COMPLETED PROJECT CASE STUDY

AQUAWRASSE: IMPROVING DELOUSING BEHAVIOUR AND EFFICACY IN FARMED BALLAN WRASSE

PARTNERS

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PROJECT LEADS

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Ballan wrasse is one of the species used as cleanerfish in salmon farming. Their natural behaviour is to pick sea lice off salmon, providing a gentle and continuous method of ridding farmed fish of parasites. This project studied the wrasse's personalities and behaviours to determine whether bolder wrasse are better at keeping salmon clean. The team also explored the best and most comfortable conditions for Ballan wrasse to perform this role. The resulting knowledge can be used to breed the most effective wrasse for use at sea. This will help reduce both the need for wild-caught wrasse and for alternative treatments. The project is an important milestone in the domestication and responsible use of Ballan wrasse for addressing the sea lice challenge in the UK and globally.

ANTICIPATED BENEFITS

- A better understanding of salmon delousing by Ballan wrasse, thanks to research into fish personality and interaction with farmed salmon.
- Improved husbandry practices during the hatchery phase and after deployment at sea, resulting in better fish health and welfare.
- Fewer wild-caught wrasse are needed due to the increased efficacy of farmed Ballan wrasse.
- Reduced need for pharmaceutical or mechanical sea lice treatments.

This project generates new knowledge to support wrasse husbandry during the hatchery phase and post-deployment. The aim is to improve performance and delousing efficacy while also giving an insight into the mutual behaviour and interactions between Atlantic salmon and wrasse.

BACKGROUND

Sea lice (*Lepeophtheirus salmonis*) present one of the biggest health issues in Atlantic salmon production and a challenge for the sustainable expansion of the sector. In the past decade, the use of wrasse to control sea lice has become an important component of integrated pest management strategies.

The continued use of wild-caught wrasse is not viable for the salmon farming sector in the long run, and farmed production is widely accepted as the way forward. While sector-led research to date has focussed on developing effective production methods for cleaner fish species and overcoming health challenges to produce robust, healthy fish, there is a demand for new research to address remaining bottlenecks to supply high-quality fish that are effective delousers.

Considerable gains in delousing efficacy can be achieved by improving the behavioural qualities of farmed wrasse, as well as providing a pen environment that enables and encourages effective delousing.

> *Cleaner fish are unique amongst farmed fish in that they are produced for their behaviour, so it makes sense that we focus our research on improving their natural instinct for delousing. While personality in fish is not a new concept, this is the first time it is applied to solve a real-world problem in aquaculture, and by working with nature we are aiming to improve the sustainability of cleaner fish as a method to control sea lice.*
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> ***Dr Adam Brooker, Research Fellow, University of Stirling's Institute of Aquaculture***

Personality is influenced by both genetic and environmental factors. It can be modified through selective breeding and/or environmental manipulations from early developmental stages. Therefore, one of the aims of the project was to assess the personality of individual fish, and correlate personality to pen behaviour as a proxy to delousing in both farmed and wild wrasse deployed in open pens.

Behaviour research to date has focused on the wrasse themselves, while the behaviour of salmon cohabiting with wrasse had remained undocumented. This project studied both wrasse and salmon behaviour concurrently, and tested the hypothesis that delousing is facilitated through a change in salmon behaviour.

Research through a previous SAIC-funded project investigated farmed Ballan wrasse behaviour and welfare after deployment, and results have shown that acclimatisation before deployment encourages natural behaviour, similar to the effective delousing behaviour of wild wrasse. This approach is now being widely adopted by Scottish producers, although more research and improvements are still required.

However, delousing performance in salmon pens can be variable, with farmed individuals showing a wide variety of behaviours. Tank-based delousing trials completed in this project have confirmed this individual variability. Ballan wrasse display complex behavioural patterns including hierarchy, dominance, territoriality, and most likely different personalities. These may, to some extent, explain the apparent high variability in delousing activity between farmed individuals, which can be traced back to farming practices in the hatchery.

