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RESEARCH PAPER

Land-use and Fish Condition Changes for Over a Decade on Konya Closed Basin, are External Anomalies Affecting the Fish Condition?

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Abstract

Anthropogenic pressures on freshwater ecosystems are growing and pose serious threats to endemic fish species. This study aimed to identify the effects of anthropogenic pressures on an endangered species, *Pseudophoxinus crassus* (Ladiges, 1960), in the semi-arid Konya Closed Basin of Türkiye. The distribution area of the species was found to be reduced and the only population sampled exhibited a high incidence of external anomalies. It is hard to identify the origin of the anomalies; however, abrupt increases in salinity and reductions in dissolved oxygen values indicate that intensive agricultural activities might be responsible for the pressures on the population. Additionally, temporal changes in population growth parameters were identified and the effects of anomalies on fish condition factors were examined. Fulton's condition factor was found to be reduced among specimens with external anomalies. This relationship between anomalies and fitness as reflected by the condition factor was also observed for two other endemic species. It is unclear whether these anomalies affect feeding behaviours, although specimens with lower condition factors are more likely to exhibit anomalies. However, it is clear that without the rehabilitation or restoration of natural habitats, fitness reduction will severely affect the statuses of populations, particularly in cases of isolated populations.

Introduction

Freshwater ecosystems cover less than 1% of the planet's surface area but they harbour a significant portion of the planet's species. With growing demands for freshwater resources, the anthropogenic pressures on these ecosystems are significantly increasing. The major threats to these habitats can be classified as over-exploitation, pollution, habitat degradation, flow modification, and species invasions (Dudgeon et al., 2006). Another important emerging problem is climate change (Reid et al., 2019), which threatens the anthropogenic use of water and biodiversity. Climate change will affect water temperatures, stream flows, and water levels, leading to water quality changes (Jiménez Cisneros et al., 2014; Reid et al., 2019). Freshwater fish populations of arid and semi-arid regions, where these effects of climate change will be most significant, are expected to face extinction or become more vulnerable (Collares-Pereira & Cowx, 2004).

The Konya Closed Basin is located on the Central Anatolian Plateau of Türkiye, surrounded by the Taurus Mountain Belt from the south. The basin is classified as semi-arid with a high risk of desertification (Türkeş et al., 2020). Annual precipitation is about 398 mm, less than the overall average precipitation in Türkiye (Altınbilek & Hatipoğlu, 2020). The basin's freshwater potential is low. However, compared to other basins of Türkiye, the Konya Closed Basin has the greatest fish endemicity with 28 endemic fish species (Çiçek et al., 2018; Yılmaz et al., 2021). Most of these species are under threat according to the International Union for Conservation of Nature.

Pseudophoxinus crassus (Ladiges, 1960) is an endangered endemic fish species in the Tuz Lake Subbasin of the Konya Closed Basin. This species is threatened by dams, pollution, and drought (Freyhof, 2014). Agricultural areas dominate the basin within the distribution area of this fish, and agrochemicals used in industrial agriculture are known to be toxic to aquatic organisms and cause developmental anomalies (Piha et al., 2006; Di Renzo et al., 2011; Maurya et al., 2019). Furthermore, intensive water drainage for irrigation causes habitat loss and changes in the physicochemical properties of the water (Moss, 2008). Agricultural pollutants may cause sudden mortality among fish populations or may accumulate in abiotic and biotic elements (Austin, 1999) and cause stress for fish, disrupting their normal metabolism (Tyus, 2011). Long-term exposure to stressors might harm the health and survival of fish populations (Barton et al., 1998). In addition to environmental stressors, inbreeding depression can cause developmental anomalies, especially in isolated populations (Madsen et al., 1996; Yousefian & Nejati, 2008; Charlesworth & Willis, 2009; Monson & Sadler, 2010).

The condition factor, or the relationship between fish weight and length, is a simple yet valuable indicator for determining the physical health of a fish population. Developmental anomalies generally cause a decrease in fitness-related traits such as growth, fecundity, and survival (Møller, 1997). Therefore, monitoring changes in the condition factor is a matter of importance in both aquaculture (Aydin et al., 2012; Prestinicola et al., 2013; Amoroso et al., 2016) and conservation studies (Sato, 2006; Yamamoto et al., 2013; İnnal et al., 2019). Negative correlations between condition factors and developmental anomalies can be observed in aquaculture studies where sample sizes and incidence rates are generally high (Taylor et al., 2013). However, negative correlations between anomalies and condition factors might not be observed in wild populations due to more limited sample sizes (Madsen et al., 1996; Sato, 2006) or the relatively diverse genetic structure of natural populations compared to aquacultural populations.

In this study, we conducted research to examine the distribution and population status of *P. crassus* in the Konya Closed Basin. We quantified the land use in the distribution area and observed a high incidence of external anomalies in the population of interest. We report the details of those anomalies in this study. Additionally, we investigated the relationship between external anomalies and fish condition factors, hypothesising that specimens with external anomalies would have reduced fitness and that fitness reduction would increase with the increasing intensity of anomalies. In order to determine whether sympatric species exhibited similar external anomalies, we selected two other endemic native species from this area, *Gobio microlepidotus* Battalgil (1942) and *Squalius cappadocicus* Özuluğ & Freyhof (2011), to be evaluated for external anomalies, as well.

Materials and Methods

Study Area

The Konya Closed Basin, the fourth largest basin in Türkiye in terms of surface area, is a semi-arid basin with limited water resources. Over the years, its contribution to Türkiye's surface water potential has decreased, reaching 1.3% in 2020 according to the General Directorate of State Hydraulic Works (DSİ, 2022). Factors such as high evaporation, low precipitation, and variable water permeability at the bottom of the basin due to karstic structures have affected the surface flows.

The General Directorate of State Hydraulic Works determined nine different subbasins in the Konya Closed Basin by considering features such as surface precipitation area, groundwater recharge area, and geological, hydrogeological, and aquifer structures. For this study, we digitised and delineated those subbasins using the map of Tunçok and Bozkurt (2015) and intersected that map with CORINE land cover maps for the years 2000 and 2018 (European Environment Agency, 2000, 2018), calculating the CORINE Level 1 percentages for the subbasins. We identified inland water resources using sampling locations and historical records (Figure 1) and we performed spatial analyses using the tools provided in QGIS 3.14 (QGIS Development Team, 2020).

