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Seal diet at salmon net fisheries



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Diet of seals at salmon nets

Introduction

Photo-identification studies have demonstrated the existence of seals that specialise in foraging at salmon bagnets and that the majority of seal sightings at salmon nets are made up of these specialist seals (Harris 2012; Konigson *et al.* 2013; Harris *et al.* 2014). These seals regularly return to nets and depredate salmon, have been observed to use more than one net fishing site and have been identified in multiple years (Harris 2012; Konigson *et al.* 2013; Harris *et al.* 2014). Lethal control of seals at nets is employed by salmon net fishers in Scotland and is expected to remove such seals. A number of reasons have been put forward for the survival of these seals from low levels of lethal control to seal behavioural traits that make them harder to shoot (Harris 2012). Interestingly, another study also noted the considerable difference that existed between the size / sex ratio of seals killed at nets and recovered for diet sampling and the size / sex ratio of seals seen at nets by observers (SMRU 1983). Suggesting that the seals recovered for diet analysis were not the seals regularly seen at nets.

In Scotland the number of identified net specialist seals is low, typically one or two seals per site (Harris 2012). During specific months land-based observations recorded net specialist seals removing salmon from nets at rates of 0.24 per hour (Harris 2012) and underwater video examining a relatively smaller area revealed depredation rates of 0.16 per hour (Harris *et al.* 2014a). Given the low number of seals concerned and the high rate of salmon depredation it is feasible that at specific times these seals may be obtaining all their energy requirements from salmon at net sites.

However, to date, digestive tract analysis of seals shot and sampled at Scottish salmon nets has not supported the existence of seals specialising on salmonids. Detection of salmonid prey in shot seals has been low, with only 19% – 22% of carcasses having salmon remains, and the diet of sampled seals was generally dominated by other prey species (Rae 1968; Pierce *et al.* 1991; Harris 2012). The small number of carcasses that did contain salmonids typically contained other prey items that likely did not come from salmon nets, implying that these seals were not specialising in targeting salmon nets (Pierce *et al.* 1991; Harris 2012). These results appear to challenge the underlying management assumptions that lethal control is targeted at those individuals depredating salmon from nets.

However, Harris (2012) suggests that these results from past and present diet studies are in part driven by different forms of sampling bias. Firstly, a large proportion of the sampled seals have been the result of bycatch, whereby seals have entered the salmon trap and have been unable to find their way out again before being shot. In fact, of the 16 seals sampled in 2005-2008 and reported in Harris (2012), eight seals (3 grey seals & 5 harbour seal pups) came from seals trapped inside salmon nets and none of these held any evidence of salmonid prey. Seals that habitually return to the nets are known to be able to negotiate the chambers of salmon traps, and so this suggests that the bycaught proportion of the diet samples may be from naïve seals unfamiliar with the chambers of a salmon net. Furthermore, seals killed inside nets are easily recovered whereas seals shot outside the net may be too difficult to recover, introducing another possible bias (Harris 2012). Finally, there may be some potential bias associated with differences in the “availability” of seals to be shot, perhaps as a result of behavioural traits (Harris 2012).

If significant sampling bias exists, as suggested by Harris (2012), then present and past assessments of the diet of seals sampled from salmon nets are unlikely to be representative of the diets of those seals that habitually return to salmon nets. Despite the limitations, diet information from seals shot at salmon nets is one method in gauging the effectiveness of seal management approaches both lethal

and non-lethal. Given killing seals is controversial ensuring this process is as selective as possible is important and that lethal control is targeted to those seals causing damage rather than the seal population in general.

Co-operation from net fishers and inland salmon fisheries allowed seals killed under licence between 2005 and 2013 to be recovered for dietary analysis when carcasses were accessible. The presence of salmon or trout in samples from carcasses enables an assessment of the prevalence of salmonids in the diet of seals. In this report we make such an assessment for seals killed at coastal nets, and compare our results with those from seals sampled from rivers (n=16) in 2005-2013 (Graham *et al.* 2011 and SMRU unreported data) and to the DNA extracted from seal scat samples (n=182) from haul-out sites in the Cromarty Firth and Findhorn Bay during 2003 and 2005 (Matejusova *et al.* 2008). Based on the current study we provide evidence and suggest methods for improving the selectivity of lethal control.

Methods

Carcasses were either sampled on site by SMRU or transported to SAC for necropsy. Gastro-intestinal tracts (GITs) were placed into separate bags and stored at -20°C.

