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Buoyancy of Atlantic cod (*Gadus morhua*) larvae in relation to spawning experience: first and second time spawners

by

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Abstract

Changes in larval buoyancy of captive Arcto-Norwegian cod were observed in relation to developmental stage and maternal influence. In this paper the detailed results of a two-year study on larval buoyancy undertaken on the same individuals, i.e. as recruit (first-time) and repeat (second-time) spawners, are presented. Relevance of buoyancy in terms of vertical and horizontal drift is discussed.

Introduction

The lack of success in prediction of year-class strength may to some extent arise from insufficient knowledge of processes affecting egg and larval survival (see recent reviews in Chambers and Trippel, 1997). As noted, the quality of eggs and larvae (cf. definition of "quality" given in Brooks *et al.*, 1997) and their capacity to develop properly are often mentioned along with the spatial distribution and transport as important factors. Buoyancy, i.e., the ability of these pelagic early life stages to float in sea water, is on one hand a positive criterion of quality *per se* (Brooks *et al.*, 1997), and on the other hand a key variable in drift models (Sundby, 1991). For Atlantic cod (*Gadus morhua* L.), egg buoyancy has been extensively studied by many research groups on both sides of the Atlantic Ocean (see Kjesbu *et al.*, 1992; Nissling *et al.*, 1994; Ouellet, 1997 and references therein). Obvious stock differences seem to exist, exemplified most clearly by the extreme high water content of the Baltic Sea cod eggs resulting in very buoyant eggs (Thorsen *et al.*, 1996). In contrast to this wealth of information, no through buoyancy data seem to exist for the larval Atlantic cod stage. One partial reason for this shortage in data might be that any standard, precise measurement of larval buoyancy in the laboratory is complicated by the fact that the larvae are non-stationary, i.e. swimming actively around in the density- (salinity-)

gradiated column normally used for this type of work.

Larvae develop active mechanisms to control their position in the water column involving the swimbladder and the fins. However, the time between hatching and formation of relevant body structures lasts generally several days. During this time period, being inversely related to environmental temperature, neutral buoyancy of the larvae seems to be very much under the same regulatory principles as outlined for egg buoyancy (see Thorsen et al., 1996). Thus, larval buoyancy is expected to vary with developmental stage. Furthermore, as egg size and quality in Atlantic cod are influenced by maternal factors such as female size, spawning experience (recruit vs. repeat spawners) and egg batch number, each adult female spawning from 5-20 batches per season (Kjesbu *et al.*, 1996; Solemdal *et al.*, 1996; Solemdal *et al.*, 1997), larval buoyancy is hypothesized to undertake complex but detectable changes in response to several of these variables.

During the 1997 spawning season a specific analysis was made addressing techniques for accurate and precise measurements of larval buoyancy and presenting series of data, including both individual-, seasonal- and stage-specific aspects. Because of this complexity, this analysis was limited to recruit spawners only. Results are in process of submitting.

In 1998, we repeat the analysis with the same females and following the same procedure and then we compare the results of larval buoyancy between first and second time spawners to address the question if the spawning experience affect the buoyancy of larvae and their possible consequences in their survivor. Results are presented in this paper.

Material and methods

Capture and holding of fish

The Atlantic cod used in this experiment were trawled in the southwestern part of the Barents Sea in May 1996 by the R/V "Johan Hjort", Institute of Marine Research (IMR). The catch was immediately screened for fish of about 60 cm in length and any healthy-looking specimens put into tanks supplied with running sea water. The geographical position of the trawl station indicated that all specimens were Arcto-Norwegian cod, the stock of present interest, to be held separate from the Norwegian Coastal cod staying along the coast of Norway. Maturity ogives studied prior to the cruise (Jørgensen, 1990; Marshall et al., 1998) also strongly indicated that the fish collected should be immature but some were expected to enter vitellogenesis in the coming fall. Back in the laboratory in Bergen after a few days, the fish were transported in an oxygenated tank from the research vessel to a 30-m^3 sea-water, outdoor tank. Any additional number of fish injured during transportation were removed. The remaining about 100 ones were weighed at intervals of 2-3 months for whole body weight (to the nearest g) and measured for total length (to the nearest cm) (no growth data are presented). The group was held on a moderate ration of dry-pelleted feed (Felleskjøpet, Norway: "torskefôr", see Svåsand *et al.*, 1996 for chemical composition) of 0.25% per g

body wet weight and day (cf. Kjesbu *et al.*, 1991). An exception to this feeding protocol was a plentiful supply during the first months in captivity as most fish were initially in poor condition. Water temperature was stable at 8° C (intake at 120 m depth in fjord). In January 1997, each individual was staged for maturity (immature or prespawning) using gonad catetherisation and/or gentle stripping of abdomen (release of milt). Specimens used in the further experiment were weighed and length measured just before and after completion of spawning.

Collection of eggs

The 200 m^3 sea-water Circular tank (CT) at the IMR was used in the detailed monitoring of natural spawning activity. One female and male were placed in each of 10 identical chambers (CT1-10) established inside the tank. To limit the extensive amount of egg material available, eggs from every third batch from eight females (Table 1) were used in the further production of larvae and measurements of buoyancy. Experimental facilities and egg production protocol used are detailed elsewhere (e.g. Kjesbu *et al.*, 1996). Briefly, all eggs from each spawning event were collected (the settling out of eggs was insignificant) and measured for total volume (ml). A subsample was subsequently taken and recorded for fertilization rate, mean egg diameter (50 single eggs, precision: +/- 10 µm) and dry weight (50 pooled eggs, in µg). The number of eggs spawned was given from the total egg volume and the egg diameter (mm) using an already established formula (*op. cit.*). As previously defined (Kjesbu *et al.*, 1990), the portion of the total number of eggs spawned (PES) for a given batch number was calculated as the ratio between the cumulative egg number spawned at that step in the spawning cycle and the total number of eggs spawned throughout the whole spawning period (i.e. from first to last batch). No food were provided while the fish were kept inside in the Circular tank. Exactly the same females were used in both experiments in 1997 and in 1998, but one male were replaced (the original one died).

