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**Observation of fish schooling behaviour in open-water
habitat of a man-made reservoir via visual census**

Ph.D. Thesis

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Annotation:

The research presented in this thesis contributes to broadening of the knowledge on free-living adult fish schools and behavioural patterns in a temperate freshwater reservoir. The behaviour of fish in the pelagic zone is rather poorly studied. Naturally, schooling tendency varied between species mostly due to increasing vulnerability to predation. Heterospecificity in schools was not a rare phenomenon, mainly for vulnerable species that shared the same space and food niche with a predatory less attractive species. The individual needs fluctuate as factors might be reconsidered in short time periods and most probably are reflected in behavioural responses. Individual responses are also reflected in the distribution of the fish in the reservoir and density in particular habitat. There is a “critical density” that triggers the formation of fish schools, followed by a slowing increase in density of fish clusters (observed units). This corresponds to increasing proportion of fish in a school and declining proportion of singletons. The trend of count of clusters tended to have an upper limit that should result in constant count of fish clusters after reaching a particular fish density. In other words, fish in the habitat maintain maximal distances even when the density increases. During high density periods the distances are kept by school formation. Overall the usage of the visual census as presented, proved to be a convenient tool for observation and assessment of freshwater fish. It has been demonstrated that the method can obtain comparable results to hydroacoustic survey amounts as well as purse seining.

Declaration:

[English]

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Declaration of originality

The co-authors fully acknowledge that Michaela Holubová is the first author of all papers presented. Most of the data processing as well as most of the statistical analyses were performed by Michaela Holubová. She also made a major contribution to writing the manuscripts. All papers contain original results. All co-authors consent to the publication of the papers in the dissertation of Michaela Holubová and one author hereby support his statement with his signature.

RNDr. Jiří Peterka, Ph.D.

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Introduction

Individuals belonging to the same species are naturally attracted to each other resulting in the emergence of aggregations. Approximately half of all fish species (Osteichthyes) form schools, some species only during early life phases, some through all their life (Shaw 1978). Aggregations of fish are observed in various complexity and structure. Fish aggregations composed of three or more fish are often called “shoals” or “schools” (Kennedy and Pitcher 1975; Partridge 1982; Pitcher and Parrish 1993). A necessary distinction was made by Pitcher (1983). “Shoals” are defined as unoriented social fish groups of independent individuals, while “schools” are oriented and polarised groups performing synchronous movements. Depending on motivation some fish species form mostly shoals other form mostly schools. (Pitcher 1998). Life strategy of particular species in a specific ontogeny stage must correspond to the strategy that is the most beneficial at that particular time period. Therefore, duration of all types of aggregations vary in between species depending on time and life phase.

Pros and cons of schooling behaviour

Maintaining assemblages brings various benefits as well as threats to their members (Helfman et al. 2009). Every group member evaluates the outcome of their presence in the assemblage separately and decide on current condition of the individual (e.g., state of satiation or individual personality; Sogard & Olla, 1997; Jolles et al., 2019). The prime evolutionary reason for aggregative behaviour is thought to be an anti-predator strategy (Pitcher and Parrish 1993), nevertheless, fish schooling is also supposed to bring several foraging and energy benefits. Although the levels of benefit will vary with the cohesion and polarisation of whole group (Maury 2017).

Energetic output of an individual in a group decreases due to a lower water resistance (Herskin and Steffensen 1998; Pavlov and Kasumyan 2000; Marras et al. 2015). In dense marine schools of ideal shape of lattice, fish are supposedly able to save up to 65 % of energy by swimming in neighbour’s wakes (Weihs 1975; Helfman et al. 2009). On the other hand, the energetic benefits have so far been proved only in migratory fish schools with fast swimming but not in non-migrating or shoaling species (reviewed in Maury 2017). Schoolmates can simply feel more secure when in group (Magurran and Pitcher 1983; Magurran et al. 1985), and therefore, have a reduced need for vigilance and more time for foraging (Bertram 1978; Magurran et al. 1985; Morgan 1988). Singletons experience more stress resulting in larger energy cost and slower growth (Davis and Olla 1992; Peuhkuri et al. 1995).

The major benefit is the anti-predator advantage of schooling. Groups provide “safety in numbers” (Kiltie 1980; Hager and Helfman 1991) as the number of fish “dilutes” the probability of attack on an individual (Hamilton 1971), which decreases with every additional member (Bertram 1978; Foster and Treherne 1981; Pitcher and Parrish 1993). Fish in schools are also able to detect a predator earlier (“Many eyes effect”; reviewed in Roberts, 1996), but the approach of a predator is not always followed by a flight reaction (Ydenberg and Dill 1986; Gerking 1994). Large schools also offer the predator multiple targets which confuses the predator’s ability to focus on a particular individual (“confusion effect”; Partridge, 1982; Landeau & Terborgh, 1986; Pitcher & Parrish, 1993). For all school members it is beneficial to be the same in looks and size (Rodgers et al. 2011). Actually, fish tend to follow this assortment in an attempt to override the “oddity effect” (Ohguchi 1978, Landeau & Terborgh 1986, Theodorakis 1989). The presence of odd fish enhances the success of the predator on the whole school (Landeau & Terborgh 1986, Theodorakis 1989). Size assortment also helps to maintain synchronisation of the group (Pitcher & Parrish 1993). Smaller individuals which might not be able to keep pace with the rest of the school, disrupt the school’s compactness and lag behind, and therefore are attacked preferentially (Parrish 1989). Large groups are also more conspicuous for predators (Alexander 1974). On the other hand, according to Larsson (2009, 2012), in highly synchronized groups the noise produced by motion can be reduced, as a result the group might be seen as a single object. The optical properties of water make the school equally visible as a single individual (Murphy 1980; Pitcher and Parrish 1993). However, predators are able to detect the prey by other means, for example, by chemical signatures (De Bose and Paul 2014).

Prey in groups occurs in fewer places and by doing this it makes itself rare (Vine 1971), on the other hand, more individuals means increased conspicuousness (Krause and Godin 1995). Nevertheless, if a predator is able to handle only one prey (as is the case in most freshwater piscivores), the rest of the group has the opportunity to escape (Pitcher and Parrish 1993) but some species can react by higher compactness of the school or perform evasive movements (Pitcher and Parrish 1993). When a predator attacks the school, individuals try to occupy the inner part of the aggregation (“Selfish herd phenomenon”; Hamilton, 1971), but in fact, fish in the middle are more prone to predation due to longer information flow (Parrish 1989).

School members experience enhanced foraging success (Krebs and Davies 1993; Day et al. 2001; Helfman et al. 2009), especially in large groups, they are more successful and faster in locating quality food resources than their single conspecifics (Milne et al. 2005; Helfman et al. 2009). Moreover, the time between finding food

patches shortens with every other searching schoolmate (Pitcher et al. 1982; Street and Hart 1985). Unskilled school members can benefit from watching other conspecifics finding a food source (Krebs 1972; Magurran 1984; Laland and Williams 1997). In contrast to the anti-predation benefits that are higher in bigger schools, foraging benefits in very large schools are accompanied by increasing competition (Bertram 1978). In fact, some species such as central mudminnows (*Umbra limi*) did not showed a preference for bigger shoals (Jenkins and Miller 2007). Size of schools is affected by individual preferences that can change depending on the physiological state of a fish (as hunger, size, species, parasitism, illnesses, presence of shelters, predation threat etc.; Pitcher and Parrish 1993; Milne et al. 2005). Schools can gain foraging benefits only if the prey is patchily-distributed (Pitcher and Parrish 1993). Maury (2017) concluded that species normally staying in aggregations in experimental conditions reached larger size than in natural conditions meaning that aggregations limit their foraging.

The presence of mating partners is another evolutionary advantage of gregarious organisms (Makris et al. 2009). Some fish species form aggregations solely during the spawning period (Turner and Pitcher 1986). But as in foraging, large numbers consequently also implies higher competition (Alexander 1974).

Another disadvantage of aggregating is the risk of parasites and diseases transmission. Usually fish avoid parasitized conspecifics (Dugatkin et al. 1994), although the important factor is the species of parasite and its reproductive strategy that affects the fish health and behaviour (reviewed in Barber et al., 2000). In directly transmitted parasites such as *Argulus* sp. the rate of parasite infection does not differ between schools of various sizes (rainbow trout; Mikheev 2009), in fact, the presence of *Argulus* enhanced the schooling behaviour in sticklebacks (Poulin and FitzGerald 1989). On the other hand, fish loaded with helminths can suffer from impaired vision which lowers the ability for keeping pace with the school and makes the individual an easy target for predators (reviewed in Barber et al. 2000).

Predation on fish schools

Predator-prey interaction is a never-ending cycle of evolutionary adaptations, prey seem to be one step ahead in “arm races” since they risk more (loss of life) than predators (loss of a meal; Dawkins and Krebs 1979). Prey fish species evolved various defences against predators, from which particularly interesting is schooling behaviour (Magurran and Pitcher 1987). Although several predators seem to outrun the prey by targeting aggregations (Pitcher and Parrish 1993). Some predatory species

have developed unconventional skills or organs for stunning (e.g., speeding in tuna, tail slapping in killer whales, elongated snout in sawfishes, swordfish, marlin or thresher shark tail; Breder 1967; Pitcher and Parrish 1993; Domenici et al. 2000) or group attacks that disrupt the coherence of the group (Major 1978). In addition, solitary predators such as Northern pike (*Esox lucius*) deliberately separate individuals from schools (Magurran and Pitcher 1987).

Prey have evolved various methods of anti-predatory defences by implementing physical, behavioural and sensual traits. Predators inhabiting freshwater habitats are usually gape limited, therefore, body shape is one of the essential features (Nilsson and Brönmark 2000). The shape of a prey's body determines its edibility and vulnerability (Hambright 1991; Nilsson and Brönmark 2000). Predators do not favour prey of maximum edible size but prefer much smaller sizes (Juanes and Conover 1994; Nilsson and Brönmark 2000) considering that a large prey means longer handling time and possible danger for the predator itself (Nilsson and Brönmark 1999). Apart from body shape, the presence of firm or spiny structures seems to lower the attractiveness to predators (Peter Eklöv and Hamrin 1989; Christensen and Persson 1993). It has been noted that armoured prey were eaten by predators in smaller sizes than the non-armoured prey (Dörner and Wagner 2003). Therefore, slender-bodied (Hambright 1991; Nilsson and Brönmark 2000) unarmoured fish (Dörner and Wagner 2003) are more prone to predation than deep bodied and armoured fish, which should be able to reach a size refuge from predation in shorter time period. Due to this factor, antipredator mechanisms differ between taxonomic groups; cyprinids being rather vulnerable species utilize grouping behaviour as predator protection to a greater extent than morphologically advanced species such as percids (Christensen and Persson 1993).

Predators are able to detect the prey not only by visual means but also by chemoreceptors. But prey can also be informed on predator presence by alarm substances released by harmed prey and choose according reaction as tighter schooling formation or flight (Helfman et al. 2009). Species prone to predation might choose to form heterospecific schools with species that are not a predator target, as suggested in "protector-species" theory (bird nesting colonies; Pius and Leberg 1998). Not only can the vulnerable species visually disappear into the school, but also its odour can be disguised by the protector species scent. Presence of a predator may be the trigger for the emergence of schools as a behavioural response to threat (Relyea 2001; Doucette et al. 2004), yet there are cases where schooling prevailed with no predators in the habitat. When previous generations were exposed to the predator pressure this behaviour remained encrypted in the descendants genes even after

disappearance of the threat from the habitat ("Ghost of predation past"; Brown & Vincent 1992; Gliwicz & Jachner 1992). Other anti-predatory behavioural responses have been showed in laboratory reared fish, moreover, specimens from the environment with and without previous contact with predators displayed various reactions (Magurran 1990). This proves the genetic memory of anti-predatory behaviour.

Composition of fish schools

Every school member gains benefits as well as experience the costs for remaining with the group, such benefits for every individual must outweigh the costs (Lima and Bednekoff 1999). These vary throughout the life of an individual, therefore, aggregations may appear at different life stages in a species-specific manner. Some species aggregate only during early life stages, whereas others form large groups also in adulthood, while some adults gather together only for spawning or wintering (Helfman et al. 2009).

Being in a school simply reduces the probability of an attack *per capita* (Foster and Treherne 1981). Supposedly the most beneficial schools consist of single species (Landeau and Terborgh 1986). Schools of identical individuals means confusion for predator senses (Pitcher and Parrish 1993), but size similarity is also an important factor. The preference for shoals composed of individuals with identical body size was proved (Krause and Godin 1994) even in heterospecific shoals (Blakeslee et al. 2009). Size is important for maintaining coherence of a school therefore all members gain benefits if they form a compact group. Smaller individuals might have trouble to keeping up with the pace and therefore, end in trailing positions. Such individuals lagging behind might be deprived of potential energy advantages of school membership, such as lower energy expensed or lower metabolic rate ("calming effect", Parker 1973).

Mixed species groups are not a rare phenomenon in the wild (Peres 1993; Stensland et al. 2003; Semeniuk and Dill 2006; Maury 2017) which also occurs in other animal classes (Krebs 1973; Allan and Pitcher 1986; Fitzgibbon 1990; Peres 1993; Hino 2000). Although visually dissimilar school member is an easy target and can disrupt the cohesion leading to a higher predator success on the group as a whole (Landeau and Terborgh 1986; Theodorakis 1989; Parrish 1989). From an individual's point of view, the risk of this oddity in the heterospecific school is still smaller than the threat of predator encounter when single (Bakun and Cury 1999). The benefits such as less need for vigilance and lower stress probably override the "oddity effect"

(Peres 1993). For a species with lower abundance a heterospecific school can act as a “transport vessel” until encountering a conspecific school or achieve enough conspecifics to create a school of their own. Increasing numbers of conspecifics in the school lowers the oddity effect (Mueller 1977). Possible benefits from heterospecific schooling differ between species (Hobson 1963; Ward et al. 2002). Species in the heterospecific school in the minority might be at a disadvantage (Bakun and Cury 1999), and are more likely to be separated (Wolf 1985). If the school is composed of more species which prefer various food sources, the advantages of schooling increases with every member and even more so better if the school is composed of subgroups divided by species (Allan 1986). Such schools can also benefit from varying sensory abilities of the included species (Morse 1977).

Ecological context in temperate reservoirs

In some countries, such as the Czech Republic, there is a very small amount of natural lacustrine habitats and artificial water bodies represent more than 50 % of the water surface, and therefore are utilised for various uses such as water supply, or recreational purposes (reservoirs), or for aquaculture (ponds). Temperate freshwater impoundments, such as the Římov Reservoir, undergo periodic changes (including two periods of mixing of the whole water column) that affect nutrient accessibility as well as viability of inhabitants in the environment. One of the main attributes is water temperature (Vašek et al. 2004, 2008). Increasing temperature during the spring mixes the winter stratified layers. Warming of the epilimnion results in settling of the summer stratification, which is disrupted during autumn due to lowering temperatures and lake stratifies again during winter in reverse stratification (Kalff 2002). Fish inhabiting such a reservoir react to these changes, during mixing and the winter period, individuals are scattered throughout the water column until the summer stratification period when they concentrate in the warm upper layer (approximately 5 m under the water surface; Čech & Kubečka 2002; Vašek et al. 2004). This goes hand in hand with oxygen stratification that also depends on mixing of the water column and during summer stratification the oxygen levels normally decrease with the depth from surface to the bottom, depending on the depth of the water body. The increased activity of organisms also changes the transparency, which is highest during the “clear water” phase (end of May – June) due to grazing activity of zooplankton and decreases during summer (in Římov Reservoir “clear water” means transparency up to 5 m Secchi Depth, while during summer it is lower than 2 m). During autumn the transparency increases, but due to thermal destratification the fish are scattered

throughout the water column. All these factors have to be taken into consideration when planning the observations.

Canyon-shaped reservoirs gradually change from a lotic to a lacustrine environment. Depending on the location of the reservoir on the river, the fish community can differ in capability to adapt and utilize the lacustrine zone of reservoirs (upper versus lower river course; Fernando & Holčík 1991). In contrast to lakes, reservoirs were created very recently and animals that became residents have not had enough time to properly specialise to all habitats they offered, various species have different pre-adaptations for utilisation of the new environment (Fernando and Holčík 1991). Less adaptable species stay in the near-shore areas or riverine-like upper parts of the reservoir (Vašek et al. 2004).

Canyon-shaped reservoirs are characterised by a very high proportion of steep banks. The shallow littorals represent only a small area, which means a low proportion of freshwater vegetation and few hiding places for animals, particularly in the lower parts. Moreover, water plants growth is affected by fluctuations in water levels and the presence of power plants, as in the case of the Římov Reservoir (Vašek et al. 2008). The pelagic zone, therefore, presents a large area of habitat for aquatic organisms. Paradoxically, the pelagic zone of reservoirs is the least studied freshwater habitat mainly due to its low commercial importance. In deep water systems the pelagic habitat contains a very important food resource – planktonic crustaceans and fish might change their habitat to take advantage of this rich habitat (Čech and Kubečka 2002; Jarolím et al. 2010; Říha et al. 2015).

Fish community changes both on the horizontal (between pelagic and littoral habitat) as well as the vertical scale (Bohl E. 1980; Järvalt et al. 2005; Muška et al. 2012; Říha et al. 2015). In addition, fish perform diel habitat shifts. Depending on the ontogenetic stage, preference for a particular habitat may change due to the difference in foraging preferences and predator threats (Werner and Hall 1988). Substantially, adults favour the pelagic zone during day time and most of them migrate to the littoral zone during the night (Říha et al. 2015). Fish choose the best daytime to effectively forage with minimal risk, therefore juveniles who are more vulnerable to predation show a more or less reversed trend to the adults and occupy the epipelagic zone during the night (Říha et al. 2015). Although freshwater predators such as pike usually hunt during low light periods (Harvey 2009) where they are less conspicuous, the potential predation threat in the pelagic habitat during the day time may not be negligible because of the absence of shelters (Pitcher 1986), even in systems with low predator levels. Adults should experience a lower predation threat than juveniles, but even less

vulnerable species, such as deep bodied bream, use anti-predatory tactics, such as school formation which is “a form of cover seeking” (Williams 1964). Schooling behaviour is however a daylight phenomenon (Whitney 1969; Milne et al. 2005). When fish take shelters, there is no other need for schooling whereas when they need to forage out of the safe area schooling or shoaling may take place. During night time the schools and shoals mostly disperse and aggregating is mostly replaced by individual behaviour (Helfman 1981b; Axenrot et al. 2004). Some species may also form night time resting aggregations, although only a very few fish species are able to keep some kind of cohesion in the school but the compactness is still lower than during the day time (Hobson 1968).

Aquatic environment undergoes changes not only seasonally but also daily. Solar irradiance patterns affect the foraging abilities of fish using visual detection and cause habitat switches during the dial cycle (Ryder 1977; Jarolím et al. 2010). Dial cycle patterns might be temporarily disrupted by variation in weather conditions. Cloud cover can significantly lower the levels of solar irradiance to substantially different levels when compared to sunny days. Such changes can alter the behavioural patterns of aquatic organisms. Attraction of fish to shaded areas have been previously stated (Helfman 1981a), similarly, under-average light levels means absence of direct sunlight which might be favoured by fish, luring them from other habitats to the epipelagic zone due to lower conspicuousness (Helfman 1981a), non-necessity for UV avoidance (Kerry and Bellwood 2015), or better foraging conditions (Zaret and Suffern 1976). Weather conditions like storms with strong winds and rain can affect the currents as well as light refraction which make fish more prone to predation and potential higher energy costs (Helfman 1981a; Johansen et al. 2007). Behaviour, therefore, is plastic and individuals must adapt their reaction to fluctuations in the environment (Fréon and Misund 1999)

(Mis)use of fish schooling by human activities

Vast aggregations of fish function as attractants for larger predatory animals. The aggregative tendencies of fish, however, have been widely utilised also by commercial fisheries. Unfortunately, increasing demands on fish yield for human consumption resulted in unsustainable fishery levels. These practices have already taken their tolls in decline and collapse of several wild populations of targeted fish species in the ocean (Hart and Reynolds 2008). Overfishing also caused an alteration in species composition of fish schools (Cury et al. 2000). Decline in densities lead to changes in fish communities that can be observed as higher emergence of heterospecific schools due to low abundance of conspecifics (Fréon and Misund

1999). The economic importance of marine commercial fishing side-lined the research on fish in the freshwater habitats (Milne et al. 2005; Guillard et al. 2006), and consequently also the knowledge on fish schools which is rather sporadic, when compared to marine aggregations (Fréon and Misund 1999; Bertrand et al. 2006). Fisheries do not directly harm freshwater aggregations as commercial aquaculture is mainly conducted in artificial ponds. Sport fishing and illegal angling activities tend to target the predator species which, can result in overpopulation by “coarse fish”. This not only means changes in competition for food resources for planktivory species, but can also result in lower water quality and the need for biomanipulation actions for pest fish removal (reviewed in Scharf 2007).

Aims of the study

This PhD. thesis aims to shed some new light on fish behaviour in an artificial man-made water body, the Římov Reservoir, with a particular interest in fish schooling behaviour (Paper I, II and III), interactions between fish and abiotic components of their environment (Paper III) and other fish species (Paper I, II and III). The part of this thesis is the compares the efficiency of the method employed – underwater video recording (UVC) and the regularly used monitoring gear – purse seining and hydroacoustics (Paper IV).

Paper I

Aims: (i) to provide a basic description of fish schools in the pelagic habitat of a freshwater temperate reservoir to obtain a real picture of schools, particularly school sizes and species composition; (ii) to explore inter-species differences, schooling tendencies and species-specific preferences that are supposed to differ due to the varying vulnerability to predators; (iii) to observe the occurrence of heterospecific schools and determine differences with conspecifics schools; (iv) and to determine the tendency of co-occurrence of particular species and encounter probabilities of species and school size with implications to species-specific aggregative tendencies.

Paper II

Aims: to unveil the relationship between fish density and attributes of schooling behaviour in the open water habitat of a temperate freshwater reservoir by describing the actual state from acquired video recording data.

Paper III

Aims: (i) to demonstrate influence of variations in environmental conditions on the presence of fish in the pelagic habitat with particular focus on fish schooling, inter-specific difference and predatory-prey species difference, and (ii) to outline possible predator-prey density relationship and their outcome for attributes of schooling behaviour. Possible implications for schooling behaviour are emphasised and discussed.

Paper IV

Aims: (i) to compare the accuracy of UVC use in three seasonal periods, (ii) to compare the differences between fish densities obtained by hydroacoustics and UVC and (iii) to compare the difference in species composition between UVC and purse seine.

Results

This Ph.D. thesis is composed of three original papers published in international scientific journals (Paper I, II and IV) and one manuscript submitted (Paper III).

The social behaviour of animals is a well-studied topic, although poorly understood in freshwater systems. This thesis comprises studies on fish densities and schooling attributes based on video recordings captured over a total of 34 days (16 h per day) in the pelagic habitat of the Římov Reservoir, Czech Republic. In **Paper I**, the composition of fish schools, species-specific schooling tendencies and preferences of fish were observed. Four species were identified as school-forming species, specifically bream, bleak, roach and perch. Although only 40% of the individuals observed formed schools (school size 3–36 individuals). However conspecific schools prevailed, 20% of individuals formed heterospecific schools. Bleak was the only species that schooled strictly with conspecifics. Even though heterospecific schools were fewer, they significantly exceed the conspecific schools in size (number of individuals). The proportion among species in heterospecific schools was always uneven with one species being more abundant. Highest inclination for schooling was observed in bleak as well as lowest probability of encounter. Species morphology and body size affect the gregarianism levels, with larger and morphologically advanced fish being less sociable and vulnerable species needing more the protection against predators.

