Evaluation of an acoustic telemetry transmitter designed to identify predation events

Edmund A. Halfyard*,[1] (iD), D. Webber[2], J. Del Papa[1], T. Leadley[1], S. T. Kessel[1], S. F. Colborne[1] and A. T. Fisk[1]

[1]*Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, Ontario, Canada N9B 3P4; and* [2]*Vemco Ltd., 20 Angus Morton Drive, Bedford, Nova Scotia, Canada B4B 0L9*

Summary

1. The field of acoustic telemetry has evolved rapidly and now permits the remote sensing of animal behaviour, movement, physiology and survival in environments, and species not previously possible. However, an inability to detect when a telemetered animal is consumed by a predator can complicate accurate interpretation of the telemetry data. In this paper, we describe the efforts taken to test the two generations of a novel prototype acoustic telemetry transmitter designed specifically to detect predation.

2. Testing involved either staged predation events where tagged prey (Rainbow Trout *Oncorhynchus mykiss* and Yellow Perch *Perca flavescens*) were fed to captive Largemouth Bass *Micropterus salmoides*, or false-positive testing where prey fish were tagged and held without the risk of predation. Metrics of interest were (i) the rate of correctly identifying the predation events, (ii) signal lag (i.e. the time required to detect a predation event), (iii) tag retention time in the predator's gut, and (iv) the rate of false-positive triggering in both live and dead prey fishes.

3. Staged predation events were successfully identified in 61/65 and 52/55 trials for generation 1 and 2 tags, respectively. Signal lag time was reduced in generation 1 tags (generally between 1 and 9 h) relative to generation 2 (3–29 h); although signal lag was highly variable. A generalized linear mixed model (GLMM) indicated strong evidence that signal lag and tag retention were both negatively correlated with water temperature, but were not affected by prey species and only slightly by individual predator traits. There was preliminary evidence that prey size may be an important determinant of both signal lag and tag retention. False-positives in live fish were absent after 120 days for generation 1 tags ($n = 31$), however, the false-positive rates were significantly higher (10/44) after only 66 days for generation 2 tags. False-positives in dead fish showed that 20% of the generation 2 predation tags would falsely trigger 2–3 days post-mortem.

4. Testing of the novel predation tags was encouraging, however, further testing is recommended. Predation tags will be an important contribution to the field of acoustic telemetry, thus, permitting the improved data interpretation and less-subjective estimates of predation rates in biotelemetry studies.

Key-words: predator, prey, tracking, trophic interactions

Introduction

The field of acoustic telemetry is evolving rapidly, facilitating the study of increasingly small animals in increasingly challenging environments (Cooke *et al.* 2013; Hussey *et al.* 2015). Acoustic telemetry has been used to study animal movement, behaviour, habitat use, survival, and trophic interactions (Heupel & Webber 2012). Like all technologies, the analysis of acoustic telemetry data requires careful consideration of the limitations of the equipment. For the acoustic telemetry, the issues of gear performance (e.g. detection efficiency, Melnychuk 2012; Kessel *et al.* 2014) and the impact of affixing transmitters to animals (e.g. Cooke *et al.* 2011; Wagner *et al.* 2011) have justifiably received considerable attention. Consequently, there have been significant technological advances in acoustic

telemetry such as more efficient coding schemes and smaller and more powerful transmitters with increased battery life.

A limitation receiving far less attention is the assumption that the data represent the location, movements or behaviour of the intended study animal. A common scenario that violates this assumption is when tagged animals are consumed by a predator and the data collected represent the location, movements or behaviour of the predator so long as the tag is retained. For example, Beland *et al.* (2001) noted a series of abnormal movements in an actively tracked (i.e. using a mobile receiver) Atlantic Salmon *Salmo salar* smolt. Further investigation led to the recovery of the tag in the stomach of a Striped Bass *Morone saxatilis* caught by a nearby angler. Similarly, suspicions of predation were confirmed when tagged trout, *Salmo trutta*, were recovered from the stomachs of predators following electrofishing (Jepsen *et al.* 1998; Jepsen, Pedersen & Thorstad 2000). Unfortunately, the ability to directly confirm the predation events is frequently limited, leaving researchers

*Correspondence author. E-mail: eahalfyard@hotmail.com

with an unknown mix of valid (prey) and invalid (predator) data.

There have been several attempts to separate predator and prey by identifying the suspect patterns in telemetry data sets, however, these *a posteriori* approaches have relied on either ancillary sensor data, such as temperature or depth (Thorstad *et al.* 2011), or assumptions of what constitutes 'normal' prey behaviour when interpreting two-dimensional position data (Perry *et al.* 2010; Buchanan *et al.* 2013). Recently, Romine *et al.* (2014) tracked both prey and predator inside a high-density gridded two-dimensional positioning system and used a multi-variate mixture model to (i) describe the movements of predator and prey, and (ii) identify the suspected predation events where prey behaved similar to predators. A similar approach was used by Gibson *et al.* (2015) where data from the tagged prey and predators were separated using a cluster analysis, however, their study occurred within a linear riverine system and with a less dense receiver array.