AIMS

This project aimed to improve our understanding of a unique trait in aquaculture and livestock production (delousing) and wrasse behaviour in association with hatchery acclimatisation, personality and interaction with salmon, in comparison to wild-caught Ballan wrasse.

The objectives of the project were:

1. Testing and validation of personality screening of Ballan wrasse to determine the population personality structure of farmed and wild wrasse to provide a baseline of current stocks. The hypothesis tested in this project is that fish with a proactive personality phenotype cope better with transfer and adopt a 'wild type' delousing behaviour.
2. Investigate wrasse/salmon mutual behaviour and the impact of personality on delousing in a commercial salmon net-pen site. The hypothesis being tested is that delousing is facilitated through a change in salmon behaviour. This will inform the development and refinement of the net-pen habitat to facilitate delousing.
3. Compare behaviour (delousing and mutualism with salmon) between experienced wild wrasse and naïve farmed wrasse.
4. Test the effect of a routine treatment (e.g. freshwater bath) on behavioural response (coping) of both wild and farmed Ballan wrasse and salmon.

EXPERIMENTAL STUDY

Individual behaviour can be used to categorise fish personality from proactive (bold) to reactive (shy), which defines their response to – and their ability to cope with – stimuli. Personality has been studied in many fish species but very little has been done on cleaner fish, despite their striking behavioural traits.

The definition of boldness or proactiveness is not well defined in fish but is generally considered to be an individual's propensity to take a 'risk', while shy or reactive fish are more likely to exhibit freezing or flight behaviour. Shy fish are also less likely to explore new areas or to approach novel objects.

Studying both wrasse and salmon behaviour concurrently could reveal whether it is a form of mutualism where both the cleaner (Ballan wrasse) and client (salmon) modify their behaviour to allow delousing, or whether the relationship is a one-sided exploitation with the salmon being passive clients.

The project involved a single large study comparing the behaviour of two groups of Ballan wrasse; one of wild origin and one of farmed origin. Fish were behaviour-tested to assess their personality and then acoustic-tagged and released into commercial salmon pens. Atlantic salmon were also acoustic-tagged to study their behaviour during cohabitation with the wrasse.

The investigation was performed at a commercial sea farm comprised of a group of 12 pens between June and August 2021. The study used two adjacent pens at one end of the group. One pen was stocked with wild Ballan wrasse (1.5% wrasse:salmon ratio) and the other with farmed wrasse (4.8% wrasse:salmon ratio).

A Passive Acoustic Tag (PAT) system was used to record the positions of acoustic-tagged fish during each trial. The acoustic tags emit at a single frequency and each one is programmed with a unique, user-defined pulse rate interval to allow tag identification. The 3D positioning of each tag pulse is achieved by measuring the time delay to at least four hydrophones and triangulating its position. An array of eight underwater hydrophones was deployed around the perimeter of the experimental pens, with four surface hydrophones suspended at 1m depth below the pen walkway and four bottom hydrophones in custom-built cradles suspended at 15m depth and pointing into the pens.

A population of farmed wrasse reared at Otter Ferry Seafish were acclimatised according to tested and validated protocols. Wild wrasse were caught by local creelers. Both groups of fish were transported by road. For three weeks before start of the trial, they were held in separate keep nets within empty pens adjacent to the experimental pens, with artificial kelp and feed blocks supplied.

In preparation for behaviour testing and acoustic tagging of the wrasse, 57 wild and 67 farmed wrasse were hand-netted from the keep net, moved onshore and kept in 1,000L tanks on a flow-through system with aeration and artificial kelp hides.

Thirty fish from each group were selected and individually behaviour tested before being acoustic tagged. Each fish was placed into a 750L static tank filled with seawater and after a 10-minute

acclimatisation period, a GoPro video camera mounted above the tank was used to record the behaviour of each fish for 5 minutes. Then, a novel object consisting of several LEGO bricks was placed into the tank and the behaviour of the fish recorded for a further 5 minutes.

