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Tracking escaped salmon back to the farm by adding rare earth elements to the feed

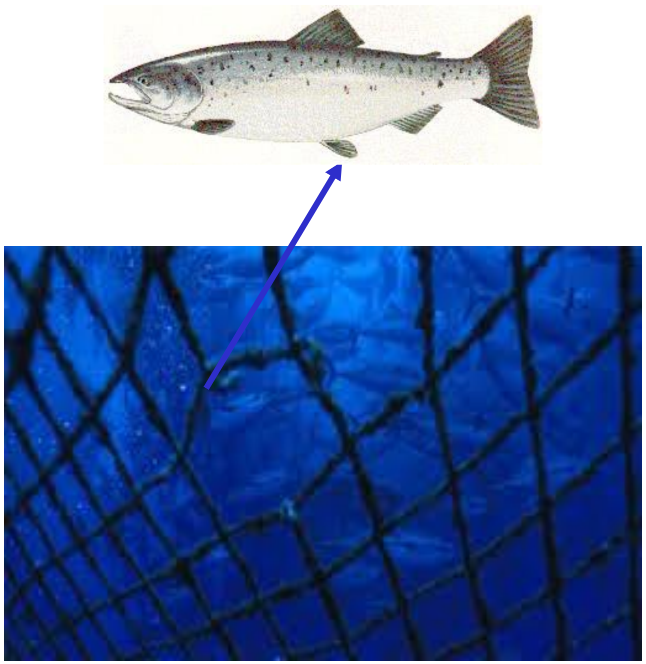
by

Magny S. Thomassen, Marta Perez, Jens-Erik Dessen, Kjell-Arne Rørvik and Yngvar Thomassen

Why is tracking escaped salmon important?

- **Farming of Atlantic salmon is an expanding industry in Norway, over one million tons were produced in 2012**
- **A major challenge is the large number of farmed salmon that escape into the wild. This is perceived as a threat to their wild conspecifics. The main concerns are related to the possible interbreeding with wild salmon, and potential spread of pathogens and diseases to wild salmon**
- **To reduce the number of escapees Norwegian authorities mandate immediate reporting and recapture efforts after escape events, and there are penalties for the breach of these regulations. Despite the legal obligations there is evidence of unreported escapes which may be unintentional (fish farmers not aware of it) or intentional (fish farmers with-holding information after escape incidents).**
- **Thus, there is increasing opinion about the need to develop a method for labeling farmed fish in order to identify the origin of escapees, and potentially use it as a management tool to detect aquaculture sites in need of better husbandry practices and to prosecute fish farmers breaching the regulations.**

