Settling velocity and total ammonia nitrogen leaching from commercial feed and faecal pellets of gilthead seabream (*Sparus aurata* L. 1758) and seabass (*Dicentrarchus labrax* L. 1758).

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Abstract

The physico-chemical characteristics of particulate wastes of *Sparus aurata* and *Dicentrarchus labrax* were investigted. Changes in dimensions, settling velocity and total ammonia nitrogen (TAN) leached from commercial feed pellets was investigated after soaking. Also, the settling velocity and TAN leached from faecal pellets of these fish were assessed at 15 and 25ºC. The settling velocity of feed pellets was influenced positively by pellet weight and negatively by immersion length as a result of changes in pellet dimensions after soaking. The settling velocity of faecal pellets was determined by pellet weight. The experimental design did not allow identifying any consistent effect of water temperature on settling velocity. TAN leaching over time from feed and faecal pellets was successfully explained by means of a first order kinetic equation. For feed pellets, water temperature significantly affected the speed of the process and the time at which the maximum TAN leached was reached, but did not influence the maximum TAN leached. Leaching was related to feed pellet size, so the smaller the pellet, the higher the leaching. TAN leaching from faecal pellets was greater per unit weight than in feed pellets. However neither water temperature nor fish species influenced on TAN leaching from faeces.

Keywords: aquaculture; particulate wastes; settling velocity; leaching; *Sparus aurata*; *Dicentrarchus labrax*.

1. Introduction

Marine aquaculture has experienced a rapid development in the Mediterranean since 1970, with gilthead seabream (*Sparus aurata* L. 1758) and seabass (*Dicentrarchus labrax* L. 1758) now intensively cultured in most coastal countries. This expansion has been accompanied by an increasing social sensitivity with respect to the potential short and long term impacts on the marine environment. Fish rearing produces a substantial quantity of particulate organic wastes, mainly faecal pellets and uneaten food that settle in the vicinity of the farms. Several studies have examined the geochemical and biological consequences of this supply of organic matter on the benthos (Aguado-Giménez and García-García, 2004; La Rosa et al., 2004; Hellou et al., 2005). Uneaten food is the main contributor among the particulate wastes loaded by fish farms (Beveridge et al., 1991; Chen et al., 1999b). Most of the information regarding particulate wastes loading refers to Atlantic salmon. In the 1980´s, it was estimated that the food loss rate during salmonid ongrowing was as high as 200-300 g kg^{-1} of the supplied food (Gowen and Bradbury, 1987). New diet formulations, and improvements in diet production processes and husbandry operations have lowered the conversion factor and also the wastes loaded, 50-150 g kg^{-1} now being the food loss rate most often reported (Findlay and Watling, 1994; Beveridge et al., 1997; Cho and Bureau, 1997) although Cromey et al. (2002) mentions 30 g kg^{-1}. As regards seabream and seabass culture, there is no information available in the scientific literature about uneaten food looses. Producers consulted estimated it around to be 50-100 g kg^{-1} on average in offshore conditions but, in agreement with Reid et al. (2009), this quantity is varies widely from operation to operation and even from day to day.

Several studies under laboratory conditions reported that 250-300 g kg^{-1} of ingested food is voided as faeces (Butz and Vens-Cappell, 1982). Just as with food losses, a continuous improvement in diet elaboration has led to a gradual reduction of faecal discharges to about 100-250 g kg^{-1} of ingested food (Cho et al., 1994; Talbot and Hole, 1994). In Mediterranean fish farming, 300-400 g of faeces are released into the environment per kilogram of fish produced (Dosdat, 2001).

Both uneaten food and faecal pellets have particular features and undergo a series of physico-chemical changes while dispersing and settling that could influence the spatial range of dispersion of particulate wastes and the net organic load reaching the seabed. According to Gowen and Bradbury (1987), particle settling velocity together with current speed and depth, determine the horizontal distance of particles reaching the bottom, and this obviously depends on particle size (Sutherland et al., 2006). In addition, these wastes release nutrients (leaching) while dispersing and settling. There are several studies that have looked at the settling velocity of feed and faecal pellets and nutrient leaching from faeces in salmonids (Findlay and Watling, 1994; Elberizon and Kelly, 1998; Chen et al., 1999a,b; Chen et al., 2003), but such information is scant for Mediterranean cultured fishes (Vasallo et al., 2006; Magill et al., 2006). Physical changes which relate to the removal or re-distribution of waste have received less attention and only a few studies concerned with salmonids, have investigated leaching from feeds during sinking (Phillips et al., 1993) and the removal of salmonid waste by wild fish (Felsing et al, 2002) and in the Mediterranean for wild fish around seacages (Fernández-Jover et al., 2007) are available. However, increased knowledge of the dynamic of particulate wastes before settling on the seabed could be useful for analysing any environmental impact and for improving the accuracy of waste dispersion models.

This study aims to determine under defined laboratory conditions some physico-chemical characteristics of gilthead seabream and seabass solid wastes as they disperse through sedimentation including settling velocity, size and weight changes and total ammonia nitrogen (TAN) leaching of a variety of feed pellets, and settling velocity and TAN leaching of faecal pellets.

2. Materials and methods

2.1. Feed pellets assays

The feed pellets used in these assays are part of a range of extruded commercial feedstuffs used for gilthead seabream and seabass ongrowing. Hereafter, we refer to the different feed types according to the nominal diameter of the cylindrical feed pellets: 2mm (FP2), 4mm (FP4a and FP4b), 6mm (FP6) and 8mm (FP8). The proximate composition of the different feed pellets was determined. Dietary moisture was determined by drying samples at 110 ºC for 24 h. Crude protein was estimated by Kjeldahl method, with 6.25 as conversion factor. Crude fat was obtained by diethyl ether extraction (SOXTEC System-HTC). Nitrogen free extracted material (NFE) was calculated as [100 – (% protein + % fat + % ash)]. Total ash was obtained by heating at 550 ºC for 18 h. Gross energy was estimated following the Miglavs and Jobling (1989) coefficients: 23.6 kJ g^{-1} for protein, 38.9 kJ g^{-1} for fat and 16.7 kJ g^{-1} for carbohydrate. Protein / Energy ratio was also calculated. Three samples of each pellet type were analyzed. Values of crude protein, crude fat, ash and NFE are expressed as g kg^{-1} dry weight.

Diameter, length, weight and density were determined in 15 pellets of each pellet type submerged for 0, 1, 5, 10, 15, 30 and 60 minutes at two different water temperatures representatives of winter and summer conditions in the Mediterranean (15 and 25 ºC). After immersion, the pellets were placed on absorbent paper for 60 s to eliminate excess water. The diameter and length were determined using a digital gauge (precision ± 0.01 mm) and the pellets were weighed using an analytical balance (precision ± 0.1 mg). Changes in size or weight were expressed as % of initial size or weight.

