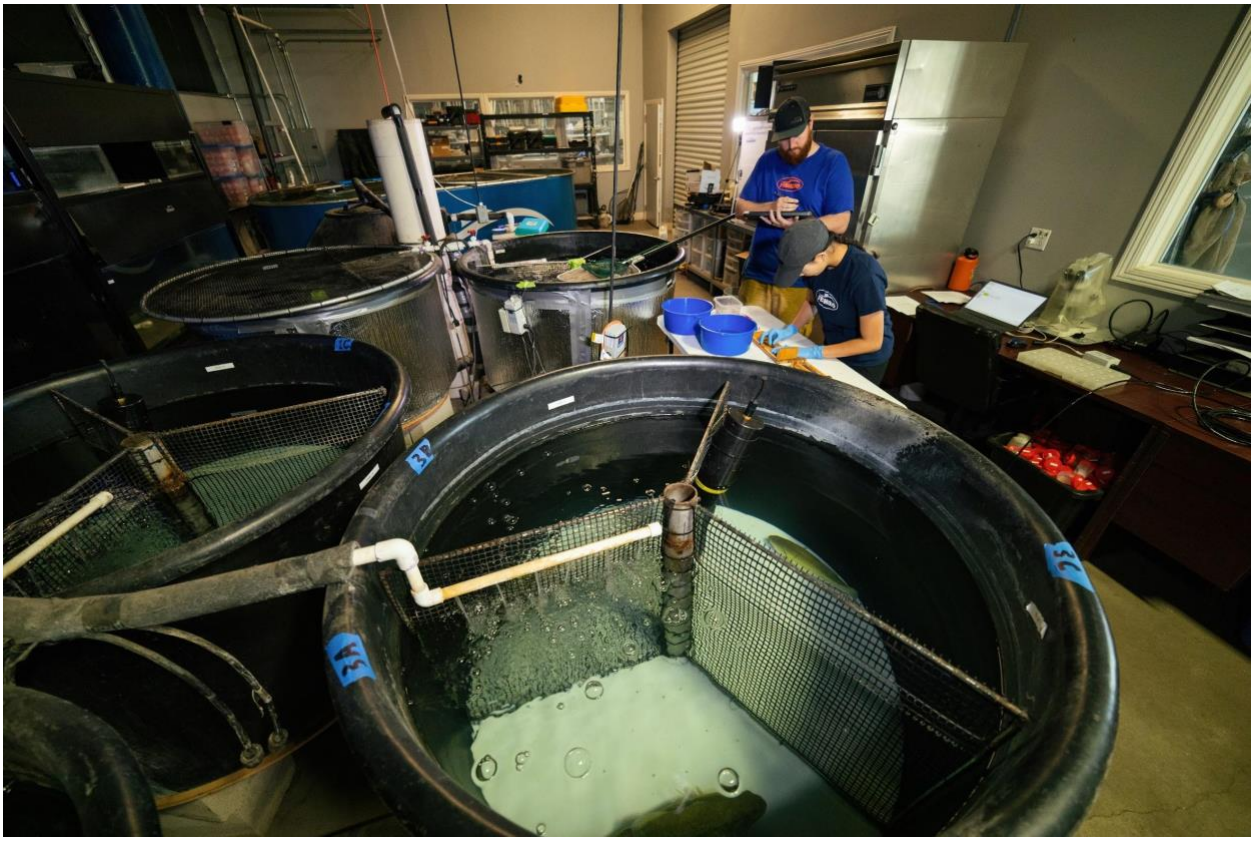
V3D Predation Tag Testing



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Overview

FISHBIO performed a comprehensive trial of the V3D predation tag (Innovasea, Nova Scotia, Canada) to quantify performance metrics for the novel transmitter's corrosive trigger and efficacy detecting a predation event. The transmitters each weighed 0.33 g in air, had a length of 15.5 mm and a diameter of 4.0 mm. The tags are equipped with a trigger mechanism designed to dissolve during digestion of the tagged specimen in the event of predation. The V3D transmits a unique identification number (ID) when first activated, which switches to a new ID when the predation trigger reacts with the acidic environment of the digestive system, thus allowing researchers to passively discern a predation event. The two tag states are differentiated by the last four numbers in the ID being either even (untriggered) or odd (triggered), indicating corrosion of the trigger mechanism and presumably a predation event. Six independent trials, using tagged rainbow trout (*Oncorhynchus mykiss*; "prey") and largemouth bass (*Micropterus salmoides*; "predator"), were performed over a six-week period to evaluate baseline trigger rates, time to trigger following a predation event, and time to expulsion of the V3D tags by the predator. Trials were performed under two different temperature regimes (18.5ºC and 23.5ºC) to evaluate the influence of water temperature and related digestion process on tag performance.

Methods

Efficacy and reliability of the V3D predation tag (0.33 g in air, 15.5 mm L x 4.0 mm diameter) were assessed under controlled conditions, using three different experiments:

1) "Bare control" trial

In this trial, tags (n = 10 per temperature treatment) were activated, submerged in an aquarium maintained at 23.5°C and subsequently monitored for a period of three weeks (21 days) or until trigger events were recorded. Following completion of the first trial, the experiment was repeated with an additional ten tags at an average temperature of 18.5°C.

Bare tags used in the immersion experiment were placed in numbered bins within the control aquarium to keep them isolated from one another. Monitoring of the immersed bare tags was performed passively using the Innovasea HR3 acoustic receiver over the course of all trials (see Figure 1).

2) "Tagged control" trial

This trial was intended to evaluate the false positive rate for tags implanted in two groups of juvenile rainbow trout. A total of 20 individual *O. mykiss* rainbow trout were surgically implanted (intraperitoneal) with the V3D transmitter. Prior to tagging, transmitters were activated individually, their activation confirmed with a HR3 receiver, and sorted into numbered holding bins. Tags and surgical equipment were sterilized using a betadine solution prior to each surgery. Fish were anesthetized by immersing them in a bath containing dissolved Alka Seltzer Gold. Once anesthetized (indicated by loss of equilibrium), each individual's weight (in grams) and length (fork length [FL] in mm) were recorded. Fish were then placed ventral side up in a surgical cradle. A small 4-5 mm incision was made using a 3 mm micro-scalpel, approximately 5-10 mm anterior to the pelvic girdle, and approximately 2 mm off the central midline. The transmitter was placed into the body cavity and the incision closed with approximately 1-2 drops of water-activated surgical glue (3M VetBond™). During fish recovery, transmitter function was verified again to ensure the tag had not been triggered during the tagging process.

