AQUATIC SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

2019

VOLUME: 34 ISSUE: 2

E-ISSN 2602-473X

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Publisher

İSTANBUL UNIVERSITY PRESS

for submussion instructions, subcription and all other information visit http://dergipark.org.tr/ase

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Aims and Scope

Aquatic Sciences and Engineering is an international, scientific, open access periodical published in accordance with indepen¬dent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University Faculty of Aquatic Sciences and it is published quarterly on January, April, July, and October. The publication language of the journal is English and continues publication since 1987.

Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

The scope of the journal includes but not limited to; aquaculture science, aquaculture diseases, feeds, and genetics, ecological interactions, sustainable systems, fisheries development, fisheries science, fishery hydrography, aquatic ecosystem, fisheries management, fishery biology, wild fisheries, ocean fisheries, biology, taxonomy, stock identification, functional morphology freshwater, brackish and marine environment, marine biology, water conservation and sustainability, inland waters protection and management, seafood technology and safety.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of aquatic sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Aquatic Sciences and Engineering is indexed in Clarivate Analytics Web of Science Emerging Sources Citation Index (ESCI), Clarivate Analytics Zoological Record, Biological Abstracts, BIOSIS Previews, TUBITAK ULAKBIM TR Index and CAB Abstracts.

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at https://dergipark.org.tr/ase. The journal guidelines, technical information, and the required forms are available on the journal's web page.

All expenses of the journal are covered by the İstanbul University Faculty of Aquatic Sciences. Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the İstanbul University Faculty of Aquatic Sciences, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

All published content is available online, free of charge at https://dergipark.org.tr/ase. Printed copies of the journal are distributed to the members of the İstanbul University Faculty of Aquatic Sciences, free of charge.

Editor in Chief: Prof. Devrim MEMİŞ
Address: İstanbul Üniversitesi Su Bilimleri Fakültesi Yetiştiricilik Anabilim Dalı Ordu Cad. No:8 34134 Laleli / İstanbul, Türkiye
Phone: +90 212 4555700/16448
Fax: +90 212 5140379
E-mail: mdevrim@istanbul.edu.tr

AQUATIC
SCIENCES and ENGINEERING

Contents/İçindekiler

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2019; 34(2): 39-45 • DOI: https://doi.org/10.26650/ASE2019545494

Research Article

Kyphosis in *Barbus pergamonensis* (Cyprinidae-Actinopterygii) from Dalaman Stream Flowing to the Mediterranean Sea

Deniz Innal[1*], Evsen Yavuz Guzel[2], Omer Gürkan Dilek[3], Seyit Ali Kamanli[1]

Cite this article as: Innal, D., Yavuz-Guzel, E., Dilek, O. G., & Kamanli, S. A. (2019). Kyphosis in Barbus pergamonensis (Cyprinidae-Actinopterygii) from Dalaman Stream Flowing to the Mediterranean Sea. *Aquatic Sciences and Engineering, 34*(2), 39–45.

ABSTRACT

Skeletal deformities are relatively well described in both cultured and wild fish. These diseases are observed in many fish species and occur due to environmental and genetic factors. It negatively affects the biological performance and commercial value of fish. For this reason, this study attempts to quantify and identify kyphosis in the natural populations of *Barbus pergamonensis* Karaman, 1971 collected from Dalaman Stream (Gölhisar/Burdur) and tends to find a possible relationship between these anomalies and several types of pollutants present in the environment. The current study found that the environmental pollutants may represent a potential risk to induce kyphosis in the natural populations of *B. pergamonensis*.

Keywords: Bergama barbel, pollution, skeletal deformities, pharmaceutical, pesticide

ORCID IDs of the authors:
D.I. 0000-0002-1686-0959;
E.Y.G. 0000-0002-8029-9254;
O.G.D. 000-0002-5717-3928;
S.A.K. 0000-0002-9118-4591

[1]Burdur Mehmet Akif Ersoy University, Department of Biology, Burdur, Turkey
[2]Çukurova University, Fisheries Faculty, Basic Sciences Department, Balcalı-Adana, Turkey
[3]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Anatomy, Burdur, Turkey

Submitted:
27.03.2019

Revision Requested
26.04.2019

Last Revision Received
06.05.2019

Accepted:
12.05.2019

Online published:
24.05.2019

Correspondence:
Deniz Innal
E-mail:
innald@yahoo.com

INTRODUCTION

It is widely known that many anatomical diseases occur in fish due to the environmental and genetic factors (Jawad & Ibrahim, 2018). In recent decades, fishery biologists have become more interested in skeletal deformity diseases of wild and reared fish (Kužir et al., 2015). Many types of deformities (Afonso et al., 2000; Sato, 2006; Jawad et al., 2017; Jawad & Ibrahim, 2018) have been reported from the variety of fish species living in the different habitats (Iwasaki et al., 2018). It was reported that these deformities affect mostly the ribs, fins, cranium and vertebral column of fish (De La Cruz-Aguero & Perezgomez-Alvarez, 2001). These diseases have negative effects on the biological performance of fish as well as decreasing the commercial value (Raja et al., 2016; Majeed et al., 2018). In wild fish, skeletal deformities that occur in any life stage of fish can cause them difficulties such as defending their territory (Sato, 2006; Majeed et al., 2018), competing for a mate (Sato, 2006), and decreasing production performance (Noble et al., 2012). In farmed fish, these injuries can affect the organism by reducing growth (Hansen et al., 2010), limiting their feeding ability (López-Olmeda et al., 2012; Okamura et al., 2018), increasing the infection (Janakiram et al., 2018) and mortality rate (Jara et al., 2017). Moreover, these negative effects of the skeletal deformities inevitably cause reasonable economic loss in the fish farms (Boglione, 2013a; Yıldırım et al., 2014).

There are a variety of reasons for the occurrence of the skeletal deformities in fish. These are mostly known as scolosis, spondylolisthesis, lordosis (dorsal curvature, ˅ shape), and kyphosis (ventral curvature, ^ shape (Yıldırım et al., 2014; Jawad et al., 2017). Although there are many reports of lordosis (Afonso et al., 2000; Fjelldal et al.,2009; Gorman et al., 2010), the rate of occurrence of kyphosis is less common than lordosis (Jawad et al., 2017). Like lordosis, kyphosis can also appear in both pre-haemal and haemal positions (Boglione et al., 2013a). All these deformities are linked to genetic and epigenetic factors (Nguyen et al., 2016). Some environmental parameters, such as salinity, sudden changes of water temperature and turbulence, oxygen content, organic compounds, radiation, heavy metals, industrial effluents, stress, food deficiency, parasitism (Dionísio et al., 2012; Fridman et al., 2012; Kolarevic et al., 2013) and pollution are among the most considerable epigenetic factors (Sfakianakis et al., 2006). In addition, pollution is considered as a useful index for evaluation of the frequency of deformities in fish (Boglione et al., 2013b).

Bergama barbel, *Barbus pergamonensis* Karaman, 1971, belonging the family Cyprinidae is a freshwater fish species distributed in the fresh waters of Turkey and Greece (Kottelat & Freyhof, 2007). The distribution areas of this fish in Turkey are reported to be from the Aegean drainages, the Bakır and Menderes Rivers and the drainages of Muğla (Kottelat & Freyhof, 2007). *Barbus pergamonensis* was reported as being heavily affected by agricultural activities, domestic and industrial pollution from the industry (Freyhof & Kottelat, 2018). Therefore, the aim of this study is to identify and quantify the skeletal deformities of *B. pergamonensis* collected from Dalaman Stream (Gölhisar/Burdur/Turkey) as well as finding a possible relationship between the deformities of this species and several types of pollutants that occur in this region.

MATERIAL AND METHODS

Specimen collection

The individuals of *B. pergamonensis* ranging from 3.5 cm to 20 cm were collected from the Dalaman Stream from three different stations [1-) 37°13’36.53”N, 29°32’57.66”E; 2-) 37°14’39.39”N, 29°31’44.33”E; 3-)37°15’10.14”N, 29°30’09.29”E, Gölhisar/Burdur/ Turkey] between June 2015 and June 2016. In total, 54 individuals were caught after seven sampling events carried out using electrofishing. All fish samples used in the current study were anaesthetized with MS-222. Fish samples were fixed in 4% formaldehyde solution and then deposited in 70% ethanol in the fish collection of the Ichthyology Laboratory at the Department of Biology, Burdur Mehmet Akif Ersoy University. Specimens were measured to the nearest 0.1 cm for standard length (SL) and weighed to the nearest 0.001 g for body weight.

X-ray treatment

In order to determine skeletal deformities of specimens, the fresh fish samples were individually x-rayed using a Fujifilm FCR Prima T®-Tokyo, Japan at Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Clinics. Specimens were x-rayed at the exposure time 100 kVp, 30 mAs, 3 second from the lateral positions of each sample to demonstrate the exact nature of the deformities.

Non-metallic inorganic parameters, dissolved metals, pharmaceutical and pesticide analysis in water samples

Many chemical parameters of Dalaman Stream were also analysed in order to determine the relationship between the pollution and kyphosis occurring in fish in the studied area. These includes non-metallic parameters, dissolved metals, pharmaceutical compounds from variety of drug groups and pesticides. Non-metallic inorganic parameters and dissolved metals shown in Table 1 were analysed in ALS Laboratory in Czechia. All methods used for the analysis of non-metallic inorganic parameters and dissolved metals/ major cations were shown in Table 1. Solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) were used for the pesticide and pharmaceutical analyses.

For pharmaceutical analysis, 1 L surface water sample was filtered using glass fibre filters (GF/F, Whatman). After filtration, samples were adjusted to pH 2 with H_2SO_4 (98wt %). Then, solid phase extraction was conducted for pharmaceutical analysis using the method of Guzel et al. (2018). First, internal standard diazepam-d5 were added to the water samples. Then, Oasis HLB cartridges (500 mg, 6 cc) were conditioned with 5 ml dichloromethane (DCM), 5 ml methyl tert-butyl ether (MTBE), 5 ml methanol (MeOH) and 5 ml ultrapure water. Water samples were then added to the SPE cartridge at a flow rate of 15 ml/min and it was washed with 5 ml ultrapure water. Next, sample loading cartridges were dried under vacuum for 1 hour. Eluent was taken with 5 ml of MeOH and 5 ml 10:90 (v/v) MeOH/MTBE. The eluent was dried with nitrogen and then dissolved with 1 ml MeOH. Extracted samples were analysed by using LC/MS-MS.

Solid phase extraction for pesticide analysis was made by using modified method of Hladik et al. (2008). Internal standard dimethoate-d6 were added to the 1 L water samples. Oasis HLB cartridges (500 mg, 6 cc) were conditioned with 10 mL ethyl acetate, 10 mL MeOH and 5 mL of ultrapure water. Water samples added to the SPE cartridge at a flow rate of 10 ml/min. Then, samples were loaded to cartridges and it was dried under vacuum for 1 hour. Eluent was taken with 12 ml of ethyl acetate. The eluent was dried with nitrogen and then dissolved with 1 ml MeOH. Extracted samples were analysed by using LC/MS-MS.

Instrumental analysis was performed with Shimadzu CBM-20A ultra flow liquid chromatography, Shimadzu SIL-20A/HT auto sampler system and Shimadzu 8040 mass spectrometry. Pharmaceutical compounds were separated using pentafluorophenylpropyl (PFPP) column (Allure 50x2.150 mm, 5µm, Restek, Bellefonte, PA, USA) and pesticide compounds were separated by using Shimpack Column, FC-ODS 150X2.0 mm, Kyoto, Japan. Pharmaceutical and pesticide compounds were detected with multiple reactions monitoring (MRM) mode. Total run time for pharmaceutical method and pesticide method were 18 and 20 minutes respectively.

RESULTS AND DISCUSSION

The skelatal deformities of the fish samples

The spinal curvature (kyphosis) was found in two (3.7%; A and B in Figures 1, 2) out of 54 adult fish sampled from three different stations in Dalaman Stream. The Standard length (SL) of these two samples were measured as 12.3 cm (A) and 12.1 cm (B) respectively (Fig. 1 and 2). In fish A, the vertebral anomaly was found between 17 and 47 vertebrae. Whereas the spinal anomaly was involved between 16 and 32 vertebrae in fish B. Although kyphosis in the caudal vertebrae of both samples were diagnosed, no external lesions were observed on these body of deformed specimens.

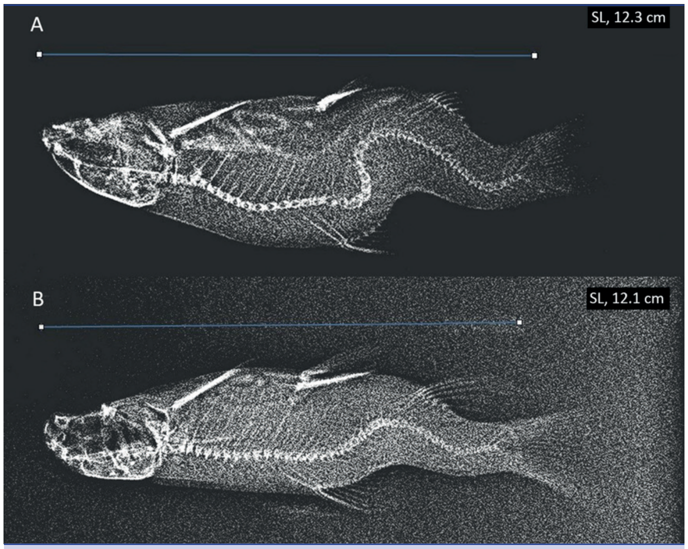

Figure 1. Radiological images of two *Barbus pergamonensis* samples diagnosed with kyphosis; (A) 12.3 cm SL; (B) 12.1 cm SL.

Figure 2. Photography images of two *Barbus pergamonensis* samples diagnosed with kyphosis; (A) 12.3 cm SL; (B) 12.1 cm SL.

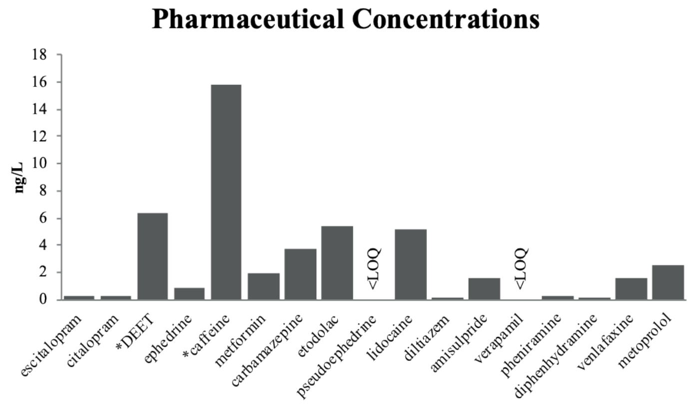

Figure 3. Pharmaceutical concentrations of water samples (DEET and caffeine concentrations reduced 10 times, <LOQ: Lower than quantitation limit).

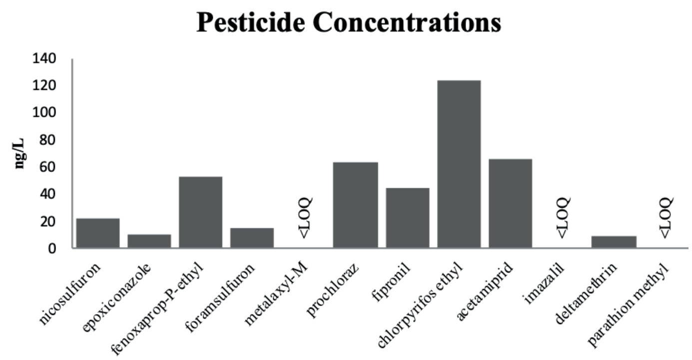

Figure 4. Pesticide concentrations of water samples (<LOQ: Lower than quantitation limit).

Determining the chemicals, pharmaceutical and pesticide in water samples

Non-metallic inorganic parameters and dissolved metals are given in Table 1.

In the current study, 10 out of 14 non-metallic parameters and 11 out of 26 dissolved metals were found higher than normal values (see Table 1). 156 pharmaceutical compounds from variety of drug groups including painkillers (analgesics, anti-inflammatories and antipyretics), antibiotics, andantibacterials, cardiovascular drugs (ß- blockers), hipolipidemics, central nervous system drugs, stimulants and illicit drugs were investigated in water samples**.** 17 of these pharmaceuticals (escitalopram, citalopram, DEET, ephedrine, caffeine, metformin, carbamazepine, etodolac, pseudoephedrine, lidocaine, diltiazem, amisulpride, verapamil, pheniramine, diphenhydramine, venlafaxine, metoprolol) were detected in ng/L values (Fig. 3).

In addition, 144 pesticides that include herbicides, insecticides, acaricides, fungicides were analysed and 12 of them (nicosulfuron, epoxiconazole, fenoxaprop-P-ethyl, foramsulfuron, metalaxyl-M, prochloraz, fipronil, chlorpyrifos ethyl, acetamiprid, imazalil, deltamethrin, parathion methyl) were detected in the water samples (Fig. 4).

The results of non-metallic inorganic parameters were given in Table 1. Ammonia and ammonium ions as N, ammonia and ammonium ions as NH4 and nitrite as N were found lower than method detection limits. According to Turkey Surface Water Quality Regulations, nitrates and total kjeldahl nitrogen as N concentrations were found as 2nd grade water quality (less

Table 1. Non-metallic and dissolved metals inorganic parameters

No	Parameter	Limit of reporting (mg/l) (LOQ)	Method	Result (mg/l)
		Nonmetallic Inorganic Parameters		
1	Ammonia and ammonium ions as N	0.040	W-NH4-SPC	<LOQ
2	Ammonia and ammonium ions as NH4	0.050	W-NH4-SPC	<LOQ
3	Chloride	1.00	W-CL-IC	568
4	Inorganic Nitrogen as N	0.500	W-NING-SPC	0.876
5	Nitrates	0.27	W-NO3-SPC	3.88
6	Nitrite + Nitrate as N	0.060	W-NNO-SPC	0.876
7	Nitrites	0.0050	W-NO2-SPC	<LOQ
8	Organic Nitrogen as N	0.50	W-NORG-SPC	0.70
9	Orthophosphate	0.040	W-PO4O-SPC	0.049
10	Total Kjeldahl Nitrogen as N	0.50	W-NKJ-PHO	0.70
11	Total Nitrogen as N	1.0	W-NTOT-CC	1.6
12	Nitrate as N	0.060	W-NO3-SPC	0.876
13	Nitrite as N	0.0020	W-NO2-SPC	<LOQ
14	Orthophosphate as P	0.010	W-PO4O-SPC	0.016
		Dissolved Metals / Major Cations		
1	Aluminium	0.010	W-METAXFL1	<LOQ
2	Antimony	0.010	W-METAXFL1	<LOQ
3	Arsenic	0.0050	W-METAXFL1	<LOQ
4	Barium	0.00050	W-METAXFL1	0.0243
5	Beryllium	0.00020	W-METAXFL1	<LOQ
6	Boron	0.010	W-METAXFL1	0.016
7	Cadmium	0.00040	W-METAXFL1	<LOQ
8	Calcium	0.0050	W-METAXFL1	42.6
9	Chromium	0.0010	W-METAXFL1	0.0019
10	Cobalt	0.0020	W-METAXFL1	<LOQ
11	Copper	0.0010	W-METAXFL1	<LOQ
12	Iron	0.0020	W-METAXFL1	<LOQ
13	Lead	0.0050	W-METAXFL1	<LOQ
14	Lithium	0.0010	W-METAXFL1	0.0044
15	Magnesium	0.0030	W-METAXFL1	31.9
16	Manganese	0.00050	W-METAXFL1	<LOQ
17	Molybdenum	0.0020	W-METAXFL1	<LOQ
18	Nickel	0.0020	W-METAXFL1	0.0039
19	Phosphorus	0.010	W-METAXFL1	0.025
20	Potassium	0.015	W-METAXFL1	1.43
21	Selenium	0.010	W-METAXFL1	<LOQ
22	Silver	0.0010	W-METAXFL1	<LOQ
23	Sodium	0.030	W-METAXFL1	6.66
24	Thallium	0.010	W-METAXFL1	<LOQ
25	Vanadium	0.0010	W-METAXFL1	0.0022
26	Zinc	0.0020	W-METAXFL1	<LOQ

contaminated water) (Anonymous, 2016). And total nitrogen as N and orthophosphate as P concentrations were found as 1st grade water quality (high quality water). But for the ECE (Economic Commission for Europe) Standard Statistical Classification of Surface Freshwater Quality for the Maintenance of Aquatic Life total nitrogen as N was found as class IV (UNECE, 1994)

Twenty-six metals and major cations were analysed in water samples (Table 1). Most of the detected metals and major cations were lower than the quantification limits. According to ECE Standard Statistical Classification of Surface Freshwater Quality for the Maintenance of Aquatic Life only chromium was classified as class II, the other quantified metals were classified as class I.

To the best of our knowledge, a few biological studies (Gaygusuz et al., 2013; Erk'akan et al., 2014) have been conducted on this species (Freyhof & Kottelat, 2018). Kyphosis which is a skeletal deformity of fish was studied by many researchers (Hansen et al., 2010; Boglione et al., 2013a; Hayes et al, 2013; Jawad & Ibrahim, 2018). In addition, there are some reports on kyphosis in cyprinid species from different regions (Bogutskaya et al., 2011). The adverse effects of skeletal deformities on the fish health and economy were also reported in detail in the previous studies (Yıldırım et al., 2014; Nguyen et al., 2016). In recent years, although the number of studies on wild and cultured fish health have been increasing in Turkey, there are limited studies on the skeletal deformities of fish such as cultured sharpsnout seabread *Diplodus puntazzo* (Yıldırım et al., 2014) and silverside *Atherina boyeri* (Jawad et al., 2017). In the current study, kyphosis was detected in the *Barbus pergamonensis* population of Dalaman Stream. It is the first study that kyphosis was detected in the wild population of this fish. In addition, there is no kyphosis report from other fish species from Dalaman Stream.

Previous reports discussed the reasons of skeletal deformities which are genetic and environmental factors (Quigley, 1995, Divanach et al., 1996; Divanach et al., 1997; Sfakianakis et al. 2006; Harris et al., 2014). Micropollutants in the aquatic habitats is one of the major issues worldwide (Eggen et al., 2014). These micropollutants have based on the anthropogenic effects, such as pharmaceuticals, pesticides, personal care products and industrial chemicals (Luo et al., 2014). It is known that all these micropollutants have toxic effects on aquatic species (Morasch et al., 2010; Pochodylo & Helbling, 2015; Minguez et al., 2016). Aquatic communities can be dramatically affected by the cocktail of low concentrations of micropollutants (Morash et al., 2010). The effects of cocktail micropollutants can be much higher than the effect of a single micropollutant.

In the current study, the relationship between *B. pergamonensis* and anthropogenic factors occurred in the habitat of this species were emphasised. The pollution effecting the aquatic environment has also been increasing in Turkey with the rising industrialisation and other reasons (Deniz Innal Pers. Obs.). In the study area of the current research, these reasons can be referred for many reasons. First, the studied area has been used as a cultivated area for the production of carrot, sugar beet, maize, green beans and wheat. In order to maintain the agricultural activities in the region, the local people and industries are using variety of chemicals (Deniz Innal Pers. Obs.). Another reason for the pollution can be the treatment facilities in the area. It was observed that the discharge of a treatment facility has been performing via Dalaman Stream. Furthermore, it was detected that the waste from the marble factory and other mining facilities have been carried to this aquatic system. The road construction on the highway between Gölhisar and Çavdır changed the structure of Dalaman Stream. Moreover, the construction of a reservoir on the system for the aim of irrigation has also adverse effect on this problem. The last two reasons caused the decrease of water flow of Dalaman Stream.

