

Does tagging transparent fish increase predation risk? A laboratory study with glass eel (*Anguilla anguilla*) and sea bass (*Dicentrarchus labrax*)

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Abstract

Barriers in the estuaries of the rivers prevent the immigration of glass eels (*Anguilla anguilla*) arriving on the European coast every spring. This leads to an unnatural accumulation of migrating glass eels below the barriers, and this may lead to additional losses in glass eels by piscivorous fish. The proportion of predation losses can be estimated using mark-recapture techniques and abundance estimates in combination with stomach content analysis of piscivorous fish. Nonetheless, whether tagging transparent glass eels increases predation risk and what the digestion rate of glass eel is in piscivorous fish are unknown. This study aimed to determine whether there is an increased predation risk for tagged glass eel; it also studies glass eel digestion status in piscivorous fish after appointed time frames. A laboratory experiment with 48 trials was conducted. Tagged (visible implanted elastomer, VIE) and untagged glass eels were exposed to small (19.1–24.4 cm) and large (31.9–43.5 cm) sea bass (*Dicentrarchus labrax*) during a 2 h trial. In 48% of the trials, successful predation was present and 13% showed clear predation attempts in which bass did not capture glass eels. No significant difference was found in predation rate between tagged and untagged glass eels and between red and blue tagged glass eels. Large sea bass preyed more, but all sizes consumed glass eel under laboratory conditions. Stomach content analysis showed intact glass eel bodies 4–6 h after ending the 2 h trial and parts of glass eel bodies up to 16–18 h. This study showed that tagging does not increase predation in mark-recapture studies using VIE-tags in transparent glass eel. It also shows that the proportion of predation in relation to local glass eel abundance can be estimated if stomach content analysis is conducted within 4–6 h after predation.

KEYWORDS

glass eel, mark-recapture, predation, sea bass, stomach content, VIE-tag

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1 | INTRODUCTION

European eel (*Anguilla anguilla* L.) is an economically important species and is listed on the IUCN red list as critically endangered (Pike *et al.*, 2020). In the North Sea, recruitment series across Europe indicate that current recruitment is only 2.1% of 1960–1979 rates (ICES, 2020). This decline may have many contributing factors, and most of these are anthropogenic, such as overexploitation (Dekker, 2000; Dekker, 2003), migratory barriers resulting in habitat loss or fragmentation (Feunteun, 2002; Tesch, 2003; van Puijenbroek *et al.*, 2019) and changes in oceanic conditions and atmosphere regime shift because of climate change (Borges *et al.*, 2019; Friedland *et al.*, 2007; Miller *et al.*, 2016). Nevertheless, glass eels arrive each year at coastal areas attempting to inhabit freshwater habitat or estuaries.

Increased predation, as an indirect cause of mortality in addition to migration barriers, has so far received little attention in studies of the decline in glass eel recruitment. Extra predation pressure, however, might contribute to recruitment losses especially during peaks in the migration in combination with unnatural accumulations at man-made barriers. For example, predation risk may be increased by man-made barriers that cause migratory delay, resulting in longer exposure time (Boulêtreau *et al.*, 2020; Newton *et al.*, 2019). For example, along coastal and inland barriers, piscivorous fish, *e.g.*, the European sea bass (*Dicentrarchus labrax*) and cod (*Gadus morhua*), and freshwater species, *e.g.*, pike perch (*Sander lucioperca*) and perch (*Perca fluviatilis*), are present and are the potential predators. Nonetheless, whether glass eels are part of the typical food spectrum of these fish are, to the best of the authors' knowledge, unknown. The authors do know that predation has been suggested for Japanese glass eel (*Anguilla japonica*) (Miyake *et al.*, 2018), especially during peaks of migration. In addition, predator fish such as Japanese sea bass (*Lateolabrax latius*) are found near Japanese glass eel accumulations (Miyake *et al.*, 2019) and authors have suggested that glass eel predation is present (Miyake *et al.*, 2018). Predation by perch (*P. fluviatilis*) and pumpkinseed (*Lepomis gibbosus*) on young yellow eel is suggested near eel ladders, but not observed (Drouineau *et al.*, 2015). One reason that knowledge about glass eel predation in general is limited may be that piscivorous fish have a short digestion time (Miyake *et al.*, 2018). Therefore, knowledge about digestion rates is needed to prevent false negatives in stomach content analysis.

