Video surveillance methods to evaluate individual feeding response in rainbow trout (*Oncorhynchus mykiss*, Walbaum)—implications for feeding regime optimisation

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Abstract

Precisely analysing and optimising feeding regimes is central to salmonid growth performance and delivery of special diets. The current study developed novel video surveillance methods and analysis techniques to assess individual feed intake and minimum pellet intake (MPI) in individually identified juvenile rainbow trout, *Oncorhynchus mykiss*. Three trials were conducted to test the impact of short-term starvation ($N=112$ [16 tanks, 7 fish per tank], average weight= 27.1 ± 3.4 g, age= 119 days), portion numbers per feeding ($N=105$ [15 tanks, 7 fish per tank], average weight= 22.8 ± 2.1 g, age= 99 days) and varied numbers of daily feeding events ($N=84$ [12 tanks, 7 fish per tank], average weight= 32.4 ± 3.3 g, age= 133 days). All trials were carried out in a recirculating aquaculture system with 20 tanks held at $15 \pm 0.5^\circ\text{C}$. All individuals were code-tagged and high quality video images were taken and analysed to identify all feeding interactions. Individual trout feeding activity under different feeding regimes could be precisely analysed with the video methods developed. Moving from one to two daily feeding events doubled pellet intake per fish from 27.4 ± 5.8 to 52.8 ± 11.5 pellets. Pellet intake (58.8 ± 24.2 pellets) did not increase at three daily feeding events but became more variable across fish. MPI nearly doubled to 30 pellets in fish receiving two daily feeding events (MPI₃₀: chi-squared = 8.74, df = 2, $p = 0.01$). Short-term starvation had no influence on intake (28 ± 8 pellets/fish) or MPI. Increasing portion number from one (27.8 ± 7.4 pellets fish⁻¹) to two (31.1 ± 7.4 pellets fish⁻¹) or more did not significantly increase the number of ingested pellets. Adjusting the feeding regime by increasing daily feeding events to two, possibly combined with multiple portions, can increase pellet intake and reduce the heteroscedasticity of pellet intake. The methods presented in this study are viable for analysing feeding regimes for juvenile rainbow trout and controlled feedstock/supplement delivery. Implications for analyses with other species and for vaccination optimisation are discussed.

Keywords: Salmonid · Video · Feeding regime · Fish welfare · Visual tags

Introduction

Feed delivery and feeding regime optimisation are key factors affecting the performance of fed aquaculture and are of particular importance in salmonid farming (Cho 1992; Guzel and Arvas 2011). Achieving high total diet intake and limiting diet wastage are essential to optimise growth of animals and minimise cost in terms of feed use (Ang and Petrell 1998; Cho and Bureau 2001). Equally important is ensuring the uptake of feed as evenly as possible across the entire fed population in order to reduce uneven growth, development of dominances and the need for grading. Even and sufficient feed intake across the entire fish population is also essential to ensure animals are appropriately dosed when fed special supplementary diets or when receiving oral vaccinations or veterinary treatments. There remains, however, a lack of established methods to measure and analyse feeding regimes at an individual level under replicable and realistic holding conditions.

Most research to date on topics such as feeding methods or alternative feedstocks have been performed using groups of fish, rather than individual feeding, as determinants. Individual feed intake by Arctic charr (*Salvelinus alpinus*) was determined indirectly by Brännäs and Alanärä (1993) using automatic feeders and feeding trigger release as measures for individual feed intake. Ten years earlier, Talbot and Higgins (1983) determined individual feed intake of juvenile Atlantic salmon (*Salmon salar*) using iron-marked pellets and radiographic spectroscopy. Both these methods however have their disadvantages, which include the exclusion of fish interactions and high levels of uncertainty due to indirect counting of feed intake or the impacts of fish euthanasia prior X-ray scans.

Visual determination of video-recorded feeding events can be used to determine direct individual intake in vivo. Drawbacks are the limited holding densities due to overlapping fish during the feeding process and the challenge of determining the identity of individuals within the treatment. De Verdal et al. (2017) used this visual approach to determine the individual feed intake of Nile tilapia, *Oreochromis niloticus*. In their study, pellets were fed to the fish pellet-wise and video records of the feeding event were evaluated post-feeding. The number of fish per tank can be increased by visually marking the fish for better differentiation or location. An example is presented by Olsen and Vøllestad (2001) who marked 0-age brown trouts *Salmo trutta* with visual implant elastomer (VIE) tags. The tag with VIE caused negligible mortality (0.5%) and long lasting visibility. Of all three methods, video is considered the most conservative. Video analyses of feeding may also be hindered in salmonids such as rainbow trout if the low stocking densities required lead to aggressive fish behaviour which can interact with feeding activity (Ellis et al. 2002).