In this study, pollution parameters in the studied area have been detected in detail. In total, 17 pharmaceutical and 12 pesticide concentrations were found over the threshold values. Caffeine as a pharmaceutical concentration was detected at the highest concentration (158.51 ng / L). Caffeine concentration is newly used as an anthropogenic effect indicator. Because caffeine is used only by humans (Sauvé et al., 2012). Similarly, in some studies, the concentration of caffeine was found to be in the highest concentrations (Yu & Cao, 2016; Guzel et al., 2018). When considered the caffeine level found in the current study, it can be supposed that the anthropogenic effect plays a main role for this reason. Whereas the highest concentrated pesticide was detected as chlorpyrifos ethyl (123.40 ng/L). One of the most frequently detected compounds was the specific metabolite of chlorpyrifos in wastewaters which indirectly and/or directly reaching to surface waters (Gracia-Lor et al., 2017). These pesticides are sold from different companies which is mainly used to eradicate several pests including worms and insects. Pesticide usage is highly toxic for the non-target organisms such as fish (Ogunfowokan, et al., 2012; Qu, et al., 2011). Ecotoxicological studies show that such pollutants can cause undesirable effects on fish even at very low concentrations (Faggiano et al., 2010; Ogunfowokan et al., 2012). These kind of detrimental effects of kyphosis can result in death of fish species through impaired metabolism. In the study, the detected pharmaceutical, pesticide and inorganic compound concentrations were low but there may be negative effects on the living organisms when all the substances are present at the same time in the water.

CONCLUSIONS

Environmental pollutants may represent a potential risk to induce spinal deformities in natural populations of *B. pergamonensis*. As a result, the incresing pollution and the detrimental chemical contents releasing from the differet systems to Dalaman Stream should be firmly decreased in order to prevent the health problems of the aquatic organism living in the region. Furthermore, the emergent skelatal deformities of *B. pergamonensis* and potentially other fish species living in the region seem to be a serious problem for the future of aquatic organisms in Dalaman Stream. Consequently, the further studies should focus on the prevention of these deformities and the main reasons of these health problems in detail.

Acknowledgements: We acknowledge the two anonymous reviewers for their time, considerations and comments which improved this paper.

Ethics Committee Approval: This study was carried out in accordance with animal welfare and the ethics of trial. All procedures were performed in accordance Law on Veterinary and Medical Activities and National Animal Welfare Act. Therefore ethical approval was not required.

Conflict of Interests: Authors declare that there is no conflict of interest.

REFERENCES

Afonso, J. M., Montero, D., Robaina, L., Astorga, N., Izquierdo, M. S. & Gińes, R. (2000). Association of a lordosis-scoliosis-kyphosis deformity in gilthead seabream (*Sparus aurata*) with family structure. *Fish Physiology and Biochemistry* 22, 159-163. [CrossRef]

Anonymous. (2016). Yüzeysel su kalitesi yönetimi yönetmeliğinde değişiklik yapilmasina dair yönetmelik, http://www.resmigazete.gov.tr/eskiler/2015/04/20150415-18.htm (Access Date: 2 May 2019).

Boglione, C., Gavaia, P., Koumoundouros, G., Gisbert, E., Moren, M., Fontagné, S. & Witten, P. E. (2013a). Skeletal anomalies in reared European fish larvae and juveniles. Part 1: Normal and anomalous skeletogenic processes. *Reviews in Aquaculture* 5, 99-120. [CrossRef]

Boglione, C., Gisbert, E., Gavaia, P., Witten, P. E., Moren, M., Fontagné, S. & Koumoundouros, G. (2013b). Skeletal anomalies in reared European fish larvae and juveniles. Part 2: main typologies, occurrences and causative factors. *Reviews in Aquaculture* 5, 121-167. [CrossRef]

Bogutskaya, N. G., Zuykov, M. A., Naseka, A. M. & Anderson, E. B. (2011). Normal axial skeleton structure in common roach *Rutilus rutilus* (Actinopterygii: Cyprinidae) and malformations due to radiation contamination in the area of the Mayak (Chelyabinsk Province, Russia) nuclear plant. *Journal of Fish Biology* 79, 991-1016. [CrossRef]

De La Cruz-Aguero, J. & Perezgomez-Alvarez, L. (2001). Lordosis in topsmelt *Atherinops affinis* (Ayers, 1860) (Teleostei: Atherinopsidae). *Revista De Biologia Marina Y Oceanografia* 36(1), 109-110. [CrossRef]

Dionísio, G., Campos, C., Valente, L. M. P., Conceição, L. E. C., Cancela, M. L. and Gavaia, P. J. (2012). Effect of egg incubation temperature on the occurrence of skeletal deformities in *Solea senegalensis*. *Journal of Applied Ichthyology* 28, 471-476. [CrossRef]

Divanach, P., Boglione, C., Menu, M., Koumoundouros, G., Kentouri, M. & Cataudella, S. (1996). Abnormalities in finfish mariculture: an overview of the problem, causes and solutions. Sea-bass and Sea Bream Culture: Problems and Prospects, Verona, Italy, October 16-18. *European Aquaculture Society, Oostende, Belgium* 45-66.

Divanach, P., Papandroulakis, N., Anastasiadis, P., Koumoundouros, G. & Kentouri, M. (1997). Effect of water currents on the development of skeletal deformities in sea bass (*Dicentrarchus labrax* L.) with functional swimbladder during postlarval and nursery phase. *Aquaculture* 156, 145-155. [CrossRef]

Eggen, R. I., Hollender, J., Joss, A., Schärer, M. & Stamm, C. (2014). Reducing the Discharge of Micropollutants in the Aquatic Environment: The Benefits of Upgrading Wastewater Treatment Plants. *Environmental Science & Technology* 48, 7683-7689. [CrossRef]

Erk'akan, F., Innal, D. & Özdemir, F. (2014). Length–weight relationships for five Cyprinid species in Turkey. *Journal of Applied Ichthyology* 30, 212-213. [CrossRef]

Faggiano, L., De Zwart, D., García-Berthou, E., Lek, S. & Gevrey, M. (2010). Patterning ecological risk of pesticide contamination at the river basin scale. *Science of the Total Environment* 408, 2319-2326. [CrossRef]

Fjelldal, P. G., Hansen, T., Breck, O., Sandvik R., Waagbø, R., Berg, A. & Ørnsrud, R. (2009). Supplementation of dietary minerals during the early seawater phase increases vertebral strength and reduce the prevalence of vertebral deformities in fast-growing under-yearling Atlantic salmon (*Salmo salar* L.) smolt. *Aquaculture Nutrition* 15, 366-378. [CrossRef]

Freyhof, J. & Kottelat, M. (2018). *Barbus pergamonensis*. The IUCN Red List of Threatened Species; Downloaded on 30 November 2018.

Fridman, S., Bron, J. E. & Rana, K. J. (2012). Ontogenic changes in the osmoregulatory capacity of the *Nile tilapia Oreochromis niloticus* and implications for aquaculture. *Aquaculture* 356-357, 243-249. [CrossRef]

Gaygusuz, Ö., Aydın, H., Emiroğlu, Ö., Top, N., Dorak, Z., Gaygusuz, Ç. G., Başkurt S. & Tarkan, A. S. (2013). Length–weight relationships of freshwater fishes from the western part of Anatolia, Turkey. *Journal of Applied Ichthyology* 29(1), 285-287. [CrossRef]

Gracia-Lor, E., Castiglioni, S., Bade, R., Been, F., Castrignanò, E., Covaci, A., González-Mariño, I., Hapeshi, E., Kasprzyk-Hordern, B., Kinyua, J., Lai, F. Y., Letzel, T., Lopardo, L., Meyer, M. R., O'brien, J., Ramin, P., Rousis, N. I., Rydevik, A., Ryu, Y., Santos, M. M., Senta, I., Thomaidis, N. S., Veloutsou, S., Yang, Z., Zuccato, E. & Bijlsma, L. (2017). Measuring biomarkers in wastewater as a new source of epidemiological information: Current state and future perspectives. *Environment International* 99, 131-150. [CrossRef]

Guzel, E. Y., Cevik, F. & Daglioglu, N. (2018). Determination of pharmaceutical active compounds in Ceyhan River, Turkey: Seasonal, spatial variations and environmental risk assessment. *Human and Ecological Risk Assessment*. [CrossRef]

Hansen, T., Fjelldal, P., Yurtseva, A. & Berg, A. (2010). Possible relation between growth and number of deformed vertebrae in Atlantic salmon (*Salmo salar* L.). *Journal of Applied Ichthyology* 26, 355-359. [CrossRef]

Harris, M. P., Henke, K., Hawkins, M. B. & Witten, P. E. (2014). Fish is fish: the use of experimental model species to reveal causes of skeletal diversity in evolution and disease. *Journal of Applied Ichthyology* 30, 616-629. [CrossRef]

Hayes, A. J., Reynolds, S., Nowell, M. A., Meakin, L. B., Habicher, J., Ledin, J., Bashford, A., Caterson, B. & Hammond, C. L. (2013). Spinal deformity in aged zebrafish is accompanied by degenerative changes to their vertebrae that resemble osteoarthritis. *PLoS ONE* 8, e75787. [CrossRef]

Gorman, K. F., Handrigan, G. R., Jin, G., Wallis, R. & Breden, F. (2010). Structural and micro-anatomical changes in vertebrae associated with idiopathic-type spinal curvature in the curveback guppy model. *Scoliosis and Spinal Disorders* 5, 1-13. [CrossRef]

Hladik, M. L., Smalling, K. L. & Kuivila, K. M. (2008). A multi-residue method for the analysis of pesticides and pesticide degradates in water using HLB solid-phase extraction and gas chromatography-ion trap mass spectrometry. *The Bulletin of Environmental Contamination and Toxicology* 80,139-144. [CrossRef]

Iwasaki, T., Inoue, N., Teruya, K. & Hamasaki, K. (2018). Osteological development and deformities in hatchery-reared longtooth grouper (*Epinephelus bruneus*): Vertebral column, dorsal-fin supports and caudal-fin skeleton. *Aquaculture Research* 49, 3245-3257. [CrossRef]

Janakiram, P., Geetha, G. K., Sunil Kumar, D. & Jayasree, L. (2018). Aetiological studies on mixed infection of Abdominal segment deformity disease (ASDD) and *Enterocytozoon hepatopenaei* (EHP) in cultured *Litopenaeus vannamei*. *International Journal of Fisheries and Aquatic Studies* 6, 19-26.

Jara, B., Abarca, M., Wilson, R., Krapivka, S., Mercado, A., Guiñez, R. & Marchant, L. (2017). Qualitative analysis of cartilaginous jaw element malformation in cultured yellowtail kingfish (*Seriola lalandi*) larvae. *Aquaculture Research* 48, 4420-4428. [CrossRef]

Jawad, L., Akyol, O. & Sağlam, C. (2017). Consecutive repetition of lordosis-kyphosis in silverside *Atherina boyeri* Risso, 1810 collected from a wild population in Homa Lagoon, Izmir, Turkey. *Archives of Polish Fisheries* 25, 117-122. [CrossRef]

Jawad, L. A. & Ibrahim, M. (2018). Environmental oil pollution: a possible cause for the incidence of ankylosis, kyphosis, lordosis and scoliosis in five fish species collected from the vicinity of Jubail City, Saudi Arabia, Arabian Gulf. *International Journal of Environmental Studies* 75, 425-442. [CrossRef]

Kolarevic, J., Selset, R., Felip, O., Good, C., Snekvik, K., Takle, H., Ytteborg, E., Bæverfjord, G., Asgard, T. & Terjesen, B. F. (2013). Influence of long-term ammonia exposure on Atlantic salmon (*Salmo salar* L.) parr growth and welfare. *Aquaculture Research* 44, 1649-1664. [CrossRef]

Kottelat, M. & Freyhof, J. (2007). Handbook of European freshwater fishes. Publications Kottelat, Cornol and Freyhof, Berlin.

Kužir, S., Maleničić, L., Stanin, D., Trbojević, T. T., Alić, I. & Gjurčević, E. (2015). Description of head deformities in cultured common carp (*Cyprinus carpio* Linnaeus, 1758). *Veterinarski Arhiv* 85, 437-449.

López-Olmeda, J., Noble, C. & Sánchez-Vázquez, F. (2012). Does feeding time affect fish welfare? *Fish Physiology and Biochemistry* 38, 143-152. [CrossRef]

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J. & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment* 473-474, 619-641. [CrossRef]

Majeed, Z., Ajab, Z., Zuberi, A., Akther, S. & Muhammad, A. (2018). Meristic variations and skeletal deformities in natural population of mahseer fish, *Tor putitora* (Hamilton, 1822). *Iranian Journal of Fisheries Sciences* 17, 208-216.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H. & Halm-Lemeille, M. P. (2016). Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. *Environmental Science and Pollution Research* 23, 4992-5001. [CrossRef]

Morasch, B., Bonvin, F., Reiser, H., Grandjean, D., De Alencastro, L. F., Perazzolo, C. & Kohn, T. (2010). Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: micropollutant removal between wastewater and raw drinking water. *Environmental Toxicology and Chemistry* 29, 1658-1668. [CrossRef]

Nguyen, N. H., Whatmore, P., Miller, A. & Knibb, W. (2016). Quantitative genetic properties of four measures of deformity in yellowtail kingfish *Seriola lalandi* Valenciennes, 1833. *Journal of Fish Diseases* 39, 217-228. [CrossRef]

Noble, C., Hernán, A., Jones, C., Damsgård, B., Flood, M. J., Midling, K. O., Roque, A., Saether, B. S. & Yue Cottee, S. (2012). Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare. *Fish Physiology and Biochemistry* 38, 61-83. [CrossRef]

Ogunfowokan, A. O., Oyekunle, J. A. O., Torto, N. & Akanni, M. S. (2012). A study on persistent organochlorine pesticide residues in fish tissues and water from an agricultural fish pond. *Emirates Journal of Food and Agriculture* 24, 165-184.

Okamura, A., Horie, N., Mikawa, N., Yamada, Y. & Tsukamoto, K. (2018). Influence of temperature and feeding regimes on growth and notochord deformity in reared *Anguilla japonica* leptocephali. *Fisheries Science* 84, 505-512. [CrossRef]

Pochodylo, A. & Helbling, D. E. (2015). Target screening for micropollutants in the Hudson River Estuary during the 2015 Recreational Season, New York State Water Resources Institute.

Qu, C. S., Chen, W., Bi, J., Huang, L., & Li, F. Y. (2011). Ecological risk assessment of pesticide residues in Taihu Lake wetland, China. *Ecological Modelling*, 222, 287-292. [CrossRef]

Quigley, D. T. G. (1995). A lower jaw deformity in juvenile and adult Atlantic salmon (*Salmosalar* L.) *Bulletin of the European Association of Fish Pathologists* 15, 206-209.

Raja, M., Raja, R. K., Ramkumar, R., Kavitha, M., Aiswarya, D., Deepak, P. & Perumal P. (2016). First report on the occurrence of abnormal vertebrae-containing giant danio-fish, *Devario aequipinnatus* (McClelland, 1839) in Stanley Reservoir of Cauvery River Tamil Nadu (India). *International Journal of Fisheries and Aquatic Studies* 4, 528-531.

Sato, T. (2006). Occurrence of deformed fish and their fitness-related traits in *Kirikuchi charr*, *Salvelinus leucomaenis japonicus*, the southernmost population of the genus *Salvelinus*. *Zoological Science* 23, 593-599. [CrossRef]

Sauvé, S., Aboulfadl, K., Dorner, S., Payment, P., Deschamps, G. & Prévost, M. (2012). Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. *Chemosphere* 86, 118-123. [CrossRef]

Sfakianakis, D. G., Georgakopoulou, E., Papadakis, I. E., Divanach, P., Kentouri, M. & Koumoundouros, G. (2006). Environmental determinants of haemal lordosis in European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquaculture* 254, 54-64. [CrossRef]

UNECE. (1994). Standard Statistical Classification of Surface Freshwater Quality for the Maintenance of Aquatic Life. In: Readings in International Environment Statistics, United Nations Economic Commission for Europe: United Nations, New York and Geneva, 53-64.

Yıldırım, Ş., Çoban, D., Süzer, C., Fırat, K. & Saka, Ş. (2014). Skeletal deformities of cultured sharpsnout seabream (*Diplodus puntazzo*) larvae during early life development. *Veterinary Journal of Ankara University* 61, 267-273. [CrossRef]

Yu, H. & Cao, W. (2016). Assessment of pharmaceutical and personal care products (PPCPs) of Dalong Lake in Xuzhou by concentration monitoring and bio-effects monitoring process. *Environmental Toxicology and Pharmacology* 43, 209-215. [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2019; 34(2): 46-50 • DOI: https://doi.org/10.26650/ASE2019537099

Research Article

Comparative Histological Observation of Liver Tissue Before and After Reproduction in Male and Female Frogs (*Pelophylax ridibundus*)

Ahmet Alkaya[1*], Hülya Şereflişan[1]

Cite this article as: Alkaya, A., & Sereflisan, H. (2019). Comparative Histological Observation of Liver Tissue Before and After Reproduction in Male and Female Frogs (Pelophylax ridibundus). *Aquatic Sciences and Engineering, 34*(2), 46-50.

ABSTRACT

Histomorphological structure of the liver of *Ranidae* species during the reproduction period contribute significantly to the production rate in the frog culture studies. In this study, the cytoplasmic status of hepatocytes, lipid and melanin pigment in liver parenchyma of adult male and female *Pelophylax ridibundus* (Pallas, 1771) were investigated before and after reproduction. For histological studies, adult frogs were collected from nature and placed in production ponds to examine the reproduction process. In this study, twenty four breeding male frogs, with average weight 36.63±12.84 g and length 69.29±9.15 mm, and twenty four breeding females with average weight and length of 56.61±19.59 g and 79.54±7.07 mm were used. Samples from the liver tissue of frog were taken in 2015 during (March) and after reproduction (June) period. Histologically pictures of liver parenchyma were taken from the as homogeneous fields as possible. Before the reproduction, it was found that the liver parenchyma of the female frogs were cytoplasmically intense, with a low amount of melanin pigment and lipid droplets. Similarly, male frogs liver parenchyma and hepatocytes were found to be cytoplasmically intense, containing a low amount of melanin pigment and lipid droplets. Both female and male frogs completed the reproduction in April and May. After reproduction period, a great amount of melanin pigment in the liver parenchyma was observed and the cytoplasmic density of the hepatocytes decreased. As a result, cytoplasmic decrease in hepatocytes, structural changes and increase in melanin pigments supports the idea that glycogen and lipids stored in the liver parenchyma are used for reproduction.

Keywords: *Pelophylax ridibundus, Ranidae,* liver tissue, hepatocyte, histology, reproduction

ORCID IDs of the authors:
A.A. 0000-0003-2117-7799;
H.Ş. 0000-0002-2510-3714

[1]Iskenderun Technical University, Marine Sciences and Technology Faculty, Iskenderun, Hatay, Turkey

Submitted:
07.03.2019

Revision Requested
08.05.2019

Last Revision Received
14.05.2019

Accepted:
21.05.2019

Online published:
28.06.2019

Correspondence:
Ahmet Alkaya
E-mail:
ahmtalkaya674@gmail.com

INTRODUCTION

Examining the histological changes is one of the important research areas of biology and aquaculture laboratories (Wester & Canton, 1991). In addition, knowing the histophysiological structure of *Ranidae* species which are economically valuable and commonly cultivated contributes significantly to the reproduction rate of this species (Bambozzi et al., 2004; Arauco, De Stefani, Nakaghi & Oliveira-Bahia, 2007). Before and after reproduction differences in liver or gonads and alterations caused by environmental effects can be followed by histological studies in amphibians (Gernhofer, Pawet, Schramm, Müller & Triebskorn, 2001). The liver plays a number of indispensible functions in many body physiological processes such as the digestion of nutrients from other parts of the body and also a storage area for the nutrients. Therefore, it is perfect organ of a key to understand the health and nutritional status of the animals (Akiyoshi & Inoue, 2012). On the other hand, liver tissue is a very good reflector of health management caused diseases and infections in food processing and zootechnics (Hipolito, Leme & Bach, 2001; Hipolito, Martins & Bach, 2004). However, information about the normal and abnormal functions of the amphibian liver is still limited. Thus, most liver disorders are known only by a retrospective diagnosis or autopsy (Crawsha & Weinkle, 2000).

Chen et al. (2011) noted in *Rana chensinensis* the increase in glycogen and lipid reserves before the reproduction and hibernation period and reported that the increase in glycogen and lipid reserves between reproduction and hibernation period altered depending on reproductive strategy. The increase in melanin pigment in liver parenchyma is inversely related to glycogen and lipid concentration. It is thought that glycogen, lipid and other substances are stored in the liver for the energy needed in the reproductive period. Furthermore, these substances are used in the initial formation of egg-sperm as well as energy-requiring activities such as cell division and mating (Cadeddu & Castellano 2012; Cayuela et al., 2014; Mentino, Mastrodonato, Rossi & Scillitani, 2014).

Mentino et al. (2017) discovered the seasonal changes in glycogen, lipid and melanin pigment in the liver of frog (*Pelophylax esculentus*). They found that melanin pigment in the liver tissue reach its maximum value during May and June which could be related with the reproductive activities. Asisi (1991) reported the degenerative changes in some liver hepatocytes after an apoptotic process such as vitellogenesis and pointed out the fact that that melanins mediated indirectly to apoptosis. Melanin pigments in the frog liver at the time of hibernation were reached more than the active period after the hibernation (Purrello et al., 2001). In addition, the reason for the high amount of melanin pigments before hibernation was reported as the support of pigments by two mechanisms: reproduction or hypertrophy (Barni et al., 2002).

This study was aimed to the morphological evaluation of the histological changes that occurred before and after reproduction of the liver of male and female edible water frog *Pelophylax ridibundus* (Pallas, 1771) which has economic importance. Also, knowing the changes in the histomorphological structure of the liver of this species during the reproduction period are thought to contribute significantly to the production rate in the frog culture studies.

MATERIALS AND METHODS

In this study, an edible frog (*P. ridibundus*), which has an economic importance, was used. The adult female and male frogs used in the study were collected around Gölbaşı Lake in Kırıkhan District of Hatay in March 2015. The collected adult frogs were transferred to the frog farm established in Aydincik in plastic boxes. The adult female and male frogs were placed in breeding ponds of 6 m^2. For this study, twenty four breeding male frogs, with average weight 36.63±12.84 g and length 69.29±9.15 mm, and twenty four female frogs with average weight and length of 56.61±19.59 g and 79.54±7.07 mm were used. For histological studies, frogs (n=48) were taken from the ponds before reproduction (March) and after reproduction (June) periods and they were brought to the research laboratory of the Faculty of Marine Sciences and Technology of Iskenderun Technical University. Then, histological samples were taken from liver tissue.

The female and male frogs were kept in ice containing containers for 30 minutes and then treated with 25 ml chloroform spilled cottons for the competition of anesthesia procedure in the laboratory. Then, the dissection procedure was carried out and the removed liver tissues were immediately stored in 10% formalin solution. Samples were then dehydrated through a series of graded alcohols, cleared in xylene, infiltrated, and embedded into paraffin. Liver paraffin wax blocks were cut into 4 μm thick sections, stained with hematoxylin and eosin (H&E), and then they were examined under Olympus CX 41 microscope (Akiyoshi & Inoue, 2012; Seixas Filho et al., 2013). Images were captured Olympus DP 20 digital camera. Pictures of liver parenchyma were taken the most homogeneous fields possible.

RESULTS AND DISCUSSIONS

In the liver of *P. ridibundus* is a single-layer plate and it contained hepatocytes with a multi-faceted structure and round core in the middle. Also, hepatocytes arranged in clusters and these structures were surrounded by sinusoid networks of liver cordon; portal venular, hepatic arterial and bile ducts were observed to be separated from each other by the presence of traditional. In histological observations of female frogs, the liver parenchyma was cytoplasmically intense, contained low amounts of melanin pigments and lipid droplets before reproduction (march) period (Figure 1, 2). Similarly, the livers of male frogs, liver parenchyma and hepatocytes were cytoplasmically intense, containing low amounts of melanin pigment and lipid droplets (Figure 3, 4).

