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Dependence of pH values in the digestive tract of freshwater fishes on some abiotic and biotic factors

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ABSTRACT

The values of pH in the digestive tracts of 20 freshwater fish species inhabiting various Russian Federation waterbodies were studied. Only in six species (*Coregonus lavaretus*, *Coregonus migratorius, Catostomus catostomus, Carassius gibelio, Rutilus rutilus, Leuciscus leuciscus*) out of 20 species, the differences in pH values between different regions of the intestine were significant. Feeding habits, feeding frequency and gut fullness in fish affected pH values. Temperature was one of the most important factors affecting pH values. During cold seasons (spring and fall; average water temperature: 8–10 °C and 5–6 °C, respectively), pH values in fish guts were higher than in summer (water temperature 22–25 °C) for *C. gibelio, Perca fluviatilis, Cyprinus carpio, L. leuciscus,* and *R. rutilus* from the Chany Lake. Similar results (lower pH values in intestine at higher water temperatures) were also obtained for *C. gibelio* in warmer years in comparison to colder years in the same waterbody and in *L. leuciscus* and *P. fluviatilis* in the different waterbodies with different water temperatures. It is hypothesized that dependence of pH in fish gut on temperature may serve as a regulatory mechanism for maintaining the activities of hydrolytic enzymes at the required level for their successful functioning.

INTRODUCTION

Studies on the digestive physiology of fish are extremely important for better understanding the nature of their adaptations to dietary changes, as digestion supplies the organism with nutrients required for different biological functions. The key role in food utilization depends on the activity of digestive enzymes present in various regions of the digestive tract. The analysis of the efficiency of fish digestion under natural and aquaculture conditions requires of detailed studies on various aspects of food hydrolysis in the gut, including the influence of temperature and pH on the activity of digestive enzymes among others, since enzyme activity mainly depends on these two factors (Izvekova et al., 2013). The majority of research dealing with analysis of activity of digestive enzymes starts with determining the optimal pH values for their functioning. These optimal values differ considerably depending on different groups of digestive enzymes (alkaline and acid proteases, lipases, carbohydrases, etc) and fish species (Lazo et al., 2007; Izvekova et al., 2013; Solovyev et al., 2015; Pujante et al., 2016).

In general terms, pH values in fish stomachs are within the range of acid values; whereas in the intestine, these values range from neutral to weakly alkaline pHs (Pegel', 1950). Pepsin is one of the most important gastric proteases. For example, in various fish species such as Nile tilapia *Tilapia nilotica,* sharptooth catfish *Clarias gariepinus,* redfish *Sebastes mentella,* seabream *Sparus aurata*, turbot *Scophthalmus maximus* and Monterrey sardine *Sardinops sagax caerulea*, the optimal values of pH for pepsin are comprised between 2 and 4 (Moriarty, 1973; Uys & Hecht, 1987; Munilla-Moran & Saborido-Rey, 1996; Castillo-Yaneza et al., 2004). However, the values of pH in the gastric juice may vary considerably from pH 1.6–2.0 to the alkaline range, such variations may be determined not only by the species considered and between individuals of the same species (Barrington, 1957; Fange & Grove, 1979; Sorvachev, 1982), but also by their feeding habits (Pegel', 1959) and the stage of the digestion process (Maier, 1984; Deguara et al., 2003). Regarding the intestine, the values of pH of this section of the digestive tract in different species like goldfish *Carassius gibelio*, ide *Leuciscus idus*, wild carp *Cyprinus carpio*, perch *Perca fluviatilis*, and zander *Sander lucioperca* are close to neutral or weakly alkaline, but seldom approach to the acidic range, regardless of the fact of having a stomach or being agastric species (Solovyev et al., 2015). Thus, the different level of pH values could effect on the activity of digestive enzymes as it was shown in numerous *in vitro* studies (Solovyev et al., 2015; Concha-Frias et al., 2016; Pujante et al., 2016 among others). Alkaline proteases may actively function in a broad range of pH values (Alarcon et al., 1998; Solovyev et al., 2015). The optimal pH values for intestinal alkaline proteases range from 8 to 10 and they could be species-specific, as it was demonstrated for Asian bony tongue *Scleropages formosus*, *S. sagax caerulea*, Mayan cichlid *Cichlasoma urophthalmus* and three-spot cichlid *Cichlasoma trimaculatum* (Natalia et al., 2004; Castillo-Yaneza et al., 2004; Cuenca-Soria et al., 2013; Toledo-Solís et al., 2015). In particular, the optimal pH values for trypsin range from 8 to 9 in different fish species such as Atlantic halibut *Hippoglossus hippoglossus*, Dover sole *Solea solea*, turbot *S. maximus* and red drum *Sciaenops ocellatus* (Glass et al., 1989; Lazo et al., 2007); whereas for chymotrypsin, the optimal pH value is close to 8 in red drum *S. ocellatus* (Applebaum et al., 2001). However, it was shown that both trypsin and chymotrypsin may exhibit higher level of proteolytic activity at pH values ranging from 9 to 10 in Prussian carp *C. gibelio* (Jany, 1976), and the same is true for other proteases like elastase and collagenase in other fish species (Hidalgo et al., 1999). In addition, the optimal pH values for another important hydrolase, the α-amylase, are also widely variable, which may be attributed to the presence of several isoforms of this digestive enzyme in many fish species. For instance, the study of five different Mediterranean sparid species (red porgy *Pagrus pagrus*, common pandora *Pagellus erythrinus*, blackspot seabream *P. bogaraveo*, bogue *Boops boops* and annular seabream *Diplodus annularis*) revealed several peaks of α-amylase activity at pH values ranging from 4 to 9 (Fernandez et al., 2001), whereas for different freshwater species from the Chany Lake were revealed two peaks of α-amylase activity between 7 and 9 (*C. gibelio*, *L. idus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca*) (Solovyev et al., 2015).

Often, the optimal pH values for digestive enzyme functioning determined under laboratory conditions do not coincide with the physiological values of pH in the digestive tract. It is worth noting that the pH optimal values for the activities of digestive enzymes may also differ depending on the substrate used in each study, which is especially true for proteolytic enzymes (Kapoor et al., 1976). Thus, the lack of coincidence of the pH values optimal for digestive hydrolases with their physiological values determines moderate activity of the enzymes and, hence a reduction in the efficiency of digestion (Sorvachev, 1982, Solovyev et al., 2015). This is why the correct interpretation of the interactions of several enzymes necessitates the determination of the physiological pH values in the digestive tract of fish. At the same time, published studies generally lack the systematic data concerning physiological values of pH from the digestive tracts of fish from various ecological groups and inhabiting different water bodies. The publications dealing with the analysis of the natural factors affecting the above mentioned values are absent either.

The goal of the present paper was to characterize the values of pH in different regions of the digestive tract of various freshwater fish from different limnetic habitats and determine dominant factors (*e.g.*, seasonal and interannual fluctuations of water temperature, feeding preferences, level of gastric and intestinal fullness, and feeding frequency) affecting these changes in pH. Along with this goal, the main tested hypothesis was to estimate the influence of water temperature in the variability of pH in fish gut as an important abiotic factor. For this purpose, we have studied several fish species from different Russian water bodies where there is a large variation in interannual environmental conditions, especially water temperature that greatly varies between seasons.

MATERIALS AND METHODS

Area of study and biological material

Adult fish used in the current study were obtained from different Russian Federation water bodies: Lake Teletskoye (51°79′N; 87°26′E); Lake Baikal (52°00′N; 106°11′E); the estuarine area of the Lake Malyie Chany – the Kargat River (54°37′N; 78°13′E) and Kolyma River (69°05′N; 160°08′E) (Fig. 1).