Following the behaviour test, each fish was acoustic tagged. A total of 30 farmed wrasse and 29 wild wrasse were tagged. 50 Atlantic salmon were also tagged; 25 per trial pen. Once deployed, tag signals were received by the hydrophones continuously until the tag batteries expired after several months.

Site husbandry followed the usual commercial routines and protocols. A wrasse hide was suspended at one corner of each pen at 3-8m depth, and feed blocks offered at each end of the hide and in the corner of the pen opposite to the hide. Mean sea lice counts were obtained weekly on 10 salmon per pen. Salmon and cleaner fish mortalities were removed and recorded daily.

DATA ANALYSIS

To analyse the behaviour test videos, individual frames were extracted from each 10-minute test video. These were processed and analysed using R. The calculated parameters for each fish were: latency to the novel object (time to first approach within a set distance), minimum and mean distance to the novel object, movement time, number of moving and static bouts, distance travelled, mean velocity, maximum velocity, and latency to first movement after the novel object was added.

One-way Analysis of variance (ANOVA) was used to test for significant differences between wild and farmed wrasse and personality groups. Fish were categorised as proactive, intermediate or reactive based on the minimum distance that each fish approached the novel object.

To analyse cohabitation, acoustic data were processed to identify tags and calculate fish positions, which were saved as a list of tag numbers and Coordinated Universal Time (UTC) stamps with three-dimensional Cartesian coordinates. Specifically, a noise filter was used to extract individual tag signals and then a 3D algorithm was used to calculate the position of each individual tag pulse based on simultaneous detections from at least four hydrophones.

Solar status was assigned to each tag detection based on their time stamp as dawn, day, dusk and night, as well as tidal status. Likewise, based on their Cartesian coordinates, each tag detection was categorised into one of five locations within each pen: bottom, hide corner, empty corner, edges, and centre.

Home ranges for each wrasse were estimated from bivariate normal fixed kernel utilisation distributions, which were calculated over a 0.5 × 0.5m resolution grid. Changes in home ranges were investigated by plotting the daily cumulative 95% kernel utilisation distributions for each fish over the period of the study.

The threshold for wrasse/salmon interaction events was when two tag signals for salmon and wrasse were within 0.5m of each other and the time difference between the signals was less than the period of the shortest tag signal of the pair.

RESULTS

During the tank-based personality test, changes in behaviour in response to the novel object showed that none of the wild wrasse froze in response to the novel object, whereas 20.5% of the farmed wrasse froze. Freezing is considered to be an anti-predatory response shown by many prey species and is considered a characteristic of a reactive personality. An equal number of wild wrasse (24.1%) showed increased and decreased activity in response to the novel object, whereas more farmed wrasse showed decreased activity (30.8%) than increased activity (10.3%) in response to the novel object.

Latency to the novel object was significantly less for wild than farmed wrasse at a minimum distance to the object of 30cm and 20cm, but there was no significant difference at 10cm. The minimum distance approached was less in wild fish and they moved for longer with a higher mean velocity. There was, however, no significant difference between wild and farmed wrasse in the mean distance to the novel object or the latency to first movement after the object was added.

These results indicate that there are differences in wild and farmed wrasse behaviour. As the farmed wrasse originate from wild broodstock and there has been no selective breeding in farmed wrasse, it can be assumed that these differences are due to the domestic rearing environment. These differences in behaviour were not unexpected. The natural habitat of the wild wrasse is far more complex and dynamic than the constant and predictable environment of the hatchery. In the natural environment, wild wrasse are required to actively forage and will regularly interact with their environment, encouraging proactive behaviours.