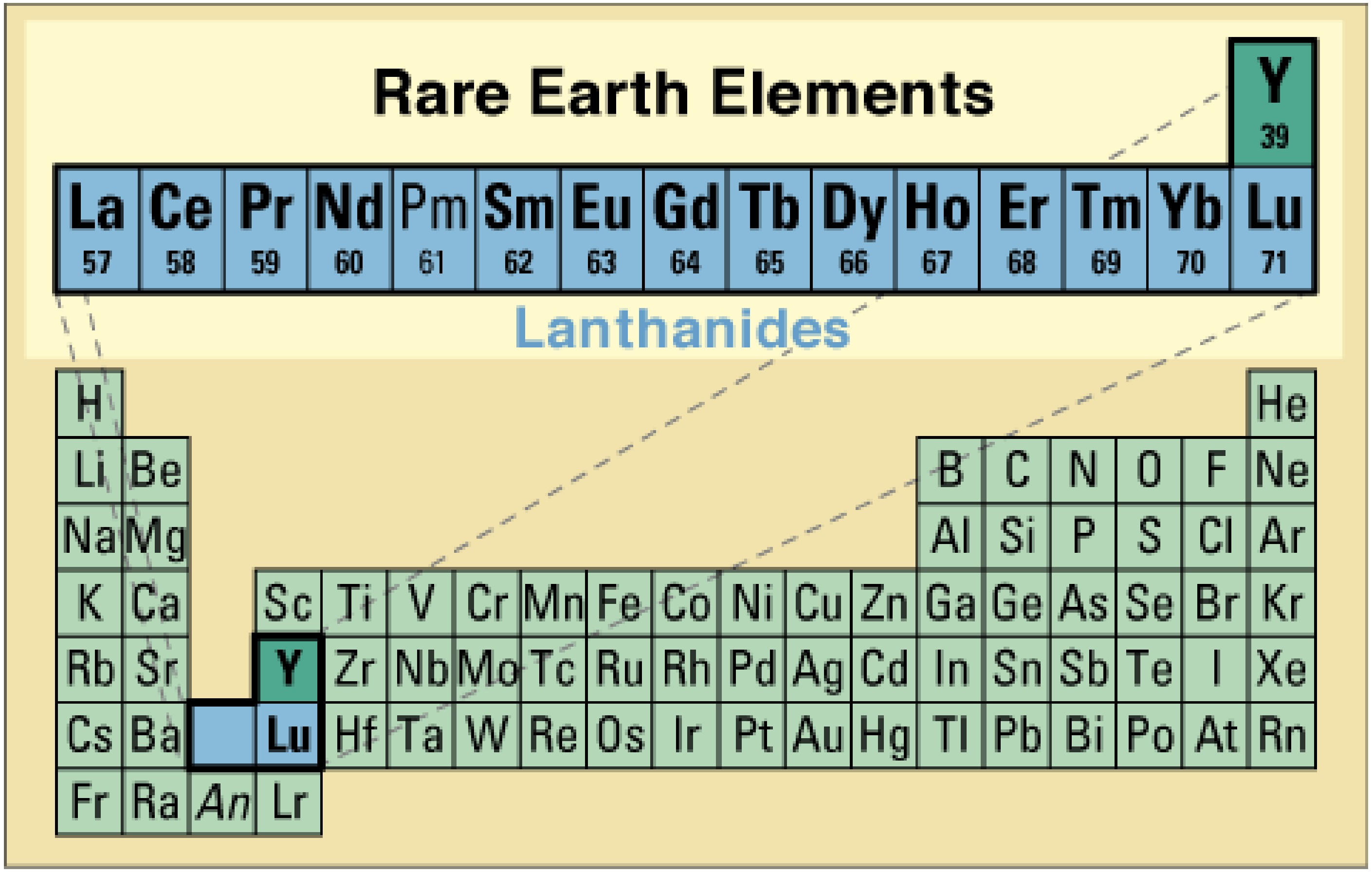
So the object of this project was to develop a method that, both easily and cheeply, can distinguish farmed from wild salmon, and at the same time track the salmon back to the farm.



Why using rare earth elements (REEs)?

- A series of marking techniques is now being evaluated, including physical tags, bar-code and genetic marks, among others
- Chemical marking is considered as good alternatives
- It offers the possibility to mark large groups of fish and individual handling is not required, which reduces labor-intensity and improves animal welfare.
- Rare earth elements are found in the bone structures of fishes, but in very low concentrations. Most of these elements are non-radioactive, easy to handle and have been shown to have a long retention time in bone. Besides, in comparison with other elements, the REEs are generally considered to be of low toxicity