Paper II explores the issue of schooling from a population point of view and focuses on the relationship between density and schooling features. ‘Critical density’ of fish in the habitat is a trigger for school formation. Increasing density of fish positively influenced the school size as well as counts of schools and proportion of schooling individuals. Counts of clusters (observed units in time, including singletons, pairs and schools) and cluster size increased with a slowing trend, which implies formation of larger groups in order not to be frequent.

Paper III is a pilot study investigating the correlation between environmental factors and the density of fish in the epipelagic habitat. Water temperature was confirmed to have a positive correlation with all observed categories: non-predatory, predatory, single non-predatory and schooling fish, as well as freshwater bream and roach. De-trended solar irradiance showed negative correlation with density of non-predatory fish, freshwater bream and European perch. Similarly, sunshine duration was negatively correlated with the density of predatory fish. Precipitation was positively correlated with single non-predatory fish and European perch, whereas

wind strength was negatively correlated with density of schooling fish. In addition, there was a positive correlation between density of predatory fish and density of single non-predatory fish and counts of observed clusters. In conclusion, fish density showed a relationship with abiotic factors as well as density of predators.

Paper IV presents a comparison of visual census with traditional fish sampling methods, which can often cause damage to the ecosystem or be selective. This study proposes the use of the UVC as a valid sampling method even in the systems with higher trophies. Species composition did not differ between purse seining and camera. In the same manner, standardised fish abundance did not differ between hydroacoustics and camera. This implies that camera not only brings comparable results on species composition and density of daytime assemblages, but also is un-invasive and can bring novel insights on behavioural patterns.

General discussion and conclusions

Some species such as cyprinids, even though they are of riverine origin, seemed to adjust well to unusual habitats and have been found to occupy the pelagic habitats of the reservoir during this monitoring (**Paper I-IV**). Abundant presence of planktonic crustaceans in the habitat offers the rarely exploited resource niche to which these species have managed to adapt to (Vašek et al. 2008). But given the riverine origin the observed school sizes were frequently smaller than 10 members and less frequently larger (up to 36 members; **Paper I**). Four out of nine fish species found in the pelagic habitat joined into schools (**Paper I**), however, the schooling tendencies differed between species. Cyprinids showed a higher inclination for schooling than percids (here represented by European perch). Several factors can affect the individual decision for school joining, e.g., individual personality or physical state such as hunger, stress or disease/parasite presence (Barber and Huntingford 1996; Harcourt et al. 2009; Jolles et al. 2019). The schooling behaviour among cyprinid species was least performed by bream followed by roach (40 and 58 % of individuals in schools, respectively). On the other hand, schooling was extensive in bleak, which occurred almost always in schools and formed exclusively conspecific schools, unlike the rest of the schooling species, i.e., freshwater bream, roach and European perch (**Paper I**). The amount of conspecifics in the habitat plays a role in school parameters (Krause et al. 2000; Hoare 2000). Some species more than others tended to form heterospecific schools if the sympatric species of similar ecology were present (**Paper I**). However, bleak utilised the pelagic habitat, but the differences in body size and foraging ecology with other species were insufficient to form heterospecific schools (**Paper I**). Roach, on the other hand, is more prone to predation due to their slender body with no firm structures (Nilsson and Brönmark 2000; Dörner and Wagner 2003), and therefore, occurred in heterospecific schools with the highest numbers (particularly in co-occurrence with bream that share similar body length and foraging strategy) (**Paper I**). Formation of heterospecific schools enables vulnerable species with lower densities to temporarily enjoy the anti-predatory benefits of a school when the abundance of the conspecific individuals is low (Peres 1993), although such groups are more likely to split (Wolf 1985). After reaching certain level, individuals of less numerous species might choose to create a school of their own. Due to this, the observed proportions of species in the heterospecific schools were never identical (**Paper I**). The formation of schools also depends on the total count of individuals in the habitat (**Paper II**). The increase in the count of fish in the habitat meant a higher encounter rate (**Paper I**), until reaching a specific level, i.e. “critical density” (**Paper II**) that triggers the formation of schools. This goes hand in

hand with a decline in the proportion of singletons and an increase in the proportion of schooling fish while the count of clusters (all observed fish units) showed a stabilizing trend.

Schooling promotes evasion from predators, therefore, the prey tends to be rarely available by making aggregations concentrating themselves in one spot (Ioannou et al. 2011). Common species are imprinted in predator brains as prey on which they focus their attention during foraging (“search image”; Ishii and Shimada 2010). There is a particular level of individuals in the habitat when the distribution changes from scattered to coordinated aggregations called “critical density” (Makris et al. 2009). This phenomenon was observed in freshwater (**Paper II**) and marine fish (Makris et al. 2009), as well as insects (locusts; Buhl et al., 2006). The anti-predatory strategy increases with school size, but so does the competition among schoolmates, therefore it is assumed that the foraging benefits are highest in smaller schools (Pitcher and Parrish 1993), the optimal size will depend on the abundance of the resource. Predator senses are somehow limited and a school with fewer members can be below the detection capability of the predator therefore, practically invisible (Maury 2017). Since most of the observed schools were rather smaller (avg. 5.6 ind.), it is questionable whether they reach the detectable size. In addition, large aggregations usually attract several predatory species (Botham and Krause 2005; Botham et al. 2005) that in the ocean are able to consume most of the fish in the school particularly when some of the predators are able to catch more individuals at once. Paradoxically, aggregating seems counter-productive for prey fish but from an ecosystem point of view prevents overpopulation (Maury 2017). The low abundance of predators in the freshwater systems, often caused by anglers, enables planktivorous species to overpopulate the ecosystem.

Effects of environmental factors

Aquatic realm undergoes changes, depending on climatic zone, seasonality, some vary on a daily basis and others repeat rather irregularly. In a temperate zone, temperature of water affects the ectothermic organisms on a long-term scale (Weetman et al. 1998; Vašek and Kubečka 2004; Vašek et al. 2008). During the summer stratification period fish mostly stick to the upper warm layer, whereas in the rest of the season fish are more or less scattered throughout the water column. During this monitoring, raising water temperature was correlated with an increase in densities of several studied categories, i.e., non-predatory fish, predatory fish, single non-predatory fish, schooling fish counts and two of the three most frequent species: freshwater bream and roach (**Paper III**). Higher activity of predators can be a trigger

for higher prey activity including higher employment of anti-predatory tactics (Weetman et al. 1998).

Some environmental factors undergo changes on a more or less regular pattern, such as light levels that follow diel and seasonal development but is altered by current cloud coverage. Detrended solar irradiance has been found to be negatively correlated with the densities of non-predatory fish and two of the three most frequent species (freshwater bream and European perch), resulting in higher fish density during under-average irradiance levels (**Paper III**). The avoidance of direct sunlight by fish has also been confirmed by other authors (Jones 1956; Helfman 1979, 1981a; Järvalt et al. 2005; Sajdlová et al. 2018). The higher density of non-predatory fish during periods of lower irradiance levels seems not to be connected to anti-predatory strategy since predators' densities were higher during days with shorter sunshine duration (counts of hours with direct sunlight) (**Paper III**) and mostly are known to prefer low light periods for foraging.

Weather conditions are rather irregularly occurring factors, but still can affect the habitat utilization of fish. Wind strength proved to be negatively correlated with the density of schooling non-predatory fish and density of European perch, while precipitation showed a positive correlation with the density of single non-predatory fish and density of European perch (**Paper III**). The two factors are more likely to cooccur and can be hard to consider separate effects. Stormy weather however has been previously stated to alter the distribution of reef fish assemblages possibly followed by flight (Johansen et al. 2007; Munks et al. 2015). Adjusting to such changes in the water environment is probably energetically costly and results in ineffective foraging (Bowen and Allanson 1982; Johansen et al. 2007).

Note on methodological improvement

Up to the present time, fish communities of water bodies have been sampled by the means of various net types. Most of the traditional gear (e.g., gillnetting, beach seining, purse seining, trawling) includes physical handling of fish, which is frequently harmful. For example, gillnetting is mostly fatal for fish (Winfield et al. 2009), it produces various results due to the difference in activity of particular species over a period of exposition (Olin et al. 2016), or is prone to selectivity (Prchalová et al. 2013) which can result in over- or underestimations of some species, or cohort of fish (Prchalová et al. 2008, 2009; Říha et al. 2012). Most of the gear is applicable for night surveys as nets can be visually detectable and evaded by fish when used during highly illuminated periods (Vašek et al. 2009). Flaws in sampling methods are

frequently solved by using a combination of gear types, but all of them include the physical handling of the fish resulting in trauma accompanied with high mortality. Some of the traditional gear can also damage the surrounding environment (Baker et al. 2016), not to mention the undesired catch, involving large crustaceans or water birds in water bodies in a temperate European climate. Recently the need for use of un-invasive means of research has been emphasised. The advances in non-destructive methods in fish survey began with the acoustic monitoring but they lack the species determination possibility (Maclennan and Holliday 1996) and can be biased since fish echoes might be mistaken with bubbles (Ostrovsky 2009). Acoustic survey is also used for observations of fish schools (e.g. Soria et al. 2003; Paramo et al. 2010) although a complement method is necessary for species determination (Massé et al. 1996). The use of UVC (underwater visual census) method is convenient as a complement to acoustic survey.

UVC methods offers a non-destructive approach, which includes direct observation of divers, or use of remote cameras (Boom et al. 2014). Use of remote cameras is particularly convenient, because recordings can be re-analysed, which lowers the possibility of errors that is higher for example in divers observations. In addition, recent advances enables for example, movement detection (Salman et al. 2019), size measurements (Neuswanger et al. 2016) or species recognition (Siddiqui et al. 2018).

As pointed out in **Paper IV**, camera observations can bring as accurate data as traditionally used gear, and if applied with proper consideration it can be utilised in a wide range of habitats including temperate freshwater ecosystems with fluctuating visibility. Observed species as well as densities acquired by camera were comparable with both purse seining and acoustic data. It has already been widely used in monitoring species inhabiting habitats such as coral reefs (Wilson et al. 2018) or streams (Jordan et al. 2008). The advantage of a remote camera is the authenticity of the observation omitting the disruption that can alter the behaviour during the diver's observation or boat presence (Harvey and Fletcher 2001; Soria et al. 2003; Wilson et al. 2018). Moreover, a camera can unveil unknown behavioural patterns to promote the understanding of natural processes and bonds in the habitat (**Paper I, II and III**) and can be purchased for relatively low costs (Letessier et al. 2015).

Conclusions

This thesis contributes to a wider understanding of free-living fish schools in the freshwater reservoir. The results indicate that anti-predatory function is the main trigger for schooling behaviour. The vulnerability of the species and presence of ecologically similar sympatric species plays important factors affecting the school composition, vulnerable species being rather obligated schoolers and if in lower densities are more willing to form schools with sympatric species of similar ecology. Non-vulnerable species form schools facultatively and are not as frequent in heterospecific schools. The schools are being formed after reaching a “critical density”, suggesting that fish clusters maintain maximal distances between each other probably due to the need of maintaining rarity status as potential prey. The density of fish in the pelagic habitat fluctuates in time and is correlated to the changing environmental factors, particularly fast changes in weather conditions could result in varying fish densities.

Pelagic habitats of European freshwater bodies are often dominated by cyprinid species and school formation suggests it is an evolutionary advantage for the dominance of cyprinid fish in later succession phases. More in situ studies on this matter should be encouraged for greater understanding of functioning of the systems as these relations are crucial for comprehension of predator–prey interactions that drives the structure of aquatic ecosystems. The knowledge on this matter is important to proper selecting of sampling gear. UVC, employed in this study, could be used as a complement of hydroacoustic sampling or with proper planning as a substitute of the traditional gear as camera cloud obtain comparable results without harming the fish or the ecosystem and on-going technical advances offers much more opportunities for future research.

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Research articles

Paper I

Species-specific schooling behaviour of fish in the freshwater pelagic habitat: An observational study.



Species-specific schooling behaviour of fish in the freshwater pelagic habitat: an observational study

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Abstract

Social living of animals is a broadly occurring phenomenon, although poorly studied in freshwater systems, fish schooling behaviour is an excellent example. The composition of fish schools, species-specific schooling tendencies and preferences of adult fish were studied in the pelagic habitat of the Římov Reservoir, Czech Republic. Video recordings captured over a total of 34 days (16 h per day) in the clear water period of three seasons were analysed. From four species identified as school-forming species – bream, bleak, roach and perch, 40% of the individuals observed formed schools of 3–36 individuals. Although conspecific schools prevailed, 20% of individuals formed heterospecific schools, except bleak that schooled strictly with conspecifics. Schools were composed of individuals of similar body size and life strategy. Heterospecific schools were significantly larger than conspecific schools and showed uneven proportion among species, that is, one species being more abundant when the school dimension increased. Probability of encounter in bleak was lowest and proved highest inclination for schooling. Gregarianism levels depended on species morphology and body size, with larger and morphologically advanced fish tending less to sociability. This indicates that the antipredator function of schooling behaviour is intensified with increasing vulnerability of the species.

KEYWORDS

antipredator behaviour, bleak, bream, perch, roach, social behaviour

1 | INTRODUCTION

Group living is a phenomenon that occurs broadly across the Animal Kingdom (Pitcher & Parrish, 1993; Seghers, 1974). Fish schools are believed to have appeared primarily as an antipredator strategy (Pitcher & Parrish, 1993). Based on the level of compactness, schools gain various other benefits (Maury, 2017) such as foraging (Day *et al.*, 2001; Helfman *et al.*, 1997; Krebs & Davies, 1993) and energetic advantages (Herskin & Steffensen, 1998; Marras *et al.*, 2015; Pavlov & Kasumyan, 2000), as well as the location of potential mates (Makris *et al.*, 2009). Fish schools can endure only if the benefits of grouping outweigh the costs (Lima & Bednekoff, 1999). For example, greater conspicuousness to predators, competition for resources such as

spawning partners, reduction in foraging efficiency or reduced oxygen supply (in dense schools of thousands of individuals) increase with an increase in group members (Alexander, 1974; Bertram, 1978; Maury, 2017; McFarland & Moss, 1967). Also stability and coherence of a school should increase with school size (Halleutique *et al.*, 2007). Because there are multiple affecting factors, groups can be dismissed and reassembled during the day, but such continuance requires visual contact among group members as indicated by Bohl (1980) and therefore it is predominantly a daylight phenomenon (Whitney, 1969; Milne *et al.*, 2005).

Species-specific characteristics such as body structure affect group formation (in birds; Griesser *et al.* 2011). Body size is important to decide whether to join a group. Inability to maintain coherence

lowers the benefits; moreover lagging individuals might be deprived of possible energetic advantages (Parker, 1973). School size might depend on the number of body-size matching individuals, particularly in organized schools (Hoare, 2000; Krause *et al.*, 2000b). In the case of their deficiency among conspecifics in the habitat, the enhanced formation of heterospecific groups might be the result as observed in tamarins (Peres, 1993). Constitution of body, specifically body depth (Hambricht, 1991; Nilsson & Brönmark, 2000) and presence of armour or spiny structures (Dörner & Wagner, 2003), can affect the proneness to predation, particularly in gape-limited predators (Hambricht, 1991; Nilsson & Brönmark, 2000), that would prefer easier prey (Juanes & Conover, 1994; Nilsson & Brönmark, 2000).

Reservoir fish that are originally riverine species rarely manage to adapt for utilization of pelagic food resources (Kubečka *et al.*, 2014), although in several freshwater bodies, including the Římov Reservoir, enhanced utilization of the pelagic habitat has been reported (Čech & Kubečka, 2002; Diekmann *et al.*, 2005; George & Winfield, 2000; Jarolím *et al.*, 2010; Říha *et al.*, 2008).

Availability of food resources is one of the factors affecting the distribution of fish within the water body. In canyon-shaped reservoirs, the shallow littoral represents only a small area with hiding places particularly in lower parts (Vašek *et al.*, 2008). On the contrary, the pelagic zone of reservoirs offers a greater volume of potentially rich resources in a habitat with fewer competitors, because only very few originally riverine species have managed to adapt. The enhanced utilization of the pelagic habitat by zooplanktivores of Římov Reservoir (Čech & Kubečka, 2002; Jarolím *et al.*, 2010; Říha *et al.*, 2015) could also favour school formation as a form of "cover seeking" in a habitat with no shelters (Williams, 1964). Occupants of the pelagic habitat might not have many opportunities of meeting a school due to the generally large volume of a pelagic zone. Therefore, they must make a decision for joining a school before predator appearance. The transition of individuals between groups is possible when the schools meet (Krause *et al.*, 2000b), although the probability of group encounters is likely to be lower in the pelagic zone than in the littoral areas due simply to incomparable volumes of the habitats. Availability of shelters in the littoral habitat probably causes other behavioural responses in preference to schooling (Jolles *et al.*, 2018). The body size of fish also affects the probability of encountering similar body-sized conspecifics (Hoare, 2000). Large individuals, which are generally fewer in number than smaller juveniles, have a lower chance of encountering similar body-sized conspecifics, particularly in the pelagic habitat, so the decision to join a school must be rapid. Understanding of schooling behaviour in the pelagic zone of freshwater ecosystems is rather sparse (Milne *et al.*, 2005) compared with marine ecosystems where their high economic importance has resulted in increased attention (*e.g.*, Sund 1935). Recent studies were focused mostly on laboratory experiments (*e.g.*, Gaffney & Webster, 2018; Krause *et al.*, 2000a) and theoretical models (Couzin *et al.*, 2002; Hensor *et al.*, 2005) which might not bring as accurate results as field observations could, but those are also still lacking (reviewed in Krause *et al.*, 2000a).

The aim of this pilot study was (a) to provide a basic description of fish schools in the pelagic habitat of a freshwater temperate reservoir to obtain a real picture of schools, particularly school sizes and species composition; (b) to explore inter-species differences, schooling tendencies and species-specific preferences that are supposed to differ due to the varying vulnerability to predators; (c) to observe the occurrence of heterospecific schools and determine differences with conspecifics schools; (d) and to determine tendency of co-occurrence of particular species and encounter probabilities of species and school size with implications to species-specific aggregative tendencies.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was carried out in the canyon-shaped Římov Reservoir situated in the south of the Czech Republic (48,848 N, 14,845 E; 170 km south of Prague). The reservoir was built on the Maše River as a drinking water supply site for adjacent areas. The total length of the reservoir is approximately 12 km with a maximum area of 210 ha, volume $33 \times 10^6 \text{ m}^3$, and maximal surface elevation of 471 m a.s.l. Mean and maximal depths of the reservoir are 16 m and 45 m, respectively. Theoretical retention time is approximately 100 days. The reservoir is dimictic, and summer stratification is established from April to October. In spring, water transparency (Secchi depth) reaches 5–6 m during the clear water phase, which is followed by a lower transparency summer period with transparency declining to 1–2 m. The dam part of the reservoir is mesotrophic, whereas the riverine part is eutrophic (Hejzlar & Vyhánek, 1998). Cyprinid species dominate in the fish community of the reservoir, namely bream (*Abramis brama*), roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*), along with European perch (*Perca fluviatilis*) (Říha *et al.*, 2008). Several predatory species can be found in the pelagic zone, specifically asp (*Leuciscus aspius*), wels catfish (*Silurus glanis*), Northern pike (*Esox lucius*) and pike-perch (*Sander lucioperca*) (Prchalová *et al.*, 2008).

The Římov Reservoir is highly convenient for studying the behaviour of pelagic inhabitants as previously demonstrated by several authors (Čech & Kubečka, 2002; Jarolím *et al.*, 2010; Peterka *et al.*, 2006). Steep shores and water-level fluctuation in the reservoir are the reason for a limited littoral zone with a lack of macrophytes, particularly in the lower part of the reservoir (Vašek *et al.*, 2004). Such conditions should enhance the use of the pelagic zone by fish (Vašek *et al.*, 2008). Nonetheless, during the clear water phase, fish of the pelagic habitat might be more jeopardized by predators due to high transparency levels increasing the encounter rate (Turesson & Brönmark, 2007).

2.2 | Camera set-up

An underwater video camera (SplashCam Delta Vision HD B/W, OCEAN SYSTEMS) was used for data recording. The camera was

mounted on a 5 m-long metal bar attached to a buoy floating on the surface and secured by two anchors in a fixed position. Previous experiments showed a 45° tilt of the camera towards the surface to be the most effective position to obtain the highest possible contrast in the visual field (silhouettes of fish positioned against the bright surface) (Peterka *et al.*, 2006). In this set-up, the camera took up approximately 65 m³ of the epilimnetic layer. The set-up was situated in the pelagic habitat of the lower most part of the reservoir, where the depth reached 30 m, at a distance of approximately 100 m from the reservoir bank. The camera was permanently connected to a computer situated in the floating boat shed anchored at the shore for storage of the recordings.

2.3 | Data processing

Considering that a high water transparency level is necessary for obtaining quality video recordings, the observations were conducted during the "clear water" phase, from May to early June (31 May 2005 – 5 June 2005, 29 May 2012 – 5 June 2012, 7 May 2014 – 3 June 2014) (Longo & Floeter, 2012). The recording took place during daylight conditions because schools are reported strictly during daytime due to the need of a visual contact among school members (Whitney, 1969), resulting in 16 h of video per day. During one week in 2014 (21–26 May) visual conditions were poor due to heavy rain, and consequently these data were excluded from the analysis. The camera output data were captured using an AVS video editor (version 7.0.1.258; Online Media Technologies Ltd., 2013). The recorded files were automatically split and saved every 20 or 60 min and afterwards analysed using the video-editing software Avidemux (version 2.6.10;

Mean, 2008). The fish were identified by species (Figure 1). Each observation was considered as an independent record unless repetition of the same individual was apparent. Some individuals, mostly perch, took an interest in the camera (example snapshot in Figure 1c, d) or circled around it, disappearing and reappearing before the camera at very short intervals. Such a repeated record occurred within 2 min was considered as a repetitive observation (therefore, excluded), and an observation longer than 2 min was considered as independent one. In total 263 repeated observations of same individuals were omitted from the records (specifically 204 perch observations, 27 bream, 20 bleak, 6 roach, 5 asp and 1 catfish). Except for several occurrences of fish fry, that were not included in this analysis, all observed individuals were considered as adults on the basis of their previously reported prevalence in the epipelagic zone during daytime (e.g., Muška *et al.*, 2013; Vašek *et al.*, 2009). Each observation was classified as a singleton, a pair or a school (Figure 1). Observed groups of fish are called "schools" because of polarization and coherence; no shoals were observed because shoals mainly occur in the littoral zones, whereas in the pelagic habitat the absence of shelters pushes fish to form coordinated and coherent "schools" as antipredator protection (reviewed in Pavlov & Kasumyan, 2000). A pair of fish behaves differently to a school; according to Partridge (1982), a pair has only a leader and a follower, whereas in a group of three all fish adjust to one another. Based on this, pairs were treated separately in the analyses. In most cases the period between observations of fish was several minutes, and the coincidental observation of several individuals was recorded as a school if the coherence and polarization of all individuals in the school was indisputable; otherwise the individuals were considered independent. Interindividual distance among school members estimated from video recordings, that is, distance between front

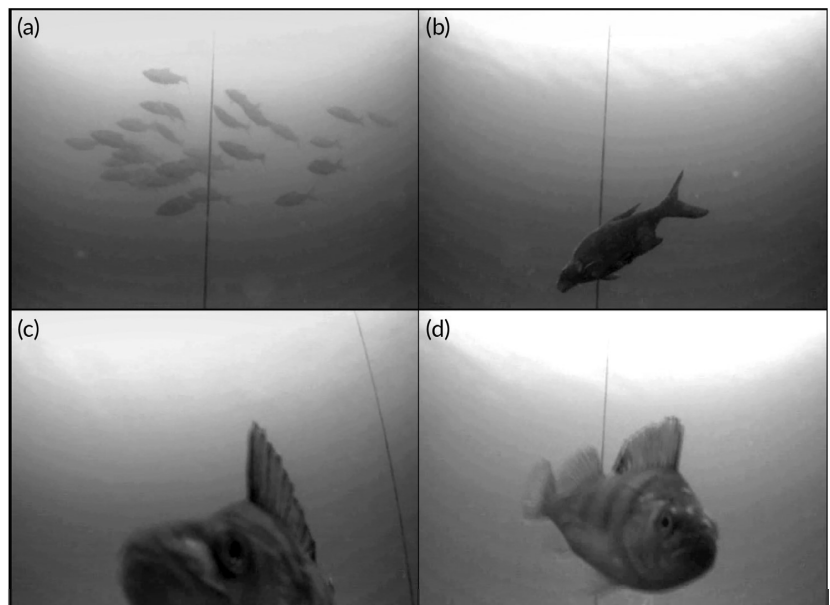


FIGURE 1 Snapshots from video footage taken by Jiří Peterka, study co-author. (a) School of roach, (b) bream performing gulping, (c) and (d) perch inspecting the camera

and tailing individuals, was mostly approximately the length of fish body. Even fish lagging more than one body length behind the school but clearly followed the school trajectory were counted as a school member. Observed schools were distant enough from the camera to claim that the vast majority of encounters provided recordings of whole schools. Because observations were performed out of the spawning period, fish pairs were treated separately because they do not share all properties with defined “schools” and can be easily dismissed, even though the pairs can become the schools including one other member more.

