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Effects of *Pan*-I genotype and family cross on oxygen consumption rate among juvenile Atlantic cod (*Gadus morhua*)

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Abstract

Atlantic cod has been proved to be adapted genotypic and behavioural to different environmental temperatures. We compared the oxygen consumption rate (VO_2/M) of juvenile Atlantic cod from both different family crosses and with different *Pan*-I genotypes acclimated to 4ºC, 8ºC and 12ºC and when exposed to an acute thermal challenge. No differences were found between genotype at any acclimation temperature or between families at 8ºC (T_{ac}) however differences were found among families at 4ºC and 12ºC (T_{ac}) although they do not seem to be associated with maternal size or studied conditions. The results suggest that *Pan*-I locus is not a perfect marker for migratory behaviour and that genetically inherit abilities from parental can produce differences between their spawning though further studies need to be done to assess this inherit conditions.

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Introduction

Although fish stocks historically have been considered to be a continuous unit recent studies have demonstrated the existence of metapopulations or behavioural types formed by local populations stocks or life history groups adapted to special environment conditions such as temperature or salinity (for review see Conover et al. 2006) and to different behaviour migration. These groups show own morphological characteristics and own behaviour characteristics but their main characteristics are shared by all the specie (Swain et al. 2001; Marcil et al. 2006; Jorgensen et al. 2008) so they are not considered a different specie. These differences have become an indicator of marine fish stock structure among commercial species (Begg et al 1999).

Atlantic cod (*Gadus morhua*) is both widely distributed and of great economic importance throughout the North Atlantic (Kurlansky, 1998) especially in countries with well developed fisheries such as Iceland and Norway. The reduction in production, size and the decrease of catches between the last years has led to considerable concern about the long-term sustainability of the cod stocks. Atlantic cod is also an example of fish species that shows local adaption to specific environmental condition and subdivision within stocks. Differences in growth (van de Meeren et al. 1994; Purchase and Brown 2001; Hutchings et al. 2007), survival (Hutching et al. 2003; Hutching et al. 2007), food conversion efficiency and energy allocation (Purchase and Brown 2001; Salvanes et al. 2004), metabolic rates (Grabowski et al. 2009) have been demonstrated within stocks across the North Atlantic. For example, a recent study found that growth rates of Atlantic cod from the North East Arctic / Barents Sea (NEA) were much lower than counterparts in the North Sea (NS) and the NEA also had reduced fertility compared to that of NS (Portner et al. 2001). It appears that different populations of cod are optimized for different thermal regimes.

A particularly interesting aspect of local adaption in Atlantic cod is the interaction between depth, temperature and the pantophysin (*Pan*-I) locus. The *Pan*- I locus codes information for integral membrane protein expressed in cytoplasmatic transport vesicles (Windoffer et al. 1999; Brooks et al. 2002) and it is affected by temperature, salinity and depth as forces of selection (Sarvas and Fevolden 2005, Case et al. 2005). Specimens from cod collected by Jónsdóttir et al (2002) showed different depth distribution that latter was attributed to a genotypic distribution for the *Pan*-I and a better adaption to different temperatures. *Pan* -I^{AA} genotype showed a greater abundance in the coastal behaviour cod while the *Pan*-I^{BB} genotype was principally manifested on individuals with deep-water or frontal behaviour even though can be found in both depth areas (Palsson and Thorsteinsson 2003). Individuals with *Pan*-I^{AB} seem to be able to act both as costal and frontal behaviour individuals. Pampoulie et al (2006) confirmed these results and proved separation between northern and southern Icelandic cod due to different thermal environments. Costal-water behaviour individuals does not experience significant temperature or depth changes throughout the year and don't use to go deep than 200 meters while frontal behaviour individuals may experience changes of 3-4°C

