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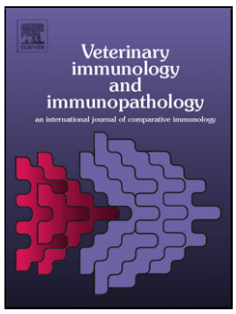

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Long-term implantation of acoustic transmitters induces chronic inflammatory cytokine expression in adult rainbow trout (*Oncorhynchus mykiss*).

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Highlights

- Presence of an acoustic transmitter did not influence surgical site healing rate
- Tags were encapsulated inside the peritoneum as early as 15 days post-surgery
- Spleen and surgical site cytokine expression was not influenced by tag presence
- Tag presence increased inflammatory cytokine expression in peritoneal immune cells
- At day 70 mature females had lower cytokine gene expression when compared to males

Abstract

Telemetry transmitters are frequently used in studies of wild fish migration and behavior. Although the effects of surgically implanted transmitters on survival, tag retention, healing and growth have been studied, there has been little research regarding the potential immune response induced by these transmitters. In the current study, mature rainbow trout received either surgical implantation of an acoustic transmitter or a sham surgical procedure. These fish were then sampled over a 10-week period so that pro-inflammatory cytokine expression in the spleen, peritoneal cavity lymphocytes and muscle at the surgical site could be analyzed. There were no significant differences in transcript expression for the spleen and muscle tissue between fish that had a transmitter and those that received the surgical procedure alone. However, transmitter presence significantly increased the expression of IL-6, IL-1β and TNFα in the peritoneal cells at 10 weeks indicating that tagged fish may be coping with chronic inflammation. Furthermore, tagged male fish had a higher inflammatory response in 10-week peritoneal lavage samples when compared to their tagged mature female counterparts, providing some evidence that mature female rainbow trout may have suppressed immune function when sexually mature. Externally, fish appeared to heal at similar rates regardless of the presence or absence of the transmitter, but the tag itself was prone to encapsulation and adhesion to the body wall and/or surgical site. This suggests that fish tagged with large intraperitoneal implants may not behave similarly to their wild counterparts. This research could aid in the development of improved telemetry tags that are more biocompatible, economical and better able to track fish behavior/movement.

Keywords
Rainbow trout, acoustic transmitter, tagging effect, cytokines, encapsulation, inflammation

1. Introduction

The development and successful use of telemetry tagging technologies in the 1950s (reviewed by Hockersmith and Beeman, 2012) has enabled researchers to track and examine large numbers of wild animals in their natural environments. Since then, biotelemetry has revolutionized our knowledge regarding the movements and migrations of numerous species (Bevan et al, 2002, Madisen et al, 2002, Fuller et al, 2003, Logan and Sanson, 2003, Jacobsen et al, 2017) with a heavy emphasis on wild fish populations. The migration and behavior of wild fishes is of particular interest as this information is essential in conservation efforts, improving species fitness, and predicting population responses to environmental changes, whether it be from natural or anthropogenic sources (Lucas and Baras, 2000, Cooke, 2008, Farrell et al, 2008). As this is the case, several different electronic tagging methods have been established for fish research including gastric insertion, external mounting, and intracoelomic implantation using either radio, satellite, archival or acoustic tags (Cooke et al, 2013, Thorstad et al, 2013). For extensive long-term studies, intracoelomic implantation of acoustic tags is the main tagging method used (Cooke et al, 2011) due to the affordability, ability to operate in both freshwater and saltwater systems (radio waves do not propagate in saltwater), and versatility (reviewed by Crossin et al, 2017). Furthermore, acoustic tags also have the capacity to record environmental, physiological, spatial, and behavioral data (reviewed by Crossin et al, 2017). Yet despite the valuable information that can be obtained from acoustic telemetry research, one of the earliest and most reasonable concerns has been the impact of tag implantation on fish welfare.

Studies using telemetry transmitters rely on the premise that the implanted tags do not influence fish behavior, survival, and/or physiology. Otherwise, any conclusions regarding the tagged individuals may not be an accurate representation of wild fish populations. This has led to numerous experiments aimed at determining the impact of telemetry tags on surgical healing, survival, physiology, behavior and growth on several fish species (Connors et al, 2002, Brown et al, 2006, Caputo et al, 2009, Collins et al, 2013, Rozynski et al, 2017). But because biotelemetry studies involve numerous variables that are difficult to regulate across experiments, many of the final conclusions of these studies have been inconsistent. Nevertheless, when it comes to transmitter effects in the long-term study of salmonid species, some reliable trends do emerge. Survival does not appear to be influenced by tag presence in salmonids, but sometimes the surgical procedure itself can result in an increase in the prevalence of infectious disease (Adams et al, 1998, Robertson et al, 2003, Brown et al, 2006, Collins et al, 2013).

Furthermore, when it comes to growth, telemetry tags are more likely to influence the development of juvenile salmonids with less of an impact observed in adults (Moore et al, 1990, Adams et al, 1998, Robertson et al, 2003, Brown et al, 2006, Collins et al, 2013). Finally, it appears that transmitter presence has little impact on the behavior and swimming performance of young salmonids unless the tag requires external antennae (Robertson et al, 2003, Anglea et al, 2004, Brown et al, 2006). The combined conclusions of these studies support the prevailing notion that electronic tags do not significantly alter the performance of tagged salmonids when returned to the wild.