Body mass values for were obtained from pelagic gillnets (nets with 12 mesh sizes, 5–55 mm, 3 m height during and 4 mesh sizes, 70–135 mm, 3 m height) during the standard August sampling in the three seasons observed by camera (2005, 2012 and 2014; Table 1).

2.4 | Statistical analyses

Redundancy analysis (RDA), a multivariate linear method (CANOCO 5; Šmilauer & Lepš, 2014), was used for the description of the species preferences for division into group size classes (singles, pairs, schools). To include the individuals forming heterospecific aggregations, the counts of individuals of a species in group size classes per sighting were used that were log transformed $y' = \log_{10}(y + 1)$ and centred by species for obtaining zero average. The significance of the relationship between species and their occurrence in group size class was tested by the Monte Carlo permutation test (999 permutations). Apart from the focus species, asp was added to the analysis due to its relatively high abundance (>50 ind.).

A Mann–Whitney *U*-test was used to compare heterospecific and conspecific schools, including the comparison between species, due to the violation of normality assumption (Shapiro test – heterospecific schools: $P < 0.001$; conspecific schools: $P < 0.001$) and uneven number of observations.

For analysis of heterospecific schools a general linear model was used to find a difference in the increase in count of individuals between major and minor species. Major species is considered a species in every heterospecific school where the proportion of individuals exceeds that of the other species. Accordingly, a minor species is considered a species with a lower proportion of individuals than other species. Counts of individuals were log-transformed, and an *F* test of the interaction between major and minor species and school size (number of individuals in a school) was used. In cases of schools

containing two minor species, the proportions of the minor species were merged into one for the analysis due to low occurrence (two cases).

Association of each pair of species and general tendency for species to co-occur with other species were tested using the methodology of Kiffner *et al.* (2014). For analyses, 473 clusters were used. Null model was created from 5000 of randomization of co-occurrence matrix using `sim9()` function from the R package `EcoSimR` (Gotelli *et al.*, 2015).

For determining encounter probability the camera was used as a focal fish, and for every encounter, the number of individuals (singletons, bream, perch, roach, bleak, non-predatory species, predatory species) or units (pairs and schools) that were visible in front of the camera was counted. Probability to encounter a targeted group was calculated as hourly averaged values per 100 m³ (count of individuals or schools) per all observation seasons.

The statistical and graphical analyses were performed using the statistical software STATISTICA (version 13; TIBCO Software Inc., 2017), CANOCO 5 (version 5.01; Šmilauer & Lepš, 2014) and R project (version 3.3.1; R Core Team, 2015) including the packages `beanplot` (Kampstra, 2008), `ggplot2` (Wickham, 2009), `robustbase` (Rousseeuw *et al.*, 2015) and `EcoSimR` (Gotelli *et al.*, 2015).

3 | RESULTS

A total number of 3576 fish individuals belonging to nine species were recorded during the monitoring. Of all individuals 3% were predatory species, in particular asp, wels catfish, Northern pike and pike-perch. None of the predatory species formed either schools (aggregations maintaining coherence and polarized swimming) or pairs (except two cases where mixed pairs composed of roach and asp were observed). Four species, namely bream, roach and bleak, along with European perch, that occupied the pelagic habitat of the Řimov Reservoir have been identified as school-forming species. A total number of 3387 individuals of school-forming species were recorded on video footage during the observation periods. The most abundant species occurring in the video recordings was bream comprising 54.2% of all observed individuals, followed by European perch (24.7%), roach (17.4%) and bleak (4.8%).

Almost half of the observed schooling species individuals (47%) occurred unaccompanied, and 53% sought company of one or more individuals. Accompanied individuals were observed in pairs (24.5%) and in schools (≥ 3 ind.) (75.5%). School size ranged from 3 to

TABLE 1 Schooling details and morphological characteristics for adults of each species

	Proportion of schooling individuals (%)	Avg. cluster size (ind.)	Weight (g)	Deep body	Spines
Bleak	98	4.69	193	–	–
Roach	58	2.18	265	–	–
Bream	40	1.61	669	+	–
Perch	12	1.18	164	–	+

36 members, and the average school size was $5.6 \pm 84\%$ (mean \pm S.D.) individuals.

3.1 | Schooling tendency

RDA of species (four cyprinids and one percid) preferences for the group size classes (singles, pairs, schools) showed a statistically significant difference ($F = 65, P = 0.002$; Figure 2). Group size classes explained 5.4% of the total variation in the species clustering data. Bleak showed the highest inclination for school formation, roach and bream preference was lower and the inclination of perch to school formation was the lowest. Being a predatory species, asp showed a preference to remain single. The proportion of individuals engaged in schooling presented an identical order as shown in results from RDA (Table 1; Figure 3). Pairs, considered as stepping stones between singles and schools, showed a consistent proportion among the species (14%–16%); only pairs of bleak showed the proportion of 1%. In cyprinid species (bleak, roach, bream) a decline in average body size of the species corresponded with an increase in schooling tendency (Table 1). Bream and perch possess advanced morphology (see Table 1) that could explain their lower schooling tendency.

3.2 | Species composition and size of schools

Schooling fish, for example, individuals gathered in aggregations showing coherent and polarized swimming movements, engaged predominantly (80.6%) in conspecific schools (202 schools and 1066 individuals); however, 19.4% of individuals were involved in heterospecific schools (33 schools and 257 individuals). Individuals of bream, roach and perch were observed in heterospecific schools, whereas bleak formed exclusively conspecific groups.

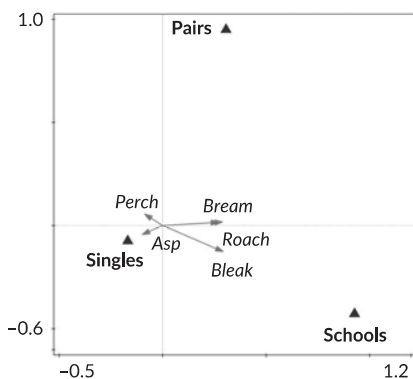


FIGURE 2 Ordination biplot of species-specific preferences for grouping size class (singles, pairs, schools) from the redundancy analysis (RDA). Triangles denote centroids of each size class. Species arrows pointing in a same direction indicate the positive correlation for a particular size class

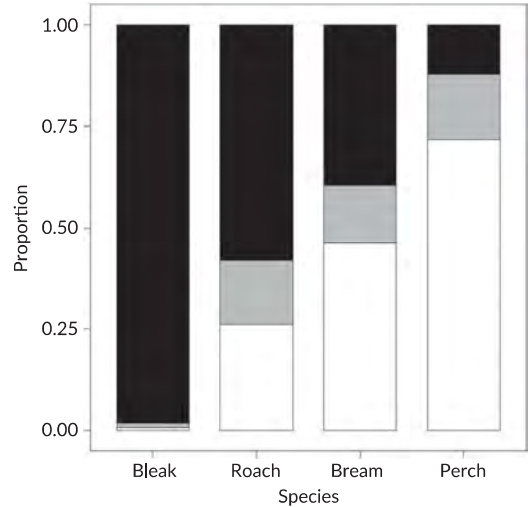


FIGURE 3 Proportion of individuals in size classes (singletons, pairs, schools) with respect to species. White for singletons, grey for pairs and black for schools

In heterospecific schools, all combinations of the three schooling species were observed; however, all three species together occurred in only 6.1% of cases, and the majority of cases were two-species schools. Most frequently observed were a mixture of bream and roach (72.7%), bream and perch (15.3%) and roach and perch (6.1%). The species proportions were never equal; one species always dominated. Regardless of species identity, the proportion of the major species increased with school size; correspondingly, the proportion of the minor species decreased with school size. The slope of the increase in the number of individuals in the school and school size differed significantly between major and minor species (general linear model,

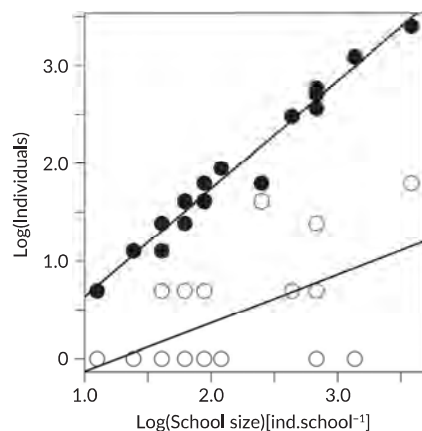


FIGURE 4 Relations between count of individual of major and minor species and size of heterospecific schools: major species – full circle, minor species – empty circle

$F_{1,60} = 32.39, P < 0.001$); major species increased more sharply than minor species (Figure 4). Particular species that dominated in the count of individuals varied between schools; roach was the major schooling species in 51.5% of schools, bream in 39.4% and perch in 9.1%.

Nevertheless, conspecific schools dominated in number, and heterospecific schools prevailed in proportion of large schools (>10 individuals; heterospecific: 52.5%, conspecific 26.4%). Likewise, sizes of conspecific and heterospecific schools differed significantly in count of school members (Mann–Whitney U -test: $U = 2410, N_1 = 202, N_2 = 33, z = -2.637, P = 0.008$) with heterospecific schools exceeding the sizes of conspecific schools [$7.8 \pm 91\%, 5.3 \pm 78\%$ (mean \pm S.D.)]. In like manner, the significant difference between school sizes of conspecific and heterospecific schools (Figure 5) was proved in schools that included bream (Mann–Whitney U -test: $U = 1280, N_1 = 116, N_2 = 30, z = -2.599, P = 0.011$) and roach (Mann–Whitney U -test: $U = 364, N_1 = 39, N_2 = 28, z = -2.360, P = 0.020$). In perch, the difference between heterospecific and conspecific school size was not significant (Mann–Whitney U -test: $U = 61, N_1 = 19, N_2 = 10, z = -1.765, P = 0.077$), and bleak did not engage in heterospecific schooling.

A tendency for a species to co-occur with other specific species was found to be significant only for bream and roach ($p < 0.001$). Similarly, tendency to form heterospecific schools was significant in bream and roach ($P = 0.004$ and $P = 0.015$, respectively).

Of all species, roach showed the highest inclination to form heterospecific schools (41% of schools that included roach were heterospecific; in bream, 20.5%; in perch, 34.5%; see Figure 6 for school

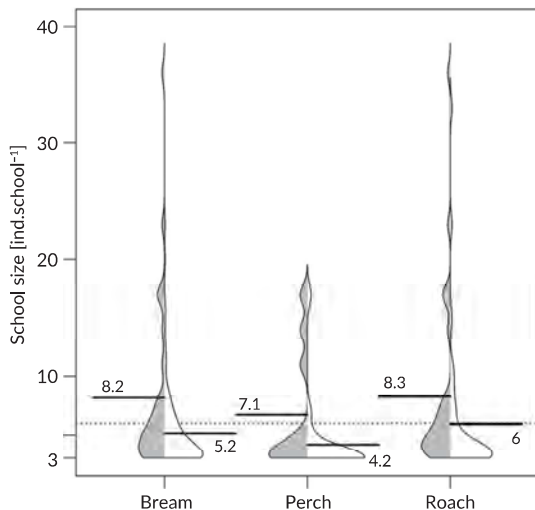


FIGURE 5 Relative frequency of observed school sizes in heterospecific and conspecific schools in relation to species. Mean school sizes are marked as thick lines, the dashed line is mean size of all schools, grey fill is for heterospecific schools and white fill is for conspecific schools. Total counts – bream: heterospecific $n = 116$, conspecific $n = 30$; perch: heterospecific $n = 19$, conspecific $n = 9$; roach: heterospecific $n = 39$, conspecific $n = 28$

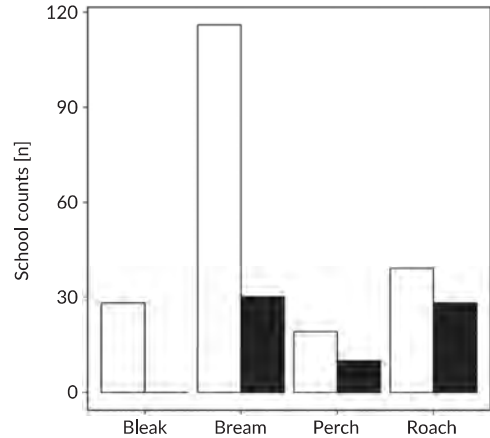


FIGURE 6 Barplot showing count of conspecific (white bars) and heterospecific schools (black bars) with respect to species

count comparison). The mean size of heterospecific schools that were joined by roach was similar to that of bream and higher than that of perch [roach: heterospecific $8.3 \pm 90\%$, conspecific $6 \pm 99\%$; bream: heterospecific $8.2 \pm 89\%$, conspecific $5.2 \pm 65\%$; perch: heterospecific $7.1 \pm 70\%$, conspecific $4.2 \pm 77\%$ (mean \pm S.D.)] (Figure 5).

3.3 | Encounter probability

Probability that focus fish would encounter another singleton was higher than to encounter a school or a pair of fish, which were very similar (Table 2). Probability of encountering a non-predatory species was naturally much higher than that of predatory species. Particularly

TABLE 2 Probabilities of encountering certain category

Category	Probability of encounter (per $h.100 m^{-3}$)	S.D. (%)
Singletons (ind.)	8.22	46
Pairs (n)	1.21	64
Schools (n)	1.14	94
Bream (ind.)	8.48	71
Perch (ind.)	4.06	82
Roach (ind.)	3.00	125
Bleak (ind.)	0.54	360
Non-predatory species (ind.)	16.11	68
Predatory species (ind.)	0.52	87

Abbreviations: ind., individuals.

Note: The average numbers are presented in individual/school per hour and $100 m^3$ of upper pelagic layer together with standard deviation values.

high probability was to encounter bream, but much lower to encounter perch, roach and bleak (Table 2).

4 | DISCUSSION

Schooling behaviour of adult individuals of various species was examined in the pelagic zone of the Římov Reservoir using a visual method that represents an effective tool for studying behavioural aspects. Canyon-shaped reservoirs are interesting ecosystems presenting gradual transitions between rivers and lakes. Such man-made water bodies hold species of riverine origin from which only a very few have been able to adapt to a lacustrine environment (Fernando & Holčík, 1991) and most of them utilize either littoral or river-like habitats in upper parts of the reservoir (Vašek *et al.*, 2004). Observed school sizes were relatively small (max 36 ind.) because of the limited amount of body-size matching conspecifics that occur in the pelagic habitat. Only a proportion of species found in the pelagic habitat of the study site actually utilized the habitat in contrast to lakes that are considerably older ecosystems and, therefore, can contain obligative pelagic species, which are more likely to form larger schools. Small individuals (juveniles or sub-adults) which occur in the higher numbers of size-matching individuals evade the habitat during the daytime because of high predatory risk as noted by Říha *et al.* (2015). Gliwicz and Jachner (1992) suggested that fear of predators pushes them out of the epipelagic habitats ("ghost of predation past").

Four out of nine fish species formed schools during the study period. Nonetheless, schooling tendency varied between species with diverse proportions of schooling and non-schooling individuals as well as school sizes and willingness to form heterospecific groups. The proportions of fish in pairs were similar among three schooling species (roach, bream and perch) and did not significantly change with increasing density of fish (Holubová *et al.*, 2019b). The low presence of pairs implies that they present just "stepping stones" towards schools. With no shelters present, antipredator strategy is needed in the pelagic zone; even though it is occupied by a negligible amount of predatory species, only a very small amount of them would prey on fish of the daytime community (Juanes & Conover, 1994; Nilsson & Brönmark, 2000). Although the most abundant species in the pelagic habitat during daytime was bream followed by bleak, roach and perch (for details see Říha, 2012), the highest schooling tendency was displayed by bleak which were found almost exclusively in schools.

In cyprinid species, the proportion of schooling individuals increased with vulnerability of the species which corresponds to the extent of advanced morphology and the gape size of the predator. Gape-limited predators seek rather slender-bodied prey without body structures, whereas morphologically advanced species are preferred in smaller body sizes or avoided (Dörner & Wagner, 2003; Krause *et al.*, 1998; Moody *et al.*, 1983; Nilsson & Brönmark, 2000). Accordingly, the smallest and most vulnerable species (bleak) proved to have the highest schooling tendency as well as lowest probability of encounter, which makes this species rather rare but not low in abundance (e.g., Říha, 2012). Bream, on the contrary, showed a low

schooling tendency; the body-size refuge from predation is reached in the shortest timeframe of all the observed species due to its deep body (Webb, 1986). A similar assumption can be made about perch with spiny structures. Such species might form the schools for higher efficiency in food resource search or hydrodynamic advances (Pitcher & Parrish, 1993). Slight hydrodynamic benefits seem probable in bleak or roach schools, in contrast to bream which frequently performs sinusoidal swimming that breaks the ideal shape (lattice constitution; reviewed in Pavlov & Kasumyan, 2000).

The highest probability of species encounter was in bream, just as their reported abundance in the pelagic habitat (Říha, 2012). There was also a higher probability of encountering a singleton than a school; although schools are preferentially targeted by predators (Krause & Godin, 1995), small schools can be undetectable by predators (Maury, 2017). This fact raises the question as to what is a detectable size of school in the freshwater environment, and whether the freshwater schools (average school size 5.6 individuals in this study) are big enough to be detected sooner than singletons. Probability of encountering a singleton, pair or school should also vary depending on the time of the day (De Kerckhove *et al.*, 2015; Makris *et al.*, 2009).

Bleak was the only species showing a strict preference for formation of conspecific schools, which are most beneficial to their members because they confuse predators by forming multiple targets (Landeau & Terborgh, 1986) of identical prey (Krause & Godin, 1994). Although most of the observed schools consisted of conspecifics, some were composed of more than one species. Wilson (2000) noted that the occurrence of heterospecific groups might be higher in the environment with the absence of shelters; therefore, high predation risk might shift school composition preferences (e.g., Mathis & Chivers, 2003) to increased heterospecific schooling (in this study this was observed in bream, perch and roach). Heterospecific schools were not as numerous but exceeded the conspecific schools in school sizes, which proves their antipredator function (Semeniuk & Dill, 2006), because small group defences might not be so effective (Hager & Helfman, 1991). In the case of low-abundance species, the efficient school size can be reached solely through heterospecific assemblages (Peres, 1993), although there is a higher probability of breaking up (Wolf, 1985). In this study, roach, whose abundance in the pelagic habitat was relatively small, formed heterospecific schools most frequently. Vulnerable species, such as roach, are better at assessing the school size and, given a choice, they prefer the larger schools (Krause *et al.*, 1998). Moreover, schools of phenotypically similar species can have the benefits of the confusion effect and lower threat from being odd (Krause *et al.*, 2000a; Blakeslee *et al.*, 2009). Potential costs reduce with an increasing number of conspecifics present in the school (Ward *et al.*, 2002). Turesson and Brönmark (2004), however, reported null confusion effect of roach schools on predators (contrary to Neill & Cullen, 1974); the simple benefit arising from larger amounts of other possible targets for predation seems enough to trigger the creation of heterospecific schools even in the marine habitat (Bakun & Cury, 1999; Cury *et al.*, 2000).

The proportion of species in heterospecific schools was never equal as reported in other littoral and pelagic fish assemblages (Springer, 1957; Krause *et al.*, 2000b). Even though the ideal conditions in heterospecific groups are believed to be when an identical number of conspecific species is present, the proportion of major species increased, whereas the proportion of minor species decreased with an increase in school size. In such a case, benefits or costs are uneven, and the individuals with a small number of conspecifics spend more time being vigilant and, therefore, less time feeding (heterospecific herds of gazelles; Fitzgibbon, 1990). This situation can spring from a low chance of conspecific encounter (particularly in the case of roach) in the pelagic habitat. Heterospecific schools are likely to be divided as a result of passive segregation when various swimming speeds occur (Wardle, 1975), or when a predator is encountered (Wolf, 1985). The other thing is that minority species could be taking advantage of heterospecific schooling in search for conspecifics and leave the heterospecific school when they meet a conspecific one or when they reach a count of conspecific large enough to create their own school. A lower proportion of heterospecific schools as well as the absence of equal proportions of the species could imply an unstable existence of such schools and emphasize the preference for conspecific schooling. As for major species, the most abundant species, bream, numerically prevailed in the count of individuals, though in the count of schools the most frequently repeated major species was roach, despite the fact that its abundance was noticeably lower. This is in accordance with higher vulnerability of roach and higher need for antipredation tactics.

Co-occurrence tendency of roach and bream that was observed either could be based on similar living strategies, including sharing time, spatial and food-resource niche (Vašek *et al.*, 2008; Vašek & Kubečka, 2004) or can be explained by protector-species theory described in bird nesting colonies (Pius & Leberg, 1998), meaning that vulnerable species can seek protection in a group of morphologically advantaged species that are avoided by predators. Although predatory species have been confirmed to be able to recognize and follow the odour of their prey (Atema *et al.*, 1980; Pohlmann *et al.*, 2001), the odour of majority species could haze that of vulnerable species mainly in cases where there is a low proportion of vulnerable species. Members of heterospecific schools experience a higher cost of the oddity effect particularly in cases of uneven proportions of species (Landeau & Terborgh, 1986). Visibility in the study site peaks during the “clear water” period when the transparency increases up to 6 m but declines later in the summer when the transparency decreases to 1–2 m. Therefore, the predator recognition of the species in a school limited by the distance from the school may depend on the olfactory system, or lateral line (Pohlmann *et al.*, 2001), but neither has been researched properly due to obvious difficulty in realization. Location of prey by olfaction and lateral line will vary depending on the water body morphology, especially water currents. As for visual conditions, it has been shown (*e.g.*, Turesson & Brönmark, 2007) that decline in water transparency lowers the chances of encountering prey. Despite the fact that animals in large groups are supposed to attract the attention of predators (Pitcher & Parrish, 1993), optical properties of water

should result in identical chances of schools and singletons being located (Murphy, 1980). Aggregated individuals are harder to find as being in a large group at one place makes them rare (Ioannou *et al.*, 2011). Behavioural responses to changes in predation risk and connected features as vigilance, stress or cease of feeding activity can also be the result of individual heterogeneity based on personality or physical condition of individual fish (Jolles *et al.*, 2019). A particular state, then, can either enhance or lower the tendency for school forming as well as willingness to form heterospecific aggregations.