Larval buoyancy measurements

About 10-60 milliliters (approx. 500 eggs ml^{-1}) of eggs from each selected batch (Table 1) were incubated in one of several aquaria (1-10l) placed together in a large temperature-regulated water bath (5 C, precision: ± 0.1C). Each aquarium being individually supplied with UV- and sand-filtered sea water (i.e., the Biotest-system, IMR) (Serigstad, 1997). Dead eggs were removed by means of a siphon. Live eggs that showed examples of ciliates/bacteria on the outer egg surface were as far as possible also removed. Hatched larvae were considered free from these microorganisms. However, only larvae that were healthy and representative for a given batch were used in the further buoyancy study.

Larvae (n =50) taken from tabled batches were anaesthetized and staged. The cod larvae staging system of Fossum (1986) supplemented with details of organogenesis in Hunt von Herbing *et al.* (1996) were used. Selected larvae (n=20) in an identical phase of development were placed in a 50 ml beaker with filtered, 5 C sea water and 0.5 ml of metomidate (Mattson and Riple, 1989) added. Following 30 min of exposure to

this anaesthetic, one by one of the larvae were carefully transferred by a specially-made pipette for buoyancy measurements. The reminding, few larvae were placed in another, similar beaker to check mortality and time to recover.

Anaesthetized larvae were introduced into one of two tubes in a density-gradiated column (Martin Instruments Ltd, UK). All examinations were undertaken in normal fluorescent light but a dark film to avoid direct sun light onto the column covered laboratory windows. The gradient was established by using commercially made seawater salt (Instant Ocean, U.S.A.) and distilled water, and stabilized at 7° C (precision: +/- 0.5 C). For every group of larvae a new density gradient was made and bulbs properly cleaned. The water bath in which the tubes were placed contained antibiotics and fungicides to avoid ongrowing. Within each tube, a number of four to six density bulbs with specific density given in four digits calibrated at 23° C (Martin Instruments) and covering the below range observed for larvae were used as references. Linear regressions between the vertical positions (to the nearest 0.1 cm) of bulbs and their specific densities were always highly significant (r^2 close to 1).

The position of each larva was recorded at 15, 25, 30 and 35 min, but using the 30 min data as reliable position. Individual larvae that demonstrated a fluctuating path between 25 and 35 min were excluded: classified to be moribund or awaking. The positions of the bulbs were recorded at 30 min. After all recordings were done the tube was emptied and every larva collected. Each larva was subsequently reexamined under the binocular microscope attached to a video camera and an Olympus image analyzer, and the photographic file stored.

All linearly transformed larval specific gravity (density) data were adjusted to a temperature of 5° C (a typical environmental temperature met by these types of larvae in the field). Every observation was checked for quality and in some cases extremes removed. Based on a reevaluation of all statistical information for the whole experiment an observation was defined as an outlier when its value exceeded five times the standard error of the normal distribution for that set of larvae.

Experimental design and statistics

For test of cod larval buoyancy in relation to developmental stage, larval specific gravity was recorded every day from the time of hatching to the time of stage 7 using the Fossum scale. The analysis was made in six females at batches 3, 9 and 15 (Table 1). Density were compared using a post hoc Tukey HSD test.

For test of cod larval buoyancy in relation to maternal factors, larval specific gravity was recorded early in development, stage 1 or 2, and late in development, stage 5, using six females and studying batches 3, 6, 9, 12 and 15 (Table 1).

For test of cod larval buoyancy in relation to batch number, relevant larval specific gravity data from the above two designs were used.

As there were examples of females that spawned less than 15 batches (Table 1), the outlined two experimental designs could not always be fully followed. In several cases stage-specific data were supplemented with information on organogenesis, particularly the swimbladder, and calculations of corresponding day-degrees.

In 39 cases was possible to record larvae from the same female, batch and stage and those sets of larvae were used to compare buoyancy between years. These comparisons were made using a student t-test. Finally, data from these subtasks were pooled and interpolated to create a mesh plot using inverse distance weighting of stage-batch-density triplet data. The pooled of each year data were adjusted also to a polynomial curve:

$$Z = ax + by + cxy + dx^2 + ey^2 + fx^2y^2 + g$$

Where Z is the density value, x the stage and y the batch number (recoded from one to five). All statistical analysis were made using Statistica (StatSoft, Inc., 1995).

Results

Larval specific gravity increased steadily, i.e. the larvae became less buoyant, during the first early stages of development in both years: first and second spawners (Figure 1). For the following stages 4-6 the specific gravity reminded nearly constant, but then started to decrease gradually (more buoyant larvae) from stage 6 to 8. In the latter situation 80-100 % of the yolk sac was resorbed. As no food was provided some of these larvae were probably starving. This phenomenon in combination with the introduction of a likely functional swimbladder from stage 7 onwards (Fossum, 1986) indicate that the density data for the oldest larvae should be treated with some caution.

For first time spawners (1997 experiment), late-season larvae were found to be heavier in terms of specific gravity than early-season larvae (Figure 1A). This applied to almost all of the stages tested. The noted difference between batch 3 and 9 were smaller than between each of these two and batch 15. At stages 3-4 the density of batch 3 and 9 were found to be almost similar. From stage 1 to stage 3 the buoyancy decreased more in batch 3 than in batch 9 and 15.

For second time spawners, the above picture described is different and no defined pattern in relation with the batch is observed (Figure 1B). Although there were differences at each stage between set of larvae, not always the heavier or lighter larvae belonged to the same batch, thus for example, larvae from one batch 3 were heavier than other batches at stage 4 but lighter at stage 2. It can be said that the moment on the spawning season (batch) when the larvae were born has no influence on density of larvae.

For the buoyancy data presented several significant differences in mean density existed (ANOVA) (Table 2). For the first year spawners, the high F-value for batch 15 of Female 6 is due to extremely-heavy stage 6 larvae. For early-season larvae (batch 3) there appeared to be a significant difference both within early stages (1-3) and between early and late stages (4-6) but not within late stages (Tukey HSD post hoc test) (Table 2). In middle- (batch 9) and late-season batches (batch 15) there were no difference within either early or late stages, but, similarly, between early and late stages. An exception to this trend is the above mentioned batch 15-Female 6 data where a significant difference occurred between stage 2 and 3. Mean buoyancy of larvae in stages 7 and 8 were insignificantly different to those for larvae in early stages but there were in most cases significant differences between stage 6 and later stages.

