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Buoyancy of Atlantic cod larvae in relation to developmental stage and maternal influences

F. SABORIDO-REY*, O. S. KJESBU[1] AND A. THORSEN[1]

CORRESPONDING AUTHOR: FRAN@IIM.CSIC.ES

In this experimental study on Atlantic cod, Gadus morhua, buoyancy was found to vary significantly with season, developmental stage, egg size and maternal condition. Before the start of the experiments, wild fish were caught in the Barents Sea and acclimatized to laboratory conditions. Pairs (n = 8), one female (recruit spawner) and one male, spawned naturally in large, indoor compartments of a circular tank and every third egg batch (out of up to 19 batches collected per female) was incubated in aquaria. Following sedation (50 mg l^{-1} metomidate hydrochloride, 30 min), individual larval specific gravity was precisely reported (30 min) using a density-graduated column. Larval specific gravity (mean σ_t) increased steadily during the first early stages of development (0–25 day-degrees), remained nearly constant during the following stages (25–35 day-degrees), but then decreased gradually (35–55 day-degrees). In general, late-season larvae were significantly less buoyant, i.e. showed higher mean σ_t, than early-season larvae. Mean σ_t was negatively correlated with female condition and egg size and dry weight. Possible implications of these findings for drift and survival are discussed.

INTRODUCTION

The lack of success in predicting the recruitment of fish may to some extent arise from insufficient knowledge of processes affecting egg and larval survival (Chambers and Trippel, 1997). In this context, the quality of eggs and larvae [cf. definitions given in Brooks *et al.* (Brooks *et al.*, 1997)] and their capacity to develop properly are often mentioned as important factors along with the corresponding oceanographic spatial distribution and transport. Buoyancy, defined as the ability of these pelagic early life stages to float in sea water, is often used as a positive criterion of quality (Brooks *et al.*, 1997), but is also as a key variable in drift models [see, for instance, Sundby (Sundby, 1991)].

For Atlantic cod (*Gadus morhua* L.), a highly fecund, multiple batch spawner, egg buoyancy has been extensively reported on by many research groups [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 low-density eggs (Thorsen *et al.*, 1996). In contrast to this wealth of information, little published buoyancy data exist for the larval Atlantic cod stage (Sclafani *et al.*, 1997), especially in relation to traits of the individual, mother fish (maternal effects) or time of spawning (seasonal effects) (Solemdal, 1997). For instance, larger cod females are known to produce larger eggs and thereby larger larvae (Marteinsdottir and Begg, 2002), while there is a significant depletion of body reserves during the course of spawning and a general reduction in egg size (Kjesbu *et al.*, 1996; Lambert and Dutil, 2000). In the work of Yin and Blaxter, buoyancy patterns from hatching to point-of-no-return of benzocaine-anaesthetized larvae of cod, Atlantic herring (*Clupea harengus*) and flounder (*Platichthys flesus*), measuring rising and sinking rates, are described pointing to large inter-specific differences (Yin and Blaxter, 1987a). One partial reason for this shortage in comprehensive fish larval buoyancy data might be that any precise recordings are complicated by the fact that the larvae need to be adequately immobilized as they are normally actively swimming around in the density-(salinity-) graduated column (Coombs apparatus) typically used today for any buoyancy work. In Sclafani *et al.* (Sclafani *et al.*, 1997), MS-222 was added to anaesthetize pooled samples of cod larvae while they were being kept inside this column.

Larvae are thought to develop active mechanisms to influence their position in the water column, involving the swimbladder and the fins (Hunt von Herbing and Boutilier, 1996; Hunt von Herbing *et al.*, 1996). However, the time between hatching and formation of relevant body structures is generally several days (Fossum, 1986). 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 (Thorsen *et al.*, 1996). Thus, larval buoyancy is expected to vary with developmental stage. Including maternal and seasonal effects, larval buoyancy is hypothesized to undertake complex but detectable changes in response to several of these variables.

In this paper on Atlantic cod, we sedated larvae of known origin with metomidate hydrochloride, previously used successfully on larger fish (Mattson and Riple, 1989), and measured their buoyancy (or, more correctly, specific gravity) in Coombs apparatus, specifically addressing maternal (length, weight and condition), seasonal (spawning time) and stage-specific aspects. Because of this complexity, this analysis was limited to first time (recruit) spawners only (Solemdal, 1997; Trippel *et al.*, 1997). It is believed that this type of insight should be highly relevant to other marine, multiple batch spawners as well, such as haddock (*Melanogrammus aeglefinus* L.) (Hislop *et al.*, 1978).

METHOD

Capture and holding of fish

The Atlantic cod used in this experiment were trawled in the south-western 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 ~60 cm in total length and any healthy-looking specimens put into tanks onboard supplied with running sea water. The geographical position of the trawl station indicated that all specimens were Northeast Arctic cod, the most important cod stock in the North-east Atlantic, and separated presently from the Norwegian Coastal cod staying closer to or along the coast. Criteria for selection of fish size were based on studies of maturity oogives (Jørgensen, 1990; Marshall *et al.*, 1998), suggesting that all individuals included were immature but, at least some, were expected to start developing gonads in the coming fall. In Bergen, the fish were transported in an oxygenated tank from the ship to a 30 m^3 seawater outdoor tank. Injured fish were removed. Those remaining, ~100, were weighed at intervals of 2–3 months for whole body weight (to the nearest gram) and measured for total length (to the nearest centimetre). Only pre-spawning and spent female data are presented. During the first months in captivity, the fish were fed in excess as most of them were initially in rather poor condition. After that period, they were held on a moderate ration using the same dry-pelleted feed ['torskefór'; Felleskjøpet, Norway; see Svåsand *et al.* (Svåsand *et al.*, 1996) for chemical composition], but 0.25% per g body wet weight per day [cf. (Kjesbu *et al.*, 1991)]. Water was taken from 120 m in the fjord and was stable at ~8°C. In January 1997, each fish was sexed and staged for maturity (immature or pre-spawning) by gentle stripping of the abdomen for release of any milt and, if negative, by gonad catheterization.

Collection of eggs and maternal features

A 200 m^3 seawater circular tank at the IMR was used to monitor individual natural spawning activity: one female and one male were placed in each of 10 identical chambers established inside the tank, but the present buoyancy study was limited to eight representative pairs (Table I). Water temperature ranged from 8.7 to 9.2°C. Experimental facilities and egg sampling protocol were as detailed elsewhere (Kjesbu *et al.*, 1996). Briefly, all eggs from each spawning event were collected (the settling out of eggs was considered insignificant based on previous studies) and measured for total volume (millilitres). 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 micrograms) (Table I). The number of eggs spawned was given from the total egg volume and the egg diameter (millimetres) using an already established formula (Kjesbu *et al.*, 1996). As previously defined in Kjesbu *et al.* (Kjesbu *et al.*, 1990), the portion of the total number of eggs spawned (PES) for a given batch was calculated as the ratio between the cumulative egg number spawned up to the reference time point and the total number of eggs spawned, i.e. from first to last batch. No food was provided while the fish were spawning.

Each of the eight females used in the experiment was weighed (to the nearest gram) at the beginning of the spawning season, i.e. when they were placed in the individual chambers described above. Individual weight was recorded again at the end of the spawning season. Three corresponding Fulton's condition factors (K), i.e. weight × 100 /length^3, were then calculated: initial K (at the beginning of the spawning season), final K (at the end of the season) and mean K (the arithmetic mean of initial and final K). Data from female and egg attributes are presented in Table I.