Tagged rainbow trout were held separately from untagged fish in a control tank (see Figure 1). Ten rainbow trout (mean FL = 133.95 mm [SD = 18.4], mean weight = 32.3 g [SD = 13.9]) were tagged and held at 18.5°C for three weeks (21 days). After completion of the first trial, the tagged rainbow trout were euthanized (CO_2 immersion, using Alka Seltzer Gold tablets). Transmitters were extracted from rainbow trout to assess the state of the trigger mechanism, tag location within the body, and any incidental glue residue on the transmitter. Ten new rainbow trout were then tagged with new transmitters and held for another three weeks (21 days) at 15°C. Water temperature was lowered in weeks 4-6 because rainbow trout showed signs of physical stress due to warm water temperature. At the completion of week 6, trout were euthanized, and the tags were examined as described above.

3) “Feeding Trial”

Rainbow trout were selected as the prey species in this study because they were readily available from a local hatchery in a size range suitable to accommodate tags. Also, they were considered an applicable species for this test, as future research uses for the V3D transmitters are expected to focus on juvenile salmonids, such as Chinook salmon (*Oncorhynchus tshawytscha*), with similar physiology. To evaluate tag performance in the event of predation, rainbow trout were implanted with the V3D transmitter and fed to predators (largemouth bass, *Micropterus salmoides*).

Specific metrics recorded across trials included the time to “trigger" (defined as the duration of time between consumption and corrosive mechanism's trigger [code switch]), as well as the rate and type (defecation vs. regurgitation) of tag expulsion by predators.

Tanks housing predatory fish were monitored 24 hours a day for the duration of the study with overhead cameras to capture predation events and the expelling of transmitters. The tanks were illuminated during the day by sunlight and facility lighting. At night, the cameras switched to infrared so as not to interfere with the diurnal cycle of the predators. Video files were recorded in one-hour blocks and stored on site for future review.

Trials were performed across two temperature regimes, based on the assumption that water temperatures would affect metabolic rate (e.g., Molnár and Tölg 1962, Volkhoff and Rønnestad 2020), and consisted of a “cool” water treatment (average temperature = 18.5^{o}C), and a “warm” water treatment (average temperature = 23.5^{o}C). Temperature was monitored continuously using HOBO temperature data loggers (Pro v2, Onset Computer Corporation, Bourne, MA) to record the fluctuations in temperature throughout the study period.

Largemouth bass used in the study (n = 9) were tagged with floy tags for identification, measured (FL, in mm) and weighed (grams) at the beginning of each temperature trial group. Bass were held in numbered screened enclosures during feeding trials. Each temperature treatment consisted of three feeding trials using eight tags per trial (tagged rainbow trout). Each feeding trial was conducted independently over the course of one week starting on Monday and ending Sunday, for three consecutive weeks. In total, 24 bass feedings were conducted per temperature regime, and 48 feedings across all six trials. We used a total of nine largemouth bass for the study and chose individuals for each trial based on their readiness to consume prey. The predators were not fed outside the feeding trials to ensure maximum appetite when the tagged rainbow trout was introduced. The ninth bass (not selected for the feeding trial) was fed an untagged rainbow trout to avoid underfeeding the predator. Warm temperature feeding trials (weeks 1-3) were conducted at water temperatures averaging 23.5^{o}C (SD = 0.6), the cool temperature trials (weeks 4-6) were performed under an average temperature regime of 18.5^{o}C (SD = 0.3). Tagging for prey fish was performed the day of the scheduled feeding trial, and tagged rainbow trout were held in numbered holding tanks for three hours prior to predator feeding (see Figure 1). Before feeding, proper tag function was verified with the HR3 receiver to ensure the tag was not accidentally triggered during the tagging process. The surgery procedure for acoustically tagged prey fish followed the same surgical methods as those performed during the “Tagged Control” trials.

Monitoring the predators after feeding was conducted using visual inspection of the enclosures and video review. Each morning, all predator enclosures were inspected for any expelled transmitters and cleaned of excess detritus. Upon discovery of an expelled transmitter, video footage was reviewed to record the time of the expulsion event and the expulsion type (defecation or regurgitation). We then identified the transmitter using the HR3 receivers within the predator tanks and examined the trigger on the transmitter as well as glue exposure. Following identification and assessment of the transmitter, we photographed and stored the transmitters in numbered bins for later reference.

Table 1. Descriptive statistics (mean and standard deviation [SD]) for fork length (mm) and weight (g) of largemouth bass and rainbow trout used in each feeding trial, and corresponding temperature (°C) measurements.

Trial	Prey FL (mm)	Prey Weight (g)	Predator FL (mm)	Predator Weight (g)	Temp Cool Trial (°C)	Temp Warm Trial (°C)
1	127.5 (5.4)	24.2 (4.8)	342.4 (17.9)	758.7 (141.8)	-	23.7 (0.3)
2	120.1 (8.6)	20.0 (3.9)	345.8 (15.2)	764.0 (136.7)	-	23.7 (0.3)
3	123.4 (6.1)	22.6 (3.6)	341.0 (18.1)	765.6 (138.1)	-	22.1 (1.2)
4	137.0 (15.3)	31.4 (10.0)	340.6 (17.1)	739.3 (126.3)	19.0 (0.6)	-
5	133.0 (14.6)	31.0 (9.2)	341.0 (17.7)	738.6 (125.3)	18.4 (0.2)	-
6	148.6 (11.1)	38.0 (8.2)	346.8 (15.8)	775.7 (128.1)	18.4 (0.2)	-

