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Gametogenesis of the oyster *Crassostrea gigas* in southern Ireland

S. Steele* and M.F. Mulcahy†

*Biological Sciences, Royal Holloway, University of London, Egham, Surrey, UK, TW20 0EX.
E-mail: s.steele@rhbnc.ac.uk. †Department of Zoology and Applied Ecology, National University of Ireland, Cork

The Pacific oyster *Crassostrea gigas* (Mollusca: Bivalvia) was introduced to Ireland in 1965 and is farmed at many sites around the coast. The reproductive biology of 1377 oysters from two sites on the south coast of Ireland was examined from April 1996 until December 1997 for variations in maturation rate and condition indices. Qualitative data were compiled by staging gonadal development using histological sections. Environmental parameters of temperature, dissolved oxygen and chlorophyll-*a* levels, as well as parasites and pathology were monitored. Unusually high sea temperatures led to oysters in Dungarvan (site 1) spawning in both years of the study. Although sea temperatures were significantly higher, oysters in Cork Harbour (site 2) did not spawn but instead reached ripeness and then started a process of gametic degeneration called resorption. Lack of spawning was not attributed to environmental conditions monitored but was tentatively attributed to levels of pollutants in the water. Oyster condition in Cork Harbour was significantly affected by the presence of blistering due to tributyltin levels in the water and also by *Polydora* sp. (Polychaete) in the shell. Oyster condition in Dungarvan was not affected by the presence of the exotic species *Mytilicola orientalis* (Copepoda: Cyclopoida).

INTRODUCTION

The Pacific or Japanese oyster, *Crassostrea gigas* (Thunberg) is native to the Pacific Ocean and is found in the sea of Okhotsk, in Japan, in Korea and along the Pacific coast of America from Alaska through to California. *Crassostrea gigas* has been cultured in Japan for centuries. The Ministry of Agriculture, Fisheries and Food introduced it to Britain in 1965. The species was introduced into Ireland and growth trials were carried out in the 1970s (Purcell, 1988). Production of the oyster in Ireland has increased from 60 tonnes in 1980 to 4500 tonnes in 1996, substantially increasing its economic importance.

In Ireland, commercial cultivation of *C. gigas* relies on seed supplied from hatcheries and on half grown oysters imported from the continent. Some spatfalls of *C. gigas* have been recorded around the British Isles (Spencer et al., 1994), but in Ireland oysters either fail to spawn or larvae do not develop to metamorphosis (G. Burnell, personal communication). *Crassostrea gigas* has been introduced to many countries where it has grown but failed to spawn (Berg, 1969; Bernard, 1974; Mann, 1979; Robinson, 1992). Unspawned oysters are thought to start a process of gametic degeneration known as resorption or atresia. While there is a good level of knowledge concerning gonadal development, no published studies could be found of the process of gametic degeneration in *C. gigas*. The purpose of this study was, therefore to examine, the hitherto, unstudied pattern of reproduction in the Pacific oyster in Ireland, and to examine the factors that influence the reproductive cycle. The reproductive cycle can be influenced by a number of environmental conditions such as temperature, salinity (Muranka & Lannan, 1984) and levels of nutrition (Deslous-Paoli et al., 1982). Alternatively, it may be influenced by exposure to environmental contamination by pollutants (Lowe & Pipe, 1986; Maung Myint & Tyler, 1982) and parasite loading (Korringa, 1951; Thain & Waldock, 1986).

MATERIALS AND METHODS

Sites and sampling period

From April 1996, monthly samples of between 20 and 30 oysters were collected from two sites on the south coast of Ireland at low spring tides (Figure 1). The oysters in Dungarvan, came were supplied as seed from Guernsey Sea Farms, Guernsey and measured (±SE) 9.20 ±1.31 cm long (maximum anterior–posterior difference) and weighed 64.32 ±15.11 g at the start of the trial, the Cork Harbour oysters were supplied as seed from Seasalter, Kent and measured 9.29 ±1.75 cm long and weighed 90.84 ±31.11 g at the start of the trial. Both groups of oysters were 2-y old at the start of the trial. Dates were recorded as Julian Days with 1 January 1996 as day 1.

Environmental parameter recording

At each site, a temperature logger, supplied by Guernsey Sea Farms, was placed in the oyster bags which recorded and stored the ambient temperature every thirty minutes.

Chlorophyll-*a* measurements were taken every month and were evaluated using the methods of Vollenweider (1969), however due to problems with equipment, there are gaps in the data.

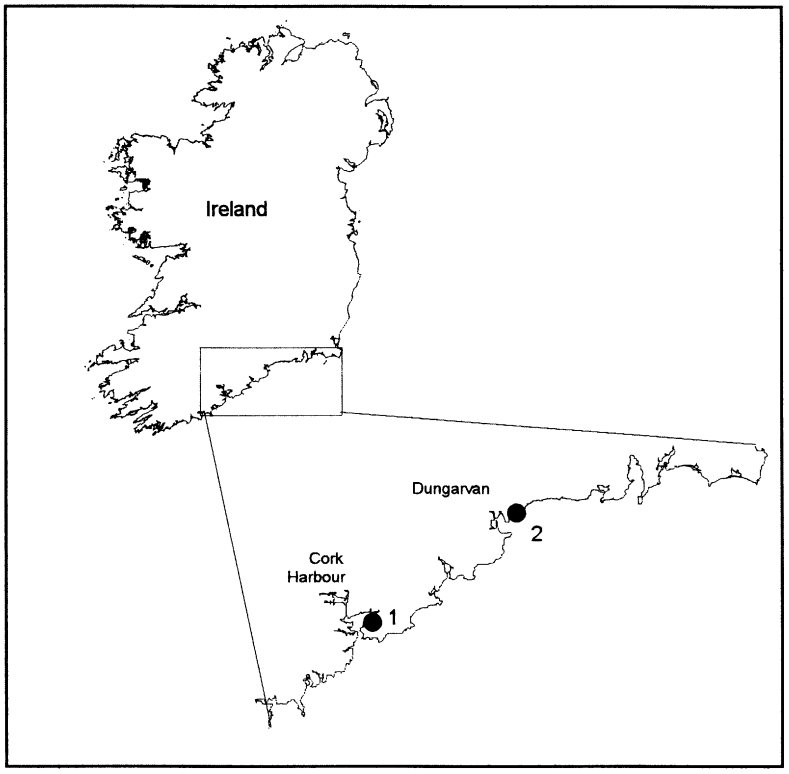

Figure 1. Map of the study area: (1) Cork Harbour, (2) Dungarvan.

