Behaviour of Atlantic cod (*Gadus morhua*) larvae: an attempt to link maternal condition with larval quality

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Abstract

Egg quality has been linked to maternal condition in several species of fish. Such inter-female and/or inter-batch differences in egg quality should be visible in the larvae that hatch from them. In an attempt to characterize possible links between the condition of Atlantic cod (*Gadus morhua*) females and that of their progeny, we tested the hypotheses that female nutritional status, size and/or incubation temperature are related to larval quality (growth rate and behaviour). Fertilized eggs were obtained from discrete spawning couples that had been maintained under controlled feeding and temperature regimes. Eggs and larvae were handled so as to maintain those from any given male-female cross and spawning event in isolation. Temperature was held at 6 °C and photoperiod was 14 h L:10 h D. The treatments applied to cod females were maternal ration x maternal temperature history (in 1995) and, in a second experiment, maternal ration x maternal size (in 1996). Three of the five or six male-female pairs from each of the four ration-temperature treatment groups, or ration-size treatment groups were targeted as sources of fertilized eggs. Larvae from these groups were subsampled at various intervals and their size and dry mass measured. Specific growth rate (SGR) was calculated from these data. Silhouette (shadow) video photography (SVP) was used to record the behaviour of cod larvae originating from these females-egg groups. SVP observations of cod swim paths were analysed frame-by-frame to extract the three-dimensional swim path coordinates of each larva. These swim paths were decomposed and analysed for swim speeds, durations and distances, "pause" (non-active displacement) duration, and vertical and horizontal changes of direction. The significant relationships between larval SGR and the swim path variables extracted from our behavioural observations support their use as indicators of

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larval performance-quality. While the incubation temperature of cod females was not related to our behavioural proxies of larval performance, there was at least an indication of a link with maternal size and ration, although the behavioural responses were inconsistent and, therefore, difficult to interpret. We conclude that, at least under non-extreme conditions (i.e., very low or very high temperatures and/or nutritional condition and/or size), the condition of cod females does not strongly affect the behavioural performance of the larvae that they produce. This conclusion must be qualified by saying that the behaviour of cod larvae from even a single male-female cross and egg batch is highly variable and that this confounds (and possibly masks) differences in the overall performance of larval groups.

Introduction

The concept of spawning stock biomass (SSB) – a fundament of fish population assessments – has recently been refined to include the observation that females do not all contribute equally to the fecundity of a population (Hislop 1988; Chambers et al. 1989; Solemdal 1997; Ouellet et al. 1997; Trippel et al. 1997; Dutil et al. 1998, 1999; Marshall et al. 1998; Marteinsdottir and Steinarsson 1998; Trippel 1999; Marteinsdottir and Begg 2002; Koster et al. 2003; Rätz and Lloret 2003). To succeed with this refinement of SSB, the "quality" of eggs and larvae (*sensu* Brooks et al. 1997), and not only the quantity, must be measurable, and strongly and consistently correlated with some index of spawner condition. Even in the simplest case of a species that releases all (or most) of its gametes at one time, this is a challenging assignment. It is even more difficult for species such as Atlantic cod (*Gadus morhua*), in which females are highly fecund determinate spawners that release their eggs in batches (Kjesbu 1989). Despite this, there have been several attempts to characterize the relationships between the condition of cod females and the number and quality of eggs that they produce (Kjesbu 1989; Kjesbu et al. 1991; Kjesbu 1994; Kjesbu and Holm 1994; Solemdal et al. 1995; Kjesbu et al. 1996; Chambers and Waiwood 1996; Marteinsdottir and Steinarsson 1998; Trippel 1998; Ouellet et al. 2001). Although the results of these studies have been inconsistent (reviewed by Ouellet et al. 2001, and see Discussion), it appears that, at least for females in good condition, (1) 17–19 egg batches are spawned per female over a period of four to six weeks, (2) the number of eggs liberated in each batch is variable, but is usually highest for the groups near the middle of the female's spawning cycle, (3) egg size and dry mass decrease from the first to the last batch, (4) egg diameter of the first batch is positively correlated with female length and (5) there is an inverse relationship between egg diameter and egg mortality. Further, cod females with high condition factors produce more previtellogenic oocytes, and use a larger fraction of them during vitellogenesis, than females with low condition factors (Kjesbu et al. 1991). Overall fecundity is also related to female condition; females with low condition factors produce fewer eggs than those with high condition factors (Kjesbu et al. 1991).

Inter-female and/or inter-batch differences in egg quality should be visible in the larvae that hatch from them. In yellow perch (*Perca flavescens*), older-larger females produced larvae that were shorter, but with larger yolk sacs and higher energy reserves than larvae from younger-smaller females (Heyer et al. 2001). These larval characteristics translated into differential survivorship,

although not differential growth (Heyer and Miller 2004). Female sea bass (*Dicentrarchus labrax*) in poor nutritional condition produced a much higher percentage of non-viable larvae than females in good condition (Cerda et al. 1994). This was also the case for Nile tilapia (*Oreochromis niloticus*) (Gunasekera et al. 1996). In the Japanese flounder (*Paralichthys olivaceus*), the percentage of normal larvae, survival of larvae at 3 days post hatching (DPH), and an index of starvation tolerance, were all positively correlated with levels of n-3 highly unsaturated fatty acids, and of arachidonic acid, in the diets of females (Furuita et al. 2000, 2003).

Female condition was positively correlated with feeding success of cod larvae, and larvae from older-larger females exhibited higher specific growth rates (at 15 DPH) (Marteinsdottir and Steinarsson 1998; Marteinsdottir and Begg 2002). Eggs liberated towards the end of a given female's spawning cycle (i.e. those from the last few egg batches) appear to produce larvae whose overall activity is less than that for larvae from earlier egg batches (Fig. 11 in Solemdal 1997, drawn from the unpublished results of A.B. Skiftesvik). Because activity and feeding rates translate into rates of growth and survivorship, these observations imply that cod larvae hatching from different egg batches, and/or from different females, may be more-or-less viable. In an attempt to further characterize possible links between the condition of cod females and that of their progeny, we tested the hypotheses that female nutritional status, size and/or incubation temperature are related to larval quality (e.g. growth rate, and as defined by Brooks et al. 1997). To succeed, we first had to establish that behavioural observations (overall activity, swim speed, swim path length, etc.) could be used as indicators of larval quality – observations that poor and/or sick groups of larvae are moribund, and behave differently from good and/or healthy groups (e.g. Skiftesvik et al. 1993), established that this was feasible. The work reported upon here further establishes a link between the behaviour of cod larvae and their quality and, with this as a basis, tests the hypothesis that female condition is related to the quality of the larvae that they produce.

The relationships between female cod and their larvae were evaluated in laboratory experiments conducted in 1995 and 1996. Material from these same experiments was used for complementary studies on female reproductive physiology-fecundity, and on egg quality (Lambert and Dutil 2000; Ouellet et al. 2001). The results on larvae, reported here, completes the life history stages that were evaluated in this project's attempt to trace maternal effects from pre-spawned gametes through to larvae.

Materials and Methods

Brood stock. Adult Atlantic cod used as brood stock in these experiments were captured by trawl from the St. Lawrence Estuary in June 1994 and 1995. After capture, and prior to experiments, the fish were maintained under natural photoperiod and temperature in 7.5 m^3 tanks at the Maurice Lamontagne Institute, Mont-Joli, Québec, Canada. During this period, animals were fed to satiation three times weekly on a diet of frozen capelin (*Mallotus villosus*).

Each year, 400 cod were divided into four groups of 100 fish that were transferred to separate 7.5 m^3 tanks in September. During the first experiment (referred to hereafter as the 1995 experiment), brood stock was further separated into two groups which were maintained under two temperature

regimes. In the first, cod were acclimated to 10 °C and the temperature was then gradually decreased (from September 1994 through January 1995) to a minimum of 6 °C. Afterwards, temperature was kept constant throughout spawning, which began in April 1995. In the second temperature regime, cod were acclimated to 6 °C and temperature was then decreased to 2 °C. Afterwards, temperature was kept constant throughout spawning. The temperature regimes applied are realistic of those to which cod from this geographic region would be exposed during the period preceding the reproductive season (i.e. gonad maturation) (Green and Wroblewski 2000). During the second experiment (referred to hereafter as the 1996 experiment), temperature was maintained at 4–5 °C throughout.

