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Under pressure: comparing in situ and boat tagging methods using time-to-event analyses

Jessica A. Keller[1]* , Danielle Morley[1], Jennifer L. Herbig[1], Paul Barbera[1], Michael W. Feeley[1,2]
and Alejandro Acosta[1]

Abstract

Background: With the increase in telemetry studies over the past decade, improving understanding of how different tagging methods influence the probability of presence in a receiver array is important in maximizing the resulting data. Disappearance from the array may be due to mortality from surgery complications, tag loss, predation, or emigration. Internally tagging fish on a boat can cause barotrauma injuries, increased stress from prolonged handling times, or predation after fish have been released back into the water. Conducting in situ internal acoustic tagging at depth of capture removes barotrauma stresses and simplifies the release method, which may improve fish survival and decrease risk of disappearance from the array. In this study, we used 8 years of acoustic tagging data to determine if the tagging method (in situ versus on the boat) influenced the likelihood of a fish being detected 4 and 6 days after the tagging event.

Results: At 6 days after tagging, Kaplan–Meier survival curves revealed that the probability of presence for fish tagged on the boat was 66% compared to 90% for fish tagged in situ. Tagging method was the only variable to significantly affect probability of presence based on Cox proportional hazards models, with fish tagged in situ ~ 75% less likely to disappear from the array compared to fish tagged on the boat at both 4 and 6 days after tagging. Examining tagging methods separately, handling time only marginally influenced probability of presence of boat-tagged fish and no variables had a significant effect on probability of presence of in situ tagged fish.

Conclusions: In this study, tagging method was the only variable to significantly affect the probability of presence for internally tagged fish. Other factors had little to no influence, but correlation of variables limited what factors could be included in the models. Implanting internal acoustic tags in situ is not a practical method for every species and for every environment, but given the increased probability of presence demonstrated here, we strongly suggest it be considered where applicable.

Keywords: In situ tagging, Acoustic telemetry, Time-to-event analysis

Background

Acoustic telemetry has become a widely accepted method for collecting animal movement data in the marine environment [16]. Data collected from acoustically tagged fish can be used to investigate a

wide variety of biological and ecological questions. For example, acoustic telemetry data have provided answers to broad ecological questions, such as revealing previously unknown migration patterns for certain species [12, 28] and have also been used to answer more localized questions, such as patterns of habitat use in a specific location [15, 18]. Although acoustic telemetry studies have been rapidly increasing in number over the past 20 years [6], there are far fewer studies that examine the influence of tagging methodology

*Correspondence: jessica.keller@myfwc.com
[1] Florida Fish and Wildlife Conservation Commission, Fish and Wildlife
Research Institute, 2796 Overseas Highway, Suite 119, Marathon, FL
33050, USA
Full list of author information is available at the end of the article

on acoustic telemetry results [9]. This information is needed because the physical act of tagging a fish can affect the outcome of the tagging event, potentially change the behavior of that fish, and even influence the interpretation of results.

In traditional mark/recapture studies, external tags are inserted into the dorsal musculature. Traditional mark/recapture studies are a cost-effective method of tagging animals, but these studies may provide only limited spatial information, require the recapture of tagged fish, and often have low success rates [25]. To overcome some of these limitations, researchers use more advanced tags, such as acoustic tags. While externally attaching acoustic tags has been accomplished through a wide array of techniques, these tags can be quickly shed, causing a truncation of data collection [17]. An alternative method of acoustic tagging that increases tag retention is internally inserting the tag, usually through surgery [45]. However, internally tagging a fish on a boat may increase the chances of fish mortality post-release due to stress from barotrauma, changes in water and air temperature, prolonged fish handling, or the amount of time the fish spends at the surface [46]. Even with survival from the tagging surgery, there is still the potential for physical trauma (bruising, bleeding, or acute damage) or physiological disturbance (changes affecting gasses, blood, and pH levels) associated with stress from capture or mishandling [13, 36, 47].

Additionally, newly tagged fish may be more susceptible to predation, especially if the fish must travel through the water column before reaching a protective habitat [27]. Acoustic tagging release method experiments have demonstrated that fish descender devices, which return fish to depth, can increase fish survivorship [3], but devices that both return a fish to depth and protect against predators (e.g., cages) are best for increased fish survivorship [27, 46]. However, a review of barotrauma treatment (venting and descending devices) in catch-and-release studies revealed inconsistency about which method is most beneficial in reducing or treating barotrauma, likely because of differences in environmental or species-specific physiological variables and differences in assessment methods [11].

All tagging methods have the potential to cause health issues for the fish, so decreasing the risk of health issues should be a priority when planning a tagging project. In the case of acoustic telemetry, it is often difficult to determine the fate of fish when they are no longer detected. While effects of the tagging procedure can cause mortality through physical trauma, the fish may also experience mortality via predation or fishing pressure, lose the transmitter, or immediately emigrate from the tagging area. A tagging procedure that minimizes stress to fish may also minimize the risk of these events by shortening the surgery recovery period and reducing the risk of both predation and emigration by releasing the fish directly into its natural sheltering habitat.

We suggest consideration of conducting in situ internal acoustic tagging of fish at depth of capture to remove barotrauma stressors and simplify the release method. Tagging fish in situ with acoustic transmitters at the same depth as capture and release has become more popular as studies have shown the negative effects of barotrauma on fish survival [31, 32]. Externally tagging fish in situ has been reported for multiple studies [1, 2, 34], and internally tagging fish in situ has become more common. For example, Starr et al. [38] captured deep-sea green spotted rockfish (*Sebastes chlorostictus*) at depths between 100 and 200 m, brought them up to 20 m where they were internally tagged, then lowered them back down to depth and released them. Lindholm et al. [22] used saturation diving from the Aquarius underwater laboratory in the Florida Keys to internally tag fish, and Tuohy et al. [43] used closed circuit rebreathers. Recently, Feeley et al. [12], McCallister et al. [26], Bryan et al. [4], and Keller et al. [18] all describe internally tagging reef fish in situ.

While there has been comparison of discard mortality between in situ and boat-tagged fish using external tags [31], to our knowledge there has been no study directly comparing the results of internally tagging fish on a boat versus in situ. In this study, we use 8 years of acoustic tagging data and our personal experiences with surgically implanting transmitters in fish to examine (1) the influence of tagging method (in situ versus on the boat) on detectability of a fish post-surgery and (2) the effects of other tagging variables (e.g., handling time and surgeon experience) that might influence detection probability after release.