GIT processing:

GIT were thawed overnight at room temperature. All surfaces and equipment were cleaned using a 3% Decon 90 solution, this process was repeated between samples. Using new disposable scalpels and new disposable gloves to avoid any cross contamination, the stomach and oesophagus were then carefully separated from the rest of the sample. Up to 3 subsamples of stomach contents were taken for DNA extraction. This was done using a fresh sterile scalpel per subsample to make a 5cm cut through the stomach wall. A 1.5ml Eppendorf was then used to extract approximately 1ml of the contents. Where possible, samples were taken from the oesophagus, the upper and lower stomach, and stored at -20°C until DNA extraction.

Using a scalpel, the entire stomach and oesophagus were then cut open and the contents collected in a high-walled metal tray. A picture was taken to document the amount and colour of the contents before work continued. The presence of pink salmonid flesh was also noted at this stage. The empty stomach was carefully hosed down over a set of sieves (4mm, 710µm & 500µm) to make sure that all hard parts were collected. Stomach contents were carefully transferred into the sieves in small portions, washed using conventional dishwashing detergent and checked for hard parts. Undigested items such as vertebra, jaws and skulls, cephalopod beaks, otoliths, fish scales and sea louse (*Lepeophtheirus salmonis*) were extracted. When the entire contents had been checked, otoliths were transferred into Eppendorf tube and beaks were stored in a 75% IMS solution for later identification. A series of pictures was taken of all the remaining hard parts, including overview and close-up shots of jaws, skulls and vertebra. Hard parts, with the exception of otoliths, scales and beaks, were then discarded.

After cleaning the re-useable equipment and surfaces using the 3% Decon 90 solution, the large intestine was carefully separated from the small intestine and transferred into a metal tray. A subsample was taken of any faecal matter contained within the large intestine for DNA extraction following the method described above. The large intestine was then cut open and its contents

collected in the tray. The washing process and the collection of hard parts followed the method outlined for the stomach.

The small intestine was then carefully cut open a few centimeters at a time with a pair of fine scissors and washed in a deep plastic tub filled with water to clean it of any hard-parts which would settle out at the bottom of the deep tub. The water from the plastic tub was then carefully poured into the sieves to extract hard-parts from the remaining material. These were documented and stored as described above. The work area and equipment was then washed in a Virkon S solution, and the work surfaces cleaned using the 3% Decon 90 solution.

DNA extraction:

DNA was extracted using Qiagen QIAmp DNA Stool Mini Kits. Using sterile inoculation loops, between 180 – 220µg of each sample were transferred into sterile 2ml Eppendorfs. The extraction process then followed the Qiagen protocol. Extracted DNA samples were stored at -20°C for up to a week until the extraction process was completed for all samples.

DNA analysis:

The extracted genomic DNA was supplied to Xelect Ltd. and tested using quantitative PCR (qPCR) to detect the presence of DNA using three qPCR assays designed for seals (*Halichoerus grypus* and *Phoca vitulina*), Atlantic salmon (*Salmo salar*) and trout (*S. trutta*) DNA according to Matejusová *et al.*, 2008. All qPCR analysis was performed in triplicate using a 1:10 dilution of genomic DNA in a 10 µl reaction containing 1 µl diluted DNA, a species specific Taqman® gene expression qPCR assay and Brilliant III mastermix (Life Technologies™, UK). Target DNA was amplified using the following amplification conditions: - 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Fluorescence was measured throughout amplification for the FAM reporter probe and a cycle threshold (Ct) value reported for each sample, where lower Ct values indicates a greater amount of target DNA in the test sample. A Taqman® Internal Positive Control (IPC) (Life Technologies™, UK) was used to check for PCR inhibitors in the DNA samples (1:10 dilutions) where VIC reporter probe measurement and comparisons of the VIC Ct value between test samples and a no template control can be used to determine if PCR inhibition has occurred, therefore a true negative result can be distinguished from PCR inhibition.

Hard-part analysis:

Salmonid otoliths were identified using Harkonen (1986), Leopold *et al.* (2001) and the first author's salmonid otolith reference collection. The remaining otoliths and beaks were identified to the family level and the number and frequency of occurrence reported. Sea louse were confirmed as *L. salmonis* by Prof. C. Todd (University of St. Andrews).

Results

Thirty-six (inc. 16 previously reported in Harris 2012) seals were sampled from salmon net fisheries 2005-2013 (Table 1). The spread of carcasses over this period was not even, with no seals sampled in 2009 and 2011. Of the thirty-six, thirty were grey seals and six were harbour seals (1 adult and 5 pups). Thirteen seals were killed between April and June and twenty three from July to September. All were from bagnet fisheries except two harbour seal pups that were from sweepnet fisheries. Four seals were from the East Coast seal management zone while all others were from the Moray Firth

(<http://www.scotland.gov.uk/Resource/Doc/295194/0112738.pdf>). Twelve seals were bycaught and subsequently culled by the fishery.

