Using stable isotopes to investigate reasons for divergence in body

Annelie Evers

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Using stable isotopes to investigate reasons for divergence in body condition of cod in the Baltic Sea

Annelie Evers, Lunds Universitet, 2013

Trots återhämtning av Atlantisk torsk (*Gadus morhua)* sen de tidigare låga värden under 1990-talet så gäller detta bara i vissa områden i Östersjön. Det blir problematiskt då det blir hög konkurrens om föda som leder till svält hos torsken. Den här artikeln undersöker ifall den dåliga konditionen som uppvisats hos vissa torskar kan förklaras med hjälp av annorlunda habitat eller födoval. För att kunna undersöka detta har en stabil isotop analys av kol och kväve utförts på torskar från två lokaler i Hanö bukten, Aspö och Nogersund. Från isotop analysen av vävnad ska skillnader i vilken trofisk nivå predatorn jagar på att kunna utläsas från kvävesignalen och även ifall födokedjan är bentisk eller pelagisk kunna utläsas från kolsignalen. Denna information har jämförts med torskens kondition. Det signifikanta sambandet som påvisades var att torsk av lägre kondition hade högre förhållande av kväve isotoper vilket skulle peka på att de födosöker på en högre trofisk nivå än de torskar med högre kondition. En anledning är att det har olika habitat med olika föda. En annan anledning till detta kan vara att torsk som svälter uppvisar en berikad kvävesignal och detta kan då påverka värdet. På grund av att påverkan av svält är okänd går det inte att se ifall konditionen påverkar från vilken trofisk nivå torsken födosöker. Det var ett negativt signifikant förhållande mellan kolsignal och kondition i Nogersund och en signifikant skillnad mellan kolvärde och hög och låg kondition vilket kan visa att torsk med lägre kondition rör sig i andra habitat än de med högre kondition. Resultatet av den här studien visar att det kan finnas ett förhållande mellan kondition och diet och habitat men det behövs mer studier för att bevisa detta.

Introduction

Evidence suggests that 90% of the global oceans large predatory fishes have been lost since fishing became industrialized in the 1950s (Myers and Worm 2003). Such development can have a large impact on worldwide ecosystems since the removal of large marine predators will resonate further down the food web, affecting organisms on lower trophic levels (Casini et al. 2009). Since the late 1980s the Baltic Sea has suffered an ecosystem shift likely caused by a large decrease in the abundance of Atlantic cod (*Gadus morhua*) which can be attributed to a combination of overfishing and unfavourable conditions for breeding (Casini et al. 2009). This dramatic decrease in cod abundance has been mirrored by a subsequent increase in the abundance of European sprat (*Sprattus sprattus*), a key source of food for the species (Casini et al. 2009). This is suggested to have lead to a restructring of the ecosystem in the Baltic Sea from a cod-dominated to a sprat-dominated state (Österblom et al. 2010). Previous abundance of herring (*Culpea herengus*), another prey species of cod has instead decreased. The cause of this could be the increase of sprat since the two species have a large diet overlap meaning they have inter-specific competition for food resources (Casini et al. 2011). The condition of both sprat and herring has decreased during the restructure due to density-dependent mechanisms connected to the high density of sprat (Casini et al. 2011). Such developments may have potential ramifications for cod since they will be feeding on prey of lower quality and therefore possibly not gaining sufficient nutrition, as proposed by the debated junk food hypothesis (Österblom et al. 2008).

Despite this development cod has nevertheless recently begun to recover from the low numbers observed during the early 1990s; however the population is still only at half the size of its historic high levels during the early 1980s (Eero et al. 2012). It appears recovery is not evenly spread out across the Baltic Sea Proper. The abundance of cod in the Bornholm basin is almost at the highest level since the historic high, while most remaining Baltic cod stocks have not changed significantly since the 1990s (Eero et al. 2012). A problem with this development is that the majority of herring and sprat inhabit areas north of the Bornholm basin, which is outside the current geographic range of the Baltic stocks of Atlantic cod (Eero et al. 2012). The biomass of sprat and herring in the Bornholm basin is only 10-15% of the total biomass, providing inadequate food supply for cod (Eero et al. 2012). As the population of cod in the Bornholm basin is rapidly increasing and food availability is low the prey/predator ratios are declining which leads to reduced condition and starvation of cod in this area and also increased cannibalism (Eero et al. 2012). Evidence suggests the decrease in weight and body condition amongst larger cod in the Bornholm basin since 2007 is most likely due to food limitation (Eero et al. 2012).

However, it appears that the competition for resources do not result in an overall decrease in the body condition of cod. A study conducted by Ovegård et al. (2012) found that depending on the fishing gear used fish of different condition were caught. Nets caught cod of generally good condition while baited gear types (hook and trap) caught fish with lower body condition (Ovegard et al. 2012). The catchability of different gear types differ since nets catch fish in movement while baited gear types (hook and trap) attract fish instead. This could mean either that cod of low condition who are actively searching for prey are more attracted to the bait (Ovegård et al. 2012) or that the different gear types catch cod with different life-history strategies, for example different habitats and foraging techniques (Sherwood 2010).