Methods

Fish tagging

Fourteen species of groupers (Serranidae) and snappers (Lutjanidae) were internally tagged with acoustic transmitters (tag life: 417–1825 days) from 2008 to 2016 by Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWRI) staff in the Florida Keys and Dry Tortugas, Florida (Fig. 1). During the 8 years of tagging examined in this study, fish were captured using either hook and line or underwater baited traps. Hook-and-line captured fish were brought to the boat where they were surgically implanted with acoustic transmitters and returned to depth by divers. Fish caught in underwater traps (times between checking and rebaiting traps were approximately 1–12 h) had their tags inserted in situ by a team of divers. A detailed description of boat and in situ tagging and acoustic array designs can be found in Feeley et al. [12], Herbig et al. [15], and Keller et al. [18].
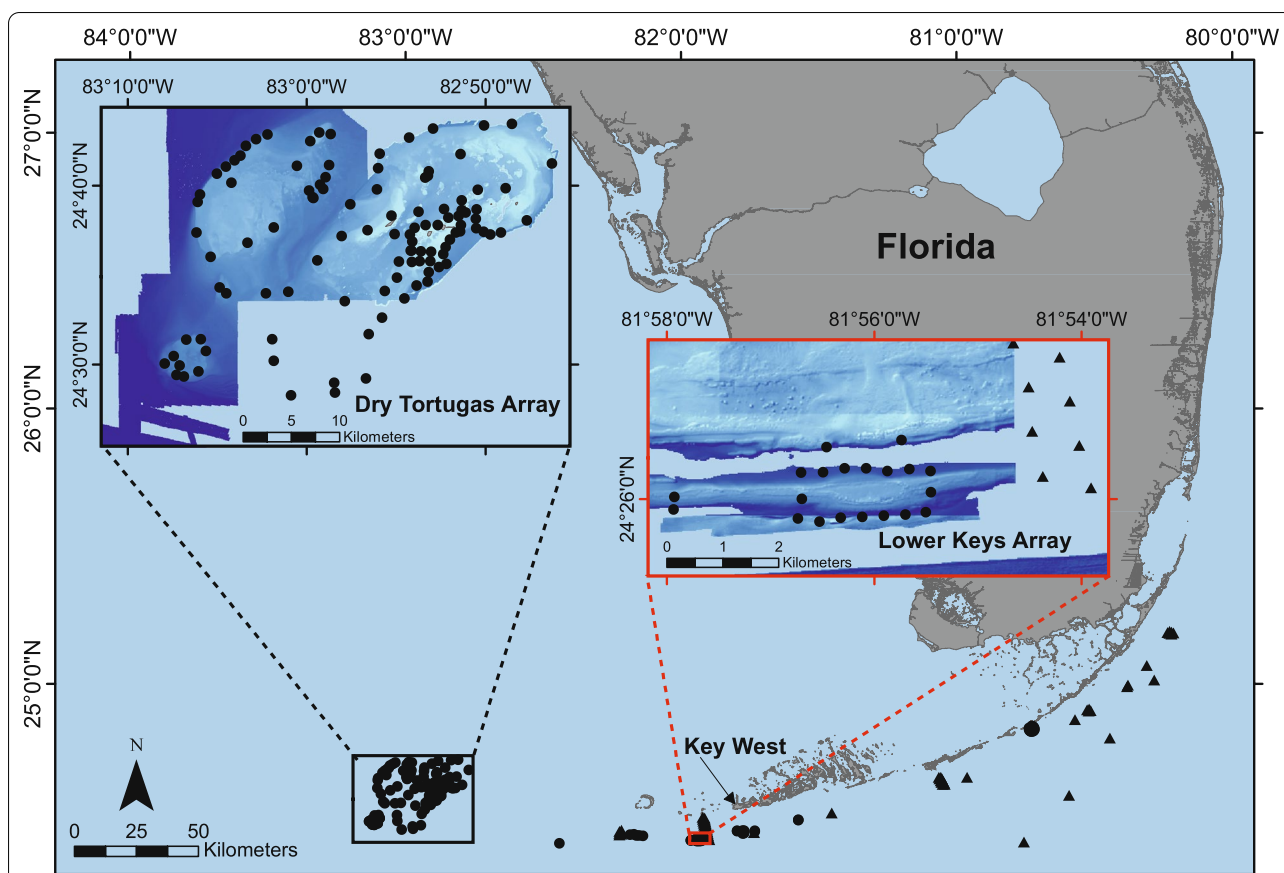
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Fig. 1 Fish tagging locations and acoustic receiver arrays in the Lower Florida Keys and Dry Tortugas. Fish and Wildlife Research Institute receivers are represented as circles and Integrated Tracking of Aquatic Animals in the Gulf of Mexico receivers are represented as triangles. Bathymetry data in the subplots shows depth as a gradient from light (shallower) to dark (deeper) blue (0–132 m). Receiver location depths ranged from 3 to 43 m. Receivers depicted on this map were deployed during different times from 2008 to 2018, depending on the study. Details regarding receiver timing can be found in [12, 15, 18]. Bathymetry data is a part of the Florida Keys National Marine Sanctuary Digital Atlas [40]

The surgical procedures during boat and in situ tagging were similar with the exception that anesthesia (AQUI-S, 50% isoeugenol; aqui-s.com) was not used in situ. We noticed that fish were calm once they were turned ventral side up and their behavior was similar to that of fish that had been anesthetized. Tonic immobility is commonly used as an alternative to chemical-based anesthesia for surgical implantation in elasmobranchs [19, 37] and after a few initial efforts, we realized that use of chemical-based anesthesia for tagging fish in situ was not necessary or beneficial and did not allow a quick and immediate release of the fish after surgery was completed. Acoustic tagging procedures in this study were approved by Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute and every effort was made to follow accepted standards and ensure the ethical treatment of captured fish.

Tagging variables

During both tagging methods, the following tagging variables were recorded: species, total length of fish (TL), depth of capture, tagging location, handling time, tagging time, and name of the person performing the surgery. Tagging time was the total time of the tag implantation surgery, while handling time was either the total time the fish was on the boat or total time the fish was handled underwater. Handling times on the boat included the time it took for fish to show effects of anesthesia before surgery and time for the fish to regain its equilibrium and display other signs of recovery before being released by divers. "Fight" times for fish caught via hook and line were not recorded, so analyses examined only the amount of time the fish was on the boat. Tagging location was the general location of the tagging, either the Lower Florida Keys or the Dry Tortugas (Fig. 1), and was included to account for any environmental differences

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between locations. Habitat types within the two tagging locations were similar and contained low- to mid-relief continuous coral reefs and patch reefs.

The family name of fish, either Serranidae or Lutjanidae ($n = 2$), was also included as a covariate to examine differences in detectability based on physiological differences between taxonomic groups while avoiding overparameterization of the models because of the high number of species ($n = 14$). Depth of capture was also recorded, though for fish tagged on the boat, the depth recorded was that of the seafloor as it was not possible to determine the exact depth in the water column the fish was first caught on hook and line. Total length of fish at time of capture was transformed into a two-factor variable called "fish size" to compare small and large fish without the confounding factor of fish family. Mean total lengths were determined separately for each family and used as a threshold value. Fish were designated as "small" if their length was below this value for the respective family and "large" if their length was equal to or greater than this value.

The variable month was used to examine whether time of year influenced the risk of disappearance. Water temperature varies throughout the year and could affect a fish's health during surgery and after release. Fish behavior (e.g., courtship/spawning behaviors) also varies throughout the year and between families, which can influence the likelihood of a fish emigrating from the area.

To determine if the tagging experience of the surgeon had an influence on the risk of disappearance, a variable called "surgery count" was created. This variable was determined by ordering the tagging data by date and named surgeon (regardless of tagging method) for all fish tagged in this study that had surgeon name recorded. This information was used to assign each fish a surgery count number based on how many surgeries the surgeon had previously completed. For example, surgery count had a value of one if it was the first fish the surgeon had ever tagged or a value of 17 if it was the 17th fish a surgeon had tagged. The data included in this study includes all, or nearly all, of each surgeon's internal tagging experience until the end of the study period or until the person left the agency. A few surgeons had some prior experience internally tagging fish, but were unable to accurately quantify their experience, so all surgery counts in this study started with a value of 1.