In salmonids, short-term starvation has been investigated as a feeding delivery method to reduce labour costs (Kindschi 1988), increase food efficiency (Azodi et al. 2015) and test for compensatory growth (Weatherley and Gill 1981). Individual pellet intake has not been the main focus in the cited studies, though refeeding after starvation periods increases size variation in juvenile rainbow trouts, which can be assigned to uneven individual feeding (Kindschi 1988). Furthermore, Kindschi (1988) did not find significant differences in growth performance, but that daily feed intake (DFI) of the deprived fish was higher than the DFI of daily fed fish during refeeding. This was also the case for juvenile rainbow trout exposed to long-term starvation periods. Even starvation phases up to 6 weeks did not lead to changes in final fish weight or cortisol levels (Sumpter et al. 1991). No detailed information is present for the optimal trade-off between maximal DFI and inhomogeneity of individual pellet intake during single feeding.

Whilst starvation experiments can have durations of days and weeks, the term feeding frequency describes the intervals between two feeding sessions mostly within 1 day. Grayton and Beamish (1977) tested different feeding frequencies (1 meal/2 days–6 meals/day) for rainbow trout and found best results, in terms of fish growth, is achieved when applying one or two meals per day. Alanärä (1992) also concluded that increasing numbers of daily feeding events increase fish stress during feeding for rainbow trout fed in net-pens using automatic feeders. Rasmussen et al. (2007) showed that for rainbow trout, an increase in feeding frequency is directly correlated to the degradation of fin condition and thereby fish welfare.

There is a dearth information in the literature on the number of feed portions added, i.e. most studies use single food addition during the feeding procedure. Whilst information on portion size for rainbow trout feeding is a well-reported parameter (Alanärä 1992; Bailey and Alanärä 2006; Hung and Storebakken 1994), information on the effects of different numbers of portion addition during a feeding event is lacking. If presented it pertains to feeding strategies such as automated versus hand feeding (Alanärä 1992; Azzaydi et al. 1998). Evaluable effects of portion numbers for salmonids are present in a study by Alanärä (1992) who tested different feeding strategies such as time controlled restricted, restricted demand and unrestricted demand feedings which presents one restricted portion, multiple portions with small portion size and multiple portions with large feed amounts. Their results showed that an increased number of portion of small quantities leads to significantly better food conversion rate and less pellet waste.

Individual feed intake under varying feeding regimes is uninvestigated for salmonids. Whilst the general principles and relations of growth and feeding regime are well understood and described based on group performance over extended feeding periods, the literature still lacks detailed experimental results based on the individual feeding response to variations in regime.

The aim of this study is to develop exact methods to observe and quantify rainbow trout feeding response. The combination of visual tagging and high definition video recording analysis are developed to allow quantification of the pellet intake of individual rainbow trout during feeding events. These methods are then applied and validated in a series of controlled feeding experiments.

Materials and methods

Facility, tanks and water quality

Experiments were conducted in the laboratories and aquaria of the Centre for Aquaculture Research (ZAF) at the Alfred-Wegener-Institute Helmholtz-Centre for Marine and Polar Research, Bremerhaven. The recirculating aquaculture system (RAS) used for all experiments consisted of 20 individual tanks with clear-glass fronts, a cooling element, a moving-bed nitrification bio-filter and a foam sheet filter. Tank dimensions were 48 × 38 × 49cm (length, width, height). The number of tanks used was modified in accordance with each of the three experimental designs outlined in the following. A photoperiod of 8 h daylight and 16 h darkness was applied at all times and exclusively during the period of video recorded feeding, an additional UV-light (405nm, SMD5050, 8.4W/tank) was applied.

Water temperature was maintained at 15 ± 0.1 °C; water replacement rate and airflow were adjusted at 4.2%/min and 300 L/h for all tanks to obtain identical experimental conditions.