for short periods of time while they swim up and down through depth changes during the feeding season so that would indicate that concentration of food would be the reason of this activity (Palsson and Thorsteinsson 2003). Even thought there exists an overlap in depth during the spawning seasons it is not known if both behavioural types interact genetically. The existence of a relationship between the *Pan*-I genotypes and the behavioral types of Icelandic cod suggests that the *Pan* -I expression might be linked to traits that express migratory ability during the feeding season, therefore leading to the existence of differential behavioral units (Pampoulie et al 2008) and it has also been related to the rate of growth in both larval and adult stage (Jónsdóttir et al. 2002; Imsland and Jónsdóttir 2003, Case et al. 2002). These behavioral groups inhabiting distinct thermal environments may differ genetically and as such may be expected to exhibit differences in their thermal physiology (Beacham et al. 2002; Billerbeck et al. 2001; Conover and Present 1990; Ruzzante et al. 2000). Coastal behaviour individuals inhabit warmer waters so are expected to have better results at higher temperatures, while deep water behaviour individuals, should have better results in colder water and should be better to rapid temperature changes (Palsson and Thorsteinsson 2003). Admittedly the relationship between the *Pan*-I locus and the observed behavior pattern is not perfect since studies found individuals with *Pan*-I^{AA} acting like frontal and *Pan*-I^{BB} acting as costal behavior cod. That would indicate that *Pan*-I locus may merely reflect the association of other unknown genes that are the real responsible of the migratory behavior of cod (Pampoulie et al 2008). Additionally the effects of the genotype could be easily masked by maternal effects, non-genetics factors contributed to the offspring phenotype by the mother that does not result from the action of their own genes and the interaction of those genes and the environment (Bernardo et al 1996), for example as seen by Marteinsdóttir and Steinarsson (1998) size and viability of eggs is related with female conditions. A good example of this could be the existence of different behavioral groups of cod that has also been shown in Norwegian waters where they are considered as two distinct stocks, namely the NEA cod with similarities with the Icelandic frontal cod and NC cod with similarities with the Icelandic costal cod. The spawning areas of NC cod are located in several coastal regions but also within the same spawning areas where NEA cod spawns (Jakobsen et al. 1987) what means that they overlap in depth during the spawning area as the Icelandic cod.

We compared the weight-specific oxygen consumption rate (VO_2/M) of juvenile Atlantic cod using *Pan*-I locus as proxy for behavioral type. Starting from the idea that Icelandic cod differ not only in behaviour but also in the response to different thermal challenges, our primary objective was to evaluate the relative roles of *Pan*-I genotype and family cross on the relation between temperature and VO_2/M in Icelandic cod both individual at acclimation and when exposed to an acute thermal challenges that may show a better fitness for the individuals that act as frontal fish.

Methods

Sampling

Juvenile Atlantic cod subjected to study were the offspring of parental of five adult males and six adult females caught using gill nets from commercial fishing vessels on April 17, 2009 at the east coast of the Vestman Islands in one of the main spawning area of the south coast of Iceland (63 º 45'N, 20 º 00'W) (Fig. 1). Crosses between different genotype fish were performed in order to have 6 different families, three with *Pan* -I^{AB} and three with *Pan* I^{BB}. The parental were caught in closer areas so it was assumed that they all had the same migratory behaviour. The extraction of eggs and sperm was produced by pressing the abdominal cavity of ripe and running individuals. *In vitro* fertilization was carried out aboard the vessel and fertilized eggs from each male-female cross were kept in 1-L containers and stored on ice for transport to the Marine Research Laboratory (MRL) of Grindavik.

FIG.1 Map of Iceland and the spawning area where parental were caught on April 17, 2009.

Larva rearing

Cod larvae were hatched two 25-L flow-through water (0.5 L min^{-1}) tanks with three families in each one at a temperature of 8.0±1.0º C for two weeks. In each tank, two of the families were marked with a fluorescent substance that was trapped inside the otolith nucleus. Tanks were emptied to be 10 litres and the marking product was supplied at a concentration of 250-300 (mg l^{-1}) for 6-8 hours. One family was marked alizarin complexone (ALZ), another with oxytetracycline (OTC) and the third one with nothing (Reinert et al.1998). After marking the cod larva were moved to 150-L flow-through (0.5 L min^{-1}) tanks at 12.0 ±1.0º C and were fed to excess with rotifers three times a day following Marcil et al. (2006) and Hutchings et al. (2007) protocol. Fish were allowed to grow over a 90-day period before proceeding with the experiment.

Experimental design

Individuals (*n*=180) were randomly assigned to the different acclimation temperatures (T_a) 4.0±1.0º °C, 8.0±1.0º °C, and 12.0±1.0º °C (Fig. 2) and allowed to acclimate to that temperature for 10 days prior to the experiment. These temperatures were chosen because approximate the range of temperatures that young-of-year cod could encounter in Icelandic waters (Astthorsson et al. 2007).

