Fisheries Oceanography
November 2016, Volume 25, Issue 6, Pages 582-597
http://dx.doi.org/10.1111/fog.12174
http://archimer.ifremer.fr/doc/00346/45676/
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Size, permeability and buoyancy of anchovy (*Engraulis Encrasicolus*) and sardine (*Sardina Pilchardus*) eggs in relation to their physical environment in the Bay of Biscay

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Abstract :

The size and specific gravity of eggs of marine pelagic fish partly determine their dispersal and survival. Using an original dataset of anchovy and sardine eggs, sampled in spring over the last decade in the Bay of Biscay, we provide a parameterization of these properties on ambient water temperature and salinity. We used the density gradient column for measurement of egg specific gravity. The column was also filled with homogeneous water for sinking velocity experiments. For anchovy, these experiments confirm that the effect of egg permeability through the chorion could be neglected when modelling sinking, while it has to be considered for sardine, its perivitelline space representing 78.6% (±6.2%) of the total egg volume, as opposed to 5–10% for most teleosts species. We estimated a coefficient of permeability of the chorion of 0.0038 mm s−1. However, permeability should not affect the measurement of sardine egg specific gravity in a gradient column, provided a minimum duration before reading is respected for equilibrium to be reached. In relation to their environment, we found that the egg specific gravity is largely determined by sea surface salinity for both species, whereas egg size is weakly but significantly impacted by temperature, for sardine only. On average, the estimated difference in specific gravity between egg and surface water is −0.92 σT for anchovy and −1.06 σT for sardine. The detailed parameterization of the relationship between eggs and water properties should prove useful, in particular to modellers dealing with the dispersal of fish early life stages.

Keywords : Keywords: anchovy, Bay of Biscay, density gradient column, egg buoyancy, egg permeability, egg size, sardine

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INTRODUCTION

Fish egg properties have a fundamental impact on their dispersal and on the survival of initial stages of life. Size and specific gravity are key among those properties. Size may be related to recruitment to late larval stage (Chambers, 1997) through its potential link with egg quality, that will determine the size and capacity of young larvae to be active, grow, and survive, or by direct relationship between size and mortality. Specific gravity is the main driver of the egg vertical distribution (Sundby and Kristiansen, 2015), defining the water mass in which they will be advected, which eventually determines connectivity patterns from spawning areas.

For analysis and integration of these dispersal processes, models of egg vertical distribution have been developed with several objectives. A first one is the interpretation of eggs distribution for better ecological understanding (Sundby, 1983; Adlandsvik et al., 2001). A second one is management, with methods of stock assessment based on total egg abundance (Petitgas et al., 2009). In the case of CUFES sampling (Continuous Underway Fish Egg Sampler, Checkley Jr et al., 1997) an extrapolation is required over the water column from observed surface abundance (Boyra et al., 2003; Curtis et al., 2007). In a third objective, vertical distribution models are also embedded in three dimensional circulation models to study connectivity patterns between spawning areas and later stages habitat and nurseries (Parada et al., 2003; Huret et al., 2010; Ospina-Álvarez et al., 2012).

In these models, egg specific gravity, and to a lesser extent size, are the most sensitive biological parameters (Petitgas et al., 2006), in addition to the water column physical properties. For most species there is a lack of descriptive information on egg properties and mostly on what control their variability in time and space. They vary

between and within species, and seem to be under the direct or indirect influence of the external environment during oogenesis.

The size of eggs spans over a well described range at the species level (Munk and Nielsen, 2005). Besides a natural phenotypic variance on a genetic basis, a maternal effect was also demonstrated, with larger fish spawning larger eggs for cod (Kjesbu, 1989; Vallin and Nissling, 2000) or haddock (Trippel and Neil, 2004). Similar pattern has been observed with maternal condition, together with (Marteinsdottir and Steinarsson, 1998), or instead of mother size (Chambers and Waiwood, 1996). Egg size may also be affected by the batch number within a spawning season, with a decrease as the season advances (Kjesbu et al., 1992; Trippel and Neil, 2004; Vallin and Nissling, 2000). But size may also be under direct influence of temperature during oogenesis from a decoupling between growth and development in the response to temperature (Chambers, 1997).

The specific gravity of eggs also shows some variability. The osmolarity of the embryo and yolk is constant and equal to the one of maternal plasma (Davenport et al., 1981), with variability of specific gravity mostly arising from variable fluid content after hydration process (Craik and Harvey, 1987). Through this maternal control, specific adaptations to regional marked hydrography differences were observed, this is the case between brackish Baltic waters and marine Atlantic waters for flounder (Solemdal, 1967) or cod (Thorsen et al., 1996), or between Mediterranean and Bay of Biscay waters for anchovy (Ospina-Álvarez et al., 2012). But more subtly, some adaptations were also mentioned within a region or population, again seemingly under indirect environment control through the maternal plasma. Coombs et al., (1985) noted that sardine eggs in the Bay of Biscay were always positively buoyant relatively to sampled sea water. In the same region, Goarant et al. (2007) observed a significant correlation between the egg specific

gravity of anchovy and the salinity at sampling station. Additionally, the egg specific gravity may slightly evolves throughout development with processes detailed in Jung et al. (2014). This evolution pattern was described for both sardine (Coombs et al., 1985) and anchovy (Ospina-Álvarez et al., 2012).

Our two species of interest in this study display strong distinctive features (Munk and Nielsen, 2005) despite their taxonomic proximity. This make them ideal case studies for comparison, as well as good candidates for generalisation of our results to other species. Anchovy (*Engraulis encrasicolus*) eggs have a prolate spheroid shape. The ellipsoid shape may have an effect on its sinking velocity depending on its orientation as compared to a sphere of equivalent volume (Coombs et al., 2004), even if this effect was estimated small for anchovy eggs (Boyra et al., 2003; Petitgas et al., 2006). Sardine (*Sardina pilchardus*) eggs are large with an unusually large perivitelline space, occupying about 80% of the total egg volume whereas it is generally lower than 10% for most other species, among them anchovy. Experiments with the density gradient column (Coombs, 1981) suggest that this could have some effects on observed sinking velocity (Coombs et al., 2004) and more embarrassing on measured specific gravity (Coombs et al., 1985). The chorion, the outer membrane separating the perivitelline space from the surroundings, is described as being permeable to salt and water (Davenport et al., 1981). Thus, egg specific gravity is only determined by the ovoplasm and the degree of hydrolysation of yolk prior to ovulation (Craik and Harvey, 1987), as well as by the amount of material invested in the chorion (Jung et al., 2014). As the ovoplasm regulates its osmolarity (Davenport et al., 1981), the measured egg specific gravity should not be affected by the exchange through the chorion. This is true provided that a sufficient duration is accorded to the experiment for an equilibrium between the perivitelline space and the surrounding water to be reached, at the

115 equivalent depth of the ovoplasm plus chorion specific gravity.

116 Considering the importance of egg properties -size and specific gravity- on their *in-*
117 *situ* fate, and that those properties are under the influence of seawater physical properties,
118 the primary objective of the present study is to provide description and parameterisation of
119 those relationships for anchovy and sardine in the Bay of Biscay. We used a large dataset of
120 observed egg size and specific gravity in the Bay of Biscay, with an update for anchovy and
121 a fully new dataset for sardine. The density gradient column of Coombs (1981) was used
122 for both specific gravity and sinking velocity experiments. As accurate measurement of
123 sardine egg specific gravity seems to remain challenging, the sinking experiments were
124 performed in order to better quantify the effect of egg permeability, which constitutes a
125 secondary objective of the paper. Besides providing useful parameterisation to models of
126 egg dispersal, the experiments and analysis we conducted restore consistency in the
127 ambiguous literature on quality of specific gravity measurements of sardine eggs, as well as
128 in the non explained higher correlation found in Goarant et al. (2007) between anchovy
129 eggs and sea surface salinity, rather than with sea surface density.

MATERIAL AND METHODS

Egg sampling

Eggs were sampled during the PELGAS small pelagic survey in the Bay of Biscay in May, onboard the R/V Thalassa, from 2005 to 2014 for anchovy (number of stations = 62) and for 2009 to 2015 for sardine (n=27). Sampling stations are shown on Fig.1. They are located from the south of the bay mostly for anchovy, to the bay of Douarnenez at the tip of Brittany (48°N – 4.5°W) for sardine, the locations being representative of the main areas of distribution of the eggs of both species. They also cover a large variability of environmental conditions, with a north to south temperature gradient in spring and a coast to offshore salinity gradient in relation to the Gironde and Loire river plumes. Eggs were sampled following the same methodology as Goarant et al. (2007), using the 'Carré Net' (Bourriau, 1991) hauled at a maximum speed of 2 knots in the mixed layer for a maximum duration of 10 min. Hauling allowed a sufficient number of eggs to be collected at stations for latter size measurement, specific gravity as well as sinking experiments. The counterpart is certainly a higher number of dead or moribund eggs in the sample. Precautions were taken to avoid any bias from including damaged eggs in the experiments : first by gently taking the sample from the collector to the container, second the sample was gently stirred to ensure selecting only the most buoyant eggs, and third by not considering the remaining moribund eggs when in the density gradient column, where they appeared opaque and sank quickly to the bottom before any height reading were performed.

At each sampling station a CTD (SBE19+v2, Seabird) cast was performed providing water temperature, salinity and then density profiles. For correlation with egg specific gravity, the sea water properties were averaged between 3 and 5m to obtain

representative values of the surface mixed-layer, where the majority of the eggs are expected to be found (Boyra et al., 2003; Coombs et al., 2004). 3m is the minimum depth for which CTD values are available at all stations. Shallower, swell often hinder the correct quality of data acquisition.

Egg size measurements

The eggs were measured onboard with a binocular 50x magnification, offering an accuracy of 0.1 mm. Anchovy egg is a prolate spheroid then the two dimensions were measured. For sardine, both egg diameter and vitellus diameter were measured even if the latter was performed only in the case early developmental stages of eggs were available, i.e. stages 1 to 3 of the classification proposed by Ahlstrom (1943), thus until beginning of gastrulation. 20 eggs were measured per stations.

