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Round goby gut contents

Title

Egg predation on native fish by invasive round goby revealed by species-specific gut content DNA analyses

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Author contributions

For DNA analyses, EL and IAK designed experiments, EL organised and performed fieldwork, laboratory experiments and analysed the data. For mark-recapture studies, RM, and PEH organised and performed fieldwork and analysed the data. HPJ performed field work and provided native fish samples and data on native fish. IAK, PEH, EL, KB, JW, and PBH wrote the manuscript.

Funding

This work was funded by the Federal Office for the Environment, Switzerland; the Canton Basel-Stadt; the lottery funds of the Cantons Solothurn, Aargau and Basel-Landschaft.

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Abstract

1. Conservation of riverine fish typically aims at improving access to spawning grounds and the restoration of longitudinal connectivity requires substantial investments. However, the removal of migration barriers also enables the upstream invasion of non-native species into spawning areas, with potential negative effects on recruitment of threatened freshwater fish through egg or fry predation.

2. Detecting egg predation is often challenging. Visual gut inspections are thought to underestimate predation on soft material such as eggs and fry, which hampers the discovery of predators preying upon these life-stages. For soft materials, molecular approaches may therefore offer a more sensitive tool for detection.

3. Here, we uncover such a macroscopically invisible conservation issue caused by predation of invasive round goby *(Neogobius melanostomus)* predation on eggs or fry of threatened common nase (*Chondrostoma nasus*) in Switzerland.

4. In addition, this manuscript presents species-specific molecular assays for five more valuable native fish, including endangered salmonid and cyprinid river spawners, and confirms the applicability of the assays in a series of laboratory and field feeding experiments involving eggs and fish tissue. The manuscript also provides a guiding tool for conservation managers regarding the use and applicability of different molecular approaches in gut-content analysis.

5. Our results inspire recommendations for local conservation measures such as a temporary reduction of round goby densities at the spawning site prior to the spawning period, and demonstrate how the targeted application of species-specific molecular markers can inform freshwater fish management.

Keywords

Neogobius melanostomus, population recruitment, reproduction, common nase, *Chondrostoma nasus*, invasion management

Introduction

Conservation target: freshwater fish recruitment

Migratory species often have high socio-cultural importance and an exceptional value attached to conserving their migrations (Meretsky, Atwell, & Hyman, 2011). At the same time, they are particularly vulnerable, since they depend on connected habitats and open migration corridors. Many riverine freshwater fish species are gravel spawners and therefore migrate from major rivers or the sea into tributaries to reproduce. Migration barriers are one of the greatest threats to reproduction by impairing spawning migrations and thus population recruitment (Ignatius & Haapasaari, 2018). Hydropower dams constitute such migration barriers and are of particular importance in Switzerland where electricity supply relies heavily on run-of-the-river hydropower plants. In appreciation of the associated conservation issues, spawning sites of so-called 'national importance' have been mapped by federal authorities for migratory species of the River Rhine's tributaries (Kirchhofer, Breitenstein, & Guthruf, 2002; Zbinden & Hefti, 2000) (**Table 1**). The importance of these species is reflected by effected and planned investments of 627 million € between 2009 and 2027 in the River Rhine and its tributaries alone. These investments mainly go into measures of stocking and securing access to spawning sites, such as building fish ladders and removing dams (Bölscher, van Slobbe, van Vliet, & Werners, 2013)**, Figure 1**).

English name	**Common barbel**	**Common nase**	**Grayling**	**Brown trout**	**Atlantic salmon**	**European chub**
Latin name	*Barbus barbus*	*Chondrostoma nasus*	*Thymallus thymallus*	*Salmo trutta fario*	*Salmo salar*	*Squalius cephalus*
German name	Barbe	Nase	Äsche	Forelle	Lachs	Döbel/Alet
IUCN Read List of Threatened Species 2001	Near Threatened	Critically Endangered	Vulnerable	Near Threatened	Regionally Extinct	Least concern
Protected according to Berne Convention	No	Yes	Yes	No	Yes	No
Local spawning season / fry emergence	May-July	March-May	March-May / June	October-January / March - June	October-January / March-June	April-June

80

81 ***Table 1.***

82 *Iconic / protected / locally relevant freshwater fish for which assays were developed in this study. Source for*

