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The behavioural repertoire of Arctic charr (*Salvelinus alpinus* (L.)) in captivity: a case study for testing ethogram completeness and reducing observer effects

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Abstract – In the last 20 years, research has been directed towards possible differences in the mating behaviour of species belonging to the Salmonidae family that may reproductively isolate wild populations from escaped hatchery or farmed fish. Despite these studies, a detailed description of the overall behavioural repertoire of Salmonidae species from wild and farmed environments is still lacking. Furthermore, although Arctic charr has been described as the most variable between all vertebrate species, possible behavioural plasticity outside of the breeding season has not been widely investigated, and a complete ethogram for Arctic charr not in breeding condition is currently unavailable. This study presents the first complete ethogram of captive Arctic charr behaviour outside of the breeding season. The completeness of this ethogram was validated based on the Behavioural Accumulation Curves methodology, a reliable and easy to use tool for assessing the best compromise between sampling effort and ethogram completeness. Additionally, a new way of presenting an ethogram has been proposed and validated using a dichotomous key to describe behaviour types. This proved to be a more effective operational tool for identifying Arctic charr behaviour than the ethogram. The dichotomous key of behaviour led to a significantly less ambiguous identification of behavioural units, thus reducing observer, recording errors and enhancing accuracy. This study therefore represents an effective step forward to a more in-depth and rigorous comparison of Arctic charr behavioural adaptation between and within artificial and natural settings.

Key words: ethogram; dichotomous key of behaviour; behavioural accumulation curves; fish; captivity

Introduction

Arctic charr (*Salvelinus alpinus* L., 1758) is the northernmost freshwater fish with over 50,000 populations recorded worldwide, from Scandinavia to Canada, Russia, Iceland, British Isles and Alps (Klemetsen et al. 2003). The native distribution of this Salmonidae species matches closely the last glaciation in the Holarctic (Johnson 1980; Beddow et al. 1998; Klemetsen et al. 2003). Arctic charr is therefore specialised in the extreme as it thrives where no other fish do, such as in freshwaters of the highest northern latitudes (40N°–82N° latitude), the highest altitudes (2344 m a.s.l. in Oberer Plenderlesee, Austria) and of the greatest water depths (up to 220 m depth in Loch Ness, Scotland) (Shine et al. 1993; Hofer & Medgyesy 1997; Klemetsen et al. 2003; Adams et al. 2010). This large variation of habitats is reflected by high diversity in its genetic constitution and high levels of polymorphism (Klemetsen et al. 2003), such that Arctic charr has been defined as the most variable of all known vertebrates (Adams et al. 2010).

Regarding the conservation status of wild populations, Arctic charr is classified as Least Concern in the IUCN Red List (Freyhof & Kottelat 2008). In Europe, Arctic charr is not listed as a priority or Annex II species under the EU Habitat's Directive

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(92/43/ECC) and it is therefore not offered direct protection (Igoe & Hammar 2004). However, populations are disappearing in most countries and European populations have undergone a serious decline (Igoe et al. 2003). The main threats to remaining Arctic charr populations include water pollution, acidification, abstraction, afforestation, competition with nonnative fish species, global warming, exploitation and aquaculture (Igoe et al. 2003; Winfield et al. 2008).

In Ireland, for example, approximately one-third (24) of all known native populations (70) are most likely extinct (Igoe et al. 2003): Arctic charr has therefore been classified as vulnerable in the Irish Red List of 2011 (King et al. 2011) and is now protected under national legislation. Also, a number of lakes with Arctic charr also contain either Annex I or Annex II species, so the designation of special areas of conservation should impart some protection to the Irish resident Arctic charr, whose populations are monitored yearly by Inland Fisheries Ireland, the state agency responsible for the protection, management and conservation of Ireland's inland fisheries and sea angling resources (Igoe & Hammar 2004; Rooney et al. 2014).

In the last decades, native wild Salmonidae populations have declined worldwide while hatcheries and farming activities have increased (Esteve 2005). Atlantic salmon (*Salmo salar* (L., 1758)) entered commercial aquaculture in 1973, and since 1998 its aquaculture biomass exceeded that of wild populations (Gross 1998). For this species, escapees from farms raise significant concerns about the genetic and ecological integrity of wild populations (Gross 1998). Arctic charr farming is not as extensive as that of Atlantic salmon; however, it is currently successfully farmed in Iceland, Canada, Sweden, Norway, Finland, Estonia, USA (West Virginia) and Ireland (Thorarinsdottir 2013). Iceland has the biggest production of farmed Arctic charr, and this has more than doubled in the last decade, passing from around 1300 tonnes in 2004 to up to 3300 tonnes in 2013 (Runarsson 2013).