As Ce Dy Er Eu Gd Ho La Lu Nd Pr Sc Sm Sr Tb Tm U Y Yb

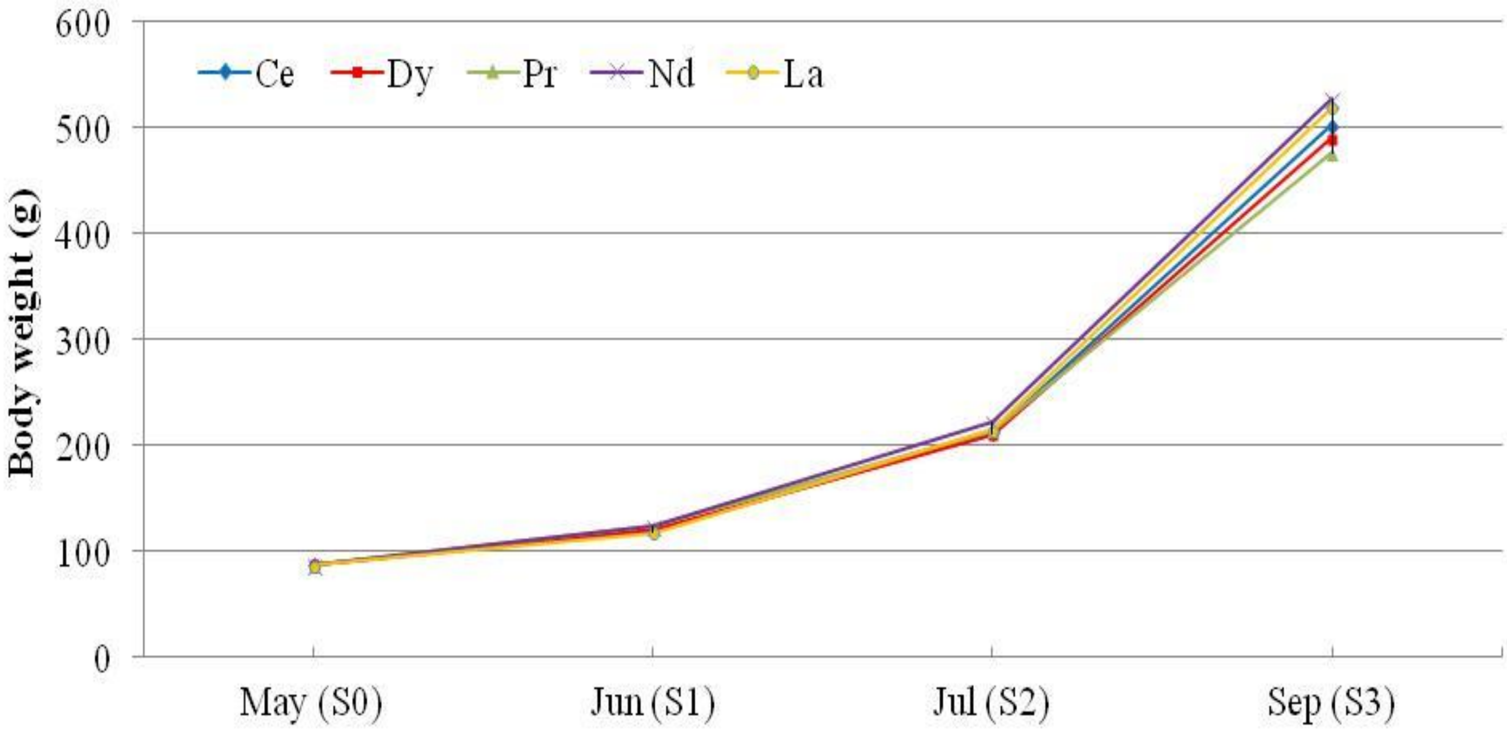


Project description

- **We decided to study the possibility of marking the scales of smolt shortly after transfer to seawater, by testing out chlorides of five different elements:**
- **Lanthanum(La), Cerium(Ce), Praseodymium(Pr), Neodymium(Nd) and Dysprosium(Dy).**
- **In our first feeding study with 1-year old salmon smolt, we used 250 mg/kg feed**
- **The chlorides were dissolved in water and added on top of a regular commersial feed using a blender. After drying the feeds for one day, rapseed oil was added, to prevent leakage of the elements.**

- **Atlantic salmon 1-year old smolt with a mean initial body weight of 87.3 ± 1.6 g were placed into 10 square tanks each of them containing a total of 50 fish. The tanks were supplied with seawater at ambient temperature with an average of 9.4 °C. The fish were acclimatized to tank environment and fed a commercial diet before the onset of the experiments.**
- **The trial consisted of a 10-week labeling period during which a REE-supplemented diet was administered, followed by a 2-month “dilution” period with the fish being fed untreated commercial feed.**
- **The five experimental diets were randomly assigned to duplicate tanks. The fish were reared under continuous light throughout the experimental period (light:dark 24:0).**