Sampling Methods

From April to November 2016, we conducted monthly physicochemical water sampling within the designated range. We used the YSI-6600D device to measure temperature (°C), pH, dissolved oxygen (mg/L), and salinity (ppt) in the water bodies where *P. crassus* was present.

Additionally, we conducted fish sampling in June 2016, surveying 41 streams and 7 lakes in the Konya Closed Basin. We used an electrofishing device (SAMUS 726 MP) to sample wadeable streams and gill nets in lakes (Permit Date/Number: 30.05.2016/35853172150-1719). Single-pass electrofishing was conducted covering all habitats (riffles, runs, and pools), and areas of 100 m^2 to 400 m^2 were covered according to the widths of the water bodies. In all reservoirs (Mamasın, İvriz, Gödet, Derebucak, and Deliçay) two standard (DIN EN 14757) benthic multi-mesh gillnets (30 m) and a set of regular gillnets (mesh size: 70, 90, and 110 mm; 100 m each) were used. In Lake Suğla, these efforts were doubled, and in Lake Beyşehir 13 multi-mesh gillnets and 4 regular gillnet sets were used. Nets were cast overnight for 12 hours. Following capture, we immediately fixed the specimens in 4% formalin and later transferred them to a 70% alcohol solution.

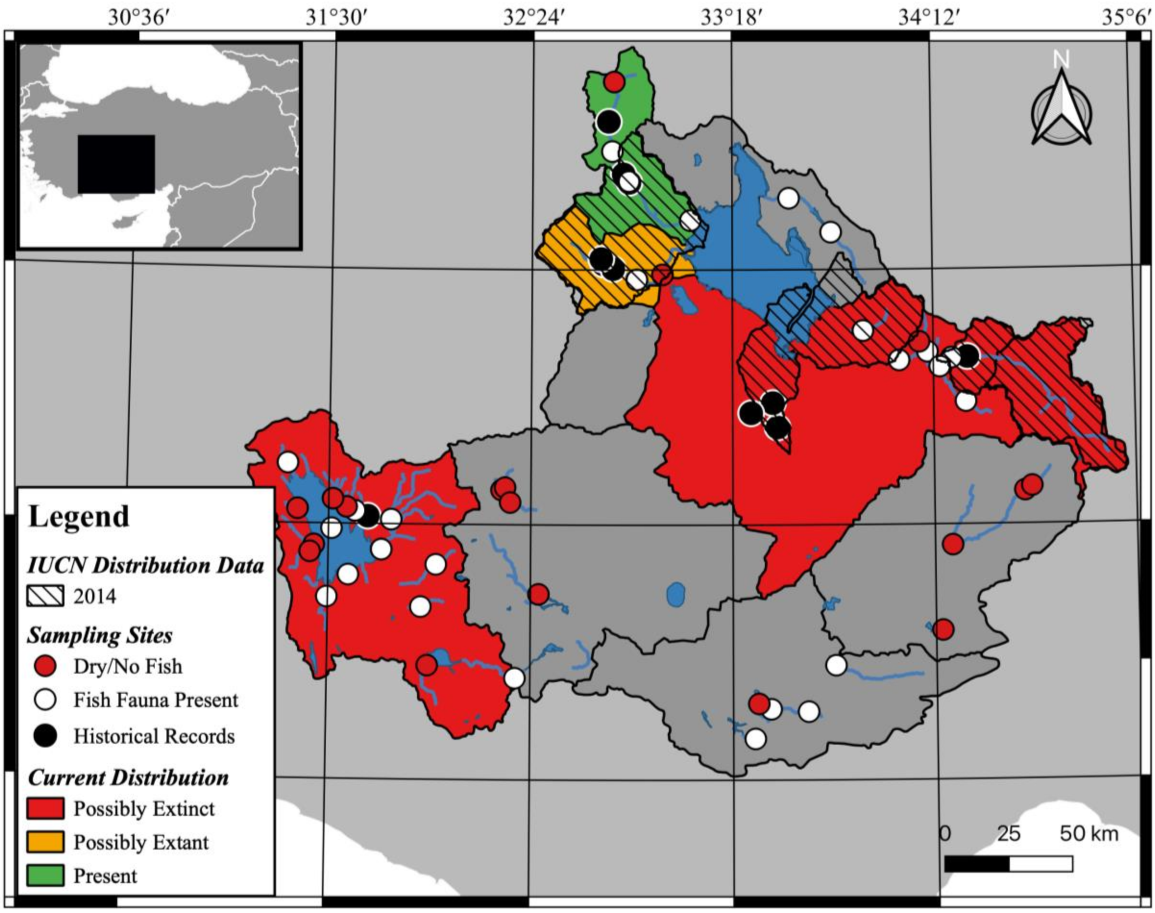

Figure 1. Historical and current records of *P. crassus* in Konya Closed Basin (sampling range: red and white dots; historical records: black dots; IUCN distribution data: black dashed lines)

Analytical Methods

We identified the species according to Atalay (2005), Naseka et al. (2006), and Özuluğ and Freyhof (2011). Sex determination was carried out based on pelvic fin length following the protocols of Atalay (2005) and Yoğurtçuoğlu et al. (2016). Total length (TL), fork length (FL), and standard length (SL) were measured using a digital calliper and rounded to the nearest millimetre, while total weight was rounded to the nearest milligram. Specimens were examined for external anomalies using the classifications of Smith et al. (2002).

To calculate length-weight relationships, we used the logarithmic transformation of Weight = a × Total Length^{b} (Ricker, 1975). We also calculated Fulton's condition factor from fork length K = (W/FL^3) × 100 (Fulton, 1904); and the relative condition factor (K_n) using K_n = W/aFL^n (Le Cren, 1951). We conducted all calculations in R 4.0.2 (R Core Team, 2020) using the FSA package (Ogle et al., 2020). We compared the obtained length-weight relationships and condition factors with data from 2003 provided by Yoğurtçuoğlu et al. (2016) and Özel (2005), respectively.