While transmitters appear to be essentially benign in salmonids, their presence has been shown histologically to induce inflammation in fish (Marty and Summerfelt, 1986, Lucas, 1989). This immune reaction can be a confounding factor in biotelemetry studies, but it is important to recognize the critical role of this response for both the survival and homeostatic maintenance of vertebrates. Inflammation is necessary to combat tissue damage of any type whether it be from infection, trauma, foreign objects, or toxins. Though there are many important components involved in a successful inflammatory response (reviewed by Martin and Leibovich, 2005, Newton and Dixit, 2012), the initiation and perpetuation of inflammation is governed primarily by the pro-inflammatory cytokines produced by damaged cells and/or responding immune cells. The three classical pro-inflammatory cytokines are interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)α. Upon tissue damage, keratinocytes and fibroblasts release IL-1β inducing fever, T cell proliferation and increasing vascular permeability (Feghali and Wright, 1997). Meanwhile resident mast cells (MCs) degranulate in response to the mechanical trauma (reviewed by Watkins et al, 1995) releasing a variety of inflammatory mediators including, but not limited to, TNFα and IL-6 (reviewed by Theoharides et al, 2012, Wulff and Wilgus, 2013). The released cache of inflammatory mediators also aids in vascular permeability as well as the activation/recruitment of circulating immune cells which produce more IL-1β, IL-6 and TNFα. Leukocytes (primarily neutrophils) that are normally restricted to blood vessels then gain access to the site of tissue injury and attempt to eliminate any invading targets (reviewed by Medzhitov, 2008). If this acute inflammatory phase is successful, it is followed by a resolution and repair phase mediated mainly by tissue-resident and recruited macrophages that shift the response from pro-inflammatory to anti-inflammatory (reviewed by Serhan and Savill, 2005). However, if the acute phase cannot eliminate/resolve the impending threat, such as with an undegradable foreign device, chronic inflammation ensues. While fish immune systems are not as well characterized as mammalian equivalents, they possess all of the same pro-inflammatory cell types and cytokines. Because it would be both impossible and irresponsible to eradicate the

inflammatory response in wild fish populations, the development of telemetry tags that limit this reaction would be ideal.

Surprisingly, although surgical implantation of telemetry tags should induce an inflammatory response in fish, there has been little to no research regarding their influence on the teleostean immune system. With biotelemetry studies consistently reporting tag recovery rates as low as 13-41% (Crossin et al, 2008, Donaldson et al, 2009, Mathes et al, 2010) it is possible that inflammation results in increased tag expulsion and/or mortality in fish released to the wild. The goal of the present study was to explore the pro-inflammatory immune response in rainbow trout that received surgical implantation of an acoustic tag. To this end, the inflammatory cytokines IL-1β, IL-6 and TNFα were selected for this study as they play roles in both acute and chronic inflammation in mammalian models (reviewed by Feghali and Wright, 1997, Gabay, 2006) and appear to have comparable functions in fish (Buchmann et al, 2004, Iliev et al, 2007, reviewed by Zou and Secombes, 2016). External healing rate and encapsulation of the implanted tags were also observed throughout the 10-week trial. Obtaining a deeper understanding of teleostean inflammation in response to implanted telemetry tags could aid in the development of improved transmitters that are more biocompatible, economical and better able to track wild fish populations. Additionally, this study represents the first attempt to understand the immune response arising from telemetry transmitters despite their widespread use in biotelemetry studies.

2. Methods

2.1 Fish

Sixty mature rainbow trout (mean ± SD fork-length of 37.4 cm ± 3.4 cm) were maintained in 1700 L flow-through tanks at 13°C at the University of Waterloo, Canada. All fish were kept and handled under a permit from the University of Waterloo Animal Care Committee according to CCAC guidelines.

2.2 Tagging and surgical procedure

All tagging and surgical procedures were meant to mimic field conditions as described previously by Wagner et al, 2011 and Mulder et al, 2018. Specifically; fish were anaesthetized with benzocaine and measured to the nearest millimeter following loss of equilibrium. An incision just wider than the diameter of the tag was made on the mid-ventral surface, anterior to the pelvic girdle. A pre-sterilized dummy tag (Vemco V9T, 36 × 9 mm, 2.2 g in water, Vemco Ltd., Shad Bay, Canada) was then inserted and pushed posteriorly. The incision was closed with two nonabsorbable silk sutures (Sofsilk™ Tyco Healthcare , Norwalk, Connecticut, USA). The surgery took less than 2 minutes and fish were allowed to recover from anaesthesia prior to returning to the tank. Immediately following the surgical procedure,

tagged fish were transferred to an individual 1700 L flow-through tank, while sham surgery fish were moved to a separate 1700 L flow-through tank.

2.3 Sample collection

Prior to surgery, 6 fish were randomly selected and euthanized with an overdose of benzocaine so that spleen, peritoneal lavage and muscle tissue at the intended location of surgery could be collected as day 0 controls. Following the surgical procedure, identical samples were collected from 9 tagged fish, and 7 sham surgery fish on days 2, 15 and 70.

The peritoneal lavage samples were collected as described by Zekikoff et al (1991). Briefly, following the removal of blood from each fish through the caudal vein, an abdominal incision was made on the ventral surface so that the peritoneal cavity could be rinsed with 10 mL of cold Dulbecco's Phosphate Buffered Saline (DPBS, Gibco). The lavage fluid was collected and centrifuged at 400 x g for 15 min at 4^{o}C. After washing the cell pellet with 5 mL of cold DPBS, the supernatant was removed, and cell pellets were frozen at -80^{o}C. Upon dissection of spleen and muscle samples, tissues were diced into small pieces, placed into 5 mL Eppendorf tubes and immediately flash frozen in liquid nitrogen. These tissue samples were then transferred and stored at -80^{o}C until further use.

