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AQUAculture infrastructures for EXCELlence in European fish research 3.0

Deliverable 4.1
Review of biotelemetry in aquaculture research

Version 1.2

WP 4
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Contents

1. Objective

The objective of deliverable 4.1 was to review the use of biotelemetry and biosensors in aquaculture research based on information gathered from the task participants and existing knowledge from literature. Based on this information, a set of recommendations on when biotelemetry may be a useful tool in aquaculture research, and how it should be used considering experimental settings, such as water type, unit size/type, animal species, and number of animals.

2. Background

Biotelemetry and biosensors are tools that can be used to track individual animals, and that are applied by equipping animals with devices convivially referred to as tags. Tags range from devices which only function by enabling humans to visually distinguish tagged individuals (e.g., external markers, or using a specific reader for PIT-tags), to devices that measure some property in or near the animal using sensors and either internally store or transmit the results to the user. Tagging methods have been used in fish research for decades originally most often with the aim of generating knowledge relevant for fisheries, stock assessments and preservation/environmental protection issues. However, the use of such methods towards aquaculture has been increasing rapidly in recent years, resulting in the emergence of new and innovative solutions for individual fish monitoring on the market. This has in turn led to increased interest for using such methods within the AQUAEXCEL 3.0 project, resulting in the creation of task 4.1.2 which aims to obtain an overview and guidelines in the use of such methods.

This work considers only active sensor tags, i.e., units that either store raw/processed sensor data internally (Data Storage Tags) or that transmit such data using radio or acoustic signals (transmitter tags) as these can generate data series describing the individual histories of the fish carrying tags. This ability makes biotelemetry and biosensors likely components in the intelligent fish monitoring systems of the future that will aspire to achieve the goals of Precision Fish Farming (PFF, Føre et al., 2018).

3. Methodology

3.1. Questionnaire among the task participants

The first step in task 4.2.1 was to formulate an online questionnaire designed to collect inputs on past (and present) experiences in using biosensors and telemetry in aquaculture research. To ensure that the questionnaire would provide useful inputs for the further work in the task on acquiring a best practice for using such tools, the task management group (i.e., NTNU, HCMR and JU) agreed upon a set of questions that covered various aspects of such experiments. The queries were grouped in five categories that mirror different phases/stages of biosensor/telemetry experiments: "Part 1: Experimental aims", "Part 2: Experimental/physical setup parameters", "Part 3: Experimental design parameters", "Part 4: Data processing methods" and "Part 5: Experimental outcomes".

There were 10 contributions in total submitted to the portal, and all submissions were anonymous. In hindsight, it would have been reasonable to also include the institution name of the submitters, but this is only a minor issue since the methods and approaches are the important elements here.

We will in the following present the outcomes of the questionnaire by iterating through the different phases/stages and commenting on the outcomes. Appendix A contains the full responses of all contributors.

3.2. Literature studies

The outcomes from the questionnaire were complemented by reviewing selected review articles and other relevant scientific publications, with the intent of identifying additional perspectives on the use of telemetry as a tool in aquaculture research. This was done by inspecting recent review works done on biotelemetry/biosensors for inputs within the five main categories in the questionnaire. Similarities and dissimilarities between the questionnaire and literature outcomes are discussed in the following section. The main article investigated is Macaulay et al. (2021) which is a recent study that explored 83 separate telemetry/biosensor studies in aquaculture and based on this reviewed the benefits, problems and solutions of such methods as a research tool for aquaculture. The authors had a particular focus on mortality and sub-lethal impacts of the tagging procedure. Other recent review-type articles such as Fahlman et al. (2021) and Brijs et al. (2021) were explored for inputs on aspects less thoroughly covered by Macaulay et al. (2021), particularly methods for visualizing and processing data.

4. Results and Discussion

4.1. Outcomes of the questionnaire

4.1.1. Part 1: Experimental aims

Q1: Experiment name

All contributors provided a unique name for their study, such that we could distinguish the different experiments in the analyses.

Q2: Main hypotheses/research questions

All contributors responded with their respective hypotheses/research questions, with four studies being aimed at testing and validating the AEFishBIT as a tool for monitoring fish in aquaculture and six studies using various tag types (two of these used the AEFishBIT) to study the biological/ecological responses of fish. The high percentage of studies testing of the AEFishBIT was not unexpected considering that several of the task partners are involved in the testing of this device which was developed during the preceding AQUAEXCEL2020 project. Testing was conducted both to verify its function for new species (i.e., Atlantic salmon), to assess the functionality of the tag for different species and to evaluate potential adverse impacts of the tagging procedure.

The biological/ecological studies had various aims, including monitoring of welfare, locomotion, energetics, growth, metabolism, stress and feeding in various species. All these studies used activity as an indicator, together with heart rate, respiration or depth.

Q3: Parameters targeted with biotelemetry/biosensors (e.g., activity, depth, heart rate, ...)

There were six studies using the AEFishBit which all focused on activity and respiration frequency as the main parameters, while the other studies focused on either swimming activity and heart rate (two studies) or swimming activity and depth (two studies).

Q4: If relevant: statistical analyses used before trial (e.g., statistical power, ...)

None of the studies reported using statistical analyses before doing the trial. This might be because the active use of these tools in aquaculture production units is still a relatively new concept, rendering the data foundation on which to make statistical observations rather sparse. An alternative explanation is that the query was not posed in the proper manner

Q5: Negative effects of tagging expected before the trial (e.g., mortality vs untagged fish, reduced health/feeding,...)

Nine contributors provided inputs on eventual negative effects. Five of these reported that no effects were expected at all, but two of them also informed that this was evaluated in hindsight by biochemistry and growth assessment. One study reported that no effects were expected after improving the method. There was just one study that reported an expected effect of tagging in the form of hyper/hypo activity. Two other inputs on this point described the observed negative effects, and were thus included in the evaluations in the final section (i.e., “Experimental outcomes”) instead.