Data validation

Before inclusion in analyses, fish that did not have complete tagging procedure data were excluded from the dataset. Detection data was then validated for fish included in analyses. To validate detection data, fish with only one detection were excluded because a single detection could be caused by transmission collisions or noise and is not considered reliable [35]. Detections from fish that were detected only on one receiver were examined to determine whether detections were the result of normal fish activity or from a tag loss or mortality event. Consistent detections regardless of hour or day or a pattern of higher detections during a particular diel period could be due to a tag sitting on the seafloor with changes in environmental conditions throughout the day influencing transmission success. If detections were suspected to be due to a tag sitting on the seafloor, those detections were removed.

Time-to-event analysis

We used time-to-event analysis (also called survival analysis) to compare the risk of tagged fish disappearing from the receiver array between the two tagging methods. The Cox proportional hazards model is a common analytical method for assessing what variables cause the most risk to survival in discard mortality and delayed mortality studies [3, 7, 33]. We used the Cox proportional hazards model to examine which tagging variables most influenced the risk of fish disappearing from the receiver array. In this study, acoustic telemetry data could only provide presence/absence data, making it difficult to determine mortality (i.e., an end of detections could be due to emigration, predation, harvest, etc.). As presence/absence data is unable to determine mortality, time-to-event analyses examined the risk of fish disappearing from the receiver array for the final time (i.e., the date of last detection) rather than risk of mortality.

Before covariates were included in the model, they were tested for covariance with other variables via a correlation matrix and Pearson's test of correlation. All categorical variables were two-factored and were changed to binary outputs to allow for point-biserial correlation. Variables that had statistically significant correlation with each other ($p < 0.05$) were not included in the same hazards model.

The influence of tagging procedure variables on the risk of disappearance from the array was examined at two different time periods, 4 and 6 days after surgery. The two time periods were chosen based on literature suggesting 4–6 days post-surgery to be a time frame when events (mortality, tag loss, or emigration) are most likely due to tagging artifacts [27, 42, 43]. Any shorter time frame may miss delayed effects from tagging and longer time frames increase the chance of mistaking behavioral events for those related to the tagging procedure. We decided to examine risk of disappearance at both 4 and 6 days after tagging to determine if there were any differences between these two time frames. For each fish, the last day

of its detection was used to calculate the number of days it was present before it disappeared from the array. For example, if a fish was last detected 5 days after the tagging procedure, it was marked as having an event at day 5.

If a fish was detected past the 4- or 6-day mark, the number of days it was considered present was truncated to 4 or 6 days, depending on which time frame was used for analysis. For example, if a fish was not detected on day 6, but was detected more than once on day 50, it was considered alive and present on day 6. Long gaps in detections may have occurred as fish often left receiver coverage area before returning, but all fish included in this study were regularly detected and did not display suspicious data such as getting detected the first few days after tagging and only having one subsequent detection a year later.

To increase the chances of determining the most accurate activity level of a tagged fish, detection data from acoustic receivers deployed throughout the Florida Keys during 2008–2018 (not just the receivers in the array where the fish was tagged), were included to determine a fish's status. Detection data came from receivers owned by FWRI and the Integrated Tracking of Aquatic Animals of the Gulf of Mexico (iTAG) network ([24]; Fig. 1).

To visually compare the probability of presence and number of disappearances between the two tagging methods, Kaplan–Meier survival curves were created using the "survival" package [41] in R [29]. To examine what factors were most influential on risk of disappearance, Cox proportional hazards models were calculated at both 4 and 6 days after tagging also using the "survival" package. Model selection started with the full model using all covariates that were not correlated with tagging method (the main variable of interest) or each other. The best-fitting model was chosen through a backwards stepwise search, starting with the full model, and model selection was based on lowest Akaike information criterion with a correction for small sample size (AICc) using the "MASS" package [44]. Beyond the initial Cox proportional hazards model examining the influence of tagging method on risk of disappearance, two secondary hazards models were performed to assess which variables influenced risk of disappearance for (1) fish tagged on the boat and (2) fish tagged in situ.

Hazard ratios from the hazards model provide information on the covariates' effects on risk of disappearance. Hazard ratios above a value of 1 indicate an increase in risk of disappearance compared to a reference point, whereas a value below 1 indicates a reduction in risk. For categorical covariates, a hazard ratio less than 1 is the proportional reduction in risk compared to the reference level and vice versa. For continuous covariates, a hazard ratio less than 1 is the proportional reduction in risk of a

disappearance as the value of the covariate increases by 1 unit. A hazard ratio greater than 1 is the proportional increase in risk of a disappearance as the value of the covariate increases by 1 unit. The farther away a hazard ratio is from 1, the greater the influence of that covariate. A hazard ratio near 1 means that covariate has a marginal effect on the risk of disappearance (and thus the probability of presence) while a hazard ratio at 1, or a confidence interval that crosses 1, has no effect on the risk of disappearance [20].

Independence between residuals and time is an assumption of the proportional hazards model [20] and was checked in all models by testing the correlation between scaled Schoenfeld residuals and time using the "cox.zph" function in the "survival" package. A statistically significant correlation of Schoenfeld residuals and time rejects the null hypothesis and violates the assumption of the proportional hazards model.

Results

Of the fish tagged from 2008 to 2016, 194 were internally tagged grouper and snapper. Fish with incomplete tagging information ($n=87$) were excluded and two additional fish were excluded from analyses because they had only a single detection. Data from fish detected at only one location were examined, but no fish had continuous, consistent detection patterns suspected of resulting from a discarded tag. Receivers in both the Lower Keys and Dry Tortugas arrays were often spaced farther apart to try to maximize coverage area and therefore most receivers did not have overlapping coverage. Since most fish detected at a single location were groupers or small snappers that were not expected to make long-distance movements, smaller movements could have been missed because of spacing between receivers. Therefore, since examined data did not display suspicious patterns, no fish detected at only a single location was excluded from analyses. In total, 105 fish from 14 different species were included in analyses (35 boat tagged and 70 in situ tagged; Table 1).

The mean total length was 57.0 ± 1.4 cm (mean $\pm$ SE) for Lutjanidae and 64.6 ± 1.7 cm for Serranidae. These values were used to determine whether the variable of "fish size" was "small" or "large". Mean handling time and depth of capture varied between tagging method, but mean tagging time was similar (Table 2).