During spawning, Salmonidae species share common behavioural patterns and courtship signals (Esteve 2005). While females select nesting areas (redds) on gravel beds where they dig a series of depressions for egg incubation (Groot 1996; Esteve 2005, 2007), males divide their time between competing with other males and courting nesting females (Esteve 2005). Fine divergence in courtship displays as well as in other behavioural traits can play an important role in sexual isolation between closely related species (Tinbergen 1951; Kitano et al. 2007). As a consequence, in the Salmonidae family, research has been directed towards possible behavioural differences in mating that may reproductively isolate wild populations from escaped hatchery or farmed fish (Fleming & Gross 1993; Fleming et al. 1996; Chebanov & Riddell 1998; Berejikian et al. 2000; Fleming & Petersson 2001; Esteve 2005). Although the literature on Salmonidae breeding behaviour is quite extensive (Jones 1959; Frost 1965; Gaudemar & Beall 1999; Esteve 2005, 2007), a detailed description of the overall behavioural repertoire of species belonging to the Salmonidae family, as well as a standard, shared terminology in ethograms is still lacking.

Phenotypic plasticity is a trait subject to natural selection and evolutionary trend (West-Eberhard 1989). Behavioural plasticity outside of the breeding season has a large impact on activities, which are crucial for fish survival, such as feeding (Werner et al. 1981; Dill 1983; Ehlinger 1989a,b, 1990; Croy & Hughes 1991). For example, in the threespine stickleback (*Gasterosteus* spp.), behavioural plasticity significantly affects food searching efficiency (Day & MacPhail 1996). Arctic charr shows an enormous phenotypic plasticity in its ecology and biology throughout its natural range (Doherty & McCarthy 2004; Adams et al. 2010), but possible behavioural plasticity outside of the breeding season has not been widely investigated and a complete ethogram for Arctic char not in breeding condition is currently unavailable. Filling this knowledge gap is the starting point for facilitating phylogenetic, phenotypic and ecological comparisons within the Salmonidae family and between Arctic charr populations.

An ethogram is a set of comprehensive descriptions of the behavioural units that characterise the behavioural pattern of an animal species, and it should be the starting point of any ethological study (Lehner 1996). There are a number of factors that an ethogram should target, where each behavioural unit should be precisely defined and objectively labelled. Behavioural units should also be homogeneous, mutually exclusive and independent of each other (Cohn & MacPhail 1996; Martin & Bateson 2009). When compiling an ethogram a compromise must be reached between sampling effort and the completeness of the catalogue (i.e. the ethogram should be exhaustive) (Martin & Bateson 2009). Behavioural Accumulation Curves (BAC), a methodology that was originally developed for investigating floristic and faunistic inventories completeness (Colwell & Coddington 1994; Gotelli & Colwell 2001; Dias et al. 2009), are an effective tool for analysing ethogram completeness. The use of such a standardised methodology allows direct comparison between studies that describe behavioural repertoires and lend rigour to those comparisons (Dias et al. 2009).

The scientific study of animal behaviour assumes that observations reflect what animals are actually doing (Marsh & Hanlon 2004). Several potential observer effects may severely impair the accuracy and precision with which behaviour is addressed (Lehner 1996): (i) Hawthorne effect: where the visual presence of the observer may influence animal behaviour; (ii) observer error: caused by many factors, including observer inexperience and poorly defined behavioural units; (iii) observer bias: due to strong conscious or unconscious expectations about the outcome of an experiment; and (iv) recording errors: due to poor technique and equipment (Lehner 1996). When compiling an ethogram multiple efforts have to be made both for assessing its completeness and for minimising observer effects.

The aims of this paper were to (i) provide the first complete ethogram of Arctic charr behaviour in captivity outside of the breeding season; (ii) test the completeness of the ethogram using the BAC method (Dias et al. 2009); (iii) suggest a new way of presenting an ethogram, that is in the form of a simple dichotomous key of behaviour descriptions (i.e. a key in which each question has two possible answer; Osborne 1962); and (iv) test the effectiveness of the dichotomous key of behaviour in enhancing accuracy and precision of the investigation.

