Dependence of RNA:DNA ratios and Fulton’s K condition indices on environmental characteristics of plaice and dab nursery grounds

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A B S T R A C T

This field study showed a lack of a correlation between a morphometric (Fulton’s K) and biochemical (RNA:DNA ratio) condition index in juvenile plaice (Pleuronectes platessa) and dab (Limanda limanda) studied to assess habitat quality in four sandy beach nursery grounds in Galway Bay, Ireland. Based on monthly surveys from June to September in 2008 and 2009, fish growth, indicated by RNA:DNA ratios and Fulton’s K, displayed considerable spatio-temporal variability. Site-related patterns in Fulton’s K for plaice and dab were consistent between years whereas RNA:DNA ratios displayed annual and interspecific variability among nursery habitats. This indicates a higher sensitivity of RNA:DNA ratios to short-term environmental fluctuations which is not apparent in Fulton’s K measurements of juvenile flatfish. Generalized Additive Modelling (GAM) revealed non-linear relationships between the condition indices and (biotic and abiotic) habitat characteristics as well as diet features, derived from gut content analyses. Density of predators, sediment grain size and salinity were the most important predictors of both condition indices. Temperature also affected condition indices in dab whereas plaice condition indices varied with depth. Diet features did not contribute to the explained variability in the models predicting RNA:DNA ratios whereas certain prey groups significantly improved the explained variability in the models predicting Fulton’s K of plaice and dab. The value of both indices for assessing fish condition and habitat quality in field studies is discussed. These findings aid understanding of the biological and physical mechanisms promoting fast growth and high survival which will help to identify high quality nursery areas for juvenile plaice and dab.

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1. Introduction

The identification of essential nursery habitat for flatfish has gained importance as a result of changing estuarine and coastal environments. Natural and human-induced impacts are known to affect the structure and functioning of these essential nurseries (Blaber et al., 2000; Cabral et al., 2001; Whitfield and Elliott, 2002; Martinho et al., 2007; Courrat et al., 2009; Rochette et al., 2010). As a consequence, reduced juvenile fish growth and survival in disturbed coastal zones are widespread (Vasconcelos et al., 2007; Vinagre et al., 2008; Amara et al., 2009). This reduction in growth and survival combined with increasing fishing pressures can impact the population size of commercially exploited fish stocks (Désaunay et al., 2006; Hermant et al., 2010). Therefore, coastal zone management is important to maintain high quality of nursery habitat increasing the probability of fish survival to maturity.

The need to define high quality nurseries has prompted investigations of the habitat characteristics which enhance individual fish growth. Primary determinants of rapid growth in juvenile flatfish are considered to be physiochemical conditions (e.g. temperature, salinity, dissolved oxygen) (Phelan et al., 2000; Amara, 2004) in combination with food availability (Burrows, 1994; van der Veer et al., 1994), predator density (Burrows and Gibson, 1995; Gibson et al., 1995), substratum characteristics (Gibson and Robb, 2000; McConnaughey and Smith, 2000) and the presence or absence of competition (Alvarez et al., 2006; Rooper et al., 2006). It is accepted that higher growth rates will successively limit susceptibility to predation and starvation which ultimately results in higher survival rates and year-class strength (Houde, 1989; Gibson, 1994).

The application of indicators of fish condition is essential for the assessment and protection of nursery habitat quality. Fulton’s condition factor K (known as Fulton’s K) is extensively used in fisheries research as a morphometric condition index and provides a useful tool to examine overall growth (Suthers, 1998). A limitation of this classical indicator is its insensitivity to recent events in the...
life of fish, such as feeding history. In contrast, the ratio of ribonucleic acid to deoxyribonucleic acid (RNA:DNA ratio or R/D) was developed as a sensitive indicator for recent growth in marine organisms (Bulow, 1970; Buckley et al., 1999). The premise for the application of this index is the fairly constant concentration of DNA in a normal somatic cell whereas RNA concentration varies in proportion to protein synthesis (Chicharo and Chicharo, 2008). Nucleic acid based condition indices provide a method to infer nutritional condition for various fish species based on their response to changes in feeding activity (Buckley, 1984; Malloy and Targett, 1994; Gwak and Tanaka, 2001; Johnson et al., 2002; Peck et al., 2003; Mercaldo-Allen et al., 2006, 2008; Ciotti et al., 2010). R/D has been particularly useful in larval fish research since starvation processes are known to play a significant role in the larval phase, characterized by exponential growth rates and intensive mortality (Hovenkamp, 1990; Chicharo et al., 1998). Comparison of R/D in fed and food-deprived laboratory-reared fish as well as in wild fish allows accurate estimates of the proportion of starved fish in their natural habitat which can be linked to environmental events and recruitment success (Clemmesen, 1994; Kooker et al., 1997). In combination, biochemical and morphometric condition indices are useful for determining the overall nursery role of a habitat.