The fish behaved well. We observed no differences in growth between diets, and no mortality



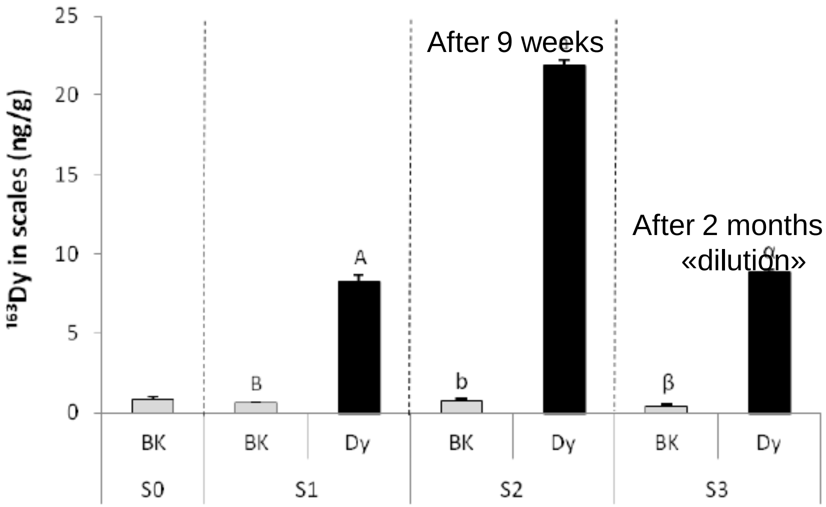
Thermal growth coefficient (TGC) for each of the dietary groups (means ± SEM; n = 2) within the different experimental periods

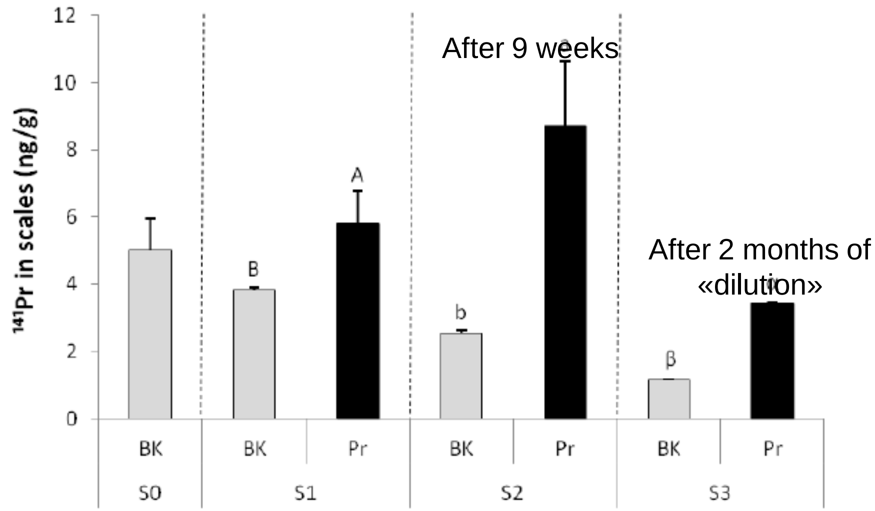
	TGC							
	Period	Ce	Dy	Pr	Nd	La	*P-value*	R^2
	Labeling period	3.23 ± 0.07	3.13 ± 0.19	3.15 ± 0.05	3.36 ± 0.29	3.40 ± 0.22	0.79	0.25
Exp. 1	Dilution period	2.88 ± 0.16	2.72 ± 0.16	2.69 ± 0.16	2.94 ± 0.10	2.88 ± 0.19	0.74	0.28
	Total period	2.99 ± 0.08	2.85 ± 0.17	2.84 ± 0.12	3.08 ± 0.02	3.03 ± 0.19	0.65	0.34
		MT-1	**MT-2**	**MT-3**	**MT-4**			
	P1 Labeling period	2.39 ± 0.11	2.04 ± 0.16	1.97 ± 0.04	2.34 ± 0.02		0.10	0.76
Exp. 2	P2 Labeling period	2.91 ± 0.36	3.40 ± 0.12	3.26 ± 0.01	3.05 ± 0.40		0.64	0.32
	Total labeling period	2.74 ± 0.21	3.01± 0.12	2.88 ± 0.00	2.82 ± 0.29		0.79	0.2