For second spawners, it can be observed that the differences between early and

late stages are sharper as reflected by the higher values of F statistic from the ANOVA (Table 2, cont.). The general pattern is, nevertheless, similar to the first spawners, i.e. density increase in the earlier stages and trend to be constant at stages 5 and 6.

In 39 cases larvae density were measured at the same stage, batch and female in 1997 and 1998. Those sets of larvae were compared to assess changes in buoyancy between first and second time spawners. Figures 2 to 7 show the mend density and standard deviation for each plot at each stage and the results of student t-test. At early stages (stage 1 to 4) the differences occurred at late-season larvae (batches 9 to 15) but at stages 5 and 6 the differences occurred at early-season larvae (batches 3 and 6).

All the data recorded for each year were pooled and interpolated to draw 3D mesh plots. Figure 8 shows 3D mesh plots for first and second time spawners. It can be observed that both years there were a similar tendency of larvae being heavier at later stages as described above. However while in 1997, first time spawners, density of larvae was dependent also of the batch; in 1998, second time spawners, such dependence didn't exist. Thus, for first time spawners, density changed sharply at early season larvae (batch 3), but the change was less sharp at late season larvae (batch 15). The change in larvae density between stage 1 and 5 was similar in second time spawners, irrespective of the batch.

To show better the trend of the buoyancy in relation with developmental stage and maternal influence (batch), the pooled data for each year was fitted to a polynomial curve. Plots are shown in Figure 9 where it is possible to observe better the pattern described. At batches 6 and 9 the density was similar between first and second time spawners and at stages 6 also the density became similar. In general larvae were heavier in the first spawners, except for batch 3.

Discussion

Buoyancy have been shown as an important factor on survival of marine fish eggs and larvae (Sclafani *et al.*, 1993; Nissling *et al.*, 1994; Lough *et al.*, 1996). The importance of the spatial distribution and transport of the early stages for recruitment of fish stocks has been pointed out by many authors. For example, Hjort, already in 1914, suggested that eggs and larvae drift determine if larvae reach suitable nursery grounds, thereby increasing their chance of survival producing a permanent loss from the population. This loss could be due to a high mortality of both eggs and larvae in an inadequate environment (Iles and Sinclair, 1982), as well to the transport from one area to other and the posterior recruitment in a different stock (Hansen and Buch, 1986).

In the spatial distribution, the vertical one is of particular importance. Vertical distribution of eggs determines where the larvae hatch, and, later, vertical distribution of larvae determines where they develop. It can occur in a productive layer where their food is produced or in layers less productive influencing their survival and posterior recruitment (Cushing, 1982). At the same time, as stated by Sundby (1990), considering the large vertical variations of the horizontal flow field, it is evident that the vertical distribution of eggs and larvae is important for their horizontal transport and spreading and their fate with respect to survival.

Our results show the Arcto-norwegian cod larvae buoyancy varies significantly with developmental stage and with maternal effect in recruit spawners while vary only with developmental stage in repeat spawners. This trend is opposite to that observed in eggs (Kjesbu *et al.*, 1992) showing that the strategy for eggs and larvae are different, although the experiment of Kjesbu *et al.* was conducted on coastal cod.

Cod larvae develop the swimbladder and the capacity of active swimming activity around stage 6 (Fossum, 1986; Hunt von Herbing, 1996) and hence their capacity to catch preys in the water column. From this stage buoyancy is a less important factor in the survival of the larvae but before that stage their buoyancy will determine the position on the water column. If we consider the mean salinity of sea water between Lofoten and Barents Sea being about 25-26 σ_t, most of the larvae of first spawners were positive buoyant since most of the eggs were spawned at batches between 6 and 12. However for second time spawners most of the larvae were close to neutral buoyant. Exceptions to this pattern are the larvae at early stages (1 and 2) with a positive buoyancy and late stage (5 and 6) with negative buoyancy. An explanation to this pattern, for early stages, could be related with the position of the eggs at the moment of hatching (eggs at later stages have higher specific gravity, see Anderson and Brad de Young, 1994 and Kjesbu, pers. observations) or/and with the current regime present in the area. For latter stages a higher specific gravity can increase the possibility of the larvae of being in a highest concentration area of zooplankton when the feeding ability become available. The increase of the specific gravity in relation with developmental stages have been showed by other authors in cod (Tilseth and Strømme, 1976; Ellertsen *et al.*, 1980; Yin and Blaxter, 1987) and other species (Blaxter and Ehrlich, 1974; Yin and Blaxter, 1987; Liu *et al.*, 1993). It was suggested that changes in the protein, lipid and water content result in differential buoyancy on the larvae throughout ontogeny (Blaxter and Ehrlich, 1974). Sclafani *et al.*, 1993 suggested that larval condition and buoyancy can explain most patterns of vertical migrations observed in the field. The decrease of the specific gravity after that stage 6 is in agreement with the results of Tilseth and Strømme (1976) and it seems related with the starvation of the larvae.

It is difficult to assess the influence of the difference in buoyancy between first and second spawners' larvae. Larvae density of recruit spawners is affected by maternal effect (batch) at early stages, being the larvae heavier at later batches. At late stages density was practically equal throughout the batches. In second time spawners there was no trend on density in relation with maternal influence. However, around batch 9, where the bulk of the eggs were spawned, density was similar between first and second time spawners. It was shown that smaller fish produces smaller eggs (Kjesbu *et al.*, 1991) and the egg size is also dependent of the batch, being smaller at early batches. This differences could be affecting also to larval quality and hence to the buoyancy. In first time spawners the quality of the larvae at early and late batches could be considerably less that those of the central part of the spawning period. Nevertheless, although there were no correlation between density and batch in second time spawners, the variance in density at stage 1 in second time spawners was considerably higher that in first time spawners. Similar results were recorded in Norwegian coastal cod eggs (Kjsebu *et al.*,

1992). Repeat spawners produce larvae more widely vertically distributed, and hence they will be subjected to greater horizontal spreading.