In situ tagged fish had a lower probability of disappearing from the array compared to fish tagged on the boat, based on Kaplan–Meier survival curves (Fig. 2). Six days after tagging, the probability of presence of fish tagged on the boat was only 66% while the probability of presence of fish tagged in situ was 90%. The null hypothesis that there was no difference between the two methods in the

Table 1 Number of fish in time-to-event analyses by species, tagging method, and size

Tagging method and fish species	Total fish	Small	Large
Boat tagged	**35**	**19**	**16**
Black grouper, *Mycteroperca bonaci*	8	5	3
Gray snapper, *Lutjanus griseus*	1	1	–
Mutton snapper, *Lutjanus analis*	14	2	12
Red grouper, *Epinephelus morio*	1	1	–
Yellowfin grouper, *Mycteroperca venenosa*	1	–	1
Yellowtail snapper, *Ocyurus chrysurus*	10	10	–
In situ tagged	**70**	**28**	**42**
Black grouper, *Mycteroperca bonaci*	17	6	11
Dog snapper, *Lutjanus jocu*	6	–	6
Gag, *Mycteroperca microlepis*	2	–	2
Gray snapper, *Lutjanus griseus*	8	8	–
Mutton snapper, *Lutjanus analis*	13	–	13
Nassau grouper, *Epinephelus striatus*	8	6	2
Red Hind, *Epinephelus guttatus*	1	1	–
Rock Hind, *Epinephelus adscensionis*	1	1	–
Scamp, *Mycteroperca phenax*	4	3	1
Schoolmaster, *Lutjanus apodus*	1	1	–
Yellowfin grouper, *Mycteroperca venenosa*	8	1	7
Yellowmouth grouper, *Mycteroperca interstitialis*	1	1	–
Total	**105**	**47**	**58**

Table 2 Mean values ± SE for handling time, tagging time, and depth between boat and in situ tagging methods

	Handling time (min)	Tagging time (min)	Depth (m)
Boat tagged	72.17 ± 6.02	6.37 ± 0.35	13.29 ± 2.14
In situ tagged	9.30 ± 0.31	6.96 ± 0.27	24.61 ± 0.94

probability of presence from day of tagging to 6 days after tagging was rejected by a log-rank test ($p = 0.002$).

Fish size, tagging time, and surgery count were the only variables not significantly correlated with tagging method (the main variable of interest) or each other and therefore were the only variables included in the initial Cox proportional hazards models (Fig. 3). Variables that were significantly correlated with tagging method or with each other could not be included in the hazards model without compromising model results. Because of correlation among variables, model selection using backward stepwise selection started with a full model including only tagging method, tagging time, surgery count, and fish size. Backward stepwise model selection found the best-fitting hazards model (based on lowest AICc) at the 4-day mark to include tagging method and tagging time as covariates, while at the
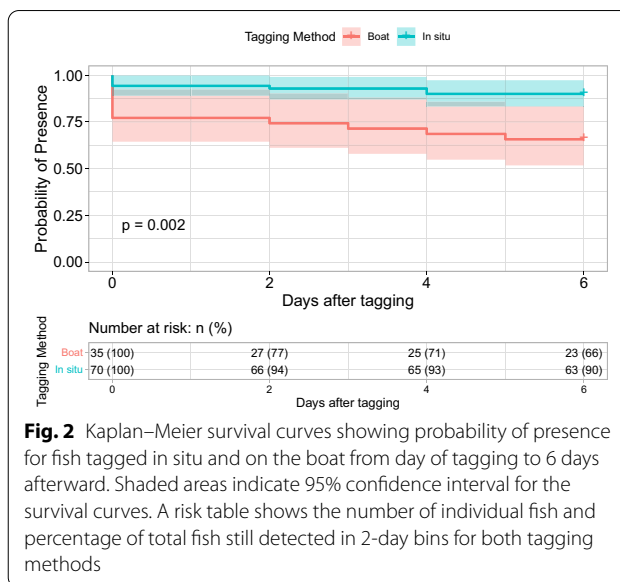

Fig. 2 Kaplan–Meier survival curves showing probability of presence for fish tagged in situ and on the boat from day of tagging to 6 days afterward. Shaded areas indicate 95% confidence interval for the survival curves. A risk table shows the number of individual fish and percentage of total fish still detected in 2-day bins for both tagging methods
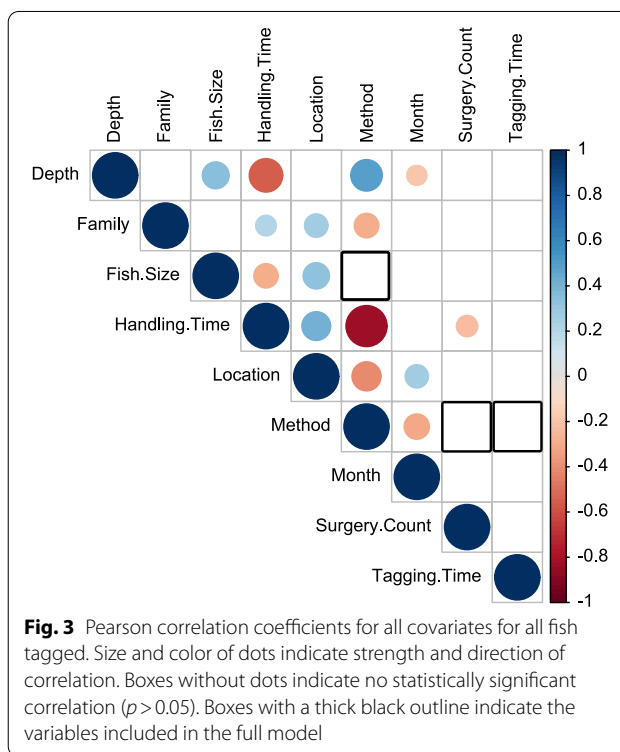

Fig. 3 Pearson correlation coefficients for all covariates for all fish tagged. Size and color of dots indicate strength and direction of correlation. Boxes without dots indicate no statistically significant correlation ($p > 0.05$). Boxes with a thick black outline indicate the variables included in the full model

6-day mark, tagging method was the only variable in the best-fitting model (Table 3).

Hazards model results for the best-fitting models indicate that fish internally tagged in situ were 76% less likely (one minus the hazard ratio) to disappear from the array 4 days after tagging and 75% less likely to disappear 6 days after tagging compared to fish tagged on the boat (Table 4). Tagging time was also included in

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Table 3 Cox proportional hazards models with AICc stepwise model ranking for both 4 and 6 days after tagging

Days after tagging	Hazards model	AICc	ΔAICc	χ^2	df	p
4	Method + tagging time	131.19	0.00	11.11	2	0.004
	Method	131.47	0.28	8.98	1	0.003
	Method + tagging time + surgery count	131.72	1.53	11.92	3	0.008
	Method + tagging time + fish size + surgery count	134.83	3.64	11.12	3	0.010
	Null	137.52	6.33			
6	Method	166.68	0.00	9.78	1	0.002
	Method + tagging time	167.58	0.90	10.82	2	0.004
	Method + tagging time + fish size	169.68	3.00	10.85	3	0.010
	Method + tagging time + fish size + surgery count	171.83	5.15	10.87	4	0.030
	Null	173.38	6.70			

All models include probability of presence as the dependent variable and list independent variables included in each individual model. All fish were included in these models ($n = 105$). p-values are from the log-rank test

Table 4 Cox proportional hazards models results for the best-fitting model at 4 and 6 days after tagging

Days after tagging	Factor	Coefficient	SE	Hazard ratio	95% CI	p
4	Tagging method: boat	Reference	–	–	–	–
	Tagging method: in situ	− 1.43	0.55	0.24	0.08–0.70	0.009*
	Tagging time	− 0.19	0.12	0.83	0.65–1.05	0.120
6	Tagging method: boat	Reference	–	–	–	–
	Tagging method: in situ	− 1.38	0.48	0.25	0.10–0.64	0.004*

For the categorical covariates, the hazard ratio is the proportion of risk compared to the reference covariate. For the continuous covariates, the hazard ratio is the proportion of risk with an increase of 1 unit (e.g., 1 min). A variable with a hazard ratio close to a value of 1 has a marginal effect and a confidence interval (CI) spanning 1 has no effect on hazard. All models include probability of presence as the dependent variable and list independent variables included in the best-fitting models. p-values are from the log-rank test and asterisks indicate statistical significance

the best-fitting 4-day model, though the 95% confidence interval spanned 1, indicating there was no effect. For all covariates in both best-fitting models, the proportional hazard assumption was supported by a nonsignificant relationship between scaled Schoenfeld residuals and time. To visually compare each covariate's risk to fish survival, hazard ratio forest plots were produced from the full and best-fitting models, and plots for 4 days after tagging are shown as an example (Fig. 4a, b).