The following species and their size in standard length (SL) were sampled from the following sites: 1) the estuarine area of the Chany Lake – Kargat River (hereinafter Chany Lake): *Carassius gibelio* (*n* = 65, SL 255–276 mm), *C. carassius* (*n* = 3, SL 170–186 mm), *Leuciscus idus* (*n* = 25, SL 230–249 mm), *L. leuciscus* (*n* = 47, SL 140–210 mm), *Cyprinus carpio* (*n* = 11 SL 167–235 mm), *Rutilus rutilus* (*n* = 44, SL 150–260 mm), *Sander lucioperca* (*n* = 5, SL 210–286 mm), *Perca fluviatilis* (*n* = 52, SL 178–200 mm), and *Esox lucius* (*n* = 3, SL 342–441 mm); years of sampling: 2011, 2012 and 2014 (from April to August and in October) at a mean depth of 1.5 m. Water temperature and pH values in the Chany Lake were 8–12 ºC (pH 7.6–8.0) during April and May, 20–25 ºC (pH 7.3–7.8) from June to August, and 6–8 ºC (pH 7.5–7.8) in October. 2) The Lake Teletskoye: *Coregonus lavaretus pravdinellus* (*n* = 12, SL 130–145 mm), *C. lavaretus* (*n* = 7, SL 150–179 mm), *L. leuciscus* (*n* = 6, SL 170–180 mm), *Thymallus arcticus* (*n* = 2, SL 200–220 mm) *and Abramis brama* (*n* = 2, SL 335–395 mm). In September 2012, the water temperature in upper horizon ranged from 15 to 17 ºC, pH was 7.5 and depth of 3–7 m. 3) The Lake Baikal: *P. fluviatilis* (*n* = 11, SL 175–280 mm), *L. leuciscus* (*n* = 9, SL 160–190 mm), *Coregonus migratorius* (*n* = 18, SL 260–350 mm), and *Thymallus arcticus baicalensis brevipinnis* (*n* = 4, SL 340–360 mm) were caught in July 2013 at a water temperature ranging from 8 to 12 ºC, pH 7.2–8.0 and depth of 5–15 m. 4) The Kolyma River: *L. leuciscus* (*n* = 7, SL 160–190 mm), *Coregonus peled* (*n* = 3, SL 260–350 mm), *C. lavaretus* (*n* = 14, SL 200–250 mm), *Coregonus nasus* (*n* = 9, SL 350–450 mm), *Catostomus catostomus* (*n* = 7, SL 400–460 mm) and *Acipenser baerii* (*n* = 7, SL 400–500 mm). Fish were sampled in July 2014 when the water temperature was in average 12–14 ºC, pH 7.3–7.9 and depth of

1–3 m. Due to logistical problems linked to the wide geographical area, samples could not be taken at the same period of time in similar years in the different water bodies considered in this study.

Fish were caught using fishing gears of various mesh sizes depending on the water body considered. Thus, a gill net of mesh size of 20–70 mm was used in the Chany and Teletskoye lakes and Kolyma River, whereas trawl nets of mesh size of 40 mm were used in the Lake Baikal. Immediately after removing fish from nets, alive specimens were placed in plastic containers filled with ice water and transported to the laboratory where fish were measured for their standard length (SL) to the nearest mm. Water temperature and pH were measured with HI 8314 portable pH meter with temperature and pH electrodes (Hanna Instruments, USA).

Determination of pH values

Fish were euthanized by a blow on the head and their gastro-intestinal tracts removed immediately and kept on ice at 0-4 ºC, and the degree of fullness of their digestive tract (stomach and intestinal regions) assessed as follows: low – gut is empty or containing a negligible amount of food particles (less than 1-2% from the total possible volume of food that could occupy the intestine), high – there are a lot of food particles in the gut. Values of pH were measured within the first 5 to 10 min after euthanasia with a HI 8314 portable pH meter with a HI 1083 B microelectrode of 3 mm of diameter (Hanna Instruments, USA). It should be stressed that food items were not eliminated from the gastrointestinal tracts of fish before pH value measurements. Depending on the structure of the digestive tract, pH values were measured in the stomach, pyloric caeca and intestine (anterior, middle and posterior intestinal segments). In coregonids (Coregonidae) and graylings (Salmonidae) that are characterized by their V-shaped stomachs, pH values were measured in the cardiac/fundic and pyloric regions (between 2 and 3 pH measurements per region). In those fish species with long intestine like *C. gibelio* and *C. catostomus*, up to eight measurements were performed along the intestine.

Relationship between digestive pH values and biotic and abiotic factors

In this work, we have analyzed the relationship between the pH values in fish gut and different factors that may affect them, such as i) the region of intestine where the measurement was taken, ii) the degree of fullness of the digestive tract (stomach and intestine), iii) seasonal changes of temperature within the same water body, iv) temperatures in different water bodies and v) feeding habits of fish species.

In particular, in order to evaluate the influence of different water temperatures during the vegetation season, a period considered when the studied area was not covered by ice (from the end of April to October), five species of fish (*P. fluviatilis, C. gibelio, C. carpio, R. rutilus, L. leuciscus*) were used as descriptors from the Chany Lake. The same species captured during the summer period were chosen for studying the relationships between the level of gut fullness, feeding habits, and pH values in the gut. The classification of fish species into different feeding groups was based on the previous studies (Kanaya et al., 2009; Solovyev et al., 2014), and on the analysis of gut contents under a dissected microscope as described in Solovyev et al. (2014).

In order to study the interannual variability of pH values, *P. fluviatilis* and *C. gibelio* were selected from the Chany Lake. Samples for *P. fluviatilis* (2011: n = 6, SL 178–199 mm; 2012: n = 8, SL 153–197 mm; 2014: n = 7, SL 138–204) and *C. gibelio* (2011: n = 5, SL 255–276 mm; 2012: n = 14, SL 200–234 mm; 2014: n = 5, SL 182–240 mm) were collected from July to August in 2011, 2012 and 2014.

L. leuciscus (n = 32; SL 170-200 mm; all waterbodies) and *P. fluviatilis* (n = 22, SL 180-290 mm; Chany and Baikal lakes) were used as model species for studying the effect of different water bodies with different temperature regimes on the gut pH values when the trophic activity of these species was maximal. Due to the low level of variation of pH values among different waterbodies, the influence of this factor on the pH in fish gut was not considered. The period of capture of these species from the Chany Lake was June-July (2012), whereas for the Kolyma River, Lake Teletskoye, and Lake Baikal it was mentioned above. *L. leuciscus* (2012: n = 10; SL 180-210 mm), *L. idus* (2012:

n = 6; SL 230-240 mm), *R. rutilus* (2012: n = 11; SL 170-220 mm) and *C. gibelio* (2012: n = 9; SL 220-270 mm) from the Chany Lake were used for determination of the effect of chyme on the level of pH values in fish gut. The measurements were done in intestines with chyme; then, the same intestines were immediately cleaned from chyme and measurements were repeated again. Chyme was collected from different parts of intestine and the level of pH was estimated. The influence of fish size (SL) on the level of pH was not analyzed because of the lack of enough different body size groups or year classes for comparative purposes. The factor "fish size in SL" was included in the MANOVA (Table 5) to show the unimportance of given size variability in studying fish species (groups).

Statistical analysis

Values from different measured variables are presented as means ± standard error of the mean (SEM), as well as their minimum and maximum values. The level of pH in various parts of digestive tract was compared between sampling dates by the Wilcoxon rank-sum test. The correlation analysis between variables was conducted by means of a R Spearman rank test using the STATISTICA software package, version 8 (StatSoft Inc., Tulsa, OK; www.statsoft.com). To assess the statistical significance of the effects of the above-mentioned, the One-way ANOSIM at $P < 0.05$ with followed by pairwise uncorrected comparison (where it was needed) was used (Hammer et al., 2001). Multifactor analysis of variance (MANOVA) was used for estimating the level of variability described by (coefficient of covariance) season, part of intestine, fullness of stomach and intestine, feeding frequency and body length in pH from five species from the Chany Lake (IBM SPSS Statistics for Windows, Version 20.0). In all cases, statistical significance was considered at $P < 0.05$.

RESULTS

pH variability along the gut

There was a considerable variability in terms of pH values in the stomach and intestine of the different fish species considered in this study (Table 1). The largest differences in pH values between species were recorded in the stomach that ranged from 1.54 in *T. arcticus baicalensis brevipinnis* to 7.97 in *C. migratorius*. In this sense, Table 1 shows a large interespecific and intraspecific variability of gastric pH values, even though the most common average gastric pH values were found between 5.0 and 6.0, comprising 63.6% of the studied species. In addition, in coregonids and graylings both having a V-shaped stomach, mean pH values in the cardiac/fundic region were not statistically significant different (pH 5.47) than in the pyloric segment (pH 5.85) (ANOSIM, $P > 0.05$).