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Table I.- Number of batches spawned by each female used in this paper. Batches analyzed and their PES[1] and larvae stages measured in each batch and each year for first and second spawners

Female n.	n° of batches		Batch		PES		Larvae stages													
	first	second	first	second	first	second	first								second					
							1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1	13	12	3	3	19.0	17.6	X	X	X	X	X	X						X	X	X	X	
			6	6	43.3	44.8			X							X			X			
				9		77.7									X				X			
			12	12	96.1	100.0		X			X											
2	16	18	3	3	11.7	14.6	X	X	X	X	X	X					X	X	X	X	X	
			6	6	33.0	33.5	X				X					X			X			
			9	9	56.6	53.5		X			X				X				X			
			15	15	99.3	93.3		X	X		X	X				X	X	X	X			
3	12	15	4		24.7			X														
			6	6	47.7	39.8	X				X					X			X			
				9		61.5									X							
			12	12	100	85.9		X			X				X				X			
				15		100.0									X				X			
4	12	18	9	9	83.1	45.7		X	X	X	X	X	X		X	X	X	X	X	X		
5	14	9	3	3	11.5	28.4		X			X				X	X	X	X	X	X		
			6	6	32.2	80.2	X				X				X				X			
			9	9	66.6	100.0		X			X				X				X			
			12		94.2		X				X											
6	17	20	3	3	13.5	9.1				X	X					X	X	X	X			
			6		39.8		X				X											
			9		60.9		X				X											
			12		76.1		X				X											
			15	15	93.7	71.5		X	X	X	X	X	X	X	X	X	X	X	X			
7	19	14		6		38.8									X				X			
			9		50.2		X				X											
			12		71.5			X			X											
			15		90.0		X		X	X	X	X	X	X								
8	16	15	3	3	14.5	11.3	X								X							
			6	6	37.3	31.3		X			X				X				X			
			9	9	57.6	55.2	X	X	X	X	X	X			X	X	X	X	X	X		
				12		79.2									X							

[1] Proportion of eggs spawned

Table 2. - Results of the Tukey HSD post-hoc comparison to test differences of mean buoyancy in relation with developmental stage. Data for first spawners.

		Post Hoc Comparisons						ANOVA
		Stage						
		1	2	3	4	5		
Batch **3** Female 1	2	**						F = 24.70 p<0.000
	3	**	n.s.					
	4	**	**	n.s.				
	5	**	**	**	n.s.			
	6	**	**	*	n.s.	n.s.		
		1	2	3	4	5		
Batch **3** Female 2	2	*						F = 20.33 p<0.000
	3	**	n.s.					
	4	**	**	n.s.				
	5	**	**	n.s.	n.s.			
	6	**	**	n.s.	n.s.	n.s.		
		2	3	4	5	6		
Batch **9** Female 4	3	n.s.						F = 18.95 p<0.000
	4	**	**					
	5	**	**	n.s.				
	6	**	**	n.s.	n.s.			
	7	n.s.	n.s.	**	**	**		
		1	2	3	4	5		
Batch **9** Female 8	2	n.s.						F = 7.94 p<0.000
	3	n.s.	n.s.					
	4	**	*	*				
	5	**	*	*	n.s.			
	6	**	*	*	n.s.	n.s.		
		2	3	4	5	6	7	
Batch **15** Female 6	3	*						F = 23.79 p<0.000
	4	*	n.s.					
	5	*	n.s.	n.s.				
	6	**	**	**	**			
	7	n.s.	n.s.	n.s.	n.s.	**		
	8	n.s.	**	**	**	**	**	
		1	3	4	5	6	7	
Batch **15** Female 7	3	n.s.						F = 13.37 p<0.000
	4	**	**					
	5	**	**	n.s.				
	6	**	**	n.s.	n.s.			
	7	**	n.s.	n.s.	n.s.	n.s.		
	8	n.s.	n.s.	**	**	**	*	

n.s. .- not significant
* significant at p<0.05
** significant at p<0.01

Table 2 (cont.). - Results of the Tukey HSD post-hoc comparison to test differences of mean buoyancy in relation with developmental stage. Data for second spawners.

		Post Hoc Comparisons					ANOVA
		Stage					
		4	5	6			
Batch **3** Female 1	5	*					F = 65.20 p<0.000
	6	**	**				
	7	n.s.	*	**			
		3	4	5	6		
Batch **3** Female 2	4	n.s.					F = 74.58 p<0.000
	5	**	**				
	6	**	**	n.s.			
	7	**	**	n.s.	n.s.		
		1	2	3	4	5	
Batch **9** Female 4	2	*					F = 83.11 p<0.000
	3	n.s.*	n.s.				
	4	**	n.s.	n.s.*			
	5	**	*	n.s.*	n.s.*		
	6	**	n.s.*	n.s.*	n.s.	n.s.	
		1	2	3	4	5	
Batch **9** Female 8	2	*					F = 313.44 p<0.000
	3	**	n.s.*				
	4	**	**	n.s.			
	5	**	**	**	n.s.*		
	6	**	**	**	n.s.*	n.s.	
		1	2	3	4		
Batch **15** Female 6	2	n.s.*					F = 24.25 p<0.004
	3	n.s.*	n.s.				
	4	*	n.s.	n.s.			
	5	**	n.s.	n.s.	n.s.		
		1	2	3	4		
Batch **3** Female 5	2	n.s.					F = 13.37 p<0.000
	3	**	**				
	4	**	**	**			
	5	**	**	**	n.s.		

n.s. .- not significant
* significant at p<0.05
** significant at p<0.01
n.s.*.-significant with a comom t-test