In both the 1995 and 1996 experiments, brood stock were fed upon different dietary regimes (details presented in Dutil and Lambert 2000) in an attempt to generate females in a range of pre-spawning nutritional condition. In the 1995 experiment, cod females were randomly assigned to the different feeding regimes, irrespective of their size. However, in the 1996 experiment, cod females were partitioned into two size classes (<55 cm: 51.2 ± 1.19 cm and >55 cm: 60.9 ± 2.99 cm). For each of these size classes, one group of females was fed on a low ration, and another on a high ration (Lambert and Dutil 2000). The condition factor (Fulton's K), expressed as the ratio between total weight (g) and length^3 (fork length in cm) multiplied by 100, was used as an index of condition for pre-spawning females. In cod, Fulton's K is a strong indicator of nutritional condition and energy reserves (Lambert and Dutil 1997a, b). Females were sacrificed 15 d after the production of their last egg batch. Fork length, total weight, and gutted liver and gonad weight were measured.

Spawning, egg collection and egg characteristics. During each experiment, females were paired with males in 1.2 m^3 circular tanks. This allowed for egg production by each female to be monitored throughout their spawning cycle, and for every batch of eggs released by females to be collected individually. A complete description of the holding conditions and methods of egg collection and sampling is presented in Lambert and Dutil (2000) and in Ouellet et al. (2001).

The spawning activity of 17 females in the 1995 experiment, and 21 females in the 1996 experiment, was characterized. For each spawning event, the volume (ml) and number of eggs released in each and every batch was measured. Mean egg diameter, dry mass and energy content for each batch were measured (Table 1 in Ouellet et al. 2001). In the 1995 experiment, total lipid and total yolk protein content were also measured for each batch of eggs. Weighted mean egg diameter and dry mass were calculated for each female by normalizing the means for each batch by the number of eggs in that batch. Details of the analytical techniques used to obtain these data are presented in Ouellet (1997) and Ouellet et al. (2001).

Source of animals and experiments on larval behaviour. Three of the male-female pairs – from each of the four ration-temperature treatment groups (in 1995), or ration-size treatment groups (in 1996) – were targeted as sources of larvae. Two egg batches from each of these females were collected: the second or third (typically of relatively poor quality) and the fifth or sixth (typically of relatively high quality). As much as possible, these were the same spawning pairs and egg groups studied by Ouellet et al. (2001). A total of 21 groups of larvae were studied (Table 1).

Table 1. *Summary data on Atlantic cod (Gadus morhua) females, egg batches, and larvae which were the source of material for the growth and behavioural experiments reported upon here. Designation = female number-egg batch number; Treatment t_1 1 = high ration; Treatment t_1 2 = low ration; Treatment t_2 1 = 6 °C; Treatment t_2 2 = 2 °C; Treatment t_2 3 = large female; Treatment t_2 4 = small female; Condition Initial = pre-spawning conditionfactor (Fulton's K); Condition Final = post-spawning condition factor (Fulton's K); Incubation time = time (in days) between spawning and 50% hatching of eggs; Fecundity = % fertilized eggs (from visual estimate of a sub-sample); Initial volume = volume of eggs produced; SGR of larvae, BL = body length; BW = body weight. (–) denotes no data available. Data on adults are drawn from Lambert and Dutil (1999), and on eggs from Ouellet et al. (2001).*

		Adult females							Eggs				Larvae	
		Treatments		Condition		Incubation time	Fecundity	Initial volume	Fresh weight egg^{-1}	Dry weight egg^{-1}	Energy	Egg diameter ±SD	SGR (% BL or BW d^{-1})	
Designation	Year	t_1	t_2	Initial	Final	(d)	(%)	(ml)	(mg)	(µg)	J egg^{-1}	(mm)	(mm)	(µg)
B08-02	95	1	2	1.02	0.77	13	95	127.0	1.9	96.9	1.73	1.43 ± 0.04	1.60	11.68
B04-06	95	2	2	1.11	0.82	13	95	156.0	1.9	94.4	1.55	1.38 ± 0.03	(–)	(–)
B04-09	95	2	2	1.11	0.82	12	60	50.0	1.6	70.6	1.26	1.31 ± 0.04	1.09	5.50
B15-05	95	2	1	1.13	0.78	13	80	169.0	1.3	64.9	1.15	1.27 ± 0.04	0.39	0.85
B15-01	95	2	1	1.13	0.78	12	80	58.0	1.7	70.3	1.26	1.37 ± 0.03	(–)	(–)
B01-01	95	2	2	0.88	0.64	12	95	52.0	2.3	116.2	1.97	1.44 ± 0.06	1.52	6.99
B01-03	95	2	2	0.88	0.64	13	100	138.0	(–)	(–)	(–)	1.50 ± 0.03	1.98	10.87
B01-07	95	2	2	0.88	0.64	14	70	175.0	1.9	87.4	1.66	1.38 ± 0.07	0.65	3.65
B13-02	95	1	1	1.18	0.80	15	80	119.0	2.3	95.8	1.79	1.46 ± 0.06	2.01	6.317
B13-05	95	1	1	1.18	0.80	(–)	(–)	(–)	1.8	82.7	1.46	1.36 ± 0.03	(–)	(–)
B19-05	95	1	1	1.05	0.77	13	90	100.0	1.7	90.7	1.50	1.35 ± 0.04	1.89	5.07
B19-09	95	1	1	1.05	0.77	15	10	(–)	1.2	56.3	1.03	1.22 ± 0.04	0.91	3.17
B07-03	95	1	2	1.08	0.70	14	50	61.0	2.3	103.1	1.74	1.46 ± 0.03	(–)	(–)
B07-06	95	1	2	1.08	0.70	15	95	110.0	1.8	74.9	1.35	1.37 ± 0.03	0.88	4.11
B03-03	96	2	4	0.84	0.60	14	100	44.0	2.1	130.4	2.34	1.48 ± 0.02	(–)	(–)
B03-07	96	2	4	0.84	0.60	12	99	25.8	1.7	110.1	2.02	1.43 ± 0.03	2.80	16.56
B05-04	96	1	3	1.29	0.75	12	95	228.0	2.3	147.4	2.96	1.61 ± 0.04	2.04	10.13
B05-06	96	1	3	1.29	0.75	14	80	216.0	2.2	143.5	2.83	1.54 ± 0.03	1.88	0.09
B06-06	96	1	3	1.29	0.78	12	95	567.0	(–)	(–)	(–)	(–)	2.24	9.14
B08-03	96	2	4	0.82	0.61	15	90	19.4	1.7	95.4	1.67	1.40 ± 0.13	1.79	8.26
B13-03	96	1	4	1.05	0.68	13	99	96.6	2.0	120.0	2.35	1.45 ± 0.03	2.32	13.64
B16-03	96	2	4	1.01	0.66	11	99	139.0	2.0	117.9	2.28	1.47 ± 0.03	2.59	14.86
B17-03	96	1	4	1.19	0.82	14	90	86.2	2.4	152.0	2.72	1.48 ± 0.04	1.92	9.23
B20-03	96	1	4	0.88	0.62	14	90	267.0	2.5	122.7	1.85	1.72 ± 0.20	2.29	10.29
B20-07	96	2	3	0.88	0.62	15	99	67.5	1.6	95.6	1.78	1.37 ± 0.06	2.06	11.09
B21-06	96	1	3	1.17	0.72	13	100	104.0	2.3	135.3	2.56	1.54 ± 0.05	3.08	21.35

Eggs were incubated in black 60 l round-bottom tanks (at 6 ± 1.0 °C). Larvae were transferred to fresh 60 l black tanks at 3 DPH (just prior to first exogenous feeding). The rearing basins were stocked with algae (*Nannochloropsis sp.*) (green water technique), and larvae were fed nutritionally enriched (INVE Aquaculture Super Selco®) rotifers (*Brachionus sp.*) at 7 ml^{-1}. Eggs and larvae were handled so as to maintain those from any given female and batch in isolation. Larvae were cultured at 6 °C on a 14 h L:10 h D photoperiod at a crepuscular-level light intensity of 1.20 μE sec^{-1} m^{-2} (diffuse light from overhead fluorescent lamps).