The livers of female and male frogs, which completed reproduction in April and May were examined histologically in June and intense amount of melanin pigment was detected on liver parenchyma (Figures 5, 6, 7). Parallel to this, obvious changes in the shape of hepatocyte cells were observed and cytoplasmic density

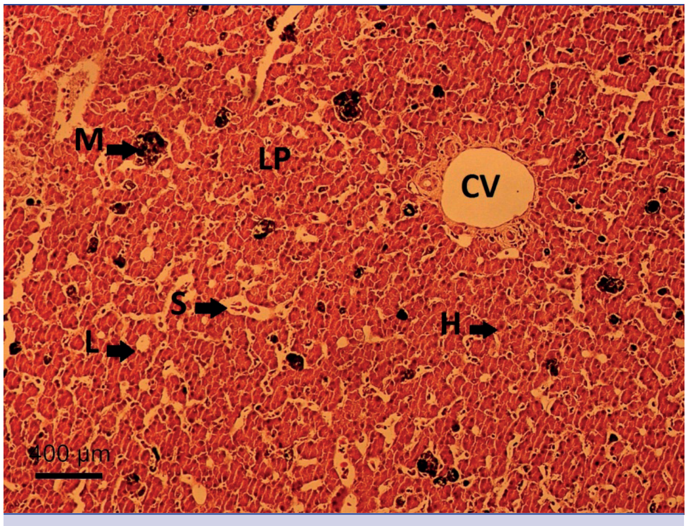

Figure 1. Low amounts of melanin pigments and lipid droplets in the liver of female frog before reproduction (H: Hepatocyte, M: Melanin, CV: Central vein, S: Sinusoid, L: Lipid, LP: Liver parenchyma. Stain: H & E, Magnification: 10X, original).

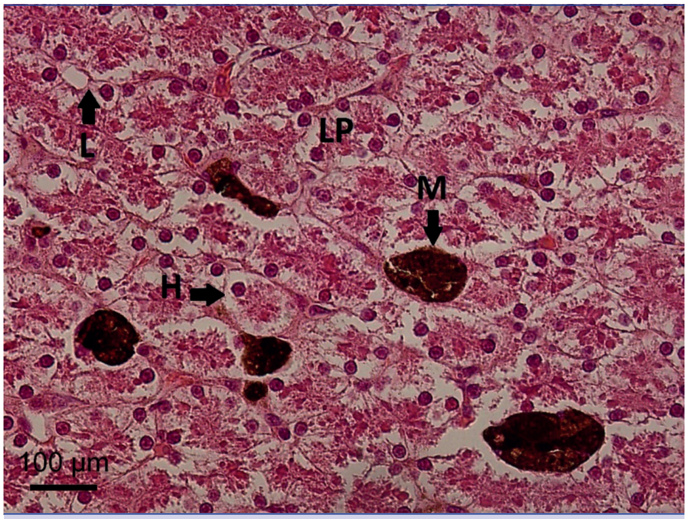

Figure 2. Low amounts of melanin pigments and lipid droplets in the liver of female frog before reproduction (H: Hepatocyte, M: Melanin, L: Lipid, LP: Liver parenchyma. Stain: H & E, Magnification: 40X, original).

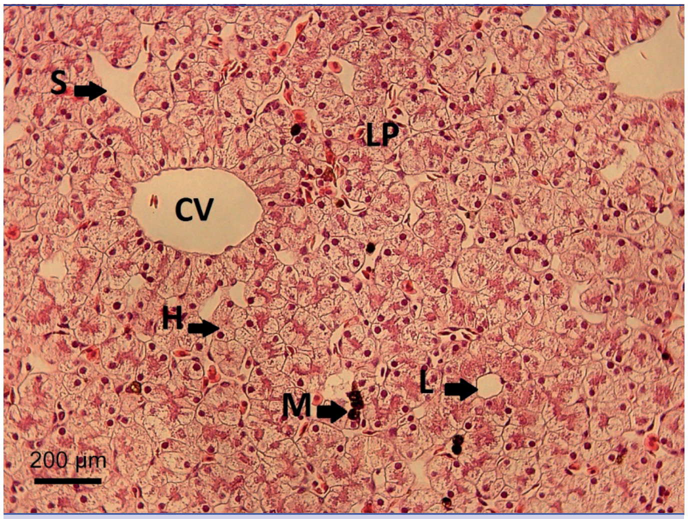

Figure 3. Low amounts of melanin pigments and lipid droplets in the liver of male frog before reproduction (H: Hepatocyte, M: Melanin, CV: Central vein, S: Sinusoid, L: Lipid, LP: Liver parenchyma. Stain: H & E, Magnification: 20X, original).

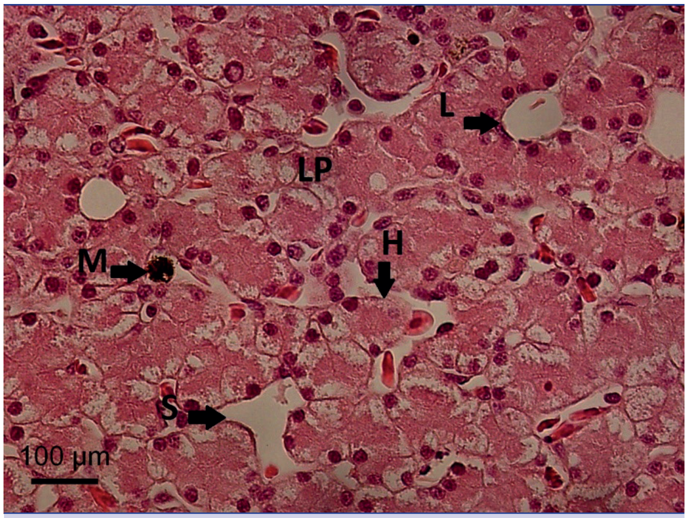

Figure 4. Low amounts of melanin pigments and lipid droplets in the liver of male frog before reproduction (H: Hepatocyte, M: Melanin, L: Lipid, LP: Liver parenchyma. Stain: H & E, Magnification: 40X, original).

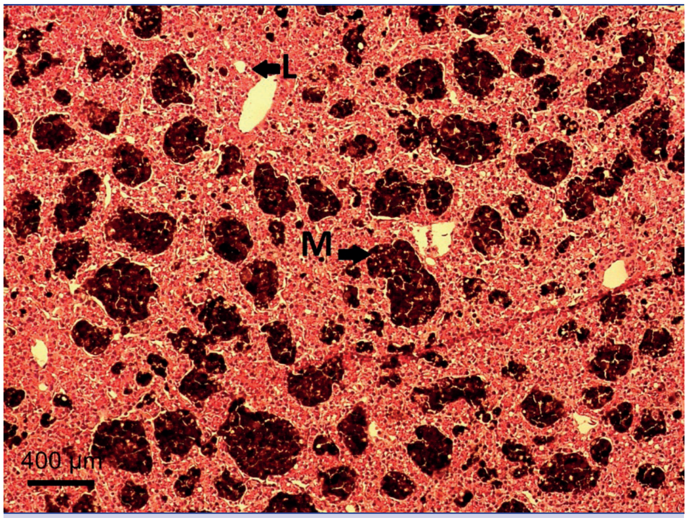

Figure 5. High amounts of melanin pigments and lipid droplets in the liver of female frog after reproduction (L: Lipid, M: Melanin. Stain: H & E, Magnification: 10X, original).

of hepatocytes was decreased compared to pre-reproduction period in both male and female individuals (Figure 6, 8). As a result of cytoplasmic decrease, structural changes and increased melanin pigments in hepatocytes; support the idea that glycogen and liver parenchyma stored in hepatocytes are used in reproduction.

The liver is an important organ that serves as a storage for glycogen and lipids, particularly in temperate climatic conditions for hibernation species and manages the change in seasonal amounts of these two substances (Dinsmore & Swanson 2008; Fenoglio, Bernocchi & Barni,, 1992; Pasanen & Koskela, 1974; Singh & Sinha, 1989). Haar and Hightower (1976) reported that *Notophthalmus viridescens* hepatocytes contain great amount of lipid and glycogen inclusions. Chen et al. (2011) reported in anuran that glycogen and lipid reserves increased before the reproductive period and hibernation and linked the increase between hibernation and beginning of the reproductive period with reproductive strategy. In pre-reproduction period (March), is determined that the liver parenchymal and density of hepatocytes in cytoplasm was intense for both male and female breeding frogs and melanin pigments and lipid droplets in the liver pa-

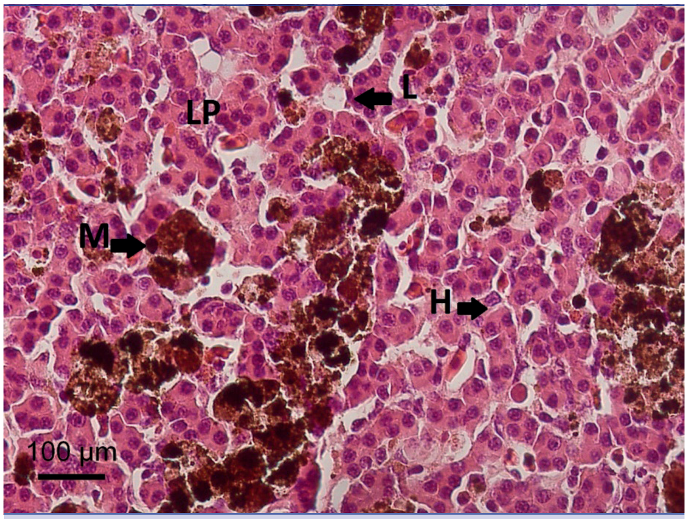

Figure 6. High amounts of melanin pigments and lipid droplets in the liver of female frog after reproduction (H: Hepatocyte, M: Melanin, L: Lipid, LP: Liver parenchyma. Stain: H & E, Magnification: 40X, original).

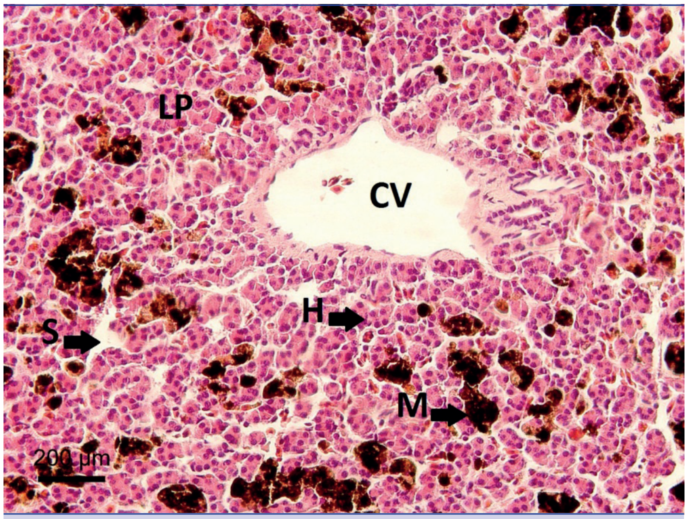

Figure 7. High amounts of melanin pigments in the liver of male frog after reproduction (H: Hepatocyte, M: Melanin, CV: Central vein, LP: Liver parenchyma. Stain: H& E, Magnification: 20X, original).

renchyma was found to be low. Therefore, it was concluded that the breeding frogs have the necessary energy reserves for reproduction and these reserves vary depending on the breeding strategy.

The concentration of glycogen and lipids is inversely related to the increase of melanin pigment in the liver cell and during the mating period of the anurans, glycogen, lipid and other substances stored in the liver were used for synthesis of egg-sperm formation related substances (Cadeddu & Castellano 2012; Cayuela et al., 2014; Mentino et al. 2014). In another study, it was reported that degenerative changes in some liver hepatocytes may be seen after an apoptotic process such as vitellogenesis (Assisi, 1999). It is known that melanins mediate indirectly to apoptosis during vitallonegenesis (Barni et al., 2002; Purrello et al., 2001). In this study, after the reproduction of the liver of the frogs was observed histologically and detected a large number of melanin pigment on the liver parenchyma. In addition, both male and female frogs showed significant changes in the shape of hepatocyte cells and decreased cytoplasmic density of hepatocytes.

As a result, these histomorphological changes observed in the liver parenchyma; support both the idea of glycogen and lipid in the liver parenchyma or hepatocyte are used in reproduction and liver parenchyma or hepatocyte are inversely related with melanin pigment abundance. Barni et al. (1991) reported the increase in the melanin area in the liver depending on increase in hypertrophy and melanin synthesis in hibernating frogs and linked this situation inversely with glycogen storage in hepatocytes.

Mentino et al. (2017), examined the seasonal changes in the glycogen *P. esculentus* and reported that the melanin pigments in the liver reached its maximum in May and June, which may be related to reproductive activities. Similarly, in this study, melanin pigments in the liver were found to be a large number and the increase in melanin pigment area is thought to be related to reproduction.

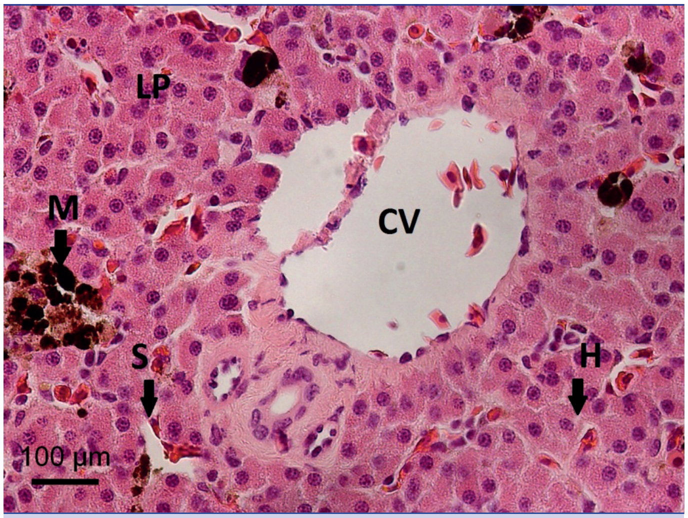

Figure 8. Cytoplasmic density of hepatocytes male frog after breeding (H: Hepatocyte, M: Melanin, CV: Central vein, LP: Liver parenchyma. Stain: H&E, Magnification: 40X, original).

CONCLUSION

As a result of cytoplasmic decrease, structural change and increase in melanin pigments on liver parenchyma after the reproduction; lipids, protein and glycogen that stored in the hepatocytes were used for reproduction. Also, this study will contribute to the production rate for frog culture by shedding light on the preparation of pellet food rations according to the metabolic energy needs of the frogs in the frog breeding period.

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

Ethics Committee Approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

REFERENCES

Akiyoshi, H. & Inoue, A. M. (2012). Comparative histological study of hepatic architecture in the three orders amphibian livers. *Comparative Hepatology*, 11(1), p. 1-8. [CrossRef]

Arauco, L. R. R., De Stéfani, M. V., Nakaghi, L. S. O. & Oliveira-Bahia, V. R. L. D. (2007). Histology of kidney, liver and intestine of bullfrog tadpoles (*Rana catesbeiana*) fed with diets containing propolis. *Ciência Rural*, 37(5), 1436-1441. [CrossRef]

Assisi, L., Autuori, F., Botte, V., Farrace, M. G. & Piacentini, M. (1999). Hormonal control of "tissue" transglutaminase induction during programmed cell death in frog liver. *Experimental Cell Research*, 247(2), 339-346. [CrossRef]

Bambozzi, A. C., Seixas-Filho, J. D., Thomaz, L. A., Oshiro, L. M. Y., Braga, L. G. T. & Lima, S. L. (2004). Efeito do fotoperíodo sobre o desenvolvimento de girinos de rã-touro (*Rana catesbeiana* Shaw, 1802). *Revista Brasileira de Zootecnia*, 33(1), 1-7. [CrossRef]

Barni, S. & Bernocchi, G., (1991). Internalization of erythrocytes into liver parenchymal cells in naturally hibernating frogs (*Rana esculenta L.*). *J. Exp. Zool.,* 258(2), 143-150. [CrossRef]

Barni, S., Bertone, V., Croce, A. C., Bottiroli, G., Bernini, F. & Gerzeli, G. (1999). Increase in liver pigmentation during natural hibernation in some amphibians. *The Journal of Anatomy*, 195(1), 19-25. [CrossRef]

Barni, S., Vaccarone, R., Bertone, V., Fraschini, A., Bernini, F. & Fenoglio, C. (2002). Mechanisms of changes to the liver pigmentary component during the annual cycle (activity and hibernation) of *Rana esculenta. L. Journal of Anatomy,* 200(2), 185-194. [CrossRef]

Cadeddu, G. & Castellano, S. (2012). Factors affecting variation in the reproductive investment of female treefrogs, *Hyla intermedia. Zoology,* 115(6), 372-378. [CrossRef]

Cayuela, H., Besnard, A., Bonnaire, E., Perret, H., Rivoalen, J., Miaud, C. & Joly, P. (2014). To breed or not to breed: past reproductive status and environmental cues drive current breeding decisions in a long-lived amphibian. *Oecologia,* 176(1), 107-116. [CrossRef]

Chen, W., Zhang, L-X. & Lu, X. (2011). Higher pre-hibernation energy storage in anurans from cold environments: A case study on a temperate frog *Rana chensinensis* along a broad latitudinal and altitudinal gradients. *Annales Zoologici Fennici*, 48(4), 214–220. [CrossRef]

Crawshaw, G.J. & Weinkle, T.K. (2000). Clinical and pathological aspects of the amphibian liver. *Seminars in Avian and Exotic Pet Medicine*, 9(3), 165-173. [CrossRef]

Dinsmore, S. C. & Swanson, D. L. (2008). Temporal patterns of tissue glycogen, glucose, and glycogen phosphorylase activity prior to hibernation in freeze-tolerant chorus frogs, *Pseudacris triseriata. Canadian Journal of Zoology*, 86(10), 1095-1100. [CrossRef]

Fenoglio, C., Bernocchi, G. & Barni, S. (1992). Frog hepatocyte modifications induced by seasonal variations: a morphological and cytochemical study. *Tissue and Cell*, 24(1), 17-29. [CrossRef]

Gernhofer, M., Pawet, M., Schramm, M., Müller, E. & Triebskorn, R. (2001). Ultrastructural Biomarkers as Tools to Characterize the Health Status of Fish in Contaminated Streams. *Journal of Aquatic Ecosystem Stress and Recovery*, 8, 241-226. [CrossRef]

Haar, J. L. & Hightower, J.A.A. (1976). Light and electron microscopic investigation of the hepatic parenchyma of the adult newt, *Notophthalmus viridescens. The Anatomical Record*, 185(3), 313-323. [CrossRef]

Hipolito, M., Leme, M. C. M. & Bach, E. E. (2001). Lesões anátomo-histopatológicas em rãs-touro (*Rana catesbeiana* Shaw, 1802) associadas à deterioração da ração. *Arquivos do Instituto de Biologia,* 68(1), 111-114.

Hipolito, M., Martins, A. M. C. R. P. F. & Bach, E. E. (2004). Aspectos bioquímicos em fígado de rãs-touro (*Rana catesbeiana* Shaw, 1802) sadias e doentes. *Arquivos do Instituto Biológico,* 71(2), 147-153.

Mentino, D., Mastrodonato, M., Rossi, R. & Scillitani, G., (2014). Histochemical and structural characterization of egg extra-cellular matrix in bufonid toads, *Bufo bufo* and *Bufotes balearicus*: Molecular diversity versus morphological uniformity. *Microscopy Research and Technique,* 77(11), 910–917. [CrossRef]

Mentino, D., Scillitani, G., Marra, M. & Mastrodonato, M. (2017). Seasonal changes in the liver of a non-hibernating population of water frogs, *Pelophylax kl. esculentus* (Anura: Ranidae). *The European Zoological Journal*, 84(1), 525-535. [CrossRef]

Pasanen, S. & Koskela, P. (1974). Seasonal and age variation in the metabolism of the common frog, *Rana temporaria L.* in northern Finland. *Comparative Biochemistry and Physiology Part A: Physiology,* 47(2), 635-654. [CrossRef]

Purrello, M., Scalia, M., Corsaro, C., Di Pietro, C., Piro, S. & Sichel, G. (2001). Melanosynthesis, differentiation, and apoptosis in Kupffer cells from *Rana esculenta. Pigment cell research,* 14(2), 126-131. [CrossRef]

Seixas Filho, J. T., Hipólito, M., Pereira, M. M., Martins, A. M. C. R. P. F., Rodrigues, E. and Mello, S.C.R.P., (2013). Liver histopathological changes in breeding bullfrogs. *Acta Scientiarium Animal Science,* 35:461-465. [CrossRef]

Singh, R. P. & Sinha, R. C. (1989). Seasonal changes in energy reserves in the common frog, *Rana tigrina. The Japanese Journal of Physiology*, 39(6), 969-973. [CrossRef]

Wester, P. W. & Canton, J. H. (1991). The usefulness of histopathology in aquatic toxicity studies. *Comparative Biochemistry and Physiology. C, Comparative Pharmacology and Toxicology,* 100(1-2), 115-117. [CrossRef]

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2019; 34(2): 51-60 • DOI: https://doi.org/10.26650/ASE2019547010

Research Article

Testing Linear Regressions by StatsModel Library of Python for Oceanological Data Interpretation

Polina Lemenkova[1*]

Cite this article as: Lemenkova, P. (2019). Testing Linear Regressions by StatsModel Library of Python for Oceanological Data Interpretation. *Aquatic Sciences and Engineering, 34*(2), 51-60.

ABSTRACT

The study area is focused on the Mariana Trench, west Pacific Ocean. The research aim is to investigate correlation between various factors, such as bathymetric depths, geomorphic shape, geographic location on four tectonic plates of the sampling points along the trench, and their influence on the geologic sediment thickness. Technically, the advantages of applying Python programming language for oceanographic data sets were tested. The methodological approaches include GIS data collecting, data analysis, statistical modelling, plotting and visualizing. Statistical methods include several algorithms that were tested: 1) weighted least square linear regression between geological variables, 2) autocorrelation; 3) design matrix, 4) ordinary least square regression, 5) quantile regression. The spatial and statistical analysis of the correlation of these factors aimed at the understanding, which geological and geodetic factors affect the distribution of the steepness and shape of the trench. Following factors were analysed: geology (sediment thickness), geographic location of the trench on four tectonics plates: Philippines, Pacific, Mariana and Caroline and bathymetry along the profiles: maximal and mean, minimal values, as well as the statistical calculations of the 1st and 3rd quantiles. The study revealed correlations between the sediment thickness and distinct variations of the trench geomorphology and sampling locations across various segments along the crescent of the trench.

Keywords: Programming language, Python, Statistical analysis, Pacific Ocean, Hadal trenches, Mariana Trench, oceanology, marine geology

ORCID IDs of the authors:
P.L. 0000-0002-5759-1089

[1]Ocean University of China, College of Marine Geo-sciences. 238 Songling Road, Laoshan, 266100, Qingdao, Shandong Province, People's Republic of China

Submitted:
30.03.2019

Revision Requested
22.05.2019

Last Revision Received
13.06.2019

Accepted:
13.06.2019

Online published:
26.06.2019

Correspondence:
Polina Lemenkova
E-mail:
lemenkovapolina@stu.ouc.edu.cn
pauline.lemenkova@gmail.com

INTRODUCTION

Multiple approaches and GIS methods have been used so far to model ocean seafloor, the most unreachable part of the Earth. These include echo sounding (Smith, & Sandwell, 1997), CTD (conductivity-temperature-depth profiler) technique (Taira et al., 2005), acoustic methods, continual profiling with single-beam systems and bottom coverage capability, multi-beam swath-mapping systems (Dierssen, & Theberge, 2014), classic approaches of the GIS mapping and other tools of geoinformatics (Fujie et al., 2006), remote sensing images analysis, navigation charts and data modelling using schematic cross-sections of the subduction zones (e.g. Schellart, 2008), statistical modelling using R and packages, e.g. dplyr, ggplot2, PMCMR, car (Reid et al., 2018). Of all these, statistical modelling of the oceanological data sets by means of R and Python programming languages is the most cost-effective for investigating hadal trench geomorphology.