Thermal growth coefficient (TGC) for each of the dietary groups (means ± SEM; n = 2) within the different experimental periods

	MT-1	MT-2	MT-3	MT-4	p-value	R^2
Labeling period 1	2,39 ± 0,11	2,04 ± 0,16	1,97 ± 0,04	2,34 ± 0,02	0,10	0,76
Labeling period 2	2,91 ± 0,36	3,40 ± 0,12	3,26 ± 0,01	3,05 ± 0,40	0,64	0,32
Total labeling period	2,74 ± 0,21	3,01 ± 0,12	2,88 ± 0,00	2,82 ± 0,29	0,79	0,20

After feeding with the «marking» feed for 9 weeks, scales were analysed by ICP-MS, and the results were good!





All groups had significantly higher levels than the background level, even after 2 months further feeding on a commersial feed, and the difference was highest when the background levels were low, as seen for dysprosium.
It was also interesting to see that even when the levels in the marked fish became lower after 2 months, the ratio to the bacground level was the same!

Dy: Ratio 35,7 after marking, 35,6 after 2 months of «dilution»
Pr: Ratio 3,3 after marking, 3,1 after 2 months of «dilution»

In our next experiment we used the other main type of smolt, called 0-smolt

In this experiment we tested out to different levels of marker added to the feed, and also a mixture of two elements.

The experiment was further run at lower water temperature since this smolt type is transferred to sea water during fall/winter. (6,6-8,6 C)