We also analysed the model's variance to assess the differences in relative condition factor among the anomalous groups (relative condition factor: ~ FL × anomalous group). As a post hoc test for ANOVA, least-square means corrected for changing length were calculated and compared within groups to define the anomaly-presenting groups from which significant differences arose. We considered additional species to further clarify the impacts of external anomalies on condition factors. First, all sampled individuals exhibiting anomalies were filtered, and from among them, we selected two endemic fish species with adequate sample sizes: *Gobio microlepidotus* Battalgil (1942) and *Squalius cappadocicus* Özuluğ & Freyhof (2011).

We used the base package to perform the analysis of variance and we used the emmeans package (Lenth, 2020) in R 4.0.2 (R Core Team, 2020) to compare the models of anomalies and condition factors.

Results

Of the 41 surveyed streams, 18 were dry or contained no fish. The sampling sites and relevant historical records are presented in Figure 1. We found *P. crassus* at only one locality within the Cihanbeyli-Yeniceoba-Kulu/b subbasin (Subbasin-b). Although we cannot confirm the absence of the species elsewhere due to our limited sampling efforts, we speculate that there has been a decline in the likelihood of its presence.

According to IUCN presence criteria, we classified the species' presence in the Cihanbeyli-Yeniceoba-Kulu/a subbasin (Subbasin-a) as 'Possibly Extant' and in the Aksaray-Misli subbasin (Subbasin-c) and Beyşehir subbasin (Subbasin-d) as 'Possibly Extinct' due to significant habitat losses.

We sampled a single population of *P. crassus* from the downstream segment of Subbasin-b. The fish assembly of this segment comprises *P. crassus* (n: 131, 82%), *Oxynoemacheilus eregliensis* (n: 9, 6%), *Anatolichthys anatoliae* (n: 4, 3%), *Seminemacheilus ekmekciae* (n: 14, 9%), and *Cobitis turcica* (n: 2, 1%). We were not able to sample any fish from the upstream segment of the river (Figure 3).

The physicochemical properties of the stream segments and their discharge point (Samsam Lake) from April to December 2016 are shown in Figure 2. The land-use map for 2018 is presented in Figure 3, while Table 1 displays the percentage changes in land cover between 2000 and 2018 in the sampling area.

We sampled 115 specimens in the headwaters of Samsam Lake, identifying 41 individuals as juveniles (FL of <84 mm). Of the remaining specimens, 38 were identified as male and 36 were identified as female (1:0.95). Male specimens had standard lengths ranging from 71 to 149 mm, while female specimens had standard lengths ranging from 70 to 128 mm. A summary of the comparison of length-weight relationship parameters between the population in 2003 (Yoğurtçuoğlu et al., 2016) and in 2016 is presented in Table 2.

Of the 115 examined specimens, 57 (50%) had single or multiple external anomalies (Figure 4). Juveniles had significantly fewer anomalies than adults, with a frequency of 17%. The frequency of anomalies was 82% for male specimens and 53% for females. Counts of individuals with a single anomaly were similar among adults (19 males and 16 females), whereas the number of male individuals with multiple anomalies was significantly higher (12 specimens versus 3). Individuals with anomalies and the distribution of their standard lengths are summarised in Table 3.

The count of external anomalies in male specimens (n=51) was significantly higher than that in females (n=23) and juveniles (n=8). We classified the recorded anomalies into eight groups (Table 4), and the most frequent anomaly type was a deformed fin (35%), followed by mild erosion of the fin (28%).

To compare the condition factors of the population between 2003 (Özel, 2005) and 2016, the mean Fulton condition factor was calculated from fork length and found to be 1.79, 1.78, and 1.72 for the female, male, and total specimens (including juveniles), respectively. Condition factors are known to fluctuate throughout the