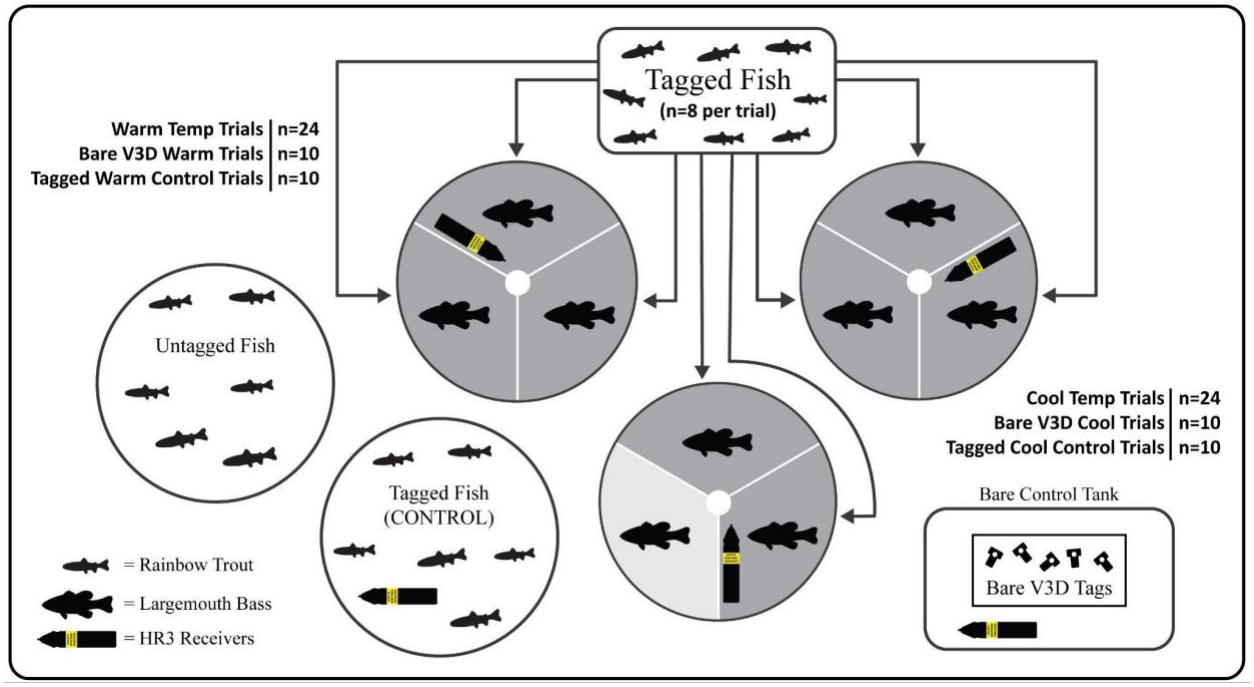

Figure 1. Conceptual diagram of laboratory setup for testing of V3D tags with HR3 receivers using Largemouth bass (*M. salmoides)* predators and Rainbow trout (*O. mykiss*) as prey. Eight tagged fish were individually fed to a predator for each weekly trial (three trials per temperature regime, at 23.5ºC and 18.5ºC). Ten Rainbow trout were surgically implanted as an experimental control and held for three weeks at each of the two temperature regimes. Ten bare tags were submerged in water in a separate tank at each temperature regime and monitored until trigger events were recorded.

Data Analysis

Data analysis and visualizations were all performed using the open-source R programming software (R Core Team 2021). Statistical analysis was performed on only two of three datasets generated ("Bare Control" and "Feeding Trials"), as no trigger events were recorded in the tagged control trials. All statistical methods were implemented with the "stats" package in R (package "stats"; R Core Team 2021).

In the "Bare tag control" experiment we pooled trigger times for each of the three trials within each of the temperature regimes. The Shapiro-Wilk test for normality showed the data was normally distributed (*P*-value = 0.089), so we applied Welch's two-sample t-test to compare differences in trigger times between the two temperature regimes tested.

In the "Feeding trial" experiment we tested for differences in trigger times in predators across the two temperature regimes. The feeding trial datasets did not conform to normality (Shapiro-Wilk, *P*-value = 0.004), therefore we applied the Wilcoxon Rank-Sum test, a non-parametric extensions of a t-test, which test for differences in the median trigger times between temperature regimes. Trials within each of the experimental temperature regimes were pooled prior statistical analysis.

Lastly, we performed a simple linear regression to evaluate the relationship between relative prey size (expressed as a fraction of the predator's body weight) and the duration between prey consumption and trigger activation.

Results

Bare Control Tag Trials

Contrary to our expectation, all (100%) of bare control tags were triggered after prolonged submersion in water. In the warm temperature treatment (23.5°C; n = 10), all tags were triggered after 309 hours, with the first trigger recorded 196 hours after activation (Figures 2, 3). Trigger times were slightly, but significantly longer in the cool temperature regime (18°C; n = 10; Welch's two-sample t-test; *P*-value<0.001), with the first trigger recorded 267 hours after activation and 100% of tags triggered after 383 hours. Mean trigger times were 340 hours (SD = 36.8) for the cool treatment and 252 hours (SD = 47.9) for the warm treatment. Data were normally distributed (Shapiro-Wilk, *P*-value = 0.089).