Ammonia, nitrate, and nitrite were controlled throughout animal holding and during experiments. Values did not exceed 0.12 mg L^{-1} , 0.2 mg L^{-1} and 50 mg L^{-1} , respectively. Oxygen levels during the trials were $88 \pm 6\%$ on average throughout the experimental trials and never fell below 82% .

Experimental fish and feeds

Rainbow trout fingerlings (*Oncorhynchus mykiss*) were obtained from a commercial fish farm (Die kleine Fischzucht, Geseke, Germany). After arrival, fish were acclimated and were stocked in a 550-L holding tank attached to the system until experiments were carried out. During that period, fish were hand-fed a commercial floating diet (F-1P Classic LT/F 2.5mm, Skretting) twice daily until visual satiation. Mean fish weight and length were $27.1 \pm 3.4 \text{ g}$ — $13.8 \pm 0.5 \text{ cm}$, $22.8 \pm 2.1 \text{ g}$ — $13.7 \pm 0.5 \text{ cm}$ and $32.4 \pm 3.3 \text{ g}$ — $14.9 \pm 0.5 \text{ cm}$ for the starvation, portions and daily feeding events experiments, respectively.

Sampling, VIE tagging and MPI

For each experiment, fish were taken from the acclimation tank, weight and length measured and fish of adequate size were anaesthetised with tricaine methanesulfonate (MS-222, 100 mg/L , 90 s, Sigma Aldrich) for tagging.

Fish were marked behind the eye and alongside the dorsal fin with visual implant elastomer tags (VIE, Northwest Marine Technology, Inc.). Different colour combinations were applied to allow each fish to be identified during the video evaluation. Directly after tagging, marked fish were distributed homogeneously amongst the glass tanks in which the trials were performed. Fish were allowed to settle into the glass tanks and recover from any tagging stress for a period of 10 days. Observation and visual estimation of fish behaviour was carried out over the first 7 days to ensure normal response, also during feeding and further 3 days was added to ensure full acclimation.

Prior the evaluation feeding, the camera setup (Sony A7sII, 60fps, ISO4800; Sigma 12-24 @18mm, f2.8) was arranged in front of the glass tanks and video recording was started approximately 1 min before feeding. After 5 min, the video recording was stopped. A preliminary analysis of the video files showed that within the first 100 s of feeding, most of the pellet uptake occurred. Hence, for the evaluation of the videos, the first 3 min of each feeding event was evaluated by following the tagged fish and counting the consumed pellets for each fish. Pellets that were disgorged by the fish were subtracted from the total pellet intake. When the portions were added to the glass tanks, a wide spread of the pellets over the whole water surface was ensured to present a maximise feeding area. Furthermore, any aggressive behaviour amongst fish was recorded.

To classify and evaluate the different feeding scenarios, the maximum pellet intake (MPI) was established in this study. Whilst the ingestion rate, which can be calculated directly and indirectly, only shows the overall intake of a cohort or an individual, the MPI represent the ratio of fish that consumed a minimum of x pellets.

Method validation trials

In order to apply and validate the video recording and analysis methods developed above, controlled feeding experiments were established to test the following feed delivery parameters:

- 1) Short-term starvation periods of varying lengths
- 2) Portion numbers per feeding
- 3) Number of daily feeding events

Data in terms of feed pellet intake per individual are presented in the following with the aim of identifying delivery methods to optimise homogeneity of feed intake and increase minimal pellet uptake.

Starvation period

In this experiment, a total 16 of the maximal 20 tanks and a total of 112 fish were used in the system. Seven VIE tagged fish with an average weight of 27.1 ± 3.4 g (rearing density = 2.3 kg m^{-3}) were acclimated for 10 days in each of the experimental glass tanks. During that period, the seven fish per tank were fed 0.8% body weight (bw) fish^{-1} twice daily. Fish in four tanks (in quadruplicates) were then starved for 0 (control C), 1 (S1), 2 (S2) or 3 (S3) days before being fed once in a single portion with the standard diet (as above) at a rate of 2% bw fish^{-1} (39 pellets fish^{-1}). This feeding event was recorded on video and fish consumption of pellets (uptake, disgorging) and interactions (aggression) was evaluated post-experimentally.