2.4 Primer Design and Sequence Validation

Primers were designed to determine transcript expression of rainbow trout pro-inflammatory cytokines IL-1β, IL-6 and TNFα as well as the housekeeping gene control, EF1α (see Table 1). Several housekeeping gene primers were developed but EF1α was selected for all samples studied as it was observed to have consistent Cp values across a subset of all samples for each tissue type (data not shown). All primer sets were designed so that at least one of the primers spanned an intron/exon junction.

For sequence validation of primer sets, the following PCR parameters were used: denaturation at 95^{o}C for 2 min, followed by 40 cycles of denaturation at 94^{o}C for 30 sec, 30 sec at 60^{o}C and extension at 72^{o}C for 30 sec. A final extension at 72^{o}C was carried out for 8 min. The amplified PCR products were separated on 2% agarose gels containing 2% GelGreen (Biotium Inc.). The resulting products were cloned into the pGEM®-T Easy vector, transformed into *Escherichia coli* XL1-blue cells and the resulting plasmids from 8 clones were extracted as previously described by Semple et al (2017). Plasmids were sequenced using SP6 primers at the TCAG sequencing facility (Sick Kids Hospital, Toronto, Ont.). The resulting sequences were then analyzed using the Basic Local Alignment Search Tool (BLAST) software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm sequence identity.

2.5 RNA extraction and cDNA synthesis

Depending on the sample, RNA was extracted from either 80-100 mg of tissue or from peritoneal lavage cell pellets using 1 mL of Trizol (Life Technologies) as described by the manufacturer. To remove any contaminating genomic DNA, RNA samples were treated with DNase I (Thermo Scientific). RNA samples were then quantified using the Take3 plate of a Synergy H1 plate reader (BioTek Instruments) and were stored at -80^{o}C until further use. Complementary DNA (cDNA) was synthesized from 500 ng of total RNA using the qScript cDNA Supermix (Quanta Biosciences) in accordance to the manufacturer's instructions. For a no template control, 500 ng of RNA suspended in 20 uL of DEPC water was included in the cDNA synthesis reaction without reverse transcriptase.

2.6 *qRT-PCR Reactions*

All qRT-PCR reactions were 10 µl and contained: 2.5 µl of cDNA (25 ng/µl diluted 1:10 in RNase free water), 2x LightCycler® 480 SYBR® Green I Master (Roche), and forward and reverse primers (Sigma Aldrich) to a final working concentration of 0.25 µM. The sequences for all primer sets are outlined in **Table 1**. All qPCR reactions were completed on the LightCycler® 480 II (Roche). Each experimental sample was run in triplicate. For each plate, triplicate wells of a calibrator, no template control and RNA only control were also present. The program used for all qRT-PCR reactions was as follows: pre-incubation at 95^{o}C for 10 min followed by 40 cycles of denaturation at 95^{o}C for 10 sec, annealing at 60^{o}C for 5 sec and extension at 72^{o}C for 8 sec. A melting curve was completed for every run from 65^{o}C to 97^{o}C with a read every 5 sec. Product specificity was determined through single PCR melting peaks. Data were analyzed using the ΔΔCt method while incorporating individual primer efficiencies into the equation as described by Pfaffl (2001) and is presented as the average of 6-9 fish with the standard error. Specifically, gene expression was normalized to the housekeeping gene (EF1α) and expressed as fold change over the day 0 control group. After confirming normal distribution and equal variance, a two-way ANOVA was individually completed for each cytokine analyzed using the statistical software Statistica (StatSoft, Tulsa, OK).

3. Results

3.1 Impact of acoustic transmitters on surgical healing

Whether fish received an acoustic tag or the sham surgical procedure alone, the healing rate at the surgical site did not appear to vary between conditions (**Figure 1**). At Day 2, there were no differences in external appearance between fish that received a tag and those that only underwent the sham surgery (**Figure 1A**). At Day 15, fish appeared to display some minor inflammation around the sutures for both surgical groups (**Figure 1B**). By Day 70, almost all fish had lost one or both of the sutures that were inserted during the surgical procedure and the wound was completely healed (**Figure 1C**). There were no

observable differences in feeding or movement behaviour between the two surgical groups. There was one mortality in the tagged group two days following the surgical procedure. No other mortalities occurred throughout the duration of the trial.

3.2 Encapsulation of acoustic tags

Following surgical implantation of the acoustic tags, after 15 days it was observed that most transmitters were completely encapsulated by a thin membrane and the surrounding adipose tissue of the gut (**Figure 2A**). This observation was also noted at Day 70 (**Figure 2B**) in the vast majority of individuals sampled. Furthermore, the encapsulation occurred regardless of whether the individual was sexually mature or not.

3.3 Inflammatory Cytokine Expression

At the site of surgery, there was very little inflammatory immune stimulation of IL-1β, TNFα and IL-6 at the transcript level (**Figure 3A**). There were no notable differences between timepoints or between individuals that received the tag when compared to those that only received the sham surgery. In the spleen samples, individuals that received a tag displayed higher variability at Day 2 in IL-6 and IL-1β gene expression when compared to their untagged counterparts (**Figure 3B**) but this variable induction was not observed to be significant. No other differences were observed at any other timepoints analyzed for the spleen samples.

When observing the inflammatory cytokine expression of the peritoneal lavage cell pellets, there were significant expression differences between tagged and untagged individuals at all time points analyzed (**Figure 3C**). IL-6 was observed to be significantly upregulated in tagged individuals only on days 2 and 70 while TNFα presented significant upregulation on Day 70 alone. IL-1β transcript levels were found to have significantly increased expression in tagged individuals for all time points studied.

An interesting observation was that on day 2, only the individuals that received the acoustic tag had a peritoneal cavity filled with fluid exudate. The fluid was removed from the peritoneal cavity prior to rinsing with 10 mL of PBS and was generally observed to be 4 mL or greater depending on the size of the fish.