Video census methods (UVC), as an underwater video camera, proved to be a relevant tool for surveying freshwater fish populations (Krause *et al.*, 2005; Holubová *et al.*, 2019b). Traditionally used gear for fish sampling still uses nets that can be harmful for both fish and surrounding environment (Baker *et al.*, 2016). UVC enable one to monitor the environment without causing physical harm to fish or habitat (Baker *et al.*, 2016) and can be used in a wide spectrum of environments (Neuswanger *et al.*, 2016), where fish behave naturally and are not disrupted by the presence of a diver or boat (Harvey & Fletcher, 2001; Olsen *et al.*, 1983; Soria *et al.*, 2003; Wilson *et al.*, 2018). This way, cameras can provide information on behavioural traits and sociability (Peterka *et al.*, 2006). A big advantage is that it is relatively cheap. On the contrary, particular light and clearance levels are necessary for obtaining quality pictures (Marini *et al.*, 2018; Peterka *et al.*, 2006); in dense populations it could be hard to count the individuals or determine the species (Marini *et al.*, 2018); distant individuals might also be problematic (Bozec *et al.*, 2011). Another drawback is its inability to detect cryptically living species (Baker *et al.*, 2016). Long-term-used cameras also need maintenance because they can become overgrown with algae which prevent the recognition of species (Marini *et al.*, 2018). When used properly, an UVC proved to be a promising tool for obtaining more complex data than previously acquired by traditional methods (Holubová *et al.*, 2019a); with further innovations suppressing the shortcomings such as automatic detection and recognition systems (Siddiqui *et al.*, 2018) or stereo cameras enabling the sizing of objects (Neuswanger *et al.*, 2016), UVC techniques might surpass traditional invasive methods (Ellender *et al.*, 2012; Holubová *et al.*, 2019a).

5 | CONCLUSION

This study represents one of the first attempts at studying adult fish schooling behaviour in a temperate freshwater reservoir. The results contribute to a deeper understanding of the biology and behaviour of species occupying the least-explored freshwater habitat of the freshwater bodies – the pelagic zone – with particular attention to species schooling specificities as to willingness to form heterospecific schools. It is highly probable that the reason behind the school formation, particularly in vulnerable species, is its antipredator function as widely mentioned in the literature. Nonetheless species not threatened by predation might use the foraging benefits of schools as a means to quickly locate a quality food source. The question posed is whether the food intake is higher in school-forming individuals of bream than

non-school forming. In conclusion, schooling behaviour most probably forms and endures based on actual needs of the individual, and changes in ambient as well as internal factors likely cause various individual responses.

This study suggests that further research on the free-living schools is necessary to obtain more coherent knowledge on the fish school formation, species specificities and factors affecting the composition of schools. The use of underwater video technologies seems to be an appropriate complement to hydroacoustic methods. Together they present a non-invasive approach to fish research with hydroacoustics providing quantity and video quality, including the recognition of behavioural aspects.

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AUTHOR CONTRIBUTIONS

H.M. wrote the article, processed and analysed the data and participated in data collection. B.P. advised on statistical analyses. P.J. invented the design, supervised and participated in data collection together with Č.M. and V.M. All contributing authors provided valuable commentaries to the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Paper II

Density dependent attributes of fish aggregative
behaviour

Density dependent attributes of fish aggregative behaviour

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ABSTRACT

Grouping behaviour, as fascinating as it is unclear, has lately drawn the attention of numerous researchers. While most of the authors focused their work on a mechanistic approach to the matter of schooling, this study explores the issue from a population point of view. Present camera observation study on the fish community carried out in the epipelagic habitat of a European temperate reservoir in the Czech Republic explored the relationship between density and aggregative features of predominantly cyprinid fish stock. Results demonstrated that schooling behaviour is triggered by the 'critical density' of fish in the habitat. School size as well as counts of schools and proportion of schooling individuals increased with the density of fish. Counts of clusters (observed units in time, including singletons, pairs and schools) and cluster size, on the other hand, showed a slowing tendency to increase. The slower increase implies the tendency of fish for not being frequent but rather to create larger groups. Altogether, our findings suggest that fish density is a triggering factor in the formation of large fish schools. As the tendency of cyprinid species for school formation could be an evolutionary advantage responsible for dominance in later succession phases of water bodies, we suggest that more in situ studies should be encouraged for the proper understanding of the ecological interactions that drive the structure of aquatic ecosystems and for ensuring unbiased assessment.

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INTRODUCTION

Fish aggregative behaviour has been puzzling people for decades. Schooling behaviour is mainly considered as an antipredator strategy (*Pitcher & Parrish, 1993*) convenient particularly for species inhabiting the environment with the lack of shelters such as the epipelagic habitat. This habitat often contains an important food source for zooplanktivores = the planktonic crustaceans. In Římov Reservoir (Czech Republic), mainly adult zooplanktivorous fish inhabit the pelagic environment during the daytime (*Říha et al., 2015*) when they can be highly conspicuous for predators; therefore, it is convenient for them to seek protection via schooling behaviour (*Williams, 1964*), although

the vulnerability to predators differ with respect to species. There is a growing body of evidence that predators prefer preying on aggregations than on individuals (*Botham & Krause, 2005*). Especially in a marine environment, large aggregations attract numerous predators and, if localised, the overwhelming majority is often consumed (reviewed in *Maury, 2017*). Still, the existence of schools proves the tendency of organisms to form a patchy distribution and its advantageousness. Being a member of the school brings various other benefits, such as lower risk of being captured (*Hamilton, 1971*), increase in detection time by predator (*Vine, 1971; Ioannou et al., 2011*), faster sighting of approaching predator (*Godin, Classon & Abrahams, 1988*), faster location of quality food resources (*Krebs & Davies, 1993*), etc. Although the sighting distance by predators increases with the number of individuals in the group, the benefits from the group are still several times higher than the risk of predation; this is true especially for freshwater-system piscivores which manage to handle no more than few, usually only one, prey fish at once (*Ruxton & Johnsen, 2016*).

The emergence of fish schools is noted to be dependent on the density of conspecific individuals (*Okubo, 1986*). In natural conditions, however, the absence of conspecifics might enhance the formation of heterospecific schools with visually and ecologically similar species (*Ward, Axford & Krause, 2002*). The presence of marginal number of individuals is a trigger which drives looser aggregations into dense schools. School size and composition can repeatedly fluctuate within a short time span (*Radakov, 1973*). Evered and Seghers (cited in *Seghers, 1981*) noted that various ambient causes as variations in the encounter rate of conspecific individuals or sympatric species (*Okubo, 1986; Croft et al., 2003*), or the state of hunger within group (*Robinson & Pitcher, 1989*) can trigger merging or division of groups (*Okubo, 1986; Gueron, 1998*). Similarly, the presence or absence of predators can affect the duration of schools as well as their cohesion (*Tien, Levin & Rubenstein, 2004*); especially in the case of heterospecific schools, the duration of school is more likely to be lower (*Wolf, 1985*).

Research on freshwater fish schooling was rather neglected (*Milne, Shuter & Sprules, 2005*) mainly due to low economic importance in comparison with marine habitat. Nevertheless, countless laboratory studies (*Wright et al., 2003; Hoare et al., 2004; Hensor et al., 2005*) and theoretical models (*Okubo, 1986; Gueron & Levin, 1995; Gueron, 1998*) have been conducted in order to unveil details on fish schools and shoals including density dependence, yet field observations are still sporadic. Moreover, focus organisms are mostly small bodied species or juvenile individuals (*Wright et al., 2003; Guillard et al., 2006*). Paradoxically, non-field results may bring inaccurate information as noted by *Hensor et al. (2005)*, who compared the shoaling tendency of banded killifish (*Fundulus diaphanous*) in shallow habitat and laboratory with simulation models. They proved that neither models nor laboratory results reflect the actual situation in the field. Artificial environment can affect the behaviour by various stimuli apart the one that is being studied as have been concluded by *Reebs (2002)*, therefore in situ observations are worth pursuing in order to obtain a 'true picture'.

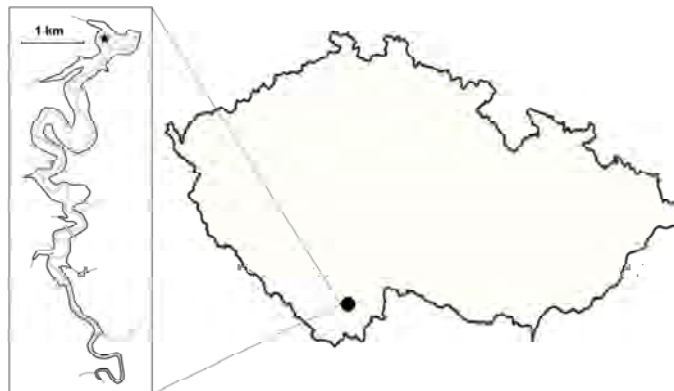


Figure 1 Map of Europe with marked reservoir location. Star is for study site.

Full-size  DOI: [10.7717/peerj.6378/fig-1](https://doi.org/10.7717/peerj.6378/fig-1)

The goal of this in situ study was to unveil the relationship between fish density and attributes of schooling behaviour in the open water habitat of temperate freshwater reservoir by describing the actual state from acquired video recording data.

METHODS

Study area

The study was conducted in the dam area of canyon-shaped Římov Reservoir built on the Malše River as a drinking water supply for adjacent areas (48.848N, 14.845E; Czech Republic; Fig. 1), therefore no public access is allowed. Researchers of Institute of Hydrobiology are allowed to enter to Římov Reservoir with the permit by Vltava River authority, contract number 300/7225. The total length of the reservoir is approximately 12 km with the max area of 210 ha, volume $33 \times 10^6 \text{ m}^3$, and maximal surface elevation of 471 m a.s.l. Mean and maximal depth of the reservoir is 16 and 45 m, respectively. Reservoir is dimictic with summer stratification established from April to October. Water transparency (Secchi depth) reaches up to six m during the ‘clear water’ phase whereas summer period transparency is rather low (less than two m). Trophic of the reservoir decreases from eutrophic riverine to mesotrophic dam part (Hejzlar & Vyhnálek, 1998). Cyprinid species dominate the community of the reservoir, namely freshwater bream (*Abramis brama*), roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*), along with European perch (*Perca fluviatilis*) (Říha et al., 2008). Several predatory species can be found in the pelagic area, specifically asp (*Leuciscus aspius*), wels catfish (*Silurus glanis*), Northern pike (*Esox lucius*), and pike-perch (*Sander lucioperca*) (Prchalová et al., 2008).

Littoral areas are extremely limited particularly in the lower part near to the dam. Steep shores and seasonal water level fluctuation are responsible for deficiency of submerged aquatic macrophytes (Vašek et al., 2009; Čech et al., 2012). The lack of shelters and need for search for food in pelagic zone should be favourable for fish school formation.

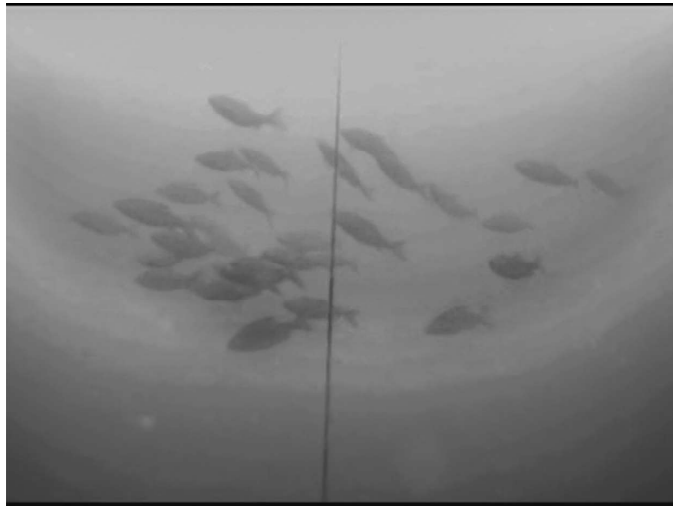


Figure 2 Snapshot from video footage taken by Jirí Peterka, study co-author. School of roach individuals passing above the camera. [Full-size !\[\]\(1679558f37f6db0dd8360a2a7e913e90_img.jpg\) DOI: 10.7717/peerj.6378/fig-2](https://doi.org/10.7717/peerj.6378/fig-2)

Camera set-up

An underwater video camera (SplashCam Delta Vision HD B/W; OCEAN SYSTEMS, Everett, Washington, USA) used for data recording was mounted on a five m-long metal bar which was attached to a buoy floating on the water surface and secured by two anchors in a fixed position. Previous experiments showed 45° tilt of the camera towards the surface to be the most effective position in order to obtain the highest possible contrast in the visual field (silhouettes of fish positioned against the bright surface; snapshot of actual footage in Fig. 2) (Peterka, Vašek & Matěna, 2006). In this set up, camera took up approximately 65 m³ of the epilimnetic layer. The set-up was situated in the pelagic habitat of the dam part, where depth reached 30 m, in the distance of approximately 100 m from reservoir bank. The camera was connected to a computer situated in the floating boat shed for the recordings storage (for scheme see Fig. 3).

Data collection and processing

The observations were conducted out of the spawning period during the ‘clear water’ phase, May–early June (31 May 2005–5 June 2005, 29 May 2012–5 June 2012, 15 May 2014–31 May 2014, but not all data were used for the analysis, see below). This period being typical for high water transparency even in systems with higher trophy due to grazing activity of planktonic crustaceans (Lampert *et al.*, 1986) is convenient since high transparency is essential for obtaining good quality data using visual census. The recording took place during daytime conditions and 16 h (from 5 AM to 9 PM) of video per day were obtained. During 1 week (May 21–26) in 2014, visual condition were inconvenient and consequently the data excluded from the analysis. The camera output data were captured using the AVS video editor (<https://www.avs4you.com/>). Recorded files were

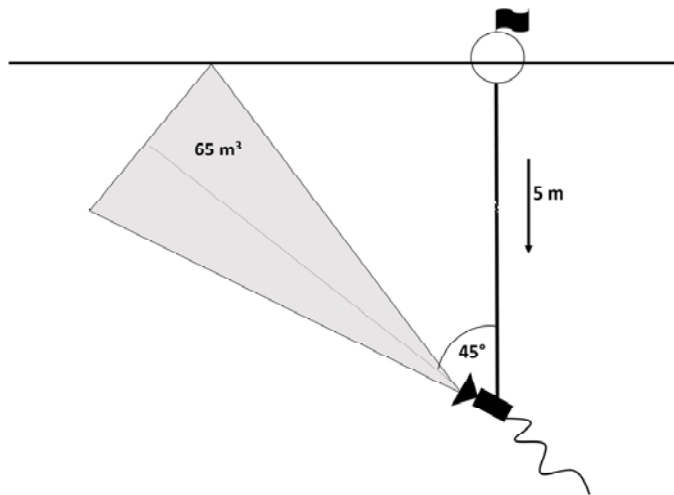


Figure 3 Scheme of the camera set-up.

Full-size  DOI: 10.7717/peerj.6378/fig-3

automatically split and saved every 20 or 60 min and afterwards analysed by means of video editing software Avidemux (<http://fixounet.free.fr/avidemux/>). Each observation was considered as an independent record unless the repetition of the very same individual was apparent. Some individuals, mostly perch, took interest in camera or cycled around, disappearing and reappearing before camera in very short intervals. If such a repetition occurred, a record in frequency shorter than 2 min was regarded as a repetitive observation (not counted) and an observation in a frequency longer than 2 min was considered as independent one. In total, 263 repeated observations of same individuals were omitted (specifically 204 perch observations, 27 bream, 20 bleak, six roach, five asp and one catfish). Except for several occurrences of fish fry that were not included in this analysis, all observed individuals were considered as adults considering obvious body size and reported prevalence in the epipelagic during daytime (Vašek *et al.*, 2009; Muška *et al.*, 2013). Observed fish were categorised as singletons, pairs or schools. Observed groups of three and more fish were called 'schools' because of polarisation and coherence; we did not observe any shoals since we feel that in freshwater habitat shoals are mainly issue of littorals, whereas in pelagic habitat the need for food search pushes fish to form 'schools'. A pair of fish behaves differently to a school; according to Partridge (1982), there is only leader and follower, whereas in a group of three all fish adjust to each other. Based on this we treated pairs separately. Though in most cases the period between observations of fish was several minutes, coincidental observation of several individuals was recorded as a school if the coherence and polarisation of all individuals in the school was indisputable; otherwise, the individuals were considered independently. Inter individual distance between school members estimated from video recordings, that is, distance between front and tailing individuals, was mostly about length of fish body, but we counted as a school member even fish lagging more than one body length behind the

school if they clearly followed school trajectory. Observed schools were distant enough from camera to claim that vast majority of encounters provided recordings of whole schools. All categories (i.e. singletons, pairs and schools) were summed as clusters for analyses of aggregation. With a few exceptions, species were distinguishable in the videorecordings. Because of the presence of heterospecific schools, the data were not separated by species especially since there is an evidence that the species in the heterospecific groups adjust to each other (Tang et al., 2017). Moreover, size of heterospecific group probably undergoes different pressures and might be more easily dismissed (Wolf, 1985). On several occasions technical difficulties during video recording caused loss of video data, therefore data from only 24 days of observation were used (days with more than three missing hours were not included into analysis). Missing hours in these days (6 days with one missing hour, 2 days with two missing hours, and 1 day with one three missing hour) were supplemented with average hourly values to obtain 16 h of observation.

Several models (basic linear level-level, log-level, log-log, exponential growth and logistic grow curve with carrying capacity) were tested to find the best fit for modelling the density dependence of aggregation attributes. For the analyses, the values summarised per day were used in case of fish density, cluster counts, school counts and counts of individuals in size categories (singletons, pairs and schools). In case of school and cluster sizes values were averaged per day.

Best fit for the relationship between cluster size (cluster is observed unit of any size) and fish density was obtained by log-log model, as well as count of schools. Count of clusters and fish density was fitted with log-level model. A linear regression proved best fit for modelling relationships between schools size and fish density, as well as relationship of proportions of fish in size categories (singletons, pairs and schools). Counts of fish in size categories (singletons, pairs and schools) and fish density were fitted with the log-log models. If logarithmic transformation was used in the analysis, the values for graphical presenting were back exponentiated for better understanding of analyses output. In all analyses best fitted models were chosen on the basis of the lowest value of Akaike Information Criterion. For demonstrating 'critical density', hourly proportions of fish in categories (singletons, pairs and schools) were used (322 h of observation). Statistical analyses and graphical visualisation were conducted in R project statistical computing software, using packages stats (R Core Team, 2017), ggplot2 (Wickham, 2009) and stargazer (Hlavac, 2018).

RESULTS

During our observation in the epilepagic habitat of the Římov Reservoir a total number of 3,174 fish were captured on video footage. The apparent majority (95%) of the recorded individuals belonged to the species that formed schools, namely freshwater bream, roach, European perch and bleak (Fig. 4). The remaining fish were common carp (*Cyprinus carpio*) and predatory species (3%), specifically asp, Wels catfish, Northern pike and pike-perch. School size ranged from three to 36 members. Smaller school

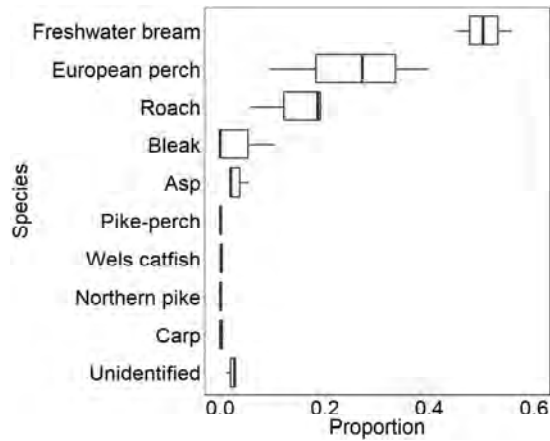


Figure 4 Boxplot showing relative species composition during three-season observation. Median values (thick lines), upper and lower quartiles (boxes), minimum and maximum values (whiskers).

Full-size [DOI: 10.7717/peerj.6378/fig-4](https://doi.org/10.7717/peerj.6378/fig-4)

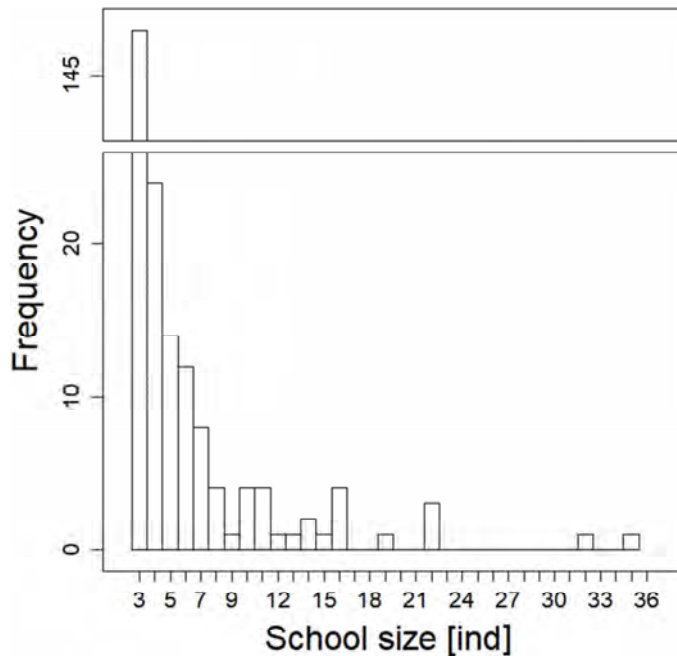


Figure 5 Histogram showing observed frequencies of fish schools of particular size.

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sizes prevailed in the recordings (see Fig. 5) with the average school size $5.6 \pm 84\%$ (mean \pm SD) individuals. Schools were often composed of more than one species; therefore, the analysis not differentiating species was performed.