Larval buoyancy measurements

Eggs from every third batch of eight females (Tables I and II) were incubated. About 10–60 ml of eggs (~500 eggs

Table I: General data regarding females and eggs used in this paper

Female								Eggs				
Female	Length (cm)	Initial wt (g)	Final wt (g)	Mean condition factor	No. of batches	Spawning period (days)	Batch	Spawned vol. (ml)	% fertilized	Mean diameter (μm)	Mean dry wt (μg)	PES[a]
1	72	3906	2886	0.910	13	24	3	269	99	1415	105	19.0
							6	341	100	1373	105	43.3
							12	195	100	1300	80	96.1
2	74	4372	3156	0.929	16	30	3	309	60	1449	113	11.7
							6	353	100	1393	105	33.0
							9	426	100	1364	99	56.6
							15	79	100	1275	75	99.3
3	60	1872	1332	0.742	12	22.5	4	191	99	1351	98	24.7
							6	216	99	1341	97	47.7
							12	97	100	1271	81	100
4	65	2768	2162	0.898	12	23	9	325	100	1356	95	83.1
5	70	3440	2513	0.868	14	27.75	3	240	100	1416	115	11.5
							6	260	99	1418	108	32.2
							9	462	100	1367	101	66.6
							12	250	96	1305	89	94.2
6	68	2701	2130	0.768	17	32	3	193	100	1404	99	13.5
							6	252	100	1374	100	39.8
							9	154	94	1337	93	60.9
							12	136	100	1311	85	76.1
							15	115	98	1260	78	93.7
7	69	3054	2033	0.774	19	40	9	271	100	1324	93	50.2
							12	195	98	1274	90	71.5
							15	147	100	1231	79	90.0
8	73	3618	2802	0.825	16	32	3	319	100	1450	117	14.5
							6	376	100	1420	113	37.3
							9	182	100	1378	102	57.6

[a]Proportion of eggs spawned.

Table II: Cod specific gravity, σ_t [mean and SD (in italics)] for each female, batch and stage analysed

Female	Batch	Stage															
		1		2		3		4		5		6		7		8	
1	3	22.31	1.15	23.91	1.67	24.40	0.97	25.44	0.93	25.86	0.82	25.67	0.78				
	6					23.70	1.21										
	12			22.89	1.25					25.06	0.99						
2	3	21.39	1.65	23.03	1.71	24.40	1.06	25.18	0.61	24.86	0.64	24.78	0.81				
	6	23.08	1.26							25.36	0.74						
	9			22.51	1.09					31.02	0.76						
	15					24.26	0.97			26.64	0.82	25.73	0.97	26.02	0.92		
3	4			23.22	1.17												
	6	23.09	1.06							25.90	0.86						
	12			24.89	1.32					25.99	1.21						
4	9			23.62	0.66	24.18	0.91	25.53	1.70	25.94	1.06	26.33	1.06	24.00	1.07		
5	3			23.28	1.37					29.51	2.39						
	6	22.84	0.98							26.96	1.18						
	9			22.92	1.13					24.89	0.70						
	12	23.08	1.90							28.03	0.94						
6	3							25.80	0.68	26.67	0.48						
	6	21.70	1.62							26.75	0.75						
	9	22.35	1.99							27.42	0.62						
	12	24.34	1.30							26.76	0.74						
	15			25.17	1.23	26.10	0.86	26.11	0.69	26.10	0.58	27.75	0.49	25.44	1.09	24.37	1.41
7	9	23.03	1.29							28.81	1.11						
	12			25.05	1.81					28.89	1.33						
	15	24.59	1.46			25.40	1.97	27.09	0.65	27.01	1.12	27.08	0.91	26.04	1.70	24.56	1.21
8	3	23.65	1.77														
	6			23.48	1.37					25.73	1.03						
	9	23.71	1.33	24.13	0.83	24.27	1.48	25.64	1.63	25.82	1.17	25.63	1.54				

ml^{-1}) from each selected batch were put into one of several aquaria (1–10 l) placed side by side in a large temperature-regulated water bath (5°C, precision: ±0.1°C). Each aquarium was individually supplied with UV- and sand-filtered sea water, i.e. the IMR Biotest system (Serigstad, 1997). Dead eggs were removed each day by means of a siphon. Live eggs that showed signs of infection were also, as far as possible, removed.

Larvae (n = 50) taken from the above selected batches were anaesthetized (more correctly, sedated, see Discussion) and staged. The larval cod staging system of Fossum (Fossum, 1986) supplemented with additional details in Hunt von Herbing *et al.* (Hunt von Herbing *et al.*, 1996) was used (Table III). Larvae in an identical phase of development were placed in a 50 ml beaker kept on ice and containing 50 mg metomidate hydrochloride l^{-1} 5°C filtered sea water. More precisely, 0.5 ml of a stock solution of 5 g of metomidate hydrochloride (Norsk Medisinaldepot, Oslo) and 35 g of antifoam (Dow Corning 1510 Antifoam, Lindberg and Lund AS, Oslo) per litre of distilled water [based on Mattson and Riple (Mattson and Riple, 1989)] was added to the beaker. According to the manufacturers (Dow Corning Corporation, 2002) antifoam agent consists of water, polydimethylsilaxane and methylcellulose, together accounting for 75% to 80% of the weight. Following 30 min of exposure to this bath, 25 larvae were carefully transferred by a pipette to the buoyancy apparatus described below. The above selected length of time of exposure and concentration of metomidate were determined in a series of small-scale tests.

Larval buoyancy was determined measuring the larval specific gravity in a density-graduated column (Coombs apparatus). Introductory trials indicated that the larvae needed to stay in the density-graduated column for 30 min before any consistent record of larval specific gravity

Table III: Cod larval staging system

Stage 1	The yolk sac is egg shaped. The eyes are incompletely pigmented (brownish). The gut is tube formed and thick walled, being smooth on the inside. This stage is seldom found in the field because of its short duration. Duration 0–1/4 day after hatching.
Stage 2	The yolk sac is spherical. The eyes are incompletely pigmented (greyish–brownish). The gut is tube formed and thick walled, smooth on the inside, 1/4–2 days after hatching (5ºC).
Stage 3	The yolk sac is elliptical, the eyes completely pigmented (black), the wall of the gut is thin and smooth on the inside. The gut can be distended and is separated into gut and rectum. The liver is round, the larval jaw is overshot and unangled. Swimbladder is a small distinct sac, dorsal to intestine and covered with pigment. This stage is present in the period 2–3 days after hatching (5ºC).
Stage 4	The yolk sac is cylindrical, the wall of the gut is thin and smooth on the inside, but can be irregular against the hindgut. The liver is irregular, the jaws are equal in length, or the jaw is overshot. The lower jaw is angled and the basis of the preorbial fin is found at this angle or in front of it. The swimbladder is a distinct sac covered dorsally by chromatophores; not yet functional. This stage is present in the period 3–4 days after hatching (5ºC).
Stage 5	The yolk sac is cylindrical or wedge shaped. The largest vertical diameter through the yolk sac is larger or equal to the myotomal height measured above the swimbladder. The inside of the gut is irregular. The liver is irregular. The larva has an underhung jaw, and the angle in the lower jaw is found in front of the preorbial fin. The mouth is functional. Swimbladder is an enlarged rounded sac, dorsal pigmentation is dense; not yet functional. This stage is present in the period 4–6 days after hatching (5ºC).
Stage 6	A remnant of the yolk sac is present. The largest vertical diameter through the yolk sac is less than the myotomal height measured above the swimbladder. The mouth is functional, and the larva is found with gut content. This stage is present in the period 6–8 days alter hatching (5ºC).
Stage 7	The yolk sac is either empty or some small granules of yolk mass can be seen. This stage is present in the period 8–12 days after hatching (6–7ºC).
Stage 8	The cell layers which enclosed the yolk sac are reduced to a string under the gut. This stage is present in the period 10–16 days after hatching (6–7ºC).

could be reported. Although larvae did sink continuously, it appeared that they generally sank relatively fast for the first minutes but hardly noticeably after 20–30 min. In the main study, the position of each larva was recorded at 15, 25, 30 and 35 min, but analysing statistically only the 30 min data. Individual larvae that demonstrated a fluctuating path between 25 and 35 min were excluded; they were considered to be moribund or awakening.

