School of Doctoral Studies in Biological Sciences

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Faculty of Science

Ecology of top fish predators, European catfish and asp, with consequences to fish communities

Ph.D. Thesis

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Annotation:

The dissertation thesis focuses on predator ecology in artificial water bodies. **Paper I** deals with the importance of chemical cues for predator-prey interactions in an aquatic environment. Here, I demonstrate that the ability to detect chemical cues represents a survival benefit for prey species. **Paper II** points out gillnet methodological bias, which may have subsequent repercussions in field evaluation of a predator's presence and assessment of larger fish abundance in general. **Papers III** and **IV** focus on asp *Leuciscus aspius* spawning grounds. In **Paper III**, I demonstrate how males maximize their spawning chances by early arrival and in **Paper IV**, I evaluate the predation pressure of asp prey, *Alburnus alburnus*, directed on asp eggs.

Declaration [in Czech]

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List of papers and author´s contribution

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I designed and conducted the laboratory experiments and participated in field sampling, statistically analyzed the data and prepared the manuscript with the help of co-authors.

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Declaration of originality

The co-authors fully acknowledge that Marek Šmejkal is the first author of all papers presented. Most of the data processing as well as most of the statistical analyses were performed by Marek Šmejkal. He 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 Marek Šmejkal and two authors hereby support this statement with their signature.

RNDr. Jiří Peterka, Ph.D.   Prof. Jan Kubečka, Ph.D.

Contents

Introduction

How I came to study these topics

My bachelor´s and master´s studies focused on the association of fish with littoral habitats in reservoirs (Šmejkal *et al.* 2014). While I succeeded in describing patterns of fish littoral habitat association in my master's thesis, I was missing the causal consequences driving fish to distribute themselves in that pattern, for it is often difficult to discriminate among major drivers such as temperature, food availability and predation avoidance in large-scale field studies (Garner *et al.* 1998; Metcalfe *et al.* 1999; Brönmark *et al.* 2008). Hence, I decided to combine field descriptive data with an experimental setup that could explain the patterns found in our observations.

One of the major advantages of studying fish is that it is feasible to set up aquarium experiments. This allows us to manipulate the factors under study and to make stronger inferences regarding the causal chain of events (Ahnesjö et al. 2008). In the littoral habitat association study, the fish with morphological defences (perch *Perca fluviatilis*, ruffe *Gymnocephalus cernua*) were more inclined to share their habitat with their predators (pike *Esox lucius* and asp *Leuciscus aspius*) compared to fish species lacking these defences (roach *Rutilus rutilus*, bream *Abramis brama*) (Šmejkal *et al.* 2014). Species relying on their defences may afford to be closer to their natural enemies and be less alert in general (Abrahams 1995; Andraso & Barron 1995). However, how can those more vulnerable species actually avoid encounters with predators in an environment with such restricted visual range? The answer may be provided by the concept of chemical cues, which I attempted to test in my subsequent work.

The role of chemical cues has been emphasized since the discovery of the alarm cue - Schreckstoff in 1938 by Karl Ritter von Frisch (Frisch 1938) - and it is currently believed to be one of the major drivers of fish behavioural decisions (Ferrari *et al.* 2010; Stensmyr & Maderspacher 2012). Whether the species that has a lower ability to detect chemical cues also has a higher susceptibility to predation, was to my knowledge at that time an unanswered question. In **paper I**, I performed experiments with three prey species (perch, roach and rudd *Scardinius erythrophthalmus*) to observe their response to dietary cues. As a model predator, I chose the European catfish *Silurus glanis* for two reasons. Firstly, we had performed a manipulative study

where catfish were introduced into a lake and apparently changed the community structure of the tested prey species. Secondly, catfish forage in low-light and turbid conditions, hence it may be advantageous for prey species to detect predators in such environment with limited visual range (Bruton 1996; Hartman & Abrahams 2000).

Fish community data collected for my master thesis and **Paper I** came from standard gillnet sampling. The standardized series of mesh sizes covers almost all size groups of commonly catchable fish species (Prchalová *et al.* 2009b). However, larger individuals of big species are usually missing (especially in the case of catfish, which was otherwise very abundant in the lakes sampled for **Paper I**). The interpretation of biased data is not an easy task, given the fact that the estimate of the largest fish has the lowest reliability due to the scarceness of any catch. European fish community studies frequently rely on the gillnet sampling standard (CEN 2005; Argillier *et al.* 2012; Blabolil *et al.* 2016; Poikane *et al.* 2017), and often do not consider the catch bias as important when interpreting results based on the European gillnet standard (Mehner 2010). To identify the imperfection of the European gillnet standard, we analyzed data from large-mesh gillnet samples, which provided evidence of the presence of large fish missed by the European gillnet standard, and identified potential biases in the European sampling design (**Paper II**).

The third study included in my thesis focuses on asp, a large predatory fish in European waters, and targets particularly the sex differences in the occupation of the spawning ground between males and females. Variance in reproductive success within a sex may generate sexual selection. When some individuals are more successful than others in competition for mates, and this success depends on the expression of a specific trait, this trait is under sexual selection (Ahnesjö *et al.* 2008). In this particular case, I investigated a species that has no parental care, forms no permanent pairs and the spawning within a population is polygynandrous, i.e. males may acquire multiple females and females may acquire multiple males in one spawning season. Here, the characteristic studied and subjected to sexual selection is the arrival strategy, since early arriving males may have the possibility to mate with more females than late arriving males (Canal *et al.* 2012; Morbey *et al.* 2012). In the example of the asp mating system in **Paper III**, I demonstrated how males precede females in their arrival and leave the spawning ground

later than females to maximize their spawning success on both a seasonal and a daily basis.

In **Paper IV**, I evaluate the impact of bleak *Alburnus alburnus* that prey on asp eggs in the fluvial spawning ground, which was used as a model environment in a previous paper. During our field study in 2014 related to **Paper III**, we observed that the most common prey fish of asp, bleak, occurs in large numbers at the spawning ground and potentially feeds on asp eggs. This may represent a good example of predator-prey role reversal; hence I decided to investigate this topic further using gut content analysis and underwater cameras installed on the asp spawning ground and evaluate the potential impact on asp eggs.

The role of chemical cues in aquatic environments

Limited visual detection range has shaped the sensory evolution of aquatic organisms (Sorensen & Wisenden 2015). In many instances, the visual ability to detect danger or a mate is restricted by the environmental conditions (e.g. turbidity, dense macrophytes coverage, caves and crevices). Hence, fish adapted through their life history the ability to communicate by non-visual senses, and probably the most important is chemosensory communication (Ferrari *et al.* 2010). Species-wide and species-specific chemicals enable fish to communicate between individuals, and during the spawning period provide precise information about males' and females' readiness to spawn (Dulka *et al.* 1987; Sorensen *et al.* 1988; Sorensen & Wisenden 2015).

The most investigated category of chemical communication are alarm and diet cues. Various fish species possess the ability to produce and perceive chemicals that warn them about oncoming danger (Brown *et al.* 1995; Ferrari *et al.* 2010). Their skin contains alarm substances, or alarm cues, which are released into the water when a fish is injured by predator. If a shoal of fish encounters a space activated with alarm cues, behavioural changes proportional to the danger are expressed by the fish (Mathis & Smith 1993; Brown *et al.* 2004). Furthermore, when the predator digests its prey, diet cues exude from the predator's skin, gills or faeces and these are also perceived by many fish species and reacted upon (Brown *et al.* 1996; Harvey & Brown 2004). Both mechanisms of danger identification may enhance the

survival ability of individuals, for they provide an informational advantage over their natural enemy.

The predator-prey relationship is an arms-race, and in this particular one, the predator has found a counter-response to chemical cues. For instance, a pike that has ingested prey containing an alarm substance defecates outside its foraging territory, therefore minimizing labelling its major hunting spot. However, when a pike digests prey without any alarm substances, no such behavior is observed (Brown, Grant *et al.* 1995; Brown *et al.* 1996). Although pike cannot conceal diet cues altogether, this behavior seems to decrease the distance in which its prey finds its foraging territory dangerous.

Despite the enormous body of evidence about chemical cues from the laboratory, there was a general lack of field studies providing a link between chemical cue perception and survival. By combining laboratory and field data, Paper II provides indirect field evidence that the perception of chemical cues may improve the survival chances for a species.

Gillnet methodology and the importance of unbiased sampling: what are we currently missing?

Unbiased fish sampling is a difficult task for the scientific community, yet it is necessary for a sound interpretation of data in ecology. To reveal the true species composition, abundance and biomass in a lake or reservoir, it is crucial to sample all relevant habitats (Prchalová *et al.* 2009a; Blabolil *et al.* 2017). While the goal may seem relatively easy to achieve, each species has a different catchability for the given sampling gear, which makes the estimate ultimately biased (Hamley 1975). Furthermore, only gillnets can feasibly be deployed in every lacustrine habitat (with the exception of habitats with dense macrophyte cover). Other gear such as trawls, electrofishing and beach seining, which would otherwise be more suitable due to their absolute catch per unit effort, have limited usage in deep water or in structured habitats (Prchalová *et al.* 2009b; Říha *et al.* 2012, 2015).

However, the proportion of a given species in the catch is not only related to the actual abundance, but also reflects fish activity, shape and body protrusions that make the catch more likely (Hamley 1975; Kurkilahti *et al.* 2002). Hence, fish have to be active, of the right shape and size to be successfully caught in gillnets in high numbers. When these criteria are not met, inactive fish or fish with an elongated shape and good locomotion

abilities are likely to be underestimated or missing in the catch compared to spiny and active fish (Prchalová *et al.* 2010). This extreme bias is demonstrated by the European eel *Anguilla anguilla* that does not entangle in gillnets due to its smooth elongated body, together with its locomotion abilities so that another sampling method is additionally required to characterize the eel population in a given water body, or a characteristically entangled gillnet may be counted as a sign of eel presence (Prchalová *et al.* 2013). Despite the obvious flaws of this gear, gillnets are an essential part of community sampling, since they are relatively easily deployed into all lacustrine habitats and standard monitoring can be performed continental-wide in various water body types (Argillier *et al.* 2012).

Fish become entangled in gillnets by their teeth, gills or the deepest part of their body (Hamley 1975; Kurkilahti *et al.* 2002). Therefore, each mesh size has an optimum fish size and on both sizes of the axis the probability of catch decreases. Even though for some fish species this curve has a log-normal distribution, meaning that fish larger than optimum can still be caught with some likelihood, scientists should aim to cover the whole fish size spectrum by implementing appropriate mesh sizes (Hamley & Regier 1973). In order to do so, the European standard was developed with 12 mesh sizes in a geometric series with a ratio of 1.25 and a mesh size range from 5 to 55 mm (Appelberg *et al.* 1995; CEN 2005). The question remains whether the decision to terminate the geometric mesh size series at 55 mm was appropriate in European waters, given some large fish predators such as European catfish inhabit these waters (Kottelat & Freyhof 2007). We address that question in **Paper II**, where we demonstrate that the European standard underestimates very common large fish and the geometric series should be extended to achieve a better sampling design of the fish size spectrum.

The pros and cons of being a male: seasonal and daily protandry in a cyprinid fish

The sex with higher variability in reproductive success is generally under stronger selective pressure; hence this sex has to adapt to maximize their mating chances (Ahnesjö *et al.* 2008). In species where the females invest more into reproduction (sum of pre- and post-reproductive expenses), it is the males who have to maximize their effort (Jonsson *et al.* 1991; Olsson & Madsen 1996). Just by virtue of encounter probability, an earlier approach to

mating grounds before females should provide more mating chances (Morbey 2000; Morbey & Ydenberg 2001). This phenomenon known as protandry occurs in many species across the animal kingdom. Several hypotheses explain this phenomenon, I will here restrict the list to the two most likely in the studied system of asp. The mate opportunity hypothesis states that just by virtue of higher probability of female encounter, it is advantageous to arrive on the mating site earlier than females (Morbey 2002). The rank advantage hypothesis claims that an early arrival is advantageous primarily for early males who can occupy and defend high-quality territories, which in turn brings an advantage to the owner due to easy access to multiple females in lek systems (a place where males gather during the reproductive season and compete to attract females; Morbey *et al.* 2012; Apollonio *et al.* 2014). The advantage of early arrival is especially apparent in non-territorial polygynandrous fish species, where the male's reproductive success depends solely on the number and quality of fertilized eggs (Sorensen & Wisenden 2015).

While early arrival may have several positive consequences for reproductive success, it may not be altogether beneficial for the individual male. The majority of species usually reproduce in the period that precedes the richest part of the year, so that their offspring are born into the most productive season. Therefore, early arrival is oftentimes accompanied with harsh environmental conditions and the early arriving sex may, as a consequence, suffer from excessive mortality (Møller 1994; Olsson & Madsen 1996). On the other hand, an early arriving male has to be in good condition, and hence the degree of his protandry may thus represent an honest signal of his qualities.

While protandry on a seasonal basis has been observed in the past for various animal groups (Gerhardt 1991; Olsson & Madsen 1996; Alcock 1997; Morbey 2000; Kokko *et al.* 2006), daily earlier arrival of males on the breeding ground has not been observed for a vertebrate species. The only study which describes this phenomenon is that of Dawson's burrowing bees, where males are active early in the day and search for later emerging females (Alcock 1997). However, in systems where individuals migrate for a certain amount of time away from the mating site and hence the mating site is recreated each day, there is the potential for daily occurrence of protandry.

Predator-prey role reversal in asp spawning ground

Although predator-prey relationships are mostly displayed simply as a situation where a predator enhances its own fitness at the prey's expense, there are situations where this relationship may be easily reversed. For instance, many predatory vertebrate ectotherms have a small initial size that could easily become prey for invertebrates and vertebrate omnivores (Bailey & Houde 1989; Deblois & Leggett 1991). Despite the short-term availability of small sized predators and their eggs, their prey may utilize them to an enormous extent due to their numerical advantage (Levine 1981). Moreover, since eggs are an important rich food source, they may in the short-term be preferred over the ordinary food source of the prey (Fuiman *et al.* 2015).

In comparison with the classical predator-prey relationship, in this short-term reversal the prey does not rely on the small-sized predator food source for the whole growth period, and hence the population may grow on prevalent food well above the carrying capacity of the small-sized predator food source (Power 1992). Hence, eggs and small-sized predators can be subjected to considerable feeding pressure (Ellis & Nash 1997; Fox *et al.* 2012). This can lead to destabilization of predator-prey dynamics or keep the predator population at low levels (Levine 1981; Leggett & Deblois 1994; Köster & Möllmann 2000).

Aims of the thesis

The Ph.D. thesis addresses the behavioral and methodological aspects of catfish and asp ecology. The first section compares the species-specific ability to detect chemical cues and the actual survival and community development in a lake experiment before and after catfish stocking (**Paper I**). The second section evaluates the bias of gillnet sampling design for large fish species. This study should highlight the importance of extending the European standard in order to quantify correctly the relative abundance of predators such as adult asp and catfish (**Paper II**). The third section (**Paper III** and **IV**) addresses two aspects of asp reproduction occurring in early spring in fast flowing rivers. **Paper III** focuses on how males and females time their arrival on and departure from the spawning ground. **Paper IV** evaluates the mortality of asp eggs foraged upon by bleak, a small cyprinid species that is a major prey species for adult asp.

Paper I

Aims to: (i) investigate the species-specific ability of prey to detect the chemical cues of a catfish by olfaction by examining subsequent behavioral changes (activity, shoal cohesion and use of refuge) for three common prey species, perch, roach and rudd, (ii) evaluate the prey preferences of catfish when exposed to the same three prey species in an experimental laboratory setting and (iii) analyze gillnet sampling data obtained from a manipulative field study conducted in Milada Lake, Czech Republic, before and after catfish stocking.

Paper II

Aims to: (i) estimate the threshold fish size above which the European Standard gillnet is ineffective for estimating fish community biomass and abundance using selectivity curves approach, (ii) compare the fish community biomass spectrum obtained by European Standard gillnet to that obtained by trawl, purse seine and large mesh gillnet, (iii) identify species for which the large mesh gillnet sampling is essential, and (iv) analyze the sampling bias of European Standard gillnet.

