Short Communication

Invasive goby larvae: first evidence as stowaways in small watercraft motors

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Abstract

Aquatic invasive species (AIS) are a major threat to aquatic ecosystems worldwide. Despite management efforts, human assisted dispersal continues to distribute AIS within and across waterbodies. An understudied translocation vector for AIS, especially for invasive fish, are the cooling systems of small watercraft motors. Here, we investigate the contents of boat motor cooling systems for the presence of invasive goby larvae in a collaboration with local boat owners. Because of the exclusively nocturnal drift activity of goby larvae, to collect samples we drove boats in the first hours after sunset. For an estimate of the translocation potential, we quantified drift density of goby larvae as well as boat traffic after sunset. We found a goby larva in a boat motor once in 30 boat drives of 1–2 hours duration each. Peak drift densities of goby larvae were 2.5 per 100 m^3, which is comparable to previously reported data. Recreational boats were active after sunset throughout the reproductive season of invasive gobies and are therefore a realistic translocation vector for goby larvae. Additionally, evidence of fish and other animals inside boat motor cooling systems, gathered from online boating forums, demonstrates the potential of AIS transport in small watercraft. Translocation inside motors is especially likely for in-water transport of boats, which should be a management focus in interconnected aquatic systems.

Key words: anthropogenic transport, biosecurity, invasive fish, *Neogobius melanostomus*, non-indigenous species, translocation, vector

Introduction

The role of recreational boats in the translocation of aquatic invasive species (AIS) has been well established for plants, invertebrates and microbes (Ashton et al. 2014; Clarke Murray et al. 2011). Most studies focus on biofouling organisms as potential hitchhikers on the outside of recreational boats and on boat trailers (Rothlisberger et al. 2010; Ulman et al. 2019). Residual waters on recreational boats are of concern as well, because of their vector potential for mobile species – for example, Campbell et al. (2016) and Darbyson et al. (2009) confirmed the presence of AIS in bilge waters. However, to fully estimate and manage the risk of translocation by small watercraft, investigating motor cooling water systems in addition to bilge water or other standing water compartments is important. Bilge water originates from passive processes (water spilling on the deck and draining into the bilge), while cooling water is actively sucked into the motor from the water column. The potential for uptake and survival of propagules should therefore differ between the two types of residual waters. Problematically, the insides of boat motors are often hard to access and therefore their potential to harbour AIS has been poorly characterized. While some studies have investigated the residual cooling water for AIS (Johnson et al. 2001; Minchin et al. 2006; Montz and Hirsch 2016), all these studies have taken their samples when the boats were out of the water. This sampling strategy might be realistic if one only focuses on overland-transport, but it neglects potential translocation within a connected system of waterbodies, or in a marine system.

Transport of small watercraft within systems of interconnected waterbodies is an important secondary transport mechanism, as it can help AIS to overcome migration barriers like dams or waterfalls via locks or boat lifts (Rahel 2007; Kelly et al. 2012). Organisms inside of cooling systems of boat motors might have increased chances of survival than those in standing waters, because aeration and temperature could be more favourable in cooling systems while the motor is still submerged, than they are in small volumes of residual waters during overland transport (Havel and Stelzleni-Schwent 2000; Johnson et al. 2001). However, sampling cooling systems while the boat is inside of the water can be challenging, because most openings for draining water are below the water surface or require tools to reach, which in turn might cause hesitance of boat owners to grant access to the cooling systems.

The round goby *Neogobius melanostomus* (Pallas, 1814) is one of the most prominent aquatic invasive fish in Europe and North America. Inside of navigable waters, ballast water transport of commercial ships is considered its main translocation mechanism (Hensler and Jude 2007; Kotta et al. 2016), but it also continues to spread in waters without commercial shipping (Bronnenhuber et al. 2011). Active upstream migration of fish is often prevented or hindered by dams and weirs, even if there are fish passes around them (Rahel 2007). For bottom-dwelling fish like the round goby, high flow rates within upstream fish passes might make a passage unlikely (Wiegleb et al. 2020). Secondary transport mechanisms are therefore probable to promote their dispersal in waters without commercial shipping (Bronnenhuber et al. 2011). Round gobies have pelagic larval and juvenile stages, which are present in the water column throughout the reproductive season of the gobies from dusk until dawn (Borcherding et al. 2016; Ramler et al. 2016). The typical size of a round goby larva during their drifting stages is 6–10 mm (Borcherding et al. 2016; Ramler et al. 2016), the gape width in that size range is 0.5–1 mm (Olson and Janssen 2017). The drifting life stages are considered propagules for translocation via ballast water of commercial ships (Hensler and Jude 2007). The typical time of larval drift is between April and August and overlaps with the peak boating season in