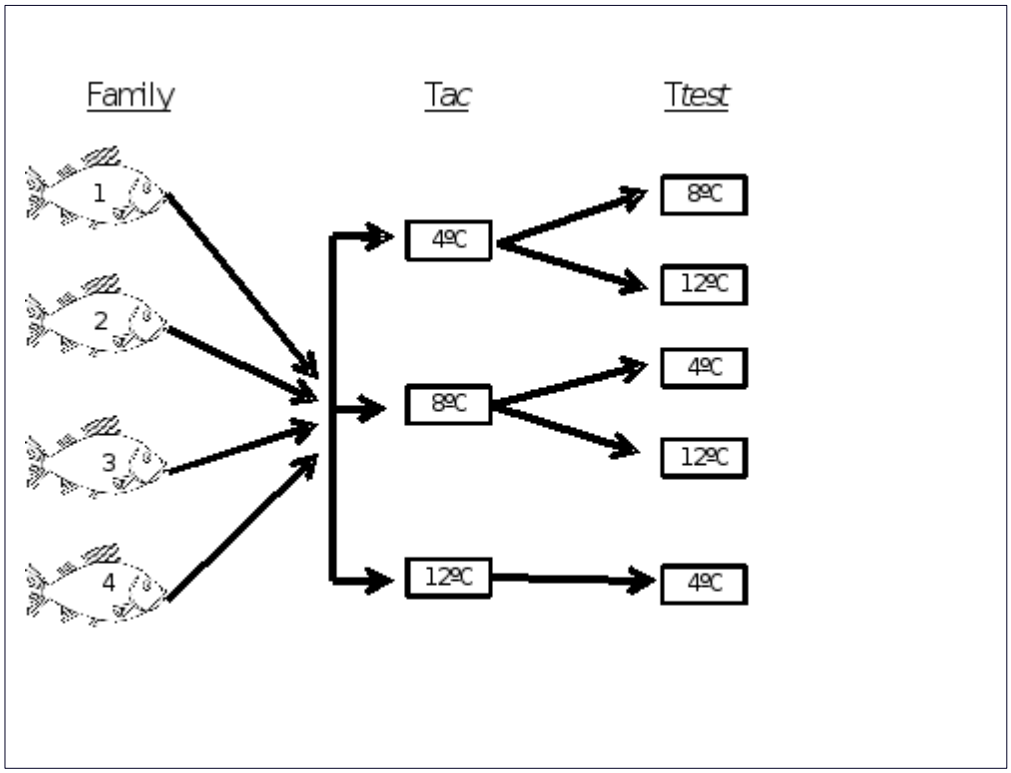

Fig 2. Diagram of the experimental design including families, acclimation temperatures (T*ac*) tanks and the different test temperatures (T*test*) tanks that the acclimation groups were moved to in order to test VO_2/M at rapid temperature changes. Family 1 (n=42) and family 2 (n=13) family 3 (n=48) and family 4 (n=13) were *Pan*-I^{AB} while only individuals from families 1(n=19) and 3(n=1) were found with *Pan*-I^{BB} genotype (Table 2).

Food was withheld for five days prior to the experiment that so an accurate measure could be made of baseline metabolic rate reducing the overestimation. After the 10-day acclimation period was completed all individuals were caught trying to cause as little stress as possible and were placed each in a 2.5-L bucket, with several holes in the lid (2 cm diameter) to allow water exchange, that were left in the tank overnight (approx. 12-14 hours) to keep the temperature constant and to allow individuals to acclimated to being held in the bucket to reduce the overestimation of metabolic rate produced by the stress. With each experimental group 6 empty control container were placed and received the same treatment as the other.

Following procedures described by Grabowski et al. (2009) experimental time, initial dissolved oxygen concentration ($DO_{control}$) and temperature (T_1) were recorded, containers were closed tightly, checked for air bubbles and made sure that none were trapped inside and left 2.5-5.0 hours to ensure detectable changes in oxygen concentration. Dissolved oxygen concentration (DO_{test}) was recorded using a digital dissolved oxygen meter (OxyGuard International, Birkerod, Denmark), temperature (T_2) (by TXL Ebro 420. ± 0.3 ºC) and total time were recorded after each trial. Then water was changed and the buckets were sealed and moved to a second test temperature. Individuals acclimated to 12ºC were moved to 4ºC tank. Individuals acclimated to 8ºC were split half to 4ºC and half to 12ºC tanks. Individuals acclimated to 4 ºC were moved half to 8ºC and half to 12 ºC. After 2.5-3.0 hours changes in oxygen concentration were detectable so we recorded a second dissolved oxygen concentration, temperature and time (Fig.2; Table 2).

After the second measurement fish were euthanized using tricaine methylsulfonate (MS-222). Total length to the nearest mm and wet weight to the nearest 0.01g were scored. Containers were washed for subsequent measurements. Both parents and experimental subjects were genotyped at the *Pan*-I loci following the protocol described by Pampoulie et al. (2006) and using the primers described by Nielsen et al. (2007). We used the Chelex/ Proteinase K extraction protocol (Walsh et al. 1991) to extract DNA from the gill filaments samples. The remaining analysis was contracted by Matís (Reykjavik, Iceland).