Measurements made on larvae. Larvae from all targeted groups were sampled at various intervals for length and weight. Larval size (standard length) was determined from measurements on live larvae examined (on ice) under a microscope. Immediately after the standard length measurements, specimens were dried at 50 °C for 24 h and then weighed on an electrobalance to the nearest 0.001 mg. Drying was continued until there was no further change in mass. For each egg batch, 10 to 12 larvae were collected from the rearing basins beginning at approximately 6 DPH. A minimum of three such measurements were obtained between 6 and 20 DPH. The mean length and dry mass measurements were used to calculate the specific growth rate for mass (SGR_w), and for standard length (SGR_l), as follows (Ricker 1979):

$$SGR_w = \frac{BM_f - BM_i}{BM_i(T_f - T_i)} \times 100 \quad \text{and,} \quad SGR_l = \frac{BL_f - BL_i}{BL_i(T_f - T_i)} \times 100$$

where, BM_f and BL_f are mean body mass (mg) and body length (mm) for the last day of measurements, BM_i and BL_i are for the first day of measurements and T_f and T_i are the ages of the larvae on the first and last day on which they were measured.

Imaging system for behavioural observations. Silhouette (shadow) video photography (SVP) (Arnold and Nutall-Smith 1974; Edgerton 1977; Browman et al. 1989; Browman and O'Brien 1992a,b; Browman et al. 1994) was used to observe the behaviour of cod larvae. SVP is superior to standard cinematographic or video imaging techniques in various ways. First, it allows filming of events in a large depth of field (in this case, approximately 20 cm) with a relatively large field of view (limited only by the size of the collimating lenses, here 15 cm). Second, magnification is independent of distance from the cameras and the resolution is very good; objects as small as 0.2 mm can be resolved. Third, image quality is unaffected by ambient light levels and the silhouette effect is attained without the use of intense light sources. As such, the behaviour of cod larvae can be observed under relatively natural conditions.

Our SVP observation system consists of two orthogonally-oriented optical rails, with the observation aquarium (20 x 20 x 20 cm) placed at their intersection (Fig. 1). The imaging optics on each rail consist of a far-red light emitting diode (LED) placed at the focal point of a 15 cm diameter biconvex collimating lens whose output passes through the aquarium. The LED's output is less than 10^{-6} W cm^{-2} – an intensity below the perceptual threshold for the red photoreceptor channel of fishes. The use of a collimated beam prevents perspective distortion and projects clear, sharp shadows of any organism (even a small and virtually transparent one) in the beam's path. Shadow

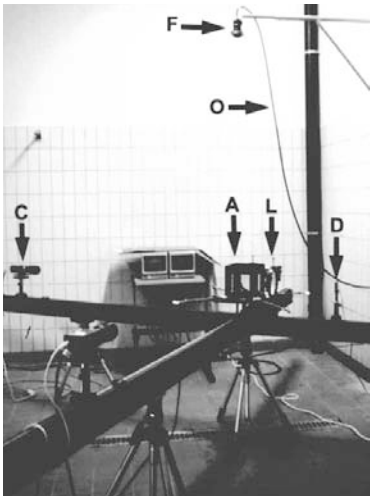

Figure 1. *Silhouette video photography system used to make behavioural observations of Atlantic cod* (Gadus morhua) *larvae. Light from a 1-kW Xenon arc lamp (not shown) is focussed onto the aperture of a UV-visible liquid light guide (O) and passes through various optical components in a filter holder (F) before reaching the test aquarium (A) in which the animals are freely swimming in a 20 cm aquarium. In the experiments reported upon here, standard fluorescent fixtures (in the ceiling above the test tank) were used as the light source. The aquarium is located at the intersection of two 3 m long optical rails. Each rail supports a far-red light emitting diode (D) placed at the focal point of a 14.5 cm diameter biconvex collimating lens (L), and a video camera (C) to image the shadows projected by the collimated beam that passes through the aquarium. Also shown are the lasers (Ar) that are used to align all of the optical components on the rails prior to a given set of experiments. The red spot at the far end of one of the rails is the light emitted by the diode. (Reprinted from: Journal of Experimental Biology 203: 1649-1657).*

images are collected by a single lens reflex lens (Tamron 70-210 mm zoom) attached to a 0.5 inch CCD sensor video camera (Panasonic WV-BL730) and recorded using a S-VHS video tape recorder (Panasonic AG-6730). The optical components on each rail are aligned using helium-neon lasers, which also allow the vertical viewing heights and orthogonal orientation of the two rails to be established precisely. The synchronously-recorded orthogonal views allow exact determination of the three-dimensional positions of targets (larvae and/or their prey) which appear in both fields of view simultaneously. The outermost 5 cm of the aquarium walls were covered with black plastic (matte-surface) contact paper. This restricted the field of view to the central 15 cm volume of water and ensured that the behaviours observed were not influenced by surface or edge effects; only cod larvae freely swimming in the water column were imaged, and their displacements analyzed.

Overview of analytical software. The custom computer programs TRAKFISH and ANAPATHS (Racca Scientific Consulting and JASCO Research Ltd., Victoria, British Columbia, Canada) were used to provide end-to-end processing of the video records. Their principles of operation will be described here to a sufficient extent to allow full understanding of the process through which larval motion statistics are obtained, including a description of the various software configuration settings and how they affect the analysis.

TRAKFISH has its roots in a software application developed for the study of flow patterns in experimental fluid dynamics by automatic tracking of particle tracers (Racca 1985; Racca and Dewey 1988). In the original approach, the test volume of a flow channel was photographed by a high-speed cine camera through a mirror arrangement that recorded simultaneous views through perpendicular windows; the film frames were then scanned to digital images that the software analysed. In TRAKFISH, the video digitization capability is built into the software along with video tape recorder step control functions, although the program can also process pre-digitized sequences from a real-time direct-to-disk video acquisition system (Tempus-PC, Kinetic Imaging Ltd). The latter also provides the option of recording the video sequences as individual non-interlaced fields

instead of interlaced frames, which effectively doubles the time resolution of the recordings and subsequent analysis to 50 images per second for the European video standard.

TRAKFISH performs simultaneous tracking of all identifiable targets directly from dual video sequences, each showing a view of the test aquarium from a perpendicular direction through the SVP set-up. Tracking is carried out for an arbitrary number of steps specified by the user at the start of a run; the increment between processing steps is also user specifiable and can be one or more video frames (or fields) – a larger increment may be appropriate for slow moving targets with steady trajectories. To identify the locations of the targets in the field of view, the digitized video images are subjected to binarization (selection of individual pixels as belonging either to a target or to background) based on either a brightness threshold or an edge strength threshold as selected by the user. This leaves clusters of connected target pixels commonly known as blobs; due to thresholding imperfections in real life images these may contain breaks or spurious bridges between adjacent targets. Blob processing is then performed to clean up these anomalies and to exclude targets whose size (area) is outside user specified limits, after which the centroids of the accepted objects are located. In an alternative operating mode used for larger subjects, TRAKFISH can instead locate the endpoints (head, tail) of elongated targets regardless of their bending or curling. At this stage a video frame (in either view) has been reduced to a collection of identified locations expressed in pixel coordinates. These are converted to real-world two-dimensional coordinates in the frame of reference of the aquarium through scaling formulae obtained from a calibration procedure that is performed when the geometry of the SVP apparatus, or the magnification of the zoom lenses, are modified. To calibrate TRAKFISH, a transparent plastic overlay with four fiduciary marks in a rectangular configuration is placed against each front window of the aquarium; the user then identifies the fiduciary marks by mouse-clicking them in the digitized views presented by the software. The rectangles defined by the fiduciary marks can be regarded as the projection onto the two image planes of a parallelepipedal "reference volume" located inside the aquarium; a scaled coordinate system is established by entering into the software the real-world dimensions of this reference volume and its offset from the bottom corner of the aquarium at the common edge of the front windows, which is taken as the origin. The dimensions of the entire water volume in the aquarium are also specified for the purpose of displaying in the main software window a projective outline, or "virtual aquarium", within which the paths of tracked targets are plotted during a run.