Analysing the diet of cod can give information about its nutritional status, habitat choice and life history strategies, potentially providing information on stock differences. One method to investigate diet is to analyse gut content; however this approach will merely provide information about what the cod has eaten over its last hours (Pinnegar and Polunin1999). To ascertain what cod has eaten over a longer period of time stable isotope analysis could be a more informative tool and it also excludes the problem with empty guts (Michener and Kaufman 2007). Stable isotopes of carbon and nitrogen provide information on the predator’s habitat choice and trophic level.

The ratio of isotopic carbon ($\delta^{13}C$) changes little as it moves through trophic levels (Post 2002). This can be useful to distinguish between different primary sources of carbon, for example littoral or pelagic sources (Michener and Kaufman 2007). The littoral food web tends to be slightly more enriched in $\delta^{13}C$ compared to the pelagic food web as benthic algae have $\delta^{13}C$ values at -17±4‰ which is enriched compared to planktonic algae that have $\delta^{13}C$ values at -22±3‰ (France 1995).

To estimate a predator's trophic position within the food web, the ratio of stable isotopes of nitrogen ($\delta^{15}N$) are used since the consumer is enriched in $\delta^{15}N$ relative to its diet (DeNiro and Epstein 1981). In a study by Minagawa and Wada (1984) it was found that the average $\delta^{15}N$ enrichment is +3.4±1.1‰ per trophic level; however Hansson et al. (1997) found that in the Baltic Sea the average was slightly lower with enrichment of 2.4±0.5‰ per trophic level. Interestingly, changes in $\delta^{15}N$ composition can act as an indicator of changes in body condition (Hobson et al. 1993). Progressive enrichment of ^{15}N in tissue occurs during fasting or if the organism is under nutritional stress because the lighter nitrogen (depleted in ^{15}N) is not replaced (Hobson et al. 1993). This means that knowledge of life history strategies of the studied organism is required to fully be able to interpret the results (Hobson et al. 1993). The base of the $\delta^{15}N$ and $\delta^{13}C$ food webs varies considerably between different ecosystems meaning that variation in $\delta^{15}N$ and $\delta^{13}C$ cannot be appointed to changes in food web unless $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ is determined (Post 2002).

The objective of this study is to investigate whether individual diet- or habitat preferences could provide any insight into the observed variation in the body condition of Atlantic cod in the Baltic Sea. This will be investigated through an analysis of stable isotopes of nitrogen and carbon. The isotopes of nitrogen would reflect the trophic position of the cod; however, since the base level is not known this information will just be used to compare between the cod. Cod expressing low condition, due to nutritional stress, may exhibit a higher ratio of isotopic nitrogen compared to cod with elevated condition. Alternatively, cod may prefer different habitats containing different prey communities, which may affect the trophic position of

