spent on curing these diseases and the increased feed costs resulting from the fish inadequately utilizing the food provided.

Parasites, by nature of their biological development, need other living organisms in part or all of their lives for survival. Many continue their life cycles on or inside fish during which time they continuously affect the metabolism and life functions of the fish host. Parasites living in the digestive tract disrupt the secretory function of the host causing diseases, sometimes killing the host. This study was performed to investigate the *N. rutili* parasite commonly encountered in the fish species of Kahramanmaras Province, Turkey in an effort to prevent parasitic diseases that lead to economic losses related to fishing.

4. Conclusion

The success of this study will hopefully lead to more original and extensive work aimed at molecular identification of parasitic agents found in fish in all regions of Turkey.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgements

This study is summary of the second author's master thesis. This work was presented as a oral presentation at 2nd International

Congress on Multidisciplinary Studies, 4th-5th May 2018, Adana-Turkey.

Ethical Approval

All animal studies were approved by the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (KSÜZİRHADYEK) and Research Institute (Protocol number: 2017/01).

References

Aksakal, E., Erdoğan, O., 2007. PCR-RFLD Uygulamalarının Su Ürünlerinde Kullanım Alanları. Türk Sucul Yaşam Dergisi 5, 747–753.

Alp, A., Büyükçapar, H.M., Eren, A., 2003. Menzelet Baraj Gölü (Kahramanmaraş) Balıkçılığı ve Ekonomik Olarak Avlanan Balık Türleri. Kahramanmaraş Sütçü İman Üniversitesi Fen ve Mühendislik Dergisi 6, 142–154.

Anonim, 1995. Karakaya Baraj Gölü Limnoloji Raporu, T.C. Bayındırlık ve İskan Bakanlığı D.S.İ. Genel Müdürlüğü 9. Böl. Müdürlüğü Su Ürünleri Baş Müh., Elazığ, p. 58

Arda, M., 1995. Biyoteknoloji, (Bazı Temel İlkeler), Kükem Derneği Bilimsel Yayınlar Ankara, No: 3. p. 432.

Arda, M., Seçer, S., Sarıeyyüpoğlu, M., 2005. Balık Hastalıkları. Medisan Yayınevi, Ankara, p. 230.

Arı, Ş., 1999. DNA'nın Polimeraz Zincir Reaksiyonu (PCR) İle Çoğaltılması, Moleküler Biyolojide Kullanılan Yöntemler. In: Temizkan, G., Arda, M., Nobel Tıp bKitabevleri (Eds.), İstanbul Üniversitesi Biyoteknoloji ve Genetik Mühendisliğ i Araştırma ve Uygulama Merkezi (BİYOGEM); Yayın No: 1, İstanbul, 57–68

Avşar, D., Özyurt, C., 1999. X. Seyhan Baraj Gölü Balıkçılığı. Ulusal Su Ürünleri Sempozyumu. Çukurova Üniversitesi Su Ürünleri Fakültesi, pp. 225–235.

Barata, S., 2012. Karakaya Baraj Gölü Kömürhan Bölgesinden Yakalanan Bazı Balıklarda Endohelmintlerin Araştırılması. Fırat Üniversitesi Fen Bilimleri Yüksek Lisans Tezi, p. 73.

Barber, I., Poulin, R., 2002. Interactions between fish, parasites and disease. In: Hart, P.J.B., Reynolds, J.D. (Eds.), The Handbook of Fish and Fisheries. Blackwell Science, Oxford, pp. 359–389.

Bauer, O.N., 1987. Key to the Parasites of Freshwater Fishes in the Fauna of the U.S.S.R. Leningrad, p. 583.

Bej, A.K., Mahbubani, M.H., Atlas, R.M., 1991. Amplification of nucleic acids by polymerase chain reaction (PZR) and other method and their amplications. Crit. Rev. Biochem. Mol. Biol. 26, 301–334.

Bykhovskaya-Pavlovskaya, I.E., Gussev, A.V., Dubinina, M.N., Izyumova, N.A., Smirnova, T.S., Sokolvskaya, I.L., Shtein, G.A., Shulman, S.S., Spshtein, V.M., 1964. Key to Parasites of Freshwater Fish of U.S.S.R. Israeli Program for Scientific Trans-lations. Jerusalem. p. 694

Coote, U.G., 1990. Amplification of Nucleic Acids by the Polymerase Chain Reaction. Article, 57–59.