The identification of predation *via* sensor tags, *via* qualitative assumptions, or *via* quantitative *post-hoc* methods has severe limitations. First, transmitters equipped with sensors are frequently too large for use in small fish (<250 mm). Second, the sensor data approach requires that the predator and prey exhibit distinguishably different internal temperatures and/or depth usage. Third, assumptions of what constitutes 'normal' behaviour rely heavily on the preconceived notions of both prey and predator behaviour which may be invalid, particularly for plastic species in dynamic environments (e.g. diadromous species undergoing behavioural changes in estuaries) or relatively unstudied species. Fourth, it is often difficult to collect a sufficient amount of data at the temporal and spatial resolution required for behavioural analyses, particularly for long-distance migrants that infrequently traverse 'gates' of receivers. As a consequence of these limitations, few studies could identify the predation events and the widespread issue of predation on telemetered animals erodes our collective confidence in telemetry-derived information.

A prototype acoustic tag that directly detects the predation events, hereafter 'Predation Tag', has been developed by Vemco Ltd. (Amirix Systems Inc., Bedford, Nova Scotia, Canada) to automate the detection of predation events and provide an objective approach towards interpreting telemetry data confounded by predation. This predation tag does not rely on an active sensor but rather is physically altered when subjected to a predator's gastrointestinal tract; subsequently providing a new identification code. The ability to detect when a tagged animal is consumed by a predator will significantly advance acoustic telemetry and will permit a more complete and accurate interpretation of telemetry data with the data from the post-predation transmitters being censored or subjected to different analyses. This may be particularly true for sparse receiver arrays where data are infrequent and do not permit *post-hoc* differentiation of predators and prey. Further, survival estimates from the fusion of acoustic telemetry data and mark-recapture modelling (e.g. Lacroix 2008; Halfyard *et al.* 2013) will be improved by the automated estimation of predation-related mortality, thus permitting the subdivision of mortality vectors.

Prior to widespread adoption of the novel predation tag, researchers must fully understand how these data are generated and whether there are alternative interpretations of the data. Such testing will need to examine the technological and engineering efficiency of the predation tags, such as how rapidly they respond to cues of predation or repeatability of results. Additionally, these transmitters are also likely to be impacted by the biological factors known to influence digestion in fish (Bromley 1994): (i) temperature, (ii) prey type, (iii) prey size, (iv) predator size, and (v) meal size.

As such, the goals of the testing described in this paper were threefold: (i) to determine whether predation tags accurately detected the presence and timing of predation events, (ii) to determine the rate at which the predation tags falsely identified a predation event when no such predation event occurred (i.e. a false-positive), and (iii) to examine the factors that influence the lag time between the predation event and when the tags report on the predation event.

To achieve the above research goals, a series of tests were conducted on two versions of a prototype tag. Testing involved either staged predation events or an evaluation of the rate of false-positive reporting for transmitters not subjected to predation events. Staged predation events occurred at various water temperatures, using two prey species and with the consideration of small changes in prey size and predator size in an attempt to address some of the biological variability likely to generate variation in tag performance. The first tag generation (GEN1) was tested and based on these initial results, the manufacturer created a more sensitive second generation (GEN2) of the tag design in an effort to decrease the lag time between the predation event and when the tags report. In this paper, we describe the efforts made to test this novel transmitter, identify the factors affecting transmitter performance and discuss the potential benefits and limitations of this new technology.

Materials and methods

PREDATION TAG

The prototype predation tag was a modified Vemco model V5 (Bedford, Nova Scotia, Canada), 180 kHz transmitter with 143 dB acoustic power output, weighing 0·65 g (in air) and with dimensions of 4·3 × 5·6 × 12·7 mm (Fig. 1). To detect a predation event, the tag relies on the acidic conditions within a predator's stomach to digest a biopolymer. This biopolymer covers a small magnet that sits within a depression on the tags surface. Once the biopolymer is digested, the magnet is released; triggering an internal sensor to change the transmitter's identification number from the pre-predation ID to the post-predation ID. Changes to the identification number is done within the R-code acoustic coding architecture (i.e. millisecond changes in the spacing between individual pings of an eight-ping sequence), and therefore, successful detection of a predation event requires only one (although ideally several) detection of the tag. Tag signal collisions, false and missed detections are ever present in the acoustic telemetry studies (see Kessel *et al.* 2014) and will affect the predation tags and non-predation tags similarly.
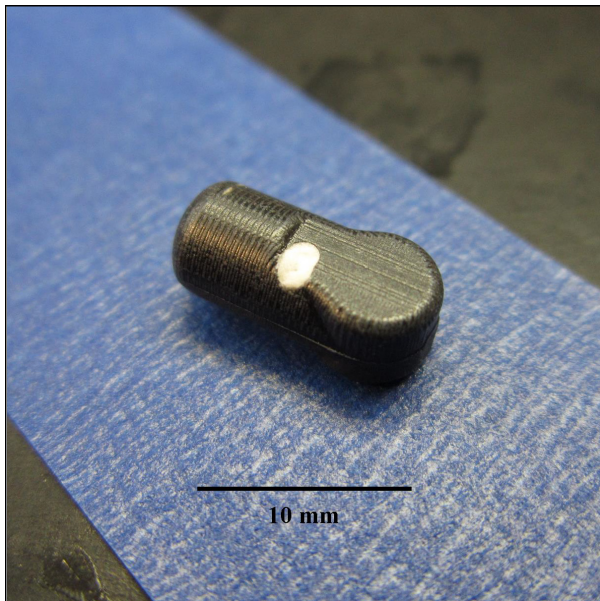
Fig. 1. A prototype predation tag. The white portion of the tag is a biopolymer designed to digest in the acidic environment of a predator's stomach. The tag transmits a 143 dB signal at 180 kHz, weighs 0·65 g (in air) and has dimensions of 12·7 × 4·3 × 5·6 mm.