Egg specific gravity measurements

We used the density gradient column of Coombs (1981) onboard the research vessel and followed the calibration and reading procedures detailed in Goarant et al. (2007). Reported accuracy in specific gravity is 0.04 σ_T (Coombs, 1981). 50 eggs were released per experiment in the column to derive specific gravity average and standard deviation. Early developmental stages of eggs were chosen, i.e. until beginning of gastrulation or stage 1 to 3 following the classification of Moser and Ahlstrom (1985) for anchovy and Ahlstrom (1943) for sardine. In any case late stages (from stage VII) were never selected since those have their specific gravity significantly increased before hatching (Coombs et al., 1985; Ospina-Álvarez et al., 2012). During the first years until 2008, temperature in the density column gradient was thermostatically controlled at 15°C. However, since temperature has

178 an effect on egg specific gravity and egg thermal expansion is not easily measured (Sundby
179 and Kristiansen, 2015, but see Coombs et al., 1985), from 2009 on, the density column
180 temperature was set at the temperature of the sea surface at station location, thus limiting
181 the effect of variation in egg temperature on the experiment results. To correct for this
182 effect in the earliest years of our sampling from 2005 to 2008, we calculated the equivalent
183 density at sea surface considering the temperature of the gradient column experiment. A
184 minimum of 4 specific gravity readings were performed in the column for the 50 eggs from
185 each station, with an interval of 1 h between readings. In the case of sardine we waited for a
186 minimum of 1 h before the first reading, as egg sinking velocity, and thus reaching
187 equilibrium, can be slow (see fig. 4 in Coombs, 1981) as compared to anchovy, due to some
188 exchange between the perivitelline space and the low density water at the top of the
189 column.
190
191 *Egg sinking velocity*
192 The density gradient column was also used to measure sinking velocities of the eggs. For
193 those experiments conducted during PELGAS surveys 2012 to 2015, columns of
194 homogeneous density were prepared with well mixed salted water. Coombs et al. (1990)
195 proposed to use sea water to measure egg buoyancy through ascent velocity in an
196 homogeneous column. The objective here is to better assess the sinking (or ascent) velocity
197 model and assess the permeability of sardine eggs. The column was then filled with a salted
198 solution of density below or close to 22 σ_T, a value *a priori* significantly lower than the
199 surface water densities in our area in which eggs are expected to be approximately neutrally
200 buoyant. The difference in specific gravity between eggs and homogeneous column can not
201 be constant since the columns are prepared before sampling, when the seawater density at

202 station is not known. This difference ranged between 1.6 and 3.6 σ_T, and the eggs were
203 travelling down the column in a reasonably small amount of time, although not too fast to
204 allow measurement. Eventually the absolute difference does not have any impact on the
205 experiment itself, provided that the column density is correctly measured, which was done
206 with a densimeter (Richter & Wiese) with 0.1 σ_T accuracy. An homogeneous column was
207 preferred to a density gradient column as used in Coombs et al. (2004) because of the
208 longer distance and time traveled by the eggs in the case of homogeneity, allowing more
209 velocity measurements per egg leading eventually to higher accuracy. For each of these
210 experiments, 6 for anchovy and 4 for sardine, 10 early stage eggs were introduced at the top
211 of the density column after size measurement, and time recorded when egg passed each
212 graduated centimeter. In parallel a classical density gradient column was performed to
213 assess the average specific gravity and its variability at the corresponding sampling station.
214 To assess the observed sinking velocities, we used an analytical model of terminal
215 velocity. Sundby (1983) first proposed the combination of Stokes's equation, with
216 Dalavalle's one (Dalavalle, 1948) when Reynold's number is higher than 0.5. We used the
217 equation of Denny (1993) who proposed a unique function allowing for continuous
218 transition and valid for Reynold's number from low to values as high as Re = 100 000. It
219 also avoids the use of a calibration constant necessary in Dalavalle's parameterisation.
220 Denny's equation is given as:
221

$$0 = 0.2 v_t^2 + \frac{6 \nu v_t}{r} + \frac{3 v_t^2}{1 + \sqrt{2 r v_t / \nu}} - \frac{4 \rho_e r g}{3 \rho_f} \quad (1)$$

222
223
224 with v_t the terminal velocity, ν the kinematic viscosity of the water, r the radius of the

sphere, g the gravity acceleration, ρ_f the density of the water, and ρ_e the egg specific gravity. This equation is solved for any difference of specific gravity between egg and surrounding water. It is more time consuming than Sundby (1983) solution, although only for the computer, but more accurate especially for high Reynold's number which can be the case in our experimental setup.

The kinematic viscosity $\nu = \mu/\rho_f$, with μ the dynamic viscosity of water, is calculated from the equation proposed in Adlandsvik (1998):

$$\mu = 10^{-3} * (1.7915 - 0.0538*T + 0.0007*T^2 + 0.0023*S) , \qquad (2)$$

with T temperature and S salinity of water, which approximates the typically used table chart with a relative error of only 1.7% (Adlandsvik, 1998).

The sinking velocity of a prolate spheroid is affected by the ratio of its axis as well as its orientation (see Hutchinson (1967). The ratio of the major to minor axis measured in our study for an anchovy egg is in the range 1.5-2.73 (mean=2.16, see Results section). Denny (1993, p.64) gives a formulation for the correction of forces acting on an ellipsoid instead of a sphere, but only for major axis >> minor axis. Hutchinson (1967, p262) provides a more generic correction for various ratio and orientation when sinking. The resulting correction ranges between 1/0.955 and 1/1.2 comparatively to the equivalent sphere, thus slightly higher velocities when in the direction of the major axis and potentially lower velocities when in other orientation. These considerations were assessed for sinking experiments of anchovy eggs.

The egg specific gravity can be expressed from the specific gravity (ρ) and volume

(V) of its respective parts (embryo + yolk, chorion -ch-, and perivitelline space -pvs-) following Jung et al. (2014) :

$$\rho_e = \left(\rho_{emb+yolk} \times V_{emb+yolk} + \rho_{ch} \times V_{ch} + \rho_{pvs} \times V_{pvs}\right) / V_e \quad (3)$$

with Ve the total egg volume. After oocyte hydration, specific gravity of embryo and yolk is known to vary only little. After fertilisation, volume of perivitelline space increases to reach its final volume, but free exchange of salt and water by diffusion are still possible through the chorion between this compartment of the egg and the external medium. Gathering variable and invariable components of the eggs, depending on their potential change of specific gravity and volume with time, gives :

$$\rho_e = \left(\rho_{emb+yolk+ch} \times \left(V_{emb+yolk} + V_{ch}\right) + \rho_{pvs} \times V_{pvs}\right) / V_e \quad (4)$$

The chorion volume could not be measured on living eggs onboard, but is assumed to be small for teleosts (1-5% of total egg volume, Sundby and Kristiansen, 2015) and constant throughout development (Jung et al., 2014). It was, thus, neglected in the equation. The chorion mass, however, as it is the heaviest component of the egg (Sundby and Kristiansen, 2015), is included in the invariable component of the egg $\rho_{emb+yolk+ch}$.

For eggs of most species, perivitelline space is within the range 5-10% of the total egg volume, and the effect of diffusion with the external medium is limited on the egg specific gravity. But for sardine, this volume is close to 80% (see result section), and then variation of ρ_{pvs} from exchange of salt and water through the chorion can have a large effect

on the egg specific gravity. To take this effect into account, we implemented a diffusion equation in the sinking velocity model for sardine, to adjust ρ_{pvs} to the density of the egg surrounding water. Based on Fick's diffusion equation of a solute through a membrane, Coombs et al. (2004) proposed the following equation for the change in specific gravity of the perivitelline space :

$$\frac{\partial \rho_{pvs}}{\partial t} = P_e \times (\rho_f - \rho_{pvs}) \quad with\ P_e = \frac{S_{ch}}{V_{pvs}} \times P_m \qquad (5)$$

with P_e the egg permeability, P_m the chorion permeability, S_{ch} the surface of exchange of the chorion and ρ_f the density of the fluid surrounding the egg.

The model sinking velocities, or egg positions in the column through time, were compared to observed egg positions in the homogeneous columns. For anchovy only Eq.1 was necessary, with ρ_e the egg specific gravity available from measurements in the associated gradient column. For sardine, the combination of Eq.1, 4 and 5 was necessary to consider the adjustment of egg specific gravity to surrounding water through time while sinking. In equation 5, P_m is unknown. Another uncertainty is the effective specific gravity of the egg when *in-situ*, or at initial time when set in an experimental column, since it is mentioned that the measured specific gravity using the gradient column may be underestimated for sardine (Coombs et al., 2004), again due to the adaptation of the perivitelline space specific gravity during the experiment. At this initial time t_0, Equation 4 can be decomposed as :

$$\rho_e(t_0) = \left(\left(\rho_e^m + \delta_{eyc}\right) \times V_{emb+yolk} + \left(\rho_e^m + \delta_{eyc} + \delta_{pvs}\right) \times V_{pvs}\right) / V_e \quad (6)$$

with ρ_e^m the measured average egg specific gravity at the station, δeyc the residual specific gravity of the egg from the station average, and δpvs the difference between actual egg specific gravity (ρ_e^m + δeyc) and specific gravity of perivitelline space at t_0.

For each egg of the 4 experiments of sinking rate performed on sardine, an estimation of the three parameters P_m, δpvs and δeyc was performed, by minimisation of the difference in egg position through time between model results and data. The R routine optim with the bounded Quasi-Newton method (Byrd et al., 1995) was used for optimisation. We then analysed the distribution of the values of estimated parameters for the whole set of eggs.

RESULTS

Egg size

Average egg size and associated variability, both on population (all measured eggs) and sample (station) scale, are given in table 1. For sardine, one interesting feature is the large size of the perivitelline space, calculated here on average to be 78.6% (+/-6.2%) of the total egg volume, with unmeasured chorion volume assumed to be part of the perivitelline space.

In an attempt to correlate egg size with environmental covariates, we could not find any significant relationship for anchovy (Fig.2). For sardine, a significant correlation ($p<0.05$) was found between both egg and vitellus sizes and sea surface temperature (Fig.3) while there was no relation between the calculated perivitelline space (total volume minus vitellus volume) and temperature. The warmer the water, the smaller the eggs and vitellus, with an estimated percentage of decrease of 7-8% between 12 and 17°C. We mention here that vitellus size was positively correlated ($p<0.01$, $n=12$) to egg size. Other tested covariates, the sea surface salinity and the egg specific gravity itself, were not significant factors of the variation in egg size (Table 2).

