

Determination of physical properties of feed pellets for Mediterranean aquaculture.

Paolo Vassallo¹, Andrea M. Doglioli^{2,3}, Fabrizio Rinaldi², Ilaria Beiso¹

¹ DIPTERIS, Department for the Study of Territory and its Resources, University of Genova, Corso Europa 26, 16132 Genova, Italy

² DIFI, Department of Physics, University of Genova, Via Dodecaneso 33, 16146 Genova, Italy

³ LPO, Laboratoire de Physique des Océans, UMR 6523 CNRS/IFREMER/UBO, UFR Sciences et Techniques, 6 avenue Le Gorgeu - C.S. 93837, 29238 Brest Cedex 3, France

Corresponding author address:

Andrea M. Doglioli,

LPO, Laboratoire de Physique des Océans, UMR 6523 CNRS/IFREMER/UBO, UFR Sciences et Techniques, 6 avenue Le Gorgeu - C.S. 93837, 29238 Brest Cedex 3, France

Phone: +33 (0)2 98 01 61 71 Fax: +33 (0)2 98 01 64 68

E-mail: Andrea.Doglioli@univ-brest.fr

Running title: Physical properties Mediterranean feed pellets

Keywords: Settling velocity, floating time, feed pellets, water adsorption, Mediterranean, waste dispersion.

Preprint submitted to Aquaculture Research

July 28, 2005

Abstract

Settled uneaten feed causes the most intense impact under sea cages and settling velocity of the feed pellets represents a key parameter for waste dispersion models. Even if some data about physical properties of feed pellets have been published in the framework of salmonid rearing, there is a complete lack of information related to Mediterranean Sea, as regards typical values of temperature, salinity and feed composition for Gilthead Sea Bream (*Sparus aurata L.*) and Sea Bass (*Dicentrarchus labrax L.*).

In this study we try to fill this lack, determining dimensions, water adsorption properties, floating times and settling velocities of a typical growing sequence of pellets for the species mentioned above, under defined laboratory conditions reproducing Mediterranean Sea water. The settling velocity increases with pellet size from 0.087, for the smallest pellet (3mm), to 0.144 ms⁻¹ for the 5 mm pellet. The biggest extruded pellet (6 mm) falls slower (0.088 ms⁻¹). The floating time before pellet's fall is found to be a critical parameter in determining settling velocity. The latter depends on pellet's size, water temperature and salinity. The examined pellets reach a 42% of weight increase after 10 minutes of immersion, while no appreciable dimension change is observed.

Our results are in part different from previous ones and could play a role in evaluating and modelling Mediterranean aquaculture environmental impact.

1 Introduction

Intensive cage aquaculture generates considerable quantities of waste, including both particulate uneaten feed, fish faeces and soluble excretory products. Settled uneaten feed pellets are assumed to be the primary cause of ecological impact on the benthos community beneath the cages (e.g. Beveridge et al., 1991; Vezzulli et al., 2003). Previous studies estimated an averaged loss of 5 % of the supplied food (Findlay & Watling, 1994) that could result in a huge amount of organic matter load on the benthos nearby the cages (Karakassis et al., 2000; Carroll et al., 2003). Furthermore a number of models has been developed for the estimation of accumulation and dispersion of organic waste from the aquaculture cages (Gowen et al., 1989; Gillibrand & Turrell, 1997; Panchang et al., 1997; Dudley et al., 2000; Cromey et al., 2002a; Doglioli et al., 2004). Settling velocity of the uneaten feed pellets has shown to be a key parameter for the accuracy of the prediction of those models. In particular when the settling velocity is of the same magnitude of the local current velocity the impact under the net pen cages can exceed critical thresholds for the fish farm wastes (Cromey et al., 2002b). Studies regarding the physical characteristics of the food pellets involved in seawater (Findlay & Watling, 1994; Chen et al., 1999a) and freshwater (Elberizon & Kelly, 1998) fish farming systems have been previously published but there is a complete lack of information regarding the characteristics of the feed employed in the rearing of Mediterranean species.

In this paper we present data of dimensions, water adsorption properties, floating times and settling velocities of a typical growing sequence involved in Gilthead Sea Bream (*Sparus aurata L.*) and Sea Bass (*Dicentrarchus labrax L.*) rearing. The ex-

periments reproduce typical Mediterranean water conditions, generally characterized by high salinity and high temperature values. The present work constitutes part of a project aimed to the development of a reliable waste dispersion model for Mediterranean marine aquaculture.

2 Material and Methods

The feed pellets employed in this experiment were produced by Coppens International (www.coppens-int.com) and are named “Marico Seabass and Seabream” growing sequence. The producer kindly provided five different kind of feed pellets both pelletized and extruded. We verified that the pellets were in good condition and not friable due to transportation and storage. In the following we will refer to the different feed types according to the nominal diameter of the cylindrical pellets: 3.5 mm and 5 mm for the pelletized ones and 3 mm, 4.5 mm and 6 mm for the extruded ones. Mean composition of the studied pellets (Table 1) was provided by the producer while the length was measured with vernier calipers (1 mm precision). Extruded pellets (3, 4.5 and 6 mm) displayed a very low standard deviation while pelletized ones (3.5 and 5 mm) showed a greater variability in length. This fact is probably due to the more elongated shape and friable composition of these latter pellets as a consequence of different production processes.