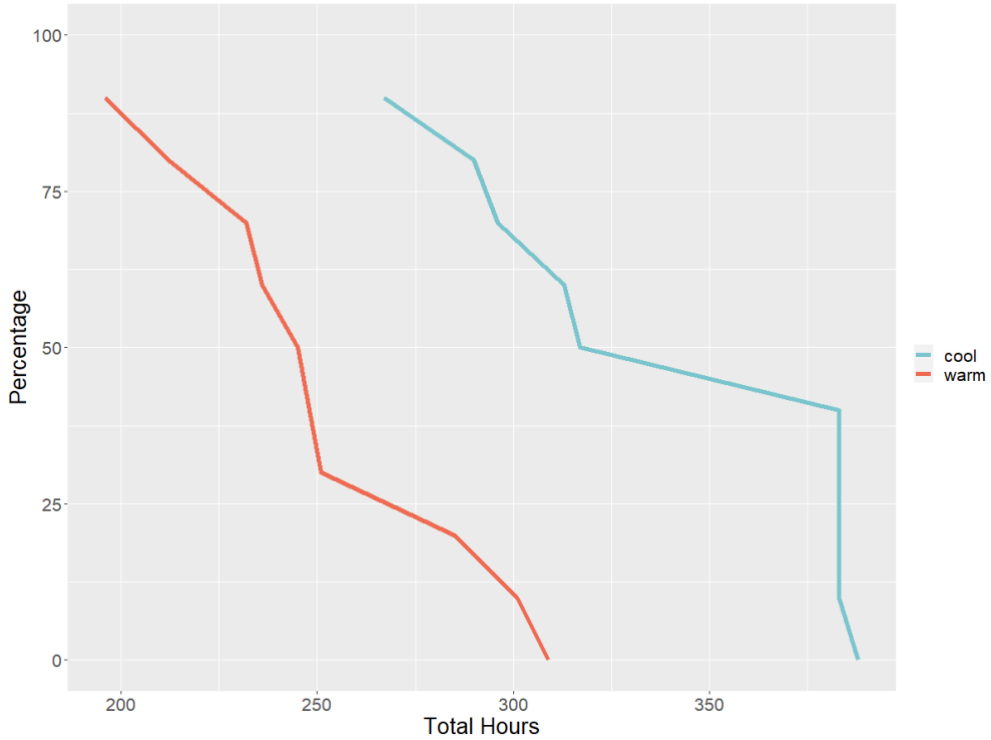

Figure 2. Decay plot showing the percentage of untriggered transmitters in control bare tags over time (warm n = 10, cool n = 10)

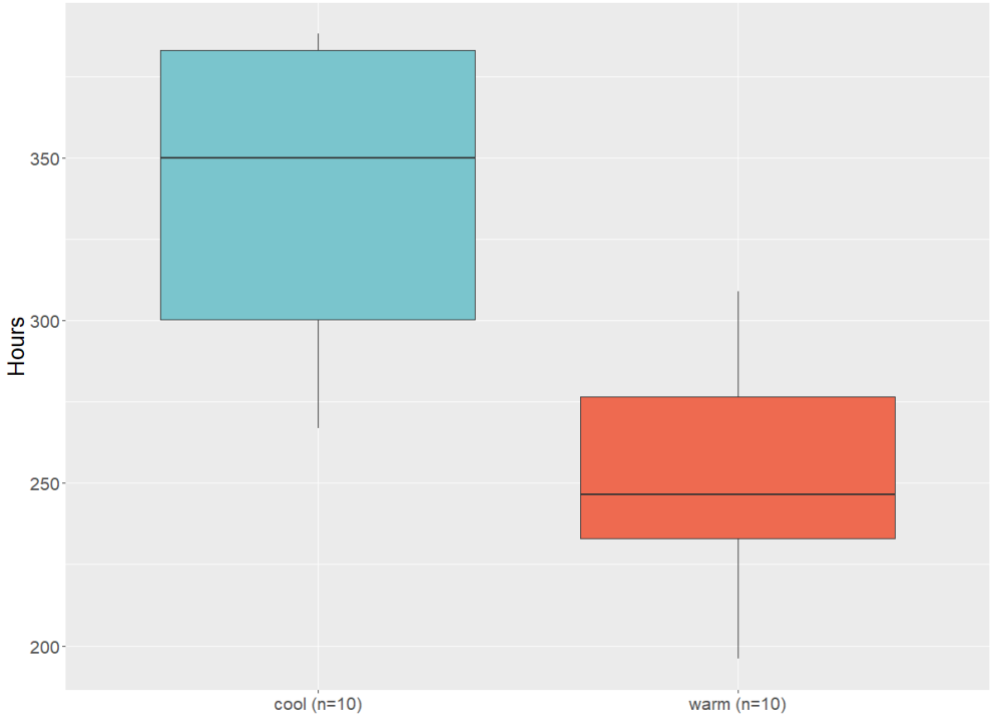

Figure 3. Median times (h) between initial activation of V3D tags and trigger activation in control tags submerged in water at two different temperature regimes. Each boxplot represents a sample size of n = 10. The solid line within each boxplot represents the median trigger time, the top and bottom of the boxes represent the interquartile range, and whiskers represent the range of samples included in the median estimate.

Tagged Control Trials

No trigger events were detected for transmitters that were intraperitoneally implanted in rainbow trout and held in the tagged control trials, irrespective of the temperature treatment (Figure 1). Following completion of the trials and after experimental specimens were sacrificed, removal and subsequent inspection of the tags revealed that 8 of the 20 transmitters (40%) showed low levels of trigger erosion. Three additional transmitters were classified as having a medium level of trigger erosion. This may indicate that the trigger mechanism slowly erodes in the peritoneal cavity, despite not being exposed to digestive processes (Figure 4).

Figure 4. Example of a V3D tag that had been implanted in a juvenile rainbow trout for a period of three weeks (left), showing signs of corrosion of the trigger mechanism (not triggered), and a new transmitter showing an intact covering of the trigger mechanism (right).

Feeding trials

Feeding trials resulted in a 100% trigger rate for transmitters in prey fish consumed by largemouth bass. In the cool temperature treatment, we estimated a median time to trigger of 14 hours (IQR: 11.50-18.25 h), while the estimated median time to trigger for the warm treatment was 6 hours (IQR; 5.75-8.25 h). The median transmitter trigger times were statistically different between temperature regimes (Shapiro-Wilk, *P*-value = 0.004, Wilcoxon Rank-Sum; *P*-value < 0.001; Figures 5, 6).

Relative prey size has a significant effect on the duration between prey consumption and tag activation in the cool (*P*-value=0.01), but not the warm water treatment (*P*-value = 0.22; Figure 7).

No tags were expelled prior to triggering. Expulsion time and type varied between temperature treatments, however within a temperature treatment there were similarities in the proportion and type of tag expulsion (Figure 8). Timing between predation and the two evacuation types showed no significant difference (Wilcoxon Rank-Sum; *P*-value = 0.68). Just over half of the tags (29 of 48; 60.4%) had a retention time of at least two days, but some tags were retained much longer and even overlapped feeding trials. Some tags (n = 14) had not been expelled by the conclusion of this study. The type of tag expulsion could not be determined for two transmitters due to a small blind spot in the camera recordings. The median time from predation to defecation was 164.8 hours (n = 12), and for regurgitation the average time was 162.0 (n = 20). While the median expulsion times were similar, we observed more regurgitations than defecation through all the trials.

Periods of tag retention by predators was highly variable; the earliest evacuation was observed 38 hours after consumption, but tag retention was noted beyond a duration of 600 hours (14 tags of the 48 tags used in the feeding trials were not expelled by the time the study had concluded). However, most tags were expelled within 200 hours of consumption (Figure 9).