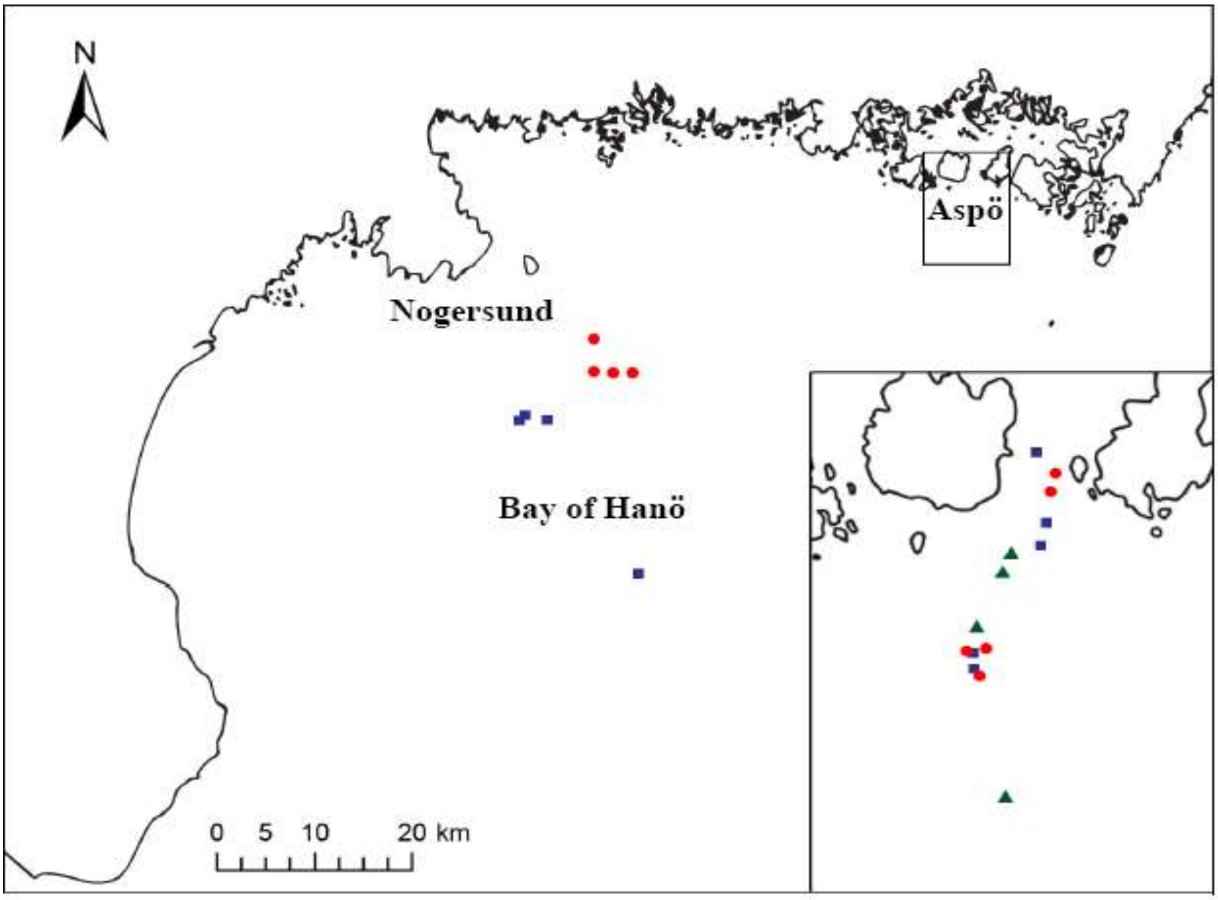

Figure1. Map over the southeast coast of Sweden where the samples were obtained in October and November 2010. The positions for sampling with gillnets (circle), traps (square) and hooks (triangle) are marked.

individual cod. The ratio of isotopic carbon will be used to see if there is any variation between Nogersund and Aspö since they likely provide different habitats (pelagic and benthic respectively).

Method

Sampling

Atlantic cod were sampled from two different areas in the Baltic Sea, off the fishing ports of Nogersund and Aspö, in October and November 2010 (figure 1). Off Nogersund, gillnets and traps were set separately by two professional fishers, meaning that the samples were taken simultaneously but not in the same location. Off Aspö one professional fisherman set three gear types (nets, traps and hooks.) Here the sampling was conducted at the same location but not simultaneously. The traps used were in strings of 8 with up to five strings used at a time, over a total length of 400m. The gillnets were set at the bottom in sets of one to five over 1000m with a stretched mesh size of 114 mm and a height of 3m. The hook gear (VMC 5757 SS16RF, MUSTAD®) was arranged along a 1440m single string with 400 baited single hooks. Both the hooks and traps were baited with Baltic herring that was cut into small pieces. For a more detailed description of the method see Ovegård et al. (2012)

Biological data

All the cod caught were measured, weighed and gutted. The cod were weighed again after the intestines were removed to attain the somatic weight. The otoliths were examined to determine age and gonads were used to determine sex. The Fulton K condition index (Bolger and Connolly 1989) was calculated with the formula below:

$$K = \frac{W}{L^3} * 100$$

Where W is the weight in grams and L is length in centimetres. The index was calculated for both the total weight and the somatic weight. White muscle tissue samples were collected from the neck. The type of tissue used for isotope analysis is relevant since Pinnegar and Polunin (1999) found that white muscle tissue was less variable than other tissues as it only contained small amounts of lipids and inorganic carbonates which cause variability.

Gut content analysis

The gut content of 50 randomly selected cod individuals caught in Nogersund, 20 cod caught with nets and 30 caught with traps, was analysed with regard to types of consumed prey. The prey were determined to species level or to relevant taxonomic group.

Stable isotope analysis

Firstly preparation of the samples for the isotope analysis was carried out. The whole workspace and the scales were sterilised with ethanol, and all the tools were placed in a cup with ethanol and then sterilised over a flame before each use. A sample of muscle tissue was isolated, weighted (range between 1,0-1,2 mg) and placed in a tin canister of known weight. The total weight of the package was thereafter determined. The opening of the tin canister was slightly sealed and the package was placed in a 96-well plate. This procedure was repeated for all individual samples. When a plate was full it was placed in an oven set to 55℃ to dry for over 24 hours, after which the dry weight of each sample if in the range between 0,2-0,6mg was noted. If the weight did not fall within this range the sample had to be redone. When the sample was of the correct weight the tin canister was folded into a square to protect the sample. The samples were thereafter analysed with isotope ratio mass spectrometry (IRMS) with the equipment Thermo DELTA V with ConFlo IV. The results of the isotope analysis, that is the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in ‰ for the individuals, were produced in an excel document. These values were then used with other biological data to find relationships and make graphs and charts.

Statistics

Linear relationship between $\delta^{15}N$ and body condition, length and weight were tested with a regression analysis. The relationship between $\delta^{13}C$ and condition was also tested with a regression analysis. If the assumption of normality and equal variance was fulfilled, T-test and ANOVAs were used when comparing groups. A T-test was conducted to test if there was a significant difference between $\delta^{15}N$ and $\delta^{13}C$ and the two different stations. Also the difference between the group Fulton K and the two groups of gear types in Nogersund, net and trap, was tested for significance with a T-test. All the statistical analyses were carried out with the statistical software IBM SPSS 21.0.