Sinking velocity

Sinking velocity experiments performed on anchovy revealed relatively constant sinking rate through the homogeneous density columns looking at their position over time (Fig. 4). Despite some variability at the individual level, with some eggs slowing down (e.g. the fastest eggs at station R0295) or accelerating (e.g. the slowest eggs at station Q0559), this was only temporary and no general trend of changing speed with time could be noticed over the whole set of experiments. The large majority of eggs were sinking in the direction

of their major axis. This is because the fluid in the perivitteline space is always heavier than that of embryo and yolk and hence give the eggs a balanced orientation. Dead or dying embryos, however, stop osmoregulation and get as heavy as the perivitelline space. Hence they lose the balanced orientation. Indeed, when sinking was not in the direction of the major axis, rate was generally abnormally high. These eggs were considered moribund, and removed from the experiments.

The model of sinking velocity with equivalent diameter is appropriate, with most egg trajectories close to the average simulated one. The 95% confident interval, provided from the measured variability in specific gravity from an associated gradient column, together with extreme values of observed egg sizes, embraces the majority of the eggs. Maximum 1 over 10 eggs in only half of the experiments are out of this confident interval. Assessing potential deviation from the spherical equivalent model only slightly modified simulated velocities. Considering this effect could partly explain the temporary low velocities of the sinking eggs of stations Q0553 and Q0559 as they are better included in the new confident interval.

For sardine experiments, a rapid decline in sinking velocities was noticed for all the eggs (Fig.5) with apparent convergence to a very low velocity. As compared to the predicted velocities of the model, with observed egg densities from an associated gradient column and egg size measurements, initial observed velocities were 2 to 10 times higher. Final measured velocities were on the opposite lower than the predicted average values, and even lower than the minimum predicted value when this minimum was positive (see experiment at station S0465).

To better assess these inconsistencies between observation and model for sardine eggs, the diffusion model introduced in the Material and Method section was used and

optimised on 3 parameters for each single egg of the four experiments. The results are shown on Fig. 6 and reveal that for each egg a solution was found. Surprisingly an egg was even ascending after a first period of sinking in the case of experiment at station R0324. Even in this extreme case an optimised parameter solution was found. The proposed solution is explained by an abnormally far lower specific gravity of the time-invariant part of the egg (embryo + yolk + chorion) as compared to the measured mean specific gravity at this station (-4 σ_T), and a permeability coefficient of the chorion not high enough to allow a rapid equilibrium of the perivitelline space with respect to the initial sinking velocity of the egg. This particular egg is however removed from the later statistical analysis on the parameters since it can be considered as an outlier.

The resulting distributions of the optimised parameters presented in Fig.7 reveal a Gaussian distribution for both P_m and δeyc, while δpvs looks log-normal with values ranging between 0 and 15. The normality of the distribution for the 2 first parameters was tested and validated using the Shapiro–Wilk test ($\alpha = 0.05$). The estimated average permeability coefficient of the chorion is P_m=0.0038 mm.s^{-1} (+/- 0.00086 mm.s^{-1}). The average deviation of egg (or embryo+yok+chorion) specific gravity to the average egg specific gravity measured at station is δeyc=0.371 σ_T (+/-0.873 σ_T).

In analogy with Fig.4 for anchovy, Fig.8 displays the average model (velocity and diffusion) estimation as well as confident interval for a single sardine experiment, revealing a good prediction of the model based on the whole population averaged permeability coefficient of the chorion, and specific gravity and size parameters specific to the station.

Egg specific gravity

For anchovy, the average egg specific gravity at station ranges between 20.85 σ_T at a

coastal plume station and 25.94 σ_T offshore. The average standard deviation locally, i.e. at stations, is equal to 0.714 (n=62). As in Goarant et al. (2007) the difference between stations tested with an ANOVA was significant. We then tested the effect of the local environment on the egg specific gravity, with surface salinity (average between 3 and 5m depth), surface density, and equivalent surface density with temperature adjusted to the gradient column temperature (Table 3). Best fit was obtained for both salinity and equivalent density, with in each case an explained variance of 70.4%, whereas for surface density only 55% is explained with higher residuals and lower F statistics. The fitted regression is shown on Fig.9 for the equivalent water density, with a slope close to 1, revealing an average negative difference in specific gravity between eggs and surface water of 0.92 σ_T. At only one station the eggs were on average negatively buoyant in surface waters.

For sardine, the average egg specific gravity at station ranges between 22.97 σ_T at a coastal plume station and 25.78 offshore (Fig.10). The average standard deviation locally, i.e. at station, is equal to 0.832 (n=27). The difference between stations tested with an ANOVA was significant. We then tested the effect of the local environment on the egg specific gravity, with surface salinity (at 3m depth), surface water density, and equivalent surface density with temperature adjusted to the gradient column temperature (Table 3). We also tested the effect of surface water density on the specific gravity of the eggs after correcting the latter by the thermal expansion coefficient proposed by Coombs et al. (1985). Best fit was obtained when using this thermal expansion coefficient (see also Fig.11), with an explained variance of 60.3%. As for anchovy, the worst fitted regression was for surface density, but when it was adjusted for temperature of the gradient column, then the fit was similar and even slightly better than for surface salinity. Fig.11 reveals a slope significantly

lower than 1, but again and similarly as for anchovy, on the range of observed values, the negative difference in specific gravity was estimated on average equal to 1.06 σ_T.

DISCUSSION

Our study quantifies relationship between both size and specific gravity of fish eggs and their environment, for anchovy and sardine. This is the first time for size in the Bay of Biscay, as well as for specific gravity for sardine. In addition several sinking experiments were conducted allowing robust estimation of the permeability of sardine eggs, while confirming that it can be neglected for anchovy eggs. Despite being taxonomically close, these two species show different characteristics in terms of size, shape and perivitelline space, providing a representative diversity of results that can be useful also for other species. While relationship of size with environment may be highly season and region dependent, that of specific gravity with sea surface density can likely be generalised to other pelagic species spawning in surface layer of stratified regions, as the result of an evolutionary adaptation.

Egg size

The size of anchovy and sardine eggs for the Bay of Biscay in spring are all within the observed range of variability given in Munk and Nielsen (2005). We also provided measurements of vitellus size for sardine and then calculated perivitelline space from the volume difference, neglecting the chorion volume. The chorion volume could not be measured on living eggs onboard, but is assumed to be small for teleosts (1-5% of total egg volume, Sundby and Kristiansen, 2015). These values are lower than 1 standard deviation of the perivitelline space (6.2%). But most important for our experiment and analysis is the fact that the chorion volume does not vary through time (Jung et al., 2014). 'At station' variability taken from 20 eggs is logically lower than variability from the whole set of

measured individuals, and we would recommend using the population estimated variability when generating variability for vertical distribution model purpose, especially when no external factors of variability applies.

This does not appear to be the case for sardine, with both its total egg and vitellus diameter decreasing with increasing surface temperature where they were sampled. Neither surface salinity ranging between 24 and 27 σ_T, nor egg specific gravity itself had any effect on the egg size. Looking for potential experimental artifact, thermal expansion of eggs could be thought of, but the results would be the opposite, with increasing temperature increasing the egg volume. Also eggs were always maintained in the sampled surface water until size measurements, preventing volume change from temperature change during experiments. Temperature can accelerate egg development, thus there could be a bias with the vitellus size decreased to the benefit of the embryo. However this should not have any effect on the egg size itself as is observed, and we also made sure to use only early stages of eggs in which embryo development is negligible.

In the absence of artifacts, and without any strong reason for direct *in-situ* temperature effect after spawning (Chambers, 1997), effects during oogenesis or indirectly through maternal influence were preferred as hypothesis. Sampling occurs every year in May, but surface temperature varies a lot at that time of the year (from 12 to 17°C at sardine sampling stations), and could either have a biological effect on egg quality, or be a proxy for more or less advanced spawning season. Decrease of the egg size along the spawning season in spring was already described at the population level for sardine in the English Channel (Southward and Demir, 1974) as well as for other clupeoids (Blaxter and Hunter, 1982) This effect was also shown at the individual maternal level for gadoids with egg size decreasing with the number of batches (Chambers and Waiwood, 1996; Kjesbu,

1989; Marteinsdottir and Steinarsson, 1998; Trippel and Neil, 2004; Vallin and Nissling, 2000). In the Bay of Biscay sardine spawning in spring occurs from February to June (Stratoudakis et al., 2007) with peak in March-April. May, especially on warm years, would then already be representative of late spawning. Late spawning season generally shows more marked decrease in egg size (Blaxter and Hunter, 1982). This could also explain the lack of observed effect on anchovy, for which May is the peak spawning season (Motos et al., 1996). Riveiro et al. (2004) also noticed a potential effect of temperature on sardine egg size from winter to summer in Galicia. They related this pattern to a correlated decrease of absolute organic content of the eggs, with the following potential ecological significance : bigger eggs with higher reserve content grow faster, which would compensate for slower development in the colder month, eventually enhancing early life stages survival. Unfortunately our type of data does not help for further conclusion on whether temperature has a direct effect during oocyte maturation whatever size, age or condition of the female, or is just a proxy of advancement of the spawning season under an effect of size and/or age structure of the spawning population. Maternal condition hypothesis is unlikely since sardine has its energy density increasing from late winter to summer in the Bay of Biscay (unpublished data). Further sampling of eggs together with biometry analysis of adults in reproduction would help disentangling the direct (oogenesis development time) and maternal effects of temperature on egg size.

Egg specific gravity is in part controlled by input of water to the oocyte during the hydration process, under the influence of maternal body fluid (Craik and Harvey, 1987) but ultimately under the influence of external medium salinity as observed between (Solemdal, 1967; Thorsen et al., 1996) or within regions (Goarant et al., 2007). Thus one would expect some variation of egg volume related to either water salinity or egg specific gravity, as a

consequence of higher hydration in low saline areas. This was indeed observed for flounder (Solemdal, 1967) herring (Alderdice et al., 1979) or cod (Kjesbu et al., 1992; Nissling et al., 1994) even if the significance on size was rather low as compared to other impacts on yolk osmolarity or chorion thickness. The relatively low observed variability of water salinity in our study may explain the lack of observed effect on egg size, i.e. change in osmolarity of yolk would imply no or undetectable change of the egg volume for our two species of interest. Comparison with other more contrasted areas such as the Mediterranean sea would be interesting.