In fish species having pyloric caeca, the levels of pH in these digestive appendages were higher than in those recorded in the stomach, but similar to those measured along the intestine. In general, pH values measured in different intestinal regions were similar in most of the studied species with the exception of *C. lavaretus, C. migratorius, C. catostomus, C. gibelio, R. rutilus,* and *L. leuciscus* that showed different pH values depending on the anterior, mid and posterior intestinal segments considered (ANOSIM, $P < 0.05$). For instance, pH values in *C. l. pravdinellus* and *C. lavaretus* from the Teletskoye Lake increased from the anterior to the posterior intestine, whereas pH values were higher in the mid intestine in this species from the Kolyma River. In *C. migratorius, C. catostomus, C. gibelio* and *L. leuciscus* from Chany Lake, the higher pH values were measured in the mid intestine, whereas in *R. rutilus* pH values decreased between the anterior and posterior intestinal regions.

pH in fish gut and water temperature

The seasonal variability of the pH values measured in the intestines of studied fish species are shown in Figure 2a. In spring with an average water temperature of 8–10 °C, the pH values in fish guts were significantly higher than in summer (22–25 °C) for all studied fish species (ANOSIM, $P < 0.01$). At fall when mean water temperatures decreased in comparison with the summer and they were between 5 and 6 ºC, pH values increased again for *C. gibelio* (ANOSIM, $P < 0.01$), *P. fluviatilis* (ANOSIM, $P < 0.01$), *C. carpio* (ANOSIM, $P < 0.01$), *L. leuciscus* (ANOSIM, $P > 0.05$). No differences were detected in the pH values between spring and autumn in *P. fluviatilis* (ANOSIM, $P > 0.05$) and *C. carpio* (ANOSIM, $P > 0.05$), whereas for *C. gibelio* (ANOSIM, $P < 0.05$) and *L. leuciscus* (ANOSIM, $P < 0.01$) these differences were statistically significant. The correlation coefficients between intestinal pH and water temperature were significant for *C. gibelio* ($R = –0.31$; $P < 0.01$; $n = 55$), *P. fluviatilis* ($R = –0.63$; $P < 0.01$; $n = 39$), *C. carpio* ($R = –0.64$; $P < 0.01$; $n = 11$), and *R. rutilus* (spring vs. summer seasons, $R= –0.59$; $P < 0.01$; $n = 44$; no data available for autumn) while for *L. leuciscus* these correlation coefficients were not significant ($R = –0.07$; $P > 0.05$; $n = 47$). The influence of such factor as “species” was significant for fish in spring (ANOSIM, $P < 0.05$, $R^2=0.10$), summer (ANOSIM, $P < 0.05$, $R^2=0.30$), and autumn seasons (ANOSIM, $P < 0.05$, $R^2=0.21$).

In the Chany Lake where both seasonal and interannual studies on the physiological pH values in the fish digestive tract were performed, no pronounced fluctuations of the water pH values were noted (pH 7.5 – 8.0). The multifactorial analysis of variance has also shown that the season, among all analyzed factors, is the factor that explains the bigger part of the variability of pH values in the majority of fish species (MANOVA, $P < 0.05$; Table 5). The only exception concerns *L. leuciscus* for which the effect of season was the second factor in order of coefficients of covariance.

The interannual variability of the pH values in three different years (2011, 2012 and 2014) in the intestines of studied fish are shown in Fig. 2b. During the considered period, water temperature tended to increase in the Chany Lake, as well as intestinal pH values that become significantly more acid in Prussian carp ($R = –0.41$, $P < 0.01$; $n = 24$), whereas it had no effect in perch ($R = –0.16$, $P > 0.05$; $n = 21$) (Fig. 2b). The frequency of full/empty gut in perch and Prussian carp was similar in those years.

L. leuciscus and *P. fluviatilis* from the Lake Baikal showed the highest pH values (7.6 – 8.3), whereas the lowest were recorded in the Chany Lake (6.6 – 7.3) (ANOSIM, $P < 0.05$; Fig. 3a,b). It is worth noting that during the summer period when fish were captured; water temperature and pH values in the Baikal, Teletskoye and Chany lakes and in the Kolyma River were 8–10 °C (pH 7.2–8.0), 15–17 °C (pH 7.5), 22–25 °C (pH 7.6–8.0) and 12–14 °C (pH 7.3–7.9), respectively. The correlation coefficient between intestinal pH and water temperature was significant for *L. leuciscus* ($R = –0.52$, $P < 0.01$; $n = 37$) and *P. fluviatilis* ($R = –0.78$, $P < 0.01$; $n = 29$), respectively.

Fullness of gut and feeding habits

The frequency of studied fish with empty and full guts and their respective pH levels is shown in Table 2. As expected, the number of studied fish with full gut was more than 50% in almost all cases. For specimens with a full stomach, the level of pH was generally lower then for ones with an empty stomach (82% of species), whereas an inverse relationship was observed when considering the level of pH in the intestine (70 % of species).

The incidence of studied fish with empty and full guts during vegetetion periods in the Lake Chany are shown in Table 3. In all cases, the average number of studied fish with full intestines was around 50% with maximum and minimum values comprised between 72.7 and 38.5%. The higher frequency of individuals with a full gut was recorded in April-May, whereas the minimum values depended on each considered species and it was not correlated to any seasonal period (ANOSIM, $P > 0.05$). In the case of gastric species, no signifiant differences were found in pH values regarding the level of stomach fullness (Wilcoxon rank-sum test; $P > 0.05$).

The relationship between the pH values in the digestive tract of fish with the levels of their gut fullness was analyzed in the most abundant fish species from the Lake Chany, since it allowed us to obtain a large number of animals with a different range of gut fullness values. In *P. fluviatilis*, gastric pH values did not vary as a consequence of gut fullness ($R = 0.72$; $P < 0.05$, $n = 18$; Wilcoxon rank-sum test; $P > 0.05$; Fig. 4 a). On the contrary, intestinal pH values, in *C. gibelio* ($R = 0.52$; $P < 0.05$, $n = 25$), *R. rutilus* ($R = 0.52$; $P < 0.05$, $n = 22$) and *L. leuciscus* ($R = 0.52$; $P < 0.05$, $n = 15$) increased as the level of gut fullness also increased (Wilcoxon rank-sum test; $P < 0.05$; Fig. 4 b), whereas for *P. fluviatilis* there was no correlation between these two variables ($R = 0.32$; $P > 0.05$, $n = 18$).

The analysis of the large number of fish species caught during the summer season in the Chany Lake enabled us to assess the infuence of fish feeding habits on intestinal pH values (Fig. 5). In this sense, intestinal pH values were different depending on the feeding habits considered (ANOSIM, $P < 0.05$). Thus, pH values increased among different feeding niches as follows: piscivorous species (*S. lucioperca, E. lucius, and P. fluviatilis*) omnivorous species (*C. carassius, C. gibelio,* and *C. carpio*) and zooplanktivorous-zoobenthivorous species (*L. idus, L. leuciscus,* and *R. rutilus*).

The level of pH values in the intestine after chyme removal was significantly lower (ANOSIM, $P < 0.05$) than in the same intact intestine still containing the chyme, as well as in the chyme for all studied species (Table 4).

DISCUSSION

pH values in the digestive tract of various fish species

A massive body of data concerning pH values in the digestive tract of different fish species has been accumulated to date. These plethora of studies have revealed that many biotic and abiotic factors influence pH values in the digestive tract of fish. Regarding biotic factors, feeding habits (Maier & Tullis, 1984) and feeding frequency (Day et al., 2014), the buffer capacity and composition of food items (Lobel, 1981), the stage of digestion process (Hlophe et al., 2014), the anatomy of the stomach and time during which food items remain in different regions of the digestive tract (Moriarty, 1973; Nikolopulou et al., 2011), the stress condition of the specimen (Moriarty, 1973), and the stage of fish development (Walford & Lam, 1993) are among the factors affecting gut pH, described so far. In addition, water pH, salinity/conductivity (Maier & Tullis, 1984), temperature (Page et al., 1976), photoperiod and season of the year (Maier & Tullis, 1984) are the main abiotic factors affecting pH values in the digestive tract of fish.