Results

Gut content analysis

The gut content of the 50 samples from Nogersund was divided with regard to consumed prey (Figure 2). The results suggest that clupeid fish constitute an important food source for cod caught in nets, but more rarely eaten by cod of lower condition. Cod caught in traps instead often exhibit empty guts or consumed benthic crustaceans such as mysids or Saudaria entemon, which is a large isopod species. These results suggest that cod caught in traps feed on species lower in the food web or not as often as cod caught in nets. As discovered by Ovegård et al. (2012), there is a significant difference in mean condition between net and trap in Nogersund (T-test, df=71, p=0,000) where cod caught in nets have better mean condition than cod caught in traps (Figure 3). This result may be applied when observing the gut content (Figure 2).

Stable isotope analysis

A total number of 141 individual cod were used in this analysis, 68 individuals from Aspö and 73 from Nogersund. There was no statistically significant relationship between $\delta^{15}N$ and body condition or between $\delta^{13}C$ and body condition when the total number of cod was analyzed. This would suggest no support for the speculation that cod of higher condition feed on prey from a higher trophic level than cod of lower condition.

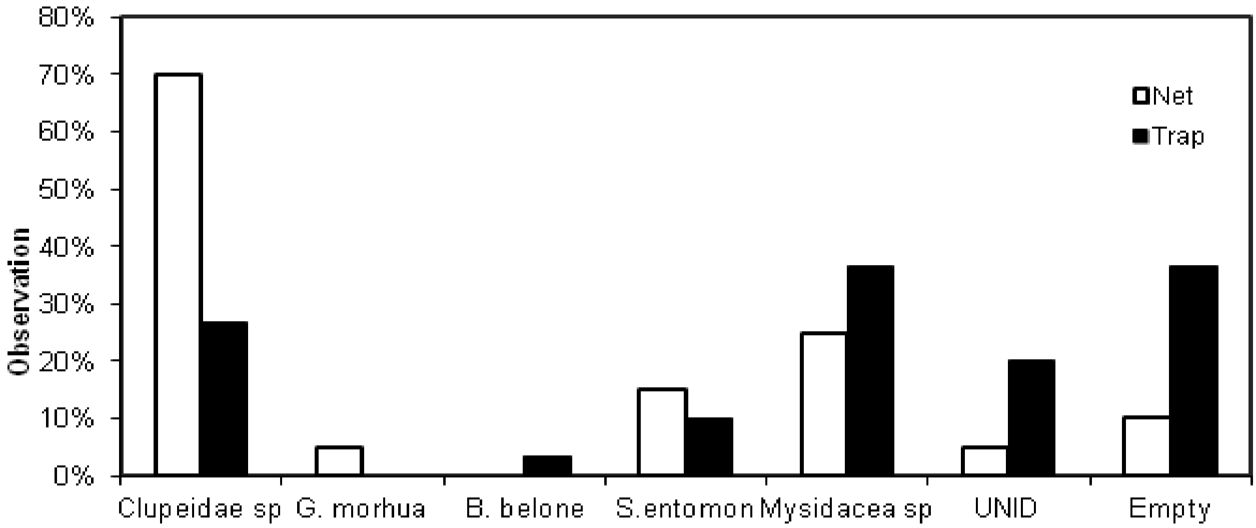

Figure 2. Bar chart of a gut content analysis of 50 cod caught in Nogersund divided into net and trap.

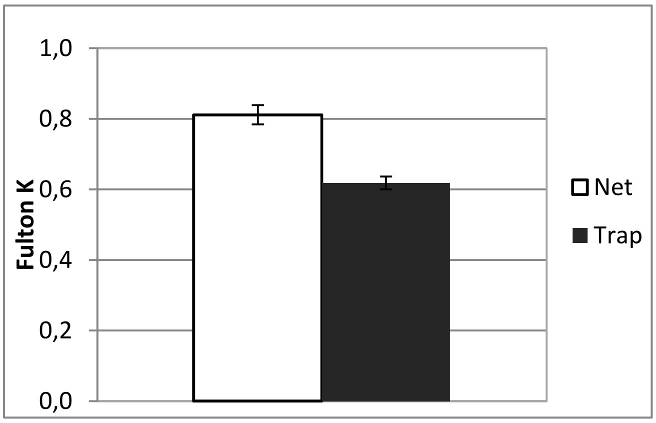

Figure 3. Bar chart over the significant difference in condition (Fulton K) between net and trap, df=71, p=0,000 in Nogersund.