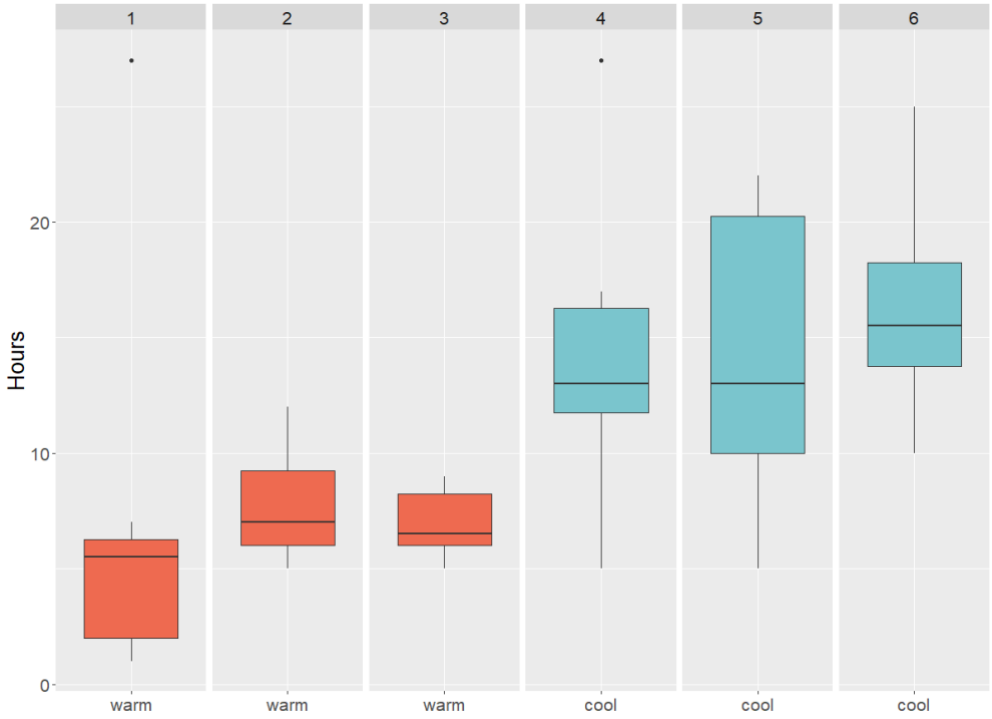

Figure 5. Observed times to trigger (h) between feeding trials (n = 8 for each). The solid line within each boxplot represents the median trigger time, the top and bottom of the boxes represent the interquartile range (25th to 75th quartile). The end of each whisker represents the reasonable min and max. Each separated point is an outlier falling outside the 95% quartile.

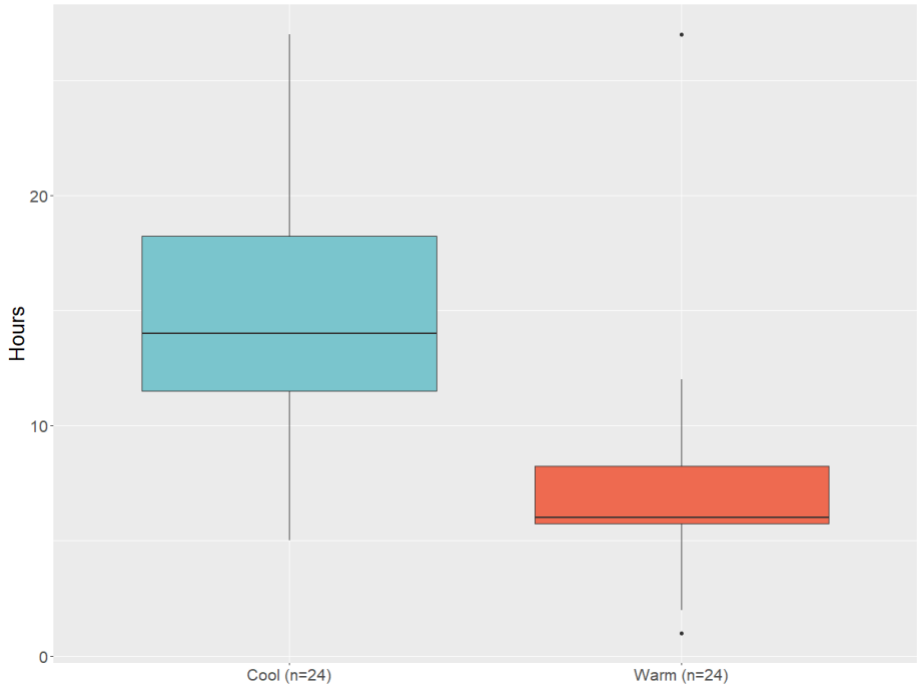

Figure 6. Hours between consumption of prey fish (rainbow trout) tagged with V3D tags by largemouth bass and transmitter trigger under two temperature regimes (n = 24 for each temperature regime).