83 *spawning and fry emergence: Office for the Environment Basel Stadt.*

84

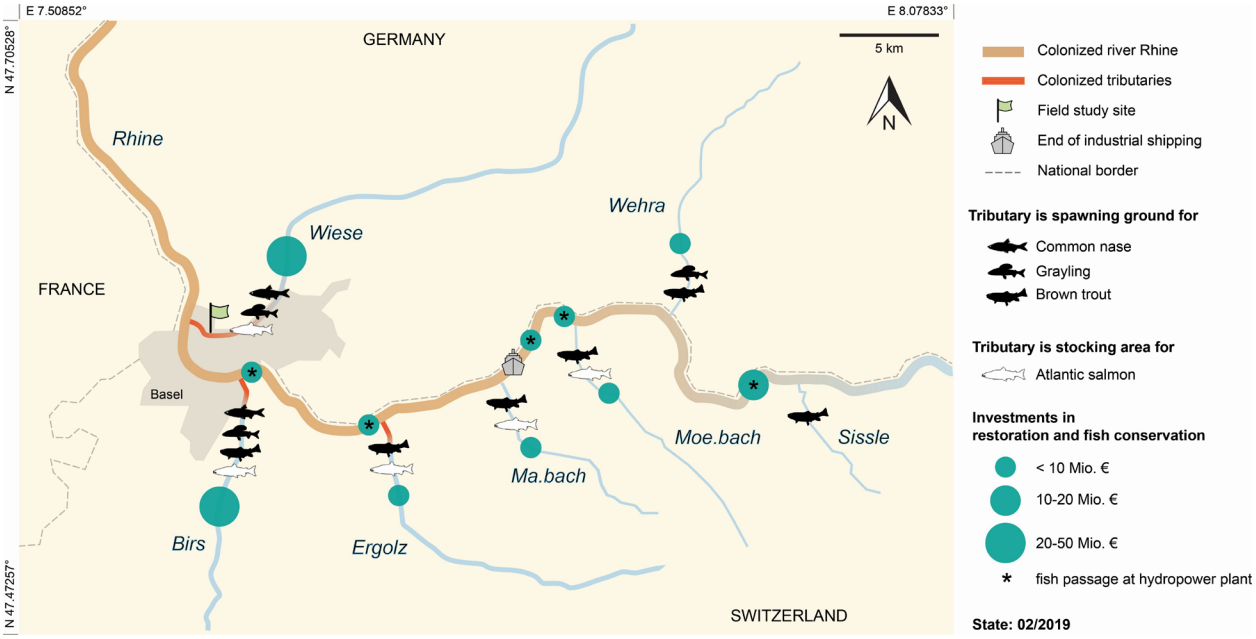

Figure 1

Map of the study area at the River Rhine in Switzerland. River sections and tributaries colonized by invasive round goby are marked with orange and red, respectively. The orange color intensity in the river Rhine reflects time since first record, with more recent colonization displayed in paler shades (Basel: 2012; close to the river Sissle: 2018). Spawning areas for fish of national importance (common nase (Chondrostoma nasus) grayling (Thymallus thymallus, brown trout (Salmo trutta), as well as areas in which the locally extinct Atlantic salmon (Salmo salar) is stocked for reintroduction are indicated by fish symbols next to the tributaries. In recent years, major investments have been made to improve the accessibility and structure of tributaries, as well as the ecological permeability of hydropower plants in the River Rhine. Sum figures of recent and planned monetary investments are indicated by green circles, with the amount reflected by the circle area.

Conservation threat from a non-native egg predator – the round goby

The efforts to improve spawning site access for migratory species have unwanted side-effects. Migration barriers not only impede spawning migrations but also protect spawning sites from invasive species dispersing from the main river. Once migration barriers for gravel spawners have fallen, the upstream invasion of potential predators and competitors poses a threat to their spawning and recruitment success.

This problem is epitomized by one of Europe's 100 worst invasive species, the round goby (*Neogobius melanostomus*). This small benthic fish is currently spreading in the River Rhine in Switzerland. Its range is now expanding into the tributaries which contain the spawning sites of several native gravel spawners (Hirsch, Thorlacius, Brodin, & Burkhardt-Holm, 2017). Round gobies consume a broad diet, but are also known as egg and fry predators. Experiments and field observations show that they prey on eggs and fry of larger fish in rivers and lakes (Chotkowski & Ellen Marsden, 1999; Fitzsimons et al., 2006; Kornis, Mercado-Silva, & Vander Zanden, 2012). In the Great Lakes, round goby predation on spawning reefs has led to severe recruitment losses of socio-economically important salmonid species (Roseman, Taylor, Hayes, Jones, & Francis, 2006). Consequently, removal efforts have been developed with the intention to decrease round goby density over spawning reefs prior to the spawning season (Wagner, Cooper, Gross, & Coffin, 2015).

The necessary evidence for conservation efforts can be gathered by molecular tools

A round goby invasion into tributaries has the potential to undermine costly conservation efforts. To decide on potential countermeasures, robust scientific evidence is required (Salafsky et al., 2019). This scientific evidence base for egg predation by round goby in the wild is difficult to establish with current methods. Diet quantifications usually rely on visual identification, but eggs and fry represent soft materials and gobies grind prey with their pharyngeal teeth thus further disintegrating these prey (Ghedotti, Smihula, & Smith, 1995). This renders such prey types visually hard to identify, which impedes the macroscopic identification in round goby stomachs.(Baker, Buckland, & Sheaves, 2014). Although eggs and fish remains are occasionally observed in round goby guts (Nichols et al., 2003; Roseman et al., 2006), visual methods may fail to report the true extent, and usually fail to provide species-level information on the prey. This situation thus requires novel tools that provide a scientific and conclusive confirmation and documentation of round goby predation on native fish species. Prey species components that are shredded beyond recognition can be identified with a variety of methods. In the context of

conservation, species-specific approaches are most useful because they require least efforts once they have been tailored to the situation (see Methods section for details).

Aims

In this paper, species-specific assays are used to detect egg predation of round goby on native nase (*Chondrostoma nasus*) and five other native species based on molecular gut content analyses. First, species-specific assays for five native species are designed (**Table 1**) and their specificity is confirmed. The method is then validated in aquarium and field feeding experiments involving fish tissues and eggs. Finally, predation of round goby on one particular species, the common nase, is tested at a spawning site in the field, with the aim to inform future conservation efforts.

Study species and study site

The nase is an endangered and protected freshwater fish that undergoes a spawning migration into tributaries. Several major spawning sites of national importance have been mapped in the River Wiese in Basel, Switzerland. At the most important site located furthest downstream, ~1000 individuals of male and female nase aggregate every year to spawn over gravel beds in 0.5 to 1m depth along a short section of river which is only 20-40m long and 20m wide (**Figure 2**; (Maier, 1997), own observations, see also the Supporting-Information-video of a nase spawning aggregation, filmed where pictures for **Figure 2** were taken). Since two years round goby are dispersing into this river, have reached the nase spawning sites (own fishing records, unpublished data, **Figure 2**), and are expected to disperse further upstream towards upstream spawning sites of nase. Based on previous research, we expect that nase reproduction is especially vulnerable to round goby predation. In contrast to salmonid winter spawners, nase spawn in spring when temperatures are higher (Maier, 1997; Zbinden & Hefti, 2000) and round goby are more actively feeding. Nase eggs are not buried, but are spawned on top of the gravel bed, where they adhere and are thus directly accessible for predators (Hofer & Kirchhofer, 1996; Patzner, Weidinger, & Rühl, 2006). Nase eggs and fry are sensitive to several external factors and losses can amount to almost 100% (Penazk & Luck, 1965 - cited in Patzner et al, 2006). For example, egg predation frequently leads to 20-30% losses (Maier, 1997), and embryonic survival is reduced by up to 20% by temperature increases of more than 5 degrees over the optimum temperature (Targoñska & Kucharczyk, 2008). Finally, studies suggest that the mortality of larvae can amount to