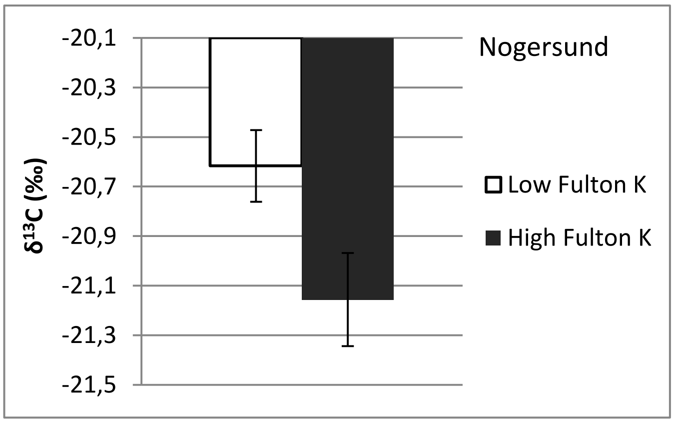

Figure 4. Bar chart over $\delta^{13}C$ in Nogersund divided into high (Fulton K 0,7-0,9) and low condition.

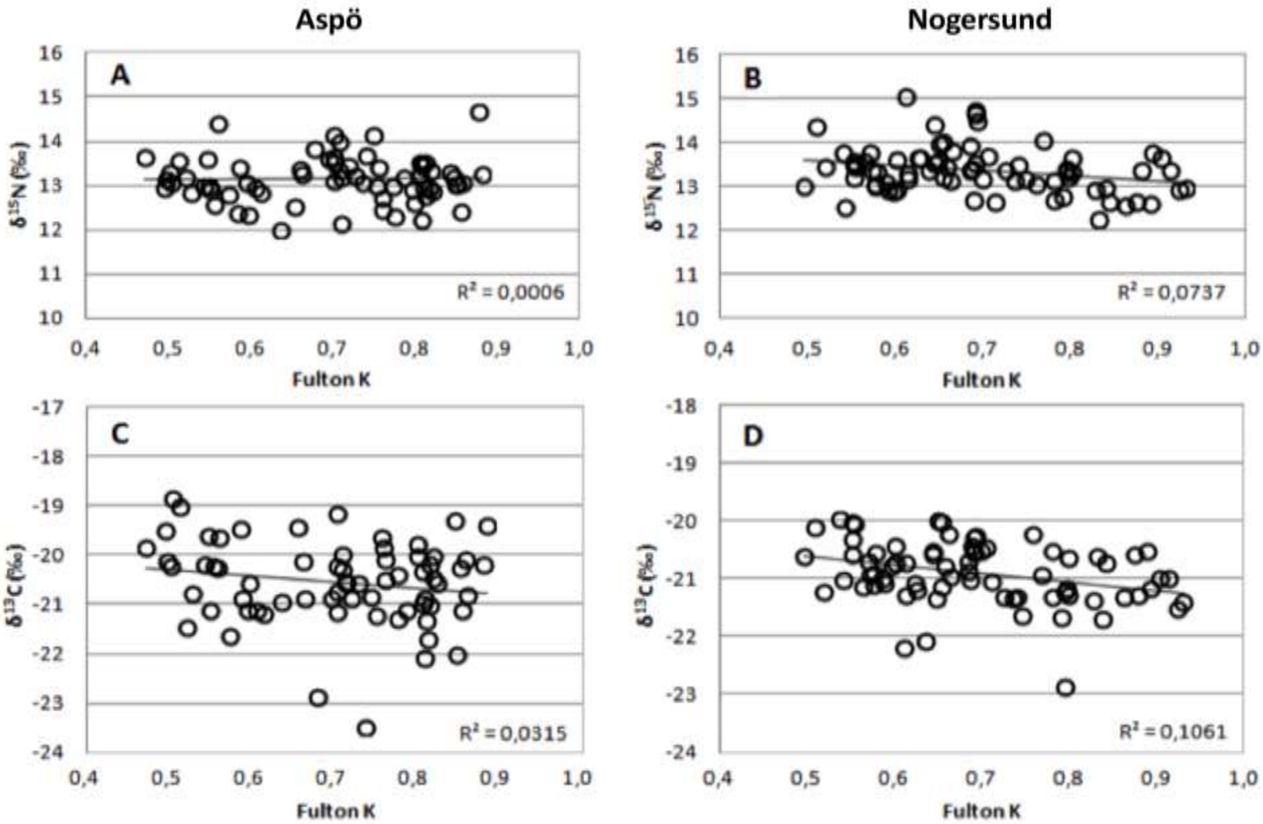

Figure 5. $\delta^{15}N$ and $\delta^{13}C$ plotted against condition (Fulton K) for both Aspö (A and C) and Nogersund (B and D). There is a significant relationship in figure B (R^2= 0,074, p=0,02) and D (R^2=0,106, p=0,005).

Analysing Nogersund and Aspö separately instead provide some interesting observations. Although no statistically significant relationships were found between condition (Fulton K) and $\delta^{15}N$ and $\delta^{13}C$ for Aspö, there was a significant negative relationship between $\delta^{15}N$ and condition for Nogersund (Linear regression, R^2 = 0,074, p=0,02) and for $\delta^{13}C$ and condition (Linear regression, R^2 =0,106, p=0,005) (Figure 5B and D). The negative relationship between $\delta^{15}N$ and condition suggests that cod of a lower condition have an enriched ratio of isotopic nitrogen compared to cod of higher condition. The values

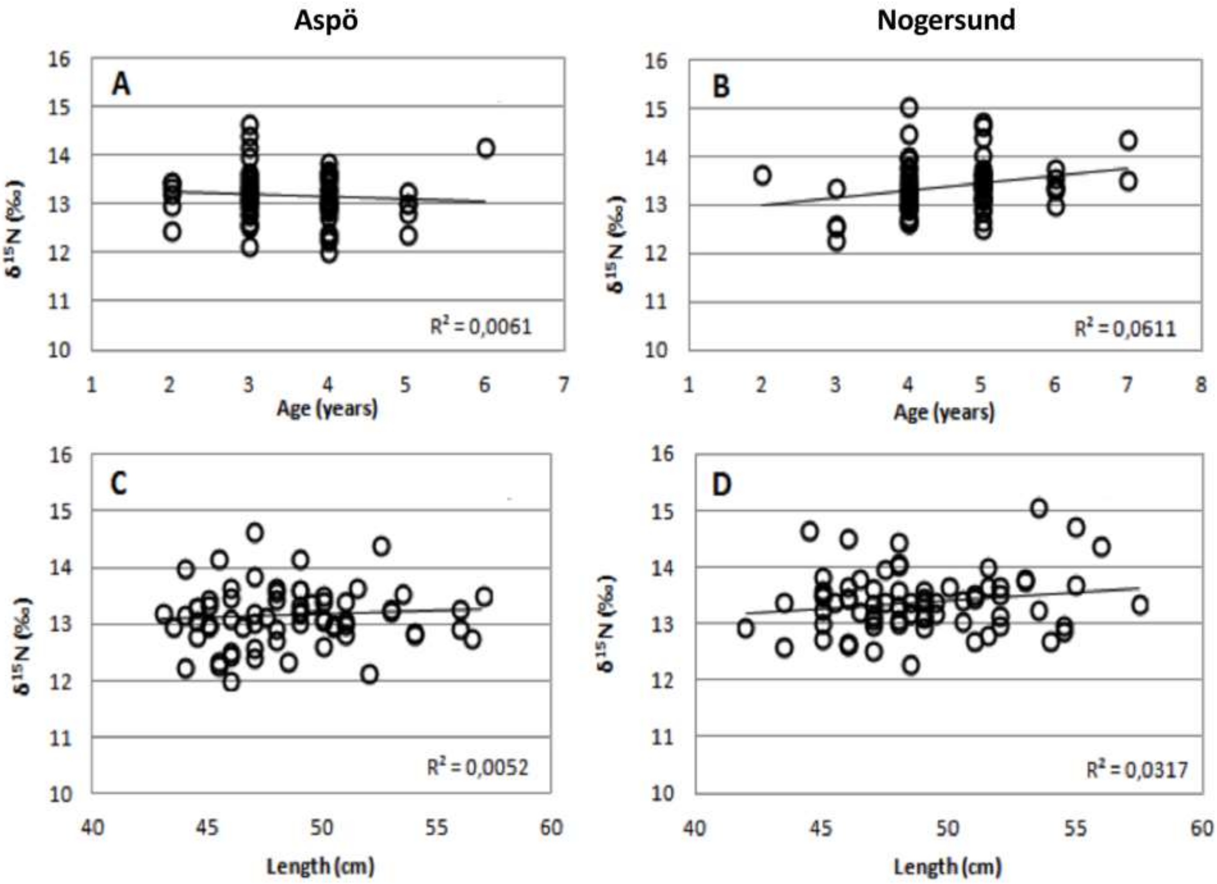

Figure 6. $\delta^{15}N$ plotted against age (A and B) and length (C and D) for both Aspö and Nogersund. Significant relationship was found for B: R^2=0,037, p=0,023

of isotopic carbon for Nogersund were divided into two groups, namely low and high condition. The low condition group comprised individuals with Fulton K values between 0,5-0,7, were as the high condition group contained individuals with Fulton K values between 0,7-0,9. When these groups were compared statistically, cod of low condition exhibited significantly higher $\delta^{13}C$ (Figure 4) meaning that cod of a lower condition in Nogersund have higher isotopic carbon value than cod with high condition. This can be due to cod of different condition moving in slightly different habitats. Cod with low condition would then be in slightly more pelagic habitats and cod with higher condition in slightly more pelagic habitats.

No statistically significant relationship was discovered between $\delta^{15}N$ and length of cod for either Aspö or Nogersund but there was a significant positive relationship between $\delta^{15}N$ and age (Linear regression, R^2=0,037, p=0,023) in Nogersund (Figure 6B). This suggests that cod of a greater age feed on prey at higher trophic level (more enriched) than younger cod.