Based on the minimum distance to the novel object, there were 19 proactive fish (3 farmed and 16 wild), 18 intermediate fish (9 farmed and 9 wild) and 9 reactive fish (6 farmed and 3 wild), and behaviour parameters were compared between wild and farmed fish within each personality group. There was no significant difference between proactive wild and farmed fish for total time moving, distance travelled, mean velocity, time of first movement, mean distance to the novel object, and latency to 30cm. Despite all proactive fish approaching within 20cm of the novel object, wild fish did have a shorter latency at this distance. Similarly, there was no significant difference between reactive wild and farmed fish for total time moving, distance travelled, mean velocity, time of first movement and mean distance to the novel object, suggesting that the wild and farmed fish within each personality group were not significantly different in terms of their behaviour in the novel object test.

More wild fish were proactive than reactive and more farmed fish were reactive than proactive, suggesting that the rearing environment strongly influences personality in Ballan wrasse. However, observed differences in personality within each group can be attributed to genetic variation, meaning that there is scope for artificial selection of personality, assuming that it is a selectable trait.

After wrasse were acclimatised and released into the pens for the cohabitation and interaction trials, it was observed that the mean swimming depth of wrasse

and salmon varied according to the time of day. Wild and farmed wrasse swam deeper during the night and shallower during the day, with intermediate depths occupied at dawn and dusk. In wild wrasse, mean depths were approximately 4-9m but after 22 days, they occupied a narrower depth range and mean depths were shallower at approximately 4-6m. In farmed wrasse, there was more variability in mean depths between days, although they tended to prefer depths of 3-8m.

Salmon also showed a strong diurnal swimming depth pattern, although opposite to the wrasse diurnal pattern: shallower depths were occupied at night and deeper depths during the day, with intermediate depths occupied at dawn and dusk. This opposite diurnal difference in swimming depths meant that wrasse and salmon were separated by depth, especially during the day.

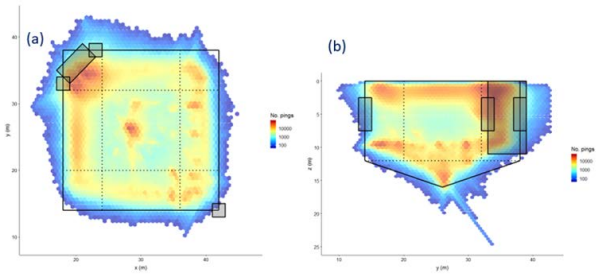
Due to this separation, there would be limited opportunities for interaction and delousing, also given that wrasse are visual feeders and tend to rest at night. Variation in activity was greatest in wild wrasse, both between different times of day and between individual fish. Activity in farmed wrasse was overall lower than in wild wrasse and there was less variation between individual fish.

For nearly all wrasse, both wild and farmed, activity levels were lowest at night and increased in the order of night < dawn < dusk < day. On most days observed, dawn and dusk were the times when wrasse and salmon occupied the same depths and cross-over during their diurnal depth migrations, both species tending to occupy the same depths in the pens and showing elevated activity levels, suggesting these times of the day are likely to be key for interaction and delousing.

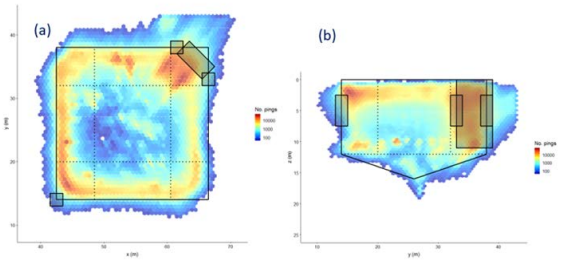
Preferred location within the pen was also assessed. As expected, for both wild and farmed wrasse, the hides and net edges were the preferred locations. Wrasse were released from the keep-nets into the hides, which were used a lot at the start of the trial, decreasing over the first week as the wrasse started to explore their new pen environment.