Water characteristics were selected on the basis of a dataset collected in four seasonal sampling campaigns during a year in four stations around the AQUA Lavagna fish farm (Ligurian Sea, NW Mediterranean) as requested to the farmers by the local

public authority. The reader is referred to Doglioli et al. (2004) for more information. A standard treatment of the data was performed and spatially averaged vertical profiles, for each season, are reported in Fig. 1. The presence of the seasonal thermocline could be clearly observed (Fig. 1A) especially in summer and in autumn when mixing process became weaker, while in winter and spring the water column resulted isothermal. The winter and spring seasons are the most rainy in Mediterranean area. Salinity profiles (Fig. 1B) show the presence at the surface of freshwater due to the proximity of the Entella river mouth (Doglioli et al., 2004). Furthermore these values agree with a number of previous works reporting CTD measurements in coastal areas of the Ligurian Sea (e.g. Astraldi & Manzella, 1983; Rossi et al., 1997). As a consequence they could be considered as representative of the North Western Mediterranean conditions.

2.1 Settling velocity measurement

Following Chen et al. (1999b) method, a 120 cm length plexiglass tube of 10 cm diameter was used to test the settling velocities of the examined particles. The transparent tube was filled with water and marked 5 cm from the top and every 50 cm from this point ahead (Fig. 2). All the apparatus was securely fixed in a vertical position and pellets were carefully laid to the water surface. The floating time τ_{float} was defined as the time needed for a pellet to fall beyond the first 5 cm. The settling velocity v_{set} was determined by manually timing the pellet fall between two marks 50 cm apart. The measurement was repeated for thirty pellets of each type, for three different temperatures (13, 18, 23 °C) and two different salinity (36 and 38 gL⁻¹) for a total of 900 measurements.

Water in the tube was filtered with a 45 μm sieve after each change of particle type while completely renewed at each salinity and temperature variation. To fix the water temperature a microwaves oven was used to warm it or alternatively a refrigerator to cool it. The stability of the water temperature was continuously checked during the experiment with a decimal degree precision thermometer. The water salt concentration was determined by mixing pure water with NaCl weighted with a microgram precision balance.

Finally after the tube was filled with conditioned water, pellets were laid one after the other and the time of fall was measured with a 0.01 second precision chronometer. Particles which came into contact with the tube wall during the fall were excluded from statistics.

2.2 Soaking experiments

Weight differences between dry feed pellets and pellets immersed in water for different time periods were examined. Since Chen et al. (1999a) showed that variation in salinity and temperature did not affect significantly the particles weight, the experiment was carried out at a single salinity (36 gL^{-1}) and a single temperature (23 $^{\circ}\text{C}$). Both these values were selected due to laboratory procedures convenience. Ten pellets of each type were randomly chosen, the diameter and the length of each particle were measured (millimeter precision) and the dry weight determined with microgram precision. Pellets were left on the surface of the water till they sank, then they were left submerged for 2, 5 and 10 minutes. At the end of the immersion period they were gently retrieved and water in excess was drained by placing pellets on an adsorbent paper. Finally

particles were re-measured and re-weighted to obtain dimension and weight increase after immersion.

2.3 Statistical analysis

The Pearson correlation analysis was performed. A four-way ANOVA test was performed to analyse the v_{set} dependence on temperature, salinity, τ_{float} and particle type (temperature, 3 levels fixed; salinity, 2 levels fixed; floating time 10-levels, random; particle type, 5 levels fixed). A three way ANOVA test was also performed to analyse the τ_{float} dependence on particles type, temperature and salinity (particle type, 5 levels fixed; temperature, 3 levels fixed; salinity, 2 levels fixed). Before performing the analysis, the variance homogeneity was assessed by Cochran's test.

All statistical tests and correlation analysis were made using the MATLAB 6-R12 statistics toolbox. In all cases the significance level was fixed to $p < 0.05$.

3 Results

3.1 Settling velocity measurements

The settling velocity of the particles was measured by manually timing the descent between two marks 50 cm apart. T-test performed never detected significant differences between velocities calculated for each sector. As a consequence in the following the v_{set} will be considered constant along the whole water column.

For each combination of particle dimension, temperature and salinity, the means and the standard deviations calculated for the settling velocities and the floating times

are reported in Fig. 3 and Fig. 4, respectively. Fig. 3 shows that the greater is the pellet dimension, the higher the velocity, except for the 6 mm pellets that show huge standard deviations probably due to air trapped at the time of pellet placement in the water. Furthermore, the highest velocities are measured for the two pelletized particles while the extruded pellets sink more slowly. The pellet dimension also influences the floating time, so that the pellets with a diameter greater than 4.5 mm soak very rapidly (Fig. 4). τ_{float} shows a negative correlation with particle dimension ($\rho = -0.36, n = 900$) and water temperature ($\rho = -0.17, n = 900$). Although the measurements show huge standard deviations examining the same kind of pellets probably due again to air bubbles.