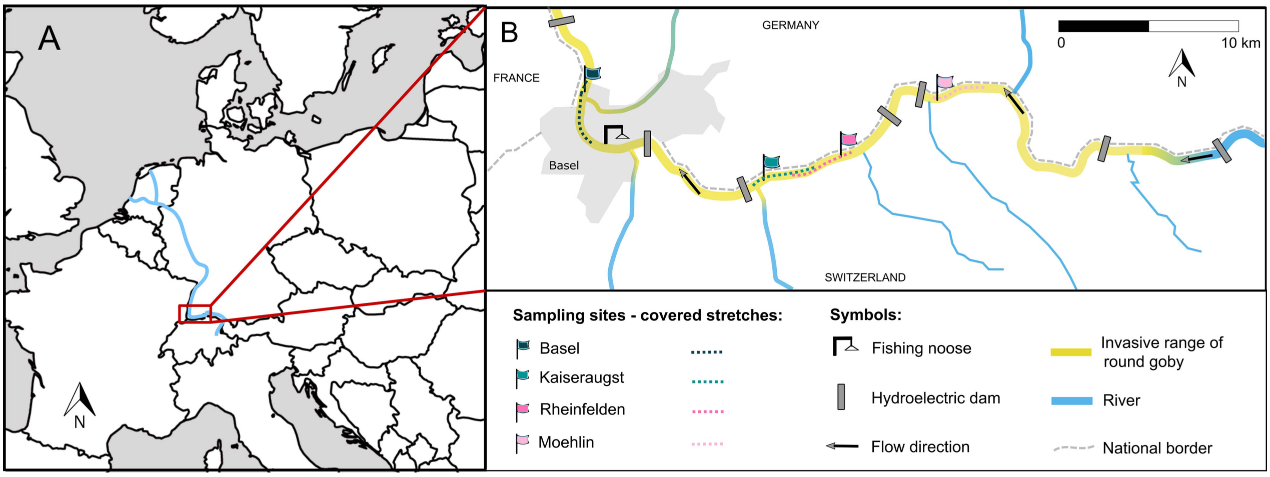

Figure 1. Map of the study locations. A: Europe showing the river Rhine (blue) and the location of the studied area (red box). B: The river Rhine and its tributaries in the study area.

Europe (Hirsch et al. 2016). The possibility of goby larvae transport in residual waters of recreational boats, however, is so far unexplored, and it is unclear how much recreational boat traffic happens during the high-risk hours after sunset.

Here, we investigate the role of motor cooling water of recreational boats in assisted dispersal of invasive ponto-caspian gobies. To study the spatial and temporal overlap with vector activity and propagule, we examine boat motor contents after drives after sunset in the river Rhine. Additionally, we determine drift densities of goby larvae at the water surface to investigate the potential for uptake into boat cooling systems. We sampled boat motors in close collaboration with local boat owners, enabling us to access the understudied cooling systems, and resulting in a mutually informative exchange of knowledge and experiences about invasive species translocation that furthered our understanding of boater behaviour.

Materials and methods

Study location

Our study took place at four locations along the High Rhine between Ryburg-Moehlin (km 144) and Basel (km 170, Figure 1). Round goby populations are established at all locations investigated.

Contact to boat owners

We sent an inquiry to 33 contacts from a stakeholder network that our working group (Program Man-Society-Environment, University of Basel) has established in the context of the ponto-caspian goby invasion in the High Rhine area since 2012 (e.g. N'Guyen et al. 2016). The inquiry asked if they a) had a suitable boat available, and b) would be willing to either lend us the boat, or drive with us (complete inquiry presented in Supplementary material Appendix 1). We defined a suitable boat as a boat with either an outboard or an inboard motor/stern drive with a minimum power of