Extraction of the sagittal otolith

As said before to assess the family cross on each individual, sagittal otoliths were marked during the early stages of growth. Both sagittal otoliths were removed, washed in water and stored dry in darkness. The extracted otoliths were include in epoxy resin (Embedding in Araldite CY 212 for electron microscopy, Agar Scientific Ltd, Essex, England) for sectioning with a precision saw (Isomet 1000, Precision saw) to expose the nucleus. If necessary, the surface was polished to improve the assessment of the nucleus following the procedure described by Secor et al. (1991). Sections were examined through a fluorescence microscope (Nikon Eclipse E800) with different fluorescence filters (Nikon VFM: UV-2A, B-2A, G-2A). The analysis of the otoliths was performed by two people and any disagreement was resolved by examining the second otolith.

Data analysis

The relative role of the *Pan*- I genotype and family cross in metabolic rates was evaluated by using ANOVA to test if oxygen consumption differed between genotypes or families at acclimation temperature (T_a) and at abrupt temperature changes (T_{test}). The weight specific oxygen consumption rate (VO_2/M) was calculated as mg of oxygen consumed per hour per gram of fish ($mgO_2g^{-1}h^{-1}$). Due to variability of body weights between the experimental groups, weight-specific values were used to standardize oxygen consumed per gram of body mass. Weight data was log-transformed to meet parametric assumptions of normality. In order to meet common-garden assumptions of homogeneity of treatments between populations one-way ANOVA was used to test for differences in mean weight.

Several ANOVA tests were used to test the null hypothesis that no differences in oxygen consumption rates between genotypes and between families existed in the different experimental groups at acclimation temperature. A first two-way ANOVA test was performed to check if weight was significant using the model: W=G+F+G:F were W was weight, G was genotype and F was family. When weight happened to be un-significant we constructed a model (VO_2=G+F) to assess the effect of both genotype and family in the oxygen consumption rate. Tests were performed to assess if differences in family weight at each acclimation temperature affected oxygen consumption rate. TukeyHSD test was done to asses which families were different when differences were found at any temperature. Only individuals with genotype Pan I^{AB} were used for the family data analysis due to the unequal distribution of families for genotype Pan I^{BB}. Maternal effects to explain family differences were assessed by a Pearson's correlation test between mean VO_2 at and different T_{test} and mean total length, total weight, gutted weight, hepatosomatic index ([liver weight/gutted weight]×100) and Fulton's condition factor (K = (gutted weight/length^3) ×100) of family mothers using α=0.05 and [r] ≥0.02.

Three-way ANOVA tests were performed to assess the relation of the family, genotype and test temperature with the changes of oxygen consumption rates when individual suffered rapid temperature changes. We did these by the model: $\Delta VO_2=T_{test}+F+G$ were ΔVO_2 the change in oxygen consumption rate and T_{test} is the test temperature that individuals were moved different from the acclimation temperature. Individual acclimated at 12ºC were tested by a two-way ANOVA (ΔVO_2=F+G) because they were all moved to the same test temperature (T_{test}=4ºC).

A sequential Bonferroni correction for the $P_{\text{-value}}$ was used to control type-I error in the ANOVAs tests. Differences were considered significant when $P_{\text{-value}} \leq 0.015$. An asterisk indicates a significant difference within the experimental group.The statistical results were obtained using R (v. 2.6.2 R Development Core Team 2008) software.

Results

Out of the 136 individuals analysed 116 exhibited were *Pan*-I^{AB} and while only 20 were *Pan*-I^{BB}. Only 4 out of 6 families were recovered at the end of the experimental period. No individuals marked with oxytetracycline (OTC) were found. Mortality rate for these families was considerate maximum. Of the 4 families for the genotype *Pan*-I^{AB} all were found whereas only two families were found for the genotype *Pan*-I^{BB}. No individuals marked with alizarin (ALZ) were found for *Pan*-I^{BB}. Maternal total length, total weight, gutted weight, hepatosomatic index and Fulton's condition factor (Table 1) were considered to asses maternal effects. A positive relation was found between weight, temperature an VO_2/M (Table 2). No differences were found in weight between families (T_{ac} =4ºC, $F_{3,46}$ =1.297, P=0.280; T_{ac} =8ºC, $F_{3,51}$ =0.0324, P=0.990; T_{ac} =12ºC, $F_{3,27}$ =2.675, P=0.067) nor between weight in genotypes at different acclimation temperatures (T_{ac} =4ºC, $F_{1,48}$ =0.057, P=0.810; T_{ac} =8ºC, $F_{1,53}$ =2.023, P=0.160; T_{ac} =12ºC, $F_{1,29}$ =2.225 P=0.146) (Fig 3)(Table 2).

