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DEFINING HABITAT CHARACTERISTICS INFLUENCING THE DISTRIBUTION, DENSITY AND GROWTH OF PLAICE (*PLEURONECTES PLATESSA*) AND DAB (*LIMANDA LIMANDA*) ON WEST OF IRELAND NURSERY GROUNDS

By

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ABSTRACT

Plaice (*Pleuronectes platessa*, L.) and dab (*Limanda limanda*, L.) are among the most abundant flatfishes in the north-eastern Atlantic region and the dominant species in shallow coastal nursery grounds. With increasing pressures on commercial flatfish stocks in combination with changing coastal environments, better knowledge of population dynamics during all life stages is needed to evaluate variability in year-class strength and recruitment to the fishery. The aim of this research was to investigate the complex interplay of biotic and abiotic habitat components influencing the distribution, density and growth of plaice and dab during the vulnerable juvenile life stage and to gain insight in spatial and temporal differences in nursery habitat quality along the west coast of Ireland. Intraspecific variability in plaice diet was observed at different spatial scales and showed a link with condition, recent growth and morphology. This highlights the effect of food availability on habitat quality and the need to consider small scale variation when attempting to link habitat quality to feeding, growth and condition of juvenile flatfish. There was evidence of trophic, spatial and temporal resource partitioning between juvenile plaice and dab allowing the co-existence of morphologically similar species in nursery grounds. In the limited survey years there was no evidence that the carrying capacity of the studied nursery grounds was reached but spatial and interannual variations in fish growth indicated fluctuating environments in terms of food availability, predator densities, sediment features and physico-chemical conditions. Predation was the most important factor affecting habitat quality for juvenile plaice and dab with crab densities negatively correlated to fish condition whereas shrimp densities were negatively associated with densities of small-sized juveniles in spring. A comparison of proxies for fish growth showed the advantage of Fulton's K for routine use whereas RNA:DNA ratios proved less powerful when short-term environmental fluctuations are lacking. This study illustrated how distinct sets of habitat features can drive spatial variation in density and condition of juvenile flatfish highlighting the value of studying both variables when modeling habitat requirements. The habitat models generated in this study also provide a powerful tool to predict potential climate and anthropogenic impacts on the distribution and condition of juveniles in flatfish nurseries. The need for effective coastal zone management was emphasized to ensure a sustainable use of coastal resources and successful flatfish recruitment to the fishery.

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Fien

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CHAPTER 1

GENERAL INTRODUCTION

Turbot
Psetta maxima
Tarbot

Megrim
Lepidorhombus whiffiagonis
Scharretong

Brill
Scopthalmus rhombus
Griet

Greenland halibut
Reinhardtius hippoglossoides
Groenlandse heilbot

Common dab
Limanda limanda
Schar

European plaice
Pleuronectes platessa
Pladijs - Schol

Lemon sole
Microstomus kitt
Tongschar

Common sole
Solea solea
Tong

European flounder
Platichthys flesus
Bot

Witch flounder
Glyptocephalus cynoglossus
Hondstong

Pictures on previous page were adapted from following links:

http://www.ispolar.is/halibut.html,
http://www.luontoportti.com/suomi/en/kalat/turbot,
http://ec.europa.eu/fisheries/marine_species/wild_species/sole_and_plaice/index_en.htm,
http://www.itameriportaali.fi/en/ajankohtaista/uutisia_muualta/2008/en_GB/brill/,
http://www.scandfish.com/ig/gallery.asp?action=viewimage&categoryid=24&text=&imageid=927&box=&shownew,%20,
http://munster-provincial-council.webs.com/fishspecies.htm,
http://www.fishbase.org/summary/speciessummary.php?id=26,
http://www.fishbase.us/photos/PicturesSummary.php?StartRow=1&ID=28&what=species&TotRec=7,
http://fishindex.blogspot.com/2009/09/european-flounder-platichthys-flesus.html,
http://www.marinespecies.org/photogallery.php?album=755&pic=2668

Trends in fish stocks, impact of fishery and environmental fluctuations

Human and environmental pressures on marine ecosystems have resulted in global declines of ecologically and economically important fish species. In recent decades, this has driven a high number of commercial fish stocks outside safe biological limits; with fishing and other sources of mortality exceeding recruitment and growth (Serchuk *et al.*, 1996; Pauly *et al.*, 2002; Myers and Worm, 2003; Thurstan *et al.*, 2010).

Overexploitation is accepted as the major cause of stock depletion and changing population dynamics. This is indicated by the phenomenon of 'fishing down marine food webs' where fish species at higher trophic levels are more intensively targeted and progressively decrease in abundance (Pauly *et al.*, 1998). Furthermore, landings of industrially fished species have increased over time but their biomass, mean fish size and proportions of large individuals have decreased, indicating the unsustainable nature of those fisheries. Genetically induced changes in life history traits due to overharvesting of large specimens may further reduce the capacity for population recovery (Walsh *et al.*, 2006). Seawater temperature has also been linked to early-life history traits and its relationship with recruitment and spawning stock biomass is demonstrated for major fish stocks (Cook and Heath, 2005). Climate change, acting on biological production and species interactions, is likely to have an additional impact on fish stocks and may explain the distributional shift of several plankton and fish species (Beaugrand *et al.*, 2002; Dulvy *et al.*, 2008; Hátún *et al.*, 2009; Brander, 2010; Engelhard *et al.*, 2011).

Anthropogenic and climate pressures are superimposed on natural fluctuations in spawning stock biomass which is known to be highly variable between years. This further complicates stock assessment and the designation of sustainable fishing quotas, which are generally determined from the spawning stock biomass. Effective fishing policy is therefore dependent on a sound understanding of the determinants of year-class strength during all stages of the ontogenetic development. The call to conduct fisheries management through an ecosystem approach also requires a greater knowledge of interactions between commercial fish species and their predators and prey.

Distribution, exploitation and management of flatfish

Flatfishes are globally distributed and are among the most important marine fish resources in the North Temperate Zone, with nearly half of global flatfish catches being exploited in the Atlantic Ocean (Pauly, 1994; Millner *et al.*, 2005). In the Northeast Atlantic region, the most abundant flatfish populations are in areas of the continental shelf where sandy or muddy sediments occur in water depths shallower than 200: the North Sea, Barents Sea, Baltic Sea and around the British Isles (Gibson, 2005). Of the 21 species present in this area (Russell, 1976), most of the commercial species belong to three families Pleuronectidae, Soleidae and Bothidae (Wheeler and Du Heaume, 1969). These species can be divided into three groups based on their contribution to total landings and economic value: large quantity landings with high value (plaice, Greenland halibut and common sole), large quantity landings with lower value (common dab, megrim, flounder, witch flounder and lemon sole) and low quantity landings with high value (turbot and brill). Time series of landings of flatfish species in Ireland since 1950 are presented in Fig. 1. Only the commercial species in the first category are managed by means of Total Allowable Catches (TACs) under the Common Fishery Policy of the European Union, based on expert advice provided annually by the International Council for the Exploration of the Sea (ICES).

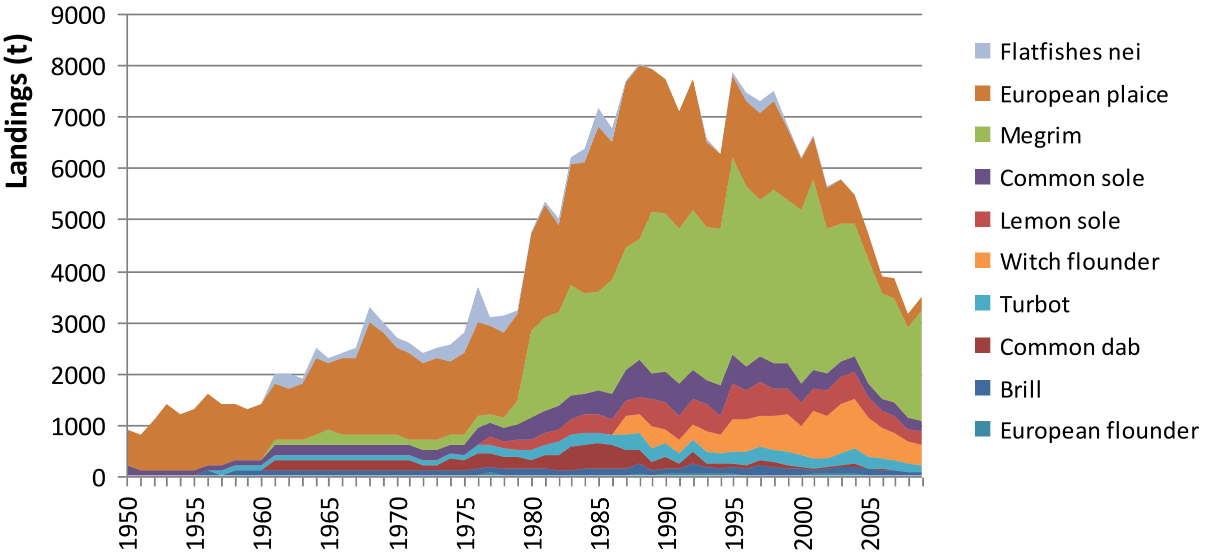

Fig. 1. Temporal trends of total landings in tonnes (t) of commercial flatfish species in Ireland between 1950 and 2009. Data source: http://www.fao.org/fishery/statistics/global-capture-production/query/en

Plaice and dab are the most abundant demersal species caught in beam trawl surveys in northeast Atlantic shelf seas (Rogers *et al.*, 1999) and are the species of interest in this thesis. Plaice have been exploited for centuries in mixed demersal and flatfish fisheries by beam and otter trawls, in directed fisheries by gillnets and seine nets and as by-catch in bottom trawl fisheries (ICES, 2011d; a; b; c). With the increasing fishing mortality from the 1950s until the late 1990s, the spawning stock biomass sharply declined below the maximum sustainable yield and plaice stocks became at risk of reduced reproductive capacity (Daan, 1997; Anon., 2011). With less intense fishing pressure since the mid-1990s, plaice stocks have shown increasing levels of spawning stock biomass in the North Sea (Fig. 2). In other management areas, including the west (ICES Divisions VIIb, c) and south-west (ICES Divisions VIIj, k) coast of Ireland (Fig. 3) and insufficient data are available for plaice stock assessment. Therefore, the measures recommended by ICES include the reduction of catch quotas and the use of larger-mesh gear to allow higher

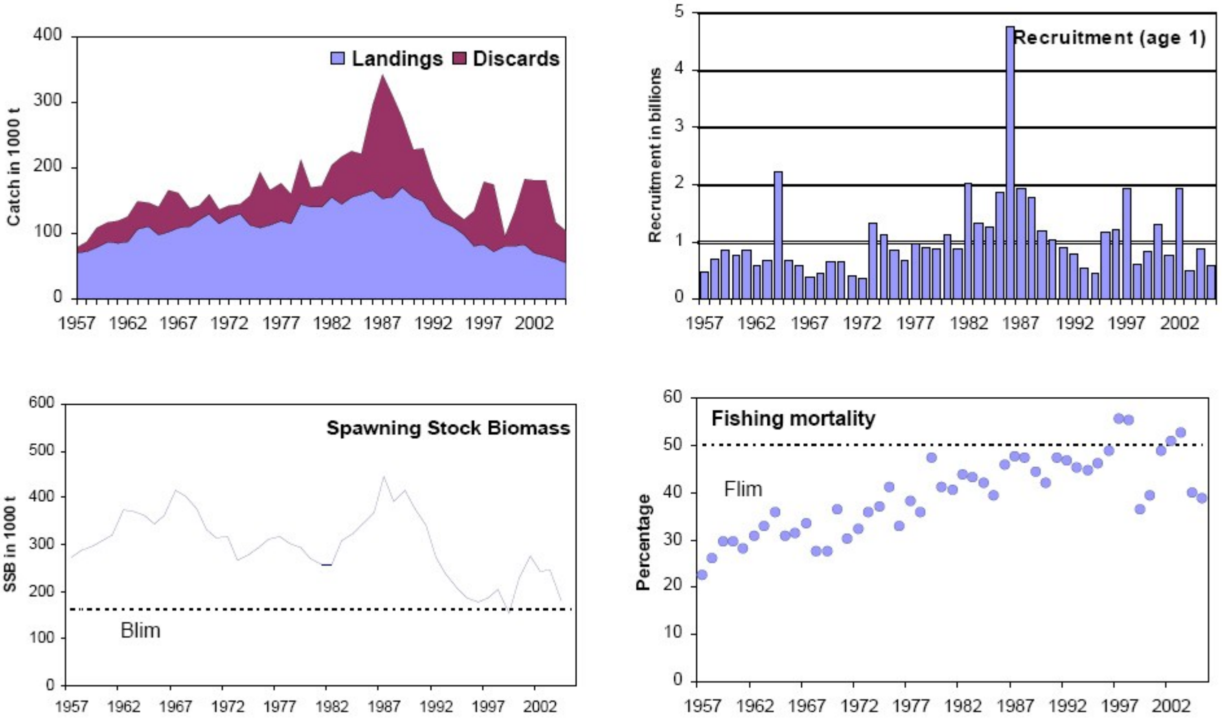

Fig. 2. Landings and discards (000 t), spawning stock biomass (000 t), recruitment at age 1 (in billions) and average fishing mortality (in %) for ages 2 to 6 for North Sea plaice between 1957 and 2006. Source: http://www.ices.dk/marineworld/fishmap/ices/pdf/plaice.pdf

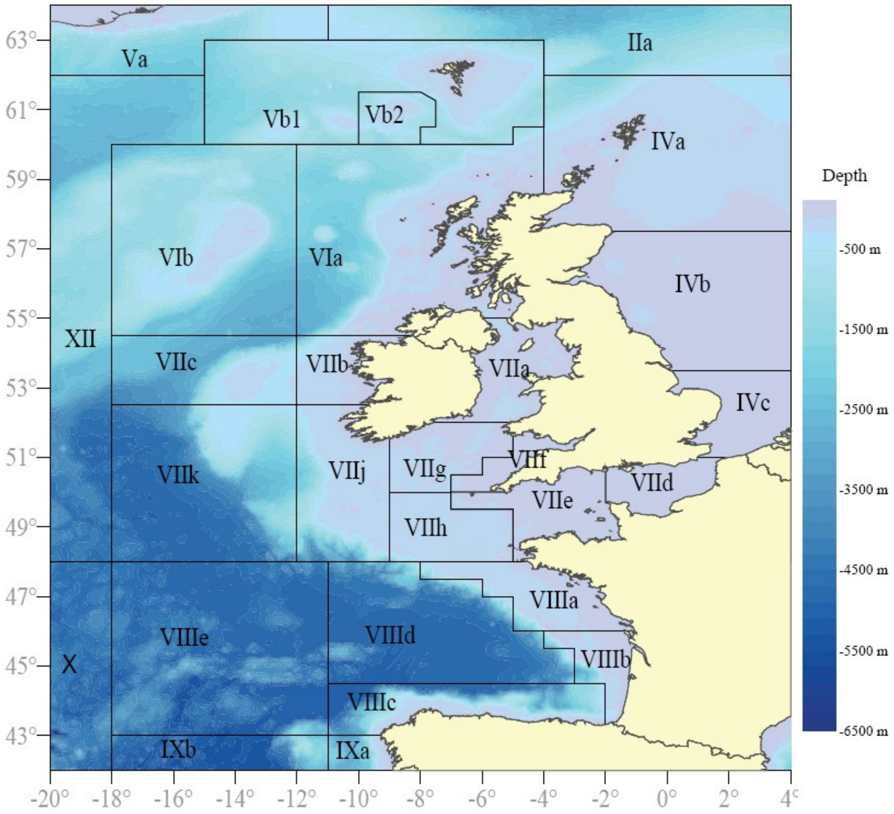

Fig. 3. ICES fishing divisions around the Irish coast. Source: The Stock Book, annual publication of the Marine Institute's Fisheries Science Services (Hjörleifsson and Pálsson), Ireland: http://hdl.handle.net/10793/27.

fishing yields (ICES, 2011e). Dab, like most other flatfish species with lower market value, are caught as by-catch in flatfish or mixed demersal fisheries and often discarded based on the availability of target species. Due to the low priority of non-target species in research, direct and indirect impacts of fishing on dab remain poorly known (Pope *et al.*, 2000). The exploitation status of this non-quota species is unknown and a lack of knowledge about population structure hinders rational management. Fisheries for formerly lower-valued dabs have developed in certain areas, e.g. the Baltic Sea, raising interest in the biological traits that determine the stock state and to predict how community structure will change in response to exploitation (ICES, 2011d). Baseline data are therefore required on the population parameters of plaice and dab stocks in the west coast of Ireland, to ensure their sustainable exploitation through effective management in the future.

On the life cycles of plaice and dab

A remarkable aspect of the life history of most flatfish is their distinct morphological appearance and utilisation of different habitats during successive life stages (Fig. 4). European plaice and common dab, like most other temperate flatfish species, spawn in offshore waters over several months (Harding *et al.*, 1978; Rijnsdorp, 1989; Miller *et al.*, 1991; Henderson, 1998). Batches of developing planktonic eggs and larvae are transported to shallow inshore areas (De Veen, 1978; Fox *et al.*, 2009). Settlement in these nursery grounds occurs at a size of about 13 mm and coincides with completed metamorphosis (Russell, 1976). The change from a symmetrical pelagic larva to a demersal flatfish requires extensive functional changes, involving flattening of the body, strong development of the unpaired fins, a marked reduction of the paired fins, eye migration and changes in pigmentation (Fuiman, 1997; Osse and Van den Boogaart, 1997; Christensen and Korsgaard, 1999; Geffen *et al.*, 2007). The juvenile demersal life stage of

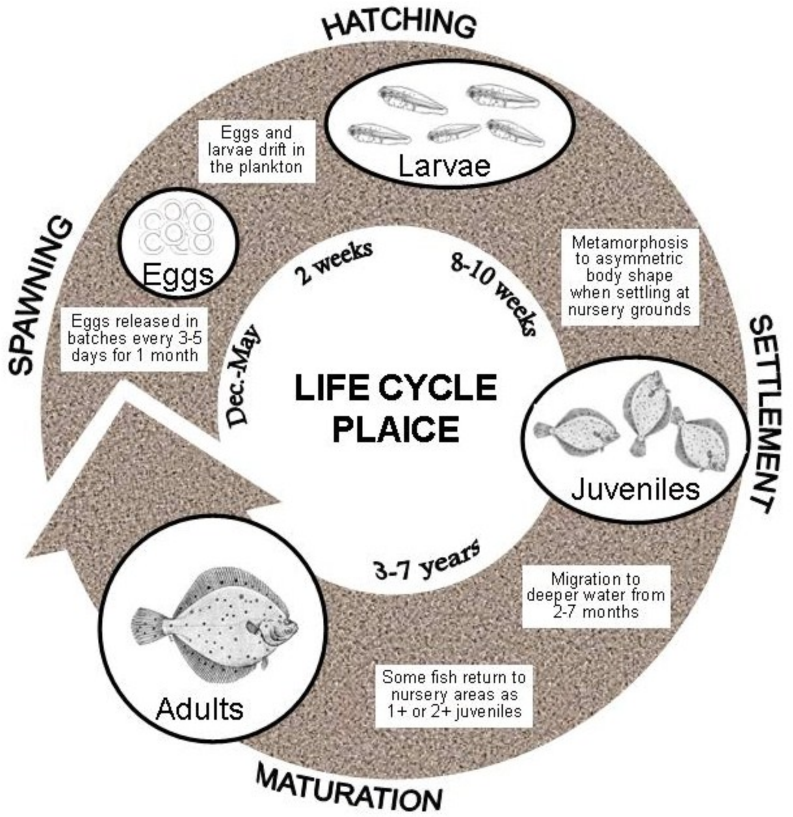

Fig. 4. General features of the life cycle of plaice, *Pleuronectes platessa*

pleuronectids is concentrated in shallow soft bottom coastal areas throughout the summer (Norcross *et al.*, 1995) whereas larger individuals successively move to deeper grounds in autumn (Gibson *et al.*, 2002). At around 2-5 years, depending on sex, plaice and dab attain sexual maturity (Rijnsdorp, 1989; Rijnsdorp *et al.*, 1992b) and undergo offshore spawning migrations to join the reproductive population. Recruitment rates show high fluctuations between years and the detection of underlying processes has gained much research attention due to the major biological and economic implications for the management of commercial fisheries. In the context of this thesis, recruitment is considered to be the estimated proportion of a cohort or year class which enters the fishery whereas year-class strength refers to the relative number of individuals at a certain time between spawning and recruitment (van der Veer *et al.*, 1994).

Recruitment variability

Processes governing fish recruitment operate at different life stages but it is generally accepted that the year-class strength of marine fishes is predominantly established during the early life stages. The fecundity of adults and the quantity and quality of eggs may drive differences in population production (Rijnsdorp, 1994; Nash and Geffen, 1999; van der Veer and Leggett, 2005) but the conditions during the pelagic stage are crucial since this is the time when mortality rates may be particularly high (Houde, 1987). Possible factors controlling larval fish survival include predation (Fox *et al.*, 2000b), starvation (Anderson, 1988; Grønkjær *et al.*, 1997) and transport conditions to the nursery areas (van der Veer *et al.*, 1998; Oshima *et al.*, 2010). There are a number of mechanisms that may be responsible for high pelagic mortality rates. According to Hjort's "critical period" hypothesis, year class strength is regulated by the capability of first-feeding larvae to find suitable amounts and types of planktonic prey, while Cushing's "match-mismatch" hypothesis proposes that year class strength depends on the coincidence between the seasonal timing of plankton production and the seasonal abundance of larvae. However, recent reviews suggest that recruitment variability is rather regulated by complex trophodynamic and physical processes acting over many temporal and spatial scales and throughout pre-recruit life (Leggett and Deblois, 1994; Houde, 2008).

Processes acting on the juvenile phase have additional regulating effects on recruitment of marine demersal (Miller *et al.*, 1991; Myers and Cadigan, 1993; Bailey, 1994) as well as reef (Caselle and Warner, 1996) and pelagic (Watanabe, 2009) fish species. Since juvenile demersal fish are spatially concentrated in restricted areas, it is found that density-dependent processes might dampen the recruitment variability generated by density-independent mortality in the pelagic period, i.e. the 'concentration hypothesis' (Beverton and Iles, 1992; Beverton, 1995). This might explain why pelagic species, which are not confined to a 2-dimensional space and which may experience a wider range of environmental conditions, show higher recruitment variability than demersal species. Other studies have not detected reduced growth and higher mortality due to crowding effects, suggesting that the carrying capacity of juvenile habitats is rarely reached (van der Veer and Witte, 1993; Nash *et al.*, 2007). The relative importance of processes acting on the juvenile life stage of flatfish is still unknown and there is a need for integrated research studying the combined effect of abiotic and biotic conditions regulating fish survival in the nursery grounds to elucidate the mechanisms responsible for the strength of a year class.

Importance of nursery habitat and imposed threats

Both the quantity and quality of nursery habitats are important factors that regulate annual levels of recruitment to the reproductive stocks (Gibson, 1994). Habitat selection by juvenile flatfish is driven by resource requirements. Individual species are restricted to a range of habitat conditions by physiological tolerances (Davenport and Sayer, 1993), feeding requirements (Piet *et al.*, 1998) or sediment associations (Moles and Norcross, 1995) which determine the quantity of suitable nursery grounds for a given species. Differential habitat requirements can therefore lead to spatial segregation between species. Studying the habitat associations of a number of flatfishes in the North Sea, Riley et al. (1981) showed that turbot and dab were rather specialized in relation to habitat, compared to plaice. The reduced surface area of suitable nursery grounds for more specialized flatfish species may result in a lower number of recruits to the adult population, according to the 'nursery size hypothesis' (Rijnsdorp *et al.*, 1992a).

High quality nursery habitats are defined as those areas in which growth, survival and fitness of juvenile fish are optimized (Gibson, 1994). Survival rates give a direct indicator of habitat quality for a given species but attaining accurate measurements involves long-term monitoring or tagging studies which are costly and time consuming. Alternatively, it has been postulated that larger ('bigger-is-better' mechanism), faster growing ('growth-rate' mechanism), and faster developing ('stage-duration' mechanism) individuals have a higher probability of survival (Chambers and Leggett, 1992; Leggett and Deblois, 1994; Sogard, 1997). These mechanisms are all based on the assumption that mortality is size dependent, delivering a benefit when minimizing the time over which individuals experience the highest mortality rates. Predation and starvation are the most important factors affecting the early life stage mortality and a reduced risk with fish size is found for flatfish species in field studies (van der Veer and Bergman, 1987; Ellis and Gibson, 1995). Laboratory research also supports these findings showing that larger juvenile flatfish are better able to avoid predation, obtain food and resist starvation which favours their survival (Post and Prankevicius, 1987; Henderson *et al.*, 1988; Gibson *et al.*, 1995; Wennhage, 2000). Whereas decreased vulnerability with increased size has been demonstrated for individual fish species, other studies highlight the complexity of prey-predator relationships within multi-species communities (Holmes and McCormick, 2010).