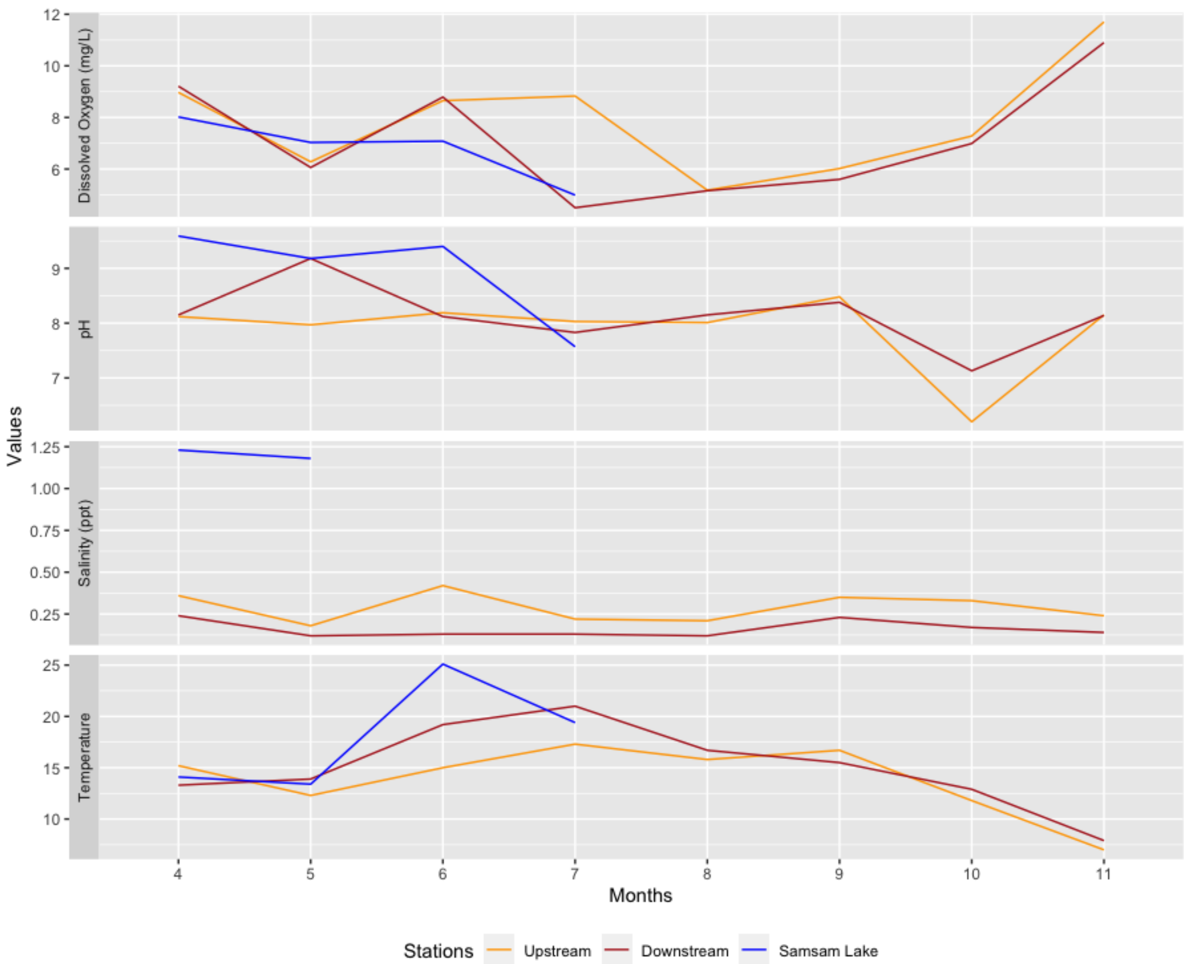

Figure 2. Physicochemical properties of the stream segments and their discharge point (Samsam Lake) between April and December 2016

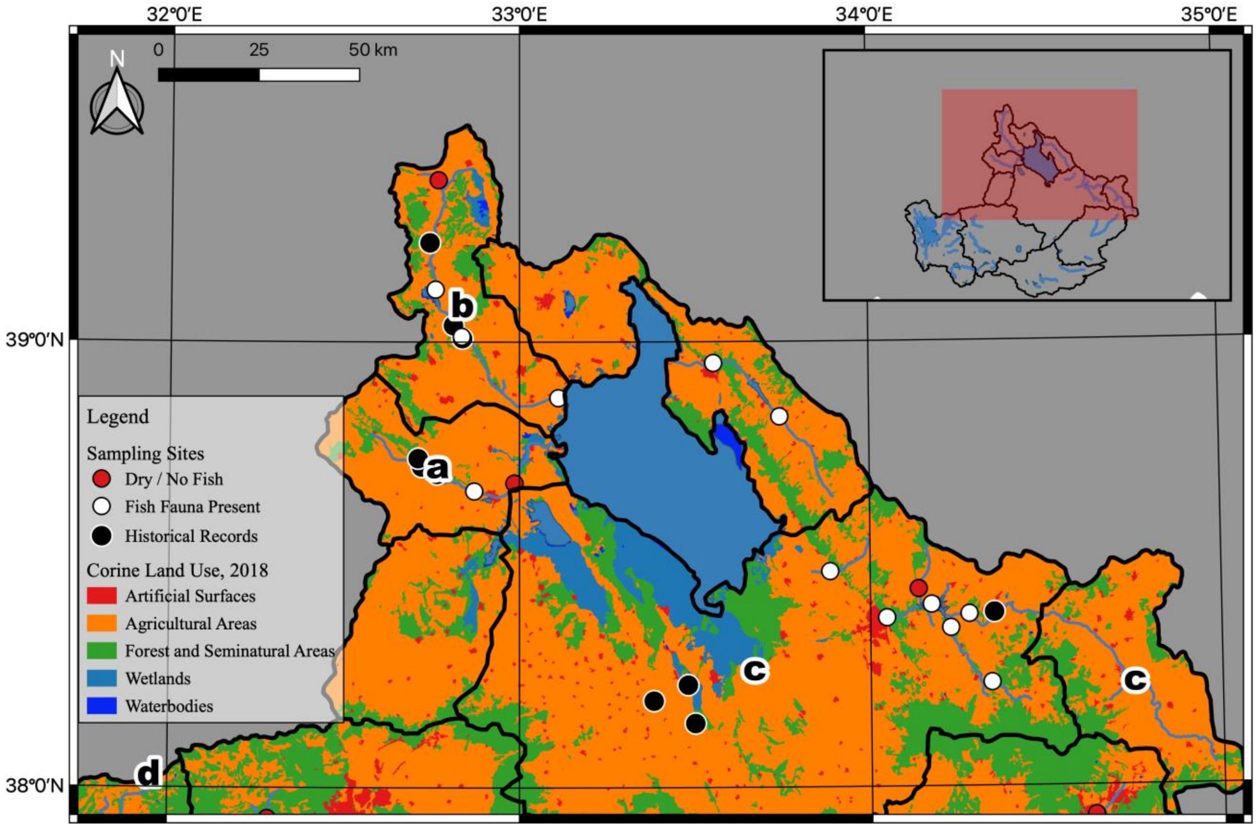

Figure 3. First-level CORINE land-use cover of the Konya Closed Basin subbasins (a-d) where *P. crassus* was present historically

Table 1. First level Corine data (2000-2018) of subbasins of Konya Closed Basin, where *P. crassus* was known to distribute

Subbasins		2000	2018	Change
Subbasin-a (Cihanbeyli-Yeniceoba-Kulu / a)	Artificial	1.9	1.7	-0.2
	Agricultural	82.3	85.9	**3.6**
	Natural	13.9	9.3	-4.6
	Wetland	1.5	2.7	1.2
	Water	0.4	0.4	0
Subbasin-b (Cihanbeyli-Yeniceoba-Kulu / b)	Artificial	2.7	2.7	0
	Agricultural	74.3	75.7	**1.4**
	Natural	20.5	17.7	-2.8
	Wetland	1.8	2.9	1.1
	Water	0.7	1	0.3
Subbasin-c (Aksaray+Misli)	Artificial	2.3	2.0	0.3
	Agricultural	67.6	65.6	**2.0**
	Natural	22.3	28.5	-6.2
	Wetland	7.1	3.4	4.3
	Water	0.6	0.5	0.1

Table 2 Length-Weight Relationship parameters of *P. crassus in* Konya Closed Basin for 2003 and 2016