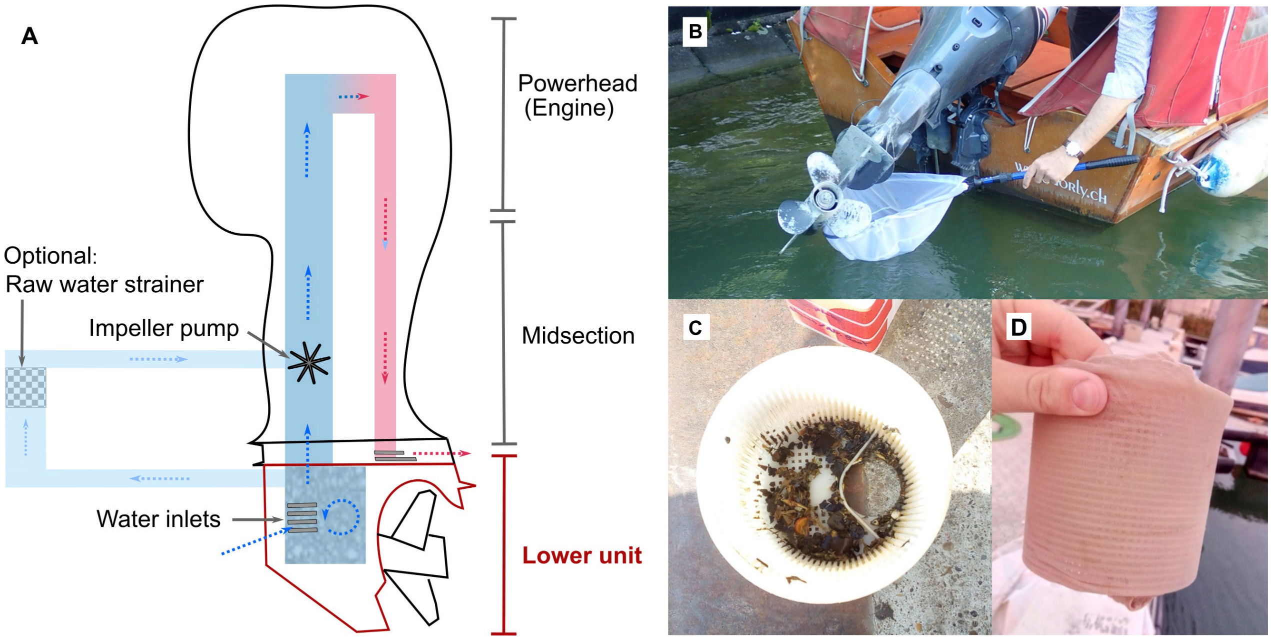

Figure 2. Boat motor water circuits and sampling. A: Simplified schematic drawing of an outboard boat motor and the water flow through the cooling system. Arrows represent flow direction. In the cavity of the lower unit (shaded), sediment and organisms accumulate if left under water. For inboard motors/stern drives (optional circuit, light blue), the engine is located inside the boat and the water circuits are therefore longer and hold more water. Additionally, they optionally include a raw water strainer to filter larger solids from the cooling water before they reach the impeller pump and the engine. B: Sampling of an outboard motor in the water using a landing net. C: Raw water strainer with contents (mesh size 1 mm). D: Additional fine-meshed filter material wrapped around the strainer for boat drives in this study. Photographs by K. Bussmann, Program MGU, University of Basel.

50–75 HP, as those motors have large enough water inlets to take up particles in the size range of drifting goby larvae (6–10 mm, Borcherding et al. 2016; Ramler et al. 2016), as well as sizeable residual water volumes.

Sampling of boat motor cooling systems and control sampling

Five boat owners were chosen at four locations (specifications in Table S1). We conducted 30 drives in total: 14 drives in Basel, five drives in Kaiseraugst, three in Möhlin, and eight in Rheinfelden (Figure 1, Table S1). All sample drives were undertaken after sunset over the reproductive season of round gobies (June–August 2020). In August, sampling only took place in Basel. In total, there were 43 hours of engine running time covering a distance of 202 km (Table S1). After clearing the initial contents of the boat motor residual waters, we drove the boat for 1–2 h on the river with speeds < 10 km/h above water. We drove the boat up- as well as downstream, while keeping the boat close (< 10 m) to shore. According to the boat owners, this drive profile is realistic for boaters enjoying sunset-drives, or anglers on night time fishing-trips.