The present field study aimed to evaluate Fulton’s K and R/D in juvenile flatfish as indicators of habitat quality in four distinct coastal nursery grounds in Galway Bay, Ireland. Plaice (Pleuronectes platessa, Linnaeus, 1758) and dab (Limanda limanda, Linnaeus, 1758) juveniles were chosen as target species since they are among the most abundant flatfish species in the north-eastern Atlantic region (Daan, 1997) as well as in coastal sandy nursery grounds in Galway Bay (De Raedemaeker et al., in press). Despite concurrent settlement in the same nursery areas, slight discrepancies exist between both species regarding morphology (Piet et al., 1998), spawning and settlement period (Steele and Edwards, 1970), depth distribution (Gibson, 1973), tidal transport (De Veen, 1978) and food selection (Edwards and Steele, 1968; Poxton et al., 1983). Therefore, an attempt to accurately link habitat quality and juvenile fish growth requires a multi-species approach to account for species-specific growth responses. In particular, following hypotheses are tested in this study: (1) both condition indices are correlated and provide the same ecological information; (2) Mean Fulton’s K and R/D co-vary spatially and temporally; (3) diet features are driving variability in nutritional condition but not in overall condition. Moreover, generalized additive modelling (GAM) is used to identify important biotic (crab density) and abiotic (temperature, salinity, sediment grain size and depth) habitat characteristics as well as diet features (gut fullness, prey diversity and numbers of polychaetes, bivalves, malacostracans and copepods) that influence Fulton’s K and R/D.

2. Materials and methods

2.1. Study area and sampling strategy

The westward facing Galway Bay is a region of high tidal energy (tidal range of 4.5 m), with an area of c. 100 km², located on the west coast of Ireland (Fig. 1). A total of four soft-bottom nursery areas which were isolated from each other by rocky shorelines were selected from within the inner Galway Bay for sampling. Ballyloughaun and Silverstrand are two small embayments in the northern section which receive a considerable freshwater input by the Corrib River and which are separated by the Mutton Island causeway. Ballyvaughan and Traught are located in the southern section of Galway Bay; the former is characterized by a substantial intertidal area whereas the latter is a small beach, located on a straight, mainly rocky coastline.

Juvenile flatfish assemblages were sampled monthly from June to September in 2008 and 2009. Due to weather conditions in June 2008, sampling was only undertaken in Ballyloughaun that month and the three other nursery areas were surveyed three weeks later in July 2008. A 1.2 m beam trawl (6 × 6 mm codend) equipped with one tickler chain was hauled by a small dinghy at a constant
velocity of 1.1 knots (34 m min⁻¹) for 2 min. This speed was chosen since it gave the largest catch of 0-group plaice using a 2-m beam trawl in Loch Ewe (Riley and Corlett, 1966; Edwards and Steele, 1968). Three replicate trawl hauls in the subtidal zone were carried out parallel to the shore to cover an area with a homogeneous depth which was recorded with an echo sounder. A mechanical flowmeter attached to the beam was used to calculate the distance towed and ultimately the swept area, which averaged 94 m² per trawl. The geographic location of the trawls was recorded using a GPS and bottom salinity and bottom temperature data were obtained at each trawl location with a CTD. All juvenile flatfish were immediately stored onboard in liquid nitrogen and transferred to a −80 °C freezer in the laboratory until further sorting and analyses. All hauled crab were counted and measured before releasing them to obtain a measure of predator abundance since crab are the most important predators on juvenile flatfish (van der Veer and Bergman, 1987). A Van Veen grab (0.025 m²) was used to collect sediment samples for particle size analysis.

2.2. Sediment processing

Particle size analysis was carried out with oven-dried sediment for all samples collected in 2008 using the method described in Bale and Kenny (2007). Sediments were divided into six fractions: silt (<63 μm diameter), very fine sand (63–125 μm), fine sand (125–250 μm), medium sand (250–500 μm), coarse sand (500 μm–1 mm) and gravel (>1 mm). Each fraction was weighed, expressed as a percentage of the total weight and the mean sediment particle size, in phi (φ) units, was calculated. The mean sediment particle size data was mapped with ArcGIS 9.2 software as a continuous surface using inverse-distance weighted averaging (IDW). This interpolation technique was applied to generate mean sediment composition data at locations of sampling in 2009, as it proved effective in other studies (Phelan et al., 2001; Stoner et al., 2001; Compton et al., 2008). Replicate sediment samples per trawl haul were pooled to obtain an average value of abiotic variables per trawl.