Sampling Year	Sex	n	Total length (cm)		Weight (g)		Regression parameters				References
			Min	Max	Min	Max	*a*	*b*	SE (*b*)	r^2	
2016	Male	38	8.7	17.96	8.1	54.3	0.0066	3.32	0.11	0.96	This study
2016	Female	36	8.4	15.16	7.4	106.7	0.0096	3.19	0.10	0.97	
2016	Juvenile	41	5	8.35	1.4	8.9	0.0057	3.45	0.08	0.98	
2016	Total	115	5	17.96	1.4	106.7	0.0085	3.23	0.02	0.99	
2003	Male	54	5.6	17.2	1.9	80.4	0.0071	3.29	0.05	0.99	Yoğurtçuoğlu et al., 2016
2003	Female	37	5.2	19.8	1.8	133.1	0.0087	3.22	0.06	0.99	
2003	Total	91	5.2	19.8	1.8	133.1	0.0075	3.28	0.04	0.99	

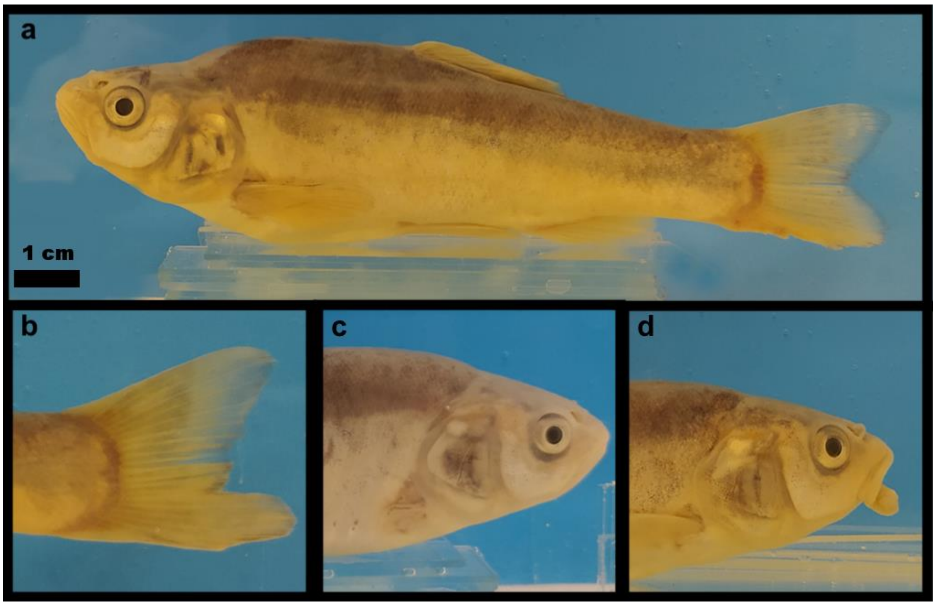

Figure 4. External anomalies: a) Specimen with multiple anomalies (head, dorsal, and caudal fins), b) unequal caudal fin lobes, c) pectoral fin missing, d) lower jaw anomaly

Table 3. Standard length distribution of *P. crassus* in Konya Closed Basin and anomaly group of specimens according to sex

Standard Length (mm)	Male				Female			
		Anomalies				Anomalies		
	n	Single	Multiple	None	n	Single	Multiple	None
70-80	3	-	-	3	4	-	1	3
80-90	5	3	2	-	6	3	-	3
90-100	10	3	5	2	9	4	2	3
100-110	11	7	2	2	14	7	1	6
110-120	6	3	3	-	2	2	-	-
120-130	1	1	-	-	1	1	-	-
130-140	1	1	-	-	-	-	-	-
140-150	1	-	1	-	-	-	-	-

Table 4. Frequency of observed external anomalies on *P. crassus*

Anomalies	Juveniles	Male	Female	Total
Mild erosion of fins	0	21	2	23
Severe erosion of fins	2	3	1	6
Tumour like growth	5	0	0	5
Nodule on fins	0	0	1	1
Deformed head	0	1	1	1
Deformed mouth	1	9	4	14
Deformed fins	0	15	14	29
Deformed scales	0	2	0	2

year, so comparisons between 2003 and 2016 were only made for similar months (Figure 5).

Differences in the relative condition factors of the anomalous groups are shown in Figure 6. According to the analysis of the variance of the model (relative condition factor: ~ FL × anomaly group), the relative conditions of the fish remain the same with increasing length (F=0.061; df: 1; p=0.80), but some groups with anomalies were found to have different condition factors (F=3.95; df: 2; p<0.05). However, when fish lengths were considered, that relationship was insignificant (p=0.56), indicating that the length distributions of specimens belonging to anomalous groups differed.

Least-square means and their standard errors of the groups were as follows: no anomalies=1.051 (SE=0.023), multiple anomalies=0.957 (SE=0.022), and single anomalies=0.995 (SE=0.016). Pairwise comparisons of K_n showed that the only significant difference was between the individuals exhibiting no anomalies and those with multiple anomalies.