FIG. 3. Mean weight of 90 days old Atlantic cod (***Gadus morhua***) caught in Iceland at acclimation temperature of 4ºC, 8ºC and 12ºC acclimation temperatures. AB groups are the individuals with *Pan*-I^{AB} and BB groups are the individuals with *Pan*-I^{BB}. No statistical differences were found between weight means among the experimental groups at acclimation temperatures (4ºC, 8ºC, 12ºC).

Effects of genotype and family on weight specific oxygen consumption rate

The null hypothesis that the genotypes AB and BB for *Pan*-I were equal at oxygen consumption rate could not be rejected at any of the treatment temperatures (T_{ac} =4ºC, $F_{1,48}$ =0.377 P=0.542; T_{ac} =8ºC, $F_{1,53}$ =4.727 P=0.034; T_{ac} =12ºC, $F_{1,29}$ =0.531, P=0.471) (Fig.3).

As said before due to the lack of individuals from family 2 and 4 among Pan-I^{BB} genotype only families with genotype *Pan*-I^{AB} were used to assess the family effect on VO_2/M. No differences were found in oxygen consumption rate between families at 8ºC (T_{ac} =8ºC, $F_{3,40}$ =3.256, P=0.031). But were found at 4ºC and 12ºC acclimation temperatures (T_{ac} = 4ºC, $F_{3,39}$ =6.006, P=0.002*; T_{ac} =12ºC, $F_{3,25}$ =5.770, P=0.004*) . Family 1 was different from family 4 (P=0.012) and family 2 from 4 (P=0.02) at 4ºC.

Family 1 was different from family 3 (P=0.017) and family 2 from family 3 (P=0.04) at 12ºC (fig.4).