Interestingly, there is no clear correlation between keep-net residency time, the period of time between the keep-net being opened and the wrasse leaving, and latency to the novel object. While the majority of fish left the keep-net within the first 24 hours, some fish from all personality groups took longer (up to 74h) to leave. The fact that many farmed wrasse begin exploring the net-pen as quickly as wild wrasse is encouraging, and illustrates the proactive behaviour of these individuals.

Farmed wrasse primarily used the top 1-5m of the net pen, other than in the hide, where they used all depths to 10m. Wild wrasse also used the top 1-5m but depths greater than 10m were also used frequently. Farmed wrasse did not use the centre of the pen, with very few tag signals in this location. However, wild wrasse did use the centre of the pen, albeit not as much as the pen edges.



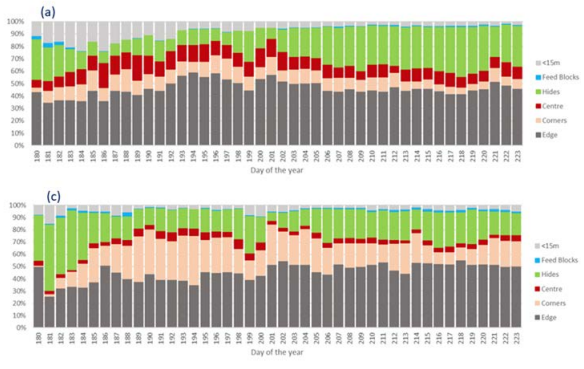
Heat maps showing number of received tag signals for wild wrasse top (a) and side (b) view.



Heat maps showing number of received tag signals for farmed wrasse top (a) and side (b) view.

Mean depths for proactive, intermediate and reactive wrasse were 5.01 ± 0.41m, 6.20 ± 0.40m and 4.13m ± 0.42m, respectively. In proactive and intermediate fish, there was a wide variation in depth between individual fish ranging from 2-12m, whereas in reactive fish there was less depth variation between individuals of 3-6.5m.

Mean activity for proactive, intermediate and reactive wrasse were 0.42 ± 0.08BL/s, 0.42 ± 0.08BL/s and 0.41m ±0.08BL/s, respectively. While there are wide variations in activity levels between fish, there is no clear difference in activity between proactive, intermediate and reactive groups.



% time spent and daily fish locations from June 2021 to August 2021 for (a) wild wrasse and (c) farmed wrasse

Salmon preferred the pen centres, with the highest tag signal density in areas over 2m from the edges. The highest signal density was a donut shape around the centre of the pen, reflecting the shoaling nature of the

salmon and their circular swimming pattern, frequently using depths from 1-12m.

Considering the differences in preferred locations between wrasse and salmon, changing the pen setup or husbandry practices to change the fishes' behaviour may result in more interaction and opportunities for delousing. Hide design and/or location could also be adjusted so that wrasse are encouraged to venture into the open pen where the salmon are swimming, or salmon could be made to swim around the hide to improve delousing opportunities.

Home ranges for wild wrasse covered more of the pen than those of farmed wrasse. For all wild wrasse, the core home range was focussed on the hide, and the home range covered most of the net pen including the pen centre. The typical core home ranges of farmed wrasse also focussed on the hide and often some of the pen edge areas, but the home ranges covered the pen corners and edges only, with very limited coverage in the pen centre.

Interestingly, the work done comparing wild and farmed wrasse found that farmed wrasse had smaller home ranges and established their home ranges later than wild wrasse, but these new results showed that farmed wrasse home ranges could be larger than those of wild wrasse, and that the time to establish the home range is similar for both groups. This may reflect the improved cleaner fish husbandry techniques that have been developed.

Regarding wrasse and salmon interactions over the study period, considered to be when both species are within 0.5m of each other, the time spent interacting was less than 1% in all wrasse. Although this figure is very low, only a very small number of the fish in the pens were tagged. Furthermore, whether wrasse and salmon are actually interacting when their tag signals are within 0.5m of each other is unknown. However, these results allow the comparison of potential interactions between wild and farmed wrasse.