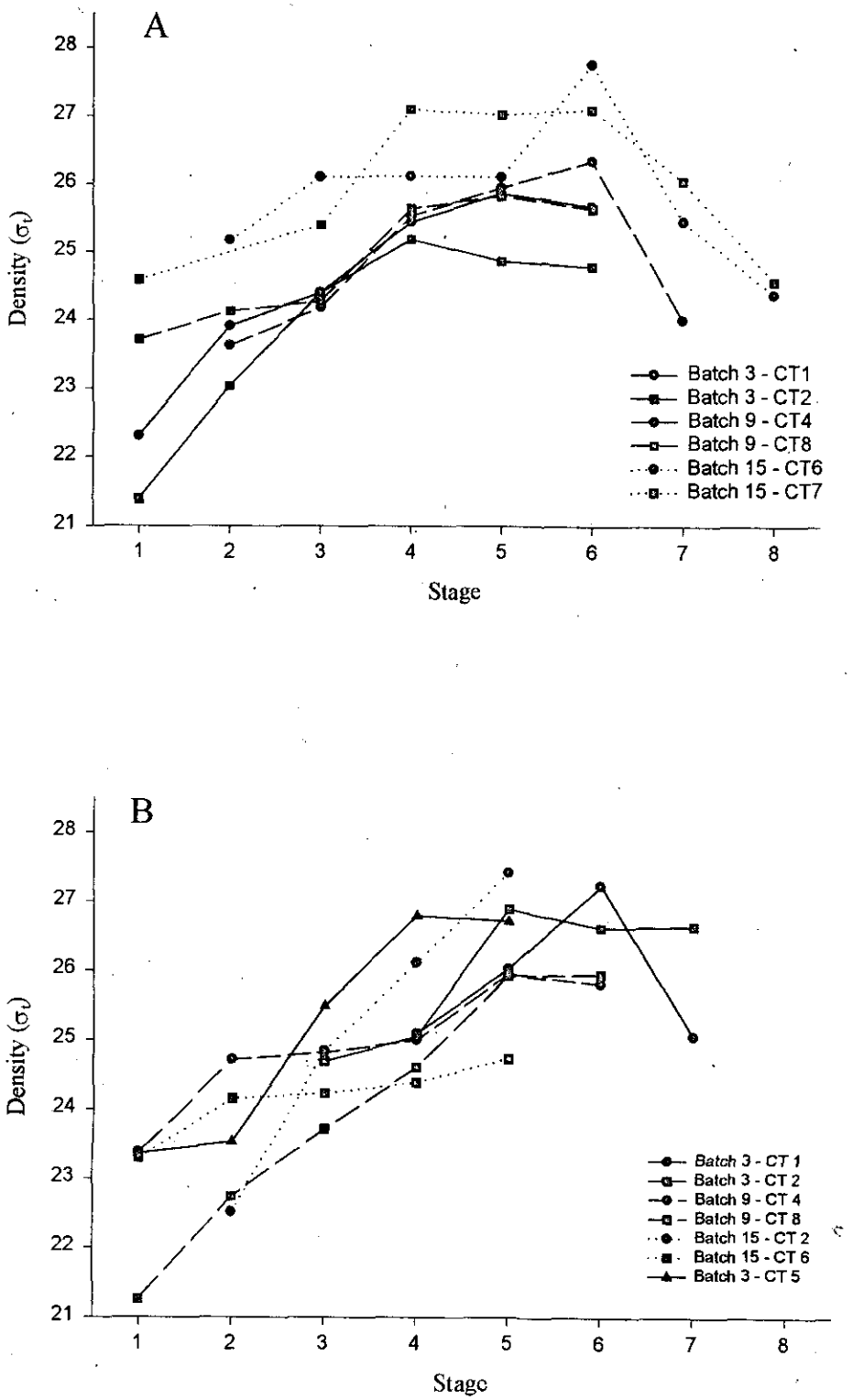

Figure 1. – Density of cod larvae at each stage in selected females and batches for first spawners (A) and second spawners (B)

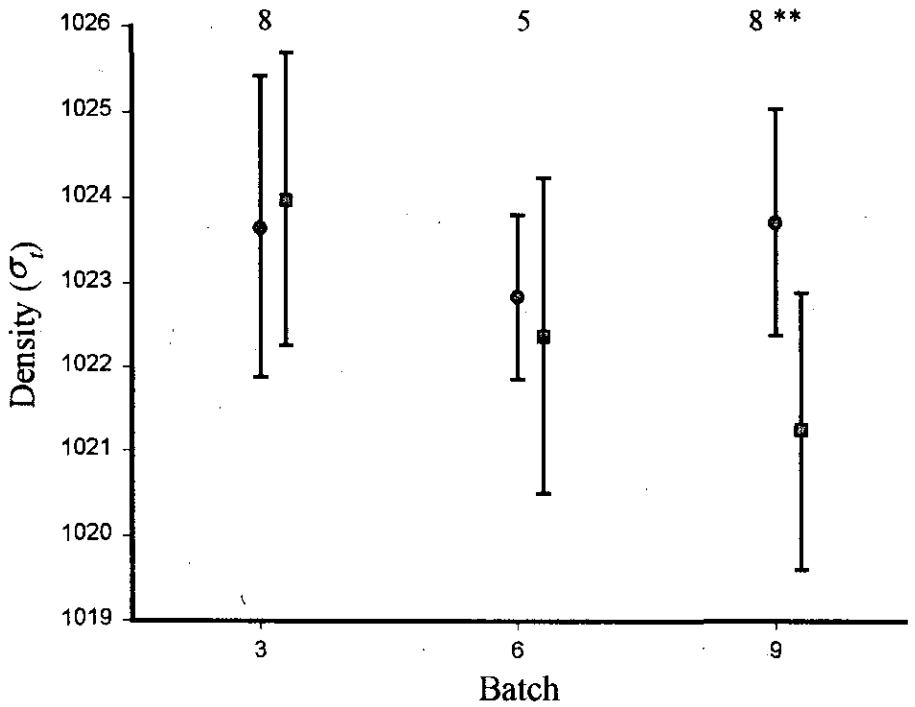

Figure 2. – Mean density and standard deviation of first spawners (circle) and second spawners (square) for larvae at stage 1. Top figures are female number. Significance: * $p<0.01$ ** $p<0.05$