To collect the samples, for outboard motors, the boat owners lifted the motor slowly, while we caught the contents exiting the water inlets using a landing net (mesh size 500 µm, Figure 2B). The filtered sample was then preserved in 100% ethanol. For boats with a sterndrive or an inboard motor and a raw water strainer, we opened the strainer before the drive and collected the content (Figure 2C). We installed a second layer of filter by wrapping a stocking around the raw water strainer (mesh size 75 µm, Figure 2D), as smaller goby larvae might be flushed through the raw water strainer (mesh size 1 mm). After each drive, we collected the contents of the raw water strainer, and then removed the stocking and emptied it in a sampling vial filled with 100% ethanol.

Additionally, we quantified the drift density of goby larvae and native fish larvae in the upper water layers during the boat drives, assuming that the likelihood of a boat motor to take up goby larvae is dependent on their occurrence per volume of water. To catch goby larvae drifting in the upper water layers, we used a plankton net (manta trawl, HYDRO-BIOS Apparate Bau GmbH, Altenholz, Germany), which was towed along the water surface 15 m behind the boat (Figure S1A, B). The manta trawl consisted of a metal frame with a mouth opening of 30 × 15 cm, two lifting bodies attached on both sides of the frame to keep the trawl at the surface, a net length of 2 m, a mesh size of 300 µm, and a removable soft net bucket to empty the contents (Figure S1A, B). A flow meter was attached on the inside of the metal frame to collect data on the amount of water filtered during each drive. After each drive, we removed the net bucket at the lower end of the plankton net and preserved the contents in sample containers (100 ml), filled with 100% ethanol.

Furthermore, we wanted to find out if goby larvae are also present in the shallow waters next to shore (< 5 m distance to shore). This information is important, because the shoreline is often the part of the river in which boats stay for longer times to load/unload the boat, or warm up the engine. We sampled shallow waters using a so-called "fishing-noose", a traditional local fishing device equipped with a fishing net, which is lowered into the water parallel to the riverbed (Figure S1 C, D). We installed a plankton net with a mesh size of 650 µm and dimensions of 3.5 × 3.5 m on the noose. We lowered the net into the water at sunset until it just touched the river bottom (depth 0.5–1.5 m) and pulled it to the shore after half an hour to search for fish and fish larvae. We euthanized all goby larvae and stored them in 100% ethanol. We repeated this procedure four times every night starting at sunset with an interval of 30 minutes each. In total, we used the fishing noose on 19 nights across the sampling season.

Quantification of boat traffic after sunset

To evaluate whether the uptake of goby larvae into boat motors after sunset was a realistic option, we collected data on boat traffic at the same time as when our sampling took place. Starting on June 24th, we recorded the presence of other boats active on the sampled stretch of the Rhine during sampling. On some dates, we were not able to count boats because of poor visibility.

Analysis of samples

In the lab, we searched all samples for fish larvae. For this, we removed all larger debris from the sample and spread out the remaining material in a thin layer, which was then systematically searched from top left to bottom right using spring steel tweezers to remove fish larvae. Each fish larva was identified under a dissecting microscope as goby larva or native fish larva and counted. Gobies were identified by the presence of a fused ventral fin, a trait that no native species exhibits (Kottelat and Freyhof 2007; Ramler et al. 2014).

We calculated the drift density per 100 m^3 of goby larvae and native fish caught in the manta trawl using the formula DD = n*100/ r*0.3*A, where DD = drift density, n = number of larvae caught, r = number of revolutions of the flowmeter, A = area of net opening (constant: 0.3 * 0.1 = 0.03; 0.1 m was the average estimated depth to which the net opening was submerged).

We counted all goby larvae caught close to shore with the fishing noose and calculated an point abundance estimate in larvae per 100 m^3 using the formula A_P = n*100/l*w*d, A_P = abundance at the time of the pull, l = length of the net, w = width of the net, and d = depth of the net below water surface.

Collection of anecdotal evidence for organisms in cooling systems

To further support our finding of fish in boat motor cooling systems and to increase the geographical scope with potentially available anecdotal evidence from international boat owners, we conducted an online search using the search engine Google. On 29. January 2021, we entered the keywords “raw water strainer” OR “outboard motor lower unit” AND “fish” OR “animal” OR “critter” OR “crab” OR “shrimp”. Within the results, we focused on boating forums or grey literature in the area of aquatic invasive species, and compiled reports, pictures and anecdotes of live and dead animals in water circuits in a supplementary document (Appendix 2).