Various studies have been reported on the geologic variations of the Mariana Trench involving uneven distribution of various geomorphic phenomena across the seafloor (e.g., Michibayashi et al., 2007; Grand et al., 1997). Amongst these, the questions of how the trench shape is varies and what are the factors affective its geomorphology are the most challenging in view of the importance of the deep ocean segments for the whole ocean environment. The distribution of elevations on the Earth or hypsography is highly uneven. Thus, the majority of the depths is occupied by deep basins (4– 6.5 km) while relatively few areas are covered by shallow zones. At the same time, a considerable pool of resources is hidden by the ocean depths which explains the actuality of the ocean research for the national economies. The limitations in marine geological methods are imposed by the high cost of the actual cruise marine expeditions. Using available open source geodata sets have removed this problem by the use of low-cost geospatial data and their processing in GIS and open source programming language R and Python. Similarly, Python based statistical set of libraries, such as NumPy, SciPy, StastModels, and Matplotlib statistical package present effective low-cost and easily available method for the marine oceanological data processing and modelling.

Regional studies of the marine geology of the trenches across the Pacific Ocean (e.g., Bello-González et al., 2018; Boston et al., 2017), modelling and predictions made upon analysis of the geophysical settings of various trench, produced by these investigators were instrumental in understanding current issues of the marine geological studies. The concepts of these reports on seafloor spreading, tectonic slab subduction, continental drift, and plate tectonics in the Pacific Ocean were analysed in the current research.

STUDY AREA AND DATA

The study area is located in the Mariana Trench, west Pacific Ocean, where the deepest place of the Earth is recorded (Theberge, 2008).

The geomorphology of the Mariana Trench was studied through the spatial and statistical analysis of the 25 cross-section bathymetric profiles digitized across the trench. Each profile has a length of 1000 km and a distance between each two is 100 km. The methodology consists of two parts: geospatial data processing and statistical analysis.

First, during the geospatial part of the research, the data were collected from the Quantum GIS project as vector layers. The attribute tables contained numerical data on bathymetry, geology, tectonic plates and geometric features of the Mariana Trench in its various segments of the geographic location: north-west, centre, south-west.

Second, during the statistical part of the research, the table in .csv format was then read into the Python environment using Pandas package. The profiles were observed using methods of the statistical modelling performed by Python programming language. During the statistical testing and experiment, several existing approaches (Box, & Tiao, 1992; Timm, 2007; Oliphant, 2007; Oliphant, 2015; Lemenkova, 2019) provided by the Python StatsModel and Matplotlib libraries were used as the core algorithms described below.

METHODOLOGY

Design matrix and model fit summary by the Ordinary Least Squares

The variables of geologic interest were stored in the table consisting of 18 rows where numeric information describes geology, bathymetry, geodesy and tectonics of the Mariana Trench. To fit most of the models covered by StatsModels Python library, the design or regressor matrix was created using existing approaches (Everitt, 2002; Box, & Tiao, 1992; Millman & Aivazis, 2011). The

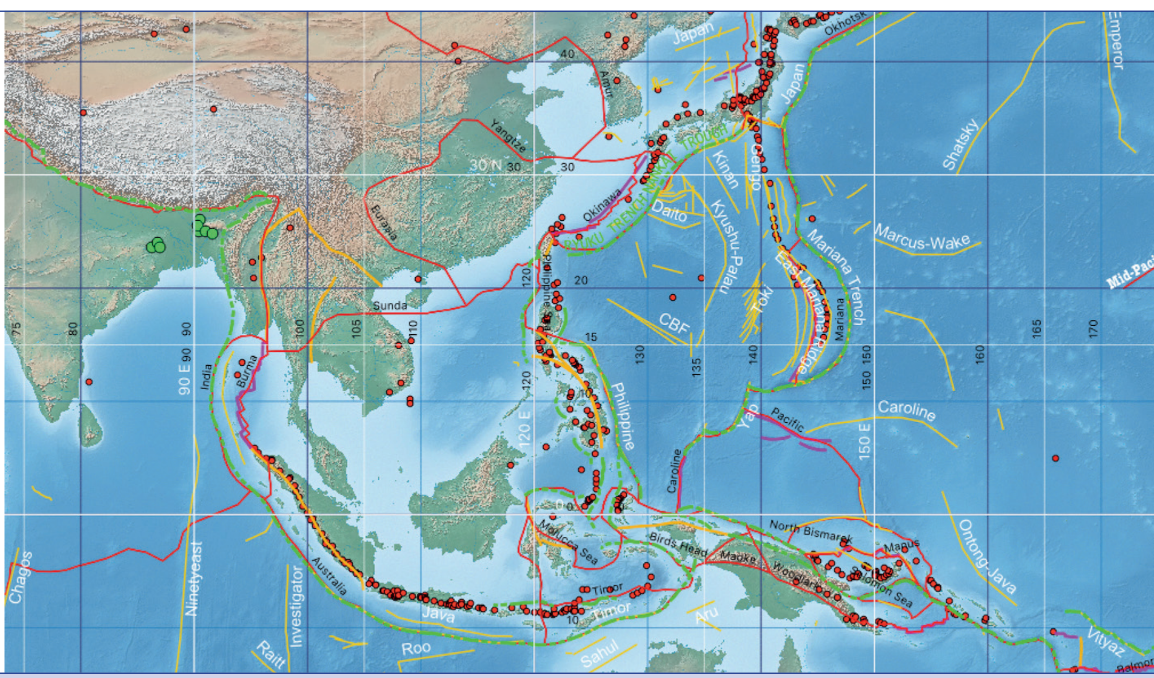

Figure 1. Study area: Mariana Trench, west Pacific Ocean (*Source: author, QGIS project*).

```python
from __future__ import print_function
%matplotlib inline
import numpy as np
import pandas as pd
import statsmodels.api as sm
from patsy import dmatrices
import os
os.chdir('/Users/pauline/Documents/Python')
df = pd.read_csv("Tab-Morph.csv")
df = df.dropna()
df[-10:]
y, X = dmatrices('profile ~ sedim_thick + igneous_volc + slope_angle',
                 data=df, return_type='dataframe')
y[:7]
X[:7]
```

	Intercept	sedim_thick	igneous_volc	slope_angle
0	1.0	132.0	112.0	25.0
1	1.0	103.0	71.0	32.0
2	1.0	96.0	0.0	51.0
3	1.0	109.0	0.0	64.0
4	1.0	127.0	3.0	52.0
5	1.0	135.0	5.0	70.0
6	1.0	142.0	0.0	55.0

```
                            OLS Regression Results
==============================================================================
Dep. Variable:                profile   R-squared:                       0.457
Model:                            OLS   Adj. R-squared:                  0.379
Method:                 Least Squares   F-statistic:                     5.881
Date:                Sun, 24 Mar 2019   Prob (F-statistic):            0.00445
Time:                        19:36:32   Log-Likelihood:                -77.241
No. Observations:                  25   AIC:                             162.5
Df Residuals:                      21   BIC:                             167.4
Df Model:                           3
Covariance Type:            nonrobust
================================================================================
                  coef    std err          t      P>|t|      [0.025      0.975]
--------------------------------------------------------------------------------
Intercept      17.9305      6.362      2.819      0.010       4.701      31.160
sedim_thick    -0.1320      0.048     -2.743      0.012      -0.232      -0.032
igneous_volc    0.0445      0.016      2.819      0.010       0.012       0.077
slope_angle     0.1328      0.115      1.151      0.263      -0.107       0.373
==============================================================================
Omnibus:                        1.669   Durbin-Watson:                   0.654
Prob(Omnibus):                  0.434   Jarque-Bera (JB):                1.095
Skew:                          -0.198   Prob(JB):                        0.578
Kurtosis:                       2.054   Cond. No.                         884.
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly specified.

Intercept       17.930487
sedim_thick     -0.131967
igneous_volc     0.044532
slope_angle      0.132841
dtype: float64
```

Table 1. Python code for design matrix 'dmatrices' (left) and OLS computation by StatsModels of Python (right), Mariana Trench data frame.

first is a matrix of endogenous variables of sediment thickness, which show the response or geological regressand on changed environmental conditions: geographic location, depth or tectonic plate.

The design matrix (Table 1, left) shows the results of the first six lines representing values of explanatory variables in a set of geological attributes, Mariana Trench.

The computing of the Ordinary Least Square (OLS) was based on the formula (1):

$$b_1 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{n} (x_i - \bar{x})} \quad (1)$$

Where,
n is the sample size;
x is a constant and a scalar regressor;
y is a random regressor, sampled together with x;
h is the number of lags being tested;

Each row of the calculated OLS coefficient estimates (Table 1, right) shows an individual bathymetric profile with the successive columns corresponding to the geologic and oceanographic variables and their specific values across the profiles.

Quantile statistics (QQ)

The used algorithm is very straightforward with a selected function of qqplot() by StatsModels to perform this task. The QQ regression is a common abbreviation for 'quantile by quantile' statistical plot. The plot shows (Figure 2) one quantile against another across various geological parameters (from left to right): A) Sediment thickness; B) Slope angle degrees; C) Pacific Plate; D); Philippine Plate E) Mariana Plate; F) Distribution of samples of igneous volcanic areas.

Technically, the plotting was performed using following code of Python for each corresponding plot:

```
ax1.plt = qqplot(df.sedim_thick, line='q', ax=ax1, fit=True,
linewidth=.5,alpha=.5,markerfacecolor='#00a497',
markeredgecolor='grey',)
```

The QQ statistics calculation has been based on the following formula (2) after Ljung, & Box (1978):

$$Q = n(n+2)\sum_{k=1}^{n} \frac{l_k^2}{(n-k)} \quad (2)$$

Where,
n is the sample size;
rho is the sample autocorrelation at lag k, and
h is the number of lags being tested.

The comparison of all the six subplots enables to analyse the form of their shape against a straight line. The quantiles are bathymetric sample observations with geologic attribute values placed in the ascending order. The QQ statistics are used over the pool of the sampling data to study their distribution. A QQ statistic is a visual representation of the quantiles of a standard normal distribution of the geological data set across the Mariana Trench, showing their variation in space.

Weighted Least Squares

A Weighted Least Squares (WLS) for the geological variables are shown on Figure 3. The approach of a weighted least squares is a standard approach in regression analysis to approximate the solution of overdetermined systems which is the case for the complex marine geological systems. The least squares algorithms has two sub-types: linear or ordinary least squares and nonlinear least squares. In the scope of this research, only the linear least squares were tested: ordinary least squares and weight-

Figure 2. Plotted QQ statistics for the data distribution: A) Sediment thickness; B) Slope angle degrees; C) Pacific Plate; D); Philippine Plate E) Mariana Plate; F) Volcanic spots.

ed least square which is a generalization of the first one. In this case, the off-diagonal entries of the correlation matrix of the geological residuals are null the variances of the observations, yet unequal along the covariance matrix.

A WLS is a statistical approach representing a special case of the generalized least squares.

The calculation is based on the principle of the Gauss–Newton algorithm that solves a non-linear least squares problem by modifying a Newton's method for finding a minimum of a function. The computation was based on the general approach of existing equation (after Björck, 1996):

$$J^T WJ \Delta \beta = J^T W \Delta y \quad (3)$$

Where
W is a diagonal when the observational errors are uncorrelated and the weight matrix;
J (t) is a transposed Jacobian matrix;
ß are unbiased estimators as linear column vectors, the entries of the Jacobian matrix;
y is a vector of the response values.

The calculations of the WLS for the data set (Table 2) were done according to the reported procedures (Seabold & Perktold, 2010; Strutz, T. (2016) by Python code snippet:

```
        # Step-1.
mod_wls = sm.WLS(y, X, weights=1./(w ** 2))
res_wls = mod_wls.fit()
print(res_wls.summary())
        # Step-2.
res_ols = sm.OLS(y, X).fit()
print(res_ols.params)
print(res_wls.params)
        # Step-3.
se  =  np.vstack([[res_wls.bse],  [res_ols.bse],
[res_ols.HC0_se],
                    [res_ols.HC1_se], [res_ols.HC2_
se], [res_ols.HC3_se]])
se = np.round(se,4)
colnames = ['x1', 'const']
rownames = ['WLS', 'OLS', 'OLS_HC0', 'OLS_HC1',
'OLS_HC3', 'OLS_HC3']
tabl = SimpleTable(se, colnames, rownames, txt_
fmt=default_txt_fmt)
print(tabl)
```

Quantile regressions

Quantile regression shows (Figure 4) the estimated conditional median and other quantiles of the response geological variables. Thus the upper two rows of the plot show (Figure 4, A, B, C, D) data distribution across tectonic plates: Pacific Plate, Philippine Plate, Mariana Plate and Caroline Plate. The lower row of the plot (Figure 4, E, F) shows data distribution for the cumulative sediment thickness and slope angle degree by profiles.

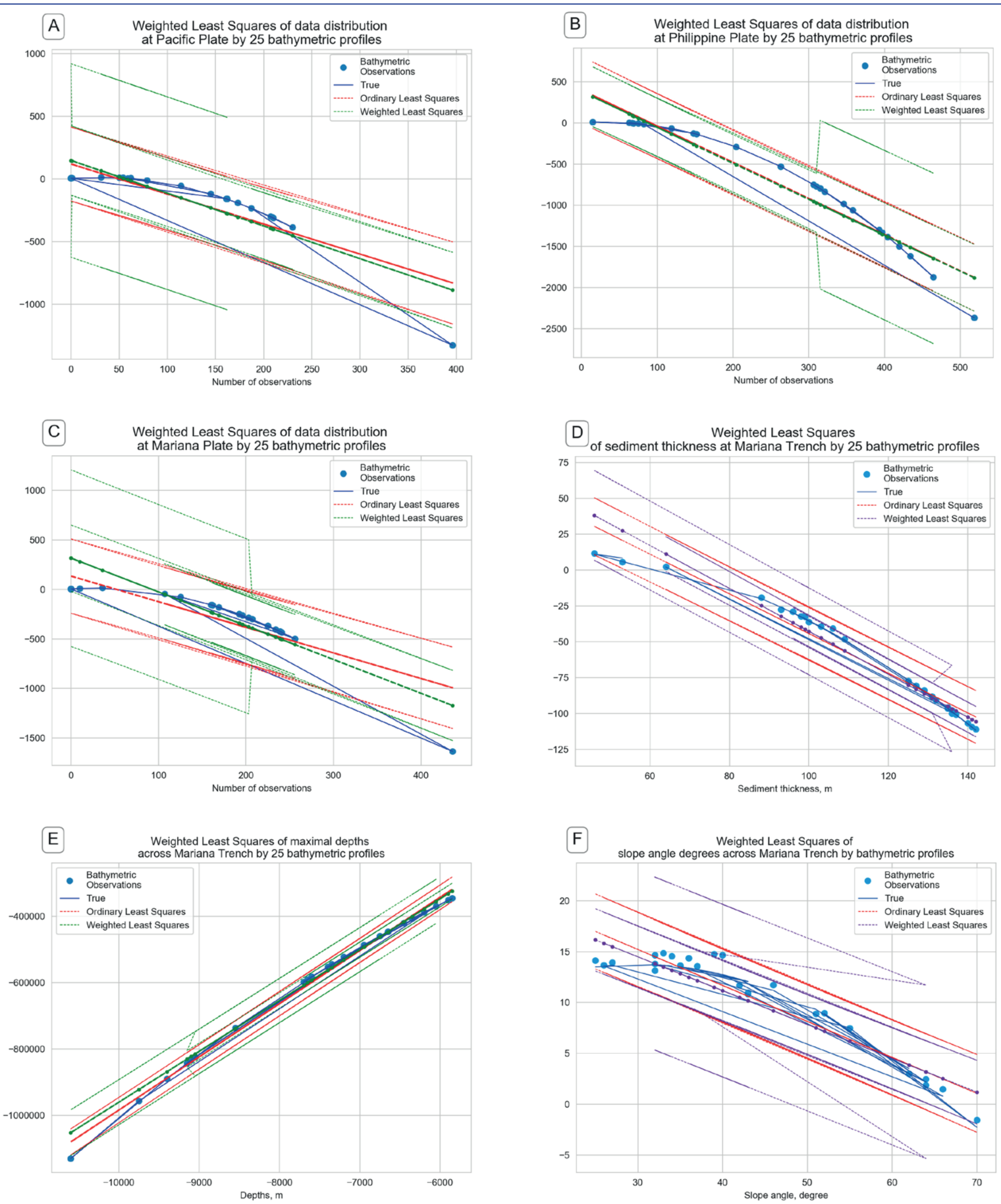

Figure 3. Weighted Least Squares plotted for data distribution: A) Pacific Plate, B) Philippine Plate, C) Mariana Plate, D) Sediment thickness, E) Depths (max); F) Slope angle degree.

The quantile regressions were plotted (Figure 4) using Python code by StatsModel:

```
    # Step-1. Least Absolute Deviation
mod = smf.quantreg('profile ~ slope_angle', data)
res = mod.fit(q=.5)
print(res.summary())
    # Step-2. Placing the quantile regression results in a Pandas DataFrame, and the OLS results in a dictionary
quantiles = np.arange(.05, .96, .1)
def fit_model(q):
    res = mod.fit(q=q)
    return [q, res.params['Intercept'], res.params['slope_angle']] + res.conf_int().loc['slope_angle'].tolist()
```

(A)

```
==== RESTART: /Users/pauline/Documents/Python/Script-038a-SM-WLS-Pacif.py ====
                            WLS Regression Results
==============================================================================
Dep. Variable:                      y   R-squared:                       0.781
Model:                            WLS   Adj. R-squared:                  0.772
Method:                 Least Squares   F-statistic:                     82.18
Date:                Mon, 25 Mar 2019   Prob (F-statistic):           4.71e-09
Time:                        14:47:33   Log-Likelihood:                -165.76
No. Observations:                  25   AIC:                             335.5
Df Residuals:                      23   BIC:                             338.0
Df Model:                           1
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const        146.6735     52.135      2.813      0.010      38.825     254.522
x1            -2.6086      0.288     -9.065      0.000      -3.204      -2.013
==============================================================================
Omnibus:                       25.519   Durbin-Watson:                   1.255
Prob(Omnibus):                  0.000   Jarque-Bera (JB):               44.284
Skew:                          -2.051   Prob(JB):                     2.42e-10
Kurtosis:                       8.068   Cond. No.                         308.
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
 specified.
[118.71874308  -2.39356004]
[146.67351771  -2.60864727]
=====================
            x1   const
---------------------
WLS     52.1346 0.2878
OLS      42.088 0.2719
OLS_HC0 52.7997 0.5709
OLS_HC1 55.0475 0.5952
OLS_HC3 62.7974 0.6996
OLS_HC3 75.3801 0.8597
---------------------
>>>
```

(B)

```
==== RESTART: /Users/pauline/Documents/Python/Script-038b-SM-WLS-Phil.py ====
                            WLS Regression Results
==============================================================================
Dep. Variable:                      y   R-squared:                       0.905
Model:                            WLS   Adj. R-squared:                  0.900
Method:                 Least Squares   F-statistic:                     218.1
Date:                Mon, 25 Mar 2019   Prob (F-statistic):           3.17e-13
Time:                        14:50:45   Log-Likelihood:                -172.93
No. Observations:                  25   AIC:                             349.9
Df Residuals:                      23   BIC:                             352.3
Df Model:                           1
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const        385.2523     67.098      5.742      0.000     246.450     524.055
x1            -4.3747      0.296    -14.768      0.000      -4.987      -3.762
==============================================================================
Omnibus:                       10.573   Durbin-Watson:                   0.805
Prob(Omnibus):                  0.005   Jarque-Bera (JB):                9.239
Skew:                          -1.101   Prob(JB):                      0.00986
Kurtosis:                       5.005   Cond. No.                         372.
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
 specified.
[403.36390193  -4.40279094]
[385.25234503  -4.37465139]
=====================
            x1   const
---------------------
WLS      67.098 0.2962
OLS      71.495 0.243
OLS_HC0 72.1853  0.3
OLS_HC1 75.2583 0.3128
OLS_HC3 77.4842 0.3246
OLS_HC3  83.229 0.3514
---------------------
>>>
```

(C)

```
==== RESTART: /Users/pauline/Documents/Python/Script-038c-SM-WLS-Maria.py ====
                            WLS Regression Results
==============================================================================
Dep. Variable:                      y   R-squared:                       0.787
Model:                            WLS   Adj. R-squared:                  0.777
Method:                 Least Squares   F-statistic:                     84.77
Date:                Mon, 25 Mar 2019   Prob (F-statistic):           3.55e-09
Time:                        14:52:41   Log-Likelihood:                -169.16
No. Observations:                  25   AIC:                             342.3
Df Residuals:                      23   BIC:                             344.8
Df Model:                           1
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const        315.5801     78.993      3.995      0.001     152.171     478.989
x1            -3.4095      0.370     -9.207      0.000      -4.176      -2.643
==============================================================================
Omnibus:                       19.030   Durbin-Watson:                   0.559
Prob(Omnibus):                  0.000   Jarque-Bera (JB):               22.681
Skew:                          -1.776   Prob(JB):                     1.19e-05
Kurtosis:                       6.025   Cond. No.                         479.
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
 specified.
[134.33527365  -2.58357379]
[315.58013093  -3.40945923]
=====================
            x1   const
---------------------
WLS     78.9928 0.3703
OLS     56.3427 0.3135
OLS_HC0 72.9913 0.6456
OLS_HC1 76.0986 0.6731
OLS_HC3 85.9408 0.7792
OLS_HC3  101.88 0.9424
---------------------
>>>
```

(D)

```
Python 3.7.2 Shell
RESTART: /Users/pauline/Documents/Python/Script-038d-SM-WLS-sedim_thickness.py
                            WLS Regression Results
==============================================================================
Dep. Variable:                      y   R-squared:                       0.972
Model:                            WLS   Adj. R-squared:                  0.971
Method:                 Least Squares   F-statistic:                     790.6
Date:                Mon, 25 Mar 2019   Prob (F-statistic):           2.58e-19
Time:                        15:18:49   Log-Likelihood:                -84.895
No. Observations:                  25   AIC:                             173.8
Df Residuals:                      23   BIC:                             176.2
Df Model:                           1
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const        107.0704      6.600     16.224      0.000      93.418     120.723
x1            -1.4981      0.053    -28.117      0.000      -1.608      -1.388
==============================================================================
Omnibus:                        0.189   Durbin-Watson:                   0.819
Prob(Omnibus):                  0.910   Jarque-Bera (JB):                0.341
Skew:                          -0.169   Prob(JB):                        0.843
Kurtosis:                       2.538   Cond. No.                         676.
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
 specified.
[94.22878922 -1.38481116]
[107.07042334  -1.49808383]
=====================
            x1   const
---------------------
WLS      6.5996 0.0533
OLS      7.1957 0.0624
OLS_HC0 10.8542 0.0878
OLS_HC1 11.3163 0.0916
OLS_HC3 12.4109 0.1004
OLS_HC3 14.2337 0.1152
---------------------
```

(E)

```
*Python 3.7.2 Shell*
===== RESTART: /Users/pauline/Documents/Python/Script-038e-SM-WLS-Max.py =====
                            WLS Regression Results
==============================================================================
Dep. Variable:                      y   R-squared:                       0.993
Model:                            WLS   Adj. R-squared:                  0.992
Method:                 Least Squares   F-statistic:                     3137.
Date:                Mon, 25 Mar 2019   Prob (F-statistic):           4.27e-26
Time:                        15:54:33   Log-Likelihood:                -277.18
No. Observations:                  25   AIC:                             558.4
Df Residuals:                      23   BIC:                             560.8
Df Model:                           1
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const       5.709e+05   2.01e+04     28.471      0.000    5.29e+05    6.12e+05
x1           153.1146      2.734     56.012      0.000     147.460     158.769
==============================================================================
Omnibus:                        2.874   Durbin-Watson:                   1.583
Prob(Omnibus):                  0.238   Jarque-Bera (JB):                2.166
Skew:                          -0.717   Prob(JB):                        0.339
Kurtosis:                       2.851   Cond. No.                     5.56e+04
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly s
ecified.
[2] The condition number is large, 5.56e+04. This might indicate that there are
strong multicollinearity or other numerical problems.
[6.18224947e+05 1.60220641e+02]
[5.70897178e+05 1.53114575e+02]
=======================
              x1    const
-----------------------
WLS      20051.5494 2.7336
OLS      19940.1136 2.5811
OLS_HC0  28789.1542 3.9103
OLS_HC1  30014.7681 4.0768
OLS_HC3  32350.8706 4.4195
```

(F)

```
*Python 3.7.2 Shell*
= RESTART: /Users/pauline/Documents/Python/Script-038f-SM-WLS-slope_angle.py =
                            WLS Regression Results
==============================================================================
Dep. Variable:                      y   R-squared:                       0.899
Model:                            WLS   Adj. R-squared:                  0.895
Method:                 Least Squares   F-statistic:                     205.7
Date:                Mon, 25 Mar 2019   Prob (F-statistic):           5.84e-13
Time:                        15:21:32   Log-Likelihood:                -53.114
No. Observations:                  25   AIC:                             110.2
Df Residuals:                      23   BIC:                             112.7
Df Model:                           1
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const         24.4614      1.109     22.063      0.000      22.168      26.755
x1            -0.3323      0.023    -14.341      0.000      -0.380      -0.284
==============================================================================
Omnibus:                        1.098   Durbin-Watson:                   2.239
Prob(Omnibus):                  0.578   Jarque-Bera (JB):                1.028
Skew:                          -0.430   Prob(JB):                        0.598
Kurtosis:                       2.502   Cond. No.                         157.
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
 specified.
[25.81457577 -0.35337792]
[24.46144126 -0.33228819]
=====================
            x1   const
---------------------
WLS      1.1087 0.0232
OLS      1.1568 0.0255
OLS_HC0  1.3046 0.0277
OLS_HC1  1.3601 0.0288
OLS_HC3  1.3952 0.0299
OLS_HC3  1.4941 0.0323
---------------------
```

Table 2. Computed results of the WLS modelling for the data distribution by plates: A) Pacific; B) Philippine; C) Mariana; D) Sediment thickness; E) Depths; F) Slope angle.