Paper III

Aims to determine: (i) whether males exhibit protandry in their seasonal and daily migration, (ii) whether males also leave later (both on a daily and seasonal basis) than females, (iii) whether males time their daily length of stay to the number of females present and (iv) whether the degree of daily protandry correlates with the daily operational sex ratio.

Paper IV

Aims to: (i) analyze whether and to what extent predator-prey role reversal occurs in the asp-bleak relationship by stomach content analysis, (ii) analyze whether egg consumption is dependent on bleak size, (iii) establish the relative effectiveness of egg consumption using underwater cameras and (iv) model the relationship between egg survival probability and the batch size of drifting eggs using underwater camera data.

Results and general discussion

Implications for management of water-supply reservoirs

In **Paper I**, the experimental evaluation of the species-specific reactions to the threat of catfish predation revealed that both roach and rudd changed their behavior under the threat of predation, although rudd unexpectedly chose to be more exposed to predation. However, we detected no behavioral reaction in perch. Furthermore, we demonstrated the negative impact of catfish targeting older age classes of rudd and perch in the experimental lake, while none of this was observed in the control lake, where only pike predator treatment was applied. Further, the analysis of roach and perch survival from the experimental lake showed higher roach survival probabilities in two out of three age classes. We propose that species-specific catfish detection and avoidance abilities may explain the decline in large rudd and perch in the experimental lake after predator stocking.

In many man-made water bodies serving as drinking water storages, effective top-down control of prey fish species is crucial for keeping the water free of excessive algae blooms. In the conditions of the Czech Republic, water management should be aimed to keep the water body in salmonid or perch phase, where the fish composition does not fully utilize zooplankton pelagic food sources due to inefficient feeding compared to filter-feeding cyprinids (Říha *et al.* 2009; Peterka & Matěna 2011) and as adults, these species becomes predators and regulate the population size of many species (Persson *et al.* 2003; Kottelat & Freyhof 2007). Prevalence of zooplankton-feeders such as roach, bream and bleak usually results in more turbid waters, which further influence the predator-prey relationship and predator foraging efficiency (Abrahams & Kattenfeld 1997; Shoup & Wahl 2009). The process of eutrophication influences piscivore-prey interactions: at least in some cases prey is the one who benefits. Pike was described as suffering from an increase in turbidity: the higher the turbidity the lower the pike reaction distance to roach and its foraging efficiency was (Ranåker *et al.* 2012). Evidence on the community level also indicates that high fish biomass and abundance are causing lower visibility due to a decrease of zooplankton

size and its filter-feeding efficiency (Brooks & Dodson 1965) and these waters have a lower percentage of piscivores (Olin *et al.* 2002; Říha *et al.* 2009).

European perch – the preferred dominant of drinking water storages - is also known to be negatively affected by reduced visibility, since perch as a visually oriented predator becomes a poor competitor to cyprinids, whose more universal foraging tactics (gulping) are, under these conditions, better than particulate feeding of perch (Persson 1986; Osse *et al.* 1997).

Paper I demonstrates that stocking of catfish into a perch-dominated lake actually supported perch removal and its substitution by roach. Hence, although catfish is commonly stocked into water bodies built for drinking water purposes, it may actually selectively remove perch from the system and indirectly support cyprinid dominance. Once cyprinid dominance is established, visibility is impaired by their filter-feeding habits and may cause a decrease in perch and pike foraging efficiency (Ranåker *et al.* 2012). While these conclusions may be preliminary and more should be done on inter-species detection abilities of chemical cues and their implications in the natural environment, this study suggests that the biomanipulation technique of predator's population enhancement is not a straightforward issue and should be done with caution.

Paper IV targets a different situation in the predator-prey relationship in an aquatic environment between asp (predator) and bleak (prey). Gut content analysis demonstrated that asp eggs were utilized in high quantities by bleak, especially in the spawning peak of the asp reproductive season. Furthermore, using underwater video, I recorded the bleak feeding efficiency on naturally drifting asp eggs as the percentage of eggs eaten. Within the 40 cm egg trajectory captured by our cameras, total egg mortality was 21.2 ± 2.2 % on average. The highest survival chances occurred among eggs drifting in aggregations, since the short drifting distance together with their aggregated distribution satiated bleak and part of the egg aggregation could attach to the spawning ground.

The studied Želivka Reservoir already has a well-established cyprinid dominance (mostly consisting of plankton-feeders) in the system, and one of the prey-regulating predators in this system is also a cyprinid – asp (Prchalová *et al.* 2008b). Because this predator spawns on a fluvial spawning ground, its whole spawning stock migrates from the reservoir into a very

small spatially delimited space and the whole asp reproductive allocation is deposited here (**Paper III**). An asp major prey species during the growth season and one of the dominants in the cyprinid-dominated waters - bleak (Prchalová *et al.* 2009a; Specziár & Rezsu 2009; Krpo-Ćetković *et al.* 2010), utilizes the eggs of their main predator during the spawning period with high efficiency. The demonstration that asp egg mortality is substantial due to effective bleak feeding is not only interesting because of the predator-prey reversal occurring in the system, but also because asp are available to bleak only for tens of seconds during the drifting phase (Šmejkal *et al.* 2017). Bleak do not utilize benthic food sources, nor was any found in their stomach content in analyses connected to this paper, and hence the substantial egg-feeding pressure occurs in a very short time of egg drift after fertilization in the water column.

Sound science rises from sound methodology

The obstacles rising from sampling of an aquatic environment result oftentimes in the usage of methods that do not fully comprehend the studied group of organisms. Especially in the case of fish, which are actively moving and avoiding the sampling gear, the correct usage of gear can dramatically improve information about the system (Kubečka *et al.* 2009). For their versatile usage, gillnets are the most widespread sampling gear in European inland waters, despite their considerable selectivity (Hamley & Regier 1973; Prchalová *et al.* 2008a). Small fish (< 80 mm) were reported to be underrepresented in European Standard gillnets (CEN 2005; Prchalová *et al.* 2009b; Olin *et al.* 2009). In **Paper II**, we demonstrate that large fish are also underestimated by European Standard gillnets. Specifically, in the case of bream, 70 % of its biomass may be allocated to individuals larger than 292 mm of standard length in some reservoirs, which is the threshold of low catchability for the largest mesh size in European Standard gillnets. In other words, the European Standard would here provide a more or less accurate estimate of a 30 % subset of the bream population, while the remaining 70 % of biomass is severely underestimated. Based on these results, we propose to implement large mesh gillnets, which simply extend the current standard by four additional mesh sizes keeping the 1.25 mesh geometric series (Appelberg *et al.* 1995; Kurkilahti & Rask 1996). Large mesh gillnets are set along with European Standard gillnets and as supplementation to improve

the precision of the large fish estimate and to provide a better baseline for interpretation of large fish data, especially in the case of large inland predators such as European catfish, for which the current standard is barely representative.

Reproductive allocation of males and females: when is protandry favored in the animal kingdom?

Paper III demonstrated that asp males arrived at the spawning ground on average approximately five days earlier than females and left four to five days later than females over two years. Both sexes performed a daily migration between a staging ground (standing water, low energy costs) and the fluvial spawning ground (high energy costs). The evening peak of an abundance of males occurred on average 1 hour 40 minutes earlier than that of females. The number of females on the spawning ground never exceeded the number of males. While the degree of protandry is hypothesized to be influenced by the operational sex ratio (ranging from 0.5 to 1 in our study), our data did not support this theory.

Earlier male arrival on the reproductive grounds compared to females seems to be a widespread phenomenon among animal taxa. A similar trend may be found among insects (Alcock 1997), fish (Morbey 2000), amphibians (Gerhardt 1991), reptiles (Wikelski *et al.* 1996), birds (Canal *et al.* 2012) and mammals (Apollonio *et al.* 2014). Protandry may be perceived as a higher investment of males in mate-finding and territory defending activities compared to females (Morbey *et al.* 2012). The male activity may be so high that they fully compensate for the female's higher energy allocations into egg development, resulting in a situation where total reproductive effort is equal or higher in males at the end of the reproductive season (Jonsson *et al.* 1991). However, not all reproductive systems favor higher male mate-finding investments, and in these systems sex-role reversal may occur (Svensson 1988; Wootton & Smith 2014).

In animal taxa where males have higher reproductive allocations due to costly parental care or where males provide females a large nuptial gift, it is the female who searches for a male and has higher activity investments into reproduction (Gwynne 1981; Clutton-Brock & Vincent 1991). In these sex-role reversal situations, females may precede males in their arrival, if such a

species aggregates for reproduction or may simply start their mate-finding activity earlier than males.

Furthermore, mate-finding investments are dependent upon differences in potential reproductive rate between males and females (Clutton-Brock & Vincent 1991; Garant *et al.* 2001; Forsgren *et al.* 2004). Due to the lower number of eggs compared to sperm production of males, it is usually the females who are limited in their potential reproductive rate. However, in species where males exhibit parental care for a prolonged period of time while females continue to reproduce, males are the more limited sex and are competed for by females (Ahnesjö *et al.* 2008).

The operational sex ratio may also contribute to the degree of mate-finding activity in a given sex. For instance, if the males become scarce in the environment, females that used to be courted by males start to actively find their mates (Forsgren *et al.* 2004). It is expected that the degree of protandry should also be dependent on the operational sex ratio in a given population and that males should arrive earlier on a given day when the operational sex ratio is male biased (Kokko *et al.* 2006). In **Paper III**, I studied this hypothesis based on the daily data of protandry. The analysis compares the difference between average male and female arrival time with the daily sex ratio on the spawning ground. While I did not find supporting evidence for this hypothesis, I do not completely reject it. The main reason is that the data points of daily protandry from days where the sex ratio is extremely male-biased are naturally impaired with very low female attendance. Therefore, the estimate of average female arrival is very sensitive to a few individuals, while the male estimate is made of tens of male arrivals and presumably more precise. I believe more should be done in future to test this hypothesis, because the operational sex-ratio should be one of the major drivers for mating behavioral decisions (Kvarnemo & Ahnesjö 1996; Weir *et al.* 2011; Székely *et al.* 2014).

Conclusions

To conclude, **Paper I** demonstrates the adaptive value of chemical cue perception in predator-prey relationships. The finding may have further impact on community ecology, and it provides an example that biomanipulation should be performed with caution. **Paper II** raises the question of correct usage of methodology and provides complementary

methodology to obtain a less biased picture of large fish. **Paper III** demonstrates seasonal and daily aspects of protandry in a model species, the cyprinid predator asp. **Paper IV** brings evidence of strong early life-history predator-prey reversal between asp and bleak. Further, the possible impact of bleak high feeding efficiency is discussed.

Future research prospects

I)   **Paper I** and **II** uses gillnet methodology, but in both instances the gillnet catch has to be treated with caution. Although gillnets simply cannot provide a reliable absolute estimate of a fish community due to their passive nature, more can be done in selectivity research, for instance the relationship between water temperature and gillnet catchability and selectivity has not been fully described. Currently gillnets are deployed in variously deep habitats, which are naturally linked with varying temperature. In the summer season, the difference between $0 - 3$ m deep habitat and > 20 m habitat can easily reach 15 °C, and hence described abundance and biomass gradients may be partly the result of decreasing fish activity with temperature (Prchalová *et al.* 2009a). While it is likely not completely the case, since acoustic surveys provide similar results (Jůza *et al.* 2012), it would still be worth investigating how the catchability changes along the temperature gradient.

II)   **Paper III** demonstrates how males precede females in their arrival on the spawning ground in order to maximize their mating efforts. It might be worth investigating whether their arrival is dependent on their age: do experienced males time their arrival better then inexperienced ones? And is it dependent also on male length? Just to clarify, some asp cease to grow after maturation in the studied system, hence there is no direct link between length and age (Šmejkal *et al.* 2016). Further questions arise from the system itself. Since asp seems not to form pairs for more than a single spawning event and seems not to be territorial, the protandry is likely driven by the mate opportunity hypothesis

(maximizing number of encountered females). Considering that males arrive on average five days before females and spend on the spawning ground an average of ten days, the mate finding effort is quite large. It would be worth looking at the advantage of early-spawned eggs and early-born asp – do they have survival advantage over individuals born later in the season?

III)   **Paper IV** demonstrates the bleak efficiency of asp egg utilization in the monitored tributary of Želivka Reservoir. Further, it would be interesting to estimate the overall number of bleak individuals migrating into the tributary and their consumption potential at a given temperature. Also, the movement of bleak between the tributary and reservoir has not been investigated yet. Does it follow the spawning movement of asp or do bleak stay in the tributary permanently?

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Research articles

Paper I

Can species-specific responses to chemical cues explain prey susceptibility to predation?

Šmejkal, M., Ricard, D., Sajdlová, Z., Mrkvička, T., Čech, M., Vejřík, L., Blabolil, P., Vejříková, I., Prchalová, M., Vašek, M., Souza, A.T., Brönmark, C., Peterka, J. (in prep.). Can species-specific responses to chemical cues explain prey susceptibility to predation?

Abstract

The perception of danger represents an essential ability of prey for gaining an informational advantage over their natural enemies. Especially in complex environments or at night, animals strongly rely on chemoreception to avoid predators. The ability to recognize danger by chemical cues and subsequent adaptive responses to predation threats should generally increase prey survival. We tested this using different species of freshwater fish and by combining small-scale experiments with a manipulative experiment in the field. First, in a laboratory experiment, we tested whether chemical cues associated with predation affected the behaviour of three common prey fish species, rudd (*Scardinius erythrophthalmus*), roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*). Further, we conducted a prey selectivity experiment to evaluate the prey preferences of a top aquatic predator, the European catfish (*Silurus glanis*). In addition, we analysed fish community data from two similar lakes (catfish stocked and catfish absent lake) inhabited by the three prey species, rudd, roach, perch and one predator species - pike (*Esox lucius*). Catfish predation caused a significant change in the prey population structure, with a decrease in the abundance of older individuals of species with absent (perch) or inadequate responses (rudd) to chemical cues.

Paper II

Biomass and Abundance Biases in European Standard Gillnet Sampling

RESEARCH ARTICLE

Biomass and Abundance Biases in European Standard Gillnet Sampling

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Abstract

The European Standard EN 14757 recommends gillnet mesh sizes that range from 5 to 55mm (knot-to-knot) for the standard monitoring of fish assemblages and suggests adding gillnets with larger mesh sizes if necessary. Our research showed that the recommended range of mesh sizes did not provide a representative picture of fish sizes for larger species that commonly occur in continental Europe. We developed a novel, large mesh gillnet which consists of mesh sizes 70, 90, 110 and 135mm (knot to knot, 10m panels) and assessed its added value for monitoring purposes. From selectivity curves obtained by sampling with single mesh size gillnets (11 mesh sizes 6 – 55mm) and large mesh gillnets, we identified the threshold length of bream (*Abramis brama*) above which this widespread large species was underestimated by European standard gillnet catches. We tested the European Standard gillnet by comparing its size composition with that obtained during concurrent pelagic trawling and purse seining in a cyprinid-dominated reservoir and found that the European Standard underestimated fish larger than 292mm by 26 times. The inclusion of large mesh gillnets in the sampling design removed this underestimation. We analysed the length-age relationship of bream in the Římov Reservoir, and concluded that catches of bream larger than 292mm and older than five years were seriously underrepresented in European Standard gillnet catches. The Římov Reservoir is a typical cyprinid-dominated water body where the biomass of bream > 292mm formed 70% of the pelagic trawl and purse seine catch. The species-specific relationships between the large mesh gillnet catch and European Standard catch suggested that the presence of carp (*Cyprinus carpio*), European catfish (*Silurus glanis*), tench (*Tinca tinca*) or bream warrants the use of both gillnet types. We suggest extending the gillnet series in the European Standard to avoid misinterpretation of fish community biomass estimates.

Introduction

Developing a tool capable of producing an unbiased picture of lentic fish communities became a task of increasing urgency as biotic and abiotic characteristics of the environment started being evaluated by standardized methods across Europe [1,2]. In recent decades, gillnet sampling methodologies evolved from single mesh gillnets into multimesh gillnets and became one of the key tools for assessing fish biomass, abundance and species composition of lentic communities [2,3]. When compared to other methods for estimating fish abundance and biomass, gillnets have advantages such as relatively low sampling costs, ease of use and possible deployment in a variety of lentic water habitats [4]. The data obtained from standardized sampling procedures may be used for large scale comparisons across various water bodies and serves as the main tool for interpreting differences in fish communities [5–7]. During the development of lentic water metrics to support the Water Framework Directive [8], certain large fish species were included as indicators [2]. Because data obtained by European Standard gillnets (ESG hereafter) have limits in representative catchable size of fish [4], improvements to gillnet sampling methodology are required to avoid biased sampling results.