Fundamental knowledge of habitat characteristics directly affecting the distribution, growth and survival of juvenile fish in Irish coastal waters is lacking. Identifying the role of biotic and abiotic habitat characteristics for the survival success of juvenile fish is crucial to predict how environmental change could alter the species-habitat association in coastal areas. This is particularly important given recent deterioration in coastal habitats due to extensive exploitation, anthropogenic impacts and climate warming. A thorough understanding of these ecological interactions determining juvenile fish success can thus prevent further deterioration and loss of juvenile flatfish by supporting the sustainable management of coastal zones (Beck *et al.*, 2001; Riou *et al.*, 2001; Maxwell *et al.*, 2009; Rochette *et al.*, 2010). Since the study area in Galway Bay has been designated as a candidate Special Area of Conservation (SAC), this new knowledge on coastal habitat ecology can play a key role in SAC monitoring and management in the future.

Indicators for growth rate of flatfish and spatio-temporal variability

Habitat quality can be indirectly examined by assessing fish growth rates. Various proxies for growth are used in fisheries research. Otolith microstructure analysis is probably the most widespread technique to yield individual growth rate measures. Otoliths are calcium carbonate structures in the inner ear of the fish and daily depositions allow determination of the timing (hatch and settlement date) and duration (larval and juvenile duration) of critical life history events (Stevenson and Campana, 1992). Whereas the number of increments reflects the total age of the fish, daily increment widths correspond with the growth rate of fish (Karakiri *et al.*, 1989; Karakiri and von Westernhagen, 1989). Relating otolith microstructure information with environmental conditions can reveal valuable information on spatial and temporal differences in stock structure, larval drift patterns, oxidative stress, growth and mortality during specific moments in the early life history of fishes (Al-Hossaini *et al.*, 1989; Rijnsdorp *et al.*, 1990; Karakiri *et al.*, 1991; Berghahn, 2000; Morales-Nin, 2000; Reichert *et al.*, 2000).

Various morphometric and biochemical condition indices have also proven to be valuable proxies for fish growth. Fulton's condition factor K is based on the length-weight relationship and is a comprehensive index of overall growth (Suthers, 1998). This widely used morphometric index is based on the assumption that heavier fish of a given length are in better condition and it provides a good indicator of habitat quality (Suthers, 1998; Gilliers *et al.*, 2004). A limitation of this classical indicator is its insensitivity to recent events in the life of fish, such as feeding history. In contrast, biochemical indices (RNA:DNA ratio) are developed as sensitive indicators for recent growth and nutritional condition in marine organisms. The underlying principle is that the amount of DNA remains constant within individual cells of an organism whereas RNA levels increase in response to protein synthesis and thus growth (Bulow, 1970; Buckley *et al.*, 1999). Since nucleic acid based condition indices respond over relatively short time scales, they prove highly valuable to detect environmental changes in habitat quality after 1-3 days.

Growth of the early life stages of fish varies considerably both temporally and spatially due to a combination of biotic and abiotic conditions. Habitat characteristics which are known to affect growth rate of juvenile flatfish are physiochemical conditions (Phelan *et al.*, 2000; Amara, 2004), food availability (Burrows, 1994; van der Veer *et al.*, 1994), predator density (Burrows and Gibson, 1995; Gibson *et al.*, 1995), substrate characteristics (Gibson and Robb, 2000;

McConnaughey and Smith, 2000) and competition (Burke *et al.*, 1991; Rooper *et al.*, 2006). On the Irish west coast, recent investigations have focused on identifying the factors determining early pelagic life history traits of plaice (Allen *et al.*, 2008), turbot (Haynes *et al.*, 2011) and flounder (O'Neill, Pers. Comm.). However, little attention has focused on the link between the juvenile stage and habitat characteristics, except for a few studies looking at general trends in growth dynamics of plaice and turbot (Haynes *et al.*, 2010). Identifying the relative importance of the primary determinants that enhance rapid growth in juvenile flatfish in west of Ireland nurseries is crucial to understand variability in habitat quality on a local scale and to predict the effect of changing environmental conditions. Comparisons of flatfish densities and growth with other geographic regions may give additional insight into effective habitat in a broader context and in the degree of varying species-habitat interactions. The development of suitable proxies for fish growth is an essential step towards assessing and protecting habitat quality. This will ensure a future supply of recruits to the reproductive population which is crucial for fisheries and maintaining ecosystem functioning.

Objectives and thesis outline

The overall aim of this thesis was to investigate the ecological mechanisms and species interactions influencing the distribution, growth and survival probability of juvenile plaice and dab which depend on marine coastal ecosystems. Understanding population dynamics during this critical life stage of these economically important flatfish species is of major importance in evaluating variability in year-class strength and recruitment to fishery. The complex relationships between biotic and abiotic habitat components are studied to gain insight into spatial and temporal differences in habitat quality of flatfish nursery grounds along the west coast of Ireland.

This thesis comprises peer reviewed research papers which are currently published or in the review process, an introduction and discussion at the start and end of the thesis respectively. Each of these chapters closely resembles the associated paper and can be read as an autonomous study. The outline, objectives and details of publication are summarized below for each chapter:

Chapter 2:

A preliminary survey was conducted to assess how morphology, feeding, recent growth and condition of juvenile plaice from nursery grounds located on the south west coast of Ireland varied over several spatial scales. The objectives were to:

- ✓ assess the degree of intraspecific variation in plaice diet and its effect on condition, growth and morphology
- ✓ explore the importance of spatial scales in field studies to evaluate quality of flatfish nursery grounds
- ✓ compare a recent growth index and a morphometric index as potential proxies for habitat quality

De Raedemaecker, F., Keating, J., Brophy, D., O'Connor, I. and Mc Grath, D., 2010. Spatial variability in diet, condition and growth of juvenile plaice (*Pleuronectes platessa*) at sandy beach nursery grounds on the south-west coast of Ireland. Journal of the Marine Biological Association of the United Kingdom 91, 1215-1223.

Chapter 3:

The role of macrobenthic food availability in west of Ireland nursery grounds as a determinant of habitat quality for plaice and dab was evaluated. Feeding habits of plaice and dab during early development were described in light of potential interspecific competition. The relative importance of environmental characteristics as structuring factors in macrobenthic communities was explored and related to spatial variability in plaice and dab condition. The objectives were to:

- ✓ assess the intra- and interspecific variation in diet of plaice and dab and its effect on fish condition
- ✓ evaluate the potential for food competition between juvenile plaice and dab during development in the post-settlement phase
- ✓ investigate the role of macrobenthic food availability as a determinant of habitat quality

De Raedemaecker, F., O'Connor, I., Brophy, D., and Black, A. 2011. Macrobenthic prey availability in flatfish nursery grounds and the potential for food competition between 0-group plaice and dab. Journal of Fish Biology Accepted.

Chapter 4:

Limited information exists on the combined effect of physical and biological habitat characteristics that influence small-scale differences in the distribution and growth of plaice and dab. Data on temporally and spatially fluctuating density/condition of juvenile fish and biotic/abiotic habitat features were collected in nursery grounds in Galway Bay to determine habitat requirements promoting high abundance and growth of juvenile flatfish. The objectives were to:

- ✓ identify good habitat quality nursery grounds on the west coast of Ireland in terms of higher abundances, and/or larger juvenile flatfish
- ✓ provide a mechanistic understanding of the association between biotic and abiotic habitat features and the density and condition of juvenile fish

- ✓ explore further ecological mechanisms allowing co-existence of two flatfish species in Galway Bay nursery grounds

De Raedemaecker, F., Brophy, D., O'Connor, I. and Comerford, S. *In review*. Habitat characteristics promoting high density and condition of juvenile flatfish at nursery grounds on the west coast of Ireland. Journal of Sea Research.

Chapter 5:

Habitat quality of nursery grounds in Galway Bay was assessed using Fulton's K and RNA:DNA ratio as condition indices and by adopting a multi-species approach. Spatial and temporal fluctuations in nutritional and morphological condition were explored in relation to diet features and habitat characteristics. The comparison of explained variability in condition indices in combination with consistency over time was used to highlight strengths and weaknesses of both condition indices. The objectives were to:

- ✓ evaluate Fulton's K and RNA:DNA ratio in juvenile fish as indicators of habitat quality
- ✓ assess if feeding conditions are driving variability in condition indices
- ✓ ascertain a set of habitat characteristics explaining small-scale variability in both condition indices

De Raedemaecker, F., Brophy, D., and O'Connor, I. 2011. Comparison of spatio-temporal variability in Fulton's K and RNA:DNA ratio as condition indices of juvenile plaice and dab. Estuarine, Coastal and Shelf Science. Accepted.

Chapter 6:

An overall discussion of this thesis addresses the findings of the four studies presented. The main conclusions are presented within the context of population dynamics of 0-group flatfish in highly dynamic shallow inshore nursery grounds. The relevance of these results to the future conservation and sustainability of west of Ireland flatfish populations are discussed and recommendations for future research are considered.

CHAPTER 2

SPATIAL VARIABILITY IN DIET, CONDITION AND GROWTH OF JUVENILE PLAICE (*PLEURONECTES PLATESSA*) AT SANDY BEACH NURSERY GROUNDS ON THE SOUTH-WEST COAST OF IRELAND

Published as:

De Raedemaecker, F., Keating, J., Brophy, D., O'Connor, I. and Mc Grath, D., 2010. Spatial variability in diet, condition and growth of juvenile plaice (*Pleuronectes platessa*) at sandy beach nursery grounds on the south-west coast of Ireland. Journal of the Marine Biological Association of the United Kingdom 91, 1215-1223.

Abstract

Characterization of suitable habitat for settlement of juvenile flatfish is important for the management of nursery areas. Food availability is one important determinant of habitat quality that can affect the condition and growth, and thus survival, of flatfish. Spatial and temporal variation in diet has been widely studied for several species of flatfish. However, levels of intraspecific variation in diet at small spatial scales are relatively unknown, with most studies focusing only on large scale variability. This study investigates how diet, growth and condition of juvenile plaice, *Pleuronectes platessa*, varies over two spatial scales (10s of kilometres and 100s of metres). Juvenile plaice were collected from three beaches and from three replicate hauls on each beach using a beach seine in September 2007 and 2008. Gut content analyses of 108 juvenile plaice within the size range of 70-90 mm were carried out. Diet composition in plaice guts differed among beaches and hauls suggesting that food abundance and availability differed at both spatial scales. A significant positive correlation was observed between a morphological condition index and the prey diversity in the gut. This suggests that fish which specialize on a limited number of prey items (perhaps due to a greater abundance of certain prey) may do better than fish which feed on a wide range of prey types. Significant differences in condition were observed between hauls and between beaches, while recent and total otolith growth varied between beaches but not between hauls. The results highlight the importance of considering small scale variation when attempting to link habitat quality to feeding, growth and condition of juvenile flatfish.

Keywords

juvenile plaice*; Pleuronectes platessa*; diet; otolith microstructure; spatial variability; growth; condition

Introduction

Characterization of suitable habitat for juvenile flatfish is important for the management of nursery areas. The nursery value of habitat is commonly assessed by measuring growth and condition of juveniles. Higher condition and growth rate results in successive improvements in feeding (van der Veer and Witte, 1993), predator avoidance (Gibson *et al.*, 1995; Wennhage, 2000) and ultimately survival (Vethaak, 1992; Islam and Tanaka, 2005). It is the combination of several habitat variables that favour rapid growth. Physicochemical conditions (e.g. temperature, salinity, dissolved oxygen) are considered to be the primary determinants of distribution and growth in flatfish (Karakiri *et al.*, 1991; Reichert, 2003; Amara *et al.*, 2004) while food abundance and quality are also important factors affecting growth and condition of young fish (van der Veer and Witte, 1993; Burrows *et al.*, 1994; Gibson, 1994).

Some studies observed field caught juvenile fish that displayed growth rates similar to those of laboratory animals reared in excess feeding conditions. This suggested that an abundance of food and an absence of competition can occur on flatfish nursery grounds (van der Veer *et al.*, 1990; Karakiri *et al.*, 1991; Amara *et al.*, 2001) which has led to the formulation of the 'maximum growth/optimal food' hypothesis (Karakiri *et al.*, 1991; Reichert, 2003). However in other instances, differences in juvenile growth rate between regions are observed, which might reflect differences in food composition and availability (van der Veer and Witte, 1993; van der Veer *et al.*, 2001) and indicate spatial variability in the quality of nursery ground habitat. Spatial variation in growth rates and condition in juvenile flatfish has been observed across a range of spatial scales (1-100s of kilometres) (Glass *et al.*, 2008).

The diet of flatfish has been widely studied for the assessment of nursery quality. Some studies have examined how the diet of recently settled flatfish changes with size to determine if prey shifts occur during the ontogeny of the fish (Whyche and Shackley, 1986; Aarnio *et al.*, 1996). Others have addressed the issue of trophic niche width and examined dietary overlap and resource partitioning between different flatfish species (Beyst *et al.*, 1999; Cabral *et al.*, 2002; Vinagre *et al.*, 2005). However, levels of intraspecific variation in diet are relatively unknown; the few studies that address this focus on large-scale variability (100s of kilometres; Woll and Gundersen (2004), 3-10 km; Berghahn (1987)). The possible impact of spatial variation in diet on the growth and condition of flatfish on sandy-beach nursery areas has not yet been addressed.

This study aims to assess growth and feeding ecology of juvenile plaice (*Pleuronectes platessa* Linnaeus, 1758*)* within a restricted size range of 70-90 mm in total body length. Plaice is a commercially important flatfish in the north-east Atlantic (Millner *et al.*, 2005) and juvenile plaice are widely dispersed on nursery grounds in this region. Special attention is given to the importance of studying growth, condition and diet over different spatial scales when evaluating flatfish nursery ground quality. The first objective was to investigate the intraspecific variation in diet over two spatial scales (10s of kilometres and 100s of metres). The second objective was to assess intraspecific variation in morphology, growth and condition and determine whether spatial variation in these parameters can be linked to spatial variation in diet at the above mentioned scales.

Materials and Methods

Field work

Sample collection formed part of a long term annual flatfish survey which aims to assess and monitor the juvenile stages of commercially important flatfish populations on beaches in the south-west and west of Ireland. Those beaches are important nursery areas for flatfish populations like turbot and plaice (Haynes *et al.*, 2010). Sampling was conducted during three consecutive days in September 2007 and during the same period in 2008. Juvenile plaice were collected during spring low tide from three beaches every year (Inch, Smerwick and Ventry in 2007 and Inch, Smerwick and Brandon in 2008) and from three replicate hauls on each beach (Fig. 1). The distance between beaches ranged from 30-80 km; the distance between hauls within the same beach ranged from 300-1000 m.

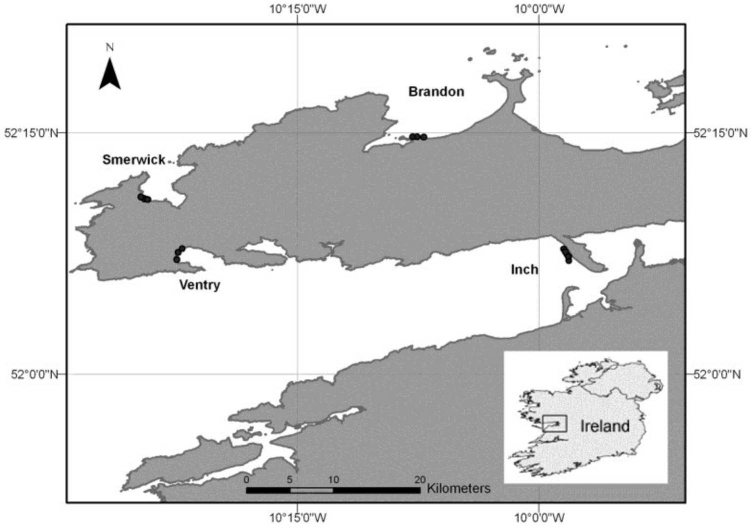

Fig. 1. Beach seine locations and position of 3 hauls per beach (per year) at nursery grounds at the south west coast of Ireland.

A beach seine of 20 m breadth with a 12 mm square mesh size was used, covering an approximate swept area of 1380 m^2. Six plaice of 70-90 mm were randomly selected from each haul and kept frozen until analysis. This size-range was chosen to allow comparison of ingested food items during a specific growth stage of fully metamorphosed plaice (Ryland, 1966).

Morphology and gut content measurements

Several morphological characteristics of a total of 108 juvenile individuals were recorded after thawing; body length, eye diameter, mouth height (Braber and de Groot, 1973b) and mouth width were measured to the nearest 0.01 mm. Mouth gape (0.01 mm^2) was calculated by multiplying mouth width and mouth height by π (Piet *et al.*, 1998). Both stomach and intestines (guts) of all juveniles were analyzed together as plaice have a rather small stomach and long alimentary tract (Beyst *et al.*, 1999). Gut content analyses of all juvenile plaice were carried out and prey items were identified under a stereomicroscope to their main taxonomic groups and counted. Total number of prey taxa and total prey abundance in the guts were measured. Stomach fullness was calculated as a percentage; length of gut containing prey divided by total gut length * 100.

Condition and Growth

Fulton's condition factor (Fulton, 1911) was calculated for each fish using the formula $K = (W/L^3)*100$, where W is fresh weight (g) and L is total length (cm). This widely used morphometric index assumes that heavier fish of a given length are in better condition and it has been proven to be a good indicator of habitat quality (Gilliers *et al.*, 2004).

Otolith microstructure analysis was used to provide a relative measure of recent growth for all juvenile plaice. The right sagittal otolith was extracted, mounted on a slide with the convex side facing upwards and embedded with crystalbond. Otoliths were polished and examined using transmitted light with a compound microscope (Olympus U-TV1X-2) and a 20 x objective lens. Image analysis software (Image Pro Plus 6.2) was used to measure the width of the 10 most recently deposited daily increments along the core-rostrum axis. The mean daily increment width over the last 10 days before capture was calculated as an index of recent growth. The otolith diameter was measured under the 4 x objective lens to obtain an index of overall fish growth.

Data Analyses

Minitab 15 was used for statistical analyses. The balanced data were tested for normality and homogeneity of variances. Prior to examining variability in plaice morphology, diet descriptors, growth and condition between beaches and hauls, a two-way Analysis of Variance (ANOVA) with beach and year in the model was carried out to examine if any variability was attributed to year-differences. In the event a significant variation between years was found, further analyses were carried out for every year separately. Two-way nested ANOVAs were performed to examine effects of beach and haul, nested within beach, on fish morphology and diet descriptors. Beach was included as a fixed factor while haul was included as a random factor (Underwood, 1997). Significant beach effects were further explored using Tukey's post hoc comparisons. Analysis of covariance (ANCOVA), with fish length as a covariate, was used to investigate if relative otolith diameter differed between plaice from different beaches or hauls. Pearson's correlation was used to determine if any correlation existed between mean diversity of gut contents and mean fish condition across all hauls.

Prey assemblage composition in the stomach of fish was analysed using the non-metric multidimensional scaling (nMDS) ordination technique of the PRIMER version 5 statistical package (Clarke, 1993). From the original samples by prey matrix (108 x 14), abundances of prey were square root transformed and the Bray-Curtis similarity coefficient was calculated for every sample to generate a similarity matrix. Differences in diet among beaches and hauls were further analysed using two-way nested analysis of similarities (ANOSIM). Where significant differences were found, particular emphasis was placed on the R-statistic values produced by ANOSIM to identify the extent of the difference. Similarity of percentages (SIMPER) was employed to determine which prey classes contributed the most to any similarities within beaches.

Results

Spatial variation in diet composition

Diet of plaice was compared between hauls and beaches based on five different prey descriptors; numbers of prey taxa, prey abundance, prey diversity, stomach fullness and prey assemblages.

Since we found variability in total prey taxa and Shannon-Wiener prey diversity between years, two-way nested ANOVA was repeated for both variables for each year separately. A two-way nested ANOVA revealed differences in gut contents on both spatial scales (Table 1). At a small spatial scale (300-1000 m), prey descriptors varied between fish from replicate hauls, except for total prey taxa and prey diversity in 2008. Prey descriptors also varied at a larger spatial scale (30-80 km) between fish collected from different beaches. A pairwise comparison revealed a significant higher prey diversity in fish from Ventry compared to Inch ($p = 0.0168$) and Smerwick ($p < 0.001$) in 2007. Differences in total prey abundance and total prey taxa in 2008 were evident across beaches but no significant differences in percentage stomach fullness were found between beaches.

Table 1. Results of two-way nested ANOVA, with hauls nested within beach, performed on prey descriptors in the guts of plaice, with mean and standard deviation for every beach in both years. Levels of significance; ^{ns}, not significant; *, $P<0.05$; ** $P<0.01$; *** $P<0.001$.

		Total taxa	**Total abundance**	**Shannon-Wiener diversity**	**% Stomach fullness**
Differences between beaches					
	Inch 2007	3.78 ± 1.59	28.39 ± 17.18	0.83 ± 0.38	56.48 ± 21.93
	Inch 2008	2.61 ± 0.70	24.39 ± 15.69	0.63 ± 0.32	59.45 ± 16.60
	Ventry 2007	4.11 ± 1.08	9.89 ± 6.09	1.21 ± 0.32	51.51 ± 29.21
	Smerwick 2007	2.39 ± 1.33	13.72 ± 9.56	0.54 ± 0.47	64.72 ± 18.13
	Smerwick 2008	1.72 ± 0.96	9.06 ± 7.25	0.32 ± 0.45	53.55 ± 17.59
	Brandon 2008	2.5 ± 0.96	20.22 ± 18.05	0.50 ± 0.40	61.16 ± 10.27
F ratio	2007	3.51 ^{ns}		6.77*	
	2008	5.33**		2.81 ^{ns}	
	2007+2008		3.40 *		0.37 ^{ns}
Differences between hauls					
F ratio	2007	2.85 *		3.05 *	
	2008	1.28 ^{ns}		2.09 ^{ns}	
	2007+2008		2.19 *		4.12 ***

The nMDS ordination plot derived from prey abundance data of the guts of fish, caught in different hauls and beaches indicated some discrete groups of prey assemblages (Fig. 2). Two-way nested ANOSIM demonstrated that prey assemblages differed significantly between hauls ($R = 0.200$, $p < 0.001$) and beaches ($R = 0.602$, $p < 0.001$). The greatest difference in prey composition occurred between fish from Ventry in 2007 and fish from other beaches in both years whereas the smallest difference was found between fish from Inch and Brandon in 2008 and between fish from Smerwick in 2007 and 2008 (Table 2).

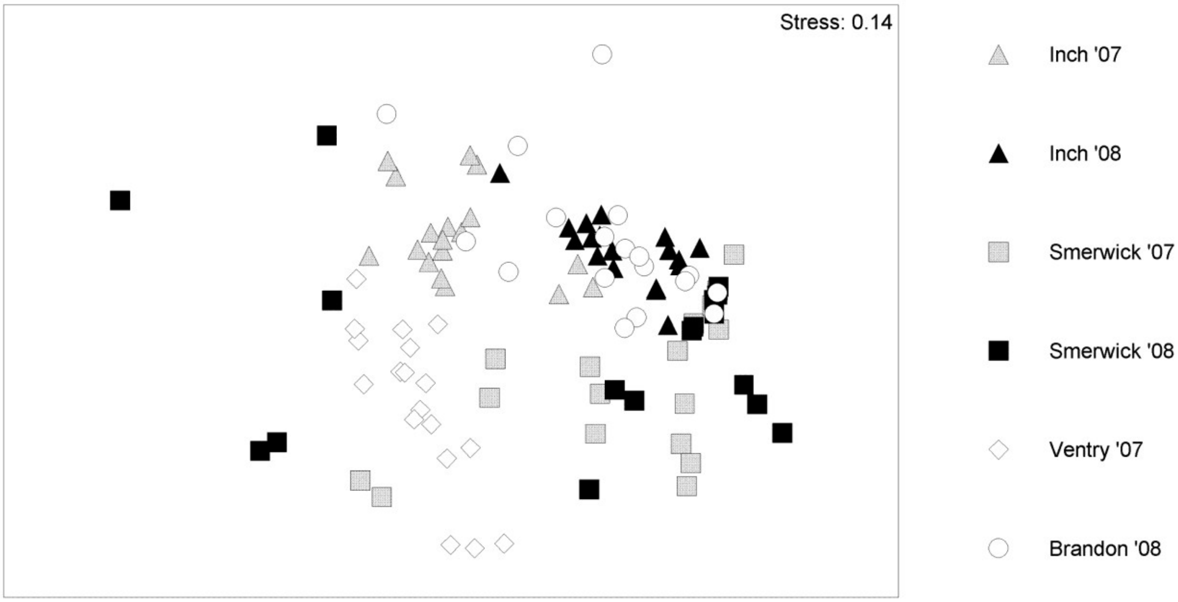

Fig. 2. Non-metric multidimensional scaling ordination plot of the prey abundance data in the guts of 108 juvenile plaice. Sampling was carried out during 6 sampling occasions divided over 4 beaches and 2 years.