The relative proportion of glass eel predated by piscivorous fish in relation to local abundance can be estimated by mark-recapture techniques. To estimate local abundance, mark-recapture methods such as alizarin red S (Caraguel *et al.*, 2015; Diekmann *et al.*, 2019; Kullmann *et al.*, 2017), oxytetracycline (Simon & Dörner, 2005), vital dyes (Briand *et al.*, 2005), coded wire tags (Simon & Dörner, 2005) and visible implant elastomer (VIE) tags (Imbert *et al.*, 2007) are used. Differences between these methods include group or individual marking, tag retention and the necessity to kill glass eels to identify recaptures (Briand *et al.*, 2005; Imbert *et al.*, 2007; Simon & Dörner, 2011; Simon & Dörner, 2005). Marking methods that can discriminate between groups, affecting neither behaviour nor survival, are preferred. When temporal and local glass eel abundance must be estimated in relation to the presence of piscivorous fish during the migration season, visible tags such as VIE-

tags may be good candidates. Experiments showed that the mortality of VIE-tagged glass eels was negligible to 1% (Drouineau *et al.*, 2015), and retention of the tags in the fish was 98.7% during a 5 month laboratory experiment (Imbert *et al.*, 2007).

Tagged and untagged glass eels are assumed to have an equal survival probability in abundance estimates of glass eel (Briand *et al.*, 2005; Diekmann *et al.*, 2019). Nonetheless, the effects of visible tags on predation risk may bias abundance estimates in case of unequal survivability. Although no clear relation between predation and VIE-tags was found for fantail darters (*Etheostoma flabellare*), largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*) and blacktail shiners (*Cyprinella venusta*) (Reeves & Buckmeier, 2009; Roberts & Kilpatrick, 2004), predation risk for fully transparent glass eels in combination with colourful VIE-tags might differ.

This research studied predation of glass eel under laboratory conditions, to test whether glass eel equipped with VIE-tags have a higher predation rate than untagged glass eel. Tagged and untagged glass eels were exposed to European sea bass (*D. labrax*) during a 2 h trial. A stomach content analysis was then executed to measure digestion status of glass eel in the sea bass. Sea bass was chosen as a potential predator because it frequently occurs along the Dutch coast during the migration season of glass eel, especially near barriers. Sea bass has an opportunistic feeding strategy that relies on available prey (Cardoso *et al.*, 2015) which might be glass eel near barriers especially during the peaks of migration. This study should provide knowledge on glass eel as prey under laboratory conditions and gives validation of the use of VIE-tags in mark-recapture studies.

2 | MATERIALS AND METHODS

2.1 | Tagging and test procedure

European sea bass ($n = 48$) were caught along the Dutch coast at Borssele (on 24 April 2020, all small individuals) and IJmuiden (29 April 2020, all large individuals) (Table 1). The fish were kept separately per catch site in 1000 l (Borssele) and 2800 l (IJmuiden) tanks, which were filled with sea water (34‰); in the tanks, the water was aerated and temperature was controlled at 11–13°C, with a light regime of 13 h light and 11 h dark, which reflects the conditions during glass eel migration in spring. To exclude stress as an interfering factor in the experiments, the fish were acclimatized for a minimum of 14 days; they were fed daily with bred sandworms (*Alitta virens*) and lugworms (*Arenicola marina*). Sea bass were only used in the experiment when

TABLE 1 Length and weight data for European sea bass (*Dicentrarchus labrax*) used in this experiment

Location	n	Length (cm)			Weight (g)		
		Avg	Min	Max	Avg	Min	Max
IJmuiden	24	36.4	31.9	43.5	467.4	312.6	733.3
Borssele	24	21.2	19.1	24.4	91.4	64.7	123.8

food intake was successful during the acclimatizing phase (Beitinger, 1990).