There is a significant difference between Aspö and Nogersund concerning $\delta^{15}N$ (T-test, df=139, p=0,013) were Nogersund have a higher mean $\delta^{15}N$ (13,38±0,12‰) compared to Aspö (13,16±0,12‰). This suggests that the cod caught in Nogersund feed at a higher trophic level than cod caught in Aspö. There was also a significant difference between Aspö and Nogersund concerning $\delta^{13}C$ (T-test, df=139, p=0,003) where Aspö was more enriched with a mean of -20,55±0,2‰ compared to Nogersund with a mean of -20,74±0,12‰. This shows tendencies towards

Aspö being more benthic than Nogersund which would be more pelagic this can also be observed in Figure 1.

Discussion

Ovegård et al. (2012) found that cod caught with nets exhibited higher condition, whereas cod caught with traps showed a lower condition, an observation illustrated here by in figure 3. Therefore the data from the gut analysis can be interpreted as differences in high and low condition, instead of net and trap. The bar chart (Figure 3), illustrates that cod with higher condition (net) consume more clupeids and juvenile cod compared to cod with lower condition (trap). The cod with lower condition eat more crustaceans and also have a higher degree of empty stomachs. This can either imply that [1)] cod with lower condition frequent habitats containing different prey communities that affect the $\delta^{13}C_{base}$, or [2)] that they cannot compete with cod of better condition. Starvation has been shown to affect the swimming performance of cod, including both sprint and endurance swimming, since muscle metabolic capacities decreases (Martinez et al. 2003, Martinez et al. 2004). Either scenario is possible.

There is however a significant negative relationship between ratios of isotopic nitrogen and the condition of cod in Nogersund. This suggests that cod with lower condition consume prey at a higher trophic level compared to cod with better condition, therefore having a higher ratio of isotopic nitrogen. Cod with lower condition have decreased swimming performance (Martinez et al. 2003, 2004) and therefore would be assumed to hunt for different prey as illustrated in figure 2. This should produce a lower ratio of isotopic nitrogen compared to cod exhibiting better condition. One possible explanation to these contradicting results could be that cod with low condition are enriched in nitrogen due to starvation (Figure 7). The excreted nitrogen in ammonia, urea and uric acid is lighter than the nitrogen in body protein. Starving animals do not replace this lighter nitrogen due to the lack of intake of dietary protein leading to progressively higher ratio of ^{15}N during the course of starvation (Hobson et al. 1993). Since there is no way of knowing to what degree this observed enrichment originates from starvation or from the trophic level of the diet it is hard to say anything about diet from the ratio of nitrogen isotopes. To better be able to examine how much of the enrichment comes from starvation amino acids can be studied using stable isotope analysis. Hobson et al. (1993) conducted a quail study where the ratio of isotopic nitrogen of amino acids did not differ between the group that was starving and the control group, so amino acids could potentially be used as a baseline. Another explanation to why the cod have a negative relationship between the ratio of isotopic nitrogen and condition could be that they have different habitats and feed on different prey. This scenario can be supported by the negative relationship between the ratio of isotopic carbon and condition in Nogersund (Figure 5D) and the significant difference between of isotopic carbon values (Figure 4) showing that cod of lower condition have a tendency to be more enriched compared to cod of higher condition, suggesting that cod of lower condition predominantly prefer costal benthic habitats.

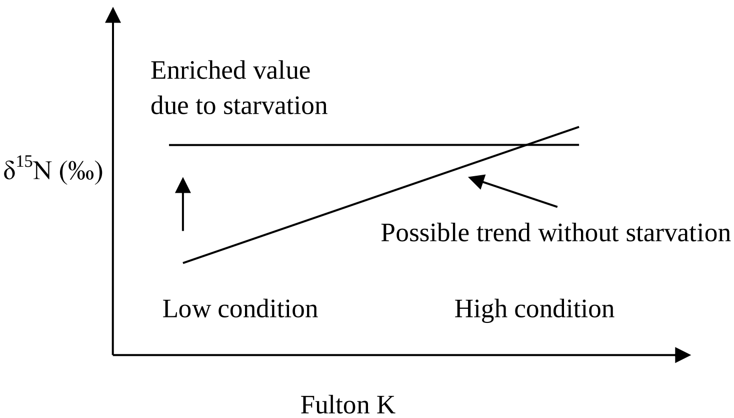

Figure 7. An illustration of possible effects of starvation on $\delta^{15}N$ values.

There was a significant difference of the ratio of carbon isotopes between Aspö and Nogersund. Cod off Nogersund had a slightly lower mean value (-20,91±0,54‰) compared to cod off Aspö (-20,55±0,84‰), results that are consistent with France's (1995) findings that benthic habitats have food chains that are more enriched than pelagic ones. Although the enrichment in France (1995) study is larger with benthic habitat values at -17±4‰ compared to pelagic values at -22±3‰ and since $\delta^{13}C_{base}$ is unknown it is hard to tell if it is different habitats.

There was not conclusive evidence supporting the notion that differences in condition of cod could be the result of differences in diet. Since it is unknown how much starvation affects the ratio of isotopic nitrogen, it remains unknown what actual trophic level individual cod preys on. Although this negative relationship could be caused by cod feeding on prey in different habitats, there is no way of disentangling the separate effects of starvation and diet enrichment. There was a significant negative relationship between the ratio of isotopic carbon and condition in Nogersund (Figure 5D) and cod of lower condition had significantly lower isotopic carbon values than cod with better condition (Figure 4) which could suggest that the cod of lower condition move in different habitats, possibly more benthic habitats even though they were caught in pelagic habitats.