Table 2. Results of ANOSIM pairwise test with R-statistic values close to unity indicating a high difference in composition of gut samples between sampling occasions and vice versa.

Pairwise comparison	R values
Smerwick 2007, Smerwick 2008	0.148
Inch 2008, Brandon 2008	0.185
Smerwick 2007, Brandon 2008	0.259
Smerwick 2008, Inch 2008	0.333
Smerwick 2008, Brandon 2008	0.333
Smerwick 2007, Inch 2008	0.444
Inch 2007, Brandon 2008	0.741
Inch 2007, Smerwick 2008	0.778
Inch 2007, Inch 2008	0.778
Ventry 2007, Smerwick 2007	0.852
Inch 2007, Smerwick 2007	0.926
Inch 2007, Ventry 2007	1
Ventry 2007, Smerwick 2008	1
Ventry 2007, Inch 2008	1
Ventry 2007, Brandon 2008	1

Table 3. SIMPER analysis showing average similarity percentages of prey composition in guts of fish within different sampling occasions. The average abundance and percentage contribution of typifying prey classes contributing >5 % to within-group similarity for the fish in the six sampling occasions are shown.

Beach - Year	Average similarity (%)	Typifying prey-classes	Average Abundance	Contributing %
Inch 2007	46.76	Amphipods	17.94	73.54
		Cumaceans	5.22	17.08
		Decapods	2.06	6.18
Inch 2008	51.94	Siphons Bivalves	17.28	73.24
		Cumaceans	4.67	18.69
		Amphipods	2.33	8.07
Ventry 2007	36.74	Amphipods	2.22	30.51
		Bivalvia	1.83	29.3
		Decapods	3.00	19.13
		Annelida	1.06	12.28
		Ostracoda	0.44	5.15
Smerwick 2007	32.87	Siphons Bivalves	9.61	78.6
		Decapods	2.44	12.12
Smerwick 2008	25.73	Siphons Bivalves	7.50	92.16
		Decapods	0.28	4.73
Brandon 2008	37.09	Siphons Bivalves	15.11	71.57
		Cumaceans	3.06	17.29
		Amphipods	1.69	10.79

SIMPER analysis performed on prey data in the fish guts detected an average similarity within beaches between 26% and 52% (Table 3). Diet of plaice from Inch is in both years characterized by a high abundance of amphipods and cumaceans; similarly to diet of fish from Brandon in 2008 but very different from diet of plaice from the other beaches. Diet of plaice from Smerwick is dominated by a high abundance of one prey item, bivalve siphons, and a small amount of decapods. Fish from Ventry in 2007 were feeding on a large variety of prey from different taxonomic classes. Pie charts in Fig. 3 graphically represent both small and large scale differences.

Intraspecific variability in plaice morphology

All fish were within the size range of 70-90 mm in total length. A two-way nested ANOVA (Table 4) showed no significant differences in fish body length between beaches or between hauls within each beach. Similarly, eye diameter (which was only measured from 2007 fish) was not significantly different between beaches or hauls. Mouth gape, standardized for fish length, differed significantly between hauls and between beaches. A pairwise comparison test showed that mouth gape was significantly larger in fish from Smerwick in 2007 compared to fish from Smerwick in 2008 ($p = 0.008$), Ventry in 2007 ($p < 0.001$), Brandon in 2008 ($p = 0.006$) and Inch in 2007 ($p < 0.001$). Fish from Inch in 2008 also showed a significantly larger mouth gape compared to fish from Inch in 2007 ($p = 0.010$) and Ventry in 2007 ($p = 0.030$).

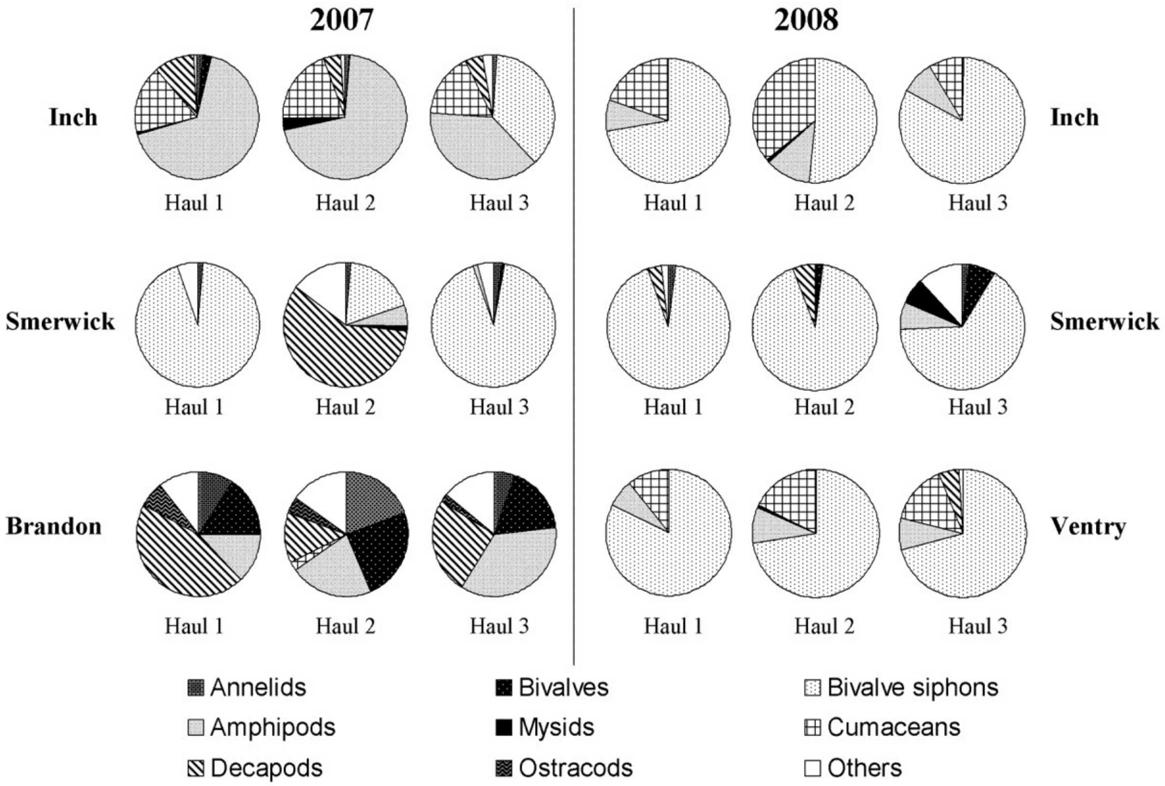

Fig. 3. Pie charts showing the taxonomic breakdown of prey items in the guts of juvenile plaice across 3 replicate hauls on 3 beaches over 2 years. Flatworms, gastropods, copepods, echinoderms and arachnids were rarely found in some guts and pooled in the group 'others'.

Spatial variation in condition and growth

In this study, Fulton's K condition index of juvenile plaice ranged between 0.86 and 1.20 g cm^{-3} in 2007 and between 0.96 and 1.40 g cm^{-3} in 2008. Fish from 2008 were in significantly better condition than those from 2007 (two-way ANOVA, $F = 98.52$, $p < 0.001$). Two-way nested ANOVA revealed significant differences in condition between beaches within both years and between hauls in 2007 but no variation between hauls was found in 2008 (Table 5). In 2007, fish with lowest condition were found in Ventry and were significantly lower compared to fish from Inch ($p < 0.001$) and Smerwick ($p = 0.001$). In 2008, fish with lowest condition were found in Smerwick, and were significantly lower to fish from Brandon ($p < 0.001$) and Inch ($p = 0.038$).

Table 4. Results of two-way nested ANOVA, with hauls nested within beach, performed on morphological characteristics of plaice, with mean and standard deviation for every beach in both years. Levels of significance; ^{ns}, not significant; *, P<0.05; ** P<0.01; *** P<0.001.

		Body length (mm)	**Eye diameter (mm)**	**Mouth gape (mm^2)**
Differences between beaches				
	Inch 2007	83.06 ± 4.92	5.30 ± 0.43	54.58 ± 9.06
	Inch 2008	81.01 ± 4.66		68.56 ± 13.50
	Ventry 2007	80.22 ± 4.52	5.33 ± 0.42	53.91 ± 13.88
	Smerwick 2007	80.39 ± 4.65	5.03 ± 0.31	78.40 ± 13.73
	Smerwick 2008	78.49 ± 4.46		60.38 ± 12.59
	Brandon 2008	78.26 ± 3.33		59.92 ± 17.29
F ratio	2007		2.19 ^{ns}	
	2007+2008	2.82 ^{ns}		11.39 ***
Differences between hauls				
F ratio	2007		1.5 ^{ns}	
	2007+2008	0.77 ^{ns}		3.71 ***

Table 5. Results of two-way nested ANOVA, with hauls nested within beach, performed on condition (Fulton's K) and recent growth rate (otolith's average increment width over last 10 days) of plaice, with mean and standard deviation for every beach in both years. Levels of significance; ^{ns}, not significant; *, P<0.05; ** P<0.01; *** P<0.001.

		Fulton's K condition (g/cm^3)	**Increment width last 10 days (μm)**
Differences between beaches			
	Inch 2007	1.02 ± 0.07	6.65 ± 1.47
	Inch 2008	1.23 ± 0.05	6.86 ± 1.02
	Ventry 2007	0.94 ± 0.06	5.19 ± 0.89
	Smerwick 2007	1.02 ± 0.06	5.94 ± 1.07
	Smerwick 2008	1.16 ± 0.11	6.28 ± 0.77
	Brandon 2008	1.27 ± 0.06	6.67 ± 0.92
F ratio	2007	13.88 ***	
	2008	8.54 ***	
	2007+2008		6.33 ***
Differences between hauls			
F ratio	2007	4.04 **	
	2008	1.59 ^{ns}	
	2007+2008		1.67 ^{ns}

Otolith diameter showed a linear increase with fish length (F = 84.40, $p < 0.0001$) confirming that otolith growth reflects somatic growth. ANCOVA did not detect differences in otolith diameter, corrected for fish length, between hauls (F = 1.42, p = 0.172) but otolith diameter differed between beaches (F = 4.19, p = 0.002). Pairwise comparisons showed that fish from Ventry in 2007 had larger otoliths than fish from Inch in 2007 ($p < 0.001$) (Fig. 4). This indicates slower growth rates over the life of fish on Ventry compared to fish on Inch. This finding corresponds with observed variation in recent growth. A two-way nested ANOVA showed that mean peripheral increment width, which ranged between 3.62 µm and 9.82 µm, was not different between hauls but differed significantly between beaches (Table 5). Recent growth was lower for fish from Ventry in 2007 compared to fish from all other beaches in both years ($p < 0.05$). In contrast to fish condition, recent growth did not differ between years, suggesting that differences in recent growth were not large enough to contribute to differences in overall condition of plaice between beaches.

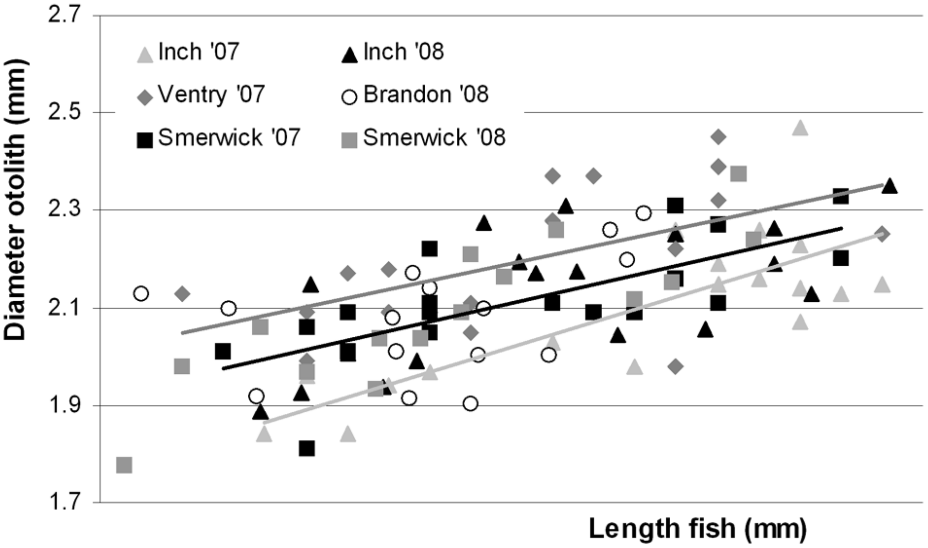

Fig. 4. Ratio of otolith diameter by total fish length for every individual, with trendlines displaying the different beaches over both years. Trendlines are presented for 2007 fish data.

Correlation between diet and condition

As described above, prey diversity in juvenile plaice guts varied between hauls within beaches. For each haul, mean condition and mean prey diversity were calculated. Pearson's correlation analysis detected significant decreases in condition with higher prey diversity (r = -0.630, p = 0.005). This significant inverse linear relationship showed that fish feeding on greater prey diversity also displayed the poorest condition (Fig. 5).

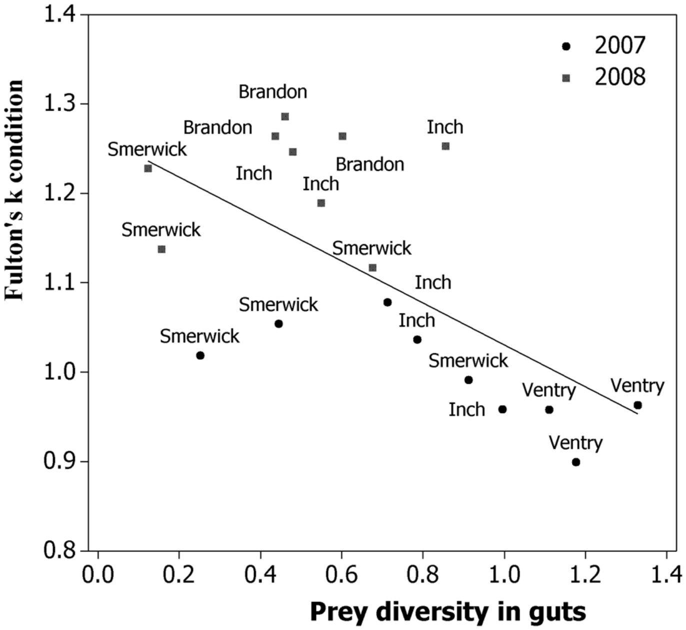

Fig. 5. Scatterplot of prey diversity in plaice guts against Fulton's K condition of plaice. Every point represents the averages of all studied plaice pooled per haul.

Discussion

Our results on food composition in plaice guts are in agreement with earlier findings. Polychaetes, molluscs and crustaceans are major food items in diet of juvenile plaice on sandy beach nursery areas (Braber and de Groot, 1973a), with an ontogenetic shift occurring in larger fish from polychaetes towards crustaceans (Edwards and Steele, 1968; Rijnsdorp and Vingerhoed, 2001). Whyche and Shackley (1986) found this change in diet occurring at a fish length of 35-40 mm, probably because at this size plaice move to deeper parts of the littoral zone where wave action is strong and food organisms such as amphipods, harpacticoids and young bivalves are more exposed. Major taxonomic groups dominating plaice diet in our study, revealed by multivariate analyses, differed among beaches. Variation in stomach fullness and prey abundance was also observed at a smaller spatial scale (between hauls) within beaches. This spatial variability in diet suggests a high degree of trophic adaptability to different prey.

Without information on the benthic fauna, it is unclear if differences in diet are the result of selective feeding behaviour and/or differences in prey distribution and availability between locations. The observed variation in the quantity and diversity of prey items occurred at a scale of 100's of metres (300 m was the smallest distance between hauls). Following the observations of Burrows *et al*. (2004) that plaice have a very high probability of remaining in a 100 m wide zone for at least one day, we can assume that plaice caught in one haul were also feeding in distinct areas compared to each other. As it is unlikely that plaice from adjacent hauls have different feeding preferences, we hypothesize that food abundance and availability differs between the haul locations.

Small-scale variation between hauls within beaches was found in Fulton's condition of plaice. Condition indices are indicative of short term fish growth and are directly linked with food (Ferron and Leggett, 1994). As discussed above, we hypothesize that food abundance and availability differed between haul locations, affecting plaice condition. The correlation between prey diversity and condition of plaice from different hauls suggested that a varied diet leads to poorer condition whereas a feeding strategy which is based on a high degree of specialization on a few prey types results in good condition. Plaice were generally in better condition in 2008 compared to 2007 and large-scale variation between beaches was found within both years. Plaice from Inch and Brandon had a better than average condition and a low prey diversity in their gut, compared to plaice from Ventry and Smerwick. Abundant food resources might be available here in combination with selective feeding (of amphipods in Inch and bivalve siphons in Smerwick) to obtain the best nutritional status. Plaice caught in some hauls in Smerwick and in all hauls of Ventry did not grow up under optimal food conditions and had a lower condition as a result. We assume that prey are less abundant in this area. Another plausible suggestion for this diet difference (maybe as a result of lower prey availability) is that fish feeding on Ventry are exploiting less mobile prey because they are in poorer condition. We observed a higher stone content in the guts of plaice from Ventry and this might indicate they have a rather endobenthic feeding behaviour.

While diet very likely caused condition and growth differences of plaice between hauls and between beaches in this study, we cannot exclude possible additional factors (working in combination) that could be driving large-scale spatial variation. The physical environment is known to affect nursery quality and subsequently fish growth. Variation in temperature between beaches might occur as there is a river inflow in Ventry beach but no freshwater input in Smerwick and Inch beaches. Optimal growth temperature for juvenile plaice is around 20 ºC when abundant food is available (Fonds *et al.*, 1992). At this temperature, the high assimilated energy from a faster ingestion is still exceeding the energy losses for metabolism which increases with temperature (Yamashita *et al.*, 2001). Sediment type also influences a fish's ability to bury itself in the substrate in order to escape predators (Gibson and Robb, 1992). The beach in Inch is more exposed than in Smerwick and Ventry, therefore, differences in sediment type are likely. However, more research is needed to investigate if differences in burying effort, and subsequently fish growth, occur between beaches. Amezcua *et al.* (2003) found significant differences in flatfish diet between sediment types. This highlights the direct influence of beach structure and exposure on composition of benthic communities. The biotic environment can affect the growth rate of plaice. Previous studies have linked spatial patterns in growth and abundance of plaice with the availability of food. Karakiri *et al.* (1989) and Berghahn (1987) reported higher growth rates of plaice in the tidal flats of a nursery area compared to plaice in the tidal channels and concluded that food limitation occurred in the channels. Poxton *et al.* (1982) found a higher abundance of juveniles in areas with an abundant benthic food supply in the Clyde Sea area. Van der Veer and Witte (1993), showed a positive relationship between food abundance and growth of plaice in the Dutch Wadden Sea. In our study, prey abundance in some haul locations could have been reduced by predator foraging (Berghahn, 1987) or other disturbances which lead to different food availabilities; e.g. beam trawling enhances the abundance of small opportunistic benthic species such as polychaetes (Rijnsdorp and Vingerhoed, 2001). Lower growth rates might also be ascribed to density dependent growth as demonstrated by Modin and Pihl (1994). In the absence of more biological or environmental data on the studied sites, we can only speculate about possible additional factors influencing variation in juvenile plaice growth and condition.

Condition indices in larval and juvenile fish respond quickly to changes in food availability and can give a good indication of the growing conditions over the previous 24 hours (Ferron and Leggett, 1994). On the other hand, otolith growth responds more gradually to changes in feeding conditions and metabolic rate and represents a running average of fish growth rather than an instantaneous measure (Campana and Neilson, 1985). This could explain why variation between hauls was observed in fish condition, but not in otolith growth rate. While patchy distribution of prey items may produce small scale variation in fish condition, over a longer time period the movement of plaice between sites within a beach would produce a more homogenous pattern of otolith growth. Moreover, diet differences between hauls do not override diet differences between beaches, as shown by a clear separation on the nMDS plot. This may reflect overall differences in habitat quality between beaches, contributing to variation in plaice growth.

In this study, dissimilarity in mouth morphology was observed; mean mouth gape differed between hauls and was higher in plaice from Smerwick compared to plaice from Inch and Ventry. No other citing of intraspecific differences in mouth gape was found in the literature and the reason for this difference here can only be speculated upon. The mouth gape has been identified by Piet *et al.* (1998) as the most important morphological characteristic affecting prey selection. As such, the observed larger mouth gape in Smerwick might be related to the high level of predation on bivalves. The jaws of plaice are well modified to biting off parts of bivalve molluscs (Yazdani, 1969). Different flatfish species adapt their behaviour to the behaviour of the prey (Holmes and Gibson, 1983) but the question remains whether juvenile plaice morphology can adapt depending on the available prey items. In stock identification studies two-dimensional measurements of the head can be useful for distinguishing fish from different areas. Further experimental work could help to establish how the availability of prey and feeding preferences influence mouth morphology.

Variation in growth and condition across replicate hauls within beaches highlights the importance of adequate replicate sampling. As juvenile plaice are active and mobile feeders but with a strong sense for site fidelity (Burrows *et al.*, 2004), studies which attempt to link the composition of the benthic fauna to flatfish dietary preferences should consider small scale variation in these parameters. There is a danger of misinterpretation if assessments of nursery ground quality are based only on broad descriptors of the benthic community and average condition or growth across a beach. Also, closer examination of how diet and condition varies within a nursery area can help to elucidate how habitat characteristics influence growth and survival of juvenile fish.

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CHAPTER 3

MACROBENTHIC PREY AVAILABILITY AND THE POTENTIAL FOR FOOD COMPETITION BETWEEN 0 YEAR GROUP *PLEURONECTES PLATESSA* AND *LIMANDA LIMANDA*

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Abstract

With increasing pressures on adult flatfish stocks, the quality of shallow coastal nurseries becomes very important to ensuring high recruitment success at the juvenile stage. An understanding of the biological and physical characteristics which benefit fish communities and the benthic prey on which they depend is therefore important to evaluate nursery habitat quality. This study aimed to describe the feeding habits of plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*) during early juvenile development and to relate differences between nursery grounds and sampling years to spatial and temporal variability in macrobenthic prey availability. The main prey taxa in the guts of both species were copepods, bivalves, amphipods, polychaetes and oligochaetes and size-related variation in diet was found. Despite considerable similarity in the prey items, differences in food composition between the two species were observed and spatial variability in diet confirmed their opportunistic feeding behaviour. A high degree of dietary overlap was found in June and decreased steadily throughout the season. The prey composition in the guts of both flatfish species largely reflected the composition of the main macrobenthic taxa in the sediment. Our overall data suggest that resources were not limiting in the littoral sandy nursery areas in the West of Ireland and no indications were found that exploitative competitive forces upon the benthic prey assemblages occurred between plaice and dab. However feeding success, Fulton's K condition, and dietary overlap showed spatial and temporal variation and were likely affected by the availability and density of macrobenthic prey.