A wide range of pH values, ranging from strongly acidic (1.5–2) to weakly alkaline (7–8) are characteristic of the stomach in fish species (Maier & Tullis, 1984; Izvekova et al., 2013; Hlophe et al., 2014). Similarly to our results obtained from 13 species from different water bodies, the values of pH in the stomach were lower than those recorded in the intestine (Maier & Tullis, 1984; Izvekova et al., 2013; Hlophe et al., 2014). In addition, many researchers have noted a significant variability of pH values in different parts of stomach. For instance, the pH is lower in the cardiac than in the pyloric part in some tilapine species (Payne, 1978), as well as in brown surgeonfish *Acanthurus nigrofuscus* (Montgomery & Pollak, 1988). In the present study, there was a remarkable variability in pH values measured in the cardiac/fundic and the pyloric regions of the V-shaped stomach in coregonid and grayling species, which resulted in the absence of significant differences between different stomach regions. Lobel (1981) noted that the fish with a thin-walled stomach, which may considerably widen in the presence of large food items, gastric pH was lower than in fish with a thick-walled stomach. This observation was also in agreement with the data on the pH from *Oreochromis mossambicus* and *Ictalurus punctatus* (Maier & Tullis, 1984). Our own data also suggested that there was a similar tendency, even though not significant [thick-walled stomach fish *i.e.* coregonids and graylings species had slightly higher, but not significantly higher pH levels (5.66 ± 1.5) than other species with thin-walled stomachs (5.09 ± 1.2) like *P. fluviatilis*, *S. lucioperca* and *E. lucius*]. Regardless of the lack of statistical differences, it may be postulated that fish species with a thin-walled stomach, which in our study were piscivorous and feed on relatively large-size prey such as other fish, should produce more HCl in the stomach in comparison with thick-walled stomach species that may feed on many different invertebrates and did not have such capacity to distend their stomachs. The similar positive correlation between HCl producing and food size was reported in leopard sharks *Triakis semifasciata* (Papastamatiou & Lowe, 2004).

There is scarce information on the pH values in the pyloric caeca from different fish species (Maier & Tullis, 1984; Izvekova et al., 2013). In the present study, pH values in these digestive appendages were close to neutral. In Mozambique tilapia *O. mossambicus*, the pH values in the pyloric caeca were neutral and differed from those measured in the stomach and in the intestine (Maier & Tullis, 1984). It has been suggested that the transition of food from stomach into the intestine, pyloric caeca play important role in the process of modifying the pH value (Montgomery & Pollak, 1988). Although the role of pyloric caeca in digestion in fish is not completely clear, the higher pH values in these digestive appendages in comparison to those recorded in the stomach (Montgomery & Pollak, 1988; Izvekova et al., 2013, present study) may be indicative of their function as an adaptation to increase the surface area, and hence the nutrient uptake capacity of fish gut as Buddington and Diamond (1986) indicated.

Generally, the range of pH variability in the intestine is narrower (pH 6–9) than in the stomach (pH 1.5–7.5). It has been shown that pH values in various fish change along the intestine; thus, the increasing in pH levels in the anterior part of intestine is aimed to neutralize the acidity of the chyme acidity by the secretion of bicarbonate ions from the accessory digestive glands (Deguara et al., 2003; Nikolopoulou et al., 2011). For instance, pH values gradually increased from the anterior to the posterior intestine in *A. nigrofuscus* and *O. niloticus* (Moriarty, 1973; Montgomery & Pollak, 1988), while in European sea bass *Dicentrarchus labrax* the reverse pattern was noted (Eshel et al., 1993). In contrast, the study on three herbivorous catfishes (*Panaque cf. nigrolineatus, Panaque nocturnus* and *Hypostomus pyrineusi*) revealed no significant differences in pH values along different intestinal segments (German & Bittong, 2009). In our study, we also observed pH variations along the intestine, even though these differences between intestinal regions were not always significant (just in 6 agastric species out of the 20 ones studied) and it did not correlate with the gut length or fish feeding habits. The wide variations in pH within regions of the intestinal tract in different fish species might be explained by several factors such as the present of protozoan gut symbionts (Montgomery & Pollak, 1988), various level of activity of alkaline phosphatase along the gut (Lalles, 2010), by differences in sampling times and procedures, as well as by the time interval between feeding and examination, since pH varies as a function of feeding time and digestion (Maier & Tullis 1984; Deguara et al. 2003).

Influence of water temperature and pH on the values of pH in digestive tract

Data on the influence of water temperature on the level of pH in fish gastro-intestinal tract are almost absent (Page et al., 1976). Some first experiments in channel catfish *I. punctatus* revealed a decrease in the intestinal pH values with increasing water temperatures, whereas this pattern was reversed in the stomach (Page et al., 1976). The authors of this study explained this phenomenon by an increase in the metabolic rate and respective increase in food consumption leading to an increase in the buffer capacity in the stomach. In addition, the increase in water temperature and consequent rise in the metabolic rate of fish, the velocity of food transport along the digestive tract was also increased and the level of pH did not reach its possible maximum (Moyano F.J., *personal communication*). According to Getachew (1989), the water acidity may affect the pH values in the digestive tract. The former author presumed that small amounts of water entering the stomach with food might change the acidity level. However, it is likely that in such case a certain compensatory mechanism may exist, since the specimens with high levels of stomach fullness exhibited quite low pH values. For example, regarding to marine fish species, $CaCO_3$ precipitation from drunken water is also important for maintenance the high pH level (9.0) in the intestine (Wilson & Grosell, 2003), since marine fish actively drink water for osmoregulatory purposes, whereas fresh water fish do not. In this particular study, the salinity of the Chany Lake, the sampling site where most of the samples were obtained for conducting this study, is low (0.8-5.3 g/L, being Na^+ and Cl^- the most important ions) and the level of pH water stable enough due to the high buffer capacity of water (Savkin et al., 2005); thus, water pH would not have a great impact on the pH values measured in the digestive tract of sampled fish, regardless of the fact that small quantities of water might have been ingested with food items.

Our study revealed that temperature affected pH values in the fish intestine. This was confirmed by data from seasonal and interannual variability of pH values in the digestive tracts of studied freshwater fishes, as well as by differences in this parameter in the fish from waterbodies differing in thermal regimes, but having close water pH values. These findings were also supported by the results from the MANOVA. Thus, our results indicated that intestinal pH values declined in parallel to the summer increase in water temperature. Besides, these values were lower in warmer years in the same water body and in other water bodies with warmer waters. This relationship may be considered as an adaptation to enable fish, as poikilothermic animals, to regulate and optimize the activity of their digestive pancreatic enzymes through concentration of hydrogen ions (*i.e.* pH) to changes in the environmental temperature. According to present results, seasonal changes in pH observed in the gastrointestinal tract of the studied species may be in average 0.8 units. In this context, it is known that the deviation of pH values from their optimum by just one unit only may lead to a 50% drop in the activities of some digestive enzymes (Hlophe et al., 2014; Solovyev et al., 2015), which may strongly affect their digestive performance. In this sense, present results may indicate that the digestive capacity of studied fish may change along the season of the year due to seasonal water temperature changes, among other factors considered in this study.

Influence of fish feeding habits on the values of pH

The diversity of structures of the digestive tract, which is strongly determined by their feeding preferences, is a characteristic of fish species with regard to higher vertebrates (Sorvachev, 1982). It has postulated that the buffer capacity of food is a factor affecting the values of pH in digestive tract to a higher extent than the type of food consumed by fish (*i.e.* algae, detritus, invertebrates, etc.)