PREDATION TRIALS

To test the overall efficacy of the predation tags, a series of staged predation events were conducted. In this paper, wild-caught Largemouth Bass, *Micropterus salmoides* (n = 12), were held in captivity and fed individual prey fish that were surgically implanted with predation tags. The total length of bass was 369 ± 35 mm (mean ± SD, range = 334–451 mm) with a mass of 716 ± 272 g (range = 410–1422 g). Two large (1·4 m × 6·9 m, water depth = 0·7 m) steel tanks with recirculating filtration systems were subdivided into 12 (six per tank) equal-sized arenas using plastic coated netting to permit the feeding of specific individuals. All fish were collected approximately 1 month prior to the first staged predation trial and were fed on a daily schedule at a rate of approximately 2–4% of their body weight. Feeding was ceased 18–24 h prior to testing. The staged predation events were video recorded by time stamped overhead high definition cameras so that the exact time of predation and tag excretion could be identified. Acoustic data were continuously recorded by Vemco VR2W acoustic receivers tuned to 180 kHz in each tank. Staged predation trials were conducted at various times of the year and captive lighting conditions followed natural light regimes.

Predation trails were conducted to examine three major parameters: (i) success: the successful identification of the predation event, (ii) signal lag: the time lag between the predation event and the first signal indicating the prey fish had been eaten (i.e. the time it takes for the original pre-predation code to change to a post-predation code), and (iii) tag retention: the retention time of the predation tag within the gut of the predator. Signal lag was calculated as the time between the observed predation event using the overhead cameras and the first tag transmission that indicated the predation event had occurred. Tag retention time was calculated as the time between the observed predation event and the observed time when the tag was evacuated from the predator. The overhead video could identify the exact moment when tags were evacuated during staged predation trials. For trials when the exact time of tag evacuation was not observed, we estimated evacuation time as the mid-point between video observations of a tag's presence on the bottom and the latest observation when the tag was not present at that position within the tank (usually several hours).

The prey species used in this study were hatchery-origin Rainbow Trout, *Oncorhynchus mykiss*, and naturalized Yellow Perch, *Perca flavescens* captured from a private pond using baited traps, seining and angling. Largemouth Bass were not fed for 48 h prior to these predation trials and were not fed again until the tag was excreted. The surgical methods followed standardized approaches common in the field of telemetry (e.g. Wagner *et al.* 2011). Surgery consisted of anaesthetizing fish using tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, WA, USA; 60–80 mg L^{-1}), followed by making a ~10 mm ventral incision immediately adjacent to the *linea alba*. Tags were inserted and directed anteriorly and away from the incision site using forceps. The incision was closed using three interrupted sutures of monofilament absorbable material (Ethicon PDS II Plus polydioxanone, Ethicon US, www.ethicon.com). Animals were permitted to recover from surgery (usually 20 min) prior to being used in the staged predation events.

Predation trials followed a repeated measures experiment design. During testing of the GEN1 tags, the influence of water temperature (factor with three levels: 12, 17 and 22 °C) and prey species (factor with two levels: Rainbow Trout or Yellow Perch) was examined. Trials using GEN2 tags examined only temperature, but at four levels (12, 16, 20, and 24 °C) and used only Rainbow Trout as the prey species. Additionally, the effect of prey size was examined by comparing two significantly ($t(18)$ = 9·5, P < 0·01) different sizes of trout at 20 °C (Table 1). In all cases, the realized water temperature was ±1 °C of the intended water temperature for each trial.

Table 1. Fork length and mass of prey used in the staged predation trials for each tag version, prey species and water temperature

Tag	Prey species	Temperature (°C)	Fork length (mm) mean ± SD	Mass (g) mean ± SD	Sample size
GEN1	RBT	12	114 ± 8	16 ± 3	10
GEN1	RBT	17	116 ± 12	19 ± 7	11
GEN1	RBT	22	119 ± 10	18 ± 3	12
GEN1	YP	12	111 ± 9	12 ± 3	11
GEN1	YP	17	121 ± 13	15 ± 6	10
GEN1	YP	22	118 ± 9	15 ± 5	11
GEN2	RBT	12	108 ± 9	14 ± 4	11
GEN2	RBT	16	123 ± 11	18 ± 5	11
GEN2	RBT (small)	20	111 ± 8	19 ± 4	11
GEN2	RBT (large)	20	152 ± 12	41 ± 9	11
GEN2	RBT	24	113 ± 6	17 ± 3	11

Generalized linear mixed models were fit to the predation trial data where individual predators were considered random effects, and temperature and prey species (GEN1 only) were considered fixed effects. GLMMs were fit independently to the dependent variables of (i) signal lag and (ii) tag retention for each tag generation. Signal lag data were typified by a few outliers and thus were modelled assuming a log-normal distribution. Tag retention data followed a log-normal distribution for the GEN1 tags and a normal distribution for the GEN2 tags. Model fitting of signal lag and tag retention data was conducted using the 'glmer' or 'lmer' functions in the package 'LME4' (Bates *et al.* 2014). All analyses were conducted using R version 3.1.1 (The R Foundation for Statistical Computing). The numerical data of temperature and prey weight were centred and scaled following z-transformation prior to modelling. The proportion of variance explained (R^2) of fully parameterized models was calculated following the methods of Nakagawa & Schielzeth (2013), which provides an estimate of model fit and the importance of the random effect terms.