99% in the first two months following hatch (Bartl & Keckeis, 2004). Even minor impacts on recruitment therefore pose a conservation threat to this species. Thus the possible predation of eggs and fry of the endangered nase at its yearly spawning site by the round goby is a relevant and suitable testbed for putting a molecular method into conservation practice.

Figure 2

Photographic depiction of the nase (Chondrostoma nasus) spawning run in the River Wiese in Basel, Switzerland. Top left picture; A co-author standing above the bridge with the white dashed line indicating the spawning area. This gives an idea of the scale of the actual spawning site is in terms of depth and widths of the River Wiese. A video filmed from the co-author's position was uploaded as a Supporting information for review, filename: 'Nase spawning aggregation April 2018 in Basel - CH.mov'. Right: A typical group of spawners located approx. equidistant to another, each individual framed by a white circle. Bottom left picture: an underwater picture of a nase with approx. 50cm total body length. Note that the underwater picture was taken outside of the spawning season and not at this site, to prevent any disturbance.

Methods

Evaluation of different molecular approaches

Three approaches (see below) with unique advantages and disadvantages are currently available for molecular gut content identification. The approaches differ with regard to the most challenging step (assay development versus data analysis) and in their specificity (detection of a species of interest versus detection of an entire community; **Figure 3**).

(1) **Species-specific approaches** detect unique and species-specific DNA sequences. They are difficult to design, but any molecular diagnostic laboratory can generate and interpret results without the need for sequencing or bioinformatic analyses. Species-specific approaches have been used to investigate prey diversity (Corse et al., 2010), but they are most useful when the aim is to investigate specific prey species.

(2) **Barcoding approaches** can be used to identify individual large prey items or to determine the diversity of gut contents, for example in lion fish *Pterois volitans* (Valdez-Moreno, Quintal-Lizama, Gómez-Lozano, & García-Rivas, 2012). They rely on the amplification of barcoding genes such as mitochondrial Cytochrome B or Cytochrome Oxidase 1, and reagents to amplify barcoding genes have been designed for many clades including invertebrates (Valentini et al., 2009). Barcoding requires reasonably intact DNA and fails on strongly digested samples. Also, predator DNA can swamp the signal and outcompete scarce prey items. For example, just 61'000 prey sequence reads were retrieved from 2'000'000 total reads for spiders (Piñol, San Andrés, Clare, Mir, & Symondson, 2014). Finally, data analysis requires sequencing to identify individual larger items or Next Generation Sequencing (NGS) and bioinformatics for analyses of diversity.

(3) **Shotgun approaches** determine prey diversity. All DNA fragments in a sample are sequenced by NGS, and the species affiliation of individual DNA fragments is then inferred bioinformatically by matching sequencing results against existing databases. In contrast to species-specific approaches, shotgun approaches require no a priori knowledge about DNA sequences of predator or prey and have been successfully applied to insects (Paula et al.,

2016). However, signals from the predator or its microbiome can outcompete scarce prey items, and data analysis requires advanced bioinformatic skills.

In the context of conservation, where bioinformatic skills and costs are limiting and the prey species of interest is usually known, as was the case for this study, species-specific approaches (1) are most recommendable.

Gut content isolation and DNA isolation

Gut contents of all gobies used in the following experiments were isolated after terminal anesthesia with Koi Med Sleep by opening the body cavity from the anus towards the pelvic fin with scissors, removing the gut, and squeezing its contents into an Eppendorf tube with 100% EtOH. Samples were stored at 4°C, with EtOH being exchanged once after several hours or on the following day. DNA extractions were performed with the DNeasy Blood & Tissue Kit from Qiagen, which yielded DNA of higher integrity than a standard Phenol Chloroform extraction as was discovered via the comparison of three extracted samples with each method.

PCR conditions

PCRs were done with FastStart^{TM} Taq DNA Polymerase from Roche in a 20 µL volume (2 µL 10x buffer, 1.6 µL dNTPs (2.5 mM), 0.4 µL forward primer (10 nM), 0.4 µL reverse primer (10 Nm), 1.25 µL BSA (20 mg mL^{-1}), 0.2 µL Polymerase (5 U μL^{-1}), 60 ng of template-DNA and ultra-pure H_2O to a total volume of 20 µL). BSA was included to alleviate potential PCR inhibition which is common in environmental samples (Adrian-Kalchhauser & Burkhardt-Holm, 2016).

Assay design

Cytochrome Oxidase I (COI) was chosen as target gene because, as of 2017, the NCBI database contained more bony fish COI sequences than other widely sequenced genes (12srDNA, 16srDNA, or Cytochrome B).