Keywords

dab; early juvenile development; feeding ecology; interspecific diet overlap; plaice

Introduction

Plaice (*Pleuronectes platessa*, Linnaeus, 1758) and dab (*Limanda limanda,* Linnaeus, 1758*)* are among the most abundant flatfish species in the north-eastern Atlantic region (Daan, 1997). Plaice are the most important flatfish for fisheries in Europe whereas dab, the smaller of the two species, are mainly exploited as a by-catch species in mixed flatfish fisheries (Gibson, 2005). The combined effect of overexploitation and climate warming has resulted in a steady decrease of these northern winter spawners in recent decades (Millner *et al.*, 2005). In some regions, this trend was preceded by a decline in juvenile abundances, indicating that impacts on recruitment have contributed to the declines in population size (Désaunay *et al.*, 2006; Hermant *et al.*, 2010). Several studies have shown that the processes governing fish recruitment variability operate at different life stages. The fecundity of adults and the quantity and quality of eggs may drive differences in recruit production (Rijnsdorp, 1994; Nash and Geffen, 1999; van der Veer and Leggett, 2005). Evidence suggests that during the larval phase, year-class strength is mainly controlled by water temperature (van der Veer *et al.*, 1990; van der Veer and Witte, 1999; Fox *et al.*, 2000a), predation (Fox *et al.*, 2000a) and transport conditions to the nursery areas (van der Veer *et al.*, 1998; Oshima *et al.*, 2010). In the post-settlement phase, it is generally accepted that the year-class strength is regulated by mortality, induced by the abiotic and biotic conditions in the nursery grounds (van der Veer *et al.*, 1990; Gibson, 1994; Beggs and Nash, 2007). Food availability is among the most important variables affecting nursery habitat quality and previous studies of feeding patterns have shown a clear link with growth, condition and survival of juvenile flatfish (Amara *et al.*, 2001; Cabral *et al.*, 2002).

The foraging ecology of juvenile flatfish differs with locality, reflecting the influence of environmental variation on food selection (Moore and Moore, 1976). Flatfish species also differ in the composition of their diet; such resource partitioning can reduce competition and is likely to be governed by morphological constraints (Piet *et al.*, 1998). Plaice and dab however have a very similar body shape with well-developed eyes adapted to their visual benthic feeding strategy. Moreover, settlement of plaice and dab has been reported in various parts of their distribution range to occur simultaneously and at the same sandy beach nursery areas (Bolle *et al.*, 1994; Gibson *et al.*, 2002). Despite those similarities, different life strategies have been adopted by both species to minimize interspecific competition. The peak abundance of settling juveniles in nursery areas lies between 1 - 4 m depth for plaice and between 3 - 5 m depth for dab (Bolle *et al.*, 1994). This may reflect differences in the optimal growing temperature for each species which is reported to be between 15-18 ºC for dab and between 18-20 ºC for plaice under laboratory conditions (Attrill and Power, 2004). Both species show a day/night cycle which strongly affects their behaviour and physiology with bottom activity restricted to daylight hours. Plaice also display a tidal rhythm of activity which is controlled by an endogenous clock, and this has been suggested as a mechanism in the redistribution of food between both species (Gibson, 1973) since dab only show a bimodal circadian activity pattern (Burrows *et al.*, 1994). More encounters between the two species are thus expected at low tide when plaice move offshore and this might intensify competition for food.

Information on the functioning of nursery grounds in the western range of the plaice and dab distribution is scarce. Dietary studies are a valuable tool for disentangling how resource partitioning occurs along trophic, spatial and temporal dimensions (Amezcua *et al.*, 2003). However there is little published research that directly relates prey availability to stomach contents in coastal nursery areas that are characterized by a gradient of environmental conditions. Looking at size-related prey preferences might also provide valuable information on interspecific differences in growth and condition and the potential for interspecific competition during particular life-stages of juvenile flatfish (Fuiman, 1997).

The present study aimed to describe the feeding habits of 0-group plaice and dab during the first months after settlement. The first objective was to explore if interspecific differences in feeding occurred throughout early juvenile development. The second objective was to relate spatial and temporal differences in macrobenthic prey availability to the feeding success and condition of plaice and dab. The third and final objective was to explore if interspecific interactions form a basis for food limitation between the studied species that use the same nursery grounds.

Materials and Methods

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 approximately 100 km^2, 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 from the Corrib River. 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.

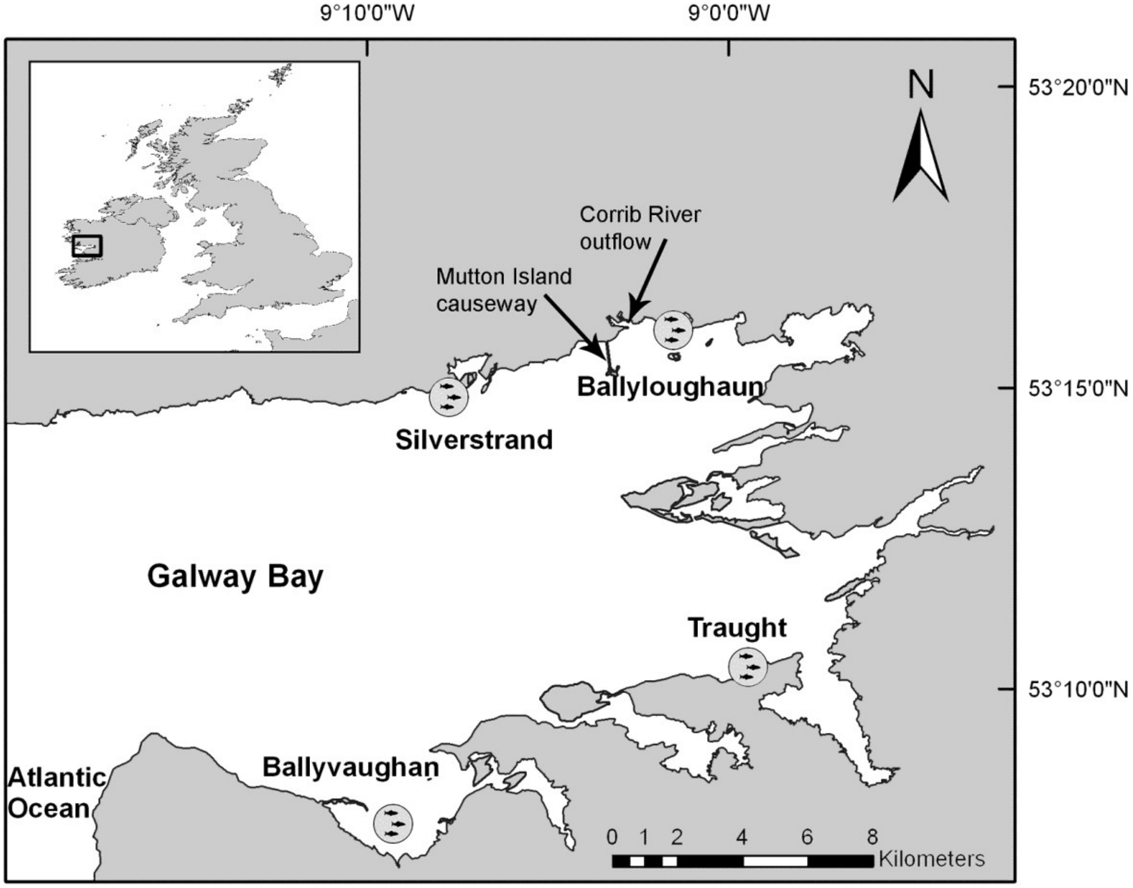

Fig. 1. Map of Galway Bay, Ireland and positioning of the four sampled flatfish nursery grounds.

Juvenile flatfish assemblages were sampled monthly from June - 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 x 6 mm codend) equipped with one tickler chain was hauled at a constant velocity of 1.1 knots for 2 min. A mechanical flowmeter attached to the beam was used to calculate the swept area which averaged 94.2 ± 16.8 m^2 per trawl. Three replicate trawl hauls were carried out both intertidally and subtidally, resulting in a total of six hauls at each site. All juvenile flatfish were immediately stored onboard in liquid nitrogen and transferred to a -80 ºC freezer in the laboratory until further sorting and analyses. The actual depth was recorded with an echo sounder and depths relative to lowest astronomical tide were calculated, expressed as 'negative depths' when working over the intertidal area. The geographic location of the trawls was recorded using a GPS. Salinity and temperature data were obtained at each trawl location with a CTD. Macrobenthos at each end of the trawl path were collected from three replicate Van Veen grabs (0.025 m^2) pooled together. A fourth grab was collected for the determination of sediment organic matter and particle size analysis (PSA).

Fish processing

All fish were counted and identified to determine the densities of young of the year (yoy) plaice and dab for each trawl (juveniles caught 100 m^{-2}). Plaice and dab were the most abundant flatfish species and three replicate specimens of each species per trawl were selected for diet analysis. The selection was based on their length, representing the first, second and third quartile of the length-frequency range so that studied fish were representative of the original length distribution in the trawl. Due to a wider distribution range of plaice (present in both intertidal and subtidal trawls) than dab (mostly found in subtidal regions), a higher number of plaice guts were studied. Standard length (L_S) was measured to the nearest mm and gently blotted fish were weighed (W) to the nearest mg. Fulton's condition factor (Fulton, 1911) was calculated for each fish using the formula $K = (W / L_S^{\ 3}) * 100$, where W is fresh weight (g) and L_S is standard length (cm). Both stomach and intestines were analysed 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. Nematoda and Platyhelminthes were also found in some gut contents, but were removed from subsequent analyses, since they may be fish parasites and not prey. 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). The relative importance of each prey item was expressed as the frequency of occurrence (% F) of each prey group in the gut of all plaice and dab and the relative numerical contribution (% N) of each prey group to total number of food items in the gut of each fish (Berg, 1979; Hyslop, 1980). The interspecific diet overlap was evaluated by Schoener Index (I_S) (Linton *et al.*, 1981; Wallace and Ramsey, 1983): $I_S = 1 - 0.5\left(\sum_{i=1}^{n} |pi_A - pi_B|\right)$, where p_{iA} and p_{iB} are the numerical frequencies of item i in the diet of species A and B respectively. Although there are no critical levels for this index, several studies have considered that diets are significantly similar if values >0.6 but this does not necessarily mean that competition for food will occur (Dolbeth *et al.*, 2008; Guedes and Araújo, 2008). All indices were calculated using the abundances of each of the main taxonomic groups occurring in the fish guts.

Sediment processing

All benthic samples were washed on a 1 mm sieve and stored in 4 % buffered formaldehyde prior to counting and identifying to the lowest possible taxon using a stereomicroscope. The macrobenthic taxa from the three replicate grabs taken at either side of the trawl were pooled before calculating the total prey density (100 m^{-2}), the density of every macrobenthic taxa, the number of taxa and the Shannon-Wiener diversity index. Organic matter was measured using the percentage loss on ignition (LOI) after burning 25 g of sediment (oven-dried at 100 °C to constant weight) in a muffle furnace at 450 °C for 6 hours. Particle size analysis was carried out with oven-dried sediment for all samples collected in 2008 using the method described in Bale & Kenny (2007). Sediments were divided into six fractions: silt (<63 µm diameter), very fine sand (63 µm - 125 µm), fine sand (125 µm - 250 µm), medium sand (250 µm - 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 size, phi, was calculated. The sediment particle size data was mapped with ArcGIS 9.2 software as continuous surfaces using inverse-distance weighted averaging (IDW). This interpolation technique was applied to generate percentage composition data at the sample locations in 2009, as it proved effective in other studies (Phelan *et al.*, 2001; Stoner *et al.*, 2001; Compton *et al.*, 2008).

Data analyses

Diet, based on the frequency of occurrence and/or numerical abundance of the different prey classes, was compared for plaice and dab. Total prey abundance, total number of taxa, Shannon-Wiener prey diversity, gut fullness and Fulton's condition were compared between beaches and years, using two-way Analysis of Covariance (ANCOVA) with standard fish length included as a covariate. Similarly total macrobenthic density, diversity and density of separate taxa were compared between beaches and years with ordinal date as a covariate. Prior to ANCOVA, data were checked for normality and homogeneity of variances and transformation of the response variables was carried out if necessary. The diet similarity between fish species and beaches was analysed through similarity relationships, using the % N of the main taxonomic prey groups for every sample. Prior to nonmetric multidimensional scaling (MDS) ordination, the dietary data were averaged per trawl, square root transformed and similarity matrices were constructed using the Bray–Curtis similarity coefficient (Clarke and Warwick, 1994b). Likewise, a similarity matrix was created for the macrofaunal density data and the correlation between the two multivariate patterns was examined using the RELATE procedure (Clarke and Warwick, 1994a). The correlation between both matrices was considered significant if the associated p value was <5 %. One-way or two-way crossed Analysis of Similarities (ANOSIM) was performed on the similarity matrices to determine whether the dietary compositions of fish differed among species and sites (Clarke, 1993). Similarity percentages (SIMPER) were used to determine which dietary categories typified particular groups and contributed to any dissimilarity between groups (Clarke, 1993). The explanatory variables that accounted most for the dietary overlap between plaice and dab were computed using a Generalized Linear Model (GLM) with normal error distribution using the R software (R Development Core Team, 2008). The variables included in the start model were year, beach, depth of trawl, ordinal date, flatfish density, total numbers and diversity of benthic prey, average gut fullness of plaice and dab. Continuous variables were pair-plotted in order to investigate multi-colinearity between the data, none was found therefore all variables were included in the model. A backwards selection using the Aikake information criterion (AIC) was used for model selection and a significance level of 0.05 was considered in all test procedures. Model validation was carried out by plotting the residuals against the different explanatory variables (Zuur *et al.*, 2009). The characterisation of the different nursery areas within Galway Bay in terms of benthic macrofauna and sediment characteristics was investigated using a Canonical Correspondence Analysis (Franco *et al.*) performed with Canoco software (version 4.5) (Ter Braak, 1989). This ordination technique determined the effect of environmental variables on the composition and spatial occurrence of benthic communities. Benthic densities were log-transformed and taxa accounting for < 1% of total occurrence were excluded prior to analysis.

Results

General feeding patterns

Only five out of 651 fish analysed (<1 %) were found with empty guts and were excluded from further analyses. The diet of plaice and dab consisted of a wide variety of benthic invertebrates (Table 1). Harpacticoid copepods made the greatest numerical contribution to the gut content of both fish species and had an equally high frequency of occurrence. Polychaetes were not abundant in the diet of dab but they were the second most ingested prey group in the guts of plaice; mainly species from the families Nereidae, Capitellidae and Spionidae. Amphipods, mainly *Gammarus* spp. and *Corophium* spp., were the second most consumed prey group for dab and had a high frequency of occurrence in plaice guts. Oligochaetes were rarely found in the diet of plaice but this prey group was third most consumed in the diet of dab. The numerical contribution of bivalves was ranked fourth in the diet of both plaice and dab. *Abra alba*, *Cerastoderme edule* and *Scrobicularia plana* were the most numerous bivalve prey species. Gastropods, cumaceans, decapods and ostracods showed a low numerical contribution to the diet of plaice and dab but their frequency of occurrence was relatively large. Isopods, echinoderms, arachnids and chironomidae were also preyed upon but at very low levels.

Table 1. Relative importance of major prey taxa to the overall diet of plaice (Pleuronectes platessa) and dab (Limanda limanda), expressed as frequency of occurrence (% F) and numerical contribution (% N). Total prey taxa, total fish analysed and average fish length ± SD are presented for both fish species.

	Pleuronectes platessa		***Limanda limanda***	
Prey taxa	% N	% F	% N	% F
Oligochaeta	0.82	4.20	13.19	37.65
Polychaeta	17.41	65.97	2.00	17.06
Siphons Bivalvia	3.53	17.02	0.08	0.59
Bivalvia	12.04	53.78	9.27	47.65
Gastropoda	0.53	8.61	0.19	8.82
Amphipoda	9.11	60.08	16.44	70.00
Mysidacea	0.18	3.15	0.00	0.00
Cumacea	9.16	50.00	1.71	30.59
Copepoda	39.93	73.53	52.25	92.94
Ostracoda	5.81	40.34	3.39	38.24
Decapoda	1.26	11.97	1.40	14.71
Isopoda	0.14	1.26	0.00	0.00
Echinoidea	0.04	0.21	0.02	0.59
Arachnida	0.05	1.68	0.07	3.53
Chironomidae	0.00	0.21	0.00	0.00
Total prey taxa	15		12	
Total fish analyse	480		167	
Average fish lengt	42.01 ± 11.72		37.11 ± 8.34	

The overall dietary composition differed significantly between both species ($R = 0.122$, $p < 0.001$) and between years ($R = 0.028$, $p = 0.038$), as demonstrated by a two-way crossed ANOSIM. The average dissimilarity between species accounted for 52.65 % (SIMPER) and this was attributed to Copepods (18 %), Amphipods (15 %), Polychaetes (15 %), Bivalves (15 %), Oligochaetes (11 %), Cumaceans (11 %) and Ostracods (10 %). Since plaice were present over a wider depth range compared to dab, a reduced similarity matrix was created for those trawls in which both species were caught. However the diet of plaice and dab at the exact same locations still proved to be different, as detected by a one-way ANOSIM ($R = 0.188$, $p < 0.001$), with an average diet dissimilarity between both species of 51.57 % (SIMPER).

Feeding efficiency, estimated by gut fullness, was slightly higher in 2009 compared to 2008; and higher for dab than for plaice (Table 2). Gut fullness also decreased significantly with fish size (Fig. 2a) and an interaction between beach and species indicated a lower feeding efficiency for plaice from Silverstrand compared to plaice from Ballyloughaun, as confirmed by the pairwise test. The total number of taxa in the diet of plaice and dab did not change significantly with the size of the fish; however a higher number of prey taxa was found in 2008 compared to 2009. An interspecific difference in total prey taxa was also found, with plaice feeding on average on a higher number of taxa than dab (Fig. 2b). There was no difference in total prey abundance between species but there was a progressive decrease with fish length and a higher prey abundance in 2009 compared to 2008 (Fig. 2c). The dietary breadth, i.e. Shannon-Wiener prey diversity index, was significantly higher in guts collected in 2008 compared to 2009 but no interspecific or spatial variation was found (Fig. 2d). The Fulton's condition for both species was significantly higher in 2009 compared to 2008 and was not affected by the length of the studied fish. Overall, plaice were in better condition than dab. No spatial differences in condition could be detected for plaice in contrast to dab which were in lower condition in Ballyloughaun compared to the other nursery grounds based on the pairwise test (Fig. 2e).

Table 2. Results of two-way analyses of covariance (ANCOVA) performed on prey descriptors of the diet and on condition of plaice and dab. Average values ± SD (averaged over two years; 2008 and 2009) are presented per beach and species if significant differences were detected between species (D, dab; P, plaice) or beaches (Bl, Ballyloughaun; Bv, Ballyvaughan, Si, Silverstrand, Tr, Traught). Interactions are shown when they proved significant (T, slope of the response variable in relation to the fish length) and results of pairwise comparisons are presented when appropriate. Levels of significance; ns, not significant; *, p <0.05; ** p <0.01; *** p <0.001.

	Gut fullness		**Total prey taxa**		**Total prey abundance (log)**	**Shannon-wiener prey diversity**		**Fulton's K Condition**	
	plaice	*dab*	*plaice*	*dab*		*plaice*	*dab*	*plaice*	*dab*
Averages between beaches									
Ballyloughaun (Bl)	65.1 ± 15.5	74.4 ± 9.5	4.4 ± 1.0	4.33 ± 1.75	3.7 ± 1.0	0.9 ± 0.4		1.84 ± 0.02	1.64 ± 0.05
Ballyvaughan (Bv)	61.3 ± 15.3	82.3 ± 10.3	5.5 ± 1.7	5.7 ± 2.0	4.2 ± 1.1			1.84 ± 0.02	1.83 ± 0.04
Silverstrand (Si)	53.5 ± 18.4	78.0 ± 14.2	5.49 ± 1.79	4.5 ± 1.3	3.5 ± 1.0			1.88 ± 0.03	1.82 ± 0.04
Traught (Tr)	57.1 ± 16.1	78.1 ± 12.6	6.00 ± 1.64	4.5 ± 1.8	3.9 ± 0.8			1.82 ± 0.04	1.81 ± 0.04
F ratio									
Year	4.13 * (2009>2008)		5.02 * (2008>2009)		44.82 *** (2009>2008)	10.44 ** (2008>2009)		118.66 *** (2009>2008)	
Standard length	35.85 *** (T = -5.99)		2.62^{ns}		28.15 *** (T = -5.31)	4.55 * (T = 2.13)		0.01^{ns}	
Beach			6.50 *** (Bl<Bv, Bl<Tr)		5.17 ** (Si<Bv)	2.56^{ns}			
Species	68.68 *** (dab>plaice)		9.10 ** (plaice>dab)		0.68^{ns}	2.84^{ns}		7.60 ** (plaice>dab)	
Beach* species	5.12 ** ($P_{Si}<P_{Bl}$)							3.84 * ($D_{Bl}<D_{Tr}$, D_{Si}, D_{Bv})	

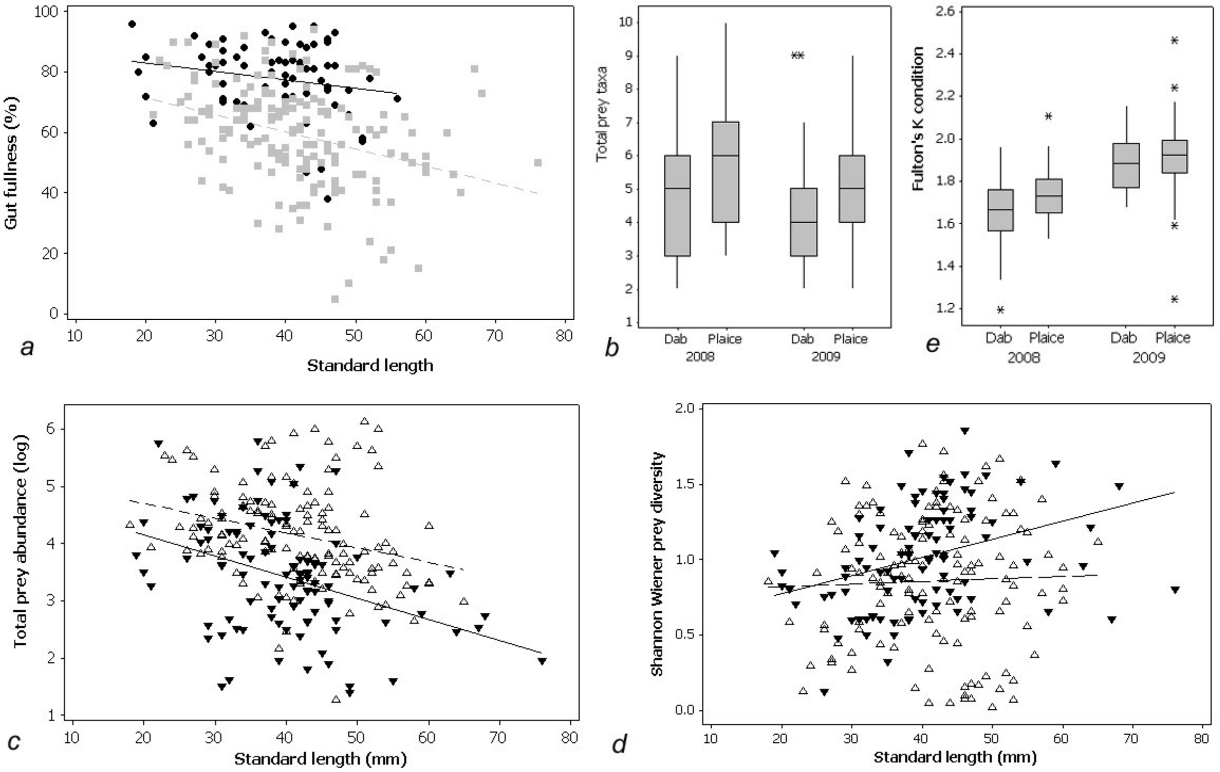

Fig. 2. **(a)** Gut fullness of plaice (■) and dab (●) in function of the fish standard length. **(b)** Interspecific and interannual differences in total prey taxa. **(c)** Total prey abundance per year (2008: ▼, 2009: Δ) in function of the fish standard length. **(d)** Shannon-Wiener prey diversity per year (2008: ▼, 2009: Δ) in function of the fish standard length. **(e)** Interspecific and interannual differences in total Fulton's K condition.