2.3. Fish processing

All fish were identified before measuring standard length to the nearest mm and weighing gently blotted fish to the nearest mg. Plaice and dab were the most abundant flatfish species and three replicate specimens of each species per trawl, if present, were selected for diet and nucleic acid analysis. The selected fish were representative of the original length distribution in the trawl. A total of 165 dab juveniles and 222 plaice juveniles ranging in length from 17 mm to 58 mm (dab) and from 21 mm to 79 mm (plaice) were studied.

Fulton’s condition factor was calculated for each fish using the formula \( K = (W/L^3)^{100} \), where \( W \) is freeze-thawed wet weight (g) and \( L \) is standard length (cm). Fish tails easily damage during beam trawling and the use of standard length when calculating this condition index is therefore recommended to aid in comparisons between studies. Fish diet analyses were conducted using both stomach and intestines together (henceforth referred to as guts) since plaice and dab have a rather small stomach and long alimentary tract (Beyst et al., 1999). Prey items were macroscopically identified to their main taxonomic groups and counted. Total number of prey taxa, total prey abundance and gut fullness were measured for each fish. The Shannon–Wiener prey diversity index was computed to provide a measure of niche breadth (Marshall and Elliott, 1997). These nutritive variables of the gut content were used as indicators for recently ingested food.

2.4. Nucleic acid determination

White muscle tissue from both sides of the fish was used to determine individual R/D based on the fluorometric methods described by Clemmesen (1993) and Caldarone (2001). A non-specific nucleic acid fluorescent dye, Ethidium Bromide, was used. Fluorescence was assessed on a Fluoroscan Ascent FL with a 355 nm filter for excitation and emission set at 592 nm using flat bottomed black 96-well microplates with 0.33 ml wells. Every tissue sample (0.013–0.030 g) was macerated on ice and homogenized in 500 μl TEN-SDS buffer (0.05 M Tris, 0.01 M EDTA, 0.1 M NaCl, 0.01% SDS, pH 8) with ø 2.0 mm and ø 0.2 mm combined glassbeads in a pulsating vortex mixer to extract nucleic acids. The homogenate was then diluted with another 500 μl TEN-SDS buffer before being centrifuged at 6000 rpm for 10 min at 4 °C. Total nucleic acid fluorescence (RNA and DNA) of two replicates of every sample was measured after adding 75 μl ethidium bromide (EB, 20 μg/ml) to 75 μl of the supernatant and 50 μl TEN buffer (0.05 M Tris, 0.01 M EDTA, 0.1 M NaCl, pH 8). Fluorescence of the DNA fraction was measured after adding 5 μl Ribonuclease A (RNAse purified from bovine pancreas, 20 U/ml) to each well and incubation in a water-bath at 37 °C for 30 min to allow enzymatic digestion of RNA. Background fluorescence was measured using two blanks of 75 μl TEN buffer. 75 μl TEN-SDS buffer and 75 μl EB and subtracted from the fluorescence values of the tissue samples. RNA fluorescence was determined by subtracting the DNA fluorescence reading (corrected for background fluorescence) from the total fluorescence value (corrected for background fluorescence). Control homogenates were prepared with fresh mussel tissue and two replicates were used in every microplate to verify the accurate reproducibility of the method. RNA and DNA content in tissue samples was calculated from calibration curves determined with a series of dilutions of pure calf-thymus DNA (Sigma) and baker’s yeast RNA (Sigma). The ratio between the slopes of RNA and DNA calibration curves was 2.15 ± 0.05 standard error (n = 21 microplates); this can be used as a standardization factor for direct inter-calibration with other studies (Caldarone et al., 2006). The R/D of each sample was determined directly as the ratio between RNA and DNA concentrations averaged over replicate readings. When the coefficient of variation between replicates exceeded 10%, samples were rerun to generate more reliable values.