Sedated larvae were introduced into one of two tubes in a density-graduated column (Coombs apparatus) (Martin Instruments Ltd, UK). All examinations were made under normal fluorescent light. The gradient was established by using commercial seawater salt (Instant Ocean, USA) and distilled water, and adjusted to 7˚C (precision: ±0.5ºC). A new gradient was made for every group of larvae. The water bath, in which the tubes were placed, contained antibiotics and fungicides to avoid any problems reading the scale on the tubes due to turbidity. Within each tube, four density bulbs with specific gravity given in four digits calibrated at 23ºC by Martin Instruments Ltd and covering the full range observed for larvae were used as references. All bulbs were properly cleaned before use. Linear regressions between the vertical positions (to the nearest 0.1 cm) of bulbs in the tube and their specific gravities were highly significant (r^2 being close to 1). The positions of the bulbs were recorded at 30 min but noticed to be stable within a couple of minutes. After all recordings were made, the tube was emptied and all the larvae collected.

All transformed larval specific gravity data were adjusted to a temperature of 5ºC, i.e. to an environmental temperature typically encountered in the field (Aure and Østensen, 1993). Based on a final evaluation 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. All reported specific gravity values are given as σ_t, which is equal to $(\rho - 1) \times 10^3$, where ρ is the specific gravity (in g cm^{-3}).

Experimental designs and statistics

The experiment was carried out to analyse buoyancy (i.e. specific gravity) variation through ontogeny of the larvae, the relationships between female and egg attributes and larval buoyancy and, finally, buoyancy in different batches. Therefore, three different experimental designs were used.

To test buoyancy in relation to developmental stage, larval specific gravity was recorded every day from the time of hatching to the time of stage 8 using the above staging scale. Six analyses were performed on three different batches (3, 9 and 15), studying in each case two females: batch 3 for females 1 and 2; batch 9 for females 4 and 8; batch 15 for females 6 and 7. Differences in larval specific gravity among developmental stages were compared using analysis of variance (ANOVA). Differences within each of the stages were assessed with a post hoc Tukey honest significant differences (HSD) test. For the rest of the batches analysed (see Table I), specific gravity was recorded at early (1–2) and late stages (5), and subsequently compared using a Student *t*-test.

The relationships between female and egg attributes and larval specific gravity were explored using Pearson correlations. For this purpose, total length, initial and final whole body weight, initial, final and mean Fulton's condition factor were used as female attributes, dry weight and diameter as egg features, and larval specific gravity at stages 1, 2 and 5, for each batch and female analysed. In addition, the specific gravities of all larvae during their first day of life after hatching were pooled for each batch and female, and named as 1st day post- hatching specific gravity.

Differences in larval specific gravity among batches at different stages were compared using ANOVA for the whole effect and post hoc Tukey HSD test to assess the differences among batches. Data on larval specific gravity were pooled for each of the batches analysed (3, 6, 9, 12 and 15), irrespective of female origin.

Finally, all data were pooled and interpolated to create a mesh plot using inverse distance weighting of stage–batch–specific gravity triplet data. All statistical analyses were performed using Statistica (StatSoft, Inc., 1995).

RESULTS

Changes in larval buoyancy with development stage

Larval specific gravity (σ_t) increased steadily, i.e. the larvae became less buoyant, during the early stages of development up to approximately stage 3–4, corresponding to 20–25 day-degrees (Figure 1). During the following stages 4–6 (25–35 day-degrees), σ_t remained nearly constant, but then started to decrease gradually (more buoyant larvae) from stage 6 to 8 (35–55 day-degrees). In the latter situation, 80–100% of the yolk sac was absorbed, while the swimbladder is known to be functional from stage 7 onwards (Fossum, 1986). However, as no food was provided in this experiment, some of these older larvae were probably starving, which might be a partial explanation for the noticed decline in σ_t.

For the six sets of larvae where σ_t was measured in each developmental stage, it was found that late-season larvae were heavier than early-season larvae (Figure 1). Thus, σ_t at batch 15 in both females analysed (6 and 7) was always higher at all stages compared with the other reported results. Values of σ_t for batches 3 and 9 were quite similar, except at stage 1. The change in σ_t from early- to late-stage larvae was higher for batch 3 than for batch 15 (Figure 1).

Further statistical analysis revealed several significant developmental effects on mean σ_t (ANOVA) (Table IV). For early-season larvae (batch 3), there appeared to be a significant difference both within early stages (i.e. stages from 1 to 3) and between early and late stages (4–6), but not within late stages. In middle- (batch 9) and late-season batches (batch 15), there was no significant difference within either early or late stages, but significant differences appeared between early and late stages. An exception to this trend is the previously mentioned batch 15–female 6 data, where a significant difference occurred between stage 2 and 3. In addition to these data, σ_t was also recorded at early stages (1 or 2) and at stage 5 in other batches and females as well, but where no detailed development studies were performed. In all of these (except again female 6 batch 15), significant differences were detected in mean σ_t between early and late stages (Table V). In five cases, σ_t was recorded in stages older than 6 (Figure 1; Table IV). Mean σ_t at stages 7 and 8 was significantly different from that for those larvae in stage 6, but declining rapidly towards values reported for larvae in early stages.

Maternal and egg influences on larval buoyancy

Some but not all maternal traits listed presently had a significant effect on larval buoyancy (Table VI). Female total length was for all stages clearly not significantly correlated with mean σ_t, while whole body weight was very close to being so at 1st day post-hatch. However, mean Fulton condition factor appeared to be significantly negatively correlated with mean σ_t (irrespective of the batch) at earlier stages but not at stage 5 (Table VI; Figure 2). At the earlier stages, the highest correlation coefficient ($r = -0.82$) was found for stage 2. Specific gravity of larvae analysed during their first day after hatching (i.e. all larvae