(A)

```
                         QuantReg Regression Results
==============================================================================
Dep. Variable:                profile   Pseudo R-squared:               0.2581
Model:                       QuantReg   Bandwidth:                       13.16
Method:                 Least Squares   Sparsity:                        22.34
Date:                Mon, 25 Mar 2019   No. Observations:                   25
Time:                        10:09:07   Df Residuals:                       23
                                        Df Model:                            1
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept     17.0000      3.450      4.928      0.000       9.864      24.136
plate_pacif   -0.0354      0.022     -1.587      0.126      -0.081       0.011
==============================================================================
      q          a         b        lb        ub
0  0.05   1.000007  0.005050       NaN       NaN
1  0.15   7.771084 -0.012048       NaN       NaN
2  0.25  15.038463 -0.038461 -0.085862  0.008939
3  0.35  15.874999 -0.037500 -0.083386  0.008386
4  0.45  16.000000 -0.033491 -0.079744  0.012762
5  0.55  17.199201 -0.035857 -0.079062  0.007349
6  0.65  17.999998 -0.037879 -0.084098  0.008339
7  0.75  25.783556 -0.057534 -0.109538 -0.005530
8  0.85  27.514284 -0.057143       NaN       NaN
9  0.95  27.500001 -0.046296       NaN       NaN
```

(B)

```
                         QuantReg Regression Results
==============================================================================
Dep. Variable:                profile   Pseudo R-squared:               0.5912
Model:                       QuantReg   Bandwidth:                       4.941
Method:                 Least Squares   Sparsity:                        8.064
Date:                Mon, 25 Mar 2019   No. Observations:                   25
Time:                        09:51:42   Df Residuals:                       23
                                        Df Model:                            1
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept      2.3059      1.574      1.465      0.156      -0.950       5.562
plate_phill    0.0463      0.005      8.649      0.000       0.035       0.057
==============================================================================
      q         a         b        lb        ub
0  0.05  2.188076 -0.002294       NaN       NaN
1  0.15  0.753246  0.043290       NaN       NaN
2  0.25  2.395155  0.040323  0.029318  0.051327
3  0.35  2.387755  0.040816  0.029346  0.052286
4  0.45  2.305933  0.046271  0.035415  0.057128
5  0.55  2.584616  0.046154  0.035049  0.057258
6  0.65  2.764206  0.048295  0.037708  0.058883
7  0.75  4.843333  0.043333  0.031794  0.054872
8  0.85  5.010830  0.043321       NaN       NaN
9  0.95  5.657380  0.044568       NaN       NaN
```

(C)

```
                         QuantReg Regression Results
==============================================================================
Dep. Variable:                profile   Pseudo R-squared:               0.4455
Model:                       QuantReg   Bandwidth:                       5.825
Method:                 Least Squares   Sparsity:                        11.46
Date:                Mon, 25 Mar 2019   No. Observations:                   25
Time:                        09:44:40   Df Residuals:                       23
                                        Df Model:                            1
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept     21.0000      1.872     11.220      0.000      17.128      24.872
plate_maria   -0.0518      0.010     -4.975      0.000      -0.073      -0.030
==============================================================================
      q          a         b        lb        ub
0  0.05   1.000005  0.002294       NaN       NaN
1  0.15   8.199082 -0.014218       NaN       NaN
2  0.25  20.606061 -0.060606 -0.081754 -0.039458
3  0.35  20.950741 -0.054187 -0.073418 -0.034957
4  0.45  21.000000 -0.053253 -0.074117 -0.032390
5  0.55  21.999999 -0.055556 -0.075791 -0.035320
6  0.65  22.000001 -0.045918 -0.069054 -0.022783
7  0.75  23.999997 -0.050459 -0.076879 -0.024038
8  0.85  24.999953 -0.050000       NaN       NaN
9  0.95  25.000000 -0.044341       NaN       NaN
```

(D)

```
                         QuantReg Regression Results
==============================================================================
Dep. Variable:                profile   Pseudo R-squared:               0.1607
Model:                       QuantReg   Bandwidth:                       12.94
Method:                 Least Squares   Sparsity:                        22.44
Date:                Mon, 25 Mar 2019   No. Observations:                   25
Time:                        09:52:54   Df Residuals:                       23
                                        Df Model:                            1
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept     12.0000      2.408      4.984      0.000       7.019      16.981
plate_carol    0.4333      0.165      2.623      0.015       0.092       0.775
==============================================================================
      q          a         b        lb        ub
0  0.05   2.000000  0.338983       NaN       NaN
1  0.15   4.000000  0.305085       NaN       NaN
2  0.25   6.000001  0.271186 -0.270035  0.812408
3  0.35   8.000018  0.237288 -0.198529  0.673105
4  0.45  11.000000  0.466634  0.113059  0.820209
5  0.55  12.999999  0.400000  0.064198  0.735802
6  0.65  15.000000  0.333333  0.019033  0.647633
7  0.75  16.999998  0.300000 -0.013342  0.613342
8  0.85  18.999994  0.217392       NaN       NaN
9  0.95  20.714286  0.142857       NaN       NaN
```

(E)

```
                         QuantReg Regression Results
==============================================================================
Dep. Variable:                profile   Pseudo R-squared:               0.2080
Model:                       QuantReg   Bandwidth:                       12.09
Method:                 Least Squares   Sparsity:                        21.97
Date:                Mon, 25 Mar 2019   No. Observations:                   25
Time:                        09:49:29   Df Residuals:                       23
                                        Df Model:                            1
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept     32.8295      9.246      3.551      0.002      13.703      51.956
sedim_thick   -0.1477      0.080     -1.843      0.078      -0.314       0.018
==============================================================================
      q          a         b        lb        ub
0  0.05   5.551728 -0.034483       NaN       NaN
1  0.15  -2.055556  0.055556       NaN       NaN
2  0.25  11.704224 -0.042253 -0.232282  0.147775
3  0.35  32.087912 -0.175824 -0.324716 -0.026932
4  0.45  30.644553 -0.144445 -0.310477  0.021587
5  0.55  32.829530 -0.147727 -0.303778  0.008324
6  0.65  33.000005 -0.142857 -0.282081 -0.003633
7  0.75  36.902437 -0.170732 -0.286019 -0.055444
8  0.85  36.999996 -0.160000       NaN       NaN
9  0.95  35.833333 -0.145833       NaN       NaN
```

(F)

```
                         QuantReg Regression Results
==============================================================================
Dep. Variable:                profile   Pseudo R-squared:              0.07835
Model:                       QuantReg   Bandwidth:                       13.89
Method:                 Least Squares   Sparsity:                        24.22
Date:                Mon, 25 Mar 2019   No. Observations:                   25
Time:                        09:49:07   Df Residuals:                       23
                                        Df Model:                            1
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept     23.2222      8.306      2.796      0.010       6.039      40.405
slope_angle   -0.2222      0.183     -1.215      0.237      -0.601       0.156
==============================================================================
      q          a         b        lb        ub
0  0.05  -0.923076  0.076923       NaN       NaN
1  0.15  -0.000022  0.062501       NaN       NaN
2  0.25   9.255824 -0.046512 -0.558346  0.465322
3  0.35  18.499998 -0.178571 -0.607333  0.250190
4  0.45  23.222223 -0.222222 -0.607558  0.163114
5  0.55  27.769232 -0.269231 -0.639233  0.100772
6  0.65  32.956522 -0.347826 -0.692941 -0.002711
7  0.75  33.565218 -0.347826 -0.671457 -0.024195
8  0.85  35.333327 -0.333333       NaN       NaN
9  0.95  33.999997 -0.281250       NaN       NaN
```

Table 3. Results of the computations for the quantile regression for sediment thickness versus geologic parameters: A) Pacific Plate, B) Philippine Plate, C) Mariana Plate, D) Caroline Plate, E) Cumulative sediment thickness and F) Slope angle degree by profiles.

```
models = [fit_model(x) for x in quantiles]
models = pd.DataFrame(models, columns=['q', 'a',
'b','lb','ub'])
ols = smf.ols('profile ~ slope_angle', data).fit()
ols_ci    =    ols.conf_int().loc['slope_angle'].
tolist()
ols = dict(a = ols.params['Intercept'],
           b = ols.params['slope_angle'],
           lb = ols_ci[0],
           ub = ols_ci[1])
print(models)
print(ols)
```

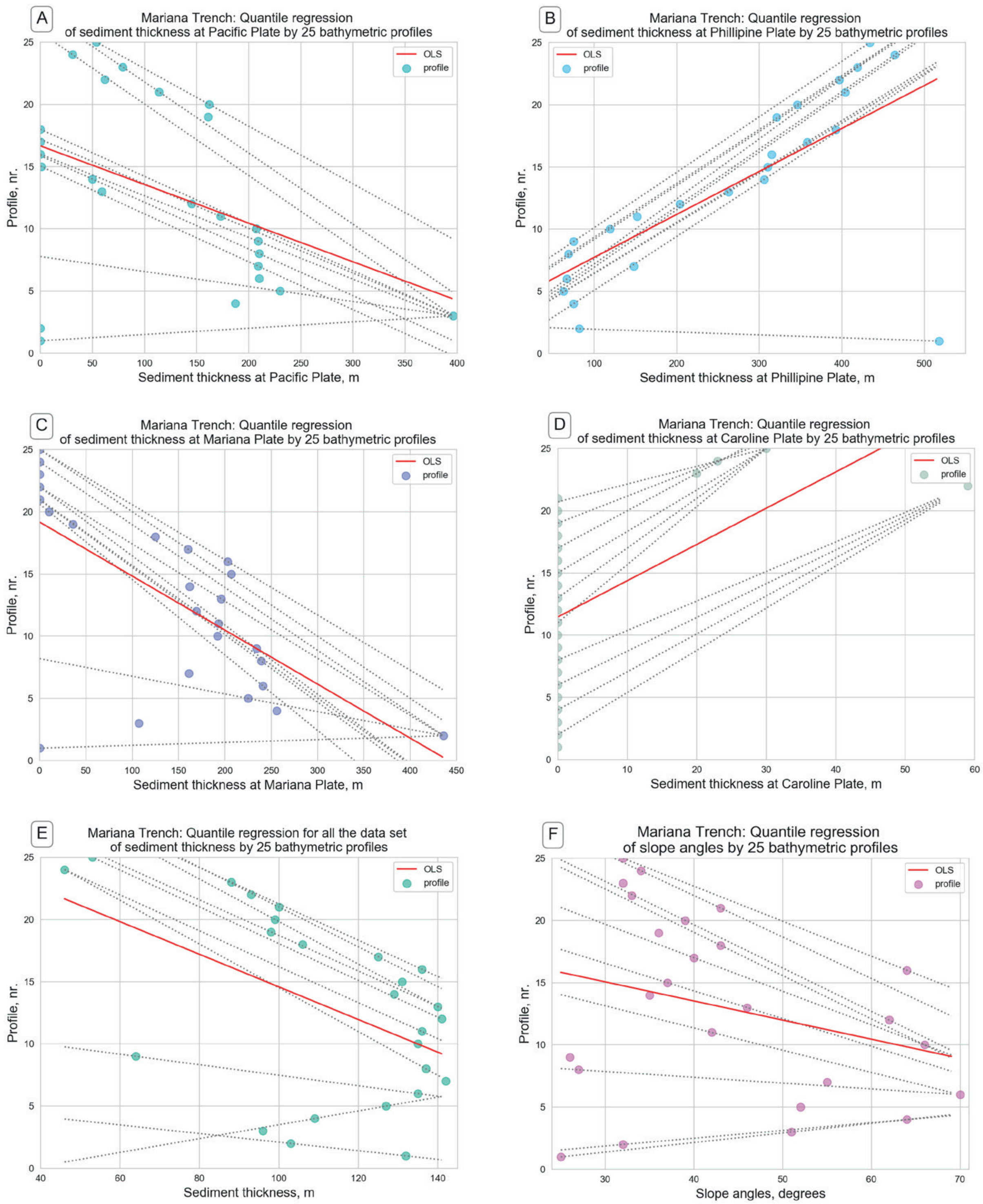

Figure 4. Quantile regression plotted for sediment thickness (m) versus geologic parameters: A) Pacific Plate, B) Philippine Plate, C) Mariana Plate, D) Caroline Plate, E) Cumulative sediment thickness and F) Slope angle degree by profiles.

Essentially, quantile regression is another approach of the linear regression tested in the current research. The quantitative results of the quantile regression are shown on Table 3, with respect to the relevant plots shown on Figure 4 (corresponding to Figure 4: A, B, C, D, E, F).

Dynamic regression model: State Autoregressive Moving Average (SARIMA)

The methodology of the dynamic regression model is based on the StatsModels embedded algorithm (Seabold & Perktold, 2010).

The abbreviation of SARIMA is the Space AutoRegressIve Moving Average model, initially developed by Ansley & Kohn, (1985). The concept of the application of the SARIMA time series estimation and post-estimation lies in the following. The changes in the variables that are taken as time lags in classic dynamic regression models are applied towards bathymetric profiles from 1 to 25. In this way, unlike in time series case when the SARIMA fits the univariate models with time-dependent disturbances, this case applies space-dependent disturbances, crossing the sampling selection from 1 to 25 (X axe).

Because the model includes both dependent and independent variables, the selected type of SARIMA was SARIMAX (see the Python code snippet below). The first group consists in changing variables that is geologic settings and bathymetry (depths). The second group (independent variables) is presented by the profiles lags that cross the Mariana Trench with the distance between each of 100 km and the length of 1000 km. This cross-section profiles are taken as independent variables. Therefore, the dependant variables differ spatially in different segments of the trench.

The model (Figure 5) fits univariate model of the geomorphic structure of the trench by independent values of the distribution of the bathymetric observation by profiles with dependent disturbances of depths.

Fitting the model was done using the Python snippet:

```
mod = sm.tsa.statespace.SARIMAX(data['sedim_thick'], trend='c', order=(1,1,1))
res = mod.fit(disp=False)
print(res.summary())
fig = sm.graphics.tsa.plot_pacf(data.iloc[1:]['Ddf.geology'], lags=25, ax=axes[1])
```

The algorithm fits a model where the disturbances follow a linear specification of the bathymetric distribution across the trench. The dependent and independent geological variables vary by profiles (Figure 5). Plotting was done using sublplot function of Matplotlib:

```
fig, axes = plt.subplots(2, 2, figsize=(15,8))
```

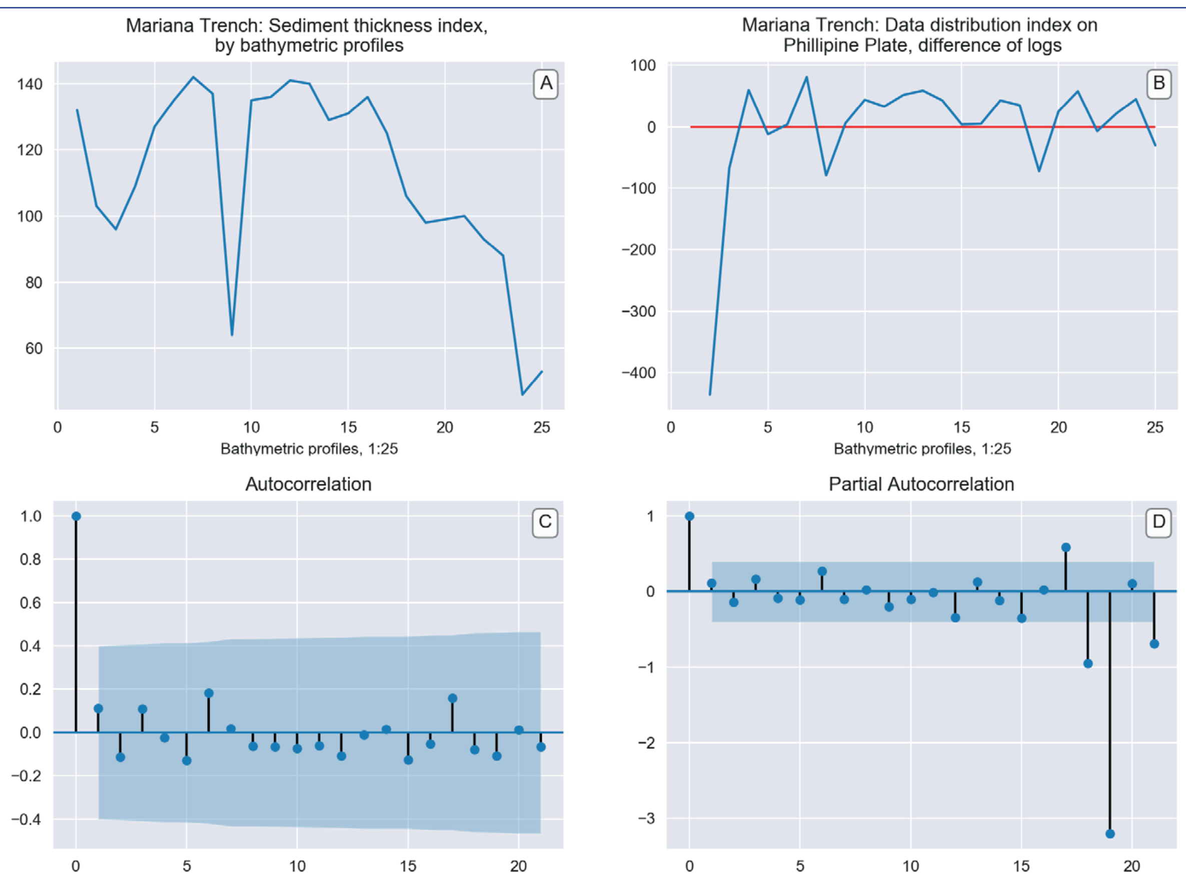

Figure 5. Plotted SARIMAX statistics for the bathymetry: A) Sediment thickness index; B) Data distribution index, Philippine Plate; C) Autocorrelation; D); Partial Autocorrelation.

Script-040-Sarimax Stata.py - /Users/pauline/Documents/Python/Script-040-Sarimax Stata.py (3.7.2)

```python
    # Step-1. Import data
os.chdir('/Users/pauline/Documents/Python')
data = pd.read_csv("Tab-Morph.csv")
data.index = data.profile
    # Step-2. Fitting the model by sediment thickness
mod = sm.tsa.statespace.SARIMAX(data['sedim_thick'], trend='c', order=(1,1,1))
res = mod.fit(disp=False)
print(res.summary())
    # Step-3. Dataset
data['sedim_thickn'] = np.log(data['sedim_thick'])
data['D.sedim_thickn'] = data['plate_phill'].diff()
    # Step-4. Graph data
fig, axes = plt.subplots(1, 2, figsize=(15,4))
axes[0].plot(data.index._mpl_repr(), data['sedim_thick'], '-')
axes[0].set_xlabel('Bathymetric profiles, 1:25', fontsize=10)
axes[0].set(title='Mariana Trench: Sediment thickness index, \nby bathymetric profiles')
axes[0].annotate('A', xy=(0.95, .92), xycoords="axes fraction", fontsize=12,
           bbox=dict(boxstyle='round, pad=0.3', fc='w', edgecolor='grey', linewidth=1, alpha=0.9))
    # Step-5. Log difference
axes[1].plot(data.index._mpl_repr(), data['D.sedim_thickn'], '-')
axes[1].hlines(0, data.index[0], data.index[-1], 'r')
axes[1].set_xlabel('Bathymetric profiles, 1:25', fontsize=10)
axes[1].set(title='Mariana Trench: Data distribution index on \nPhillipine Plate, difference of logs');
axes[1].annotate('B', xy=(0.95, .92), xycoords="axes fraction", fontsize=12,
           bbox=dict(boxstyle='round, pad=0.3', fc='w', edgecolor='grey', linewidth=1, alpha=0.9))
    # Step-6. Graph data
fig, axes = plt.subplots(1, 2, figsize=(15,4))
fig = sm.graphics.tsa.plot_acf(data.iloc[1:]['D.sedim_thickn'], lags=21, ax=axes[0])
axes[0].annotate('C', xy=(0.95, .92), xycoords="axes fraction", fontsize=12,
           bbox=dict(boxstyle='round, pad=0.3', fc='w', edgecolor='grey', linewidth=1, alpha=0.9))
fig = sm.graphics.tsa.plot_pacf(data.iloc[1:]['D.sedim_thickn'], lags=21, ax=axes[1])
axes[1].annotate('D', xy=(0.95, .92), xycoords="axes fraction", fontsize=12,
           bbox=dict(boxstyle='round, pad=0.3', fc='w', edgecolor='grey', linewidth=1, alpha=0.9))
plt.show()
```

```
                           Statespace Model Results
==============================================================================
Dep. Variable:            sedim_thick   No. Observations:                   25
Model:               SARIMAX(1, 1, 1)   Log Likelihood                -108.677
Date:                Fri, 29 Mar 2019   AIC                            225.354
Time:                        10:49:01   BIC                            230.066
Sample:                             0   HQIC                           226.604
                                 - 25
Covariance Type:                  opg
==============================================================================
                 coef    std err          z      P>|z|      [0.025      0.975]
------------------------------------------------------------------------------
intercept     -1.8019      2.957     -0.609      0.542      -7.597       3.993
ar.L1          0.2696      0.390      0.692      0.489      -0.494       1.033
ma.L1         -0.6914      0.414     -1.669      0.095      -1.503       0.121
sigma2       495.4968    197.897      2.504      0.012     107.625     883.369
===================================================================================
Ljung-Box (Q):                       18.06   Jarque-Bera (JB):                 4.25
Prob(Q):                              0.75   Prob(JB):                         0.12
Heteroskedasticity (H):               0.38   Skew:                            -0.73
Prob(H) (two-sided):                  0.19   Kurtosis:                         4.46
===================================================================================

Warnings:
[1] Covariance matrix calculated using the outer product of gradients (complex-step).
>>>
```

Ln: 28   Col: 29

Table 4. Python programming code for used SARIMAX algorithm (left) and Statespace Model Results (right). Tested case in Mariana Trench tectonics: sediment thickness across Philippine Plate.

