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Microsatellite DNA used for parentage identification of partly digested Atlantic salmon (Salmo salar) juveniles through non-destructive diet sampling in salmonids

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SHORT REPORT

Microsatellite DNA used for parentage identification of partly digested Atlantic salmon (Salmo salar) juveniles through non-destructive diet sampling in salmonids

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Abstract
Predation during early life history is an important component of fitness in salmonids. Farmed Atlantic salmon display lower survival in the wild in comparison to wild salmon; however, the underlying mechanisms remain unknown. Salmon eggs from 69 families of farmed, hybrid and wild parentage were planted into a river. Following swim-up, 760 brown trout predators were non-lethally sampled. Of the trout, 4.2% had ingested salmon fry (0–15 fry/trout). From a total of 48 salmon fry recovered from trout stomachs, 46 were successfully identified to family using microsatellites. Of the 69 planted families, 29 were represented among the predated salmon fry; however, there were no significant differences in susceptibility to predation between the three groups (farm, wild and crosses), but the power of resolution was low due to small sample sizes. Nevertheless, we have successfully demonstrated that microsatellites can be used to address natural selection via diet analysis of predators in a natural system.

Key words: Stomach analysis, non-destructive sampling, parentage identification, predation mortality, salmonids

Introduction
Natural mortality is high in salmonid fishes, especially during the early life stages (Wotton 1969). This starts immediately after swim-up, probably as a result of competition for territories and food and predation mortality associated with this behaviour (Elliott 1989, 1994). Mortality continues throughout the freshwater period due to predation from fish (Alexander 1979; Barstad et al. 1998; Vik et al. 2001), mammals (Heggenes & Borgstrøm 1988, 1991; Doloff 1993) and birds (Lindroth 1955; Feltham 1995), as well as physical factors like droughts (Elliott 1994) and freezing (Borgstrøm & Museth 2005).

Differences in individual fish behaviour may influence their risk of being preyed upon, as documented by Bachman (1984) in a study of local and stocked brown trout (Salmo trutta Linnaeus, 1758). Also, Skaala et al. (1996) found that offspring resulting from natural matings between introduced hatchery brown trout and wild sea trout in the stream Øyreselv had a lower survival rate than wild parr. Wild and farmed Atlantic salmon (Salmo salar Linnaeus, 1758) display very different growth rates under farming conditions (Glover et al. 2009). Studies conducted with simulated predators under artificial conditions showed that offspring of farmed salmon displayed higher risk-taking behaviour than offspring of wild salmon (Einum & Fleming 1997). Furthermore, field studies have revealed that offspring of native wild salmon display greater survival in the wild than offspring of farmed salmon (McGinnity et al. 1997, 2003; Fleming et al. 2000; Skaala et al. 2012). Nevertheless, while it is likely that observed differences in survival between salmon of farmed and wild genetic background are at least in

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part due to differences in susceptibility to predation, this still remains untested.

DNA-based methods have been applied to a wide range of forensic applications on both human and animal tissues. Basically, as long as the target sample is not overly degraded, DNA methods will permit identification of species and individuals. Looking specifically at stomach content analysis, DNA methods have permitted the identification of diet. For example, analysis of microsatellite DNA markers have permitted the identification of partially digested minke whale (*Balaenoptera acutorostrata* Lacepède, 1804) skin, ingested by Greenland shark (*Somniosus microcephalus* Bloch and Schneider, 1801) to individual whales included in a genetic register (Haaland et al. 2011; Glover et al. 2012b). Similarly, analysis of microsatellite DNA markers have permitted the documentation of filial cannibalism in the wild by identification of ingested larvae to the guarding father by paternal match or mismatch at multiple microsatellite loci (DeWoody et al. 2001). Furthermore, microsatellite DNA markers have been extensively used to conduct parentage testing (i.e. identification of both mother and father) in aquaculture and livestock management (Taggart 2007), to conduct parentage testing in experimental field studies of survival (e.g. McGinnity et al. 1997, 2003), and more recently to identify the origin of salmon escaping from aquaculture farms (Glover et al. 2008, 2010). However, until now they have not been used in full parentage testing (i.e. identification of both mother and father) of partly digested stomach content in predators sampled within a natural habitat.

Given the above, we set three primary objectives for the present study: (1) to quantify the frequency of salmon juveniles observed in the stomachs of brown trout predators in a natural environment; (2) to evaluate whether microsatellite DNA analysis would permit the identification of ingested salmon recaptured from brown trout stomachs to family, and thus their group of origin (i.e. farmed, hybrid or wild parentage); and finally (3) to evaluate whether salmon of farmed, hybrid and wild parentage displayed different exposure to mortality by brown trout in the natural environment.

**Material and methods**

The River Guddalselva drains into the middle region of the Hardangerfjord on the west coast of Norway. The drainage area is 37 km² and the water discharge ranges from approximately 0.5 to 16 m³ s⁻¹. The length of the river available for the anadromous species, Atlantic salmon and brown trout, is approximately 2 km, from the sea up to the waterfall at Liarefossen, which acts as a barrier to ascending fish. Above the waterfall, resident brown trout are the only naturally occurring fish species. The brown trout is a predator of juvenile fish in this section of the River Guddalselva. The European dipper (*Cinclus cinclus* Linnaeus, 1758), nesting along the river, is also known to prey on salmonid fry (Haftorn 1971).