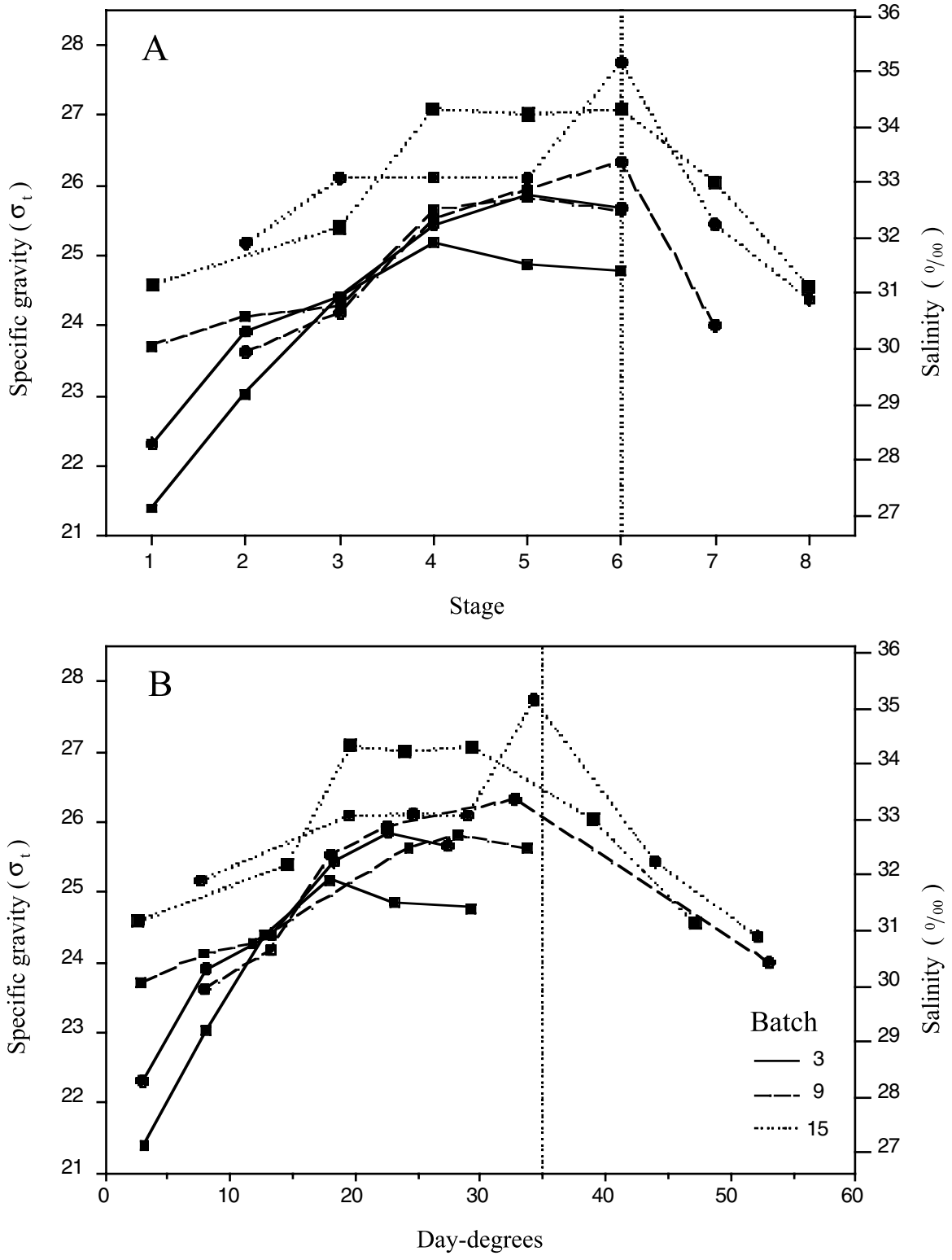

Fig. 1. Specific gravity of cod larvae at each developmental stage (**A**) and with time (day-degrees) (**B**) for batch 3–females 1 (solid line and circle) and 2 (line and square), batch 9–females 4 (dashed line and circle) and 8 (dashed line and square) and batch 15–females 6 (dotted line and circle) and 7 (dotted line and square). The vertical dotted line refers to the moment when the larval mouth becomes functional and the yolk sac is considerably reduced.

Table IV: Results of the Tukey HSD post-hoc comparison to test differences in mean specific gravity in relation to developmental stage

		Post-hoc comparisons						ANOVA
		Stage						
		1	2	3	4	5		
Batch 3	2	**						
Female 1	3	**	n.s.					
	4	**	**	n.s.				$F = 24.70$
	5	**	**	**	n.s.			$P < 0.0001$
	6	**	**	*	n.s.	n.s.		
		1	2	3	4	5		
Batch 3	2	*						
Female 2	3	**	n.s.					
	4	**	**	n.s.				$F = 20.33$
	5	**	**	n.s.	n.s.			$P < 0.0001$
	6	**	**	n.s.	n.s.	n.s.		
		2	3	4	5	6		
Batch 9	3	n.s.						
Female 4	4	**	**					
	5	**	**	n.s.				$F = 18.95$
	6	**	**	n.s.	n.s.			$P < 0.0001$
	7	n.s.	n.s.	**	**	**		
		1	2	3	4	5		
Batch 9	2	n.s.						
Female 8	3	n.s.	n.s.					
	4	**	*	*				$F = 7.94$
	5	**	*	*	n.s.			$P < 0.0001$
	6	**	*	*	n.s.	n.s.		
		2	3	4	5	6	7	
Batch 15	3	*						
Female 6	4	*	n.s.					
	5	*	n.s.	n.s.				$F = 23.79$
	6	**	**	**	**			$P < 0.0001$
	7	n.s.	n.s.	n.s.	n.s.	**		
	8	n.s.	**	**	**	**	**	
		1	3	4	5	6	7	
Batch 15	3	n.s.						
Female 7	4	**	**					
	5	**	**	n.s.				$F = 13.37$
	6	**	**	n.s.	n.s.			$P < 0.0001$
	7	**	n.s.	n.s.	n.s.	*		
	8	n.s.	n.s.	**	**	**	*	

*$P < 0.05$; **$P < 0.01$; n.s., not significant.