Size-related diet changes

Diet analysis showed that 0-group plaice and dab fed on similar prey items but size-related changes in diet composition showed interspecific variation (Fig. 3). Based on the numerical prey contribution, copepods composed most of the diet of both species, ranging from 70 % in recently settled fish down to 40 % in 40 mm plaice and in 60 mm dab. While copepods were most abundant in the diet of dab throughout the studied juvenile development, 50 mm plaice displayed a shift towards bivalves as major food item. Bivalve siphons represented a significant proportion of this component of the diet, accounting for nearly 40 % of the total bivalve abundance at a plaice length of 40 mm. In fish larger than 40 mm, siphons decreased in importance with larger fish consuming more whole bivalves. The numerical abundance of bivalves in the diet of dab remained lower throughout and siphon clippings were rarely found. The most significant difference observed between the diets of the two species was in the proportion of malacostracans in the diet; this taxon accounted for 40 % of the diet in dab at 60 mm whereas they did not exceed 30 % of the diet in plaice and this percentage decreased steadily in larger fish. Amongst malacostracans, amphipods and cumaceans were evenly abundant in the diet of plaice and the contribution of decapods, mysids and isopods was low. In contrast, malacostracans in the diet of dab were primarily amphipods and all other classes were very scarce. Polychaetes, oligochaetes and ostracods were prey of lesser abundance for both fish species. Prey items which were rarely found were gastropods, echinoderms, arachnids and chironomids.

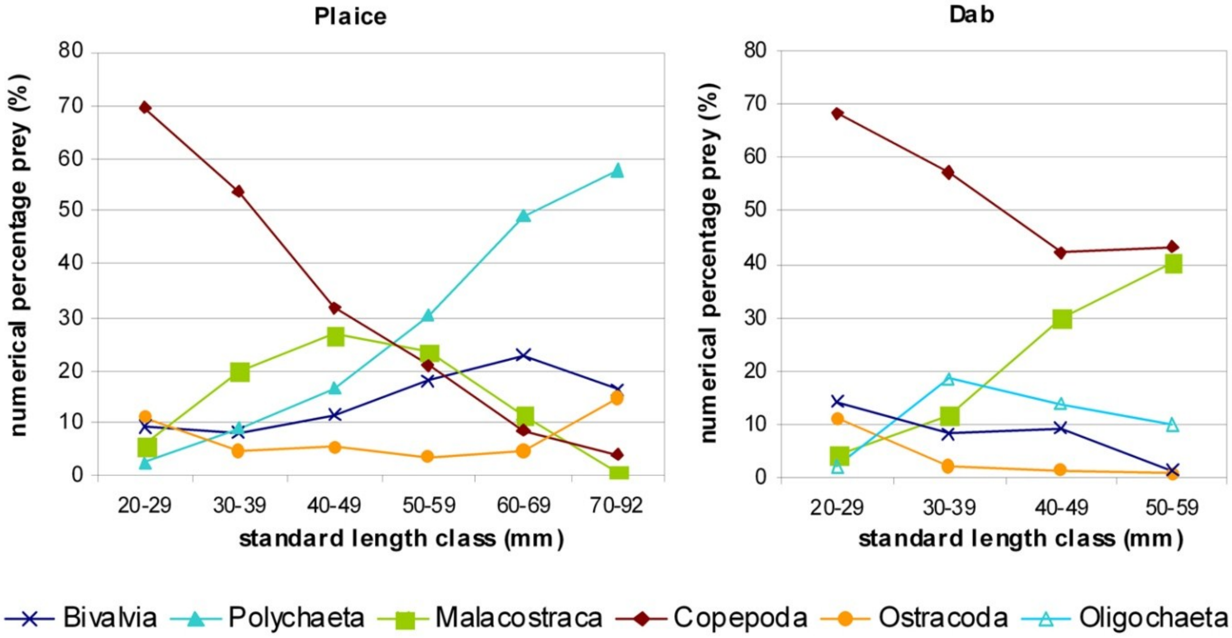

Fig. 3. Diet composition in different size classes of plaice and dab.

The shift in diet was also explored in terms of a seasonal trend but the resulting signal was weaker compared to the size-related trend. This suggests that the benthic abundance in the sediment was less likely to change with season and that prey selection was more related to the growth of the fish.

Interspecific diet overlap

A GLM showed that the variation in dietary overlap between dab and plaice was, in order of importance, explained by the ordinal date (F = 27.23, $p < 0.001$), year of sampling (F = 10.43, p = 0.0020) and beach (F = 4.57, p = 0.0060), together accounting for 63 % of the variance. Diet overlap was higher in 2008 compared to 2009 (p = 0.0021) and in both years, the dietary overlap progressively decreased with time (Fig. 4). Given the high correlation between seasonality and fish size, this most likely reflects the high degree of diet overlap between small fish and the subsequent decrease in overlap as the fish grow. The diet overlap ranges from around 0.8 in June down to 0.4 in September. We were unable to prove that other factors, such as density of juveniles and depth, had a significant effect on the diet overlap, since they were removed from the final model based on the AIC values. This suggests that partitioning of the available resources by the potentially competing fish species is most probably prevented by an abundant food supply. However the different nursery areas studied showed differences in diet overlap suggesting they differed in productivity. Fish from Ballyloughaun had a lower dietary overlap compared to Ballyvaughan (p = 0.0053), Traught (p = 0.0011) and Silverstrand (p = 0.0167).

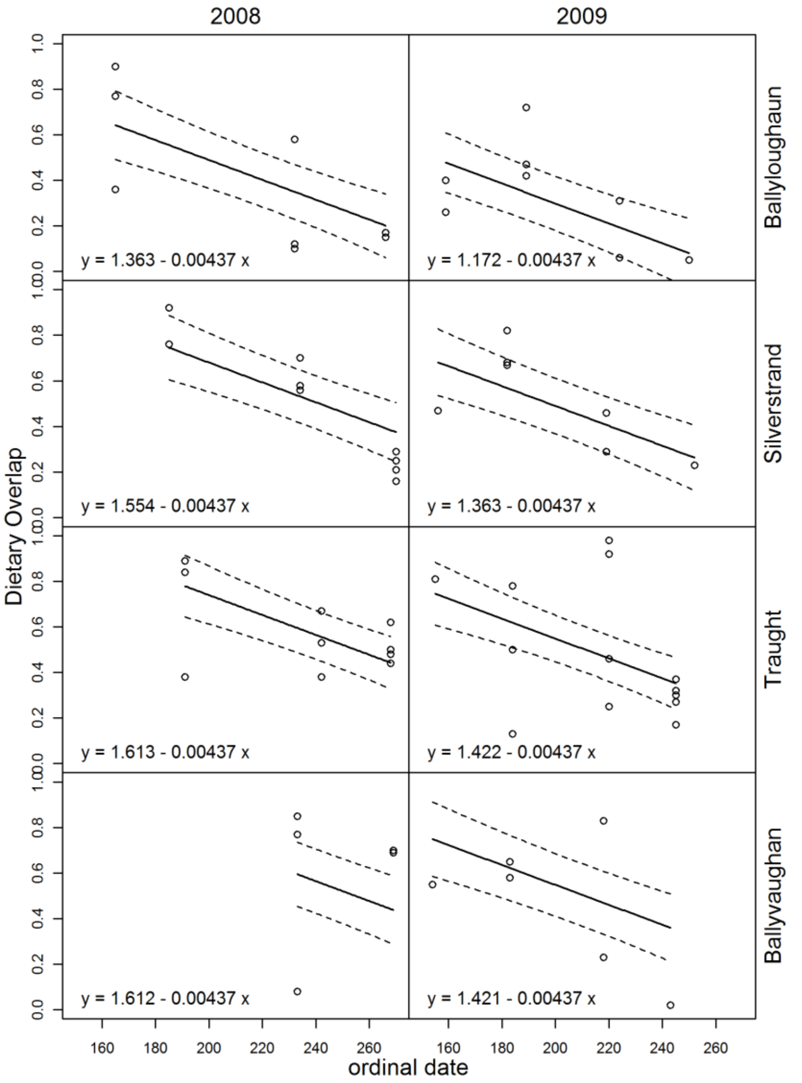

Fig. 4. Dietary overlap between plaice and dab averaged per trawl and presented for two years and four nursery areas. The models apply only over the range of the data and are indicative of linear trends present in that range. Lines show the decreasing trend in dietary overlap with ordinal date, as revealed by a GLM including ordinal date, year of sampling and nursery ground in the optimal model. 95 % Confidence limits are represented with dashed lines.

Spatial diet variability and correlation with food availability

The similarity in diet of fish from different beaches is shown in the MDS plots in Fig. 5. Plaice diets significantly differed among beaches (ANOSIM, R = 0.251, p < 0.001) and the significance level of all pairwise combinations was < 0.001. Overall dab diets also differed between beaches (ANOSIM, R = 0.126, p < 0.001). The pairwise tests revealed significant differences in dietary samples between dab collected in Ballyloughaun and Traught (R = 0.187, p <0.001) and Silverstrand (R = 0.165, p = 0.002) and between dab collected in Traught and Silverstrand (R = 0.155, p = 0.006). Relating the overall plaice and dab diet with the macrofaunal composition per trawl location revealed a significant correlation between both similarity matrices. In other words, plaice and dab diets revealed spatial differences which were related to the distinct macrofaunal communities of the different beaches (RELATE; ρ = 0.308 and p < 0.001 for plaice and ρ = 0.117 and p = 0.012 for dab).

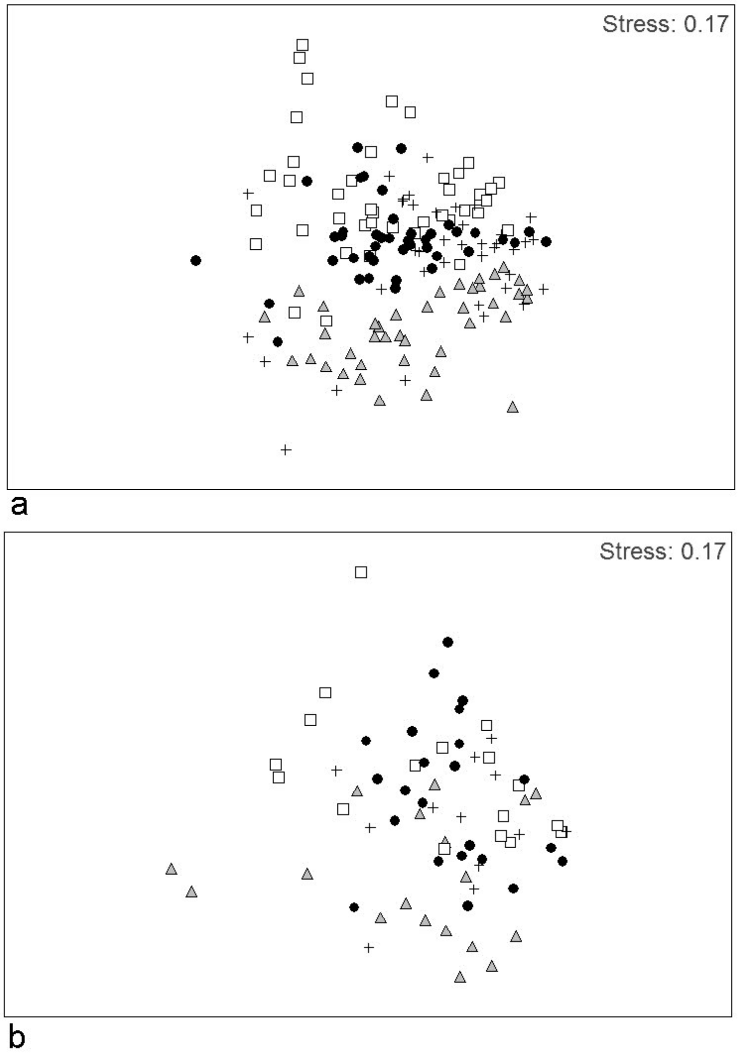

Fig. 5. Similarity in diet of plaice **(a)** and dab **(b)** averaged per trawl location in different beaches as revealed by nonmetric Multidimensional Scaling (MDS) (□: Silverstrand, ●: Traught, +: Ballyvaughan, Δ: Ballyloughaun).

The canonical correspondence analysis based on densities of benthic taxa indicated that the environmental variables significantly explained 17.6 % of the spatial variation in benthic composition (Fig. 6). Benthic samples from the same nursery grounds were grouped together and were separated from each other along the first axis with Traught clustered at the left and Ballyloughaun at the right. The environmental variables explained 96.8 % of the total variation, of which 70.2 % explained by the first axis and an additional 15.2 % by the second axis. The variables describing the particle size of the sediment explained the variation over the first axis. Ballyloughaun was characterised by a high percentage of silt, very fine sand and organic content and the associated benthic groups were mostly ostracods and polychaetes. Samples from Traught were located at the other side of the axis with a high association of fine, medium and coarse sand, corresponding to a high abundance of amphipods, cumaceans and bivalves. The second axis divided the nursery areas mainly into depth strata with the intertidal areas showing a higher abundance of hermit crabs and the subtidal areas contained more copepods and echinoids.

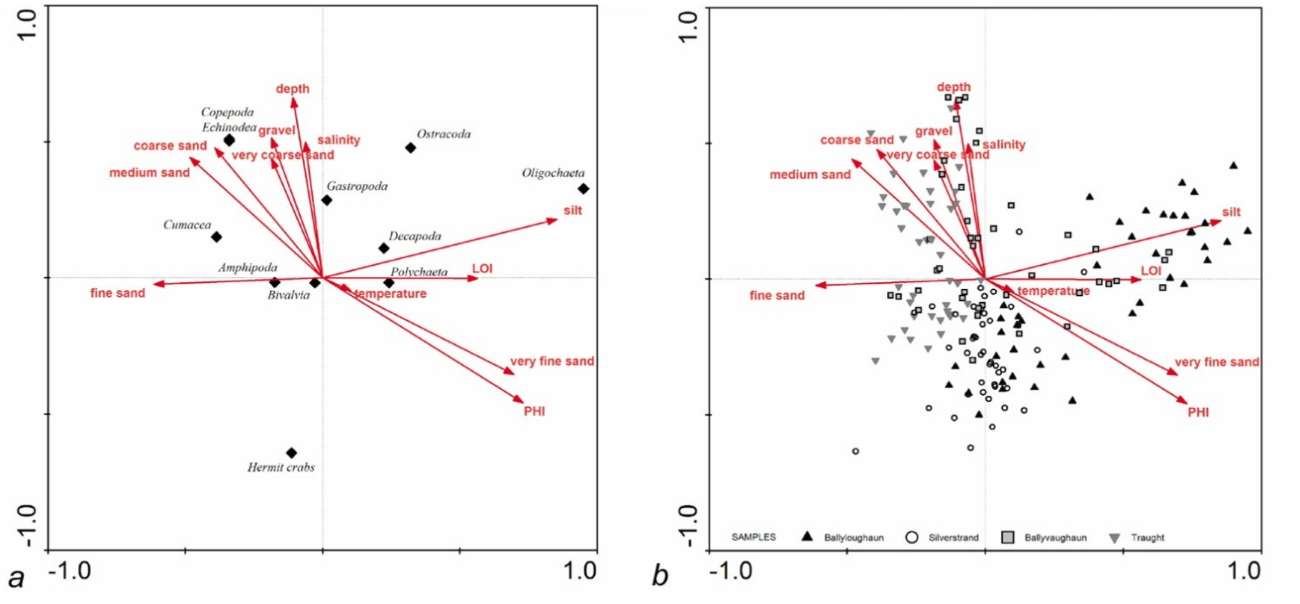

Fig. 6. Ordination diagram of the first two axes from a Canonical Correspondence Analysis (CCA) describing similarity between (a) 11 macrobenthic taxa collected in (b) 168 samples with 13 habitat descriptors represented by vectors.

Interannual (R = 0.132, $p < 0.001$) and spatial (R = 0.54, $p < 0.001$) variation in macrobenthic composition was revealed by a two-way crossed ANOSIM. Pairwise comparisons revealed significant differences between all beaches ($p < 0.001$), confirming the spatial separation of the different nursery areas of the CCA. Furthermore, the interannual dissimilarity accounted for 44.60 % (SIMPER) and was mainly attributed to bivalves (31 %), amphipods (21 %), polychaetes (16 %), gastropods (7 %), cumaceans (6 %) and hermit crabs (5 %); of which the densities were generally higher in 2009 (Table 3), as confirmed by ANOVA (Table 4). Despite these density differences, we were unable to find differences in total benthos taxa and benthos diversity between and within the sampled years. However, ANOVA revealed significant differences between nursery areas; characterizing Ballyloughaun as a nursery area with a low macrobenthic diversity and abundance on the one extreme and Traught with a high macrobenthic diversity and abundance on the other extreme.

Table 3. Mean densities (m^{-2}) ± SE of the benthic taxa in the sediment samples collected with a Van Veen grab, over two years and four nursery grounds.

	Ballyloughaun		**Ballyvaughan**		**Silverstrand**		**Traught**	
	2008	2009	2008	2009	2008	2009	2008	2009
Oligochaetes	0.0 ± 0.0	0.00 ± 0.00	31.1 ± 19.2	3.6 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.4 ± 0.3
Polychaetes	98.9 ± 12.0	137.9 ± 13.6	115.6 ± 27.6	149.4 ± 22.3	66.7 ± 15.3	46.9 ± 7.5	57.4 ± 10.5	82.5 ± 8.9
Bivalves	9.6 ± 2.1	27.4 ± 5.9	112.2 ± 23.4	209.6 ± 22.4	175.9 ± 21.5	171.8 ± 18.3	295.6 ± 46.5	580.4 ± 57.5
Gastropods	3.7 ± 1.3	1.4 ± 0.7	6.7 ± 2.2	11.4 ± 2.9	3.7 ± 1.2	3.8 ± 1.1	8.9 ± 2.8	16.7 ± 6.4
Amphipods	2.2 ± 1.2	4.2 ± 1.5	43.7 ± 10.9	157.1 ± 50.3	23.7 ± 4.6	76.1 ± 13.5	102.6 ± 17.0	154.7 ± 20.0
Cumaceans	0.0 ± 0.0	0.0 ± 0.0	5.6 ± 3.3	8.5 ± 2.8	6.7 ± 2.0	9.7 ± 3.1	8.1 ± 2.0	10.7 ± 3.5
Hermit crabs	1.1 ± 0.6	0.0 ± 0.0	0.7 ± 0.5	0.4 ± 0.3	7.0 ± 1.5	12.5 ± 3.1	7.0 ± 3.5	3.5 ± 1.5
Others	4.4 ± 1.7	1.1 ± 0.6	7.0 ± 2.3	28.8 ± 11.5	7.9 ± 3.6	0.6 ± 0.4	6.3 ± 2.1	15.6 ± 8.5

Table 4. Results of two-way analysis of covariance (ANCOVA) performed on descriptors of the macrobenthic fauna. Sampling was carried out in four beaches during a time period between June - September and average values ± SD are presented per beach and where appropriate averaged over two years. Results of pairwise comparisons are presented when appropriate (Bl, Ballyloughaun; Bv, Ballyvaughan, Si, Silverstrand, Tr, Traught) (T, slope of the response variable in relation to the fish length). Levels of significance; ns, not significant; *, p <0.05; ** p <0.01; *** p <0.001.

	Total benthos taxa	**Total benthos density (log)**		**Shannon-Wiener benthos diversity**
		2008	**2009**	
Averages between beaches				
Ballyloughaun (Bl)	2.67 ± 1.26	4.64 ± 0.58	4.99 ± 0.62	0.49 ± 0.30
Ballyvaughan (Bv)	4.83 ± 1.40	5.61 ± 0.65	6.21 ± 0.54	1.06 ± 0.30
Silverstrand (Si)	4.76 ± 1.19	5.52 ± 0.60	5.66 ± 0.52	1.06 ± 0.20
Traught (Tr)	5.19 ± 1.47	6.01 ± 0.72	6.69 ± 0.40	0.95 ± 0.28
F ratio				
Year	0.12 ^{ns}	28.89 *** (2009 > 2008)		1.66 ^{ns}
Ordinal date	1.82 ^{ns}	4.43 * (T = 2.1)		3.88 ^{ns}
Beach	31.08 *** (Bl<Bv,Si,Tr)	54.55 *** (Bl<Bv,Si,Tr & Si<Bv,Tr & Bv<Tr)		41.90 *** (Bl<Bv,Si,Tr)

Discussion

Similarity of feeding patterns

Juvenile plaice diet has been described over its geographic distribution range from Norway and Sweden (Evans, 1983; Pihl, 1985; Wennhage and Pihl, 2002; Freitas *et al.*, 2010) to the North Sea, Wadden Sea and Irish Sea (Braber and de Groot, 1973a; Whyche and Shackley, 1986; Piet *et al.*, 1998; Beyst *et al.*, 1999; Hostens and Mees, 1999; Rijnsdorp and Vingerhoed, 2001) south to France (Amara *et al.*, 2001). Major prey items reported in these studies were molluscs, crustaceans and polychaetes. This is in accordance with findings in Galway Bay, which is located at the western part of the plaice distribution. Dietary analyses of juvenile dab are less frequently reported but have shown the same major prey items (Edwards and Steele, 1968; Braber and de Groot, 1973a; Whyche and Shackley, 1986; Beyst *et al.*, 1999; Amara *et al.*, 2001; Hinz *et al.*, 2005). This is in contrast to dab diet in Galway Bay which lacked a high occurrence of (palps of) polychaetes and showed instead a higher occurrence of oligochaetes in fish >30 mm. Prey consumption as a function of fish size was similar to earlier findings showing a high feeding effort on meiofaunal crustaceans (ostracods and copepods) in newly settled fish and a shift to macrofaunal-sized prey in dab >60 mm and plaice >50 mm. In the guts of larger dab, crustaceans were more abundant whereas bivalves and/or polychaetes were more abundant in plaice guts, depending on the availability on the nursery grounds. Despite considerable similarity in the general prey items, the detected difference in food composition between the two species implies that resource partitioning on a trophic dimension occurred.

Dissimilarity of feeding behaviour

Interspecific variation in behaviour or morphology may explain the observed difference in diet composition. The experimental food capture and ingestion studies by Bels & Davenport (1996) suggested that steeper pressure gradients and higher water velocities are being developed during a plaice attack compared to a dab attack due to differences in jaw movement speeds. This might explain why plaice is more specialized at catching buried bivalves and polychaetes whereas dab are more successful at capturing mobile epibenthic prey. Morphological differences in body regions involved in prey capture show a high degree of correlation with the feeding choice of plaice and flounder (Russo *et al.*, 2008). Between plaice and dab, different hunting postures might also explain the main differences in prey items (Steven, 1930; Holmes and Gibson, 1983).

Plaice shoots upon its prey in a horizontal direction with his head only slightly raised from the bottom, being restricted in its range. Dab however adopt a hunting posture with the head slightly raised in order to scan the vicinity for food and are thus able to capture a greater range of organisms. In this study we found a higher feeding efficiency in dab than in plaice which might support this hypothesis. Other studies also reported a greater variety of food items in the diet of juvenile dab compared to plaice, reflecting the potential for better survival (Braber and de Groot, 1973a; Whyche and Shackley, 1986). This is in contrast to the results of the present study which showed a greater number of prey taxa in the diet of plaice compared to dab. However this result might have been confounded by differences in spatial distribution and length range between both species. This likely resulted from earlier settlement of plaice; March until mid June (van der Veer *et al.*, 1990), compared to dab; May until early July (Henderson, 1998; Bolle *et al.*, 2001) in addition to faster growth of plaice (Amara *et al.*, 2001). Since the number of prey taxa proved to increase with size this may also explain why prey taxa are less diverse in dab guts. Despite the similar body morphology of dab and plaice (Piet *et al.*, 1998), slight shape variations in combination with different hunting behaviour are thus likely to play a role in the mechanisms of resource partitioning between these two species.

Spatial variation in flatfish diet

A simultaneous analysis of prey consumption and availability in the environment is a good approach to illustrate the opportunistic feeding strategy of juvenile plaice and dab. Spatial differences in the diet of both species between nursery areas with characteristic substrate properties and benthic community composition, suggest that plaice and dab can alter their diet to increase feeding opportunities. These results are in agreement with earlier studies reporting intraspecific diet differences of juvenile plaice on different spatial scales (De Raedemaecker *et al.*, 2010).

Temporal variation in macrobenthic communities

Since habitat quality is accepted to be one of the major components affecting recruitment variability (Gibson, 1994), studies linking prey availability and consumption are crucial to assess how variability in benthic productivity can affect fish growth and survival. Benthic communities in fish nursery areas show generally high seasonal and yearly fluctuations (Gibson *et al.*, 1993; Aarnio *et al.*, 1996; Selleslagh and Amara, 2008; Florin and Lavados, 2010). Despite the short time series in this study, macrobenthic community composition and food availability for flatfish also varied between years; with higher densities in 2009 than in 2008. Direct causal relationships between macrobenthic densities and feeding efficiency or condition of fish cannot be drawn with certainty. However the observed higher benthic prey densities in 2009 also coincided with a higher feeding success (described as a higher gut fullness and prey abundance in the gut and a lower prey diversity and number of taxa), a lower interspecific dietary overlap and a higher Fulton's K condition. Feeding upon a larger range of food items could negatively affect fish condition (De Raedemaecker *et al.*, 2010) whereas food abundance is positively related with growth of 0-group plaice (Karakiri *et al.*, 1991; van der Veer and Witte, 1993). Therefore, we suggest that better feeding conditions in 2009, amongst other possible drivers, are very likely to explain the higher condition of plaice and dab in 2009.