Histological techniques

After collection, oysters were left to starve for 2 d to purge gut contents in seawater at a constant temperature and in darkness. Preparation of oysters for histological study followed standard procedures employed by the NOAA (Howard & Smith, 1983). Oysters were scrubbed. Morphological measurements were taken of length (maximum hinge-bill distance) and width (widest distance) using a vernier callipers in millimetres to one decimal place. Wet weight of the oysters was recorded in grams to two decimal places using a Mettler scales. The tissue of each oyster was removed from the shell, weighed and sectioned with a sharp razor blade. One transverse cut was made from the junction of the gills and palps, with a second transverse cut made a few millimetres below the first. The resulting segment of body tissue was fixed in Davidson's fluid, embedded in paraffin wax, sectioned at 6 μm, and stained in Harris' haematoxylin and counterstained in eosin (Humanson, 1979). The prepared microscope slides were examined to determine sex and stage of gametogenesis. Between three and five preparations were examined per organism.

The oysters were classified into distinct phases of gonadal maturity based on the observations made: undifferentiated (stage 0), developing (stage 1 and 2), ripe (stage 3), partially spent (stage 4), totally spent (stage 5), post spawning (stage 6) and resorbing (stage 7). The criteria taken for the classification are modifications of the staging system used by Mann (1979). Figures 2 & 3 show stage 7, resorption of the gonad. The description of the gonads and the distinct subphases of gonadal maturity for both females and males are summarized in Tables 1 & 2.

Table 1. *Description of the phases of gonadal maturity in female* Crassostrea gigas.

Stage 0 (undifferentiated)	No evidence of the presence of follicles peripheral to the digestive gland.
Stage 1 (developing—early active)	Oogonia arising from stem cells along the follicle; no free oocytes. Connective tissue is very abundant.
Stage 2 (developing—late active)	Free and attached oocytes present with distinct nuclei that stain lighter than the cytoplasm.
Stage 3 (ripe)	Free oocytes with distinct nucleus and nucleolus.
Stage 4 (partially spent)	Large numbers of free ooctyes appear but not densely compacted, occupy centre of lumen in the follicle.
Stage 5 (totally spent)	Follicle walls appear broken and follicles empty; ripe ova genital canals; some phagocytes present.
Stage 6 (post-spawning)	Follicles are collapsed and small. A large number of phagocytes appear. Residual oocytes are in process of cytolysis.
Stage 7 (resorption)	Connective tissue is apparent in follicles. Oocytes can be seen in the process of cytolysis and in some follicles there will be macrophage cells present. (Figure 2).

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Table 2. *Description of the phases of gonadal maturity in male* Crassostrea gigas.

Stage 0 (undifferentiated)	No evidence of the presence of follicle peripheral to the digestive gland.
Stage 1 (developing—early active)	Many small follicles; spermatogonia and spermatocytes numerous, no spermatozoa.
Stage 2 (developing—late active)	Follicle cells contain predominantly spermatids and spermatozoa; characteristic swirling pattern of spermatozoa, with tails toward follicle lumen, in centre of follicle.
Stage 3 (ripe)	Inter follicular tissue and germinal epithelium are inconspicuous. Follicles filled with spermatozoa oriented with tails to follicle lumen forming characteristic swirling pattern that completely fills follicle.
Stage 4 (partially spent)	Follicles are partially empty, with a large number of spermatozoids but they are not densely compacted.
Stage 5 (totally spent)	Most follicles empty or partially so with sperm evident in sperm ducts in some individuals.
Stage 6 (post-spawning)	The connective tissue develops rapidly between the collapsed follicles. Germinal cells in the lumen are cytolysed. Numerous phagocytes are present.
Stage 7 (resorption)	Connective tissue is apparent in follicles. There is some infiltration of the follicles by blood cells. The spermatocytes are still densely packed in the follicles (Figure 3).

Parasites and pathology

Occurrence of parasites in oysters was noted, as were any abnormalities. Oysters in Dungarvan are affected by the endoparasitic copepod *Mytlicola orientalis* (Mori) (Copepoda: Cyclopoida). Cork Harbour is a sea inlet with heavy shipping traffic and industrial input. Oysters from this site show characteristic shell thickening and blistering that is associated with mild TBT pollution (Minchin et al., 1996). Shell blisters were classed as mild (<25% inner shell coverage) and severe (>25% shell coverage). *M. orientalis* numbers were recorded and specimens were fixed in 70% alcohol. *Polydora* sp. infestation was classed as mild (<25% inner shell coverage) or severe (>25% shell coverage). Each oyster had an individual record for all parameters, allowing the determination of relationships between levels of infestation of parasites, condition index and morphometrics on levels of development.

Condition indices

Soft tissues, removed from the shells, were blotted and weighed to obtain total wet weight, and then were dissected for histology. The remaining tissues were weighed to obtain partial wet weight, dried at 60°C for 48 h, then re-weighed (partial dry weight). The resulting wet:dry ratio was used to estimate the total dry tissue weight (Fisher et al., 1996). The condition index was calculated as in Crosby & Gale (1990):

$$\text{Condition index} = \frac{\text{Dry soft tissue weight(g)} \times 100}{\text{Internal shell cavity capacity g}} \quad (1)$$

The shell cavity capacity of a bivalve is determined by subtracting dry shell weight (g) in air, from the total whole live weight of the animal. Crosby & Gale (1990) find that this method yields indices which assess the proportion of available internal cavity capacity utilized by a bivalve's soft body tissues. This allows comparison between metabolism directed towards calcification processes and metabolism focused towards somatic and gametic processes.

Statistics

A one sample *t*-test was used to ascertain differences between condition indices, temperatures and chlorophyll-*a* levels in the two sites. The Kendall rank-order correlation coefficients (Campbell, 1989) were calculated to ascertain the degree of association among temperature, shell length, sample date, infestation of parasites, sex, stage of maturity and condition indices at the two sites. Kendall's coefficient is a non-parametric measure of association for ordinal or ranked variables that takes ties into account.

RESULTS

Sex ratios

Of the 1377 oysters examined, there were three hermaphrodites. There were two hermaphrodites in Cork Harbour and one in Dungarvan. Sex ratios varied throughout the year and the overall proportion of male and female oysters was approximately 1:1. Percentage of females decreased after spawning (Tables 3 & 4). There were no distinguishable females in November 1996 and 1997 in Cork Harbour. Undifferentiated oysters were only observed during the winter.

Sexual cycle

In Dungarvan, at the start of the study, in April 1996, females were all stage 1 and some of the males had developed to stage 2 (Figure 2). The first stage 2 females developed in June (day 161). Ripe females developed in July (day 197) and males reached the ripe stage in September (day 257). Mass spawning occurred on the 23 September 1996 (day 266). Spawning appeared to be synchronous between male and female oysters. Post-spawning, oysters showed lysis beginning, with macrophage cells present in the gonad. Female oysters stayed in post spawning stage until early November 1996 (day 316); then resorption started. Residual gametes remained in males over a longer period then females. Males were still post spawning until March 1997 (day 432). Females started to develop gametes in early November (day 316) and 100% of females were stage 1 in March 1997 (day 432). Appearance of male gametes in late January 1997

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Table 3. *Percentage occurrence of male, female and undifferentiated* Crassostrea gigas *from Dungarvan.*

Year	Julian day	Male	Female	Undifferentiated	Hermaphrodite	N
1996	111	58	40	2	0	19
	161	54	46	0	0	21
	197	74	26	0	0	18
	229	63	37	0	0	23
	257	42	58	0	0	23
	275	59	33	8	0	24
	286	61	17	22	0	18
	316	45	20	30	0	28
	350	29	32	39	0	30
1997	389	52	41	7	0	27
	405	62	38	0	0	29
	432	63	37	0	0	27
	464	55	45	0	0	29
	492	43	57	0	0	28
	510	35	65	0	0	29
	550	45	52	0	1	29
	578	43	57	0	0	23
	627	52	32	16	0	25
	654	42	8	50	0	26
	683	32	29	39	0	28
	714	36	24	44	0	25
Average		48	39	13	0	528

N, number of specimens.