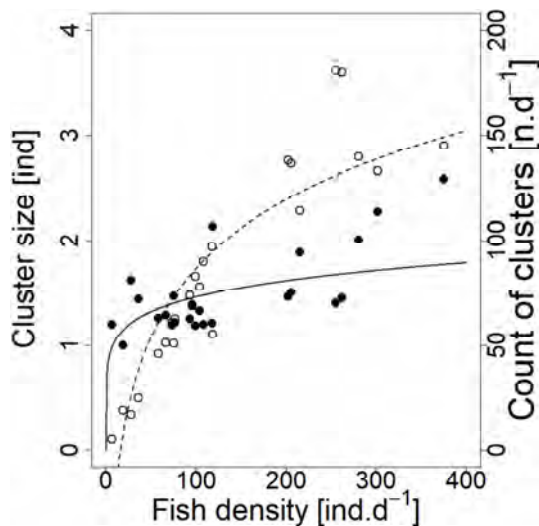


Figure 6 Relationship between cluster count (sum value per day) and cluster size (average diel value) and fish density (sum value per day). Counts of clusters—empty circle and dashed line; cluster sizes—full circle and solid line. For visualisation of log-log model of cluster size and fish density relationship, the predicted y value was back exponentiated. [Full-size !\[\]\(1663bb69f307a960345edb0e712f8c02_img.jpg\) DOI: 10.7717/peerj.6378/fig-6](https://doi.org/10.7717/peerj.6378/fig-6)

Analysed fish were organised in 2,183 clusters (comprising singletons, pairs and schools) that included 238 schools (polarised groups of three and more fish). Counts of clusters as a measure of aggregative behaviour observed in the epipelagic habitat showed a slowing increase with fish density ($R^2_{\text{adj}} = 0.796$, $F_{1,22} = 90.75$, $p < 0.0001$; Fig. 6; Table 1), as well as cluster size (cluster size: $R^2_{\text{adj}} = 0.316$, $F_{1,22} = 11.62$, $p = 0.0025$; Fig. 6; Table 1). School size and counts of schools increased linearly with observed fish density (school size: $R^2_{\text{adj}} = 0.795$, $F_{1,22} = 90.080$, $p < 0.0001$; Fig. 7A; counts of schools: $R^2_{\text{adj}} = 0.840$, $F_{1,22} = 1,201.800$, $p < 0.0001$; Fig. 7B; Table 1). Count of singletons showed slowing increase trend with fish density ($R^2_{\text{adj}} = 0.867$, $F_{1,22} = 144.2$, $p < 0.0001$; Fig. 8A; Table 1), whereas count of fish in schools increased rather exponentially ($R^2_{\text{adj}} = 0.868$, $F_{1,22} = 152.1$, $p < 0.0001$; Fig. 8A; Table 1) and count of fish forming pairs increased linearly with small slope ($R^2_{\text{adj}} = 0.585$, $F_{1,22} = 33.42$, $p < 0.0001$; Fig. 8A; Table 1). The proportion of schooling fish linearly increased with fish density ($R^2_{\text{adj}} = 0.505$, $F_{1,22} = 24.46$, $p < 0.0001$; Fig. 8B; Table 1), simultaneously, the proportion of singletons linearly declined ($R^2_{\text{adj}} = 0.564$, $F_{1,22} = 30.740$, $p < 0.0001$; Fig. 8B; Table 1). The proportion of pairs, transitions between singletons and schools, showed no significant relationship with fish density ($p > 0.05$; Fig. 8B; Table 1) and rarely exceeded the proportion of 0.2 per day. The data also shows that between densities of 10 and 30 individuals per hour the proportion of fish in schools exceeded the proportion of single fish (Fig. 9). This emergence of schooling behaviour confirms the existence of a threshold in density that stirs fish to group formation called ‘critical density’. Our results prove that fish tend to form schools after reaching a ‘critical density’, which triggers the tendency to join other individuals and form

Table 1 Best-fit regression models on fish schooling attributes and fish density.

Regression results										
Dependent variable										
	Count of clusters	Cluster size (log(x))	Count of schools (log(x+1))	School size	Singletons (log(x+1))	Fish in pairs (log(x+1))	Fish in schools (log(x+1))	Proportion of singletons	Proportion of paired fish	Proportion of schooling fish
Fish density (log)	47.227*** (4.957)	0.145*** (0.042)	0.921*** (0.083)		0.765*** (0.064)	0.881*** (0.152)	1.354*** (0.110)			
Fish density				0.016*** (0.002)				-0.001*** (0.0003)	-0.0001 (0.0002)	0.001*** (0.0003)
Constant	-130.669*** (23.168)	-0.286 (0.198)	-2.321*** (0.390)	-0.482 (0.289)	0.490 (0.298)	-1.392 (0.712)	-3.004*** (0.513)	0.728*** (0.050)	0.171*** (0.028)	0.101** (0.044)
Observations	24	24	24	24	24	24	24	24	24	24
R ²	0.805	0.346	0.847	0.804	0.868	0.603	0.874	0.408	0.029	0.527
Adjusted R ²	0.796	0.316	0.840	0.795	0.862	0.585	0.868	0.381	-0.015	0.505
F Statistic (df = 1; 22)	90.753***	11.624***	121.830***	90.084***	144.173***	33.419***	152.126***	15.137***	0.658	24.464***

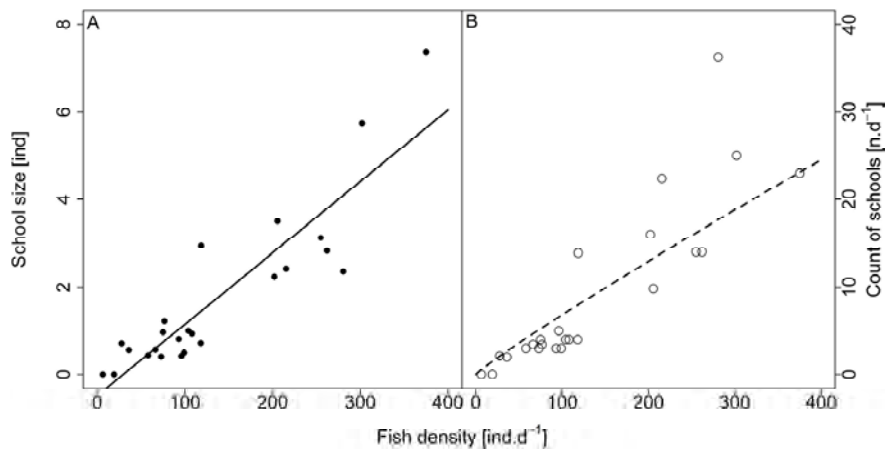
Notes:** $p < 0.05$.*** $p < 0.01$.

Figure 7 Relationship between (A) school size (average diel value) and (B) count of schools and fish density (sum values per day). Count of schools—full circle and solid line, school size—empty circle and dashed line. For visualisation of log-log model count of schools and fish density relationship, the predicted y value was back exponentiated. [Full-size !\[\]\(e1c9322a51cd0e999864da940416cebe_img.jpg\) DOI: 10.7717/peerj.6378/fig-7](https://doi.org/10.7717/peerj.6378/fig-7)

schools whereas tendency to stay alone decreases (as apparent from proportions in size categories; Fig. 8). A slowing increase in count of clusters (Fig. 6) suggests that cluster density might reach an upper limitation resulting in stabilised cluster counts with simultaneous increase in school sizes. This means that although increasing in the density, the encounter rate of schooling fish can stabilise thanks to creating larger schools.

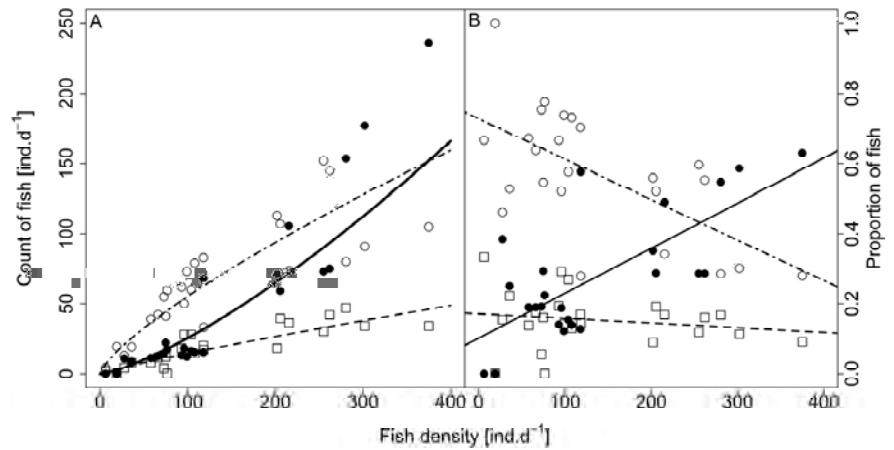


Figure 8 Relationship between (A) counts and (B) proportions of fish in size category (singletons, pairs and schools) and fish density. Singletons—empty circle and dotdash line, pairs—square and dashed line, and schooling individuals—full circle and solid line. For visualisation of log-log model of count of fish in categories (singletons, pairs and schools) and fish density relationship, the predicted y value was back exponentiated. [Full-size !\[\]\(5f471a71b78d7676bc356df190b88ab4_img.jpg\) DOI: 10.7717/peerj.6378/fig-8](https://doi.org/10.7717/peerj.6378/fig-8)

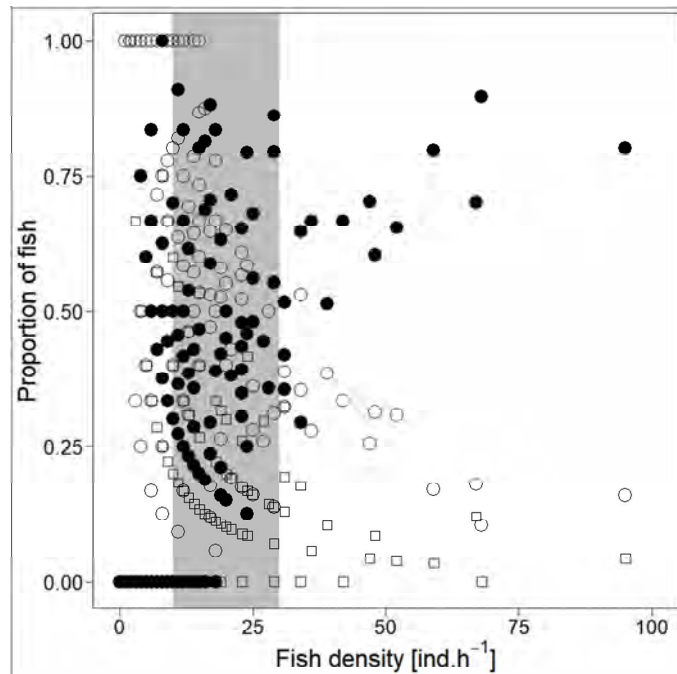


Figure 9 Demonstration of 'critical density' on relationship between proportions of fish in size category (singletons, pairs and schools; hourly values) and fish density. 'Critical density'—depicted by grey area; singletons—empty circle, pairs—square and schooling individuals—full circle. [Full-size !\[\]\(e6d8ed0e56026ff17854aa495380637d_img.jpg\) DOI: 10.7717/peerj.6378/fig-9](https://doi.org/10.7717/peerj.6378/fig-9)

DISCUSSION

Our study explored the effect of fish density on formation of fish schools in the epipelagic habitat of the European temperate reservoir. Species of the pelagic habitat occurred in corresponding densities with previous studies and school forming ones were the most abundant species (Říha, 2012). Schools were comprised mostly of cyprinid species (freshwater bream, roach and bleak) and European perch. All those species are visually oriented zooplanktivores (Lazzaro, 1987; Vašek & Kubečka, 2004) that utilise the pelagic habitat in our study site in a search for food resource–zooplankton (Vašek & Kubečka, 2004). The absence of shelters in the pelagic habitat can enhance the school formation (Magurran & Pitcher, 1983).

Several tendencies depending on fish density in the habitat were recognized in our recent study. Decrease in proportion of singletons went hand in hand with increase in proportion of fish engaged in schools. Accordingly, school sizes followed linearly increasing trend with fish density and count of clusters and cluster size showed slowing increase with fish density. Altogether, the findings confirm the hypothesis that school formation is triggered by the amount of fish present in the habitat of open water. In other words, schooling behaviour emerged at ‘critical density’ (between 10 and 30 individuals per hour), just the same as was proposed for marine populations (Makris et al., 2009; Maury, 2017). Slowing increase trend of the cluster counts together with increasing amount of schooling fish as well as school and cluster sizes suggest that clusters (observed units of fish) maintain minimal distances from each other. For fish as prey it is disadvantageous to be frequent because predators are able to remember common prey appearance and focus on them (search image; Krebs, 1978). Formation of schools ensures the scattered distribution and evasion of predators as well as faster location of food resource. In addition, the optical properties of the water makes it difficult to recognize friend to foe on long distance and it is only logical that fish encountering same or sympatric species would stick together due to dilution effect of the group (Pitcher & Parrish, 1993). A slowing increase in counts of clusters also corresponds with work of Okubo (1986) who noted that group size and group count tend to be constant. This distribution pattern could serve for limitation of the predator encounter by making themselves rare (Vine, 1971).

This study as well as others on fish (Hensor et al., 2005; Maury, 2017) and other gregarious animals (Wirtz & Lörcher, 1983; Vander Wal, Van Beest & Brook, 2013), confirmed that the key factor affecting the group size is population density. Size of observed schools reached 10 of individuals, with small schools being more frequent than larger ones which is in accordance with other studies (Seghers, 1981; Niwa, 1998; more examples from other taxa in Okubo, 1986). Freshwater school sizes are noticeably smaller than marine schools that can go to thousands of individuals. Smaller densities results in lower number of potential schoolmates. On the other hand, a higher encounter rate in freshwater than in ocean environment (compare Hoare & Krause, 2003; Misund et al., 1998), together with more heterogenous environment and possibilities to migrate to shallow areas, could be the cause for more frequent merging and splitting of

freshwater schools. Very large schools are not only exposed to higher competition for resources (Bertram, 1978), but also higher conspicuousness to predators (Turner & Pitcher, 1986) and susceptibility to disease (reviewed in Maury, 2017) and parasites infections (reviewed in Mikheev, 2009), resulting in higher mortality. Nevertheless, the tendency of animals to form large groups with increasing density is undeniable. From opposite point of view, this fact might present an evolutionary mechanism to regulate the population sizes of sympatric species to maintain the equilibrium of ecosystems (Maury, 2017). Some of freshwater bodies suffer from activities of recreational anglers that focused usually on predatory species (Scharf, 2007) which influence could be deeply underestimated (Arlinghaus, Mehner & Cowx, 2002; Lewin, Arlinghaus & Mehner, 2006). Low proportions of piscivorous fish effect ecological interactions and ecosystem structure (Goeden, 1982) even by enhancing the competition ability of gregarious species. This could lead to increase in system trophy, which is undesired for example in water bodies used as drinking water supplies such as Římov Reservoir.

CONCLUSIONS

Our results provide further evidence that the density of fish in the habitat triggers the schooling behaviour. Schooling is in the temperate climate of European water bodies broadly utilised by cyprinid species that dominate the freshwater systems in later succession phases. The question arises as to whether schooling behaviour might be the reason behind the selective advantage responsible for cyprinid dominance in the later succession phases of water bodies. This highlights the need for more ecologically complex studies including the behavioural attributes of specific organisms since they are important for a correct understanding of predator–prey interactions that drives the structure of aquatic ecosystems. Moreover, knowledge of species-specific distribution patterns and aggregative tendencies is crucial for sampling gear selection in attempts to establish the true picture of fish communities.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Michaela Holubová performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Martin Čech contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Mojmír Vašek contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Jiří Peterka conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e. approving body and any reference numbers):

Researchers from the Institute of Hydrobiology were allowed to enter Římov Reservoir with a permit from the Vltava River authority, contract number 300/7225.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the [Supplementary File](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.6378#supplemental-information>.

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Paper III

Fluctuations in pelagic fish density linked to ambient conditions.

Fluctuations in pelagic fish density linked to ambient conditions.

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Abstract

Animal behaviour has been broadly studied for its social or functional aspects but less often for understanding the interactions between organisms and their ambient conditions. A pilot observational study was performed by means of underwater camera to investigate the correlation between environmental factors and the density of fish in the epipelagic habitat of a European temperate reservoir. Explored factors confirmed a positive correlation between water temperature and all observed categories: non-predatory, predatory, single non-predatory and schooling fish, as well as freshwater bream and roach. On the other hand, de-trended solar irradiance was found to be negatively correlated with density of non-predatory fish, freshwater bream and European perch. Sunshine duration was negatively correlated with the density of predatory fish. Precipitation showed positive relationship with single non-predatory fish and European perch, whereas wind strength had a negative relationship with density of schooling fish. Furthermore, density of predatory fish was positively correlated with density of single non-predatory fish and counts of observed clusters. Altogether, findings indicate that fish density is correlated with abiotic factors and the occurrence of predators. This suggests that more ecologically complex studies should be encouraged for better understanding of ecological interactions that drive the structure of aquatic ecosystems.

Introduction

Aquatic environment is a complex system with a large number of interactions in which changes in current abiotic conditions can have more serious influences than in terrestrial habitats. Although, rather low economic importance of freshwater fisheries in relation to marine environment (Milne *et al.*, 2005) and prevalence of the use of artificial ponds in commercial freshwater fisheries resulted in minor interest in research of free-living fish communities in lakes and reservoirs despite the fact that freshwater habitat houses about 41 % of all fish species (Cohen, 1970). Fish community composition of particular habitat springs from the variety of internal preferences and external contexts of the environment (Smith & Powell, 1971). Aquatic environment is inhabited by numerous classes of organisms (from viruses to large vertebrates), whose interactions might occur on various places and levels and several factors (e.g., temperature, UV light are probable to affect the outcome of individual preferences for habitat, grouping (Leech & Williamson, 2001; Bartolini *et al.*, 2015), since every species has unique set of preferences on their habitat (Ward *et al.*, 2002). Changes in surrounding conditions can result in re-evaluation of risks and benefits or even viability in the present habitat. On that account, optimal habitat of particular species and cohort is composed of biotic and abiotic elements that varies differently on the time scale (Fréon & Misund, 1999). For example perch switch during ontogeny from zooplanktivory to piscivory (Persson *et al.*, 2004), from pelagic habitat mostly to littoral habitat (Čech *et al.*, 2007; Říha *et al.*, 2015) and from large shoals to small schools (Persson, 1988). Sociability as well depends on the ecological context as food or shelter presence (Jolles *et al.*, 2018), but stability of the environment also varies in temperate water bodies throughout the season; therefore, fluctuations are apparent on both long- and short-term scales.

Water temperature is one of the main long-term abiotic factor influencing fish (Weetman *et al.*, 1998; Vašek & Kubečka, 2004; Vašek *et al.*, 2008). This major environmental variable affects not only metabolism (Fry, 1971; Brett & Groves, 1979; Cooke *et al.*, 2003), growth (Nunn *et al.*, 2003; Gabillard *et al.*, 2005; Petrtýl *et al.*, 2015), migration (Bjornn, 1971; Jonsson *et al.*, 1990) or activity (Weetman *et al.*, 1998), but also behavioural aspects as handling time (Society *et al.*, 1986), reproduction (Kramer & Smith, 1962; Kvarnemo, 1996), school cohesion and polarization, tail beat frequency or rheotaxis (Bartolini *et al.*, 2015). Moreover, all of the features vary between species. Water bodies of temperate zone undergo changes in thermal stratification throughout the season, which have implications on life strategies in all organisms in the water body (Sommer *et al.*, 1986). Succession in thermal stratification brings also changes in abiotic attributes as transparency. When the “clear water” period is settled, high visibility may affect light sensitive organisms and alter predator-prey interactions. On the other hand, conditions during “clear water” phase resembles the actual state in oligotrophic water bodies.

The fish stock composition and activity of various cohorts in the pelagic habitat undergo changes during the diel cycle due to dissimilarities in predator pressure (Helfman, 1981b; Vašek *et al.*, 2009; Muška *et al.*, 2013; Říha *et al.*, 2015) and feeding demands in different ontogenetic stages (Randolph & Clemens, 1976; Kadri *et al.*, 1997; Alanärä *et al.*, 2001). Basically, adult fish stay in the littoral zone during night, move to epipelagic habitat after dawn and remain there during the day (Říha *et al.*, 2015). But the numbers of fish during daytime do not follow clear pattern. Proposedly, changes during daytime could partially be influenced by the fluctuation in ambient conditions, since large fish are sporadically observed offshore during stormy or rainy weather periods (Jarolím *et al.*, 2010). The importance of light intensity for foraging of pelagic and visually orientated zooplanktivores, has been implied by their exclusively daytime feeding

activity (Vašek & Kubečka, 2004) and increase of fish occurrence in highly illuminated pelagic areas (Muška *et al.*, 2018). Particularly pelagic planktivorous fish significantly reduce typical foraging and feeding behaviour under lower light intensities (Jarolím *et al.*, 2010) or even disappear from the epipelagic habitat during the severe weather conditions (Johansen *et al.*, 2007; Munks *et al.*, 2015). Particular change can happen in prey (zooplankton) distribution as well both actively or passively (Pinel-Alloul, 1995; Leech *et al.*, 2005a, 2005b). Since food availability is also important factor affecting distribution, change in prey availability can result in shift either in utilized prey species (Werner & Hall, 1988) or the consumer distribution. Furthermore, not only current solar irradiance intensity but also other faster changing factors like wind speed, and precipitation may play important roles as possible determinants of fish distribution by being unfavorable for food search efficiency (Jarolím *et al.*, 2010) or even energetically costly. Variations in cisco (*Coregonus artedii*) school distribution have been suggested as a result of windy weather (De Kerckhove *et al.*, 2015). Wind induces waves can impede fish swimming and raise its energetic expenses. Accordingly, feeding rate of fish have been reported to decelerate when heavy waves occurred (Bowen & Allanson, 1982).

As the density affects the formation of schools, the presence of the fish schools in the pelagic habitat should fluctuate in like manner (De Kerckhove *et al.*, 2015; Holubová *et al.*, 2019). Fish aggregates in schools predominately during day using vision as the primary sense to maintain the school (Whitney, 1969) and disperse during twilight as the light level decrease and individuals can't visually maintain contact with shoaling neighbours. However, it has been noted that schooling tendency depends on actual number of conspecific fish in the habitat (Holubová *et al.*, 2019). Species of the pelagic habitat not rarely incline to socializing by the means of group living (Fréon & Misund, 1999), although aggregations are particularly numerous and dense

in marine habitats (Makris *et al.*, 2009), freshwater fish tend to form much smaller assemblages (e.g., Russell *et al.*, 2004; Holubová *et al.*, 2019). Predator occurrence is an important factor in spatial structuring of aquatic inhabitants (Jackson *et al.*, 2001) and its presence can affect the fish distribution (Werner & Hall, 1988). It is well known that predators are preferentially attracted to largely aggregated prey (Botham & Krause, 2005) since they are more easily detected. Maury (2017) proposed that truly beneficial in both food income and protection against predators are smaller aggregations. Such groups are virtually undetectable by any predator sense and gain more foraging efficiency (Fonteneau & Hallier, 2015; Maury, 2017).

Our study explored possible factors altering the fish presence and densities in the epipelagic habitat of the European temperate reservoir. We hypothesize that all fish in the pelagic habitat are influenced by the environmental factors. We suppose that the presence of all fish will differ between highly illuminated and calm weather periods and rainy, cloudy or windy periods. During rainy and stormy weather conditions, the abundance of the fish in the epipelagic habitat should be low with scarce presence of schools, whereas during sunny and calm weather the abundance of the fish would be higher with high numbers of fish in schools. We tested effects of the environmental factors on non-predatory fish density, predatory fish density, non-predatory single fish density, density of schooling fish and density of three dominant species in order to determine whether these factors influence either of observed categories. Moreover, we tested the relationship between the densities of the predatory and non-predatory fish in the pelagic habitat.

The goal of this *in situ* study was (1) to demonstrate influence of variations in environmental conditions on the presence of fish in the epipelagic habitat with particular interest in fish schooling, interspecific difference and predatory and prey species difference, and (2) to

outline possible predator-prey density relationship and their outcome for attributes of schooling behaviour. Possible implications for schooling behaviour are emphasised and discussed.