3.4 Sex differences in chronic inflammatory response

Due to the variability observed in the peritoneal lavage cytokine expression at Day 70 (**Figure 3C**), transcript expression for each tissue was separated based on whether the individual was a male or a sexually mature female (**Figure 4**). Sexual maturity was assessed based on egg size and the developmental stage of the gonad following Wallace and Selman (1981) and Tyler et al (1990). When analyzing the muscle tissue at the surgical site, males were observed to have significantly higher

transcript expression in only TNFα and IL-1β when compared to mature females (**Figure 4A**) while in the spleen samples there were no significant differences between male and mature females (**Figure 4B**). In the peritoneal lavage cell pellet samples, significantly higher transcript expression in males was observed for all three of the cytokines analyzed (**Figure 4C**).

4. Discussion

4.1 Healing rate and tag encapsulation

Fish integument is a multifunctional barrier (reviewed by Glover et al, 2013 and Esteban, 2012) and as such, imperfect healing of this system following a surgical procedure could have repercussions regarding individual fitness in the wild. Presence of a foreign object within the body cavity, such as the tag itself, could influence the healing process, thus it is important to determine the impact that telemetry tags can have on surgical recovery. External healing rate at the surgical site following tag implantation has been extensively studied in a variety of fish species. These studies have revealed that there are several factors which influence healing rate such as fish species, age, environment, water temperature, tag size, and suture type (Collins et al, 2013, Lopes et al, 2016, Rozynski et al, 2017, Schoonyan et al, 2017). In the current study, the presence of the acoustic tag did not appear to influence external healing when compared to individuals who received the sham surgery alone. Although there have been many variations in experimental design and numerous variables tested (Cooke et al, 2011), it appears that tag presence does not influence surgical site healing rate in the majority of cases. With regards to adult rainbow trout, the results presented here provide further evidence that acoustic tags do not influence surgical healing rate and survival.

While external healing was not seen to be influenced by the presence/absence of transmitters, tag presence within the peritoneal cavity has been shown to induce an inflammatory response similar to the foreign body response (FBR) (Marty and Summerfelt, 1986, Lucas, 1989) and the encapsulation that is associated with medical device implantation in mammals (reviewed by Morais et al, 2010). In these mammalian models, the FBR involves an initial acute phase, lasting hours to days, followed by a subsequent chronic phase. The acute phase is characterized by the influx of neutrophils and fluid exudate to aid in matrix formation, wound cleaning and eradication of the threat (dos Santos et al, 2017). At two days post-surgery in the present study, this acute phase of inflammation was observed when excess fluid was seen in the peritoneal cavity of only tagged individuals. If the foreign object cannot be destroyed or phagocytosed, the chronic phase of inflammation follows marked by the persistent presence of lymphocytes and macrophages. These macrophages attract fibroblasts to the site

of the foreign entity and thus, enhance collagen building. The final result is the formation of a thin fibrous capsule around the foreign object in an attempt to isolate it from the local tissue environment (reviewed by Morais et al, 2010, Anderson and McNalley, 2011). Here, when the acoustic tag could not be cleared/destroyed during the acute phase of inflammation, subsequent encapsulation of the tag in a thin fibrous layer by the surrounding intestinal tissue was seen as early as 15 days post-surgery. This encapsulation has been consistently reported in multiple telemetry tag studies (Marty and Summerfelt, 1986, Lacroix et al, 2004, Welch et al, 2007, Lopes et al, 2016) and in a variety of fish species (Marty and Summerfelt, 1986, Lacroix et al, 2004, Welch et al, 2007, Lopes et al, 2016). Quite often, encapsulation precedes the ejection of the telemetry tag either directly through the body wall or via passage into the intestine and out the anus (Lucas, 1989, Moore et al, 1990, Baras and Westerloppe, 1999, Welch et al, 2007). Although only encapsulation was noted in this study, the walling off and/or ejection of foreign objects that the immune system cannot eradicate is a typical response across phyla (Anderson, 1971, Battistella et al, 1996, Lucke et al, 2014, Olsen et al, 2015). Likely with a longer trial duration, tag expulsion would have been evident in this study and is something that will be explored in future experiments.

4.2 Inflammatory cytokine expression in response to acoustic tags

Surgical implantation of acoustic tags involves the initial breach of a vital immune barrier, the integument, as well as maintenance of a foreign object within the body cavity of a fish for an extended period of time. As this procedure and the long-term presence of a foreign object would induce an inflammatory response, pro-inflammatory cytokine expression was analyzed at three relevant locations in tagged and untagged fish. At the surgical site and spleen, there were no significant differences in inflammatory transcript expression between tagged and untagged individuals at all time points analyzed in the current study. Although this has not been previously explored in salmonids, Zubaidi et al (2015) showed that inflammatory cytokine levels in mice generally remain constant in skin and muscle tissue at surgical sites. Initially, mice recruit inflammatory macrophages to the site of muscle injury, but these macrophages switch to an anti-inflammatory phenotype once they are within the injured tissue. It is believed that this phenotype-switch may be required to promote muscle healing at the site of injury (Arnold et al, 2007). A similar trend is observed in the spleen tissue of mice following surgery where the pro-inflammatory cytokine IL-6 is released into the serum initially but by 48 hours post-surgery anti-inflammatory responses are induced so as to avoid tissue destruction (Andres-Hernando et al, 2017). Perhaps there are similar processes in rainbow trout which may explain the limited inflammatory

response at these tissues observed during the study period. Further research and the development of rainbow trout cytokine specific antibodies would be required to confidently demonstrate this.