The ANOVA tests provide a quantitative confirmation of these results (Table 2, Table 3). The ANOVA tables have six columns: the first shows the source of the variability; the second shows the Sum of Squares (SS) due to each source; the third shows the degrees of freedom (df) associated with each source; the fourth shows the Mean Squares (MS), which is the ratio SS/df; the fifth shows the F statistics, which is the ratio of the mean squares; the sixth shows the p-values for the F statistics. Table 2 shows that pellet diameter, floating time and the interaction between these two factors, affect significantly the settling velocities of the particles. Temperature and salinity variations instead do not affect settling velocity. Table 3 shows that for the floating time the pellet's diameter represents again the most important factor, but in this case temperature and salinity play some role. In particular as temperature increases, the density of the water decreases causing τ_{float} to decrease.

Averaging on all trials for each type of particle (Table 4), the settling velocity

ranges from $0.087 \pm 0.008 \text{ ms}^{-1}$ to $0.144 \pm 0.011 \text{ ms}^{-1}$ for 3 mm and 5 mm pellets, respectively. The floating time ranges from $2 \pm 7 \text{ s}$ to $73 \pm 77 \text{ s}$ for 5 mm and 3.5 mm pellets, respectively. The obtained v_{set} values are in general agreement with previous studies about salmonid feed, nevertheless two differences have to be mentioned. First, our biggest pellets (6 mm) fall slower than pellets with similar dimension studied by Chen et al. (1999a) and Elberizon & Kelly (1998). Second, the smaller pellets (3 mm, 3.5 mm, 4.5 mm, 5 mm) have a higher settling velocity with respect to Findlay & Watling (1994), Chen et al. (1999a) and Elberizon & Kelly (1998).

3.2 Soaking experiment

None of the examined particles shows an appreciable dimension change after the three different periods of immersion (2, 5 and 10 minutes). The weight increase shows a positive correlation with immersion time ($\rho = 0.17, n = 150$) and a negative correlation with the diameter of the particles ($\rho = -0.86, n = 150$) as can be clearly observed in Fig. 5. In particular, the smallest pellets show the highest weight increase after immersion in agreement with previous studies (Chen et al., 1999a). The examined particles reach a maximum of 42% of weight increase after 10 minutes of immersion, revealing greater absorption properties in respect to Atlantic salmon feed. Pelletized and extruded pellets showed similar attitude to water absorption even if the pelletized particles resulted much less firm after immersion.

4 Summary and Conclusions

Physical properties of a commercial growing sequence of Sea Bass and Seabream pellets were assessed. The laboratory conditions were established in order to reproduce Mediterranean values of temperature and salinity. According to the linear Stokes' Law, a particle falls in sea water with a settling velocity depending upon its dimensions, density and viscosity of the medium. Viscosity in turn is dependent upon temperature, solute concentration and pressure. Nevertheless, as already pointed out both by Chen et al. (1999a) and Elberizon & Kelly (1998), the feed pellets settling velocity is non-Stokesian, due principally to the larger Reynolds number of the flow and the shape factor of the pellets. Furthermore, the feed composition and preparation are expected to have a key role on the water adsorption properties of the pellets. For these reasons, although some parameters values model used in salmonid marine cage aquaculture could be potentially be applied in Mediterranean (Doglioli et al., 2004), much of relevant data on the feed physical behavior are unusable.

Settling rate of salmonid feed pellets was studied by various authors. Gowen & Bradbury (1987) quote results from unpublished data of velocities of 0.09 to 0.15 ms^{-1} and then Gowen et al. (1989) used a settling velocity equal to 0.12 ms^{-1} . Findlay & Watling (1994) provided data on several North American pellet types or sizes and quoted settling rates of 0.055 ms^{-1} and 0.155 ms^{-1} for 3 mm and 10 mm dry pellets, respectively. Elberizon & Kelly (1998) showed settling velocities of freshwater salmonid pellet diets ranging from 0.05 to 0.12 ms^{-1} for 2 mm and 8 mm pellet sizes, respectively. These results are similar to settling rates found by Chen et al. (1999a), who studied the

physical characteristics of commercial pelleted Atlantic salmon feeds finding that the temperature and the salinity of sea water influence the settling velocity. In the present study a growing sequence (3 to 6 mm of diameter) for typical Mediterranean rearing species (Gilthead Sea Bream *Sparus aurata L.* and Sea Bass *Dicentrarchus labrax L.*) was studied. In order to obtain parameter values for realistic dispersion modelling, the temperature and salinity values were chosen on the basis of field data and compared with published data for North-Western Mediterranean (Astraldi & Manzella, 1983; Rossi et al., 1997, e.g.). Furthermore, even if the experimental water column was not so deep as the one used by Chen et al. (1999a), the influence of the wall drag and the bottom shear effects could be considered negligible as in Chen et al. (1999a) and Elberizon & Kelly (1998).