ESG data were recently used for analysing piscivore top down control of prey fish [5], and size spectra of lake fish assemblages [6,9]. ESG catch is used according to recent findings that fish smaller than 80mm are underrepresented [10–12] and are thus left out of subsequent size spectra analyses [6]. Although large fish are more likely to be caught in gillnets than their smaller conspecifics due to increasing swimming ability with body size [13,14], large fish are also reported to be underrepresented in standard gear catch [15–17].

Despite the usefulness of gillnet data in comparative studies, gillnets as a passive gear were repeatedly proven to be size- and species-selective [10,18,19]. Size- and species-selectivity may be based on different encounter, contact and retention probabilities [reviewed in 20]. The main nature of gillnet mechanical selectivity is based on the relationship between fish girth and mesh perimeter [21,22] or, alternatively, fish species length and mesh size [20,23,24]. In order to sample the wide range of fish sizes that compose a water body's community structure, the multimesh gillnets of Norden type consisting of 12 different mesh sizes were developed [1]. To avoid biased length-frequency distribution of samples captured by multi-mesh gillnets, ESG mesh sizes follow a geometric series from 5mm to 55mm with a ratio of 1.25 [1,24] such that the selectivity of adjacent mesh sizes overlaps [25].

The efficiency of most sampling methods is affected by fish species and size. In various sampling methods, the lowest efficiencies have been recorded for extreme sizes of fish (i.e. very small and large individuals; [26]). This fact is also true for the ESG where a biased picture for young-of-the-year and one year old fish (so-called 0+ and 1+ fish) was detected. These small fish are underestimated by gillnet catches and need adjustment before the data can be correctly interpreted [10]. Jurvelius *et al.* briefly described biased gillnet catches of large fish in their comparison of four sampling methods (gillnetting, seining, trawling and hydroacoustics, [27]). The European Standard describes the catchable fish size as a range from 40–400mm total length [4]. An exact evaluation of the ESG catch of large fish has not yet been done and is missing from the scientific literature.

The goal of this study was to investigate how the ESG catch of large fish is biased in terms of fish biomass, abundance and fish species and size composition. In order to compare the ESG with another tool capable of estimating catch rates of large fish (biomass per unit of effort, BPUE), we simultaneously deployed ESG along with a novel type of multimesh gillnet consisting of four large mesh sizes (large mesh gillnet, LMG hereafter), expanding the geometric series of the European Standard. We also compared gillnet samples with catches obtained by pelagic trawling and purse seining that do capture the full spectrum of fish sizes. We anticipated that

the largest fish of certain species would be underestimated by the ESG and that the LMG would improve their biomass estimates.

The aims of this study were: (I) to estimate the threshold fish size above which the ESG is ineffective for estimating fish community biomass and abundance using selectivity curves approach (II) to compare the fish community biomass spectrum obtained by ESG to that obtained by trawl, purse seine and LMG, (III) to identify species for which the LMG sampling is essential, and (IV) to analyse the sampling bias of ESG for large bream (*Abramis brama*), a common and widespread large fish species in Europe.

Methods

Fish sampling

Three types of gillnets were deployed during this study—single mesh gillnets (SMG hereafter), ESG and LMG. We used SMG and LMG data to describe mesh size selectivity and for fitting selectivity curves. ESG and LMG were deployed concurrently to directly compare their catches and to identify possible improvements of gillnet sampling by extending the mesh size range.

Sampling by SMG was conducted in the Římov Reservoir, Czech Republic, from 1999 to 2003. The mesh sizes used were 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43, 55, 60, 65 and 85mm (knot to knot; benthic and pelagic gillnet measured 1.5m height x 25m length and 4.5m height x 25m length, respectively, S1 Table). ESG following the European Standard Document [4] (benthic gillnet: 1.5m height x 30m length, 2.5m panels for each 12 mesh sizes; pelagic gillnet: 3m or 4.5m height x 30m length, 2.5m panels for each 12 mesh sizes) were used for sampling from 2009 to 2013 in 12 Czech and two Spanish reservoirs, and in two Czech post-mining lakes (Fig. 1, S2 Table). ESG mesh sizes follow a geometric series with a ratio of about 1.25 (5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55mm; [4]). LMG consisting of four mesh sizes extending the ESG geometric series (70, 90, 110 and 135mm; knot to knot; size 1.5m height x 40m length, 10m panels for each 4 mesh sizes) were deployed in the same habitats and localities along with the ESG. The density of large individuals is usually much lower than the density of their small conspecifics [15,28], so the length of LMG panels was made 4 times larger than ESG panels to increase the probability of capturing large individuals, and to improve the precision of abundance and biomass estimates. Both ESG and LMG were deployed in sets consisting of three gillnets joined by 30m rope. For LMG, mesh-specific catch was recorded in most years and water bodies.

Depth stratified sampling was conducted in all water bodies. Depth ranges were 0–3m, 3–6m, 6–9m, 9–12m, 12–18m and > 20m for benthic habitats; and 0–4.5m and 5–9.5m for pelagic habitats. Each water body was divided into several localities along its longitudinal axis in order to cover the gradient of fish distribution following nutrient concentration from tributary to dam [29]. Each locality was sampled to its maximum depth using the above stratification scheme. To cover both the sunset and sunrise peaks of fish activity, gillnets were deployed two hours before sunset and lifted two hours after sunrise [30].

The catch was sorted by species and standard length (SL) was measured for each individual fish. Catch data was expressed as biomass per unit of effort (BPUE, kilograms per 1000m^2) by dividing the catch biomass by the area of deployed gillnets and multiplying by 1000. For each species, subsamples of at least 50 individuals from each reservoir and year were also weighed to the nearest gram to estimate length-weight relationships used in calculation of total catch biomass.

Active gear sampling was performed in the Římov Reservoir in 2010. Pelagic trawling and purse seining were used at night in the pelagic zone to obtain independent estimates of the length frequency distributions and biomass of the whole fish community. To obtain an
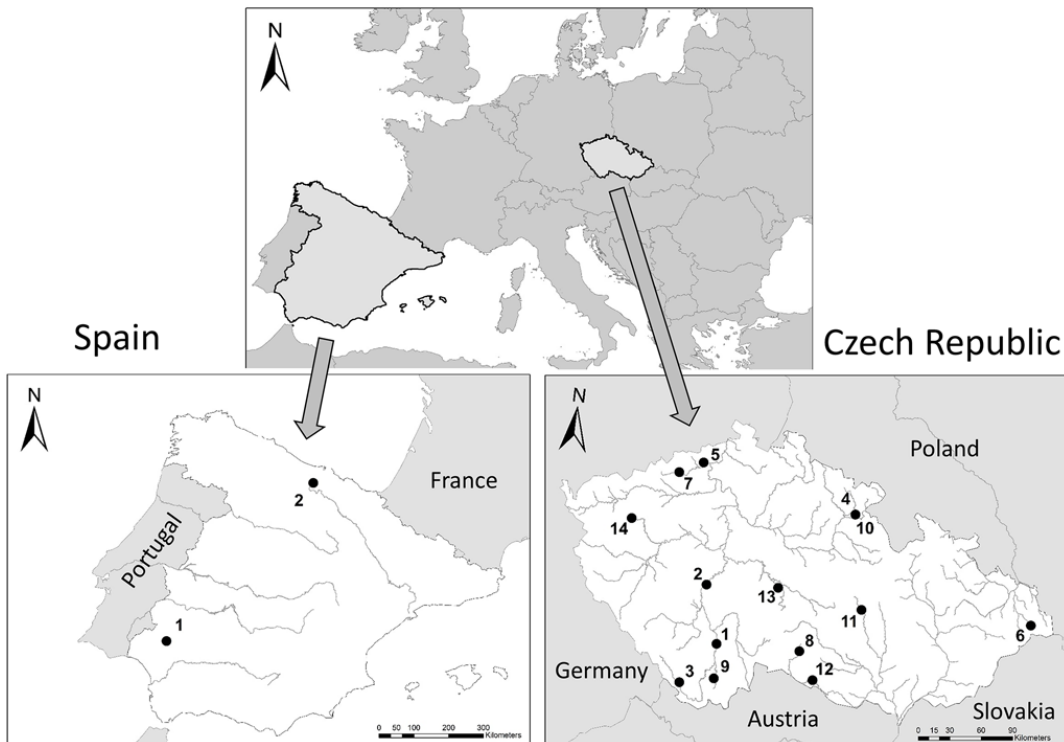
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Fig 1. Map of the Czech Republic and Spain showing the locations of the water bodies sampled in this study. Reservoirs located in Spain: 1—Aracena, 2—Ebro. Reservoirs and lake located in the Czech Republic: 1—Hněvkovice, 2—Kamýk, 3—Lipno, 4—Malá Rozkoš 5—Milada, 6—Morávka, 7—Most, 8—Nová Říše, 9—Římov, 10—Rozkoš, 11—Vír, 12—Vranov, 13—Želivka, 14—Žlutice.

unbiased sample of fish length-frequency distribution, density estimates of small fish (≤180mm) were obtained by purse seining and those of larger fish (>180mm) were obtained using the trawl [31]. This approach was chosen because of recent findings that the trawl used underestimates the fish density up to 180 mm SL [31]. The trawl opening width was 12–13.5m, and the opening height was 8m. The lengths of the main body and cod end were 38m and 11m, respectively. The mesh size in the main body was 80/40/20mm (half mesh, knot centre) from the opening toward the end and 10mm in the cod end. The purse seine net had a length of 120 m and height of 12 m. The exact position of each haul was recorded with a Garmin GPS device during the setting of the net, and the area sampled was calculated with OziExplorer software [32]. Trawl and purse seine catches were expressed as biomass per unit of effort (BPUE, kilograms per hectare).

Selectivity curves

We calculated the intersection of selectivity curves of 55mm and 70mm mesh sizes for bream to identify the threshold standard fish length where sampling by the 70mm mesh size starts being more efficient than sampling with the 55mm mesh size. The intersection of the two curves is the fish length value $\chi_{threshold}$, which resulted in the same probability density for the

Gaussian distributions for the 55mm and the 70mm mesh size. That is, we found the value $\chi_{threshold}$ such that:

$$f(x_{threshold}, \mu_{55mm}, \sigma_{55mm}) = f(x_{threshold}, \mu_{70mm}, \sigma_{70mm}) \tag{1}$$

where f is the Gaussian probability density function ($f(x, \mu, \sigma) = \sqrt{(2\pi*\sigma^2)}*\exp\left(-\frac{(x-\mu)^2}{2*\sigma^2}\right)$), $\mu_{55mm}, \sigma_{55mm}$ are the mean and standard deviation of the 55mm mesh size selectivity curve and $\mu_{70mm}, \sigma_{70mm}$ are the mean and standard deviation of the 70mm mesh size selectivity curve.

Biomass- and abundance-at-length

Biomass-at-length information was used to generate biomass spectra for ESG and LMG to determine the proportion of the fish community that was missed by ESG sampling. Length-specific BPUE (25mm length classes from 10 to 560mm) were computed for ESG and LMG and then used to compare the biomass spectrum captured by each type of gear, and to determine whether large fish were underrepresented and what improvements were achieved by the addition of LMG gear.

The length frequency distributions of fish caught by pelagic trawls and purse seines were compared to those obtained by ESG and LMG catches to evaluate whether the full spectrum of fish sizes was captured by using both ESG and LMG. We used a one-sided t-test to determine whether the mean fish length from the LMG was statistically smaller than the mean length of large fish captured by trawl (i.e. whether the LMG catch captures the whole size spectrum of large individuals). The trawl capture data used in the t-test consisted of fish individuals that were larger or equal to the smallest fish caught in the LMG.

To further examine whether the ESG captured the full spectrum of fish sizes, we calculated mesh-specific selectivity curves for bream sampled by SMG and LMG in the Římov Reservoir from 1999 to 2013. We chose bream as an example of a common and widespread species in Europe [33] because it has a wide spectrum of lengths over its long lifespan (see subsection Length-age analysis below) and is an ideal species to examine size selectivity of ESG. The catches obtained from three LMG mesh sizes (70, 90, 110mm) were used to determine whether or not some large fish were properly sampled by ESG.

To estimate whether biomass estimates derived from ESG samples were biased, and to quantify this bias, BPUE obtained from active gear and pelagic gillnet catches were compared for fish above and below the ESG standard length threshold ($SL \geq \chi_{threshold}$ and $SL < \chi_{threshold}$). To circumvent the problem arising from the fact that trawl and gillnet BPUE have different units, BPUE ratios were used to provide a measure of ESG bias. We estimated the ESG bias for large fish by computing the difference in trawl to ESG BPUE ratios for small and large individuals using the following equation:

$$\frac{BPUE_{active, SL < x_{threshold}}}{BPUE_{ESG, SL < x_{threshold}}} = \beta_{ESG} \frac{BPUE_{active, SL \geq x_{threshold}}}{BPUE_{ESG, SL \geq x_{threshold}}} \tag{2}$$

where β_{ESG} quantified the ESG sampling bias magnitude for large fish. A positive value of β_{ESG} indicated that large fish were under-sampled by ESG. To determine whether the inclusion of LMG sampling reduced the estimated bias for large fish, a similar ratio was computed for trawl BPUE and total gillnet BPUE (ESG and LMG together) for fish above and below the ESG

size threshold:

$$\frac{BPUE_{active,SL<x_{threshold}}}{BPUE_{ESG,SL<x_{threshold}} + BPUE_{LMG,SL<x_{threshold}}} = \beta_{ESG+LMG} \frac{BPUE_{active,SL\geq x_{threshold}}}{BPUE_{ESG,SL\geq x_{threshold}} + BPUE_{LMG,SL\geq x_{threshold}}} \quad (3)$$

where $\beta_{ESG+LMG}$ is the estimated bias of the total gillnet catches.

Species-specific LMG to ESG catch relationships

Species-specific benthic BPUE was computed for both ESG and LMG in all available reservoirs and years for the 17 species which had more than five BPUE records. The relationship between ESG and LMG biomasses (ESG_s and LMG_s, respectively) was then estimated for every species s using the following linear model:

$$\log(LMG_s + 1) = \alpha_s + \beta_s \log(ESG_s + 1) \quad (4)$$

The model allows the identification of 1) species for which LMG catches are not significant (intercept α_S and slope β_S values near zero), 2) species for which LMG catches are positively correlated with ESG catches (intercept value near zero and positive slope value) and 3) species for which LMG catches are significant and are underrepresented in the ESG catches (positive intercept value). These species-specific estimates allowed the identification of the types of water bodies where LMG should be deployed along ESG to representatively sample all fish sizes.

Length–age analysis

In order to evaluate the representation of large bream cohorts in terms of biomass and age classes between ESG and LMG, we examined the length-age structure of the Římov Reservoir bream population. 105 individuals caught during the 2012 sampling campaign were used for length-age analysis. Otolith reading was used for ageing fish. The relationship between *age* and *length* was estimated using a logarithmic curve of the form:

$$age = e^{(\alpha + \beta length)} \quad (5)$$

where α and β were estimated using a generalised linear model with Gamma error and a log link.

The estimated length-age relationship allowed us to identify the age of bream corresponding to a standard length of $\chi_{threshold}$ from [equation (1)](#). This standard length (*SL*) was then converted to total length (*TL*) using the following linear relationship:

$$TL = \alpha + \beta SL \quad (6)$$

where parameters α and β were estimated using 756 bream individuals for which both standard length and total length measurements were available. These individuals were captured during yearly sampling in the Římov Reservoir between 2000 and 2012. Standard lengths ranged from 31mm to 426mm and total lengths ranged from 39mm to 520mm.

All analyses were conducted using the R software version 3.1.2 [34].

Results

From 1999 to 2003, a total of 1145 SMGs, with a combined sampled area of 60543.75m^2, were deployed in the Římov Reservoir resulting in a catch of 14614 fish with a combined biomass of 1659.36kg. From 2009 to 2013, 1404 ESGs and 1221 LMGs were deployed in 16 water bodies

with a combined sampled area of 93150 m^2 and 108585 m^2 respectively, resulting in a combined ESG catch of 84502 fish with biomass of 4082.25kg, and a combined LMG catch of 1396 fish with biomass of 2011.85kg.