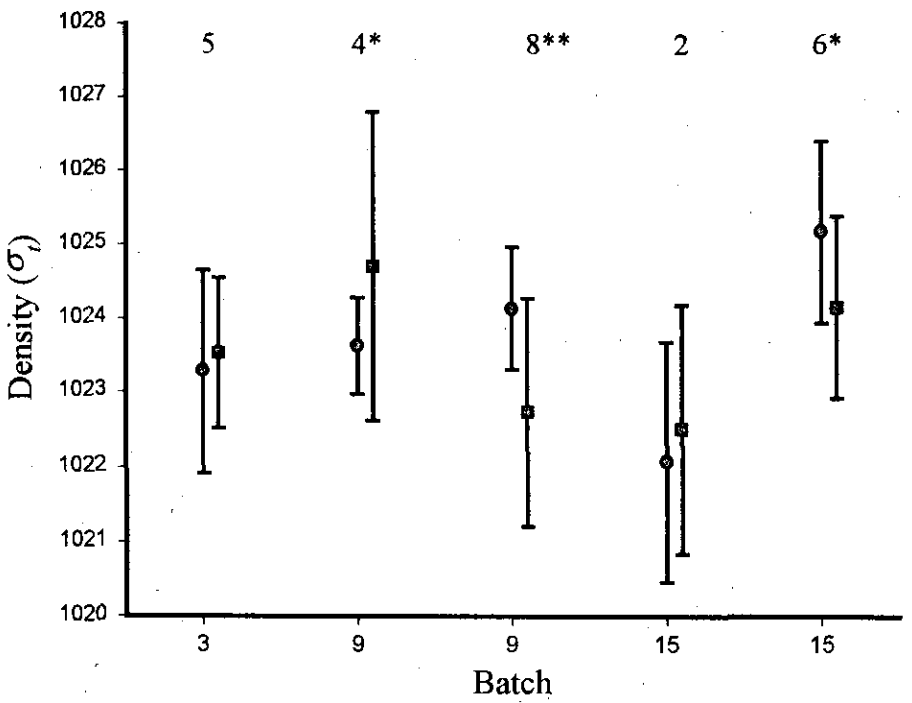

Figure 3. – Mean density and standard deviation of first spawners (circle) and second spawners (square) for larvae at stage 2. Top figures are female number. Significance: * $p<0.01$ ** $p<0.05$

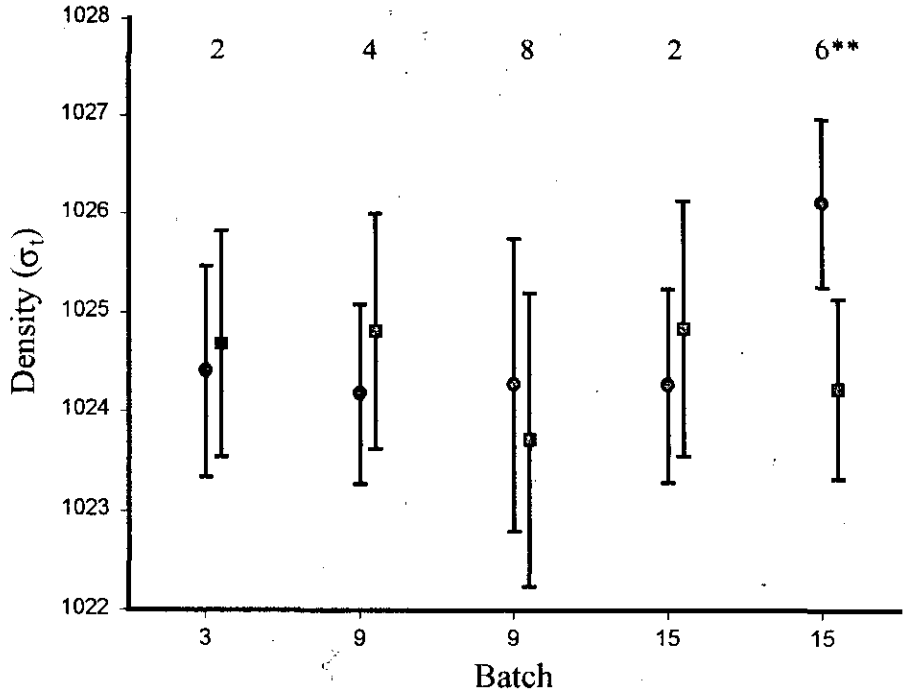

Figure 4. – Mean density and standard deviation of first spawners (circle) and second spawners (square) for larvae at stage 3. Top figures are female number. Significance: * $p<0.01$ ** $p<0.05$

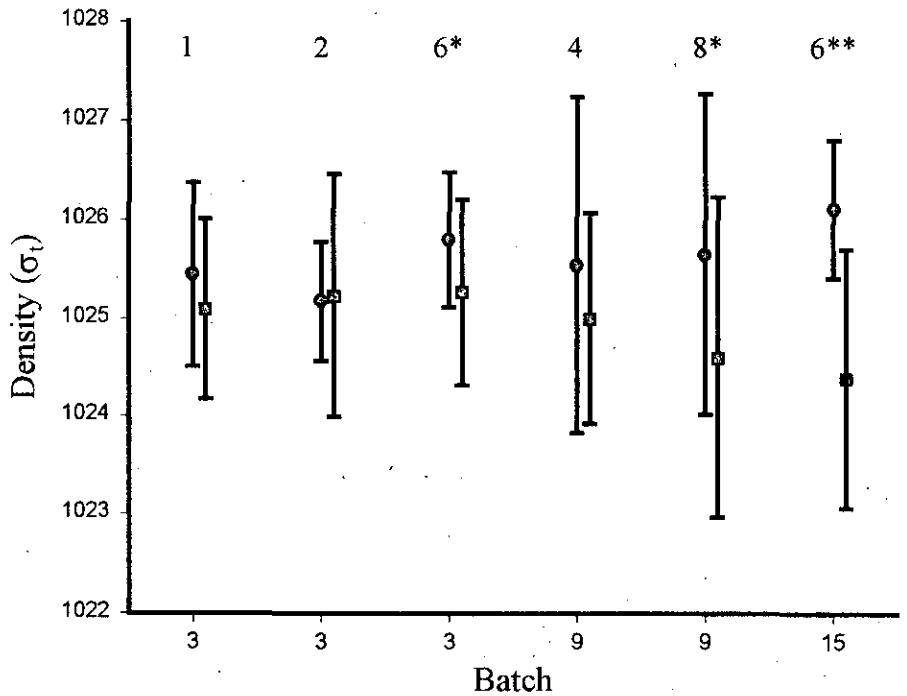

Figure 5. – Mean density and standard deviation of first spawners (circle) and second spawners (square) for larvae at stage 4. Top figures are female number. Significance: * $p<0.01$ ** $p<0.05$