Glass eels were caught along the Dutch coast and kept in 45 l aquaria (lwh: 50 × 30 × 30 cm), which were connected to a filtered saltwater (34‰) system, aerated and temperature controlled at 11°C. Maximum stocking density was c. 200 glass eels per aquarium. The aquaria cage was enriched with multiple PVC pipes (3–4 cm diameter) to prevent stress and no food was provided, because glass was kept for a maximum of only 10 days.

Glass eels were selected for transparency and anaesthetised (0.4 ml l⁻¹ 2-phenoxyethanol) before implanting a double red or blue VIE-tag of 3–4 mm in the tail with a 0.3 mm needle. Double tags were used to increase visibility compared to untagged glass eels. After that, glass eels were transferred to an aerated container to recover from the treatment and checked for normal behaviour, which was defined as regular swimming behaviour or hiding in the PVC pipes. Control groups followed the same procedure except for tagging. All glass eels were treated 20–24 h before using in the trials. No tag loss was observed, and negligible mortality was observed because of tagging or handling ($n = 1$).

Trials of 2 h were executed to test the differences in predation and behavioural response. In total, 25 trials with “blue–blue glass eels” and 23 with “red–red glass eels” were executed. Each day, two trials were conducted simultaneously in two (lwh: 200 × 200 × 70 cm) fibreglass square rounded tanks filled with 2000 l (34‰) sea water each equipped with one LED floodlight (4500 lm, 4000 K and 380–780 nm wavelength). No sediment or hiding material was placed in the tanks. Trials were observed visually (behind tarpaulins) for the first 15 min during the trial and filmed for behaviour analysis by a GoPro7 centred above the tank for the full 2 h period. To trigger predation interest, sea bass were withheld food for 66–72 h before the trial according to the following procedure: two sea bass were randomly netted and stored in a 1000 l tank in which no food was provided. After 48 h, the sea bass were transferred and individually stored in the test tank. In the test tanks, the sea bass were given 18–24 h acclimatization time. After this period, 20 glass eels (10:10 tagged:untagged) were introduced into the test tank and exposed for predation for 2 h. After 2 h, the sea bass were removed, anaesthetised using 0.4 ml l⁻¹ 2-phenoxyethanol, weighed and measured. The (remaining) glass eels were caught and counted.

2.2 | Stomach content analysis

Sea bass that showed successful predation were kept separately for stomach content flushing procedure. During this procedure the fish were anaesthetised using 0.4 ml l⁻¹ 2-phenoxyethanol, weighed and measured. The stomachs were then flushed by a hand pump (Kamler & Pope, 2001), consisting of two parallel 30 cm silicon tubes of different diameter, of which the smaller (ø5 mm) was connected to a bulb filled with sea water. The other tube (ø12 mm) allowed water and stomach content to return for analysis. This procedure was conducted after 0.5, 1, 2, 4, 8, 16 and 32 h after finalizing the 2 h trial. For each time interval three bass were used. If flushing did not

successfully return (parts of) glass eel, the fish was euthanized and stomachs (and intestines) were analysed manually by dissection to evaluate the hand pump procedure. If no glass eels were found by dissection, it was assumed that glass eels were digested. All other fish were released again into the wild.