Sinking velocity

Egg size measurements were used as input for the experiments on sinking velocities. The equation of Denny (1993) for anchovy eggs considering a sphere of equivalent volume provides good fit to the observations. The use of a shape correction together with consideration of a variable orientation of the eggs when sinking does not change much the prediction, but widens the possible range of sinking velocities slightly with few more eggs in the confident interval of the model. During the experiment most eggs were falling in the direction of their major axis, a sign of good quality (see result section). Despite removal of most suspicious eggs, there was slight deviations from perfect orientation along the sinking trajectory, which could partly explain the non fully linear sinking rate of certain eggs. Coombs et al. (2004) estimated that correcting for the shape gave a higher velocity but found a lower fit to data than when using the equivalent spherical shape. Boyra et al. (2003) estimated the effect of a shape correction on a vertical distribution model and could not find any significant effect. One possible explanation proposed is that in the field orientation is variable under mixing effect and thus individual effect of a particular orientation can not be

evidenced. Our study also confirms that in a statistical sense the consideration of shape does not significantly improve the prediction and that spherical equivalent volume is a justified approximation. For both species, after permeability correction for sardine (see below), our model of sinking velocity is able to predict both mean and variability of the sinking velocity of the eggs from a given sample.

Egg permeability

The experiments on anchovy sinking velocity did not call for any additional diffusion component between egg and external medium. This was not the case for sardine for which experiments showed a strong decrease of velocity for every single egg. To explain this and considering the properties of the different part of an egg (Craik and Harvey, 1987; Davenport et al., 1981; Jung et al., 2014), the logical hypothesis relies on perivitelline space adjusment in specific gravity to the external medium. Our model of sinking velocity and diffusion through the chorion fits well to the data, at the individual (Fig.6) or sample (Fig.8) scale and then validate this hypothesis.

Another confirmation is the normal distribution of the optimised chorion permeability coefficients. We found a mean value of 0.0038 $mm.s^{-1}$ to be compared to 0.002 $mm.s^{-1}$ found by Coombs et al. (2004) in a similar exercise but on a single experiment with few sardine eggs. Their value lies within our 95% confident interval and seems in agreement with our study. The values of optimised parameter δeyc, which refers to a degree of freedom to take into account 'at station' variability in observed egg specific gravity, also display a normal distribution. Its standard deviation 0.873 σ_T is really close to the standard deviation 0.832 σ_T of 'at station' egg specific gravity. The average of 0.371 σ_T reveals a bias although small as compared to the expected null deviation on average. Indeed, under the

hypothesis that embryo+yolk+chorion do not change in specific gravity in different medium and determine whole egg specific gravity at the equilibrium, average egg specific gravity should be equal in both sinking and density gradient experiments, and thus on average the residuals δeyc should equal zero. This parameter is well constrained at the end of the experiment when perivitelline space has come to an equilibrium and only specific gravity of embryo+yolk+chorion determines sinking velocity. It is suspected that in our experiments equilibrium is not always exactly reached when the egg reaches the bottom of the homogeneous column, which could explain this small positive bias. The optimised values of the third parameter δpvs were surprising. While values close and centered around 0 were expected, a somehow uniform distribution with values ranging between 1 σ_T and almost 15 σ_T resulted from optimisation. As perivitelline space is about 80% of the total egg volume, it is excluded that such high values are representative of *in-situ* situation. It thus means that specific gravity of the perivitelline space has increased significantly between sampling and release in the column. As eggs were maintained in surface sampled water for the whole experimental duration before release in the column, we suspect here loss of water when in contact with air either while floating in the container or when size measured in a water drop. However, this feature has only the effect of increasing the velocity at the beginning of sinking experiment and does not invalidate the diffusion model we used, with the assumption of no exchange with the ovoplasm and strong permeability of the chorion.

Figure 11 shows the estimated time for eggs to reach an equilibrium in a typical density gradient column with a typical egg specific gravity found in our area. This reveals that accounting for permeability of the chorion for anchovy is negligible given the small volume of the perivitelline space (~5 %). It also shows that convergence to an equilibrium for sardine can take more than twice the time than for anchovy, and that a minimum of 50-

552 60 mn is a reasonable duration to wait before the first height reading for sardine, as
553 compared to the 20 min duration for anchovy.
554
555 *Influence of surface water on egg specific gravity*
556 Our study updates the correlation found by Goarant et al. (2007) between anchovy eggs and
557 surface water, and provides the statistics of a similar regression for sardine. Egg specific
558 gravity is determined in the ovary prior to the ovulation under the combined result of
559 oocyte hydration and investment in heavy chorion material (Jung et al., 2014). Oocyte
560 hydration involves yolk proteolysis resulting in increasing oocyte osmolarity (Craik and
561 Harvey, 1987) and water passage through the vitelline membrane using molecular water
562 channels (Fabra et al., 2005).
563 Goarant et al. (2007) found better correlation with salinity than density. We evidenced that
564 this was only because of difference in temperature between sampled water and density
565 gradient column. By correcting for this, i.e. calculating the equivalent surface density at the
566 column temperature, we logically found equivalent statistics for both salinity and density in
567 our dataset. The explained variance (70%) is of the same order as the one found by Goarant
568 et al. (2007) with a smaller set of data. For sardine, the results are similar, with equivalent
569 density having slightly better statistics than salinity. Using the thermal expansion
570 coefficient proposed by Coombs et al. (1985) for sardine eggs, statistics get even better,
571 with 60% of the variability explained. From mechanisms behind thermal expansion based
572 on egg constituents, Sundby and Kristiansen (2015) estimated that this effect can in most
573 cases be neglected for egg buoyancy determination. However, given the accuracy of the
574 measurements expected from the density gradient column, having a similar measured
575 thermal expansion coefficient for anchovy eggs would also slightly improve the statistics

for this species. In any case, having or not the specific thermal expansion, our recommendation is to set the temperature of the gradient column to the one of the surface water, in order to maximise the accuracy when assessing relationship with the environment.

Our regression confirms that pelagic eggs found in mixed surface layers are positively buoyant, with their vertical distribution at the equilibrium in the field complementary controlled by mixing. In the range of the sampled surface density, the estimated difference in specific gravity between egg and water for anchovy is almost constant, around -0.92 σ_T. This difference is almost similar for sardine (-1.06 σ_T), but with a slight increasing trend with water density increasing. The regression residuals may have several origins. First, egg specific gravity is known to vary with development for both species (Coombs et al., 1985; Ospina-Álvarez et al., 2012). For a given stage the correlation should not change, but we can not make sure all eggs used in our experiments have the exact same stage. Second, vertical structure of the water column may be really diverse, especially in spring in the vicinity of a river plume like the Gironde. Spawning depth as well as sampling depth is obviously sensitive or in interaction with this structure, and the regression to the 3-5m layer may be affected by this. Third, we can not exclude some experimental effect in the residuals. Even if the impact of perivitelline space was shown limited if caution is taken in the reading procedure of the gradient column, its buffering effect adds some complex processes in the experiment, as well as in the potential equilibrium of the vertical distribution reached *in-situ*.

Interest for modeling application of egg vertical distribution

Size is not the most sensitive parameter in vertical distribution model of eggs, but our extensive set of measurements provides useful information on size variability in order to

eventually better fit to variability of the *in-situ* vertical distribution. Our study confirms that approximating the anchovy egg with the equivalent spherical volume is relevant, especially when assuming that *in-situ* orientation of eggs is likely to change under mixing effect. Consideration of the relationship of size with water temperature may also prove useful, for sardine at least in the Bay of Biscay, when simulations have to be made over the spawning season spanning a wide range of temperature. This temperature effect may also be informative for bioenergetics models, since relative biochemical composition is rather stable between eggs and thus the bigger it is the more energy it generally contains.

Egg specific gravity is the most sensitive factor determining position of eggs in the water column. Taking into account its relationship with local water density or salinity is also key, otherwise simulated eggs may be stuck at surface or rapidly fall at the bottom based on existing coastal gradients in the vicinity of plume areas. Salinity is only useful if the correction of temperature is applied, otherwise inconsistency between the specific gravities of the egg and local water may occur. Thus we recommend using directly the proposed regression with density, even more that it seems generic enough to be applied across regions (Ospina-Álvarez et al., 2012). Our study provides an update of it for anchovy, and a new parameterisation for sardine, together with associated 'at station' variability, again useful in reproducing correctly observed vertical distributions. These regressions are valid for first stages of egg and set the egg specific gravity at spawning, or initial condition in a model. Then, models of vertical distribution and drift need to consider relative change in the specific gravity through development (Ospina-Álvarez et al., 2012). Including permeability of the chorion in dynamic models of egg vertical distribution for species with large perivitelline space may also prove necessary to eventually improve accuracy of dispersal modelling results, but needs to be verified against *in-situ* field data.

ACKNOWLEDGEMENTS

The authors would like to thank J. Massé and M. Doray the chief scientists of the PELGAS surveys, as well as the crew of the R/V Thalassa for help in egg sampling. We also thank two anonymous reviewers for their constructive comments. This work was supported by successive research programs and among them the project SEAMAN of the ANR and EU FP7 ERA-NET SEASERA.

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632

633 **TABLES**

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636 **Table 1**. Statistics of egg size. Average sample ('at station') variability (standard deviation)

637 is given in brackets, with n_{st} the number of stations.

	Diameter (sardine) or equivalent diameter (anchovy)	Vitellus diameter	Diameter (major axis)	Diameter (minor axis)
Anchovy	0.855 +/- 0.050, n=1127 (+/- 0.036, n_{st}=48)		1.428 +/- 0.101, n=1130 (+/- 0.082, n_{st}=49)	0.662 +/- 0.049, n=1131 (+/- 0.036, n_{st}=48)
Sardine	1.608 +/- 0.113, n=587 (+/- 0.095, n_{st} =28)	0.964 +/- 0.084, n=270 (+/- 0.055, n_{st}=13)		

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Table 2. Statistics for different regression models between size of sardine eggs and environment covariates or egg specific gravity, based on 27 sampled stations. (*) indicates significativity of the model for $p<0.05$.

Covariate	Parameter	Estimate	p-value	F statistics	R^2
Sea surface salinity	α (slope)	-0.003	0.88	0.02	0.001
	β (intercept)	1.700			
Sea surface temperature	α	-0.018	0.0171*	6.53	0.207
	β	1.877			
Egg specific gravity	α	0.002	0.920	0.01	0.004
	β	1.565			

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647 **Table 3**. Statistics for different regression models between specific gravity of anchovy eggs

648 and environment covariates, based on 62 sampled stations. All models are significant for

649 $p<0.01$.