Food portions

In this experiment, a total 15 of the maximal 20 tanks and a total of 105 fish were used in the system. Seven VIE tagged fish with an average weight of 22.8 ± 2.1 g (rearing density = 2.0 kg m^{-3}) were acclimated for 10 days in the experimental glass tanks. During that period, fish were fed 0.8% bw fish^{-1} twice daily. At day 11, the video recorded portion feeding was conducted. A total of 2% bw fish^{-1} (33 pellets fish^{-1}) was divided into one (PA1), three (PA3) or five (PA5) portions, added every 30 s to the respective tanks. Treatments were repeated in five replicate tanks each.

Daily feeding events

In this experiment, a total 12 of the maximal 20 tanks and a total of 84 fish were used in the system. Seven VIE tagged fish with an average weight of 32.4 ± 3.3 g (rearing density = 2.8 kg m^{-3}) were acclimated for 14 days in the experimental glass tanks in quadruplicates. During that period, fish were fed 0.8% bw fish^{-1} twice daily. At day 15, the video recordings of the different feeding scenarios were conducted. Fish of the FE1 treatment were fed once at 9 a.m., fish of the FE2 treatment were fed twice at 9 a.m. and 3 p.m. and fish of the FE3 treatment were fed three times at 9 a.m., 12 p.m. and 3 p.m. In each feeding event, fish were provided with 2% bw fish^{-1} (46 pellets fish^{-1} or approx. 0.65 g fish^{-1}) feed.

Statistical analysis

Pellet consumption data were tested for normal distribution (RSTUDIO, shapiro.test) and variance homogeneity using the Bartlett test. When data were normally distributed and homogeneity of variance was given, a generalised linearized model was used to test for significant differences between treatments. For over-dispersed data, the negative binominal distribution was tested and used (RSTUDIO, glm.nb). When data were normally distributed but

variance was not homogeneous amongst the replicates, a Kruskal-Wallis test was performed and if significant differences were detected the Dunn's post hoc test was used to show pairwise differences within the groups. The variance of pellet consumption and all other variance data herein are presented as the standard deviation of the mean. For all tests, a significance level of 0.05 was applied. All statistical tests were conducted using R Version 3.5.0 (R_Core_Team 2018).

Results

The VIE tags allowed precise identification of all fish during the video evaluation. Further, videos gave an exact overview of fish behaviour during the feeding session. The exact uptake of individual pellets and the disgorging (if any) of pellets was able to be recorded. All aggression events amongst the fish were also clearly recorded. During the starvation trial, one fish in the control treatment was harassed by a conspecific (0.9% of all fish) at the end of the event only, thus aggressive behaviour can be excluded from the starvation trial. During the daily feeding events trial, one fish from the control treatment showed aggressive behaviour towards the conspecific during the midday feeding event (1.2% of all fish). However, the fish showing the aggressive behaviour showed low pellet intake (16 pellets during the midday feeding). As with the starvation trial, fish aggression can be excluded as a factor in the daily feeding events trial. For the time of video evaluation of the portion trial, one fish from a PA3 and PA5 treatment was harassed by a conspecific 3 and 2 times (1.9% of all fish), respectively. The fish in the PA3 treatment showed reduced pellet uptake with 2 pellets during the specific feeding event. No effect on pellet uptake was present for the fish in the PA5 treatment. This represented less than 3.2% of all feeding reactions and, as such, aggression can be disregarded for the portion trial.

Starvation period

Across all treatments, an average of 28 ± 8 pellets was consumed, which is equivalent to $1.45\% \text{ bw fish}^{-1}$ (Fig. 1). There were no significant differences in pellet intake (one-way-ANOVA, $F(3,108)=0.282$, $p=0.8$) or pellet intake variance (Bartlett-test, $K \text{ squared}=6.6688$, $\text{df}=3$, $p=0.08$) between animals starved over different time periods. When comparing homogeneity of variance against the control treatment, significant differences of variance are present for control to S2 treatment (Bartlett-test, $K \text{ squared} = 6.551$, $\text{df} = 1$, $p \text{ value} = 0.01048$). Different starvation periods had no impact on the minimum pellet intake (MPI) (Table 1). Generally, the control treatment showed the highest MPI values. Whilst in the control treatment, a pellet intake of minimum 10 pellets per fish was present for $100 \pm 0\%$ of fish, only $96.4 \pm 7.1\%$ was achieved for the S1, S2 and S3 treatment. At an MPI set at 20 pellets fish^{-1} , the control treatment was 7% (of total fish) higher than S1 and S3 and 11 % higher than the S2 treatment (Table 1).