The next step in the motion analysis by TRAKFISH is the matching of positional data in the two simultaneous perpendicular views to yield the three-dimensional spatial coordinates of targets. We shall refer to the projections in the two view planes of a particular target location in space as an *orthogonal pair*, after the term *stereo pair* used in photogrammetry to denote the corresponding views of a given feature in stereoscopic images. The correct identification of orthogonal pairs must be based solely on geometrical considerations, since visual cues such as object shape—which can be helpful in the matching of stereo pairs—provide little or no assistance in orthogonal views due to the potentially different aspect of an object seen from perpendicular directions. In the absence of parallax and misalignments the projection coordinates of orthogonal pairs would have identical values in the direction common to both views, which in the optical arrangement used here is the vertical or *z* axis. Thus, for projected coordinates (x_0, z_0) in one view, and (y_0, z_0) in the

other, the spatial location of the target would be at (x_0, y_0, z_0). Racca (1985) derived mathematical relations to reconstruct the spatial position of a target point from its orthogonal projections fully accounting for parallax and, thereby, provided a criterion to verify whether two projected points constituted an orthogonal pair. In the SVP optical system used in this work, however, images of objects within the visible volume are projected onto the perpendicular view planes with no significant parallax thanks to the collimated back lighting; the *z* coordinates of orthogonal pairs can, therefore, be assumed to be identical. In practice, small discrepancies will always exist because of the effect of slight optical aberrations and misalignments and the variability in the automated placement of a target's centroid depending upon the shape and contrast of the object in each view. TRAKFISH does, therefore, allow the user to specify a matching tolerance (in mm; 3.0 mm in the analyses reported here) that represents the maximum discrepancy between *z* values of projected points that would still be accepted as forming an orthogonal pair. Because of this finite matching tolerance, at high densities of targets it is possible that there will be more than one match candidate for a point in one of the views. In this case, TRAKFISH ranks the candidates based upon the difference in *z* values (smaller differences yielding a higher rank) and accepts up to *n* highest ranked matches, *n* being a user specified parameter denoted as the multiple match allowance. It stands to reason that *n* in principle should be unity, as only one pair of projected points can arise from a target. The concept of multiple match allowance was introduced to allow for cases where two projected points in one view coalesce into a single centroid position, thus requiring a one-to-many matching, or where the correct pairing is indeed not the one with the least *z* difference because of the positioning variability mentioned above. Allowing multiple matches has the serious drawback of potentially creating ghost targets, or incorrectly reconstructed spatial positions that would be tracked along with real targets. The rationale for allowing this to occur is that the conditions resulting in multiple matches for any given point in a view are unlikely to persist for more than a few frames as the relative positions of targets shift, and it would be far more damaging to the motion analysis to lose a track because of an incorrect match than to have occasional, short lived spurious tracks from ghost points.

The outcome of the matching step is a collection of *(x, y, z)* triplets representing the spatial location of all identified targets (and possibly some ghost targets) for the current frame. The process of tracking targets consists primarily of finding, among the spatial locations in the current frame, the most appropriate continuations to any paths that have already been followed through previous time steps, and secondarily, of attempting to start new paths out of those locations that are not assigned to existing ones. The algorithm for following a target is based upon the assumption that from one time step to the next its velocity vector does not undergo extreme changes but rather evolves gradually in both magnitude and direction. Under this assumption, the target displacement during one time step can be linearly extrapolated as an approximation of the path in the next step. Having thus set a predicted position, the algorithm searches within a surrounding sphere of radius r_{old} (user specified; 6.0 mm in the analyses reported here) for available target locations. If any are found, the existing path is extended to the one closest to the predicted position and that target location is made unavailable to other trajectories. If no successor can be found within the search sphere, the path is terminated at the previous step. The process is repeated for all established paths.

A target location that does not become assigned to an existing path is tentatively considered a

new object for which a path can be started. The beginning of a path must be handled in a different way, since a predicted trajectory step cannot be constructed. In this case the algorithm uses a larger search sphere of radius r_{new} (also user specified; 12.0 mm in the analyses reported here) surrounding the starting position; r_{new} must be made as large as the maximum expected displacement for the subjects under study in one time step, unlike r_{old} that only needs be large enough to allow for the expected deviation from a steady path. To create the first path step the algorithm looks at available target locations in the next frame that are within the search radius r_{new}; any such location could potentially be a successor. The location that is nearest to the original position is considered first. Based on this provisional step a position is extrapolated one step further as previously explained, and a second successor is sought within a radius r_{old} from it. If one is found, the two-step trajectory is accepted and added to the collection of active paths. Otherwise, the process goes back one frame and attempts to start a path using the next nearest tracer to the original position still within a radius r_{new}. The process continues until either a trajectory has been started or all available successors in r_{new} have been exhausted, in which case the new target is abandoned.

Because tracking is performed in a parallel fashion on all targets identified as video frames are processed, a handling hierarchy must be assigned to control the order in which paths are extended or started. TRAKFISH follows a hierarchy based on the current lifespan of trajectories, meaning that targets that have been followed through the largest number of steps are analysed first. Since a successor, once assigned, is made permanently unavailable to other trajectories, this scheme ensures that well-established paths will not be cut off by a new target appearing for the first time. To manage the overhead of this intricate process TRAKFISH maintains three arrays of target positions, corresponding to frames $F - 1$, F and $F + 1$ (with the understanding that "frames" here denotes images in an evenly spaced sequence, and not necessarily consecutive video frames or fields). On the first iteration, all targets in $F - 1$ are new and, therefore, undergo the two-stage start-up tracking through frames F and $F + 1$. The old $F - 1$ is then lost, F becomes $F - 1$, and a new $F + 1$ is created by processing a new pair of video frames (one for each view). On all following iterations, paths are handled in order of seniority, with newly appearing targets being considered last. Target locations in $F - 1$ that were assigned to paths have already a successor in F from the previous iteration, so their next position can be predicted and a successor sought in $F + 1$. New targets in $F - 1$ undergo two-stage tracking as usual. The frames are again shifted back one position and the process continues until $F + 1$ is the last frame in the sequence. As the analysis progresses the coordinates of tracked targets and the identifiers of the paths to which they belong are output to an ASCII file; the evolving trajectories are also displayed graphically within the outline of the "virtual aquarium".

The software ANAPATHS was developed to perform various types of analysis on the path records generated by TRAKFISH. This program includes visualization, sorting, and editing functions to facilitate certain preliminary operations such as the selection of the longest or most enduring paths and the splicing of disjoint segments of the same path (which can occur due to tracking anomalies) into a continuous trajectory. The latter function is aided by a "wizard" (automated assistant) process that identifies and presents to the user all likely splicing candidates based on the spatial and temporal nearness of their end-points, allowing the selective approval and immediate implementation of individual splices.

The analytical functions available in ANAPATHS include the computation of the fractal dimensions of paths in single planes or in three-dimensional space to provide a measurement of the morphological complexity of swim patterns. The most important feature of the software in the context of the present study, however, is the pause/movement statistical analysis of swim trajectories. This component of ANAPATHS is based on research by Collins et al. (1995) on the reliable identification of stops in search pathways, and the code for ANAPATHS incorporates an extended version (Racca Scientific Consulting) of the computer program STOPGAP (©1991-1995 by R.D. Collins and M.K. Tourtellot, used by permission) introduced in that work. STOPGAP accepts one or more trajectories, being sequences of two- or three-dimensional coordinates with a constant time interval between consecutive points, and for each trajectory it identifies stops and periods of movement. A stop is defined to occur when the subject fails to move a specified minimum distance (length criterion) over a given period of time (duration criterion); both criteria are selectable by the user (0.3 mm and 0.3 s, respectively, in the analyses reported here) and are tuned to the movement pattern of the organisms being observed. The first stage in stop identification is to tentatively classify as being part of stops those trajectory steps in which the distance covered is less than the length criterion, and the remaining ones as being part of moves. The next stage is to iteratively evaluate stop duration. With each iteration the validation interval is increased by one time step; moves lasting less than that interval are redefined to be stops, and stops lasting less than that interval are redefined to be moves. The process continues until the validation interval reaches the duration criterion. Having identified and validated the stops and the intervening move periods for a trajectory, the original STOPGAP program computes statistics (mean, standard deviation, minimum and maximum) for stop duration, distance between points during stops, move duration, and distance between points during moves. The extended version that was developed for use in ANAPATHS computes three additional quantities from the analysis of stops and moves: the path length for each move period, the change in direction after a stop (turn angle) in a horizontal plane, and the same in a vertical plane. For the purpose of measuring the turn angles, horizontal and vertical motion vectors are computed based on the overall displacement of the subject during a move period; this approach is justified if it is assumed that no significant changes in direction of swimming occur during moves. Turn angles are recorded as unsigned values, that is, the direction of turn (its possible asymmetry, or handedness) is not taken into account in the statistics. For each path analysed, the program generates statistics for these additional parameters as well as the ones originally in STOPGAP. It also computes the overall activity level, defined as the percent of time spent moving over the lifetime of the trajectory. Furthermore, the stop/move analysis code in ANAPATHS generates discrete frequency distributions for stop duration, move duration, horizontal turn angle, vertical turn angle, move length and move speed. The upper limit of the binning range for each parameter (the lower limit being always zero), and the number of binning intervals used to compute the frequency distributions, are user specifiable. The results for an analysis run (which may contain statistical results for each of several paths selected) are output to a report file in plain text form. ANAPATHS provides a function to pool the results from one or more report files into a single set of statistics for all of the paths therein. The frequency distributions in these pooled statistics are normalized to a total of unity, unlike those for individual paths that contain raw counts of moves or stops per bin. ANAPATHS also outputs the raw values used to generate the frequency distributions.