Covariate	Parameter	Estimate	F statistics	R^2
Salinity	α (slope)	0.85	142.9	0.704
	β (intercept)	-4.58		
Density	α	0.83	72.7	0.548
	β	3.55		
Equivalent density at gradient column temperature (Fig.9)	α	1.1	143	0.704
	β	-3.43		

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653 **Table 4**. Statistics for different regression models between specific gravity of sardine egg

654 and environment covariates with different corrections, based on 27 sampled stations. All

655 models are significant for $p<0.01$.

Covariate	Parameter	Estimate	F statistics	R^2
Salinity	α (slope)	0.584	27.99	0.528
	β (intercept)	4.339		
Density	α	0.538	14.55	0.368
	β	10.59		
Density with egg specific gravity corrected from thermal expansion (Fig.10)	α	0.748	38	0.603
	β	5.343		
Equivalent density at gradient column temperature	α	0.730	28.72	0.535
	β	5.751		

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659 **LIST OF FIGURES**

Fig.1. Location of stations with egg sampling in the Bay of Biscay. Stations with anchovy (a) and sardine (b) eggs used in the experiments, with corresponding sampling year. Isobaths 100, 200, 500, 1000 and 2000 are drawn, and upper left panel shows the general location of the Bay of Biscay in the Northeast Atlantic..

Fig.2. Diameter of anchovy eggs. Mean values over 20 eggs per station are plotted with associated standard deviation, for long axis (left) and min axis (right).

Fig.3. Diameter of sardine eggs (left) and vitellus (right). Mean values over 20 eggs per station are plotted with associated standard deviation.

Fig.4. Sinking experiments for anchovy eggs from six stations. Anchovy egg positions are plotted over time (dotted lines) in homogeneous density columns. Title of each panel is the name of the station, with values between brackets the density of the column and the mean egg specific gravity at station (σ_T), as measured in a gradient column (other set of 50 eggs from same sample). Continuous lines are for calculated velocities based on the mean egg specific gravity and mean equivalent egg diameter, dashed lines for mean specific gravity +/- 2 standard deviation, and max/min equivalent egg diameter, respectively. Grey dashed lines represent uncertainty associated to the variable inclination of the egg when sinking (after Hutchinson, 1967). Note the different scales of the axis between experiments, due to a smaller experiment duration for the 3 last.

Fig.5. Sinking experiments for sardine eggs from 4 stations. Sinking velocity profiles are

plotted (dotted lines) for 10 eggs in homogeneous columns. Name of station is given as panel title. Continuous lines are for calculated velocity based on mean egg specific gravity measured in an associated gradient column (other set of 50 eggs) with average egg diameter. Dashed lines are for mean specific gravity +/- 2 standard deviation, and max/min egg diameter, respectively.

Fig.6. Sinking model fitting to each single sardine egg from 4 stations. Sardine egg positions are plotted over time (empty circles) in homogeneous density columns. Title of each panel is the name of the station, with values between brackets the density of the column and the mean egg specific gravity at station (σ_T), as measured in a gradient column (other set of 50 eggs from same sample). Continuous lines are simulated velocities by the fitted model with parameters optimised for each egg trajectory (see text).

Fig.7. Probability density of optimised parameters of the model of sinking velocity for each sardine egg of the 4 experiments of Fig.6. (a) Coefficient of permeability of the chorion P_m, (b) difference in specific gravity just before release in the homogeneous column between perivitelline space and egg (δpvs) and (c) between embryo and average specific gravity of the eggs measured in the associated gradient column (δeyc) . Continuous lines are fitted gaussian distributions.

Fig.8. Sinking model result for experiment on sardine eggs from station S0465. Continuous lines are for calculated velocity based on mean egg specific gravity measured in the gradient column (other set of 50 eggs) and mean egg diameter, dashed lines for mean

specific gravity +/- 1 standard deviation, and max/min egg diameter, with the following optimised parameters : permeability of chorion (P_m averaged over all eggs of all experiments) and average difference in specific gravity at this station between the perivitelline space and the averaged measured specific gravity (at station δpvs) .

Fig.9. Relation between anchovy egg specific gravity and sea surface density. The mean egg specific gravity (σ_T) is plotted with associated 95% confident interval calculated locally at each station. The equivalent sea surface (3-5 m) density at the temperature of the density column experiment is given. The continuous line is the fitted linear regression (see Table 3), and the dotted one is the y=x line.

Fig.10. Relation between sardine egg specific gravity and sea surface density. The mean equivalent egg specific gravity (σ_T) is plotted with associated 95% confident interval calculated locally at each station. It is corrected from thermal expansion (from Coombs, 1985). The continuous line is the fitted linear regression (see Table 4), and the dotted one is the y=x line.

Fig.11. Simulation of time to neutral buoyancy in a gradient column for an anchovy and a sardine egg with same specific gravity. The minimum and maximum density of the column are 19 σ_T and 29 σ_T, respectively. The specific gravity of the egg is 24.5 σ_T. Dotted and dash-dotted curves are for an anchovy egg with and without permeability of the chorion (P_m=0.0038 mm.s^{-1}). Continuous and dashed curves are for sardine egg with average P_m and P_m +/- 2 s.d., respectively. Vertical lines indicate the timing when specific gravity of eggs comes closer to the known specific gravity than the given gradient column resolution (0.04

σ_T).

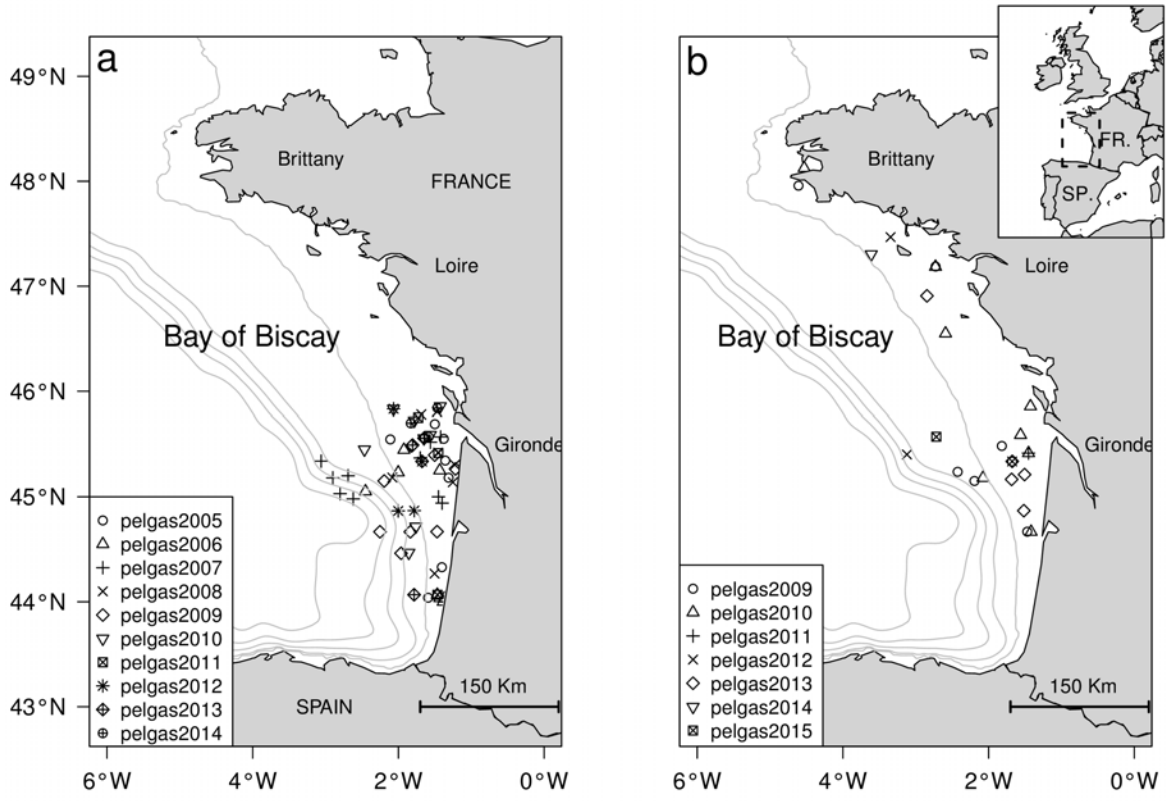

a
b
Brittany
FRANCE
Loire
Bay of Biscay
Gironde
SPAIN
150 Km
FR.
SP.
49°N
48°N
47°N
46°N
45°N
44°N
43°N
6°W
4°W
2°W
0°W
pelgas2005
pelgas2006
pelgas2007
pelgas2008
pelgas2009
pelgas2010
pelgas2011
pelgas2012
pelgas2013
pelgas2014
pelgas2015

Fig.1

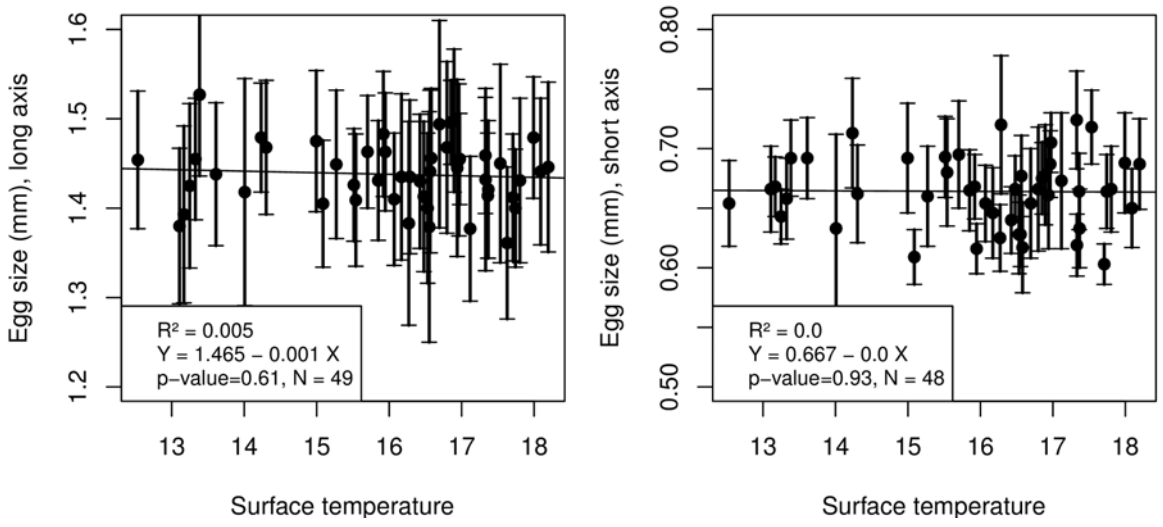

Egg size (mm), long axis
R² = 0.005
Y = 1.465 – 0.001 X
p–value=0.61, N = 49
Surface temperature
Egg size (mm), short axis
R² = 0.0
Y = 0.667 – 0.0 X
p–value=0.93, N = 48
Surface temperature