(Maier & Tullis, 1984). The values of pH in the stomach depended on the food composition. For instance, in the fish consuming food items containing calcium carbonate (shells and bones), the food entering into the stomach may be characterized by a certain buffer capacity. It was shown that feeding milkfish *Chanos chanos* on green algae, the pH value in the stomach was 1.9, whereas a diet based on crustaceans and oligochaetes mixed with sand, resulted in pH values of 6.6 (Lobel, 1981). The pH values of the stomach were recorded at 8.5 in eel-pout *Zoarces anguillaris* fed on sea urchins together with their shells. Thus, it is possible that the alkalinity of the stomach content may be determined by the alkalinity (mainly carbonate concentration) of food items or by some reflux of the intestinal content into the gastric cavity. The values of pH grew with increase in the buffer capacity of the ingested (MacKay, 1929). It has been reported that in carnivorous fish, gastric pH values were slightly higher compared to herbivorous species (Kapoor et al., 1975). In our study, coregonids and graylings from different water bodies also consumed different species of mollusks with their shells that could increase the level of pH in their stomachs, whereas this might not occur in other carnivorous species like *P. fluviatilis*, *S. lucioperca* and *E. lucius*, which are mainly piscivourous. The relationship between the values of pH in various parts of fish digestive tract and fish feeding preferences has been studied by several authors (Kapoor et al., 1975; Maier & Tullis, 1984). Our own data revealed a significant increase in the intestinal pH values the following order: carnivorous – omnivores – zooplanktivores / zoobenthivores. Maier & Tullis (1984) noted the slight insignificant increase in intestinal pH values from herbivorous and omnivores to carnivorous species. We supposed that this differences could be related to the variability in proximate composition and consequently, in the buffer capacity of different food items in the chyme. The proximate composition of food items and the buffer capacity of the chyme could also influence the pH in the intestine, because of the bicarbonate ions from liver with bile and exocrine pancreas (Deguara et al., 2003, Nikolopoulou et al., 2011) continually mix with the chyme due to peristaltic movements (Pegel', 1950). This fact could explain the significantly higher pH level in the chyme when compared with that of the intestine.

Another possible factor that could be potentially associated with and affect the gut fullness and gut pH, and consequently explain the observed results is feeding frequency of fish. It was hypothesized that the different species of fish with different feeding habits (*I. punctatus* - omnivorous, grass carp *Ctenopharyngodon idella* - herbivorous, and largemouth bass *Micropterus salmoides* - carnivorous) may have various feeding frequencies (Day et al. 2014). Indeed, the studied fish from the Chany Lake according to their feeding frequencies (maximum of the gut fullness) during 24 hours (four checking the fish nets every six hours in the Chany Lake) could be classified in four groups: fish that had maximum level of fullness in intestine in morning-day-evening (*R. rutilus*), morning (*L. leuciscus* and *P. fluviatilis*), night (*C. carpio*), and evening-night (*C. gibelio*) hours (unpublished results). The influence of “feeding frequency” as a factor was significant in summer (ANOSIM, $P < 0.05$) and spring (ANOSIM, $P < 0.05$) (autumn was not analyzed due to lack data for roach), but the ratio of explained variability was low (12% and 6%, respectively) and comparable with other factors listed in Table 5. The frequency of feeding determinates the gut fullness and consequently, the time when pH level reaches maximum/minimum or average values.

Influence of digestive tract fullness on the level of pH

Many researchers have studied the effect of gut fullness on pH values (MacKay, 1929; Norris et al., 1973; Maier & Tullis, 1984; Deguara et al., 2003; Yúfera et al., 2012; Hlophe et al., 2014). However, the results of these studies are contradictory. It is generally accepted that in the absence of food in the stomach, the pH is weakly alkaline, but its acidity increases following food consumption (Pegel’, 1950; Sorvachev, 1982; Yúfera et al., 2012). In some cases, the changes in the values of pH were noted either in all parts of the digestive tract or in a certain segment (Maier & Tullis, 1984; Deguara et al., 2003; Hlophe et al., 2014). In other cases, no changes in pH were observed after food consumption (Lobel, 1981).

Considering present data, the reported pH values for different gastric fish species from this species might be used as a reference for these populations. In particular, 82% of the considered species had their stomachs full, and their values were lower than those measured in their congeners with empty stomachs. These results are similar to those found in other species like in *Acanthurus triostegus* where the mean value of the pH in specimens with a full stomach was 4.25, whereas those with an empty stomach had pH values of 6.7 (Lobel, 1981). In contrast, no pronounced influence of stomach fullness on the gastric values of pH was found in *Z. anguillaris* (Mackay, 1929), *Caranx ignobilis* (Lobel, 1981) and *Pleuronectes platessa* (Bayliss, 1935). In addition, fasting for 19 days had no effect on the pH value in stomach of *O. mossambicus* (Maier & Tullis, 1984). A fast decline of pH values after entering the food into the stomach was found in *Lepomis macrochirus* (Norris et al., 1973), *O. niloticus* (Moriarty, 1973) and *O. mossambicus* (Maier & Tullis, 1984). In *O. niloticus,* the highest pH values of 5–7 were recorded at night in fish with empty stomachs (Moriarty, 1973). It was also demonstrated that the more food entering into the stomach, the stronger was the decrease in the value of pH (Akintunde, 1982). The patterns and rates of changes in the pH values of food entering into the digestive tract differed among different fish species. For instance, in *S. aurata* (Deguara et al., 2003) *and O. mossambicus (*Maier & Tullis, 1984), *Tilapia rendalli* and *C. gariepinus* (Hlophe et al., 2014), pH values depended on the level of stomach fullness, and they recovered to the initial pH levels after 12, 8 and 31 h, respectively. In contrast, pH levels in full and empty stomachs of *P. fluviatilis* from the Chany Lake did not vary, which could be explained by the lack of information regarding the time lapse between of food consumption and fish sampling in this species. Indeed, the significant decrease of pH level in the stomach of *P. fluviatilis* was observed only after 10 hours post-feeding (Solovyev et al., 2016). So, it has been clearly shown that even if we were able to detect different food items in the stomach, no enough time had passed for decreasing the pH levels to a certain significant level. Also, in addition, it may also be considered that sampling fish with gill nets might have led to stress and regurgitation of food (Getachew, 1989) that might have resulted in the incorrect assessment of the gut fullness and, hence of pH values in the digestive tract.

Regarding the intestine, the level of pH in full intestines was observed higher than in empty ones in ca. 70 % of the studied species. In *C. auratus*, the value of pH in the all regions of the intestine increased from 6.89 to 8.01 between 1 to 4 h after feeding, whereas pH values returned to their initial values between 6 to 7 h later (Maier & Tullis, 1984). Thus, no differences in the intestinal pH values were found between fasting specimens of *I. punctatus* and their conspecifics studied three hours after beginning of feeding, while the differences in the pH values in the stomach reached 2.5 units (Maier & Tullis, 1984). In contrast, no significant changes in pH were revealed in the intestine for *S. aurata* (except for the posterior part) (Deguara et al., 2003), *O. mossambicus* (2003; Maier & Tullis, 1984), *O. mossambicus, T. rendalli, C. gariepinus* (Hlophe et al., 2014) and *P. platessa* (Bayliss, 1935) during the whole feeding period. Such differences between studies and species remained unclear and they might be due to species-specific differences in digestive processes and their regulation among the studied species, which suggested that further research is needed to characterize the role of feeding on regulating the pH values in the digestive tract, as well as its associated regulatory physiological mechanisms.

In conclusion, this study revealed a remarkably variability in the pH values along the length of digestive tract in a wide number of fish species from different water bodies and ecological groups. Regarding the stomach, pH values varied from strongly acidic to neutral and, in some species, to weakly alkaline, which were in agreement with the function of the above-mentioned digestive organ. Among the parameters assessed, water temperature was one of the factors that strongly affected the pH values in the digestive tract of fish. This was confirmed by the decrease in the intestinal pH values during summer, coinciding with an increase in water temperatures and by lower pH values in warmer years in the same water body. Presumably, the dependence of the pH values in the fish digestive tract on the water temperature may serve as a regulatory mechanism for maintaining the activities of hydrolytic enzymes at the level providing successful digestion. In addition, we found differences in intestinal pH values depending on the feeding habits of the species; thus, a significant increase in the intestinal pH values from carnivorous, omnivores to zooplanktivores and zoobenthivores was observed in this study. Data presented in this study regarding pH values along the digestive tract of different freshwater fish species under different biotic and abiotic conditions may serve as reference value for further field and/or laboratory studies dealing with the digestive physiology of fish.