The ESG catch contained a total of 36 species and 6 kinds of hybrids while the LMG catch consisted of 18 species and 2 hybrids ([Table 1](#)). However, the bulk of the LMG catch came from eight species: bream (55.2% in numbers and 31.7% in biomass), carp *Cyprinus carpio* (30.2% and 46.7%), European catfish *Silurus glanis* (2.1% and 5.8%), asp *Leuciscus aspius* (1.9% and 3.5%), tench *Tinca tinca* (2.9% and 3.2%), rudd *Scardinius erythrophthalmus* (2.6% and 2.0%), European whitefish *Coregonus lavaretus* (1.4% and 1.5%), and pikeperch *Sander lucioperca* (1.0% and 1.4%). Although ESG were deployed in the same reservoirs and localities as LMG, two species and a hybrid (bighead carp *Hypophthalmichthys nobilis*, Siberian sturgeon *Acipenser baerii* and white bream x rudd *Blicca bjoerkna x Scardinius erythrophthalmus* hybrid) were only detected by LMG ([Table 1](#)).

Selectivity curves

From [equation (1)](#), we determined the intersection of the 55mm mesh size and 70mm mesh size Gaussian distribution curves to be 292mm for bream. The dataset used for length frequency analyses of bream included individuals ranging from 30 to 420mm in standard length. The majority of length classes were properly covered by mesh sizes used in ESG. Although there was a wide range of catchable lengths of ESG, ESG was not capable of enmeshing the larger bream also present in the community ([Fig. 2](#)). Thus, the ESG mesh size range did not representatively cover the whole size spectrum of bream.

Biomass- and abundance-at-length

The biomass spectra (BPUE-at-length) for ESG and LMG obtained from the Římov Reservoir in 2010 ([Fig. 3a](#)) indicated that ESG sampling missed a significant proportion of the fish community biomass, especially for fish greater than 300mm standard length. While the ESG BPUE peaked at a standard length of 290mm and did not capture individuals larger than 390mm, the LMG BPUE peaked at a standard length of 330mm and extended towards larger sizes up to 530mm.

The LMG length-frequencies did not significantly differ from the large fish length-frequencies obtained by trawling (one-sided t-test, p = 0.94 in the 2010 sampling year). Both pelagic trawl and LMG showed peak frequencies for larger fish (300–350mm standard length, [Fig. 3b](#)). Fish above 300mm of standard length represented 37% of abundance and 70% of biomass of the trawled and purse seined fish community. However, this peak was not recorded in ESG catches indicating a serious underestimation of fish larger than 300mm of standard length during ESG sampling ([Fig. 3b](#)).

We estimated that the ESG bias for large fish, as determined by parameter β_{ESG} in [equation (2)](#), was a 26-fold underestimation. By adding the LMG samples to the sampling design ([equation 3](#)), the bias decreased substantially to a 1.36-fold underestimation of large fish. The value of both β_{ESG} and $\beta_{ESG+LMG}$ represented the magnitude of the change in active gear to gillnet BPUE ratios for small and large fish. A value slightly above or below 1.0 can still identify an unbiased sampling strategy since there is a certain amount of measurement error in both gillnet and active gear catches.

Species-specific LMG to ESG catch relationships

Based on species-specific regressions between LMG and ESG biomasses we identified species for which deployment of LMG significantly improved gillnet-based estimates. Small- and

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Table 1. Species captured by ESG and LMG in all water bodies sampled during the study.

Family	Common name	Scientific name	ESG	LMG
Acipenseridae	Siberian sturgeon	*Acipenser baerii*		X
Anguillidae	Eel	*Anguilla anguilla*	X	
Balitoridae	stone loach	*Barbatula barbatula*	X	
Centrarchidae	largemouth bass	*Micropterus salmoides*	X	
Centrarchidae	pumpkinseed	*Lepomis gibbosus*	X	
Cobitidae	spined loach	*Cobitis elongatoides*	X	
Cyprinidae	Andalusian barbel	*Luciobarbus sclaterii*	X	X
Cyprinidae	asp	*Leuciscus aspius*	X	X
Cyprinidae	belica	*Leucaspius delineates*	X	
Cyprinidae	bighead carp	*Hypophthalmichthys nobilis*		X
Cyprinidae	bleak	*Alburnus alburnus*	X	
Cyprinidae	bream	*Abramis brama*	X	X
Cyprinidae	carp	*Cyprinus carpio*	X	X
Cyprinidae	chub	*Squalius cephalus*	X	
Cyprinidae	dace	*Leuciscus leuciscus*	X	
Cyprinidae	Ebro barbel	*Luciobarbus graellsii*	X	X
Cyprinidae	Ebro nase	*Parachondrostoma miegii*	X	
Cyprinidae	Grass carp	*Ctenopharyngodon idella*	X	
Cyprinidae	gudgeon	*Gobio gobio*	X	
Cyprinidae	hybrid asp x ide	*Leuciscus aspius x Leuciscus idus*	X	
Cyprinidae	hybrid white bream x rudd	*Blicca bjoerkna x Scardinius erythrophthalmus*		X
Cyprinidae	hybrid white bream x vimba bream	*Blicca bjoerkna x Vimba vimba*	X	
Cyprinidae	hybrid white bream x bream	*Blicca bjoerkna x Abramis brama*	X	
Cyprinidae	hybrid roach x bream	*Rutilus rutilus x Abramis brama*	X	
Cyprinidae	hybrid white bream x roach	*Blicca bjoerkna x Rutilus rutilus*	X	
Cyprinidae	hybrid roach x rudd	*Rutilus rutilus x Scardinius erythrophthalmus*	X	X
Cyprinidae	Iberian gudgeon	*Gobio lozanoi*	X	
Cyprinidae	ide	*Leuciscus idus*	X	
Cyprinidae	Prussian carp	*Carassius gibelio*	X	X
Cyprinidae	roach	*Rutilus rutilus*	X	X
Cyprinidae	rudd	*Scardinius erythrophthalmus*	X	X
Cyprinidae	Spanish nase	*Pseudochondrostoma willkommii*	X	
Cyprinidae	stone moroko	*Pseudorasbora parva*	X	
Cyprinidae	tench	*Tinca*	X	X
Cyprinidae	vimba bream	*Vimba vimba*	X	
Cyprinidae	white bream	*Blicca bjoerkna*	X	X
Esocidae	pike	*Esox lucius*	X	X
Gobiidae	tubenose goby	*Proterorhinus marmoratus*	X	
Percidae	perch	*Perca fluviatilis*	X	X
Percidae	pikeperch	*Sander lucioperca*	X	X
Percidae	ruffe	*Gymnocephalus cernua*	X	
Salmonidae	brown trout	*Salmo trutta*	X	X
Salmonidae	European whitefish	*Coregonus lavaretus*	X	X
Salmonidae	grayling	*Thymallus thymallus*	X	
Salmonidae	rainbow trout	*Oncorhynchus mykiss*	X	
Siluridae	European catfish	*Silurus glanis*	X	X

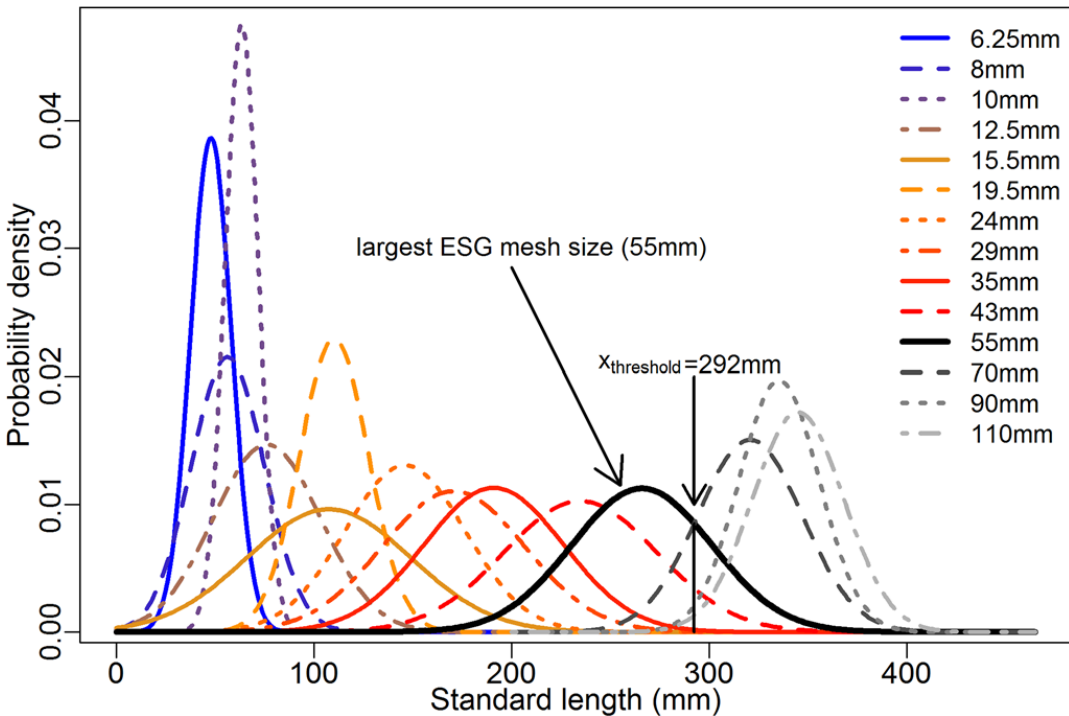doi:10.1371/journal.pone.0122437.t001

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Fig 2. Mesh-specific length frequency distributions of bream captured in SMG in the Římov Reservoir and LMG in all sampled water bodies. The selectivity curve of the 55mm mesh size (the largest mesh size of the ESG) is highlighted and the fish standard length corresponding to the intersection between the 55 and 70mm curves ($\chi_{threshold}$) is shown.

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medium-sized species (bleak *Alburnus alburnus*, roach *Rutilus rutilus* and ruffe *Gymnocephalus cernua*) had intercept and slope values that were not statistically different from zero, indicating that they were never captured in LMG. Thus gillnet-based estimates of small- and medium-sized fish communities were unlikely to be improved by LMG sampling. Larger species (carp, European catfish, tench and bream) had significantly positive intercept ($p<0.001$, $p<0.05$, $p<0.05$ and $p<0.1$, respectively) and slope values ($p<0.001$, $p<0.05$, $p<0.05$ and $p<0.01$, respectively) indicating that these species were regularly recorded in LMG catches but underrepresented in ESG samples. A higher proportion of large species in fish communities led to a larger bias in the ESG estimates, and LMG sampling improved the biomass estimates of such communities. Rudd had a significantly positive slope ($p<0.01$) and near zero intercept value ($p = 0.41$, i.e. slope is not significantly different from zero), which suggested that its LMG catch was proportional to its ESG catch.

Length-age analysis

The threshold standard length of bream ($\chi_{threshold} = 292mm$) computed from equation (1) was equivalent to a total length of 366mm, (equation 6, $TL = 4.4+1.24SL$) which corresponded to 5 year old fish (Fig. 4). Bream larger than this threshold standard length of 292mm and 5 years of age and more were not representatively recorded by ESG sampling. These large individuals
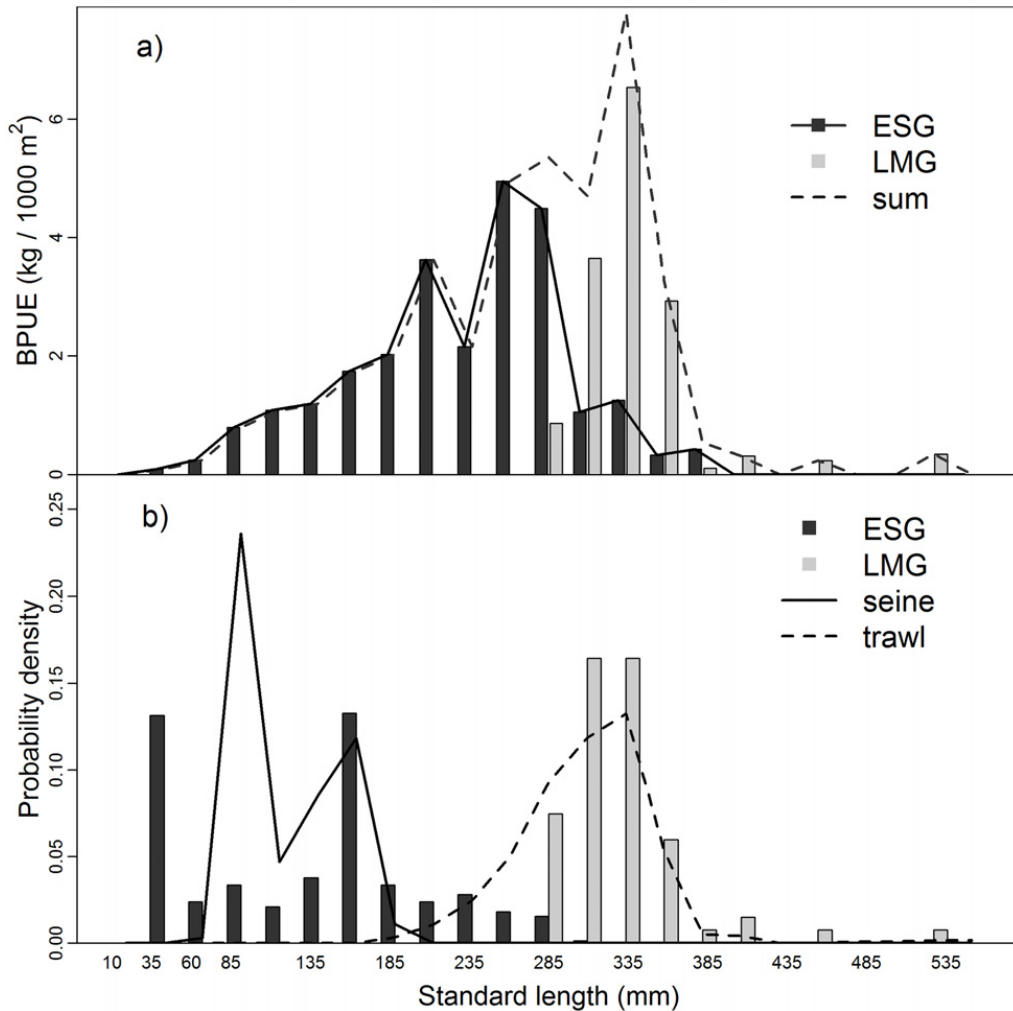
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Fig 3. Biomass spectrum and length frequency distributions of all fish species captured in the Římov Reservoir in 2010. a) Biomass spectrum for ESG and LMG in the Římov Reservoir in 2010. b) Comparison of length frequency distributions of all fish species from trawl, purse seine, ESG and LMG sampling in the Římov Reservoir in 2010.

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represented 10% of bream caught by SMG (231 out of 2290 individuals), and 35% of the bream catch weight (170 out of 486kgs). In comparison, large bream accounted for only 6% of individuals caught by ESG, but for 94% of individuals caught by LMG (38% and 96% of bream catch weight, respectively).