Behavioural observations and their analysis. Larvae were tested at 3 and 6 DPH. Three DPH was just before the initiation of exogenous feeding; 6 DPH was after. Comparing and contrasting observations made on these two days was meant to provide a baseline with respect to differences in behaviour associated with the onset of feeding (*sensu* Skiftesvik 1992). On each test day, fifty larvae from any given group were placed into the test aquarium and video taped for 30 min. This was repeated three times, with 50 new larvae observed each time (and, thus, three replicates). Due to the nature of the imaging system, the behavioural observations cannot be attributed to any single larva – they swim in and out of the field of view and, therefore, it is impossible to tell one larva from another. Further, observations on any one larva in a tank containing 50 are not independent (in a statistical sense). Thus, for statistical analysis (see below), all observations made on the 50 larvae in any given tank collapse down to a sample size of one. To minimize the possibility of subsampling artefacts, swim paths of cod larvae were extracted using TRAKFISH from two to three 5 min. segments drawn from different times during the 30 min. of observation. Thus, for any given test group of larvae, there were three replicate 30 min. observations periods, each of which was then subsampled for 10-15 min.

Swim paths extracted by TRAKFISH were analysed using ANAPATHS. Path analysis followed principles (thoroughly described in Bell 1990, pp: 281-300; O'Brien et al. 1989; 1990) that are widely applied to study the movement patterns of species in many taxonomic groups (e.g. Anderson et al. 1997; Kramer and McLaughlin 2001). For each of the 5 min. subsamples, the longest 20 tracks were identified and combined (recall that, for statistical analysis, all of these paths must be collapsed into one since they are not independent). Thus, the 40-60 longest paths for each replicate 30 min. observation period – representing a mean displacement per replicate of 2.23 ± 0.178 m in 1995 and 0.734 ± 0.10 m in 1996 – were used as the basis for the analysis. A total of 215 m of cod larval swim paths were analysed.

ANAPATHS extracted the following variables from the swim paths: durations of stops (non-active displacement, as defined and operationalized by Collins et al. 1995), lengths and durations of moves, and turn angles (decomposed into vertical and horizontal components). For each of these variables, the software produced a mean ($\pm$ standard deviation, SD) for the 120-180 longest paths from all three replicates. Average overall activity level (percent time actively swimming) and swim speed were also computed. Examples of the swim path and frequency distribution data extracted by TRAKFISH and ANAPATHS are presented in Figures 2 and 3.

Statistical analysis. This was a three-replicate experimental design. In 1995, the treatments were ration and incubation (of the spawning pairs) temperature. In 1996, the treatments were ration and female size. In evaluations of maternal influences, egg batch number was not considered as a separate treatment effect because there were no discernible differences in the behaviour of larvae from the different egg batches at 6 DPH (with the exception of swim speed, for which the difference was mildly significant by t-test, $P = 0.03$).

A model I analysis of covariance was applied to length and weight–age regression curves to determine whether larval growth (from all groups combined) was different between experiments (1995 vs. 1996). To evaluate the relationship between egg characteristics and larval growth, data from both years were pooled and the Pearson product-moment correlation coefficient, r, was applied. The behaviour of larvae at 3 vs. 6 DPH was compared using a one-way ANOVA (performed on each

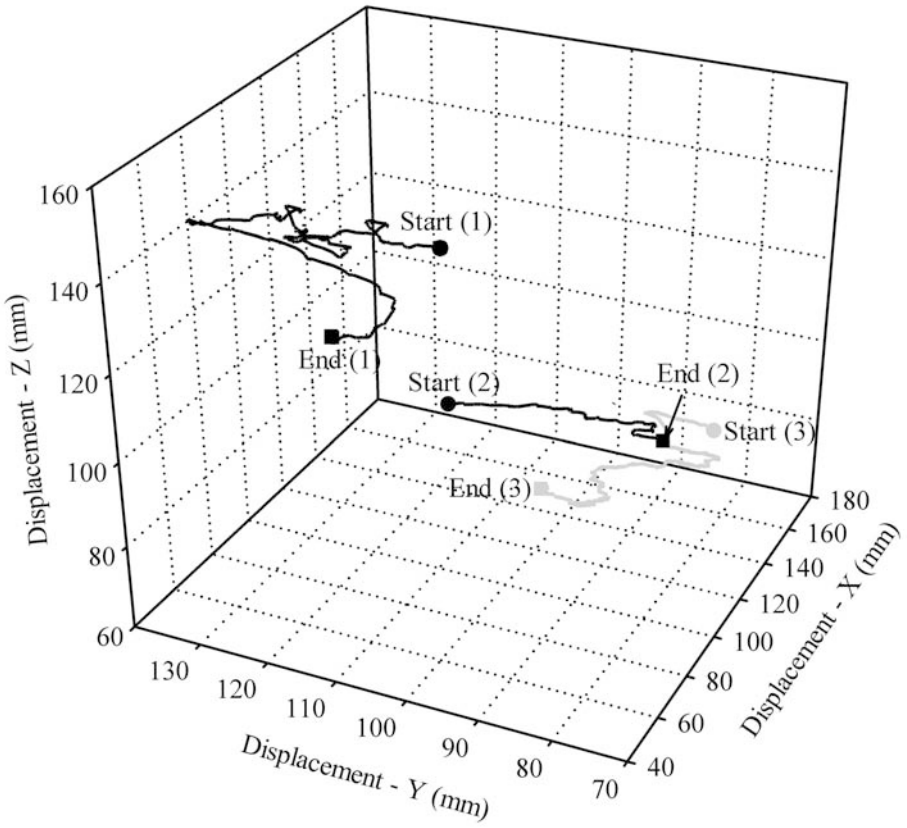

Figure 2. *Typical swim paths for Atlantic cod* (Gadus morhua) *larvae extracted using the TRAKFISH software. Swim paths 1, 2 and 3 are 355, 104 and 190 mm long respectively.*

behavioural variable). The same test was applied to compare larval behaviour (6 DPH only) in 1995 vs. 1996. Possible correlations between the SGR of cod larvae and the behavioural variables (6 DPH only) were evaluated using the Pearson product-moment correlation coefficient. Finally, the possible effects of female treatment (incubation temperature, ration, size) on larval behaviour (6 DPH) were examined by two-way ANOVA (including treatment interactions). For these latter tests, only behaviour at 6 DPH was tested because larvae were not yet feeding at 3 DPH.

Results

In order to establish the overall context for the work on larvae, the most salient of the previously published results on brood stock (Lambert and Dutil 2000), and on eggs (Ouellet et al. 2001), are summarized here. For complete details, readers are referred to these companion studies.