Entire gastro-intestinal tracts (oesophagus to anus) were collected from twenty-five seals, just the stomach was collected from a further nine seals and the stomach and colon were collected from two seals. Between 1 and 4 DNA samples were collected from each seal depending on the amount and distribution of prey material within the GIT.

During the testing of the DNA samples PCR inhibition was not detected in any sample and the samples were therefore suitable to be used for detecting the presence of seal, Atlantic salmon and trout DNA. All DNA samples tested positive for seal DNA using the assay designed to *H. grypus* and *P. vitulina* cytochrome b sequences (Matejusová *et al.*, 2008).

Four seals tested positive for trout using the assay designed to *S. trutta* cytochrome b sequence, all four seals were shot in Gamrie Bay in June 2012 or June 2013. The trout DNA signal was weak in three seals and was not detected in all the sub-samples taken from each seal. The trout DNA signal in the fourth seal was stronger and present in all four sub-samples from that seal. In two of these seals the presence of trout was confirmed visually by the presence of trout otoliths.

Eleven seals tested positive for Atlantic salmon using the assay designed to *S. salar* cytochrome b sequence. The salmon signal was not detected in all sub-samples from three of these seals. Two seals tested positive for both salmon and trout.

Table 1. Number of seals sampled from salmon net sites for the purpose of dietary analysis and the proportion that tested positive for salmonid DNA

	Grey seal ¹		Harbour seal ²	
	Juvenile	Adult	Pup	Adult
Female	4 (0.5)	18 (0.33)	2 (0)	1 (1)
Male	5 (0.2)	3 (1)	3 (0)	0

1. Grey seals were assumed to be juveniles if less than 110 kg and adults if greater than 110 kg (SMRU, 1984).

2. Harbour seals were assumed to be pups if their standard length was less than 1 m, juveniles in their first or second year if 1-1.1 m in length and adults if longer than 1.1 m (Corpe, 1996; Thompson *et al.*, 1992).

The proportion of samples that tested positive for salmon and trout were compared with results from seals shot in freshwater (Graham *et al.* 2011 and unreported SMRU data) and those from scat samples from estuarine haul-out sites (Matejusova *et al.* 2008) (Table 2).

Table 2. The number of samples and proportion of samples testing positive for salmonid DNA; from 182 estuarine haul-out scat samples from Matejusova *et al.* (2008) and 16 seals shot in rivers (Graham *et al.* 2011 and unreported SMRU data) and 36 seals killed at salmon nets during this study.

	Samples (n)	Salmon (%)	Trout (%)	Combined (%)
Nets	36	31	11	36
Estuary haul-out	182	7	8	13
River	16	19	38	50

In contrast the hard-part analysis of otoliths revealed a lower proportion of salmon and trout. Five of the net-sampled seals held salmon otoliths and two further seals held trout otoliths (Table 3). Pink salmonid flesh and salmonid sea lice were recorded in a further three net-sampled seals. When

incorporating these visual cues of salmonid prey (pink flesh and salmonid sea lice), evidence of salmonid prey through hard-part analysis was detected in a total of ten seals (28%) compared with thirteen (36%) from DNA analysis.

Of the 36 seals examined, fish otoliths and squid beaks were detected in 27 seals, 6 seals contained prey material but with no evidence of otoliths or beaks and 3 seals held no evidence of any prey. Gadids (whitefish) were the prey items encountered most frequently in seals recovered from salmon net fisheries, being detected in 16 carcasses (Table 3). The presence of salmonid otoliths can provide quantitative information on the number and size of salmon consumed or damaged. Nineteen salmon otoliths were recovered from five seals and four trout otoliths were recovered from a further two seals (Table 3).

Table 3. The number of otoliths and their frequency of occurrence within twenty seven seals sampled from salmon net sites. A further nine seals contained no otoliths or beaks.

Prey group	Frequency of occurrence	Number of otoliths or beaks
Gadids	16	120
Perciforms	14	820
Pleuronectids	14	334
Salmon	5	19
Cephalopods	3	13
Trout	2	4
Clupeids	2	17
Cottids	2	7
Unidentified	6	9*
Total	N = 27	1343

*9 otoliths were unidentified due to their small size or level of digestion; they were not thought to have come from salmonid prey.

Combining DNA and hard-part analyses, the majority of seals containing evidence of salmonid prey also held other prey items (Figure 1). One seal had evidence of only salmonid prey and one seal had evidence of only trout prey.