As part of an ongoing field experiment to investigate survival and growth of offspring of farmed and wild Atlantic salmon, eyed salmon eggs from known crossings were planted above the waterfall in the River Guddalselva. There was no natural spawning by salmon in this area. Eggs and milt from wild salmon were supplied from the Norwegian Genebank for Atlantic salmon and transferred to the Voss hatchery, as were eggs and milt of farmed...
salmon. Controlled family crosses were established and eggs incubated as single family units. At the eyed egg stage, eggs were shocked and the dead ones removed before being accurately counted. Eggs from different families and experimental groups were thoroughly mixed by agitation in several containers in order to ensure that families and experimental groups were entirely randomized prior to being transported to the River Guddalselva, where perforated plastic baskets (40 × 70 × 20 cm) were prearranged with gravel and dug down in the river bed. In each basket, eggs were divided into egg pockets containing 500 eggs each to imitate a natural redd (Barlaup & Moen 2001). In total, 205,266 Atlantic salmon eyed eggs from 69 families were planted in the winters of 2003, 2004 and 2005. After the swim-up stage, baskets were dug up and dead eggs counted to estimate egg survival. The planted stretch of the river was then repeatedly electrofished to recover resident brown trout. All captured fish were tranquillized by benzocaine and all the stomach content was flushed out by carefully inserting a thin soft plastic tube through the mouth and down into the stomach and then flushing the stomach with fresh water (Hyslop 1980). Stomach contents were collected from each trout and the remains of fish fry, digested to a varying degree, were preserved in ethanol. In order to obtain information about the size range of predators, the total length of more than 80% was measured. After the flushing and sampling of stomach contents, predators were kept in an observation tank until fully recovered, after which they were returned to the river.

Partially digested salmon fry collected from trout stomachs were distinguished from brown trout fry by remaining external colouration and size and body shape, as brown trout fry is usually larger and have a deeper body shape than salmon fry. Salmon fry were then subjected to DNA analysis for assignment of parentage to experimental group. DNA was extracted from fish fry remains using a commercial kit (Qiagen DNAeasy). Four microsatellite markers, Ssa85, Ssa202, Ssa197 (O’Reilly et al. 1996) and SSOSL85 (Slettan et al. 1995), were used to genotype all parental fish used in the egg planting and the partially digested fry samples. These analyses were conducted at the Institute of Marine Research in Bergen on an ABI 3730 Genetic Analyser. The microsatellite amplification conditions are available from the authors upon request. The four markers permitted >95% of offspring to be identified unambiguously to one of the 69 families using the family assignment program FAP (Taggart 2007). This meant that in theory, a low number of salmon in the river may share a composite genotype between two families, although this only occurred within experimental groups (i.e. farmed, wild and hybrid) and therefore would not bias estimations of predation rates at the group level.

Results

Based on counts of dead eggs in baskets, the survival to hatching was 99.1% (2003), 98.3% (2004) and 98.3% (2005). The total number of brown trout captured and length measured between June and August in 2003, 2004 and 2005 was 616. On average, 4.2% of the brown trout had Atlantic salmon fry and parr in the stomachs (Table I). Occurrence of salmon fry in brown trout stomachs varied among brown trout of different length, and there was a slight tendency for fewer salmon juveniles in the stomachs of smaller brown trout (Table I). Altogether, 48 partly digested salmon juveniles were identified in the brown trout stomachs. It was only possible to obtain a DNA profile for 46 of these juveniles, as 2 displayed heavily degraded DNA profiles. Of the 46 individuals successfully genotyped, all were successfully identified to a single family (i.e. none of these individuals displayed genotypes clashing between families). In single stomachs, the number of eaten individuals varied from 1 to 15. During July 2003, salmon juveniles were found in 5 stomachs out of 144 analysed, but these predators are not included in Table I, as length was not measured on all individuals. Therefore, altogether 760 predators were checked. One salmon fry was found in the stomach of a salmon parr 10.4 cm in length, out of 10 salmon parr that were investigated in July 2005.

Fry originating from 29 of the total of 69 families planted in the River Guddalselva were represented in the stomach samples of brown trout and Atlantic salmon (Table II). The number of identified salmon juveniles in the stomach samples ranged from one to four per family. The recorded numbers of fry from farm and hybrid origin in the stomach samples were higher than expected relative to the number of planted eggs, and the number of fry of wild origin was lower than expected (Table II). However, there were no significant differences between the three

Table I. Number of brown trout stomachs flushed and examined for fish and fish remains and number of stomachs with fish in the period June–August in 2003, 2004 and 2005.