Fig.2

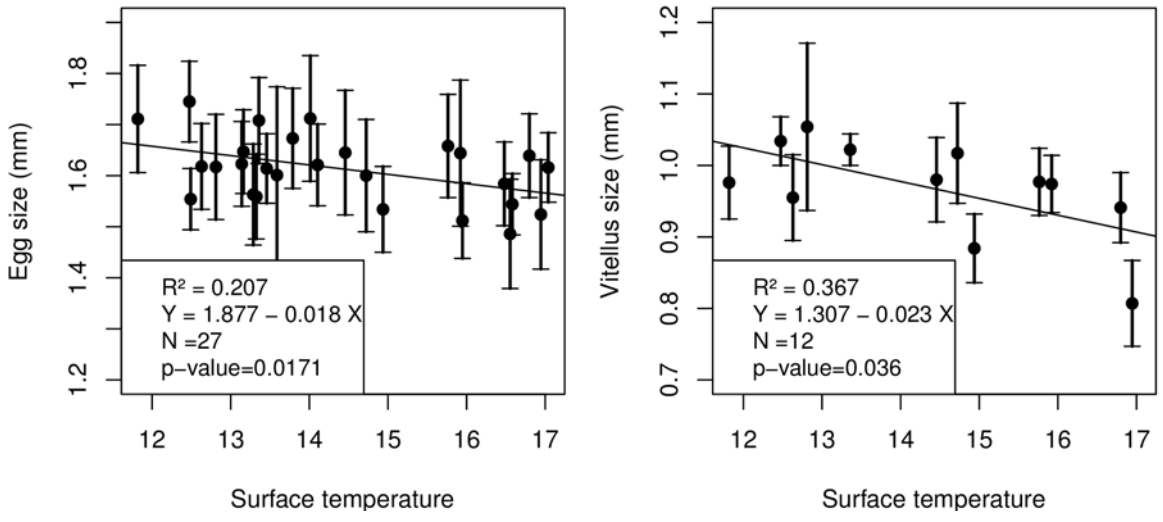

Egg size (mm)
R² = 0.207
Y = 1.877 – 0.018 X
N =27
p–value=0.0171
Surface temperature
Vitellus size (mm)
R² = 0.367
Y = 1.307 – 0.023 X
N =12
p–value=0.036
Surface temperature

Fig.3

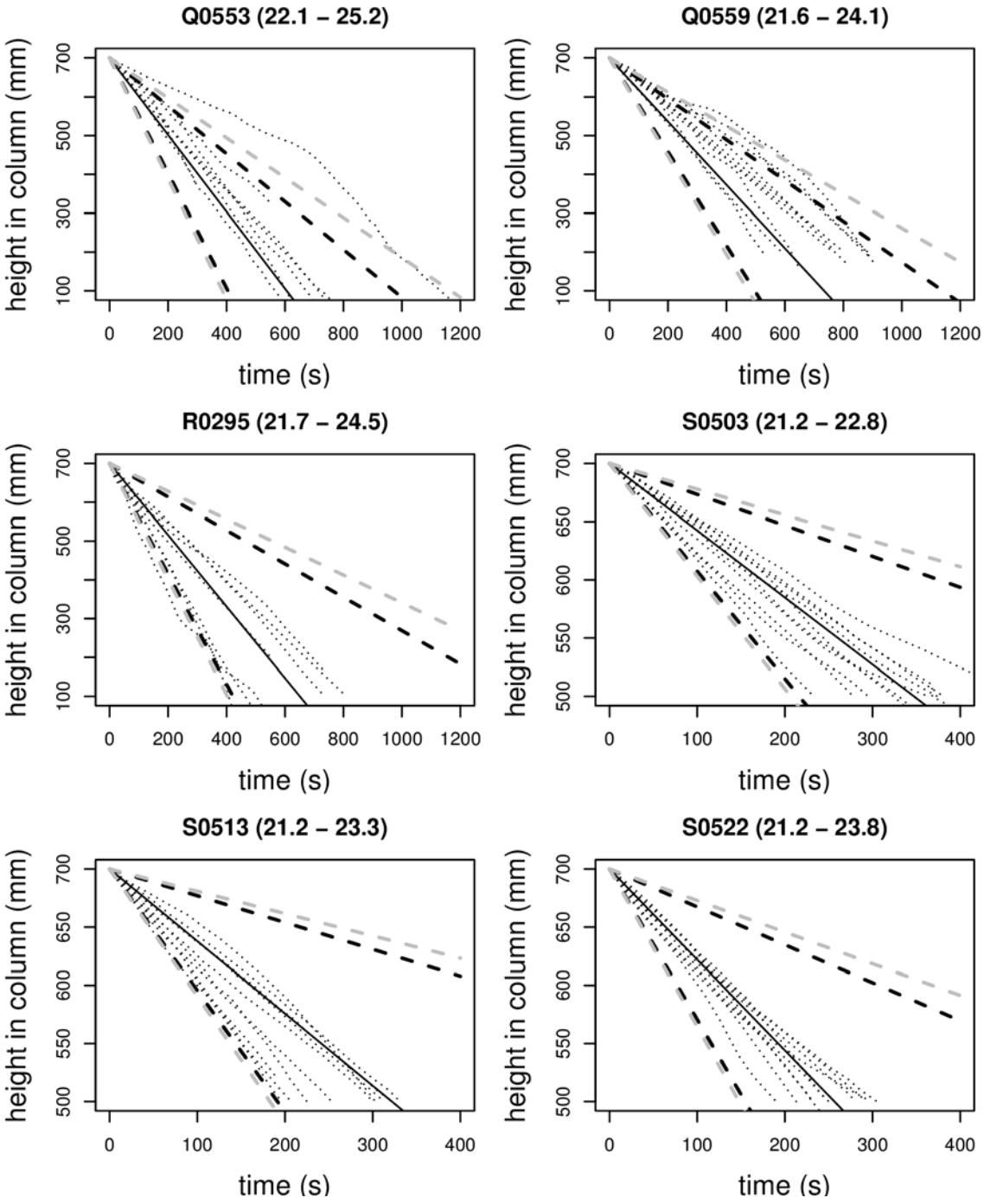

Q0553 (22.1 – 25.2)
Q0559 (21.6 – 24.1)
R0295 (21.7 – 24.5)
S0503 (21.2 – 22.8)
S0513 (21.2 – 23.3)
S0522 (21.2 – 23.8)
height in column (mm)
time (s)