2.3 | Sea bass behaviour

To link successful predation to specific swimming behaviour, a video analysis was done for each trial. In each trial, sea bass behaviour was checked to see whether there had been at least one clear predation attempt. This “predation attempt” was defined as a (clear) rapid movement towards a glass eel. In addition, activity of the sea bass was tested to validate the trial. In each trial, sea bass behaviour was analysed for (a) grid change, (b) spatial use of the tank (SPI) and (c) latency of the activity. To quantify the movement of the sea bass, the tank was virtually divided into a grid of nine equal squares (3 × 3). Analysis was done using Cowlog (Hanninen & Pastell, 2009). Each time a sea bass moved between grid cells, this was noted as “grid change” (a) and expressed as grid change per minute. Spatial use of the tank (SPI) (b) was defined as the time spent in each grid cell and expressed as SPI (Dickens, 1955; Plowman, 2003; Rose & Robert, 2013). The SPI shows an index of how an animal uses its available space, where 0 equalizes maximum use of all the grids and a 1 indicates use of only a single grid. Latency (c) was defined as the time (seconds) until the sea bass changed grid for the first time. The first 10 s after release of the glass eels were ignored because of potential disturbance.

2.4 | Data analysis predation

A binomial generalized linear model (GLM) with a logit link function was run with multiple covariates (there was no over-dispersion in the binomial GLM). The number of eaten tagged glass eels per trial was the number of binomial successes, and the number of eaten eels in total in a trial was the binomial totals (thus subtracting the number of eaten tagged eels from the number of eaten eels in total gives the number of binomial failures). With respect to the covariates, the length of the sea bass was excluded from the model because it highly correlated with weight. In addition, grid change per minute and SPI were highly correlated; grid changes per minute were chosen to be in the full model. The full model had the following covariates: latency, the time of the experiment, weight of the sea bass, grid change, days in the lab, catch site of the sea bass, colour of the tag and whether the glass eel was tagged or untagged. From this full model, covariates were selected through backward elimination by AIC. A restriction was set up in variable selection, so that the variable “tagged/untagged” was not to be eliminated. Separate analysis for comparison between covariates was done with Monte Carlo Permutation Tests (using 10⁵ simulations). *P*-values lower than 0.05 were considered to be significant.

2.5 | Ethical statement

The care and use of experimental animals (sea bass and glass eel) complied with the Dutch animal welfare laws, guidelines and policies as approved by the “Central Committee Animal experiments” following protocol 2019.D-0050.

3 | RESULTS

3.1 | Differences between tagged and untagged glass eel

In 23 (48%) of the 48 trials, one or more glass eels were eaten; in six trials (12%) a clear attempt was seen, and in 19 trials (40%) none were eaten nor an attempt made. A total of 164 glass eels (17% of the 960 glass eels exposed to predation) were eaten. Of these, 77 (16% of 480) were tagged and 87 (18% of 480) were untagged. When predation occurred, an average of 7.1 ± 5.6 (mean \pm s.d.) (36%) glass eels were eaten. In one trial, all 20 glass eels were consumed. Eight of the 23 successful sea bass ate over 50% of the glass eels within 2 h.

Successful predation was shown in 11 of the 23 trials (red) and 66 glass eels were eaten (30:36 control:tagged). In the trials with blue tagged glass eels, this was 12 of 25 trials (blue) and 98 glass

eels were eaten (57:41 control:tagged). The covariate selection of the binomial GLM through backward elimination resulted in a model with only the covariate “tagged/untagged” retained. This model revealed no significant difference between tagged and untagged glass eels ($P = 0.27$, Table 2). Additional analyses using a Monte Carlo Permutation Test showed no differences in predation between different tag-colours, neither between control and tagged groups ($P = 0.67$, Figure 1).

TABLE 2 Estimated coefficient parameters, standard errors, z-value and P-values for the binomial GLM

Term	Estimate	s.e.	z-value	P-value
(Intercept)	-0.1221	0.1565	-0.7804	0.4352
Tagged/untagged	0.2442	0.2213	1.1036	0.2698

Note: $n = 46$ observations based on 23 trials in which glass eel was eaten.