Settling velocity of dry and immersed (for 1, 5, 10, 15, 30 and 60 minutes) feed pellets was determined using a 1 m long, 0.25 m diameter methacrylate sedimentation column. Feed pellets were carefully placed with forceps just below the water surface, in the centre of the surface avoiding bubbles. The column was marked at 0.05, 0.40 and 0.75 m from the top. The first 0.05m and the final 0.25m of the column length were not considered in order to provide enough reaction time to start the timer manually, and to avoid any bottom shear effect imposed by sedimentation column bottom on pellet velocity (Chen et al., 1999a). Settling velocity was determined in 30 pellets of each type by timing the descent between two marks 0.35m along its length, at 15 and 25ºC.

For TAN leaching determination, feed samples of each type were weighted and assigned at random to one of six leaching periods (1, 5, 10, 15, 30 and 60 minutes). This wide range of immersion lengths was chosen in order to assure that settling velocity and TAN leaching estimations and the dynamic of the processes could be revealed for long settling periods that even exceeded the time needed for deposition. Feed pellets were individually dropped in different 50 ml beakers filled with filtered seawater (glass microfibre filter GF/C 0.45µm), at 15 and 25ºC. To simulate turbulence while settling, samples were gently shaken with an automatic shaker approximately at their previously determined settling velocity. Each incubation time was replicated five times. Immediately after incubation, the samples were fixed with 0.1ml of 0.5N HCl to displace the ionic balance to the soluble form NH_4^+. TAN was measured with an ion-selective electrode (ORION 9512 BN) as described in APHA

(1995). The accuracy of this method has been favourably compared with the autoanalyzer (indophenol blue method), which is the most widely used technique for ammonia determination in seawater, no significant differences being observed between them (Arango-Pulgarín and Pérez-Navarro, 2005, and references therein). Before measuring, 1ml of Ionic Strength Adjustor solution (ISA: 5M NaOH, 0.5M disodium EDTA and 10% methanol with blue colour indicator) was added to displace the ionic balance to the gaseous form NH_3, at which the electrode membrane is permeable. TAN leaching is expressed as $\mu g\ g^{-1}$ dry weight of TAN released from the samples.

2.2. Faecal pellets assays

Gilthead seabream (0.528 ± 0.122 g) and seabass (0.636 ± 0.218 g) were stocked in circular 2000L tanks supplied with running seawater (salinity 37 $g\ L^{-1}$). The tanks were part of recirculating system fitted with biological filtration and ultraviolet lamp; and the fish were allowed to acclimate to the test diet for at least 10 days. Fish were fed to satiation twice per day at 9:00 and 12:00 a.m. with FP 6 feedstuff. Fish were killed by immersion in iced seawater, and fresh faeces were collected by dissection of the distal 4 cm of the gut according to Chen et al. (1999a) just before the assays. The proximate composition of faecal pellets was determined as described in the previous section.

Once obtained, faecal pellets were partially dried on blotting paper for 10 seconds (Chen et al., 2003) and weighed prior to assays. The settling velocity and TAN leaching from faecal pellets was determined as explained in the previous section, at 15 and 25ºC. The mean faecal (± s.e.m.) pellets weight for TAN leaching assays was 0.15 ± 0.01g.

2.3. Statistical treatment of data

The proximate compositions of feed and faecal pellets were tested by one-way ANOVA, and differences between pellet types or fish species by means of the *post hoc* Student-Newman-Keuls (SNK) test. Multiple regression analyses (MRA) were performed: i) for the settling velocity of feed pellets as dependent variable, and pellet size and density, water temperature and immersion time as independent variables; ii) for weight and volume increase of feed pellets after immersion as dependent variables, pellet size, water temperature and time of immersion as independent variables; iii) for pellet density as dependent variable, water temperature and time of immersion as independent variables; iv) for settling velocity of faecal pellets as dependent variable, faecal pellet weight, water temperature and fish specie were tested as independent variables. The significance of the coefficients of the independent variables and their correlation indicated the influence on dependent variables.

TAN leaching from feed and faecal pellets was fitted by non-linear regression to the first order kinetic equation (Fernández-Jover et al., 2007):

$$y = a \cdot \left(1 - e^{-k \cdot t}\right)$$

where y is the TAN leaching (µg g^{-1} d.w.), a and k are fit parameters that represent the maximum leached TAN (µg g^{-1} d.w.) and the velocity of the process (min^{-1}) respectively and t is the immersion length (min).

To test the influence of feed pellet size and water temperature on the leaching process, MRA were performed for a, k and t_a (immersion time at which a is reached: estimated from the equations) as dependent variables.

Differences in TAN leaching of faecal pellets between fish species and water temperature were tested using the Chow test (Fernández-Jover et al., 2007):

$$F = \frac{\left[\sum S_{pool}^2 - \left(\sum S_A^2 + \sum S_B^2\right)\right]/K}{\left(\sum S_A^2 + \sum S_B^2\right)/\left(n_A + n_B - 2K\right)}$$

where $\sum S_{pool}^2$ is the residual sum of squares of the pooled samples $\sum S_A^2$ and $\sum S_B^2$ that represent the residual sums of squares for the samples A and B, respectively. K is the number of regression parameters (here K =2, slope and intercept), while n_A and n_B are the sample sizes of A and B. If the F-value exceeds the tabulated value for the F-distribution for P=0.05, K degrees of freedom for the numerator, and n_{A+} $n_B - 2K$ degrees of freedom for the denominator, the regressions lines are significantly different.

3. Results

3.1. Proximate composition of feed and faecal pellets

The results of the proximate composition analyses of feed and faecal pellets are shown in Tables 1 and 2 respectively. Feed pellet densities were very similar, with no statistical differences between them (SNK $P > 0.05$), although smaller pellets showed a slightly higher density. Differences between feed types with respect to macronutrient composition were not very outstanding. FP8 showed the lowest protein content (435.28 g kg^{-1}) and FP2 the highest content (506.84 g kg^{-1}) (SNK $P < 0.05$). The feedstuff with the lowest lipid content (187.71 g kg^{-1}) was FP2 which also showed the lowest moisture content (55.74 g kg^{-1}) (SNK $P < 0.05$). FP8 showed the highest NFE values (285.29 g kg^{-1}) and FP4b the lowest (198.62 g kg^{-1}) (SNK $P < 0.05$). Gross energy was very similar for all the feedstuffs. The P/E ratio in FP8 was also the lowest (18.56g MJ^{-1}) (SNK $P < 0.05$). Faecal pellet density was significantly lower than for feed pellets, but there were no differences between fish species, and only minor differences were observed with regard to the ash and crude protein content (SNK $P < 0.05$).