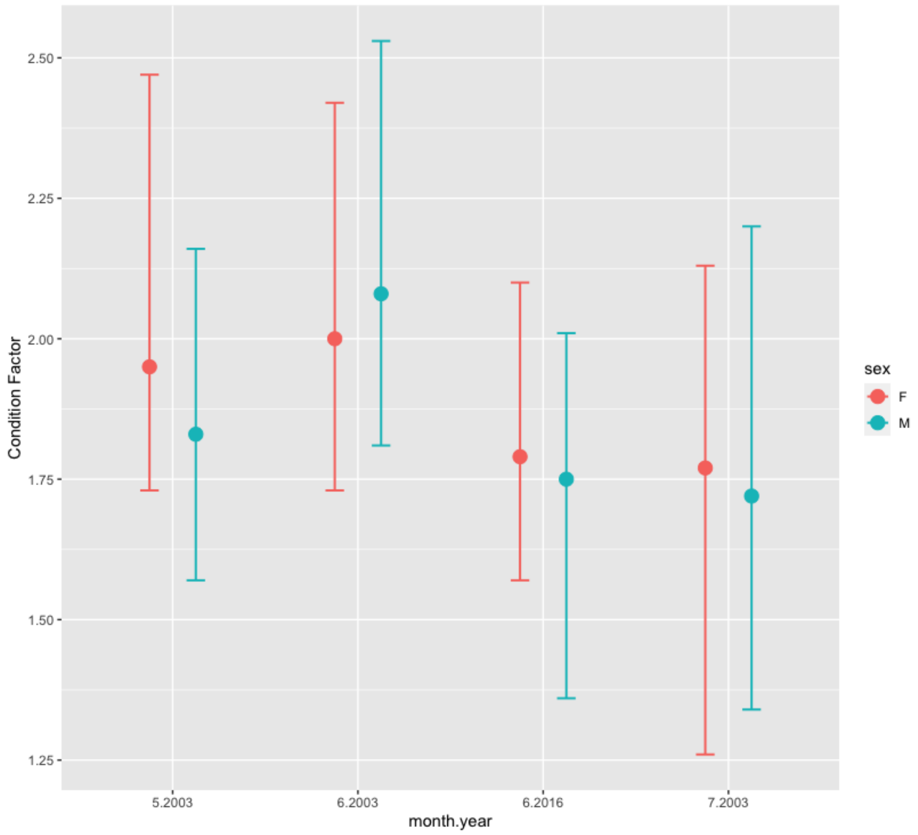

Figure 5. Fulton condition factor differences between 2003 and 2016 for *P. crassus* in the Konya Closed Basin

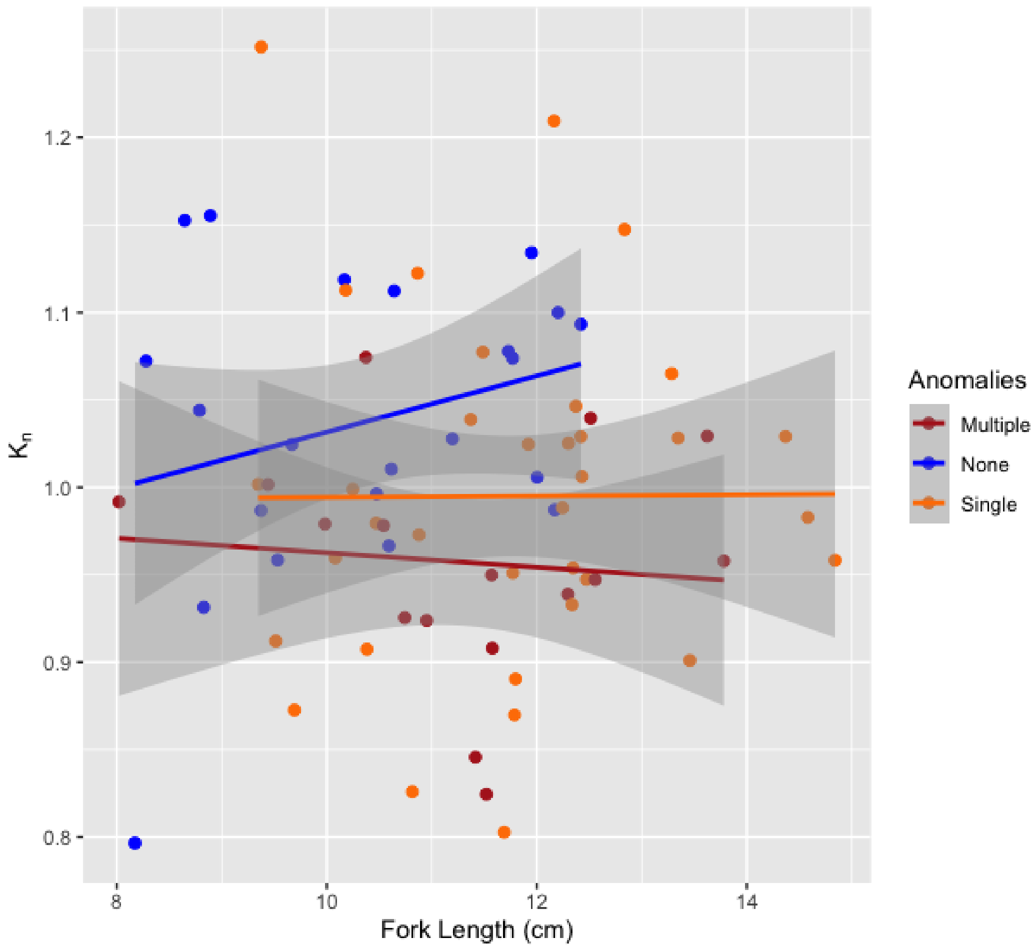

Figure 6. Relative condition factor changes with increasing length among anomalous groups

The distribution areas of *Gobio microlepidotus* Battalgil (1942) and *Squalius cappadocicus* Özuluğ & Freyhof (2011) overlap with the historical distribution area of *P. crassus*. *G. microlepidotus* was sampled from the Beyşehir subbasin (Subbasin-d) and *S. cappadocicus* from the Melendiz subbasin (Subbasin-c). The anomaly frequencies of these populations were significantly lower than that of *P. crassus*, at 25% for *G. microlepidotus* and 34% for *S. cappadocicus*.

The least-square means and standard errors of K_n among the groups were as follows: no anomalies=1.011 (SE=0.007), multiple anomalies=0.897 (SE=0.048), and single anomalies=0.990 (SE=0.013) for *G. microlepidotus* (Figure 7), and no anomalies=1.009 (0.013) and single anomalies=0.976 (0.021) for *S. cappadocicus* (Figure 8). Pairwise comparisons of K_n means showed that the only significant difference occurred between the individuals exhibiting no anomalies and those with multiple anomalies among the *G. microlepidotus* (p=0.055) specimens.