2.5. Data analyses

RNA:DNA ratios of marine organisms generally change with developmental stage (Buckley et al., 1999). Exploratory data analysis revealed a weak correlation between length and condition indices (length and Fulton’s K; plaice: ns, dab: \( r = 0.43 \) —length and R/D; plaice: \( r = -0.28 \) and dab: \( r = -0.35 \)). Therefore, the effect of fish length on the two condition indices was removed according to following equation: \( Y’ = Y - (bL) - (a - Y) \), where: \( Y’ \) = corrected condition index value, \( Y \) = original condition index value, \( Y \) = mean condition index value condition index value, \( L \) = fish length, \( b \) = slope of the regression of length and condition, \( a \) = intercept of the regression of length and condition. Regression slopes of the relationship between condition indices and fish length varied between years, therefore, length corrections were applied separately per year. It was still possible to compare the corrected condition values between years since the mean fish length of both species did not vary significantly between years (ANOVA, \( F = 0.51, p = 0.471 \) for plaice and \( F = 2.79, p = 0.097 \) for dab) (Fig. 2). Corrected indices were used in all subsequent analysis to avoid possible bias resulting from small differences in length frequency composition amongst fish of different nurseries and sampling
periods, an approach successfully conducted in previous studies (Rooker et al., 1997; Vasconcelos et al., 2009).

Pearson correlation coefficients between Fulton’s K and R/D were calculated for fish pooled as well as separated per year. Variability in condition indices between species and over time (both between and within years) was explored using Generalized Linear Modelling (GLM). Explanatory variables considered were year, species, ordinal date (including the cubic term to allow non-linear changes in time), and all possible second-order interactions. Similarly, spatial variability in condition indices of fish from different nursery areas was determined for both species using GLMs with site, year and the cubic term of ordinal date in the model. The year*ordinal date interaction was included to determine if seasonal changes in condition were consistent between years. The year*site interaction was included to explore annual consistency in condition differences between sites.

Sources of variation on fish growth and condition in the field are difficult to assess when variability in habitat conditions occurs simultaneously and interacts with each other. Generalized additive Modelling (GAM) is a useful tool which aids in understanding ecological processes regulating fish growth by establishing non-linear relationships between condition indices and the additive effect of habitat variables. GAMs were used to identify the effects of abiotic variables (temperature, salinity, sediment grain size and depth), biotic variables (crab density) as well as diet characteristics derived from gut content analyses (gut fullness, prey diversity and numbers of polychaetes, bivalves, malacostracans and copepods) on both fish condition indices per species. Prey classes included in these models were those that contributed most to the total numeric abundance in fish guts based on a detailed description of feeding patterns of juvenile plaice and dab in Galway Bay (De Raedemaeker et al., 2011). Using GAMs allowed for a smoothed relationship between response and predictor variables based on penalized regression splines (Wood, 2006). The degree of non-linearity in the relationship is presented by the effective degrees of freedom (edf); ranging from one (linear) to infinity.

GLMs were carried out to examine differences in diet characteristics between years, with fish length included as a covariate. Habitat characteristics of the studied nursery areas are presented in more detail in De Raedemaeker et al., 2011. Biotic and abiotic variables were tested for interannual variability using the non-parametric Kruskal–Wallis test. Prior to all GLM and GAM analyses, data were tested for outliers, normality and homogeneity of variances and log transformations were carried out if required (Zuur et al., 2009). Data were pair-plotted in order to investigate and eliminate multi-collinearity between independent variables. A backwards selection using the Akaike Information Criterion (AIC) was used for model selection, followed by a systematic removal of variables based on a significance level of 0.05. Model validation was always carried out by plotting model residuals against the explanatory variables and by creating partial regression plots. The goodness-of-fit of the predictors was assessed by comparing their relative contribution to the total deviance explained. All statistical analyses were carried out in the R environment (R Development Core Team, 2008).

3. Results

3.1. Correlation between condition indices

Condition indices of juvenile fish in four nursery areas in Galway Bay, collected between June until September in 2008 and 2009 displayed considerable variability. RNA:DNA ratios ranged from 2.51 to 8.81 for dab and from 1.64 to 7.74 for plaice. Fulton’s K values ranged from 1.24 to 2.24 for dab and from 1.37 to 2.70 for plaice. The Pearson correlation coefficient showed a significant correlation between Fulton’s K and R/D for both species (n = 387) when data were pooled over two years (plaice: $r = 0.15, p = 0.025$; dab: $r = 0.20, p = 0.005$).
r = 0.34, p < 0.001). However, this correlation was driven by annual differences since correlation coefficients per year were not significant for plaice and dab. Therefore both condition indices were representing different ecological information and were used complementary in further analyses.

3.2. Temporal and interspecific variability in condition indices

A GLM with year, ordinal date and species in the model (Fig. 3) revealed that both condition indices showed a comparable temporal trend within a year but these dynamics differed between 2008 and 2009 (expressed by a significant interaction between year and cubic order of ordinal date (R/D: F = 41.38, p < 0.001; Fulton’s K: F = 42.21, p < 0.001)). Condition of 0-group plaice and dab was also higher in 2009 than in 2008 for both indices (R/D: F = 56.22, p < 0.001; Fulton’s K: F = 230.81, p < 0.001).

Fulton’s K of plaice and dab did not differ (F = 3.00, p = 0.115) and no significant interaction of species with year (F = 3.58, p = 0.059) and with ordinal date (F = 4.83, p = 0.051) was detected, showing that Fulton’s K of both fish behaved identically between and within years. DNA content and protein expression is species-specific hindering comparisons of R/D between species (Dahlhoff, 2004).

3.3. Spatial variability in condition indices

After accounting for temporal effects on condition indices within a year, spatial trends in Fulton’s K and R/D varied between species (Table 1). Fulton’s K of plaice did not vary between nurseries in either 2008 or 2009 whereas Fulton’s K of dab showed spatial variation with highest values observed in Silverstrand in both years. R/D showed spatial variability between sites for both plaice and dab but no consistent trends were observed, with interactions between year and site (Table 1).

3.4. Effect of diet features and habitat characteristics on fish condition

Variability in condition indices of plaice and dab in Galway Bay was explained by diet features and biotic and abiotic habitat characteristics (Table 2). Most of the variability in R/D and Fulton’s K of dab juveniles was predicted by the same variables; sediment grain size, crab density, temperature and salinity, explaining 74% and 62% of the total deviance of the GAM respectively (Fig. 4). These variables all deviated from a linear relationship with condition to some extent. Higher Fulton’s K and R/D values were generally found in dab residing in areas with lower $\varphi$ (or larger sediment grain size), lower crab densities and lower temperatures. Fulton’s K of dab peaked at salinities between 26 and 28 whereas R/D showed an overall positive relationship with salinity. Additionally, dab with more copepods in their guts showed significantly higher Fulton’s K condition. Compared to dab, R/D and Fulton’s K of plaice juveniles was less predictable, as shown by the GAMs which explained 42% and 51% of the total deviance. The same predictors were found to drive most of the variability in Fulton’s K and R/D of plaice: sediment grain size, crab density, depth and salinity (Fig. 5). Higher Fulton’s K and R/D was generally found in plaice residing in areas with lower $\varphi$ (or larger sediment grain size), lower crab densities and larger depth, but deviations from a linear relationship were clear. Higher polychaete densities and gut fullness were also associated with higher Fulton’s K in plaice.

3.5. Yearly fluctuations in diet and habitat conditions

A high degree of variability in condition indices between years was observed (Table 1) and may be attributed to environmental differences between sampling years. Abiotic habitat descriptors did not vary between years with the exception of sampled depth (slightly higher in 2008) and salinity (slightly higher in 2009)

Fig. 3. Temporal and interspecific variability in Fulton’s K and RNA:DNA ratio of plaice and dab (pooled from 4 different nursery grounds), collected monthly between June–September over two different years. Each dot represents a fish and the fitted lines with 95% confidence intervals are the mean predicted values from a GLM with predictors; year, ordinal date (cubic relationship), species and the interactions between year with ordinal date and with species.
Compared to abiotic characteristics, diet features were highly chance variation in the timing of sampling relative to the tide. In both years, these differences are likely not represented by Fulton (Table 3). Since juvenile flatfish were sampled on average around high tide in both years, these differences are likely not represented by Fulton (Table 3).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Fulton’s K plaice</th>
<th>Fulton’s K dab</th>
<th>RNA:DNA ratio plaice</th>
<th>RNA:DNA ratio dab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>F</td>
<td>Pairwise test</td>
<td>F</td>
<td>Pairwise test</td>
</tr>
<tr>
<td>Year</td>
<td>0.91**</td>
<td></td>
<td>7.32***</td>
<td></td>
</tr>
<tr>
<td>Orbital date</td>
<td>117.98***</td>
<td>2009 &gt; 2008</td>
<td>125.79***</td>
<td>2009 &gt; 2008</td>
</tr>
<tr>
<td>Site:Year</td>
<td>19.44***</td>
<td>1.40**</td>
<td>5.33**</td>
<td></td>
</tr>
<tr>
<td>Orbital date:Year</td>
<td>8.69**</td>
<td>23.83***</td>
<td>10.33***</td>
<td></td>
</tr>
<tr>
<td>Site:Year</td>
<td>0.21***</td>
<td>1.48**</td>
<td>5.53**</td>
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</table>