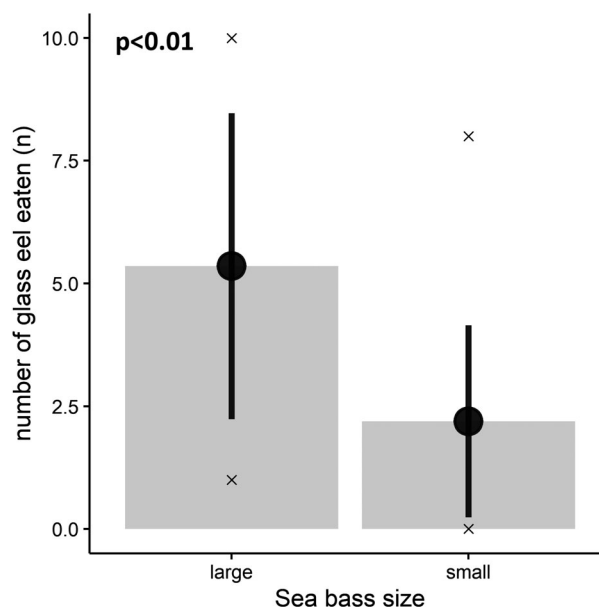


FIGURE 2 Results of predation between large ($n = 10$) and small ($n = 13$) sea basses (*Dicentrarchus labrax*). A significant difference in the number of glass eels (*Anguilla anguilla*) eaten was found between large and small sea bass ($P < 0.01$) following Monte Carlo Permutation Tests. Figure shows means (dot), s.d. (lines) and $x =$ min and max value

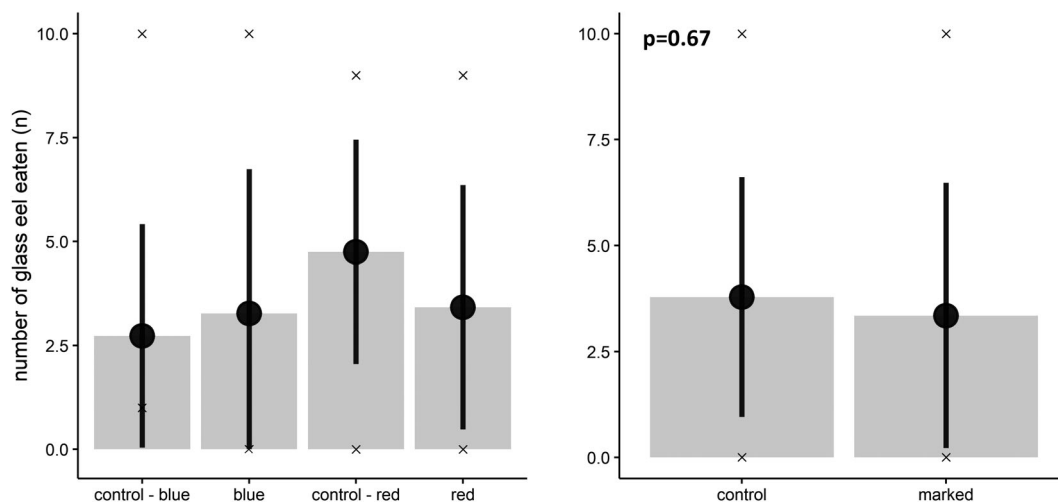


FIGURE 1 Number of tagged and untagged glass eels (*Anguilla anguilla*) eaten by sea bass (*Dicentrarchus labrax*) ($n = 23$ trials). Left: average number of glass eels eaten in trials with different colours. Right: average number of glass eels eaten in the control groups and in the marked (red and blue combined) groups. Figure shows means (dot), s.d. (lines) and $x =$ min and max value