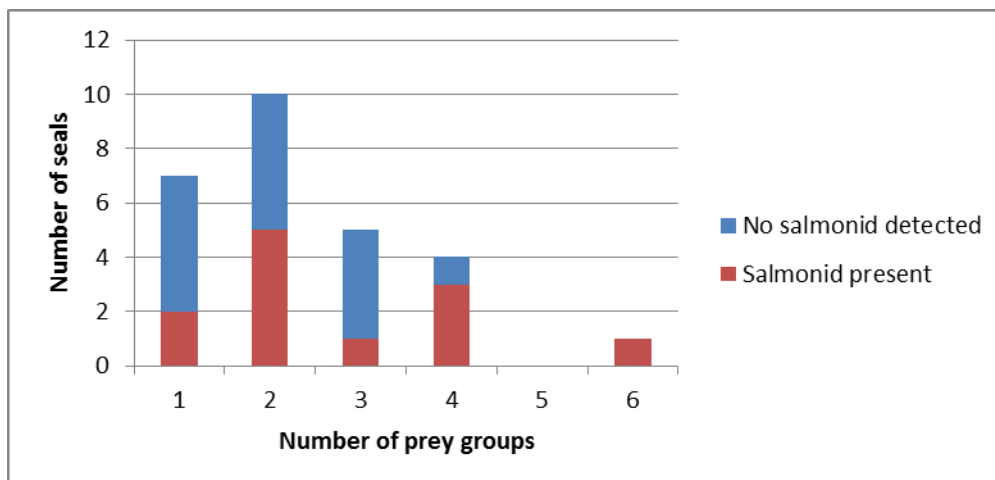


Figure 1. Number of different prey groups detected in the diet of seals killed at salmon nets from otoliths/beaks or salmonid DNA analysis.

Discussion

Gadids and perciforms (in particular sandeels) were the most prevalent prey from sampled seals. The proportion of seals that tested positive for salmonid DNA was relatively low (0.36), although this proportion was higher than reported in Harris 2012 (0.2). The increase in the proportion of salmonids in sampled seals was mainly due to the inclusion of seals from Gamrie Bay in 2012 and 2013 (n=12; proportion with salmonids = 0.58) although carcasses from Portmahomack and Scarvaig in 2012 (n=4; proportion with salmonids = 0.75) also contributed, whereas carcasses from Montrose and Balintore (n=4: 0.00) countered this increase. The presence of other prey species in the diet of most seals that contained salmon DNA (Figure 1) indicates that these seals were not solely preying on salmon at the nets. This observation may suggest that these seals may not be specialising in targeting only salmon nets (Pierce *et al.* 1991). However one seal, a large male grey seal from Portmahomack in 2012, held evidence of a diet of only salmon (6 salmon otoliths).

The use of ADDs since 2009 may also have influenced the proportion of transient versus salmon net specialist seals in the sample. For instance an ADD was trialled and used intermittently at both Portmahomack and Scarvaig in 2012 and it is possible that the use of this device may have kept 'transient' seals away from the nets and resulted in shooting becoming more selective to those seals determined to forage at nets. It is of interest that in 4 years prior to ADD use at Portmahomack, ten seals were sampled and only one seal tested positive for salmonid DNA. In the following 5 years, after ADD installation, only 2 seals were shot and both tested positive for salmon DNA. We suggest that ADDs not only reduce the need to shoot (Harris *et al.* 2014b) but, where lethal control is required, likely improve the ability of the fishery to be more selective. This maximises the benefits to salmon fisheries and minimises the costs to seal populations.

The same may be true for the increasing use of net modifications, such as steel doors that prevent seals from swimming into fish courts. In previous studies the majority of samples came from seals that found their way into the fish court of salmon nets and were unable to find their way out again (Rae 1968; Pierce *et al.* 1991; Harris 2012). As net specialist seals are known to be able to negotiate the chambers of salmon nets (Konigson 2007; Harris *et al.* 2014a) diet samples in these studies are unlikely to be representative of net specialist seals and are likely biased towards those naïve seals unfamiliar with the chambers of a salmon net. Results from the present study (2005-2013) continue to support this hypothesis as twelve seals were bycaught and only two held evidence of salmonid prey. Continuing to encourage net modifications may therefore not only improve catches (Harris *et al.* 2014a) but also reduce the bycatch and subsequent killing of 'naïve' seals, thus improving the selectivity of lethal control.