Results

Goby larvae in boat motor cooling systems

We detected one goby larva in a raw water strainer sample from a sterndrive once (sample taken after a drive on August 19th in Basel). The goby larva caught measured 7 mm total length (Figure 3). It was located in the fine-meshed filter, indicating that it moved through the raw water strainer. The goby larva looked externally unharmed when investigated under a dissection microscope. We did not find any other fish or fish larvae inside the boat motors. However, we found invertebrates inside the motors, including invasive species like the killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894), zebra mussel shells (*Dreissena polymorpha* Pallas, 1771), or the freshwater shrimp *Atyaephyra desmarestii* (Millet, 1831). The latter two species were both found in the motors after the respective boat had been to rivers in France. Given the scope of the paper, we did not identify and count the invertebrates in detail.

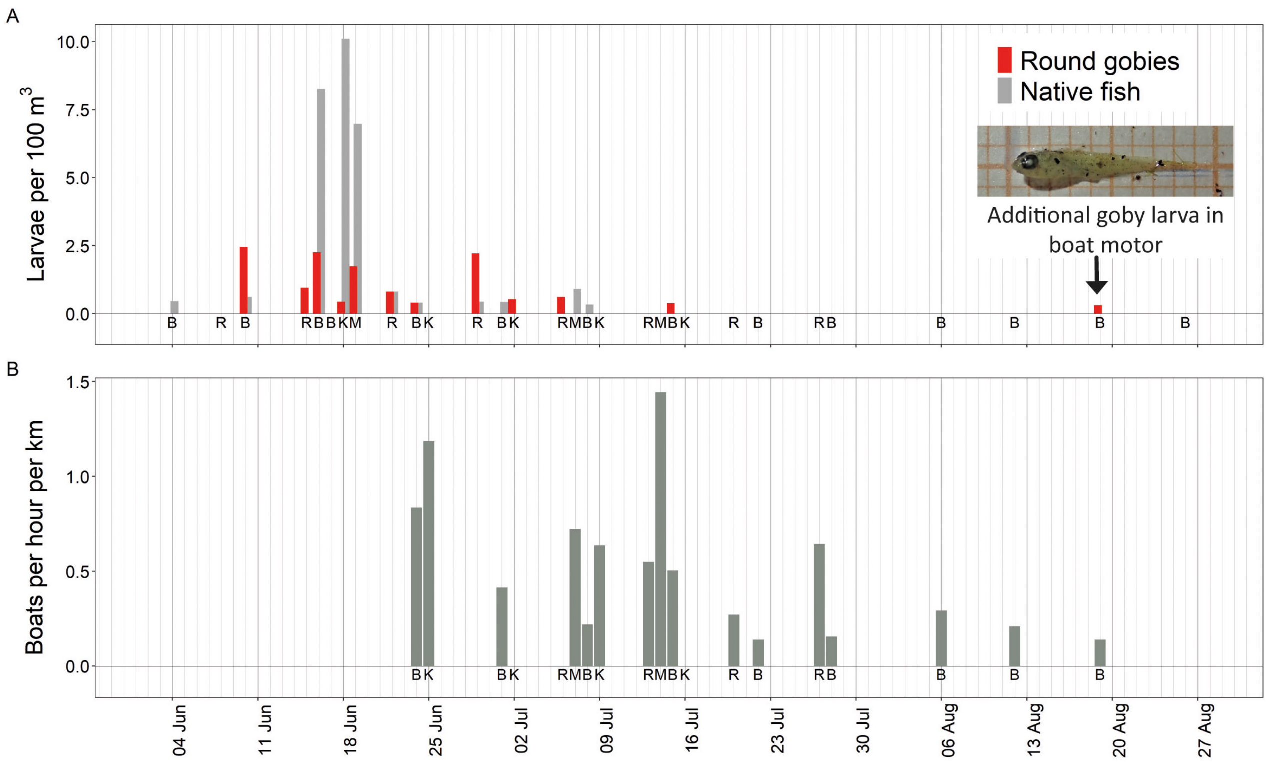

Figure 3. Results of invasive goby larvae sampling and boating activity after sunset. A: Drift density of fish larvae caught in the plankton net towed behind recreational boats after sunset at different locations along the river Rhine (B = Basel, R = Rheinfelden, K = Kaiseraugst, M = Moehlin). Boat drives only took place on dates that are indicated with a study location. Round goby larvae are presented in red, native fish larvae (species not identified) are presented in grey. On 19th August, an additional goby larva was caught in a boat motor. B: Number of recreational boats active during the 1–2 h after sunset per kilometre at different locations along the river Rhine. Quantification of boats only took place on those dates indicated with a study location. Vertical grid lines represent days. A value of zero on a date at which a sampling took place means that no fish larvae were caught on that date, respectively no boats were seen.