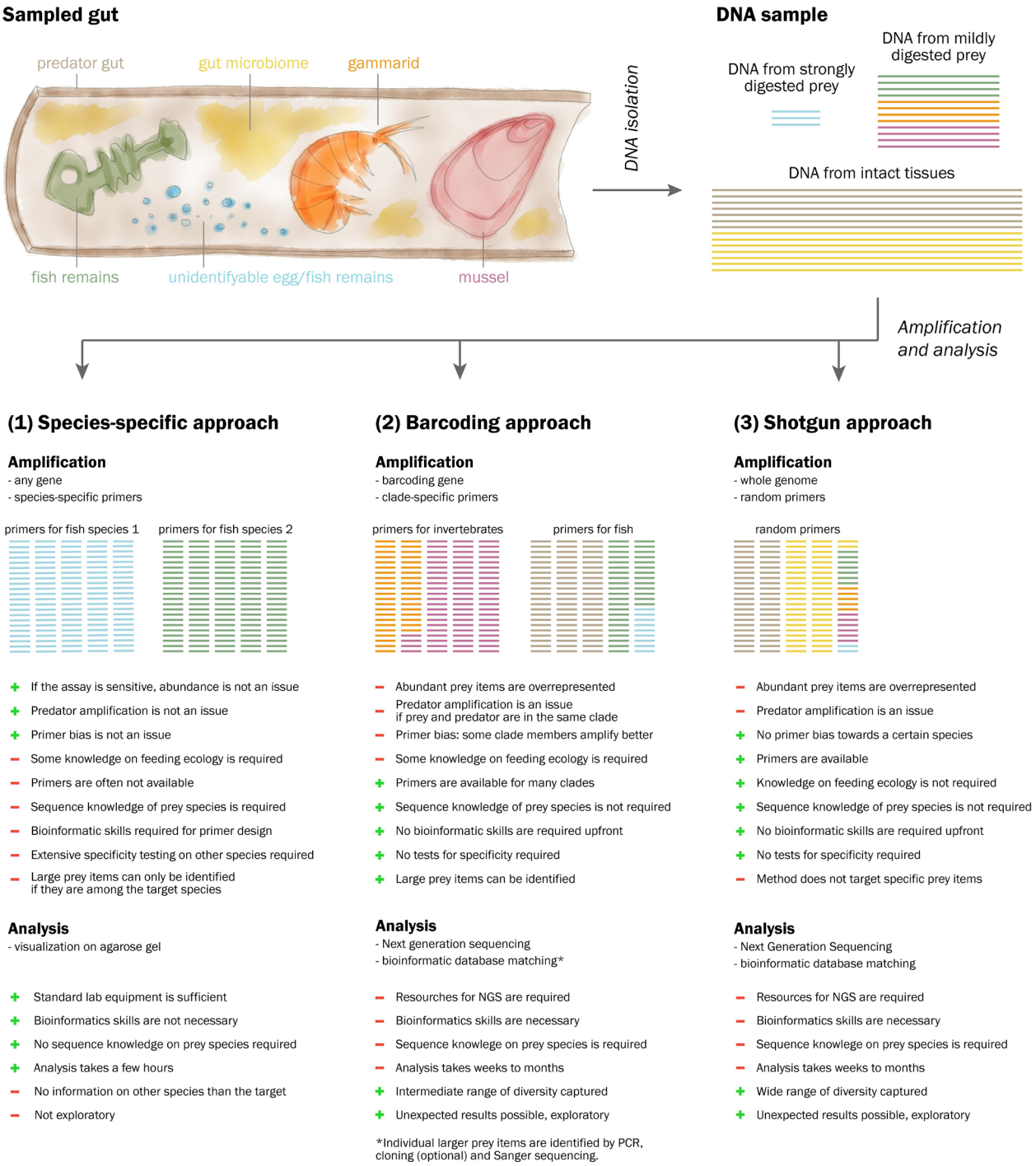

Figure 3

Overview of molecular approaches to gut content identification. In any given gut, some prey items can be identified to species level visually (such as gammarids or mussels), some prey items can be identified to higher taxonomic level (such as fish remains), and some prey items are digested beyond recognition (such as unidentifiable egg or fish

remains). Samples always also contain DNA from the predator and DNA from the gut microbiome. The amount and the fragment length of DNA isolated from gut contents depends on the degree of digestion. Species-specific approaches (1) are designed to detect the DNA of a selected prey species of interest. Barcoding approaches (2) are designed to either identify individual prey items, or to reveal prey diversity within a clade of interest. If predator and prey are phylogenetically related, predator DNA may be amplified with primers designed for the prey. Shotgun approaches (3) are designed to reveal the entire prey diversity and do not focus on a particular genomic region. The figure lists major challenges and advantages of each approach.

Hard-material invertebrate prey item as a method test

As a method test, an assay targeting a common invertebrate prey item was developed. For that we used the zebra mussel (*Dreissena polymorpha*) because it is a common prey item in round goby and because its hard shell is easy to identify visually (Özdal, 2016). COI sequences for all bivalves and gastropods present in the High Rhine (Rey et al., 2015) (Appendix S2) were retrieved from the NCBI database and aligned with the Clustal Omega online tool (Chojnacki, Cowley, Lee, Foix, & Lopez, 2017). Primers were chosen with 1) zebra-mussel specific and GC rich 3'ends, 2) primer lengths between 22 and 24 and 3) amplicon size below 300 base pairs. EL_17F ATTGGTACCAATAATACTGAGTC (5'-3') and EL_18R GCACGTATATTACCTCATGTCC, **Appendix S3**) were tested on samples from a previous fishing campaign, and results were predominantly in agreement with visual gut content inspections.

Fish assays

In a similar manner, assays for six fish species were designed: Common barbel (*Barbus barbus*), common nase (*Chondrostoma nasus*), grayling (*Thymallus thymallus*), brown trout (*Salmo trutta fario*), Atlantic salmon (*Salmo salar*), and European chub (*Squalius cephalus*). All species spawn in the investigated area, are relevant to local fisheries and/or are endangered and part of species protection programs and/or are species of local and national importance (**Table 1**). Primers were designed as above on an alignment of native locally occurring fish (**Appendix S4**). Specificity was tested on samples obtained from 'Projet Lac' (EAWAG/Ole Seehausen), local food stores, stocking companies, and routine monitoring campaigns. For Souffia (*Telestes souffia)*, brook lamprey (*Lampetra planeri)*, and the European bitterling (*Rhodeus amarus)* no samples were available (**Appendix S5**).