Methods

Study site

The study was conducted in the canyon-shaped Římov Reservoir built on the Malše River as a drinking water supply for adjacent areas (48.848 N, 14.845 E; Czech Republic, Fig 1A, 1B) with no public access. This 12 km long reservoir of 210 ha area and volume $33 \times 10^6 \text{ m}^3$ has a maximum of 45 m and mean depth 16 m. Reservoir is dimictic with summer stratification establishing from April to October. During late May to early June about one month lasting “clear water” phase establishes, in this time water transparency (Secchi depth) reaches up to 6 m due to

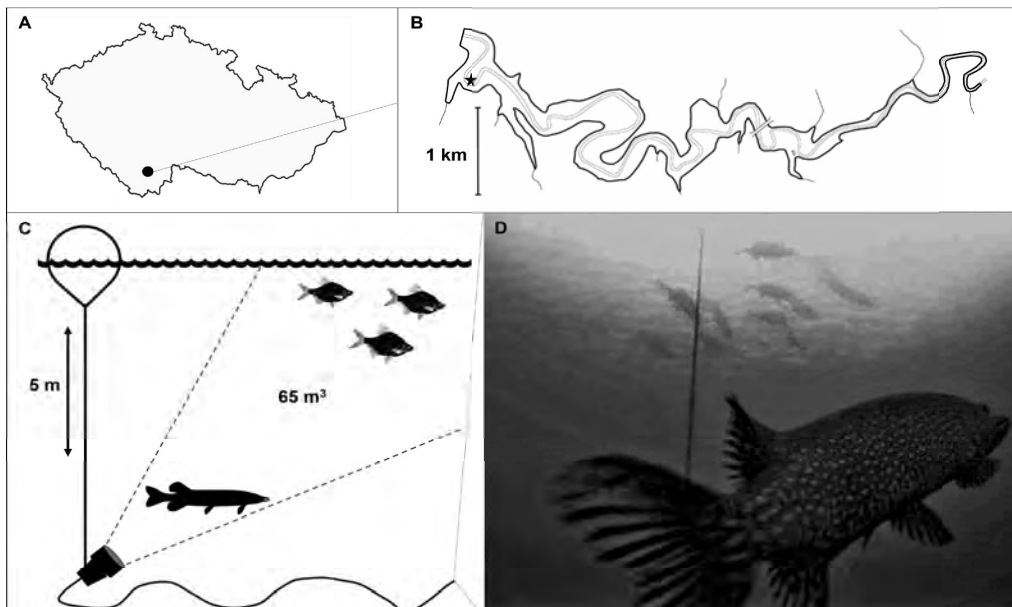


Fig 1. (A) Map of the Czech Republic. Dot indicates location of the reservoir. (B) Římov reservoir map. Star indicates study site. (C) Scheme of the epipelagic camera set-up. (D) Snapshot from recorded video. School of freshwater bream followed by Northern pike (taken by Jiří Peterka, study co-author).

grazing activity of planktonic crustaceans, mainly of genus Cladocera (Lampert *et al.*, 1986) while later in the summer period transparency decreases to approximately 2 m. The trophic state of the reservoir decreases from eutrophic in the riverine to mesotrophic in the dam part (Hejzlar & Vyhnanek, 1998). Reservoir is typical for lack of littoral areas particularly in the dam part (Vašek *et al.*, 2009; Čech *et al.*, 2012). Steep banks together with water level fluctuation cause the deficiency of submerged aquatic macrophytes in the littorals (Krolová *et al.*, 2013). Due to elongated shape and steep banks bordering the reservoir, effect of wind on wave action is minimal. Cyprinid species dominate the community of the reservoir, namely freshwater bream (*Abramis brama*), roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*), along with a percid, European perch (*Perca fluviatilis*) (Říha *et al.*, 2008). Predatory species include asp (*Leuciscus aspius*), wels catfish (*Silurus glanis*), Northern pike (*Esox lucius*), and pike-perch (*Sander lucioperca*) (Prchalová *et al.*, 2008), but their densities are rather low.

Camera set-up and data management

Data were recorded by the means of an underwater video camera (SplashCam Delta Vision HD B/W, OCEAN SYSTEMS; Everett, Washington, USA) fastened to a metal bar hanging from a buoy and secured by two anchors in a fixed position (Fig 1C). Situated in the depth of 5 m the camera's field of view captured the volume of approximately 65 m³ of the epilimnetic layer. A 45° tilt of the camera towards the surface have previously been showed as the most effective position in order to obtain the best quality images (silhouettes of fish positioned against the bright surface, Fig 1D) (Peterka *et al.*, 2006). The set-up was situated in the epipelagic habitat of the dam part of the reservoir, where depth reached 30 m, in the distance of approximately 100 m from the bank. The camera was connected to a computer situated in the floating boat shed for the recordings storage. For obtaining good quality visual data,

the observations were conducted out of the spawning period during the “clear water” phase, May-early June (31.5. - 5.6.2005, 29.5. - 5.6.2012, 15.5. – 31-5.2014, but not all data were used for the analyses), which is typical for high transparency (Lampert *et al.*, 1986). The recording took place during daytime conditions and 16 h (from 5 AM to 9 PM) of video per day were obtained. For one week (May 21.-26.) in 2014, visual conditions were unsuitable and consequently the data excluded from the analyses. The camera output data were captured using AVS video editor (downloaded from <https://www.avs4you.com>). Recorded files were automatically split and saved every 20 minutes. Avidemux software (downloaded from <http://avidemux.sourceforge.net>) was used to analyse the video footages. Each observation of fish was considered as an independent record unless the repetition of the very same individual was apparent. Some individuals, mostly European perch, took interest in the camera or cycled around, disappearing and reappearing before camera in very short intervals. If such a repetition occurred, a record in frequency shorter than 2 minutes was regarded as a repetitive observation (not counted) and an observation in a frequency longer than two minutes was considered as an independent one. In total 263 of repeated observations of same individuals was omitted (specifically 204 European perch observations, 27 freshwater bream, 20 bleak, 6 roach, 5 asp and 1 wels catfish). Except for several occurrences of fish fry (not included in our analyses) all observed individuals were considered to be adults due to obvious body size and reported prevalence in the epipelagic during daytime (Vašek *et al.*, 2009; Muška *et al.*, 2013). Observed fish were categorized as singletons, pairs or schools. Observed groups of three and more fish were called “schools” because of polarization and coherence, no “shoals” were observed since they are composed of unorganised group of individuals acting independently, what seems to be mainly issue of littorals in freshwater habitats, while in the epipelagic habitat the need for food

search pushes fish to form organised and compact “schools”. A pair of fish behaves differently to school, according to Partridge (1982), there is only leader and follower, whereas in a group of three all fish adjust to each other. Based on this we treated pairs separately. Thought in most cases the period between observations of fish was several minutes, coincidental observation of several individuals was recorded as a school if the coherence and polarization of all individuals in the school was undeniable, otherwise, the individuals were considered independently. Inter individual distances between school members estimated from video recordings, i.e., distance between the leading and trailing individuals, was mostly about one body length of fish, but we counted as a school member including fish lagging more than one body length behind the school and followed school trajectory. Vast majority of observed schools were certainly encountered as whole schools. With a few exceptions (63 ind., 2 % on total), species were distinguishable in the videorecordings.

Environmental factors

Meteorological data were recorded (10-minute intervals) with a floating hydro-meteorological station MS16 (Fiedler AMS, Czech Republic) located in the dam part of Římov Reservoir (48.8489N, 14.4868E), which included probes at 2-m height above the water surface for measurement of air temperature (TEP1, Fiedler AMS), wind speed (W2, Fiedler AMS), and solar irradiance (pyranometer CNR 1, Kipp & Zonen, Delft, the Netherlands), the rain gauge (SR02, Fiedler AMS) and a probe for water temperature at 0.5-m depth (PT100-XM, Fiedler AMS). Solar irradiance data were used to calculate sunshine duration within one day (“defined as the sum of the time for which the direct solar irradiance exceeds 120 W m^{-2} ”; WMO, 2010). Values of wind speed and solar irradiance were averaged hourly whereas values for precipitation were summed hourly. Transparency measured by Secchi disc did not fluctuate during monitoring

period and remained on the value of 5 m, except, as mentioned above, from one week in 2014 (heavy rains) that was not included to analysis. Environmental data were not available for all days of recording due to some technical difficulties, therefore those days were excluded from later analysis (see below). Variation of the factors are depicted in S1- S3.

Statistical analysis

The effect of environmental factors on the amount of the fish in the epipelagic habitat (non-predatory fish, predatory fish, singletons, schooling fish and in supplementary included separated species: freshwater bream, roach and European perch) was analysed with Generalized Estimating Equations model (*geeglm*) using the R package *geepack* (Halekoh *et al.*, 2006) in the R programming language and environment, version 3.3.1 (R Core Team, 2015). For the analysis of the density of schooling fish a total number of 208 fish schools was analysed. GEE is an extension of the Generalized linear models (*glm*; Zuoqing *et al.*, 1986) and is suitable for data that are within cluster correlated and that violates the independence assumption in conventional regression analyses and leads to type 1 errors, the GEE also allows for autocorrelation structure in the cluster. A GEE model assuming Poisson distribution was used since the data lacked the normality. A first order autoregressive model AR (1) was used in analyses assuming time dependence within each day of observation, accordingly the day variable was used as “id” argument to set clusters. Time variables were set as argument “waves” to treat the missing values in the dataset. The jackknife estimation principle was applied to avoid bias because of small number of clusters (<30) in all models (Halekoh *et al.*, 2006). Explanatory variables used in models were solar irradiance (averaged per hour W), de-trended solar irradiance (values of solar irradiance undergoes changes during diel cycle, this trend was smoothed for the analyses by centring the values on the mean, values were averaged per hour W), precipitation (mm/hour),

water temperature (measured at 7.00 AM), wind speed (hour average, m/s), sunshine duration (h/day), time and second-degree polynomial of time (in case of non-linear trend). Predictors were assumed to be independent (correlation coefficient between predictors was ≤ 0.5 both positively and negatively, except sunshine duration and detrended irradiance which correlation was 0.6 S4). Forward selection and Wald test were used to assess the overall significance of variables and produce the minimum adequate model. Evaluation of model was performed by checking the residuals against the fitted values to be randomly scattered without showing any pattern and plot of residuals was examined to check for normality and assess the appropriateness of the fitted model. For the analysis total count of 20 days of all three observation seasons were used.

For the analyses of predatory fish density relationship, the values in the dataset summarised per day were used in case of non-predatory fish density, predatory fish density, cluster counts (units of observation containing all sizes, single non-predatory fish included), school counts and density of single non-predatory fish and schooling fish. In case of school and cluster sizes values were averaged per day. On several occasions technical difficulties during video recording caused loss of video data, therefore data of only 24 days of observation were used (days with more than three missing hours were not included into analysis). Missing hours in these days (six days with one missing hour, two days with two missing hours, and one day with three missing hours) were supplemented with average hourly values for particular day to obtain 16h of observation for each day. The correlation between non-predatory fish density and predatory fish density (count of individuals per day = 16 hours of observation) was tested by various models (due to the excess of zeros following models were tested negative binomial, zero inflated Poisson, zero inflated negative binomial, Poisson, log-linear model, general linear model, exponential growth). Best fit was chosen based on Akaike Information Criterion (AIC).

All analyses were carried out in graphical and statistical computing software R Studio (RStudio Team, 2018). As the data showed over-dispersion, the best fit proved negative binomial model (*glm.nb*, package MASS; Venables & Ripley, 2002) which is also appropriate for over-dispersed count data (Lindén & Mäntyniemi, 2011). Statistical details were provided by functions *nagelkerke* (package rcompanion, Mangiafico, 2019), *rcorr* (package Hmisc, Harrell Jr & Dupont, 2020) and *Anova* (package car, Fox & Weisberg, 2019). All categories (i.e. singletons, pairs and schools) were summed as units of observations (not individuals) to a variable: clusters.

Results

Video footages captured a total number of 3 029 fish (2005: 1 563 ind., 2012: 641 ind. and 2014: 825 ind.) that occurred in the epipelagic habitat of the Římov Reservoir during our study period. Following fish species were identified: freshwater bream (1499 ind.), European perch (751 ind.), roach (521 ind.), bleak (108 ind.), asp (72 ind.), wels catfish (5 ind.), Northern pike (3 ind.), pike-perch (2 ind.) and common carp (*Cyprinus carpio*, 5 ind.). Predatory species were rather scarce and took up only approximately 3% of the recorded individuals (82 ind.), whereas non-predatory fish were comprised of totally 2 884 individuals with the apparent majority (95 % of all fish) belonging to the species that formed schools (namely freshwater bream, roach, European perch and bleak). Actual schools (n=208) were formed by 1 157 individuals (38 % of all observed fish) and were composed of up to 36 school members.

Abiotic factors

Effect of environmental factors on density of non-predatory fish

Among all evaluated environmental factors (water temperature, wind speed, solar irradiance, de-trended solar irradiance, sunshine duration, precipitation, year of observation and time and second-degree polynomial of time), only water temperature ($\chi^2=15.800$, $df=1$, $p<0.0001$) and de-trended solar irradiance ($\chi^2=10.500$, $df=1$, $p=0.001$) have been found to have a significant relationship with density of non-predatory fish (S5, S6). Density of non-predatory fish in the epipelagic habitat was negatively correlated with de-trended solar irradiance (solar irradiance above average). On the other hand, density of non-predatory proved positive correlation with raising water temperature.

Effect of environmental factors on density of predatory fish

There has been found a significant relationship between density of predatory fish and water temperature ($\chi^2=4.030$, $df=1$, $p=0.045$), together with sunshine duration ($\chi^2=24.570$, $df=1$, $p<0.0001$). Density of predatory fish was positively correlated with water temperature, whereas negatively correlated with sunshine duration (S5, S7).

Effect of environmental factors on density of single non-predatory fish

Water temperature ($\chi^2=34.700$, $df=1$, $p<0.0001$) along with precipitation ($\chi^2=6.000$, $df=1$, $p=0.014$) have been found to be significantly correlated with density of single non-predatory fish (in addition there has been a marginal significance of de-trended solar irradiance ($\chi^2=2.900$, $df=1$, $p=0.087$)). Density of single non-predatory fish increased with water temperature and with amount of precipitation. De-trended solar irradiance had marginal negative and very weak correlation with the density of single non-predatory fish (S5, S8).

Effect of environmental factors on density of schooling individuals

Density of fish in schools have been found to be significantly correlated to water temperature ($\chi^2=7.300$, $df=1$, $p=0.007$), whereas the correlation was negative in wind strength ($\chi^2=5.000$, $df=1$, $p=0.025$) and de-trended solar irradiance ($\chi^2=4.700$, $df=1$, $p=0.031$). Density of schooling fish also differed between the years of observations ($\chi^2=55.800$, $df=2$, $p<0.0001$; S5, S9).

Effect of environmental factors on species separately

The effect of environmental factors was tested on the three most abundant species: freshwater bream, roach and European perch (S10). Density of freshwater bream were positively correlated with water temperature ($\chi^2=11.420$, $df=1$, $p<0.001$) and negatively with de-trended solar

irradiance levels ($\chi^2=4.940$, $df=1$, $p=0.026$). Roach showed to have positive correlation with water temperature ($\chi^2=7.260$, $df=1$, $p=0.007$) and their densities differed between years ($\chi^2=19.610$, $df=1$, $p<0.0001$), particularly year 2012 significantly varied from 2005 in roach density (see S10). Counts of European perch showed to be negatively correlated with de-trended solar irradiance ($\chi^2=7.020$, $df=1$, $p=0.008$), but positive correlation has been found in amount of precipitation ($\chi^2=10.030$, $df=1$, $p=0.002$), moreover, the density of European perch significantly differed between years ($\chi^2=17.030$, $df=1$, $p<0.001$). Model also showed a correlation with marginal significance between European perch density and wind strength ($\chi^2=3.570$, $df=1$, $p=0.059$) and correlation between European perch density and second-degree polynomial trend of time (hour; $\chi^2=5.810$, $df=1$, $p=0.055$), showing an increase starting during early hours and decreased in the afternoon (see S10). This corresponds to the peak in density during the noon and low density during twilight periods.

Biotic factors: predator-prey interactions

A significant increasing relationship have been found between the density of predatory fish (ind. per day) and density of single non-predatory fish (ind. per day) ($F_{1,22}=6.009$, $p=0.023$; Fig. 2A) as well as counts of clusters (counts of all observed “units” per day) ($F_{1,22}=6.990$, $p=0.015$; Fig 2B). There was no significant relationship found between the density of predatory fish and density of non-predatory fish (ind. per day) ($F_{1,22}=1.785$, $p=0.195$), density of schooling fish (ind. per day) ($F_{1,22}=0.322$, $p=0.576$), counts of schools (n per day) ($F_{1,22}=0.373$, $p=0.548$), average size of schools (ind. per day) ($F_{1,22}=0.272$, $p=0.607$) and average size of clusters (observed “units” per day) ($F_{1,22}=0.017$, $p=0.899$). Detailed results are shown in Tab 1.

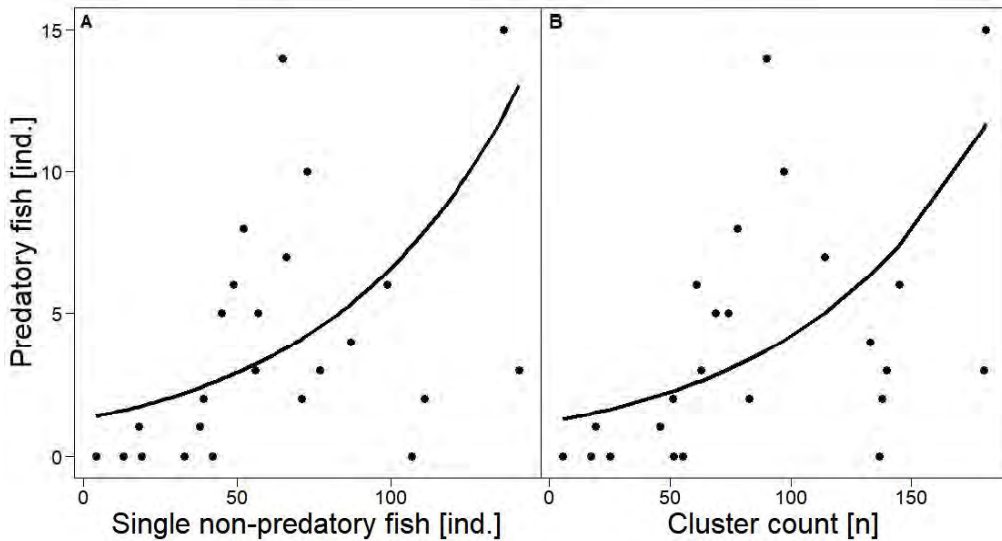


Fig 2. Relationship between counts of predatory fish and (A) counts of single non-predatory fish and (B) counts of clusters (fish units of all sizes: singletons, pairs and schools). For visualization of negative binomial model results the predicted y values were back exponentiated in both cases.

Tab 1. Biotic factors: Regression results (estimates with standard errors in parentheses) showing relationship between the counts of predatory fish and of non-predatory fish grouping attributes. Every row presents separate statistical model.

	<i>Density of predatory fish</i>			
	Estimate	Constant	theta	Pseudo R ²
<i>Density of non-predatory fish</i>	0.003 (0.002)	0.907** (0.385)	1.034** (0.430)	0.07
<i>Density of single non-predatory fish</i>	0.016*** (0.006)	0.254 (0.448)	1.262** (0.549)	0.195
<i>Density of fish in schools</i>	0.002 (0.004)	1.282*** (0.304)	0.946** (0.382)	0.125
<i>Counts of schools</i>	0.016 (0.025)	1.242*** (0.328)	0.948** (0.383)	0.014
<i>Size of schools</i>	0.065 (0.127)	1.279*** (0.322)	0.942** (0.380)	0.01
<i>Counts of clusters</i>	0.013*** (0.004)	0.171 (0.449)	1.345** (0.597)	0.226
<i>Size of clusters</i>	-0.066 (0.425)	1.474*** (0.547)	0.928** (0.373)	0.001

Note:

*p<0.1; **p<0.05; ***p<0.01

Discussion

Certain abiotic factors proved to be correlated with the densities of fish in the epipelagic habitat of our study site. Particularly important long-termed environmental factor that affects the organisms as well as other abiotic attributes is temperature. In our study, water temperature was confirmed to be positively correlated with the density of non-predatory fish, predatory fish, single non-predatory fish, schooling fish counts and two of three most frequent species: freshwater bream and roach present in the epipelagic of the Římov Reservoir. This is in accordance with generally accepted fact that fish as ectotherms are influenced by external temperature. Presence of fish in the epipelagic habitat is coupled with the raising temperature of upper layers and settling thermal stratification. Increase in water temperature boosts fish activity (Society *et al.*, 1986; Weetman *et al.*, 1998), swimming performance (Society *et al.*, 1986) or distribution sometimes even after minor changes (Biro *et al.*, 2010). In the end of the spring, the raising temperature also enhances the activity and swimming performance, therefore increase encounter rate with conspecifics as well as predators that can result in higher probability of group formation (Holubová *et al.*, 2020). Such a thing was reported in a laboratory study on *Poecilia reticulata* by Weetman *et al.* (1998), who also suggested that higher temperature could represent a cue of higher predator activity and thus higher need for antipredator protection.

Another important attribute altering distribution is light. While its intensity undergoes a daily pattern, it is being disrupted by variations in cloud coverage. Both direct levels and de-trended solar irradiance were tested, but only de-trended solar irradiance had significant negative effect on non-predatory fish and two of three most frequent species (freshwater bream and European perch), in single non-predatory fish and schooling fish the negative effect was

in marginal significance. Our results imply preference for under-average solar irradiance levels by fish in the epipelagic layer. This is consistent with previous studies showing that mostly prey fish species evade periods and habitats with high illumination but rather seek protection of shaded objects or deeper layers (Jones, 1956; Helfman, 1979, 1981a; Järvalt *et al.*, 2005; Sajdlová *et al.*, 2018), but this has been noted to be rather connected to light avoidance than predator avoidance (Kerry & Bellwood, 2015). At the same time, predator efficiency increases with light intensity (Macy *et al.*, 1998) as well as with higher water transparency (Figueiredo *et al.*, 2016). Particular light levels probably induce corresponding behaviour, which apparently results in higher fish density during the under-average irradiance periods or possible shift in utilised layer of water column. Although there has not been found a significant relationship between de-trended radiation and predator counts, there was a significant negative correlation between sunshine duration (counts of hours with direct sunlight) and predator density. Lower light levels are more convenient for piscivorous predators due to moment of surprise and the activity of predators seemed to accordingly follow the changes in the ambient conditions, giving them more time for prey search during convenient illumination.

Density of single non-predatory fish and density of European perch was positively correlated with precipitation. Also, wind strength showed negative correlation with density of schooling non-predatory fish and density of European perch. Precipitation and wind strength occur very often at the same time, but their effects are less often studied separately. Assemblages of reef fish undergo changes when fluctuations in environmental conditions connected to stormy weather occur (Munks *et al.*, 2015) and fish have often been reported to disappear from the habitat during severe weather conditions (Johansen *et al.*, 2007; Munks *et al.*, 2015). Rainy weather can impair the ability to maintain schools and result in their splitting up therefore higher

occurrence of single fish in the epipelagic when increased precipitation. Other previous studies mentioned that heavy waves during windy days was connected to decline in the feeding rate of juvenile *Tilapia mossambica* (Bowen & Allanson, 1982) and caused utilization of deeper layers by fish due to different refraction of light from waves that makes them more visible and apparent for predators (Helfman, 1981a). Conditions during such weather periods are probable to be energetically costly or ineffective for foraging therefore the schools and some species can choose to seek refuge to spare energetic expenses (Johansen *et al.*, 2007).