Quantification of propagule density and vector activity

The plankton net, which was towed behind the boat, filtered a total of 6050 m^3 of water and caught 50 larvae of native fish and 23 larvae of invasive gobies in total. Native fish larvae in the surface waters were present predominantly in June and were not present in the plankton net after mid-July, while goby larvae occurred from early June until late August (Figure 3A). For gobies, the number of larvae caught was higher in June than in July or August. Goby larvae were present in samples of all four locations, and none of the locations stood out with particularly high or low catch numbers. We only caught goby larvae with the fishing noose between mid-June and mid-July, reflecting the peak of larval drift. In those nights, the absolute number of goby larvae caught per night was between 1 and 3 (0.5–1 per pull of the noose, or 5.5–11.1 per 100 m^3).

During all but two drives on which we quantified boat traffic, there were other boats present and active after sunset (Figure 3B). Identifiable categories of boat rides were pleasure rides (people eating/drinking), water sports (swimming, water ski, wakeboarding), or angling trips (fishing rods visible).

Collection of anecdotal evidence for organisms in cooling systems

We found eleven websites which documented evidence of fish and other creatures inside of boat cooling systems and other boat compartments. Especially in boat forums there were often several reports by different users reporting findings, so that the total number of reports exceeds that of the number of websites found: There were three reports of live fish, five reports of dead fish, and eight reports of fish for which it was unclear whether they were alive or dead. Additionally, we found reports of live and dead other animals inside of cooling systems, among those were crabs, shrimp, jellyfish and even a snake. The complete results of the online search are presented in Appendix 1.

Discussion

The presence of a goby larva in the cooling system of a boat motor is an important proof of principle for our hypothesis: that invasive gobies can be translocated by small watercraft via motor cooling systems. Invasive goby translocation by small watercraft has long been assumed but to date has lacked empirical evidence (Ahnelt et al. 1998; Moskal'kova 1996; Bussmann and Burkhardt-Holm 2020). Moreover, the only mode considered for goby translocation via recreational boats so far is transport of their eggs on boat hulls similar to biofouling organisms (Adrian-Kalchhauser et al. 2017). Goby larvae as propagules transported by recreational boats have, to the best of our knowledge, never been considered in the scientific literature.

The density of round goby larvae in the uppermost layers of water in the sampled area of the river Rhine between June and August 2020 was never higher than 2.5 individuals per 100 m^3, which is in a similar range as found in some other drift-net studies (Hensler and Jude 2007; O'Brien et al. 2019). However, the long duration of round goby reproductive season makes the pick-up and viable transport and release of larvae within recreational boat engines possible at any point of time during the European summer months. Additionally, the density of round goby larvae can be much higher and vary between locations and years (Borcherding et al. 2016; Hayden and Miner 2009). Studies of larval drift at different depths indicate that larval densities can be higher in deeper water layers (depth > 2 m), while the uppermost layers hold most larvae of the smallest size class (< 9 mm), which might be especially prone for uptake by small watercraft motors (Hayden and Miner 2009; Juza et al. 2016). Data collected with the fishing noose shows that goby larvae are present in shallow waters along the shore of rivers. The abundances of goby larvae can reach numbers as high as 11 larvae per 100 m^3 when point sampling. This has ramifications for assessing the probability of larval uptake by boats: boats often remain running for a long time to warm up the engine or load/unload the boat close to the shoreline, which might increase uptake probability.

The observed numbers of recreational boats active after sunset confirm that the intake of goby larvae is not just a hypothetical risk. Angling in particular is a popular night-time activity for boat owners in the area (personal communication of various boat club members and anglers). Even if they would not move their boat to a new location during the night, fish larvae caught inside the cooling systems could stay inside and only move out after a relocation on a day afterwards. This connection highlights how important it is to gain insight into the behavioural patterns of stakeholders, who might unintentionally translocate invasive species. For example, fishing trips can be used to infer invasion risk of round goby released as baitfish (Drake and Mandrak 2014). Our study demonstrates how invasive species could be translocated not only as baitfish but also within motor engines of fishing boats. This information can inform and improve risk models evaluating translocation probabilities (e.g. Acosta and Forrest 2009; Parretti et al. 2020).