The applicability and feasibility of the assays in wild individuals were tested by field feeding. Filets of the target species was fastened inside minnow traps (one target species per trap). Traps were exposed for 5h in the local harbor Kleinhüningen (N 47.587453°, E 7.593608°) and/or in the River Rhine (N 47.570444°, E 7.583609° and N 47.560365°, E 7.620167°). The assays reliably detected ingested prey of the respective target species and, in many cases, were more sensitive than visual inspections (**Figure 4**), with the exception of European chub. While the European chub assay detected pure chub DNA reliably, amplification from six round goby gut contents failed, even though putative fish tissue was visible in one sample.

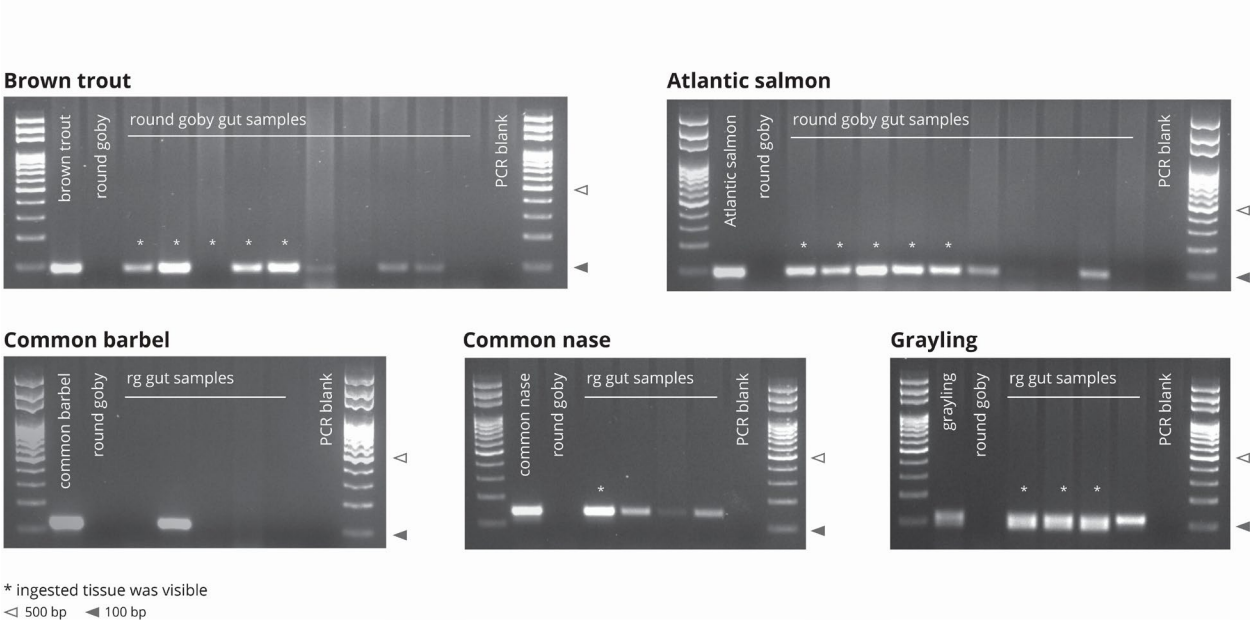

Figure 4

PCR-based detection of brown trout, Atlantic salmon, common barbel, common nase, and grayling material from the guts of wild round goby that were caught in traps baited with the respective species. A white band in the agarose gel indicates successful detection of the target species. Leftmost and rightmost lanes: size standards, arrows indicate 100bp and 500bp band. First lane: assay on pure DNA of the target species (positive control). Second lane: assay on pure DNA from round goby (negative control). Last lane: assay on water (negative control). Other lanes: assay on DNA extracted from round goby gut contents. An asterisk marks the samples in which ingested bait tissue chunks were macroscopically visible during gut content isolation.

Trout egg predation

Current efforts in trout fisheries management move away from stocking and towards enhancing natural reproduction (Spalinger, Dönni, Hefti, & Vonlanthen, 2018). To understand the potential of round goby to negatively affect those efforts, the ability of round goby to consume trout eggs as well as the ability of the trout assay to detect ingested eggs was determined in aquaria experiments. Due to the protected status of nase, nase eggs were not available for experiments. Sixty round goby were maintained in groups of 5 individuals, fed with bloodworms (chironomid larvae), and starved for two days before the feeding experiments. Brown trout eggs at the eyed egg stage (diameter ~ 4 mm) from the local cantonal fisheries association (www.basler-fischerei.ch, Hermann

Koffel) were placed in front of individual round gobies hiding in PVC tubes. Eggs were offered to large individuals first and then progressively to smaller individuals. Feeding was stopped when it became clear that individuals below 9 cm would not accept eggs. Nine individuals were found to consume eggs. After feeding they were translocated to an empty tank and sampled after time spans of 15 min (n = 2), 2 h (n = 1), ~5 h (n = 3), or ~20 h (n = 3). Two individuals received bloodworms as negative controls.