Table 4. *Percentage occurrence of male, female and undifferentiated* Crassostrea gigas *from Cork Harbour.*

Year	Julian Day	Male	Female	Undifferentiated	Hermaphrodite	N
1996	182	30	70	0	0	23
	244	39	50	11	0	18
	282	31	27	42	0	26
	296	21	32	47	0	19
	317	37	5	58	0	19
	328	20	0	80	0	20
	342	10	50	40	0	30
	354	21	21	58	0	19
1997	404	38	52	10	0	29
	417	50	40	10	0	28
	435	48	43	9	0	23
	449	56	44	0	0	18
	463	32	68	0	0	28
	479	45	52	0	1	29
	491	35	65	0	0	20
	505	45	56	0	0	27
	521	48	48	0	1	27
	539	29	71	0	0	24
	549	36	60	4	0	25
	569	39	61	0	0	23
	577	41	59	0	0	22
	586	30	57	13	0	23
	609	41	45	14	0	29
	626	25	21	54	0	28
	640	30	10	60	0	20
	655	36	14	50	0	22
	670	25	0	75	0	12
	682	27	42	31	0	26
	700	11	18	71	0	28
	713	33	17	50	0	24
Average		40	42	18	0	862

N, number of specimens.

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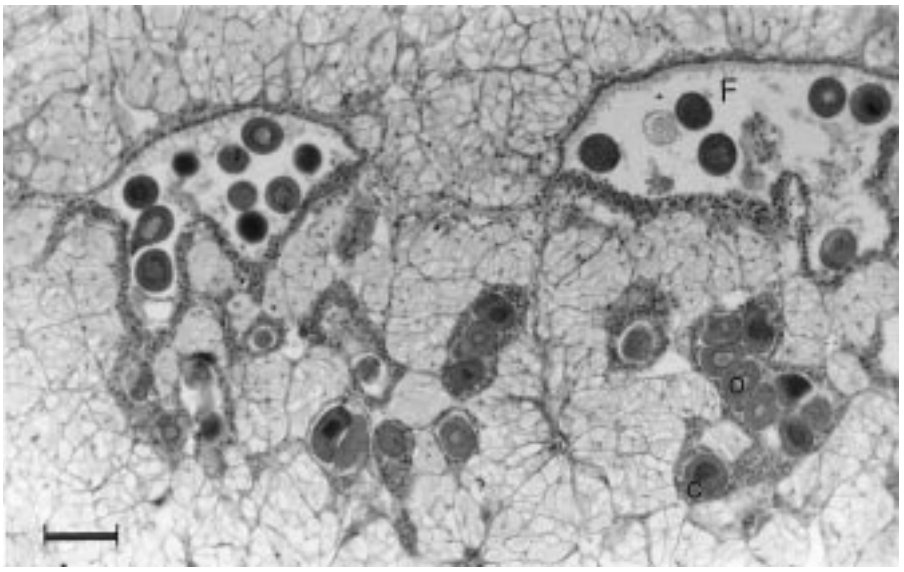

Figure 2. Female *Crassostrea gigas* in the process of resorbing gonad. This is classed as stage 7 using a modified version of Mann's (1979) staging system. Oocytes (o) are undergoing cytolysis and the cytoplasm (c) is granular. There is connective tissue in the follicles (F) with many macrophage cells (M) present. Scale bar: 0.4 mm.

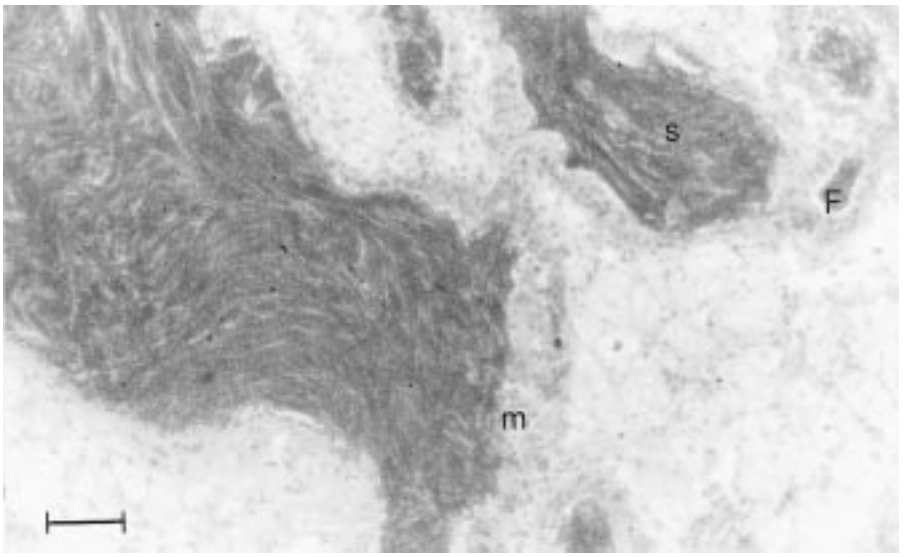

Figure 3. Male *Crassostrea gigas* in the process of resorbing gonad. This is classed as stage 7 using a modified version of Mann's (1979) staging system. There are few remaining spermatazoa (s) with many macrophage cells (m) present in the follicles (F). Scale bar: 0.5 mm.

(day 389) indicated the start of a new gametogenic cycle. Males reached stage 2 in early February 1997 (day 405) and females in early April (day 464). Ripe males and females developed in early July 1997 (day 550). Spawning occurred on 15–18 August (592–595). Oysters entered post-spawning stage after spawning had occurred. As winter progressed, both male and female oysters started resorbing gametes.