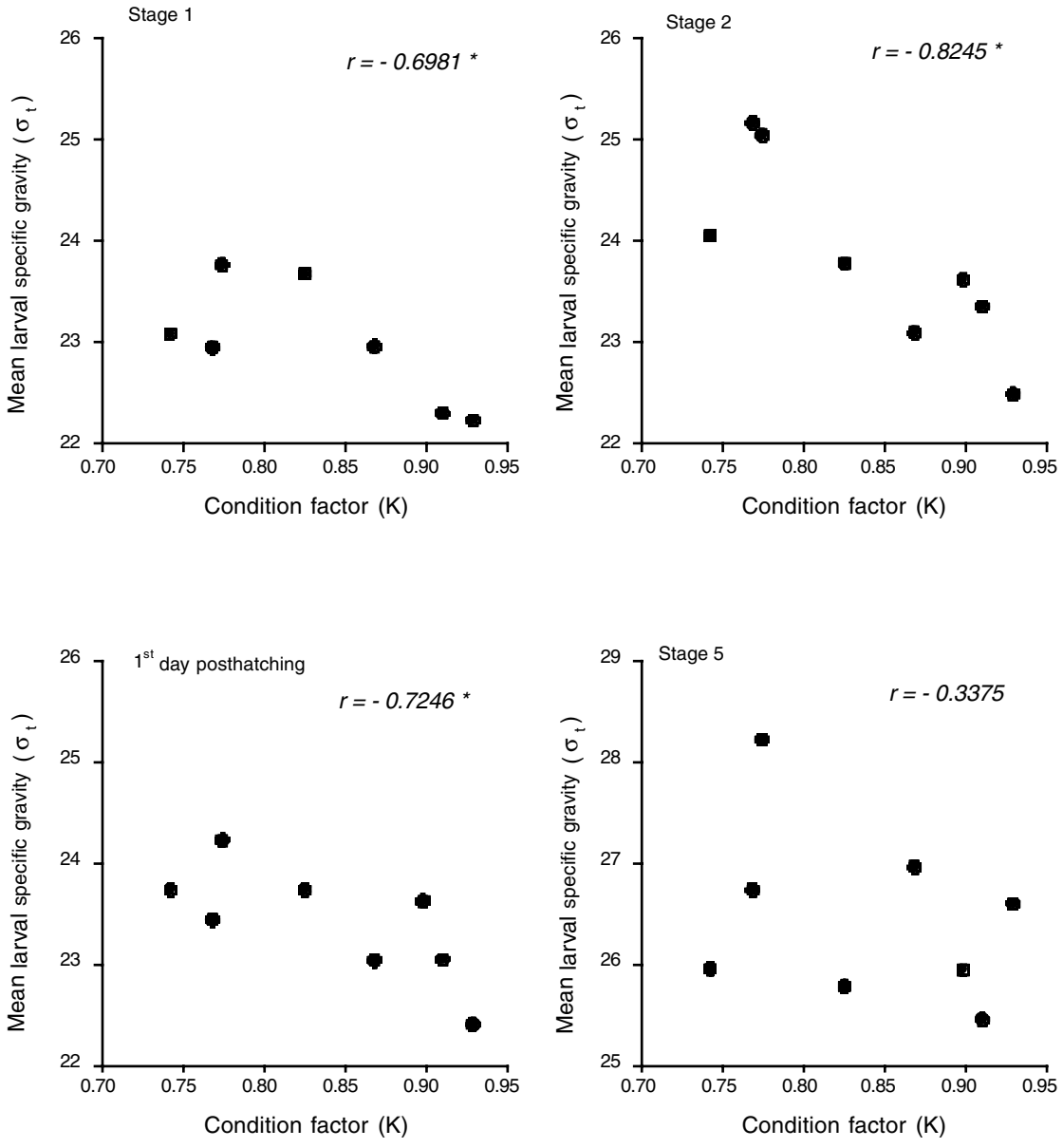

Fig. 2. Relationships between mean cod larval specific gravity and female mean Fulton's condition (*K*) at different larval developmental stages. Note that the stage 5 plot has different scaling on the *y*-axis.

at stage 1 and some at stage 2) also showed a significant negative correlation with mean Fulton condition factor. Significant correlations were also found between initial and final condition factor and mean σ_t at stage 2 (Table VI).

Bigger eggs resulted in more buoyant larvae at earlier stages (Table VI; Figure 3). Egg diameter and mean σ_t were significantly correlated at earlier stages (stages 1 and 2) and 1st day post-hatch, but not at stage 5. Egg dry weight and mean σ_t showed high negative *r* values at earlier stages, but were significant only at 1st day post-hatch.

Seasonal changes in larval buoyancy

Larval specific gravity increased as spawning season evolved (Figure 4; Table VII). Eggs produced by females early in the spawning season were significantly larger and heavier than those produced towards the end of the spawning season (ANOVA; $P < 0.01$ in both cases). Probably correlated with this fact, examples of significant

Table V: Results of Student's t-test on differences in mean specific gravity between early (stage 1 or 2) and old (stage 5) larvae

Female	Batch	Mean density at stage			P
		1	2	5	
1	3	22.31	23.91	25.86	**
	12		22.89	25.06	**
2	3	21.39	23.03	24.86	**
	6	23.08		25.36	**
	9		22.51	31.02	**
	15		22.07	26.64	**
3	6	23.09		25.90	**
	12		24.89	25.99	*
4	9		23.62	25.94	**
5	3		23.28	29.51	**
	6	22.84		26.96	**
	9		22.92	24.89	**
	12	23.08		28.03	**
6	6	21.70		26.75	**
	9	22.35		27.42	**
	12	24.34		26.76	**
	15		25.17	26.10	n.s.
7	9	23.03		28.81	**
	12		25.05	28.89	**
	15	24.59		27.01	**
8	6		23.48	25.73	**
	9	23.71	24.13	25.82	**

*$P < 0.05$; **$P < 0.01$; n.s., not significant.

Table VI: Pearson correlation coefficients between attributes of females, eggs and mean larval specific gravity

	Stage 1		Stage 2		1st day post-hatching		Stage 5	
Female length	–0.236	0.613	–0.390	0.342	–0.499	0.208	0.062	0.885
Female initial weight	–0.445	0.317	–0.623	0.099	–0.664	0.073	–0.045	0.916
Female final weight	–0.453	0.308	–0.624	0.099	–0.679	0.064	–0.184	0.662
Female initial *K*	–0.650	0.114	**–0.828**	0.011	–0.700	0.053	–0.175	0.679
Female final *K*	–0.661	0.083	**–0.744**	0.034	–0.686	0.06	–0.498	0.209
Female mean *K*	**–0.698**	0.048	**–0.825**	0.012	**–0.725**	0.042	–0.338	0.414
Egg diameter	**–0.561**	0.046	**–0.614**	0.026	**–0.590**	0.003	–0.143	0.524
Egg dry weight	–0.510	0.075	–0.503	0.080	**–0.497**	0.016	–0.005	0.981

The significance level is in italics; bold values are significant correlations ($P < 0.05$). K is Fulton's condition factor, the mean values refer the midpoint of initial (pre-spawning) and final (spent) values.

differences in mean σ_t (ANOVA; $P < 0.01$) were detected between batches at all stages analysed (1–7 and 1st day post-hatch). More precisely, at early stages (1, 2 and 1st day post-hatch) there were no significant differences in mean σ_t between batches 3, 6 and 9, but there were between these three batches and late-season batches (12