Q6: Abstract describing experiment (if existing)

Nine of the studies provided abstracts which describe the experiments more elaborately. These can be read at full length in appendix A.

4.1.2. Part 2: Experimental/physical setup parameters

Q7: Experimental unit type

Contributors could here choose from a list of four options: tank, cage, raceway or aquarium. Out of the nine that responded to this query, most (77.8%) reported using tanks while the rest used cages:

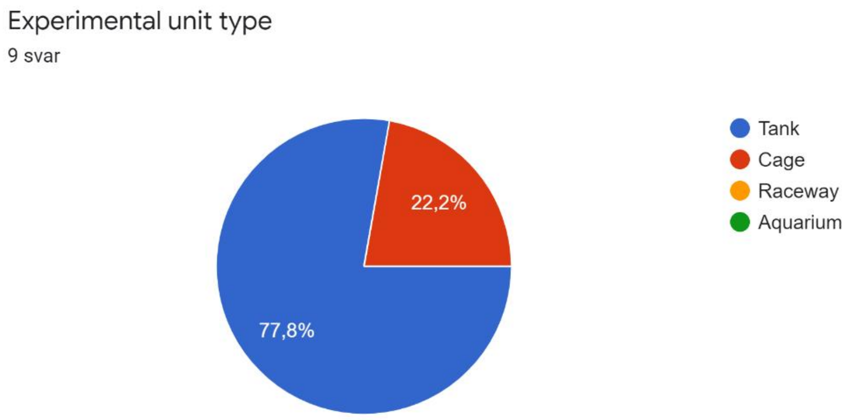

Figure 1: Pie chart describing the distribution of the reported studies between different experimental unit types

Q8: Unit dimension (e.g., diameter, depth, length, volume,...)

All responders reported the unit dimensions used in their experiments. As implied by the response to the preceding question, most of the studies were conducted in tanks, either circular or square shaped. The tank volumes reported were 90, 500, 3000, 4000 and 5600 L, with around 3000 L being the most frequent as it was featured in five of the studies. Two studies were conducted in sea-cages and thus featured larger unit volumes. One of these studies reported using square shaped cages that measured 5 x 5 x 5 m and 12 x 12 x 10 m, while the other cage based study used full-scale cages with 50 m diameter and about 30 m depth.

Q9: Indoors or outdoors

All responders reported whether the study was conducted inside or outside, resulting in a 50/50 split between the two options:

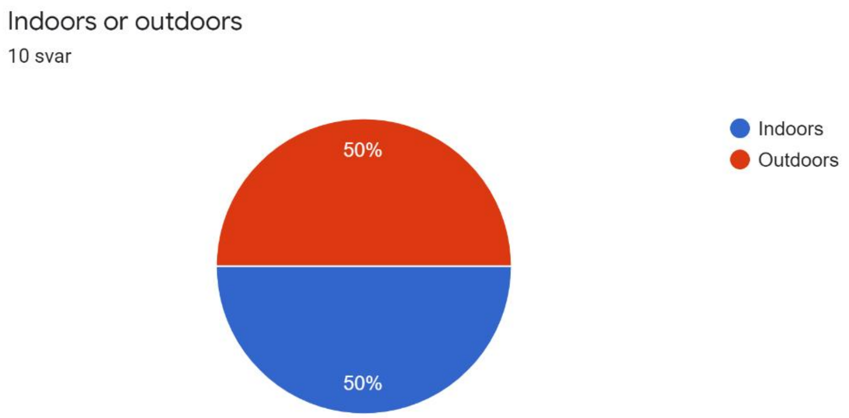

Figure 2: Pie chart showing the distribution of whether the reported studies were conducted indoors or outdoors.

Q10: Species (e.g., Atlantic salmon, Seabass, sea bream,...)

In total, the studies reported only three different species: Atlantic salmon, sea bream and seabass. Of these, five studies used salmon, four used sea bream, while three used seabass, with some studies featuring two species:

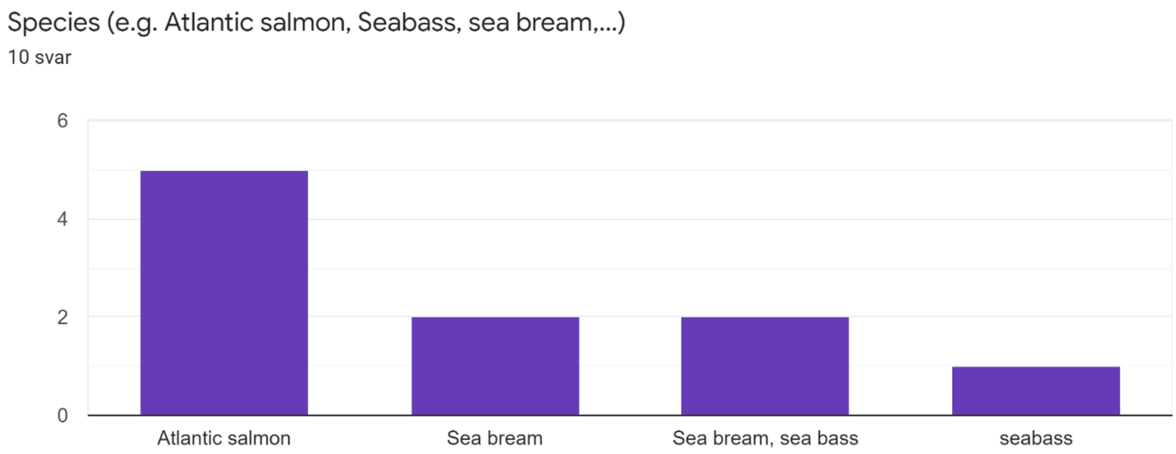

Figure 3: Bar chart showing the number of studies per fish species reported for the 10 studies

Q11: Population size parameters (e.g., number of fish, biomass in kg, individual fish size,..)