In contrast to the Dungarvan site, oysters in Cork Harbour did not spawn in either 1996 or in 1997. On the first sample date in June 1996 (day 182); 19% of females and 14% of males were ripe. There was a gap of 62 d until the next sample date, and in late August (day 244), the majority of males (72%) and females (63%) were resorbing. There was no observation of spawning and the tissues in the gonad were compacted and full of resorbing gametes. The resorption of unspawned gametes took six months in the males (September 1996 – March 1997) and four months in the females (September 1996 – January 1997) (Figure 4). No females were observed in late November 1996 (day 328). Stage 1 females developed in early December (day 342); The number of developing females increased until early February (day 404), when all females examined were stage 1. Stage 1 males developed in January 1997 (day 377). Development of stage 2 males and females began in late March (day 449). Both males and females were ripe by early July (day 549). Twenty days later 21% of females and 11% of males were resorbing. No spawning was observed. Resorption continued for males

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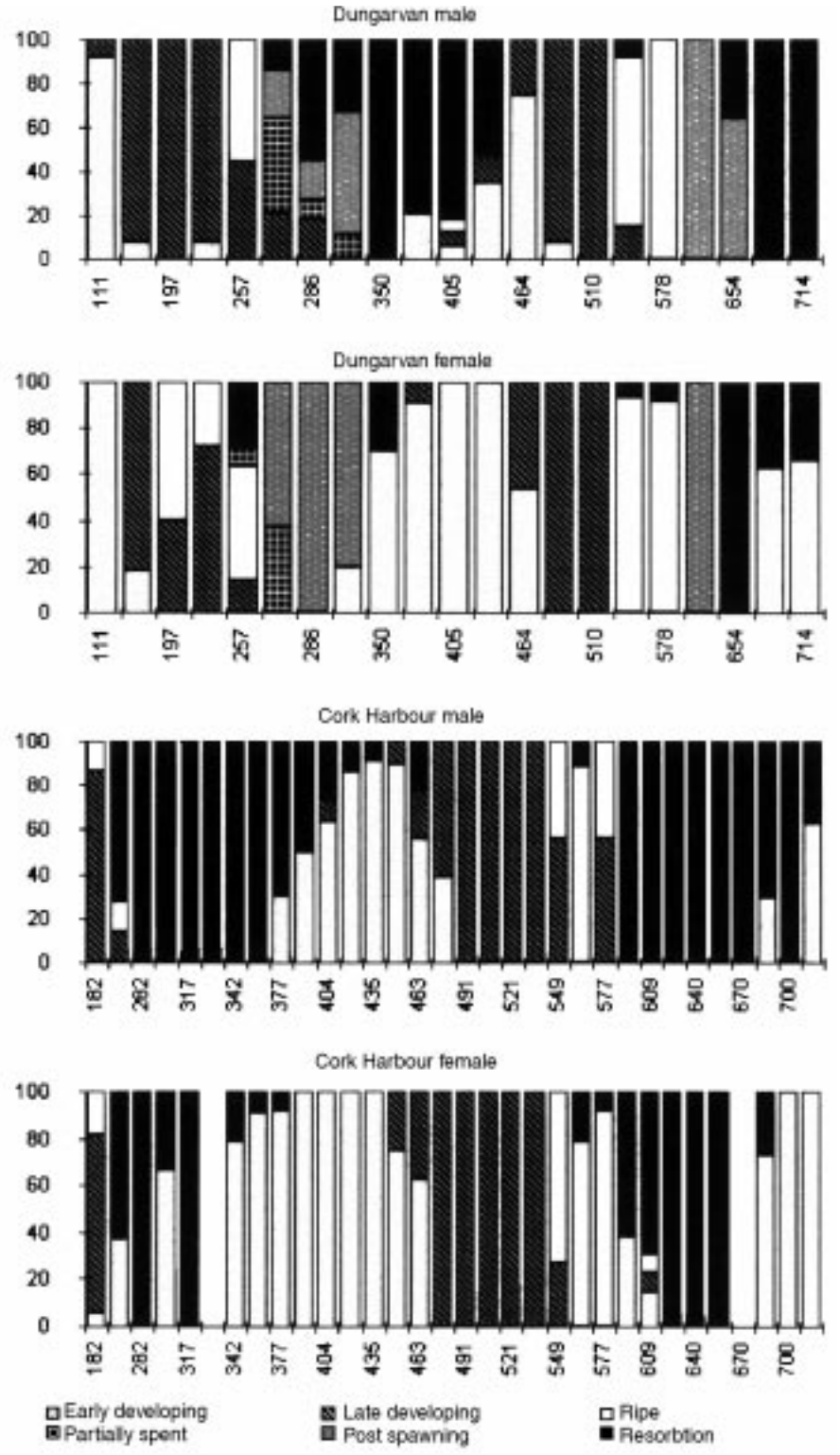

Figure 4. The percentage frequencies of developmental stages in male and female *Crassostrea gigas* from Dungarvan and Cork Harbour. Gonadal development stages expressed with Mann's stages (1979).

until the end of sampling in December 1997 (day 713), with some stage 1 males developing in mid November. The majority of females were resorbing until late October (day 655) but there were stage 1 females at the end of July and the beginning of August (day 577 and 586). No females were observed at the end of October (day 670), and the majority of females until December were stage 1.

Biometric measurements

At the start of the study, oysters in Dungarvan measured (±SE) 9.2 ±0.27 cm and weighed 64.32 ±3.15 g. The Cork Harbour oysters measured 9.29 ±0.32 cm and weighed 90.85 ±6.15 g. Oysters grew over the period of the study. There was a significant difference in length in Cork Harbour (one-way ANOVA, $F=6.60$, $P<0.001$) and in Dungarvan (one-way ANOVA, $F=13.70$, $P<0.001$)