Settling velocities of Marico Sea Bream and Sea Bass feed pellets range from $0.087 \pm 0.008 \text{ ms}^{-1}$ to $0.144 \pm 0.011 \text{ ms}^{-1}$ for 3 mm and 5 mm pellets, respectively. Increasing the pellets nominal diameter the settling velocity increases, except for the 6 mm pellets. The pelletized feed sink faster than extruded pellets, probably due to its elongated shape and different composition and preparation (in particular, a smaller percentage of fat). Temperature and salinity differences between the different trials, show that the seasonal temperature and salinity variability seems to have a negligible influence on the settling velocity. Nevertheless temperature and salinity play some role in the floating time determination. The latter ranges from $2 \pm 7 \text{ s}$ to $73 \pm 77 \text{ s}$ for 5 mm and 3.5 mm pellets, respectively. We have measured, for the first time, the floating time since the ANOVA test showed that it affects significantly the settling velocity. The reason of this fact could be found in the observed weight increment of pellets immersed

in the water at the surface before they start to fall. The soaking experiment provide a quantitative estimate of this process, pointing out that the phenomenon is stronger for smaller particles. Thus, it could be said that the influence of temperature and salinity on the settling velocity is indirect via τ_{float} . Furthermore, τ_{float} can be seen as the period during which the fish has the highest probability to reach the feed. According to this, the bigger τ_{float} the lesser the uneaten feed percentage will be. However, a quantitative calculation of this link is very hard to achieve but knowing the τ_{float} value provides already a precious information for model calibrations and validation processes.

Finally, the present study provides important information for aquacultural wastes dispersion modeling. A realistic dispersion model would then have to consider: a) the diameter of the actual feed distributed to fishes; b) the seasonal variation of temperature. Collaboration with farmers, essential for nutritional data collection and hydrological measurements will be useful to improve aquaculture impact predictions. This recommendations will be followed in next steps of our project to develop a reliable waste dispersion model for Mediterranean marine aquaculture on the basis of the POM-LAMP3D numerical model (Doglioli et al., 2004).

Acknowledgements

The authors warmly thank Marcello Magaldi for the enlightening discussions on the paper subject, for his precious contributions and for the irreplaceable help in paper drawing. We also acknowledge Luca Lanza (Department of Environmental Engineering, University of Genova) that provided the experimental apparatus, Corrado Ratto for the interest in our research, Mauro Fabiano and the Marlab laboratory that kindly hosted us and the apparatus, West Waters Srl that provided pellets samples and Aqua Srl for the temperature and salinity dataset for the Ligurian Sea.

References

- Astraldi, M. and Manzella, G. (1983). Some observations on current measurements on the east ligurian shelf, Mediterranean Sea. *Cont. Shelf Res.*, 2:183–193.
- Beveridge, M., Phillips, M., and Clarke, R. (1991). A quantitative and qualitative assessment of wastes from aquatic animal production. In Brune, D. E. and Tomasso, J. R., editors, *Advances in World Aquaculture Volume 3*, pages 506–533. World Aquaculture Society, Baton-Rouge, USA.
- Carroll, M., Cochrane, S., Fieler, R., Velvin, R., and White, P. (2003). Organic enrichment of sediments from salmon farming in norway: environmental factors, management practices, and monitoring techniques. *Aquaculture*, 226:165–180.
- Chen, Y., Beveridge, M., and Telfer, T. (1999a). Physical characteristics of commercial pelleted atlantic salmon feeds and consideration of implications for modeling of waste dispersion through sedimentation. *Acquaculture Int.*, 7:89–100.
- Chen, Y., Beveridge, M., and Telfer, T. (1999b). Settling rate characteristics and nutrient content of the faeces of Atlantic salmon, *Salmo salar* L., and the implications for modelling of solid waste dispersion. *Acquaculture Res.*, 30:395–398.
- Cromey, C., Nickell, T., and Black, K. (2002a). DEPOMOD-modelling the deposition and the biological effects of wastes solids from marine cage farms. *Aquaculture*, 214(1-4):211–239.
- Cromey, C., Nickell, T., Black, K., Provost, P., and Griffiths, C. (2002b). Validation of

- a fish farm waste resuspension model by use of a particulate tracer discharged from a point source in a coastal environment. *Estuaries*, 25(5):916–929.
- Doglioli, A., Magaldi, M., Vezzulli, L., and Tucci, S. (2004). Development of a numerical model to study the dispersion of wastes coming from a marine fish farm in the Ligurian Sea (Western Mediterranean). *Aquaculture*, 231(1-4):215–235, doi:10.1016/j.aquaculture.2003.09.030.
- Dudley, R., Panchang, V., and Newell, C. (2000). Application of a comprehensive modeling strategy for the management of net-pen aquaculture waste transport. *Aquaculture*, 187:319–349.
- Elberizon, I. and Kelly, L. (1998). Settling measurements of parameters critical to modelling benthic impacts of freshwater salmonid cage aquaculture. *Aquaculture Research*, 29:669–677.
- Findlay, R. and Watling, L. (1994). Toward a process level model to predict the effects of salmon net-pen aquaculture on the benthos. In *Modeling Benthic Impacts of Organic Enrichment from Marine Aquaculture*, Canadian Technical Report of Fisheries and Aquatic Sciences 1949: xi + 125 p. B.T. Hargrave.
- Gillibrand, P. and Turrell, W. (1997). The use of simple models in the regulation of the impact of fish farms on water quality in Scottish sea lochs. *Aquaculture*, 159:33–46.
- Gowen, R. and Bradbury, N. (1987). The ecological impact of Salmon farming in coastal waters: A review. *Oceanographic and Marine Biology: An Annual Review*, 25:563–575.