Discussion

Thirty-eight freshwater fish species are known to be distributed in the Konya Closed Basin and 28 of those are endemic (Çiçek et al., 2018; Yılmaz et al., 2021). As seen in Figure 3 and Table 1, agricultural areas dominate the land usage in all subbasins where the considered species are known to be distributed. From among 48 sampling localities, *P. crassus* was only sampled from a single locality in the Cihanbeyli-Yeniceoba-Kulu/b subbasin (Subbasin-b). This subbasin had the lowest reduction in natural areas and the lowest increase in agricultural areas (Table 1) between 2000 and 2018. The related percentage changes in land-use cover might seem low, but these percentages should be interpreted carefully. Natural habitat losses, especially in riparian zones, might exert significant impacts on aquatic fauna (Sweeney et al., 2004; Richardson et al., 2010). Furthermore, in addition to changes in the total agricultural area, crop diversity and changes should also be evaluated to accurately visualise the full pressure of agriculture on water resources. Changes in crop diversity might significantly increase the water usage (Yılmaz et al., 2021).

Nearly 44% of the streams in which sampling was conducted were dry or without fish. Regarding the physicochemical characteristics of the water in Subbasin-b (Figure 2), the dissolved oxygen content dropped drastically in May and July in the downstream segment and Samsam Lake following the rise of water temperatures. Before the second more significant drop in June, the salinity values of the upstream sampling area had nearly doubled, reaching 0.42 ppt. Increases in

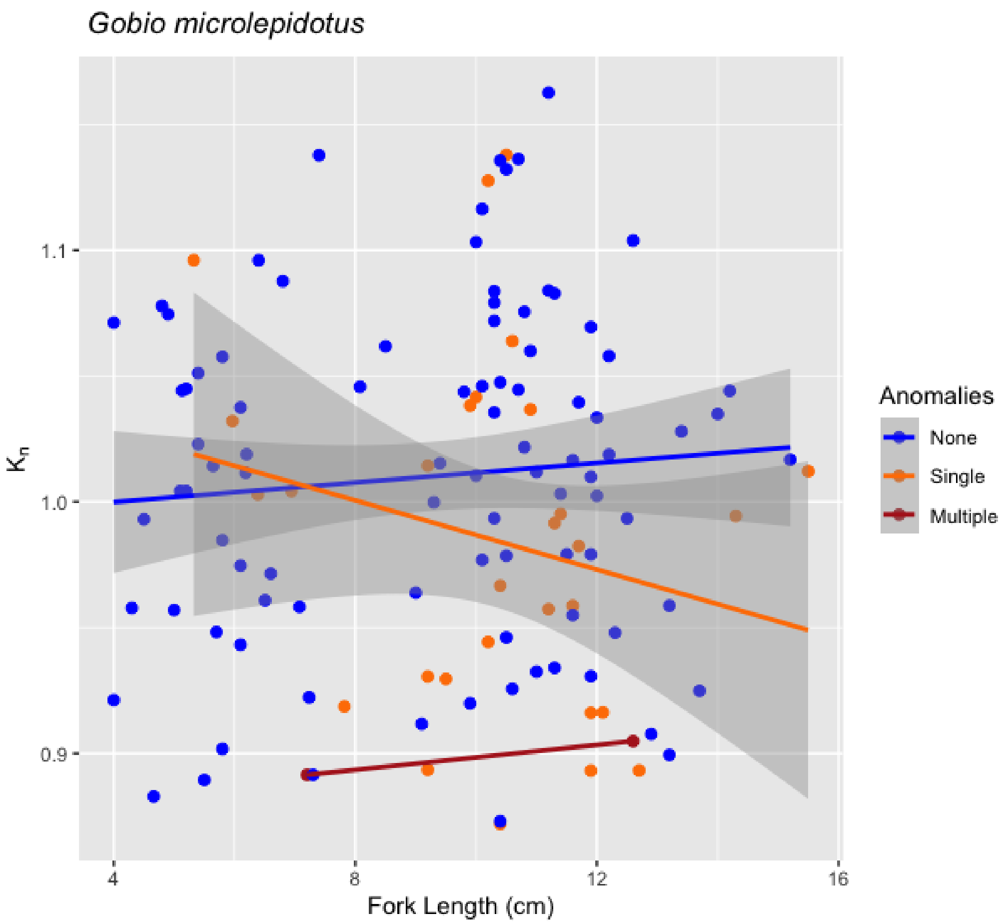

Figure 7. Relative condition factor changes with increasing length among anomalous groups of *G. microlepidotus*