In any foreign body response, it is the foreign object that is responsible for the chronic inflammation observed in the surrounding tissue. As acoustic tags are surgically implanted within the peritoneal cavity, lavages were conducted at this site to collect the surrounding cells in both tagged and untagged individuals. At all timepoints analyzed, tagged individuals showed a significant increase in inflammatory cytokine expression with a reduction observed at day 15. This likely represented the acute (day 2) and chronic phases of inflammation (days 15 and 70). In mammals, quite often the FBR is studied using subcutaneous biomaterials but there have been some studies utilizing the peritoneum (Mooney et al, 2010, Peters et al, 2011, Mooney et al, 2014, Christo et al, 2016). After surgically implanting sterile cubes into the peritoneal cavity of mice, Mooney et al (2010) observed a typical FBR reaction with encapsulation occurring at 2 days post-surgery, a phenomenon that is generally observed later when tagging fish. As teleosts have been reported to have delayed immune responses when compared to mammalian counterparts, later encapsulation was to be expected. Additionally, when foreign objects are implanted into the peritoneum of mice and rats, an enhanced inflammatory response is seen in the peritoneal cells of only those individuals that received the foreign object (Mooney et al, 2010, Peters et al, 2011). In mice, acute inflammation is observed within hours of foreign device implantation while chronic inflammation is observed after 7 days (Christo et al, 2016). Though the time points may differ between species, it appears that tag implantation and the observed inflammation that it induces within the peritoneal cavity is a teleostean version of the FBR.

In medical studies using mammalian models, the FBR occurs in response to implants created from several different materials including those that are hydrophilic, hydrophobic, hard, soft, polymeric, metallic and ceramic materials (reviewed by Ward, 2008 and O'Brien, 2011). Though this list is extensive, it is possible that telemetry transmitters could be made from a material that reduces the FBR and the associated inflammation. In particular, finding a material that diminishes the adsorption of proteins would avoid recognition by macrophages and thus, would prevent/inhibit encapsulation (reviewed by Ward, 2008). Previous studies have shown that biomaterials created from the zwitterionic material, carboxybetaine, adsorb less than 0.3 ng/cm^2 of protein from 100% blood plasma or serum (Ladd et al, 2008). When hydrogels were created from this material and then surgically implanted into mice, both the foreign body and inflammatory responses were significantly reduced for the entirety of a three month trial (Zhang et al, 2013). The external casings of the Vemco acoustic tags used in this study consisted of epoxy, from which notoriously hydrophilic polymers are synthesized. As described above,

hydrophilic compounds have been shown to adsorb protein heavily (Kastantin et al, 2011, Swartzlander et al, 2014) which may provide an explanation regarding the significant inflammation observed as a result of tag presence. With this consideration, perhaps improvements to the external material of acoustic tags would limit the inflammatory response that was observed in the current study. Because not all acoustic tag compositions are the same, a promising future study would be to compare the inflammatory response raised against tags developed by different companies. Though further research is required, ensuring that telemetry tags are smaller and created from low protein adsorbing material may prevent chronic inflammation and eventual tag ejection, thus improving tag recovery rates and data collection.

4.3 The impact of sexual maturity on inflammatory gene expression

In teleosts, immune suppression during sexual maturation is a well-studied phenomenon that has been supported by several different experiments (Nakanishi, 1986, Hou et al, 1999, MacKenzie et al, 2006). Furthermore, the hormones associated with sexual maturation have been shown to drastically impact immune function in the majority of fish species (reviewed by Yada and Nakanishi, 2002). The female sex hormone, 17β-estradiol (E2), plays an essential role in the egg production of all oviparous vertebrates, including fish (Anderson et al, 1996). When fish are exposed to varying concentrations of this hormone, E2 has been consistently shown to suppress the inflammatory response and increase susceptibility to pathogen infection (Wang and Belosevic, 1994, Seemann et al, 2013, Wenger et al, 2014). In the present study, tagged sexually mature females were observed to have suppressed inflammatory cytokine expression when compared to their tagged male counterparts. At appropriate biological levels, this induced suppression can actually be protective, as a damaging inflammatory response could result in the destruction of valuable eggs and a potentially significant reduction in reproductive fitness. Additionally, because mature eggs are in close proximity to the organs of the peritoneal cavity, they could be jeopardized in the crossfire when mounting an immune reaction against infectious agents and/or a foreign object such as a telemetry tag. Thus, the suppressed immune response observed here in mature females was likely a result of “immune privilege” (MacKenzie et al, 2006) to protect the germ line.

5. Conclusions

Biotelemetry studies reveal important information about the lifestyle, distribution and breeding patterns of wild fish populations. Though their contributions to our understanding of fish ecology and behaviour have been significant, biotelemetry does not come without risks to the animal being tagged. The results of the current study show that the implantation of acoustic transmitters, though not

influencing external healing rate, do induce chronic inflammatory cytokine expression in peritoneal lavage cell pellets. At 15 and 70 days post-surgery, tags were consistently observed to be encapsulated providing more evidence for the chronic phase of inflammation that is associated with the FBR. Furthermore, sexual maturity appeared to play a role in inflammatory status indicating that special care should be taken to avoid tagging mature females. This study represents the first time immune function was compared between tagged and untagged individuals and has revealed that acoustic tag implantation stimulates an immune response in tagged fish. As a result, future studies should focus on developing smaller telemetry transmitters from a material that limits the FBR. These technological improvements would ensure more accurate assessments of fish populations, greater tag return rates and thus, larger sample sizes per experiment.

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Table 1: Primer sequences used for qRT-PCR analysis of pro-inflammatory cytokines in rainbow trout.