Limitations

We cannot be certain that the goby larva that we found in the raw water strainer was still alive in the motor, because we only found it during analysis in the laboratory. To prove the actual translocation potential of boat motors, the documentation of a live fish larva would be necessary. We have ample reason to believe that survival in motor cooling systems is possible: The goby larva we found looked externally unharmed upon examination of the sample. Fletcher et al. (2017) documented the successful transport of a living juvenile fish through an impeller bilge pump, which is a similar pumping system as used in boat motors. Furthermore, some of the collected anecdotal evidence (see Appendix 1) speaks for the possible survival of organisms in cooling water circuits and raw water strainers. Additionally, we found numerous other live organisms in the boat motor cooling systems and strainers over the course of this project, as well as during former projects looking at motor interiors (Amt für Umwelt und Energie Kanton Basel Stadt 2019).

The quantification of boat traffic during the nights in the area was circumstantial and did not include the weekend nights, when traffic is likely to be highest. While we can show that boat traffic after sunset is not an exception, we acknowledge that our data likely underestimates the true extent of after-sunset boating activities.

Minimizing translocation risk of cooling systems

The risk of taking up a round goby larvae at surface drift densities as determined in our study might seem low, as we only found a single goby larvae in one out of 30 drives. However, the uptake risk of an individual boat is determined by multiple factors like motor type and size, motor running time, and speed. For example, a standard impeller pump for a boat motor up to 400 HP nominally pumps 80 l/min at 1500 rpm (4.8 m^3 per hour), while its maximum pumping capacity is 102 l/min (6.12 m^3 per hour). If driven for 2 h during peak drifting season (2.5 goby larvae per 100 m^3), a boat would take up 0.24–0.31 goby larvae. While these numbers are low for any individual boat, the probability of uptake can become substantial if taken cumulatively across all boats moved from invaded to uninvaded areas. For example, in the year 2013, there were between 10 and 5200 boat transports per weir in the Rhine upstream of the current invasion front (Figure 1), with the highest numbers at a weir very close to the ecologically and economically valuable Lake Constance (Hirsch et al. 2016).

Measures against the translocation of invasive species in the inside of boat motors and residual waters could include: motor flushing devices ("muffs") as mandatory boat equipment, check-points with information and instructions for boats at harbours or locks, or (mobile) boat cleaning stations (Horvath 2008). We forwarded the outcomes of this study to relevant authorities in Switzerland, resulting e.g. in adjustments in a newly launched information campaign about translocation risks of recreational boats and measures for prevention to specifically include the insides of boat motors (AWEL Zuerich 2020).

Conclusions

The residual water in the insides of boat motors present an understudied potential for secondary translocations AIS. The finding of invasive goby larvae inside the cooling water system of a recreational boat motor is important to consider in order to develop effective measures against translocation of AIS, especially during in-water transport of boats. Anecdotal evidence suggests the repeated occurrence and viability of organisms inside the cooling systems. In addition, management recommendations for boat cleaning often neglect to include flushing of cooling systems. However, there are simple methods to avoid unintentional translocation of AIS in cooling systems: tilting the motor to remove most residual water whenever stationary, flushing the motor with hot water, and educating boat owners about the hidden organisms inside the motors and effective measures for the different types of motors could help preventing the further spread of AIS, especially across biogeographical barriers.

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Author's contribution

KB: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation; writing - original draft, writing – review and editing. PEH: research conceptualization, sample design and methodology, writing – review and editing. PBH: research conceptualization, sample design and methodology, funding provision, writing – review and editing.

Ethics and permits

Ethics approval was not required according to Swiss law. The offices for environment and energy of the cantons Basel-Stadt and Aargau granted permits for using the manta trawl and fishing noose to catch fish.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Inquiry sent to boat owners to ask for collaboration on the project.
Appendix 2. Anecdotal evidence for organisms in boat motors from online boat forums.
Figure S1. Figure showing control sampling methods.
Table S1. Data on sampling locations, sampling dates, and boat types used for sampling.

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