Portion trial

There were no significant differences in pellet consumption or variance between fish fed different portion sizes across three treatments (GLM, neg.binom., $\vartheta=32.95$, $\text{Intercept}=3.3238$, $p=0.11$ OR ANOVA, $F(2,98)=2.281$, $p=0.108$). During the portion experiment, four of the 105 marked fish (3.8%—consuming zero pellets) showed abnormal swimming behaviour and

Fig. 1 Summary of the starvation experiment, the y-axis presents the ingested pellet fish⁻¹. The triangle presents the treatment mean, circles present outliers

denied feed uptake. These individuals were excluded from the data analysis. It is important to note that the exclusion of these fish do not change the results but reduced the data noise (Fig. 2).

An average of 27.8 ± 7.4 pellets was ingested on the addition of one portion (PA1), an average of 31.1 ± 7.4 pellets were ingested in treatments offering three or five portion per feeding event. Consequently, the feed intake per fish is $1.71\% \text{ bw fish}^{-1}$ for the one portion treatment and $1.91\% \text{ bw fish}^{-1}$ for all other treatments. Concomitant to the effect of different starvation periods, the portion number has an influence on the MPI. Whilst for all treatments more than 88% of fish ingested a minimum of 20 pellets fish⁻¹, 15% and 17% less fish ingested a minimum of 25 or 30 pellets fish⁻¹ when offered only one portion (Tables 2).

Daily feeding events

During the first feeding event in the morning, fish ingested an average of 28 ± 11 pellets ($1.19\% \text{ bw fish}^{-1}$) across all treatments. At the end of the feeding trial, fish in the FE1 treatment ingested 27.4 ± 5.8 pellets, fish fed twice daily (FE2) ingested 52.8 ± 11.5 pellets ($2.24\% \text{ bw fish}^{-1}$) and fish fed three times daily (FE3) ingested 58.8 ± 24.2 pellets ($2.5\% \text{ bw fish}^{-1}$). A Kruskal-Wallis-test revealed significant differences between the treatments (chi-squared=46.27, df=2, $p<0.01$; Fig. 3). Fish receiving only one feeding event per day ingested

Table 1 Percent of fish ($N=112$) that consumed a minimum of 10, 20 or 30 pellets during the feeding event of the starvation trial

MPI (% bw fish ⁻¹)	C [% fish] $\pm$ SD	S1 [% fish] $\pm$ SD	S2 [% fish] $\pm$ SD	S3 [% fish] $\pm$ SD
10—(0.52 %)	100 $\pm$ 0	96.4 $\pm$ 7.1	96.4 $\pm$ 7.1	96.4 $\pm$ 7.1
20—(1.04 %)	96.4 $\pm$ 7.1	89.3 $\pm$ 7.1	85.7 $\pm$ 0	89.3 $\pm$ 7.1
30—(1.56 %)	42.9 $\pm$ 11.7	46.4 $\pm$ 29.5	35.7 $\pm$ 18.4	35.7 $\pm$ 18.4

Fig. 2 Summary of the portion experiment, the y-axis shows the ingested pellet of each individual. The triangle presents the treatment mean, circles present outliers

significantly less diet (Dunn's test: $p < 0.01$) whilst no differences of mean pellet intake was present between fish receiving two or three feeding events daily (Dunn's test: $p = 0.92$).

Pellet uptake variance was not homogenous between the treatments (Bartlett-test, K square = 64.492, $df = 11$, $p < 0.01$). Furthermore, the inspection of the standard deviation in relation to the mean ingested pellets showed that feeding once or twice daily results in a relative standard deviation of 21.2 and 21.8% of the mean ingested pellets, whereas feeding three times daily leads to a relative standard deviation of 41.2% of the pellet mean intake.

Generally, the use of more than one feeding event per day roughly doubles the average pellet intake of fish (Fig. 3). Unfortunately, the standard deviation increases concomitantly resulting in higher data noise. At the end of the feeding trial, fish receiving only one feeding event consumed $1.19\% \text{ bw fish}^{-1} \text{ day}^{-1}$, $2.27 \text{ bw fish}^{-1} \text{ d}^{-1}$ and $2.62 \text{ bw fish}^{-1} \text{ d}^{-1}$ for the treatments with two and three daily feeding events, respectively.