Pearson correlation coefficients were also calculated for all covariates when splitting the data into fish tagged on the boat and fish tagged in situ. Handling time and depth were the two main covariates of interest. However, since these two covariates were correlated for fish tagged on the boat (Fig. 5a), they could not be included in the same hazards model. As a result of these correlations, two full models were created for fish tagged on the boat: (1) one with depth and all variables not correlated with each other, and (2) one with handling time and all variables not correlated with each other.

For in situ tagged fish, handling time and depth were not correlated with each other, but depth was correlated with fish size and both depth and handling time were correlated with month (Fig. 5b). Three full models were created for in situ tagged fish: (1) one with handling time, depth, and all other variables not correlated with each other, and (2) one with handling time, fish size, and all other variables not correlated with each other, and (3) one with month and all non-correlated variables.

Handling time was the only covariate included in the best-fitting model for boat-tagged fish at 4 days after tagging (Table 5). At 6 days after tagging, both handling time and family were included in the best-fitting model. Handling time was significant in both best-fitting models; however, the hazard ratio was very close to 1, meaning the effect of handling time on hazard of disappearance was marginal (Table 6). A hazard ratio of 2.55 for family Serranidae in the 6-day model indicated an increase in risk compared to Lutjanidae. However, this covariate had a wide confidence interval that crossed 1, indicating no significant effect of hazard and a somewhat unreliable point estimate [20]. For all covariates in both best-fitting models, the proportional hazard assumption was supported by a nonsignificant relationship between scaled Schoenfeld residuals and time.
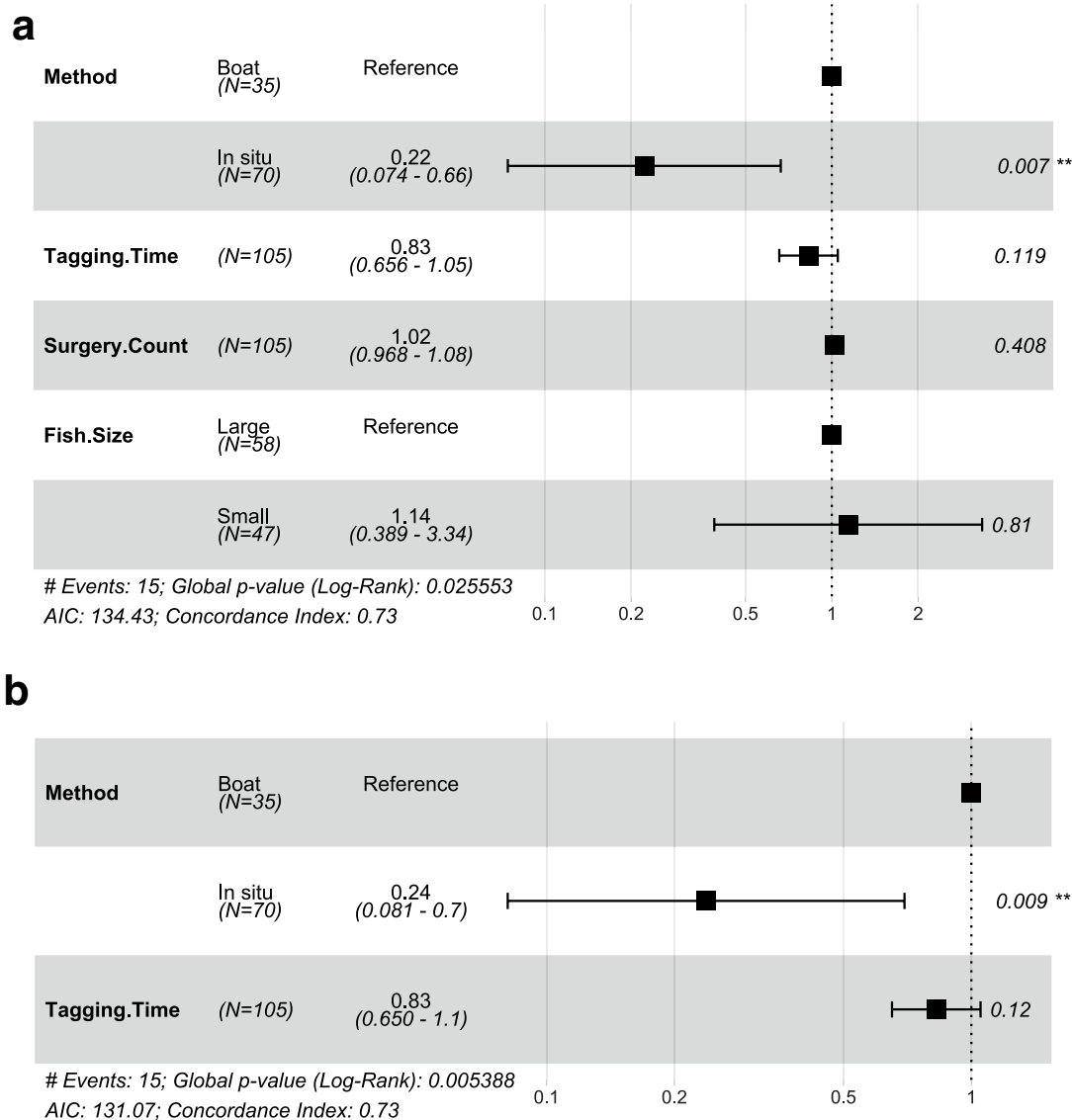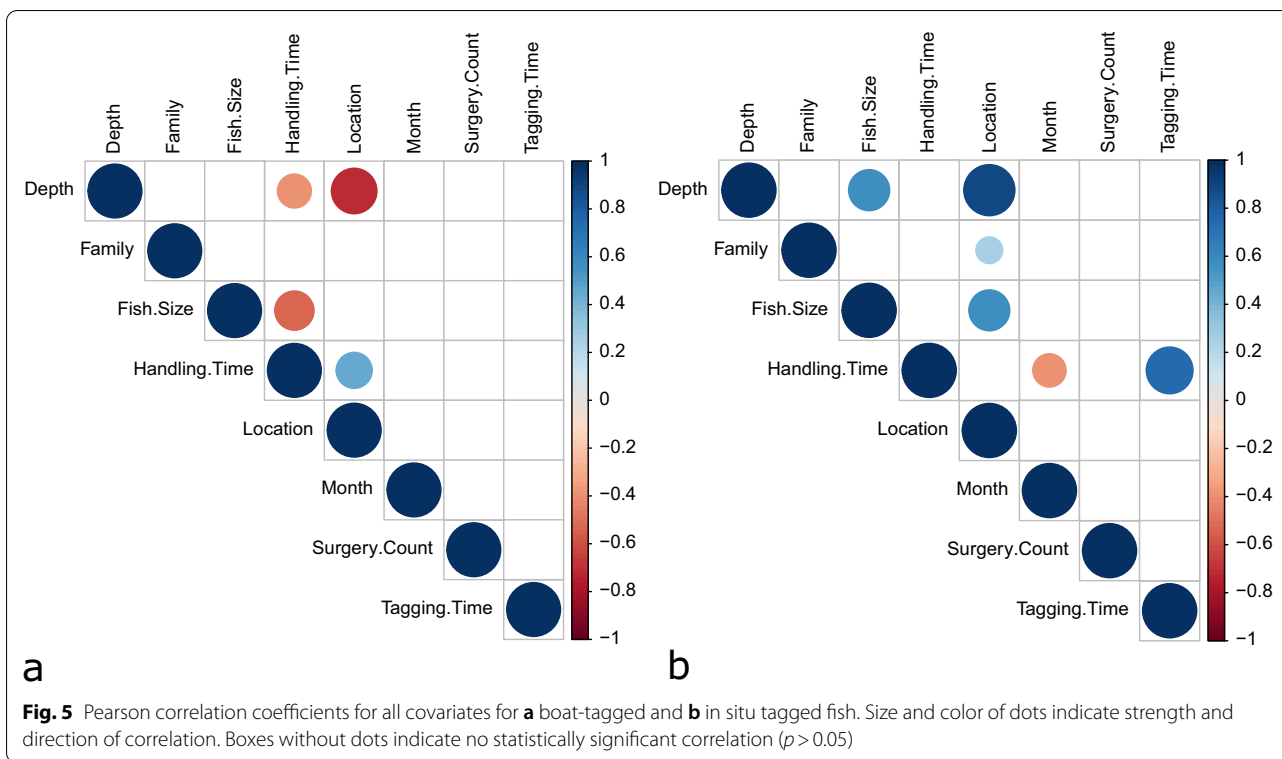
Fig. 4 Forest plots showing covariate hazard ratios at 4 days after tagging for the **a** full model and **b** best-fitting Cox proportional hazard model. Hazard ratios and 95% confidence intervals in comparison to reference points are shown for each covariate with *p*-values listed on the right-hand side of the plots (asterisks indicating statistical significance). Hazard ratio values less than 1 (represented by the dotted line) indicate a reduction in risk of disappearance from the array while values greater than 1 indicate increased risk of disappearance. Covariates with hazard ratios close to 1 have a marginal effect on hazard and confidence intervals crossing 1 indicate no significant effect