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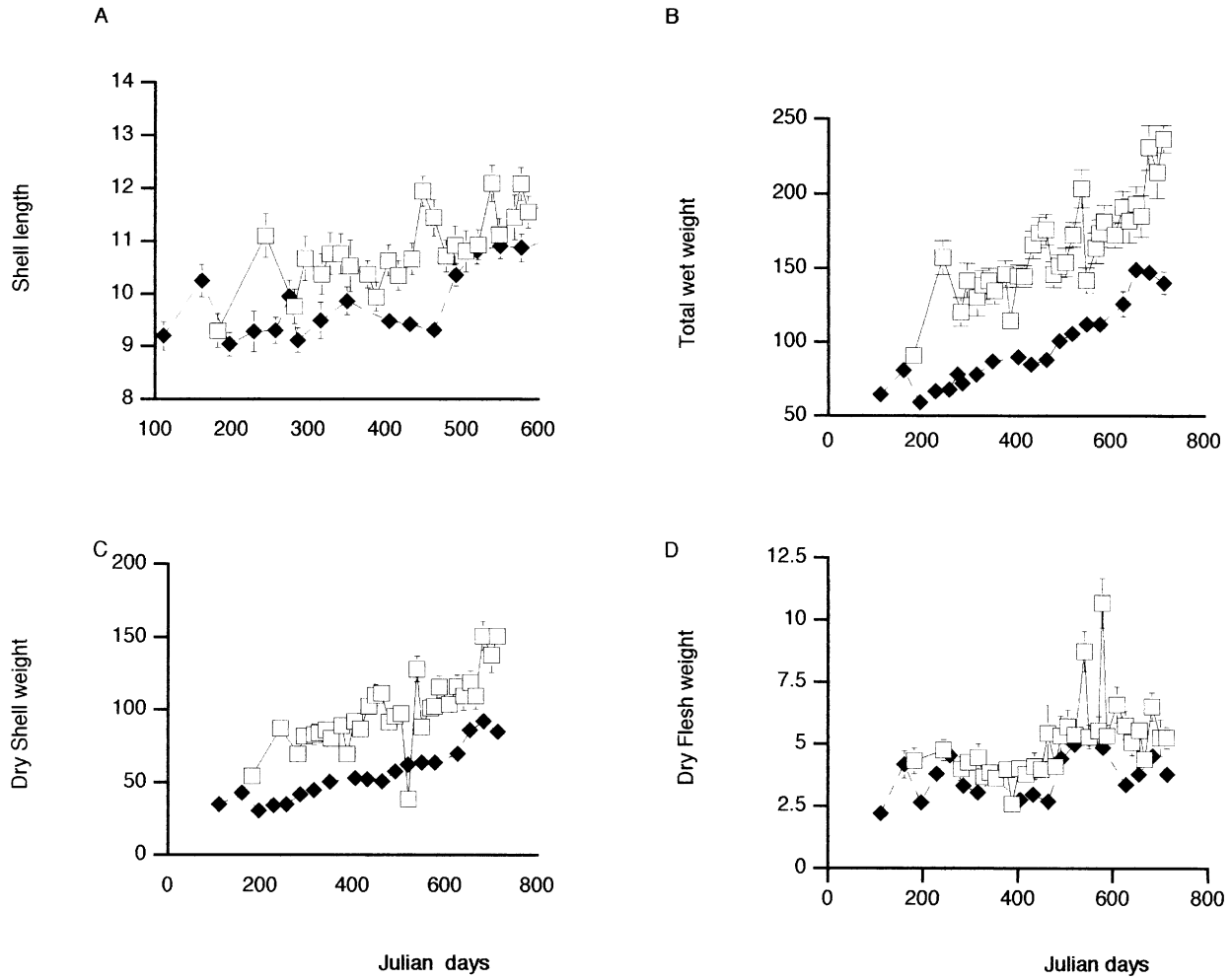

Figure 5. Mean biometric measurements of sampled individuals of *Crassostrea gigas* from Dungarvan and Cork Harbour. (A) Shell length (mm); (B) total wet weight (g); (C) dry shell weight (g); (D) dry flesh weight (g).

over the period of the study. Changes in length and total wet weight over time are illustrated in Figure 5. By the end of the study, in December 1997, Dungarvan oysters measured 11.60 ±0.233 cm and weighed 140.06 ±7.72 g and Cork Harbour oysters measured 13.42 ±0.256 cm and weighed 236.72 ±9.36 g.

Condition indices

Averaged monthly index values in Dungarvan showed a marked seasonal cycle, with a wide range in values (Figure 6). Index values were generally higher in the summer before spawning. In 1996, condition reached a maximum in mid September (day 257) of 136.48 ±3.45. Spawning occurred at the end of September (day 266). Oysters in Dungarvan were in low condition after spawning until early March 1997 (day 432). Condition rose from March to a maximum of 113.08 ±5.24 in early June (day 520). Index values decreased after spawning to a minimum value of 59.84 ±2.96 in mid September 1997 (Day 627).

Cork Harbour oysters followed annual cycles similar to Dungarvan with maximum values during the summer. However, indices in Cork Harbour were significantly lower than Dungarvan (one sample *t*-test, $t = -62.26$, $P < 0.0001$). Cork Harbour indices also showed a smaller range in values, with a maximum condition of 103.89 ±9.77) during the sampling period. In 1996 and 1997, condition gradually decreased in Cork Harbour as oysters resorbed their gonad.

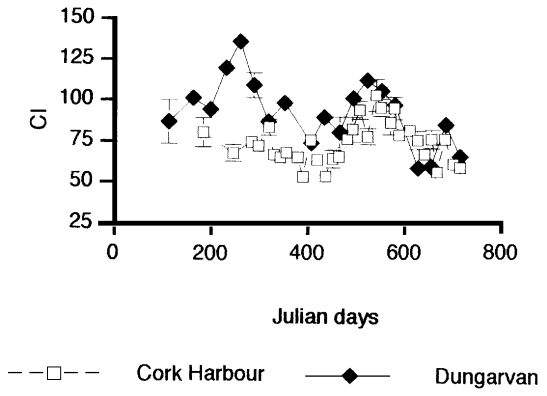

Figure 6. Seasonal variation in the condition index measured from *Crassostrea gigas* collected in Dungarvan and Cork Harbour. Condition index (Crosby & Gale, 1990) was calculated as the ratio of dry tissue weight multiplied ×1000 to internal shell cavity volume.

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Table 5. *Percentage of* Crassostrea gigas *from Dungarvan, southern Ireland infested with* Mytilicola orientalis *and with* Polydora *sp.*

N	% of oysters infested with *Mytilicola orientalis*	% of oysters infested with *Polydora* sp.	Julian day
23	8.70	26.09	111
29	10.34	27.59	161
19	0.00	57.89	197
22	18.18	77.27	229
24	20.83	79.17	257
26	7.69	92.31	275
20	10.00	85.00	286
20	10.00	95.00	316
30	13.33	73.33	350
30	10.00	83.33	389
30	16.67	76.67	405
27	29.63	85.19	432
30	13.33	70.00	464
28	17.86	85.71	492
29	27.59	89.66	520
28	10.71	100.00	550
29	27.59	93.10	578
26	11.54	76.92	627
29	24.00	72.00	654
29	14.00	100.00	683
30	00.00	97.00	714

N, number of specimens.

Table 6. *Percentage of* Crassostrea gigas *from Cork Harbour, southern Ireland infested with blistering and* Polydora *sp.*

N	% of oysters with blisters	% of oysters with *Polydora* sp.	Day
23	39.13	8.70	182
20	55.00	25.00	244
26	65.38	42.31	282
19	63.16	31.58	296
20	35.00	35.00	317
20	60.00	40.00	328
30	40.00	36.67	342
20	55.00	25.00	354
29	10.34	17.24	377
28	71.43	53.57	388
30	70.00	46.67	404
28	32.14	60.71	417
25	40.00	52.00	435
20	45.00	70.00	449
28	53.57	21.43	463
30	36.67	46.67	479
46	19.57	17.39	491
28	53.57	25.00	505
20	65.00	0.00	521
25	48.00	24.00	539
27	51.85	44.44	549
22	54.55	40.91	569
24	70.83	29.17	577
27	55.56	77.78	587
30	60.00	80.00	609
28	67.86	78.57	626
25	44.00	84.00	640
26	31.00	92.00	655
25	32.00	56.00	666
28	57.00	64.00	682
28	43.00	93.00	700
30	67.00	90.00	713

N, number of specimens.

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Table 7. *Kendall correlation matrix for* Crassostrea gigas *situated in (A) Dungarvan and (B) Cork Harbour. The significance of the coefficient indicates the direction of the relationship, and its absolute value indicates the strength with larger absolute values indicating stronger relationships. Possible values range from −1 to 1.*

A. *Dungarvan*.	Condition index	Date	Shell length	*M. orientalis* infestation	*Polydora* sp. infestation	Sex	Stage	Temperature
Condition index		−0.224**	−0.104**	0.003	0.022	0.143**	0.030	0.223**
Date	−0.224**		0.355**	0.050	0.258**	−0.096**	0.059	−0.022
Shell length	−0.104**	0.355**		0.022	0.107**	−0.006	−0.009	0.069*
M. orientalis infestation	−0.003	0.050	0.022		0.095*	0.042	−0.029	0.027
Polydora sp. infestation	0.022	0.258**	0.107**	0.095*		−0.043	0.058	0.053
Sex	0.143**	−0.096**	−0.006	0.042	−0.043		0.072	0.145**
Stage	0.030	0.059	−0.009	−0.029	0.058	0.072		0.094**
Temperature	0.223	−0.022	0.069*	0.027	0.053	0.145**	0.094**	

B. *Cork Harbour*.	Shell blisters	Condition index	Date	Shell length	*Polydore* sp. infestation	Sex	Stage	Temperature
Shell blisters		−0.082**	0.021	−0.005	0.009	−0.086**	−0.054	0.062*
Condition index	−0.082**		0.032	0.044	−0.100**	0.170**	0.167**	0.186**
Date	0.021	0.032		0.255**	0.296**	−0.048	0.096**	0.339**
Shell length	−0.005	0.044	0.255**		0.037	0.013	0.009	0.109**
Polydora sp. infestation	0.009	−0.100**	0.296**	0.037		−0.125**	−0.046	0.049
Sex	−0.086**	0.170**	−0.048	0.013	−0.125**		0.394**	0.100**
Stage	−0.054	0.167**	0.096**	0.009	−0.046	0.394**		0.308**
Temperature	0.062	0.186**	0.339**	0.109**	0.049	0.100**	0.308**	

*, correlation is significant at the 0.05 level (two-tailed); **, correlation is significant at the 0.01 level (two-tailed).

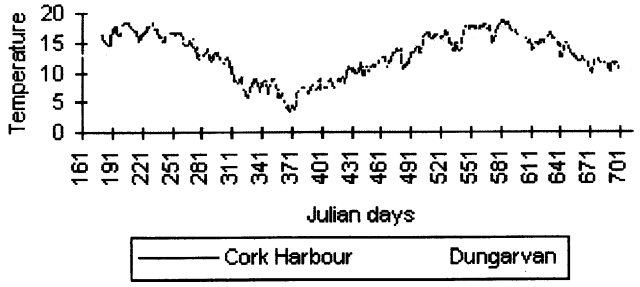

Figure 7. Mean daily sea temperatures in Cork Harbour and Dungarvan extracted from data recorded on a Guernsey Sea Farms temperature logger.

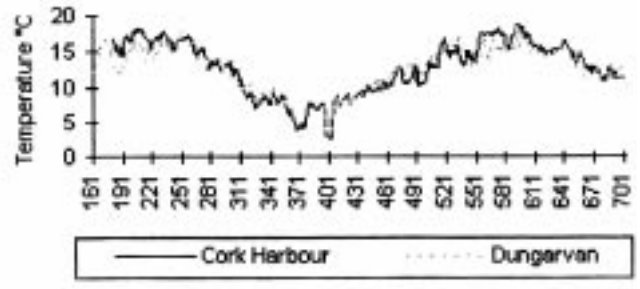

Figure 8. Mean daily temperatures in Cork Harbour and Dungarvan recorded every 30 min on a Guernsey Sea Farms temperature logger.

Parasite levels and pathology

In Dungarvan 14.38% of oysters were infested with *Mytilicola orientalis*. Numbers of *M. orientalis* averaged per oyster was 0.6. One oyster contained 20 *M. orientalis*. Infestation did not appear to have a seasonal cycle and ranged from 0 to 29.63% (Table 5). Cork Harbour oysters contained no *M. orientalis*. Using a Kendall correlation matrix, there was no significant interaction between *M. orientalis* and the condition, sex or stage of the oyster (Table 7).

In Dungarvan 73.48% of oysters and 47.15% in Cork Harbour were infested with *Polydora* sp. (Tables 5 and 6). There was considerable variation in the percentage of oysters infested, ranging in Dungarvan from 26 to 100% and in Cork Harbour from 0 to 84%. In Cork Harbour, *Polydora* sp. and condition of the oysters showed a significant negative correlation (Kendall tau-B$=-0.1$, $P<0.001$). In Dungarvan (Table 7) there was a significant positive correlation between *M. orientalis* and *Polydora* sp. (Kendall tau-B$=0.095$, $P<0.005$). The inner valves of 54.9% of the oysters from Cork Harbour contained blisters. Blisters in the shells showed a significant negative correlation with the condition of the oysters (Kendall tau-B$=-0.82$, $P<0.001$). Between 10 and 71% of oysters from monthly samples in Cork Harbour contained blisters. Interestingly, significant negative correlation occurred between the sex of the oysters in Cork Harbour and the blistering and levels of *Polydora* sp. in the shells (Table 7). Undifferentiated oysters contained less blistering and less *Polydora* sp. tubes than males and males and undifferentiated oysters contained less blistering and less evidence of *Polydora* sp. tubes than females. In Dungarvan there was no significant correlation between levels of *Polydora* sp. in the shell and the sex of the oyster. No oysters from Dungarvan showed shell blistering.

Environmental parameters

Temperature

Daily mean temperatures from June 1996 to December 1997 recorded in Dungarvan and Cork Harbour are shown in Figure 7. These mean temperatures include air and sea temperature. Figure 8 shows mean sea temperatures. There is a significant difference between sea temperatures in the two sites (t-test, $t=10.87$, $P<0.000$). In Dungarvan, the sea temperature reached 17.55°C on 7 September 1996 (Julian day 251) and 18.45°C on 13 August 1997 (Julian day 591). In Cork Harbour, the temperature reached 18.15°C on 25 July 1996 (Julian day 207) and 18.65°C on 17 August 1997 (Julian day 1997). The minimum temperature in Dungarvan was 2.70°C on 6 February 1997 (Julian day 403) and in Cork Harbour the minimum temperature was 2.75°C on the same day.

*Chlorophyll-*a*, oxygen and salinity measurements*

Chlorophyll-*a*, oxygen and salinity measurements are presented in Table 8. Dungarvan had a mean salinity of 33.9 psu and Cork Harbour had a lower mean of 30.8 psu. The mean oxygen level in Dungarvan and Cork Harbour was 13.1 $\mu g\,l^{-1}$. The chlorophyll-*a* level displayed marked seasonal changes. The mean chlorophyll-*a* level in Dungarvan was 9.88 $\mu g\,l^{-1}$ and 9.49 $\mu g\,l^{-1}$ in Cork Harbour. There was no significant difference between chlorophyll-*a* levels in Cork Harbour and Dungarvan (t-test $t=-0.14$, $P=0.89$). Peaks in chlorophyll-*a* were observed in Dungarvan on 14 September 1996 (16.60 $\mu g\,l^{-1}$ on day 258) and 4 June 1997 (26.30 $\mu g\,l^{-1}$ on day 521). Oysters in Dungarvan spawned nine days after the 1996 bloom. No August measurement of chlorophyll-*a* was taken in 1997. In Cork Harbour, algal blooms were observed in September 1996 (16.6 $\mu g\,l^{-1}$) and in October 1997 (17.9 $\mu g\,l^{-1}$).

Table 8. *Mean salinity (psu) and chlorophyll-*a *($\mu g\,l^{-1}$) measured in Dungarvan (D) and Cork Harbour (CH) from June 1996 until December 1997*

	Chlorophyll-*a* ($\mu g\,l^{-1}$)		Salinity (psu)	
Month	D	CH	D	CH
July 1996	13.2	8.0		
August 1996				
September 1996	16.6	16.6	34.4	29
October 1996	6.1	10.0	35	32.9
November 1996				
December 1996				
January 1997				
February 1997				
March 1997				
April 1997	5.5	9.0		
May 1997	2.5	8.1		
June 1997	26.3	6.0		
July 1997	4.3	7.0		
August 1997				
September 1997	10.3	4.3		
October 1997	5.8	17.9		
November 1997	32.1	28.8		
December 1997	8.2	8.0	34	30.5

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DISCUSSION

The proportion of male, female and undifferentiated oysters varied temporally, in both Dungarvan and Cork Harbour. Proportions of males and females decreased over the winter and oysters went into a resting period. The mean proportion in Dungarvan was 49% males, 39% females and 12% undifferentiated. In Cork Harbour, the proportion was 33% male, 41% female and 26% undifferentiated. In other studies; the proportions of male to female oysters have also varied. Ruiz et al. (1992) found that there were more females than males after spawning and at the onset of gametogenesis. In Dungarvan, at the onset of gametogenesis there was a very high proportion of females. The transition from category of undifferentiated to male is less distinct than undifferentiated to female, which may lead to females being identified earlier in the reproductive cycle. Katkansky & Sparks (1966) working in Washington State found 86% males to 14% females (N=455). In their study, oysters were collected once a year and the high ratio of males may be due to the stage of gametogenesis. Katkansky & Sparks (1966) do not refer to numbers of undifferentiated oysters. Paniaga-Chavez & Acosta-Ruiz (1995) working in Mexico found proportions of 70% male, 24% female and 4% undifferentiated. The study was carried out over a period of 11 months with a sea temperatures that reached a maximum of 23°C. This result corresponded with 67% males and 33% females (N=328) in a study in Virginia held at 8–15°C (Sphigel, 1989). However, Sphigel (1989) found a ratio of 46 males to 46 females at 30°C. In this study, hermaphrodites accounted for 0.23% of the oysters studied. There has been variance in numbers of hermaphrodites found in *Crassostrea gigas* (Table 9). The variance in sex ratios between studies highlights the need for an understanding of the mechanisms of sex change in *C. gigas*. This variance could be due to the use of different physiological taxa of *C. gigas*, temperature effects, different handling or sample size and location.

Table 9. *Numbers of hermaphrodites in* Crassostrea gigas *in different studies*.

	Hermaphrodites	N
This study	3	1298
Paniaga-Chavez & Acosta-Ruiz (1995)	13	440
Mann (1979)	0	1140
Berg (1969)	1	382
Katkansky & Sparks (1966)	9	552

Introduced oysters have failed to spawn in the UK, Washington state and in British Columbia (Berg, 1969; Bernard, 1974; Katkansky & Sparks, 1966; Mann, 1979; Quayle, 1969). Temperature is a major regulator in gametogenesis in marine bivalves (Sastry, 1979). The method of control of temperature over the reproductive cycle is complex, with many studies now showing that gametogenesis is a function of temperature intensity and time rather than the attainment of a critical temperature (Muranka & Lannan, 1984). Mann (1979) described the relationship between development and temperature using the equation:

$$D = d(t_i - t_0) \quad (2)$$

where D is day degrees, d is number of days to attain a ripe state, t is the temperature to which animals are exposed and t_0 is the temperature below which no evidence of gonad development is found. Mann (1979) found that for *C. gigas*, D=592, t_0 is 10.55°C. Wilson & Simmons (1985) adapted this equation to:

$$D = \sum_{i=1}^{n} (t_i - t_0) \quad (3)$$

where t_i is the temperature of the water. Mann's (1979) equation was found to give an accurate estimate of the time of ripening of *C. gigas* by Ruiz et al. (1992), working in El grove, Spain. In the present study, while the equation showed that the sea temperatures were high enough to allow ripening, oysters reached ripeness earlier then predicted. In 1996, computation of day degrees was not possible as temperatures were above 10.55°C when sampling. Temperatures in Cork Harbour rose above 10.55°C on 10 April 1997 (day 466) and in Dungarvan on 31 March 1997 (day 456) and the predicted time of ripening for Cork Harbour was the 31 August 1997 (day 609) and for Dungarvan was the 4 September 1997 (day 613). However, Dungarvan oysters were ripe by 3 July 1997 (day 550) and spawned between the 15 and 18 August 1997 (day 593–596). Cork Harbour oysters attained ripeness on the 2 July 1997 (day 549) and started to show signs of resorbing gametes on 22 July 1997 (day 569). Both sites attained ripeness earlier than expected in Mann's (1979) prediction. Rate of gametogenesis and temperature of spawning vary all over the world (See Ruiz et al., 1992 for review). Prior to this study, it was thought that *C. gigas* introduced to Ireland did not usually reproduce due to low sea temperatures. The spawning of oysters in Dungarvan may have been due to exceptionally warm weather. 1996 was a warmer than average summer (Met Eireann, 1996) and 1997 was the warmest year recorded since the 1950s (Met Eireann, 1997). In Dungarvan the maximum recorded temperature was 17.55°C in 1996 and 18.45°C in 1997. Sea temperatures in Cork Harbour reached above 18°C in 1996 and in 1997. Cork Harbour temperatures were significantly higher than Dungarvan, leading to the conclusion that something other then temperature could influence the lack of spawning and resorption of gametes in Cork Harbour.