Common nase egg predation at natural spawning sites

Next, the consumption of common nase egg or fry was tested at a natural spawning site in the field. Round goby were sampled with minnow traps and by electrofishing at a local spawning site in the River Wiese (N 47.581812°, E 7.591157°; **Figure 2**). For conservation reasons, electrofishing and intense trapping efforts were restricted until after hatch. Common nase eggs require around 180 day degrees to develop, which corresponds to 10-16 days in local conditions. Larvae then remain on site for another 10 days. Spawning took place from the 14th to the 20th of April 2018. Traps were set at the river banks from 16th of April to 16th of May and emptied every 2-4 days, while electrofishing was carried out on the 25th of April upstream from the spawning site, and on the 16th of May (when larvae were expected to have emerged), upstream and downstream from the spawning site. 50 round goby were caught with both approaches combined. In addition, 10 round goby were caught with traps at a nearby commercial harbor as negative control. In the harbor, nase are occasionally caught but no nase spawning occurs.

Management options and required resources

Round goby densities at the common nase spawning site are available from 2016 and 2017, the two years preceding this work. In 2016, a mark-recapture study was performed between the 14th September and 10th October 2016. Round gobies were marked with pit tags and population density was determined with the Lincoln-Peterson estimator for a 2-sample closed-population model (Bagenal & Tesch, 1978). In summer 2017, the Office for Environment and Energy, canton Basel-Stadt, conducted an electrofishing campaign at the site, targeting large species for relocation in the course of a renaturation project, and as a by-product caught hundreds of round goby.

Results

Trout egg predation by round goby

Round goby larger than 9 cm total length accepted trout eggs as prey. Individuals smaller than 9 cm standard length (n = 5) were not able to swallow trout eggs (~ 4 mm diameter) and/or did not consider them as prey. Individuals ingested up to 14 eggs, but more commonly 6-8 eggs. Trout eggs could be detected from the guts 21 h after ingestion, also when eggs were no longer macroscopically visible (**Figure 5**). Longer time periods were not tested for lack of animals. In our sample, animals larger than 9 cm standard length were predominantly male (n = 8), however, one female was included, and likewise consumed eggs.

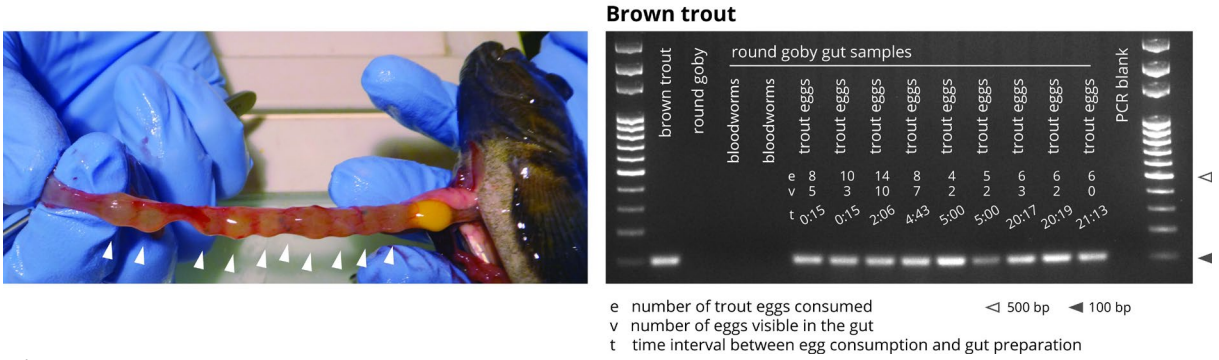

Figure 5

Detection of trout eggs from round goby fed with trout eggs in fish tanks. Left panel, gut of a round goby with ten ingested eggs and a piece of corn, dissected 15 minutes after feeding. Right panel, PCR-based detection of trout eggs from round goby guts. A white band in the agarose gel indicates successful detection of the target species. Leftmost and rightmost lanes: size standards, arrows indicate 100bp and 500bp band. First lane: assay on pure DNA of brown trout (positive control). Second lane: assay on pure DNA from round goby (negative control). Last lane: assay on water (negative control). Other lanes: assay on DNA extracted from round goby gut contents. e (eggs): number of trout eggs consumed by the individual. v (visible): number of eggs visible in the gut during dissection. t (time): time elapsed between egg consumption and gut preparation.

Nase egg predation by round goby at a spawning site of national importance

Even though our sampling campaign was spatially and temporally restricted to locations downstream from the spawing site and to the time after fry emergence, several round goby sampled had consumed eggs or larvae of

the common nase. Despite the sampling limitations, which were instigated to avoid disturbing spawning and negative impacts on recruitment, four out of fifty gut samples tested positive for common nase, two of them strongly (4115 and 4152) and two of them weakly (4120 and 4150; **Figure 6**). All four positive round goby individuals were caught close to the spawning site. Samples from further downstream as well as all control samples from the nearby harbor were tested negative. Samples were also tested for presence of grayling and European chub, two species that spawn at the same time but further upstream, but all samples were tested negative for these two species (data not shown).