Interaction was often high at dawn or dusk in many fish, both wild and farmed, and night-time was consistently the period of lowest interaction.

For both wild and farmed wrasse, interactions were primarily focussed in the area in front of the hides. Net edges were also locations with high levels of interaction. There was also a focus of interaction at 10m depth along the pen edge, whereas interaction at depth was very low in farmed wrasse. While interaction occurred throughout the net-pen in wild wrasse, there was almost no interaction in the pen centre in farmed wrasse.

A clear distinction between salmon swimming peaks during interactions was also observed when cohabiting with wild wrasse, with the lower peak representing salmon that are slowing down in response to the proximity of a wrasse, possibly to facilitate delousing. This is the first evidence that delousing is a mutual behaviour, with salmon seeking to be deloused, rather than a one-sided interaction with no behaviour modification by the salmon. In contrast, salmon with farmed wrasse showed only a slight decrease in swimming speed during interaction events but there were no two distinct peaks as in salmon with wild wrasse. This suggests that there may be a slight response in salmon with farmed wrasse, but farmed wrasse do not appear to elicit the same response in salmon as wild wrasse.

IMPACT

The project was ambitious from the outset and aimed to deliver a number of significant outcomes, most of which have been achieved.

The personality study of individual wild and farmed Ballan wrasse has confirmed that wild-caught wrasse are more proactive than the hatchery-reared, which is likely due to the uniform environment of the farmed wrasse. It was difficult to relate the results of the behaviour test with the fishes' behaviour in the pen, although there does appear to be some loose correlations. Personality in fish is complex, and a more complex test would elucidate more behaviour parameters that are components of personality, such as activity, anxiety, sociality and aggression (Whittaker et al., 2021). Furthermore, the behaviour test could only be performed once before the fish were acoustic tagged. Personality is defined as repeatable behaviour in a specific situation, and repeating the test would confirm personality in each fish. Both of these improvements (a more complex test and repeatable behaviour) are being investigated in the SAIC-funded CleanGain project, a follow-on to this project.

The behaviour of wild and farmed wrasse in the pens was comparable in many aspects, including depth, activity, and the time taken to establish a home range. In both wild and farmed wrasse, the hide is a focal point of activity. This is an improvement on the results of a previous hydroacoustic study where farmed wrasse behaviour was not consistent with wild wrasse behaviour (Brooker et al., 2019). This is encouraging and suggests that improvements in recent years in hatchery rearing protocols and net pen husbandry are producing better-quality farmed wrasse and improved behaviour. The main differences between wild and farmed wrasse were diurnal variation in activity (greater variation in wild wrasse) and pen locations (farmed wrasse stayed close to edges but wild wrasse covered the whole pen).

While wrasse preferred the pen corners, edges and hides, salmon primarily shoaled in the pen centre. Comparing the behaviour of wrasse and salmon suggests that the important periods for interaction are dawn and dusk, when the diurnal depth pattern of each species crosses over and the primary locations for interaction are in front of the hides and pen edges, and this is likely when/where delousing occurs. A key finding from the study is that salmon slow down when they are interacting with wild wrasse and, to a limited extent, with farmed wrasse. This is strong evidence that delousing is a mutual symbiotic behaviour, with both species modifying their behaviour during interactions, and this should be investigated further and exploited to promote better delousing.

Breeding and rearing wrasse in captivity results in different selection pressures than in wrasse in the wild. Desirable 'wild' traits could be lost or reduced in captivity without careful selective breeding. Useful traits in the wild, such as proactive and inquisitive personalities and grazing behaviour, may be promoted by natural selection, whereas they may confer no

benefit to wrasse in captivity. Therefore, it is essential to identify and select such traits to safeguard them for breeding future generations, to avoid losing 'wild' traits in farmed wrasse.