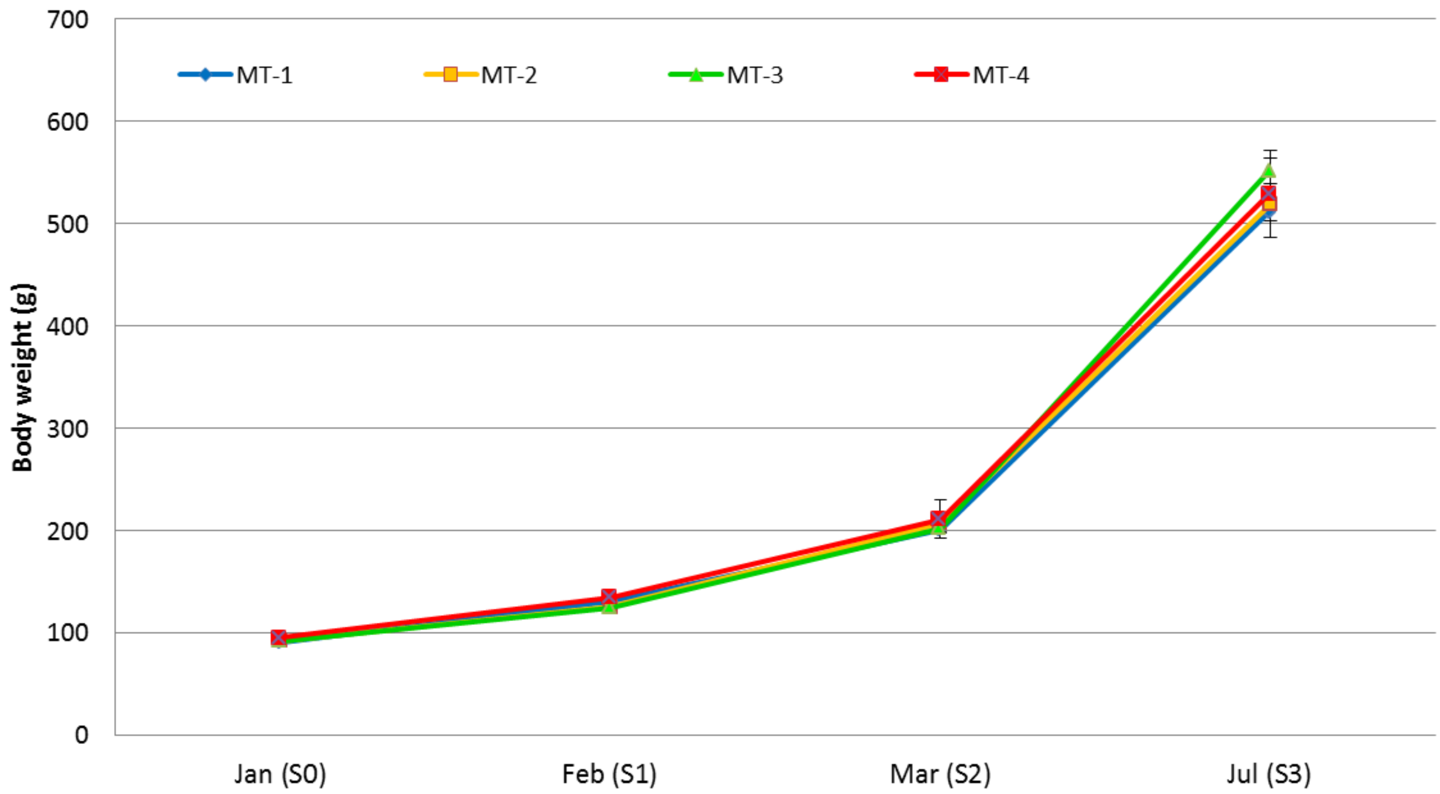
Feed 1: 125 mg Dy/kg

Feed 2: 250 mg Dy/kg

Feed 3: 125 mg Pr/kg

Feed 4: 125 mg Pr + 125 mg Dy /kg

As seen for the 1+ smolt, there were no significant differences in growth with the 0-smolt, and no mortality.



Thermal growth coefficient (TGC) for each of the dietary groups in the labeling trial with 0^+ smolt within the different