Discussion

The current version of the European Standard document (2005) states that "the location of each gillnet in the lake is determined in such way that the total catch should constitute an
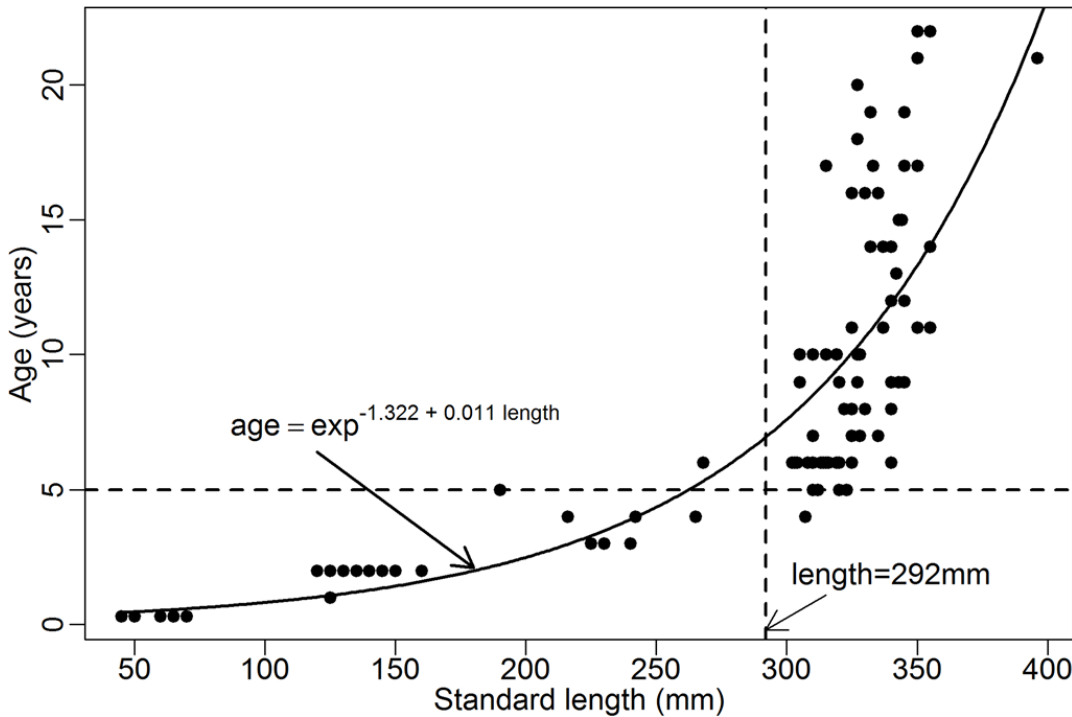
Fig 4. Bream length-age relationship in the Římov Reservoir, 2012. Vertical and horizontal lines indicate standard length of 292mm and age of five years, respectively, the threshold length and age above which bream is underrepresented in ESG catch.

unbiased sample of the catchable part of the fish assemblage in the lake. '*Catchable*' fish means active fish species within a range of about 40mm to 400mm which are usually caught in gill-nets." We provided evidence of the fact that sampling by European Standard methods biases estimates of fish even smaller than 400mm of total length (367mm of total length in case of bream). Further, we showed that these fish constituted a considerable part of the fish assemblage in some water bodies. Because certain large fish species were recently included in the evaluation metrics of lentic waters within the Water Framework Directive [8], we recommend the addition of large-mesh gillnet sampling to avoid biased sampling results.

Obtaining a correct estimate of fish community length-frequency distribution requires representative fish samples. A major hurdle stems from the fact that all fishing gears provide biased results. The most pronounced biases in length-frequency distributions are for individuals of extreme sizes [26] and the largest individuals from a water body are often not detected by common sampling gear [16]. In the case of active fishing gear, large fish are able to escape from the moving gear because of their greater swimming capacity compared to smaller individuals [35,36]. Gillnet size-selectivity is strongly dependent on their design [37]. When comparing different gear for fish sampling, a number of studies identified an underestimation of large fish by ESG [15,17,27]. The latter study [17] suggests using 70mm mesh size gillnets in alpine lakes to increase the catchability of large fish and to improve estimates of size distribution and

biomass of fish communities. We showed that simultaneous deployment of large mesh gillnet would improve the precision in length-frequency distribution estimates.

Holmgren & Appelberg [15] pointed out that additional sampling effort, beyond what is required for smaller fish, would be needed to obtain reliable biomass estimates for large fish. In the case of LMG design, we extended the length of large mesh panels from ESG's 2.5m to 10m for several reasons. First, we expected that large fish would be less numerous than their smaller conspecifics due to the fact that natural mortality in fish is highest during early stages of their life history (so-called type III survivorship curve, [28]). Second, we assumed that gillnet saturation was more pronounced as the size of fish increase because of the disturbance that caught fish cause to their surroundings [38–40]. Third, we aimed to achieve a high precision in our estimates of large fish since large fish can compose a considerable part of the overall biomass in certain fish communities [27,41].

The composition of the fish community in a water body was shown to be mostly dependent on latitude, altitude, nutrient concentration and morphology [6,42,43]. The recent findings of Emmrich *et al.* [9] show that in general, average fish size declines from north to south. On a finer scale, we can identify several types of water bodies with distinct fish assemblages: deep vendace (*Coregonus albula*) lakes, shallow roach lakes and ruffe lakes [42]. A high proportion of large fish is found in alpine lakes [9,44], in bream-dominated lowland reservoirs of Central Europe and in northern shallow eutrophic lakes [45,46]. The lakes chosen for the development of ESG were based on sampling of predominantly small species [1,24,39]. Therefore, the largest mesh size from ESG geometric series is insufficient for sampling of large fish assemblages.

The data used in this study were collected predominantly in reservoirs, man-made water bodies with a number of anthropogenic pressures that are deteriorating aquatic communities [47]. Large fish are often the most prone to human influences and disappear from impacted ecosystems faster than small ones [48,49]. The large proportion of small fish observed while sampling reservoirs using gear that underestimates large fish may lead to the false assumption that large fish are only a minor part of the fish community. However, large fish are an integral part of ecosystem functioning [50] and may serve as an important indicator of human disturbances. Moreover, large trophy fish naturally attract the interest of the general public [16] and they have the potential to shape the rest of aquatic communities through predation [51] and very often represent the bulk of fish biomass [52]. For all the above reasons, the information about large fish must not be omitted in future standardised sampling.

33 European countries are currently using the European Standard or are bound to use it in near future [4]. Despite the fact that the standard is regularly debated by the European Committee for Standardization, there is a risk that such methodology could be implemented without proper evaluation of its potential shortcomings in water bodies where large fish are present. This study highlights that the European Standard underestimates the large fish community. Therefore, we suggest extending the European Standard mesh series with the additional larger mesh sizes used in this study. The large mesh gillnets should be used in water bodies where presence of larger fish species (e.g. bream, carp, European catfish, tench) is expected. The suggested modification to the sampling standard will ensure that the portion of the fish community that is composed of large fish is appropriately sampled and that biomass estimates for whole water bodies are representative of the entire fish community.

Supporting Information

S1 Dataset. Data file. Spreadsheet containing all the data required to reproduce the figures and analyses presented in the manuscript.
(XLSX)

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S1 Table. Single mesh gillnet catch and effort summary. Summary of gillnet effort (number of nets), sampled area and number of fish recorded in each SMG mesh size, Římov Reservoir, 1999–2003.
(DOCX)

S2 Table. Multimesh gillnet catch and effort summary. Summary of the sampling in 2009–2013 in the different water bodies analysed in this study (CZ—Czech Republic, SP—Spain). For each water body, the year of sampling (Year), total gillnet effort (Nets), effort with ESG and LMG nets (ESG nets and LMG nets, respectively), the total number of fish (ESG and LMG fish) and biomass of fish recorded (ESG and LMG biomass in kg) are reported.
(DOCX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: JK JP MP. Performed the experiments: MŠ MP MŘ MM PB MČ MV TJ AMH LE JP JK. Analyzed the data: DR MŠ MP JK. Contributed reagents/materials/analysis tools: JK. Wrote the paper: MŠ DR JK MP TJ MV MM.

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Paper III

Seasonal and daily protandry in a cyprinid fish

SCIENTIFIC REP⚙RTS

Seasonal and daily protandry in a cyprinid fish

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In polygynandrous mating systems, in which females limit reproductive success, males can increase their success by investing in courtship. Earlier arrival at the spawning ground compared to when females arrive may increase their opportunities in competitive mating systems. In this study, we used passive telemetry to test whether a male minnow known as the asp, *Leuciscus aspius*, times its arrival at spawning grounds relative to the arrival of females. Males arrived in a model stream approximately five days earlier than females on average and left four to five days later than females over two years. Both sexes performed a daily migration between a staging ground (standing water, low energy costs) and the fluvial spawning ground (high energy costs). Fish abundance peaked twice a day, with a major peak at sunset and a minor peak at sunrise and with the evening peak abundance for males occurring 1 hour 40 minutes earlier than that of females. The number of females on the spawning ground never exceeded the number of males. While the degree of protandry is hypothesized to be influenced by the operational sex ratio (ranging from 0.5 to 1 in our study), our data did not support this theory.

Successful reproduction at least once in each individual's lifetime is crucial for every living organism. Sex-dependent chances of reproduction vary among mating systems and types of parental care[1, 2]. The most intense competition for mates occurs in the sex that make up the majority of the breeding population and has the higher reproductive potential[3, 4]. In most animal species, females are the sex with the limiting reproductive rate, which favours male intra-sexual competition and active courtship[5, 6]. Strong male competition is especially predominant in mating systems in which males do not participate in parental care or feed females[7–9].

In polygynandrous mating systems, males typically exhibit high levels of mate-finding activity to increase their reproductive success[5]. Reproductive gatherings in certain areas help males in their search for females; however, male intra-sexual competition is intense in such systems[10, 11]. Examples of reproductive gatherings in which male success differs considerably among individuals include lek systems and many fish spawning grounds[12–14]. In these mating systems, male behaviours that increase the probability of successful mating are under strong sexual selection[15].

Although there are a wide variety of types of reproductive gatherings, we still see common patterns among different mating systems. In species in which mating occurs at the gathering site and that have conventional sex roles, males exhibit protandry, generally arriving at the breeding ground before females during the breeding season[16–18]. This early arrival is used to establish intra-sexual hierarchies and territories in many types of reproductive gatherings and lek mating systems, and thus, early arriving males may achieve a higher rank and consequently have access to more females (rank advantage hypothesis)[16, 19, 20]. Alternatively, protandry may simply result in maximizing the number of mating opportunities by merely allowing males to encounter a higher number of females during the mating season (mate opportunity hypothesis)[21, 22]. While protandry has positive effects on male fitness, early arrival can reduce survival because harsh environmental conditions are common at the beginning of the mating season[21, 23, 24]. Finally, a male-biased operational sex ratio should generally promote protandry, as the competition for mates is very high in male-dominated systems[22].

The length of the ready-to-mate period is usually sex-specific. While males are prepared to breed over a relatively long period in a given season, female receptivity determines when mating actually occurs[25, 26]. Therefore, a possibly beneficial strategy for males is to always be ready and wait for the chance to mate[21, 22, 27]. However, daily energy expenditure by males is high during the reproductive period. In male Atlantic salmon, *Salmo salar*, the energetic costs of mating counterbalance the six-times-higher investment in gonadal development by females[10, 28].

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Hence, if the mere presence at the breeding site is costly in comparison to the cost of remaining in the surrounding environment (e.g., due to male competition or a hostile environment), the allocation of energy by males should be aimed at the specific seasonal and daily periods when the chances of encountering a receptive female are the highest[29]. Furthermore, daily male arrival should be dependent on the current operational sex ratio, and daily protandry should occur especially when the sex ratio is strongly male-biased[17, 22].

Here, we studied the sex-specific seasonal and daily aspects of the timing of reproductive migrations in an iteroparous fish, *Leuciscus aspius* (asp), Cyprinidae, inhabiting European lentic and lotic environments. The asp spawn following a polygynandrous mating system typical for the majority of cyprinid species, and its spawning grounds are restricted to fast flowing rivers[30, 31]. Males actively search for receptive females in the spawning grounds. The act of spawning consists of one or multiple males vigorously chasing a female and terminates in the simultaneous release of eggs and milt near the surface of the water. The negatively buoyant, adhesive eggs drift in the water current and eventually stick to the stony ground. Asps do not form permanent pairs during spawning, which results in an open contest for receptive females[32]. There is no parental care, i.e., reproductive costs only involve gonadal development and the energetic costs associated with migration to and movement at the spawning grounds. In the system we studied, the entire reproductively active population migrates daily from the staging ground in a reservoir (standing water, low energy costs) to the spawning ground in a short tributary (fast-flowing water, high energy costs). Such a system represents an ideal model for the study of both seasonal and daily aspects of protandry because all individuals migrate to a space-limited spawning ground ideal for passive telemetric monitoring. In this study, monitoring tiles were used to ensure that the spawning ground is well-defined and the asp do not spawn below the monitoring site. In order to test the male and female difference in timing of arrivals and departures on the spawning ground, 433 males and 316 females were captured and individually tagged with passive integrated transponders (PIT tags) in 2014 and 2015. In subsequent spawning seasons, passive telemetry systems were installed in spatially delimited spawning ground for the month-long spawning season and altogether, 351 tagged individuals from previous years were detected. Timing of individual arrivals and departures on the spawning ground were recorded by the passive telemetry systems.

The goals of this study are specifically to test I) whether males exhibit protandry in their seasonal and daily migration, II) whether males also leave later (both on a daily and seasonal basis) than females, III) whether males time their daily length of stay to the number of females present and IV) whether the degree of daily protandry correlates with the daily operational sex ratio.

Results

Monitoring coverage of the spawning ground.   The monitoring sites were significantly different, and the uppermost egg monitoring site was the most used spawning ground (likelihood ratio test: χ^2 (df = 2) = −359.8, p < 0.001; mean number of eggs ± SD - fast 70 ± 68, medium 40 ± 46, slow 1 ± 1). Furthermore, the single likelihood tests demonstrated that the slow site was significantly different than the fast site (Wald test: χ^2 (df = 1) = 73.4, p < 0.001) and the medium site (Wald test: χ^2 (df = 1) = 32.4, p < 0.001). Based on these results, we conclude that studied reproducing asps had a spatially delimited spawning ground and that the spawners had to pass at least one antenna to enter the spawning ground (Fig. 1).

Seasonal protandry, day of leaving and movement at the spawning ground.   Ninety-nine tagged males and 51 tagged females were detected by the antennas in 2015, and 171 males and 101 females were detected in 2016. When present on the spawning ground, males were detected approximately twice as frequently per hour than were females, indicating their higher level of mate-finding activity. Generally, males invested approximately three times as much time to spawning as females in both years (in terms of both hours and days). When measured from the first day of monitoring in a given year, males arrived for the first time at the spawning ground on average five days earlier and left four to five days later than females (Table 1, Fig. 2). The male-female comparisons were almost identical in both seasons despite the fact that we increased the precision of monitoring by deploying a third antenna system in the spawning ground.

Daily protandry, time of leaving, male length of stay and operational sex ratios.   The distributions of the daily presence of males and females were significantly different (Rank envelope test, N = 351, p = 0.005), indicating that more males than females arrive before sunset and more females than males arrive at night in relative numbers (Fig. 3).

A Spearman's rank-order correlation revealed a positive relationship between male length of stay and number of females on the spawning ground in the evening period (r_s (38) = 0.394, p = 0.014; Fig. 4), while the relationship was not significant in the morning period (r_s (24) = 0.234, p = 0.271).

The operational sex ratio was male-biased during both the evening and morning periods (evening median = 0.79 with interquartile range IQR = 0.70–0.88, morning median = 0.88, IQR = 0.73–1). Male daily protandry was not correlated with the operational sex ratio during either the evening or morning period (evening period: GLM ratio F = 0.21; df = 1; p = 0.649; morning period: GLM ratio F = 0.01; df = 1; p = 0.952).