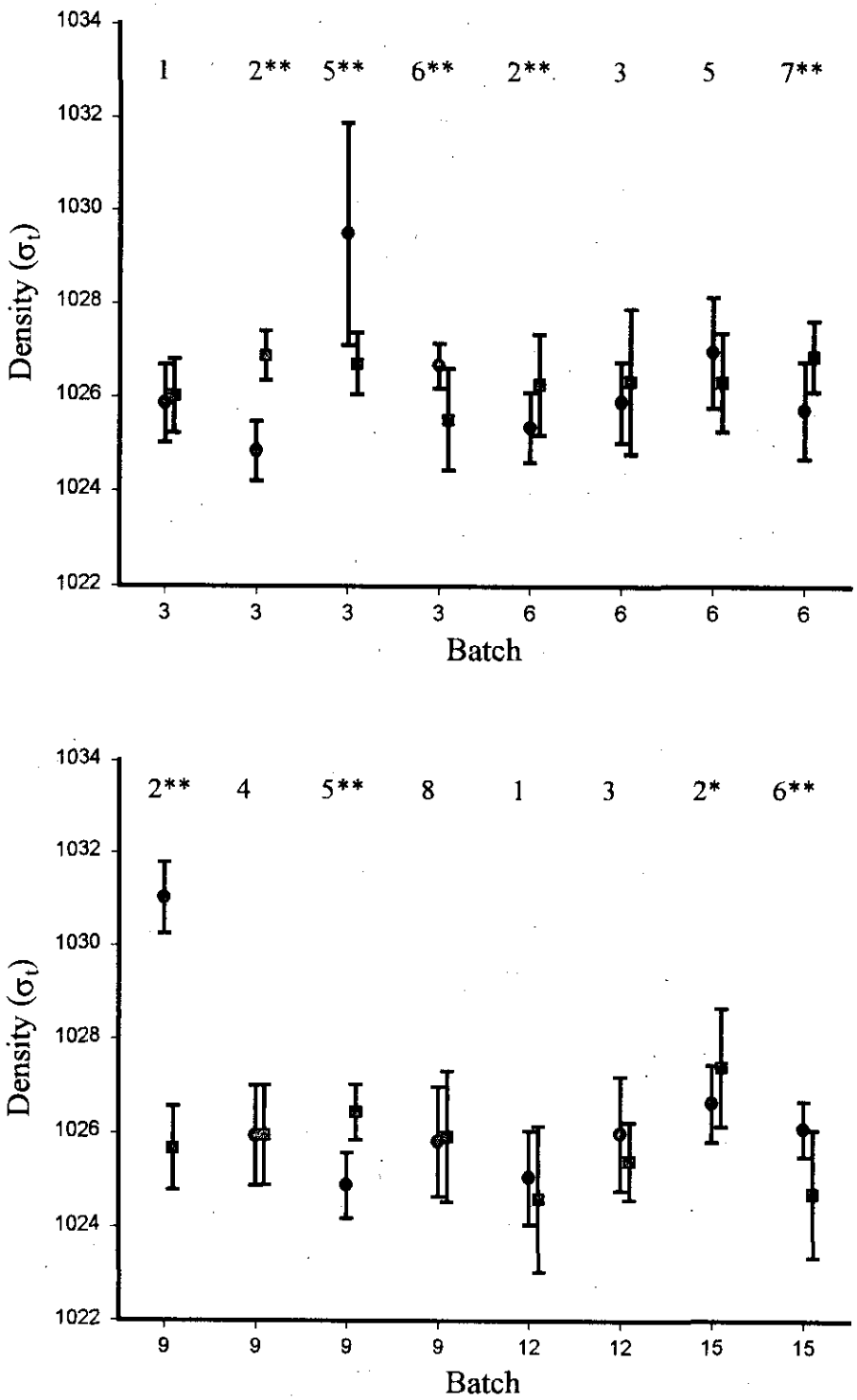

Figure 6. – Mean density and standard deviation of first spawners (circle) and second spawners (square) for larvae at stage 5. Top figures are female number. Significance: * $p<0.01$ ** $p<0.05$

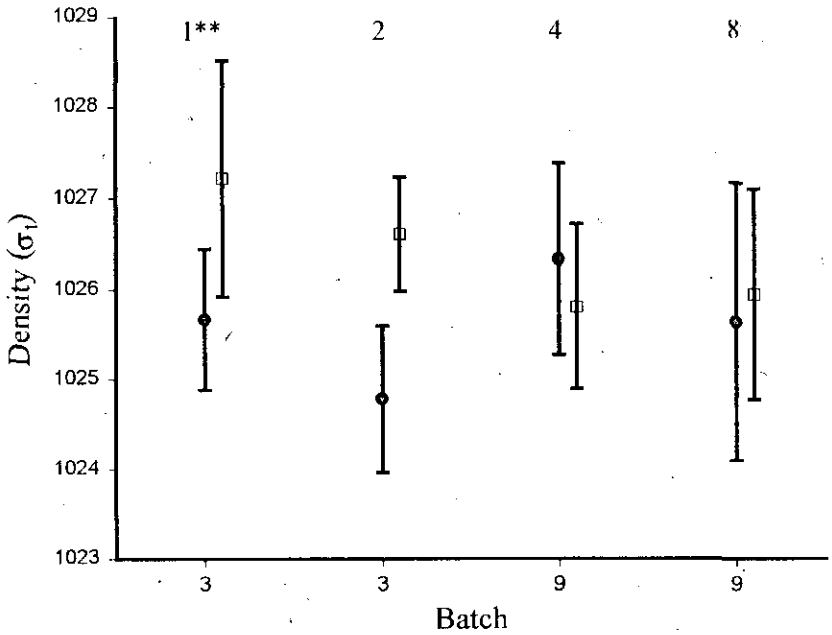

Figure 7. – Mean density and standard deviation of first spawners (circle) and second spawners (square) for larvae at stage 6. Top figures are female number. Significance: * $p<0.01$ ** $p<0.05$