Several models with varying fixed terms (all combinations, *see* Appendices for full list) were fit and ranked by Akaike Information Criterion (AIC) which addresses the trade-off between over- and under-parameterized models by formally weighing the trade-off between model bias and model variance (Burnham & Anderson 2004). Parameter estimates were model averaged using all candidate models with delta AIC values within 10 of the most parsimonious candidate model.

FALSE-POSITIVE TRIALS

The false identification of a predation event when no such event occurred has the potential to overinflate the estimates of predation and under-estimate survival rates. Two field scenarios under which false-positives could impact the data interpretation are when tags report a predation event but the tagged fish are alive and not consumed or when tagged fish die of causes not related to consumption by a predator.

To test the rate at which intact predation tags incorrectly identify the predation events while in a live and mobile fish, we implanted Rainbow Trout with tags. The fish were held in a similar tank as described above without the risk of predation. Receivers affixed to the bottom recorded transmissions from the tags. Fish were fed daily *ad libitum* and the tank was checked for mortalities a minimum of 3 days per week.

False-positive testing of the GEN1 tags ($n = 31$) occurred over 120 days (December–April) at an ambient water temperature of 10.8 ± 1.3 °C. False-positive trials using GEN2 tags ($n = 44$), persisted for 66 days (June–September) at an ambient water temperature of approximately 17.0 °C (based on the tank chiller controls).[1] The fork lengths of the Rainbow Trout used in the live false-positive trials were 17.8 ± 1.2 and 12.9 ± 1.5 for GEN1 and GEN2 trout, respectively.

In an effort to determine whether a dead and decomposing fish could falsely trigger the tag, and thus to report a predation event, we implanted the GEN2 predation tags into 15 recently killed Rainbow Trout. A similar test was not completed for the GEN1 tags. The surgical procedures were identical to those described above except that the

trout had been euthanized immediately prior to surgery. Tagged trout were placed into one of the two aerated 105 L bins held at 20 °C. Tags were monitored for 120 h using a receiver placed in each tank.

Results

PREDATION TRIALS

A total of 65 and 55 staged predation trials were successfully completed for the GEN1 and GEN2 tags respectively. Failure to complete an additional seven trials was the result of (i) predators refusing to eat (early in the trials), or (ii) a failure to observe the predation event (i.e. delayed consumption occurring at night). The overall rate at which the predation tags correctly identified the predation events was 94% (61 of 65) for GEN1 tags and 95% (52 of 55) for GEN2 tags.

Of the GEN1 tags tested, >90% exhibited a signal lag of less than ~29 h (Table 2, Fig. 2). Signal lag was dramatically reduced in the GEN2 tags, with >90% of the tags triggering in less than ~9 h (Table 2, Fig. 3). For both GEN1 and GEN2 tags, the remaining tag trials ($5/60^2$ and 4/52, respectively) were typified as outliers for which there was no evidence of the potential causes.

Several candidate models showed the utility in describing the GEN1 signal lag data (Appendix S1-A, Supporting Information). Model-averaged results suggest that the fixed-effect term of standardized ambient water temperature was important (coeff. = −0.386, SE = 0.082, Z-value = 4.686, $P < 0.001$), but not the fixed effect of within-trial standardized prey mass (coeff. = 0.006, SE = 0.044, Z-value = 0.142, $P = 0.887$), nor the fixed effect of prey species (coeff. = 0.016, SE = 0.074, Z-value = 0.214, $P = 0.831$). Conditional R^2 of the fully parameterized model was 0.55, of which the random effect term accounted for only 0.10. Similarly, signal lag of the GEN2 tags while considering only the 'small' trout was described by more than one model (Appendix S1-B). Signal lag of the GEN2 tags was related to standardized ambient water temperature (coeff. = −1.563, SE = 0.426, Z-value = 3.672, $P < 0.001$), but not the effect of standardized prey mass across the range tested (coeff. = 0.066, SE = 0.275, Z-value = 0.239, $P = 0.811$). Conditional R^2 of the fully parameterized model was 0.99, of which the random effect term accounted for 0.10. A comparison of 'small' (~19 g) and 'large' (~41 g) Rainbow Trout during the paired trials at 20 °C suggest that signal lag was significantly less in small trout (coeff. = −1.510, SE = 0.285, Z-value = −3.070, $P < 0.001$), with a signal lag of 2.9 ± 1.9 h for smaller trout compared with 7.8 ± 7.6 h for larger trout (Table 2, Fig. 3, Appendix S1-C). Conditional R^2 of the model including the trout size term was 0.98, of which the random effect term accounted for 0.34.