Structuring effects of environmental characteristics on benthic faunal composition

Shallow nursery areas are known to have higher productivity than adjacent non-nursery areas (Wouters and Cabral, 2009) but macrobenthic communities within nurseries in Galway Bay showed a high degree of spatial variability in composition. Abiotic variables explained 18 % of the variability in benthic assemblages in this study, which is relatively low compared to other areas (Wouters and Cabral, 2009) although a high proportion of unexplained variability in benthic composition is generally detected. Sediment granulometry and depth were the main structuring factors, as reported in other studies (Ysebaert *et al.*, 2003). This link is reflected in the association of a dominance of silt and very fine sand with high numbers of polychaetes at the nursery ground of Ballyloughaun. Sediment properties may directly influence the distribution or survival of flatfish species through effects on burial capacities and predator avoidance (Stoner and Abookire, 2002; Ryer *et al.*, 2004) as well as through their substrate-mediated food habits (McConnaughey and Smith, 2000). This site was also characterized by higher sediment organic matter, which had a negative effect on total taxa and abundance of macrobenthos, implying a relatively high organic disturbance as described by empirical models (Pearson and Rosenberg, 1978). The density decrease in suspension feeders and selective deposit feeders in Ballyloughaun can also be related to higher organic input (Weston, 1990; Grall and Chauvaud, 2002). This could explain why dab, which are morphologically less adapted to subsurface-feeding, have a lower condition in this nursery ground. Temperature and salinity are both important structuring factors commonly reported in the literature, especially in estuarine environments (Ysebaert *et al.*, 2003; Dethier and Schoch, 2005) but were not significant in our study, possibly due to a lack of variation in these variables across the sampled nursery grounds. The inclusion of variables such as food, competition and predation of macrobenthos might have increased the explained variability in their community composition in Galway Bay, in accordance with evidence from other regions (Möller, 1986; Defeo *et al.*, 1997; Peeters *et al.*, 2004; Van der Wal *et al.*, 2008). The study of spatial and temporal dynamics of macrobenthic communities in these shallow sandy nursery grounds proved valuable to understanding biological and physical processes responsible for (interspecific, spatial and temporal) differences in condition and feeding success of demersal fish.

Potential competition for food resources

Comparative studies on diet preferences have shown various degrees of dietary overlap between flatfish species, generally increasing with similar morphology (Aarnio *et al.*, 1996; Darnaude *et al.*, 2001; Vinagre *et al.*, 2005; Rooper *et al.*, 2006; Dolbeth *et al.*, 2008; Guedes and Araújo, 2008; Martinho *et al.*, 2008; Russo *et al.*, 2008; Florin and Lavados, 2010). Small dab and plaice caught in June in Galway Bay had a high diet overlap (0.8), suggesting the possibility of interspecific competition (Cabral *et al.*, 2002). However, both species were mainly feeding on meiofauna and predation is unlikely to depress meiofaunal populations (McIntyre, 1968; Tito de Morais and Bodiou, 1984). The high diet overlap in June did therefore likely not imply competition but did rather refer to a morphological restriction to small prey which are highly abundant in the environment.

An increase in food niche-overlap can occur when a food supply diminishes, potentially leading to increased competition especially when fish densities are high (Thorman, 1982). Reduced benthic productivity in 2008 coincided with higher dietary overlap between dab and plaice. Therefore, partitioning of available resources by the likely competing fish species may have been more apparent in 2009 whereas fish may have expanded their diet to be sufficiently fed in 2008. However it is unlikely that food limitation occurred during 2008 since the numbers of fish with empty guts were so small and since juvenile flatfish densities had no significant effect on the dietary overlap. Therefore no evidence was found that interspecific competition occurred and juvenile densities were not high enough to influence dietary overlap. The effect of density on dietary overlap between juvenile plaice and dab was investigated for the first time in this study and it would be interesting to assess this relationship in more densely populated nursery grounds.

Interspecific diet overlap also varied between nursery grounds, possibly because of differences in productivity and dominance of faunal groups which could influence the feeding success and hence the degree of resource partitioning. At first glance, lower dietary overlap in fish from Ballyloughaun seems surprising since this site had the lowest benthic diversity and density. Given the fact that the macrobenthic community was mainly composed of polychaetes and that dab is less successful in catching this type of infauna, this might explain the food niche segregation between both species in this nursery ground. The possibly restricted or more energy demanding feeding of dab in Ballyloughaun is also implied by the lower condition of dab compared to plaice at this nursery ground. Competitive interactions may be inferred from niche widths and dietary overlaps (Thorman and Wiederholm, 1986) but our study suggests that good habitat quality for one flatfish species might not necessarily imply the same for another flatfish species, and is reflected in a low dietary overlap. We emphasize that the interaction between food availability, dietary overlap and the interspecific differences in feeding success and condition has practical limitations for using dietary overlap as a measure of habitat quality.

Although only four nursery grounds were sampled intensively, our overall data suggest that resources were not limiting in the littoral sandy nursery areas in the west of Ireland and no indications were found that competitive forces between plaice and dab upon the benthic prey assemblages were likely to result in recruitment variability. However habitat quality, indicated by feeding successes and Fulton's K condition, showed spatial and temporal variation and was likely affected by, amongst other possible factors, the productivity of macrobenthic communities.

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CHAPTER 4

HABITAT CHARACTERISTICS PROMOTING HIGH DENSITY AND CONDITION OF JUVENILE FLATFISH AT NURSERY GROUNDS ON THE WEST COAST OF IRELAND

Submitted as:

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Abstract

Coastal zones are essential nursery habitats for most juvenile flatfish species. Understanding the habitat requirements promoting high abundance and growth of juvenile flatfish is important for evaluating nursery habitat quality. The present study aimed to assess nursery ground quality for the most common flatfish species; dab and plaice, in Galway Bay, on the west coast of Ireland. Monthly surveys were carried out in the period after peak settlement over two years. Variability in flatfish density and condition was explained in relation to biotic and abiotic habitat characteristics, differing within and between distinct nursery grounds. Dab were concentrated in deeper waters, were negatively associated with shrimp densities and salinity and their abundance showed a decrease from June to September combined with interannual variation. Plaice densities were highly associated with shallower depths and high polychaete and shrimp densities and were negatively related with increased seaweed cover and organic content. Most of the variability in Fulton's condition was explained by the same set of variables for both species; year and densities of crab and malacostracans. This analysis revealed important ecological mechanisms allowing the co-existence of two flatfish species in nursery grounds. However, high quality nursery grounds for dab and plaice differed and anthropogenic and climatic impacts on flatfish nurseries are likely to have a different impact on plaice and dab populations. Knowledge gained about the quality of nursery habitat for commercially important fish species provides a basis for mapping essential flatfish habitats to inform management plans for coastal areas.

Keywords

juvenile flatfish; *Pleuronectes platessa*, *Limanda limanda*, habitat requirements; small-scale variability; density; predator densities; Galway Bay, eastern Atlantic

Introduction

Nursery grounds for many flatfish species, including plaice and dab, are located in shallow soft bottom coastal areas (Norcross et al., 1995). Both the quantity and quality of these habitats are important factors that regulate annual levels of recruitment to the adult stocks (Rijnsdorp *et al.*, 1992a; Gibson, 1994). Habitat selection by juvenile flatfish is driven by resource requirements promoting fast growth and survival during this vulnerable life stage (Beck et al., 2001). Individual species are restricted to a range of habitat conditions by physiological tolerances (Davenport and Sayer, 1993), feeding requirements (Piet et al., 1998), sediment associations (Moles and Norcross, 1995) or vulnerability to predators (Ryer et al., 2008) and this may lead to spatial segregation or overlap between species. Fundamental knowledge of habitat characteristics directly affecting the distribution and growth of juvenile fish is crucial to support decision making for the protection and management of nursery areas (Maxwell et al., 2009).

The need for a successful approach to assessing essential habitat for juvenile fishes has lead to the development of habitat suitability models. These models use a measure of fish abundance (e.g. presence absence, counts, densities) as a response variable to relate their distribution to a set of environmental factors. The physical variables: depth, substratum and temperature have been identified as the strongest indicators of fish occurrence for numerous flatfish species over large spatial scales (Norcross *et al.*, 1995; Abookire and Norcross, 1998; Able *et al.*, 2005; Florin *et al.*, 2009). The integration into these models of biological elements of the habitat, including prey and predator abundances, allows for a more complete assessment of nursery quality (Vinagre *et al.*, 2006; Le Pape *et al.*, 2007; Nicolas *et al.*, 2007; Wennhage *et al.*, 2007). Studies on small-scale variability in the habitat use of juvenile fish within nursery areas are less common but can gain additional insight into the response of a population to environmental gradients (Allen and Baltz, 1997; Maes et al., 2004). Factors that structure habitat use of various juvenile sole species have been shown to operate at both small and larger scales (Cabral and Costa, 1999; Vinagre et al., 2009). Therefore, studies investigating small-scale variability in fish densities may contribute to the development of habitat suitability modeling at larger scales.

Most studies on the quality of nursery habitat and its effect on recruitment variability for different flatfish species are based on density measures. Areas occupied at the highest densities may represent the best available habitat and should be indicative of the species' requirements

(McConnaughey and Smith, 2000). Likewise, an uneven distribution within the area of occurrence may indicate small-scale differences in habitat quality. However, recruitment to the adult stock does not only depend on the numbers of juveniles that reach nursery areas. Other (density dependent) mechanisms affecting juvenile fish are known to dampen the variability generated in the larval period (Myers and Cadigan, 1993; Nash and Geffen, 2000; Houde, 2008). Houde (1989) showed that cohorts of fast-growing fish that reach a large size early in life experience lower cumulative, stage-specific mortality and have a higher probability to recruit. Measures of growth rate and condition of juvenile fishes may therefore reflect the probability of survival and can be used as indicators of habitat quality (Amara et al., 2009; Vasconcelos et al., 2009).

Despite the growing body of literature describing early life history parameters of flatfish, there is limited information on the combined effect of physical and biological habitat characteristics that influence small-scale differences in the distribution and growth of plaice and dab. A mechanistic understanding of the association between habitat features and growth of juvenile fish is crucial to evaluate anthropogenic and climatic impacts on flatfish nurseries and ultimately fish stocks. The objective of the present work was to assess the quality of flatfish nursery grounds in Galway Bay, on the west coast of Ireland, using data on the density and condition of juvenile fish in relation to a set of abiotic and biotic habitat characteristics. Sampling surveys during two years and covering the months after peak abundance were carried out in six locations within four distinct nurseries. The first aim was to determine small-scale spatial and temporal variation in density and condition for both species and to identify nurseries in Galway Bay that represent habitat of relatively high quality for juvenile plaice and dab. The second aim was to ascertain a set of habitat characteristics explaining small-scale variability in density and condition of juvenile plaice and dab.

Materials and Methods

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 km^2, 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. 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.

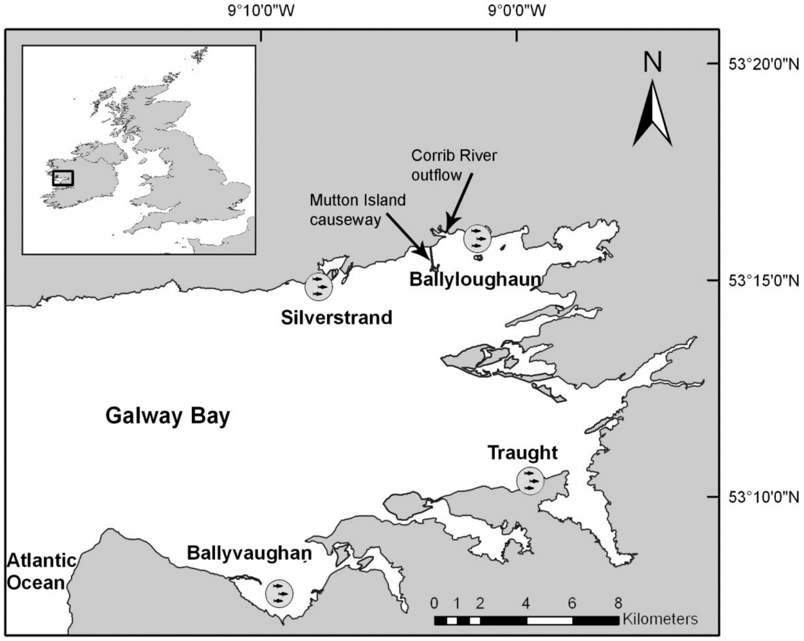

Fig. 1. Map of Galway Bay, Ireland and positioning of the four sampled flatfish nursery grounds.

Juvenile flatfish assemblages were sampled monthly from June - 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 x 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) for 2 min. This speed was chosen since it gave the largest catches of 0-group plaice using a two-meter beam trawl in Loch Ewe (Riley and Corlett, 1966; Edwards and Steele, 1968). The beam trawl was hauled parallel to the shore to cover an area with a homogeneous depth. Tidal height for every haul was calculated using tide tables for Galway Bay and the exact time of hauling. Actual depths were recorded with an echo sounder and used in combination with tidal height to obtain depths relative to lowest astronomical tide (depth LAT), which were expressed as ‘negative depths’ when working over the intertidal area. Three replicate trawl hauls were carried out both intertidally and subtidally, resulting in a total of six hauls at each site. A mechanical flowmeter attached to the beam was used to calculate the distance towed and ultimately the swept area, which averaged 94.2 ± 16.8 m^2 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 ºC freezer in the laboratory until further sorting and analyses. Shore crab (*Carcinus maenas*) and brown shrimp (*Crangon crangon*) were the most abundant predators during spring and summer in Galway Bay and all hauled specimens were counted and measured before releasing them, simultaneously with the few other occurring species of shrimp (e.g. *Palaemon serratus*) and crab (e.g. *Necora puber*). Only crab and shrimps >3 cm were assessed since laboratory experiments carried out by van der Veer and Bergman (1987) determined this to be the minimum size enabling predation on 0-group plaice. The amount of seaweed in each trawl was used to obtain a relative measure of the abundance of seaweed on the seabed, using a qualitative scale ranging from 0 (no seaweed cover) to 5 (filling a bucket of 50 l). Macrobenthos at each end of the trawl path were collected from three replicate Van Veen grabs (0.025 m^2) pooled together. A fourth grab was collected for the determination of sediment organic matter and particle size analysis.

Fish processing

All fish were counted and identified to determine the densities (individuals 100 m-2) of the different species of 0-group flatfish in each trawl. Standard length of freshly thawed and gently blotted fish were measured to the nearest mm and weighed to the nearest mg. Fulton’s condition factor was calculated for each fish using the formula $K = (W / L_s^3) * 100$, where W is freeze-thawed wet weight (g) and Ls is standard length (cm). Standard length and not total length was used in this formula since some caudal fins were partly lost during beam trawling. This should be taken into account when comparing the Fulton‘s K condition values obtained here with other studies. Because of skewed length-frequencies and the resulting deviations from a normal distribution, the median length of both species per month was estimated and used to calculate growth rates of both species in 2009 between June and August; the months in which linear growth generally occurs (Poxton et al., 1983). Since not all sites were sampled in June 2008 and since dab were not present in all sites in 2008, the growth rate calculations were not repeated for 2008.

Sediment processing

All benthic samples were washed on a 1 mm sieve and stored in 4 % buffered formaldehyde prior to counting and identifying to class level using a stereomicroscope. The main prey items of plaice and dab (polychaetes, bivalves and malacostracans) were counted and the two grabs representing one trawl haul were pooled before calculating the density of the different prey taxa per 100 m-2, as well as the density of total prey, the number of taxa and the Shannon-Wiener diversity index (Marshall and Elliott, 1997). Organic matter was measured using the percentage loss on ignition (LOI) after burning 25 g of sediment (oven-dried at 100 °C to constant weight) in a muffle furnace at 450 °C for 6 hours (Bale and Kenny, 2007). Particle size analysis was carried out with oven-dried sediment for all samples collected in 2008 using the method described in Bale & Kenny (2007). Sediments were divided into six fractions: silt (<63 µm diameter), very fine sand (63 µm - 125 µm), fine sand (125 µm - 250 µm), medium sand (250 µm - 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, phi (φ), was calculated. The sediment particle size data was mapped with ArcGIS 9.2 software as continuous surfaces using inverse-distance weighted averaging (IDW). This interpolation technique was applied to generate % composition data at the sample locations in 2009, as it proved effective in other studies (Phelan *et al.*, 2001; Stoner *et al.*, 2001; Compton *et al.*, 2008). Similar to the faunal density calculation, replicate sediment samples per trawl haul were pooled to obtain an average value of abiotic variables per trawl.

Data analyses

The R environment (R Development Core Team, 2008) was used to investigate variability in density (by generalized linear modeling (GLM)) and condition (by generalized linear mixed modeling (GLMM)) of plaice and dab. A first set of five models was developed to investigate differences in density and condition for both species in relation to nursery ground and time of sampling (including the interactions of year with ordinal date and with nursery ground to explore interannual consistency). Since flatfish densities and condition may not necessarily change in a linear fashion over time, a quadratic and cubic function of time was introduced in the model fits.

A random haul effect was included in the GLMMs explaining variability in juvenile condition to account for variation between hauls within a nursery ground. Modeling the variation of dab abundances was carried out in two steps due to the high proportion of zero catches: a logistic regression model with a logit link was first fitted to estimate the probability of presence and a Gaussian regression model was second fitted to account for the variability in (log-transformed) densities conditional to positive catches (Le Pape et al., 2007). The last model was compared with a Gamma regression model with a log link but the Gaussian regression model had a consistent better model validation. Although some authors have suggested that the use of gamma density is preferable to the use of a lognormal density for fisheries data (Myers and Pepin, 1990), others have argued that both analyses will usually produce similar results (McCullagh and Nelder, 1989).

With a second set of five models, habitat characteristics accounting for variability in condition and density of flatfish, within and between nurseries, were identified. The variables considered in these models described characteristics of prey (density of polychaetes, bivalves, malacostracans, number of species and individuals and prey diversity), predators (density of crab and shrimps), flatfish (density of plaice and dab), substrate (organic content, mean sediment particle size, seaweed coverage) and other abiotic variables (depth, depth corrected for lowest astronomical tide, bottom salinity, bottom temperature) and year. Prior to analysis, 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-colinearity 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.

Results

Spatial and temporal patterns of juvenile flatfish assemblage in Galway Bay

From the content of 72 trawls in 2008 and 96 trawls in 2009, a total of 653 (2008) and 921 (2009) age-0 flatfish plus 12 (2008) and 56 (2009) age-1+ flatfish were sampled. Six flatfish species were identified. Solea solea (Common sole), Psetta maxima (Turbot), Platichthys flesus (European Flounder) and Scopthalmus rhombus (Brill) were not further analyzed due to their low occurrence over all sampling events (<2 % of all juvenile flatfish). Plaice were the most numerous overall, representing 83 % of juvenile catch, and dab accounted for 15 % of juvenile catch. Averaged over both years, we recorded a mean density of 8.2 100 m^{-2} for plaice and 2.6 100 m^{-2} for dab in the subtidal area. Unimodal length-frequency distributions showed that plaice and dab did not settle after June and plaice attained a longer length at the end of the summer (Fig. 2). Plaice growth was on average 0.25 mm day^{-1} and dab growth averaged 0.16 mm day^{-1} in 2009. Table 1 summarizes the average fish and habitat measurements per nursery area and year.

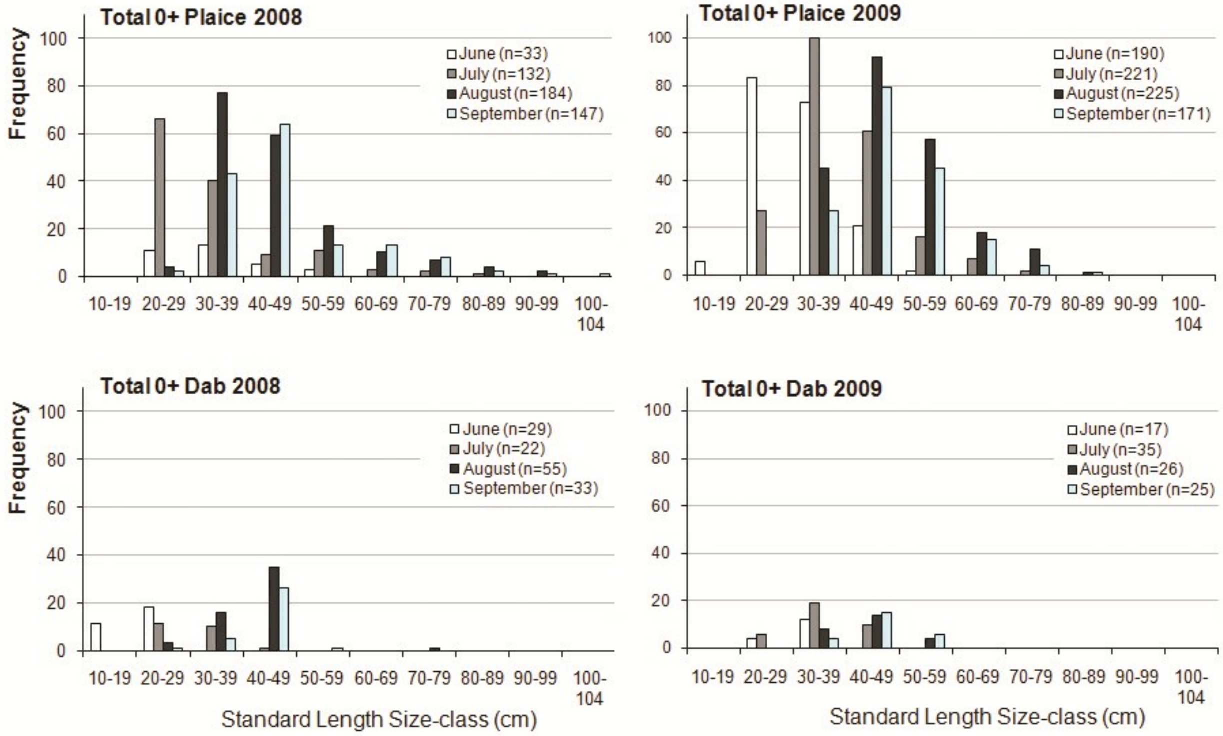

Fig. 2. Monthly length-frequency distributions of 0-group plaice and dab pooled over all sites and presented per sampling year. Total sampled fish numbers per month are presented in parentheses.

Table 1. Overall ranges and averages ± SE of fish and habitat characteristics presented per nursery ground and year and averaged over all sites per year.