3.2. Physical changes after soaking of feed pellets and settling velocities of feed and faecal pellets

MRAs for feed pellets (Table 3) showed that water temperature and pellet density had no influence on changes in the physical characteristics of the pellets or the settling velocity ($P > 0.05$). Pellet size had a positive influence on settling velocity ($P < 0.001$), while immersion time had a negative influence ($P < 0.001$). Thus, the larger the pellets the faster the settling, and the longer the submergence time for any pellet type, the slower the settling velocity (Figure 1A). Immersion time caused significant increases in weight and volume (mainly diameter) (P < 0.001) and a decreases in density of feed pellets (P < 0.001). Smaller feed pellets, with larger surface/volume ratios, had greater weight and volume increases after soaking (P < 0.001), which became greater with soaking time (Figures 1B and 1C). Smaller pellets underwent a weight and volume increase of 8.18% and 9.02%, respectively after 1 minute of immersion, and 64.21% and 59.92% after 60 minutes respectively, while larger pellets underwent a weight and volume increase of 5.19% and 3.46%, respectively, after 1 minute of immersion, and 27.63% and 23.90% after 60 minutes.

The settling velocity of faecal pellets (Figure 2) was not influenced by water temperature (P > 0.05), and was statistically similar for gilthead seabream and seabass (P > 0.05). Faecal pellet weight showed a positive correlation with the settling velocity (P < 0.001) (Table 4), being fastest in the largest faecal pellets. The settling velocities of faecal pellets were approximately 60% slower than of feed pellets for all pellet sizes and species assessed.

3.3. TAN leaching from feed and faecal pellets

TAN leaching from feed pellets were successfully described by mean of first order kinetic equations (Table 5, Figure 3A-E). Table 6 shows the results of MRAs for the parameters of the fitting equations and water temperature and pellet size. The constant a was not significantly influenced by water temperature (P > 0.05) but it was by pellet size (P < 0.01), so the smaller the pellets, the higher the maximum TAN leached (a). Respect total nitrogen in samples, % of TAN leached from larger feed pellets was 2-3 times lower than from smaller pellets, and % TAN leached from faecal pellets was 10-20 times greater than from feed pellets (Table 5). Constant k was significantly higher at 25 ºC (P < 0.05) and in smaller feed pellets (P < 0.05), while t_a was significantly larger at 15 ºC (P < 0.05) but also for smaller pellets (P < 0.05) (Table 6), so that the largest pellets at low temperature reached a later. On average, a

was reached after 60 and 45 minutes at 15 and 25 ºC respectively.

TAN leaching from gilthead seabream and seabass faecal pellets was also described by means of first order kinetic equations (Table 6 Figure 4A-B). A Chow F-test comparison of the regression parameters (Table 7) showed that there were no significant differences in leaching for gilthead seabream and seabass faecal pellets ($P > 0.05$). Water temperature only had a significant effect on leaching from seabass faeces at 15 *versus* 25 ºC ($P < 0.05$) as shown by the pairwise comparision. The maximum leached TAN (a) from faecal pellets was around 3-fold higher per unit weight than from feed pellets. Also, leaching velocity (k) and time to reach a level (t_a) were faster for faecal pellets per unit weight than for feed pellets.

4. Discussion

4.1. Physical changes after soaking of feed pellets and settling velocities of feed and faecal pellets

The settling velocity of feed pellets was between 0.068 and 0.136m s^{-1} for the diameters of 2–8 mm in our assays. These values are largely similar to the range of settling velocities of 0.087–0.144 m s^{-1}reported by Vassallo et al. (2006) for 3-5 mm seabream and seabass feed pellets, although in this case the pellets were larger non-extruded pellets, which have a greater propensity to sink that the extruded pellets used during this study. There is considerably more information on settling velocity available for salmonid feedstuffs, which are normally extruded pellets. In any case, settling velocity of salmon feed pellets is similar to that found in this study. Findlay and Watling (1994) reported settling velocities ranging from 0.055 – 0.155 m s^{-1} for 3–10 mm pellets; Elberizon and Kelly (1998) indicated settling velocities of 0.05–0.12 m s^{-1} for 2 and 8mm pellets; and Chen et al. (1999b) recorded settling velocities of 0.058–0.109 m s^{-1} for 2-8 mm pellets. The settling velocity of an object depends on many factors relating to the object itself and to the medium in which it is settling, such as pellet weight, shape, floating or porosity, and temperature, salinity, density, viscosity or pressure in the case of seawater, although Elberizon and Kelly (1998) and Chen et al. (1999b) suggested that this influence does not comply with the Stokes´ Law. Vasallo et al. (2006) revealed that pellet size and its floating time prior to sinking were key factors to explain settling velocity. The influence of pellet weight was also identified by other authors (Elberizon and Kelly, 1998; Chen et al. 1999b, Sutherland et al., 2006). In this study, we not only found that initial pellet size determined settling velocity but also, unlike Chen et al. (1999b), that velocity changed as the pellets sank due to physical transformations that the pellets underwent. As immersion time increases, pellet weight also increased, but contrary to expectations, settling velocity did not increase. Pellet volume, especially diameter, and density also increased with time of immersion. It was therefore hypothesized that pellet weight increase was due to hydration and this caused a volume increase and shape change, causing a greater influence on settling velocity than weight because of greater friction produced, and a higher resistance to fall. Weight increment after soaking was higher in smaller pellets, as Chen et al. (1999b) and Vasallo et al. (2006) noticed, but these authors did not observe the dimension and shape changes that we saw, probably because their immersion periods were shorter or because they simply did not measure the pellets after soaking (a diameter increase of 10% in a 6mm diameter pellet is negligible to the naked eye). Elberizon and Kelly (1998) also mentioned the increased density of trout feed pellets after immersion in fresh water, but they did not provide data on weight and dimension increases nor on the settling velocity after immersion. In the present study, floating time was not considered (unlike Vasallo et al., 2006) because observations show that under industrial rearing conditions, water motion is dynamic, feed is not supplied slowly and methodically, while large number of fish moving and eating voraciously, the result being that pellets tend to sink immediately. Seawater density depends on both temperature and salinity. No clear effect of seawater temperature or salinity was found on the settling velocity of feed pellets in our experiment. Nor was it in the recent literature (Elberizon and Kelly, 1998; Vasallo et al. 2006), probably because, as the above authors state, the range of parameters studied was not so critical to the settling velocity. Despite this, we observed greater but non significant settling velocities at low temperature, as Chen et al. (1999b) noted. These authors suggested that this could be due to the influence of temperature on pellet density, although in our experiments we found that seawater temperature did not affect pellet density.

Regardless of pellet weight and in agreement with Chen et al. (2003), the settling velocities of faecal pellets were much lower than that observed for of feed pellets, due to the lower density of the faecal pellets. Water temperature (with the exception of seabass for 15 and 25 ºC) showed no effect on settling velocity, as in the case of feed pellets. In this study, the settling velocity of faecal pellets ranged from 0.022 to 0.075 m s^{-1} in faeces of 0.02–0.74 g wet weight, there being no differences between gilthead seabream and seabass in this respect. For salmon faeces, Chen et al. (1999a, 2003) showed a great variability in settling velocity: 0.053–0.066 m s^{-1} in faeces of 0.04–0.09 g wet weight, and 0.051–0.064 m s^{-1} in faeces of 0.13–0.22 g wet weight. These authors suggested that faecal pellet mass is not a good predictor of settling velocity. Our results (Figure 2) also showed noticeable variability, but they were significantly influenced by faeces wet weight, so the heavier the faecal pellet, the faster the settling. Magill et al. (2006) reported much slower settling velocities for a wide weight range of gilthead seabream and seabass faecal pellets (average 0.005 and 0.007 m s^{-1} respectively), but they studied almost the total fractionating particles (macro and micro-particles at maximum pixel resolution) by means of computer image analysis. Fish faecal pellets have high water content so their nature in seawater is very close to liquid (Vita et al., 2004). Their shape is very variable and not correlated with fish size (Magill et al., 2006). While feed pellets are stable in seawater for long time, faecal pellets tend to fractionate into smaller particles and even become disaggregated pieces which positive buoyancy (Chen et al., 1999a; Magill et al., 2006). Such disaggregating can be caused by the turbulence created by fish swimming under high density rearing conditions. In short, establishing predictions for the settling velocity of faecal pellet is complicated since these friable particles can settle as fast as some medium-size feeding pellets (this study), while micro-particles may show a very slow settling rate or even remain suspended. This erratic behaviour has been successfully integrated into a deposition model by Magill et al. (2006), the most accurate model available at the moment. If large intact or semi-intact faecal pellets and feed pellets are able to reach the seabed, then the settling velocities measured in this study and the study mentioned above, along with the influence of variables such as feed and faecal pellet weight and changes in dimensions of feed pellets while sinking, should be taken into account for waste dispersal modelling purposes. These settling velocities and models using them suggest that uneaten food is dispersed and settles closer to the farms, while faecal particles are more widely dispersed (Doglioli et al., 2004). Fractionated particles from feed pellets and feed pellet dust have still not been studied in terms of buoyancy, flocculation, settling velocity and dispersion, but should be included in deposition models.

4.2. TAN leaching from feed and faecal pellets

TAN leaching from feed and faecal pellets were successfully explained by fitting data to a first order kinetic equation, which permitted us to derive the dynamic of the TAN leaching process. Maximum leached TAN (a) proved to be independent of water temperature, for feed and faecal pellets. Smaller feed pellets leached more TAN than expected since their surface/volume relationship, and hence their contact with seawater, is greater than larger pellets. However, in feed pellets the speed of the process (k) and time in which a value was reached (t_a) were significantly influenced by temperature the higher the temperature, the faster the process and the shorter the t_a. That is to say, water temperature affected the speed of TAN leaching and the immersion length until reaching the maximum was reached, but did not influence the maximum level reached, demostring the influence of temperature in accelerating some biochemical processes. In every feed and faecal pellet type, TAN leaching was very fast during the first few minutes, and the smaller the feed pellets, the faster the process. The only reference found in the literature about leaching from feed pellets is that of Fernández-Jover et al. (2007), who also showed that leaching was faster during the initial stages, but who obtained lower *a* and higher *k* values than ours in feed pellets of non-specified size. They also found that water temperature significantly and positively influenced both *a* and *k*, but this comparison is not entirely valid since both methods for measuring TAN and the degree of replication differed between the respective studies.

In agreement with Chen et al. (2003) who postulated that leaching from faeces is a rapid process, we found that TAN leaching from faeces was three times faster/greater per unit weight than feed pellets. These results agree with Fernández-Jover et al. (2006). For modeling purposes Chen et al. (2003) proposed that leaching values over ten minutes are sufficient for faeces produced from extruded salmon feeds. Fernández-Jover et al. (2006) suggested that ten to twenty minutes is more suitable for seabream and seabass faeces and their feeds. According to our results, maximum leached TAN from gilthead seabream or seabass faecal pellets is not reached until fifteen to thirty minutes, while forty five to sixty minutes is necessary for feed pellets. In any case, it is expected that feed and faecal pellets settled on the seafloor before the maximum leachable TAN (*a*) is reached, although this, obviously depends on water depth and current velocity. Fernández-Jover et al. (2006) ascertained that leaching from faeces was a temperature and species-dependent variable, showing that leaching was faster at low temperatures. Our experimental design did not allow us to demonstrate that TAN leaching from faeces was temperature-dependent, although, in our case, t_a was always reached more quickly at 25ºC. The fact that fish faeces were so labile (Tlusty et al., 2000; Vita et al., 2004) and leach so fast may have obscured the effect of temperature. As regards to species-dependence, the faecal pellets from gilthead seabream and seabass were qualitatively similar and, as both species ate the same food, it is not to be unexpected that TAN leaching from their faeces was similar.

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Table 1: Physical characteristics and proximate composition of the feed pellets used in the experiments (mean ± s.e.m.). Different superscript in the same row indicates statistical differences between pellet types (SNK, $P < 0.05$). Macronutrients and energetic indices are referred as dry weight. NFE: nitrogen-free extracted material. P/E: crude protein / gross energy ratio.

Pellet types	FP 2	FP 4a	FP 4b	FP 6	FP 8
Diameter (mm)	2.50 ± 0.11	4.22 ± 0.09	4.07 ± 0.46	5.42 ± 0.17	8.07 ± 0.02
Length (mm)	2.43 ± 0.05	4.59 ± 0.09	4.21 ± 0.16	7.07 ± 0.16	8.19 ± 0.11
Weight (mg)	14.10 ± 0.50	72.10 ± 1.90	68.20 ± 1.90	187.30 ± 5.60	360.10 ± 7.60
Density (kg m^{-3})	1071.85 ± 29.65^{a}	1119.42 ± 15.06^{a}	1125.36 ± 16.49^{a}	1069.85 ± 25.36^{a}	1069.77 ± 15.06^{a}
Ash (g kg^{-1})	66.03 ± 0.77^{a}	78.29 ± 0.30^{b}	85.17 ± 0.81^{c}	78.74± 0.36^{b}	58.35 ± 0.34^{d}
Moisture (g kg^{-1})	55.74 ± 0.66^{a}	69.75 ± 0.39^{b}	63.40 ± 0.81^{c}	73.55 ± 0.72^{d}	75.11 ± 0.47^{d}
Crude protein (g kg^{-1})	506.84 ± 2.65^{a}	489.98 ± 0.91^{a}	494.87 ± 2.52^{a}	485.75 ± 2.36^{a}	435.28 ± 17.75^{b}
Crude fat (g kg^{-1})	187.71 ± 6.25^{a}	218.04 ± 3.18^{b}	215.57 ± 0.24^{b}	214.82 ± 1.19^{b}	216.33 ± 0.74^{b}
NFE (g kg^{-1})	235.51 ± 3.16^{a}	207.80 ± 2.06^{ab}	198.62 ± 1.91^{b}	214.43 ± 3.15^{ab}	285.29 ± 17.01^{c}
Gross energy (MJ kg^{-1})	23.19 ± 0.13^{a}	23.51 ± 0.07^{a}	23.38 ± 0.03^{a}	23.40 ± 0.05^{a}	23.45 ± 0.11^{a}
P / E (g protein MJ^{-1})	21.85 ± 0.13^{a}	20.84 ± 0.04^{a}	21.16 ± 0.14^{a}	20.76 ± 0.14^{a}	18.56 ± 0.94^{b}

Table 2: Physical characteristics and proximate composition of faecal pellets used in the experiments (mean ± s.e.m.). Different superscript in the same row indicates statistical differences between species (SNK, $P < 0.05$). Percentages are referred as dry weight. NFE: nitrogen free extracted material.

Faecal pellets	*Sparus aurata*	*Dicentrarchus labrax*
Diameter range (mm)	3.01 – 7.50	3.19 – 7.92
Length range (mm)	3.20 – 14.50	3.60 – 14.90
Weight range (mg)	23.10 – 648.00	29.12 – 740.00
Density ($kg\ m^{-3}$)	1021.43 ± 12.28^{a}	1018.29 ± 12.97^{a}
Ash ($g\ kg^{-1}$)	24.10 ± 1.06^{a}	36.85 ± 1.38^{b}
Moisture ($g\ kg^{-1}$)	892.91 ± 0.98^{a}	892.25 ± 1.46^{a}
Crude protein ($g\ kg^{-1}$)	215.91 ± 1.01^{a}	207.10 ± 1.44^{b}
Crude fat ($g\ kg^{-1}$)	43.47 ± 5.88^{a}	43.97 ± 3.99^{a}
NFE ($g\ kg^{-1}$)	714.61 ± 5.76^{a}	709.16 ± 6.92^{a}

Table 3: Results of the multiple regression analyses for settling velocity, weight and volume increase and density changes of feed pellets after immersion.

	Dependent Variables: y							
	Settling Velocity ($m\ s^{-1}$)		Weight Increase (%)		Density (%)		Volume Increase (%)	
Independent Variables	Coefficients	S.E.	Coefficients	S.E.	Coefficients	S.E.	Coefficients	S.E.
Intercept: *a*	5.749^{**}	1.749	22.616^{***}	2.019	1.085^{*}	0.019	24.632^{***}	2.878
Immersion time (*It*): *b*	-0.035^{***}	0.004	0.514^{***}	0.034	-0.001^{***}	0.000	0.653^{***}	0.063
Temperature (*T*): *c*	$-0.135^{n.s.}$	0.154	$0.164^{n.s.}$	1.381	$0.010^{n.s.}$	0.013	$1.727^{n.s.}$	1.969
Pellet size (*Ps*): *d*	0.906^{***}	0.039	-2.722^{***}	0.338	0.008^{***}	0.003	-3.420^{***}	0.483
Density (*D*): *e*	$0.866^{n.s.}$	1.598	-	-	-	-	-	-
R	0.95		0.91		0.54		0.90	
R^2 adj	0.91		0.83		0.25		0.79	
F-ANOVA	226.61^{***}		95.73^{***}		7.65^{***}		75.90^{***}	
n	70		60		60		60	

Fitting equation: $y = a + b \cdot (It) + c \cdot (T) + d \cdot (Ps) + e \cdot (D)$

*P < 0.05; **P < 0.01; ***P < 0.001; n.s. non-significant.

Table 4: Results of multiple regression analysis for settling velocity of faecal pellets as a function of fish species, water temperature and faecal pellet wet weight.

Dependent Variable *y*: Settling Velocity (m s^{-1})		
Independent Variables	Coefficients	S.E.
Intercept: *b*	3.439^{***}	0.211
Species (*S*): *c*	$0.037^{\text{n.s.}}$	0.159
Temperature (*T*): *d*	$-0.245^{\text{n.s.}}$	0.184
Faecal pellet weight (*Fp*): *e*	4.642^{***}	0.656
R	0.67	
R^2	0.44	
F-ANOVA	31.03^{***}	
n	116	

Fitting equation: $y = b + c \cdot (S) + d \cdot (T) + e \cdot (Fp)$

*P < 0.05; **P < 0.01; ***P < 0.001; n.s. non-significant.

Table 5: Results of the non-linear regression (1st order kinetic equation) for TAN leaching (*y*) of feed and faecal pellets as a function of immersion length (*t*). t_a is estimated from the equation as the immersion time at which *a* was reached.

	15 ºC					25 ºC				
Feed pellet	*a* (μg g^{-1})	*k* (min^{-1})	R^2 ESS F-ANOVA	t_a(min)	*a* respect total nitrogen in the pellets (%)	*a* (μg g^{-1})	*k* (min^{-1})	R^2 ESS F-ANOVA	t_a(min)	*a* respect total nitrogen in the pellets (%)
FP 2	296.31^{**}	0.0437*	0.95 22.72 74.43^{***}	52.69	0.365	278.06^{***}	0.0917^{**}	0.96 24.38 86.61^{***}	25.11	0.343
FP 4a	249.34^{***}	0.0394^{***}	0.99 7.41 581.23^{***}	58.44	0.318	234.33^{*}	0.0664^{*}	0.83 33.86 19.65^{*}	34.68	0.299
FP 4b	292.28^{**}	0.0315^{*}	0.97 16.45 124.80^{***}	73.10	0.369	114.93^{***}	0.0526^{**}	0.97 7.09 135.35^{***}	43.77	0.145
FP 6	78.13^{**}	0.0399^{*}	0.94 6.24 68.32^{**}	57.71	0.101	135.75^{**}	0.0455^{*}	0.91 14.40 38.33^{**}	50.81	0.175
FP 8	72.15^{**}	0.0361^{*}	0.93 6.02 55.39^{**}	63.78	0.104	139.86^{*}	0.0304^{*}	0.92 12.43 45.27^{**}	75.74	0.201
Faecal pellets										
Sparus aurata	921.15***	$0.1063^{*.}$	0.92 142.14 21.78^{**}	23.26	2.666	834.36^{***}	0.1463^{*}	0.90 99.56 36.95^{**}	15.53	2.415
Dicentrarchus labrax	791.09**	0.0758^{*}	0.84 125.92 20.93^{**}	28.57	2.387	888.42^{***}	0.1452^{*}	0.85 140.33 22.47^{**}	15.87	2.681

Fitting equation: $y = a \cdot \left(1 - e^{-k \cdot t}\right)$ *P < 0.05; **P < 0.01; ***P < 0.001; n.s. non-significant

Table 6: .Results of multiple regression analyses for the maximum TAN leached (*a*), speed of the leaching process (*k*) and time in which *a* is reached (t_a) as a function of water temperature and feed pellet size. t_a is estimated from the equation as the immersion time at which a was reached.

	Dependent Variables *y*					
	a ($\mu g\ g^{-1}$)		*k* (min^{-1})		t_a (min)	
Independent Variables	Coefficients	S.E.	Coefficients	S.E.	Coefficients	S.E.
Intercept: *b*	357.547^{***}	52.503	0.063^{*}	0.011	2.639^{***}	0.239
Temperature (*T*): *c*	$-17.056^{n.s.}$	38.245	0.019^{*}	0.008	-0.501^{*}	0.200
Pellet size (*Ps*): *d*	-33.314^{**}	9.375	-0.005^{*}	0.002	0.432^{*}	0.148
R	0.80		0.80		0.82	
R^2 adj	0.55		0.54		0.59	
F-ANOVA	*		*		*	
n	10		10		10	

Fitting equation: $y = b + c \cdot (T) + d \cdot (Ps)$

*P<0.05; **P<0.01; ***P<0.001; n.s. non-significance.

Table 7: Chow test F-values for the pairwise comparisons of TAN leaching from faecal pellets of *Sparus aurata* (S) and *Dicentrarchus labrax* (D) at 15 and 25 ºC.

Pairwise combinations	Tabulated $F_{(2, 56)} = 3.15$
S_{15} - S_{25}	$0.317^{n.s.}$
S_{15} - D_{25}	$0.635^{n.s.}$
S_{15} - D_{15}	$2.426^{n.s.}$
S_{25} - D_{25}	$0.204^{n.s.}$
S_{25} - D_{15}	$1.378^{n.s.}$
D_{15} - D_{25}	3.848^{*}

*P < 0.05; **P < 0.01; ***P < 0.001; n.s. non-significant

Figure captions

Figure 1(A-C): (A) Settling velocities of the different feed pellets after increasing immersion length; (B) mean weight increase (%) and (C) volume increase of feed pellets after immersion at 15 and 25ºC. Mean ± s.e.m.

Figure 2: Settling velocities of faecal pellets of different weight from *Sparus aurata* and *Dicentrarchus labrax* at 15 and 25ºC, and predicted values from the equation $y = b + c \cdot (S) + d \cdot (T) + e \cdot (Fp)$.

Figure 3(A-E): TAN leaching (mean ± s.e.m.) from the different food pellets types after immersion at 15 and 25ºC, and predicted values from the equation $y = a \cdot \left(1 - e^{-k \cdot t}\right)$. (A): FP 2; (B): FP 4a; (C) FP 4b; (D) FP 6; (E): FP 8.

Figure 4(A-B): TAN leaching (mean ± s.e.m.) from the faeces of (A) *Sparus aurata* and (B) *Dicentrarchus labrax* after immersion at 15 and 25ºC, and predicted values from the equation $y = a \cdot \left(1 - e^{-k \cdot t}\right)$.

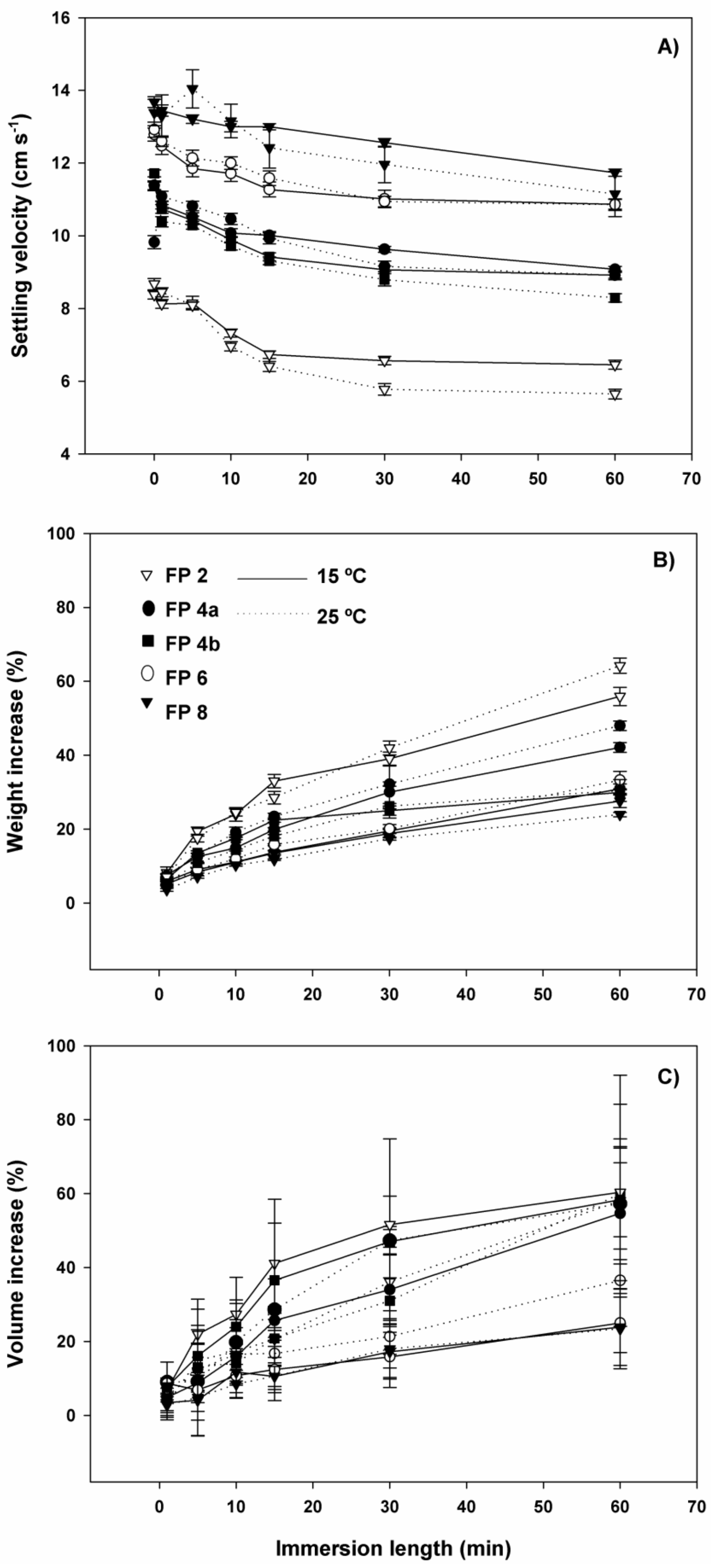
A)
Settling velocity (cm s^{-1})
16
14
12
10
8
6
4
0
10
20
30
40
50
60
70
B)
Weight increase (%)
FP 2
FP 4a
FP 4b
FP 6
FP 8
15 ºC
25 ºC
100
80
60
40
20
0
C)
Volume increase (%)
Immersion length (min)

Fig1

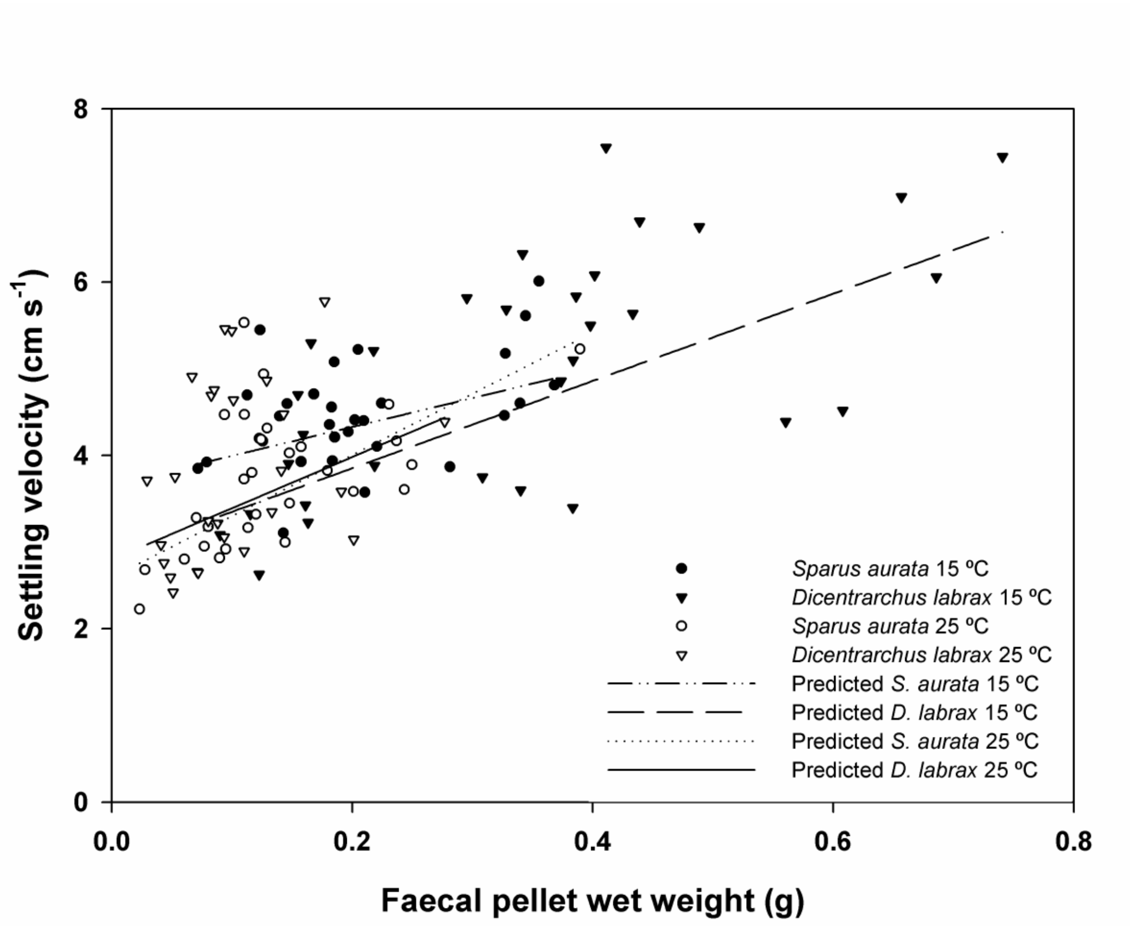
Settling velocity (cm s^{-1})
Faecal pellet wet weight (g)
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
● *Sparus aurata* 15 ºC
▼ *Dicentrarchus labrax* 15 ºC
○ *Sparus aurata* 25 ºC
▽ *Dicentrarchus labrax* 25 ºC
Predicted *S. aurata* 15 ºC
Predicted *D. labrax* 15 ºC
Predicted *S. aurata* 25 ºC
Predicted *D. labrax* 25 ºC

Fig 2

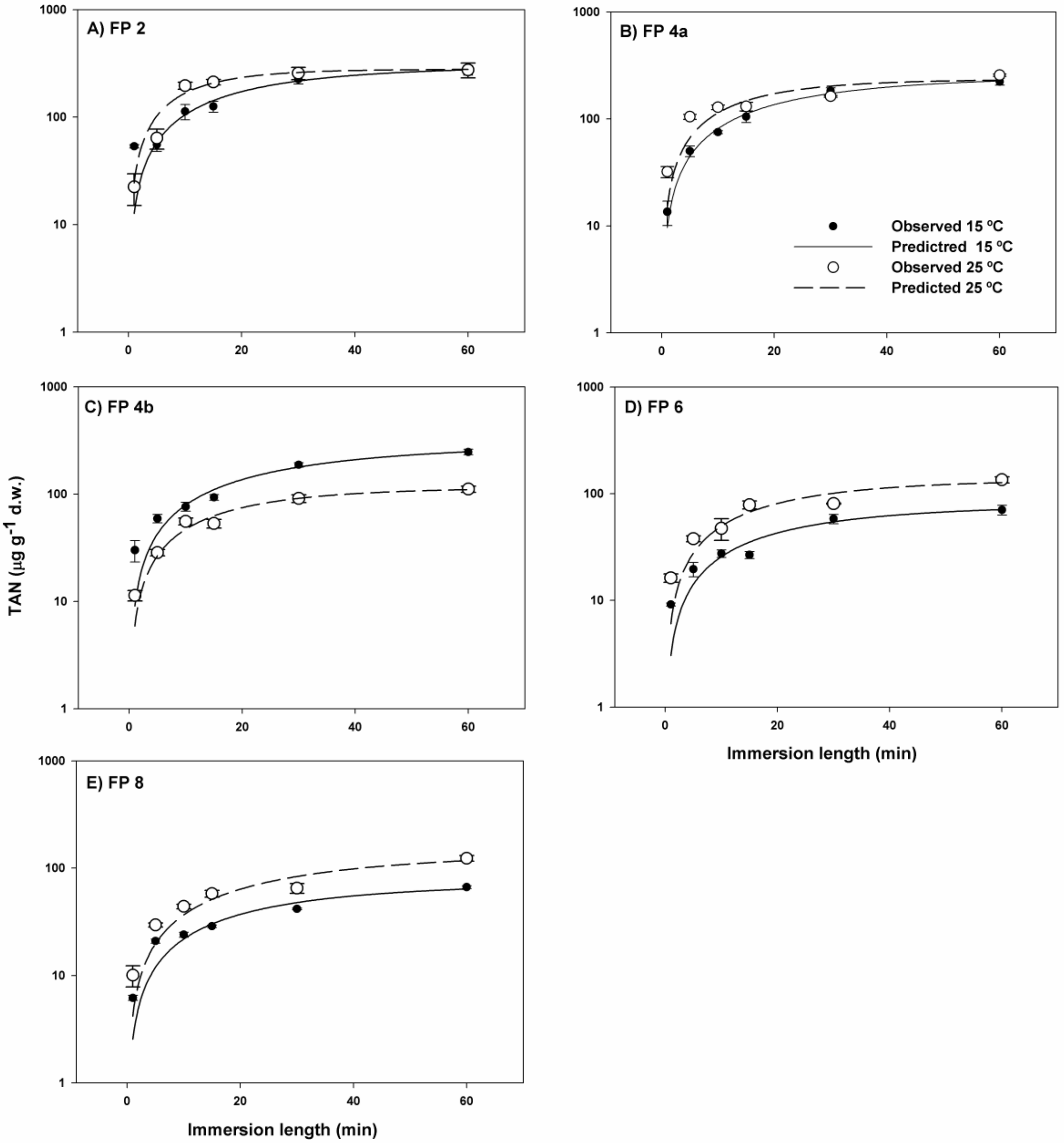
A) FP 2
B) FP 4a
C) FP 4b
D) FP 6
E) FP 8
TAN (µg g^{-1} d.w.)
Immersion length (min)
1000
100
10
1
0
20
40
60
Observed 15 ºC
Predictred 15 ºC
Observed 25 ºC
Predicted 25 ºC

Fig 3

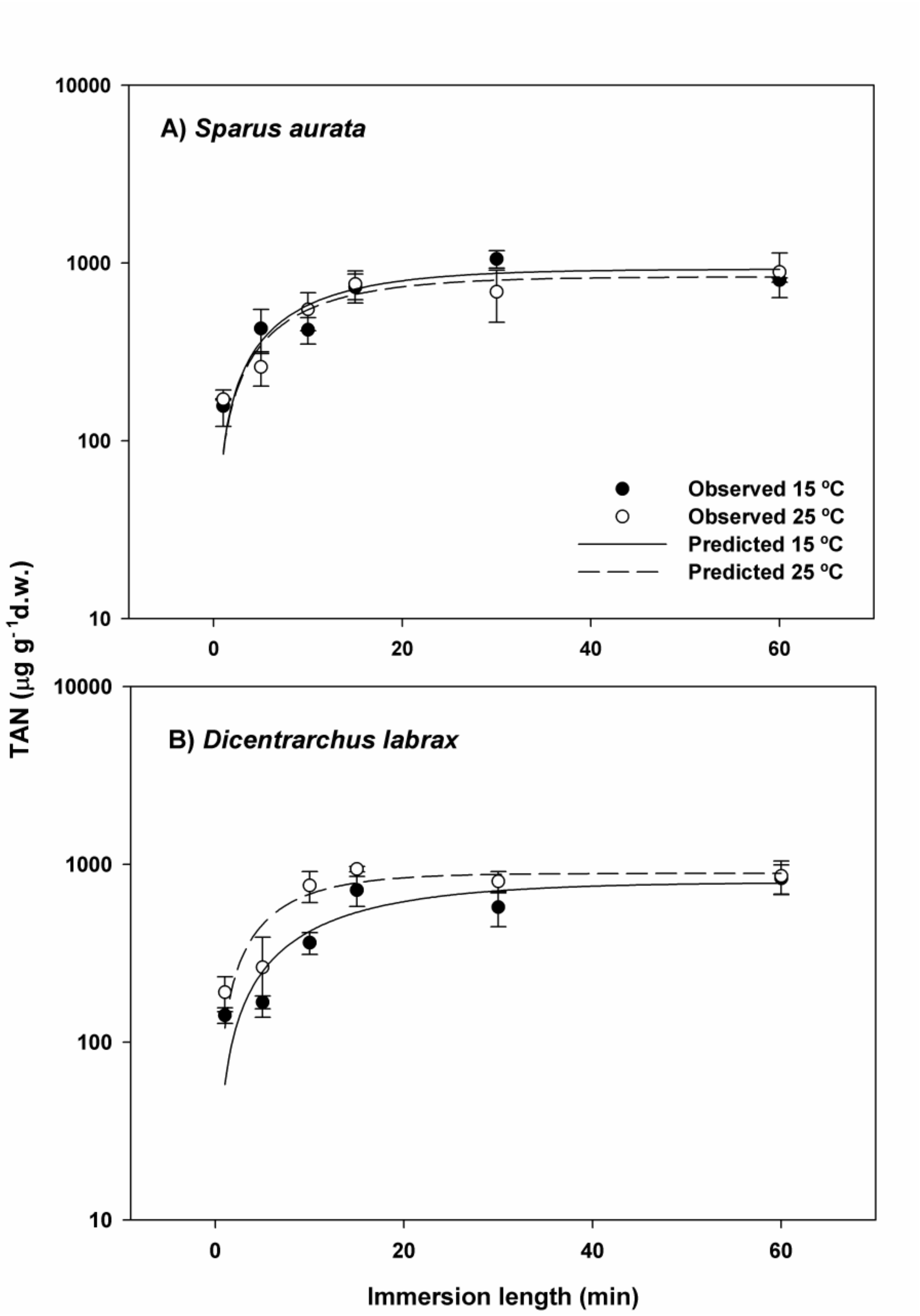
A) Sparus aurata
B) Dicentrarchus labrax
TAN (µg g⁻¹d.w.)
10000
1000
100
10
0
20
40
60
Immersion length (min)
Observed 15 ºC
Observed 25 ºC
Predicted 15 ºC
Predicted 25 ºC

Fig 4