For in situ tagged fish, month was the only covariate included in the best-fitting model at 4 days after tagging and both month and fish size were in the best-fitting model at 6 days after tagging (Table 7). However, the global log-rank tests examining significance of the models overall were nonsignificant in both cases. The hazard ratio confidence intervals crossed one for all covariates in the best-fitting models, indicating no effect. Additionally, the particularly wide confidence interval for fish size in the best-fitting 6-day model indicated the point estimate was unreliable (Table 8). For all covariates in both best-fitting models, the proportional hazard assumption was supported by a non-significant relationship between scaled Schoenfeld residuals and time.

Fig. 5 Pearson correlation coefficients for all covariates for **a** boat-tagged and **b** in situ tagged fish. Size and color of dots indicate strength and direction of correlation. Boxes without dots indicate no statistically significant correlation ($p > 0.05$)

Discussion

Using an historic dataset of 8 years of fish tagging by Florida Fish and Wildlife Conservation Commission scientists in the Florida Keys, we examined factors a posteriori that influence probability of presence of internally tagged fish. At 6 days after tagging, Kaplan–Meier survival curves demonstrated that fish tagged in situ had a 25% higher probability of detection within the acoustic array (i.e., 75% lower probability of disappearing) compared to fish tagged on the boat. Tagging method was the only factor affecting the risk of disappearance when we examined the initial Cox proportional hazards model assessing the probability of presence of all fish.

In this study, there were several variables that could not be included in the initial hazards model because of correlation, but their inclusion and limited influence in the subsequent boat-tagged and in situ tagged models reinforced that the tagging method was the most important factor in risk of disappearance from the array. Acoustic tracking data of in situ surgically tagged fish from two other telemetry studies also demonstrate the benefit of tagging method, indicated by high survival rates (97–100%) post-surgery [4, 43]. The decrease in probability of presence of boat-tagged fish could be due to several factors. As described in Feeley et al. [12] and Herbig et al. [15], all fish tagged on the boat were returned to the

seafloor via divers after they recovered from anesthesia to ensure a safe return to the reef. Nevertheless, the tagged fish may still have suffered from barotrauma, increased stress from being caught by hook and line, increased handling times (which could also increase the impacts of barotrauma), or long-lasting effects of anesthesia. All these factors could increase risk of boat-tagged fish disappearing from the array.

At the start of in situ tagging, we decided not to use anesthesia at depth because we discovered that fish held ventral side up with their eyes covered were just as calm as anesthetized fish. We also deemed it advantageous to avoid unnecessarily impairing the tagged fish's response to predators once the fish was released into sheltering habitat after surgery. Using anesthesia during fish surgeries may not always be the best course of action in every situation, and all potential effects on the fish must be considered [8, 23]. We believe the benefits of reduced total handling time for fish tagged in situ versus fish tagged on the boat, which usually included a recovery period (mean ± SE: In situ = 9.30 ± 0.31 min, Boat = 72.17 ± 6.02 min), coupled with immediate release into protective habitat and the lack of pressure and temperature changes, explain the increased probability of presence within the acoustic array for in situ tagged fish. The increased probability of presence of fish

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Table 5 Cox proportional hazards models examining probability of presence for fish tagged on the boat ($n = 35$) at both 4 and 6 days after tagging

Days after tagging	Hazards model	AICc	ΔAICc	χ^2	df	p
4	Handling time	66.00	0.00	4.17	1	0.04
	Handling time + family	67.08	1.08	5.76	2	0.06
	Tagging time	68.13	2.13	2.37	1	0.10
	Null	68.26	2.26			
	Handling time + tagging time + family	68.60	2.60	6.96	3	0.07
	Tagging time + surgery count	69.12	3.12	3.79	2	0.20
	Tagging time + surgery count + family	70.64	4.64	5.06	3	0.20
	Handling time + tagging time + month + family	70.67	4.67	7.30	4	0.10
	Tagging time + surgery count + month + family	72.31	6.31	5.84	4	0.20
	Handling time + tagging time + surgery count + month + family	72.91	6.91	8.34	5	0.10
	Depth + tagging time + surgery count + month + family	74.85	8.85	5.86	5	0.30
	Depth + tagging time + surgery count + month + fish size + family	77.79	11.79	5.90	6	0.40
6	Handling time + family	77.51	0.00	8.28	2	0.02
	Handling time	77.55	0.04	5.43	1	0.02
	Handling time + month + family	78.13	0.62	10.32	3	0.02
	Month	79.66	2.15	3.22	1	0.07
	Handling time + tagging time + month + family	79.75	2.24	11.22	4	0.02
	Family + month	79.85	2.34	5.43	2	0.07
	Tagging time + month + family	80.73	3.22	6.96	3	0.07
	Null	81.06	3.55			
	Tagging time + fish size + month + family	81.61	4.10	9.98	4	0.06
	Handling time + tagging time + surgery count + month + family	82.28	4.77	11.73	5	0.04
	Tagging time + surgery count + month + family	84.22	6.71	9.33	5	0.10
	Depth + tagging time + surgery count + month + family	87.07	9.56	9.36	6	0.20

Models are ranked by AICc values. All models include probability of presence as the dependent variable and list independent variables included in each individual model. *p*-values are from the log-rank test

Table 6 Cox proportional hazards models results for best-fitting models (lowest AICc) of boat-tagged fish at both 4 and 6 days after tagging

Days after tagging	Covariates	Coefficient	SE	hazard ratio	95% CI	p
4	Handling time	− 0.02	0.01	0.98	0.96–0.99	0.048*
6	Handling time	− 0.02	0.01	0.98	0.96–0.99	0.016*
	Family: Lutjanidae	Reference	–	–	–	–
	Family: Serranidae	0.94	0.60	2.55	0.79–8.23	0.116

All models include probability of presence as the dependent variable and list independent variables included in each individual model. *p*-values are from the log-rank test and asterisks indicate statistical significance

tagged in situ compared to fish tagged on the boat indicates that any negative effects due to the lack of anesthesia are minimal and do not outweigh the benefits of in situ tagging.

There were several surprising results presented from the analysis of this data. Some of the variables that were originally believed to impact the probability of detection were either not influential in the hazards models or had the opposite effect. For example, we hypothesized

that additional handling time would increase the risk of a tagged fish having an event. The models, however, suggested an increase in 1 min of handling time slightly decreased the risk of disappearance for boat-tagged fish, though the confidence intervals neared or crossed 1 in all cases, indicating little to no effect. Handling time may not have been very influential in risk of disappearance, but it is still surprising that increased time may have reduced risk, especially as handling times for fish tagged

Table 7 Cox proportional hazards models examining fish survival for fish tagged in situ ($n = 70$) at both 4 and 6 days after tagging

Days after tagging	Hazards model	AICc	ΔAICc	χ^2	df	p
4	Month	40.38	0.00	3.26	1	0.07
	Fish size + month	41.49	1.11	4.51	2	0.10
	Handling time	41.65	1.27	2.34	1	0.10
	Depth + month	41.99	1.61	3.96	2	0.10
	Null	42.19	1.81			
	Handling time + fish size	42.30	1.92	3.78	2	0.20
	Handling time + family	42.74	2.36	3.25	2	0.20
	Fish size + family + month	43.24	2.86	5.05	3	0.20
	Depth + family + month	43.76	3.38	4.46	3	0.20
	Handling time + fish size + family	43.79	3.41	4.45	3	0.20
	Handling time + depth + family	44.77	4.39	3.41	3	0.30
	Tagging time + fish size + family + month	45.21	4.83	5.09	4	0.30
	Handling time + fish size + surgery count + family	45.57	5.19	4.52	4	0.30
	Tagging time + depth + family + month	45.92	5.54	4.49	4	0.30
	Handling time + depth + surgery count + family	46.97	6.59	3.44	4	0.50
	Tagging time + fish size + surgery count + family + month	47.53	7.15	5.11	5	0.40
6	Fish size + month	57.43	0.00	5.69	2	0.06
	Fish size	57.84	0.41	3.17	1	0.08
	Handling time + fish size + surgery count	57.87	0.44	5.41	3	0.10
	Fish size + surgery count	58.37	0.94	4.45	2	0.10
	Month	58.70	1.27	2.03	1	0.20
	Null	58.86	1.43			
	Fish size + surgery count + month	58.90	1.47	6.48	3	0.09
	Depth + month	59.52	2.09	3.5	2	0.20
	Handling time + fish size + surgery count + family	59.54	2.11	5.67	4	0.20
	Surgery count	60.17	2.74	1.15	1	0.30
	Depth + surgery count	60.92	3.49	1.72	2	0.40
	Fish size + surgery count + family + month	60.95	3.52	6.68	4	0.20
	Depth + surgery count + month	61.02	3.59	3.94	3	0.30
	Handling time + depth + surgery count	62.10	4.67	2.32	3	0.50
	Tagging time + depth + surgery count + month	62.88	5.45	4.62	4	0.30
	Tagging time + fish size + surgery count + family + month	63.19	5.76	7.5	5	0.20
	Handling time + depth + surgery count + family	64.08	6.65	2.64	4	0.60
	Tagging time + depth + surgery count + family + month	64.97	7.54	4.87	5	0.40

Models are ranked by AICc values. All models include probability of presence as the dependent variable and list independent variables included in each individual model. *p*-values are from the log-rank test

Table 8 Cox proportional hazards models results for best-fitting models of in situ tagged fish

Days after tagging	Covariates	Coefficient	SE	Hazard ratio	95% CI	*p*-value
4	Month	0.59	0.36	1.82	0.89–3.69	0.10
6	Month	0.34	0.24	1.41	0.89–2.24	0.14
	Fish size: Small	1.44	0.84	4.23	0.82–21.84	0.08

All models include probability of presence as the dependent variable and list independent variables included in each individual model. *p*-values are from the log-rank test

on the boat were high compared to other tagging studies [3, 39] and minimizing handling of fish has been reported as one of the most important considerations in implantation surgeries [30]. For in situ tagging, handling time was not included in best-fitting models, but that may be because there was little variance in the handling times of fish tagged in situ.

Fish size was not a significant factor in the best-fitting models. Fish tagged in this study varied between 32 and 107 cm TL, a range large enough that differences in probability of presence because of size were expected. We hypothesized that smaller fish would be at a higher risk of disappearance because they might not have the same energy resources to aid in recovery as larger fish or they may be more susceptible to predation. However, this was not supported by the models. "Fish size" was included in the best-fitting model for in situ tagged fish at 6 days after tagging but was not significant, and a large spread in the hazard ratio confidence interval (0.82–21.84) represented high uncertainty in the hazard ratio estimate. While there are likely species-specific differences (e.g., body type, stress tolerance) that influence probability of presence of different sized fish, in our study fish size did not significantly affect probability of presence post-tagging. Also, fish less than 20 cm FL have been shown to have high survival from internal tagging [21], suggesting that surgery and implantation of appropriately sized tags for the body size of the fish may not impair survival, regardless of fish size.

Fish family was also not a significant covariate, although differences in tolerance to barotrauma and handling were seen among species. The high number of species in this study (which originally led to overparameterization in models) and the unevenness of species between tagging methods resulted in grouping species by family. There are physiological differences between species of the same family, and grouping them by family may have masked these differences and may help explain why family did not affect probability of presence. Although an in-depth look at the effect of tagging method on species was not possible in this study, a planned, paired study with fewer species may find that fish species influences probability of presence. For any tagging project, factors such as body type, susceptibility to barotrauma, and stress tolerance should be considered when deciding on the appropriate tagging method.

Water temperatures and differences between water and air temperatures vary throughout the year and could affect the risk of a fish disappearing from the array, but month as a proxy for temperature did not have a significant influence in either boat-tagged or in situ tagged models. Life history traits of the fish families (e.g., the time of year that they reproduce) are also linked to time of year and water temperature, and thus linked to the variable month. Although time of year was not a significant in this study it should also be considered when deciding how to maximize fish survival and probability of presence when setting up a tagging project.

Experience of the surgeon has been shown to influence tag retention and survivorship [5, 10], but this was not seen in our study. Surgery count was not in any best-fitting model, but was a covariate in the third best-fitting model for both the initial model at 4 days after tagging and the in situ model at 6 days after tagging. Although the effect was not statistically significant, both models indicated an increase in surgery count decreased risk of fish disappearance. Consistent training among surgeons may have reduced the effect of surgery count enough so that other variables had larger influences. Before the start of a tagging project, surgeons practiced the tagging procedure with guidance from an experienced team member, and initial training was provided by a professional plastic surgeon (D. Hawtof, personal communication). Additionally, less experienced surgeons were always paired with an experienced surgeon during the tagging procedure to provide guidance during both boat and in situ tagging. The training and oversight throughout the tagging procedure may have helped to reduce the effect of surgeon experience on risk of disappearance.

Hazards models were analyzed at both 4 and 6 days after tagging because there was discrepancy in the literature about how long after surgery to examine survival related to the tagging procedure. Results between these two time periods were similar (e.g., hazard ratios for tagging method in the initial model differed by only 0.015), but we felt it was informative to present both cases. Although differences between the results at both time periods were minimal, they are still interesting to note because they show how the dynamics of fish survival may subtly shift over time. The maximum time after surgery that a fish was still detected was just over 4 years (data from this fish was analyzed in [12]), and a possible future direction of work could include examining longer term differences in detection patterns between different tagging methods, particularly if the goal is to maximize data acquisition. Analyzing probability of presence on a longer time frame may find different factors which are more influential than those found in this study, but consideration should be given to whether disappearances from the array past 6 days post-surgery are a result of the tagging procedure or from a natural behavioral event.

This study was not originally designed to rigorously examine all factors affecting boat and in situ tagged fish. Therefore, some variables may not have proven as influential as they would in a more systematic study. Depth was expected to have more of an influence on hazard because

detrimental effects of barotrauma have been shown to increase with depth of capture [14]. In this study, depth may not have been the best representation of the severity of barotrauma. Boat-tagged fish were generally caught using hook and line and were not necessarily caught from the seafloor, which was the depth recorded, and furthermore, mean depth of capture of boat-tagged fish was significantly shallower compared to in situ tagged fish (Wilcoxon two-sample rank test, $p < 0.05$). Fish that were near the surface or mid-water column when caught on hook and line would have a greater depth recorded than where they were physically caught in the water column, meaning that they may have exhibited fewer signs of barotrauma than if they had actually been caught at the depth recorded. Fish tagged in situ were caught via baited traps on the seafloor, so the depth of capture was the depth of the trap and the actual depth of the fish when it entered the trap. Additionally, after the start of in situ tagging, this method was preferentially chosen over tagging on the boat at deep sites and only a few fish were captured, tagged, and released from the boat in deep water (> 30 m) and in situ tagged fish had a higher overall sample size compared to boat-tagged fish. The method of tagging was based on what was deemed best for survival of the fish and logistically made sense for each tagging event. If boat tagging data had included more fish captured from deep water, resulting in no discrepancy in mean depth per tagging method, we hypothesize depth would have had a larger effect on survival. Additionally, comparing risk of disappearance between tagging methods where boat-tagged fish were either released at the surface or brought back down to depth for release (the method used for all boat-tagged fish in this study) would further explore the effects of depth, barotrauma, and release method.

Fight time, location of hook placement on fish, whether the fish exhibited signs of barotrauma, whether a fish with barotrauma was vented before its return to depth, and overall health of the fish at time of release were not often recorded and thus were not included as covariates in the hazards models. The amount a time a fish spent in a trap could influence the fish's health and probability of presence after tagging, but the time a trap was baited does not equal the time a fish was in a trap (a trap may have been baited and left for 12 h, but a fish may have entered the trap at hour 11). Use of cameras for identifying how long fish were in traps and recording specifics on condition upon release would provide more details on why risk of disappearance was higher in boat-tagged fish.

This study demonstrated some of the limitations of a posteriori-designed study including correlated variables and missing data (particularly during the initial stages of conducting acoustic telemetry research when it was not known what supplementary data would be beneficial to record). However, despite these caveats, we believe that this historic dataset using over 100 internally tagged fish representing 14 different species surgically tagged by 11 different scientists was robust enough to demonstrate that tagging method had an effect on risk of fish disappearance. Telemetry studies are expensive to conduct, both in terms of effort and money, and often outcomes are not known until months later when the data can be downloaded. Therefore, it is critical to have a better understanding of how tagging method influences probability of presence (which can be considered a proxy for fish survivorship). By examining the effects of tagging methods, we can inform future telemetry studies, thereby increasing the usefulness of telemetry data.

This study has shown that there were clear benefits to tagging fish in situ, though due to high correlation among variables we are unable to pinpoint the source behind decreased risk of disappearance compared to boat-tagged fish. The lack of barotrauma, shorter handling time, lack of anesthesia, and use of baited traps instead of hook and line may all have played a role in increasing an in situ tagged fish's probability of presence. We understand that this in situ method may not always be practical. In situ tagging can increase the amount of effort spent to tag each fish because of the logistics required and the time limitations when tagging at deeper depths. Some target species may be located outside recreational diving limits, which can increase effort and cost for specialized training and dive gear. Also, in this study we used baited traps to capture fish in situ, but other methods should be considered if the species of interest is unlikely to be captured in this manner. Furthermore, studies that rely on anglers to supply the tagging subjects or focus on species found in very shallow water may not find in situ tagging feasible or worthwhile. However, we suggest that all aspects of the tagging procedure, from tagging method (e.g., on a boat vs. in situ) to use of anesthesia, method of capture, and method of release be carefully considered for each target species to reduce stress to the fish, and maximize success of the study.

Conclusions

In this study, fish internally tagged in situ with acoustic tags were ~75% less likely to disappear from the receiver array (i.e., mortality, emigration, or tag loss event) shortly after tagging compared to fish brought to the surface and tagged on a boat. Other factors had little to no influence on probability of presence after tagging, but correlation of variables limited what could be included in hazards models. Despite the limitations due to correlation, fish had a higher probability of being present and a lower probability of disappearance when tagged with the in situ methods presented. Therefore,

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when designing a telemetry study, serious consideration of tagging method (in situ versus on a boat) and other variables (capture method, release method, use of anesthesia, etc.) should be evaluated because they play a critical role in the overall health of fish and success of the study. Implanting internal acoustic tags in situ is not a practical method for every species of interest and for every environment, but given the increased probability of presence demonstrated here, we suggest it be considered for fish species where applicable.

Authors' contributions
JK analyzed the data. JK, DM, JH, MF, and AA contributed to the interpretation of results and JK, DM, JH, AA, MF, and PB contributed to the writing of the manuscript. PB, MF, DM, JK, AA, and JH performed fieldwork. AA, DM, and MF secured funding. All authors read and approved the manuscript.

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available by request in the FWCDL repository (https://f50006a.eos-intl.net/F50006A/OPAC/Details/Record.aspx?BibCode=5386179).

Ethics approval and consent to participate
Acoustic tagging procedures in this study were approved by Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute and every effort was made to follow accepted standards and ensure the ethical treatment of captured fish. This research was conducted under scientific research permits issued by the Florida Keys National Marine Sanctuary (FKNMS-2008-015, FKNMS-2013-040, FKNMS-2013-040-A2, FKNMS-2018-047) and the Dry Tortugas National Park (DRTO-2008-SCI0003, DRTO-2010-SCI-0006).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 2796 Overseas Highway, Suite 119, Marathon, FL 33050, USA. [2] National Park Service, South Florida/Caribbean Inventory and Monitoring Network, 18001 Old Cutler Rd Suite 419, Palmetto Bay, FL 33157, USA.

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