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Table 1. Values of pH in the fish digestive tract (mean ± SE)

Fish species	Water body	Part of the digestive tract					
		Stomach	Pyloric caeca	Part of intestine			Mean for intestine
				anterior	middle	posterior	
E. lucius	Chany Lake	6.20 (4.65-6.84)	–	6.23±0.07 (6.01-6.41)	6.19±0.05 (6.05-6.34)	6.27±0.09 (6.05-6.50)	6.23±0.07 (6.01-6.50)
P. fluviatilis	Lake Baikal	5.94 (3.41-7.59)	7.43±0.05 (6.93-8.00)	7.55±0.06 (7.07-8.11)	7.64±0.07 (6.82-8.20)	7.48±0.09 (6.70-8.19)	7.56±0.07 (6.82-8.20)
	Chany Lake ***	4.16 (2.70-7.34)	6.67±0.07 (6.12-7.15)	6.59±0.05 (6.06-7.14)	6.67±0.05 (6.25-7.25)	6.67±0.04 (6.28-7.20)	6.64±0.05 (6.06-7.25)
S. lucioperca	Chany Lake	5.72 (4.00$^{¥}$ - 7.30$^{€}$)	6.75±0.09 (6.60-6.90)	6.72±0.06 (6.53-6.76)	6.81±0.09 (6.61-7.04)	6.91±0.19 (6.50-7.60)	6.81±0.11 (6.53-7.60)
A. brama	Lake Teletskoye	–	–	6.28±0.14 (6.88-7.70)	7.57±0.11 (7.22-7.88)	7.21±0.09 (6.84-7.41)	7.35±0.07 (6.84-7.88)
C. carassius	Chany Lake	–	–	6.97±0.09 (6.74-7.35)	7.00±0.11 (6.74-7.29)	7.18±0.18 (6.50-7.61)	7.04±0.13 (6.50-7.61)
C.gibelio	Chany Lake	–	–	6.84±0.03^{a} (6.28-7.96)	6.93±0.03^{b} (6.51-7.68)	6.84±0.03^{a} (6.09-7.58)	6.87±0.03 (6.09-7.96)
C. carpio	Chany Lake	–	–	6.63±0.04^{a} (6.30-7.12)	6.58±0.03^{b} (6.32-6.90)	6.59±0.05^{ab} (6.23-6.97)	6.60±0.04 (6.23-6.97)
L. idus	Chany Lake	–	–	7.12±0.04 (6.76-7.68)	7.29±0.05 (6.79-7.86)	7.18±0.06 (6.65-8.02)	7.20±0.05 (6.76-8.02)
L. leuciscus	Lake Baikal	–	–	7.78±0.11^{a} (6.65-8.22)	8.31±0.09^{b} (7.35-8.77)	8.26±0.12^{b} (7.06-8.87)	8.11±0.10 (6.65-8.87)
	Lake Teletskoye	–	–	7.57±0.08 (7.12-8.00)	7.73±0.09 (7.18-8.06)	7.78±0.11 (7.01-8.25)	7.69±0.06 (7.01-8.25)
	Chany Lake	–	–	7.18±0.05^{b} (6.66-7.81)	7.40±0.07^{a} (6.84-8.07)	7.25±0.05^{b} (6.71-7.77)	7.28±0.06 (6.66-8.07)

	Kolyma River	–		–	7.23±0.08^{a} (6.84-7.64)	7.51±0.08^{b} (7.04-8.16)	7.55±0.05^{b} (7.14-7.80)	7.43±0.05 (6.84-8.16)
R. rutilus	Chany Lake	–		–	7.12±0.05^{b} (6.60-7.80)	7.31±0.05^{a} (6.75-7.89)	6.97±0.04^{c} (6.55-7.72)	7.14±0.04 (6.55-7.89)
C. lavaretus	Lake Teletskoye	ND	4.34/(2.22-6.12)	ND	7.20±0.07^{a} (6.80-7.36)	7.43±0.09^{ab} (7.11-7.60)	7.49±0.14^{b} (7.12-7.78)	7.30±0.07 (6.80-7.78)
	Kolyma River	5.22/(2.12-6.90)	5.85/(4.01-7.10)	ND	7.29±0.06^{a} (6.36-8.10)	7.49±0.06^{b} (6.55-8.32)	7.35±0.05^{ab} (6.43-8.04)	7.38±0.07 (6.36-8.32)
C. l. pravdinellus	Lake Teletskoye	ND	5.36/(2.90-7.72)	ND	7.20±0.17 (6.76-7.60)	7.36±0.17 (7.01-7.70)	7.24±0.18 (6.92-7.65)	7.26±0.06 (6.76-7.70)
A. baerii	Kolyma River	3.90/(2.09-6.79)*		ND	6.54±0.06 (6.38-6.73)	6.56±0.05 (6.40-6.69)	6.49±0.06 (6.25-6.66)	6.50±0.05 (6.25-6.73) /7.00±0.06** (6.58-7.69)
C. catostomus	Kolyma River	–		–	6.57±0.04^{a} (6.10-7.72)	7.23±0.09^{b} (6.24-8.02)	7.07±0.07^{c} (6.54-7.80)	6.95±0.07 (6.10-8.02)
C. migratorius	Lake Baikal	5.25/(2.22-7.97)	5.98/(2.22-7.83)	–	7.85±0.11^{a} (6.44-8.62)	7.90±0.11^{a} (6.67-8.63)	7.57±0.10^{b} (6.83-8.23)	7.77±0.10 (6.44-8.63)
C. nasus	Kolyma River	5.64/(2.17-6.91)	5.8/(2.83-6.94)	ND	6.86±0.08 (6.20-7.53)	6.93±0.07 (6.50-7.59)	6.85±0.05 (6.53-7.07)	6.88±0.04 (6.20-7.59)
C. peled	Kolyma River	6.12/(4.92-6.73)	6.02/(4.34-70)	ND	7.27±0.11 (7.05-7.49)	7.45±0.13 (7.04-7.79)	7.22±0.08 (7.05-7.38)	7.31±0.07 (7.04-7.79)
T. arcticus	Lake Teletskoye	ND	3.10/(1.90-5.98)	ND	7.02±0.11 (6.91-7.13)	7.08±0.15 (6.93-7.23)	7.25±0.30 (6.94-7.55)	7.12±0.10 (6.91-7.55)
T. a. baicalensis brevipinnis	Lake Baikal	4.41/(1.54-7.38)	5.90/(3.50-7.16)	7.09±0.09 (6.67-7.46)	7.42±0.10 (6.96-7.83)	7.46±0.10 (7.17-7.85)	7.46±0.12 (7.04-8.06)	7.45±0.06 (6.96-8.06)

* – in parentheses: minimal and maximal values; ** – pH in the spiral valve; *** – the values for summer time; “–” – lack of sector (part); ND – No data. The lowercase letters denote statistically significant differences among different parts of gut (Wilcoxon rank-sum test, P<0·05).

Table 2. Frequency (in %) and pH (mean) of full and empty (negligeble ammount) digestrive tracts in fish from different water bodies.

Species	Stomach		Intestine	
	Empty / pH	Full / pH	Empty / pH	Full / pH
Chany Lake				
S. lucioperca	60 / 6.47	40 / 4.2	60 / 6.74	40 / 6.92
E. lucius	66.6 / 6.58	33.3 / 4.65	100 / 6.22	0
L. idus	–	–	15.8 / 7.24	84.2 / 7.22
C. carassius	–	–	66.6/6.88	33.3/7.32
Lake Teletskoye				
C. lavaretus	20.0 / 5.91	80.0 / 3.83	0	100 / 7.30
C. l. pravdinellus	63.6 / 7.32	36.4 / 3.4	25.0 / 7.00	75.0 / 7.35
T. arcticus	50 / 4.27	50 / 1.93	50 / 7.30	50 / 6.93
L. leuciscus	–	–	16.7 / 7.18	83.3 / 7.79
A. brama	–	–	0	100 / 7.35
Kolyma River				
C. peled	66.6 / 6.79	33.3 / 4.63	66.6 / 7.19	33.3 / 7.58
C. nasus	77.8 / 5.39	22.2 / 6.39	44.4 / 6.79	55.6 / 6.85
C. lavaretus	78.6 / 5.48	21.4 / 4.16	21.4 / 6.93	78.6 / 7.46
A. baerii	14.3 / 5.37	85.7 / 4.29	85.7/0*|6.53/	14.3/100*| 6.45/6.91
L. leuciscus	–	–	28.6 / 7.48	71.4 / 7.41
C. catostomus	–	–	33.3 / 7.12	66.6 / 6.95
Lake Baikal				

P. fluviatilis	14.3 / 6.91	85.7 / 5.20	14.3 / 7.43	85.7 / 7.64
C. migratorius	77.8 / 5.23	22.2 / 7.50	50 / 7.33	50 / 8.04
T. arcticus baicalensis brevipinnis	0	100 / 5.90	0	100 / 7.44
L. leuciscus	–	–	0	100 / 8.11

* **-** *intestine /* the spiral valve; "–" – lack of sector (part); ¥ - for fish with V-shaped stomach the means are calculated for cardiac/fundic and pyloric segments together.