Dal, A., 2006. Atatürk Baraj Gölü (Adıyaman)'nde Yetiştiriciliği Yapılan Gökkuşağı Alabalığı (Oncorhynchus mykiss)'nda Parazitolojik Araştırmalar. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, p. 60

Dick, T.A., Choudhury, A., 1995. Phylum Nematoda. In: Woo, P.T.K. (Ed.), Fish Diseases and Disorders, Chapter 11, Volume 1, Protozoan and Metazoan Infections, CAB International, Cambridge, UK. pp. 415–446.

Dörücü, M., İspir, Ü., 2005. Keban Baraj Göl'ünden Avlanabilen Balık Türlerinde İç Paraziter Hastalıkların İncelenmesi. Fırat Üniversitesi, Fen ve Mühendislik Bilimleri Dergisi, pp. 400–404.

Duman, E., Çelik, A., 2001. Atatürk Barajı Gölü Bozova Bölgesinde Avlanan Balıklar ve Verimlilikleri. Ege Üniversitesi Su Ürünleri Dergisi 18 (1–2), 65–69.

Ekingen, G., 1983. Tatlı Su Balık Parazitleri, Fırat Üniversitesi Su Ürünleri Yüksek Okulu F.Ü. Basımevi, Elâzığ. No:1 p. 253.

Geldiay, R., Balık, S., 1996. Türkiye Tatlısu Balıkları (Ders Kitabı), IV. Baskı, Ege Üniversitesi Su Ürünleri Fakültesi Yayınları No:46 Ders Kitabı Dizini No: 16, Ege Üniversitesi Ege Meslek Yüksekokulu Basımevi, Bornova/ İzmir, p. 532.

Goméz, A., Serra, M., Carvalho, G.R., Lunt, D.H., 2002. Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution 56 (7), 1431–1444.

Grabda, J., 1991. Marine Fish Parasitology: An Outline. Pwn-Polish Scientific Publishers, Warszawa, p. 306.

Güralp, N., 1981. Helmintoloji. Ankara Üniversitesi. Veteriner Fakültesi Yayınları. 368s.

Hoffman, G.L., 1967. Parasites of North American Freshwater Fishes. University of California Press, Berkeley and Los Angeles, p. 486.

Karabulut, C., 2009. Keban Baraj Gölü'nde Dört Farklı Bölgeden (Koçkale, Pertek, Çemişgezek, Keban) Avlanan Aynalı Sazan (Cyprinus carpio L., 1758)'da Endohelmintlerin Araştırılaması. Yüksek Lisans Tezi, Fırat Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı, Elazığ, p. 29.

Karaman, Z., 2011. Kalecik Baraj Gölü (Karakoçan-Elazığ)'nde Avlanılabilen Balıklarda Endohelmintlerin Araştırılması. Fırat Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, p. 66.

Kennedy, C.R., 1974. A. checklist of British and Irish freshwater fish parasites with notes on their distribution. J. Fish Biol. 6, 613–644.

Kır, İ., Tekin Özan, S., 2005. Işıklı Baraj Gölü (Denizli)'nde Yaşayan Turna Balığı (*Esox lucius* L., 1758)'nın Endoparazitleri, Mevsimsel Dağılımları ve Etkileri. Türkiye Parazitoloji Dergisi 29 (4), 291–294.

Král'ová-Hromadová, I., Tietz, D.F., Shinn, A.P., Špakulová, M., 2003. ITS rDNA Sequences of *Pomphorhynchus laevis* (Zoega in Müller, 1776) and P. lucyi Williams and Rogers, 1984 (Acanthocephala: Palaeacanthocephala. Syst. Parasitol. 56, 141–145.

Lin, C.K., Tsen, H.Y., 1996. Use of two 16S DNA targeted oligonucleotides as PZR primers for the specific detection of salmonella in foods. J. Appl. Bacteriol. 80, 659–666.

Marx, J.L., 1988. Multiplying genes by leaps and bounds. Science 240, 1408–1410.

Merdivenci, A., 1984. Klinik Parazitoloji, Beta Basım Yayın Dağıtım A.Ş. Osman Aytaç Matbaası, İstanbul, p. 399.