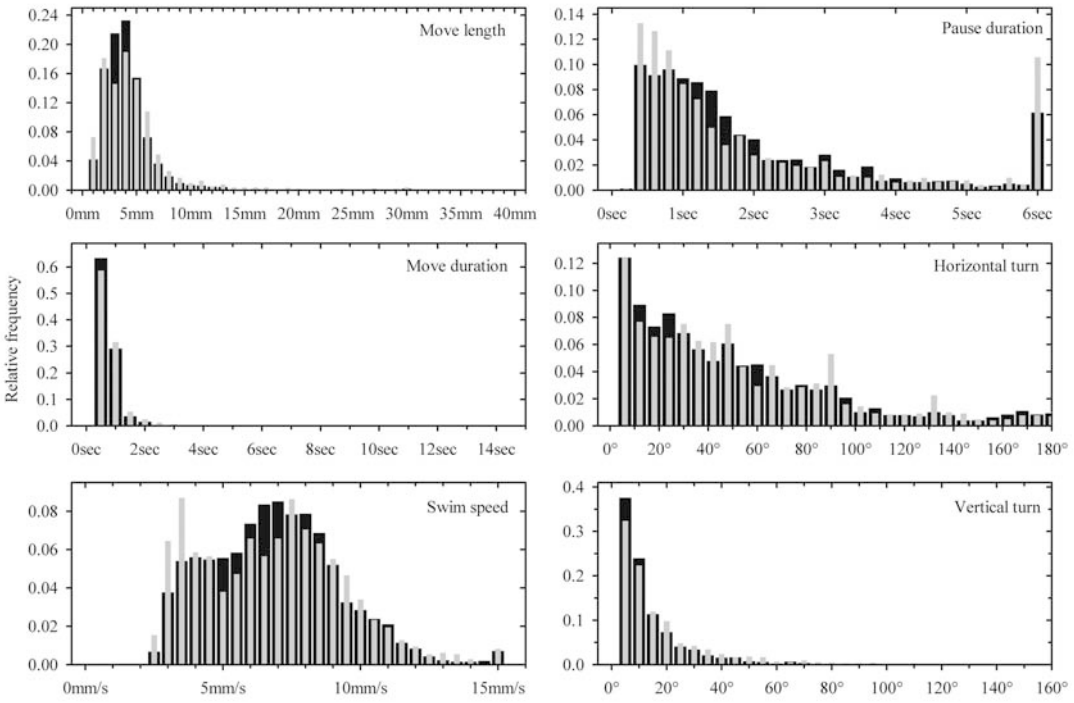

Figure 3. *Frequency distributions for the behavioural variables extracted using TRAKFISH and ANAPATHS software on the same group of Atlantic cod* (Gadus morhua) *larvae at 3 and 6 days post hatch (DPH). Black bars = 3 DPH; grey bars = 6 DPH.*

Female size and condition. Despite the differences in incubation temperature, and the partitioning of females into two size groups, the size of pre-spawning females was not discernibly different in the 1995 vs. the 1996 experiments. Female pre-spawning and post-spawning condition (Fulton's K) ranged from 0.66–1.24 in 1995, and 0.48–0.92 in 1996 (Table 1). Female length, and pre- or post-spawning condition were not correlated within either experiment. Post-spawning condition differed between years, with the mean being slightly lower in 1996 (0.66 ± 0.08) than in 1995 (0.76 ± 0.11) (Table 1). Smaller fish, and fish in lower condition, were better represented in the 1996 experiment (Table 1).

Relationships between female size and condition and egg production, viability and hatching success. The mean realized fecundity, mean number of egg batches, and number of days between batches all indicate that the fish were at least second time spawners, and their spawning performance was similar in both experiment years. The mean number of egg batches, realized fecundity, and the average interval between batches, were not discernibly different between years. Although there was a positive correlation between pre-spawning condition of females (maturing at 6 °C) and weighted mean egg diameter, dry mass, and energy content, this relationship was no longer statistically dis-

cernible when the only female with a low condition factor was excluded from the analysis. There was no relationship between female size and weighted mean egg diameter, weighted mean egg dry mass, nor energy content. While mean egg dry mass per batch declined over the spawning sequence of individual females, no definite trends in total egg dry mass per batch were observed. However, the batch number in the spawning sequence did have a significant negative effect on cod egg diameter and dry mass. The total lipid/protein ratios of eggs from different batches were not discernibly different, nor were they related to female pre-spawning condition. Total egg dry mass per batch of individual females during spawning was not related to post-spawning somatic, protein, or lipid reserves. Neither mean egg diameter nor dry mass were correlated with egg survival. Nor was there any relationship between egg survival to hatch and female pre-spawning condition. However, there was some indication that late embryos and larvae hatching from eggs produced by females in poor condition were less viable. Hatching success was independent of batch number and was not related to egg diameter or dry mass. Finally, female pre- and post-spawning condition and hatching success were not correlated in either experiment.

Larval growth. A total of 775 cod larvae were measured and weighed over the two experimental seasons. Larval groups from the 1996 experiment generally grew better than those from the 1995 experiment (analysis of covariance for body length, P = 0.037, and for body weight, P = 0.007) (Table 1, Figs. 4 and 5). Overall, the growth rates of larvae in these experiments (Table 1) were comparable to those reported for cod larvae in other laboratory-based work (e.g. Gamble and Houde 1984; Yin and Blaxter 1986; Bolz and Lough 1988; Otterå 1993). This supports the validity of the methods applied, and of analyses based upon the growth rate data.

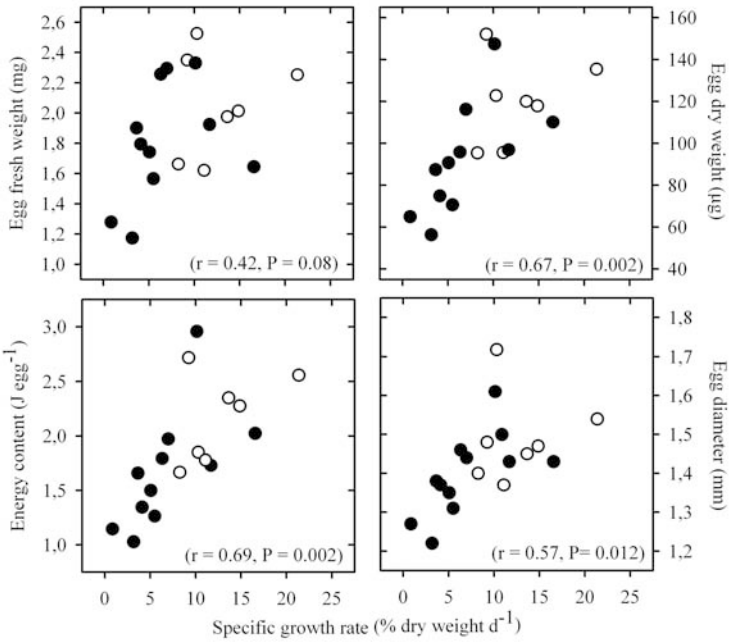

Figure 4. *The relationship between the specific growth rate of Atlantic cod* (Gadus morhua) *larvae, as percent body length and percent body dry mass per day, and the characteristics of the eggs from which they hatched (1995 (filled circles) and 1996 (open circles) experiments combined). r = the Pearson-product moment correlation coefficient at probability P.*

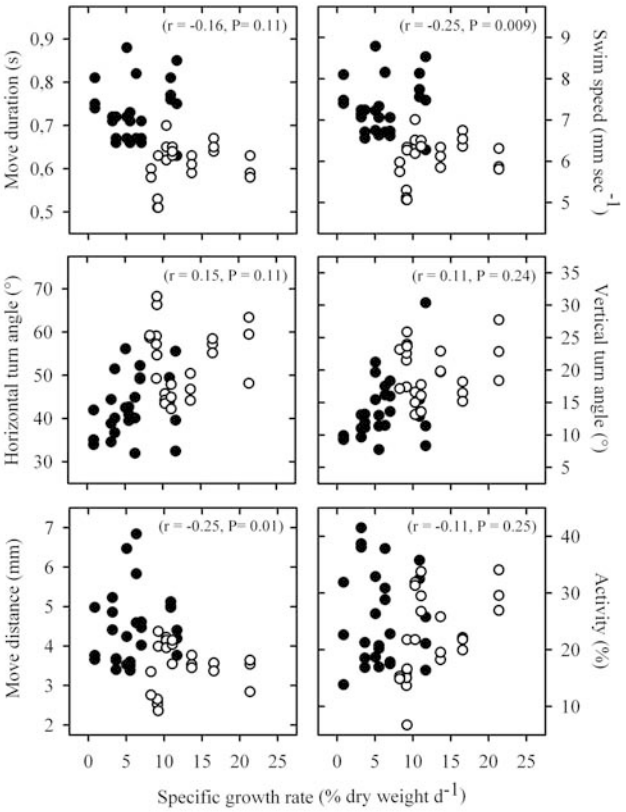

Figure 5. *The relationship between the specific growth rate of Atlantic cod (Gadus morhua) larvae, as percent body length and percent body dry mass per day, and their behaviour (1995 (filled circles) and 1996 (open circles) experiments combined). r = the Pearson product-moment correlation coefficient at probability P.*

Relationship between larval growth, egg characteristics and female treatments. There were statistically discernible (Pearson product-moment correlation coefficients) positive correlations between larval growth and egg diameter, dry weight and energy content (Fig. 4). A consistent relationship was exhibited for egg fresh weight, although it was not significant. There was no statistically discernible relationship between female pre- nor post-spawning condition factor (Fulton's K) and the growth of the larvae that they produced (Pearson product-moment correlation, $P > 0.05$). Nor was there any relationship between female ration, size, and/or incubation temperature and the growth of their larvae (one-way ANOVA, $df = 1$, $P > 0.05$ in all cases).