Gene	Primers (5' to 3')	Amplicon Length (bp)	Accession Number
IL-1β	**F:** CCACAAAGTGCATTTGAAC **R:** GCAACCTCCTCTAGGTGC	155	AJ298294.1
IL-6	**F:** CTTCTACACGCTATCTCTCACTC **R:** CGTCTGTCCCGAGCT	128	NM_001124657.1
TNFα	**F:** GTGCAAAAGATACCCACC **R:** CACTGCACGGTGTCAG	108	AJ278085.1
EF1α	**F:** CGCACAGTAACACCGAAACTAATTAAGC **R:** GCCTCCGCACTTGTAGATCAGATG	134	NM_001124339

Figure Legends:

Figure 1: Representative pictures depicting the rate of healing at the surgical site. Pictures were taken of each fish at the surgical site on Day 2 (A), Day 15 (B) and Day 70 (C) so that the rate of healing could be compared between fish in both the presence and absence of a telemetry tag.

Figure 2: Representative pictures presenting tag location and encapsulation. Tag encapsulation was first observed 15 days post-surgery (A) in all fish sampled. At 70 days post-surgery (B) encapsulation was also consistently observed regardless of whether the fish was sexually mature or not.

Figure 3: Pro-inflammatory cytokine gene expression in muscle tissue at the site of surgery (A), spleen tissue (B) and peritoneal lavage cell pellets (C) of tagged and sham surgery fish. All timepoints represent the fold change of at least 6 individuals. Significant differences between tagged and untagged individuals of the respective cytokine are indicated ($p<0.05$). Vertical error bars represent the standard error.

Figure 4: Sex-based differences in pro-inflammatory cytokine transcript levels at 70 days post-surgery. Cytokine expression was separated based on the sex of the fish in the muscle tissue at the surgical site (A), spleen (B) and in the peritoneal lavage cell pellets (C). Significant differences between males (n=3) and sexually mature females (n=4) are indicated by * ($p<0.05$) and ** ($p<0.01$). Vertical error bars represent the standard error.

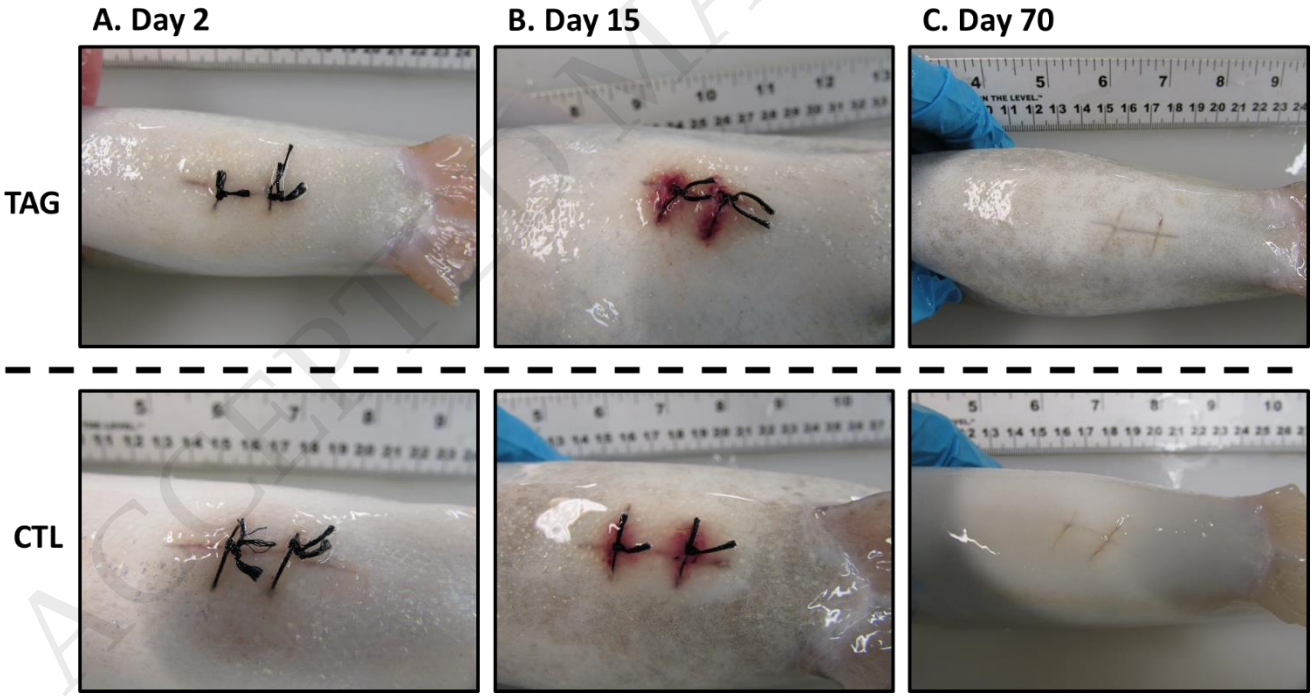


Figure 1

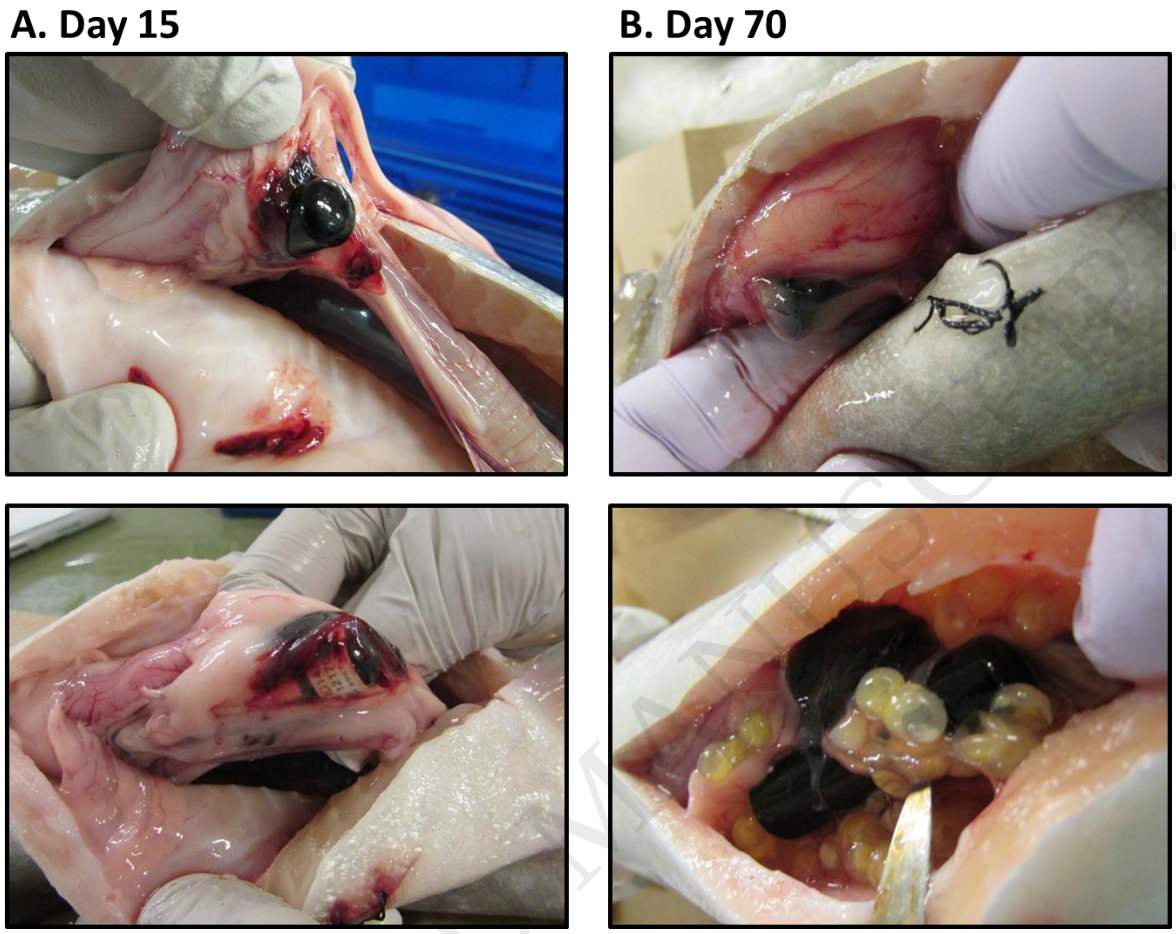
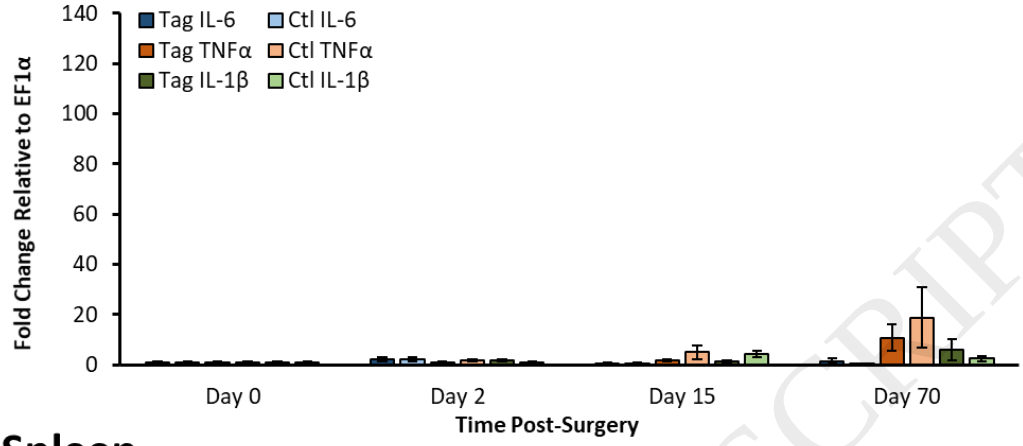
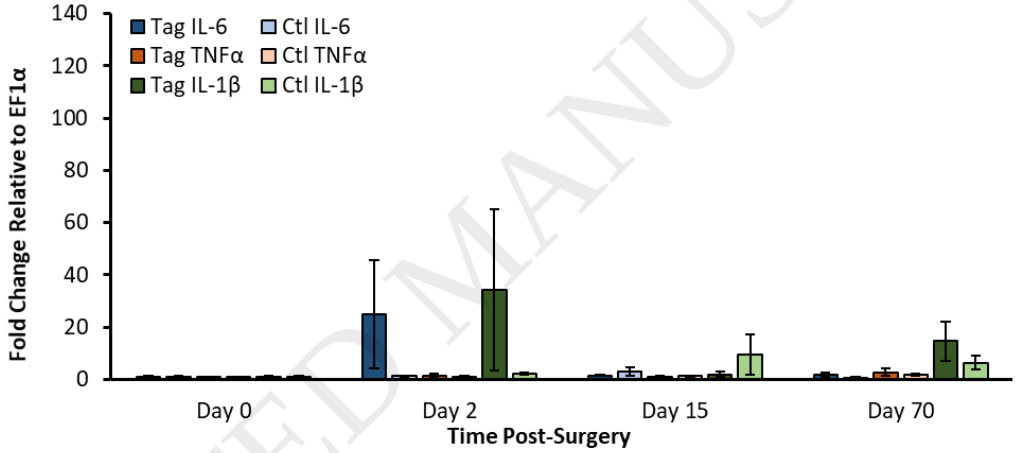


Figure 2

A. Surgical Site



B. Spleen



C. Peritoneal Lavage

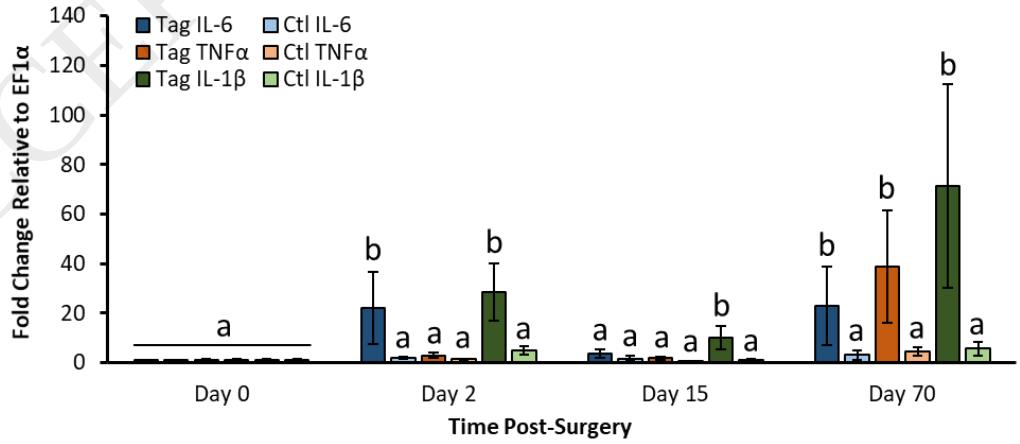


Figure 3

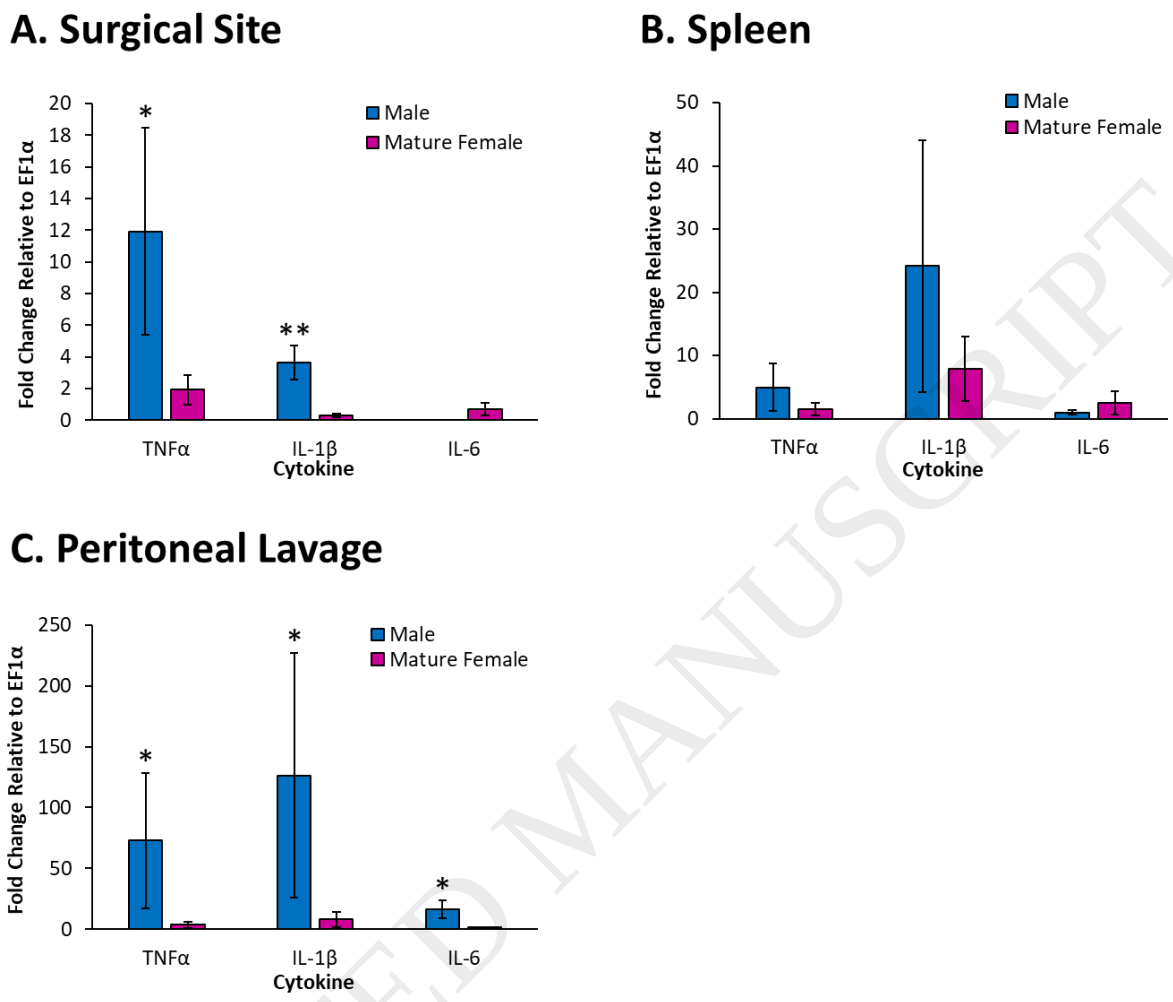


Figure 4