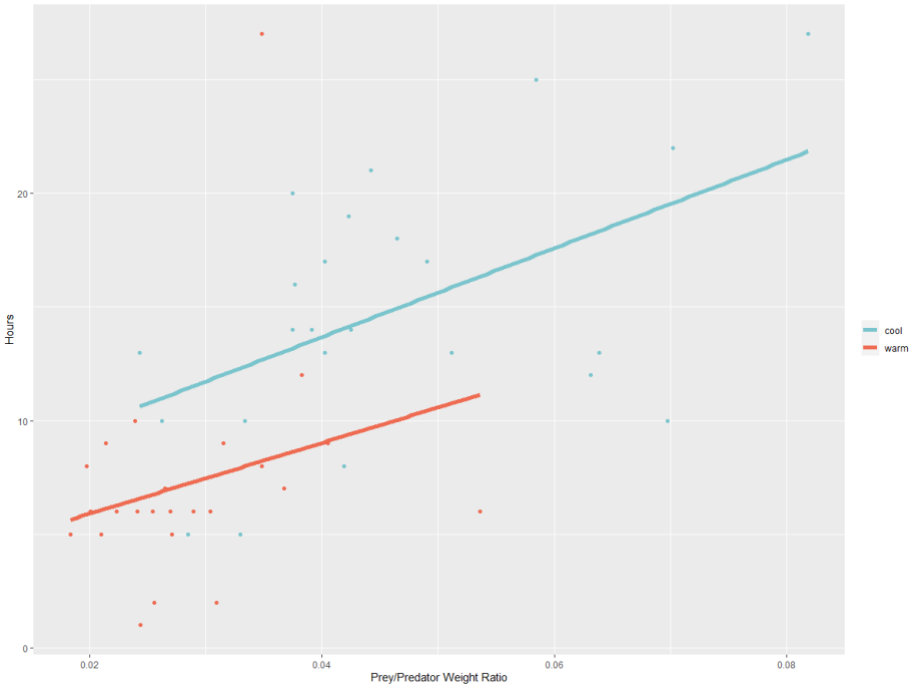

Figure 7. Linear regression model applied to prey/predator weight ratio and trigger times (hours), indicating a significant positive relationship between relative prey size and trigger activation in the cool-water trials (slope=195.2, intercept =5.9, *P*-value=0.01). No statistically significant relationship was found for the warm-water trials (slope =155.8, intercept =2.8, *P*-value= 0.22).

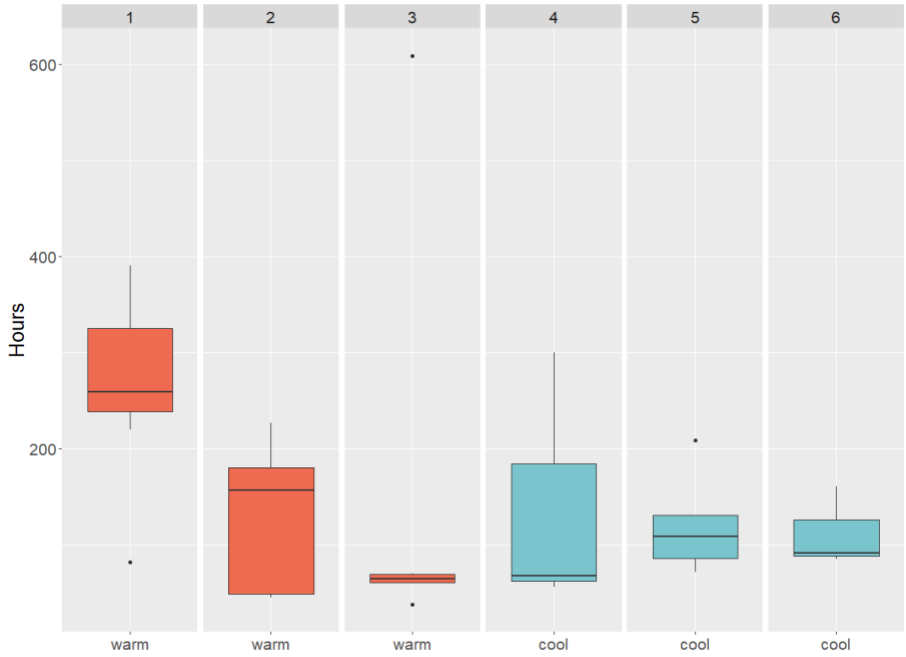

Figure 8. Time (h) from predation to observed expulsions for each trial by temperature. Note that all tags from all trials were expelled before the conclusion of the study leading to different sample sizes in each trial (Trial 1, n = 7; Trial 2, n = 7; Trial 3, n = 6; Trial 4, n = 4; Trial 5, n = 5; Trial 6, n = 3).

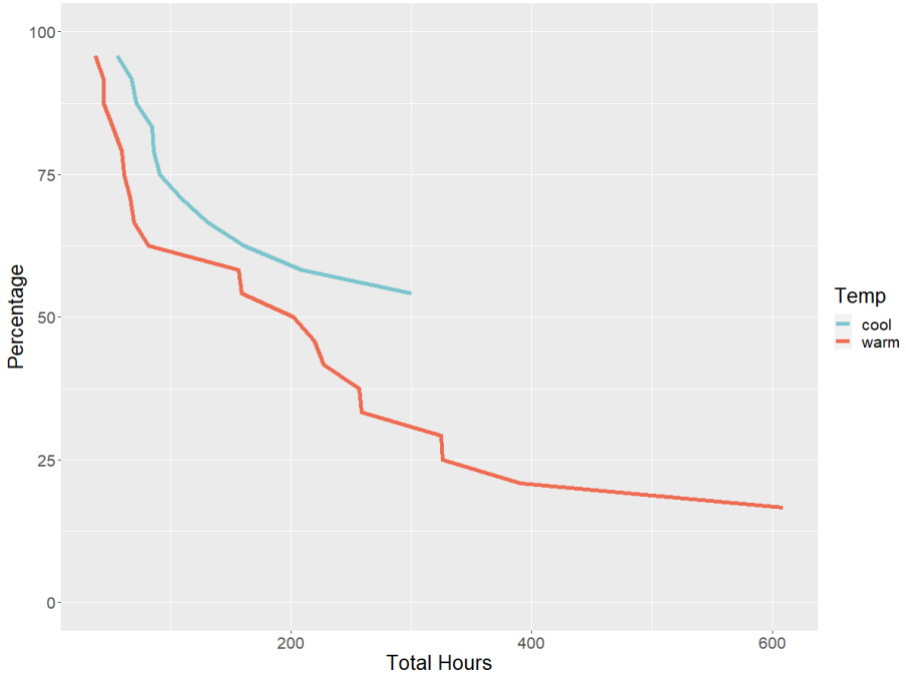

Figure 9. Decay curve showing the percentage of tags that are retained by largemouth bass through time separated by temperature treatment (warm n = 20, cool n = 12).

Discussion

In this study, we used three different experiments to assess the performance of the V3D transmitters. Each experiment was intended to yield important information about the transmitter performance under controlled conditions. Recommendations provided below might allow Innovasea to further improve the V3D transmitter and to provide additional guidance to future users of this technology.