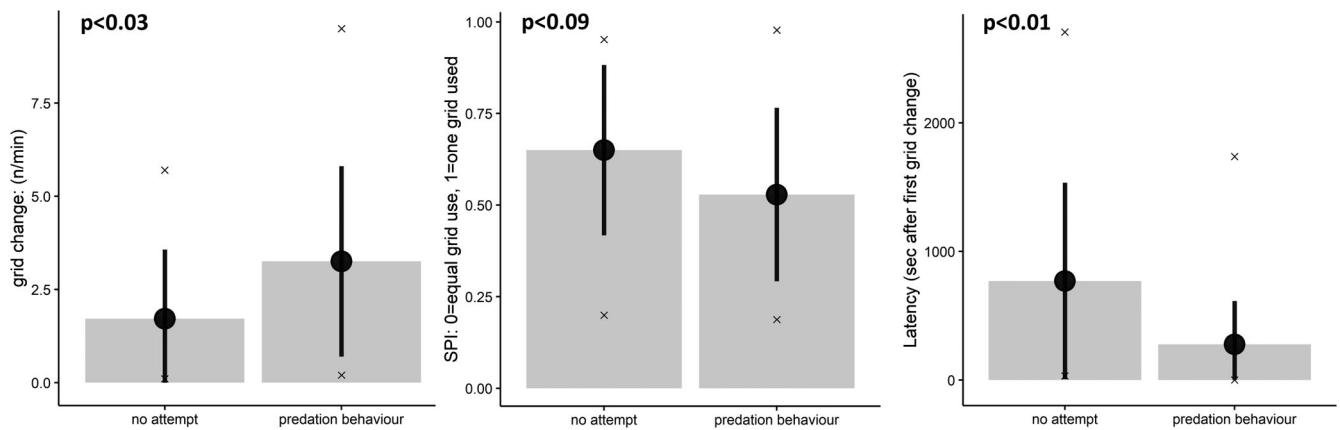


FIGURE 3 Results of behaviour comparisons (grid change, latency and SPI) of sea bass (*Dicentrarchus labrax*) showing no attempt of predation ($n = 19$), compared to sea bass that showed a clear attempt to predate on glass eels (*Anguilla anguilla*) during the experiment either successful ($n = 23$) or unsuccessful ($n = 6$). A significant difference was found for grid change and latency following Monte Carlo Permutation Tests. Figure shows means (dot), s.d. (lines) and $x =$ min and max value

3.2 | Difference between large and small sea bass in predation

Larger sea bass ate more glass eels during the trials compared to small sea basses ($P < 0.01$, Figure 2). Nonetheless, successful predation, independent of the total number of glass eels eaten, was slightly higher for small sea bass: 13 of 24 trials (54%) using small ($n = 13$) sea bass showed successful predation in comparison to 10 of 24 trials (42%) using large ($n = 10$) sea bass. In general, large sea bass showed slightly less interest in eating glass eels, but they ate more than small sea bass.

3.3 | Sea bass behaviour

To verify sea bass behaviour, three groups were identified. The first group showed active and successful predation ($n = 23$), the second group was unsuccessful but showed clear attempt(s) at predation during the trials ($n = 6$) and the third group showed no response/no interest ($n = 19$). For further analysis, the first and second groups were combined ($n = 23 + 6$) and identified as bass with “predation behaviour.” The third group was identified as “no attempt.” Sea bass showing predation behaviour were more active and less restricted to a specific location in the tank compared to sea basses that showed no predation behaviour (grid change $P < 0.03$ and latency $P < 0.01$, Figure 3). Nonetheless, the groups showed no statistically significant difference in SPI ($P < 0.09$).