Fig.4

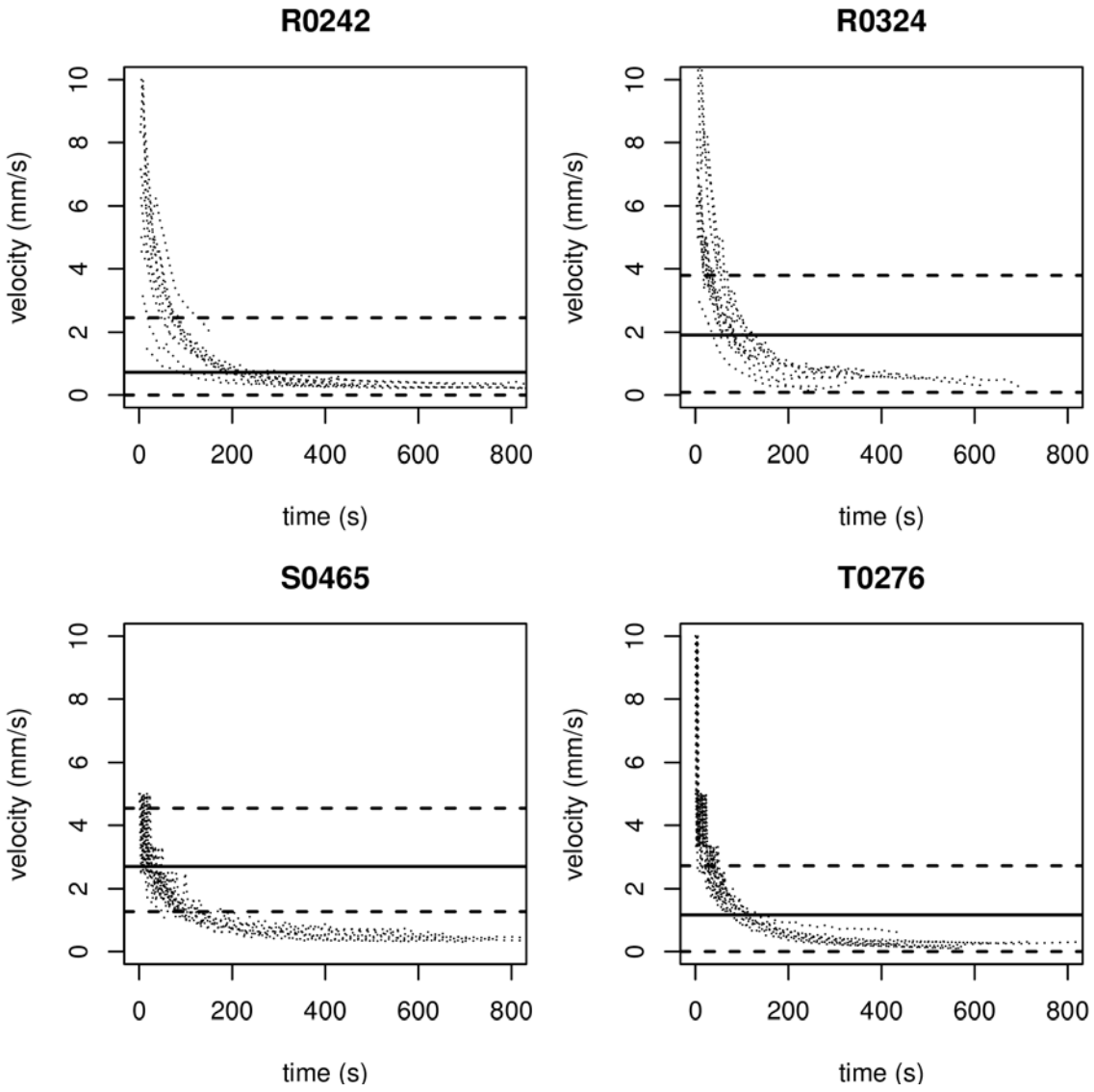

R0242
R0324
S0465
T0276
velocity (mm/s)
time (s)
0
2
4
6
8
10
0
200
400
600
800

Fig.5

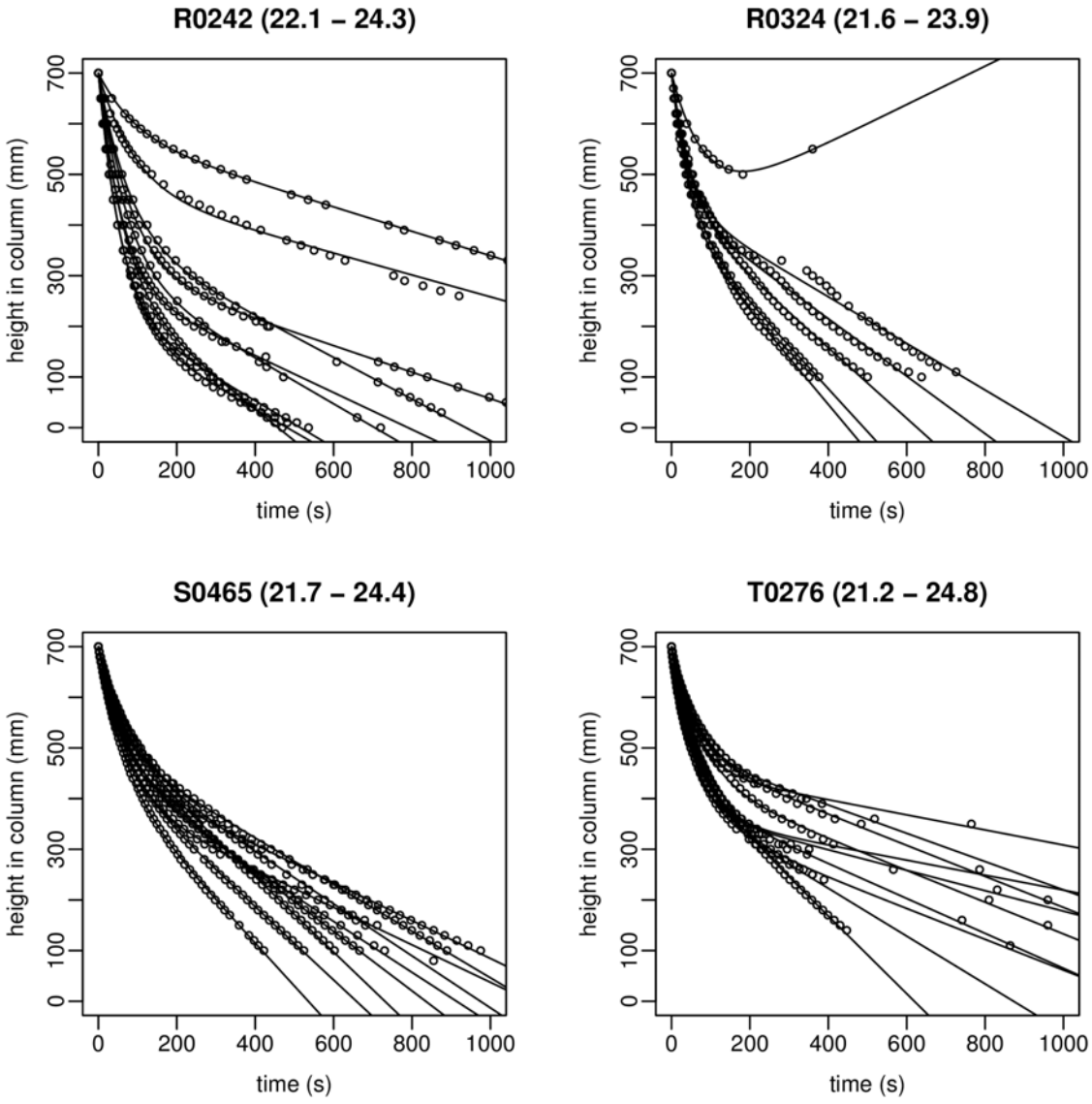

R0242 (22.1 – 24.3)
R0324 (21.6 – 23.9)
S0465 (21.7 – 24.4)
T0276 (21.2 – 24.8)
height in column (mm)
time (s)
0
100
300
500
700
0
200
400
600
800
1000

Fig.6

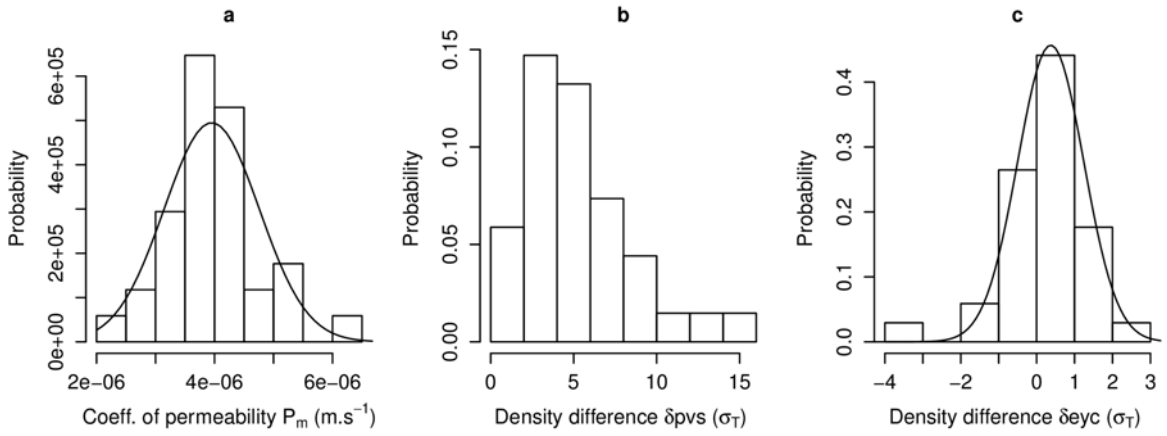

a
Probability
0e+00
2e−05
4e−05
6e−05
2e−06
4e−06
6e−06
Coeff. of permeability P_m ($m.s^{-1}$)
b
Probability
0.00
0.05
0.10
0.15
0
5
10
15
Density difference δpvs (σ_T)
c
Probability
0.0
0.1
0.2
0.3
0.4
−4
−2
0
1
2
3
Density difference δeyc (σ_T)

Fig.7

Fig.8

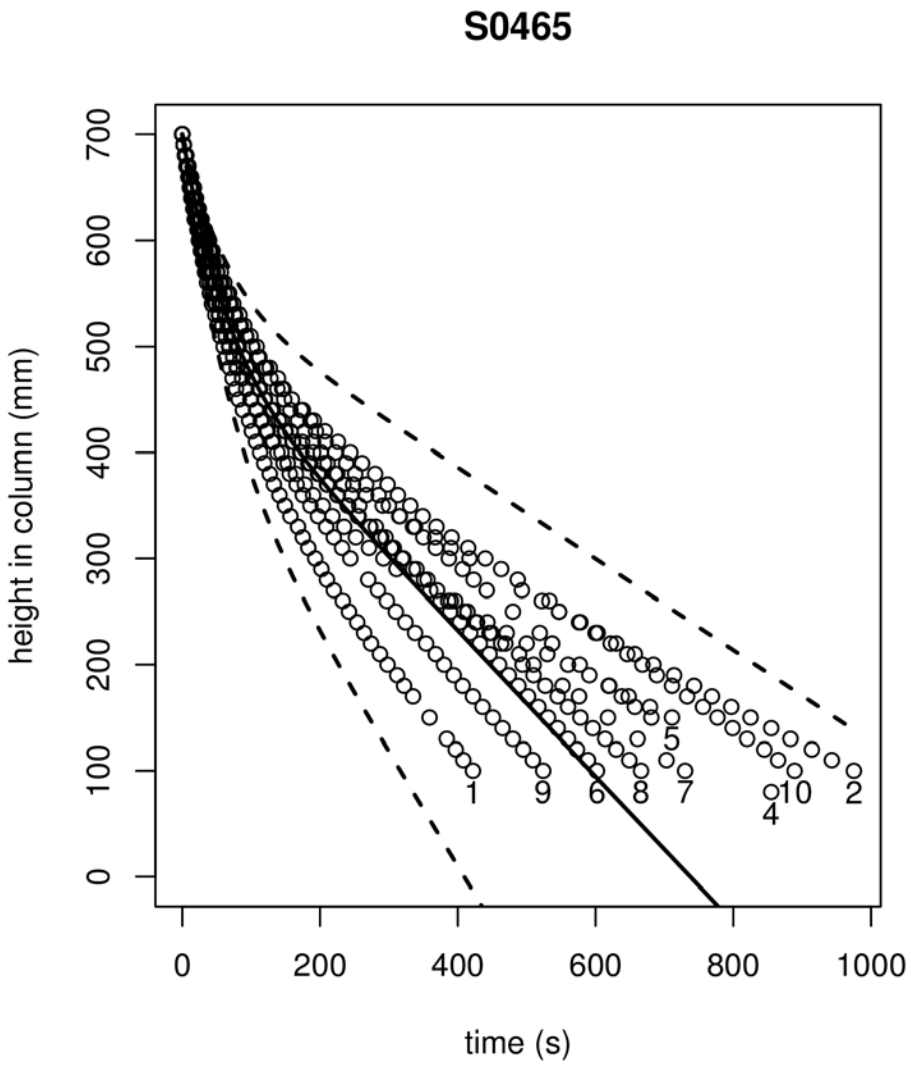

S0465
height in column (mm)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
time (s)
1
9
6
8
7
5
10
4
2