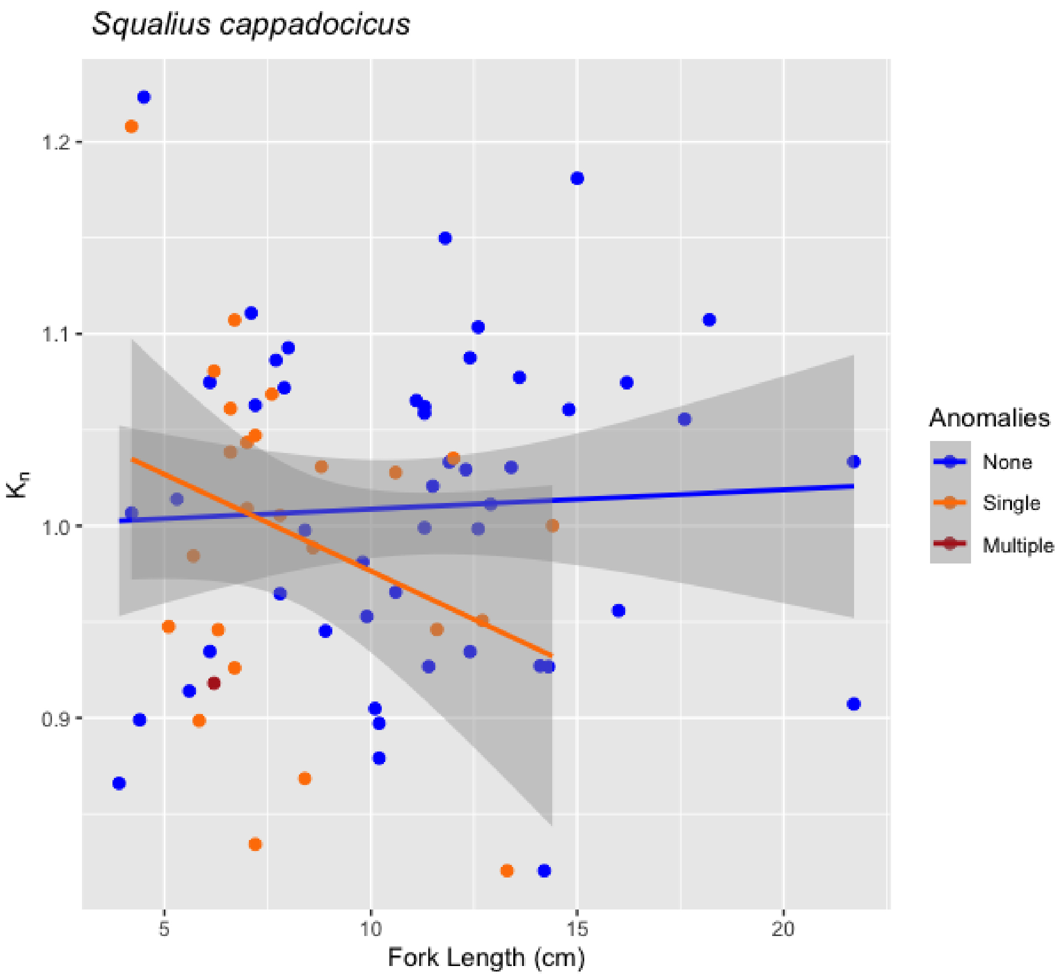

Figure 8. Relative condition factor changes with increasing length among anomalous groups of *S. cappadocicus*

temperature and salinity decrease the oxygen solubility of the water (Verberk et al., 2011). However, oxygen solubility does not entirely explain low oxygen values. According to percentage saturation values calculated from temperature, salinity, and dissolved oxygen with DOTABLES v. 3.6 (USGS, 2018), saturation dropped to nearly 54%. The pollution of the water most probably causes this oxygen deficiency, and if this pollution is coupled with toxic pollutants, which is possible in agricultural areas (Moss, 2008; Matae-Sagasta et al., 2017), the effects of the toxic pollutants may increase in areas with low oxygen levels (Lloyd, 1961). All of these effects can cause developmental anomalies in fish (Sindermann, 1979; Sloof, 1982; Longwell et al., 1992; Barker et al., 1994). A high frequency of anomalies such as deformities, erosions, lesions, and tumours (i.e. DELT anomalies) indicates chemically induced sublethal stress (Iowa DNR, 2001). Habitat degradation coupled with bacterial infections, extreme physicochemical changes, and chemical pollutants may be related to fin erosion in wild fish populations (Latremouille, 2003). Physiological changes, genetics, ageing, nutrition, and other factors may also be related to skeletal deformities (Boglione et al., 2013). On the other hand, deformation anomalies may be connected to developmental instabilities, which may also be associated with pollution or more complex deformations such as pug-headedness (Valentine, 1975; Slooff, 1982).

The population under study exhibits intensive external anomalies and its growth seems to be impaired. Specimens smaller than 8.35 cm could not be sexed in this study. Therefore, comparisons between the datasets (2003 and 2016) could be conducted most accurately based on total individuals. Compared to the records from the previous year (see Table 2), the exponent b value of the total individuals seems to have decreased. However, the length and weight distributions of the compared datasets were not identical. The length distribution of the 2003 dataset ranges between 5.2 and 19.8 cm, while that of the 2016 dataset ranges between 5 and 17.96 cm. Difference between the length distribution occurs on the largest individuals (maximum (x, y) coordinates). A few outliers in this region would not affect the regression line. However, as data points in this region increase, the model may be significantly altered (Stevens, 1984). Therefore, it is not easy to speculate whether these observed differences were due to condition changes or to certain artefacts in the regression analysis.

Özel (2005) reported that March and April constituted the reproduction period for this population and showed that the average condition factor for all specimens was highest in June. However, according to our results, the condition factor of the population in June 2016 was significantly lower than that reflected by the 2003 data (Figure 5), showing much more resemblance to the July values in the previous dataset. Because the 2003 data include only minimum, maximum, and average values, it is hard to interpret the significance of the difference in the means of condition factors. However, a significant change in the distribution of condition factors is visible in Figure 5.

Based on the results presented here, we found partial support for our hypotheses, which can be summarised as follows: anomalies affect the condition factors of fish, but the intensity of anomalies does not affect the magnitude of the change in condition factor. We anticipated that multiple but relatively small anomalies such as fin erosion would not have effects on the organism as significant as those of a single life-impairing anomaly such as jaw deformation. Internal anomalies should also be considered to obtain a clearer picture of the effects of anomalies, and all anomalies must be assessed separately. However, significant results in that regard would require more specimens, which would be more difficult and perhaps unnecessary, and thus unethical, from the point of view of wild, endangered populations. The significance of the present results has also been affected by the relatively small distribution range of $K_n$and random errors from model fitting. Therefore, increasing the sampling effort to assess anomalies separately may not decrease the statistical significance as expected.

The endangered species *P. crassus* is one of the 28 endemic fish species of the Konya Closed Basin. In one of the few populations of this species, we compared the growth and condition of the population structure across 13 years and reported extensive morphological anomalies. We showed that these anomalies affect the condition of the fish and possibly make this species more vulnerable to environmental and climate changes. These anomalies were thought to be associated directly or indirectly with habitat use and habitat change.

The Konya Closed Basin is a unique habitat with high biodiversity and it is under serious threats of salinisation (Jeppesen et al., 2020; Yılmaz et al., 2021), drought (Topak & Acar, 2010; Doğan, 2013; Dolsar, 2015; Yılmaz, 2017), and pollution (Ucan & Dursun, 2009; Aydın et al., 2012). These threats and human dependence on agriculture are making the environmental management of the basin difficult. However, responsible agricultural applications could establish a middle ground between species conservation and human needs.

Ethical Statement

Work conducted on fish species in this study was approved by the Republic of Türkiye Ministry of Agriculture and Forestry (Permit date/no: 30.05.2016/35853172150-1719).

Author Contribution

The study was conceived and designed by MK and FM. MK, FM, and SVY were in charge of collecting data and conducting research. The manuscript was drafted by MK and FM. The manuscript was reviewed and revised by MK, FM, and SVY. The final manuscript was read and approved by all authors.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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