<table>
<thead>
<tr>
<th>Length-class (cm)</th>
<th>Number examined</th>
<th>Number with fish in stomach</th>
<th>Predator frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0–9.9</td>
<td>76</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>10.0–14.9</td>
<td>364</td>
<td>13</td>
<td>3.6</td>
</tr>
<tr>
<td>15.0–19.9</td>
<td>156</td>
<td>9</td>
<td>5.8</td>
</tr>
<tr>
<td>20.0–31.9</td>
<td>20</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Total</td>
<td>616</td>
<td>26</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Table II. Number of farm, hybrid and wild salmon families and eggs planted in the River Guddalselva and number of families and fry identified by DNA microsatellites in stomach samples of brown trout and salmon parr.

<table>
<thead>
<tr>
<th></th>
<th>Farm</th>
<th>Hybrid</th>
<th>Wild</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planted families</td>
<td>26</td>
<td>23</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>Recaptured families</td>
<td>12</td>
<td>10</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>Planted individuals</td>
<td>75,600</td>
<td>66,153</td>
<td>63,513</td>
<td>205,266</td>
</tr>
<tr>
<td>Recaptured individuals</td>
<td>19</td>
<td>16</td>
<td>11</td>
<td>46</td>
</tr>
<tr>
<td>Expected number of</td>
<td>16.9</td>
<td>14.8</td>
<td>14.2</td>
<td>45.9</td>
</tr>
<tr>
<td>individuals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

groups (Chi squared test, $\chi^2 = 1.08$, df = 2, $P = 0.58$), but the power of resolution was low due to the small sample sizes. The 14 fry successfully identified in one trout stomach originated from 12 different families, all of which originated from cohort 3 planted in winter 2005. Out of these, seven were of wild origin, three were hybrids and four were farmed.

Discussion

The present study successfully identified the parentage of partly digested salmon fry in stomach contents of brown trout and, therefore, also the number of prey of farm, hybrid and wild origin. Thus, we have demonstrated that in combination with stomach flushing of predators, DNA microsatellites can be used for parentage identification even in partly digested stomach contents in fish by a non-destructive sampling of predators.

Molecular-genetic methods, including PCR-based techniques, have previously been used in a variety of studies for the identification of prey species (Scribner & Bowman 1998; Jarman et al. 2002; Symondson 2002; Matejusova et al. 2008), but, to our knowledge, the present study is the first to identify individual prey to family level. It has been argued that for identification of prey species, molecular techniques may give a less biased picture of the diet composition than what is obtained by direct visual inspection of stomach or faeces content (Symondson 2002). In a study of salmonid prey in scats of grey seal (Halichoerus grypus Fabricius, 1791) and harbour seal (Phoca vitulina Linnaeus, 1758) Matejusova et al. (2008) consistently detected the presence of prey in more scats by a DNA technique than when only hard-part analysis was used. In our study, only whole fish or partly digested fish were analysed and this probably underestimates the amount of prey fish eaten by brown trout, since small juveniles without scales will be very quickly digested (Brabrand 1995). Thus, the 4.2% of brown trout observed with salmon fry in their stomachs probably represents the level of predation displayed by this species on salmon parr. Farmed escaped salmon have successfully introgressed in some native populations (Skaala et al. 2006; Glover et al. 2012a), but their offspring generally displayed reduced survival in the wild from eyed egg to smolts when compared to the offspring of wild and hybrid salmon (McGinnity et al. 1997, 2003; Fleming et al. 2000). In a recent study on survival of farm, wild and hybrid salmon from the eyed egg to the smolt stage in River Guddalselva, Skaala et al. (2012) found pronounced differences among families and that egg size had an important impact on survival. However, the causal explanations, such as different predation mortality, have not been investigated. Bachman (1984) found higher mortality of introduced trout compared to native trout and explained the difference by increased predation due to higher activity and more exposure to predators. Similarly, in a laboratory study Einum & Fleming (1997) found differences between offspring from wild and farmed salmon in aggression and in response to a predator, where farmed salmon spent less time in shelter after predator attack. Such differences may be caused by selection for growth rate and possibly behavioural changes following farming selection (Glover et al. 2009). It was expected that these differences would change feeding behavior and predator avoidance in the wild, thereby making farm and hybrid offspring more vulnerable to predators like brown trout and European dippers. However, although offspring of farm and hybrid salmon were slightly over-represented compared to offspring of wild salmon, as prey in brown trout stomachs in the present study their occurrence was not significantly different to that of wild fry. Clearly, despite extensive efforts to quantify predation by brown trout, the frequency of sampled individuals that had preyed upon the hatching salmon fry was low and, thus, it was not possible to gain large enough sample sizes to robustly test whether differential mortality existed between the groups. Nevertheless, these results demonstrated that 29 of 69 families were preyed upon, and salmon of farmed, hybrid and wild parentage were more or less equally preyed upon. In order to gain a large sample size, more extensive sampling of brown trout would have to be conducted. Nevertheless, the predation of brown trout on Atlantic salmon fry is clearly demonstrated in this study.

In summary, the present study has demonstrated the feasibility of predation studies by a combination of non-destructive sampling of predators and DNA-based parentage identification. This extends the use of DNA microsatellites beyond traditional applications and may open up new studies on early life history, predation mortality and comparisons of survival and fitness among fish families and groups.
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