Fig.9

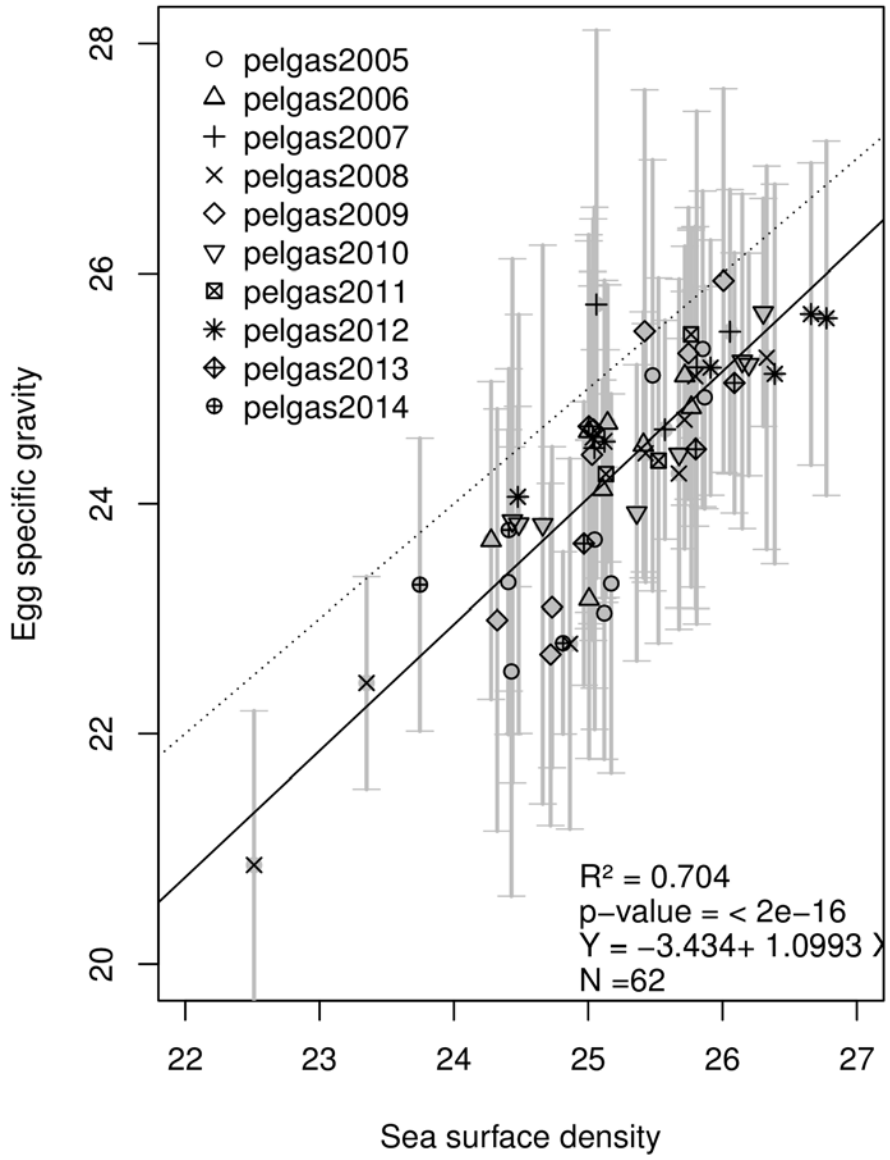
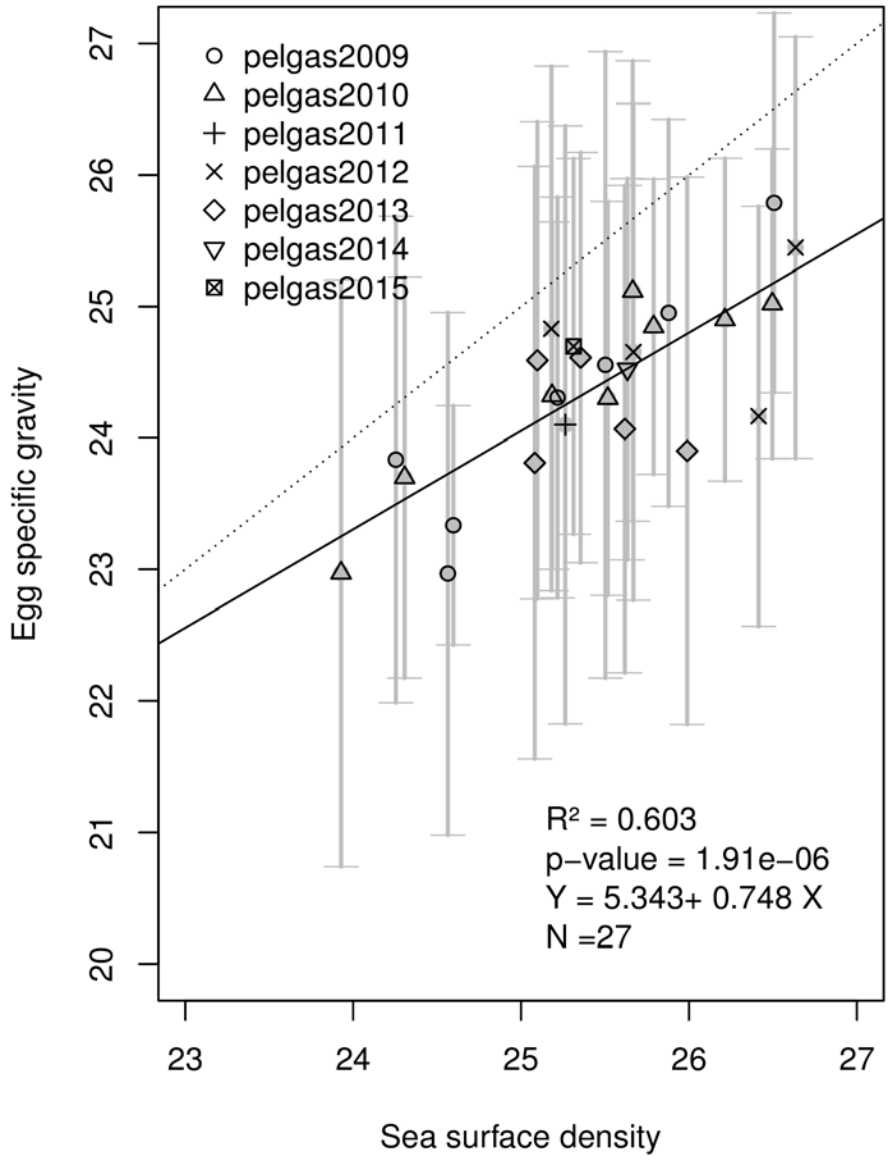

pelgas2005
pelgas2006
pelgas2007
pelgas2008
pelgas2009
pelgas2010
pelgas2011
pelgas2012
pelgas2013
pelgas2014
R² = 0.704
p−value = < 2e−16
Y = −3.434+ 1.0993 X
N =62
Egg specific gravity
Sea surface density
20
22
24
26
28
22
23
24
25
26
27

Fig.10


pelgas2009
pelgas2010
pelgas2011
pelgas2012
pelgas2013
pelgas2014
pelgas2015
R² = 0.603
p−value = 1.91e−06
Y = 5.343+ 0.748 X
N =27
Egg specific gravity
Sea surface density
20
21
22
23
24
25
26
27
23
24
25
26
27

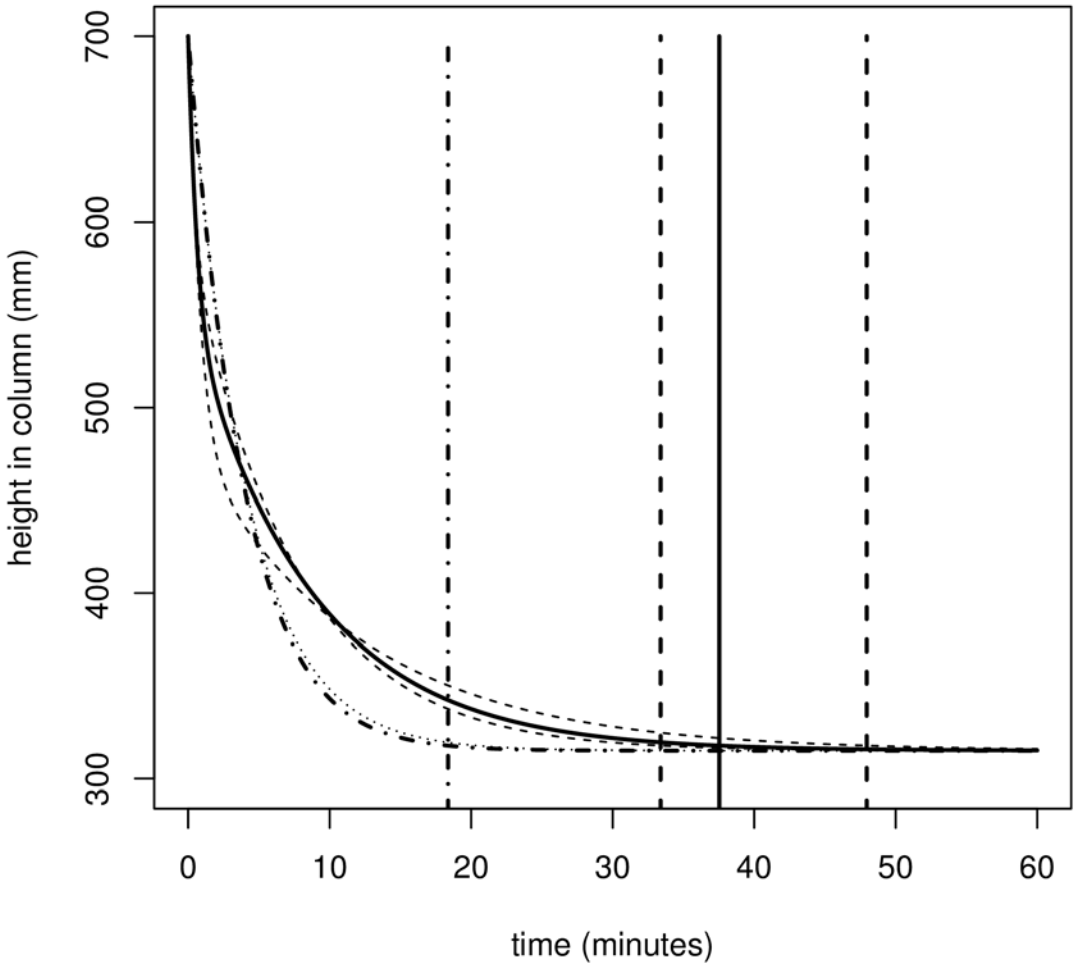

height in column (mm)
700
600
500
400
300
0
10
20
30
40
50
60
time (minutes)

Fig.11