Materials and methods

Fish collection and maintenance

Forty-five Arctic charr specimens (24 months old, 25 males, 20 females, average weight 500 g) were purchased in April 2013 from the Organic Aquaculture Enterprise Stofnfiskur Ltd (Corandulla, Co. Galway, Ireland). All the forty-five specimens were full sibling of the fifth farm generation of originally wild native Icelandic population. Eggs hatched at Stofnfiskur Ltd on 5 Feburary 2011; as fish grew, they were transferred to tanks of various capacities (15 tanks of 1 m^3, 10 of 2 m^3, 35 of 12 m^3, 5 of 140 m^3 and 5 of 170 m^3 capacity) with an average stocking density of 50 $kg \cdot m^{-3}$ without sex isolation. For the first 6 months after first feeding, photoperiod was 24 h daylight; then, natural photoperiod was allowed. Fish were fed daily with fish pellets ‘Ephico Alpha’ manufactured by BioMar in Denmark. The same food regime was used in Galway-Mayo Institute of Technology (GMIT) throughout the duration of the experiment.

At GMIT, fish were kept at the stocking facility of the ‘Total Energy Solutions for Sustainable Aquaculture’ (TESSA) project: this consists of an aquaculture recirculating system (ARS) of three fibreglass tanks (3600 l in total, 1.4 m diameter and 0.7 m high). Fish were provided with well-oxygenated freshwater maintained at 12 °C (T_{min} = 10.2 °C, T_{max} = 14 °C) using an external chiller; water chemical parameters (pH, nitrates, nitrites, phosphates and ammonia) were checked on a daily basis. A 14L:10D photoperiod was used from the collection of the fish (April 2013) and for the duration of this study (May 2013).

Data collection and ethogram preparation

To compile the ethogram, Arctic charr behaviour was observed in one of the stock tanks of the TESSA project in GMIT during May 2013 for a total of 1125 min (equally distributed over the daylight hours) using underwater video recordings. Recordings were obtained in a tank with 10 males and 10 females using an underwater camera (Jetview Electronics, JFCM26-B) from which output was digitised (Pinnacle Dazzle DVD Recorded Plus) and then recorded in.avi format using a personal computer running Pinnacle studio v.12. Videos were observed on a television (Samsung LE32B45), and, using an ad libitum sampling (Lehner 1996), the ethogram was compiled by the author (M.B.) both in the traditional fashion (i.e. a comprehensive description of named behaviours) and as a simple dichotomous key of behaviours (Osborne 1962) (see Results section 1 and 2).

Dichotomous key reliability

Five behavioural units (swimming, resting, circling, bite attempts and resting with spread fins) were randomly selected within the ethogram by the author (M.B.). These were shown to 23 independent observers (ranging from final year undergraduate students to PhD students at GMIT, Galway, Ireland). None of these 23 observers were familiar with Arctic charr behaviour prior to the experiment.

Each observer was provided with both tools (i.e. ethogram and dichotomous key of behaviour) and was asked to identify each behavioural unit using only one specific tool. The assignment of the tool to be used for the identification of each behavioural unit was randomised before the experiment by the author (M.B.) between and within observers, resulting in a total of 57 behavioural units identified with the ethogram and 57 behavioural units with the dichotomous key (23 observers; each observer identified two behavioural units with one tool and three behavioural units with the other tool; total of 57 identifications with each tool). Observers were spaced throughout a lecture room, and they were not allowed to speak to each other for the duration of the experiment. Each observer was provided with the ethogram, dichotomous key of behaviour, a hard copy questionnaire and a watch (used by each student to measure the time taken for each behavioural unit identification).

The use of cell phones or personal laptops was not allowed. The author (M.B.) showed one video at a time using a projector connected to a personal computer. Each video depicted one of the five randomly selected behavioural units, and the next video was shown only when all the undergraduate observers had identified the behavioural unit. PhD student observers also carried out the experiment using the same videos and tools (ethogram, dichotomous key and questionnaire) but in isolation one from each other and using personal computers. For each behavioural unit identified, observers were asked to fill in a short questionnaire in which they indicated: (i) the tool used; (ii) the name of the identified behavioural unit; (iii) the time spent identifying behaviours; and (iv) how often they had to read the same passage and/or start again (ranked on a scale from 0 = never to 4 = often). At the end of the experiment, observers were asked to answer a direct question regarding which tool they found easier to use.