The retention of tags within the gut of Largemouth Bass was similar between GEN1 and GEN2 tags (Table 2, Figs 4 & 5). Several candidate models showed utility in describing the

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[1]Tags used in the GEN2 trial had been either (i) recovered from an 11 day false-positive where a pump failure resulted in the complete mortality of trial fish, or (ii) recovered from concurrent dead fish trials. All reused transmitters were recovered within 24 h post-mortem, were gently washed in warm water (without soap, detergents, disinfectants or sterilants) and were subsequently re-deployed in live Rainbow Trout.

[2]An additional trial was completed (i.e. 61 of 65 attempted) where the tag successfully identified the predation event, however, the time of consumption was not recorded (due to darkness).

Table 2. Summary of signal lag (time between predation and signal) and tag retention (time between predation and tag excretion) data for each permutation of tag generation, ambient water temperature and prey species tested

Tag version	Temperature (°C)	Species	No. failures	Signal LAG		Tag RETENTION	
				Mean ± SD (range) (hrs)	CV (%)	Mean ± SD (range) (hrs)	CV (%)
GEN1	12	RBT	0/10	22·3 ± 8·7 (11·8–41·5)	39	191·8 ± 30·5 (144·1–242·7)	16
GEN1	17	RBT	4/11	23·0 ± 14·3 (10·5–48·1)	62	107·5 ± 36·6 (72·0–202·7)	34
GEN1	22	RBT	0/12	9·2 ± 5·1 (3·4–17·9)	55	66·5 ± 6·9 (57·0–80·9)	10
GEN1	12	YP	0/11	26·5 ± 15·7 (14·6–71·5)	59	192·3 ± 42·0 (128·1–276·0)	22
GEN1	17	YP	0/10	13·2 ± 8·5 (3·1–28·4)	64	97·5 ± 24·7 (66·3–142·0)	25
GEN1	22	YP	0/11	12·2 ± 2·5 (9·0–16·5)	20	60·6 ± 21·4 (26·8–107·3)	35
GEN2	12	RBT	1/11	9·1 ± 10·1 (2·6–34·2)	110	162·3 ± 29·2 (141·5–219·0)	18
GEN2	16	RBT	0/11	3·5 ± 1·6 (2·0–6·5)	44	101·1 ± 19·9 (77·0–134·8)	20
GEN2	20	RBT (Small)	0/11	2·9 ± 1·9 (0·9–7·7)	65	75·6 ± 20·2 (31·9–94·7)	27
GEN2	20	RBT (Large)	1/11	7·8 ± 7·6 (2·4–27·2)	97	115·3 ± 23·3 (83·4–155·1)	20
GEN2	24	RBT	1/11	3·1 ± 1·7 (1·4–7·6)	56	n/a	n/a
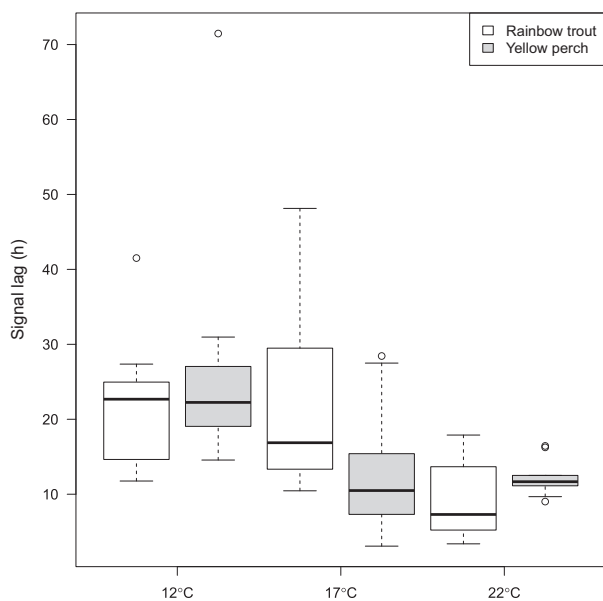
Fig. 2. Boxplot of signal lag (time between predation and signal, hours) for generation 1 tags as influenced by ambient water temperature (°C) and prey species. Boxplots represent the 25th and 75th percentiles ('box'), the median (bold horizontal line inside the box), values within 1·5 interquantile units (dashed bars), and outliers (open circles). Sample sizes in parentheses.
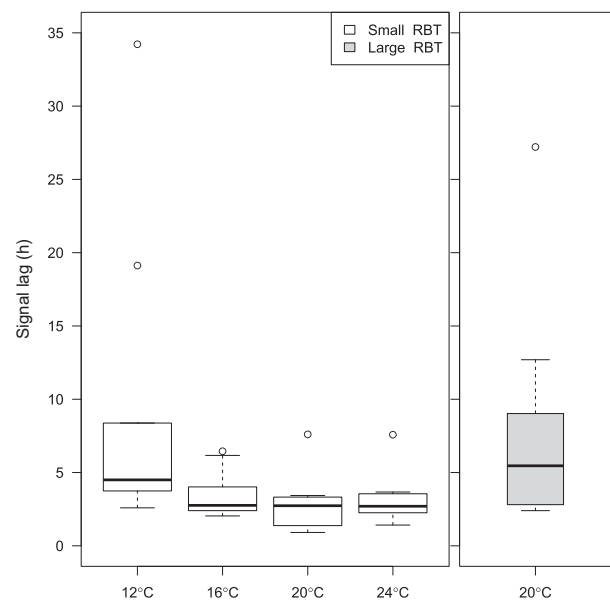
Fig. 3. Boxplot of signal lag (time between predation and signal, hours) for generation 2 tags as influenced by ambient water temperature (°C) using Rainbow Trout prey. Also shown are the results of the 20 °C trial using large (mean = 41 g) Rainbow Trout. Boxplots represent the 25th and 75th percentiles ('box'), the median (bold horizontal line inside the box), values within 1·5 interquantile units (dashed bars), and outliers (open circles). Sample sizes in parentheses.