All responders reported parameters describing the population size. Reported individual fish sizes were 300-500g, 30-150 g, 292.5±20.5 g, 161.0±14.9 g, 917.0±37.2 g, 645.1±49.3 g, 200-300 g, 100-120 g, 70-130 g, 237-514 g, 760 g, 3 kg, 2100 g and 3077+/- 477 g. This implies that the fish sizes used in the studies were mostly below 1 kg, with only three studies using fish exceeding 1 kg in individual weight. This is again not unexpected since most of the studies were conducted in tanks, where it is often more feasible to do experiments using small fish.

Fish density was also reported for most of the studies and covered a range of different densities: 11.6 kg m-3, 6-8 kg m-3, 8-10 kg m-3, 14 kg m-3, 9-12 kg m-3, 11-12 kg m-3 and 12 kg m-3. The studies that did not report fish density reported numbers/gross biomass instead, from which it is possible to derive fish densities, with e.g., 392 tonnes per cage in the full scale experiment, and 8000 fish in the small scale cage experiment. Details on the number of fish per different tanks/treatments/phases can be read in the specific responses given in Appendix A.

Q12: Water type

All contributors responded on the water type applied. 90% reported using seawater, while one study reported that freshwater was used.

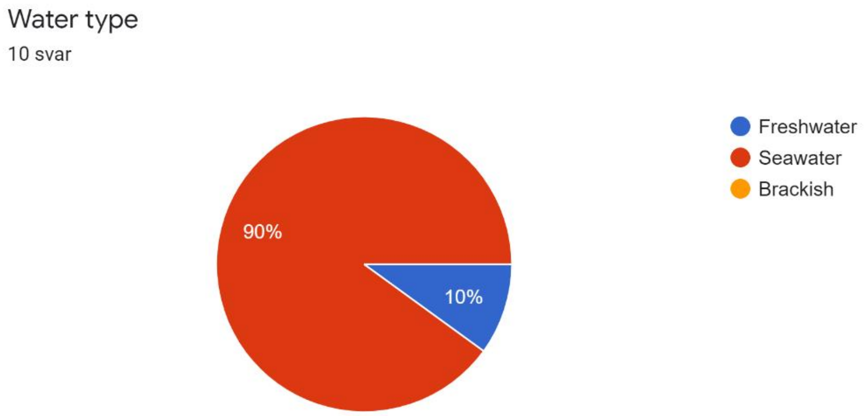

Figure 4: Pie chart showing the distribution of water types across the reported experiments.

Q13: Geographic/temporal conditions (e.g., Latitude, season, time of the year, time of the day,...)

Nine responders had replies to the query on geographic/temporal location. The experiments were spread across a large proportion of Europe, spanning from 40° 5'N; 0° 10'E in Castellon, Spain, to Tromsø, Norway at 69.5° N, 18.56° E. Most of the contributors also provided the time of year for their experiments, which in sum covered most of the year, showing that the collected descriptions did cover experiments during all seasons except spring/early summer: March, July, February, October- February, January-March and October-December.

Q14: Experimental settings (e.g., water temperature, light conditions, ...)

All responders provided information on the experimental settings. Most (seven) reported using natural light conditions, while two studies reported using artificial light, as a treatment parameter between tanks or to provide continuous illumination. Water temperatures reported ranged from 4 to 12° across studies, with some using the natural water temperatures from the ambient environment/influx water. One study also reported that oxygen saturation was kept at 97.7%, while another reported that the fish were exposed to varied water quality to simulate good vs. poor RAS conditions.

Q15: Duration of the experiment (e.g. to-from date, total number of days,...)

There was much variation in the duration of the reported experiments. Two studies reported using short time scales (2 hr) for tunnel experiments and then 2 day trials for free swimming. One of these also had a 1 week period afterwards to review the impact of attachment. The rest of the studies spanned several days: two days (four studies), four days (three studies), 25 days, 62 days, 73 days and 141 days. Similarly to the geographic spread of the experiments, this illustrates the variation in the types of studies reported.

4.1.3. Part 3: Experimental design parameters

Q16: Tag types used (i.e., manufacturer, type name, data types, data storage vs. transmitter tags, ...)

All responders reported the tag types used, and these included both transmitter tags and data storage tags. Six studies reported using the data storage tag AEFishBit, three studies used transmitter tags from Thelma Biotel, two studies used data storage tags from Star Oddi, and one study reported using VEMCO tags.

Q17: Auxiliary equipment used to collect data (e.g., acoustic receivers, DST-reader units,...)

All responders reported using auxiliary equipment. Five studies reporting using a custom made device for communicating with and charging the AEFishBit, two studies reported using logging equipment from Thelma Biotel (TBR-700), two reported using Vemco devices (VR2W and VR100), while two reported using the communication box from Star Oddi.

Q18: Sampling regime (e.g., sampling interval per individual, value range for measured parameters, measurement period, duty cycling,...)

All studies reported on the applied sampling regime focusing either on raw sensor sampling rates or data point rates. Reported sensor sampling rates ranged from 1 Hz for depth sensors (sampling period of 130 s or more), through 5-20 Hz for accelerometers in transmitter tags (sampling period of 30 s) to ECG-sampling at 80-100 Hz (7.5-15 s sampling period). Data point rates were naturally generally lower than the sensor sampling rates as each data point was typically based on a series of raw sensor samples. Respiratory frequencies and physical activity values computed by the AEFishBit were typically generated and stored over 2 min periods with 15-30 min inter-measurement intervals depending on the length of the study period (i.e., a duty cycle of 2/15 or 2/30). Activity and depth transmitter tags were reported to transmit activity proxies at between 220-380 s or 4 min intervals, while heart rate DSTs were said to register heart rate values at either 10- or 2-min intervals. Some also reported details on how the stored/transmitted proxies were computed from raw sensor data, see appendix A for details.