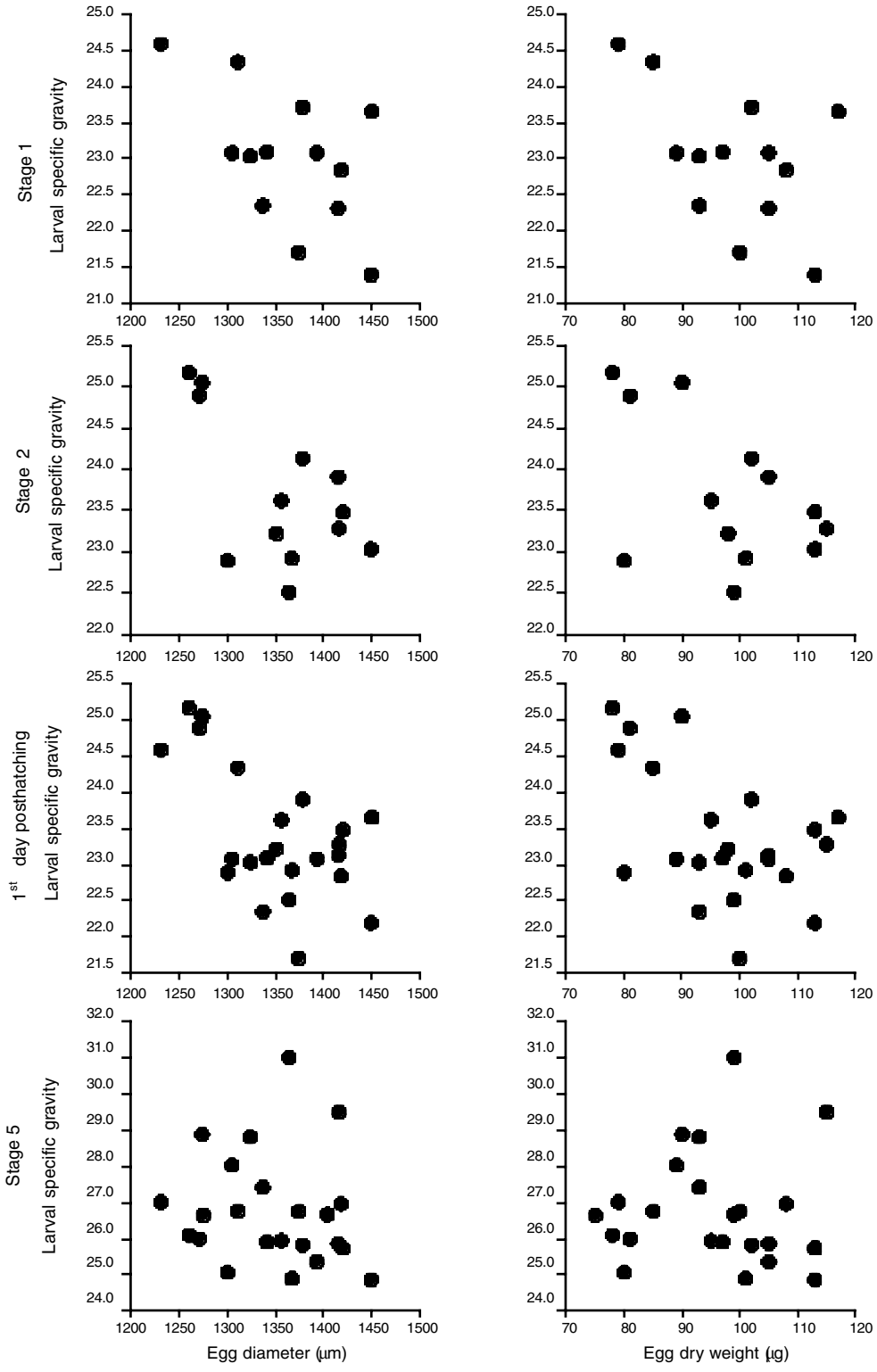

Fig. 3. Relationships between mean cod larval specific gravity at different developmental stages and mean egg diameter and dry weight. Note that there are differences in *y*-axis scale among plots.

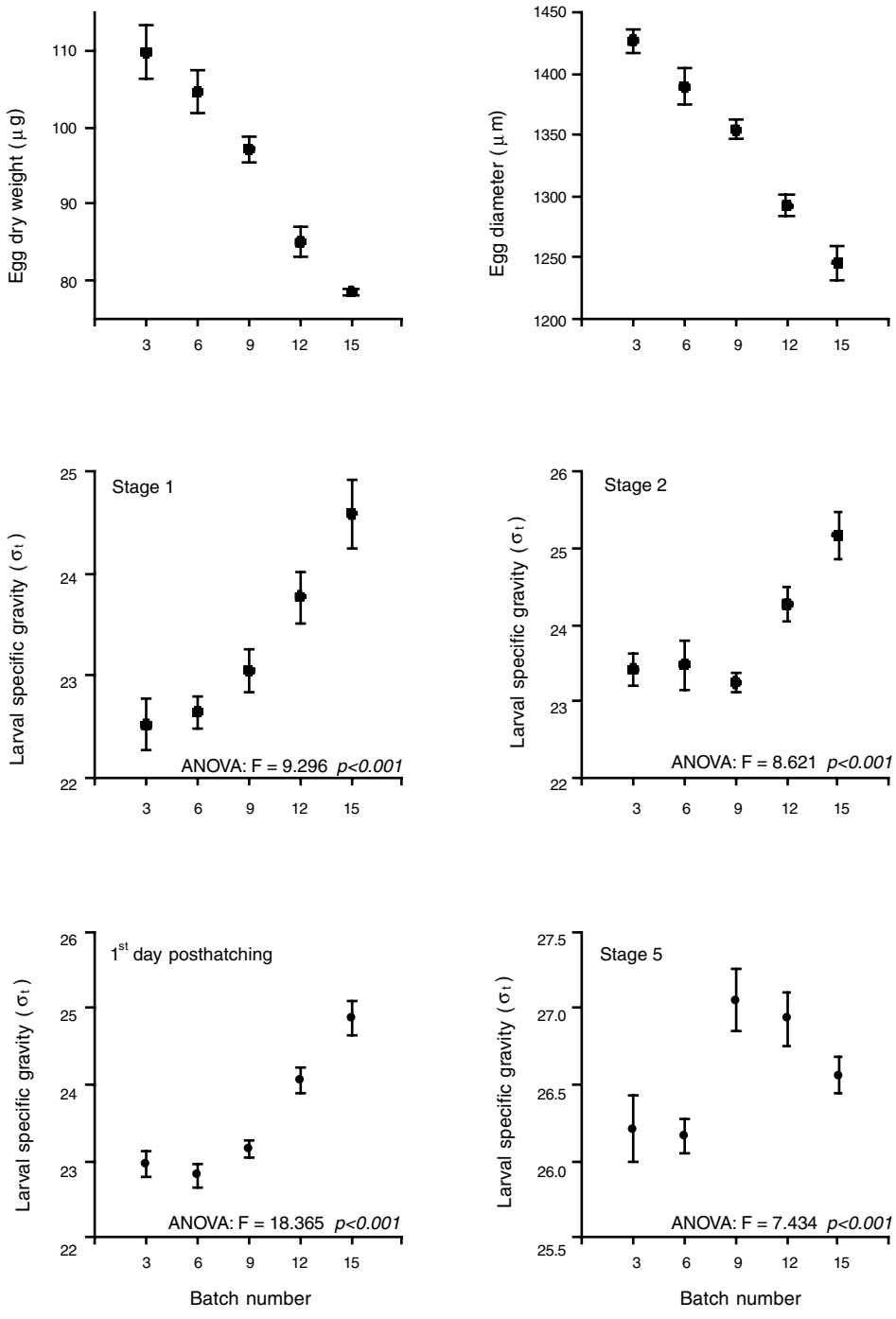

Fig. 4. Mean (± SEM) egg diameter, egg dry weight and specific gravity of cod larvae from selected batches. Specific gravity is shown at the selected stages. Note that there are differences in *y*-axis scale among plots.

and 15). Also, differences occurred between batches 12 and 15, except for stage 1 ($P = 0.063$; close to being significant). At a later stage (stage 5), no differences were found either between batches 3 and 6, or between batches 9 and 12, but significant differences were detected between these two pairs. At this stage, batch 15 was significantly different only from batch 9.