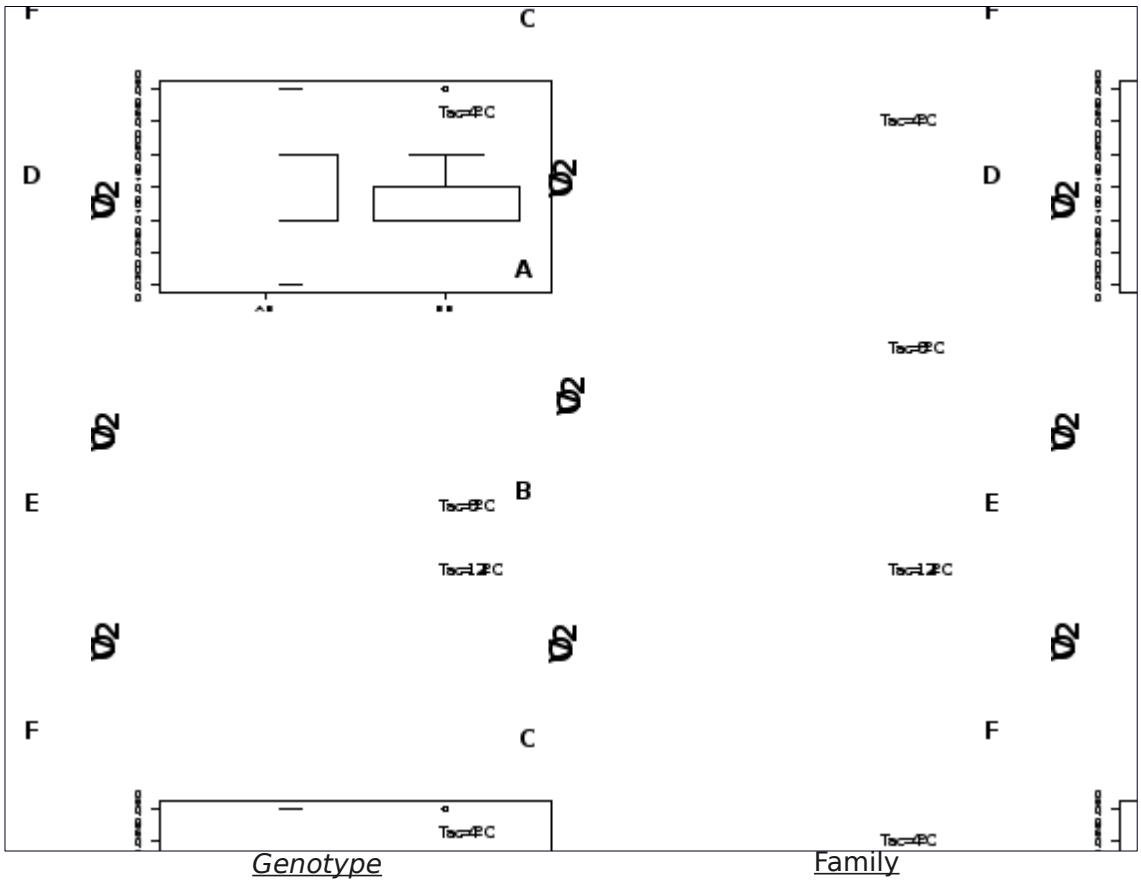

FIG.4. Oxygen consumption rate (VO_2 as $mgO_2g^{-1}h^{-1}$) for each genotype and family at 4ºC, 8ºC and 12ºC acclimation temperatures. "A","B" and "C" show VO2/M of individuals split according to the *Pan*-I genotype at acclimation temperature of 4ºC, 8ºC and 12ºC respectively. "D","E" and "F" show VO_2/M of individuals split according to the family crosses at acclimation temperature of 4ºC, 8ºC and 12ºC respectively. No statistical differences were found between genotypes and no differences were found between families at T_{ac}= 8ºC. Whereas differences were found between families 1-4 and 2-4 at T_{ac}=4ºC and between families 1-3 an2-3 at T_{ac}=12ºC.

Oxygen consumption rate at rapid temperature changes

No differences were found between families(T_{ac} =4ºC, $F_{3,44}$ =1.069, P=0.371; T_{ac} =8ºC, $F_{3,49}$ =2.128, P=0.108; T_{ac} =12ºC, $F_{3,26}$ =2.457, P=0.360) or between genotypes (T_{ac} =4ºC, $F_{1,44}$ =0.022 P=0.882; T_{ac} =8ºC, $F_{1,49}$ =2.195, P=0.144; T_{ac} =12ºC, $F_{1,26}$ =0.865, P=0.08) when individuals were exposed to rapid temperatures variations(Table 2) (Fig.5). However despite of no statistical result confirms it family two has higher

oxygen consumption rate at lower temperature and when was moved to colder temperature and family three has the lower VO_2 at any temperature but the higher when it was moved from cold to warmer temperature (T_{ac}= 4ºC to T_{test} = 12ºC).