Discussion

Currently, sex-dependent reproductive timing in fish has been studied mostly in species with a certain level of parental care[10, 26, 33–35], whereas investments of species spawning in aggregations with no parental care have not received much scientific attention. However, these mating systems are very suitable for testing protandry – there is no permanent pair formation, and therefore, males should maximize the number of spawning events and the number of acquired females as the reproductive success of males depends solely on the number and quality of eggs he fertilizes[36]. From this point of view, mating opportunities should increase more steeply with increasing protandry and reproductive activity than in species in which pairs are formed early in the season and in which
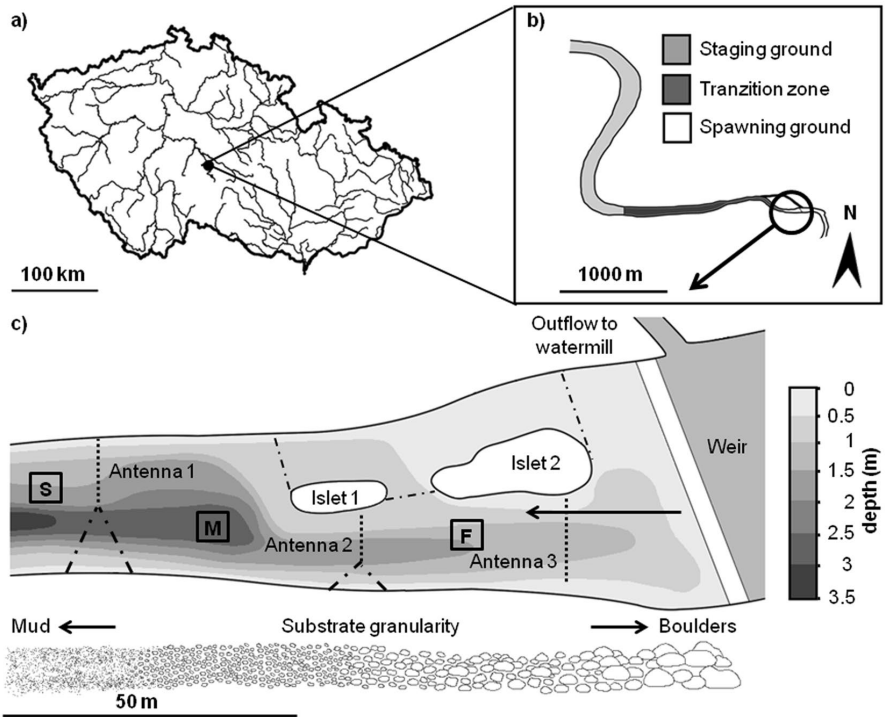
Figure 1. Schematic representation of the position of Želivka Reservoir in the Czech Republic (**a**), approximate position of staging ground and transition zone trough which fish migrate (**b**), and detail of monitored spawning ground with the experimental setup (**c**). Placement of antenna systems is indicated by dotted lines. Antennas 1 and 2 were deployed in 2015–2016 and the additional antenna 3 in 2016. Nets guiding asps into antennas are indicated by dot-dashed lines. Squares S, M and F represent sites for asp egg monitoring in 2016 where 4 tiles were placed at each site (S - slow, M - medium and F – fast current). Arrow shows the direction of flow. The figure was generated by the software ArcMap, version 10.2.2[53].

Variable	Season	Mean		Median		Q₁		Q₃		Wilcoxon	N	P
		M	F	M	F	M	F	M	F			
Detections per hour	2015	27	15	18	15	14	12	22	17	1640.0	150	<**0.001**
	2016	55	25	28	25	22	19	46	30	6342.0	272	<**0.001**
Hours	2015	44	17	33	17	16	11	70	23	1331.5	150	<**0.001**
	2016	67	17	53	14	31	9	90	22	1928.0	272	<**0.001**
Days	2015	10	3	9	3	6	2	14	4	798.5	150	<**0.001**
	2016	11	3	10	2	7	2	14	3	1563.0	272	<**0.001**
Arrival day No.	2015	7	13	6	15	2	7	13	17	3794.0	150	<**0.001**
	2016	14	19	14	17	11	16	16	20	13154.0	272	<**0.001**
Departure day No.	2015	20	16	19	17	17	16	22	18	1460.5	150	<**0.001**
	2016	27	22	27	20	22	17	33	24	4788.0	272	<**0.001**

Table 1. Summary of movement at the spawning ground, seasonal protandry and times of leaving in the 2015 and 2016 seasons. Mean values, medians, first (Q_1) and third quartiles (Q_3), values from the one-sided Wilcoxon rank-sum test, sample sizes (N) and p-values are presented in the table. Significant p-values are given in bold.

surplus paternity can be achieved only by mating with subsequently acquired females during the season or via extra pair paternity[37, 38]. In our study, high male investments into reproduction are demonstrated by their length of stay and by their greater movement at the spawning ground.

Over the course of the spawning season, asp males preceded females by five days on average in their seasonal arrival time and left four to five days later. Because fish sex can only be ascertained with certainty during the
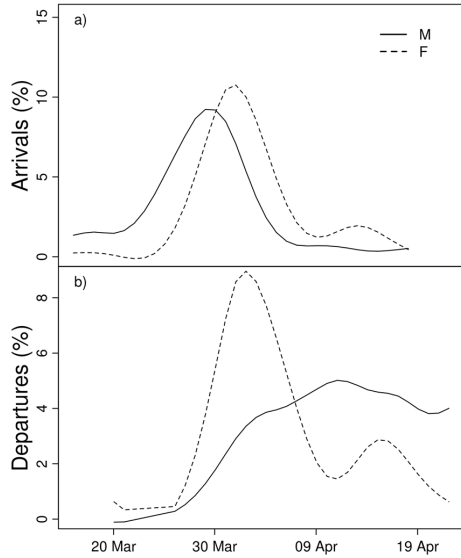
Figure 2. Arrival (**a**) and departure dates (**b**) in 2016 plotted for females (F) and males (M) separately. Lines in panels (**a**) and (**b**) represent the percentage of arriving and departing individuals in a given day. Raw data are provided in Supplementary Fig. S1.
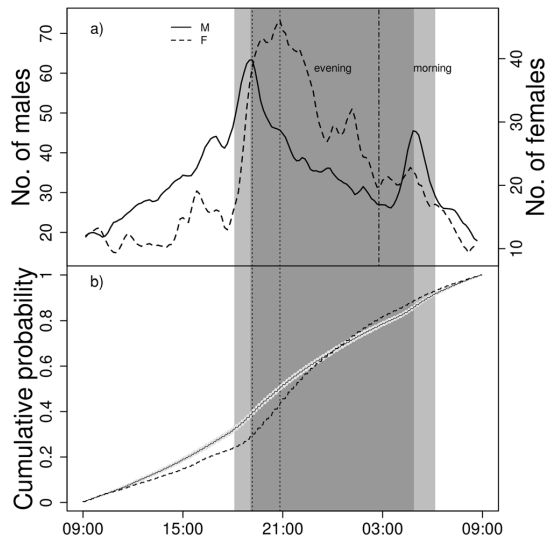

Figure 3. The male and female presence on the spawning ground in 24-hour cycles starting at the lowest observed attendance (**a**), and the rank envelope test comparing general fish presence with female presence at the spawning ground (**b**). In the upper panel, the weighted male and female curves were standardized to the same height for better comparison. Moving average with 30-minute interval was used for the plot. Male and female peaks of presence are indicated by dotted lines. Division between morning and evening period defined as points with the lowest abundance is indicated by dot-dashed line and by starting time of the plot. In the lower panel, any departure of the data from the global envelope proves the rejection of the null hypothesis that the female curve is the same as male curve. The light grey area demonstrates the shift between sunset and sunrise during the monitoring period.
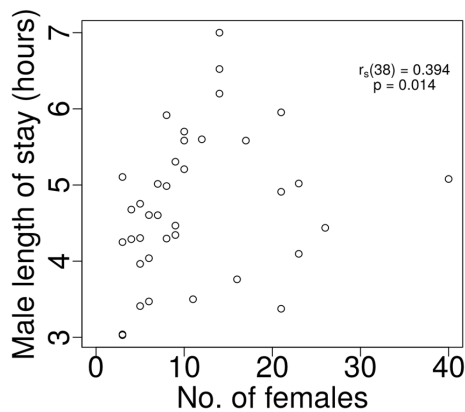
Figure 4. The relationship between male length of stay and the number of females on the spawning ground in the evening spawning period.

spawning season[39], individual fish were tracked in the spawning season following tagging to avoid any influences of electrofishing and manipulation on their spawning behaviour. Although this enabled us to precisely analyse the sex-specific differences in both seasonal and daily arrival times, the analyses are biased towards experienced individuals as their first spawning season could not be monitored because of the nature of our methodology. Young individuals may not time their arrival to the spawning ground as precisely as experienced fish, as has been demonstrated in pike, *Esox lucius*[40]. The tagging of juveniles and subsequent sex determination based on behaviour at the spawning ground may shed light on any differences between inexperienced and experienced spawners.

The longer period of activity at the spawning ground by males should favour protandry, as has been assumed in a model for salmon[41]. This requirement of protandry is fulfilled in the asp mating system, in which males stay at the spawning ground for 10 days on average, which is three times longer than females stay. Male departure is most likely determined by exhaustion and a lack of milt. We do not think that protandry in asps is driven by the differential susceptibility hypothesis, which postulates that the larger sex arrives first due to its greater resistance to harsh environmental conditions[42]. Asp males are generally smaller than females (see the lengths of tagged males and females in the methods section), and hence, the larger female body size would predict that females, not males, should arrive earlier in the spawning season. Instead, we believe that sexual selection drives the male arrival strategy and their length of stay at the spawning ground.

Research on seasonal protandry has provided evidence that this phenomenon may positively affect male fitness in several ways. Protandry may result in a higher number of sired offspring because early-arriving females are commonly in prime body condition and may produce more offspring than late-arriving females[11, 43]. In mating systems where pairs are formed early in the season, the early-arriving male may form more than one pair in the mating season[21] or may achieve more offspring via extra-pair paternity[37]. Additionally, early-acquired offspring may have higher chances of survival, possibly giving the offspring higher chances of being recruited into the breeding population[44]. Furthermore, greater seasonal protandry should be favoured when female arrival is protracted[41]. This was indeed the case in our study, which showed that the spawning season lasted for approximately one month in both years, while the average female spent only three days in the spawning ground. However, we do not have individual mating success data, and hence, further research is needed to reveal the consequences of early seasonal arrival for individual males.

Protandry may have positive effects on fitness not only seasonally but also daily. In Dawson's burrowing bees, males are active early in the day and search for later-emerging females[45]. In the asp mating system studied here, the proximity of standing water and the requirement of a fluvial spawning ground presents an energetic trade-off, which likely results in daily migration between the spawning ground, with its associated high energetic costs, and the staging ground located in the standing water, with its lower associated energetic costs. Males arriving earlier to the spawning ground may encounter more opportunities to mate with newly ovulating females. Although we have no data on the duration of ovulation in females, many females spent very little time in the spawning ground, suggesting that this period was most likely very closely related to the actual female spawning time. Hence, it would indeed be advantageous for males to arrive before females on a daily basis. Our data demonstrate that males arrived earlier in the evening peak of asp spawning activity, and males stayed longer in the morning than females. Although the daily data may suggest a certain mismatch between male and female presence, we have to emphasize that the actual estimated number of females never exceeded the number of males at the spawning ground.

The daily pattern of fish presence, with evening and morning peaks, may be driven by internal mechanisms similar to those found to synchronize spawning in goldfish *Carassius auratus* reviewed in Sorensen & Wisenden[36]. In the goldfish mating system, fish are reported to spawn from morning to noon, whereas asp spawning activity was observed throughout day and night, with an uneven number of spawners and with the highest effort around sunset and sunrise. Asp females spent an average of six hours at the spawning ground and paid it three visits, possibly temporarily running out of eggs ready for ovulation during each visit. The relative synchrony of these

female visits seemed to create the distinct peaks of female spawning activity. Hence, male timing should take into account the high number of mating chances at these hours but should also consider the other periods of the day where there are more limited chances to mate.

While males adjusted their length of stay according to the number of females in the spawning ground, the actual increase in time was not very large and was only significant in the evening period. We assume that males were limited by milt storage in their sperm ducts during the periods with many females due to excessive mating and eventually returned to the staging ground to restore the necessary amount of milt[46].

Because fish capture was strongly dependent on their reproductive activity (fish captured in the staging ground were present or future active spawners), our day-to-day catches reflect the operational sex ratio better than the adult sex ratio in the asp population. Due to the very short-term presence of females compared to that of males, the adult sex ratio could be more reliably assessed only based on recaptures in the following spawning season. Hence, the adult sex ratio could be estimated only for 2014 and 2015, when it was 0.47 and 0.42, respectively[32]. However, females are likely to skip a spawning season[9, 47], which may consequently cause the overestimation of the number of females in the system due to their relatively lower recapture rate compared with that of males. For these reasons, we instead used the operational sex ratio in our analyses.

Conclusions

In animal species in which the male has a higher potential reproductive rate and does not provide any parental care, it is the male who has to invest more energy in courting behaviours. Our study presents field evidence of the seasonal and daily aspects of protandry in asps. To our knowledge, this study provides the first evidence of daily protandry in a vertebrate, the extent of which seems not to be driven by changes in the operational sex ratio at the breeding ground.

Materials and Methods

Study site. The study was conducted in the largest water-supply reservoir in Central Europe, Želivka Reservoir, which is located at 49°57′84″ N, 15°25′16″ E in the Czech Republic. The reservoir has one large inlet, the Želivka River. A weir located on the river just above the reservoir blocks further upstream migration and restricts asp spawning to a 100-m long and, on average, 22-m wide stream site (Fig. 1). The bottom of the transition zone between the standing water and the upstream spawning site consists of clay deposits that are unsuitable for asp spawning and egg development.

Fish capture, tagging and migration recording. Individual spawning asps were captured at the staging ground using an electrofishing boat (electrofisher EL 65 II GL DC, Hans Grassel, Schönau am Königsee, Germany, 13 kW, 300/600 V) from 26 March to 3 April 2014 and from 4 to 11 April 2015. The fish were anaesthetized with benzocaine, and their total length (TL), sex and weight were recorded. The males were distinguished based on milt release during the tagging period, breeding tubercles and a slender body, whereas females had no tubercles, a robust body and predominantly released eggs at the end of the tagging period. After removing 3–4 scales, a 4–5 mm vertical incision was made 3 cm posterior to the pelvic fin, and a PIT tag (Oregon RFID, half-duplex, length 32 mm, diameter 3.65 mm, weight 0.8 g, ISO 11784/11785 compatible) was inserted into the body cavity. No sutures were used to close the incision, according to a previously described cyprinid tagging methodology[48, 49]. The tagged individuals were released immediately after recovery from anaesthesia. Altogether, 221 males (597 mm TL ± 53 mm SD) and 135 females (635 ± 75 mm) were tagged in 2014, and 212 males (565 ± 42) and 181 females (595 ± 62) were tagged in 2015. The tagged individuals represented a substantial part of the mature asp population in the studied reservoir; the population size of spawning individuals, based on mark-recapture estimates, was 979 males and 1107 females in 2014[32].

The movements of the tagged individuals were monitored via passive telemetry using Oregon RFID antenna systems (LF HDX RFID readers). The PIT tag is energized each time a fish passes the antenna and then emits an individual code that is recorded and stored together with the date and time. Two (three in 2016) RFID antennas were installed in the main tributary of the Želivka Reservoir 50 m apart (Fig. 1), and their time settings were synchronized. The first and second antenna systems were installed in the downstream and middle sections of the spawning ground, respectively, and in 2016, a third antenna was installed in close proximity to the weir to increase the coverage and probability of fish detection at the spawning ground. The river topography allowed us to only cover half of the river, so we guided the asp to swim through the 10-m wide antennas using nets with a 40-mm mesh size (Fig. 1). Fast currents prevented the installation of guiding nets and their attachment to the third antenna, so one guiding net was attached to the island only, and hence, fish could swim close to the left bank of the river without being detected. The system recording frequency was set to 10 energize/receive cycles s^{-1}, and the systems were tested daily to ensure that they were scanning their entire detection range. Monitoring began prior to the beginning of asp spawning (25 March 2015 and 16 March 2016) and continued until the majority of the asp disappeared from the spawning ground (21 April 2015 and 22 April 2016).

Monitoring of asp eggs. We monitored egg density on standardized concrete tiles (Fig. 1) to delimit the size of the spawning ground in 2016. A hole was drilled in the centre of each tile, which was then attached to a rope with a float to facilitate monitoring. At three sites in the spawning ground, a total of 12 concrete tiles (40 × 40 cm) were deployed. The surrounding spawning substrate consisted of large stones and pebbles. The depth and length profile was measured at each site, and water flow was estimated as fast (34 ± 9 cm s^{-1}), medium (27 ± 7 cm s^{-1}) or slow (16 ± 4 cm s^{-1}) for each egg monitoring site based on the daily total water flow data recorded by the river authority (Povodí Vltavy). The tiles were checked daily for new eggs at 8 am and 7 pm, and eggs were removed after being counted.

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The field sampling and experimental protocols used in this study were performed in accordance with the guidelines of and with permission from the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic (Ref. No. CZ 01679). All methods were approved by the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic.