- Gowen, R., Bradbury, N., and Brown, J. (1989). The use of simple models in assessing two of the interactions between fish farming and marine environment. In DePauw, N., Jaspers, E., Ackefors, H., and Wilkins, N., editors, *Aquaculture - A Biotechnology in Progress*, pages 1071–1080. European Aquaculture Society.
- Karakassis, I., Tsapakis, M., Hatziyanni, E. Papadopoulou, K., and Plaiti, W. (2000). Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES Journal of Marine Science*, 57(5):1462–1471.
- Panchang, V., Cheng, G., and Newell, C. (1997). Modeling hydrodynamics and aquaculture waste transport in Coastal Maine. *Estuaries*, 20:14–41.
- Rossi, G., Di Fesca, V., Cerrati, G., and Baldi, A. (1997). Analisi in situ ed in continuo di parametri conservativi nel tratto di mare di fronte al fiume entella. Technical report, ENEA. RTI/AMB/GEM-MAR/97/10/RL2/B.1.1.2/01.
- Vezzulli, L., Marrale, D., Moreno, M., and Fabiano, M. (2003). Sediment organic matter and meiofauna community response to long-term fish-farm impact in the Ligurian Sea (Western Mediterranean). *Chemistry and Ecology*, 19(6):431–440.

List of Figures

1	Vertical profiles (depth reported in meter) of A. temperature ($^{\circ}\text{C}$) and B. salinity (gL^{-1}) measured around a fish farm in Ligurian Sea	19
2	Schematic representation and picture of the cylinder employed in the settling velocity determination	20
3	Settling velocities (ms^{-1}) of Marico Sea Bream and Sea Bass feed pellets which differ in size and diet formulation under a range of environmental conditions. Error bars represent standard deviations. Nominal diameter marked with an asterisk identify pelletized particles	21
4	Floating time (s) of Sea Bream and Sea Bass Marico feed pellets which differ in size and diet formulation under a range of environmental conditions. Error bars represent standard deviations. Nominal diameter marked with an asterisk identify pelletized particles	22
5	Weight increase in percentage of Sea Bream and Sea Bass Marico feed pellets after different period of immersion. Nominal diameter marked with an asterisk identify pelletized particles	23

Nominal diameter (mm)	3	3.5*	4.5	5*	6
Length mean (std) (mm)	3 (0)	9 (2)	4 (0)	10 (3)	7 (1)
Protein (%)	42.0	44.0	46.0	47.0	46.0
Fat (%)	18.0	11.0	24.0	12.0	20.0
Fibre (%)	1.7	2.5	0.8	1.5	1.2
Ash (%)	8.8	9.8	10.8	10.7	10.2

Table 1: Characteristics of Marico Seabass and Seabream growing sequence pellets. Nominal diameter and composition as declared by the producer, length measured during the experiment. Nominal diameter marked with an asterisk identify pelletized particles.

Source	Sum. Sq.	D.F.	Mean Sq.	F	Prob
Diameter	0.01017	4	0.00339	12.21	< 0.0001
Temperature	0.00028	2	0.00028	1.01	0.3155
Salinity	< 0.00001	1	< 0.00001	1	0.9582
τ_{float}	0.00196	9	0.00098	3.52	0.0304
Diameter*Temperature	0.00118	8	0.00015	0.53	0.8344
Diameter*Salinity	0.00063	4	0.00016	0.57	0.6842
Diameter* τ_{float}	0.00798	18	0.00044	1.6	0.0569
Temperature*Salinity	0.00042	2	0.00021	0.76	0.4698
Temperature* τ_{float}	0.00098	9	0.00011	0.39	0.9381
Salinity* τ_{float}	0.00010	5	0.00002	0.07	0.9967

Table 2: Analysis of variance for settling velocity. In bold significant values.

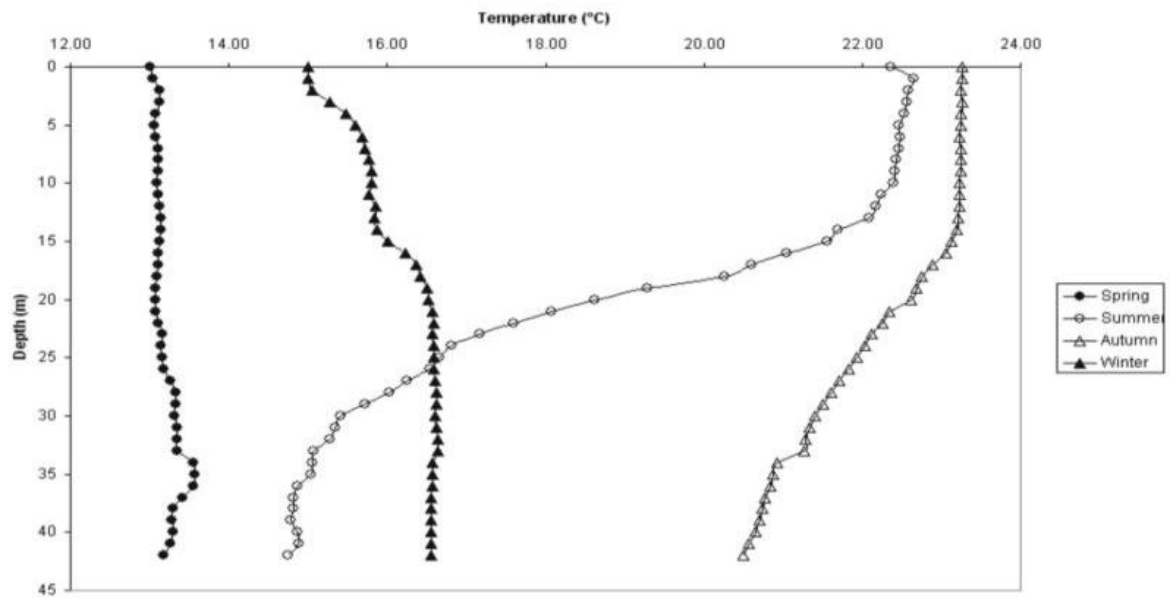
Source	Sum. Sq.	d.f.	Mean Sq.	F	Prob
Diameter	108565.2	4	27141.3	70.25	< 0.0001
Temperature	4134.3	2	2067.1	5.35	0.0050
Salinity	1707.1	1	1707.1	4.42	0.0361