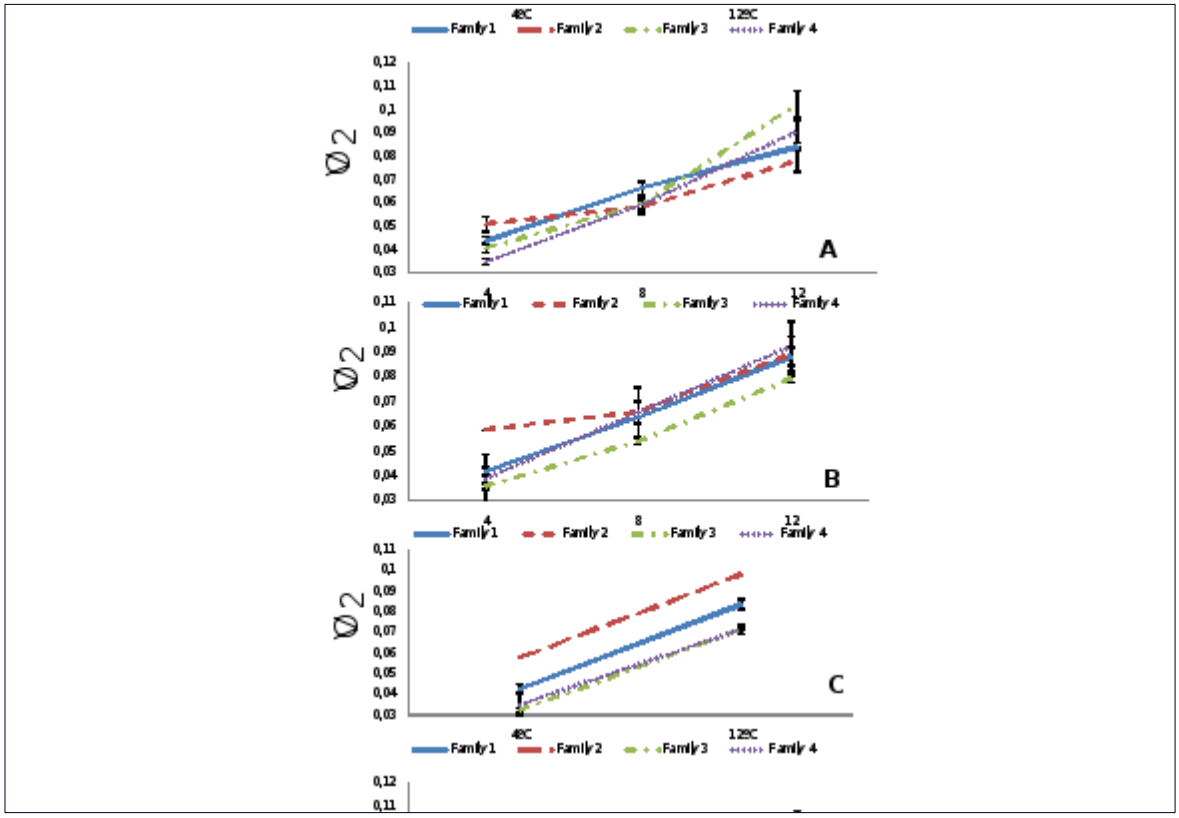

FIG.5. Weigh-specific oxygen consumption rate (VO_2 as $mgO_2g^{-1}h^{-1}$) reaction norms at acclimation temperature (T_a) and at test temperature (T_{test}) of Atlantic cod (*Gadus morhua*). "A" represents VO_2 of individuals acclimated at 4ºC (T_a) and moved to 8ºC and 12ºC (T_{test}). "B" represents VO_2 of individuals acclimated at 8ºC (T_a) and moved to 4ºC and 12ºC (T_{test}). "C" represents VO_2 of individuals acclimated at 12ºC (T_a) and moved to 4ºC (T_{test}).

Maternal effects on oxygen consumption rate

We evaluated the relationship between maternal conditions and VO_2 expressed by those effects. At 8ºC acclimation temperature no differences were found between families so it was assumed no maternal effects were relevant. At 4ºC and 12ºC acclimation temperature we found statistical differences as said before but no relation was found

between mean VO_2 at this acclimation temperatures (T_{ac}=4ºC,T_{ac}=12ºC) mother total length (T_{ac}=4ºC, *P*=0.102; T_{ac}=12ºC, *P* =0.313), weight (T_{ac}=4ºC, *P* =0.058; T_{ac}=12ºC, *P* =0.262), hepatosomatic index T_{ac}=4ºC, *P*=0.726; T_{ac}=12ºC, *P*=0.944) or Fulton's condition factor (T_{ac}=4ºC, *P*=0.413; T_{ac}=12ºC, *P* =0.538)(Table 1).

Table . Characteristics of family mothers of the experimental individuals of Atlantic cod (*Gadus morhua*) and Pearson's correlation coefficient (*r*) and *P-value* compared with the mean VO_2 of each family at 4ºC and 12ºC acclimation temperature were differences were found between families.

Family	1	2	3	4	*r* T_{ac}=4ºC	*r* T_{ac}=12ºC	*P* T_{ac}=4ºc	*P* T_{ac}=12ºC
Length (mm)	880	860	890	970	-0.898	-0.686	0.102	0.313
Weight (g)	7555	5510	6865	9285	-0.941	-0.737	0.058	0.262
HSI	0.147	O.077	0.099	0.061	0.273	0.055	0.726	0.944
Fulton's K	1.108	0.866	0.973	1.017	-0.586	-0.461	0.413	0.538

Discussion

No clear effects of the *Pan*-I genotype and the family cross were found on the oxygen consumption rate due we didn't have all the genotypes since we lack of *Pan*-I^{AA} and the value of the intermediate genotype *Pan*-I^{AB} is not clear. The bad distribution of the families among the groups was an important factor to assess a relationship with maternal effects. Also parental behaviour was not completely assessed and both could had the same behaviour even carrying different *Pan*-I genotype as described in Pampoulie (2008).

Problems may appear when we try to compare different studies about VO_2 consumption rate due to the differences in methodology can cause differences in the accuracy of the study (Peck and Buckley 2008). Our results may have some overestimation for VO2/M as they include an unknown specific dynamic action that was tried to eliminate by withholding the feeding. The stress during capture and testing was reduced by the rapid recovery of stress of juvenile cod (Artigas et al. 2005) and by the acclimation time in the respirometry chambers so stress was not an important contribution to our overestimation. The variability between weight and oxygen consumption rate does not suggest a strong interaction between temperature, weight and metabolism despite of what we initially thought, however our experiment wasn't designed to assess this relation.