Statistical analyses on the results of the questionnaire were performed using the statistical package STATISTICA 8 (StatSoft, Dell). Nonparametric statistics were used because assumptions for parametric tests were not met (data were ordinal and non-normal; Shapiro test, $P = 0.00$).

Comparisons between the ethogram and the dichotomous key reliability were made with the Mann–Whitney U-test (two tailed; alpha level of 0.05).

Is the ethogram exhaustive? BAC analysis

An instantaneous sampling (Lehner 1996) on a time grid of 2 min ($N = 376$) was carried out on 752 min of underwater video recordings, collected as previously described. All video recordings were observed on a television (Samsung LE32B45): the occurrence of all behavioural units (as defined in the ethogram) performed by the fish framed by the camera was scored every two minutes on a previously prepared ad hoc matrix using a personal computer. In particular, data were entered in a matrix in which the rows represented the observed behavioural units (scored as 1 for presence and 0 for absence) and the columns represented the sampling units. This matrix was then loaded onto EstimateS, a free software application for Windows and Macintosh available at http://viceroy.eeb.uconn.edu/ EstimateS (Colwell 2006). EstimateS allows for calculating the mean number of new behavioural activities observed for each sampling unit (2 min) accumulated up to the total sample size. To prevent the form of the accumulation curves being affected by temporal biases in sampling effort, data were randomised.

Results

Arctic charr ethogram

Locomotion

Swimming: to move from one point to another, the fish resorts to a subcarangiform locomotion (Moyle & Cech 2004). This type of locomotion involves the rhythmic undulations of the rear part of the body accompanied by rhythmic beats of the caudal fin. To equilibrate the body in the water column the pectoral fins may be spread.

Emphasised swimming: similar to swimming, but in this case, the body and tail movements are more conspicuous and fast.

Swimming in place: fish continuously undulates the rear part of the body and rhythmically beats the caudal fin; pectoral fins may undulate at the same time as the tail beats. This does not result in any change of position: the fish always maintains its position at the same point of the water column, usually against the water flow.

Sprint: a fish that is swimming instantly increases its swimming speed as a result of more conspicuous tail beats.

Glide: with spread pectoral fins, fish moves slowly in the water column without changing directions as a result of slow and relatively low-intensity pectoral fin quivers.

Change direction: while swimming, thanks to a vigorous tail shake, the fish changes direction.

Stationary positions

Resting: the fish is in a stationary position, usually on the bottom, but sometimes, this behavioural unit occurs even in the water column. Movement is not detectable. Pectoral fins are close to the body.

Resting with spread fins: this behaviour is similar to resting, but in this case, pectoral fins are spread or the fish is settled with the tips of the pectoral fins touching the bottom.

Low-intensity quivering: the fish is in resting position close to the bottom, and it quivers the pectoral fins continuously with a relatively low repetition rate.

Quivering: the fish is in resting position close to the bottom, and it quivers the pectoral fins continuously with a relatively fast repetition rate. In this case, the body also quivers slightly.

Quivering with tail movements: this behaviour is similar to quivering, but in this case, the fish moves the tail too. Tail beats are relatively low in intensity and in repetition rate.

Quivering in the water column: this behaviour is similar to quivering but is performed in the water column.

Resting with tail movements: similar to swimming in place, but in this case, the tail beats are less vigorous and occur with a really low repetition rate (at maximum one tail beat every 2 s). In contrast with the swimming in place behaviour, in this case, the tail moves in an approximately circular pattern.

Social interactions and display
Showing the belly: the fish, close to the bottom, turns upside down: the belly is facing the surface, and the body is shaken by continuous and vigorous oscillations from the pelvic fins to the tail. Then, the fish turns in a normal position (dorsal fin facing the surface) and after a very short period (up to 1 s) the fish turns upside down again, repeating this pattern up to 3 or 4 times.

Circling: two individuals swim in a circular pattern, one following the other's tail from a very short distance. Swimming is relatively slow.

Approach with quivering: when two fish are resting close to each other (i.e. <1 body length apart), one approaches the other while quivering the pectoral fins. The approach is slow: the propulsion is given by the energy of quivering.

Shakes: when two fish are resting close to each other (i.e. <1 body length apart), one starts to rapidly shake the body from tail to pelvic fins with a relatively fast repetition rate.

Chasing: two individuals swim together, but not in a circular pattern, one following the other's tail from a very short distance. Swimming is rapid and the mouth of the chaser is very close to the tail of the chased fish.

Bite attempt: two individuals are performing either the swimming in a circle or the chasing behaviour. The follower approaches the flank of the followed fish and attempts a bite. This behaviour can be repeated more than once if the followed individual does not rapidly escape.

Escape: one fish swims rapidly away from a conspecific, as a result of strong movements of the tail, the pectoral fins and the body, in any direction that can provide an escape route.

Pushing: an individual approaches another individual who is in a resting position. The approaching individual forces the other to move by pushing with the snout on the other individual's flank.

Feeding
Prefeeding: before the feeding behaviours are performed, probably elicited from visual or chemical food stimulation, the fish starts to convulsively shake the entire body, from the pelvic fins to the tail.

Feeding in the water column: the fish eats the food on the surface or in the water column. The approach to food is relatively fast, and the fish usually swims towards the food while it sinks in the water column.

Feeding on the bottom: the fish eats the food on the bottom. The approach to the food is slower than in the feeding in the water column behaviour. The fish approaches the food from an upper position, the fish body is at an angle, with the mouth on the bottom grabbing the food and the tail in the water column. The fish may repeat this behaviour to eat a second particle of food: rarely, it eats several food particles in the same sequence.

Dichotomous key of Arctic charr behaviour

The first step necessary when using the dichotomous key is to decide to which of the subsequent groups (A, B or C) the observed behaviour belongs. Once the group is identified, the next step is to follow the identification instructions and steps of the dichotomous key. For a detailed description of the behavioural units identified, the ethogram may be consulted.
GROUP A: the behaviour is not performed towards/with other individuals; go to step 1.
GROUP B: food is involved in the performance of the observed behaviour; go to point 13.
GROUP C: the behaviour involves more than one individual (i.e. social behaviour). Social behaviours also include those behaviours that are performed by one individual, but they are oriented towards another (e.g. visual display); go to step 15.

1. (group A) The fish moves from one point to another in the water; go to step 2.

   The fish is always at the same point of the water column or it is always at the same point on the bottom; go to step 6.

2. (1) Body movements are detectable; go to step 3.

   Body movements are not detectable; GLIDE.

3. (2) Fish is moving with a constant speed; go to step 4.

   Fish is moving with a clearly detectable acceleration pattern; SPRINT.

4. (3) Fish body movements appear proportionate to the activity it is performing (i.e. no emphasised movements are detectable); go to step 5.

   Fish body movements are emphasised; EMPHASISED SWIMMING.

5. (4) While the fish is moving, it maintains the same direction; SWIMMING.

   While the fish is moving, it changes direction; CHANGE DIRECTION.

6. (1) Body movements are not detectable; go to step 7.

   Body movements are detectable; go to step 8.
7. (6) The pectoral fins are close to the fish body; RESTING.

   The pectoral fins are spread; RESTING WITH SPREAD FINS.
8. (6) The main movements are performed by the tail; go to step 9.

   The main movements are performed by the pectoral fins; go to step 10.
9. (8) Fast and vigorous tail beats; SWIMMING IN PLACE.

   Slow tail beats; RESTING WITH TAIL MOVEMENTS.
10. (8) Tail is moving; QUIVERING WITH TAIL MOVEMENTS.

    Tail is not moving; go to step 11.
11. (10) The behaviour is performed in the water column; QUIVERING IN THE WATER COLUMN.

    The behaviour is performed on the bottom; go to step 12.
12. (11) Fast beats of the pectoral fins; QUIVERING.

    Slow beats of the pectoral fins; LOW-INTENSITY QUIVERING.
13. (group B) The fish takes food; go to step 14.

    The fish does not take food; PREFEEDING.
14. (13) The behaviour is performed in the water column; FEEDING IN THE WATER COLUMN.

    The behaviour is performed on the bottom; FEEDING ON THE BOTTOM.
15. (group C) The behaviour involves swimming; go to step 16.

    The behaviour does not involve swimming; go to step 19.
16. (15) The fish is swimming in a circular pattern; CIRCLING.

    The fish is not swimming in a circular pattern; go to step 17.
17. (16) The individual observed is chased a short distance by another; ESCAPE

    The individual observed is chasing another; go to step 18
18. (17) Bite attempt; BITE ATTEMPT

    No bite attempt; CHASING
19. (15) A physical contact between two individuals is detectable; PUSHING.

    No physical contact is detectable; go to step 20.
20. (19) The behaviour is directed towards another individual; APPROACH WITH QUIVER.

    The visual display or the behaviour is not directed towards another individual; go to step 21.
21. (20) The dorsal fin is always facing the water surface; SHAKES.

    The fish belly is facing the water surface for at least a fraction of the behavioural unit; SHOWING THE BELLY.

Dichotomous key reliability

Neither of the two proposed tools for the identification of Arctic charr behaviours (i.e. ethogram and dichotomous key) lead to faster identification of one behavioural unit ($N = 57$; $U = 1521$; $P = 0.55$). On average, the time taken to identify a behaviour was the same between the two methodological tools used (ethogram: mean = 2.16 min; min = 1 min; max = 5 min; SD = 0.9. Dichotomous key: mean = 2.13 min; min = 1 min; max = 4 min; SD = 1.0).

Results showed no significant difference in the frequency with which an observer had to read the same passage twice and/or start again between the two tools ($N = 57$; $U = 1596$; $P = 0.86$).

Significant results were found when comparing the preference for one specific tool; in particular, 65% of the observers preferred to rely on the dichotomous key of behaviour rather than on the ethogram, considering it less ambiguous and easier to use ($N = 23$; $U = 184$; $P = 0.04$).

Furthermore, the dichotomous key of behaviour allowed a more precise identification of the behavioural units: 87% of the behaviours were correctly identified with the dichotomous key while only 61% were correctly identified using the ethogram ($N = 57$; $U = 119$; $P = 0.01$).

Is the ethogram exhaustive?

As expected, the number of new behavioural units observed decreased progressively with increasing sampling effort (Fig. 1). During the 376 2-minute samples, 24 different behavioural units were observed: data fitted well with the Clench model's function, giving an estimated asymptotic repertoire size of 24.74 (a = 2.59; b = 0.10). Therefore, the observed proportion of the total repertoire was 96.9%. This means that the ethogram here presented is representative of the behaviour of Arctic charr in captivity (at least from the population and for the setting used in this study).

Discussion

Ethograms represent the starting point of any behavioural study, and they should include all the behaviours of the species observed (Lehner 1996; Dawkins 2009; Martin & Bateson 2009). However, ethograms are often generated from an operative perspective, presenting the fractions of the behavioural pattern of an animal species on which further research will be focused (Martin & Bateson 2009). Often ethograms are related to the setting in which animals are observed, that is artificial or natural setting. Prior to this study, an ethogram of Arctic charr behaviour outside of the breeding season was not available for both wild and captive populations. We present the first ethogram, inclusive of all the behavioural units observed in Arctic charr when in a farmed setting and outside of the breeding season (i.e. the most typical situation in a farm). Considering that an accurate behavioural description is a prerequisite for behavioural comparison, this ethogram may serve as a starting point for comparing captive behaviour of closely related Salmonidae species or for comparing Arctic charr behaviour within and between wild and farmed settings. This is especially important when searching for behavioural differences that may contribute to reproductive isolation of wild and escaped populations.

Presently, a comparison with the behaviour of other Salmonidae species when not in breeding conditions is difficult to achieve considering the knowledge gaps in such work. However, circling is a well-described behavioural unit occurring in Salmonidae during the breeding season (Berst et al. 1981; Esteve 2005) and it is interesting to note that this behaviour occurs even in artificial settings when water and light parameters are different to those during the breeding season and when stock density is maintained at low levels. Finally, the ethogram presented in this paper was generated by observing Arctic charr specimens housed at low stock density (10 kg·m^{-3}). However, stock density in commercial farms can be variable and this has been demonstrated to affect fish behaviour (Jørgensen et al. 1993). In particular, Arctic charr suffer less physical damage and grow more rapidly at high-density rates (i.e. 120 kg·m^{-3}) and furthermore, following Jørgensen et al. (1993), schooling behaviour does not occur in low-density setting (i.e. 15 kg·m^{-3}). Schooling behaviour was never noticed during the present study. Considering that Backström et al. (2014) demonstrated a connection between Arctic char captive behaviour and stress responsiveness, further investigation on qualitative differences in Arctic charr behaviour when housed at different stocking densities could therefore help to reduce stress and maximise fish welfare in captivity.

Considering that animal behaviour can be defined as a continuous stream of actions (Martin & Bateson

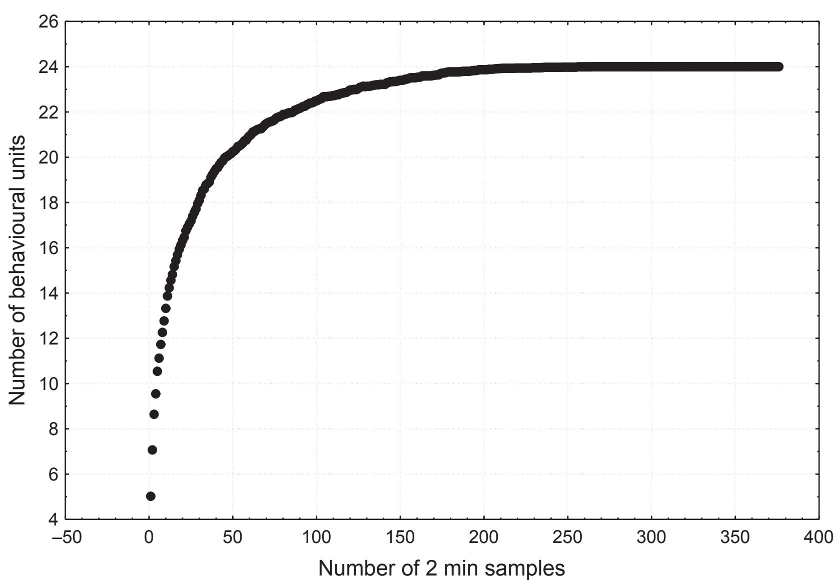

Fig. 1. Behavioural Accumulation Curve of Arctic charr behaviour in captivity outside the breeding season obtained with a total of 376 instantaneous samples taken every 2 min.

2009), researchers have to divide this stream into independent and well-defined units to measure it. One of the most challenging aspects of creating an ethogram is the assessment of the ethogram completeness. The Behavioural Accumulation Curve methodology demonstrates that the ethogram presented here covers approximately 96% of the total-behavioural repertoire of Arctic charr; therefore, it should be noted that there may still be some behaviours missing from the ethogram. For example, although we observed bite attempts, the occurrence of a bite was never observed. The present study supports the conclusion and methodology of Dias et al. (2009) proving that Behavioural Accumulation Curves are a reliable and easy to use tool for assessing the best compromise between sampling effort and ethogram completeness. The completeness of the Arctic charr ethogram has been reached with a smaller sampling effort than the one of Dias et al. (2009), probably due to a smaller resolution and a less complex behavioural pattern of the species observed Therefore, even though Behavioural Accumulation Curves represent a tool for interspecific comparison, their output appears to be strongly species and setting dependant. However, the use of such a standardised methodology could lend rigour to the comparison between studies that describe behavioural repertoires of Arctic charr in natural or artificial settings or between closely related species.

Finally, a new way of presenting an ethogram was proposed and validated during the present study. The dichotomous key of behaviour significantly reduced both observer and recording errors, leading to a more rigorous identification of the observed behaviours, even when the observers had little or no experience in animal behaviour observations. Observer error is due to many factors, including poor observer experience and badly designed tools for animal identification (Lehner 1996). Providing better designed tools is advantageous when observers are not experts in scientific terminology and methodology for animal behaviour investigations. Observer error is just one of the possible observer effects causing incorrect identification of animal behaviour (Lehner 1996; Dawkins 2009). Other sources of within observer imprecision are observer bias and the Hawthorne effect (Lehner 1996). Furthermore, it is important to reduce the variability with which different observers identify the same behaviour (Lehner 1996; Dawkins 2009; Martin & Bateson 2009; Burghardt et al. 2012). Different measures are devised to minimise as many sources as possible of variability and error in animal behaviour investigations (observer effects and interobserver reliability) and should be therefore adopted whenever possible.