Q19: Percentage of population tagged

The percentage fish tagged was reported for four studies: 0.016%, 2.25%, 20% and 24.5%. The lowest percentages were as expected in the cage studies where the population was substantially larger than for the tank studies. One study also reported the number of fish tagged in different treatments, but not as a percentage.

Q20: Replicates (e.g., replicate tanks/cages, replicates on individual level,...)

Nine studies reported the status on replicates. While some of these were on production unit level (i.e., 1 RAS tank, four replicate tanks), most reported replicates on the individual level: 6 sea bream and 6 sea bass, 5-7 animals per size class, 10 sea bream and 10 sea bass, 8 sea bream and 8 sea bass, 6-8 individuals per family, 16 fish per experimental condition, 21 individuals, 3 tagged vs 4 untagged fish per unit and 9 individual replicates. One study reported no replicates were used.

Q21: Control groups (e.g., untagged individuals in same unit, units with no tagged fish,...)

Nine studies were reported to have control groups. Two of these reported using extra tanks containing untagged individuals, while five studies reported using untagged individuals in the same tank as control. One study also included a control group that were only tagged with the same external indicators as the fish carrying electronic tags, while another reported using uninfected individuals carrying tags as a control (as the experiment was focused on disease progression and not tagged vs. untagged fish).

Q22: Independent observation methods for comparing tagged and untagged individuals (e.g., cameras, visual inspection, sonars,...)

Six studies reported using independent observation methods for comparison with tag outputs. All reported using cameras/video or direct visual inspection.

Q23: Metadata collected during trial (e.g., temperature, oxygen, video,...)

Five studies reported on the metadata collected during the studies. Four measured temperature, three measured oxygen, three reported using video, one study measured light and one study measured turbidity and suspended matter.

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4.1.4. Part 4: Data processing methods

Q24: Statistical methods applied in the analyses (e.g., methods such as ANOVA, statistical power achieved,...)

All responders described the statistical approaches used to analyse the data. While one study reported no statistics used since it was a proof-of-concept study, and one reported that the analyses are not complete yet, the others provided detailed descriptions on how statistics were used. One-way ANOVA was most common, being mentioned for four studies, while Student-t tests were employed in three studies. Three studies used Pearson correlation coefficients, three used a simple cosinor model (to analyse rhythmicity) while two reported using Holm-Sidak post-hoc test (in combination with ANOVA). The remaining methods reported were only featured in one study each and included multivariate partial least-squares discriminant analysis (PLS-DA), non-parametric Mann-Whithney U test, PERMANOVA PRIMER V 7, binary recursive partitioning approach (Vignon, 2015) and Seasonal Decomposition of time series, asymptotic time regression function. Most studies used a combination of at least two statistical approaches.

Q25: Visualisation methods and plotting applied to the data

Visualisation was reported by nine contributors, and was plotted as both time series plots and more aggregated formats such as box plots (see appendix A for more details).

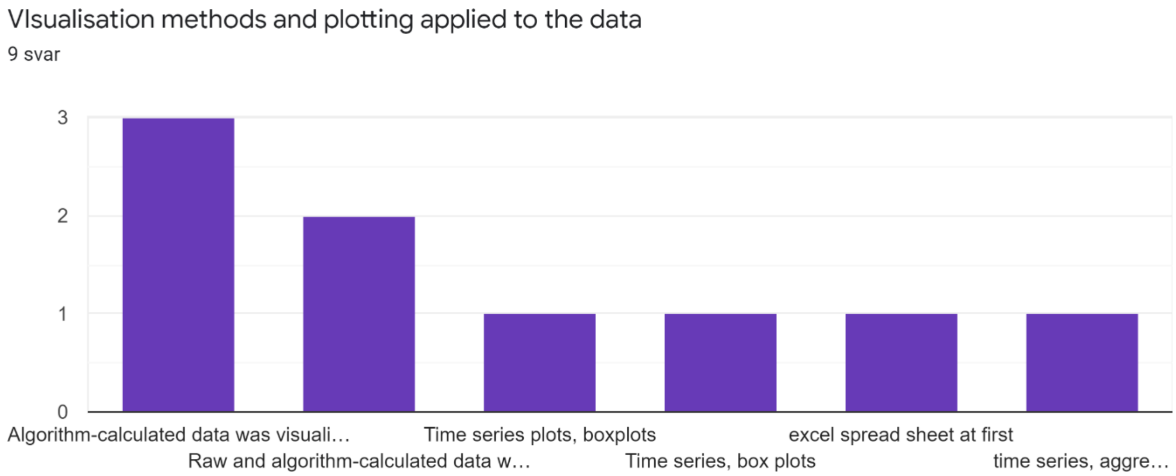

Figure 5: Bar chart of the different visualisation methods applied

4.1.5. Part 5: Experimental outcomes

Q26: Was the hypothesis confirmed?

Apart from a single study where the data processing is not done yet, all reported that the main hypothesis founding the study was confirmed. Some reported that there was some need for adjustments for the outcome to fit with the hypothesis:

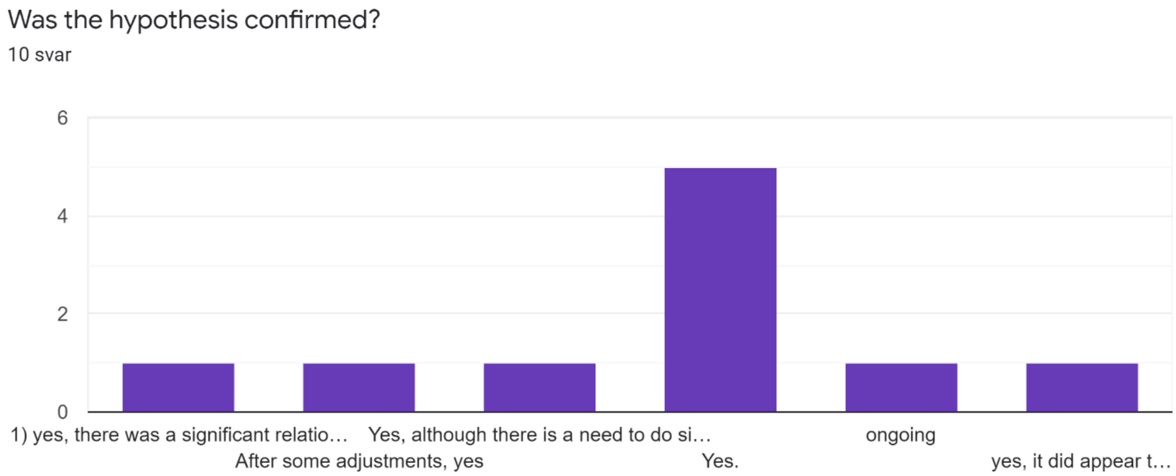

Figure 6: Bar chart showing to what extent the experiments confirmed the main hypothesis of the study