Data analysis. *Monitoring coverage of the spawning ground.* We first tested whether asps had to pass at least one antenna to reach the main spawning ground. The counts of new eggs from the three monitoring sites (Fig. 1) were used as a sign of the extent of the spawning ground, and the total number of eggs on each tile was used for the statistical analysis. Among-site comparisons were then performed using a generalized linear model assuming that egg numbers followed a Poisson distribution. The logit link was used in the Poisson model.

Seasonal protandry, day of leaving and movement at the spawning ground. Fish that were tagged and detected in the same season were excluded from the analysis due to a possible negative influence of the tagging procedure on their behaviour. Hence, fish tagged in 2014 were analysed in the spawning seasons of 2015 and 2016, and fish tagged in 2015 were analysed in the spawning season of 2016. Each fish recorded by the antennas was defined as a potential spawner. A properly installed antenna may achieve $97 \pm 1.5\%$ detectability of 32-mm tags, where missed detection may be the result of a high swimming speed and swimming in a group, or a parallel orientation of the tag to the antenna[50]. Therefore, it is possible for fish to leave the spawning ground without being detected lastly by antenna 1. Hence, we used merged detections by all antennas as a proxy for individual presence at the spawning ground and to avoid the misinterpretation of sequential records from antennas. Thanks to their active mating system where the fish frequently swam over the entire range of the spawning ground and do not occupy territories[32], fish were frequently recorded by antennas when considered as present (see results above).

In the seasonal analysis, we tested for sex-specific differences in spawning effort and specifically whether males exhibit greater mate-finding effort and are seasonally protandric by analysing the follow variables: the detection frequency per hour, the hours and days detected at the spawning ground, and the first and last day of detection (on a scale, using the number of monitoring days in a given year). To simplify the tested variables, a single detection in an hour/day was defined as sufficient to count the fish as present in the given hour/day. The detection frequency per hour was calculated from the hours in which the individual was detected at the spawning ground. Statistical comparisons were conducted using a non-parametric one-sided Wilcoxon rank-sum test. Bonferroni corrections were applied to the estimated p-values.

Daily protandry, time of leaving, male length of stay and operational sex ratios. Daily protandry was defined as an earlier daily presence of males, on average, compared with the presence of females. To test the difference between male and female timing, we used the fish presence data. Individual fish was considered as present on the spawning ground when the time between two subsequent detections did not exceed one hour. A rank envelope test was used to identify the difference between male and female presence distributions[51]. A rank envelope test consists of computing simultaneous global envelopes for every functional value. Any departure of the data from the global envelope proves the rejection of the null hypothesis under global significance. Here, the null hypothesis was that both presence functions would follow the same distribution.

Asp presence had a clear bimodal pattern around sunset and sunrise (Fig. 3). Hence, we defined the two main spawning periods (peaking at sunset and sunrise) for use in GLM tests as the periods between the two points with the lowest abundance. Within these two periods (further referred to as evening and morning according to the peaks of activity), the times of arrival and leaving were defined as the times of first and last detection within the period range, respectively. Because migration peaked around sunset and sunrise and the day length changed during the spawning season, we expressed the arrival and leaving times as the difference from the time the sun set (sunrise for the morning peak). To test whether male length of stay depends on the number of females in the spawning ground, we used a Spearman rank-order correlation coefficient in order to eliminate the effect of distant measurements and to avoid the normality assumption. The number of hours that individual males were present was related to the number of females present in a given daily period. The analysis was performed separately for the evening and morning periods. In this and subsequent analysis, we used a subset of data with at least three females in the tested period to analyse a relatively representative estimate of reproductive period.

Finally, we tested whether the degree of protandry (differential value between individual male and average female arrival times) depends on the operational sex ratio in a given period using a GLM analysis, where fish ID was included as a random variable. Protandry was calculated for each male as the difference between his arrival time and the mean female arrival time in a given period. For the computation of operational sex ratio, we assumed that all fish present on the spawning ground are ready to mate. Operational sex ratio was computed as the fraction of the number of males and females present on the spawning ground within the given day period where these numbers were first corrected for the unequal number of tagged males and females arrived over the season and sex ratio in the population.

Statistical analyses were performed using Statistica software (Statistica, Inc., StatSoft, Tulsa, Oklahoma, USA) and R software version 3.2.3[52].

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Author Contributions

M.Š., L.V., L.V., R.B., Z.S., P.B., I.V. and J.K. participated in the field work. M.Š. and L.V. designed the study. D.R., T.M., M.P. and M.Š. conducted the statistical analysis. M.Š. wrote the first draft. All authors contributed substantial feedback during manuscript preparation.

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Seasonal and daily protandry in a cyprinid fish

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Supplementary figure 1: Arrival (a) and departure date (b) in 2016 plotted for females (F) and males (M) separately. Lines in panels (a) and (b) represent the percentage of arriving and departing individuals in given day. Open triangles and circles represent individual data points, from which lines were derived.

Paper IV

Early life-history predator-prey reversal in two cyprinid fishes

SCIENTIFIC REPORTS

OPEN

Early life-history predator-prey reversal in two cyprinid fishes

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Predator-prey relationships are often perceived simply as a situation in which a predator enhances its own fitness while reducing the fitness of its prey. However, this relationship may become reversed when the prey feeds on the juvenile predator stages. We investigated this phenomenon in a model asp (*Leuciscus aspius*; predator)-bleak (*Alburnus alburnus*; prey) relationship. The adhesive asp eggs are available for bleak predation after a spawning event for only tens of seconds before they adhere to the stones, where bleak do not forage. Gut content analysis demonstrated that eggs were utilized in high quantities, especially in the spawning peak of the asp reproductive season. Furthermore, using underwater video, we recorded the bleak feeding efficiency on naturally drifting asp eggs as the percentage of eggs eaten. Within the 40 cm egg trajectory captured by our cameras, total egg mortality was 21.2 ± 2.2% on average. The highest survival chances occurred among eggs drifting in aggregations, since the short drifting distance together with their aggregated distribution satiated bleak and part of the egg aggregation could attach to the spawning ground. This study emphasizes the potential efficiency of predator egg utilization by prey, which may have further consequences in predator-prey dynamics.

Ectotherm vertebrate predators inhabiting aquatic environments commonly utilize the food web over a wider range throughout their lifetime compared to endotherms, since their initial size is usually much smaller and their growth is indeterminate[1–3]. Their vertebrate prey is frequently omnivorous, foraging on small organisms[4]. If the prey of such species is able to utilize predators' early life stages, such as eggs and larvae, the predator-prey relationship may become reversed, and predators may be foraged upon by large prey individuals[5]. Since prey is naturally numerous, a considerable proportion may be consumed, especially if a large part of the prey population forages on the early life stages of predators[5–7]. Considering the small-sized eggs and larvae available in enormous quantities in many fish groups, some prey individuals may consume more predators in their lifetime than an adult predator consumes its prey[8–10].

The occurrence of predator eggs and larvae is commonly restricted to a short post-spawning period, which is only when such a reversed situation may occur. However, the easy accessibility of this energetically rich food source may lead to a complete switch from the common diet of the prey to predator eggs and offspring, especially when reproduction occurs early in the season when other food sources are scarce[5, 11, 12].

In comparison with the long-term dependence of predators on prey availability in predator-prey systems, the short-term predator egg or larvae consumption by prey species likely does not represent a very important food source for the prey. Hence, its absence or low availability would not cause bottom-up limitations in prey populations[13]. This means that the size of the prey population is independent of the egg food source; however, prey may utilize it to a large extent[14, 15]. This can escalate into a positive loop in which high prey abundance may exert high consumption pressure on the early life stages of a predator, which consequently lowers predator recruitment. Hence, numerous prey foraging on their predator may eventually cause considerable changes in the predator-prey dynamics, keeping the predator population low in abundance or triggering more pronounced fluctuations of predators in time[5, 16, 17].

Contradicting the simple definitions of predator and prey roles in ecosystems, predator-prey reversal was discovered among many species in recent decades[14, 18, 19]. In marine benthic communities, rock lobsters, *Jasus lalandii*, usually represent a keystone predator in diverse ecosystems. However, if its common prey, the whelk (*Burnupena* sp.), greatly outnumbers the rock lobster, it may result in an alternative stable ecosystem where rock
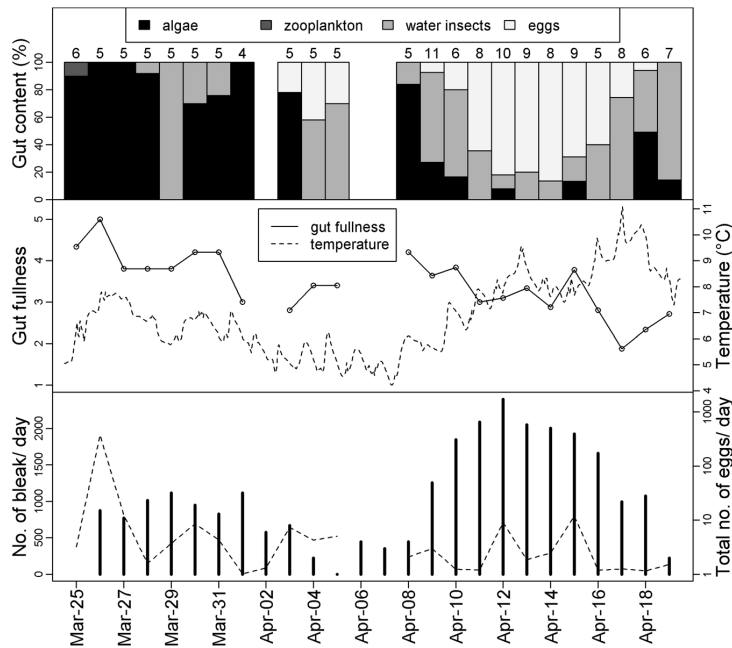
Figure 1. The gut content analysis of bleak (upper and middle panel) and electrofishing estimates of number of bleak within sampling transect (lower panel) in 2015. The numbers above the upper histogram represent the sample size for gut content analysis on a given day. Gut fullness is displayed in the middle panel on a scale of 0–5, with a value of 0 representing empty and a value of 5 fully distended. The temperature is shown by the dashed line. Bottom panel displays the total number of eggs (collected from 12 monitoring tiles, 40×40 cm, log 10) and number of bleak in the monitored transect (dashed line).

lobsters are killed by whelks, which dominate in the alternated ecosystem[14]. Wizen & Gasith experimentally demonstrated how larvae of the ground beetle *Epomis* sp. lure and kill their anuran predator, which otherwise feeds on many insect species[20]. It is likely that predator-prey reversal is most common in aquatic environments, where tiny eggs of many predator fishes are vulnerable to predation by other fish species and invertebrates[21–24]. This phenomenon is potentially very common among fishes and has possible repercussions in commercially harvested waters as well as in protected species ecology.

To investigate predator-prey role reversal, we used a simple model system of two fish species, the asp (*Leuciscus aspius*; predator) and bleak (*Alburnus alburnus*; prey), which are common cyprinid fishes inhabiting inland waters of Central and Eastern Europe[25, 26]. The asp is a long-lived, large, iteroparous predator, migrating to fluvial spawning grounds in the early spring, where it spawns above the open substrate and provides no parental care to its eggs and larvae[26, 27]. The negatively buoyant adhesive eggs are approximately 2 mm in diameter; they are spawned near the water surface and carried by the water current until they adhere to cobbles and stones on the river bottom[27]. Asps do not forage on prey fishes during the spawning season, and first attacks are observed shortly after the termination of spawning[28]. The bleak is a small, short-lived species with a superior mouth position, feeding preferably on emerging insects and zooplankton (in standing water) near the water surface[25, 29, 30]. Adult bleak are oftentimes foraged upon by asps, being their most common prey item[25, 31, 32].

In the present study, we investigate predator-prey role reversal in the asp-bleak relationship. Specifically, the goals of this study are to (I) analyse whether and to what extent predator-prey role reversal occurs in the asp-bleak relationship by stomach content analysis, (II) analyse whether egg consumption is dependent on bleak size, (III) establish the relative effectiveness of egg consumption using underwater cameras and (IV) model the relationship between egg survival probability and the batch size of drifting eggs using underwater camera data.

Results

Gut content analysis demonstrated that asp eggs were an important food source for bleak. During the sampled period, 64 out of the 147 caught bleak individuals contained eggs, with a $31 \pm 42\%$ (mean $\pm$ SD) contribution to the total amount of food. Other important food sources in this period were water insects (65 individuals, $34 \pm 44\%$), filamentous algae (54 individuals, $34 \pm 46\%$) and zooplankton (two individuals, $1 \pm 3\%$). The proportion of eaten eggs was independent of bleak size (GLM F $= 1.29$; df $= 1$; p $= 0.257$), and eggs prevailed, especially when their abundance in the environment was high (GLM F $= 8.54$; df $= 22$; p < 0.001 Fig. 1). Gut fullness was independent of bleak size (GLM F $= 0.23$; df $= 1$; p $= 0.635$), with a significant effect of the date (GLM F $= 1.74$; df $= 22$; p $= 0.031$; Fig. 1).
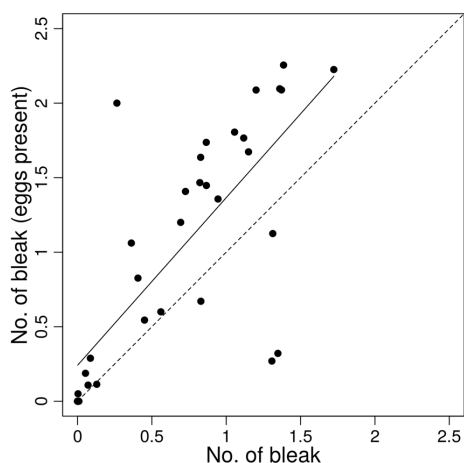
Figure 2. The relationship between the mean number of bleak in camera view during a single video recording and the mean number of bleak in camera view during the same recording when eggs were present in camera view. Dashed line indicates 1:1 ratio, solid line represents trend line and dotted lines represent 95% confidence interval.

		No. of bleak				
		1	**2**	**3**	**4**	**5**
No. of eggs	1	0.574	0.469	0.367	0.275	0.199
	2	0.576	0.471	0.368	0.276	0.200
	3	0.577	0.472	0.369	0.277	0.201
	10	0.587	0.482	0.379	0.286	0.208

Table 1. Examples of survival probability of individual eggs computed from the logit model for different numbers of bleak ranging from one to five combined with one, two, three and 10 drifting eggs.

One-tailed paired t-test between the average number of bleak and the average number of bleak when at least one egg was drifting in the camera view indicates that bleak were more numerous in the camera view when eggs were drifting (mean $\pm$ SD: 0.69 $\pm$ 0.53 vs. 1.01 $\pm$ 0.80; t (33) = 3.55, p < 0.001; Fig. 2). According to the binomial model, total egg mortality was 21.2 $\pm$ 2.2% and bleak foraging efficiency was 53.6 $\pm$ 3.2% - estimated from recordings when at least one bleak was present in the camera view (within the 0.42 m detection distance of our cameras).

Our logit model, which considers only recordings when eggs were drifting and at least one bleak was within view of the camera, reveals a high probability of eggs being consumed by bleak. The effect of the number of bleak in the model is significant (Wald test χ^2 (N = 1509) = 73.6; p < 0.001). An increasing number of drifting eggs significantly decreased the probability of being eaten for an individual egg, implying an advantage of aggregation (Wald test χ^2 (N = 1509) = 102.3; p < 0.001; Table 1; Fig. 3).

Discussion

In this study, we demonstrated that, due to high foraging efficiency, the reversed predator-prey relationship in the studied case may possibly lead to a considerable reduction in the number of eggs surviving until attachment to the substrate (i.e., first tens of seconds in the stream). The gut content analysis revealed that eggs represented a considerable part of the bleak diet in the studied period, especially during the highest spawning activity of asps. From our video records, the bleak egg-foraging efficiency was very high when eggs passed through the camera view, suggesting considerable predation pressure on the predator's eggs by its prey. Eggs drifting in dense aggregations had a higher probability of survival than those drifting alone or only in small aggregations.