Significant seasonal differences in the density of schooling fish and density of roach and European perch are probably results from some inter-annual variability. The effect of daytime (with second degree polynomial trend) was found in European perch. Although, the significance was only marginal, the fact that the trend does not copy the reported higher activity during twilight period might suggest the difference between European perch and cyprinid species. Altogether it seems that cyprinid species are less affected by changes in environmental components than European perch. This might spring from different foraging strategy, freshwater bream and roach monitored in our study utilised “gulping”, a form of filter feeding, whereas European perch was particulate feeder (all was apparent from video recordings).

Interactions between organisms are major factors structuring compositions and whereabouts of organisms of all trophic levels, especially predator-prey interactions. Predatory fish counts showed increasing tendency with single non-predatory fish and counts of clusters suggesting the possibility that predatory species are reacting to behavioural shifts of non-predatory fish, particularly single fish. It seems probable that the presence of prey attracts the predators, vast marine fish school are attractive for various predatory species, but similarly zooplankton patches could attract the zooplankters (Maury, 2017). There is a possibility that

predatory fish react to higher occurrence of single non-predatory fish as suggested by our study, or to the occurrence of younger fish in the epipelagic. During our monitoring the young-of-the-year fish were observed during the daytime apparently reacting to lower light levels of cloudy days (Holubová, unpublished data). Such a behaviour was reported by Sajdlová *et al.* (2018) in juvenile fish individuals which adjusted their circadian vertical migrations adequately to artificial light intensities. Emergence of predators therefore, might depend on the prey occurrence timing or when the schools are dispersed or not formed yet. These observations together show that circadian behaviour is a result of behavioural plasticity in reaction to environmental changes rather than inner clock.

Certain behaviour as schooling and habitat preference do not strictly follow a circadian pattern but adapt to the immediate surrounding conditions state. Grouping was found to be an effective tool against olfactory predators (Johannesen *et al.*, 2014). Species that formed schools were the most abundant ones occurring in the epipelagic habitat of the Římov Reservoir (Říha, 2012) comprising mostly cyprinids (freshwater bream, roach and bleak) and European perch. Mentioned species are visually oriented zooplanktivores (Lazzaro, 1987; Čech & Kubečka, 2002; Vašek & Kubečka, 2004) that utilize the epipelagic of our study site as a foraging habitat providing abundant zooplankton prey (Vašek & Kubečka, 2004). During unfavorable conditions, food search as well as maintenance of the school cohesion may exceed the costs over the benefits, especially if energetic advantages of schooling are only in trailing positions whereas in leading positions the energetic output is higher than in singletons (Johansen *et al.*, 2010). Our study confirmed decline in schooling individuals during increase in wind strength, this probably goes hand in hand with the higher density of singletons during increasing precipitation. This suggest that energetic disadvantage is linked with low density of fish schools

in the epipelagic during inconvenient environmental conditions, but we doubt that the energetic advantage of schooling would be the impulse for fish school formation in the Římov Reservoir because of small school sizes and looser school cohesion that was apparent from the recordings. It is more probable that spatial dilution decreasing encounter probability with predators is the aspect, which plays a key role here, especially in small species as bleak. Although the sighting distance by predator increases with number of individuals in the group, freshwater-systems piscivores manage to handle no more than few, usually only one, prey fish at once (Ruxton & Johnsen, 2016). Therefore the size of schools that was on average about 5-6 individuals per school (Holubová *et al.*, 2020) could be under detection threshold as proposed by Maury (2017). In larger species, faster food location could present the drive enhancing school formation, nevertheless, we believe that despite the low proportion of possible predators able to jeopardise adults of species like freshwater bream or roach, antipredator function is still enhancing the school formation. Especially, if we take into consideration “ghost of the predation past” theory (meaning that antipredator responses employed in juvenile age endures to adulthood even with absence of continuous predator threat, Gliwicz & Jachner, 1992) and the fact that patchiness of zooplankton have not been reported in the study site (Sed’a pers. com.), although the food search efficiency of schooling during lower solar irradiance periods would seem to be a good explanation as well. Our study did not prove direct effect of irradiance levels on aggregating and our expectation that the fish schools might be more frequent or bigger during periods of high irradiance due to higher conspicuousness was proved to be false. However, Paciorek & McRobert (2012) noted that shoaling behaviour undergoes a pattern in intensity according to light regime. Ryer & Olla (1998) claimed that it is the higher activity initiated after dawn that simply triggers the shoal or school formation, but during very low light

intensities, the schooling behaviour is impaired due to failure in maintaining the visual contact between school members (Whitney, 1969). Nevertheless, this seems to fit to our previous study that suggested the need for presence of particular amount of fish in the habitat in order to form fish schools in the first place (Holubová *et al.*, 2019). Experimental study of McCart *et al.* (1997) also showed that bluegills (*Lepomis macrochirus*) formed schools more intensely when the light levels were lower, whereas during the higher light intensities they preferred to hide in the shadows which seems to be in accordance with our observations as well. It is possible that lower light intensities make predators advantageous therefore the fish might choose to stick in the schools. Still, predator efficiency will vary correspondingly to the light regime and as well as schooling preferences, both would most probably differ throughout the season, however the conditions during “clear water” phase might be similar to oligotrophic systems and high visibility during the “clear water” phase means better fishing conditions for visually-oriented piscivorous fish (Craig & Babaluk, 1989).

Conclusions

Our results provide further evidence that certain ambient factors affect the presence and abundance of adult fish in the epipelagic habitat during daytime. There is still question whether some behavioural patterns are induced by present predator, fear of predator, shift in resource distribution or simple avoidance of inconvenient change in abiotic factors. Cyprinid species dominating in European temperate water bodies broadly utilise schooling behaviour. This arises the question whether schooling behaviour might be the reason behind selective advantage responsible for cyprinid dominance in later succession phases of newly-formed water bodies as reservoirs. This highlights the need for more ecologically complex studies including behavioural attributes of specific organisms since they are important for correct understanding of predator–

prey interactions that drives the structure of aquatic ecosystems. Moreover, knowledge on species-specific distribution patterns and between species links is crucial for sampling gear selection in attempts to establish the true picture of fish stocks. The results and the literature imply further need for long-term investigations focused on issues concerning behavioural attributes with respect to changing environmental conditions since this work highlights the complexity of bonds in freshwater habitats and points out that the systems interactions are intricate and shows still a lot of uncertainties.

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Ethics

Visual observation using underwater camera was used for gathering the data, the fish were not subjected to any experimental treatment or influenced in any way, hence the ethical information requirements are NOT APPLICABLE in this study.

Author contributions

H.M. wrote the article, processed and analysed the data and participated in data collection. HJ provided environmental data. P.J. invented the design, supervised and participated in data collection together with B.P., Č.M. and V.M. All contributing authors provided valuable commentaries to the manuscript.

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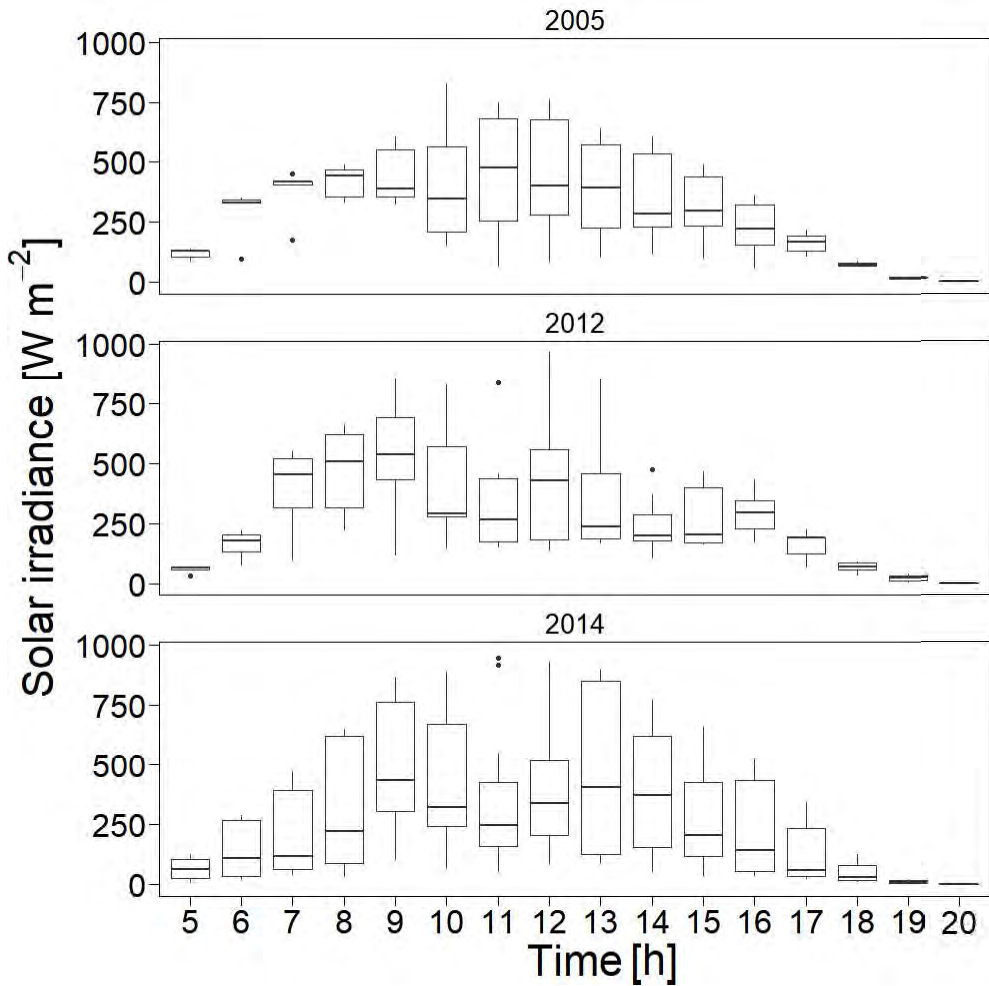
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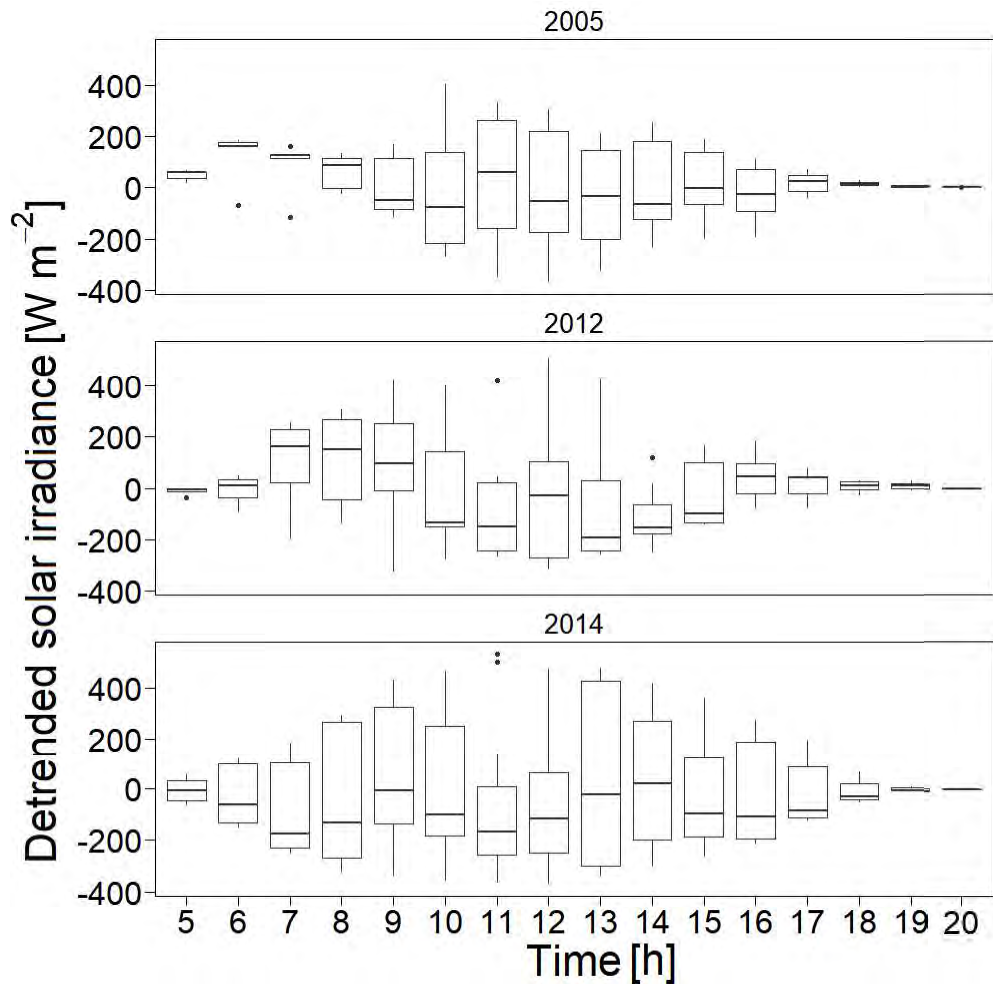
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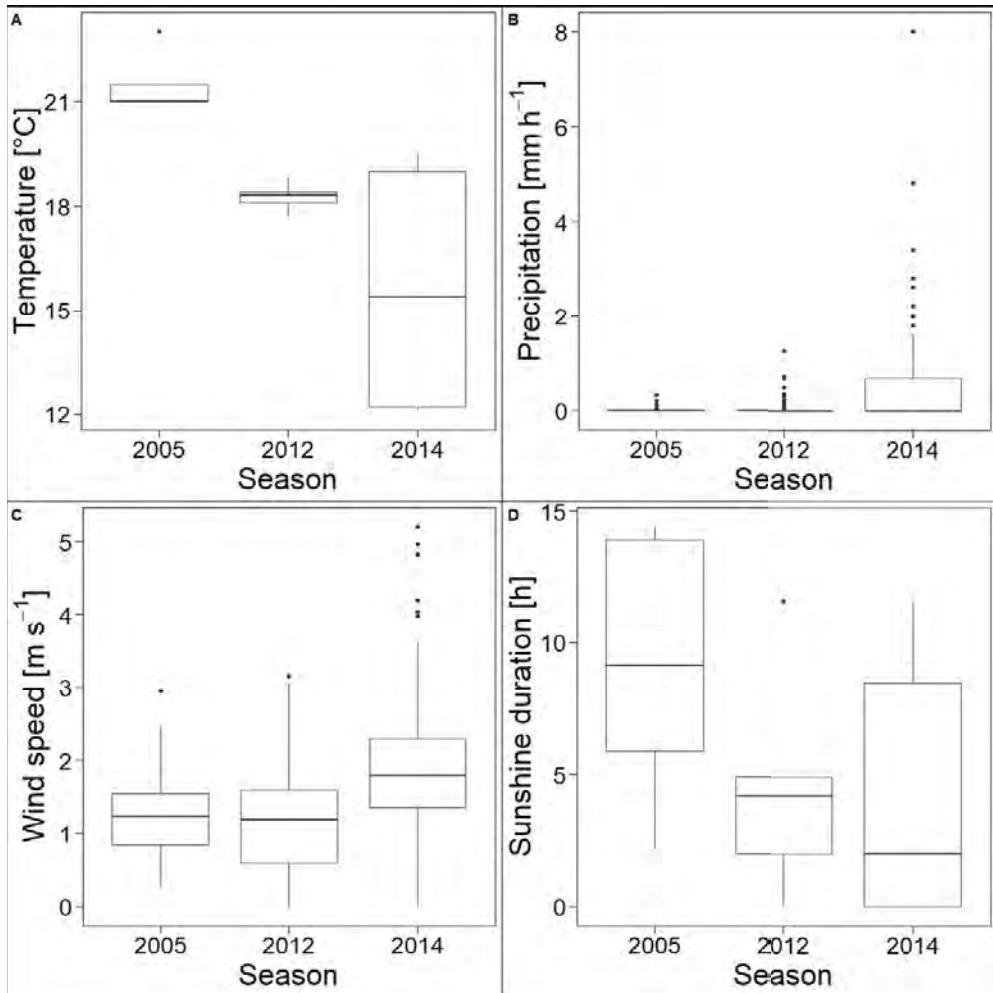
Supplementary



S1. Illustration of variability of solar irradiance among monitored seasons. Median values (thick lines), upper and lower quartiles (boxes), minimum and maximum values (whiskers adjusted for skewed distribution) and outliers (dots) are shown.



S.2. Illustration of variability of de-trended solar irradiance among monitored seasons. Zero value represents mean from observed seasons. Median values (thick lines), upper and lower quartiles (boxes), minimum and maximum values (whiskers adjusted for skewed distribution) and outliers (dots) are shown.



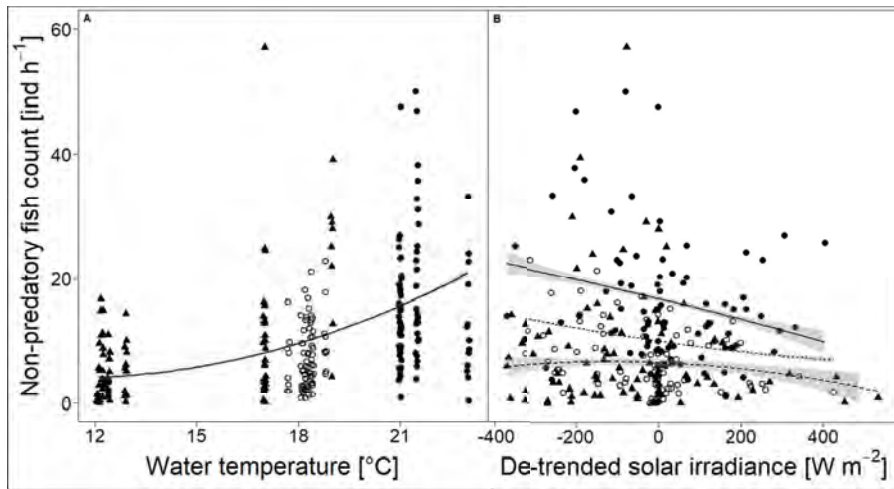
S. 3. Illustration of variability of environmental aspects among monitored seasons: (A) Temperature, (B) Precipitation, (C) Wind speed, (D) Sunshine duration. Median values (thick lines), upper and lower quartiles (boxes), minimum and maximum values (whiskers adjusted for skewed distribution) and outliers (dots) are shown.

S4. Correlation matrix for selected environmental variables. Above diagonal grey area, Pearson's correlation coefficient; below diagonal text in italics, statistical significance. Bold text indicates significant correlations at $P < 0.05$.

	Time	Sunshine duration	Precipitation	Wind strength	Temperature	De-trended irradiance
Time		-0.01	-0.01	-0.04	0	-0.03
Sunshine duration	<i>0.7954</i>		-0.39	0.04	0.16	0.61
Precipitation	<i>0.9084</i>	<i>0</i>		0.07	-0.19	-0.37
Wind strength	<i>0.4092</i>	<i>0.4811</i>	<i>0.188</i>		-0.26	0.14
Temperature	<i>0.9934</i>	<i>0.0019</i>	<i>0.0001</i>	<i>0</i>		-0.06
De-trended solar irradiance	<i>0.5398</i>	<i>0</i>	<i>0</i>	<i>0.0052</i>	<i>0.2288</i>	

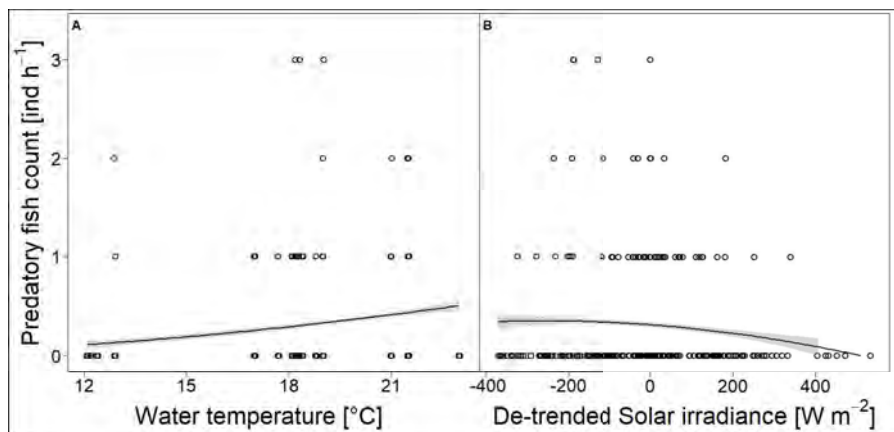
S5. Results from the GEE analysis modelling as a function of environmental variables.

	Non-predatory fish			Predatory fish			Single non-predatory fish			Schooling fish		
	Estimate	Std.err	p	Estimate	Std.err	p	Estimate	Std.err	p	Estimate	Std.err	p
<i>Intercept</i>	-0.585	0.720	0.417	-4.506	1.460	0.002	-1.300	0.460	0.005	-2.314	1.711	0.176
Temperature	0.158	0.038	<0.0001	0.208	0.083	0.012	0.154	0.024	<0.0001	0.224	0.082	0.006
De-trended solar irradiance	-0.001	0.000	<0.001				-0.0005	0.0003	0.074	-0.001	0.001	0.055
Sunshine duration				-0.120	0.026	<0.00001						
Precipitation							0.1961	0.081	0.015			
Season 2012										-1.331	0.400	0.001
Season 2014										0.844	0.683	0.216
Wind strength										-0.381	0.126	0.003
<i>Estimated Correlation Parameters</i>	0.423	0.110		0.114	0.062		0.354	0.076		0.196	0.109	

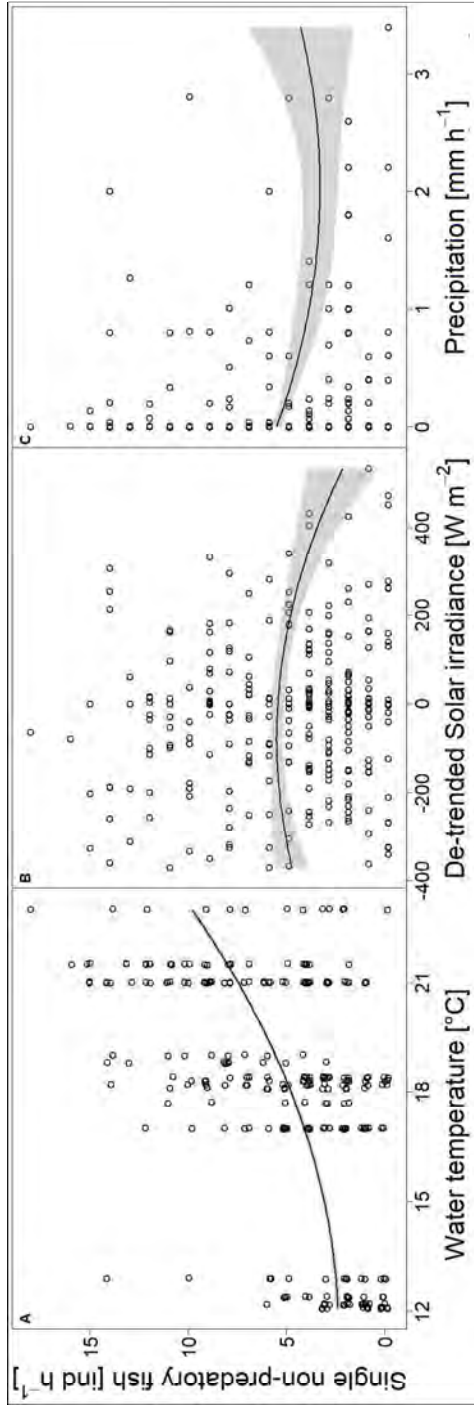


S6. Model predictions. (A) The effect of Water temperature and on the count of non-predatory fish. Thick line = model estimate; full circle = year 2005, empty circle = year 2015 and triangle = 2014; grey area = confidence interval. Y values of model result were back exponentiated in both cases and three outliers were omitted from plotting area for better visual presentation.