This study revealed ecological mechanisms affecting nutritional condition (determined by R/D) and morphometric condition (represented by Fulton’s K) of juvenile plaice and dab inhabiting dynamic coastal nursery grounds in the months after peak settlement. The lack of a direct correlation between the indices is consistent with some previous studies (Gilliers et al., 2004; Vasconcelos et al., 2009; Walther et al., 2010). Other authors reported a slight correlation between the morphometric and biochemical condition index (Amara et al., 2009). This discrepancy between studies might be related to the number of samples, the temporal and spatial extent of the studies and the developmental stage of the fish.

The relatively high range of Fulton’s K for juvenile dab (1.24–2.24) and plaice (1.37–2.70) in Galway Bay compared to other studies (Gilliers et al., 2004; De Raedemaeker et al., 2010) suggests that growth limitation was not likely to have occurred. It is not surprising that Fulton’s K did not differ between juvenile plaice and dab since this index is related to body shape; a highly comparable feature of both species of the Pleuronectidae family or righteye flounders (Piet et al., 1998). The range of R/D for dab (2.51–8.81) and plaice (1.64–7.44) could not be compared to findings from other field studies due to the rare use of nuclear acid based condition indices for juveniles of these species and practical limitations regarding inter-laboratory comparison (Caldarone et al., 2006). Without experimental laboratory calibration, we were unable to determine the critical value of R/D below which juveniles were considered to be starved with substantial limitations on growth. This critical ratio is species-specific and shows spatio-temporal and regional variability (Gwak and Tanaka, 2001; Islam et al., 2006) but typically ranges between 1 and 3, depending on temperature (Ferron and Leggett, 1994). Only 7.8% of the studied fish had R/D values smaller than 3 indicating that rates of starvation were low. However, the real incidence of individual starvation in this study was impossible to determine.

Despite the lack of correlation between morphometric and biochemical condition indices, average values of both condition indices followed a similar temporal trend within both years. Since a large part of the variability in Fulton’s K and R/D was predicted by the same set of variables for both species (sediment particle size, crab density, depth and salinity for plaice; sediment particle size, crab density, temperature and salinity for dab), it is likely that these habitat and environmental descriptors were at the origin of this trend. Crab densities negatively affected the growth of juvenile dab and plaice in Galway Bay with Fulton’s K showing a stronger association than R/D. While it is not clear why the magnitude of the response differs between the two indices, the overall effect most likely arises as a result of predation pressure, which is the most important cause of flatfish mortality during the post-settlement phase (Bailey, 1994). Predation mainly by Carcimus muens has been previously postulated as a regulating factor in juvenile flatfish densities (Edwards and Steele, 1968; Pihl, 1990; Amara and Paul, 2003). Processes such as predator avoidance, reduced activity levels, increased burying behaviour and feeding delay can lead to impaired condition in juvenile flatfish (Burrows and Gibson, 1995;
Fig. 4. GAM plots showing additive effect of each significant variable on (a) Fulton’s K and (b) RNA:DNA ratio of dab over four sites and two years (n = 165).

Fig. 5. GAM plots showing additive effect of each significant variable on (a) Fulton’s K and (b) RNA:DNA ratio of plaice over four sites and two years (n = 222).
Table 3
Interannual variability in habitat characteristics using a non-parametric Kruskal–Wallis test. Levels of significance; ns: not significant, ‘: p < 0.05, ‘‘: p < 0.01, ‘‘‘: p < 0.001.