	Ballyloughaun		**Ballyvaughan**		**Silverstrand**		**Traught**		**Average**		**Overall range**
	2008	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	
Plaice density (100 m^{-2})	8.6 ± 1.8	11.3 ± 1.7	6.2 ± 1.6	7.9 ± 1.4	5.2 ± 1.2	4.1 ± 0.6	9.8 ± 1.9	12.0 ± 1.8	**7.4 ± 0.8**	**8.8 ± 0.8**	0 - 29.6
Dab density subtidally (100 m^{-2})	5.6 ± 1.6	1.8 ± 0.5	0.9 ± 0.3	1.9 ± 0.6	2.7 ± 0.7	2.3 ± 0.6	4.9 ± 1.2	1.6 ± 0.5	**3.5 ± 0.6**	**1.9 ± 0.3**	0 - 16.3
Plaice condition (g cm^{-3})	1.74 ± 0.03	1.95 ± 0.02	1.73 ± 0.03	1.92 ± 0.02	1.81 ± 0.03	1.99 ± 0.03	1.70 ± 0.02	1.90 ± 0.02	**1.75 ± 0.01**	**1.94 ± 0.01**	1.34 - 2.45
Dab condition (g cm^{-3})	1.56 ± 0.06	1.79 ± 0.03	1.73 ± 0.06	1.94 ± 0.06	1.76 ± 0.05	1.88 ± 0.04	1.66 ± 0.02	1.89 ± 0.05	**1.66 ± 0.03**	**1.87 ± 0.03**	1.25 -2.24
Bottom salinity	30.2 ± 0.8	27.6 ± 1.1	32.0 ± 0.2	33.1 ± 0.1	26.8 ± 0.8	29.9 ± 0.7	32.0 ± 0.1	31.8 ± 0.5	**30.3 ± 0.4**	**30.6 ± 0.4**	12.1 - 34.6
Bottom temperature (°C)	15.9 ± 0.2	17.5 ± 1.0	15.5 ± 0.2	15.4 ± 0.3	15.9 ± 0.2	15.6 ± 0.4	16.3 ± 0.3	16.3 ± 0.4	**15.9 ± 0.1**	**16.2 ± 0.3**	12.4 - 28.0
Crab density (100 m^{-2})	5.8 ± 1.2	6.5 ± 0.9	11.0 ± 2.0	14.5 ± 1.8	2.6 ± 0.5	2.8 ± 0.5	7.1 ± 1.0	11.0 ± 1.7	**6.6 ± 0.7**	**8.7 ± 0.8**	0 - 41
Shrimp density (100 m^{-2})	16.1 ± 3.4	4.0 ± 1.1	28.7 ± 8.3	16.0 ± 4.6	20.0 ± 5.7	7.8 ± 1.4	20.9 ± 3.6	20.0 ± 3.3	**21.4 ± 2.8**	**12.0 ± 1.6**	0 - 100
Organic content (%)	1.14 ± 0.10	0.95 ± 0.08	1.12 ± 0.07	0.86 ± 0.05	0.98 ± 0.03	0.90 ± 0.02	0.91 ± 0.04	0.64 ± 0.03	**1.04 ± 0.03**	**0.84 ± 0.03**	0.36 - 1.77
Mean particle size (φ)	3.26 ± 0.08	3.34 ± 0.06	2.66 ± 0.09	2.53 ± 0.07	2.97 ± 0.06	2.90 ± 0.04	2.33 ± 0.04	2.23 ± 0.05	**2.81 ± 0.05**	**2.75 ± 0.05**	1.71 - 3.78
Polychaete density (m^{-2})	98.9 ± 12.0	137.9 ± 13.6	115.6 ± 27.6	149.4 ± 22.3	66.7 ± 15.3	46.9 ± 7.5	57.4 ± 10.5	82.5 ± 8.9	**84.6 ± 9.1**	**104.2 ± 8.2**	0 - 500
Bivalve density (m^{-2})	9.6 ± 2.1	27.4 ± 5.9	112.2 ± 23.4	209.6 ± 22.4	175.9 ± 21.5	171.8 ± 18.3	295.6 ± 46.5	580.4 ± 57.5	**148.3 ± 18.5**	**247.3 ± 26.3**	0 - 1230
Malacostracan density (m^{-2})	4.1 ± 1.4	4.9 ± 1.7	51.1 ± 12.8	187.1 ± 50.3	31.9 ± 5.7	86.4 ± 14.5	112.2 ± 17.1	167.5 ± 20.5	**49.8 ± 7.2**	**111.5 ± 153.7**	0 -1110
Number of benthos taxa (m^{-2})	2.9 ± 0.4	2.5 ± 0.2	4.6 ± 0.3	5.0 ± 0.3	5.2 ± 0.3	4.5 ± 0.2	5.2 ± 0.4	5.2 ± 0.3	**4.5 ± 0.2**	**4.3 ± 0.2**	1 - 9
Number of benthos density (m^{-2})	120 ± 16	172 ± 17	323 ± 43	569 ± 62	292 ± 39	322 ± 31	487 ± 54	865 ± 63	**305 ± 25**	**482 ± 36**	27 - 1600
Benthos diversity	0.51 ± 0.08	0.47 ± 0.06	1.00 ± 0.08	1.10 ± 0.05	1.03 ± 0.04	1.08 ± 0.04	0.96 ± 0.07	0.93 ± 0.06	**0.88 ± 0.04**	**0.90 ± 0.04**	0 - 1.75

Spatial and temporal variability in density and condition

Table 2 shows the effect of site, year and seasonality on the density/presence and Fulton's condition index of plaice and dab. The probability of encountering dab did not differ between sites, years or between seasons and a spatial or temporal trend was absent in both years. Dab densities did not differ between sites but were consistently higher in 2008 than in 2009 in all sites and showed a consistent decrease with season. Plaice densities did not differ between or within years but were consistently higher in Ballyloughaun and Traught compared to Ballyvaughan and Silverstrand in both years. Mean values of Fulton's K condition for dab and plaice were higher in 2009 than in 2008 and within years the condition was variable over time, as indicated by the interaction term on year and third order of ordinal date (Fig. 3 & 4). For both species there was a significant site effect; dab were in better condition in Ballyvaughan and Silverstrand whereas plaice were in better condition in Ballyloughaun and Silverstrand; this was consistent over the two years.

Table 2. Predictors explaining spatial and temporal variability in presence absence and/or density (using generalized linear modeling (GLM)) and Fulton's K condition (using generalized linear mixed modeling (GLMM)) of dab and plaice. The 1st order linearity corresponds to a linear relationship and a 3rd order to a cubic relationship in ordinal date. Sites; Bl: Ballyloughaun, Bv: Ballyvaughan, Si: Silverstrand, Tr: Traught. Levels of significance; ns, not significant; *, p <0.05; ** p <0.01; *** p <0.001.

	Dab Presence/Absence		**Dab Density**		**Plaice Density**		**Dab Condition**		**Plaice Condition**	
	Chi.sq	pairwise test	F	pairwise test	F	pairwise test	F	pairwise test	F	pairwise test
Site	4.46 ^{ns}		0.78 ^{ns}		6.39 ***	Bl,Tr> Bv,Si	21.66 ***	Bv,Si> Bl,Tr	3.82 *	Bl,Si> Bv,Tr
Ordinal Date	1.78 ^{ns}		10.63 ** (1st order)		2.52 ^{ns}		46.93 *** (1st order)		5.07 * (3rd order)	
Year	1.52 ^{ns}		11.74 **	2008> 2009	0.52 ^{ns}		93.87 ***	2009> 2008	211.90 ***	2009> 2008
Ordinal Date : Year	0.05 ^{ns}		0.18 ^{ns}		0.74 ^{ns}		20.79 *** (3rd order)		5.55 * (3rd order)	
Site : Year	1.98 ^{ns}		2.71 ^{ns}		0.66 ^{ns}		1.33 ^{ns}		1.02 ^{ns}	

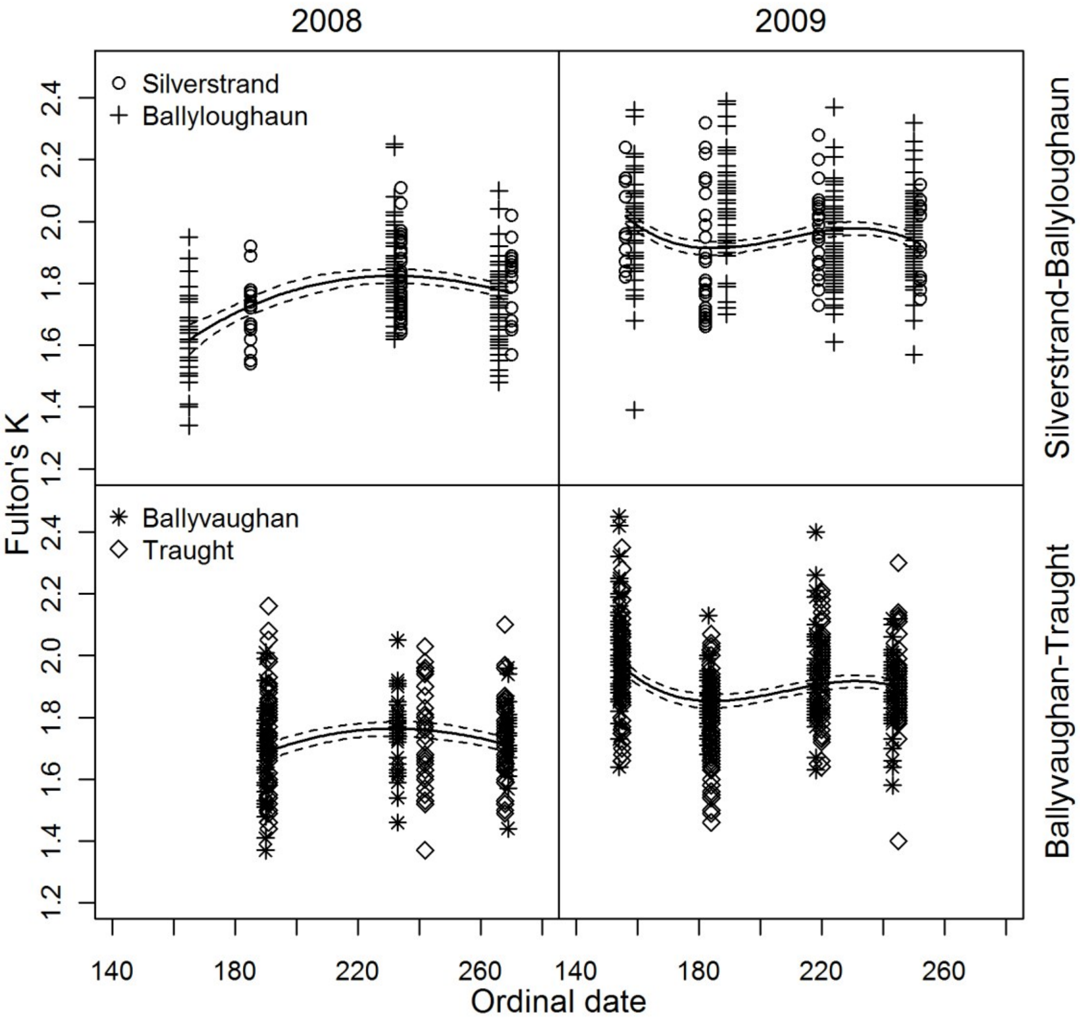

Fig. 3. Fulton's K condition of plaice collected in four different nursery grounds and over two different years. Each dot represents a fish and the fitted lines with 95 % confidence intervals are the mean predicted values from a GLMM with predictors; site, year, ordinal date (cubic relationship) and the interaction between year and ordinal date (as described in Table 1).

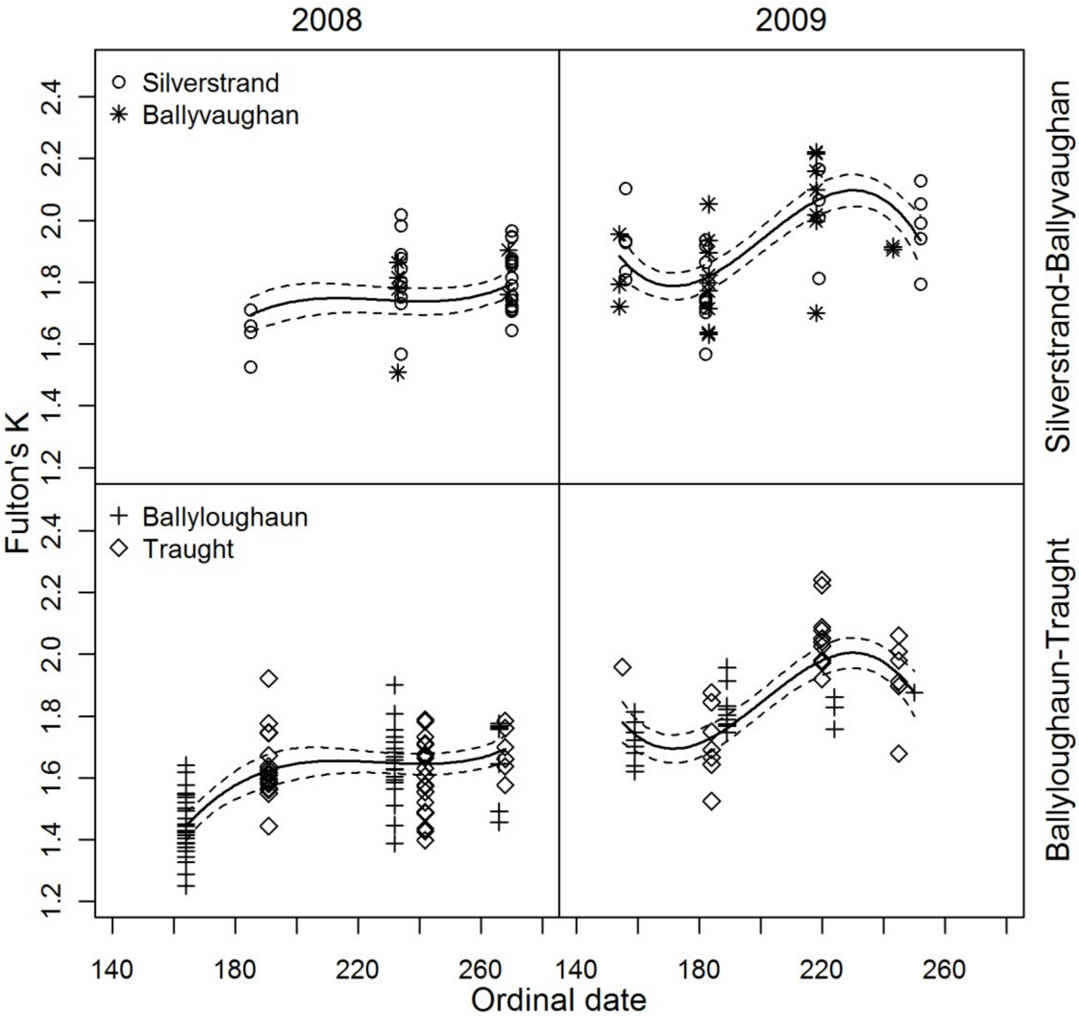

Fig. 4. Fulton's K condition of dab collected in four different nursery grounds and over two different years. Each dot represents a fish and the fitted lines with 95 % confidence intervals are the mean predicted values from a GLMM with predictors; site, year, ordinal date (cubic relationship) and the interaction between year and ordinal date (as described in Table 1).

Habitat characteristics explaining variability in density

As described above, no differences in dab presence and density were detected between nursery grounds when only ordinal date, site and year were included in the model. However small-scale variability within nursery grounds was found in dab presence and density based on habitat characteristics, explaining 35 % and 39 % respectively of the optimal model (Table 3 & Fig. 5a). Most of the variability in the probability of encountering dab was predicted by depth; with dab more likely to occur in the subtidal area. Ordinal date was the second most important explanatory variable; the probability of encountering dab increased as the season progressed. The addition of site significantly improved this optimal model suggesting that other variables not measured in this study contributed to variation in dab distribution between nursery grounds.

Table 3. Goodness-of-fit statistics for the GLMs fitted to predictors of small-scale variability in the presence absence and/or density of dab and plaice. Variables shaded in grey become insignificant upon addition of site in the optimal model and are thus not attributing to within-site variability but explain differences in the response variable between sites. Predictors are ordered with increasing explanatory power based on AIC values when comparing the information loss after every predictor is alternatively removed from the optimal model.

	Parameters		Estimate	Std. Error	z value	*p*	Deviance	% Expl	AIC
Dab presence/absence									
NULL							228.86		154.02
	Intercept		-5.065	1.348	-3.758	<0.001			
	Ordinal date		0.013	0.006	2.336	0.01949			
	Depth	Subtidal	3.313	0.457	7.247	<0.001			
RESIDUAL							148.02	35.32	
Dab density									
NULL							3.82		-27.24
	Intercept		1.953	0.367	5.325	<0.001			
	Salinity		-0.019	0.009	-2.125	0.03742			
	Shrimp density (Log)		-0.140	0.579	-2.424	0.01814			
	Year	2009	-0.169	0.054	-3.133	0.00259			
	Depth		0.097	0.024	4.014	<0.001			
	Ordinal date		-0.004	0.001	-4.984	<0.001			
RESIDUAL							2.33	39.07	
Plaice density									
NULL							22.88		120.41
	Intercept		0.581	0.179	3.249	0.0014			
	Organic content		-0.210	0.093	-2.270	0.02455			
	Weed		-0.050	0.020	-2.460	0.01495			
	Polychaete density (Log)		0.198	0.074	2.689	0.00793			
	Shrimp density (Log)		0.149	0.053	2.835	0.00517			
	Depth LAT		-0.102	0.028	-3.593	<0.001			
RESIDUAL							18.53	19.02	

Small-scale variability in dab densities (Table 3 & Fig. 5b) were mainly driven by ordinal date, with numbers decreasing from June to September. Depth was the second most important variable for predicting dab densities, with higher numbers occurring in deeper waters. Densities were higher in 2008 and decreased with increasing numbers of shrimps and with increasing salinity. Site was not significant in this optimal model suggesting that habitat features driving small-scale differences in dab densities were similar across the sampled sites.

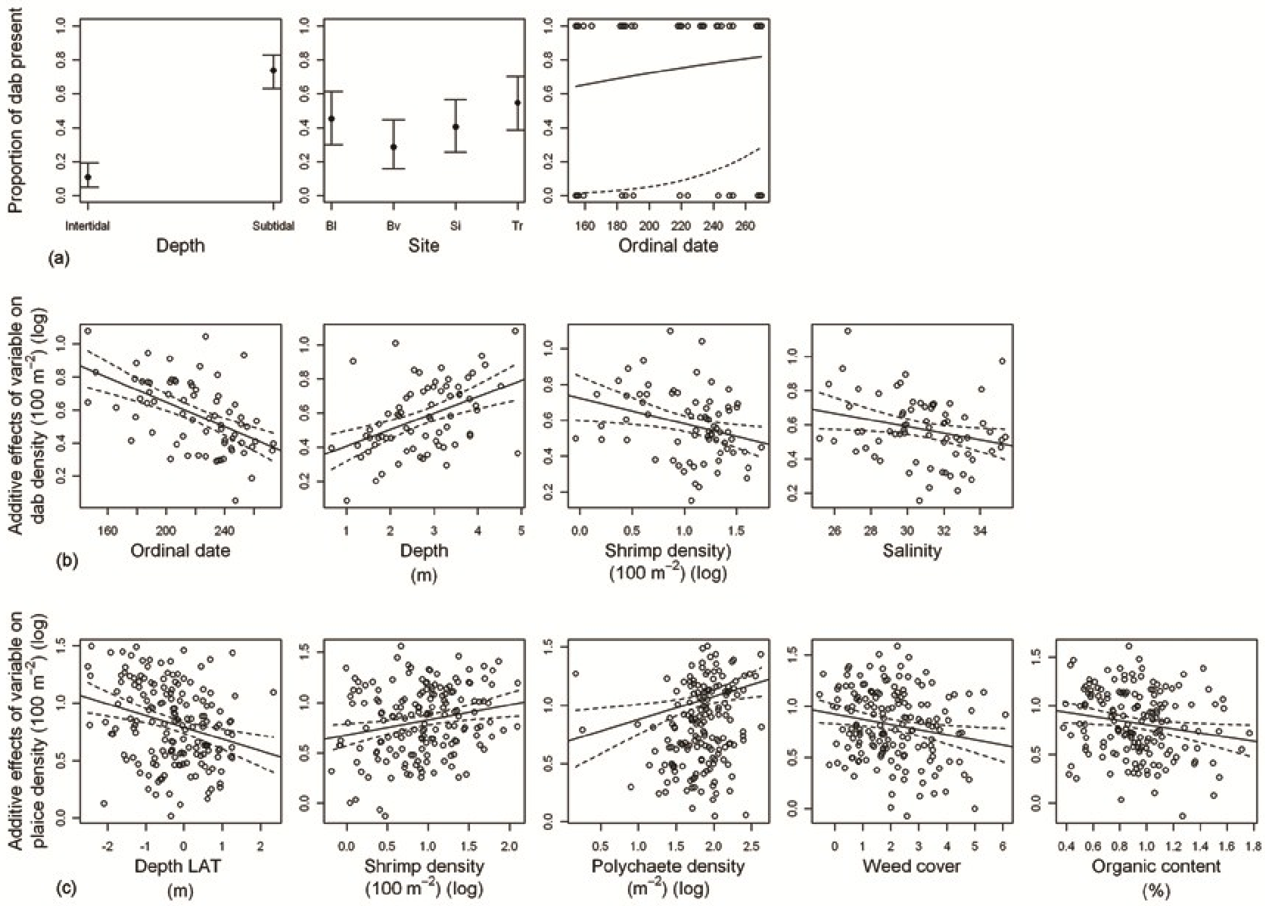

Fig. 5. (a) Proportion of dab presence and absence in a total of 168 trawl hauls over four sites and two years, separated by depth stratum, site (using Clopper-Pearson method) and by sampling time throughout the post-settlement period (using a binomial GLM with dashed line: intertidal area, solid line: subtidal area), **(b)** GLM plots showing additive effect of each significant variable on positive dab densities per haul (n = 71) over four sites and two years, (c) GLM plots showing additive effect of each significant variable on plaice densities (n = 162) over four sites and two years.

Compared to dab, small-scale variation in plaice distribution is more unpredictable, as suggested by the optimal model which only explained 19 % of the total variability in plaice density (Table 3 & Fig. 5c). In contrast to dab densities which were more associated with actual depth, variability in plaice density was best described by the depth relative to the lowest astronomical tide, with lower plaice densities found in subtidal areas. The density of shrimps was the second most important variable in the model, with high numbers of this predator associated with high numbers of plaice. Plaice density was also found to increase with polychaete density and to decrease with algal cover and organic content. However, the organic content, polychaete and shrimp densities explained overall differences in plaice densities between sites and did not contribute to small-scale variability within sites. In this respect, only depth and algal cover proved to explain variability in plaice density over larger spatial scales between distinct nursery areas within Galway Bay.

Table 4. Goodness-of-fit statistics for the GLMMs fitted to predictors of small-scale variability in Fulton's K condition of dab and plaice. Predictors are ordered with increasing explanatory power based on AIC values when comparing the information loss after every predictor is alternatively removed from the optimal model. The mean sediment particle size was correlated with the abundance of malacostracans, bivalves and total prey (-0.7, -0.8 and -0.9 respectively) within the areas occupied by dab, but no significant correlations were detected within the larger spatial range of plaice.

	Parameter		Estimate	Std. Error	t value	*p*	Deviance	% Expl	AIC
Dab condition									
NULL							8.80		-243.38
	Intercept		1.744	0.106	16.501	<0.001			
	Malacostracans density (Log)		0.054	0.023	2.385	0.0183			
	Crab density (Log)		-0.108	0.038	-2.828	0.0053			
	Year	2009	0.088	0.029	3.022	0.0036			
	Polychaete density (Log)		0.164	0.044	3.729	<0.001			
	Depth		-0.047	0.012	-3.883	<0.001			
	Organic content		-0.193	0.047	-4.106	<0.001			
RESIDUAL							2.39	72.85	
Plaice condition									
NULL							40.76		-1247.37
	Intercept		1.813	0.021	84.330	<0.001			
	Crab density (Log)		-0.061	0.021	-2.916	0.0036			
	Malacostracan density (Log)		-0.024	0.008	-2.899	0.0038			
	Year	2009	0.209	0.015	13.723	<0.001			
RESIDUAL							21.51	47.23	

Habitat characteristics explaining variability in Fulton's K condition

As described above, year and seasonality attributed to condition differences of plaice as well as dab, with fish showing higher conditions in 2009. Inclusion of habitat characteristics in the models (Table 4 & Fig. 6) showed that low flatfish condition was associated with high numbers of crab. High densities of malacostracans were associated with a low plaice condition and a high dab condition. Variability in plaice condition was explained by the previous three predictors only, while dab condition also increased with decreasing depth and organic content (which is positively co-linear with particle size in the dataset of dab condition) and with increasing polychaete densities. Densities of potentially competitors (other flatfish species) had no effect on plaice or dab condition. The factor site did not reduce the residual deviance of the optimal model and thus did not contribute to any additional variability in dab and plaice condition. The optimal model predicting the variability in dab condition is more complete (73 %) than the one for plaice condition (47 %).