RESULTS AND DISCUSSIONS

The conducted statistical data modelling and several types of the regression analysis were aimed to compare the variance in the geological data sets of the Mariana Trench explained by the complex interplay of the geomorphic, geological and oceanological attributes of the data and the bathymetric factors of the location of several segments of the trench in various parts of the Pacific Ocean. Using StatsModels library of Python, in particular several linear models of the correlation between various factors were computed, analysed and explained by the groups of variables.

Tested environmental variables of the Mariana Trench include four main geological factors (location on the tectonic plates, slope steepness degree, sediment thickness of the layer and bathymetric depth and submarine volcanism) and attributes of the 25 cross-section bathymetric profiles (mean values, maximal depths, median values and two quartile sub-division of the data sets) and the shared variances between environment and attributes. Shared variance arises due to correlations between the factor of sediment thickness and slope angle degree, e.g. because the attributes of the sediment accumulation are influenced by the canyon shape apart from the directly or indirectly depending on the oceanological conditions of the submarine currents.

The graphical output shows normal data distribution as demonstrated by (Figure 2), where each QQ profile has a given value for data distribution by quantiles across the trench profiles. The distribution of the geologic residuals is shown on Figure 3 showing particularly data correlation for several cases: frequency of the data distribution by Pacific Plate, Philippine Plate, Mariana Plate, sediment thickness, and range of the bathymetric depths taken for the maximal values, and finally, geomorphological shape as slope angle degree. The results shown on Table 2 represent the computed numerical values of the previous graph (Figure 3).

The results on the Quantile regression (Figure 4) show the conditional median of the response geologic variable given changing bathymetric values with movement southwards across the Mariana Trench. Thus, the upper plots shows (Figure 4, A, B, C) data distribution across tectonic plates: Pacific Plate, Philippine Plate, Mariana Plate and Caroline Plate. The lower plot (Figure 4, D, E, F) shows data distribution for the Caroline Plate and cumulative sediment thickness and slope angle degree by profiles.

The numerical explanation of the Figure 4 with corresponding sub-plots is presented in Table 3. The Figure 5 shows dynamic regression model using Python function embedded in StatsModel: State Autoregressive Moving Average. Finally, Table 4 shows the Python code that was used to perform the procedure of SARIMAX and the resulting output table. The model shows autocorrelation of the data by bathymetric profiles. The results demonstrated a correlation between the geological variables and geospatial location of the samplings across Mariana Trench, which proves the interplay between multiple factors affecting its geomorphology.

CONCLUSIONS

While the usage of the traditional methods of geoinformatics and spatial analysis is, beyond doubts, strongly recommended for any research in geoscience, there is another powerful tool for the geospatial data processing other than GIS, sometimes overlooked or skipped by the geographers: a data modelling by use of Python or R programming languages. Python, an open source free programming language is highly suitable for the statistical analysis in geoscience research, since it has a powerful statistical and math libraries, e.g. StatsModel, highly effective for scientific computing and used in the current research. The functionality of Python language and StatsModel, tested in this work, is proved to be highly effective for the statistical analysis of the geo-marine sets.

The proposed approach of the Python based statistical analysis enables accurate and efficient computation and modelling of the large data sets in marine geology and oceanology. A challenge in the evaluation of geological big data sets (that is, several thousand of observation points as in this case, 12.590 samples) concerns the difficulty in manual identifying a correct algorithms in the computations and data distribution analysis.

The necessity to apply a precise machine learning algorithms is recently increased in geographic sciences with respect to the importance of choosing an effective method for data visualization and computation. The solutions to the mentioned above problems are provided by Pandas data frames: for example, optimizing structure of the data, selecting the correct parts from the whole data frame (columns, rows in the data arrays) and plotting. Based on the presented results, the application of Python programming language is strongly recommended in geoscience research as an addition to the traditional GIS methods.

Conflict of Interests: The author declares no conflict of interest.

Funding: This research was funded by the China Scholarship Council (CSC), State Oceanic Administration (SOA), Marine Scholarship of China, Grant Nr. 2016SOA002, Beijing, People's Republic of China.

REFERENCES

Ansley, C. F. & R. J. Kohn. (1985). Estimation, filtering, and smoothing in state space models with incompletely specified initial conditions. *Annals of Statistics* 13, 1286–1316. [CrossRef]

Bello-González, J. P., Contreras-Reyes, E. & Arriagada, C. (2018). Predicted path for hotspot tracks off South America since Paleocene times: Tectonic implications of ridge-trench collision along the Andean margin. *Gondwana Research*, 64, 216–234. [CrossRef]

Boston, B., Moore, G. F., Nakamura, Y. & Kodaira, S. (2017). Forearc slope deformation above the Japan Trench megathrust: Implications for subduction erosion. *Earth and Planetary Science Letters*, 462, 26–34. [CrossRef]

Björck, A. (1996). Numerical methods for least squares problems. SIAM, Philadelphia. ISBN 0-89871-360-9. [CrossRef]

Box, G. E. P.; Tiao, G. C. (1992). *Bayesian Inference in Statistical Analysis*. New York: John Wiley and Sons. ISBN 0-471-57428-7. (Section 8.1.1). [CrossRef]

Dierssen, H. M. & Theberge Jr. A. E. (2014). Bathymetry: History of Seafloor Mapping. Encyclopedia of Natural Resources, Taylor & Francis. [CrossRef]

Fujie, G., Ito, A., Kodaira, S., Takahashi, N., & Kaneda, Y. (2006). Confirming sharp bending of the Pacific plate in the northern Japan trench subduction zone by applying a traveltime mapping method. *Physics of the Earth and Planetary Interiors*, 157, 72–85. [CrossRef]

Grand, S. P., Hilst, R. D. van der, & Widiyantoro, S. (1997). Global Seismic Tomography: A Snapshot of Convection in the Earth. *GSA Today*, 7(4), 2–7.

Lemenkova, P. (2019). Processing Oceanographic Data by Python Libraries NumPy, SciPy And Pandas. *Aquatic Research*, 2(2), 73-91. [CrossRef]

Ljung, G. M. & Box, G. E. P. (1978). On a Measure of a Lack of Fit in Time Series Models. *Biometrika*. 65 (2): 297–303. [CrossRef]

Michibayashi, K., Tasaka, M., Ohara, Y., Ishii, T., Okamoto, A., & Fryer, P. (2007). Variable microstructure of peridotite samples from the southern Mariana Trench: Evidence of a complex tectonic evolution. *Tectonophysics*, 444, 111–118. [CrossRef]

Millman, K. J. & Aivazis, M. (2011). Python for Scientists and Engineers, *Computing in Science & Engineering*, 13, 9-12. [CrossRef]

Oliphant, T. (2015). *Guide to NumPy* (2 ed.). CreateSpace. ISBN 978-1517300074.

Oliphant, T. E. (2007). *Python for scientific computing*. Computing in Science & Engineering 9(3), 10-20. [CrossRef]

Reid, W. D. K., Cuomo, N. J., & Jamieson, A. J. (2018). Geographic and bathymetric comparisons of trace metal concentrations (Cd, Cu, Fe, Mn, and Zn) in deep-sea lysianassoid amphipods from abyssal and hadal depths across the Pacific Ocean. *Deep-Sea Research Part I*, 138, 11–21. [CrossRef]

Schellart, W. P. (2008). Subduction zone trench migration: Slab driven or overriding-plate-driven? *Physics of the Earth and Planetary Interiors*, 170, 73–88. [CrossRef]

Seabold, S. & Perktold, J. (2010). Statsmodels: Econometric and statistical modeling with python. Proceedings of the 9th Python in Science Conference.

Smith, W. H. F., & Sandwell, D. T. (1997). Global Sea Floor Topography from Satellite Altimetry and Ship Depth Soundings. *Science*, 277, 1956–1962. [CrossRef]

Strutz, T. (2016). *Data Fitting and Uncertainty (A practical introduction to weighted least squares and beyond)*. Springer Vieweg. ISBN 978-3-658-11455-8.

Taira, K., Yanagimoto, D., & Kitagawa, S. (2005). Deep CTD Casts in the Challenger Deep, Mariana Trench. *Journal of Oceanography*, 61, 447–454. [CrossRef]

Theberge, A. (2008). Thirty years of discovering the Mariana Trench. *Hydro International*, 12, 38–39.

Timm, N. H. (2007). *Applied Multivariate Analysis*. Springer Science & Business Media, 695 p. ISBN: 978-0-387-95347-2. [CrossRef]

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2019; 34(2): 61-66 • DOI: https://doi.org/10.26650/ASE540928

Research Article

Impacts of Cycled Dietary Protein Ratios on the Growth Performance and Somatic Indices of Meagre (*Argyrosomus regius*)

Derya Güroy[1], Onur Karadal[2]

Cite this article as: Guroy, D., Karadal, O. (2019). Impacts of cycled dietary protein ratios on the growth performance and somatic indices of meagre (Argyrosomus regius). *Aquatic Sciences and Engineering, 34*(2), 61-66.

ORCID IDs of the authors:
D.G. 0000-0002-8254-1403;
O.K. 0000-0002-6241-5039

[1]Department of Aquaculture, Armutlu Vocational School, Yalova University, 77500, Armutlu, Yalova, Turkey
[2]Department of Aquaculture, Faculty of Fisheries, İzmir Kâtip Çelebi University, 35620, Çiğli, İzmir, Turkey

Submitted:
16.03.2019

Revision Requested
22.04.2019

Last Revision Received
25.04.2019

Accepted:
25.04.2019

Online published:
28.06.2019

Correspondence:
Derya Güroy
E-mail:
dguroy@yahoo.com

Onur Karadal
E-mail:
onur.karadal@ikc.edu.tr

ABSTRACT

Protein is both an essential and a costly nutrient in aquafeeds. In this study, the effects of different cyclic protein ratios on growth performance, biological indices, and profitability of meagre (*Argyrosomus regius*) were investigated. Diets which included 48, 44, and 40% protein ratios were prepared, and the experimental groups were designed by cyclical periods of 48% for 12 weeks (P48, control), 40% for 6 weeks + 48% for 6 weeks (P40/48), and 44% for 6 weeks + 48% for 6 weeks (P44/48). The study was carried out with three replicates for 12 weeks in nine 450 L fiberglass tanks connected to a recirculating marine water system. Meagre with an average weight of 49.93±0.35 g were kept with 10 fish per tank. At the end of the study, the best weight gain was observed in the P48 group compared to the other treatments ($P<0.05$). However, no statistical differences were found between the specific growth rates and feed conversion ratios of the experimental groups ($P>0.05$). In addition, both condition factor and viscerosomatic index were higher in P44/48 and P40/48, respectively ($P<0.05$). In conclusion, aquafeeds with a protein content of 48% can be circulated for short periods as part of 40% protein diets.

Keywords: Marine aquaculture, fish nutrition, cycled feeding, profitability, *Argyrosomus regius*

INTRODUCTION

Protein is a dietary nutrient that directly affects growth, weight gain, and biological parameters in fish. Protein requirements of fish are influenced by several biotic and abiotic factors, including species, size, age, physiological situation, diet quality, protein to energy ratio, temperature, salinity, etc. (Tacon and Cowey, 1985; NRC, 2011). Determination of daily protein amounts per animal is particularly necessary in culture conditions (Kureshy and Davis, 2002). Dietary protein deficiency prevents fish from reaching their maximum growth values and excessive protein in the feed is not suitable both economically and environmentally (Lovell, 1989). Nevertheless, protein is considered as a limiting factor in aquafeeds, because of its high costs (Gatlin III et al., 2007). For this purpose, researchers have focused on decreasing protein ratios (especially fish meal proteins) in aquafeeds using various methods such as increasing the amount of plant-based raw materials (soybean meal, corn meal, etc.), using alternative animal-based protein sources (squid meal, krill meal, etc.), and applying different feeding strategies (feeding level, feeding frequency, feeding rate, etc.). However, some of these methods are not suitable for certain fish species. For example, carnivorous fish need a diet including high amounts of animal-based proteins and increasing the level of plant-based ingredients in their aquafeed depresses the growth of fish (Hartviksen et al., 2014). The best method for these species is to apply specific feeding regimes, including cycled nutrition combined with diets containing different protein levels.

Feeding is an important operation in aquaculture because it accounts for the largest share of expenses in the production process. This share might be balanced with the application of proper feeding regime (Güroy et al., 2017). Feeding management depends on different factors, including species, size of fish, physical conditions of facilities, farmer/staff experience. Regular feeding regimes should specify an integrated schedule of these factors. Regulating the optimum feeding schedule for cultured species may improve the growth performance, survival rate, and feed utilization by decreasing feed wastage (Jiwyam, 2010; Okorie et al., 2013). One of the basic applications of a proper feeding regime is the cycling of aquafeeds or feed ingredients. Some compensatory growth studies have been carried out on different commercial fish species, including rainbow trout (*Oncorhynchus mykiss*), gilthead sea bream (*Sparus aurata*), and European sea bass (*Dicentrarchus labrax*) (Quinton and Blake, 1990; Eroldoğan et al., 2008; Türkmen et al., 2012) which focus on feeding the fish with restricted/cycled periods or starvation. These kinds of studies aimed to minimize consumption of feed by decreasing or limiting and cycling the portions and thus, improving profitability. In other feeding regime studies, the ratio of important nutrients such as protein in the diet was reduced instead of the feeding rate. For example, Gaylord and Gatlin III (2001) carried out a study with diets which included different protein and energy ratios on channel catfish (*Ictalurus punctatus*).

Meagre (*Argyrosomus regius*) is a high potential marine fish which is popular in Mediterranean aquaculture due to its valuable meat quality and flavour (Vallés and Estévez, 2013; Güroy et al., 2017). This species has been readily accepted for culturing by farmers because of its exceptional natural features in culture conditions such as its high growth performance (Monfort, 2010; Ribeiro et al., 2013). Also, meagre shows advanced survival rates, optimum feed conversion ratio, and a more rapid growth rate than gilthead sea bream (*S. aurata*) and sea bass (*D. labrax*) in aquaculture conditions (Barata et al., 2016; Emre et al., 2016). The market share of meagre is increasing day by day. Determining the specific feed needs and feeding regimes of meagre is important for the expansion of market share.

The aim of this present study was to investigate the influence of diets composed of different protein ratios by giving cyclical portions to meagre (*Argyrosomus regius*). Thus, it is predicted that using a combination of cyclical feeds alternating low and high protein content may reduce feed costs if it leads to positive effects on growth performance.

MATERIALS AND METHODS

Experimental design and diets

Three protein levels were determined (40, 44, and 48%) and cycled feeding regimes were applied in this study. The control group (P48) was continuously fed with diets containing 48% protein during the experiment. P40/48 and P44/48 groups were fed

Table 1. Formulation and proximate composition of the experimental diets

Ingredients	P40	P44	P48
Fish meal[1]	18.80	25.00	34.00
Corn gluten[2]	14.50	14.50	14.50
Dehulled soybean meal[3]	11.00	11.00	11.00
Poultry by-product[4]	6.00	6.00	6.00
Feather meal[5]	2.40	2.40	2.40
Wheat gluten[6]	2.50	2.50	2.50
Wheat flour[7]	17.56	12.82	9.00
Sunflower seed meal[8]	12.00	11.00	8.00
Fish oil[9]	13.65	13.65	12.15
Vitamin premix[10]	0.20	0.20	0.20
Mineral premix[10]	0.15	0.15	0.15
Choline chloride[10]	0.10	0.10	0.10
Dicalcium phosphate[10]	1.14	0.68	0.00
Chemical Composition			
Crude Protein (%)	41.32	44.44	48.78
Crude Lipid (%)	18.12	18.72	18.14
Crude Ash (%)	5.31	5.86	6.61
Crude Fibre (%)	4.04	3.75	3.10
NFE (%)*	29.96	26.44	23.27

[1]Anchovy fishmeal, Sibal Feed Mill Company, Sinop, Turkey
[2]Cargill, İstanbul, Turkey
[3]Kırcı Soya Company, Balıkesir, Turkey
[4]Sibal Feed Mill Company, Sinop, Turkey
[5]Sibal Feed Mill Company, Sinop, Turkey
[6]Cargill, İstanbul, Turkey
[7]İpek Wheat Company, Nevşehir, Turkey
[8]Sibal Feed Mill Company, Sinop, Turkey
[9]Anchovy fish oil, Sibal Feed Mill Company, Sinop, Turkey
[10]DSM Nutritional Products, İstanbul, Turkey
*Nitrogen-free extract (NFE) = 100 - (protein + lipid + ash + fibre)

with diets of 40% and 44% protein ratios in the first half (initial 6 weeks) of the experiment, respectively, and then during the second half of the experiment (last 6 weeks), these groups were fed with diets containing 48% protein. All experimental diets with 40, 44 and 48% dietary protein were formulated using fish meal, plant ingredients, fish oil and vitamin-mineral mix (Table 1). Dietary ingredients were mixed in a food mixer (Dirmak Food Equipment, IBT-22) with water until a soft slightly moist consistency was achieved. This dough was then cold press extruded (La Monferrina, PTM P6) to produce discs of 4 mm in diameter. The moist pellets were then fan-dried and stored at -18 °C until use.

Rearing system and fish

Meagre were obtained from a commercial hatchery in Muğla, Turkey and transported to the Marine and Freshwater Fish Unit of Armutlu Vocational School, Yalova University, Turkey. Prior to the feeding trials, the fish were transferred to the 450-L tanks and fed a commercial sea bass diet (45% protein, 18% lipid, 2.5% fibre, 7% ash; Sürsan Inc., Turkey) for two weeks for acclimation. Nine 450-L circular tanks within a 25000-L recirculation seawater system were annexed to a 650-L sand filter, bag filter, and ultraviolet light unit. A partial seawater exchange (15% total system volume) was performed twice each week. The water flow rate was 5 L/min and water quality was monitored daily. Water temperature was maintained at 22.7±1.01 °C and dissolved oxygen at 8.12±0.29 mg/L (Handy Polaris, Oxy-Guard). Average pH was recorded at 7.89±0.17 (HANNA HI 9125), salinity at 21.2±0.10 ppt (Atc Refractometer), and ammonia at 0.13±0.02 mg/L (Hach Lange DR 2800). The recirculation system was housed in a climate-controlled unit with controlled photoperiod 12:12 h (light:dark). Meagre (mean body weight 49.93±0.35 g) were randomly distributed over nine fiberglass tanks at a density of 10 fish per tank. Fish were hand-fed to near satiation three times a day (09:00, 13:00, and 17:00) for 84 days. Feed was carefully administered by dropping a few pellets into the tanks until the feeding activity ceased.

Evaluation of growth performance

Fish were weighed biweekly in bulk after suspending feeding for 1 day. All fish were anesthetized with clove oil (50 mg/L) during the weighing procedure (Barata et al. 2016). Growth parameters, including feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) were calculated using the following equations: FCR = feed intake / weight gain, SGR = 100 x [(Ln final fish weight) - (Ln initial fish weight)] / experimental days, SR = 100 x (total fish count - dead fish count) / total fish count.

Measurement of somatic indices

Three fish per tank were randomly selected, individually weighed, and then dissected to obtain the viscera to determine biological parameters. The somatic indices, including condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and mesenteric fat index (MFI) were calculated using the following formulae: CF = 100 x (final body weight / fork length^3), VSI = 100 x (viscera weight / final body weight), HSI = 100 x (liver weight / final body weight), MFI = 100 x (body fat weight / final body weight).

Economic analysis

Economic analyses in terms of economic conversion ratio (ECR) and economic profit index (EPI) were developed by Sánchez-Lozano et al. (2007). These parameters were determined using the following formulae: ECR = feed conversion ratio x feed price, EPI = (final body weight x fish sale price) - (economic conversion ratio x weight gain).

The cost of feed ingredients was as listed by index mundi (IndexMundi, 2018). Meagre sale price was calculated at 4.5 €/kg.

Statistical analysis

The Shapiro-Wilk W test and Levene test were examined to verify normality and homogeneity of variance before further analysis was undertaken. All data was subjected to one-way analysis of variance (ANOVA), after proving the normality and homogeneity of the data. Tukey's multiple range test was used to rank groups when different interaction was found using Statgraphics Centurion XVI (Statpoint Technologies Inc., The Plains, VA) statistical software (Zar, 1999). All data was presented as "mean±standard error" of the mean calculated from all replicates. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSIONS

Growth parameters recorded for 6 weeks and 12 weeks of the study are given in Table 2. The mean weight at the 6th week of the P48 group was significantly different to the P44/48 group ($P<0.05$). Feed intake at the 6th week of the P48 group was high-

Table 2. Growth parameters of meagre fed with diets containing different protein ratios by cyclical feeding regimes for 6 weeks (6w) and 12 weeks (12w)

	P40/48	P44/48	P48
Initial mean weight (g)	49.59±0.60	49.09±0.75	50.22±0.22
Mean weight at 6w (g)	69.02±1.41^{ab}	65.15±1.26^{a}	74.04±1.83^{b}
Mean weight at 12w (g)	86.04±0.87^{a}	82.77±2.28^{a}	94.25±1.71^{b}
Feed intake at 6w (g)	73.64±0.16^{a}	75.79±0.84^{a}	83.37±4.23^{b}
Feed intake at 12w (g)	74.85±0.66^{a}	79.03±0.49^{a}	85.82±2.42^{b}
Specific growth rate at 6w (%/day)	0.85±0.07	0.73±0.02	0.99±0.15
Specific growth rate at 12w (%/day)	0.71±0.03	0.67±0.02	0.81±0.08
Feed conversion ratio at 6w	1.26±0.10	1.47±0.05	1.13±0.13
Feed conversion ratio at 12w	1.32±0.04	1.50±0.06	1.26±0.08
Survival rate (%)	96.67±3.33	93.33±3.33	93.33±3.33

In the same line, different letters indicate statistical significant differences (P<0.05) among the treatments er than both the P40/48 and P44/48 groups (P<0.05). There were no statistical differences in the specific growth rates and feed conversion ratios at the 6th week between the experimental groups (P>0.05). The highest mean weight and feed intake values at the 12th week were recorded in the P48 group (P<0.05). There were no statistical differences in the specific growth rates and feed conversion ratios at the 12th week or in survival rates between all groups (P>0.05).

In Figure 1, weight increments of the meagre are shown week by week. The very close mean weights were recorded in the 4th week in the P48 and P40/48 groups with 65.88 and 65.76 g, respectively. The mean weight of P40/48 dramatically deviated from the P48 after the 6th week. The mean weights in different weeks of P44/48 never reached that of either P48 or P40/48 groups during the study. The weight gains of experimental groups were noted as 44.02, 36.75, and 33.68 for P48, P40/48, and P44/48 groups, respectively (Figure 2).

The biological indices of the meagre fed with the different protein level diets are listed in Table 3. The condition factor (CF) of P48 was significantly different to the P44/48 (P<0.05). The highest viscerosomatic index (VSI) was found in the P40/48 group (P<0.05). No differences were observed in the hepatosomatic index (HSI) and mesenteric fat index (MFI) between the groups (P>0.05).

Economic analysis of this experiment is presented in Table 4. No significant difference was found in economic conversion ratios (ECR) across all groups (P>0.05). The economic profit index (EPI) of P48 was significantly different from P44/48 (P<0.05).