Mujakić, I., 2014. Molekularna Karakterızacıja Kukaša (Acanthocephala) Iz Crıjeva Slatkovodnıh Rıba. Sveučilište u Zagrebu Prirodoslovno-Matematički Fakultet Biološki Odsjek. Graduation Thesis, p. 70.

Mullis, K.B., 1990. The unusual origin of the polymerase chain reaction. Sci. Am. 262 (4), 56–65.

Near, T.J., Garey, J.R., Nadler, S.A., 1998. Phylogenetic relationships of the acanthocephala inferred from 18S ribosomal DNA sequences. Mol. Phylogenet. Evolut. 10 (3), 287–298.

Pala, M., 1996. Keban Baraj Gölü Ova Bölgesi'nde Kullanılan Balık Yakalama Aletlerinin Yapısı ve Av Verimlilikleri. Fırat Üniversitesi Fen Bilimleri Yüksek Lisans Tezi, p. 69.

Quirke, P., 1992. Molecular Biology and Infections of the Gut. Gut 33, 1441–1443.

Sağlam, N., Sarıeyyüpoğlu, M., 2002. *Capoeta trutta* Balığında Rastlanan *Neoechinorhynchus rutili* (Acanthocephala)'nin İncelenmesi. Türkiye Parazitoloji Dergisi 26, 329–331.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239, 487–491.

Sarabeev, V., Tkach, Ie, Sueiro, R.A., Leiro, J., 2020. Molecular data confirm the speciєs status of *Neoechinorhynchus personatus* and *N. yamagutii* (Acanthocephala, Neoechinorhynchidae) from the atlantıc and pacific grey mullets (Teleosteı, Mugilidae). Zoodiversity 54 (1), 1–10.

Sheridon, V.M., Pratt, I., 1964. The life history of *Neoechinorhynchus rutili* and its development in the intermediate host (Acanthocephala: Neoechinorhynchidae). J. Parasitol. 50 (3), 394–400.

Taylor, G.R. 1993. Polymerase chain reaction: basic principles and automation. In: McPherson, M.J., Quirke, P., Taylor, G.R. (Eds.), PZR: A Pratical Approach. Oxford University Press, Oxford, pp. 1–14.

Tekin Özan, S., Kır, İ., Ayvaz, Y., Barlas, M., 2006. Beyşehir Gölü Kadife Balığı (*Tinca tinca* L., 1758)'nın Parazitleri Üzerine Bir Araştırma. Türkiye Parazitoloji Dergisi 30 (4), 333–338.

Tınar, R., 2006. Helmintoloji, Nobel Yayın No: 965 Fen ve Biyoloji Yayınları Dizisi: 48, Nobel Yayın Dağıtım, 1. Basım, Ankara, p. 588.

Topçu, A., 1993. Van Yöresinde Bulunan Sazan (Cyprinus carpio L., 1758)'ların Sindirim Kanalı Helmintleri. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı, Doktora Tezi, p. 59.

TÜİK, 2018. Türkiye İstatistik Kurumu. Su Ürünleri İstatistikleri. https://www.tarimorman.gov.tr/sgb/Belgeler/SagMenuVeriler/BSGM.pdf.

Türkmen, H., 1990. İznik Gölü'ndeki Sazan (Cyprinus carpio L., 1758) ve Akbalıkların (Rutilis frisii Nordmann, 1840) Sindirim Kanalı Helmintleri. İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, Parazitoloji Anabilim Dalı, Doktora Tezi, p. 60.

Uzunay, E., Soylu, E., 2006. Sapanca Gölü'nde Yaşayan Sazan (*Cyprinus carpio* Linnaeus, 1758) ve Karabalık (*Vimba vimba* Linnaeus, 1758)'ın Metazoan Parazitleri. Türkiye Parazitoloji Dergisi 30 (2), 141–150.

Williams, H., Jones, A., 1994. Parasitic Worm of Fish. TaylorFrancis Ltd, London, p. 584.

ZMO, 2017. Su Ürünleri Raporu. URL (erişim tarihi: 27.01.2019). http://www.zmo.org.tr/genel/bizden_detay.php?kod=27302andtipi=17andsube=0.