Table 3: Analysis of variance for floating time. In bold significant values.

Nominal diameter (mm)	3	3.5*	4.5	5*	6
v_{set} mean (ms^{-1})	0.087	0.118	0.103	0.144	0.088
(std)	(0.008)	(0.008)	(0.009)	(0.011)	(0.030)
τ_{float} mean (s)	69	73	29	2	12
(std)	(50)	(77)	(40)	(7)	(35)

Table 4: Means and standard deviations of settling velocity and floating time calculated without considering temperature and salinity influences. Nominal diameter marked with an asterisk identify pelletized particles.

A



B

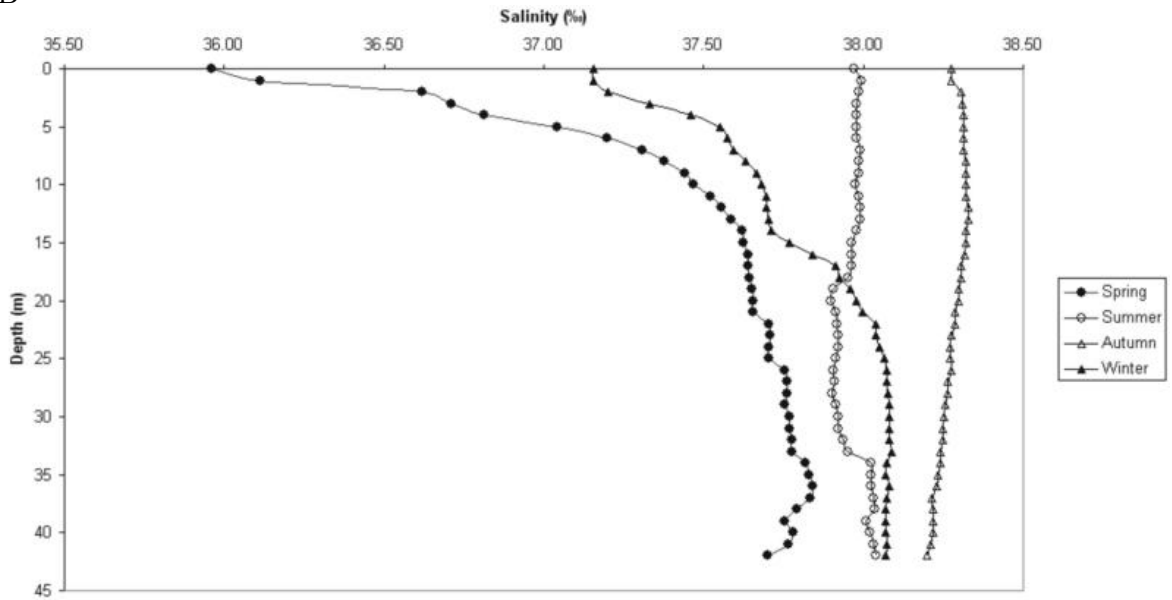


Figure 1:

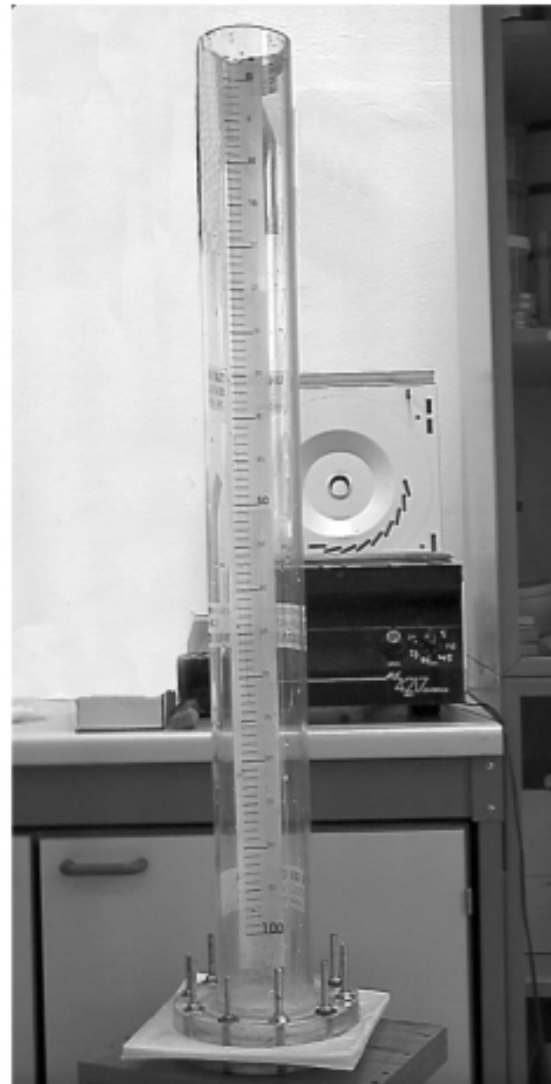
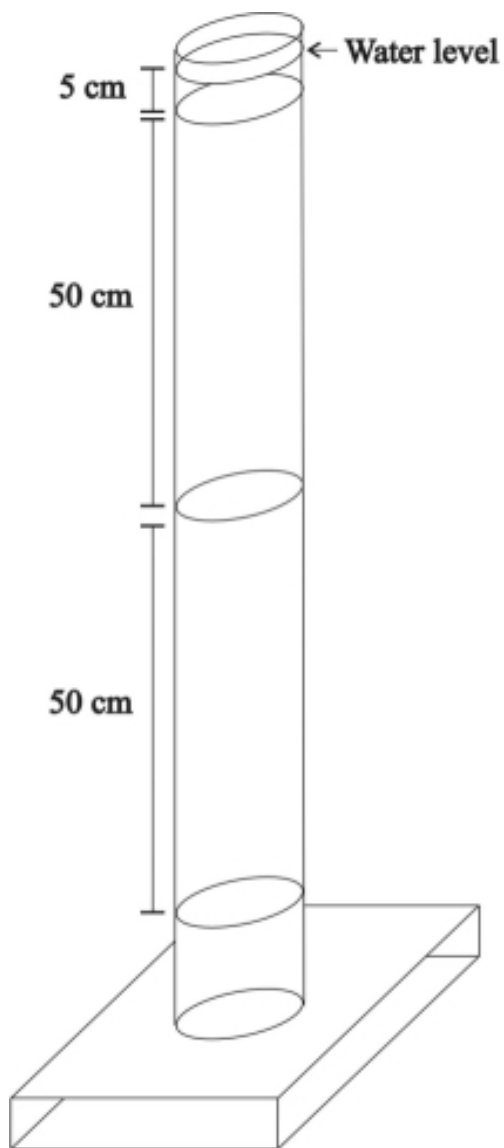


Figure 2:

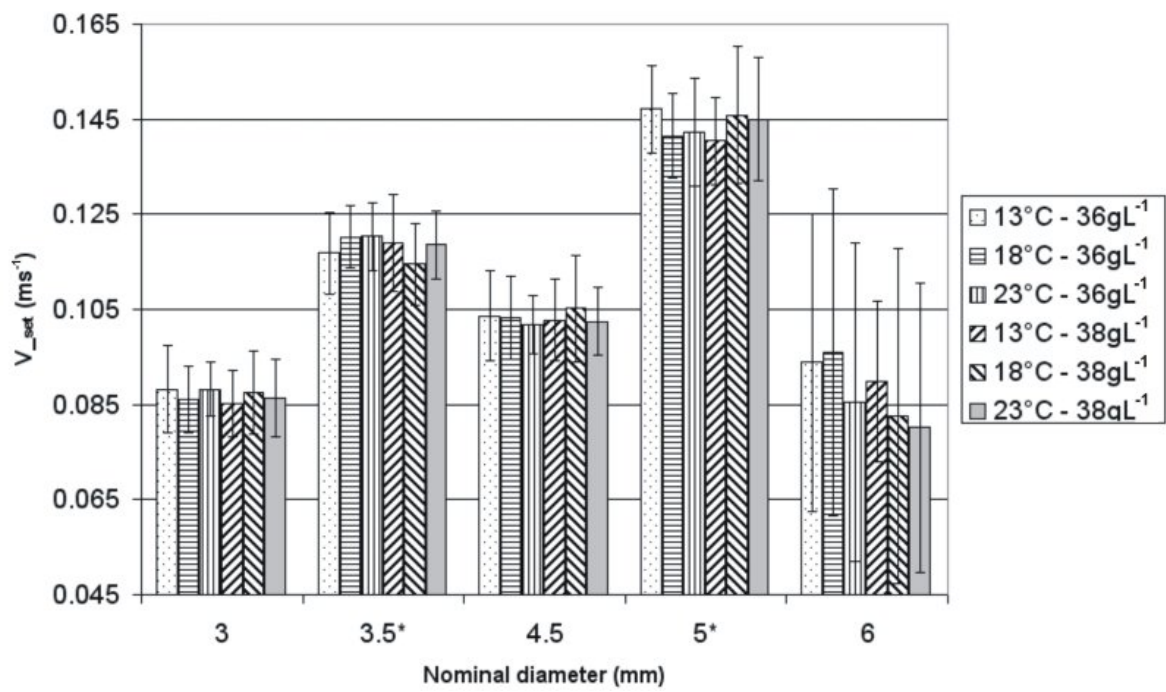


Figure 3:

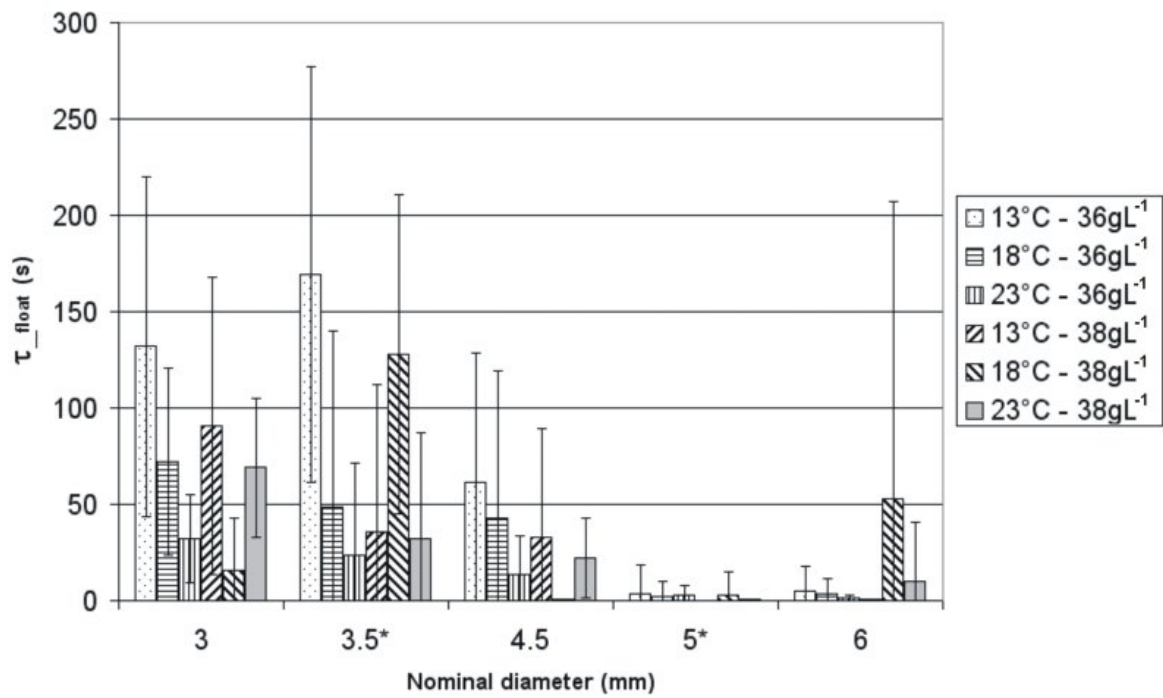


Figure 4:

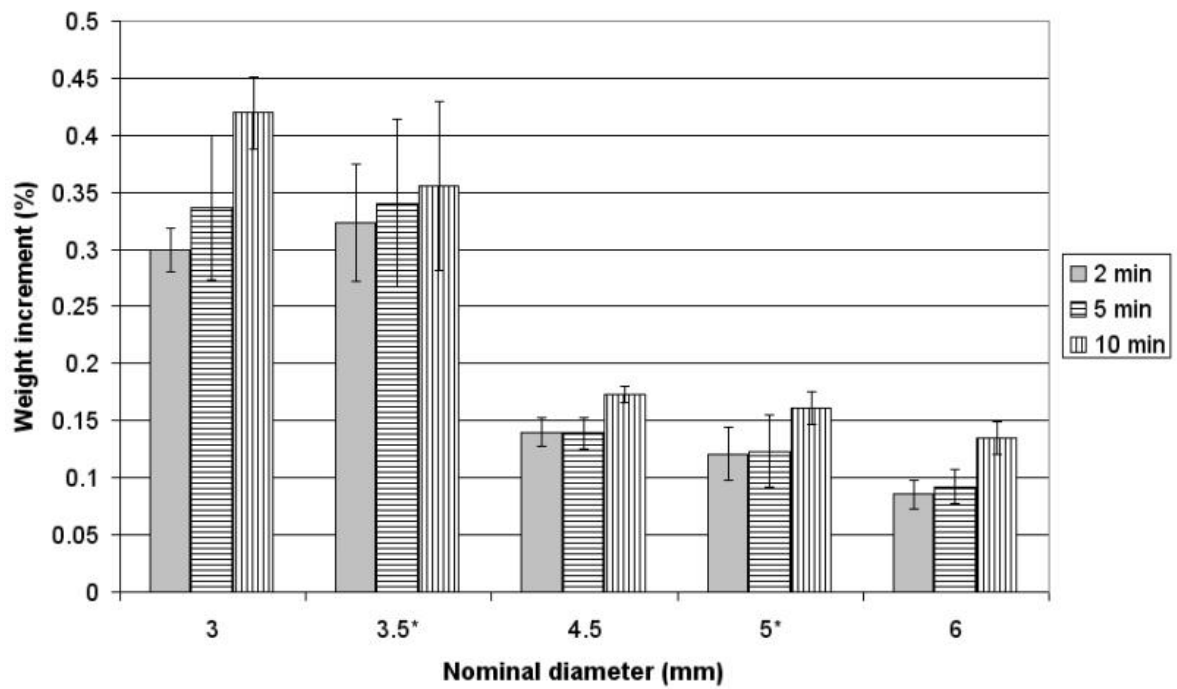


Figure 5: