The concept of fecundity regulation in plaice (*Pleuronectes platessa*) tested on three Irish Sea spawning populations

J. Kennedy, P.R. Witthames, and R.D.M. Nash

Abstract: The fecundity of European plaice (*Pleuronectes platessa*) in the Irish Sea between 2000 and 2004 was estimated during the spawning season for fish in the three main spawning areas (Liverpool Bay, the Cumbrian coast, and the western Irish Sea) and one small spawning group on the west coast of the Isle of Man. Fecundity was also estimated during September of 2003 and 2004. The aim of this was to assess the variability in fecundity between areas and years in the Irish Sea and also to identify when differences in fecundity become apparent in the maturation cycle. There were variations in fecundity on both the temporal and spatial scales. The greatest variation in fecundity between years occurred in the western Irish Sea, whereas there was no variation between years in the southeastern Irish Sea (Liverpool Bay). There was no difference in fecundity between areas or years during September. The maximum fecundity in plaice is determined by the total weight of the fish at the end of follicle recruitment in the ovary, and differences in the fecundity of each population are the result of different levels of down-regulation in the period between the end of follicle proliferation and spawning.

Résumé : Nous avons estimé la fécondité de la plie (*Pleuronectes platessa*) de la mer d'Irlande de 2000 à 2004 durant la saison de reproduction dans les trois régions principales de fraie (baie de Liverpool, côte de Cumbrie et mer d'Irlande occidentale), ainsi que pour un petit groupe de reproducteurs sur la côte occidentale de l'île de Man. La fécondité a aussi été estimée en septembre 2003 et 2004. Notre étude vise à évaluer la variabilité spatiale et annuelle de la fécondité dans les diverses régions de la mer d'Irlande; elle cherche aussi à déterminer le moment dans le cycle de maturation où apparaissent les différences de fécondité. Il y a des variations de fécondité tant à l'échelle temporelle que spatiale. La plus grande variation annuelle dans la fécondité se produit dans la mer d'Irlande occidentale, alors qu'il n'y a pas de variation annuelle dans le sud-est de la mer d'Irlande (baie de Liverpool). Il n'y a pas de différences annuelles ou spatiales de fécondité en septembre. Chez la plie, la fécondité maximale est déterminée par la masse totale du poisson à la fin de la période de recrutement des follicules dans les ovaires et les différences de fécondité dans les diverses populations résultent de divers niveaux de régulation descendante durant la période entre la fin de la prolifération des follicules et le début de la fraie.

[Traduit par la Rédaction]

Introduction

Spawning is a very costly activity with large investments of energy into egg production and the behaviour related to spawning (Rijnsdorp 1990; Smith et al. 1990). The total fecundity of a population represents the maximum number of potential recruits to the population and is affected by many factors. The maternal parent must balance resources between maximizing reproductive output and also conservation of resources for survival after spawning. Resources must also be partitioned between growth and reproduction. Rijnsdorp (1990) proposed a hypothetical model of the mechanism of surplus production based on physiology postulating that surplus production is prioritised into building up reserves followed by reproduction and somatic growth. The allocation to reproduction then reaches a level of saturation resulting in greater allocation to somatic growth. This allocation is known to be affected by age and total length of the fish, with lower amounts of energy being invested in somatic growth with an increase in age (Jørgensen and Fiksen 2006). It has been seen that subpopulations of European plaice (*Pleuronectes platessa*) in the Irish Sea that have a higher surplus production have a higher reproductive investment (Nash et al. 2000). Variation in fecundity of plaice has been

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shown to occur on both spatial (Bagenal 1966; Nash et al. 2000) and temporal scales (Bagenal 1966; Horwood et al. 1986; Rijnsdorp 1991) and can be affected by food level (Horwood et al. 1989). However, Rijnsdorp (1994) stated that relative fecundity (number of follicles per unit of body weight) is constant over a wide range of population abundances and levels of surplus energy, with a decrease in relative fecundity only occurring at high population levels. The absolute level of reproductive investment will still increase when surplus energy increases because of a larger body size attained through an increase in somatic growth.

Plaice have a determinate spawning strategy (Hunter et al. 1992) in which the annual fecundity of an individual female is determined before the onset of the spawning season (Urban 1991; Murua and Saborido-Rey 2003) with the development of the ovary beginning several months before spawning (Dawson and Grimm 1980; Rijnsdorp 1989). During spawning, eggs are released in batches (the realised fecundity) that are recruited into final maturation at intervals of 2 to 5 days over a period of 4 to 6 weeks (Rijnsdorp 1989).

Fecundity estimates of plaice taken in 1995 from the Irish Sea exhibited spatial variation, with fish from Liverpool Bay having the highest fecundity and fish from the western Irish Sea having the lowest (Nash et al. 2000). Nash et al. (2000) also showed that fecundity in Liverpool Bay and the Cumbrian coast in 1995 was not significantly different from estimates taken in 1953 (A.C. Simpson, unpublished data). This has also been the case in Cardigan Bay for the years 1953 and 1988 (Horwood 1990). No studies have examined fecundity of plaice over a number of consecutive years in the Irish Sea.

In this paper, we refer to the unit of fecundity as a follicle, i.e., the oocyte and its surrounding somatic tissue (Tyler and Sumpter 1996). Before maturation, the ovary contains pre-vitellogenic follicles, which consist of an ooplasm surrounded by oolemma zona radiata and a follicle containing no yolk protein (Tyler and Sumpter 1996). The annual maturation cycle starts when extra-ovarian proteins (vitellogenin) are sequestered, processed, and packaged via the follicle into oocytes, which become known as vitellogenic follicles (Tyler and Sumpter 1996). The follicles enter final maturation in batches and after ovulation release eggs expelled from the ovary for fertilization. The number of eggs released is the realised fecundity.

Several species have been shown to recruit more follicles than are taken to full development, e.g., Atlantic herring (*Clupea harengus*) (Kurita et al. 2003), turbot (*Scophthalmus maximus*) (Bromley et al. 2000), Atlantic cod (*Gadus morhua*) (Kjesbu et al. 1991; Armstrong et al. 2001), and sole (*Solea solea*) (Armstrong et al. 2001). Fecundity is then down-regulated by atresia in relation to available energy reserves (Kurita et al. 2003). Atresia has also been shown to occur in prespawning plaice, but this was at a low prevalence (proportion of fish with atresia) and intensity (number of atretic follicles per gram of body weight in females with atresia) (Armstrong et al. 2001).

During this study, the fecundity of plaice caught in the three main spawning areas of the Irish Sea (Liverpool Bay, the Cumbrian coast, and the western Irish Sea) over several years was estimated. In 2000, the intensity and prevalence of

atresia was also quantified. Using the current estimates and data available from 1953 and 1995, differences between years and effects of body condition and muscle condition (muscle water content) on individual fecundity were examined. Whole body condition was calculated as it is an indication of the level of stored energy available to the fish, and a high water content in the muscle is an indication of protein depletion (Stirling 1976; Costopoulos and Fonds 1989). Fecundity estimates were also taken in September to investigate whether down-regulation of fecundity occurs between the period of vitellogenic follicle recruitment and spawning.

Materials and methods

Collection of samples

Fish were caught by trawl during the spawning season (between late January and early May) in 2000, 2001, 2003, and 2004 and during September in 2003 and 2004 from the three main spawning areas in the Irish Sea: Liverpool Bay, the Cumbrian coast, and the western Irish Sea (Nash et al. 2000) (Fig. 1). Fish were also caught from a small spawning group approximately 6 miles west of the Isle of Man in 2004 (Ellis and Nash 1997) (Table 1). Data were also available for 1953 (A.C. Simpson, unpublished) and 1995 (Nash et al. 2000). In 2000 and 2001, the ovaries were preserved whole in 3.7% formalin. In 2003 and 2004, the ovaries were weighed at sea and a follicle sample was taken from the middle section of the lighter ovary (M2) (to distinguish between the two ovaries, the heavier ovary was termed ovary 1 and the lighter ovary was termed ovary 2) and preserved in 3.7% formalin. Ovaries containing hydrated oocytes, which were easily identified because of their large size, were excluded from the analysis to avoid including fish that may have previously spawned during the year in question. The ovaries from 2000 were examined histologically for the presence of postovulatory follicles (which indicate that eggs have been ovulated and the fish is likely to have spawned during the current spawning season (Hunter and Goldberg 1980)) to validate the spawning or nonspawning status assessed from the presence or absence of hydrated eggs.

Homogeneity of ovary

To test whether the ovaries were homogenous with respect to follicle diameter and density (number of follicles per gram of ovary), samples were taken from the anterior (A), middle (M), and posterior (P) of ovary 1 and ovary 2 of 20 fish. The mean follicle diameter and density were measured in each piece, and the mean for the whole ovary was determined after weighing according to the mass of the anterior, middle, and posterior pieces. From the results, it was decided to standardize the sampling of ovaries to the middle section of the lighter ovary (M2).

Estimation of fecundity

The estimation of fecundity was based on the gravimetric method (Hunter et al. 1989) but was also intercalibrated with the autodiametric method (Thorsen and Kjesbu 2001). The autodiametric method works on the principle that follicle density is directly proportional to mean follicle diameter, linked by a power curve relationship. Follicle counts and

Fig. 1. (*a*) Map of the Irish Sea showing the main plaice (*Pleuronectes platessa*) spawning areas (shaded areas; Liverpool Bay, the Cumbrian coast, Cardigan Bay, and the western Irish Sea) in the Irish Sea. The small spawning area west of the Isle of Man is also shown. (*b*) Map of Europe showing the location of the Irish Sea.
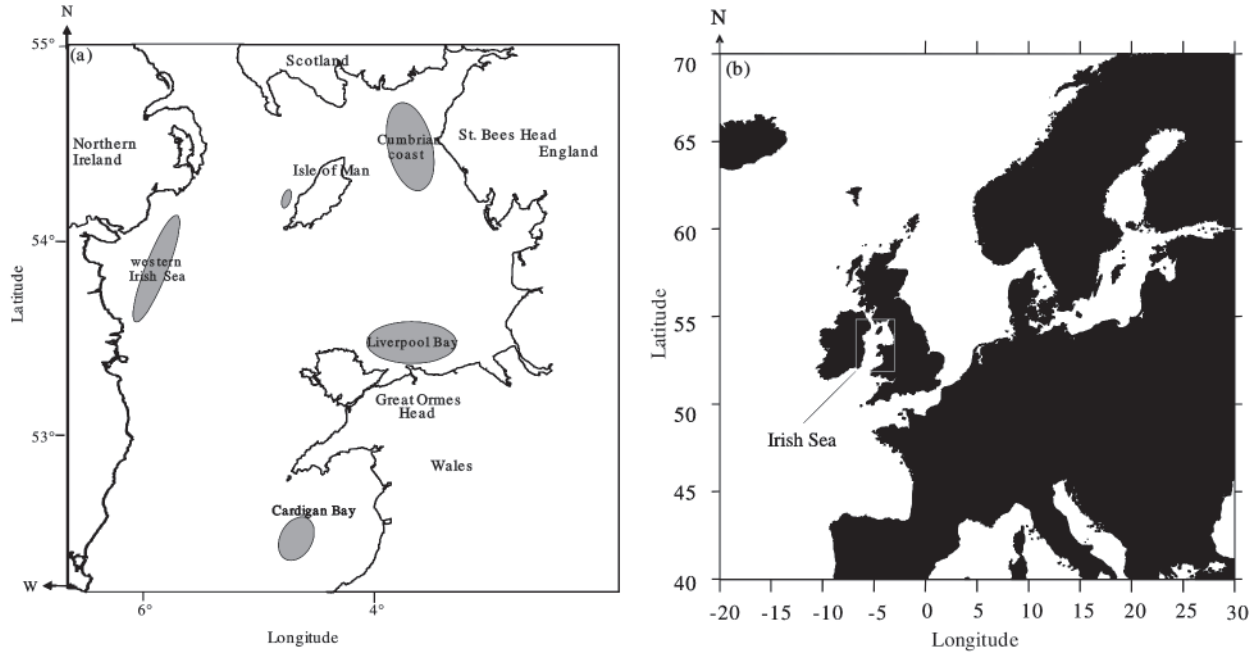
Table 1. Summary of sample size of plaice (*Pleuronectes platessa*) after removal of individuals containing hydrated oocytes (*n*), length range (minimum/maximum; cm), ln(fecundity) – ln(total length) relationship (*F–L*) and respective R^2, ln(fecundity) – ln(total weight) relationship (*F–W*) and respective R^2 for year, sampling time, and area.

Year	Sampling month	Area	*n*	Length range	Average	*F–L* relationship	R^2	*F–W* relationship	R^2
1953	SS	LB	28	22/35	28	$\ln F = -1.55 + 3.73\ln L$	0.78		
	SS	CC	95	22/49	32	$\ln F = -3.48 + 4.25\ln L$	0.81		
1995	SS	LB	42	22/41	27	$\ln F = -3.13 + 4.20\ln L$	0.85	$\ln F = 3.98 + 1.26\ln W$	0.90
	SS	CC	95	22/49	32	$\ln F = -3.48 + 4.25\ln L$	0.81	$\ln F = 4.42 + 1.18\ln W$	0.81
	SS	WIS	44	21/43	27	$\ln F = -4.32 + 4.43\ln L$	0.84	$\ln F = 2.71 + 1.43\ln W$	0.89
2000	SS	LB	42	21/40	28	$\ln F = 3.28\ln L$	0.55	$\ln F = 5.08 + 1.07\ln W$	0.63
	SS	CC	71	22/42	30	$\ln F = -1.59 + 3.80\ln L$	0.84	$\ln F = 4.61 + 1.16\ln W$	0.90
	SS	WIS	89	20/48	30	$\ln F = -1.39 + 3.72\ln L$	0.89	$\ln F = 4.75 + 1.15\ln W$	0.91
2001	SS	LB	52	21/43	29	$\ln F = -2.59 + 4.06\ln L$	0.79	$\ln F = 3.92 + 1.27\ln W$	0.84
	SS	CC	46	21/45	31	$\ln F = 0.21 + 3.20\ln L$	0.80	$\ln F = 5.14 + 1.04\ln W$	0.84
	SS	WIS	85	16/42	28	$\ln F = -2.43 + 3.95\ln L$	0.84	$\ln F = 3.55 + 1.32\ln W$	0.91
2003	SS	LB	52	19/35	26	$\ln F = -2.59 + 4.06\ln L$	0.79	$\ln F = 3.92 + 1.27\ln W$	0.84
	SS	WIS	13	23/45	30	$\ln F = -2.45 + 4.02\ln L$	0.94	$\ln F = 4.20 + 1.23\ln W$	0.96
	September	LB	50	23/43	31	$\ln F = 1.69 + 3.86\ln L$	0.85	$\ln F = 4.49 + 1.22\ln W$	0.90
	September	CC	54	26/49	32	$\ln F = -1.53 + 3.81\ln L$	0.85	$\ln F = 4.04 + 1.29\ln W$	0.89
	September	WIS	25	22/37	26	$\ln F = -3.16 + 4.27\ln L$	0.87	$\ln F = 3.52 + 1.39\ln W$	0.85
2004	SS	WIS	58	20/50	31	$\ln F = -2.98 + 4.16\ln L$	0.88	$\ln F = 4.09 + 1.25\ln W$	0.91
	September	WIOM	35	25/44	31	$\ln F = -1.61 + 3.84\ln L$	0.72	$\ln F = 4.58 + 1.22\ln W$	0.84
	September	LB	53	23/43	32	$\ln F = -1.76 + 3.87\ln L$	0.79	$\ln F = 4.53 + 1.22\ln W$	0.80

Note: SS, spawning season; LB, Liverpool Bay; CC, Cumbrian coast; WIS, western Irish Sea; WIOM, west of Isle of Man. Data from 1953 are from A.C. Simpson (unpublished data) and from 1995 are from Nash et al. (2000).

measurements were performed using a PC-based image analysis system Aphelion (ADCIS France) with commercially available software GFA (Pilkington Image Analysis Systems). Follicle samples were stained with periodic acid and Schiff's reagent to improve the identification of follicles during image analysis. Follicles that were not identified automatically were measured manually. The threshold between vitellogenic and previtellogenic follicles was set at 200 μm as suggested by evidence from follicle size distributions in plaice (Horwood 1990) and is the size at which follicles be-

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come vitellogenic in several other fish species with pelagic eggs (Kjesbu and Kryvi 1989; Carnevali et al. 1992; Hunter et al. 1992). The relationship between follicle density and diameter was calibrated using the gravimetric and auto-diametric methods for 88 fish caught in the Irish Sea in January and September 2004. These samples encompassed a range of mean follicle diameters from 269 to 1170 μm. An exponential regression line was fitted to the data. The relationship was tested by comparing the fecundity estimates of 10 pairs of ovaries (which were not included in the regression) that were analysed using both the gravimetric and the autodiametric methods. Because of differences in the follicle diameter at different positions in the ovary, the relationship was calibrated to follicles from the M2 portion of the ovaries.

To check if the realised fecundity was similar to the potential fecundity, ovaries collected in 2000 were examined histologically for the prevalence (the proportion of mature females in which atresia takes place) and intensity (the number of atretic follicles per gram body weight in females with atresia) of atresia. This method measured the standing stock of atretic follicles and tested whether the estimated fecundity would equal the realised fecundity and if the potential fecundity estimates would need to be adjusted for more accurate estimates of realised fecundity. This was assumed to be represensive of 10 days of production, as duration of the atretic stage of plaice is unknown but has been reported to be 10 days for cod (Kjesbu et al. 1991). The actual loss of fecundity (follicles·g^{-1}·day^{-1}) was estimated using the following equation:

(1)   actual loss of fecundity =

intensity × prevalence / duration of atretic stage

Histological sections were processed using ascending concentrations of ethanol and ending with resin polymerised using a PFTE mould. Sections were then examined for atretic follicles and the intensity was calculated using the disector method (Sterio 1984). This involved taking several sections at least one-third the distance of the maximum follicle diameter apart. For each section, the number of vitellogenic and atretic follicles were counted. Follicles that were present in more than one section were only counted once, which removed the bias resulting from vitellogenic follicles being larger and hence having a greater probability of appearing in a specific section.

Muscle tissue

Muscle tissue samples were taken from the dorsal surface of the fish for estimation of water content. The samples were stored in a 2 mL Eppendorf-type tube and frozen until analysis. The tissue and the tube were thawed, weighed to the nearest 100 μg, and dried at 60 °C until a constant weight was achieved. The microtube was then cleaned and reweighed. The percentage water was then calculated using the following equation:

(2)   percentage water =

$(OW - TW) - (FW - TW)/(OW - TW) \times 100$

where TW is tube weight, FW is final weight (tissue plus tube), and OW is original weight (tissue plus tube).

Fish condition

Relative condition index (K_r) was used as the measure of fish condition rather than Fulton's condition factor because Fulton's factor tends to increase with increasing length (Morgan 2004). Condition was assessed for total weight (whole body condition) and total weight minus ovary weight (somatic condition). A similar relationship was also used to calculate fecundity index (F_r).

(3) $K_r = W/W_p$

(4) $F_r = F/F_p$

where W is total weight, W_p is predicted body or somatic weight from the length–weight relationship from all stage 4 (ripe but not spawning) fish sampled in 2001, F is actual fecundity, and F_p is predicted from the length–fecundity relationship from all stage 4 fish sampled in 2001 (Table 1).

The length – total weight and length – somatic weight relationships from all stage 4 (ripe but not spawning) fish sampled in 2001 ($n = 183$) were

(5)   total weight $= 0.0064 \times$ length$^{3.1622}$

($R^2 = 0.88$, $P < 0.001$)

(6)   somatic weight $= 0.0093 \times$ length$^{3.0094}$

($R^2 = 0.89$, $P < 0.001$)

Relative fecundity was also calculated for each fish using the equation

(7)   relative fecundity = fecundity / total weight

Weight was a better predictor of fecundity; however, length was chosen as the independent variable in comparison of fecundity between areas as ovary weight is a significant component of total weight and fecundity. Weight also varies throughout the year and changes during fasting in December, before spawning, whereas there is no change in length during fasting. Fecundity index was analysed against somatic index as the ovary can make up a significant proportion of the total weight and there is a strong relationship between fecundity and ovary weight. The change in relative fecundity with follicle diameter was analysed using fish caught from Liverpool Bay as there were no differences in fecundity between years for this population. Gonadosomatic index (GSI) was calculated for fish when ovary weight was available; this was calculated using the equation

(8)   GSI $= O/W \times 100$

where O is ovary weight and W is total fish weight.

Follicle size distributions

The change in the follicle size distributions through ovary maturation was examined using the modal size of the follicle size distributions as an indicator of the stage of ovary development.

Statistical analysis

All statistical tests were carried out using Statistica 6.1 (StatSoft Inc. 2002). Differences in mean follicle diameter and follicle density between ovaries and between sampling sites within an ovary were tested using analysis of variance (ANOVA). All length, weight, and fecundity values were

Table 2. Two-way analysis of variance comparing differences of follicle density (top) and mean M2 follicle diameter (bottom) between sampling sites in ovaries of plaice (*Pleuronectes platessa*).

	SS	df	MS	*F*	*P*
Follicle density					
Intercept	114.707	1	114.707	11 010.18	<0.001
Sampling site	0.0364	2	0.0182	1.75	0.181
Fish	0.2528	37	0.0068	0.66	0.919
Error	0.7605	73	0.0104		
Follicle diameter					
Intercept	18 518	1	18 518	1 267 346	<0.001
Sampling site	0.66	4	0.17	11	<0.001
Fish	131.00	18	7.28	498	<0.001
Error	255.59	17 492	0.01		

Note: SS, sum of squares; df, degrees of freedom; MS, mean square.

ln-transformed to achieve normal distribution of data. Differences between the two fecundity estimation methods were tested using the Student's *t* test for dependent samples. Linear regression models were fitted to the fecundity–length and fecundity–weight ln-transformed data for all areas and years. Fecundity differences between areas and years were tested for using analysis of covariance (ANCOVA) with length as the independent variable. Forward stepwise regression was used to find the best predictor of fecundity with length, weight, and somatic condition as predictor variables. Least-square means of fecundity, with the removal of the effect of fish length, were calculated for each population in each year using analysis of covariance in Statistica 6.1 (StatSoft Inc. 2002).

Results

Homogeneity of ovary

There was no significant difference in the mean follicle density between sampling sites in the ovaries (two-way ANOVA, $P > 0.05$; Table 2); however, there were differences in the mean follicle diameter between sites (two-way ANOVA, $P < 0.001$; Fig. 2; Table 2). It was decided to standardize the sampling of ovaries to the middle portion of the smaller ovary (M2).

Calibration of mean follicle diameter against follicle density

There was a significant positive relationship between mean M2 follicle diameter and follicle density (Fig. 3; Table 3). An exponential regression line was fitted to follicle density and mean M2 follicle diameter data for the samples collected in 2004 (exponential regression, $R^2 = 0.97$, $n = 91$, $P < 0.001$) and used for the calculation of follicle density from the measured mean M2 follicle diameter from other samples. The regression function was

$$(9) \qquad FD = 54506 \, e^{-3.3607MFD}$$

where FD is follicle density and MFD is mean M2 follicle diameter.

There was no significant difference in fecundity estimations between the gravimetric and autodiametric method

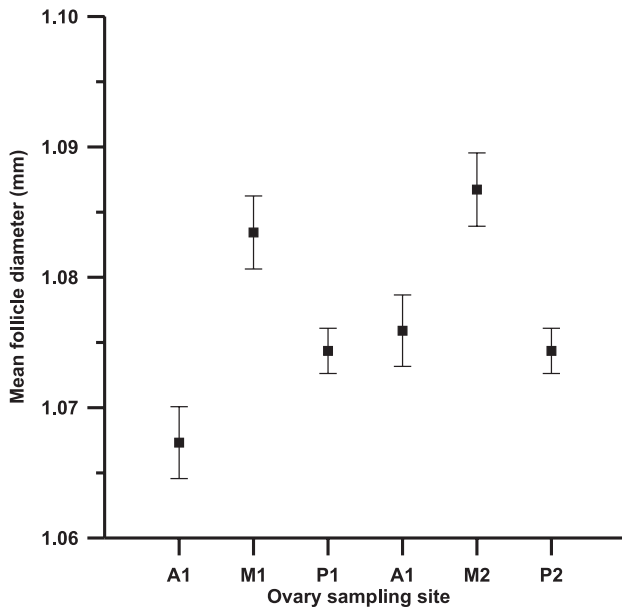(*t* test for dependent samples, $t = 0.07$, $n = 10$, $P > 0.05$) (Fig. 4).

Fecundity, fish size, and condition

Linear regression models were fitted to the fecundity–length and fecundity – total weight ln-transformed data for each area and year (Table 1). Total weight was the best predictor of fecundity for all groups except for fish in the western Irish Sea in September 2003 where length alone was the best predictor. Fish condition had a small influence in 5 of the 17 groups and length exerted an influence in 8 groups (Table 4). There was an increase in the fecundity index with somatic condition for fish in 2000, 2001, 2003, and 2004 but not 1995 (Table 5) (the fish in 1953 were not tested for this as ovary weight was unavailable). Somatic condition index decreased with increases in follicle diameter for fish caught in the spawning season during 2000 and 2001; however, this relationship was very weak. This relationship was not present in the data from 2003 or 2004 (Table 6).
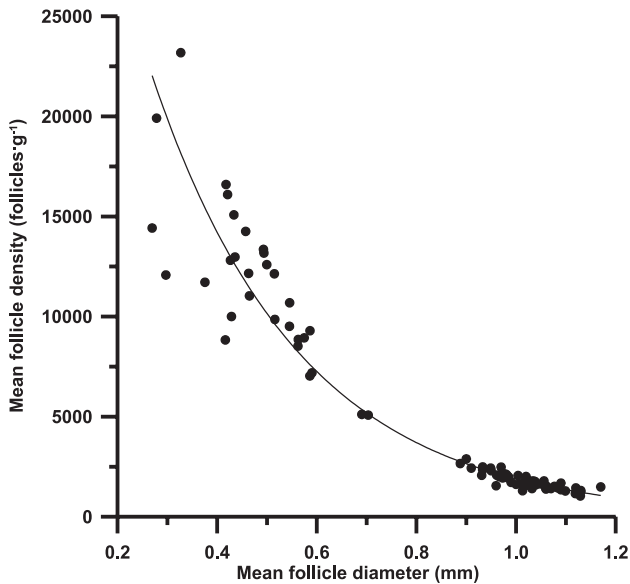
There was a weak but significant negative effect of whole body condition on muscle water content for the fish caught in September 2003 (correlation analysis, $R^2 = 0.08$, $n = 128$, $P < 0.001$) (Fig. 5) and September 2004 (correlation analyses, $R^2 = 0.40$, $n = 52$, $P < 0.001$) and fish caught during the spawning season in 2004 (correlation analyses, $R^2 = 0.05$, $n = 91$, $P = 0.017$) (muscle water content was not measured before September 2003). There was no effect of muscle water content on fecundity.

GSI increased with follicle diameter for fish caught in September and during the spawning season in 2000, 2001, 2003, and 2004 (Table 7). There was an increase in mean M2 follicle diameter with fish length in the Cumbrian coast in 2000, in Liverpool Bay in 2000, in all three areas in 2001 (data combined), and in the western Irish Sea and west of the Isle of Man in 2003 (data combined) (Table 8). This was not tested for data in 1953 and 1995 because of follicle sizes being unavailable. The prevalence of atresia in fish caught in the spawning season during 2000 was 11%, with an average intensity of 5.31 follicles·g^{-1} of fish weight (Fig. 6). These results gave an overall average loss of fecundity of 0.06 follicles·g^{-1}·day^{-1} for the previous 10 days. As this value was low, atresia levels were assumed to have no significant effect on estimated fecundity.

Fig. 2. Mean follicle diameter at each sampling site in the ovaries of Irish Sea plaice (*Pleuronectes platessa*) (A, anterior; M, middle; P, posterior; 1, heavier ovary; 2, lighter ovary). Error bars indicate ±1 standard error.

Fig. 3. The relationship between follicle density and mean follicle diameter in the middle section of the lighter ovary in plaice (*Pleuronectes platessa*) sampled in the Irish Sea in January and September 2004. Line shows fitted exponential regression line.

Table 3. Univariate test of signifigance of follicle diameter versus mean follicle density in middle section of the smaller ovary in plaice (*Pleuronectes platessa*).

	SS	df	MS	F	P
Intercept	3.51×10^9	1	3.51×10^9	1184	<0.0001
Follicle size	2.17×10^9	1	2.17×10^9	733	<0.0001
Error	2.55×10^8	86	2.96×10^6		

Note: SS, sum of squares; df, degrees of freedom; MS, mean square.

Fig. 4. Fecundity estimate for 10 plaice (*Pleuronectes plaressa*) using the gravimetric (shaded bars) and autodiametric method (open bars).
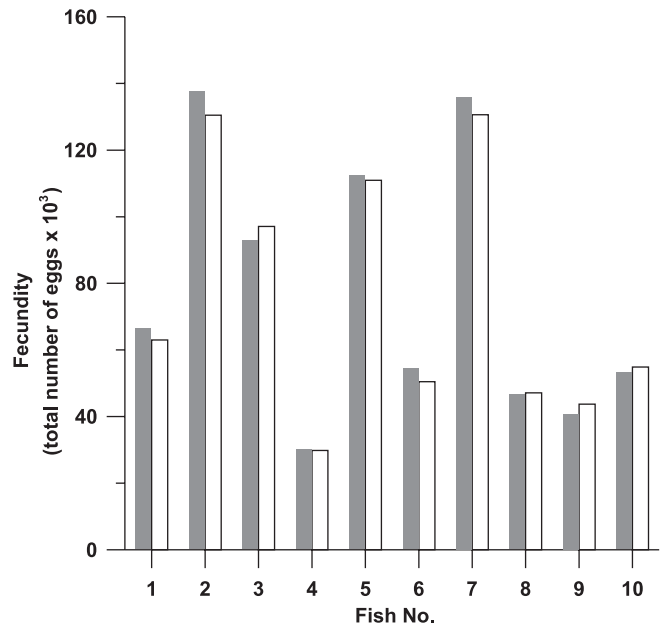
2004. The rank in fecundity between areas changed from 2000 to 2001 (Fig. 8), with fish from Liverpool Bay having the lowest fecundity of the three in 2000 to having the greatest in 2001. There was no difference in fecundity between fish from Liverpool Bay and the western Irish Sea in 2003. There was a difference in fecundity between fish from the western Irish Sea and the Isle of Man population in 2004 (Fig. 8), with fish from the latter having a higher fecundity than those from the western Irish Sea. The highest fecundity was found in fish from the Cumbrian coast in 2000 and the lowest was in fish from the western Irish Sea in 1995. The fecundity differed in these two groups by 44%, 39%, and 33% for fish of 30, 35, and 40 cm, respectively.

Fecundity in September

The distribution of follicle diameters was generally tailed towards the smaller follicle sizes, with continuity in the frequency distribution of follicle diameters between the previtellogenic and vitellogenic follicles in many of the samples (Figs. 9a–9c).

The fecundity determined in September for fish in all three areas was significantly higher than all fecundity estimates taken in previous years during the spawning season (Fig. 7). There were no significant differences in the fecun-

Interannual and interareal differences in fecundity

There were significant year effects on fecundity in the Cumbrian coast (ANCOVA, df = 4, *P* < 0.001) (Fig. 7a) and the western Irish Sea (ANCOVA, df = 5, *P* < 0.001) (Fig. 7b); however, there was no interannual difference in fecundity in fish caught in Liverpool Bay (ANCOVA, df = 5, *P* > 0.05) (Fig. 7c).

There were significant areal effects on fecundity in 1995 (previously reported in Nash et al. (2000)), 2000, 2001, and

Table 4. Results from stepwise regression showing the variables included to give the best predictor of fecundity for each spawning group of plaice (*Pleuronectes platessa*) for each year sampled, the variables included in the model, the variance explained by each variable (if any), and the total variation explained by the model (total R^2).

Year	Sampling period	Area	Variables	R^2 explained by S	R^2 explained by L	Total R^2
1995	January–February	LB	W, L		0.03	0.92
		CC	W, S, L	0.04	0.04	0.89
		WIS	W			0.88
2000	January–February	LB	W, L		0.04	0.67
		CC	W			0.90
		WIS	W			0.91
2001	January–February	LB	W, S, L	0.02	0.01	0.87
		CC	W			0.84
		WIS	W, S, L	0.02	0.02	0.93
2003	January–February	LB	W			0.84
		WIS	W			0.96
	September	LB	W			0.90
		CC	W			0.89
		WIS	L		0.87	0.87
2004	January–February	WIS	W			0.91
		WIOM	W, S, L	0.03	0.04	0.90
	September	LB	W, S, L	0.15	0.04	0.98

Note: LB, Liverpool Bay; CC, Cumbrian coast; WIS, western Irish Sea; WIOM, west of Isle of Man; W, total weight; S, somatic condition; L, length.

Table 5. R^2, n, and P values for correlation analyses of fecundity index against somatic condition for plaice (*Pleuronectes platessa*) caught in the Irish Sea during the spawning season in 1995, 2000, 2001, 2003, and 2004.

Year	R^2	n	P
1995	—	181	>0.05
2000	0.03	202	0.004
2001	0.41	183	0.001
2003	0.17	26	0.020
2004	0.07	93	0.005

Table 6. R^2, n, and P values for correlation analyses of somatic index against follicle diameter for plaice (*Pleuronectes platessa*) caught in the Irish Sea during the spawning season in 2000, 2001, 2003, and 2004.

Year	R^2	n	P
2000	0.07	202	<0.001
2001	0.07	183	<0.001
2003	—	26	>0.05
2004	—	93	>0.05

Fig. 5. The correlation between fish condition and muscle water content in Irish Sea plaice (*Pleuronectes platessa*) sampled in September 2003. Line shows linear regression.

dity among the three areas in 2003 (ANCOVA, $P > 0.05$) or between 2003 and 2004 in Liverpool Bay (ANCOVA, $P > 0.05$); therefore, a common linear regression model was fitted for length (linear regression, $R^2 = 0.871$, $n = 183$, $P = 0.001$) and weight (linear regression, $R^2 = 0.873$, $n = 183$, $P = 0.001$) (Fig. 10). There was a positive correlation between fish length and follicle diameter (linear regression,
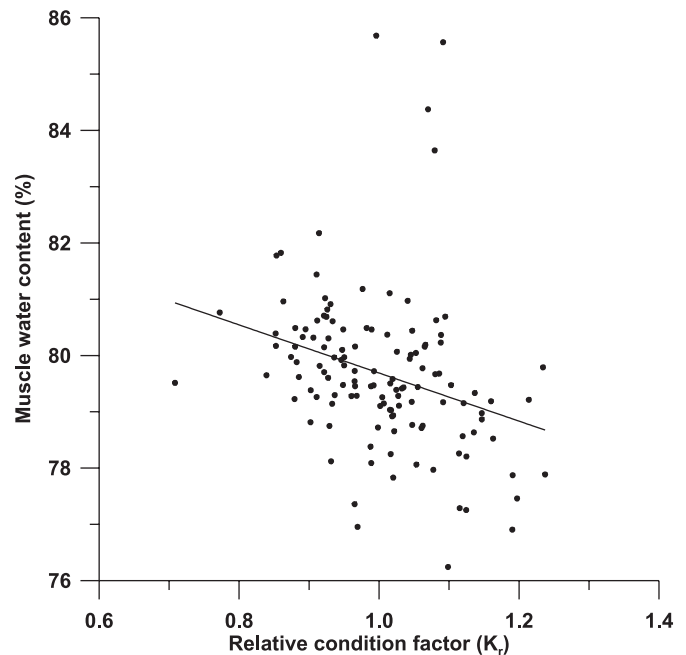
$R^2 = 0.14$, $n = 183$, $P > 0.001$) (Fig. 11). For fish with a mean M2 follicle diameter less than 700 µm, there was a positive correlation between follicle diameter and relative fecundity (fecundity/total weight) (linear regression, $R^2 = 0.18$, $n = 125$, $P > 0.001$) (Fig. 12a). This became a negative correlation when mean M2 follicle size was greater than approximately 700 µm (linear regression, $R^2 = 0.087$, $n = 103$,

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Table 7. R^2, n, and P values for correlation analyses of gonadosomatic index (GSI) against follicle diameter for plaice (*Pleuronectes platessa*) caught in the Irish Sea during September 2003 and during the spawning season (SS) in 2000, 2001, 2003, and 2004.

Year	R^2	n	P
September 2003	0.72	184	<0.001
SS 2000	0.14	202	<0.001
SS 2001	0.19	183	<0.001
SS 2003	0.34	26	<0.001
SS 2004	0.16	93	<0.001

Table 8. R^2, n, and P values for correlation analyses of M2 follicle diameter against total length for plaice (*Pleuronectes platessa*) caught in the Cumbrian coast (CC), Liverpool Bay (LB), and western Irish Sea (WIS) during the spawning season in 2000, 2001, 2003, and 2004.

Year	R^2	n	P
2000			
CC	0.10	71	0.004
LB	0.10	42	0.020
WIS	—	89	>0.05
2001	0.03	182	0.007
2003	0.16	26	0.020
2004	—	93	>0.05

Note: Data from the three areas are combined for years 2001, 2003, and 2004.

Fig. 6. Intensity of atresia in the ovaries of Irish Sea plaice (*Pleuronectes platessa*) containing atretic follicles caught during the spawning season in 2000.

$P = 0.001$) (Fig. 12*b*). Relative fecundity appears to plateau when the mean M2 follicle diameter reaches approximately 1.0 mm.
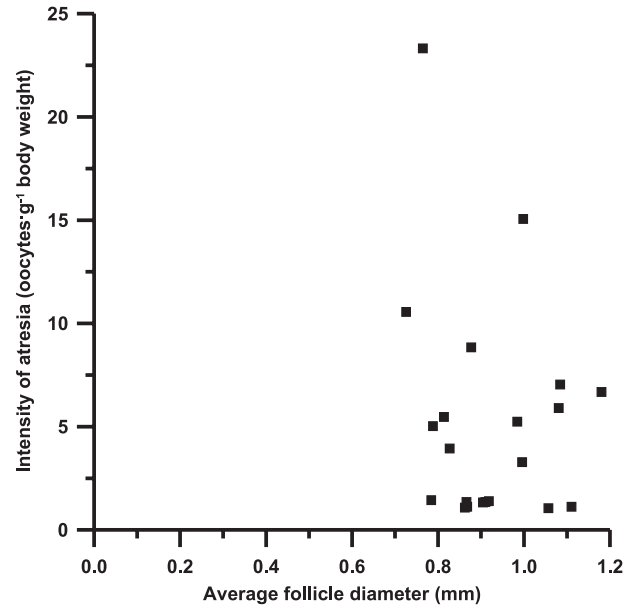
Follicle size distributions

The vitellogenic follicle size distributions during early vitellogenesis began as unimodal (Fig. 9*a*), then as the modal size increased, which represents progression through ovary development, a bimodal distribution formed (Fig. 9*b*), which then became a skewed distribution (Figs. 9*c*, 9*d*, 9*e*). The skewness towards smaller follicle sizes increased as the mode of the distribution increased until it reached approximately 600–700 µm. The skewness then decreased with increases in the mode of the size distribution (Fig. 13), with the distribution becoming unimodal (Fig. 9*f*). The distribution again becomes bimodal at the start of spawning, representing the hydration of a batch of follicles (Figs. 9*g*, 9*h*).

Discussion

As in cod (Thorsen and Kjesbu 2001), the autodiametric fecundity method in conjuction with image anlysis was a very useful and efficient method for the estimation of plaice fecundity. The method gave results that were comparable with those of the gravimetric method and also gave information on follicle size distributions to assess the stage of ovary maturation. The follicle size distributions also provided reliable identification of fish that had commenced spawning

(because of the identification of ovulated eggs) during the spawning season in question. In addition, the same relationship between follicle diameter and mean M2 follicle density could be used for fish from the start of follicle recruitment right through to spawning fish. It must be noted that there was much greater variability in the relationship at smaller follicle sizes and that the comparison between the gravimetric and autodiametric methods was made using ovaries from fish that were caught during the spawning season. The relationship also differed from the relationship in Thorsen and Kjesbu (2001), which consisted of a power curve through the data, whereas in the present study, there was an exponential regression line. Friedland et al. (2005) concluded that the relationship between follicle size and density in American shad (*Alosa sapidissima*) was too imprecise to provide useful fecundity estimates. They suggested that the poor precision may be due to their sample handling and preservation technique but also may reflect species differences in ovarian development and ovarian anatomy, which may be why there is a difference between the relationship for the present study and that of Thorsen and Kjesbu (2001). This method, as applied in this paper, is deemed appropriate for the estimation of fecundity in individual plaice that have advanced ovaries and for the estimation of population fecundity in plaice with less advanced ovaries. However, it must be applied with caution when estimating fecundity of individual fish close to the start of maturation because of the larger variance in the relationship at smaller follicle sizes.

Previous fecundity studies have used Gilson's to preserve the ovaries and break down the ovarian tissue (Nash et al. 2000; Friedland et al. 2005), whereas during the present study, we use formaldehyde. Gilson's is a digestive fluid that is much more toxic and causes greater shrinkage in follicle size than formaldehyde. Tissue preserved in Gilson's is also not suitable for histological examination. The requirements

Fig. 7. Least square means of the fecundity of plaice (*Pleuronectes platessa*) caught from (*a*) the Cumbrian coast, (*b*) the western Irish Sea, and (*c*) Liverpool Bay from 1953 to 2004 during the spawning season and in September. Results from September are indicated by an "s" after the year on the *x* axis. Error bars show ±1 standard error.
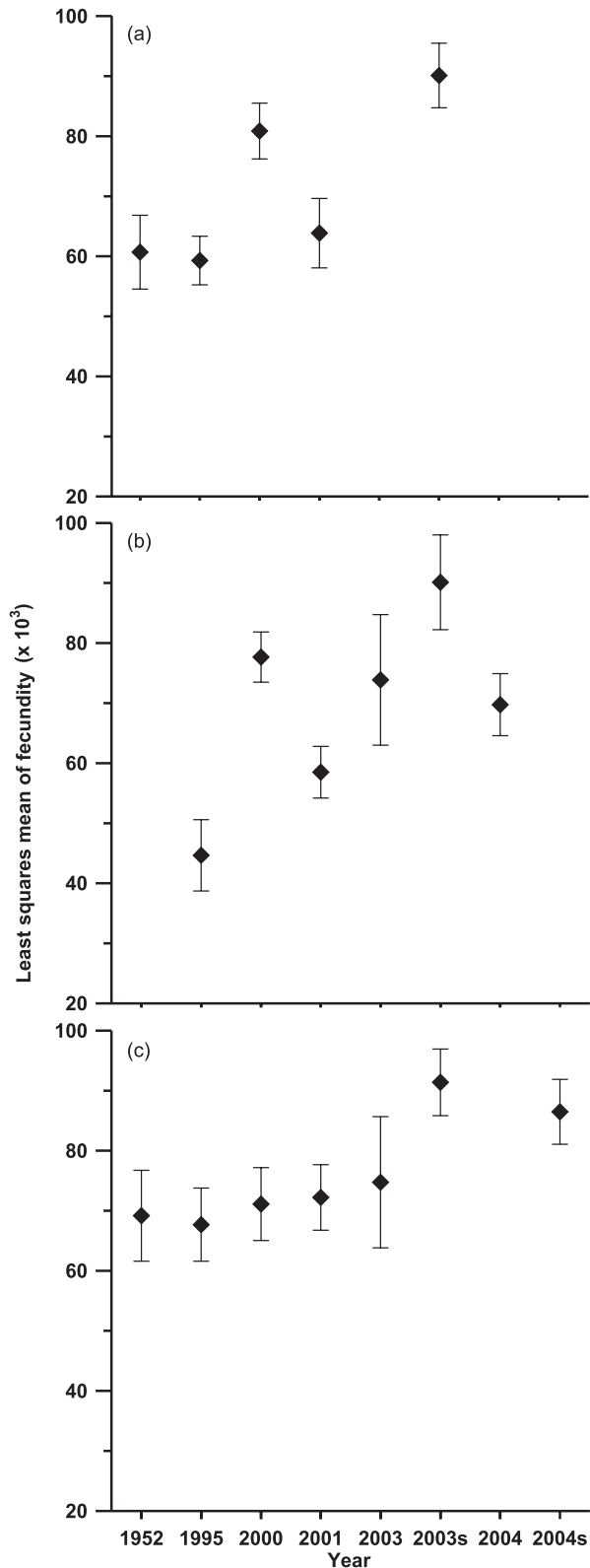
Fig. 8. Least square means for fecundity of plaice (*Pleuronectes platessa*) caught during the spawning season in 2000 (◆), 2001 (▲), and 2004 (■) from the Cumbrian coast (C), Liverpool Bay (L), the western Irish Sea (WIS), and west of the Isle of Man (WIOM). Error bars show ±1 standard error.
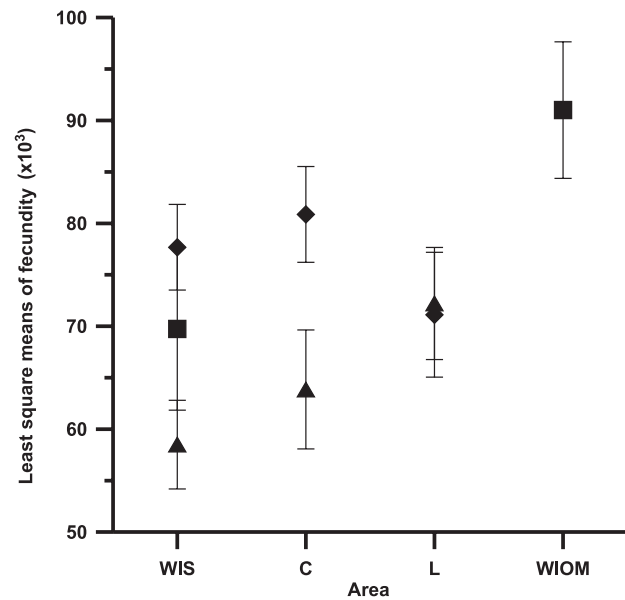
of the present study required the use of formaldehyde, and this was deemed to have no effect on the results as fecundity estimates from the present study were similar to those taken in 1995 (Nash et al. 2000) where Gilson's fixative was used.

No differences were found in the mean follicle density between ovaries or within an ovary; however, differences were found in the mean follicle diameter between ovaries and between parts of the ovary. This difference in mean follicle diameter between sites in the ovary was very small and could be due to the large number of follicles measured. Previous work by Nichol and Acuna (2001) found that follicle density in yellowfin sole (*Limanda aspera*) differed among areas of the ovary but there was no difference in mean follicle diameter between ovaries or within ovaries. Ma et al. (1998), who did a detailed study on ovary homogeneity in herring using bootstrapping, also found no difference in follicle diameter between ovaries and different parts of the ovary.

Muscle moisture content was inversely related to condition, which is in agreement with current knowledge. Condition factor is a good estimate of energy content of plaice (Costopoulos and Fonds 1989), and it is known that increased white muscle water moisture is an indication of protein depletion (Stirling 1976). The relationship showed a larger variation, and so the effect (if any) on fecundity was undetectable.

Fecundity increased with weight and length in individual fish, and weight was the best predictor of fecundity, with a small influence of length and condition independent of weight in some populations. This is in agreement with Koops et al. (2004), who found a similar result with cod and brook trout (*Salvelinus fontinalis*). Ovary weight can be a significant proportion of the total weight, and there is a very strong correlation between ovary weight and fecundity. There was a positive relation between somatic condition and

Fig. 9. Follicle size distributions of plaice (*Pleuronectes platessa*) caught from the Irish Sea. Each panel supplies data on the fish from which the sample was taken. MFD, mean M2 follicle diameter (mm); MoFD, modal follicle diameter (mm); GSI, gonadosomatic index; FPG, follicles per gram ovary tissue.
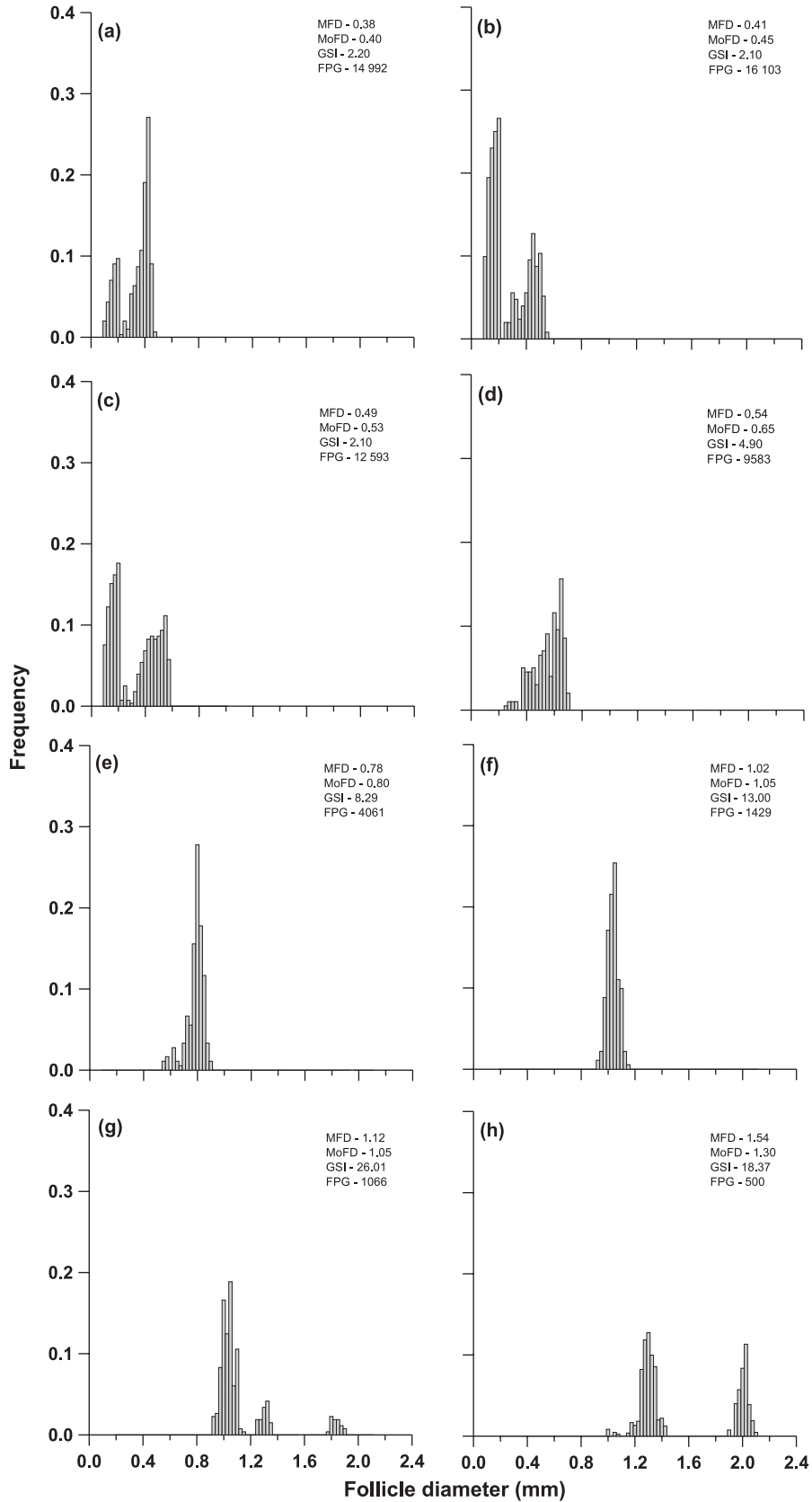
Fig. 10. Relationship between weight and fecundity of plaice (*Pleuronectes platessa*) sampled during September from Liverpool Bay in 2003 (×), the Cumbrian coast in 2003 (▽), the western Irish Sea in 2003 (◆), and from Liverpool Bay in 2004 (+). Line shows power regression line for all data combined ($\ln(Y) = 1.31 \ln X + 3.95$, $R^2 = 0.87$).
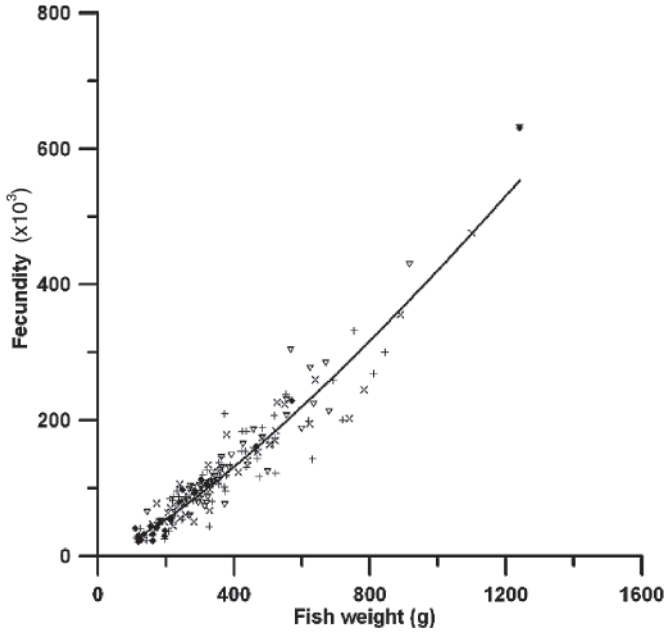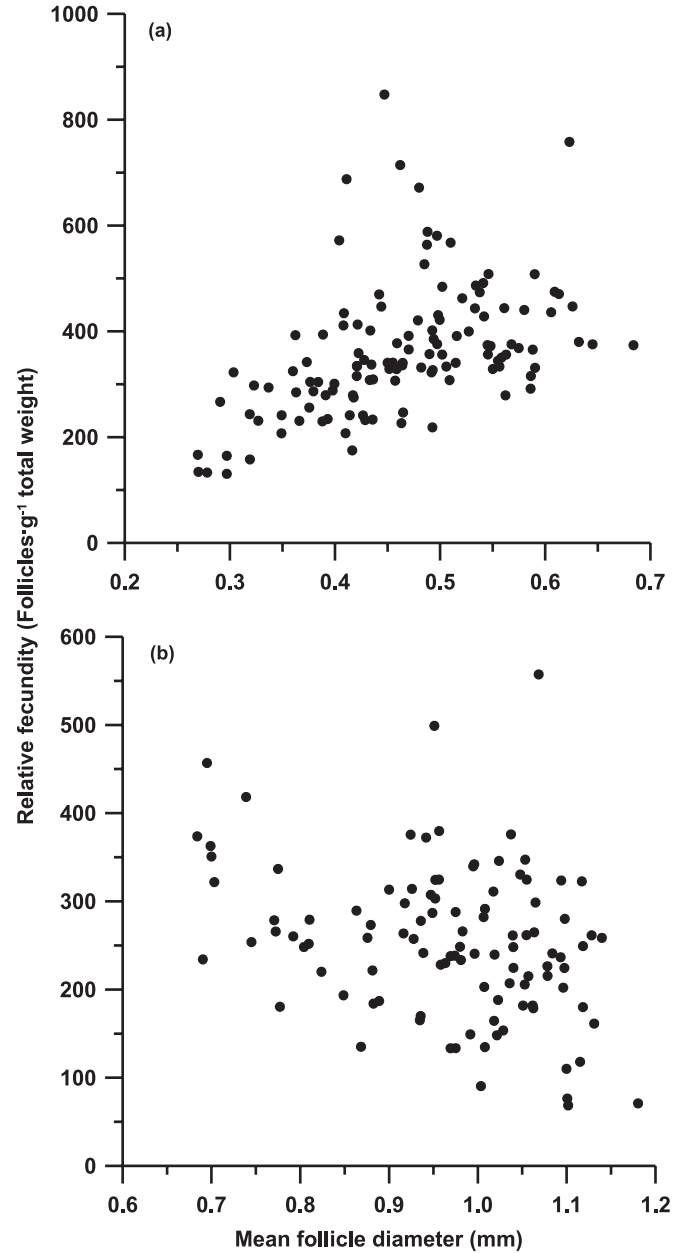

Fig. 11. Scatterplot of fish length and mean M2 follicle diameter for plaice (*Pleuronectes platessa*) sampled in September 2003.

Fig. 12. Scatterplot of mean M2 follicle diameter and relative fecundity of plaice (*Pleuronectes platessa*) sampled from Liverpool Bay in (*a*) September and (*b*) January.
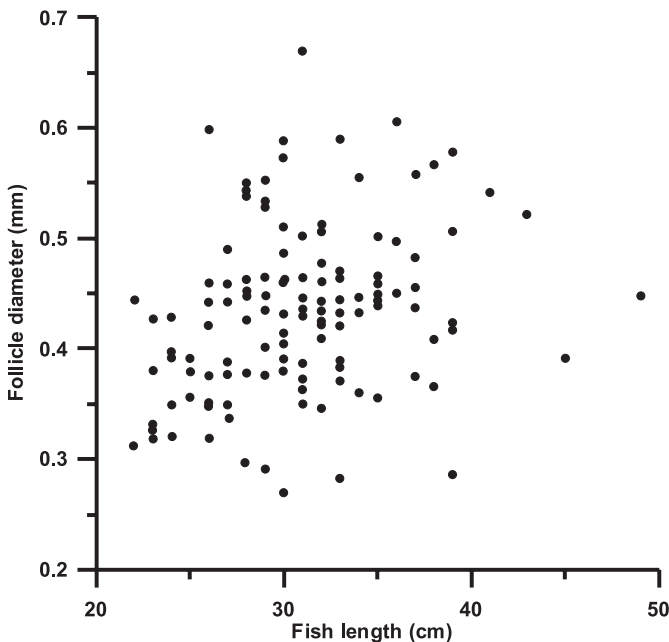
fecundity index, i.e., fish in better condition had a higher fecundity than fish in poorer condition. Condition only had an effect on fecundity independent of weight in a small number of the groups and showed only a small effect. Weight in plaice can be a good indicator of energy reserves as a large part of plaice total weight is made up of muscle, which is the main reserve store for lipids. Weight was also a much better predictor than length (which is a poor predictor of energy reserves). With a very close correlation between weight and fecundity, it can concluded that energy stores have a very strong influence on fecundity.

Somatic condition decreased with increasing follicle diameter for two of the four years during the spawning season. This relationship had a high variance and may be why it was not detectable during 2003 and 2004 when sample numbers were much lower. This relationship indicates that fish are using stored resources for ovary development, which is agreement with Rijnsdorp (1990) who estimated that up to 50% of the gonad growth in plaice is subsidised from body reserves built up during the growing period. Fish that have a

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Fig. 13. Scatterplot of skewness of follicle size distributions versus mode of follicle size distribution of plaice (*Pleuronectes platessa*).
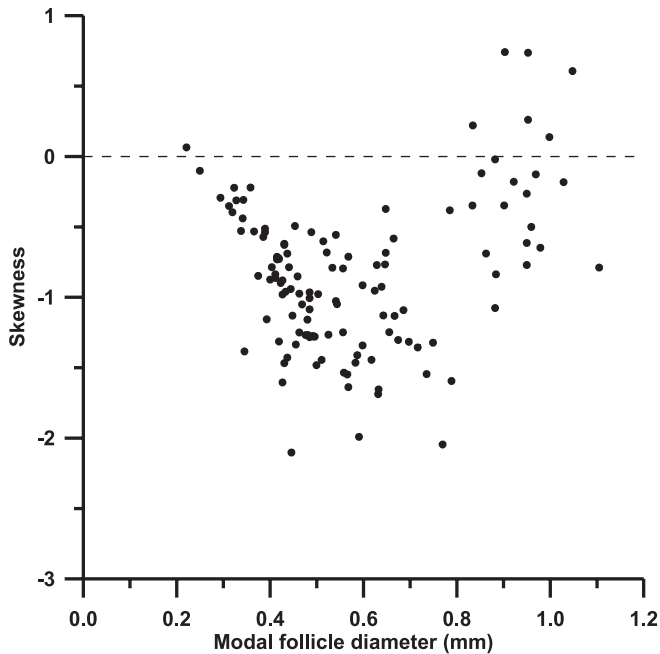
higher intake of food during ovary development use less of their resources for ovary development (Kennedy 2006), which may explain the high variation in the relationship as individual fish may get different levels of food and may cease feeding at different times. From the results, it can be seen that as ovary maturation proceeds, somatic condition will decrease as a result of using energy stores for ovary development and routine metabolism, resulting in a decrease in weight and also fecundity (see below).

This is the first time that interannual variations in fecundity have been reported in the Irish Sea; however, there are only two previous studies on this in the literature: Nash et al. (2000) and Horwood (1990), who found no difference in the eastern Irish Sea and Cardigan Bay, respectively, between 1953 (A.C. Simpson, unpublished data) and their respective sampling years. There were also differences in fecundity among the three spawning areas and differences in the variance exhibited between years. Fish from the western Irish Sea showed the greatest variation between years, and fish from Liverpool Bay showed no variation between years. The variations in the western Irish Sea are probably due to variations in prey abundance (total amount of prey and composition of prey) or prey quality after fecundity proliferation has ended (see below). This could indicate that prey abundance or the nutritional quality of the prey during autumn is much less variable between years in Liverpool Bay than in the western Irish Sea. Population density of plaice is higher on the western Irish Sea compared with the east; thus, prey abundance may be more sensitive to changes in population level. Feeding level has previously been shown to affect fecundity in plaice (Horwood et al. 1989; Kennedy 2006), and variations in fecundity have been linked to changes in population density in plaice (Bagenal 1973). Bagenal (1973) also linked variations in population density and fecundity in witch flounder (*Glyptocephalus cynoglossus*) and Norway

pout (*Trisopterus esmarkii*), and changes in population fecundity have been linked to changes in food level in Arcto-Norwegian cod (Kjesbu et al. 1998).

At the time of sampling in September, plaice were at the stage of early vitellogenesis and were still recruiting follicles, as evident from the presence of continuity in follicle sizes between the previtellogenic and vitellogenic follicles and the increase in fecundity with mean M2 follicle diameter (indication of progress through maturation). During the autumn months, plaice build up reserves and so gain weight (Rijnsdorp 1990). As plaice are still recruiting follicles in September and there is a close correlation between weight and fecundity, with no difference in the relationship between areas, it is clear that follicles are recruited over a period of time in line with increases in weight. The maximum fecundity will then be determined by the fish's weight at the end of follicle recruitment. Similar results have been found for cod whereby there is a positive correlation between the weight of the fish and the number of previtellogenic follicles entering the circumnuclear phase during the postspawning phase. There is also a positive correlation between the production of previtellogenic follicles during the autumn and the condition factor of the fish (Kjesbu et al. 1991).

There is a good relationship between weight and fecundity during the spawning season as the weight of the ovary makes up a significant component of the total weight of the fish. This is not so for fish in September, which shows that a fish's fecundity is decided on the basis of the fish's body size rather than body weight being a result of a fish's fecundity. The results are in agreement with the model proposed by Rijnsdorp (1990) hypothesizing that when surplus production was above a minimum level, a fish will build up body reservesnecessary for winter metabolism and will produce an amount of eggs proportional to body size. A similar mechanism is present in cod, which is also a highly fecund, determinate spawner. For an individual cod, weight during the early period of vitellogenesis is the best predictor of fecundity (Skjæraasen et al. 2006).

Fecundity estimates taken in September were generally higher than those taken during the spawning season. There was also a negative relationship between follicle size and relative fecundity in fish with a mean M2 follicle diameter greater than 700 µm, which indicates that down-regulation of fecundity occurs and the level of down-regulation is affected by the condition of the fish. This is evident from the positive relationship between somatic condition and fecundity index. Atresia has been shown to occur in cod (Kjesbu et al. 1991), sole (Witthames et al. 1995), turbot (Bromley et al. 2000), and herring (Kurita et al. 2003) and is hypothesized to be a method of fine-tuning fecundity in relation to available energy reserves (Kurita et al. 2003). Atresia in plaice is low during the spawning season (Armstrong et al. 2001; present study), which means that the decrease in fecundity occurs before the spawning season commences. The recruitment of more follicles than are typically used for spawning will allow the fish to have an increased fecundity if feeding conditions are good. However, if food availability is low later in the season and all follicles cannot be sustained, the fish can reabsorb follicles by atresia without experiencing heavy energetic losses, as the follicles are reabsorbed and the nutrients are presumably available for recycling (Bromley et al.

2000). A similar method for the control of optimum egg production has been seen in captive Norwegian coastal cod whereby they produce too many follicles before spawning and this unsustainable production is subsequently re-absorbed during the spawning season, with fish in good condition spawning more eggs than fish in poor condition (Kjesbu et al. 1998).

Plaice have been shown to segregate into discrete feeding aggregations during the summer nonbreeding season (Hunter et al. 2004), and tagging studies in the Irish Sea have shown very limited movement of plaice between the eastern and western area (Dunn and Pawson 2002). Because of this segregation, plaice in the different areas will experience different feeding conditions and population densities. With differing food levels, the level of atresia will differ between populations: populations with decreased feeding conditions will have a greater incidence of atresia (Bagenal 1969; Wootton 1973; Kjesbu et al. 1991), resulting in the observed differences in fecundity between areas and years. Fish size will still remain a good proxy for fecundity within an area: fish in the same area will experience similar conditions during this period, and therefore, any changes in the weight of individual fish within the area will be similar for fish across the whole area.

There is an effect of sampling date on fecundity estimates because of the down-regulation that occurs in the period before spawning. The fecundity of fish from west of the Isle of Man was high compared with other areas. This is probably due to the fish being sampled in early January, which was much earlier in the spawning season in comparison with the other areas and years. Fecundity appears to level off when the mean M2 follicle diameter reaches approximately 1.0 mm. Many of the fish sampled from west of the Isle of Man had a mean M2 follicle diameter lower than this and so more down-regulation of fecundity may have occurred before the fish began spawning.

Mean M2 follicle diameter increased with fish size when sampled both in September and during the spawning season, which has been documented previously for plaice in Cardigan Bay (Horwood 1990). This suggests that larger fish started maturation at an earlier date and will probably be in spawning condition sooner and may begin spawning sooner (Kjesbu 1994). Larger plaice are known to spawn earlier in the year than smaller plaice in Cardigan Bay (Horwood 1990) and in the North Sea (Simpson 1959; Heessen and Rijnsdorp 1989). As fish increase in size, they invest proportionally less in somatic growth and more into reproduction (Bromley 2000). It is therefore not surprising that larger fish begin ovary development sooner than smaller fish as the larger fish will spend less time on somatic growth during the summer months and so can begin ovary development at an earlier time. By beginning ovary development at an earlier date, larger fish will have a greater amount of time for follicle growth to take place and so may use this time to produce larger eggs; larger female plaice have been observed to produce larger eggs (Fox et al. 2003; Kennedy et al. 2007).

From the follicle size distributions, it can be inferred that previtellogenic follicles are recruited in batches into vitellogenic follicles over a period of time. Recruited batches then increase in size through vitellogenesis. As the first batches go through vitellogenesis, more batches are recruited resulting in the distribution of follicle diameters becoming skewed towards the smaller sizes. Follicle recruitment appears to cease when the lead cohort reaches approximately 0.6–0.7 mm (this is supported by the relationship between follicle diameter and density changing from a positive to a negative relationship when follicle diameter reaches approximately 0.7 mm). The later cohorts then begin to "catch up" with the leading cohort until there is a single modal peak distribution. The follicles then increase in size until spawning, preserving the single modal distribution. During spawning, batches of follicles are hydrated and increase in size. It appears that a hydrated batch is not always spawned before the next batch begins hydration.

Horwood (1990) observed that several plaice had bimodal distribution in follicle sizes, which was the first time that this had been documented in plaice, and suggested that this was due to a second burst in egg production. As these fish were caught in the autumn, the observed follicle distribution was probably the result of continuing follicle recruitment.

In conclusion, fecundity varies on the temporal and spatial scales in the Irish Sea, with plaice from the western Irish Sea having the greatest interannual variability and plaice from Liverpool Bay showing no significant differences between years. The maximum fecundity is determined by the weight of the fish at the end of follicle proliferation. This is then down-regulated by atresia in the time between the end of proliferation and spawning. It is believed that the degree of the down-regulation is affected by the availability of food and that different degrees of down-regulation in different areas and years cause the observed differences in fecundity. Larger fish are generally farther ahead in ovary development than smaller fish and most likely to spawn earlier in the spawning season.

Acknowledgements

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