data (Appendix S1-D). Model-averaged results suggest that the fixed-effect term of ambient water temperature was significant (coeff. = −0·451, *SE* = 0·030, *Z*-value = −14·83, *P* < 0·001), but not the fixed effect of within-trial prey mass

(coeff. = −0·019, SE = 0·032, *Z*-value = 0·592, *P* = 0·554), nor the fixed effect of prey species (coeff. = −0·019, SE = 0·042, *Z*-value = 0·459, *P* = 0·646). Conditional *R*² of
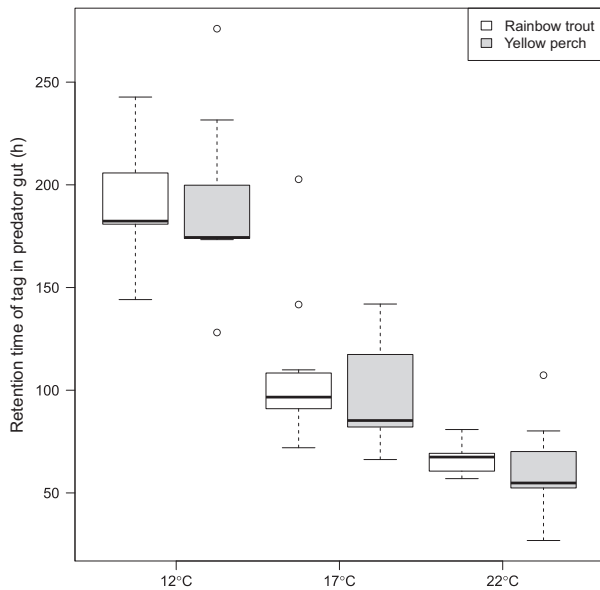
Fig. 4. Boxplot of tag retention (time between predation and tag excretion, hours) for generation 1 tags as influenced by ambient water temperature (°C) and prey species. Boxplots represent the 25th and 75th percentiles ('box'), the median (bold horizontal line inside the box), values within 1·5 interquantile units (dashed bars), and outliers (open circles). Sample sizes in parentheses.

Fig. 5. Boxplot of tag retention (time between predation and tag excretion, hours) for generation 2 tags as influenced by ambient water temperature (°C) using Rainbow Trout prey. Also shown are the results of the 20 °C trial using large (mean = 41 g) Rainbow Trout. Boxplots represent the 25th and 75th percentiles ('box'), the median (bold horizontal line inside the box), values within 1·5 interquantile units (dashed bars), and outliers (open circles). Estimates of tag retention at 24 °C were not completed. Sample sizes in parentheses.

the parameterized model was 0·21, of which the random effect term accounted for 0·02. Similarly, when comparing the models of GEN2 tags (Appendix S1-E) while considering only 'small' trout (i.e. not the single trial at 20 °C with 'large' trout), tag retention was negatively related to water temperature (coeff. = −1·482, SE = 0·194, Z-value = 7·31, $P < 0.001$), but not the fixed effect of within-trial prey mass (coeff. = −0·021, SE = 0·097, Z-value = 0·213, $P = 0.831$). Conditional R^2 of the parameterized model was 0·67, of which the random effect term accounted for <0·01. Results from the paired trials at 20 °C suggest that the signal tag retention was significantly less in small trout than large trout (coeff. = −1·644, SE = 0·442, Z-value = 3·722, $P < 0.001$, Fig. 5, Appendix S1-F). The conditional R^2 of the parameterized model was 0·58 of which the random effect term accounted for 0·11.

The signal lag times represented only 16 ± 10% (GEN1) and 5 ± 5% (GEN2) of the tag retention times, suggesting that there was ample opportunity for tags to trigger prior to tags being excreted. There was no obvious pattern in the ratio of signal lag to tag retention with respect to water temperature.