experimental periods (P1 and P2 refer to first and second phase within the labeling period).

	TGC							
	Period	**Ce**	**Dy**	**Pr**	**Nd**	**La**	***P-value***	**R^2**
	Labeling period	3.23 ± 0.07	3.13 ± 0.19	3.15 ± 0.05	3.36 ± 0.29	3.40 ± 0.22	0.79	0.25
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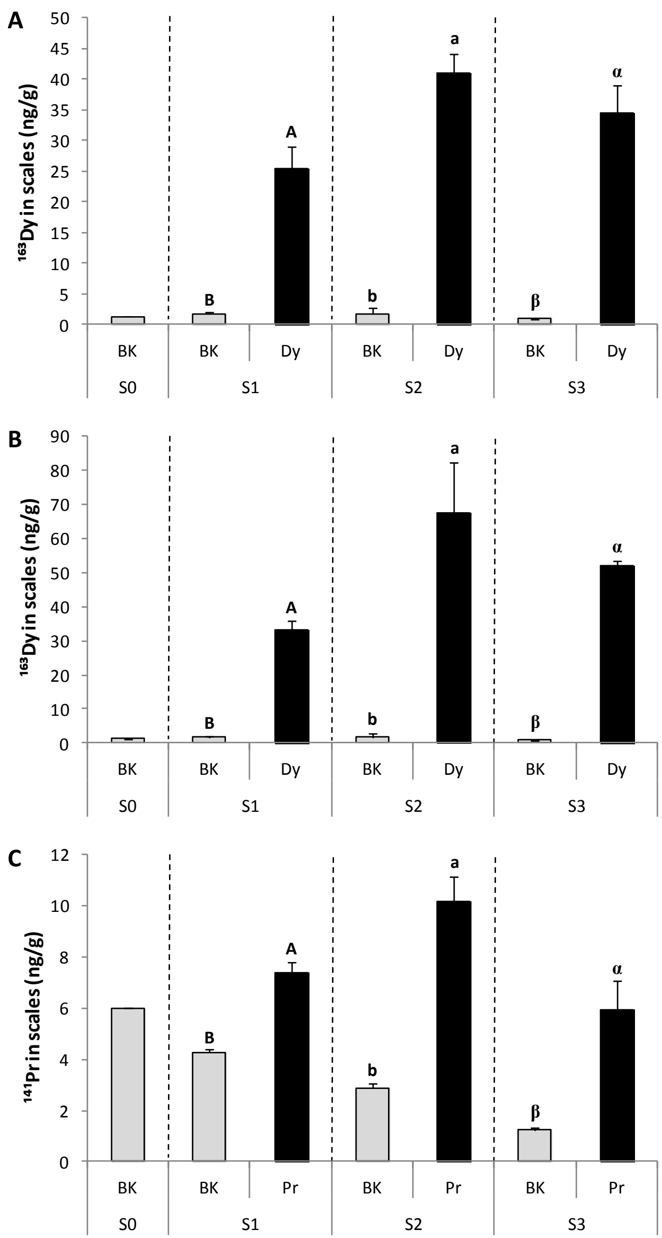
Marking of scales