The increase in daily feeding events increased the MPI. All fish ingested a minimum of 10 pellets throughout the treatments and fish being fed twice even reached an MPI of 20 pellets for all fish. For both, MPI of 30 and 40 pellets/fish, a significantly reduced number of fish was detected in the FE1 treatment, compared to the FE2 and FE3 treatments (MPI₃₀: chi-squared = 8.735, $df = 2$, $p = 0.01$; MPI₄₀: chi-squared = 8.307, $df = 2$, $p = 0.02$) (Table 3).

Table 2 Percent of fish ($N = 105$) that consumed a minimum of 10–30 pellets during the feeding event of the portion trial

MPI (% bw fish ⁻¹)	PA1 [% fish] ± SD	PA3 [% fish] ± SD	PA5 [% fish] ± SD
10—(0.62)	94.3±7.8	91.4±7.8	97.1±6.4
15—(0.93)	91.4±12.8	88.6±12	97.1±6.4
20—(1.24)	88.6±12	88.6±12	97.1±6.4
25—(1.54)	65.7±12.8	88.6±12	82.9±12
30—(1.85)	42.9±14.3	57.1±17.5	60±18.6

Fig. 3 Summary of the daily feeding event experiment, the y-axis shows the ingested pellet of each individual. The triangle presents the treatment mean, circles present outliers

Discussion

There is a dearth of reliable and applicable methods to provide information on feed uptake and feeding regimes at an individual level for finfish. There is also a lack of reliable data on individual fish feeding response to different feeding regimes and no quantification of evenness of diet uptake across fed groups of fish. The current study developed and applied a novel methodology to analyse individual diet uptake in Rainbow trout. The video method applied with extremely low-impact individual tagging of fish proved highly effective, although it is a time intensive tool to determine individual food uptake. It allowed the development of direct measures for evenness of diet uptake across a group of individual fish and provides insights into the efficacy of various known feeding regimes/methods considered viable for improving diet uptake.

Portion size, short-term starvation and daily feeding events are amongst a number of methods previously used as parameters to attempt to influence feed intake amongst farmed fish. The results in the present study demonstrated that the feeding procedure for a particular feeding event could be optimised by adjusting the food supply methods. Whilst short-term starvation (trial 1) and portion numbers (trial 2) effected no significant differences in feed

Table 3 Percent of fish ($N=84$) that consumed a minimum of 10–40 pellets during the feeding event of the daily feeding event trial

MPI (% bw fish ⁻¹)	FE1 [% fish] $\pm$ SD	FE2 [% fish] $\pm$ SD	FE3 [% fish] $\pm$ SD
10 (0.44)	100 $\pm$	100 $\pm$ 0	100 $\pm$ 0
20 (0.87)	92.9 $\pm$ 8.3	100 $\pm$ 0	97.1 $\pm$ 6.4
30 (1.30)	53.7 $\pm$ 18.4**	91.4 $\pm$ 19.2	91.4 $\pm$ 12.8
40 (1.74)	3.6 $\pm$ 7.2*	77.1 $\pm$ 16.3	74.3 $\pm$ 18.6

Significant differences within each MPI group are given by an asterisk (** $p<0.01$, * $p<0.05$)

intake homoscedasticity for rainbow trout, a clear statement of the effect of different feeding regimes and their advantages can be made using MPI.

Starvation was ineffective in improving diet uptake. Equally, in this study, the distribution of the first 20 pellets each fish consumed was more homogeneous without starvation or with short starvation periods. Research on starvation periods has been linked to compensatory feeding and food efficiency. Information on individual food consumption has not been stated so far or was determined indirectly with automatic feeders (Brännäs and Alanärä 1993). In the current study, one feeding event in the morning during the starvation trial was used for video evaluations. With a notional second food addition in the evening, hyperphagia could have been amplified and differences observable. Similar results were observed by Azodi et al. (2015), who tested refeeding after short-term starvation (1–3 days) on 17g rainbow trout fingerlings. However, feed intake was determined indirect by feed intake of all fish. No significant effects were found even though daily feed intake of deprived groups (at feeding) tended to be higher than the continuously fed control group. They concluded “that rainbow trout has the ability to achieve full compensatory growth during short-term starvation and re-feeding periods.”