Behaviour of larvae at 3 vs. 6 DPH, and as related to growth rate. There were statistically discernible differences between the behavioural variables (except for move duration) on 3 vs. 6 DPH (Table 2, Fig. 3). Since the larvae were not feeding at 3 DPH, but were at 6 DPH, this demonstrates the validity of using these behavioural variables as indicators of changes in larval behaviour.

Swim speed, turn angles associated with repositioning movements, and move distances were all discernibly different in larvae from the 1995 vs. the 1996 experiment (Table 3). Since larvae from 1996 exhibited higher growth rates, this observation demonstrates a link between growth and at least some of the behavioural variables. The negative correlation between swim speed, move dis-

Table 2. *Mean (±SD) values for each of the behavioural variables measured on all groups of Atlantic cod (Gadus morhua) larvae at 3 vs. 6 days post hatch (DPH) and the results of a one-way ANOVA (df = 1) testing for differences in these variables between the two days (both experiment-years combined).*

Behavioural variable	3 DPH	6 DPH	F	P
Move distance (mm)	3.5 ± 1.0	3.9 ± 1.0	5.20	0.024
Move duration (s)	0.6 ± 0.1	0.6 ± 0.1	1.26	0.264
Swim speed (mm s^{-1})	6.1 ± 1.1	6.5 ± 1.0	6.80	0.010
Pause duration (s)	2.7 ± 1.2	2.1 ± 1.0	9.68	0.002
Horizontal turn angle (°)	55.9 ± 16.4	49.1 ± 9.7	8.65	0.004
Vertical turn angle (°)	19.6 ± 6.7	17.2 ± 5.4	5.53	0.025
Activity (%)	19.7 ± 9.7	24.3 ± 9.2	8.12	0.005

Table 3. *Mean (±SD) values for each of the behavioural variables measured on all groups of Atlantic cod (Gadus morhua) larvae in the 1995 vs. 1996 experiments (6 days post hatch) and the results of a one-way ANOVA (df = 1) testing for between-year differences in these variables (all maternal treatments combined).*

Behavioural variable	1995	1996 (n=35)	F	P (n=32)
Move distance (mm)	4.3 ± 1.1	3.5 ± 0.7	11.9	<0.001
Move duration (s)	0.6 ± 0.1	0.6 ± 0.1	0.2	0.7
Swim speed (mm s^{-1})	7.1 ± 0.9	5.9 ± 0.6	34.5	<0.001
Pause duration (s)	0.3 ± 0.2	0.3 ± 0.2	0.02	0.9
Horizontal turn (°)	44.0 ± 7.1	54.7 ± 9.1	29.0	<0.001
Vertical turn (°)	14.5 ± 4.7	20.2 ± 4.6	25.0	<0.001
Activity (%)	24.6 ± 10.0	23.6 ± 8.3	0.4	0.5

tance and growth rate (Fig. 5) is consistent with this, and further supports the validity of applying these behavioural variables as proxies of larval quality-performance.

Relationship between larval behaviour and maternal condition and incubation temperature. There were no statistically discernible relationships between female incubation temperature (i.e., in the 1995 experiment) and any of the behavioural variables measured, although the effect on swim speed was close to significance (P = 0.06) (Table 4). Further, there were no treatment interaction effects (female ration with incubation temperature) on larval behaviour in the 1995 experiment. Female ration had a significant impact upon larval activity in the 1995 experiment (Table 4), but not in the 1996 experiment (Table 5). Conversely, female ration had a significant impact upon larval swim speed in the 1996 experiment (Table 5), but not in the 1995 experiment (Table 5). Vertical turn angle (but not horizontal turn angle) was significantly affected by female ration in both years (Tables 4 and 5), while pause duration was only significantly affected in 1996 (Table 5). In the 1996 experiment, female size had a significant effect on larval activity, horizontal turn angle and pause duration (Table 5). Further, there was a significant treatment interaction effect (female ration with female size) on larval swim speed and horizontal turn angle (Table 5).

Table 4a. *Mean (±SD) values for each of the behavioural variables measured on groups of Atlantic cod (Gadus morhua) larvae produced by brood stock reared on different rations and at different temperatures in the 1995 experiment-year. R = brood stock ration; T = brood stock incubation temperature.*

	Treatment			
	R		**S**	
Behavioural variable	***High (n = 14)***	***Low (n = 18)***	***Large (n = 18)***	***Small (n = 14)***
Move distance (mm)	4.6 ± 0.3	3.9 ± 0.3	4.6 ± 0.3	4.0 ± 0.3
Move duration (s)	0.6 ± 0.02	0.6 ± 0.03	0.6 ± 0.03	0.6 ± 0.02
Swim speed (mm s^{-1})	7.2 ± 0.2	7.2 ± 0.3	7.6 ± 0.3	6.8 ± 0.2
Pause duration (s)	1.7 ± 0.3	2.3 ± 0.4	1.7 ± 0.4	2.3 ± 0.3
Horizontal turn angle (°)	43.6 ± 1.8	41.6 ± 2.2	40.1 ± 2.2	45.1 ± 1.8
Vertical turn angle (°)	15.9 ± 1.2	12.0 ± 1.4	12.2 ± 1.5	15.7 ± 1.2
Activity (%)	29.0 ± 2.3	21.1 ± 2.7	27.4 ± 2.8	22.7 ± 2.2

Table 4b. *Results of a two-way ANOVA (df = 1 for all comparisons) testing for between-treatment differences in the behavioural variables measured on all groups of Atlantic cod (Gadus morhua) larvae in the 1995 experiment-year. R = brood stock ration (testing high vs. low); T = broodstock incubation temperature (testing high vs. low); R x T = treatment interaction.*

	Treatment effect tested					
	R		**S**		**R x T**	
Behavioural variable	**F**	**P**	**F**	**P**	**F**	**P**
Move distance (mm)	3.10	0.09	2.49	0.13	0.38	0.54
Move duration (s)	6.75	0.01	0.52	0.48	1.46	0.24
Swim speed (mm s^{-1})	0.01	0.93	3.93	0.06	0.49	0.49
Pause duration (s)	2.08	0.16	1.27	0.27	0.30	0.59
Horizontal turn angle (°)	0.53	0.47	3.00	0.09	2.12	0.16
Vertical turn angle (°)	4.37	0.05	3.54	0.07	0.55	0.46
Activity (%)	4.90	0.03	1.72	0.20	0.13	0.73

Table 5a. *Mean (±SD) values for each of the behavioural variables measured on groups of Atlantic cod (Gadus morhua) larvae produced by brood stock differing in size and reared on different rations in the 1996 experiment-year. R = brood stock ration; S = female size.*

	Treatment			
	R		**S**	
Behavioural variable	***High (n = 14)***	***Low (n = 18)***	***Large (n = 18)***	***Small (n = 14)***
Move distance (mm)	3.5 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	3.5 ± 0.2
Move duration (s)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Swim speed (mm s^{-1})	5.8 ± 0.1	6.3 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
Pause duration (s)	2.4 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	2.4 ± 0.2
Horizontal turn angle (°)	55.9 ± 2.0	51.8 ± 2.2	50.8 ± 2.0	57.0 ± 2.2
Vertical turn angle (°)	22.4 ± 1.0	17.1 ± 1.0	18.8 ± 1.0	20.8 ± 1.1
Activity (%)	21.7 ± 1.9	25.0 ± 2.1	27.2 ± 2.0	19.5 ± 2.1

Table 5b. *Results of a two-way ANOVA (df = 1 for all comparisons) testing for between-treatment differences in the behavioural variables measured on all groups of Atlantic cod (Gadus morhua) larvae in the 1996 experiment-year. R = brood stock ration; S = female size.*