Overview

For synthesis and visualization of the previously described

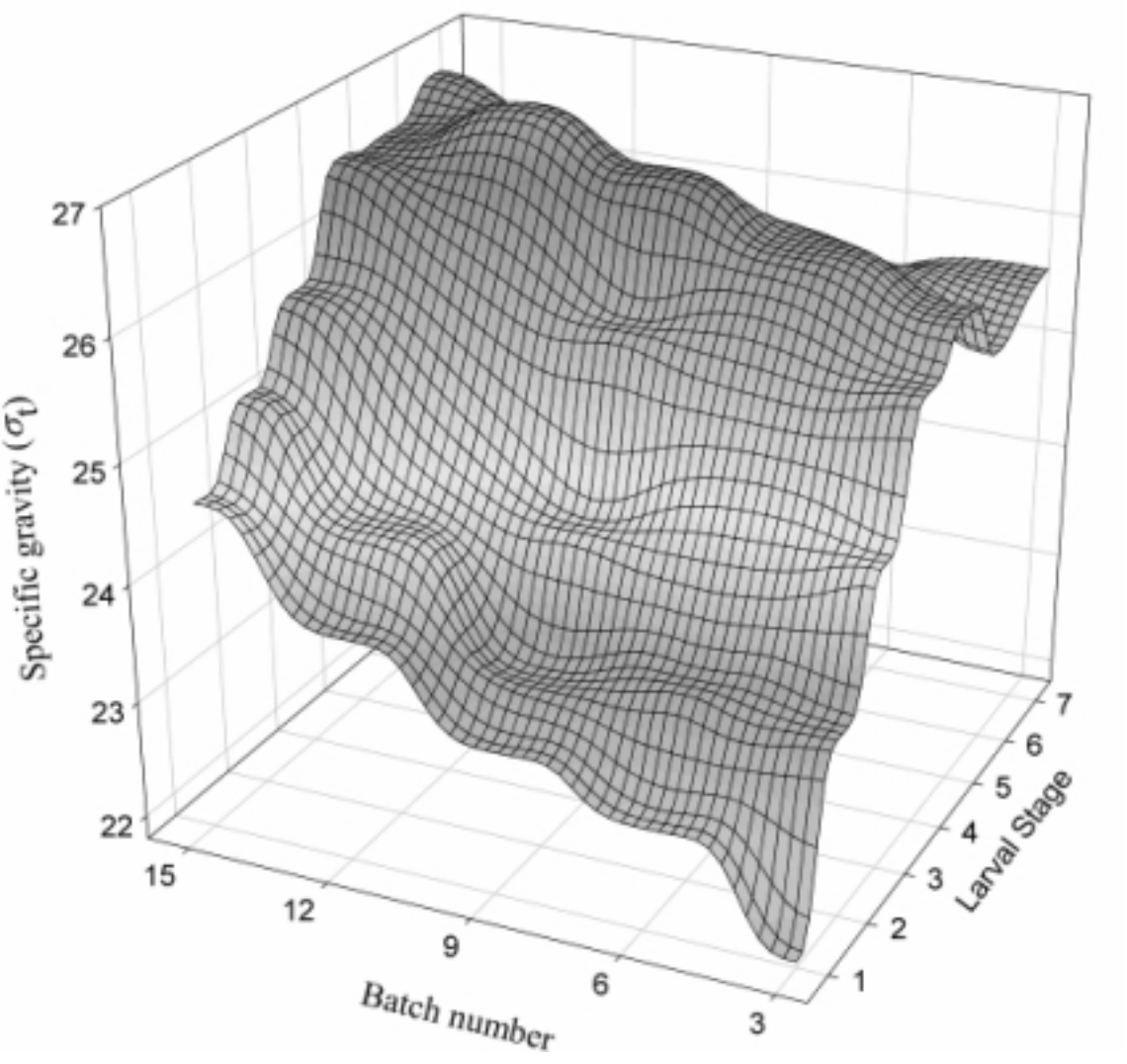

Fig. 5. Three-dimensional mesh plot for all present cod larval specific gravity data pooled and interpolated in relation to larval stage and batch number.

patterns, all buoyancy data were pooled and interpolated to create a mesh plot using batch and larval stage as independent variables (Figure 5). As expected from the above, separate analyses, a general, progressive increase in larval specific gravity was seen along both of these two axes. Thus, the most buoyant larvae (mean $\sigma_t \approx 21$) were newly hatched ones (stage 1) from early-season eggs and the less buoyant larvae (mean $\sigma_t \approx 27$) were older ones (stage 6) from late-season eggs. The increasing pattern in specific gravity was more or less constant with season and larval stage, although the differences were greatest at early stages. In agreement with earlier results, the three-dimensional plot showed a fall in mean σ_t following stage 6.

DISCUSSION

Based on the literature, the buoyancy of Atlantic cod larvae is expected to be the result of multiple mechanisms, including physical, morphological and physiological factors varying in relative strength over time. The vertical distribution of marine fish eggs is known to depend on ambient water density (a function of salinity and temperature), turbulent mixing (wind speed) and buoyancy of individual eggs (Sundby, 1991). Logically, the same principles should apply to larvae prior to the formation of body structures that may influence their specific location in the water column, namely the swimbladder, and morphological characteristics necessary for swimming activity.

Table VII: Results of the Tukey HSD post-hoc comparison to test differences in larval specific gravity in relation to batch number at each developmental stage

	Batch	Batch			
		3	6	9	12
Stage 1	6	0.6651			
	9	0.0809	0.1497		
	12	**0.0001**	**0.0003**	**0.0227**	
	15	**0.0000**	**0.0000**	**0.0003**	0.0632
Stage 2	6	0.8774			
	9	0.5322	0.5515		
	12	**0.0027**	**0.0471**	**0.0001**	
	15	**0.0000**	**0.0008**	**0.0000**	**0.0274**
1st day	6	0.5041			
post-hatching	9	0.3597	0.1145		
	12	**0.0000**	**0.0000**	**0.0000**	
	15	**0.0000**	**0.0000**	**0.0000**	**0.0076**
Stage 5	6	0.8649			
	9	**0.0001**	**0.0000**		
	12	**0.0076**	**0.0012**	0.1390	
	15	0.2427	0.1425	**0.0089**	0.1761

See also Figure 4. Numbers are the *P* values for each analysis.

Measurements of buoyancy might still be relevant after this time, particularly as inflation of the swimbladder does not necessarily overcome negative buoyancy (Hoss *et al.*, 1989). Another issue is that Shelbourne (Shelbourne, 1956) and recently Nissling and Vallin (Nissling and Vallin, 1996) both concluded that buoyancy of pelagic teleost larvae is also regulated by the appearance of subdermal spaces of low density. Furthermore, Blaxter and Ehrlich demonstrated that changes in protein, lipid and water content result in differential buoyancy forces acting through early stages of ontogeny (Blaxter and Ehrlich, 1974). In this line, the building of body structures such as bones and muscles and the corresponding reduction of yolk sac should negatively alter larval buoyancy.

The buoyancy of the present cod larvae decreased steadily from hatching up to stage 6 (35 day-degrees), then progressively increased. The latter might relate to swimbladder formation and proximate body composition. Our experiment ended at ~7–8 days post-hatch (stage 6–7), although the swimbladder might not have been fully functional until 2–3 days later (Hunt von Herbing *et al.*, 1996). Another reason for ending the experiment at this step in the development was that none of the larvae was given food; therefore, effects of starvation *per se* could start to occur. Note also that cod larvae have been shown to have a functional mouth and gut at stage 6 (Hunt von Herbing *et al.*, 1996). The general effect of starvation on larval buoyancy is believed to make the organism more buoyant (Blaxter and Ehrlich, 1974; Tilseth and Strømme, 1976; Yin and Blaxter, 1987a). On the other hand, Sclafani *et al.* (Sclafani *et al.*, 1997) stressed the importance of any loss of osmoregulation capacity that may reduce the ability to float. In Sclafani *et al.*, cod larvae were continuously exposed to MS-222 while being kept in the density- graduated column (Sclafani *et al.*, 1997). To our knowledge, metomidate (hydrochloride) has not previously been used to temporarily inactivate fish larvae for subsequent measurements of buoyancy. This chemical worked very well, but any true comparison of this anaesthetic with others, such as MS-222 and benzocaine, was, however, considered beyond the scope of this study. Metomidate is known to have very few negative side-effects, a high safety margin and considered to have a hypnotic instead of an analgesic effect (Mattson and Riple, 1989; Brattelid, 1998).