The results of this present study clearly show that meagre need high protein levels in their diets and that decreasing the protein level (in their diets or partial feeding regimes) directly affects growth performance. In this study, cycled feeding with diets containing 40 and 44% protein levels were not as efficient as continuously feeding with 48% protein. Meagre is a fish species that

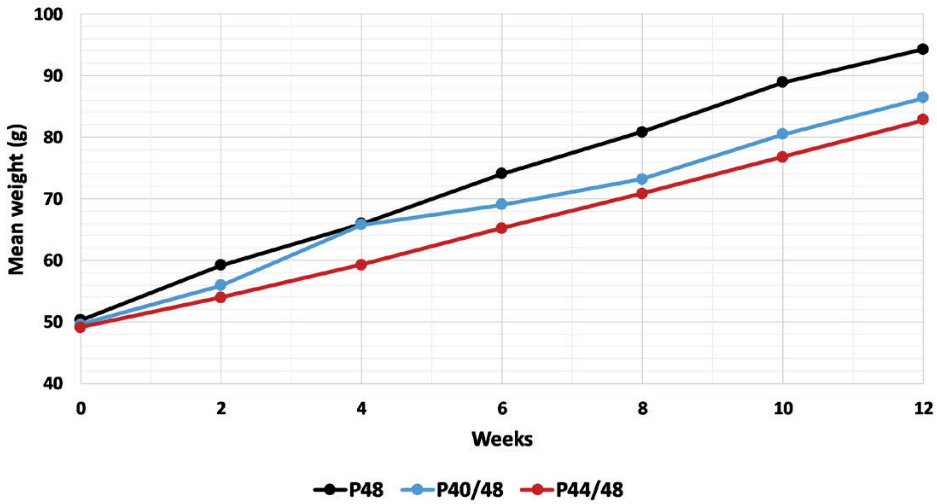

Figure 1. Mean weight increments of experimental groups of meagre during the 12 weeks.

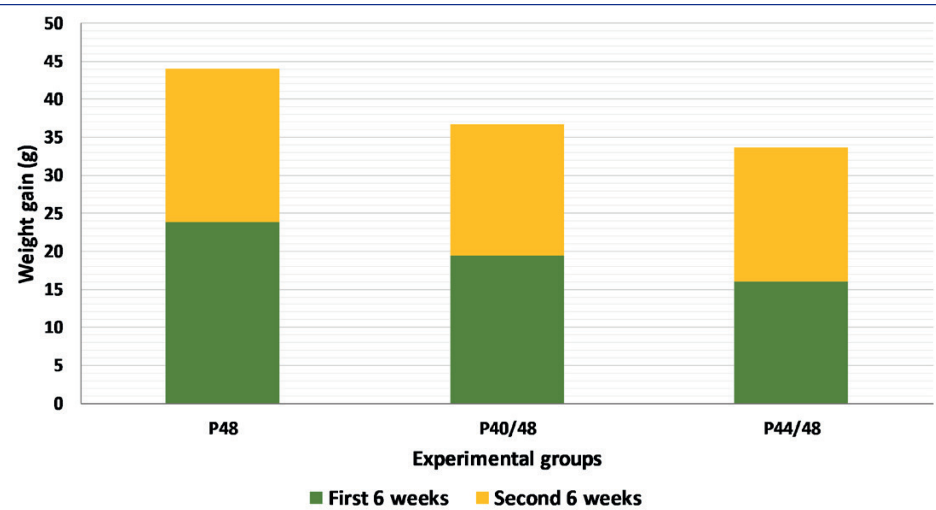

Figure 2. Weight gains of experimental groups of meagre during the 12 weeks.

Table 3. Somatic indices of meagre fed with diets containing different protein ratios by cyclical feeding regimes for 84 days

	P40/48	P44/48	P48
Condition factor (%)	1.37±0.01^{ab}	1.39±0.02^{b}	1.31±0.02^{a}
Viscerosomatic index (%)	5.88±0.77^{b}	5.14±0.17^{a}	5.16±0.23^{a}
Hepatosomatic index (%)	0.65±0.04	0.69±0.03	0.80±0.05
Mesenteric fat index (%)	0.04±0.01	0.05±0.01	0.08±0.02

In the same line, different letters indicate statistical significant differences (P<0.05) among the treatments

Table 4. Profitability analysis of meagre fed with diets containing different protein ratios by cyclical feeding regimes

	P40/48	P44/48	P48
Economic conversion ratio (€/kg)	1.34±0.04	1.58±0.07	1.38±0.09
Economic profit index (€/fish)	3.39±0.03^{ab}	3.19±0.10^{a}	3.64±0.08^{b}

In the same line, different letters indicate statistical significant differences (P<0.05) among the treatments

needs high dietary protein levels in the range of 45-50% (Chatzifotis et al., 2012; Güroy et al., 2017).

The WG and FI of the P40/48 and P44/48 groups were found continuously lower than the P48 group during the experiment. Kureshy and Davis (2002) stated that protein requirement depends on the optimum protein ratio of the diet and inferior levels of protein might be recompensed by a higher feed intake. Nevertheless, in this study, dietary protein level and feed intake increased linearly. This could be explained by size of the fish. In previous studies carried out on several carnivorous marine fish, including yellowtail (*Seriola dumerilii*), Korean rockfish (*Sebastes schlegeli*), and parrotfish (*Oplegnathus fasciatus*) it was stated that 45-50% was the optimum protein ratio in the juvenile stages of these species (Jover et al., 1999; Cho et al., 2015; Kim et al., 2017). However, Craig and Helfrich (2017) declared that the protein requirements of fish were related to the life stages of the species. The authors also noted that older fish need lower levels of dietary protein. Interestingly, however, although there was a quantitative difference between the SGR and FCR parameters of the groups at the midpoint and at the end of the experiment, they were found to be significantly similar (P>0.05). These parameters are used to specify the efficiency level of the feed or feeding regime in aquaculture (Craig and Helfrich, 2017). From this point of view, it is thought that the outputs of the study are useful for proving the importance of growth parameters in the condition of cyclical feeding regime with diets including different protein levels.

The CF displayed an inverse relation with the mean weights and feed intakes at the 6th and 12th weeks in this study. In contrast, Kim et al. (2001) recorded a parallel tendency between the CF and growth parameters in Korean rockfish (*S. schlegeli*). In a study carried out with grouper (*Epinephelus coioides*), the CF and VSI were improved at the optimum protein level (Luo et al., 2004). Yang et al. (2003) revealed that the VSI of Taiwan barb (*Spinibarbus hollandi*) was decreased with increased protein levels. The VSI was not enhanced with the highest growth (P48) in this current study. Kim et al. (2001) reported that the HSI of Korean rockfish (*S. schlegeli*) was improved with increased protein levels. Although there is no statistical difference between the groups, similar results were found in this present study (HSI ranged between 0.65 and 0.80). Lee et al. (1993) revealed that enhanced HSI was a sign of increased protein levels. An inverse record has been presented on the Atlantic cod (*Gadus morhua*) by Grisdale-Helland et al. (2008).

Fish meal and fish oil are the most expensive raw materials in aquafeeds. However, the carnivorous fish species need high quality animal protein sources in their diets. Decreasing the protein levels of diets means improving the profitability (Sweilum et al., 2005; Güroy et al., 2017). In this study, the EPI of P48 was higher than the other groups, but significantly related to P40/48. This situation might lead to a decrease in the production profitability in the culture of early stages of meagre, but this result may be useful for broodstock management because adult fish need less protein levels in their diets.

CONCLUSION

It can be concluded that meagre need high levels of protein in their diets and this species cannot compensate for reduced levels. Low levels of protein limited the growth performance and biological parameters of the fish. It is useful to compare the present study with previous protein level studies because this particular type of study has not been examined before. As mentioned above, the outputs of this study may be beneficial in adult feeding or broodstock management in the meagre culture and for future studies related to cyclical feeding regimes with important nutrients in the diets.

Conflicts of interest: The authors have no conflicts of interest to declare.

Ethics committee approval: This study was carried out in accordance with animal welfare and the ethics of experimentation.

Acknowledgements: Some parts of this study were presented in the 18th National Fisheries Symposium, İzmir, Turkey as a poster presentation on September 1-4th, 2015.

REFERENCES

Barata, M., Soares, F., Aragão, C., Almeida, A.C., Pousão-Ferreira, P. & Ribeiro, L. (2016). Efficiency of 2-phenoxyethanol and clove oil for reducing handling stress in reared meagre, *Argyrosomus regius* (Pisces: Sciaenidae). *Journal of the World Aquaculture Society*, *47*(1), 82-92. [CrossRef]

Chatzifotis, S., Panagiotidou, M. & Divanach, P. (2012). Effect of protein and lipid dietary levels on the growth of juvenile meagre (*Argyrosomus regius*). *Aquaculture International*, *20*(1), 91-98. [CrossRef]

Cho, S. H., Kim, H. S., Myung, S. H., Jung, W. G., Choi, J. & Lee, S. M. (2015). Optimum dietary protein and lipid levels for juvenile rockfish (*Sebastes schlegeli*, Hilgendorf 1880). *Aquaculture Research*, *46*(12), 2954-2961. [CrossRef]

Craig, S. & Helfrich, L. (2017). Understanding fish nutrition, feeds, and feeding. Virginia Cooperative Extension, Publication 420-256. College of Agriculture and Life Sciences, Virginia Tech, Blacksburg, 6 p.

Emre, Y., Kurtoğlu, A., Emre, N., Güroy, B. & Güroy, D. (2016). Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and haematological parameters of juvenile meagre, *Argyrosomus regius*. *Aquaculture Research*, *47*(7), 2256-2265. [CrossRef]

Eroldoğan, O. T., Taşbozan, O. & Tabakoğlu, S. (2008). Effects of restricted feeding regimes on growth and feed utilization of juvenile gilthead sea bream, *Sparus aurata*. *Journal of the World Aquaculture Society*, *39*(2), 267-274. [CrossRef]

Gatlin III, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E. J., Stone, D., Wilson, R. & Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research*, *38*(6), 551-579. [CrossRef]

Gaylord, T. G. & Gatlin III, D. M. (2001). Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). *Aquaculture*, *194*, 337-348. [CrossRef]

Grisdale-Helland, B., Shearer, K. D., Gatlin III, D. M. & Helland, S. J. (2008). Effects of dietary protein and lipid levels on growth, protein digestibility, feed utilization and body composition of Atlantic cod (*Gadus morhua*). *Aquaculture*, *283*(1-4), 156-162. [CrossRef]

Güroy, D., Karadal, O., Güroy, B., Mantoğlu, S., Çelebi, K., Şimşek, O., Eroldoğan, O. T., Genç, M. A. & Genç, E. (2017). The effects of dietary protein levels with amino acid supplementation on the growth performance, haematological profile and histology of meagre (*Argyrosomus regius*) in two different size classes. *Aquaculture Research*, *48*(12), 5751-5764. [CrossRef]

Hartviksen, M., Bakke, A. M., Vecino, J. G., Ringø, E. & Krogdahl, Å. (2014). Evaluation of the effect of commercially available plant and animal protein sources in diets for Atlantic salmon (*Salmo salar* L.): digestive and metabolic investigations. *Fish Physiology and Biochemistry*, *40*(5), 1621-1637. [CrossRef]

IndexMundi. (2018). IndexMundi - Country Facts, <www.indexmundi.com/commodities> (Accessed Date: 05.08.2018).

Jiwyam, W. (2010). Growth and compensatory growth of juvenile *Pangasius bocourti* Sauvage, 1880 relative to ration. *Aquaculture*, *306*, 393-397. [CrossRef]

Jover, M., Garcia-Gómez, A., Tomas, A., de la Gándara, F. & Pérez, L. (1999). Growth of Mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing different levels of protein and lipid. *Aquaculture*, *179*(1-4), 25-33. [CrossRef]

Kim, K. W., Kim, K. D., Han, H. S., Moniruzzaman, M., Yun, H., Lee, S. & Bai, S. C. (2017). Optimum dietary protein level and protein-to-energy ratio for growth of juvenile parrot fish, *Oplegnathus fasciatus*. *Journal of the World Aquaculture Society*, *48*(3), 467-477. [CrossRef]

Kim, K. W., Wang, X. J. & Bai, S. C. (2001). Reevaluation of the optimum dietary protein level for the maximum growth of juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research*, *32*(1), 119-125. [CrossRef]

Kureshy, N. & Davis, D. A. (2002). Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, *204*(1-2), 125-143. [CrossRef]

Lee J. Y., Kang Y. J., Lee S. M. & Kim I. B. (1993). Protein requirements of the Korean rockfish *Sebastes schlegeli*. *Journal of Aquaculture*, *6*(1): 13-27.

Lovell, T. (1989). Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York. [CrossRef]

Luo, Z., Liu, Y. J., Mai, K. S., Tian, L. X., Liu, D. H. & Tan, X. Y. (2004). Optimal dietary protein requirement of grouper *Epinephelus coioides* juveniles fed isoenergetic diets in floating net cages. *Aquaculture Nutrition*, *10*(4), 247-252. [CrossRef]

Monfort, M. (2010). Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. In *Studies and Reviews*. General Fisheries Commission for the Mediterranean. FAO, Rome, Italy.

NRC. (2011). *Nutrient requirements of fish and shrimp*. National Research Council (NRC), National Academies Press.

Quinton, J. C. & Blake, R. W. (1990). The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. *Journal of Fish Biology*, *37*(1), 33-41. [CrossRef]

Ribeiro, L., Soares, F., Quental-Ferreira, H., Gonçalves, A. & Pousão-Ferreira, P. (2013). Portuguese research studies meagre production in earthen ponds. *Global Aquaculture Advocate*, *16*, 38-40.

Okorie, O. E., Bae, J. Y., Kim, K. W., Son, M. H., Kim, J. W. & Bai, S. C. (2013). Optimum feeding rates in juvenile olive flounder, *Paralichthys olivaceus*, at the optimum rearing temperature. *Aquaculture Nutrition*, *19*(3), 267-277. [CrossRef]

Sánchez-Lozano, N. B., Vidal, A. T., Martínez-Llorens, S., Mérida, S. N., Blanco, J. E., López, A. M., Torres, M. P. & Cerdá, M. J. (2007). Growth and economic profit of gilthead sea bream (*Sparus aurata*, L.) fed sunflower meal. *Aquaculture*, *272*(1-4), 528-534. [CrossRef]

Sweilum, M. A., Abdella, M. M. & Salah El-Din, S. A. (2005). Effect of dietary protein-energy levels and fish initial sizes on growth rate, development and production of Nile tilapia, *Oreochromis niloticus* L. *Aquaculture Research*, *36*(14), 1414-1421. [CrossRef]

Tacon, A. G. & Cowey, C. B. (1985). Protein and aminoacids requirements. In P. Tytler & P. Calow (Eds.), *Fish energetics, new perspectives* (pp. 155-183). Croom Helm, London and Sydney. [CrossRef]

Türkmen, S., Eroldoğan, O. T., Yılmaz, H. A., Ölçülü, A., Kiriş İnan, G. A., Erçen, Z. & Tekelioğlu, N. (2012). Compensatory growth response of European sea bass (*Dicentrarchus labrax* L.) under cycled starvation and restricted feeding rate. *Aquaculture Research*, *43*(11), 1643-1650. [CrossRef]

Vallés, R. & Estévez, A. (2013). Light conditions for larval rearing of meagre (*Argyrosomus regius*). *Aquaculture*, 376-379: 15-19. [CrossRef]

Yang, S. D., Lin, T. S., Liou, C. H. & Peng, H. K. (2003). Influence of dietary protein levels on growth performance, carcass composition and liver lipid classes of juvenile *Spinibarbus hollandi* (Oshima). *Aquaculture Research*, *34*(8): 661-666. [CrossRef]

Zar, J. H. (1999). *Biostatistical analysis*. 4th edt, Upper Saddle River: Prentice-Hall Inc. 929 p.

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2019; 34(2): 67-73 • DOI: https://doi.org/10.26650/ASE495721

Research Article

Seasonal Variation of Zebra Mussel (*Dreissena polymorpha* Pallas, 1771) Colonization on Turkish Narrow-Clawed Crayfish (*Astacus leptodactylus* Eschscholtz, 1823) in Lake Eğirdir, Turkey

Mehmet Cilbiz[1], **Zübeyde Hanol Bektaş**[1], **Kadir Çapkın**[1], **Barış Çağdaş Hatipoğlu**[1], **Mehmet Ali Kaya**[1]

Cite this article as: Cilbiz, M., Hanol Bektaş, Z, Çapkın, K., Hatipoğlu, B. Ç., & Kaya, M. A. (2019). Seasonal Variation of Zebra Mussel (Dreissena polymorpha Pallas, 1771) Colonization on Turkish Narrow-Clawed Crayfish (Astacus leptodactylus Eschscholtz, 1823) in Lake Eğirdir, Turkey. *Aquatic Sciences and Engineering*, *34*(2), 67-73.

ABSTRACT

In this study, the hosting status of *Astacus leptodactylus,* which has one of the highest economic value of the inland waters fisheries of Turkey, dependent on zebra mussels (*Dreissena polymorpha*) was studied. Seasonal and sexual differences of zebra mussel prevalence, density situation on crayfish and some morphometric properties of zebra mussels retrieved from host were determined. Field works were carried out in January, April, July and October of 2015 with 8 fishing operations. 200 fyke net with different mesh sizes were used in sampling process. Sex of the crayfish and if they have mussels on them were determined by organoleptic observation. *Mann-Whitney U, Kruskal Wallis* and *Chi-Square* tests were used for evaluating of nonparametric data. A total of 4024 crayfish were caught and *D. polymorpha* was determined on 624 of them. The annual prevalence was found as 15.51% for both sex groups and it is higher in female (19.81%) than in males (9.82%). The highest mean abundance was found to be 1.93 in the spring season. The mean weights of the mussels were 0.2277 g and the average shell size was 12.3802 mm indicating a strong exponential relationship between weight and shell size.

Keywords: Invasive alien species, freshwater ecosystems, aquatic invasions

ORCID IDs of the authors:
M.C. 0000-0002-7686-7570;
Z.H.B. 0000-0003-1050-9620;
K.Ç. 0000-0002-6822-6896;
B.Ç.H. 0000-0003-4185-1330;
M.A.K. 0000-0002-1802-0232

[1]Eğirdir Fisheries Research Institute, Isparta, Turkey

Submitted:
11.12.2018

Revision Requested
12.01.2019

Last Revision Received
15.04.2019

Accepted:
15.04.2019

Online published:
28.06.2019

Correspondence:
Mehmet Cilbiz
E-mail:
mehmetcilbiz@gmail.com

INTRODUCTION

The freshwater zebra mussels *D. polymorpha* have become one of the most dominant species in many lakes and rivers of Europe, since it started spreading from the Caspian area at the beginning of the 19th century (Stanczykowska, 1977). It is a relatively small mollusk (25-34 mm long) which is native to the Black Sea basin and can form dense colonies in the hard and soft substrates of waterbodies. Over the last decades, the zebra mussel has invaded and spread across Eurasia and North America (Higgins and Vander Zanden, 2010). Some agencies such as fishing equipment ballast water, boat hulls, aquarium dumping, and perhaps by waterfowl has mediated to introducing successfully to several continents of *Dreissena* sp. (Reynolds and Donohoe, 2001).

Zebra mussels have a short maturation time (1-2 years), as well asthe ability for high dispersion and a really high fecundity (large number of eggs produced) (Higgins and Vander Zanden, 2010). Zebra mussels have high filtering capability and their seasonal production of free-swimming veliger larvae which will attach to hard substrates with their byssus, a strong filamentous structure secreted by bivalves. This species has caused much disruption both economically and ecologically on unexploited freshwater system of the worldwide (Reynolds and Donohoe, 2001).

Monotypic colonization is the typical characteristic of zebra mussels. Once established, zebra mussels have proven nearly impossible to remove. Therefore, their control is of considerable interest (Reynolds and Donohoe, 2001; Johnson and Carlton, 1996). Zebra mussels are physiologically strong organisms. They live longer than many other bivalves. An environment like Great Lakes which has the greatest diversity of freshwater bivalves with 297 species (Ricciardi et. al., 1998) and constitutes an extremely suitable habitat for zebra mussels. *D. polymorpha* shares characteristics of many successful invasive species: rapid growth, prolific reproduction at an early age (0-1+) and a relatively short life span. This usually provides the potential for rapid exponential growth of zebra mussel populations within a waterbody (Frances, 2006). This suggests that juveniles are capable of making decisions of where to colonize. One factor in deciding where to colonize is the threat of predation. Interior substratum is not suitable for Dreissenids using of protection and live, but rocky substrata is a very good alternative for that intended use (Thorp et. al., 1998). There are many zebra mussels predators in North American waters, for instance crayfish by Stewart et. al., (1998) and sunfish by Molloy et al. (1997) and Magoulick and Lewis (2002). Predator and prey interactions are very strongly in the biological communities (Polis and Strong, 1996).

In the previous studies, which were carried out on crayfish and zebra mussels relationship, (*I*) the connection location of zebra mussel on the crayfish was researched by Berber et al. (2018) and (*II*) predation effect of crayfish on the zebra mussels was investigated (Martin and Corkum ,1994; Perry et al. 1997; Reynolds and Donohoe, 2001; Kutluyer et al., 2013). However, there are no studies connected with the hosting status of natural crayfish stock for invasive zebra mussels by evaluating of seasonally.

We aimed to determine the seasonal and sexual differences of Zebra Mussel prevalence and density on crayfish, and also investigated some biometric characteristics of zebra mussels which were extracted from *A. leptodactylus*.

MATERIAL AND METHOD

The fieldworks were conducted in January, April, July and October of 2015 with 8 fishing operations. 200 fyke net with 14, 26, 34 and 42 mm mesh size were used in the fishing trial. Fyke nets were harvested once every two days. The sex of the crayfish and whether or not they carried mussels on them were determined by organoleptic observations. Morphometric measurements of crayfish were determined with 0.01mm sensitivity using digital caliper according to Rhodes and Holdich (1984). In the evaluating of nonparametric data *Mann-Whitney U, Kruskal Wallis* and *Chi-Square* tests were used. In addition, Dunn's test was utilized for multiple comparisons. *Pearson Correlation* test was used to determine the relationship between the morphometric measurements of crayfish and the total number of Dreissena they carry. *R* (v3.3.3) based *RStudio* (v1.0136) program was used for all statistical calculations. Prevalence, mean intensity and mean abundance values have been modified from Bush et. al., (1997) as follows.

$$Prevalence = \frac{\text{Number of crayfish with mussel}}{\text{Number of total caught crayfish}} x100$$

$$Mean\ Intensity = \frac{\text{Number of total mussel}}{\text{Number of crayfish with mussel}}$$

$$Mean\ Abundance = \frac{\text{Number of total mussel}}{\text{Number of total caught crayfish}}$$

RESULT AND DISCUSSION

Of the 4024 crayfish examined, Dreissena was seen at 624 (454 female and 170 male), but not at 3400 (1838 female and 1562 male). There is statistical difference between sex groups by hosting or not (p<0,05) (Table 1). Minimum, maximum and mean number of zebra mussel collected from crayfish are given Table 2. Statistical differences were found between male and female crayfish with regard to the carrying of zebra mussels (p<0,05); presence of Dreissena on females is higher than on males. The annual prevalence was found to be 15.51 but showed great seasonal differences. The prevalence was highest in female in the spring season (%44.99). The host situation of crayfish for Dreissena was of minimum level in the autumn and winter months (Table 4). Mean density has shown statistical differences between sampling seasons (p<0,05). Mean abundance was highest in the spring months. It is thought that the cause of this density is due

Table 1. Hosting status of crayfish by different sex groups

Sex Groups	Dreissena (+)	Dreissena (-)	X^2	*p*
♀♀	454	1838	75,185	0,000
♂♂	170	1562		

Table 2. Mean density of D. polymorpha which are hosted by A. leptodactylus

Sex Groups	Mean±SE	*Min. (n)*	*Max. (n)*	*Man.Whit (U)*	*Wilcoxon (W)*	*p*
♀♀	4.41±0,24	1	43	32934	47469	0,004
♂♂	3.56±0,33	1	28			

*Calculations were conducted only host crayfish

Table 3. *D. polymorpha* presence on *A.leptodactylus* by season and sex groups

Season	Sex Groups								
	♀♀			♂♂			♀♀+♂♂		
	+	-	Prv. (%)	+	-	Prv. (%)	+	-	Prv. (%)
Spring	368	450	44.99	123	199	38.20	491	649	43.07
Summer	86	772	10.02	45	516	8.02	131	1288	9.23
Autumn	-	81	-	2	76	2.56	2	157	1.26
Winter	-	535	-	0	771	0.00	-	1306	-
Annual	454	1838	19.81	170	1562	9.82	624	3400	15.51

(+: . *D. polymorpha* positive, -. *D. polymorpha* negative, *Prv:* Prevalence)

Table 4. Mean density and abundance of D. polymorpha which are hosted by A. leptodactylus

Season	Total Number of		Total Caught Crayfish	Mean Density	Mean Abundance	Number range of Dreissena	
	Host Crayfish	Dreissena				Min. (N)	Max. (N)
Spring	491	2203	1140	4.49 ± 0.23^{ab}	1.932	1	43
Summer	131	384	1419	2.93 ± 0.34^{a}	0.270	1	28
Autumn	2	20	159	10.0 ± 9.00^{b}	0.125	1	19
Winter	0	0	1306	0	0	0	0
Total	624	2607	4024	4.177	0.647	0	43

Table 5. Presence of mean density *D. polymorpha* on crayfish by length class

Length Groups	n	Dreissena (+)	Dreissena (-)	Mean±SE	Min.-Max.
3-6	21	21	140	2.10 ± 0.42^{a}	1-9
7-9	543	543	2380	4.25 ± 0.21^{b}	1-43
>10	60	60	879	4.25 ± 0.61^{ab}	1-19

to the fact that Dreissenan and crayfish life cycle are not in the period of shell change. Mean density was calculated as 10.0 for the autumn season as highest value. It is thought that, this result stemming from very poor data which gained for autumn season.