While farmed wrasse are routinely acclimatised immediately prior to deployment, considerable improvements in farmed wrasse behaviour may be achieved by continuous environmental enrichment throughout the hatchery phase. Such modifications may include variable current and feeding regimes, including time and depth of feeding, and a variety of food sources. Exposure and acclimatisation to salmon and sea lice would be difficult in the hatchery, but experimentation with artificial model salmon and sea lice could be worthwhile. While these environmental modifications may compromise production objectives (e.g. rapid growth and low mortality), there may be an acceptable level of trade-off to produce more effective delousers.

Some results from the project were presented at the Aquaculture UK conference (Aviemore, May 2022) as part of the Innovation with Impact session hosted by SAIC. This project and its dissemination have encouraged regular discussions with cleaner fish managers from Scottish salmon producers to share current best practices for cleaner fish, which have been improved as a result of the Aquawrasse project.

The project has confirmed that there are different behaviours in individual wrasse (personality) and that these affect their behaviour in the net pen. Future work should explore further these differences in behaviour to (a) confirm the personality baseline of farmed stocks, (b) confirm the effect of personality on delousing, (c) develop an effective protocol for identifying desirable personality traits for selective breeding, and (d) explore hatchery rearing techniques to modify the behaviour of farmed wrasse (behavioural plasticity).

ADDITIONAL INFORMATION

REFERENCES

Brooker, AJ, Davie, A, Leclercq, E, Zerafa, B & Migaud, H (2019) Pre-deployment acclimatisation of farmed ballan wrasse (*Labrus bergylta*) to sea-pen conditions promotes behaviour analogous to wild conspecifics when used as cleaner fish in Atlantic salmon (*Salmo salar*) farms. Aquaculture 520, 734771.

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AQUAWRASSE IN THE PRESS

It has long been thought that people's personality traits determine how suited they might be to different types of jobs. This project takes the concept a step further by looking at how it might apply to a particular species of fish. The project team will undertake a series of tests to determine whether the boldness of individual Ballan wrasse makes them more adept at picking sea lice parasites from salmon.

The researchers will study wild and farmed Ballan wrasse behaviour by introducing a new object into their tank and video-tracking individual cleaner fish. They will then monitor the wrasses' reactions to the object – how quickly they interact with it – and correlate this with hatchery rearing conditions, acclimatisation protocols, and how they later perform when presented with the opportunity to clean salmon on actual fish farms.

In addition to the personality study, the team will use hydroacoustic tracking, where underwater hydrophones are used to locate the position of tagged fish to understand and compare the behaviour (in terms of both delousing and interaction with salmon) of experienced wild wrasse and those produced in hatcheries. The same technique will be used to understand salmon behaviour by, for example, identifying whether the salmon swim closer to the wrasse and slow down in a bid to encourage them to clean.

> *"Ballan wrasse are very special fish – they are very clever and demonstrate fantastic behaviour. If you walk by a tank, they will take an interest in you and physically watch you and follow. They are unique in many ways; each has their own personality and we want to correlate which traits – namely boldness and shyness – translate into Ballan wrasse being reliable and effective at delousing salmon."*
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> ***Professor Herve Migaud, University of Stirling's Institute of Aquaculture.***

Drawing on a range of veterinary, rearing, and research skills, the project will also explore the optimal conditions for Ballan wrasse to perform their role across a range of variables, including their feed, the set-up and 'furniture' in pens, as well as the ratio of salmon to cleaner fish.

> *"Wrasse have been an integral part of our production since 2014 and they have provided the business with a strong performance platform to grow salmon as naturally as possible. The natural behaviour of the wrasse isn't taught, nor is it forced, but it is allowing the animal to express their natural behaviour in comfortable setting and under the full care of our site teams."*
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> ***Lewis Bennett, Cleaner Fish Manager, Loch Duart***