The role of the vertical position of the larvae in their likely survival has been discussed elsewhere (Yin and Blaxter, 1987a; Sclafani *et al.*, 1993, 1997; Anderson and de Young, 1994; Gotceitas *et al.*, 1996; Lough *et al.*, 1996; Grønkjær and Wieland, 1997), but larvae should obviously ideally develop in layers where the conditions are optimal, i.e. in terms of salinity, oxygen content, predators and concentration of prey. Energetically, it should also be an

advantage to be neutrally buoyant, keeping a low level of general activity. In the first few days after hatching, cod larvae do not move very much by themselves and obtain oxygen only from cutaneous diffusion (Hunt von Herbing and Boutilier, 1996). During the mixed-feeding period, i.e. from 6 days post-hatch at 5°C for Newfoundland cod larvae (Hunt von Herbing and Boutilier, 1996), higher costs of activity are incurred in maintaining the position in the water column, as the swimbladder is believed, as discussed above, to be not yet fully functional. Because marine fish larvae have very low initial feeding rates (Yin and Blaxter, 1987b), conservation of energy is essential at that time. Thus, larvae with a higher relative content of yolk at the beginning of the exogenous feeding period should be in a better position to survive. In addition, Pepin *et al.* (Pepin *et al.*, 1997) stated the significance of a close relationship between environmental temperature and growth rates of larvae; larvae living in an optimal temperature range will grow faster and reach the exogenous feeding period at a larger size and hence with higher prey-catching capabilities (Otterå, 1993).

This study shows that North-east Arctic cod larvae are clearly buoyant just after hatching, showing a density <25 σ_t, while the relevant environmental density (Skrova and Eggum hydrographic stations) ranges from ~26.5 σ_t in the surface layer (coastal water) to ~27 σ_t in the transition layer (~150 m) between the coastal water and the deeper Atlantic water (Aure and Østensen, 1993). Owing to the loss of the very compact eggshell (~1.2 g cm^{-3} or 200 σ_t) (Kjesbu *et al.*, 1992) at hatching, the originating larva is expected to be more buoyant than the egg. Baltic cod larvae in the days just after hatching are known to float close to the surface (Nissling *et al.*, 1994), i.e. well away from any anoxic bottom layers. This general high-up-in-the-water strategy can be an advantage for the North-east Arctic cod larvae in another way as the northward coastal current speed is typically highest near the surface (Aure and Østensen, 1993). The low larval specific gravity at the early stages implies that the larvae move passively upwards while body development occurs. During the ascent, eyes and jaws develop, so that by the time the larvae reach the near surface layers, where suitable food items are found, they are functionally capable of feeding exogenously (Grønkjær and Wieland, 1997; Ådlandsvik *et al.*, 2001). As the larva moves upwards, its specific gravity increases and finally balances the surrounding environment; larvae at stages 4–6 (when the mouth becomes functional) are neutrally buoyant. These types of changes in cod larval buoyancy have been reported earlier in other areas such as the North Sea (Yin and Blaxter, 1987a) and the Baltic Sea (Nissling and Vallin, 1996), as well as for Norwegian coastal cod (Ellertsen *et al.*, 1980) and North-east Arctic cod (Tilseth and Strømme, 1976). The passive movement towards the surface is probably also an advantage from an energetic point of view, to limit locomotion and hence visibility to predators during early development, as hypothesized in blue whiting (*Micromesistius poutassou*) by Ådlandsvik *et al.* (Ådlandsvik *et al.*, 2001). Active diurnal vertical migration has been shown for many fish larvae [see (Coombs *et al.*, 2001) and references therein], but generally only for the older ones with more developed locomotory capabilities. Relatively small larvae showed little evidence of diurnal migration in different species (Ellertsen *et al.*, 1980; Nissling *et al.*, 1994; Ådlandsvik *et al.*, 2001; Coombs *et al.*, 2001). In Norwegian coastal cod (Ellertsen *et al.*, 1980), locomotory activity increased as larvae developed, with a maximum at days 4–9 after hatching, which in our study corresponds to stage 5–8. Thus, for younger larvae, buoyancy may clearly be one of the most important factors influencing vertical distribution.

We found that maternal weight and condition factor, egg diameter and egg dry weight were all negatively related to larval specific gravity, but this was not always statistically significant. Several of these predictors were obviously interrelated. For instance, female cod size has been found to be positively related to egg diameter (Kjesbu, 1989; Kjesbu *et al.*, 1996; Trippel, 1998; Vallin and Nissling, 2000; Marteinsdottir and Begg, 2002). This was not found by Chambers and Waiwood (Chambers and Waiwood, 1996), probably due to a narrow size range of females (Vallin and Nissling, 2000). Pepin *et al.* also found a positive correlation between egg size and larval size, although temperature was the most important factor in explaining variance in cod larval size (Pepin *et al.*, 1997). But for buoyancy, probably more important than larval size is initial yolk sac size, which is expected to be proportional to egg size (Knutsen and Tilseth, 1985; Miller *et al.*, 1995). Furthermore, egg size is negatively correlated to neutral egg buoyancy (Nissling and Vallin, 1996; Vallin and Nissling, 2000). However, Marteinsdottir and Begg found in Icelandic cod a positive correlation between egg size and egg density (Marteinsdottir and Begg, 2002). In any case, larval specific gravity just after hatching should be closely related to egg density.

Changes through ontogeny showed different patterns for different batches. Larvae just after hatching were positively buoyant in all batches, but the late-season larvae were less so. Larval specific gravity increased as spawning season evolved, which is a reflection of the decreasing trend observed in egg size and egg dry weight, following the correlation explained in the previous paragraph. However, although the larger eggs and the lower specific gravity larvae are produced at the beginning of spawning, at stage 5 larvae from early batches are still slightly positively buoyant, while larvae from middle and late batches

show a more clear tendency to be neutral buoyant with sea water during most of the time period prior to consumption of the yolk sac.

As the larvae develop, yolk sac consumption and the formation of the body structures lead to larval specific gravity depending more on their own body density, which is determined by the ontogeny and therefore influenced more by the environment than by egg attributes; a few days after hatching, egg and female characteristics were no longer significantly correlated with larval specific gravity. However, the maternal effect until that point might have influenced the life history of the larvae. Larger eggs produced by females with high condition factor produce larvae with a bigger yolk sac and with a lower specific gravity, distributed at shallower depths where the chance for more favourable conditions is greater, both in terms of physical environment and food availability for future larvae. Positive relationships were detected between egg and larval size characteristics and egg and larval viability in cod (Solemdal *et al.*, 1995; Kjesbu *et al.*, 1996; Marteinsdottir and Steinarsson, 1998; Nissling *et al.*, 1998; Trippel, 1998). In particular, larvae that hatched from the largest eggs initiated feeding earlier and expressed a higher incidence of feeding during the first days of feeding (Marteinsdottir and Steinarsson, 1998). In older larvae, feeding success might be a critical variable in determining their specific gravity and then their vertical distribution in the water column (Sclafani *et al.*, 1997). In younger larvae, prior to the exogenous feeding period, buoyancy is determining by yolk sac size, ontogeny and other variables as shown above. Different quality aspects may also play an important role in determining larval buoyancy, but this is still poorly studied. The fact that the females in the present study were recruit spawners can be of importance for the conclusions drawn, as repeat spawners and recruit spawners seem to differ in many respects (egg and larval size and viability, and seasonal egg buoyancy variation) (Kjesbu *et al.*, 1992, 1996; Solemdal *et al.*, 1995; Marteinsdottir and Steinarsson, 1998; Trippel, 1998). Therefore, one obvious way to proceed would be to test for buoyancy differences between larvae from recruit and repeat spawners, but also to model the vertical and horizontal drift patterns in a realistic scenario.

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