We found no principal differences between the two smolt types.

And both with Dy and Pr we got significant marking also with125 mg/kg

And we found significant marking even after 4 months of «dilution».

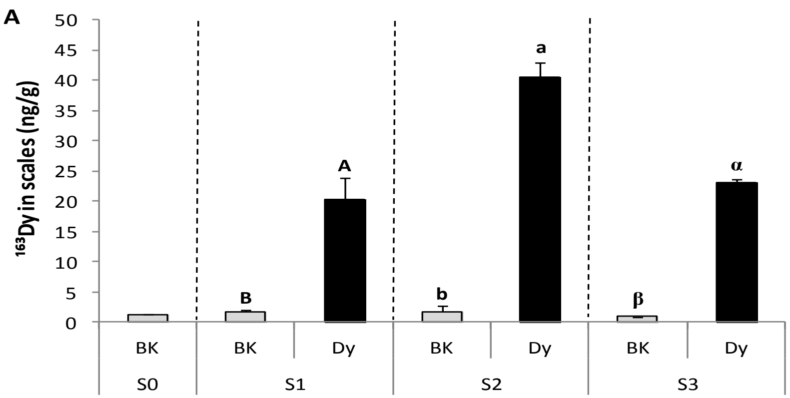


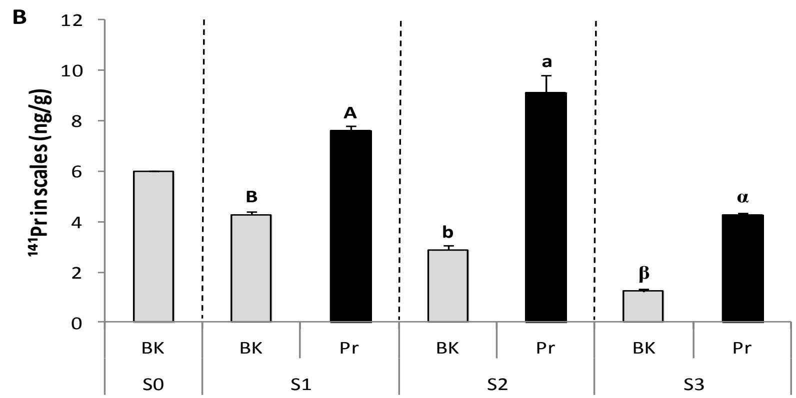
Dy 125 mg/kg

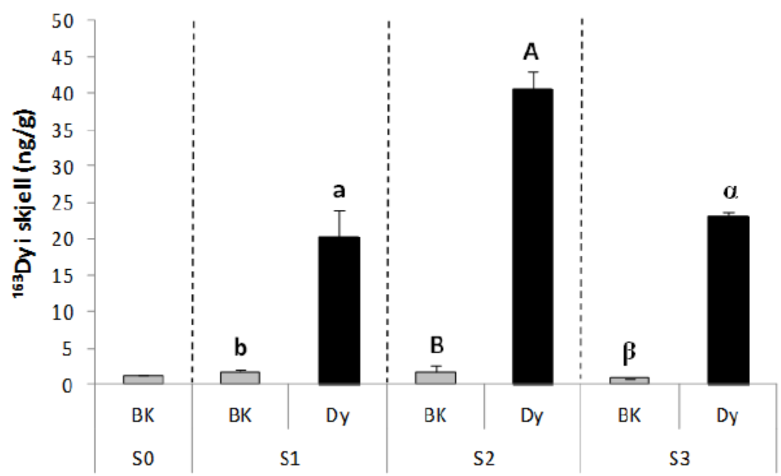
Dy 250 mg/kg

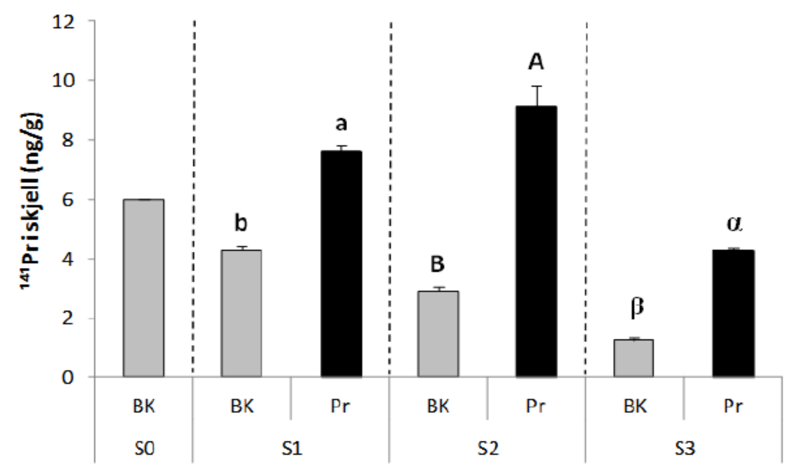
Pr 125 mg/kg

Feeding with a mixture of two REEs (Dy and PR) gave no reduction in incorporation.

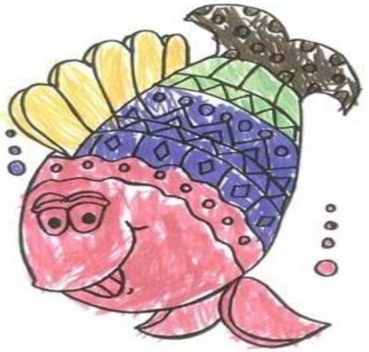








Conclusion



By adding small amounts (125-250 mg/kg) of rare earth elements (REEs) to the salmon feed for just a few weeks we were able to mark both 1- and 0- smolt, and the label survived for several months afterwards in the sea.

This feed addition did not seem to have any negative effects on the fish.

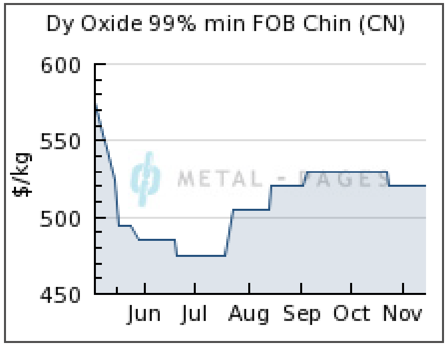
Easily?

- Scales can be sampled and put into an envelope easily, so everyone can do this

- Very little is needed, so this can be done without killing the fish

- The sample preparation before the ICP-MS analysis is not complicated

Cheeply?



How much «marking feed» is needed?: 120 g/fish

How much REE?: 125 mg x 0,12 kg = 15 mg

500 US dollar/kg

0,5 US dollar/g x 0,015 g = 0,0075 US dollar/fish

Thanks to the project group!

and to FHF for the financing!