Bare Control Tags

The bare control tag trial was used to establish whether the V3D transmitters would activate when exposed to water for extended periods of time. This is an important consideration during tagging studies and might occur in two different scenarios: the V3D tag is shed by a tagged salmonid (or other prey fish) before the battery is exhausted, or a tagged fish dies after tagging, which would eventually cause the tag to be exposed to water, leading to false positives in both cases. We found, under laboratory conditions with consistent water temperatures during the each feeding trials and pH of approximately 8.0, that all V3D transmitters were triggered by exposure to water, regardless of temperature treatment.

While tag shedding and mortality of tagged fish is typically low in most tagging studies, it is important to recognize that false-positive triggering events may result, resulting in a positively biased estimate of predation rate. Temperature had a significant effect on time to trigger, as bare tags triggering faster in the warm treatment compared to the cool treatment. However, both the warm and cool treatments were conducted in a pH environment of 8.0, so no inferences about the relationship between trigger time and pH could be drawn. It may be valuable to better understand tag performance under varying pH and temperature treatments, ideally with a strong experimental study design with sufficient contrast in explanatory variables.

Tagged Control

During the two three-week trial periods, no tags had triggered by the end of each trial. This indicates that no false-positive triggering events would be expected to occur for at least three weeks post-tagging. However, at the conclusion of each trial, there was some evidence of trigger erosion on the implanted transmitters. We estimated that the tag life of the tags were at least six weeks (42 days), as tags used in the first feeding trial were still transmitting at the conclusion of the study. Tagged trout were only held for half of that time, so some uncertainty remains about whether, and when, tags implanted in trout would have triggered.

Feeding Trials

In all feeding trials, we observed a 100% trigger rate with a mean trigger time over all trials of 11.3 hours (SD = 6.5 h, range = 1 - 27 h). A similar study conducted using PDAT tags (Schultz et al. 2017) only showed a 90% trigger rate that showed a predation event and a mean activation rate

of 59.2 hours (SD = 28 h, range = 22 - 140 h). Schultz et al. (2017) observed that none of their tags triggered within 24 hours of being consumed and that some tags were expelled prior to triggering. In this study, 46 of 48 (96%) V3D tags triggered within 24 hours of being consumed. The two tags that did not trigger within 24 hours triggered shortly thereafter at around 27 hours. Additionally, no transmitters were expelled by largemouth bass prior to being triggered.

Halfyard et al. (2017) reported that about 94% and 95% of tags were successfully triggered between two different generations of predation tags. In the same study, the time to trigger ranged from 1 to 29 hours depending on which tag was used. The V3D tag triggered at a higher rate (100%) than both previous studies and with the same approximate time to trigger as Halfyard et al. (2017).

Notably, we observed a significant relationship between relative prey size and trigger time at the cool temperature regime, but not for the trials conducted at warmer temperatures. This is likely attributable to the larger range in prey sizes used during the cool water trials, compared to the more limited range in prey during the warm water treatment. However, as larger prey were used in the cool trials, the potential interaction effect between relative prey size and temperature regime warrants further investigation in future experiments.

We observed that most largemouth bass used in the feeding trials did not immediately pass their tags either from defecation or regurgitation. In the warm trial, 50% of largemouth bass retained the V3D tags around 200 hours (just over 8 days). The observation period for the cool trials was not sufficient to estimate the time to 50% retention, however, based on the increased time to evacuation in the cool trial, we expect it would be slightly longer than the warm trial, perhaps around 9 or 10 days. Largemouth bass retained their tags longer during cool trials compared to warm trials and temperatures used in these trials, approximately 18.0ºC and 23.5ºC, respectively, might be considered to be the upper temperature conditions that tagging studies might occur in. Therefore, tag retention times in field applications would likely be longer than those observed in this study. This has important implications for the detection of predation events, as predators with a triggered transmitter would have a higher chance of being detected by passive or active methods. However, some researchers have reported that some piscivorous predators of Bloater (*Coregonus hoyi*) retained larger V9DT-2x transmitters from 1 to 194 days with 30% of predators retaining tags for more than 150 days (Klinard et al. 2019).

Caveats and Future Study Improvements

The scope of the current study was similar in scope to other studies (i.e., Schultz et al. 2017) and provided important information about the performance of the V3D tags. The performance of this particular transmitter, to our knowledge, has not been widely assessed as the previous generations of predation tags have. In this study, 48 total feeding trials were conducted using rainbow trout (prey) and largemouth bass (predator), which is slightly less than the total number used in Schultz et al. (2017). In that study, a total of 60 feeding trials were used to assess transmitter performance of PDAT tags in trials between striped bass (predator) and Chinook salmon (prey).

An important potential shortcoming of the current study that must be acknowledged relates to surgery closure methods. We opted to use bio adhesive instead of sutures for wound closure before realizing that the bio adhesive may have an adverse effect on tag activation. We believe that our precautions to reduce exposure of the transmitter to bio adhesive worked well, we would likely opt for suture closure in future evaluations to eliminate this potential source of error.

Future tag testing should also seek to standardize prey size to the extent possible. As the trials progressed, prey items used in the later trials were larger, on average, due to limited prey availability. Using larger prey in the last three trials could have led to delayed activation of the V3D transmitters during the cool temperature treatment (Figure 9). However, this issue did not affect the conclusion that trigger times were significantly different between temperature treatments.

Lastly, due to time constraints, we were unable to observe evacuation of transmitters following the cool temperature trials for the same amount of time as we did for the warm temperature trials. As warm trials were conducted, the remaining three weeks of the study period could be used to observe transmitter evacuations. When the cool trial ended, we were only able to observe transmitter evacuations for seven days after the conclusion of the final week of feeding trials (Figure 8). We recommend that future studies allow for a prolonged observation period for both groups of predators.

Other Considerations for Future Studies

Several biological and physical parameters that were not tested or controlled for in this evaluation, but may affect tag performance, warrant further investigation.

Latent tagging mortality

Under consideration of our observation that tags may trigger when exposed directly to water, latent tagging mortality of study specimens and subsequent post-mortem tag activation should be quantified. This could easily be accomplished by tagging a recently euthanized trout (or other species of interest) and submerging the tagged fish in water until trigger events are recorded. While relevant to field studies, this aspect is probably of lesser importance than the effects of the surgical procedure, as detection of a tag activated in this manner (at least via stationary receivers) is relatively low. However, roving surveys would not be able to distinguish between actual predation events and false positives.