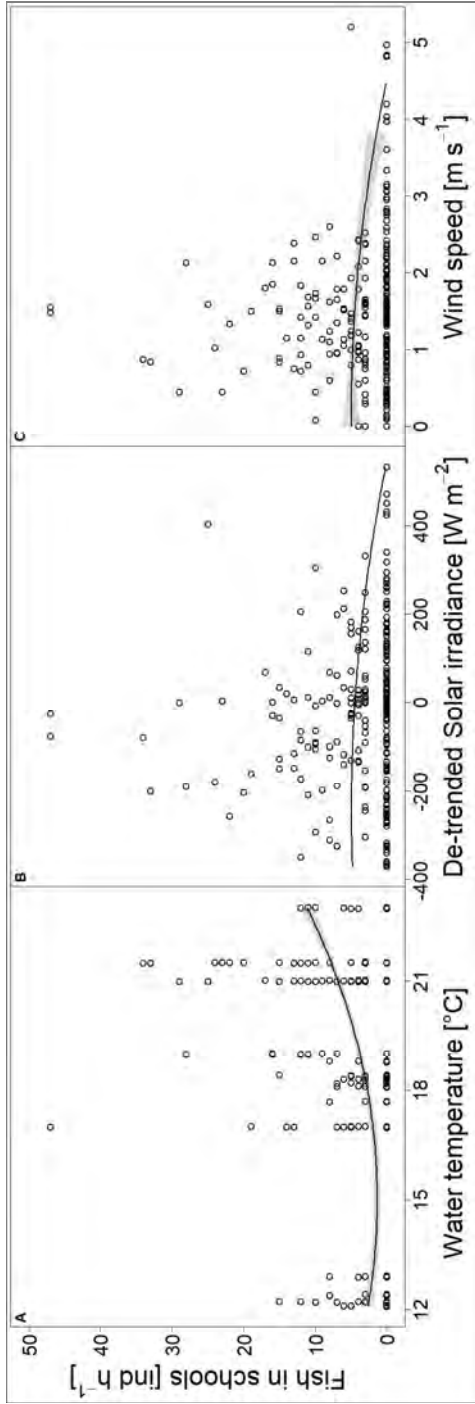
(B) The effect of De-trended solar irradiance on the count of non-predatory fish. Thick line = model estimate for year 2005, dotted line = model estimate for year 2012 and dashed line = model estimate for year 2014; full circle = year 2005, empty circle = year 2015 and triangle = 2014; grey area = confidence interval. Y values of model result were back exponentiated in both cases and three outliers were omitted from plotting area for better visual presentation.



S7. Model predictions. The effect of (A) Water temperature and (B) De-trended solar irradiance on the count of predatory fish. Thick line = model estimate, grey area = confidence interval. Y values of model result were back exponentiated in both cases and three outliers were omitted from plotting area for better visual presentation.



S8. Model predictions. The effect of (A) Water temperature, (B) De-trended solar irradiance and (C) Precipitation on the count of single non-predatory fish. Thick line = model estimate, grey area = confidence interval. Y values of model result were back exponentiated in both cases.



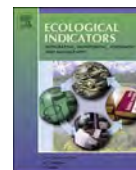
S9. Model predictions. The effect of (A) Water temperature, (B) De-trended solar irradiance and (C) Wind strength on the count of single non-predatory fish. Thick line = model estimate, grey area = confidence interval. Y values of model result were back exponentiated in both cases.

S10. Results from the GEE analysis modelling as a function of environmental variables for separate species.

	Freshwater bream			Roach			European perch		
	Estimate	Std. err	p	Estimate	Std. err	p	Estimate	Std. err	p
<i>Intercept</i>	-1.098	0.815	0.178	-4.540	1.758	0.010	1.797	0.229	<0.0001
Temperature	0.150	0.044	0.001	0.268	0.081	0.001			
De-trended solar irradiance	-0.001	0.000	0.034				-0.001	0.000	0.016
Precipitation							0.414	0.188	0.028
Season 2012				-1.209	0.589	0.040	-0.580	0.182	0.001
Season 2014				1.028	0.665	0.122	-2.094	0.281	<0.0001
Wind strength							-0.225	0.100	0.025
poly(Hour, 2)							1.811	1.291	0.161
poly(Hour, 2)							-3.455	1.142	0.003
<i>Estimated correlation parameters</i>	0.238	0.074		0.080	0.104		0.239	0.106	

Paper IV

On the use of a visual census in surveying fish communities in lentic water bodies



On the use of a visual census in surveying fish communities in lentic water bodies



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ABSTRACT

Fishing survey gear often causes damage to the ecosystem and can be size or species selective. Use of an underwater video camera (UVC) omits these flaws, and can bring accurate information on species composition and density of daytime assemblages, moreover it can discover unknown details about behavioural patterns. This study presents a model situation and proposes the use of the UVC as a valid sampling method even in the systems with higher trophies. No significant difference in species composition was found between traditionally used survey gear – purse seining and camera; nor was there a difference in the standardised fish abundance between hydroacoustics and camera when surveying an open water habitat of a temperate freshwater reservoir.

Invasive methods are generally used during monitoring of fish communities. Usually several more methods are combined to complement each other, since various approaches have been reported to have specific selectivity with respect to size and species of fish (Prchalová et al., 2009; Říha et al., 2012; Vejřík et al., 2016). Traditional sampling methods mainly include nets that can damage the surrounding environment and are particularly harmful to fish (Baker et al., 2016). Increase in damaging impact of fishing activities (Cooke and Cowx, 2004) and the need for protection of the bottom structures (Turner et al., 1999) enhance the importance of the use of non-invasive methods. Hydroacoustics is frequently used in research surveys, but species identification using this gear is rather challenging (Maclelland and Holliday, 1996), particularly when fish in freshwater do not always form conspecific schools (Krause et al., 2000).

Use of underwater video census by means of direct observation by divers or remotely operated vehicles equipped with cameras has already been promoted for application in coral reef ecosystems (e.g. Boom et al., 2014; Wilson et al., 2018). UVC is applicable in both littoral and pelagic habitats, whereas net gear (such as beach seine, purse seine, trawl, gillnets: benthic, pelagic, bathypelagic) is applicable in specific water habitats. Some visual surveys such as baited video recording or diver observation have been criticized for producing biased results focused on predatory species (Watson et al., 2005) and underestimation due to the disruption of fish by divers, or inaccurate sizing of fish due to the distortion of the water-air interface, or due to the skills of

the diver (Harvey and Fletcher, 2001). Stationary video camera is not biased by evasive behaviour and escaping fishing nets, it is less demanding on expenses and manpower, allows species identification and does not bring any harm to the aquatic ecosystems. Although the camera fails to detect cryptically living species (Baker et al., 2016), it allows long-term monitoring of ecosystems and observation on variations in the species composition (Boom et al., 2014; Wilson et al., 2015). However, in a species-rich environment, there is a higher chance of species misidentification (Baker et al., 2016), in a species-poorer environment such as freshwater systems in a temperate climate, the probability of misidentification decreases. Particular light levels are required for obtaining quality results, this is accomplished by choosing an appropriate time of day and specific water transparency levels (low turbidity) (Baker et al., 2016). Even though, the use of a camera in the dark is not effective unless provided with artificial light, the use during the day is not biased and poses a great complement to traditionally used gear that possess various flaws during daytime surveying (for comparison see Table 1.). Another limitation is the time consuming data analysis (Willis et al., 2000), on the other hand, recorded data are available for another processing (Boom et al., 2014) and do not suffer from subjectivity as more processors can double check the output.

This study aimed to compare a) the accuracy of UVC use in three seasonal periods, b) differences between fish densities obtained by hydroacoustics and UVC and c) difference in species composition between UVC and purse seine.

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Table 1
A comparison of limitations and negative effects among traditionally used gear types in fish sampling surveys.

Sampling gear	Method use limitations			Method destructiveness		Limitations for resulting outputs		
	Habitat	Saturation	Day time use	to fish	to environment	Fish evasiveness	Species selectivity ⁷	Species recognition
Mobile visual census ¹	all	no effect	day	none	none	little effect	little effect	usually possible
Stationary visual census ¹	all	no effect	day	none	none	no effect	no effect	usually possible
Mobile hydroacoustics	pelagic ²	no effect	day-night	none	none	little to medium effect ⁵	little effect	usually not possible ⁸
Stationary hydroacoustics	pelagic ²	no effect	day-night	none	none	no effect ⁶	no effect	usually not possible ⁸
Electrofishing	littoral	no effect	day-night	low to high	none	significant effect	significant effect	possible
Benthic gillnetting	benthic	significant	dusk-dawn ³	high	usually low	significant effect	significant effect	possible
Pelagic gillnetting	pelagic	significant	dusk-dawn ³	high	low	significant effect	significant effect	possible
Beach seining	littoral	less important	day-night	low to medium ⁴	high	significant effect	no effect	possible
Purse seining	pelagic	less important	day-night	low to medium ⁴	none	significant effect	no effect	possible
Benthic trawling	benthic	less important	day-night	medium to high ⁴	high	significant effect	significant effect	possible
Pelagic trawling	pelagic	less important	day-night	medium to high ⁴	none	significant effect	significant effect	possible

¹ Highly affected by water transparency.

² In special cases littoral/sub-littoral or benthic observations also possible.

³ Twilight periods of crucial importance.

⁴ Strongly dependent on fish species, amount of catch, tow duration, material of the net etc.

⁵ Boat/vessel construction and engine type play the role.

⁶ In some cases fish species and used acoustic frequency dependent.

⁷ Fish behavior dependent all the time.

⁸ Possible with limitations when e.g. high frequency and multi-frequency sonars used.

The study site Římov Reservoir (48°51'N, 14°29' E; South Bohemia, Czech Republic), is a temperate freshwater reservoir of meso- to eutrophic character (Hejzlar and Vyhánek, 1998) and is canyon-shape. The study was carried out during June, August and October 2005. Both hydroacoustic and camera observation were recorded between 5.00 and 21.00 (16 h per day) during June 1.-5., August 9.-12. and October 12.-14. (camera only October 12.) 2005, whereas purse seining hauls were carried out only in August 2005 (8.-9.) between 5.00 and 21.00.

For video observation, an underwater camera (SplashCam Delta Vision HD B/W; Everett, Washington, USA) was attached to a metal rod and installed 5 m under the water surface. This set-up was fixed to a buoy and moored by two anchors. The camera was inclined 45° towards the water surface to ensure the easiest species recognition (dark silhouettes against bright water surface) and the highest possible volume of water in the recordings as noted by Peterka et al. (2006b), for scheme see Holubová et al. (2019). Most of the fish ignored the presence of the camera set-up, only European perch showed curiosity towards the UVC set-up. Several times, obviously the same fish appeared in front of the camera, such records were omitted from the dataset.

Hydroacoustic data were acquired by means of a bottom-based (38 m depth) uplooking scientific echosounder, Simrad EY 500, along with an ES 120-7G circular split-beam uplooking transducer (nominal angle 7.1°), signal frequency was 120 kHz, pulse length 0.1 ms, pulse interval 0.2 s and output power 63 W (for detailed method description see Jarolím et al., 2010). Changes in fish density were monitored throughout the whole water column and during all seasonal periods investigated, but for comparison of hydroacoustic data and camera observations only the upper 5 m of epipelagic layer were used, excluding fish individuals smaller than 100 mm (TL; target strength larger than -41.17 db). While hydroacoustics sampled approximately 78 m³ of the epipelagic layer (above 5 m depth), the camera sampled 65 m³ of water volume. Counts of individuals from hydroacoustics and camera were standardized to a volume of 100 m³ for comparison.

Purse seining was carried out by a 120 m long and 12 m high net (for more details see Říha et al., 2012). A total of ten purse seining hauls were used solely for comparison of species proportion with camera observations, therefore, all the data from entire sampled volume was used (from 12 m high net, since most of the fish concentrates in the upper 5 m and less than 10% of fish reached deeper than 5 m as apparent in the hydroacoustic data).

Vertical profiles of temperature (°C) and dissolved oxygen (mg l⁻¹) were measured with a calibrated OXI 196 probe (WTW, Germany).

Video recordings were evaluated using Avidemux video software (<http://fix.ounet.free.fr/avidemux/>). The graphical and statistical analyses were carried out with R software (<http://www.r-project.org/>).

The total fish counted from camera recordings was 1364, 2663 fish from hydroacoustic observation and 91 fish from purse seining. This data was subsequently analysed. It is well known that lentic water systems undergo a stratification cycle throughout the season. Thus, water temperature and oxygen profiles differed between seasonal periods (compared in 1A). Recorded acoustical abundances differed between months of observation (Kruskal-Wallis ANOVA: $\chi_2^2 = 6.2654$, $p < 0.05$; Fig. 1C). Accordingly, recorded fish in camera data differed as well (Kruskal-Wallis ANOVA: $\chi_2^2 = 7.4085$, $p < 0.05$; Fig. 1C). Although the absence of fish from the camera data during the August observation resulted from low transparency conditions, the seasonal development is responsible for the difference between June and October. Ongoing stratification during spring induced the fish to begin to concentrate in the upper layers and throughout the summer period fish utilized mainly the upper 5 m of the epipelagic layer, as apparent from the hydroacoustic records (in June 88% of fish gathered in the epipelagic, average fish depth was 3.2 m, in August 90% of fish occurred in the epipelagic, average fish depth was 2.7 m; Fig. 1B). Whereas during the autumn mixing period, fish were dispersed in the water column and the lower proportion remained in the upper 5 m layer (in October only 32% of all fish were in the upper layer and the average fish depth was 8.8 m) as shown in Fig. 1B. This is why the sampling timing must be properly considered in order to obtain unbiased results.

In meso- and eutrophic temperate systems, like the Římov Reservoir, the visual conditions vary throughout the season. During the summer period, transparency typically decreases to as low as 2 m (Secchi depth) due to algal bloom whereas highest transparency is reached during the "clearwater" phase (5 m). The autumn period is unsuitable for active net methods or video recording due to the dispersion of fish in the water column. In the summer period, the active methods are suitable but in systems with higher trophy, the low transparency of the water makes it impossible to use the camera, moreover species determination from recordings was insufficient (the species of 47% of observed fish were unable to be determined). For the use of visual observation methods late spring is the best period since the clear water makes the visual conditions suitable for observation (Peterka et al., 2006b) and the ongoing stratification triggers the assembly of fish in the epipelagic layer. The counts of fish observed during June did not significantly differ between the camera and

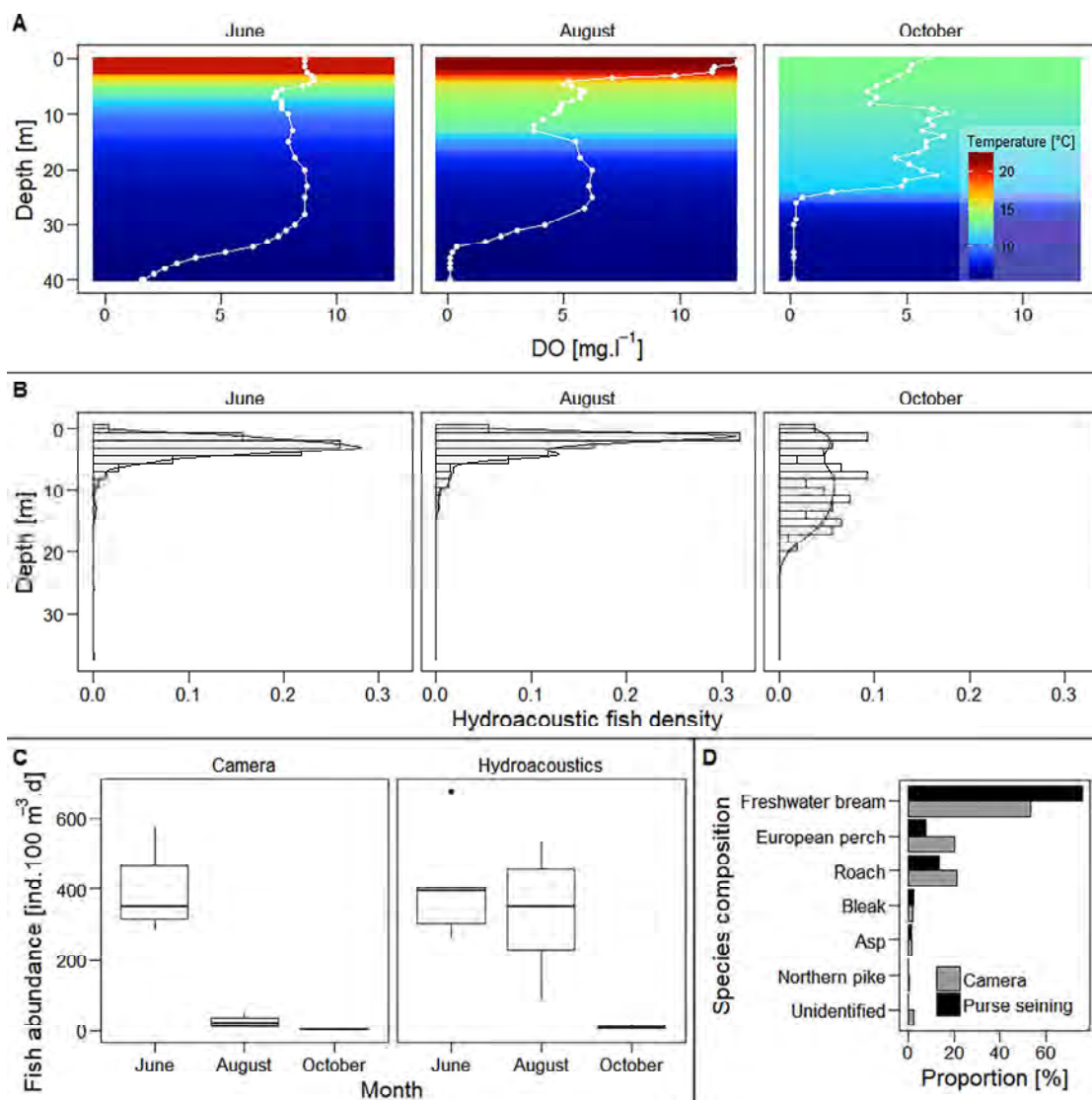


Fig. 1. A) Vertical profile of water temperature (background colours) and dissolved oxygen (DO; white dots) showed for June, August and October 2005. B) Density of fish from hydroacoustic data in various depth layers showed for June, August and October 2005. C) Boxplots showing fish abundance comparison of hydroacoustic and camera observation with respect to month of observation. Median values (thick lines), upper and lower quartiles (boxes), minimum and maximum values (whiskers adjusted for skewed distribution) and outliers (dots) are shown. D) Species composition comparison between camera observation in June and purse seining catch in August.

hydroacoustics (Kruskal Wallis ANOVA: $\chi_1^2 = 0.011$, $p = 0.917$, Fig. 1C).

The most abundant species was freshwater bream (*Abramis brama*), followed by roach (*Rutilus rutilus*), European perch (*Perca fluviatilis*) and bleak (*Alburnus alburnus*) respectively. Less abundant was asp (*Leuciscus aspius*), and Northern pike (*Esox lucius*). Species composition of fish stock that was captured by camera and purse seining did not differ (paired Mann Whitney-U: $V = 18$, $p = 0.578$), but recorded fish assemblage contained one extra species (Northern pike) than the purse seine catch (camera – six species, whereas the purse seine catch five species, Fig. 1D).

By comparison with the frequently used sampling gears, hydroacoustics and purse seining, the results in this study demonstrate that

during good visual conditions the use of an underwater video camera can provide accurate information on fish stock composition and density in lentic environments and should be encouraged due to minimal impact on the ecosystem and introduces no bias from net evading individuals (for comparison of the survey gears pros and cons see the Table 1). Although Baker et al. (2016) concluded lower species richness in using a camera compared to beach seining in marine coastal habitats, during this survey in the pelagic habitat, the camera captured more species than purse seine, moreover a different study using visual census recorded up to 9 species (Holubová et al., 2019).

The use of visual methods is more applicable during daytime, this is when the fish can visually evade the passive methods such as gillnets, and can even escape from the active gear - netting. Proper timing of the

survey is also a very important factor. Fish species composition sampling is most appropriate to perform during the period when most of the individuals concentrate in the upper layer, which is the summer season in temperate reservoirs (Blabolil et al., 2017; Čech and Kubečka, 2002; Jarolím et al., 2010). Although the hydroacoustic surveys can record the whole column, the lack of species identification calls for the need of additional survey methods.

As far as we are aware, this is the first attempt to engage the UVC as a survey method in reservoir with higher trophy. Direct videorecording proved to provide equally good estimates as in oligotrophic environments (Wilson et al., 2015). Several factors can play a role in fish detectability using a camera with transparency being the most important, therefore, timing must be properly considered. The results also demonstrate that use of an underwater camera can present an effective tool for sampling daytime fish community composition in lakes and reservoirs. Moreover the use of a camera is ideal to shed some light on yet unknown species-specific behaviour (e.g. gulping and sinusoidal swimming pattern performed by freshwater bream; Čech and Kubečka, 2002; Peterka et al., 2006a), or aggregating tendencies (Holubová et al., 2019; Krause et al., 2000). For use of this method in the field, it is highly advantageous to complement the optical gear by hydroacoustic survey, which would ensure the quantitative measurements for comparison of various parts of the season or between different water bodies. UVC method is particularly convenient in the monitoring of protected areas and rare or endangered species (Ebner and Morgan, 2013). However, the characteristic of the monitored habitat must be taken into account when developing the sampling scheme depending on the fish density, species richness and size of water body. More stationary video set-ups would be preferred in species rich habitats, littoral areas, particularly with dense macrophyte coverage. For vast pelagic habitats the stationary visual method might not be an effective tool for sampling since the results from such a camera were poorer when the density of the fish was low (Wilson et al., 2015), therefore even multiple stationary UVC set-ups might not produce accurate estimates.

Present upgrades of visual methods such as automated underwater vehicles (Seiler et al., 2012), automated systems for species recognition (Huang et al., 2012) or stereo cameras (Neuswanger et al., 2016) give the use of a camera lots of possibilities for further research. Together visual methods promise to obtain such details as in-situ description of inter- and intra-species interactions, their sizes, swimming speeds, and dispersion or encounter rates. Moreover, video tracking systems (Delcourt et al., 2009) will provide shorter data handling time, which would ease the long-term monitoring. Knowledge on patterns of various species and age cohorts might be applicable during surveys and bio-manipulating processes.

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Ethical approval

All applicable international, national and institutional guidelines for the care and use of animals were followed by the authors.

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Statement of significance

This study is an addition to methodological comparisons for fish survey, highlighting the use of a non-invasive approach of underwater video census, which presents a good addition to monitoring of fish densities. Furthermore, observations by camera can shed some light on yet unknown behavioural attributes and promise many other possibilities for future research.

Declaration of Competing Interest

None.

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Cirriculum Vitae

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Research Papers not included in the dissertation

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