FALSE-POSITIVE TRIALS

After 120 days, none of the GEN1 tags ($n = 31$) had falsely triggered to indicate a predation event and all Rainbow Trout had retained the tags within their intraperitoneal cavities. The trout had grown an average of 2·8 ± 0·7 cm and 21·5 ± 5·4 g, representing a 15% length gain and a 36% mass gain. The false-positive trials for live fish using GEN2 tags were partially compromised by the competing risks of tag expulsion; a phenomenon not observed in the GEN1 trials. In total, 23% (10 of
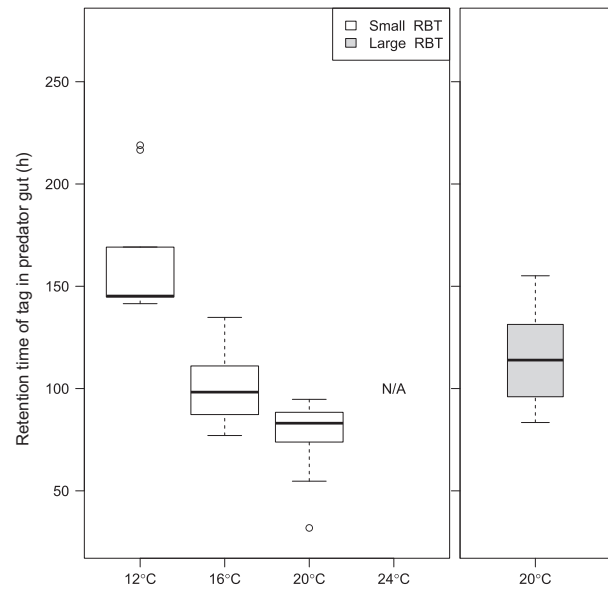
44) of the tags had been expelled by the end of the 66-day trial. These tag expulsions occurred between days 43 and 62; determined by the tank cleaning schedule. A total of 43% of all tags (19 of 44) had falsely triggered. The proportion of expelled tags that had falsely triggered (30%) was not significantly different (z-test, Z = 1·0, $P = 0.34$) than tags which remained in the trout (47%). False triggering occurred on average 47·0 ± 11·2 days post-tagging. With the exception of a single early false-positive (15·3 days), all other events occurred after day 34.

A total of 3 of 15 (20%) of the GEN2 predation tags falsely identified a predation event in the dead and decomposing fish. The times to falsely trigger were 45, 51 and 75 h post-mortem. At the end of the study, the trout were enveloped by bacteria and their abdomens were distended, however, the sutures were generally intact and the incision remained closed.

Discussion

In this study, we provide initial evidence that the prototype Vemco predation tags is promising for field studies. Predation tags are not likely to falsely identify a predation event for short term (5–6 weeks) deployments at low water temperatures, can correctly identify the most predation events on timelines suitable for most research, and provide the ability to identify mortality unrelated to predation if the tag is found stationary shortly post-mortem.

The novel predation tags appear to work well based on the laboratory testing where 94–95% of all the predation events were correctly identified. Further investigation of the

incidences when the tags failed to report the predation event suggest that these errors may be related to the prototype nature of the tags. For example, all four of the failures of the GEN1 tags were from a single production batch and one tag failed to transmit entirely. These tags were produced by hand in small batches (~15) and despite standardized production methods and materials, human error and inconsistencies may have influenced the tag performance. The automation of tag manufacturing is likely to reduce the variability in the tag performance. Further, the signal lag times of GEN2 tags were significantly reduced compared with the GEN1 tags, however, the false-positive rates increased. This likely represents a trade-off between these two parameters with more sensitive tags producing the results observed in the GEN2 tags. This trade-off will need to be addressed in future in the design of predation tag.

Water temperature was an important determinant of both signal lag and tag retention and is likely reflective of its impact on fish metabolism; for which significant literature exists (e.g. Persson 1981; Bromley 1994). In the case of surgically implanted predation tags, a predation event can only be detected once the prey fish is digested enough to reveal the predation tag, following which the predation tag's biopolymer must be digested. To this end, the effect of temperature on fish digestion rate is likely to affect the signal lag of predation tags. Additional study on the effect of temperature on tag performance should be conducted to further develop this relationship, particularly at low (i.e. <12 °C) water temperatures.

Our initial examination of tag performance relative to prey type, prey size and predator size, failed to show a significant influence on the predation tag performance. However, the testing described in this study employed small sample sizes and mixed-effect model power was not clear. Therefore, additional work is warranted considering the literature linking gastric activity in fishes to prey energy density (Andersen 1999), prey shape, texture, structure and size (Bromley 1994), and the effect of total meal size (Persson 1981; dos Santos & Jobling 1991; Andersen 1999). Further, the low R^2 values associated with the random effect terms of the GLMM models suggest that the variables associated with the individual predator traits, such as predator size or individual digestion characteristics, had only minor effects on the tag performance in this study. Again, additional testing using a larger size range of predators or other species is warranted given evidence of size-related effects on gastric activity in fishes (e.g. Swenson & Smith 1973; dos Santos & Jobling 1995) or interspecific and intraspecific variation in metabolism (e.g. Clarke & Johnston 1999; Killen, Atkinson & Glazier 2010).

Tag retention times within the gut of Largemouth Bass reported in this study were longer than those reported by Schultz, Kumagai & Bridges (2015) who fed tagged dead Chinook Salmon to free-ranging Striped Bass that voluntarily consumed the prey. At a mean water temperature of 23·3 °C, retention of their acoustic tags (0·5 g, 13·5 × 6·1 mm) averaged 43·2 ± 11·8 h. This is substantially more rapid when compared with the Rainbow Trout trials using GEN1 tags at 22 °C (66·5 ± 6·9 h) and trials of GEN2 tags at 20 °C

(75·6 ± 22·0 h). This discrepancy may be related to differences in predator species, predator size or the effect of meal size, which is unknown for the Striped Bass described by Shultz *et al.* (2015). Additionally, metabolism likely differs between free-ranging and captive predators which may affect tag retention rates. Further, the predators in our study were not fed following the predation event until the tags were excreted whereas the free-roaming predators of Schultz, Kumagai & Bridges (2015) likely continued to feed; presumably aiding tag passage. Other factors, such as prey size (131–165 mm) and water temperature (22–26 °C), were approximately similar, the prey species are closely related and likely have similar energy densities (<15% difference, Roby *et al.* 2003).