	Treatment effect tested					
	R		**S**		**R x S**	
Behavioural variable	**F**	**P**	**F**	**P**	**F**	**P**
Move distance (mm)	0.53	0.48	0.92	0.35	3.01	0.09
Move duration (s)	0.12	0.73	1.39	0.25	0.66	0.43
Swim speed (mm s-1)	6.77	0.02	0.001	0.98	6.14	0.02
Pause duration (s)	4.44	0.04	6.49	0.02	0.33	0.57
Horizontal turn angle (°)	1.94	0.17	4.47	0.04	7.18	0.01
Vertical turn angle (°)	13.74	<0.001	1.95	0.17	1.19	0.29
Activity (%)	1.39	0.25	7.27	0.01	0.04	0.84

Discussion

Relationships between female size and condition and egg production, viability and hatching success. Despite the strong influence of female size and nutritional condition on realized fecundity observed in these experiments (Lambert and Dutil 2000), there were only limited effects of maternal nutritional condition on egg survival and hatching success (Ouellet et al. 2001). Total fecundity (all egg batches) and egg dry weight were significantly lower in poor-condition females and female cod with high pre-spawning condition factors were in better post-spawning condition, and lost a lower proportion of their somatic mass and energy reserves, than did females in poor condition (Lambert and Dutil 2000). Although the total mass of eggs produced was positively correlated with the pre-spawning condition of females (in the 1996 experiment), there was no trend in the total egg dry mass per batch (Ouellet et al. 2001). Further, there was only a limited impact of egg characteristics (or batch number) on their viability and hatching success. Although this appears to contradict some of the previous work on cod, not all of that work is completely consistent (e.g. see the seemingly conflicting results in Solemdal et al. 1995 vs. 1998, and as reviewed by Ouellet et al. 2001). Overall, the results of our experiments are consistent with the findings of several analogous studies that investigated the relationships between egg characteristics and hatching success in fishes (as summarized in Table 3 of Ouellet et al. 2001).

Published observations on the relationships between the condition of female cod and the characteristics of their eggs are somewhat contradictory (Chambers and Waiwood 1996; Trippel 1998; Kjesbu et al. 1996; Marteinsdottir and Steinarsson 1998). These studies, which are thoroughly reviewed and discussed by Ouellet et al. (2001), indicate that it may be some combination (one or more) of female size, female condition, female age, and/or female spawning history that are correlated with the phenotypic characteristics of eggs. Just which of these is the most appropriate predictor remains unclear, and is possibly species- and/or stock-specific.

Relationship between larval growth and maternal condition, size and incubation temperature. The positive correlations reported here between cod larval growth rate and egg diameter are consistent with

the long-held and well-documented contention that differences in egg quality – whatever their origin – are transmitted to the larvae that hatch from them (e.g. Chambers and Leggett 1996; Marteinsdottir and Steinarsson 1998 and references cited therein). Since there is a relationship between female condition and egg quality (albeit sometimes weak and/or inconsistent), it follows that this relationship should extend to larvae. Nonetheless, direct evidence of maternal effects on the phenotypic characteristics of fish larvae (e.g. size at hatch, feeding rate, growth rate, buoyancy, DNA content) is limited, and results, at least in part, from the positive correlation between egg size and the size of larvae at hatching (Bengston et al. 1987; Pepin and Miller 1993; Heath and Blouw 1998; Marteinsdottir and Steinarsson 1998; Benoît and Pepin 1999; Heyer et al. 2001; Marteinsdottir and Begg 2002; Saborido-Rey et al. 2003; Heyer and Miller 2004). Despite these precedents, there was no relationship between female condition, size, and/or incubation temperature and larval growth in this study.

Relationship between larval behaviour and maternal condition and incubation temperature. Maternal effects may be defined as "…nongenetic influences derived from parental phenotypes or environments that have an impact on offspring phenotypes." (Heath and Blouw 1998). Under this definition, maternal effects on the behaviour of their offspring are likely common, diverse and possibly adaptive (Bernardo 1996; Mousseau and Fox 1998a,b). To paraphrase Heath and Blouw (1998), this is so "…because behaviour is possibly the most immediate and malleable source of interaction between the phenotype of an organism and its environment." Thus, since natural selection acts upon phenotypes, "…we expect adaptive evolution to be common at this interface." When the connection between egg size and larval characteristics (discussed above) is superimposed upon this, maternal effects on the behaviour of larvae are to be expected.

Earlier work demonstrated quantifiable changes in behaviour associated with the transition from endogenous to exogenous feeding (Skifetsvik 1992), and also that the behaviour of sick larvae was different from those that were healthy (Skiftesvik and Bergh 1993). Thus, the variables that we measured should be sensitive enough to detect any maternal effects on the nehaviour of larvae. With the exception of move duration, all of the behavioural variables were different at 3 vs. 6 DPH. Percent activity, swim speed, and move distance were all higher at 6 DPH than at 3 DPH, while turn angles and the duration of pauses were lower at 6 vs. 3 DPH. This is all consistent with the fact that larvae were not feeding at 3 DPH, but had begun to feed at 6 DPH: feeding larvae are more active and swim faster and longer than those that are not feeding (Browman and O'Brien 1992a,b; Skiftesvik 1992). Thus, the validity of using these variables as indicators of changes in larval behaviour is supported. Swim speed and move distances were higher in larvae from the 1995 vs. the 1996 experiment, and swim speed and move distance were negatively correlated with growth rate. Since, all else being equal, larvae that move more (and swim faster) will use more energy and, thus, grow slower, these observations further support the use of behavioural variables as indicators of larval quality.

There were no strong and consistent relationships between any indicator of female condition (size, ration, Fulton's K, incubation temperature) and the behavioural variables measured. This is consistent with the lack of a relationship between larval growth rate and female condition. Close examination of the behavioural data, including each and every swim path analysed, demonstrates a high level of variability (which is consistent with the observations of Ouellet et al. (2001) on

eggs). That, in-and-of-itself, makes resolving maternal effects all the more difficult. Spawning in batches over a relatively long (4–6 week) period is thought to increase the chances that the larvae of any given female will encounter feeding conditions adequate to support rapid growth and an increased probability of survival (this in the context of hypotheses such as Cushing's match-mismatch). In this scenario, perhaps the most adaptive strategy for females – at least those that release eggs into a pelagic and highly stochastic environment – is to produce eggs and larvae with a broad range of characteristics, in each an every batch of eggs that they release. That way, at least some individuals will possess characteristics that will enable them to survive, regardless of the conditions in which they find themselves. This high level of background variability serves to mask our ability to discern any possible maternal effects on the characteristics of eggs and larvae.

Conclusion. The sources of variability in the phenotypic characteristics (e.g. vital rates) expressed by fish eggs and larvae remain poorly known, although most ascribe at least some of it to maternal effects (reviewed in Heath and Blouw 1998). Several adaptive explanations for intrapopulation variation in egg and larval characteristics have been proposed (e.g. Parker and Begon 1986; Hendry et al. 2001; Einum and Fleming 1999); all of them assume that there is a maternal effect on the egg size-offspring fitness function. Using data drawn from the literature, Einum and Fleming (2002) demonstrated that maternal effects are more pronounced in species with demersal eggs and larvae than in species with pelagic eggs and larvae (such as cod). Thus, for pelagic batch spawners such as cod, the maternal effect on egg and larval phenotypes may be small. This is a possible reason for the lack of a more consistent maternal effect on the behaviour of cod larvae in these experiments. Other plausible explanations are that (i) the majority of egg groups followed were the progeny of females with relatively high pre-spawning condition factors; (ii) the relatively small range of pre-spawning condition factors for these females; (iii) the fact that the full complement of egg groups from each female could not be followed; (iv) high variability; and several other possibilities (taken up by Ouellet et al. 2001). On the other hand, and for all of these reasons, it is also possible that there simply is no consistent and clearly discernible relationship between maternal condition and larval quality in cod. To paraphrase Browman (1999), data which do not strongly support a research hypothesis should not be summarily dismissed as incorrect or irrelevant. Such results may provide more balance for a subject area thus far supported only (or primarily) by positive results; they may indicate that a subject area is not as mature or clearly defined as previously suspected; they may show that a particular line of research is not worth further efforts; or they may indicate that our current methodologies are inadequate for producing a definitive result. In the case of maternal effects on cod early life stages, all of the above should be considered.

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