The results of this study show that there is possibly a relationship between condition, habitat diet with cod of lower condition preying and moving in different habitats compared to cod with higher condition. However more studies and more information are required to draw a conclusion. A comparison between the ratio of isotopic nitrogen in amino acids (that are not affected by starvation) and the ratio of isotopic nitrogen in muscle tissue could be conducted in order to observe how much starvation affects the results. To better interpret the relationships of the ratios of isotopic carbon and nitrogen the base values would be helpful information.

References

Bolger T, Connolly P. L. (1989) The selection of suitable indices for the measurement and analysis of fish condition, The Fisheries Society of the British Isles, Dublin

Casini, M. Hjelm, J. Molinero, J. C. Lovgren, J. Cardinale, M. Bartolino, V. Belgrano, A. Kornilovs, G. (2009) Trophic cascades promote threshold-like shifts in pelagic marine ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106 (1): 197-202

Casini, M. Kornilovs, G. Cardinale, M. Mollmann, C. Grygiel, W. Jonsson, P. Raid, T. Flinkman, J. Feldman, V. (2011) Spatial and temporal density dependence regulates the condition of central Baltic Sea clupeids: compelling evidence using an extensive international acoustic survey. Population Ecology 53(4): 511-523

Deniro, M. J. and Epstein, S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Et Cosmochimica Acta 45(3): 341-351

Eero M, Vinther M, Haslob H, Huwer B, Casini M, Storr-Paulsen M, Köster FW (2012b). Spatial management of marine resources can enhance the recovery of predators and avoid local depletion of forage fish. Conservation Letters 0:1-7

France, R. L. (1995) C-13 Enrichment in benthic compared to planktonic algae - foodweb implications. Marine Ecology Progress Series 124(1-3): 307-312

Hansson, S. Hobbie, J. E. Elmgren, R. Larsson, U. Fry, B. Johansson, S. (1997). The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. Ecology 78(7): 2249-2257

Hobson, K. A. Alisauskas, R. T. Clark, R. G. (1993) Stable-nitrogen isotope enrichment in avian-tissues due to fasting and nutritional stress - implications for isotopic analyses of diet. Condor 95(2): 388-394

Martinez, M. Bedard, M. Dutil, J. D. Guderley, H. (2004) Does condition of Atlantic cod (Gadus morhua) have a greater impact upon swimming performance at U-crit or sprint speeds? Journal of Experimental Biology 207(17): 2979-2990

Martinez, M. Guderley, H. Dutil, J. D. Winger, P. D. He, P. Walsh, S. J. (2003) Condition, prolonged swimming performance and muscle metabolic capacities of cod

Gadus morhua. Journal of Experimental Biology 206(3): 503-511

Michener RH, Kaufman L (2007) Stable isotope ratios as tracers in marine food webs: An update. In: Stable isotopes in ecology and environmental. 2nd edition. Eds. Micherner RH & Lajtha K. Blackwell Publishing; Malden, Oxford & Carlton, chap. 9

Myers, R. A. and Worm B. (2003) Rapid worldwide depletion of predatory fish communities. Nature 423 (6937): 280-283

Osterblom, H. Gardmark, A. Bergstrom, L. Muller-Karulis, B. Folke, C. Lindegren, M. Casini, M. Olsson, P. Diekmann, R. Blenckner, T. Humborg, C. Mollmann, C. (2010) Making the ecosystem approach operational-Can regime shifts in ecological- and governance systems facilitate the transition? Marine Policy 34(6): 1290-1299

Osterblom, H. Olsson, O. Blenckner, T. Furness, R. W. (2008) Junk-food in marine ecosystems. Oikos 117(7): 967-977

Ovegard, M. Berndt, K. Lunneryd, S. G. (2012) Condition indices of Atlantic cod (Gadus morhua) biased by capturing method. ICES Journal of Marine Science 69(10): 1781-1788

Pikitch, E. K. Santora, C. Babcock, E. A. Bakun, A. Bonfil, R. Conover, D. O. Dayton, P. Doukakis, P. Fluharty, D. Heneman, B. Houde, E. D. Link, J. Livingston, P. A. Mangel, M. McAllister, M. K. Pope, J. Sainsbury, K. J. (2004) Ecosystem-based fishery management. Science 305(5682): 346-347

Pinnegar, J. K. and Polunin, N. V. C. (1999) Differential fractionation of delta C-13 and delta N-15 among fish tissues: implications for the study of trophic interactions. Functional Ecology 13(2): 225-231

Post, D. M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83(3): 703-718

Sherwood, G. D. and Grabowski, J. H. (2010) Exploring the life-history implications of colour variation in offshore Gulf of Maine cod (Gadus morhua). ICES Journal of Marine Science 67(8): 1640-1649

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22362 Lund