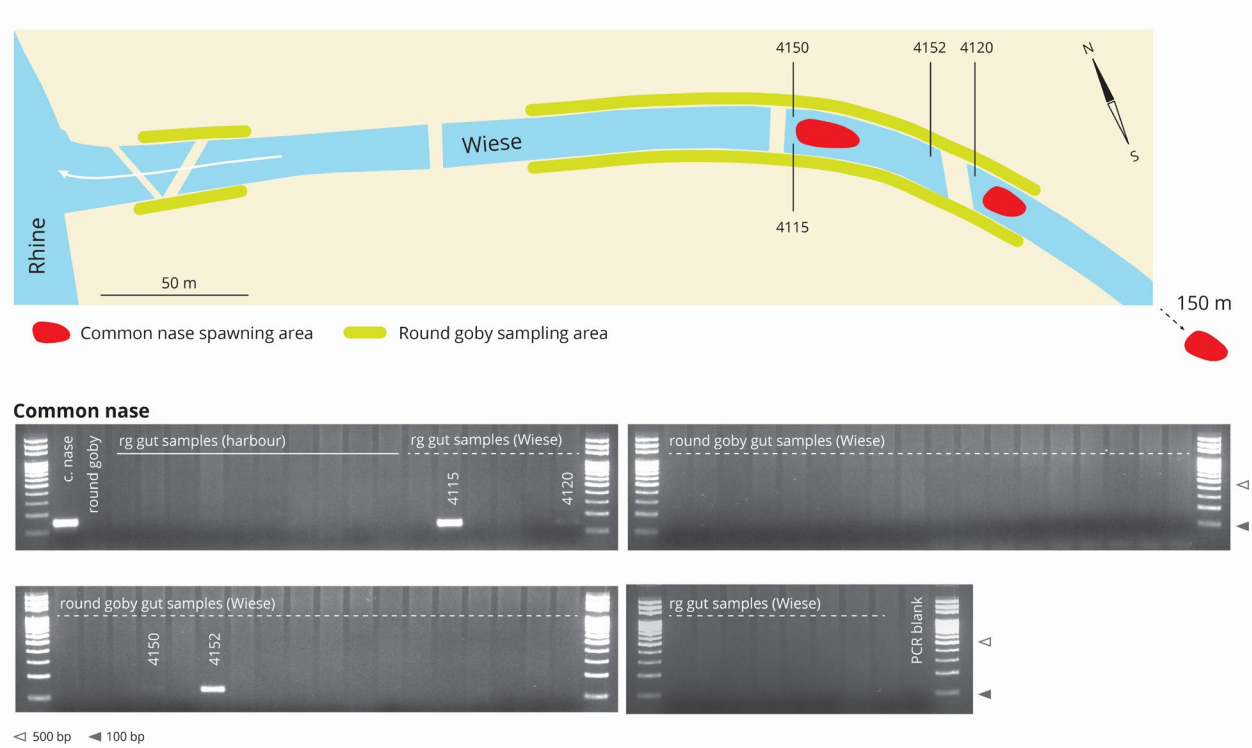

Figure 6

Round goby consume eggs of the endangered and protected common nase near a spawning site. Top panel: Map of the River Wiese, with areas of round goby fishing marked in yellow and common nase spawning sites indicated in red. Bottom panel: PCR results. A white band indicates presence of target species DNA. Leftmost and rightmost lanes: size standards, arrows indicate 100bp and 500bp band. First lane: assay on pure DNA of common nase (positive control). Second lane: assay on pure DNA from round goby (negative control). Last lane: assay on water (negative control). Gut samples (harbor): assay on DNA extracted from round goby gut contents from a nearby

industrial harbor where no common nase spawning took place. Gut samples (Wiese): assay on DNA extracted from round goby gut contents from the River Wiese. In two samples (4115 and 4152) a strong signal is visible, in two samples (4120 and 4150) a weak but repeatable (n=3) signal is visible. Note that all round goby individuals were caught after the spawning season proper and downstream of the actual spawning site in order to not disturb spawning (see methods for details).

Round goby density quantification and management options

The mark-recapture campaign revealed a maximum population density near the spawning site of ~11 round gobies per sqm. On the 20x20 m of the investigated spawning site, this corresponds to a maximum of ~4400 individuals in total. A non-quantitative sampling campaign directed at large individuals of other species in the same area in 2017 yielded hundreds of round goby.

Discussion

Our molecular approach confirms that round goby consume eggs or fry of the common nase at their natural spawning sites, and thus pose a potential conservation issue for this migratory gravel spawner. Visual gut content analysis would not have been able to discover this issue. Our tests have the potential to reveal similar "invisible" conservation threats for trout, grayling, barbel, salmon, and chub, since the assays are able to detect ingested tissue when it is no longer macroscopically visible.

Conservation implications of round goby egg predation on the nase

The data collected in this study does not allow to quantitatively predict population-scale effects of round goby on the nase. Such quantitative predictions require sound data on round goby densities, round goby consumption rates, egg availability, and the relative contributions of other factors to nase reproductive output. Such data cannot be provided due to sampling limitations. The local nase population is extremely well-protected and the knowledge gain from sampling and quantification of spawners, eggs, or fry needs to be balanced against the potential losses. Because larvae can be extremely sensitive to electrofishing, this method could also not be used in closer temporal or spatial proximity to the actual spawning. The actual number of positively tested round goby might be even higher if they could have been caught directly above the spawning site and directly during or shortly after spawning. At any case, in the absence of such further data, any attempts to make speculative quantifications of losses on the population level should be disencouraged. However, it is quite likely that the observation of 4 positive gut samples out of 50 guts analyzed substantially underestimates predation pressure due to the time and distance between the catch of the potential predators and spawning of the potential prey.

Considering the high mortality of nase eggs and larvae described in the literature (see introduction), the sensitivity of the species to adverse factors such as higher spring temperatures which are likely to increase in the near future, and the vulnerability of common nase to chemical pollution from the petro- and agrochemistry industry (Devaux et al., 2015), even a few percent loss of reproduction to round goby predation could be the proverbial nail in the coffin for nase recruitment at a given year. Accordingly, following the precautionary principle (Leung et al., 2002) and considering investments already undertaken to support the population, our data is certainly sufficient to instigate a discussion on the conservation implications of evidence for egg predation.