3.4 | Stomach content analysis

Stomach contents showed intact glass eels bodies 4–6 h after predation (given that the exact timing of predation per item within the 2 h experiment is unknown) (Figure 4). After 8 h, stomach contents included a mix of intact glass eels and parts of glass eels. After 16 h,

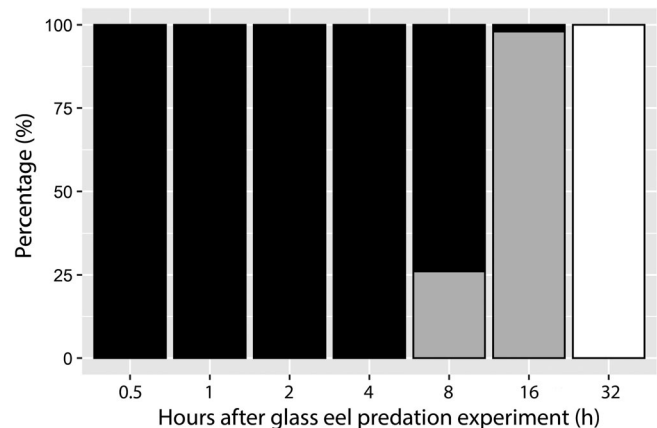


FIGURE 4 Results of stomach content analysis executed after a 2 h trial in which sea bass (*Dicentrarchus labrax*) was exposed to 20 glass eels. Each time interval 0.5–32 h contains three individual sea basses (■) complete glass eel (▒) parts of glass eel (□) nothing found

with few exceptions, stomachs contained only parts of glass eels, and after 32 h, nothing was found.

4 | DISCUSSION

4.1 | Predation risk of tagged glass eel

This study showed that there was no increased predation risk for tagged glass eels by European sea bass. Therefore, the assumption for mark-recapture studies that VIE-tagged glass eel have equal predation mortality as untagged glass eel seems justified. Moreover, the experiment was conducted in a worst case scenario for exposing tagged glass eel (i.e., light similar to day conditions, equal ratio tagged untagged, no hiding opportunities and no other prey fish).

A similar experimental set-up found similar results using 10 tagged and 10 untagged hatchery-produced age-0 fish and three different predator fish species (Reeves & Buckmeier, 2009). Their trials continued for 24 h or until 50% of the prey had been consumed, and 10 repetitions per predator species were used. As in this study, they found no predation preference for any VIE-tag colour. In addition, no significant results were found by Roberts and Kilpatrick (2004), who used yellow (VIE-tag), green (VIE-tag) and untagged fantail darters exposed to rock bass and smallmouth bass during 6–8 day trials (or after 50%–75% of the prey were eaten). The results of this study show that in addition to these tested pigmented fish, VIE-tagging transparent fish like glass eel does not increase predation rate.

VIE-tags illuminate using UV light for clear identification by professionals or volunteers. It is possible, contrary to the laboratory set-up, that VIE-tags might illuminate during sunlight hours and increase attention for piscivorous fish. Nonetheless, under natural conditions, this would not happen, as glass eels migrate after sunset (Tesch, 2003). Because, during that period, the light conditions are far from optimal, contrast (a non-transparent tag in a transparent fish) could cause higher predation risk than colour distinction. In this study, predation rate because of additional contrast was tested by selecting only transparent glass eels and tagging them with two tags. As no significant differences were found between test and control groups under light conditions, neither colour nor contrast seems to induce additional predation risk.

The trials in this study showed 48% successful predation and 13% clear attempts of predation. In contrast to those sea bass, the sea bass that showed no response (39%) waited significantly longer to show any activity (latency), and if they moved, they moved significantly less (grid change). This could be the results of their lack of interest in the glass eels, but it could also be a stress response. Based on the results and experimental set-up, the authors cannot determine why sea bass ignored the glass eels. Different personalities, such as shy or bold individuals among the test fish (Jolles *et al.*, 2016; Sih *et al.*, 2015), may explain different responses to the experimental set-up. Although based on significantly different behaviour (latency and grid change) between the two groups, stress might be the crucial factor in ignoring the glass eels. To exclude stress as an interfering factor in the experiments of this study, sea bass were only used after food intake in captivity was successful (Beitinger, 1990), thus selecting the more acclimatized individuals. Nonetheless, some sea bass may have been stressed, explaining their lack of interest in the glass eels. Some sea bass show freezing behaviour, which might indicate stress (Cerqueira *et al.*, 2020). Even if some sea bass did not predate on glass eel because of stress, it would be very unlikely that they would have shown different predation rates on tagged vs. untagged glass eel.