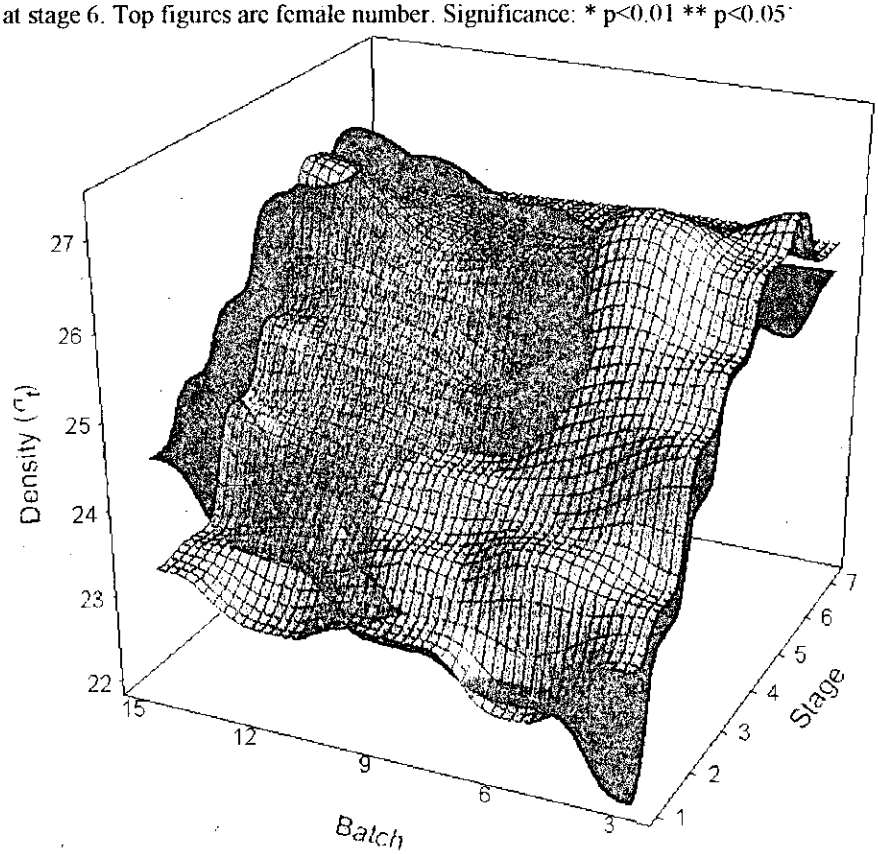

Figure 8. – 3D mesh plot for all the data pooled and interpolated. Black plot corresponds to first time spawners. Grey and grided plot are the second time spawners.

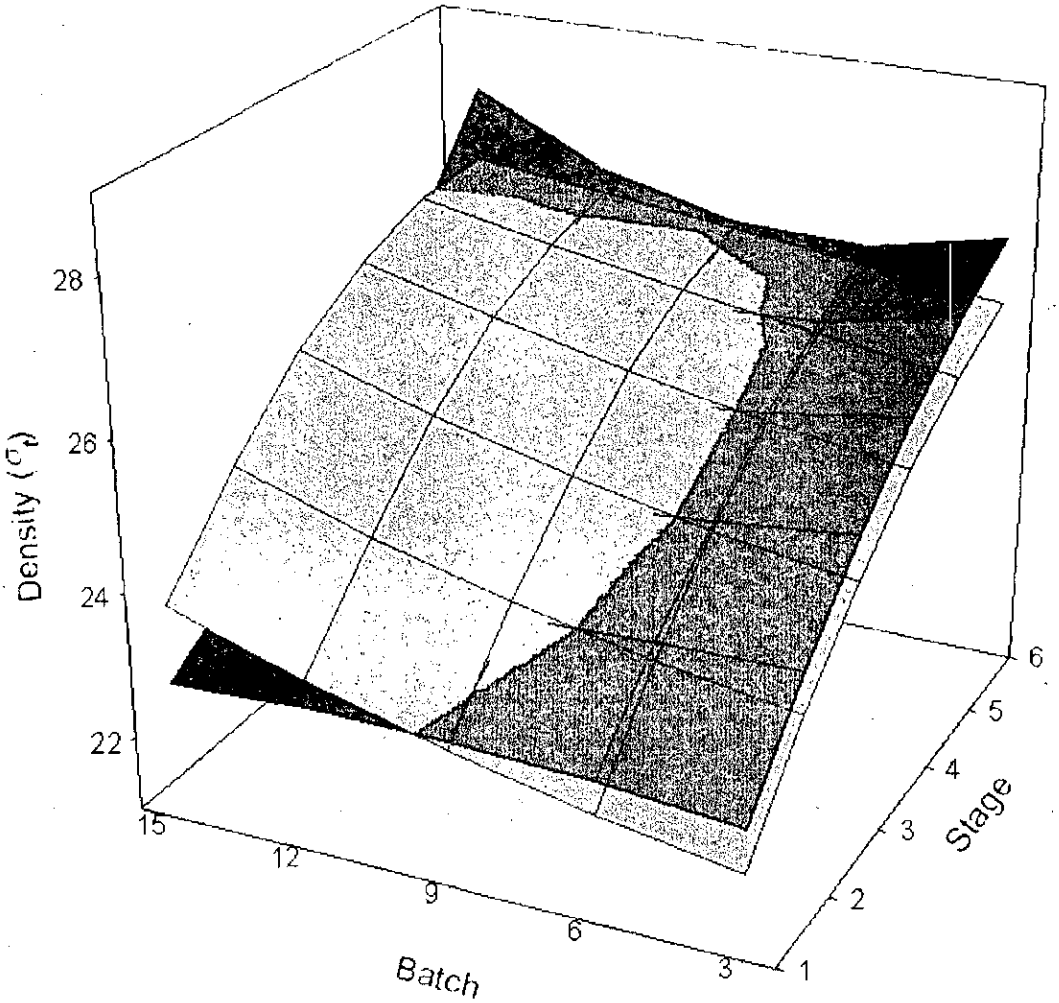

Figure 9. – 3D mesh plot for all data fitted to a polynomial curves. Light grey plot corresponds to first time spawners and black plot to second time spawners.