Q27: Did you achieve the desired data coverage? (e.g., did data collection match expectations and aims?)

All studies reported that they did achieve the desired data coverage, although one response was a little more ambiguous ("more or less"). Some of the reports on this point also described how tag data was compared with other information sources such as video recordings and respirometer data, while others outlined the implications of their data, e.g., in validating the function of algorithms for in-tag processing and detecting differences between treatments. See appendix A for more details.

Q28: Unexpected insights obtained (e.g., features in data not expected during experimental design)

All responders replied to this query, six reporting no unexpected insights during the trials. Those that did obtain unexpected insights reported experiences that were quite varied in nature: sampling rate was very variable in acoustic tags in RAS (but not to the detriment of the experiment), the tagging protocol had to be modified to accommodate a new species (salmon), all tagged fish showed very similar activity during crowding, heart rate variations were larger than expected and the collected dataset also described heart rate and activity for a mature male salmon fighting with another male salmon.

Q29: Observed tag effects (e.g., wound healing, inflammations, complexity of procedure, impacts on behaviour/physiology...)

All contributors responded to the query on observed tag effects. While three studies reported no apparent tagging effects (over a window up to 3 weeks), some studies reported development of wounds at longer term, which is to be expected for external tagging. One study used daily visual inspections of behaviour and fish exterior during the experiments to document eventual impacts of tagging but could not see any apparent adverse effects. This observation was strengthened by the condition factor and final body weight of both tagged an untagged fish being almost equal.

Another study sought to investigate eventual physiological effects of tagging by measuring selected parameters in the circulating water in the tanks. While they found no difference between tagged and untagged fish in cortisol, glucose, or lactate levels, they observed lower triglyceride levels in tagged vs. untagged individuals for small fish (<300 g) both in seabass and sea bream. Two studies using heart rate tags reported that these tags are very difficult to implant in seabass and needs a lot of practice and that there was some inflammation in the surgical wounds for salmon carrying these tags. One long term cage study reported that some fish expelled the tags through their abdominal cavity wall, and that there was some inflammation/embedding of tags into the flesh were observed, but that the mortality of tagged vs. untagged fish was similar.

Q30: Tag function (e.g., battery life vs. experiment duration, errors in data collection/download, malfunction or success, ...)

Eight contributors responded to this query, and none reported any substantial challenges with the tags applied in the studies. One study mentioned that the design of the AEFishBit was recently improved to counter some previously encountered challenges related to malfunctioning prototypes.

Q31: Datasets obtained (i.e., short description of these, preferably with a link for downloading if possible)

All contributors responded to the query on the data outcomes from their experiments. Three reported collecting only algorithm-calculated data (i.e., data resulting from processing of raw data internally in the device), while two studies reported using both algorithm-calculated and raw data.

All five of these studies used the AEFishBit. Another study using the AEFishBit also reported that both raw and processed data were collected and provided some details on both of these. Raw triaxial acceleration data was collected at 50 Hz, and then processed into two derived data types. The first of these was the respiration frequency which was found by first integrating the z-axis acceleration measured by the tag (the tag was mounted such that the z-axis of the accelerometer was normal to the gill cover) and then bandpass filtering the result (which corresponds to the operculum velocity) keeping only the spectrum 0.5-8 Hz. The number of zero crossings in this signal was then found for a fixed period, and then divided by 2 to find the number of oscillations for that period. This operation was repeated several times over a 2 min window and the averaged of all repetitions was then used to find the respiration frequency (for that 2 min period). The second data type was a proxy for physical activity (i.e., due to swimming and other bodily activities) and was found by estimating the average jerk (i.e., the derivative of acceleration) over 2 min periods. Jerk was estimated by (numerically) differentiating the averaged x- and y-accelerations. See appendix A for more details.

There were two unrelated studies that reported collecting both activity and heart rate data from acoustic tags and data storage tags, one of which had not yet processed the heart rate data (in progress). Finally, two studies reported that activity and depth data for the entire experimental periods was available, although one of these implied the data was distributed between several different file formats and rather old (from 2009) and hence not very easily accessible.

Only three studies have thus far provided links for downloading data to the benefit of task 4.2.2.

4.2. Corresponding observations from literature

4.2.1. Experimental aims

Macaulay et al. (2021) found that the parameters most commonly measured in the reviewed studies were movement and swimming behavior. This agrees with the outcomes of the questionnaire where most studies reported fish activity as the only or one of several measured parameters. Moreover, Macaulay et al. (2021) found assessment of movement and behavior in production units, responses to increased stocking density and responses to stress to be the most common study aims, while other topics (e.g., feeding, respiration and swimming activity) were reported, but by fewer studies. This also largely harmonizes with the questionnaire results, wherein welfare, locomotion, stress and feeding were among the aims reported. Brijs et al. (2021) focused more on the overall aims of studies, highlighting feeding optimization, welfare monitoring and animal-environment interactions as three main areas in which biosensors/telemetry may play a crucial role in the future of aquaculture. These three areas were also highlighted by Fahlman et al. (2021) as emerging application areas for biosensors in animal monitoring in general.

While few of the studies in the questionnaire reported expected negative effects before tagging, this was a topic covered in more details by Macaulay et al. (2021). Specifically, the authors found that some studies (e.g., Rillahan et al., 2009; Stehfest et al., 2017) did pilot trials before the actual experiments to better assess eventual negative impacts, while others kept tagged animals in smaller containment units for a while after tagging for evaluation before being released into the unit used in the study (e.g., Jurajda et al., 2016). Other aspects discussed by Macaulay et al. (2021) that were relevant for this topic were the tag weight vs. fish body weight ratio which ranged between 0.2 and 3% in the surveyed studies, and post-tagging recovery times, which were found to range from 0 to 7 days. These elements were not covered by the present questionnaire.

4.2.2. Experimental/physical setup parameters

While tanks were the preferred experimental unit among the questionnaire participants, Macaulay et al. (2021) found that cages were more common (61%) than both tanks (33%) and ponds (6%). This disparity was not unexpected as most of the respondents to the questionnaire have primary research infrastructures that are land-based tank facilities. A similar effect was seen in the geographic distribution of studies, as the questionnaires were all from Europe, while Macaulay et al. (2021) found that a relatively large proportion of the studies were conducted in North America (USA, 12%, Canada 10%).

Atlantic salmon was the most common species in the survey by Macaulay et al. (2021), mirroring the questionnaire outputs. The second and third most common species reported by Macaulay et al. (2021) were rainbow trout and Atlantic cod, contrasting with the questionnaire outputs which implied sea bream and seabass as the second and third most common species, respectively.

4.2.3. Experimental design parameters

Macaulay et al. (2021) found that keeping all tagged fish within a single unit was more common (59% of all studies) than distributing them between two (12%) or three or more (29%) production units. This resembles the outcomes of the questionnaire in which most of the replicates were on the individual level (i.e., the number of fish) rather than on the experimental unit level.

Most of the studies surveyed by Macaulay et al. (2021) used acoustic transmitter tags, while DSTs were the second most common tag type. In addition, 24% of the studies reported using external marker tags to identify fish carrying internally implanted tags. This relationship was reversed in the questionnaire outcomes, with DSTs being more common than transmitter tags. The reason for this is mainly that 60% of the respondents used the same tag (AEFishBit). This disparity was also reflected in tag deployment methods; Macaulay et al. (2021) reported that surgical implantation into the abdomen was more common (60%) than external attachment, while the questionnaire implied external attachment (as used for the AEFishBit) as more common.

Macaulay et al. (2021) found that the percentage of the total population in the study unit that carried tags averaged at 4.9% but had a median of 0.58%. This disparity is most likely caused by a few studies with a very high percentage of tagged fish. Although just four respondents to the questionnaire reported this percentage, these too varied between very low (0.016% and 2.25%) and very high (20% and 24.5%) values.

4.2.4. Data processing methods

All studies in the published literature seeking to use biosensors/telemetry to divulge new knowledge on animals apply some sort of processing method. While statistical methods of various levels of complexity are still the most common tools for this purpose, Fahlman et al. (2021) highlighted novel methods based on Machine Learning (ML) and Artificial Intelligence (AI) principles as potential game changers in data processing. Such methods may indeed be a complement to conventional knowledge-/experience-based interpretation, in potentially detecting relationships and correlations that are hard to spot through manual analyses.

The most common approach for visualizing biosensors/telemetry data is to present either individual data series, average time series or other statistical properties as time series plots. Although these will probably still be predominant methods in the future, the continuous development within visualization tools enables the development of increasingly informative and illustrative figures. Some recent studies have combined processed data curves with pictograms and text to illustrate different phases of an operation in relation to the fish data (e.g., figure 1 in Brijs et al., 2021). Other approaches summarize the main findings of a study through conceptual figures to illustrate e.g., fish responses toward variations in oxygen and temperature (figure 2 in Brijs et al., 2021) or differences in schooling patterns in response to water current strength (figure 2 in Johansson et al., 2014).

This type of figure enrichment may prove to be a useful method to better illustrate the main outcomes and conclusions of studies using biosensors/telemetry, and should thus be considered in future studies.

4.2.5. Experimental outcomes

Macaulay et al. (2021) found that most studies did not report the mortality of the untagged fish and highlighted this as an important factor for exploring increased mortality in tagged fish. This was not a query in the questionnaire, and is hence difficult to compare, but is an important topic to take into account.

Macaulay et al. (2021) found that most of the studies did not report in detail on physical effects of tagging on the fish, while a few studies either reported no apparent effects or specific effects such as physical damage, reduced growth and inflammation. Behavioral effects of the tag presence (e.g., swimming performance, food consumption, irregular swimming patterns) were more commonly reported, but not in great detail. As such, the questionnaire outcomes were perhaps more detailed than that provided in the literature, with most studies reporting not only the effects observed (e.g., wound development, behavioral changes and physiological responses). This could illustrate how some elements regarding the impacts of tagging are under-communicated in scientific publications, although the researchers have usually studied this feature.

Another post-tagging effect that was not a part of the questionnaire but that Macaulay et al. (2021) explored was mortality in tagged fish. Across the studies, the authors found correlation between study duration and mortality, and that cage studies had higher mortality rates than studies in tanks. These features could imply that biosensors and telemetry are more suitable for studies on fish in more controlled conditions (i.e., tanks) than in sea-cages where external features such as weather, strong waves/currents, pathogens and parasites can contribute to amplify the challenges caused by tag deployment and presence. Tanks were also found to have an advantage in tag retrieval, with 100% retrieval rates in all tank studies, whereas cage-based studies were found to have a retrieval rate decreasing with study duration.

5. Conclusion and recommendations

5.1. Questionnaire and literature

The questionnaire inputs were gathered from 10 studies. Although this is a lower number of studies than that covered by literature reviews (Macaulay et al., 2021, at 83 studies from 49 articles), it is within the same order of magnitude, and could hence contribute to expanding the overall picture of how telemetry and biosensors are used in aquaculture research today. While the outcomes of some questions in the questionnaire contrasted with those from the established literature, the overall impression is that the questionnaire results largely match the findings from literature. As such, the literature studies and the questionnaire outcomes may together represent a useful view into the use of such methods in aquaculture research.

5.2. Recommendations: "best practice guide"

While the questionnaire outcomes together with literature provide a good overview of the use of telemetry and biosensors in aquaculture research, the final step in this task was to use this knowledge to derive a set of simple recommendations for the future use of such tools. In the following, a simplified "best practice guide" is presented. Since many of the experimental parameters are often decided before selecting telemetry/biosensor solution (e.g., experimental hypotheses, production unit type/size, and fish species/size), the guide will not make recommendations on setting these, and rather focus on parameters of the telemetry/biosensor system/setup that are possible to adapt to the other experimental parameters/settings.

5.2.1. When to use or not to use telemetry/biosensors

A fundamental question to be asked before choosing whether or not to use telemetry/biosensors is: do you target individual animals or a group of animals? If groups are the target, it is usually more practical to use optical solutions (e.g., computer vision) or acoustic solutions (e.g., echo sounders, sonars, Didson acoustic camera). These methods have the advantages of not requiring interaction with the fish, being easy to deploy and being able to monitor a sub-volume continuously.

However, telemetry/biosensors are the only options if the aim of the study is to learn more about individual animals and particularly their behavioral or physiological histories over time. Although it can be possible to obtain such data with cameras in tanks containing few fish that are distinguishable through some visual feature, this is very difficult to achieve when populations and experimental units increase in size.

Another important element to consider is the species of fish used in the study. Not all species are equally suited for carrying such devices, and hence may need equipment specifically designed and adapted to their morphology/physiology. For instance, it is unlikely that flatfish can carry the same intraperitoneal tags as Atlantic salmon or other fusiform fish, while eels and other anguilliform fish have specific bodily features that put extra design challenges on the devices for implantation (Jepsen et al., 2002).

5.2.2. Measured parameter/sensor choice

What sensors to choose is usually largely determined by the hypothesis in question and the study objectives. For instance, if one wants to study vertical movement, depth sensors may be a more reasonable choice than accelerometers, even though you could derive some information on vertical movement based on acceleration data. Some studies also target specific fish parameters such as heart rate, thereby limiting the possible selection of sensors to those able to sense heart rate.

Another feature that could impact the choice of parameter to measure is the target species, as different species have different expected behavioral patterns. For example, many species that reside close to the water surface or that live pelagically tend to exhibit continuous swimming activity. Acceleration data from such fish can often provide good insight into the activity levels of these fish, as the dynamic component of acceleration affecting a swimming fish tends to be dominated by the tail beat action. Conversely, acceleration may be of less interest when aspiring to monitor animals that either move passively/slowly in the water mass or that are stationary for most of their lives.

A third element relevant for sensor selection is that devices containing the desired sensors are commercially available, and the unit cost of these devices. Although cost can sometimes be accounted for during the development of research project proposals, this can be a crucial issue particularly if many fish are to be tagged. Then the cost of the unit will matter, something that could limit the ensemble of eligible devices.

Finally, sensor choice may also be affected by other choices made during experimental planning and design. For instance, not all sensors are yet available in transmitter tags. Heart rate is an example of this, as this is a parameter currently only available in DSTs (e.g., Hvas et al., 2021). Conversely, some concepts actively utilize the carrier wave of acoustic tags for Doppler shift, and thereby estimating the movement speed of the fish relative to the receiver (Hassan et al., 2022). Such solutions will obviously not be possible to implement in DSTs due to their dependency on the acoustic carrier wave.

5.2.3. Transmitter or Data Storage Tag

Just like the preceding selection parameters, the choice of whether to use transmitting or storing tags depends on several factors. One of the most important of these is the size of the production unit. For small to meso-scale tanks or cages, it is often more feasible to collect the devices after experiments than what is possible in large/full scale production units as smaller units typically contain fewer untagged fish than large/full-scale production units. Moreover, the largest production units are usually parts of commercial farms, meaning that it is difficult to access the fish to collect tags outside of sorting/slaughtering events. Together these factors imply that DSTs are more suitable for small-scale controlled experiments (e.g., Warren-Myers et al., 2021), while large/full scale experiments are best served by transmitting tags (e.g., Føre et al., 2017, who had challenges locating DSTs after a full-scale trial).

Another aspect to consider when selecting between storage and transmitter tags is whether it is important to be able to access the data in real-time (or near-real-time), or if a high data density is preferred. This is a point where storage tags and transmitter tags have radically different properties and is often a key element in choosing technology type. DSTs can usually store data at higher densities than transmitter tags since data values of interest is then written directly to an internal storage medium rather than being modulated as acoustic/radio waves and conveyed over a (usually) limited communication channel. However, only transmitter tags can provide real time access to data, thus when this is important, the choice is simple. Transmitter tags also have an advantage in that it is possible to continually synchronize the data with GPS time by synchronizing the receiver units. DSTs do not have this option since they are designed to operate autonomously across the logging period and may hence experience drift in system time. Time drift is usually not a big issue in short term due to the high precision of the clock circuits in modern embedded systems but may lead to inconvenient time differences in longer studies.

A third aspect that is relevant in this context is the technological maturity and nature of the sensor/measurements that are planned. This relates to both data density requirements and the basic principles behind the measurements. When working with new sensing concepts it is often useful to start with a design based on a DST-platform, as this will provide better means of storing raw sensor data with a high data density. The raw data can then be used to derive proxy values that reflect the main dynamics of the system that is being monitored. This practice is typically used for novel (e.g., Svendsen et al., 2021) sensor technologies. Moving over to a transmitting platform often requires some sort of data compression/pre-processing before transmission and will thus be more suitable after suitable proxy values have been established. However, as previously mentioned, other solutions exploit the signal carrier wave directly, and can thus not be realized without transmitter platforms (e.g., Hassan et al., 2022).