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>H</th>
<th>Pairwise test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (m)</td>
<td>3.12 ± 1.23</td>
<td>2.53 ± 0.73</td>
<td>5.41*</td>
<td>2008 &gt; 2009</td>
</tr>
<tr>
<td>Salinity</td>
<td>30.61 ± 2.95</td>
<td>30.89 ± 3.30</td>
<td>9.55*</td>
<td>2009 &gt; 2008</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>15.85 ± 0.93</td>
<td>16.32 ± 2.30</td>
<td>0.01**</td>
<td></td>
</tr>
<tr>
<td>Sediment particle size (μ)</td>
<td>2.84 ± 0.51</td>
<td>2.73 ± 0.09</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Crab density (ind 100 m²)</td>
<td>7.92 ± 6.67</td>
<td>8.20 ± 7.11</td>
<td>0.01**</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>51</td>
<td></td>
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</tr>
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Wemholage and Gibson, 1998; Gibson et al., 2002; Maia et al., 2009). Predation risk may also explain why condition indices of dab and plaice in Galway Bay were associated with sediment grain size. Species-specific preferences in sediment composition determine burying behaviour essential for avoiding predators (Gibson and Robb, 1992; Jager et al., 1993; Stoner and Abookire, 2002). Additionally, these periods of inactivity can help reduce metabolic rates and conserve energy (Howell and Canario, 1987). The negative association of depth with juvenile flatfish condition, as observed within Galway Bay, is a common phenomenon in coastal nurseries. Depth is a factor incorporating information about sediment, composition, prey availability, competition, temperature and salinity and the complex interaction between different habitat variables.

Fish metabolism and thus condition, growth and survival are intrinsically linked to physico-chemical characteristics of the environment (Fry, 1971). This is reflected in the fact that salinity had a strong effect on both condition indices of juvenile plaice and dab in Galway Bay, with a peak in condition generally occurring at salinities between 26 and 28. Place and dab could do best at these salinities due to the minimised energetic cost dedicated to osmotic regulation and food conversion. Alternatively, it is expected that optimal growth of juvenile flatfish is highest at intermediate salinities (8–16) which correspond with the internal osmotic pressure in fish (Gaumet et al., 1995; Boeuf and Payan, 2001; Lefrançois and Claireaux, 2003). Juveniles of other flatfish species also show increased biochemical condition at higher salinities both in the field (Glass et al., 2008) and under laboratory conditions (Sampaio et al., 2007; O’Neill et al., 2011). Since the osmotic concentration of plaice usually decreases with fish length, this suggests that juvenile fish are more hypoosmotic to the surrounding area (Vlasblom et al., 1977). However, the energy costs associated with adjusting to highly variable salinity conditions must also be considered (Evans, 1993; Vinagre et al., 2008). Juveniles may minimise the energy expended on osmotic regulation by actively selecting areas with more stable, and therefore higher, salinity conditions. Alternatively, another habitat parameter which is linked to salinity may underlie the apparent association between salinity and condition. For example, a trade-off between habitat and salinity preferences is common in natural environments due to improved feeding conditions or fewer encounters with predators.

Temperature is considered the most prominent hydrological variable affecting fish condition but its effect on condition indices appeared to vary between plaice and dab in Galway Bay. Temperature has been demonstrated to be a key factor in controlling biological performance (swimming activity, feeding rate, food conversion) and consequently growth rates of various fish species (Fonds et al., 1992; Fuiman and Ottey, 1993; Lanford and Targett, 1994; Mallek et al., 1998; Lefrançois and Claireaux, 2003). Growth-related temperature optima are species-specific but small juvenile fish generally grow faster at higher temperatures (Fonds et al., 1992). The lack of a temperature effect on both condition indices of plaice might be related to their tendency to display tidal transport which may confer a higher degree of plasticity in their response to temperature fluctuations. Alternatively, the decrease in Fulton’s K and R/D of plaice with depth might have also captured the temperature effect in the explanatory models. Dab caught in waters of 20 °C in Galway Bay showed the highest values of Fulton’s K. This is slightly higher than the reported growth optimum for juvenile dab in laboratory studies (between 15 °C and 18 °C) but within the temperature limit of 22 °C, above which no growth was observed (Bolle et al., 1994). The relationship between temperature and RNA:DNA ratios of dab in Galway Bay was highly non-linear but showed a general decreasing trend, consistent with temperature-induced decreases in nutritional condition of juvenile fish reported in previous studies (Islam et al., 2006; Walther et al., 2010). Thermal dependence of RNA:DNA ratios is due to a compensatory mechanism by which a higher quantity of RNA is required to achieve a given growth rate at higher temperatures (Goolish et al., 1984). This results in higher RNA:DNA ratios in fish acclimatized to cold waters than in fish acclimated to warm waters (Islam et al., 2006). Therefore, using the biochemical condition index as a growth estimate may be biased when habitat quality of nurseries from distinct geographic regions or from different seasons is compared. Additional laboratory experiments with juvenile plaice and dab under different temperature conditions are required to standardize biochemical indices in order to eliminate the temperature dependency of this method.