Effect of predator species

Different species of piscivorous fish exhibit different predation behaviors that may affect the digestive rate of a prey item (Hunt 1960), and variation in evacuation rates among species with varying activity levels and feeding behaviors can be significant (Stehlik et al. 2021). Evacuation rates directly affect the duration of the period between tag trigger and evacuation, potentially

limiting opportunity to detect the predation event. Species of interest with life histories and migration patterns that differ substantially from those of largemouth bass include striped bass, walleye, pikeminnow and catfish.

Effect of predator-prey size relationship

Some studies have shown that digestion rates of prey larval fish increased with water temperature and decreased at larger prey sizes (Legler et al. 2010). It follows that the size of tagged prey organisms could affect the trigger time of the transmitters. Prey digestion rates could also be influenced by the size of the predator. While we found trigger time to be related to relative prey size, controlling for additional variables (similar prey sizes between temperature treatments, etc.) would be desirable. To alleviate the ambiguity associated with the predator:prey size relationship, a further study that controls for prey size could be implemented and evaluate differences, if present, in trigger times following predation. Predator size is expected to have a limited effect on evacuation rate (He and Wurtsbaugh 1993).

Trigger activation performance at low temperatures

The most frequent applications of V3D tags are expected during studies involving salmonids, which will rarely, if ever, occur at the water temperatures we tested. Thus, to better evaluate expected tag performance in field research applications, additional trials at temperature regimes between in the 4-15 ^{o}C would be valuable.

Additional Considerations for Field Applications

Potential applications may be limited by trigger and evacuation times. Based on the trigger times and evacuation times the technology may have limitations for large-scale studies where receiver distances are highly spread out. However, for species that are less mobile, maintain a small home range, or perhaps exhibit fidelity to some feature; there may be significant applications for understanding species interactions. There is likely high value in the technology for localized predation studies such as around structures, small scale high density arrays, gates and weirs.

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Table A1. Summary table of PDAT trials. Temperature indicates the temperature regime (warm: 23.5 ºC; cool: 18.5 ºC). Fish ID refers to the floy tag number of the largemouth bass used on the feeding trial. Evacuation type refers to the nature of tag evacuation by the largemouth bass, if known (based on video review). Hours to trigger refers to the amount of time elapsed since prey consumption and tag-code switch. Hours to evacuation refers to the time elapsed between predation and tag evacuation, as determined from video review.

Trial	Temperature	Tag ID	Fish ID	Hours to Trigger	Hours to Evacuation	Evacuation Type
1	warm	H307-1902-1260	227	6.9	220.8	Defecation
1	warm	H307-1902-1232	228	5.0	326.5	Regurgitation
1	warm	H307-1902-1246	230	6.8	391.8	Regurgitation
1	warm	H307-1902-1272	232	7.9	325	Regurgitation
1	warm	H307-1902-1204	233	2.5	N/A	Unknown[1]
1	warm	H307-1902-1190	238	27.5	82.5	Regurgitation
1	warm	H307-1902-1218	242	1.6	257.7	Defecation
1	warm	H307-1902-1274	243	2.6	259.3	Defecation
2	warm	H307-1902-1194	227	12.3	52.5	Defecation
2	warm	H307-1902-1220	228	6	45	Defecation
2	warm	H307-1902-1192	230	6.9	157.9	Regurgitation
2	warm	H307-1902-1234	232	10.6	227.7	Regurgitation
2	warm	H307-1902-1276	233	8.3	N/A	Unknown[1]
2	warm	H307-1902-1248	239	9.5	202	Regurgitation
2	warm	H307-1902-1262	242	6	45.9	Defecation
2	warm	H307-1902-1206	243	5.5	159.2	Regurgitation
3	warm	H307-1902-1278	228	6.5	60.2	Regurgitation
3	warm	H307-1902-1264	230	8.2	38.9	Defecation
3	warm	H307-1902-1268	232	9.6	N/A	Retained[2]
3	warm	H307-1902-1250	233	6.3	609.9	Defecation
3	warm	H307-1902-1210	238	7.3	N/A	Retained[2]
3	warm	H307-1902-1222	239	6.9	67.3	Regurgitation
3	warm	H307-1902-1236	242	9.7	70.0	Regurgitation
3	warm	H307-1902-1208	243	5	62.5	Regurgitation
4	cool	H307-1902-1252	227	13.9	N/A	Retained[2]
4	cool	H307-1902-1266	230	17.2	56.2	Regurgitation
4	cool	H307-1902-1398	232	13.5	N/A	Retained[2]
4	cool	H307-1902-1224	233	16	68.2	Regurgitation
4	cool	H307-1902-1412	238	13.1	N/A	Retained[2]
4	cool	H307-1902-1384	239	8.3	268.7	Defecation
4	cool	H307-1902-1280	242	5.5	300.3	Regurgitation
4	cool	H307-1902-1238	243	27.6	N/A	Retained[2]
5	cool	H307-1902-1406	227	10.3	72.5	Regurgitation
5	cool	H307-1902-1392	228	21.5	209.5	Regurgitation
5	cool	H307-1902-1446	230	14.9	86.6	Regurgitation
5	cool	H307-1902-1416	232	5.9	N/A	Retained[2]
5	cool	H307-1902-1404	238	20.2	109.5	Defecation
5	cool	H307-1902-1434	239	10.5	N/A	Retained[2]
5	cool	H307-1902-1388	242	22.3	131.1	Regurgitation
5	cool	H307-1902-1460	243	12.3	N/A	Retained[2]
6	cool	H307-1902-1436	227	17.3	N/A	Retained[2]
6	cool	H307-1902-1396	228	18.8	85.3	Defecation
6	cool	H307-1902-1422	230	14.2	N/A	Retained[2]
6	cool	H307-1902-1394	232	19.8	91.2	Regurgitation
6	cool	H307-1902-1408	233	13.2	N/A	Retained[2]
6	cool	H307-1902-1450	239	25.5	161.0	Defecation
6	cool	H307-1902-1410	242	10.9	N/A	Retained[2]
6	cool	H307-1902-1424	243	14.9	N/A	Retained[2]

[1] Evacuation Type could not be classified due to blind spots in the video surveillance

[2] Tag not expelled for the duration of the remaining trials and subsequent post-trial monitoring period