Whilst food deprivation leads to an increase in feed efficiency, food restrictions are better known for inducing hyperphagia (Boujard et al. 2000). In sustainable and equitable aquaculture, good fish welfare is highly beneficial, as poor fish status will lead to a decreased immune response post-vaccination and at the same time decrease resistance against diseases (Yarahmadi et al. 2016). Even though food restriction may have a significantly higher influence on feed intake and intake homoscedasticity as per Boujard et al. (2000), food deprivation as used in trial 1 is an easier method to apply in current aquaculture facilities.

Cho and Bureau (2001) reviewed different diet formulation strategies and feeding systems to reduce feed wastage, stating that the use of demand feeding with restricted portions increases pellet intake of fish and reduces feed leftovers. Moreover, they proposed that fish are given the opportunity in time and space to satisfy their hunger. Both conclusions are integrated and verified in this study. Current results showed that by the addition of only one portion, feed intake is 10% lower than by the addition of 3 or 5 portions. Remarkably, the standard deviation of the 3 and 5 portion treatments did not increase concurrently with the feed intake, leading to relatively better feed distribution amongst the multiple portion treatments. Even though the increase is not significant, the analysis of the MPI supports the trend that the application of multiple portions during one feeding event is beneficial for homogeneous feed intake. The differences in MPI between one vs. multiple food portions are conspicuous. When fed one portion, approx. 15% less animals which have eaten 30 pellets despite the delivery of exactly the same total amount of feed in all portion treatments.

Under different feeding regimes, the majority of fish eat either in the first 2 to 3 h of light or within two phases; one in the morning and one in the evening (Boujard et al. 2002). These two daily feeding event scenarios match the findings of this study, where the best homoscedasticity of feed intake and MPI was present with 1 or 2 feeding events daily. When higher MPIs are required, the use of two feeding events per day is favourable. An additional feeding event at the end of the day nearly doubles the feed intake and the standard deviation. However, the MPI of 30 pellets is thereby increased from 54 to 100% of all fish from FE1 to FE2 daily. The addition of one more feeding event per day to a total of 3 event did not increase the total feed intake per day and also unfavourably decreased intake homogeneity. It is suggested that this represents simply a further (excess) feeding event which may offer no great feeding benefit but still contributes to increased stress by feeding activity related to avidity (greed) or increased swimming speed, metabolism or competition during the feeding event. Similar feeding

behaviour was described by Alanärä (1992) when investigating the differences of demand and time restricted feeding. Alanärä (1992) concluded that intensive feeding twice a day in the morning and evening is favourable and that increased numbers of feeding events induces stress and high competition for which might also be the case for this trial. Alternatively, as Rasmussen et al. (2007) suggested, increased feeding event frequency may lead to more aggressive interactions between fish. However, significant aggression was not observed in the current study.

Increased fish stress can have a variety of sources such as increased rearing densities, aggression or food supply. An easily accessible and representative indicator is the condition of the dorsal fin (Rasmussen et al. 2007). Rasmussen et al. (2007) found increased feeding frequency leads to degraded fin condition so that lower daily feeding frequencies are beneficial for high stock densities. An influence of food supply in terms of fish condition was not found in this study possibly due to low fish density. Rearing a small number of salmonids can cause territorial behaviour and aggression and is described in a variety of studies (Alanärä and Brännäs 1996; Laursen et al. 2015; North et al. 2006). However, in the current study negligible feeding or territorial aggressions occurred during the video records. In the current study, it is difficult to predict if increases or decreases in fish numbers would have resulted in different levels of aggression as behaviour exhibits high levels of plasticity in behaviour under varying densities and holding regimes (Harwood et al. 2002). Greater numbers of fish allow for greater stress in terms of feeding competition interactions at feeding events; however, aggression may offer limited benefits to fish when feeding at higher densities (Harwood et al. 2002). Rasmussen et al. (2007) also reported lower levels of aggression at higher densities and suggested aggression may be advantage for feeding strategy at lower densities. In this study, decreased territorial behaviour may result from higher densities.

Hence, it is tentatively presumed that for higher densities ($> 2 \text{ kg m}^{-3}$) or upscaled experiments, less aggressive behaviour will occur due to the above stated knowledge of salmonids reared at high densities. Further development or practice will assumedly allow larger groups of fish to be monitored in controlled feeding using this method. Equally, the advancement of artificial intelligence and computer-aided monitoring systems promises to vastly increase the number of fish that can be accurately monitored with these methods.