Unlike other predation experiments that lasted from 24 h to multiple days (Catalano *et al.*, 2001; Reeves & Buckmeier, 2009; Roberts & Kilpatrick, 2004), the length of the experiments of this study might have been too short for some of the (shy or stressed) sea bass to get used to the experimental tanks. This makes it difficult to compare the timed experiments of this study to longer experiments where the percentage of successful predation was used instead

(Catalano *et al.*, 2001, Reeves & Buckmeier, 2009, Roberts & Kilpatrick, 2004). The experiments of this study showed that 8 of 23 sea bass ate over 50% of the glass eels within 2 h; longer experiments do not necessarily mean better results. A solution can be to quantify glass eel predation during the experiment continuously, ending the trial if 50%–75% of the glass eels are eaten. Nonetheless, because stress of the sea bass was possibly a factor influencing predation during the experiments, continuously and visually counting the glass eels during the experiments was not an option; this will influence test results, because the presence of observers could induce stress in at least some of the sea bass, and glass eels are invisible in the footage because of their transparency and size. Another option is to increase the number of glass eels offered to the sea bass and subsequently increase the duration of the experiment. To do so, a balance between number of glass eels, tank size and size of the sea bass should be considered.

The lack of a statistically significant difference could also be caused by low statistical power, given the low number of successful replicates ($n = 23$). A sample size calculation was conducted to quantify the number of required trials (each trial required 1 sea bass and 20 glass eels) to find a significant difference between predation of tagged and untagged fish given the current results. Within the experiment, the average difference in proportion of eaten glass eels was found to be *c.* 0.166 (0.417:0.583). The sample size calculation for proportions (power = 0.80, significance level = 0.05, difference in proportion = 0.166) revealed that the number of required trials was *c.* 107. Considering that only 23 of 48 showed successful predation (48%), independent of using small or large sea bass, up to 223 trials may be required. Given that the predation on untagged glass eel was even slightly higher than tagged glass eel (87 vs. 77), there was no indication that more trials with higher statistical power would result in a significantly increased rate for tagged glass eel. Furthermore, even if a possible difference in predation between tagged and untagged eel exists, it would be so small that it needs large amounts of trials, and this difference is not expected to influence results in the field and might therefore be rather uninteresting.

4.2 | Implication for field studies

Mark-recapture studies assuming similar predation risk between tagged glass eel (VIE-tag) and untagged glass eels do give valid results for abundance estimations. In addition, losses of the local glass eel abundance because of predation can be measured by stomach flushing if conducted within 4–6 h after potential predation. Predation may occur by various fish species also during resting phases of the glass eels. Nonetheless, coming back to increased predation as an indirect mortality cause in addition to migration barriers, field studies should be conducted during the evenings and dark periods to observe and quantify predation in relation to local abundance. In those periods glass eels are actively swimming in large aggregations at the surface at barriers and they may be especially vulnerable to piscivorous fish. After 4–6 h, the risk of finding only

parts of glass eels is high and stomach content analysis by sight (parts of glass eel) or DNA analysis of the stomach contents can only qualitatively determine whether glass eels were eaten by a predator fish (Miyake *et al.*, 2018). During the field studies, both small and large piscivorous individuals should be considered. In this study, both sizes of sea bass showed interest in glass eel. There was a significant difference between large and small sea bass in the number of eaten glass eels, which might be explained by the relative quantity of fish in stomachs increasing with size, and starting at 10 cm (Cardoso *et al.*, 2015).

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AUTHOR CONTRIBUTIONS

A.B.G.: funding acquisition, conceptualization, methodology, analysis, lab work, supervision and writing; W.J. and T.M.: methodology, lab work, data collection and writing; T.W.: statistical analysis; H.V.W.: supervision, review and editing.

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