Contrary to the expectation that feeding success affects nutritional condition of fish over short temporal scales, R/D variability was not driven by differences in diet features. In contrast, gut fullness and polychaete densities were significant predictors for Fulton’s K of plaice whereas Fulton’s K of dab improved with higher numbers of copepods in their diet. These prey groups partly explained the variability in overall condition between years, confirming the better foraging environment in 2009, in light of the fact that prey in the fish guts reflected their availability in the benthos (De Raedemaeker et al., 2011). Our results agree with previous...
studies where recent prey consumption and R/D were not strongly related (Stierhoff et al., 2009). A time lag between the time of food ingestion and the eventual increase in RNA and protein production may explain this lack of correlation.

The quality of near-shore habitat, with regard to its potential as a fish nursery, is in general highly variable between spatially distinct areas. This is reflected by the widely reported spatial variability in condition and growth rates of juvenile fish (Phelan et al., 2000; Kuropat et al., 2002; Islam and Tanaka, 2005; Fonseca et al., 2006; Vasconcelos et al., 2009). In this study, spatial variation in Fulton’s K of plaice and dab revealed consistent trends between sampling years, despite the fluctuating foraging environment. All investigated nursery areas provided suitable conditions for survival and rapid growth of early juvenile stages of plaice and dab, with relatively higher morphometric condition observed in dab from one particular nursery ground (Silverstrand). The temporally stable spatial trends in Fulton’s K suggest that it is a suitable index of condition for juvenile flatfish and useful for characterizing sites in terms of nursery habitat quality. In contrast, site-related patterns in R/D were not maintained from one year to another and were also highly variable among species. The fact that the sites promoting optimal condition were species-specific highlights the different mechanisms acting on nutritional condition in each species due to characteristic preferences for environmental optima. Interannual variation in relative quality among nursery grounds, based on nutritional condition of juvenile flatfish, likely occurred due to short-term variations in food availability or habitat characteristics. It is accepted that nucleic-based condition indices are more responsive to short-term fluctuations in these environmental conditions whereas this effect is ruled out when looking at overall morphometric condition of juvenile flatfish (Ferron and Leggett, 1994; Ramirez et al., 2004). The observed interannual variability of site-related patterns of R/D in juvenile flatfish in Galway Bay is in contrast with results from other studies in which R/D was not impacted by small-scale and short-term variability (Vasconcelos et al., 2009). Therefore, little general consensus regarding the integrative and representative nature of the RNA:DNA ratio as an index for nursery habitat quality is reached and additional research is suggested.

Fulton’s K proved to be a relatively easy to measure and efficient index of condition for juvenile flatfish with most of the variability explained by a clear set of abiotic and biotic habitat descriptors. Once a standardized system of measurement is used, this index allows easy comparison with other field studies and assessments over wide spatial and temporal ranges. Accurate RNA:DNA ratios are more difficult to obtain due to stricter handling requirements to avoid RNA degradation during field and laboratory processing. Moreover, the use of this method requires laboratory calibration for the effect of fish length, thermal dependence, interspecific variability and determination of critical ratio to assess starving incidence in natural conditions. Fulton’s K is therefore a more appropriate condition index to compare nursery habitat quality over a wide spatial and temporal range, with little environmental fluctuations. However, in natural environments affected by anthropogenic impacts or intense natural fluctuations, the use of R/D provides a powerful tool to detect periods of slow growth before measurable variations in somatic growth occur (Dahlhoff, 2004).

In summary, the results of the present study revealed functional relationships between environmental characteristics, diet features and variability in two condition indices of juvenile flatfish in a coastal nursery habitat. The biochemical and morphometric condition indices were not correlated and displayed considerable spatio-temporal variability. A higher sensitivity of RNA:DNA ratios to short-term environmental fluctuations was detected based on annual and interspecific variability in RNA:DNA ratios among nursery habitat whereas site-related patterns in Fulton’s K for plaice and dab were consistent between years. Abiotic and biotic habitat characteristics influenced both condition indices whereas recent prey consumption was not related to nutritional condition of plaice or dab. In contrast, certain prey groups in flatfish guts, polychaetes and copepods, were associated with higher Fulton’s K of respectively plaice and dab. These findings are important in light of the characterization of high quality nursery areas which promote fast growth and a high juvenile survival and recruitment to the adult stock.

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References


Poxton, M.G., Eleftheriou, A., McIntyre, A.D., 1983. The food and growth of 0-group flatfish on nursery grounds in the Clyde Sea Area. Estuarine, Coastal and Shelf Science 17, 319–337.