In farming situations, more than one operator can be devoted to the fish welfare control, which is of fundamental importance to guarantee fish health and stress avoidance and to maximise production (Huntingford 2004). In these contexts, behavioural shifts are often the first indicator of different kinds of stressors acting on the animals. Operators usually use a behavioural listing score, in which the incidences of abnormal behaviours, such as stereotypic behaviour, inappropriate time budgeting or the occurrence of atypical behaviour, are all parameters to be measured (Wolfensohn & Lloyd 2013). The first step for the operator is to correctly identify the behaviour observed: once the behaviour is properly identified, shifts from the normal pattern can be noticed and, if necessary, welfare measures can be rapidly put in place. In such contexts, considering that behavioural analysis can be complex, the possibility of correctly identifying a behavioural occurrence has to be maximised (Wolfensohn & Lloyd 2013) and several measures can be proposed to achieve this goal. Providing operators with better designed tools represents one of the most important solutions to this problem. The use of better tools will reduce the possibility of observer and recording errors, and at the same time, enhances interobserver reliability (Lehner 1996). The dichotomous key of behaviour has proved to be an effective and less ambiguous tool for addressing animal behaviour than behavioural catalogues, and the one proposed in this paper, in particular, could be used as a baseline for checking healthy conditions in low-density farmed populations of Arctic charr. Furthermore, because observer and recording errors may be correlated with the number of behavioural units in an ethogram (Lehner 1996) to further reduce errors, some behavioural units in the ethogram we have presented could be classified as different subunits, or intensity levels, of the same behaviour. In particular, swimming and emphasised swimming could be considered as two different intensities of the behaviour swimming. Additionally, low-intensity quivering, quivering with tail movements, quivering in the water column and quivering could be consider as different intensities of the behaviour quivering. Finally, feeding in the water column and feeding on the bottom could be considered as different subunits of the behaviour feeding. However, the current ethogram proved to lead to 61% positive identification of behavioural units, increasing up to 87% when using the dichotomous key, even for naive observers.

Finally, in the case in which behavioural observations are part of the welfare monitoring measures in farm settings, a proper training of operators is recommended in order (i) to further reduce observer errors and (ii) to increase interobserver reliability. To avoid the possibility of observer bias, on the contrary, a self-conscious effort has to be enforced by each operator to limit as much as possible the expectations about the output of the observations. Furthermore, the tool provided should not leave space for observer interpretation. In the case where animals have been exposed to different treatments, observer bias could be minimised due to 'blind' observation, that is the observer is not aware of which treatment the animals have received (Lehner 1996; Dawkins 2009; Burghardt et al. 2012). Finally, behavioural observations on video recordings rather than direct observation of the animals are recommended for several reasons (i) to avoid Hawthorne effects (Lehner 1996); (ii) to further reduce observer errors especially in naive observers, where there is a possibility of observing the recorded behaviours more than once; and (iii) to reduce recording errors.

Burghardt et al. (2012) reviewed several hundred published articles from 1970 to 2010 in five leading animal behaviour journals and found that <10% of the articles reviewed adopted measures to reduce observer bias and to enanche interobserver reliability. Good practise measures in this situation include (i) prioritising the possibility of collecting data blind with regard to comparison group (i.e. avoid observer bias); (ii) having at least one co-author or other trained investigator to independently code a subset of the data collected for interobserver reliability measures; and (iii) reporting clearly in the manuscripts methods and/or reliability measures (Burghardt et al. 2012)

In conclusion, we have presented the first complete qualitative description of low-density captive Arctic charr behaviour when not in breeding season. We have further validated ethogram completeness, and we have designed it in a fashion which minimises observer and recording errors and increases interobserver reliability.

Previous research has shown that the conditions for Arctic charr sympatric divergence occur in the wild, where individual niche specialisation drives the generation of phenotypic variation and ontogenetic processes can play a part in the early stages of speciation (Adams et al. 2010). Considering that behavioural plasticity outside of the breeding season has not been widely investigated in Arctic char, the description of its behaviour in different wild and captive conditions could provide important insights in understanding the process of sympatric divergence, also from a conservation perspective.

This study therefore represents the first step in filling in this knowledge gap, and it may be considered as a starting point for facilitating phylogenetic, phenotypic and ecological comparisons between Arctic charr populations and within the Salmonidae family.

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