382 Our data makes a local removal of round goby populations a conceivable solution to minimize negative
383 effects on recruitment of iconic or protected species. Round gobies directly below the spawning site, but not further
384 downstream, had ingested common nase larvae or eggs. Round gobies generally show high site fidelity with
385 estimated home-ranges of 5 ± 1.2 m^2 (Ray & Corkum, 2001). A study in Lake Michigan showed individuals to
386 move within a maximum of 67 m shoreline range of a release point (Wolfe & Marsden, 1998). This indicates that
387 physically removing round goby from spawning sites of national importance prior to the spawning season should be
388 further investigated as an efficacious option to minimize egg predation.
389 Based on existing population control models (N'Guyen et al., 2018), eradication of round goby in secluded
390 areas might be achieved by a long-term yearly removal of 85% of all the population's adult individuals. Our own
391 experience with sampling in 2018 and participation in the 2017 electrofishing campaign indicates that round goby
392 populations at the nase spawing site can be substantially reduced by electrofishing. It is unclear how many round
393 goby need to be removed to reduce predation pressure. However, it can be estimated that a series of consecutive
394 electrofishing campaigns can substantially reduce population density in the given setting. Three campaigns would
395 correspond to 9 whole workdays or 72 work hours. At a rate of 50 EUR per hour (average Swiss labor cost), this
396 corresponds to personnel costs of EUR 3600 per year. Although this estimate of the expected costs is coarse, it
397 allows for a simple conclusion: the costs for temporarily reducing round goby densities at the spawning site are
398 vanishingly small compared with the planned investment of more than 35 million EUR into river restoration of the
399 River Wiese over the course of 15-20 years (office for environment and energy, canton Basel-Stadt, 2015). Ten
400 million Euros have already been spent between 2016 and 2018 to restore only the downstream section, where the
401 spawning sites of the nase are located (office for environment and energy, canton Basel-Stadt, 2018).
402
403 **Methodological advancements for evidencing egg predation by invasive species**
404 Our work underscores the potential of species-specific molecular prey detection to uncover previously
405 unknown and "invisible" conservation threats. Molecular prey identification methods are increasingly used to
406 elucidate prey diversity, because they outperform visual approaches in three ways.
407 Firstly, they extend the detection window (Carreon-Martinez, Johnson, Ludsin, & Heath, 2011). For
408 example, visual identification of herring eggs in round goby stomachs is possible only during 9 h post feeding

(Wiegleb, Kotterba, Hammer, & Oesterwind, 2018). Similarly, our assays extended the detection window for eggs as well as for soft muscle tissue compared to visual inspection.

Secondly, molecular approaches reduce detection bias against soft prey items. The round goby is known to prey on a variety of taxa, including zooplankton, benthic invertebrates, small fishes, fish eggs and the larvae of small fishes, with exact diet composition depending on habitat, season, and body size (Karlson, Almqvist, Skora, & Appelberg, 2007; Kornis et al., 2012; Wiegleb et al., 2018). Commonly, diet components are determined to the "lowest possible taxon" based on structures such as shells and exoskeleton elements. This approach performs poorly on soft structures (such as larvae or eggs) or taxonomically ambiguous prey items (such as juvenile fish) and disregards amorphous masses. In our experience, up to 30 % of round goby gut contents can be categorized as amorphous mass (Özdal, 2016). Accordingly, large biases introduced by differential prey digestion are expected in visual approaches (Walsh, Dittman, & O'Gorman, 2007). Molecular approaches promise to reduce this bias, as exemplified in this study.

Thirdly, molecular approaches yield species-specific information on ambiguous prey items. Eggs found in fish stomachs usually cannot be assigned to a species with certainty, and have to be reared until hatch for visual species identification. Molecular approaches circumvent such issues.

Molecular tools for conservation

A major obstacle in nature conservation is the lack of data supporting or discouraging management. With this article, it is aimed to fill such a knowledge gap for a specific species, and provide tools for conservation managers to gather additional data, in line with a state-of-the-art conservation management framework of (Salafsky et al., 2019). Our data encourages locally and temporally restricted management of round goby at spawning sites. Conducting and reporting on such a campaign is beyond the scope of our article. However, our study's results can provide a sound basis for political decision makers, conservation managers and scientists to engage in a co-design of a research project to tackle these challenges.

Caveats and future research directions

A disadvantage of molecular methods is that they do not discriminate the ingested tissue type. Eggs, fry, or muscle tissue would all yield the same signal. Accordingly, the positive samples from the Wiese could also stem

437 from nase carcass consumption. Carcass-feeding in round goby has been described in experimental settings (Polacik,
438 Jurajda, Blazek, & Janac, 2015) and the extent of carcass feeding by round goby in the wild is at present unknown.
439 However, common nase do not die after spawning as, for example, Pacific salmon (*Oncorhynchus* spp.) do, and no
440 dead animals were observed at the site.

441 For this and other reasons, molecular approaches are unlikely to completely substitute visual stomach
442 content analyses in the future. It is rather likely that crossover approaches combining visual and molecular analyses
443 are most promising. Samples could be fixed in ethanol, large prey items could be identified visually, and amorphous
444 masses could be further processed for barcoding, shotgun, and/or species-specific approaches, depending on the
445 research question.

446

447 **Conclusions**

448 In conclusion, our results demonstrate the value of species-specific molecular markers to generate
449 conservation-relevant data. This data can be used to inform freshwater fish management. This manuscript
450 demonstrates that these assays are useful to find a tailored solution for a real-world problem, namely whether a
451 particular species or area may require protective measures in the face of predator invasions and the removal of
452 migration barriers. These assays allow to indicate predation risk with greater sensitivity and robustness than visual
453 and taxonomic approaches. Evidence gathered by the assays can then become the basis of management e.g. a
454 removal strategy, which was deemed a valuable and worthy investment considering the substantial investments into
455 restoration efforts. Our results can now enable political decision makers, practitioners, and researchers to co-design
456 and implement such effective conservation measures together.

Acknowledgements

We are grateful to Attila Rüegg for animal care and assistance in field work, to Guillaume Arnet for assistance with electrofishing and field work, to Hermann Koffel for providing trout eggs, to Ole Seehausen and Project Lac for providing fish samples, and to Mark Charran for critically reading the manuscript. The authors have no conflict of interest to declare.

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