A detailed description of the process of resorption, gametic degeneration or 'atresia' has been provided in *Pecten maximus* and *Mytilus edulis* by numerous authors, but not in *C. gigas* (Dorange & Le Pennec, 1989a,b; Lubet et al., 1987; Motavkine & Varaksine, 1983; Pipe, 1987a). Motavkine & Varaksine (1983) differentiated three types of resorption: (1) a mechanism controlling the number of cells in the acinus which has a finite capacity; (2) a 'self-cleaning' process, which prepares the gonad for a new sexual cycle which could induce the massive lysis of gametes. (3) Unfavourable environmental conditions could induce the massive lysis of gametes, thus stopping a sexual cycle, which in favourable conditions would end the spawning. The unfavourable conditions for gametogenesis could be a number of natural environmental

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conditions such as temperature (Mann, 1979), salinity (Muranka & Lannan, 1984) and food levels. Lubet et al. (1987) suggest that lysis is related to a deficit in energy due to lack of food. Gamete resorption can lead to recycling of gamete material to satisfy basal metabolism (Benninger & Le Pennec, 1991; Herlin-Houtteville & Lubet, 1975; Pipe, 1987b). There was no significant difference in chlorophyll-*a* levels between Dungarvan and Cork Harbour. Even though chlorophyll-*a* levels may not always fully represent the food available to the oyster (Wilson, 1987), it appears that there are other factors influencing gametic degeneration in oysters in Cork Harbour. The salinity in Cork Harbour was 30.8 psu, which was lower than salinity levels in Dungarvan, however, 30.8 psu is still above the salinity levels found to cause retardation of spawning in oysters (Muranka & Lannan, 1984). Hydrocarbons induce resorption in *M. edulis* and this is not due to a low energy level in the mussel (Lowe & Pipe, 1986). Hydrocarbons will destabilize lysosomes leading to tissue breakdown. Copper, zinc and cadmium exposure lead to resorption of oocytes in *M. edulis* (Maung-Myint & Tyler, 1982). Cork Harbour is an industrial area. Oysters from Cork Harbour showed abnormal and extreme shell thickening and blistering. This has been attributed to an organotin compound tributlytin (TBT) (Alzieu et al., 1980, 1982). TBT has an adverse effect on many marine organisms and in 1987, a bye-law was introduced in Ireland which prohibited, the usage of TBT on vessels under 25 m in length. Cork Harbour is a sea inlet with heavy shipping traffic and was shown in 1994 to have evidence of contamination. Development of gametes in *Ostrea edulis* was retarded in the presence of TBT (Thain & Waldock, 1986). Pollutants in the harbour, alone or combined with other effects are a tentative cause for the degeneration and resorption of the gonad. In Ireland, many areas have different parasites and virus infestations. Due to this oysters could not be transplanted and this study examined oysters originally from two different hatcheries in the UK. Difference in reproductive cycles can be due to the existence of physiologically different races of oyster (Imai & Sakai, 1961). Aquaculture of *C. gigas* in the UK and Ireland is based on two batches of 76 and 74 individuals that were imported from British Columbia to the UK in 1964 and 1972 (Child et al., 1995). These oysters are of the Miyagi strain (Gosling, 1982). The hatcheries in Guernsey Sea Farms and Seasalter received oysters from these batches. The oysters from these hatcheries were used in this study. However, Child et al. (1995) found that polymorphism occurred in some of the oysters in UK hatcheries, probably due to the migration of French oyster larvae into the UK. This may have influenced the reproductive cycle of the oysters.

Condition index is defined as 'the ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological' (Mann, 1978). There was a significant difference in the condition indices of the oysters from the two sites. Dungarvan condition indices rose with the onset of gametogenesis and dropped sharply after spawning due to the loss of body mass. Cork Harbour condition was significantly lower than Dungarvan and the condition of the oysters started to decrease in 1997 before resorption occurred. Condition has been found to be directly proportional to glycogen levels in the tissues (Galstoff, 1964). As glycogen is the primary energy substrate in the tissues, low condition can have effects on many physiological processes such as reproduction (Bayne, 1976). Maung-Myint & Tyler (1982) found that pollutants caused decrease in the condition index of *M. edulis* as well as the degeneration of the gonad. In Cork Harbour oyster condition was significantly lower in oysters with blistering and *Polydora* sp.. *Polydora* sp. is a tubicolous polychaete, generally free-living, but also found burrowing in the shells of molluscs (Williams, 1968). Korringa (1952) has shown that oysters infested with *Polydora* sp. have to expend a certain amount of energy derived from food for extra shell secretion; as well as having to live in more confined space because of blistering. Shell blistering due to TBT also requires extra energy expenditure (Thain & Waldock, 1986). The amount of gametogenic material produced, is directly related to the amount of glycogen accumulated prior to gametogenesis (Bayne, 1975) and the energy expenditure on shell growth in Cork Harbour will have affected the condition and hence the reproductive cycle of the oysters. This effect was observed in *C. virginica* infected with the parasite MSX (*Haplosporidium nelsoni)* (Barber et al., 1988). Oyster condition and sex of the oyster were found to have a significant positive relationship in both Dungarvan and Cork Harbour. Undifferentiated oysters were included in the calculation of correlation. Undifferentiated oysters had lower condition than oysters that were sexed.

In Dungarvan, oysters were infected with *Mytilicola orientalis*. *Mytilicola orientalis* is originally from Japan (Mori, 1935). It was observed first in Europe in 1977, in *C. gigas* in France, thought to be introduced from Japan or British Columbia (His, 1977). It is thought to have been introduced to Ireland in half grown French oysters (De Grave et al., 1995). It has been observed in oysters in Dungarvan but is not present in Cork Harbour. The oysters were not infested with the close relative *M. intesinalis* (Steuer), which is endemic around European coasts (Blateau et al., 1992). Intensity of *M. orientalis* infestation did not significantly affect condition indices. Opinions vary on the impact of *Mytilicola* sp. on condition (Deslous-Paoli, 1981). De Grave et al. (1995) found that there was no significant effect of *M. orientalis* on oyster condition in Dungarvan. De Grave et al. (1995) also found that the mean intensity of *M. orientalis* in Dungarvan in July, 1994 was between 1.07 and 1.48, with a maximum infestation of seven copepods per oyster. This study found a maximum of 20 copepods per oyster, with a mean intensity of 0.64. *Mytilicola* numbers in oysters have been found to vary by up to 40 copepods per oyster (Korringa, 1951). There was no association between *M. orientalis* infestation and the sex and stage of the oysters. Williams (1969) found reduced gonad size and possible retarded spawning due to infestation. Hepper (1955) found that the copepod only affects the host if environmental conditions are adverse. There was a significant positive relationship between numbers of *M. orientalis* and intensity of infestation by *Polydora*. Williams (1969) found that *Polydora* caused a decreased rate of parasitism by the copepod *M. intestinalis* in *Mytilus edulis*, possibly due to lack of food as more energy is put into shell repair when shells infested by *Polydora*.

The results of this study demonstrated that the process of maturation in *C. gigas* was as has been described for

this species elsewhere and in addition outlined the process of gonadal resorption, which occurs in unspawned oysters, and which has received little attention in the literature. Contrary to popular belief oysters at the Dungarvan site spawned in both years of the study. In Cork Harbour, even though environmental parameters of temperature and chlorophyll-*a* indicated suitable spawning conditions, the oysters did not spawn in either year of the study; one suspected reason was contamination by TBT, but this requires further study.

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