Kolyma River
Moscow
Lake Chany
Lake Teletskoye
Lake Baikal

Fig. 2 Seasonal (**a**) and interannual (**b**) variability of pH values in the fish digestive tract. Values are presented as mean ± SEM. One-way ANOSIM at $P < 0.05$

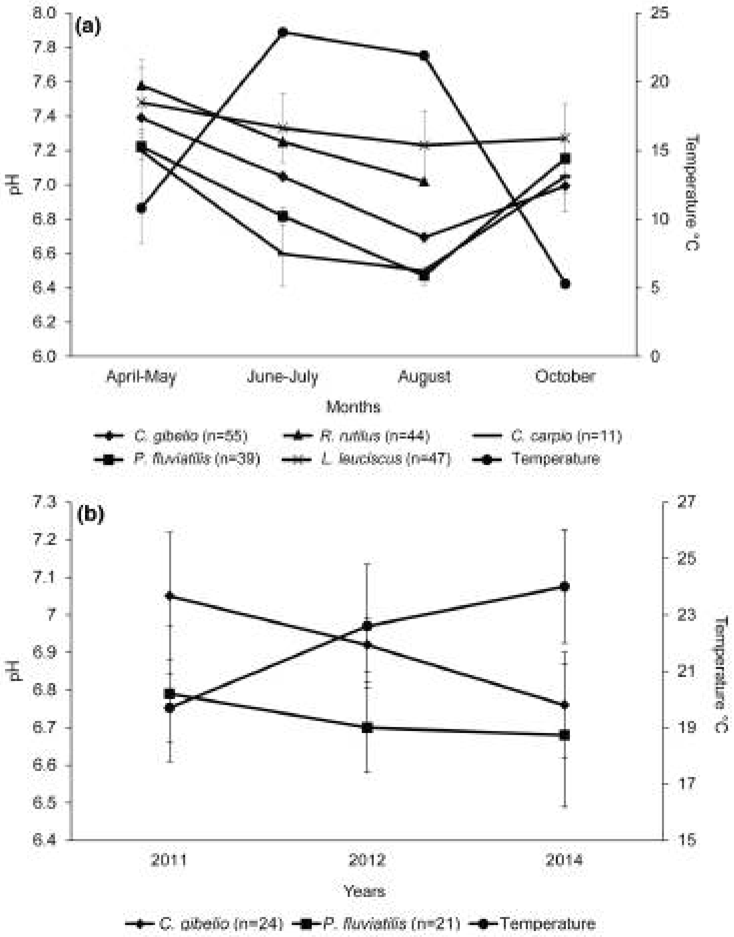

Fig. 3 Values of pH in the intestine of *L. leuciscus* (**a**) and *P. fluviatilis* (**b**) from different waterbodies. Values are presented as mean ± SEM. One-way ANOSIM at $P < 0.05$

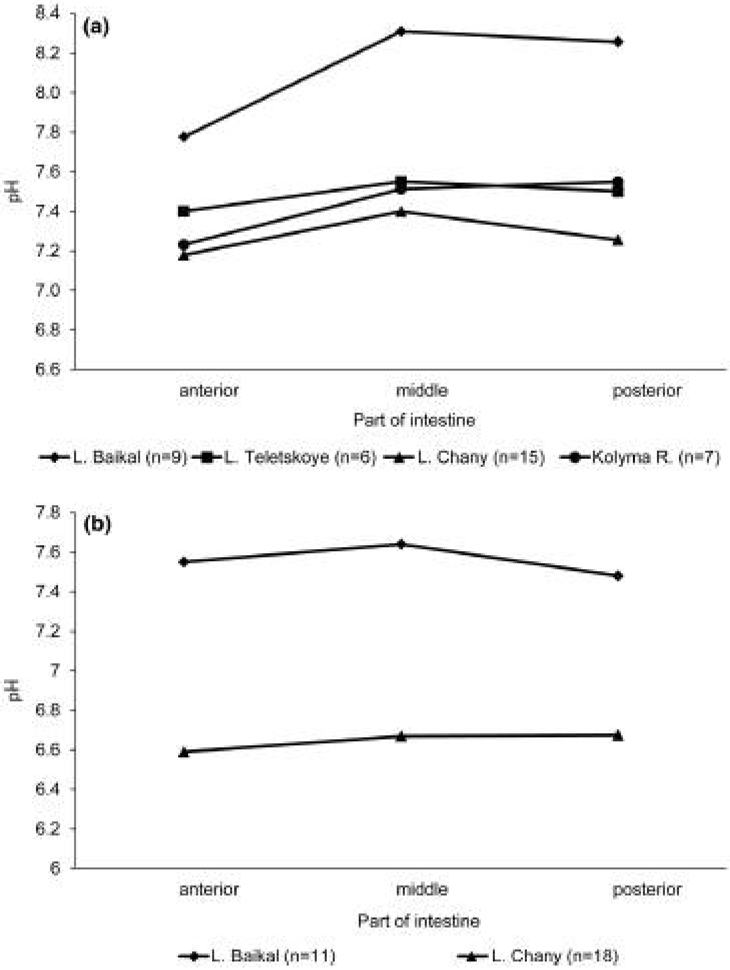

Table 3. The ratio (in %) of full and empty guts in studied fish during vegetation periods in the Chany Lake.

Species	Vegetation periods							
	April-May		June-July		August		October	
	Empty gut	Full gut	Empty gut	Full gut	Empty gut	Full gut	Empty gut	Full gut
C. gibelio	28.6^{A}	71.4^{a}	29.4^{B}	70.6^{a}	12.5^{A}	87.5^{a}	50.0^{A}	50.0^{b}
C. carpio	0.0^{B}	100^{a}	0.0^{C}	100^{a}	33.3^{B}	66.7^{b}	33.3^{A}	66.7^{b}
R. rutilus	22.3^{A}	72.7^{a}	44.4^{A}	55.6^{b}	61.5^{C}	38.5^{b}	ND	ND
L. leuciscus	28.6^{A}	71.4^{a}	50.0^{A}	50.0^{b}	60.0^{C}	40.0^{b}	46.2^{A}	53.8^{b}
P. fluviatilis	33.3*/27.8^{A}	66.7/72.2	36.4/54.5^{A}	63.6/45.4	42.9/85.7^{D}	57.1/14.3	43.3/53.3^{A}	56.7/46.7

* - *stomach / intestine;* the capital letters denote statistically significant differences among lines for empty guts (Wilcoxon rank-sum test, $P < 0.05$).

The lowercase letters denote statistically significant differences among columns for full guts (Wilcoxon rank-sum test, $P < 0.05$). ND – No data.

Table 4. pH values in the intestine with chyme, after removing chyme and in the chyme of studied fish from the Chany Lake.

Species	Intestine		Chyme
	After chyme removal	Before chyme removal	
L. leuciscus	7.19 ± 0.06^{A}	7.51 ± 0.07^{B}	7.67 ± 0.10^{B}
L. idus	6.98 ± 0.04^{A}	7.38 ± 0.06^{B}	7.59 ± 0.10^{B}
R. rutilus	7.51 ± 0.04^{A}	7.86 ± 0.04^{B}	8.15 ± 0.05^{C}
C. gibelio	6.62 ± 0.12^{A}	6.82 ± 0.17^{B}	6.91 ± 0.17^{B}

Different capital letters denote statistically significant differences among columns (ANOSIM, P < 0.05).