The presence of *D. polymorpha* on crayfish has been shown differences by length class (X^2: 81,325) (p<0,05). It is said that, small length class of crayfish were less sensitive then middle and big length class in regard to Dreissena invasion. The highest prevalence in the female was found in the 6 cm length group with 25.938% and in the males was found in 5 cm length group with 13.636%. The highest prevalence in the calculation for both sex groups was 21.023% in the 6 cm length group (Table 6). In general, the prevalence of the individuals above the 10 cm group was significantly lower than the other groups. In general, prevalence of female individuals was higher than male individuals.

Minimum and maximum number of *D. polymorpha* which were isolated from each crayfish and values of mean density and abundance are given in Table 7. It was determined that, while the number of *D. polymorpha* isolated from female crayfish was minimum 1 and maximum 43 (an individual in the 7 cm size group) in males, min 1 and maximal 28 (an individual in the 8 cm size group).

It is seen that the prevalence is very high in 6,7,8 cm length classes according to advanced length classes. The main reason for this situation is thought to be catching composition-oriented. Because the young crayfish constitute a significant portion of the prey in this group the prevalence is higher than others. In general, prevalence of female individuals was higher than male individuals. This situation can be explained by the fact that the males who tend to grow faster than females have changed the shell before the sampling period and thus they are free from the Dreissena on them.

The average density and abundance of *D. polymorpha* in female and male crayfish showed significant differences according to the length classes (p<0,05). In general, the average intensity in malesincreases in parallel to the length increase. *D. polymorpha* was not found in female crayfish above 10 cm and in male crayfish above 13 cm. Prevalence was observed more in small size

Table 6. *D. polymorpha* presence on *A. leptodactylus* by length class and sex groups

Length Class	Sex Groups								
	♀♀			♂♂			♀♀+♂♂		
	+	-	Prv (%)	+	-	Prv (%)	+	-	Prv (%)
3					1			1	
4	1	13	7.143		8		1	21	4.545
5	11	62	15.068	9	57	13.636	20	119	14.388
6	83	237	25.938	28	180	13.462	111	417	21.023
7	185	561	24.799	65	462	12.334	250	1023	19.639
8	139	479	22.492	43	461	8.532	182	940	16.221
9	25	255	8.929	16	167	8.743	41	422	8.855
10	10	100	9.091	4	87	4.396	14	187	6.965
11		64		4	64	5.882	4	128	3.030
12		37			42			79	
13		23		1	21	4.545	1	44	2.222
14		7			9			16	
15					3			3	

(+: . *D. polymorpha* positive, -. *D. polymorpha* negative, *Prv*: Prevalence)

Table 7. Mean density and abundance values by different length class and sex

Length Class	♀♀					♂♂				
	Min.	Max.	Sum	Mean Density	Mean Abundance	Min.	Max.	Sum	Mean Density	Mean Abundance
3										
4	9	9	9	9.000	0.643					
5	1	4	18	1.636	0.247	1	4	17	1.889	0.258
6	1	21	332	4.000	1.038	1	12	88	3.143	0.423
7	1	43	852	4.605	1.142	1	18	188	2.892	0.357
8	1	31	683	4.914	1.105	1	28	165	3.837	0.327
9	1	17	93	3.720	0.332	1	19	74	4.625	0.404
10	1	4	15	1.500	0.136	5	18	38	9.500	0.418
11						5	10	30	7.500	0.441
12										
13						5	5	5	5.000	0.227
14										
15										

groups as Dreissena was common in seasons when the crayfish were small in size (Table 7).

In female crayfish, there is an increase in both the number of host crayfish and the number of hosts starting from the 5 cm height class. A similar situation is observed in male crayfish. This increase has a tendency to decrease by making a 7 cm height class peak and after the 10 cm length class, both the number of host crayfish and the number of hosts decrease significantly. A similar situation is observed in male crayfish.

The correlation matrix of relations between some body measurements of crayfish and the *D. polymorpha* numbers which they carry is given in Table 8. In general, the relationship between the number of zebra mussels on the body and the different body lengths was found to be very weak.

The Dreissena keep on crayfish at the end of the winter months and spring months continue to grow on crayfish until July until beginning of shell change period. Figure 1(A) and (B) shows Dreissena colonization on crayfish and Figure 4(C) shows some examples of different sized Dreissena isolated from crayfish.

The spread and overgrowth of zebra mussels which are defined as an aquatic invasive species in its settling environment has become an important ecological problem for all countries around

Table 8. Correlation matrix between number of the *D. polymorpha* and measured body part of *A. leptodactylus*

Correlations	Total Number of *D. polymorpha*	Total Length	Carapace Length	Abdomen Length	Carapace Width	Abdomen Width
Total Number of ***D. polymorpha***	1					
Total Length	0.066	1				
Carapace Length	0.056	0.977	1			
Abdomen Length	0.069	0.974	0.903	1		
Carapace Width	0.063	0.957	0.922	0.946	1	
Abdomen Width	0.089	0.918	0.860	0.933	0.898	1

the world. According to Bobat et al. (2001), it is estimated that the spread of the zebra mussel to the other major continents from the natural spreading areas is through the bilge (ballast) waters of the ships. Access to other water sources at the places of transportation is due to their ability to attach themselves to boats. But it is not a question of intercontinental spread to Turkey. The zebra mussels naturally found in the Euphrates Basin (East-South Turkey) and breeding below the damage limit have grown extensively by finding a more suitable environment (temperature, pH, calcium, nutrients, adhesive surface, etc.) to breed due to the changing ecological conditions after dam construction. In addition, the pouring of domestic waste into the Atatürk Dam Lake increased the phosphate content in the aquatic environment, which also played a nurturing role for the mussels (Bobat et al., 2001). Another element of dissemination way may be commercial fishermen by means of carrying fishing gears from one fishing location to the another. For example, gillnets, trammel nets, crayfish fyke-nets and beach seine net etc. In addition, the sales of second-hand boats widely held in the inland waters of Turkey. Zebra mussels cause deterioration of the water quality and in the food chain in the lakes (Noonburg et al., 2011) where they are oriented towards eutrophication, which cause irreversible damage in the long run. For this reason, there is need for studies on the distribution and overproduction of mussels. Ardura et al. (2017) have developed a species-specific environmental *DNA* marker for early detection of a global invasion problem, zebra mussel, and consequently report that the method is consistent, reproducible, rapid, inexpensive and technically easy.

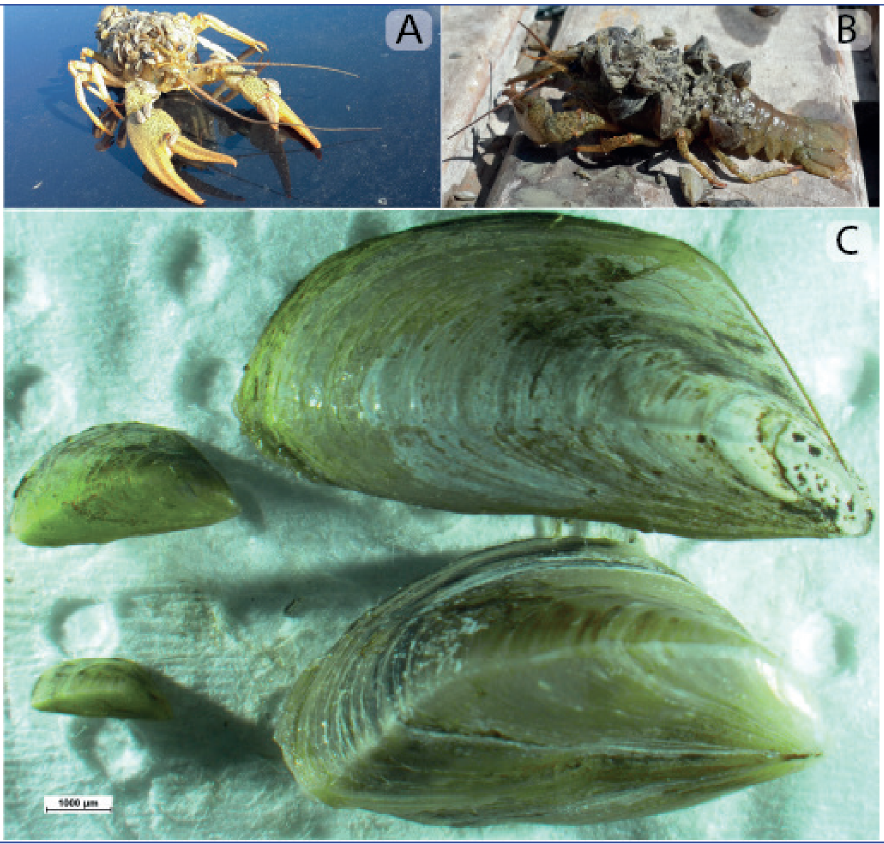

Figure 1. Different sized Dreissena samples isolated from crayfish.

It is thought that the prevalence of female individuals is higher than that of male individuals, which is generally due to the fact that male individuals with better growth performance are more likely to molt than females. Generally male crayfish are more frequently molting than females during the year are in the higher prevalence than females compared to males because they are removed from the Dreissena on them end of the moulting process.

According to Bolat (2001), male crayfish can molt 45-50 times a year and females 30-35 times (Groves, 1985; Alpbaz, 1993). In Turkey, it has been reported by some researchers that the crayfish species *Astacus leptodactylus* start to molt in June. It has been reported that immature individuals molt 8 times in the first year, 5 times in the second years and 2-3 times in the third year, after maturation males molt twice a year and females only once a year (Atay, 1984; Anonymous 1985; Erdem, 1993; Bolat, 2001).

Brazner and Jensen (2000), have reported that the number of zebra mussels per crayfish ranged from 16 to 431, attached zebra mussels ranged in size from 1.2 mm up to 12.0 mm, with a mean size of 3.6 mm. The number of colonized individuals, length range of Dressenia and number of zebra mussels for per crayfish infestation were a bit smaller than our result. This may be related to the relative motility and/or moulting characteristics of these different organisms. This kind of works can give some results to countries, understanding of seriousness of the situation and spend the budget for the struggle.

CONCLUSION

Zebra mussels cause many ecological and economical dangers in aquatic areas. Dressenia and crayfish interactions have been unexplained up until now except prey-predator relationship. In this context, recently increasing of the crayfish production in Eğirdir Lake may derive from Dreissenia based feeding regime of crayfish. Zebra mussels present crayfish a with very good camouflage ability to protect them from predators in addition to a potential food relationship. However, there are some observed dangers of zebra mussels on crayfish. Such as, (I) using extra energy for carrying of penetrated Dreissena during move in habitat and (II) deteriorating of the fyke net selectivity. While most crayfish with a normal body shape (below the minimum landing size) can easily escape the fyke net, carrying of the penetrated zebra mussel on the shell makes escaping the fyke net almost impossible. New studies are needed, especially focus on the escape of caught crayfish (carrying the zebra mussel on its shell) from the fyke net easily for sustainable stock management.

Conflicts of interest: The authors have no conflicts of interest to declare.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

Acknowledgment: This study was supported by Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies, besides which was presented in 7th National Limnology Symposium..

REFERENCES

Anonymous, A. (1985). Investigations on the Production and Population of Crayfish and Egirdir Lake. Water Product. General Directorate, Isparta Water Products Regional Director., issue. 2,21.

Ardura, A., Zaiko, A., Borrell, YJ., Samuiloviene, A., Garcia-Vazquez, E. (2017). Novel tools for early detection of a global aquatic invasive, the zebra mussel *Dreissenia polymorpha*. Aquatic Conservation: *Marine and Freshwater Ecosystems*. 27(1):165-176.

Atay, D. (1984). Crustacean Aquaculture Technic. Ankara Üniversitesi, Ziraat Fakültesi Yayınları, 194, 192 [in Turkish].

Berber, S., Ates, A. S., & Acar, S. (2018). First observation of the zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) on the narrow-clawed crayfish inhabiting in some water sources of Turkey. Su Ürunleri Dergisi, 35(1), 55-61.

Bobat, A., Hengirmen, MO., Zapletal, W. (2001). Technical, economic and ecological harmful in freshwater ecosystem: Rural Environment Yearbook Zebra Mussels.112-129.

Bolat, Y. (2001). Estimation population size of freshwater crayfish (*Astacus leptodactylus* Esch., 1823) in Hoyran region of Egirdir Lake. Süleyman Demirel University, (Phd.) Thesis 133p.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of parasitology*, 83(4): 575-583.

Brazner, J.C. and Jensen, D.A. (2000). Zebra Mussel [Dreissena polymorpha (Pallas)] Colonization of Rusty Crayfish [Orconectes rusticus (Girard)] in Green Bay, Lake Michigan. *The American Midland Naturalist*, 143(1):250-256.

Erdem, M. (1993). A study on the detection of artificially observed rates of life of eggs from Egirdir Lake Crayfish (*Astacus leptodactylus* salinus, Nordman 1842). Suleyman Demirel University, MSc Thesis, 4-17.

Frances, L. (2006). Early life stages of Dreissena polymorpha (Zebra mussel): the importance of long-term datasets in invasion ecology. *Aquatic Invasions*, 1(3): 171-182.

Higgins, SN and Vander Zanden, MJ. (2010). What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystem. Ecological monographs 80(2), pp179-196, by the Ecological Society of America.

Johnson, LE and Carlton, JT. (1996). Post-establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel Dreissena polymorpha, Ecology, 1686–1690.

Kutluyer, F., Aksu, Ö., Aksu, Ö., 2013. Kerevitlerin beslenmesinde (*Astacus leptodactylus* Eschscholtz, 1823) alternatif besin olarak Zebra midyesinin (*Dreissena polymorpha* Pallas, 1771). Bilim ve Gençlik Dergisi, 1:2, 64-70[in Turkish].

Martin, G. W., & Corkum, L. D. (1994). Predation of zebra mussels by crayfish. Canadian Journal of Zoology, 72(11), 1867-1871.

Magoulick, D.D and C.Lewis, L. (2002). Predation on exotic zebra mussels by native fishes: effects on predator and prey. *Freshwater Biology*,1908-1918.

Molloy, DP., Karatayev, AY., Buraklova, LE., Kurandina, DP., Laurelle, F. (1997). Natural enemies of zebra mussels, predators, parasites and ecological competitors. *Reviews in Fisheries Science*, 5:27-97.

Noonburg, E. G., Shuter, B. J., and Abrams, P. A. (2003). Indirect effects of zebra mussels (*Dreissena polymorpha*) on the planktonic food web. Canadian Journal of Fisheries and Aquatic Sciences, 60(11), 1353-1368. doi:10.1139/f03-116

Perry, W. L., Lodge, D. M., & Lamberti, G. A. (1997). Impact of crayfish predation on exotic zebra mussels and native invertebrates in a lake-outlet stream. *Canadian Journal of Fisheries and Aquatic Sciences, 54*(1), 120-125.

Polis, GA and Strong, DR. (1996). Food web complexityand community dynamics. *American Naturalist*, 147:813–846.

Reynolds, JD., Donohoe, R. (2001). Crayfish predation experiments on the introduced zebra mussel, *Dreissena polymorpha*, in Ireland, and their potential for biocontrol. *Bulletin français de la pêche et de la pisciculture*, 361:669-681.

Rhodes, CP and Holdich, DM. (1984). Length-weight relationship, muscle production and proximate composition of the freshwater crayfish *Austropotamobius pallipes* (Lereboullet). *Aquaculture*, 37:107-123.

Ricciardi, A., Neves, RJ and Rasmussen, JB. (1998). Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology*, 67(4): 613–619.

R Development Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 Retrived from http://www.R-project.org.

Stanczykowska, A. (1977). Ecology of Dreissena polymorpha (Pall.) (Bivalvia) in Lakes. *Polish Archives of Hydrobiology*, 24: 461–530.

Stewart, TW., Miner, JG., Lowe, RL. (1998). Quantifying mechanisms for zebra mussel effects on benthic macroinvertebrates: organic matter production and shell-generated habitat, *Journal of the North American Benthological Society*, 17 (1):81–94.

Thorp, LH., Delong, MD and Casper, AF. (1998). In situ experiments on predatory regulation of a bivalve mollusk (*Dreissenia polymorpha*) in the Mississippi and Ohio Rivers, *Freshwater Biology*, 39:649-661.

Instructions to Authors

Aquatic Sciences and Engineering is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University Faculty of Aquatic Sciences and it is published quarterly on January, April, July, and October. The publication language of the journal is English and continues publication since 1987.

Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

The scope of the journal includes but not limited to; aquaculture science, aquaculture diseases, feeds, and genetics, ecological interactions, sustainable systems, fisheries development, fisheries science, fishery hydrography, aquatic ecosystem, fisheries management, fishery biology, wild fisheries, ocean fisheries, biology, taxonomy, stock identification, functional morphology freshwater, brackish and marine environment, marine biology, water conservation and sustainability, inland waters protection and management, seafood technology and safety.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of aquatic sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), the International Council of Medical Journal Editors (ICMJE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

Manuscripts submitted to Aquatic Sciences and Engineering will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies. If required, ethics committee reports or an equivalent official document will be requested from the authors.

For manuscripts concerning experimental research on humans, a statement should be included that shows the written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

Aquatic Sciences and Engineering requires experimental research studies on vertebrates or any regulated invertebrates to comply with relevant institutional, national and/or international guidelines. The journal supports the principles of Basel Declaration (basel-declaration.org) and the guidelines published by International Council for Laboratory Animal Science (ICLAS) (iclas.org). Authors are advised to clearly state their compliance with relevant guidelines.

Aquatic Sciences and Engineering advises authors to comply with IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora for research involving plants.

All submissions are screened by a similarity detection software (iThenticate by CrossCheck).

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Each individual listed as an author should fulfil the authorship criteria recommended by the ICMJE. The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged in the title page of the manuscript.

Aquatic Sciences and Engineering requires corresponding authors to submit a signed and scanned version of the authorship contribution form (available for download through istanbul.dergipark.gov.tr/ase) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility for authorship during the submission and review stages of the manuscript.

Aquatic Sciences and Engineering requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal's Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve cases that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

When submitting a manuscript to Aquatic Sciences and Engineering, authors accept to assign the copyright of their manuscript to İstanbul University Faculty of Aquatic Sciences. If rejected for publication, the copyright of the manuscript will be assigned back to the authors. Aquatic Sciences and Engineering requires each submission to be accompanied by a Copyright Transfer Form (available for download at istanbul.dergipark.gov.tr/ase). When using previously published content, including figures, tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial and criminal liabilities in this regard belong to the author(s).

Statements or opinions expressed in the manuscripts published in Aquatic Sciences and Engineering reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility in regard to the published content rests with the authors.

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2017 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, TREND guidelines for non-randomized studies, and COREQ guidelines for qualitative studies.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at istanbul.dergipark.gov.tr/ase. Manuscripts submitted via any other medium will not be processed.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following forms during the initial submission. These are available for download at istanbul.dergipark.gov.tr/ase

- Copyright Transfer Form,
- Author Contributions Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, and highest academic degree(s) of the author(s) and ORCID ID (orcid.org)
- Grant information and detailed information on the other sources of financial support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfil the authorship criteria.

Abstract: A Turkish and an English abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract.

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text should contain Introduction, "Materials and Methods", "Result and Discussion", "Conclusion" and "References" sections.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in researches and should guide future studies. The main text should start with Introduction and end with "Conclusion" and "References" sections. Authors may choose to use any subheading in between those sections.

After the Conclusion section and before references list, information regarding conflict of interest and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Short Communication" Readers can also present their comments on the published manuscripts in the form of a "Short Communication". The main text should contain Introduction, "Materials and Methods", "Result and Discussion", "Conclusion" and "References" sections.

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤20	250	40
Review Article	≤25	250	60
Short Communication	≤5	250	20

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. List references in alphabetical order. Each listed reference should be cited in text, and each text citation should be listed in the References section. The reference styles for different types of publications are presented in the following examples.

Reference Style and Format

Aquatic Sciences and Engineering complies with APA (American Psychological Association) style 6th Edition for referencing and quoting. For more information:

- American Psychological Association. (2010). Publication manual of the American Psychological Association (6th ed.). Washington, DC: APA.
- http://www.apastyle.org

Accuracy of citation is the author's responsibility. All references should be cited in text. Reference list must be in alphabetical order. Type references in the style shown below.

Citations in the Text

Citations must be indicated with the author surname and publication year within the parenthesis.

If more than one citation is made within the same paranthesis, separate them with (;).

Samples:

More than one citation;
(Esin et al., 2002; Karasar, 1995)
Citation with one author;
(Akyolcu, 2007)
Citation with two authors;
(Sayıner & Demirci, 2007)
Citation with three, four, five authors;
First citation in the text: (Ailen, Ciambrune, & Welch, 2000) Subsequent citations in the text: (Ailen et al., 2000)
Citations with more than six authors;
(Çavdar et al., 2003)

Major Citations for a Reference List

Note: All second and third lines in the APA Bibliography should be indented.

- **A book in print:** Baxter, C. (1997). *Race equality in health care and education.* Philadelphia: Ballière Tindall. ISBN 4546465465
- **A book chapter, print version:** Haybron, D. M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (pp. 17-43). New York, NY: Guilford Press. ISBN 4546469999
- **An eBook:** Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song.* Retrieved from http://www.amacombooks.org/ (accessed 10.10.15)
- **An article in a print journal:** Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy, 14*(1), 38-48.
- **An article with DOI:** Gaudio, J. L. & Snowdon, C. T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (saguinus oedipus) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
- **Websites - professional or personal sites:** The World Famous Hot Dog Site. (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.15)
- **Websites - online government publications:** U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.15)
- **Photograph (from book, magazine or webpage):** Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.15)
- **Artwork - from library database:** Clark, L. (c.a. 1960's). *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
- **Artwork - from website:** Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.15)

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Editor in Chief: Prof. Devrim MEMİŞ
Address: İstanbul Üniversitesi Su Bilimleri Fakültesi Yetiştiricilik Anabilim Dalı Ordu Cad. No:8 34134 Laleli / İstanbul, Türkiye
Phone: +90 212 4555700/16448
Fax: +90 212 5140379
E-mail: mdevrim@istanbul.edu.tr