Transmitter tags may use either acoustics or radio signals as a communication channel, and the choice between these two will also depend on the experimental setup, venue, and design. Specifically, when working in seawater, acoustics are generally always preferred as radio signals are heavily attenuated by salt water due to the high conductivity. Moreover, acoustics has a longer transmission range (kilometers) in water than radio signals (meters), irrespective of the water properties, and may thus be preferred when transmitters are expected to migrate far from the receivers. However, this extra range comes at a cost, as radio signals are substantially faster and more cost effective than acoustics, rendering radio-based tags able to transmit data at a higher rate.

5.2.4. Tag size

While device size is determined by the manufacturer, many tag types are available in different size classes to accommodate different applications. The primary parameter for determining tag size limits is fish size, as it is imperative to minimize the degree to which the presence of the tag affects the behavioral and/or physiological dynamics of the fish. Although the common practice is that the tag weight in air should not exceed 2% of the fish weight in air based on the potential for compensatory inflation of the swim bladder (Winter, 1983; Winter, 1996), current consensus is that this rule should be considered a recommendation, and that it is important to explicitly consider tag size vs fish size for each tagging study (Thorstad et al., 2013; Macaulay et al., 2021).

Although ensuring the tag vs. fish size relationship is of primary importance, this will often, especially when working with adult fish, result in a range of tag sizes being eligible. For instance, fish that exceed 1 kg are usually large enough to accommodate most tags designed for use in fish. The final choice of tag size will thus often have to be made based on other criteria. Most of these criteria are linked to previously covered topics such as tag performance. For DSTs, performance in terms of data storage capacity and battery life will generally increase with tag size, while larger transmitter tags typically have better battery life and transmitter power than smaller tags. Cost is another element that comes into play here, although it may vary whether smaller or larger units are more expensive. In some cases, the targeted parameter will also matter in this discussion. For instance, using tags to measure the gill cover movement will often require the use of small tags to not disturb the gill motion (Martos-Sitcha et al., 2019).

5.2.5. Tag deployment method

Tag deployment can generally happen in three ways: intraperitoneally by abdominal implantation, externally by suturing the tag to the muscle tissue of the fish (often dorsally), or in the stomach by placement through the gastric system (i.e., into the mouth of the fish). Which method is best will always depend on various parameters of the experimental setup. One of the more important parameters is trial duration. If the experiment is expected to have a long duration (months), intraperitoneal implantation should be preferred as the surgical wounds can then heal completely with time, reducing the long-term impact of the tagging procedure. In contrast, external tagging is typically done by attaching the tag directly with twines, clamps or wires to the tissue of the fish, meaning that complete healing of the wounds is impossible without removing the tag, rendering the method less adapted for long-term studies.

External tagging is eligible for shorter studies, and in some cases even necessary to achieve the monitoring goal; when targeting features of the environment (e.g., oxygen levels, salinity and temperature in the water) the tag needs direct access to the environment around the fish and can thus not be placed inside the fish.

Gastric deployment, where the tag is inserted through the mouth of the fish, is only suitable for even shorter trials as fish tagged with gastric tags tend to either regurgitate the tags after hours/days or may suffer from appetite deprivation after tagging. However, this method should be considered for shorter trials as it is presumably less invasive than the other approaches in not needing surgical procedures.

5.2.6. Number of tagged fish (# or %)

A final question to answer when setting up trials using biosensors and telemetry is how many animals you wish to monitor and hence equip with tags. This is a recurring question when working with these methods, often linked with the desire to keep a balance between monitoring enough animals to achieve a representative proportion of the population and having a sufficiently large untagged population to provide a relevant situation for the tagged fish. However, this choice is often affected by factors beyond the purely scientific aims. Sometimes the unit type will only house a certain amount of animals, effectively providing an upper boundary for how many fish it is possible to tag. Although this is usually no problem in experiments in commercial scale tanks/cages, such studies may experience the opposite effect, i.e., that there are too many fish for it to be feasible to tag a large enough percentage of the population to achieve a fraction perceived to be representative of the population.

Another factor affecting this issue can be cost, as tags tend to be in a price range of €300-1000. Instrumenting many fish will thus be a costly affair, that could set some hard limits for the number of fish to be tagged in an experiment.

The surgical requirements for tag deployment may also have an impact on this aspect, as implantation can be a process that requires not only expertise, but also relatively long time per individual fish. This can be both demanding time wise and cost wise, and hence be a driver working against a high number of tagged animals

Finally, the type of sensor/measured parameter can also be of importance for choosing the number of fish to tag. To monitor variables known to vary strongly across individuals, one may need to tag a larger number of individuals to ensure representativity.

5.3. Conclusion

In conclusion, this deliverable summarizes the findings of a questionnaire on the use of biosensors/telemetry in aquaculture research that was circulated among the partners involved in task 4.2, and links this with corresponding inputs from literature. The general impression is that the aims, experimental design, processing methods and experimental outcomes of the studies reported through the questionnaire match those most commonly identified in literature. This probably implies that the consortium's approach to using biosensors/telemetry in aquaculture harmonizes with the mode of conduct found in other scientific constellations. While this in itself is interesting, the main outcome of this deliverable was to derive a set of recommend a set of "best practices" for using such tools in aquaculture. This was done by reviewing the questionnaire outcomes and relevant literature together and resulted in recommendations within six separate topics that are endemic to the world of biosensors/telemetry where individual animals need to be equipped with electronic devices. While these recommendations are not exhaustive and probably do not cover all aspects of how such tools should be used in aquaculture, they can hopefully serve as a guideline for future experiments using biosensors/telemetry.

5.4. Acknowledgements

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6. Appendix

All outputs from the questionnaire are attached after the reference list.

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