Table 5.The values of co-variation coefficients of multifactorial analysis of variance

Factor	Fish species				
	Agastric				Gastric
	Dace	Prussian carp	Roach	Common carp	Perch
Part of intestine	8.8 (10.43)*	3.0 (9.33)	5.7 (7.41)*	2.0 (0.57)	2.6 (2.05)
Fullness of stomach	–	–	–	–	4.1 (6.62)
Fullness of intestine	1.4 (3.16)	0.9 (5.27)	0.6 (1.54)	16.1 (10.75)*	0.2 (0.26)
Body length	2.4 (3.05)	0.5 (31.61)	8.0 (21.28)*	ND	2.1 (3.27)
Season	3.3 (3.74)*	16.8 (61.17)*	39.6 (160.40)*	39.0 (35.78)*	25.4 (53.10)*

In parentheses: F values for p < 0.05; “–” – lack of sector (part); ND – No data; * – the value is significant.

Fig. 1 Map of the Russian Federation indicating different sampling points (see text for detailed geographical coordinates for sampling sites)

Fig. 2 Seasonal (a) and interannual (b) variability of pH values in the fish digestive tract. Values are presented as mean ± SEM. One-way ANOSIM at $P < 0.05$

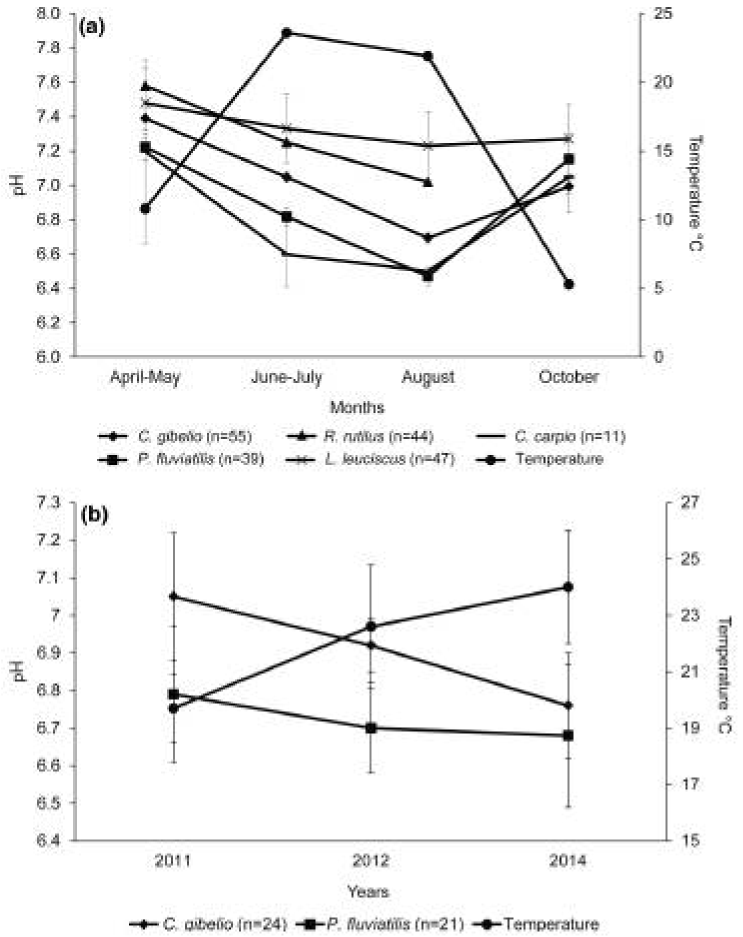

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Fig. 3 Values of pH in the intestine of *L. leuciscus* (**a**) and *P. fluviatilis* (**b**) from different waterbodies. Values are presented as mean ± SEM. One-way ANOSIM at $P < 0.05$

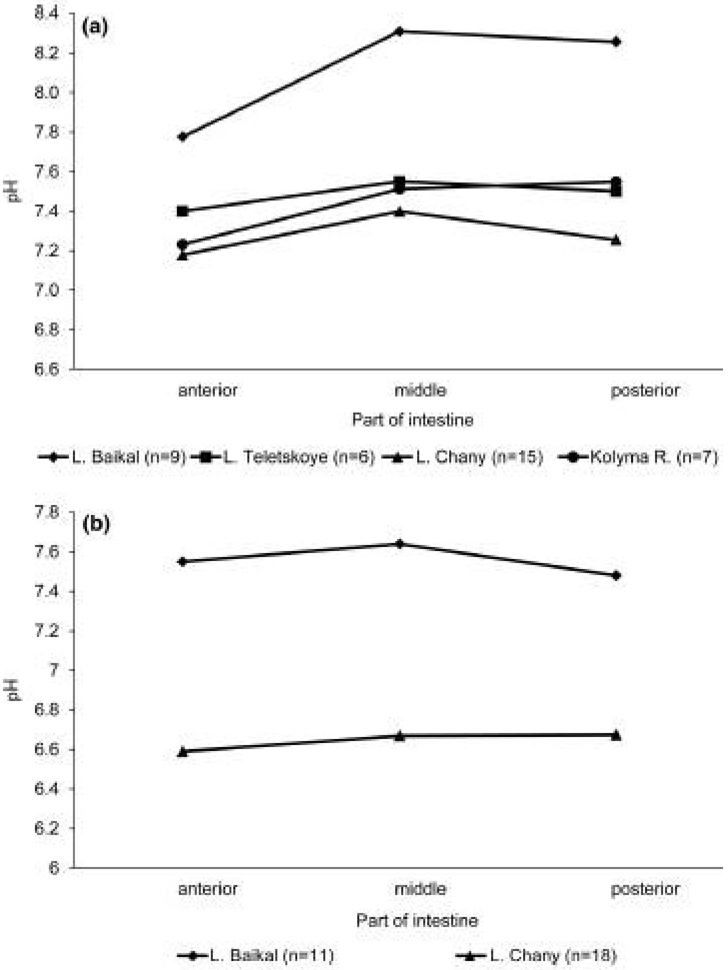

Fig. 4 Dependence of the pH values on the levels of fullness in stomach (**a**) and intestine (**b**). Values are presented as mean ± SEM. One-way ANOSIM at $P < 0.05$

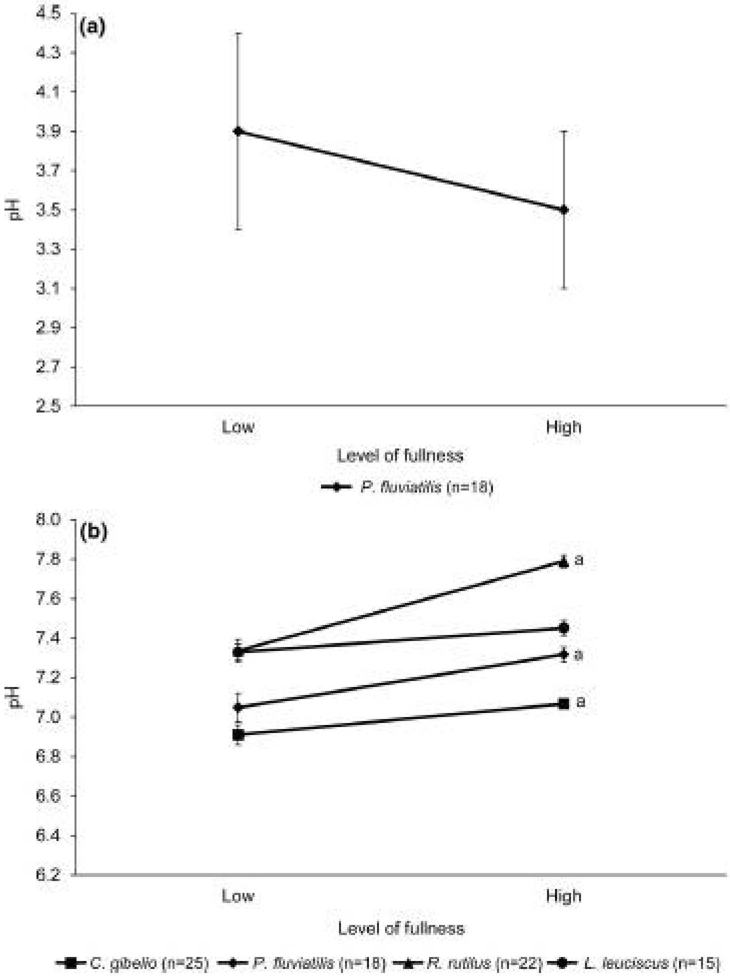

Fig. 5 Values of pH in the intestines of the fish species most common in the Chany Lake and differing in the feeding habits. Values are presented as mean ± SEM. One-way ANOSIM at $P < 0.05$