While fish predation on fish eggs has been previously described in many cases (e.g. refs [17], [21], [33] and [34]), this seems to be, to our knowledge, the only study in which adhesive eggs are utilized immediately after their release by female fish, before their attachment to the substrate. Commonly studied systems are pelagic or benthic environments, where eggs may be utilized by an egg-eating organism throughout the egg development period[22, 34–36], and hence the foraging fish may consume an enormous part of the egg production[15, 37]. Despite the short time available, bleak have been shown to utilize the eggs of its predator to a considerable extent. In 2016, the monitoring site was shifted lower due to the presence of air bubbles near the weir, and hence some of the eggs spawned below the weir could have adhered to the substrate before reaching the monitoring site. Hence, we cannot accurately estimate the total predation pressure on asp eggs by bleak, as it is very likely that their survival in the drifting stage may be higher in the uppermost part of the spawning ground, where the drifting aggregations may be
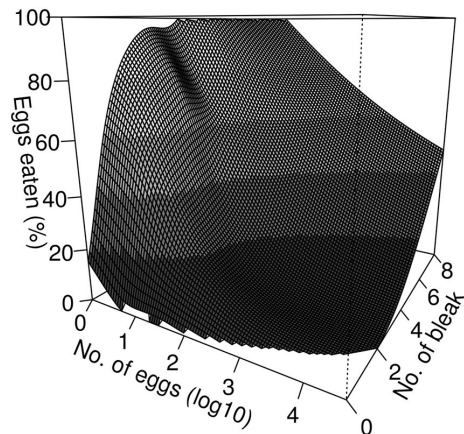
Figure 3. The 3D visualization of the relationship among number of eggs (log 10), number of bleak and percentage of eaten eggs. Shades of grey indicate modelled percentage of eaten eggs in the given scenario (the lowest number of eaten eggs – dark grey, the highest – light grey).

denser. We assume that adhered eggs are probably safe from the predation of bleak, which are adapted to foraging near the water surface and do not utilize benthic food sources[25, 29, 30]. We never observed sand or gravel in the bleak gut content analysis, which would otherwise indicate utilizing eggs that had adhered to the substrate (eggs were oftentimes covered with sand particles on monitoring tiles).

In our model, we demonstrated the advantage of drifting in dense aggregations, where there is a higher survival probability for individual eggs. Due to the short drifting period of asp eggs (tens of seconds), aggregation may minimize the individual chances of being chosen by bleak[38–40].

Female asp specific fecundity is somewhere between 27,500 and 58,500 eggs per kg of body weight, and females appear to release large quantities of eggs in each spawning event[41]. Despite the observed advantage of drifting in aggregations, most of the eggs were recorded singly, and if they appeared simultaneously, it was in very low numbers. They were likely spawned further upstream from the camera position and were diluted by the current before reaching our monitoring site. Alternatively, it is possible that some of those eggs were not actually freshly spawned but eggs that had been detached from the substrate by the strong water current. Those eggs may not be able to settle in a suitable fluvial environment due to the deactivation of the sticky glycoprotein layer with time, and hence they might not survive even if not consumed by bleak[42]. However, we could not discern between detached eggs and scattered freshly spawned eggs in our video records. Given the circumstances, the impact on recruitment may not be as severe as it appears from the bleak egg-consumption efficiency, since some of the eggs may be considered lost when detached from their optimal substrate. On the other hand, the total drifting distance before adhesion to the substrate will influence egg survival. Our estimate of egg survival was computed for the short drifting distance in the camera view, while the actual drifting distance may be several metres[28]. Hence, the proportion of eaten eggs could be considerably higher.

Although we have been counting the number of bleak in the monitored transect in 2015, bleak number seemed to not be strongly related to the egg availability inferred from the monitoring tiles (e.g. high egg abundance did not correspond with high bleak abundance). We believe that this discrepancy may be caused by the transect location: to protect the main spawning site, electrofishing was performed in the lower part of the tributary. Hence, the large number of bleak visually observed in the uppermost part could not be reached by electrofishing.

While we assume that the consumption of asp eggs is necessarily influencing recruitment, it may actually not be so straightforward[9]. Once entering the feeding regime, the early life stages may be limited by density-dependent processes, and the bleak-consumed part of the eggs may not influence actual recruitment, since the survivors may have higher food abundance due to decreased competition and hence higher condition, growth rate and survival[43]. Especially if we consider as successful reproduction offspring that actually reach the reproductive age (4–5 years in asps)[28], there may not be a difference between bleak-limited and bleak-non-limited recruitment. However, a case study of reproductive success in the common carp, *Cyprinus carpio*, in a lake system in the Mississippi river basin demonstrated that small omnivorous fish effectively control carp recruitment by feeding on eggs and larvae. Carp recruitment was high only in lakes where small omnivorous fish were nearly absent due to winterkills by hypoxia[44]. Similar to our study, a large number of eggs was found in stomachs of egg-feeding species, and eggs disappeared from the spawning ground due to excessive egg-feeding by omnivores[37].

Although the observed predator-prey reversal in the asp-bleak relationship was demonstrated in only one ecosystem, it is likely a very common phenomenon due to the reproductive migration of both species. Asp migration to the fluvial spawning ground is shortly followed by bleak migration into similar spawning grounds, where it spawns few weeks after the asp[45]. Hence, the first bleak migrants into the stream encounter an abundance of fat-rich food represented by asp eggs[46].
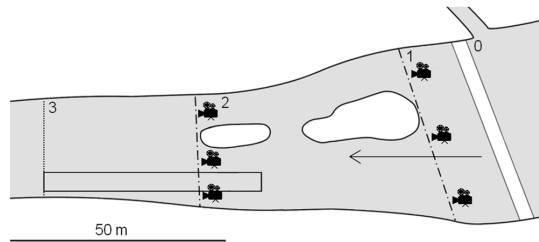
Figure 4. Schematic representation of the spawning ground located downstream of the weir. The space between the weir (0, solid lines) and the dotted line represents the estimated spawning ground[27]. Electrofishing was performed within a 50 m transect visualized by the rectangle. Dot-dashed lines represent position of video monitoring sites for 2015 (site no. 1) and 2016 (site no. 2). Within each site symbolized by camera illustration, three cameras were placed randomly. At sites no. 1, 2 and 3, water velocity was computed using daily mean water discharge data. Four monitoring tiles were deployed at each site, and eggs were counted and removed daily. Arrow shows the direction of flow. The figure was generated by the software ArcMap, version 10.2.2[51].

To conclude, we demonstrated in this study a very high prey foraging efficiency on predator eggs, which may have further consequences on the predator-prey dynamics. This study seems to be the first describing the impacts of egg-eating predators on the eggs of lithophilic spawners before adhesion to the substrate. Further, we provide a basic model showing that in this predator-prey reversed relationship; egg aggregation provides survival benefits for individual eggs. We believe that future research should focus on the assessment of the damage done to asp eggs by bleak and the benefits for prey species. In addition, and most importantly, does the reversed predation influence predator recruitment and to what extent?

Materials and Methods

Study site.   The study was conducted in the main tributary of the Želivka Reservoir, located at 49°578497′ N, 15°251671′ E, Czech Republic. The reservoir is 39.1 km long with an area of 1602 ha at a maximum water level of 381.7 m above sea level. Approximately 2000 asps migrate from the reservoir to spawn in the early spring (mid-March to mid-April) and release a vast amount of eggs and milt into a 100 m long tributary within a one-month long spawning period[28, 47]. Further upstream, the migration is restricted by a weir, and downstream, spawning is limited by an inadequate water current and the substrate composition; hence, spawning along with egg predation can be monitored within the short study site[27]. Thousands of bleak individuals were repeatedly observed in the tributary at the peak of the spawning period. This seems to be the most abundant egg-eating species in this system[28]. Hence, we focus here on this species, although other potential egg-eating species were also observed (see bleak electrofishing below).

Bleak electrofishing and gut content analysis.   Electrofishing was performed from 25 March to 19 April 2015 daily at 3 p.m. by an electrofishing boat (electrofisher EL 65 II GL DC, Hans Grassel, Schönau am Königsee, Germany, 13 kW, 300/600 230 V). Due to rainy weather conditions, sampling was not conducted on three days (2, 6 and 7 April) due to safety reasons. A 50 m long and 6 m wide transect was sampled (Fig. 4). Electrofishing was not performed further upstream in order to not damage the major spawning ground undergoing long-term monitoring.

Due to the fragile nature of bleak and the long-term sampling schedule, we decided to only count bleak to avoid damaging a large number of egg-eating individuals by hand nets (bleak scales are very easy to remove during handling). The number of bleak and other omnivorous fish species was estimated each day within the defined transect. In total, 9,589 bleak, 133 roach (*Rutilus rutilus*) and 76 bream (*Abramis brama*) were recorded. A subsample of bleak within the transect (5–11 individuals) was measured (nearest mm), weighed (nearest 0.1 g) and collected for gut analysis (standard length 108 ± 14 mm, weight 13.9 ± 6.0 g, mean $\pm$ standard deviation (SD)) during every electrofishing day (total sample: 147 bleak individuals). Fish were anaesthetised using a lethal dose of MS–222 and immediately processed in the field laboratory. A gut fullness index was assigned to each individual fish on a scale of 0 to 5; 0 indicating empty and 5 fully distended[48, 49]. Gut content was classified into four food categories: asp eggs, filamentous algae, water insects and zooplankton. The relative volume of each food category was estimated visually as a percentage of the total gut content volume of each gut.

Monitoring of asp eggs.   We monitored the egg density on standardized concrete tiles (Fig. 4) to assess the main asp spawning period. A hole was drilled in the centre of each tile, which was then attached to a rope with a float to facilitate monitoring. At three sites on the spawning ground, a total of 12 concrete tiles, 40×40 cm, were deployed. The surrounding spawning substrate consisted of large stones and pebbles. The tiles were checked daily at 7 p.m. for new eggs and were cleaned after eggs were counted.

Egg detection efficiency in video records.   In order to reveal whether an observer is able to detect all eggs drifting in the camera view, an experiment was conducted on the studied site from 8 to 9 April 2017. Random number of asp eggs (0–35) was released upstream from the camera position so the eggs drifted in the camera view. Altogether, 100 replicates were performed. Egg detection efficiency was evaluated using double-blind

methodology. The observer of the video had no prior knowledge of the number of released eggs and the same person analysed all records obtained in this study. Egg detection efficiency was estimated to be $98.05 \pm 4.40\%$ based on a comparison of counted vs. released numbers of asp eggs.

Video monitoring.   Asp spawning activity was visually observed each day and inferred from the number of eggs on the monitoring tiles. Three underwater cameras (Epoque Edivecam, Epoque World Co., Ltd., Japan) were randomly placed around monitoring sites oriented downstream to record bleak foraging on days when bleak presence at the spawning ground was visually observed and asps exhibited high spawning activity (10–12 April 2015 and 5–11 April 2016, Fig. 4). Cameras were mounted on the holder with weights and their detection range covered approximately the upper two-thirds of the water column. The distance at which bleak and eggs could be identified was 42 ± 3 cm (mean $\pm$ SD, estimated for each recording day). Recording occurred between 1 and 3 p.m. Altogether, three underwater videos (total time 02:51:45 (hh:mm:ss)) were recorded in 2015, and 31 videos (total time 29:40:45) were recorded in 2016. Our methodology was limited by air bubbles originating from the presence of the weir in 2016, which prevented the use of cameras directly below the weir, and in 2015 due to higher water discharge (Fig. 4).

Water temperature and discharge.   Along with fish monitoring, two data loggers (TidbiT v2, Onset, USA) measuring water temperature were placed in the tributary and recorded the temperature with hourly frequency in 2015 and 2016. To demonstrate slowdown of the water current contributing to the egg adhesion to the substrate, we computed water discharge for three consecutive sites along the spawning ground profile. The water velocity was estimated to be 22 ± 6 cm s^{-1} and 34 ± 9 cm s^{-1} (mean $\pm$ SD; 2015 and 2016 seasons, respectively) at the first site, 18 ± 5 cm s^{-1} and 27 ± 7 cm s^{-1} at the second site and 11 ± 3 cm s^{-1} and 16 ± 4 cm s^{-1} at the third monitoring site (Fig. 4). The estimates are based on daily data on total water discharge provided by the river authority (Povodí Vltavy, s.p.).

Ethics.   The field sampling and experimental protocols used in this study were performed in accordance with the guidelines of and with permission from the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic (Ref. No. CZ 01679). All methods were approved by the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic.

Video analysis.   The video records were used to estimate the capture efficiency of bleak. Bleak were analysed only in records where they were close enough to be discerned from different species inhabiting the studied system. In each video, every recording in which one or more separate eggs (further referred to as aggregation) were drifting was noted, along with the number of bleak within camera view and the number of successfully eaten eggs by bleak. A bleak was counted as present when it was clearly identified and at least its head was visible in the camera view (e.g., more than half of the bleak without its head in view was not counted as present, while only the head being visible was counted as present). The number of bleak in the camera view was counted every 15 seconds to estimate the bleak presence in the given record, and the average was used as a characteristic of each video record. Another average bleak number was calculated for the recordings in which eggs were observed in order to determine whether bleak are attracted by drifting eggs.

Statistical analysis.   Two general linear model (GLM) analyses with the explanatory variables bleak length and sampling date were performed to test (I) the proportion of eggs in the total diet and (II) whether gut fullness was dependent on the explanatory variables used.

To reveal whether there is a relationship between number of bleak and number of drifting eggs (egg-finding behaviour), one-tailed paired t-test was used. For each video record, the average number of bleak in the camera view in moments when eggs were drifting was computed for each video record and was related to the average number of bleak computed from counts spaced at 15 s intervals. Egg-finding behaviour could be identified by the former value being generally higher than the latter.

To study the probability of eggs being eaten, we can assume that the total number of eaten eggs (X_i) in one observed video follows the binomial distribution with parameters n_i and p, where n_i is the total number of eggs in the ith video and p is the probability of eggs being eaten. This assumption means that all eggs are taken as independent observations. This assumption is not satisfied because, for instance, the probability of catching an egg when two eggs are observed in the video differs from catching an egg when one egg is observed. Hence, we separately computed the probability (p_k) for k observed eggs drifting in the video simultaneously. The index k is taken from 1 to 10 only to avoid rare situations when the eggs satiated the bleak. The maximum likelihood estimator of p_k follows the binomial model $\hat{p}_k^l = X_i^k/n_i^k$, where X_i^k is the number of eaten eggs with k observed eggs in the video and n_i^k is the total number of times the k eggs are observed. In the binomial model, we can easily pool the observations from different samples together because $\sum_i X_i^k$ also has a binomial distribution with parameters $\sum_i n_i^k$ and p_k. In addition, we thus obtain a maximum likelihood estimator of the parameter p_k as $\hat{p}_k = \sum_i X_i^k/\sum_i n_i^k$.

To obtain an estimator of p, we must use the theorem of complete probability, i.e.,

$$\hat{p} = \sum_k \hat{p}_k * \hat{q}_k \tag{1}$$

where $\hat{q}_k = \frac{\sum_i n_i^k}{N}(N = \sum_k\sum_i n_i^k)$ is the estimate of the probability of k eggs appearing in the video. This simplifies into

100

$$\hat{p} = \frac{\sum_k \sum_i X_i^k}{N} \tag{2}$$

The standard deviation of this estimator can be computed straightforwardly using the fact that $\sum_i n_i^k$ is distributed according to the multinomial distribution. This can be simplified into $\sum_i n_i^k = n^k$ and $\sum_i X_i^k = X^k$. The standard deviation is then computed as

$$SD(\hat{p}) = \sqrt{\sum_k \left(\frac{X^k n^k N - X^k (n^k)^2 + X^k (n^k)^2 N - (X^k)^2 N + (X^k)^2 n^k + (X^k)^2 n^k N - (X^k n^k)^2}{(n^k)^2 N^3} \right)}$$
$$- 2 \sum \sum_{k<l} \frac{X^k X^l}{N^3} \tag{3}$$

The effect of the number of bleak and the number of eggs in a single video observation on the probability of predation on eggs was further evaluated using a classical logit model. Here, the observations with many eggs (more than 10) were also considered, and the dependence on the number of eggs is here taken into consideration in one factor.

Statistical analyses were performed in Statistica software (Statistica, Inc., StatSoft, Tulsa, Oklahoma, USA) and R software version 3.2.3[50].

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M.Š., L.V., D.B., R.B., P.B. and J.K. participated in the field work. M.Š. and J.K. designed the study. T.M., P.B. and M.P. conducted the statistical analysis. M.Š. wrote the first draft. All authors contributed substantial comments during manuscript preparation.

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