Due we couldn't use individuals with *Pan*-I^{AA} genotype in this study the results were strongly influenced by the role of the *Pan*-I^{AB} on the behavior. The similarity on oxygen consumption rate between individuals with *Pan*-I^{AB} and individual with *Pan*-I^{BB} may be explain by the assumption that *Pan*-I locus is not a perfect marker for migratory behaviour and also it could not be the only gen related to the migratory behaviour and the response to thermal changes (Pampoulie et al 2008). Various studies proved that Pan I^{AB} individuals can both act as coastal individual or as deep water individuals (Palsson and Thorsteinsson 2003). Therefore we cannot assess the real role of those individual in our experiment. The lower number of experimental individuals with Pan I^{BB} could affect to the statistical results. Since we worked under controlled conditions the selection forces for the *Pan*-I genotype could be the same for both genotypes and the differences could be easily masked.

The irregular distribution of the families among the experimental groups reduced the possibilities to find a strong interaction between the VO_2 and mother qualities. Family differences may be related to some unknown maternal conditions that we didn't study or some effects not passed on by the mother that need to be found in next studies. Parental origin and characteristics may provide the spawning with genetically inherited differences in oxygen consumption rate that may be expressed as interfamily and intrafamily variation. Since parental were caught at the same spawning areas they could have the same migratory behaviour and also no appreciable body differences were seen

between the mothers so it is easy to accept they all had the same origin and gave their spawning the same properties, that could be the reason why maternal effects didn't unleashed the differences in oxygen consumption rate at 4ºC and 12ºC. More studies need to be done to assess what kind of effects may be the reason of these differences and why we didn't find differences at an intermediate temperature (T_{ac}=8ºC).

It is obviously essential to find new tools to differentiate between behaviour groups and to assess if it is possible for an individual to change between behavioural types because only by the *Pan*-I genotype is not possible to establish the behaviour of an individual since as said before it is not a perfect marker. One of these new tools could be morphological differences to increase the discrimination power mainly to assess what behaviour show individuals with *Pan*-I^{AB} genotype that could have been used to separate individuals with costal behaviour from individuals with frontal behaviour in order to have a better experimental design.

Table . Acclimation temperature(T_a); Test temperature(T_{test}); number(n);total length(TL) mean(±SD); weight(W) mean(±SD); mean(±SD) weigh oxygen consumption rate VO_2/M both at T_{ac} and T_{test}.

Genotype	**Family**	**T_a**	**n**	**$TL_{(mm)}$**	**$W_{(g)}$**	**VO_2/M (T_a) *($mgO_2g^{-1}hr^{-1}$)***	**T_{test}**	**VO_2/M(T_{test}) *($mgO_2g^{-1}hr^{-1}$)***
AB	1	4ºC	14	125±2,9	17,4±1,2	0,038±0,002	8 ºC	0,071±0,004
							12 ºC	0,093±0,006
		8ºC	13	130±1,7	18,9±0,7	0,062±0,002	4 ºC	0,040±0,001
							12 ºC	0,089±0,004
		12 ºC	15	123±1,7	15,7±0,7	0,084±0,003	4 ºC	0,044±0,003
	2	4 ºC	6	131±3	20,1±1,2	0,051±0,003	8 ºC	0,058±0,003
							12 ºC	0,078±0,009
		8 ºC	5	134±2,6	19,3±1,5	0,065±0,004	4 ºC	0,062±0
							12 ºC	0,0891±0,007
		12 ºC	1	118	12.8	0,100±0	4 ºC	0,062±0
	3	4 ºC	14	128±2,2	18,9±0,9	0,040±0,002	8 ºC	0,059±0,003
							12 ºC	0,102±0,005
		8 ºC	22	130±2,2	19.24±0,9	0,053±0,001	4 ºC	0,035±0,001
							12 ºC	0,079±0,001
		12 ºC	12	131±1,8	18,43±0,9	0,071±0,002	4 ºC	0,034±0,001
	4	4 ºC	9	132±2,2	18,9±0,9	0,035±0,001	8 ºC	0,059±0,002
							12 ºC	0,091±0,006
		8 ºC	2	130±10	19,56±5	0,065±0,065	4 ºC	0,400±0
							12 ºC	0,900±0
		12 ºC	2	114±2	13,8±0,17	0,070±0	4 ºC	0,032±0,006
BB	1	4 ºC	7	128±3,2	18,4±1,4	0,043±0,003	8 ºC	0,062±0,003
							12 ºC	0,079±0,004
		8 ºC	10	134±3	21,03±1,5	0,065±0,002	4 ºC	0,045±0,002
							12 ºC	0,082±0,004
		12 ºC	2	135±1	20±0,02	0,081±0,005	4 ºC	0,040±0,001

	3	8 ºC	1	138	21,68	0,060±0	12 ºC	0,080±0

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