Another method for improving selectivity may be to prevent the lethal control of pups. In the present study five harbour seal pups were killed, each less than 14kg (comparable in size to a large otter or salmon) and these seals contained no evidence of salmonids. This sample size can be increased by a further three seals if we include seals (<20kg) that have been shot to protect inland salmon fisheries (SMRU unreported data). Again, none of these seals held evidence of salmonid prey. In addition photo-identification studies at nets and in rivers suggest that these very small seals tend to be 'transient' in their behaviour and are not habitual visitors. This suggests that these very small seals are unlikely to present a threat to salmonid fisheries and removing pups from seal licences would also likely improve selectivity.

The relatively high proportion of salmonid prey in seals from Gamrie Bay (proportion with salmonids = 0.58) is of interest as the majority were shot before the installation of an ADD. The relatively high

occurrence of salmonid prey in the diet of seals from this station may have been attributed to the high rate at which salmonids were hung in the leaders to the nets (pers. obs.). The presence of hung fish makes depredation easier for passing predators and, as a result, the nets become a rewarding foraging site. The presence of hung fish in the leaders brings seals close to land and makes shooting easier for marksmen, possibly resulting in more seals containing salmonid DNA being shot. The net fishers suggest that the high level of hung fish at this station was due to the exceptionally clear water, as they believe that more fish become hung the clearer the water (G.Pullar pers coms). Developing net modifications that reduce the chances of salmonids being hung should therefore be seen as an important part of any net modification project.

Salmon or trout DNA was detected in thirteen seals and the presence of salmonid prey in ten of these seals was confirmed visually by otoliths, pink salmonid flesh or salmonid sea lice. Had other bones i.e. vertebrae or jaw bones also been used then it is feasible that the number of detections made by hard-part analysis may have increased by one seal. Regardless, despite the DNA detection of salmonid prey being an expensive process it may be the most reliable way of detecting the presence of salmonid prey and therefore should be continued. The salmon or trout DNA signal in five seals was not detected in all DNA sub-samples from these seals, stressing the need for multiple samples to be taken from each seal to increase the probability of detecting signals. Alternatively GIT contents could be thoroughly homogenised to allow fewer DNA samples to be analysed, however, this process would likely be to the detriment of hard-part analysis as delicate structures may be destroyed. In four seals the DNA signal was not detected throughout the GIT, with salmonid DNA being detectable only in the upper or lower digestive tract.

Carcass collection and subsequent dietary analysis is continuing in conjunction with ADD studies and net modification work. The mismatch in results between diet and observational studies is of interest and we will continue to investigate this.

In conclusion the collaboration between net fishers and SMRU has led to a better understanding of seal depredation and damage, and such work will help reduce the need to shoot animals and increase the benefits to the fishery. Under the conceptual framework described by Butler (2011) a win – win scenario for both salmon fisheries and seal conservation could be realised through:

- The modification of nets to better protect catch (Lunndred *et al.* 2003; Lehtonen & Suuronen 2004; Hemmingson *et al.* 2008; Harris *et al.* 2014a).
- The removal of pups from licences as the lethal control of pups may provide little or no benefit to the fishery and carries a high cost to seal conservation.
- The use of ADDs to reduce the presence of seals at nets.

Suggested further studies:

- Contact with marksmen should be maintained to help encourage and assist where necessary the recovery of seal carcasses for dietary analysis to further increase sample size and help assess changes in diet composition following the installation of ADDs and net modifications. Carcasses can provide a source of samples for a wide range of scientific studies, biological information such as pregnancy rates in female seals are providing an additional source of life history information. Stomach and intestines should be subjected to both descriptive and hard-

part analysis as well as the molecular methods for salmonid DNA when funds permit. Monitoring the proportion of salmonid prey in shot seals will provide an indication of the selectivity of this form of control.

- The stable isotope ratios of carbon and nitrogen in seal tissues may be used to give a qualitative assessment of seal diet over a longer time period. These samples have been stored from some seals shot to protect salmon fisheries and fish farms and while as yet there are no firm plans to pursue this line of investigation, preliminary work at the SMRU has been initiated to determine how useful such samples might be in investigating longer term diet through stable isotope analysis. The collection of these tissues should be continued.
- New net designs should be developed to reduce seal – salmon fishery conflict. This can be achieved with a better understanding of salmon and seal behaviour at nets through the continued use of underwater cameras coupled with data recording by both salmon net fishers and observers. Excluding seals from entering fish courts and creating an effective barrier to prevent seals from damaging fish through the meshes of the net should be a priority. Whilst also developing methods to reduce the hanging of fish. Despite the different environmental/tidal conditions present in the Baltic it may be useful to experiment with Baltic style nets in Scottish waters to assess their practicality and adapt existing nets.
- The monitoring and data collection from sites using ADDs should be continued to improve our understanding of a wider range of ADD manufacturers and specifically the long term effectiveness of these devices.

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