No significant effects of the different feeding scenarios were present. This is supported by the literature whereby Mäkinen (1993) investigated the feeding rhythm of rainbow trout and neither continuous feeding nor diurnal peak feeding had an advantage on feeding. However, unlike the current study, a significant tank effect was present in Mäkinen's study. Grayton and Beamish (1977) tested different feeding frequencies of rainbow trout fingerlings ranging from 1 meal per 2 days to 6 meals per day and in terms of fish growth, best performance is achieved when applying one or two meals per day. Grayton and Beamish's (1977) similarly found applying two meals per day ensured fish growth (as an indirect measure of feeding success) was maximised. Similar results have been reported for larger rainbow trout (60–130g) where 8 h feeding cycles are beneficial (Landless 1976). Whilst the use of only one feeding event for rainbow trout ($\approx 155\text{g}$) is efficient for growth (Başçınar et al. 2007), the application of two daily feeding events decreases feed intake variations, which is highly beneficial for feeding regimes and delivery of special supplement diets or oral vaccines.

The video surveillance and analysis method developed herein allow us to state that MPI can be maximised and homoscedasticity of diet intake increased by feeding unstarved rainbow trout twice daily with multiple portions (3). With this applied feeding regime, the chance that each individual ingests a nominal minimum of 20 pellets ($\approx 1\% \text{ bw fish}^{-1}$) is maximised to

nearly 100%. By this recommendation, not only homogeneity and MPI of pellet uptake is maximised but also pellet wastage is minimised, leading to better cost efficiency and water quality. On the basis of the results of the starvation experiment in the current study, it is likely that results of the portion and daily feeding event experiment could be more explicit when applying absolutely no starvation period pre-feeding. Furthermore, results of the daily feeding event trial could be further optimised by the use of multiple portions. These recommendations differ from those of the FAO for homogeneous rainbow trout fingerlings growth which recommended a feeding frequency of 4 feeding events day⁻¹ with 1% bw fish⁻¹.

In addition to presenting a viable and applicable method for measuring individual feed intake in rainbow trout, the authors develop the minimum pellet ingestion (MPI) parameter. The MPI is an important tool for the optimization of feeding regimes and can also play a role in application of oral veterinary treatments or vaccines. The MPI measure is extremely useful when not only the quantity of feed intake is important but also minimal treatment or minimal vaccine intake across the entire fish population needs to be determined. To date, research on oral vaccination mainly used individual handfeeding delivery methods. It is very unlikely that the vaccination approach of, e.g. Adelman et al. (2008) or Ballesteros et al. (2014) can be reproduced at commercial scales to deliver treatments or vaccines and guarantee MPI. Diverse current research on vaccine active components (Adelman et al. 2008; Børgwald and Dalmo 2019; Villumsen et al. 2014) and their administration methods (Bowersock et al. 1999; Ellis 1998; Garinot et al. 2007) with different protective mechanisms for the vaccine particles exists. Yet, practical vaccine oral delivery methods require fundamental research outputs. This study provides methods and quantitative results related to direct determination and modification of pellet uptake. In doing so, it partially bridges the knowledge gap between the active and adjustable components of a vaccine and the fundamental feeding regime required to ensure accurate oral delivery. This lends weight to the value of the methods presented herein to accurately understand and optimise MPI under different feeding regimes to allow accurate veterinary dosing under aquaculture conditions.

Future applications of these methods are planned for other key aquaculture species and in the application and optimisation of oral veterinary treatments. Due to the presented method, the examination of, e.g. attractants, the acceptance of different transport matrixes for vaccination or supplements of new feed ingredients can be performed fish conservative, specific and fast.

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Author contribution Philip N. Just: Conceptualization, methodology, sampling and evaluation, writing of original draft, manuscript reviewing and editing. Bernd Koellner: Conceptualization and reviewing. Matthew James Slater: Supervision, methodology review, validation, manuscript review and editing.

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Data availability The authors will provide the original data if required and requested.

Declarations

Ethics approval The authors followed all ethical policies of the journal in all methods and actions. Specifically, all experiments were conducted in accordance with the German Animal Protection Act (TierSchG) and the regulations on the protection of animals used for experiments or other scientific purposes (TierSchVersV) in agreement with applicable EU directives and were approved by the Veterinary Authority of Bremen under the administrative number TV148.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no conflict of interest.

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