The identification of predation using this technology depends on ingestion of the transmitter by a predator which is detectable by a study receiver. Thus, this technology may not be suitable for identifying the predation events when aquatic predators shred or masticate their prey and fail to ingest a portion of the transmitters. Further, the issue of aerial or terrestrial predation affects all the acoustic telemetry (i.e. signals are not received when transmitters are removed from the water) and is not specific to predation tags, therefore, these considerations are not discussed in this paper.

The negative relationship between temperature and tag retention observed in this study is consistent with the previous research in the study of fish. For example, Schultz, Kumagai & Bridges (2015) showed a weak relationship between water temperature and acoustic tag retention within Striped Bass; however, water temperature ranged from only 21·4 to 26·5 °C and therefore does not represent cooler water temperatures expected to prolong tag retention. The strength of the temperature-tag retention relationship was much stronger in this study and the range of water temperatures tested here was greater. Largemouth Bass are thought to reduce feeding activity at 10 °C (Lemons & Crawshaw 1985); therefore, we did not consider conducting predation trials at lower temperatures. Additional study of tag performance at temperatures <12 °C is warranted, and will be particularly relevant for studies in temperate or polar regions.

The false-positive trials in live fish using GEN1 tags provided high confidence that predation tags would signal a predation event only when consumed by a predator. These trials, lasting 120 days, exceeded the duration of many field studies (e.g. salmon smolts, Thorstad *et al.* 2011; Halfyard *et al.* 2012). Live fish trials of using GEN2 tags were less encouraging with a false-positive rate of 43%; the onset of which began on day 15. Several aspects of these trials warrant consideration for the assessment of tag performance. First, the initial mortality of the GEN2 test fish, recovery of the tags and their subsequent reuse may have inadvertently impacted the integrity of the tags by impacting the biopolymer although no sign of degradation was immediately obvious. This failure rate may also be related to environmental factors. The GEN2 trials were conducted at warmer water temperatures (GEN1 = ~11 °C; GEN 2 = ~17 °C) which may have impacted the internal conditions of the fish (perhaps through increased metabolic processes) or the performance of the biopolymer.

The false-positive trial in dead fish using GEN2 tags provides an evidence of tag triggering in decomposing fish and despite a moderate rate (20%), the interpretation of these data in a field setting could lead to the conclusion of predation when mortality was not related to predation. This trial occurred in extreme conditions (e.g. high biological oxygen demand) and tag response in field settings may be different. For studies using dense arrays of receivers, and especially positioning arrays (e.g. Steel *et al.* 2013), or those supplementing fixed receiver arrays with frequent active tracking (e.g. Halfyard *et al.* 2012), it should be possible to identify the false triggering in decomposing fish as one would expect the transmitter to appear stationary immediately prior to a false-positive signal.

The novel predation tags described here should permit a less-subjective estimate of predation rates relative to *post-hoc* approaches (Perry *et al.* 2010; Buchanan *et al.* 2013; Romine *et al.* 2014; Gibson *et al.* 2015). Predation tags would alleviate the need for intensive labour to assign a fate to each transmitter, such as SCUBA (Karam, Kesner & Marsh 2008) or intensive active tracking (Halfyard *et al.* 2012), and may facilitate intensive study in the environments not amenable to intensive study (e.g. the open ocean, the high-arctic). An obvious application of the technology is an assessment of the spatial and temporal patterns in predation risk and mortality, which permit the refinement of population and management models and permit an assessment of the population-level impacts of predation or mortality (Pollock, Jiang & Hightower 2004). These transmitters will provide the estimates of predation-related mortality rates which can be used to partition total mortality rates (e.g. from mark-recapture analysis of mark-recapture data, e.g. Halfyard *et al.* 2013) into predation-induced mortality vs. other sources of mortality. Data such as these are informative for management decisions, conservation or recovery strategies and fundamental studies of species ecology. To this end, the increased certainty with which researchers can interpret their telemetry results should provide higher quality science products. The efficacy of the tags tested here was high with success rates and response times at a level acceptable to most studies and points to the promising developments for the use of predation tags in the near future. This study used widely distributed prey and predator species and occurred at water temperatures expected in many field settings, however, we recommend that researchers conduct validation in conjunction with field applications to determine the gear performance specific to their study species, expected predators, and environmental conditions.

Authors' contributions

Study design was completed by E.A.H., A.F. and D.W. Experimentation was conducted by E.A.H., J.D.P., T.L., S.T.K., S.F.C. and A.T.F. Data analysis and interpretation were completed by E.A.H. Manuscript drafting was done by E.A.H. with critical review by all authors.

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Data accessibility

- Data S1. Summarized signal lag data from staged predation trials
- Data S2. Summarized tag retention data from staged predation trials
- Data S3. Summarized false-positive data
- Data S4. R scripts: signal lag and tag retention analysis
- Data deposited in the Dryad Repository: https://doi.org/10.5061/dryad.017v9 (Halfyard *et al.* 2017)

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Appended tables of candidate models examined as part of generalized linear mixed-effects modelling.