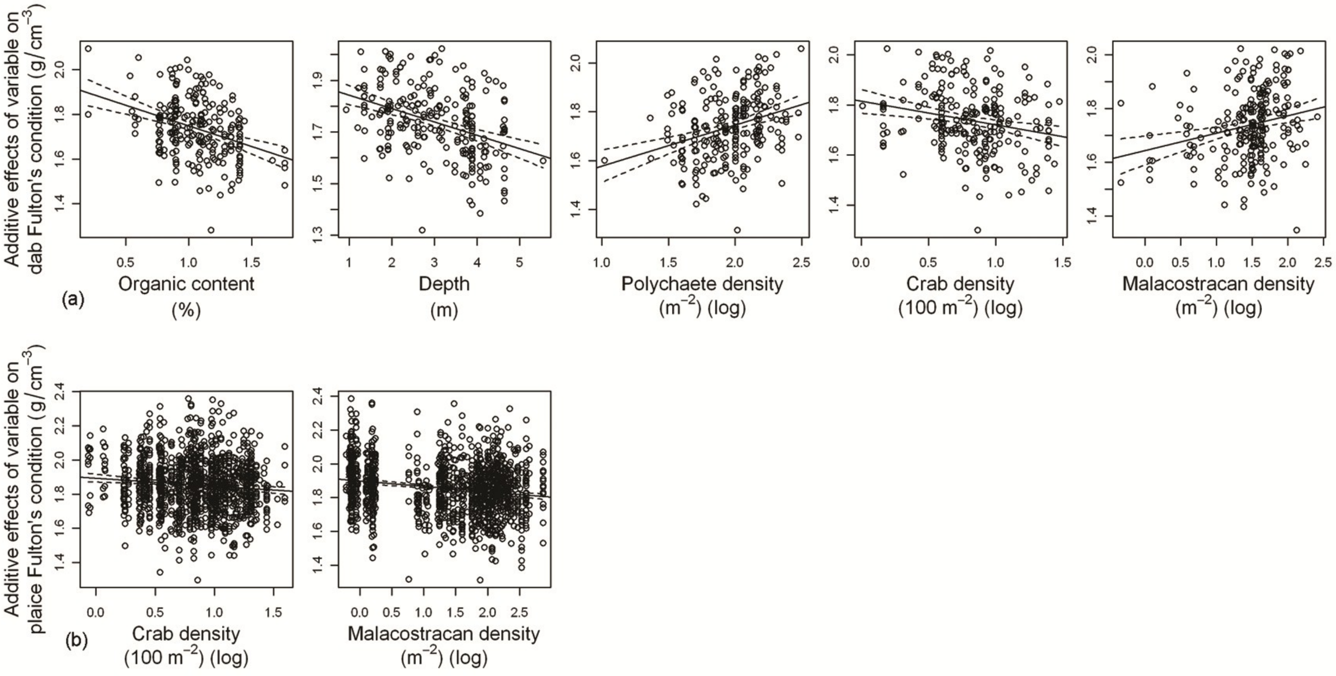

Fig. 6. (a) GLM plots showing additive effect of each significant variable on Fulton's K condition of all dab caught (n = 222) over four sites and two years during the post-settlement period, **(b)** GLM plots showing additive effect of each significant variable on Fulton's K condition of all plaice caught (n = 1253) over four sites and two years during the post-settlement period.

Discussion

Density and condition of plaice and dab in Galway Bay varied spatially and temporally and was influenced by a variety of habitat characteristics. Dab occurrence was only associated with depth and ordinal date whereas dab and plaice densities were related to a combination of physical and biological variables. Juvenile condition was mainly predicted by biological variables, with depth and organic content explaining additional variability in plaice condition. The percentage of density variability explained by habitat characteristics was similar to those reported in other studies, which often range around 20 % and seldom exceed 50 %. The nature of juvenile flatfish distribution is generally very patchy (Modin and Pihl, 1996) and it is known that physical descriptors do not always account for its variation (Le Pape *et al.*, 2003a), causing much of the remaining unexplained variability in the density models. In contrast, the explained variability in juvenile condition was higher, reaching >70 % for dab. This might reflect that fish condition is influenced by recent variation in habitat conditions which we have more precisely quantified than the complex variety of interrelated factors that cause fluctuations in fish densities. Additionally, the explained variability in density and condition was greater for dab than for plaice, possibly due to the larger sample size or distribution range of plaice.

Temporal variability in density and condition

This study recorded interannual variability in the density of 0-group dab, in the condition of dab and plaice and in the fish length of both species just after settlement. It is generally accepted that year-class strength varies greatly between years due to fluctuating environmental conditions during egg and larval development (Beverton and Iles, 1992; Gibson, 1994; van der Veer *et al.*, 2000; Beggs and Nash, 2007). A more favorable environment during the larval phase in 2009 may have resulted in the consistently higher fish condition and average fish length just after settlement. Winter and spring temperatures, as recorded from the M1 buoy outside Galway Bay, were on average 1 °C lower in 2009 than in 2008 (Marine Institute, pers. comm.) and may have contributed to the higher fish condition observed in 2009. This would be in accordance with other studies reporting a negative correlation between spring seawater temperature and year-class strength of many flatfish species (van der Veer, 1986; Pihl, 1990; Henderson and Seaby, 1994; Henderson, 1998; Haynes *et al.*, 2010). However, a more comprehensive analysis of the temperature experienced by these fish during the larval phase and a longer time series would be needed to confirm this link.

Seasonal fluctuations in flatfish densities in Galway Bay showed interspecific differences. Dab densities declined from June to September while the probability of dab occurring in the trawls increased during the same period. It is possible that the dab distribution becomes less patchy as they grow older and become more active foragers. Reported peaks in the timing of dab settlement vary widely between habitats, with densities reaching a maximum between June and October (Poxton *et al.*, 1982; Bolle *et al.*, 1994; Amara *et al.*, 2001; Amara, 2004; Beggs and Nash, 2007). Plaice densities in Galway Bay did not show yearly or seasonal fluctuations contrasting with results from previous studies, which show highest densities between April and July and a subsequent population decline (Poxton *et al.*, 1982; van der Veer *et al.*, 1990; Modin and Pihl, 1994; Nash and Geffen, 2000; Pihl *et al.*, 2000; Hjörleifsson and Pálsson, 2001; Amara and Paul, 2003; Freitas *et al.*, 2010). The rate of population decline was larger for dab than for plaice in Galway Bay. Similar results were reported in the Clyde Sea area where interspecific differences in timing of fish emigration from nursery areas were proposed to account for this variation (Poxton et al., 1982).

Environmental variables explaining variability in density and condition

Settlement of plaice and dab in nursery areas in Galway Bay appeared to be primarily delineated by depth. Settling plaice were restricted to shallow water with peak densities occurring around 1 m. This is consistent with other studies which rarely report plaice in depths >3 m (Poxton et al., 1982) and which show that plaice display a tidal rhythm, moving closer to the beach at high tide (Gibson, 1973; De Veen, 1978). Depth distributions of settling dab have not been previously studied on the west coast of Ireland and our sampling efforts were restricted to 5 m depth, where the highest dab densities were recorded. It is likely that settlement also occurs in deeper waters, as reported in offshore waters in the North Sea and Irish Sea with peak densities within 10 m but settlement extending up to 70 m depth (Bolle *et al.*, 1994; Dickey-Collas *et al.*, 1997; Beggs and Nash, 2007). The rare appearance of dab in intertidal areas in Galway Bay supports the absence of a tidal transport pattern for this species. Partial segregation between plaice and dab in Galway Bay is shown as a result of spatial partitioning of resources, which is a strategy used by morphologically similar species to reduce interspecific competition (Schoener, 1974).

Salinity is an important determinant of flatfish community structure (Schreiber, 2001) and has a larger effect on estuarine species like flounder and Senegalese sole (*Solea senegalensis*) (Cabral et al., 2007; Ramos et al., 2009). To a lesser extent it also affects distribution and growth patterns of flatfish in coastal zones due to differences in osmoregulatory capacity (Lyndon, 1994). Dab densities were slightly higher in less saline areas in Galway Bay, but a response to salinity was not reflected in plaice densities or flatfish condition. The higher dab density in less saline areas is in accordance with similar findings for plaice in the Humber estuary where younger fish used lower salinity areas to enhance growth (Marshall and Elliott, 1998). The influence of salinity on juvenile growth depends on the developmental stage (Jager et al., 1993) and this might be the reason that salinity did not explain overall variability in dab and plaice condition in Galway Bay.

Although temperature is known to be an important environmental determinant of juvenile distribution and growth (Fonds et al., 1992; Reichert et al., 2000; Yamashita et al., 2001), no direct link with flatfish density or condition was detected in this study. It is possible that fish condition was not affected by the temperature at the time of the survey, but by that in the preceding period. Alternatively, the moderating effect of temperature may have partly been expressed by time, water depth or prey and predator abundance in the resulting models. Temperature loggers at a finer temporal scale should have been used to reveal possible site differences.

The present study revealed the influence of substrate characteristics (particle size, prey abundance and organic content) on the density and condition of juvenile flatfish. It is well known that sediment structure co-varies with other, especially prey related, substrate characteristics (Gibson, 1994; McConnaughey and Smith, 2000), which was also observed in Galway Bay. The condition of dab was positively associated with particle size demonstrating the negative effect of muddy locations with consequently lower prey density. Sediment properties may directly enhance survival of demersal fish by permitting burying behaviour critical for conserving energy by reducing metabolic rates (Howell and Canario, 1987) or for escape from predators (Gibson and Robb, 1992; Jager et al., 1993; Stoner and Abookire, 2002). The absence of a relationship between plaice condition and sediment particle size suggests a wider tolerance of plaice to varying sediment substratum. Burial behavior studies showed that newly metamorphosed plaice are unable to bury in grain sizes larger than 500 µm (Riley et al., 1981) and avoid sediment with more than 10 % mud fraction (Jager et al., 1993); average grain size in the studied nurseries in Galway Bay was generally within those extremes. Sediment properties in Galway Bay had an additional impact on flatfish condition through the composition and abundance of benthic invertebrates. High polychaete and malacostracan densities were associated with high dab condition whereas plaice condition was negatively correlated with malacostracan density. This highlights the different feeding strategies of plaice and dab, with a higher constitution of malacostracans in dab diet as described by previous dietary analysis of juveniles in Galway Bay (De Raedemaecker *et al.*, 2011b). This study showed that availability of favorable macrobenthic prey is crucial for rapid growth of flatfish and its effect might possibly exceed that of sediment particle size. This is also suggested by previous laboratory experiments indicating the complexity of interrelated factors in habitat choice (Phelan et al., 2001).

The negative correlation of organic enrichment with plaice density and dab condition is most likely indicative of the deteriorated feeding circumstances associated with organic content, which generally provides information on productivity of meio-and macro-faunal invertebrates in nursery grounds (Möller et al., 1985). With low organic matter, invertebrate biomass will likely increase whereas higher values of organic matter can lead to high levels of oxygen consumption and subsequent anoxia and elimination of macrobenthos (Pearson and Rosenberg, 1978). Anthropogenic inputs of organic matter in coastal bays could therefore have a negative impact on flatfish nursery areas.

Macroalgal cover in Galway Bay had a negative effect on plaice density; this corroborates laboratory evidence of plaice actively selecting substrata free of vegetation (Wennhage and Pihl, 1994) and field reports of reduced plaice densities in areas partly covered with algae (Pihl and Van der Veer, 1992). The effect is likely attributed to reductions in feeding efficiency (Nordström and Booth, 2007) since plaice are mostly dependent on sedentary infaunal prey (De Raedemaecker, in press). Macroalgae play an important functional role in structuring benthic faunal assemblages and alter their accessibility as food source. Despite the increase of some mobile epifauna under algal cover, there is a general tendency of decreased numbers of sedentary sediment-water interface feeders (especially bivalves and tube-dwellers) and prey availability (Everett, 1994). A relationship between algal cover and dab density was not detected and, to our knowledge, has not been previously recorded in the literature. Interspecific differences in the use of intertidal areas, foraging behaviour and dominant prey type might explain the different effect of macroalgae cover on densities of the two flatfish species. Although the mechanisms (e.g. mortality due to anoxic condition, active avoidance, reduced prey availability) underlying low plaice densities in algal covered habitats in Galway Bay are uncertain, the effect could result in decreased recruitment (Pihl et al., 2005). As a consequence of increased eutrophication in shallow waters, nursery grounds are more often covered by fragmented and detached drifting vegetation (Pihl et al., 2005) which can decrease habitat complexity and cause severe oxygen deficiency if persistent in an area (Norkko and Bonsdorff, 1996). Management actions are therefore crucial to prevent deteriorating coastal habitats and the presence of algal mats in nursery areas.

Biological mechanisms affecting variability in density and condition

Predation, which is perhaps the most important cause of flatfish mortality during the post-settlement phase (Bailey, 1994), affected flatfish distribution and condition in Galway Bay. Dab densities were lower in areas with high shrimp densities, consistent with observations in the Thames estuary where shrimp predation has been postulated as a controlling factor in juvenile dab abundance (Power et al., 2000). The effect may be linked with predator avoidance as suggested by laboratory evidence that settling fish spend less time on sediments where predators are present (Wennhage and Gibson, 1998). Surprisingly, plaice densities in Galway Bay were positively correlated with shrimp densities. This may be because both demersal species were attracted to sites with abundant food supply or other favorable environmental conditions. The higher proportion of larger plaice compared to dab may explain their lower vulnerability to shrimp predation. Reduced plaice and dab condition at sites with higher crab densities was also revealed. This is in accordance with results of Burrows & Gibson (1995) which showed reduced activity levels and feeding success of plaice in the presence of predators. This would inevitably reduce fish condition. Predator-prey interactions are determined by size ratios (Ellis and Gibson, 1995) and laboratory experiments carried out by van der Veer and Bergman (1987) found that plaice have a size refuge from predation by *Crangon crangon* at 30 mm, and from *Carcinus maenas* at 50 mm. The majority of the fish sampled in Galway Bay were over 30 mm and this might explain why shrimp densities, in contrast to crab densities, did not feature as a significant variable in models explaining flatfish condition. A long-term study in the west of Scotland was also unable to detect evidence for control of plaice populations by shrimp predation (Burrows et al., 2001).

There was no evidence of inter- and intra-specific competition negatively influencing the density and condition of either species, implying that flatfish densities did not exceed the carrying capacity of the nursery grounds in Galway Bay. This is in contrast to other studies in Irish Sea where density-related processes on juvenile plaice may occur (Nash and Geffen, 2000). However, these studies have reported densities between 0.4 m-2 and 3 m-2, substantially higher than the densities observed in Galway Bay. Productivity of single nursery grounds is also suggested as an important regulator of juvenile flatfish densities (Nash et al., 2007)

Nursery habitat quality in Galway Bay

Juvenile plaice and dab from two years and four distinct nursery areas in Galway Bay showed spatial variation in Fulton's condition and density but no uniform trends were revealed for both species. Certain nursery areas showed consistently higher growth or density over the years but it was unclear whether these differences arose as a result of differences in larval supply and topographic features of the shore line (Pihl et al., 2000) or due to processes acting on the juvenile life-stage. Longer time series could elucidate the nature of the mechanisms underlying this pattern. Future research should determine the relative importance of each nursery area by their overall contribution (and contribution per unit area) of juvenile flatfish recruiting to the adult stock (Beck et al., 2001; Dahlgren et al., 2006). Either way, it is important to identify these nursery areas with a high survival rate of juvenile flatfish and consider them appropriately in coastal zone management.

Conclusion

This study provides a comprehensive look at how biotic and abiotic habitat characteristics influence the growth and density of two important flatfish species. The interaction of various substrate features showed the complex nature of these highly dynamic nursery areas. However, predation pressure, feeding opportunities and sediment grain size clearly contributed to growth differences of juvenile plaice and dab between nurseries. This study also illustrates how distinct sets of habitat features can drive spatial variation in density and condition of juvenile flatfish highlighting the value of studying both variables when modeling habitat requirements. Knowledge gained about the quality of nursery habitat for commercially important fish species provides a basis for mapping essential flatfish habitats to elucidate the causes of survival and recruitment variability and to inform management plans for coastal areas.

Acknowledgements

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CHAPTER 5

COMPARISON OF SPATIO-TEMPORAL VARIABILITY IN FULTON'S K AND RNA:DNA RATIO AS CONDITION INDICES OF JUVENILE PLAICE AND DAB

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Abstract

The growing importance of defining high quality nursery grounds for juvenile fish as a result of changing nearshore environments has resulted in the use of different indicators for fish condition and growth. This field study aimed to compare and evaluate a morphometric (Fulton's K condition factor) and biochemical (RNA:DNA ratio) condition index in juvenile flatfish to assess habitat quality in four sandy beach nursery grounds in Galway Bay, Ireland. Juvenile plaice (*Pleuronectes platessa)*, and dab (*Limanda limanda)* were surveyed monthly from June - September in 2008 and 2009 using a 1.2 m beam trawl. Sediment samples were collected using Van Veen grabs for substrate characterization and physicochemical measures and predator densities were recorded. Gut content analyses revealed recent feeding history of both species. Generalized Additive Modelling (GAM) was used to investigate non-linear relationships between condition indices and habitat characteristics and diet features. The results of the study revealed a lack of correlation between the morphometric and biochemical condition indices of plaice and dab. 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. 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 Fulton's K of plaice and dab. The usefulness of both indices for assessing fish condition and habitat quality in field studies are 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.

Keywords

habitat quality; condition indices; juvenile flatfish; *Pleuronectes platessa*; *Limanda limanda*; Ireland; Galway Bay

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 process can, in combination with increasing fishing pressures, 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), substrate characteristics (Gibson and Robb, 2000; McConnaughey and Smith, 2000) and the presence or absence of competition (Burke *et al.*, 1991; 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).

Developing indicators for fish growth is an essential step towards assessing and protecting 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 (Chícharo and Chícharo, 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, sometimes even after short periods of 1-3 days, under varying temperature levels (Buckley, 1984; Malloy and Targett, 1994; Johnson *et al.*, 2002; Peck *et al.*, 2003; Mercaldo-Allen *et al.*, 2006; Mercaldo-Allen *et al.*, 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; Chícharo *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 starved fish in their natural habitat which can be linked to environmental events and recruitment success (Clemmesen, 1994; Rooker *et al.*, 1997). In combination, biochemical and morphometric condition indices are useful for determining the overall nursery role of a habitat.

The aim of the present field study is 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 Raedemaecker *et al.*, 2011a). 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 and abiotic habitat characteristics that influence Fulton's K and R/D.

Materials and methods

Study area and sampling strategy

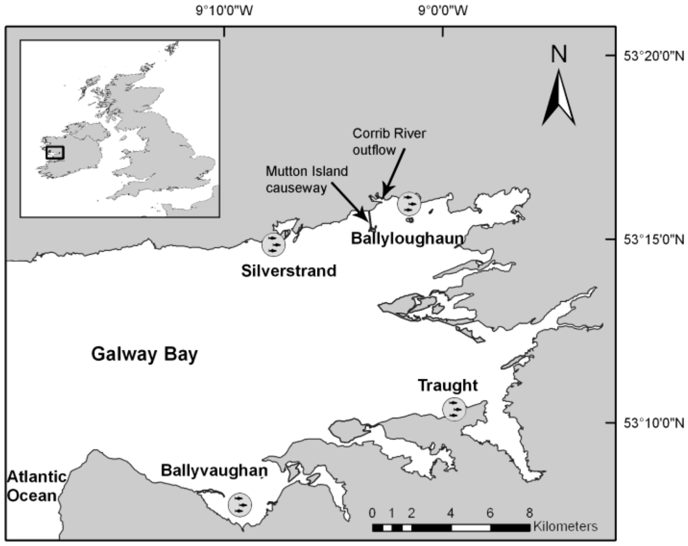

Fig. 1. Map of Galway Bay, Ireland, showing the position of four sampled flatfish nursery grounds.

The westward facing Galway Bay is a region of high tidal energy (tidal range of 4.5 m), with an area of approximately 100 km^2, located on the west coast of Ireland (Fig. 1). A total of four soft-bottom nursery areas which are 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 - September in 2008 and 2009. Due to weather conditions in June 2008, sampling was only undertaken in Ballyloughaun and the three other nursery areas were surveyed three weeks later in July 2008. A 1.2 m beam trawl (6 x 6 mm codend) equipped with one tickler chain was hauled at a constant velocity of 1.1 knots for 2 min. A mechanical flowmeter attached to the beam was used to calculate the swept area which averaged 94 m^2 per trawl. Three replicate trawl hauls in the subtidal zone were carried out at each site. All juvenile flatfish were immediately stored onboard in liquid nitrogen and transferred to a -80 ºC freezer in the laboratory until further sorting and analyses. The geographic location of the trawls was recorded using a GPS and the actual depth was recorded with an echo sounder. Salinity and temperature data were obtained at each trawl location with a CTD. A Van Veen grab (0.025 m^2) was used to collect sediment samples for particle size analysis (PSA). 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).

Sediment processing

Particle size analysis was carried out with oven-dried sediment for all samples collected in 2008 using the method described in Bale & Kenny (2007). Sediments were divided into six fractions: silt (<63 µm diameter), very fine sand (63 µm - 125 µm), fine sand (125 µm - 250 µm), medium sand (250 µm - 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, phi (φ), 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.

Fish processing

All fish were identified before measuring total length (TL) and standard length (SL) to the nearest mm and weighing gently blotted fish (W) 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 K (Fulton, 1911) was calculated for each fish using the formula $K = (W/L^3)*100$, where W is fresh weight (g) and L is standard length (cm). Care should be taken when comparing Fulton's K derived from different studies since there are inconsistencies in the use of standard length and total length when calculating this condition index. Fish tails easily damage during beam trawling and the use of standard length is therefore recommended to aid in comparisons between studies. Fish diet analyses was 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.

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 g - 0.030 g) was macerated 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 minutes 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 waterbath at 37 °C for 30 min to allow enzymatic digestion of RNA. Background fluorescence was measured using two blanks of 50 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.

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: $Y' = Y - (b * L) - (a - \hat{Y})$, where: Y' = corrected condition index value, Y = original condition index value, $\hat{Y}$ = mean 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 = 0.476 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).

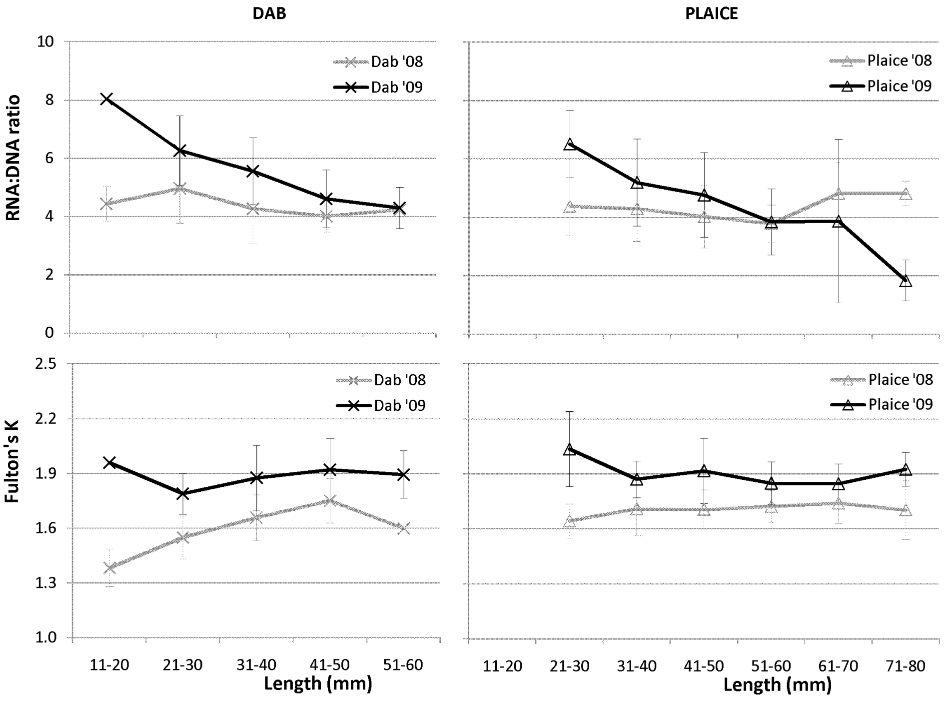

Fig. 2. Relationship between mean RNA:DNA ratios and Fulton's K values (with standard deviation) in function of 10 mm length classes of dab and plaice juveniles. Fish were collected in June-September during 2008 and 2009.

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 occur simultaneously and interact 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 Raedemaecker *et al.*, 2011b). 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 Raedemaecker et al. (2011a). 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-colinearity 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).

Results

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: $r = 0.34$, $p < 0.001$). However, this correlation was driven by annual differences since insignificant correlation coefficients were found when each year was analysed separately (plaice 2008: $r = 0.035$, $p = 0.738$; plaice 2009: $r = 0.052$, $p = 0.563$; dab 2008: $r = 0.071$, $p = 0.536$; dab 2009: $r = 0.090$, $p = 0.407$). Therefore both condition indices are representing different ecological information and are used complementary in further analyses.

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 were different in 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. Values of R/D were statistically not compared between species since DNA content and protein expression is species-specific hindering comparisons between species (Dahlhoff, 2004).

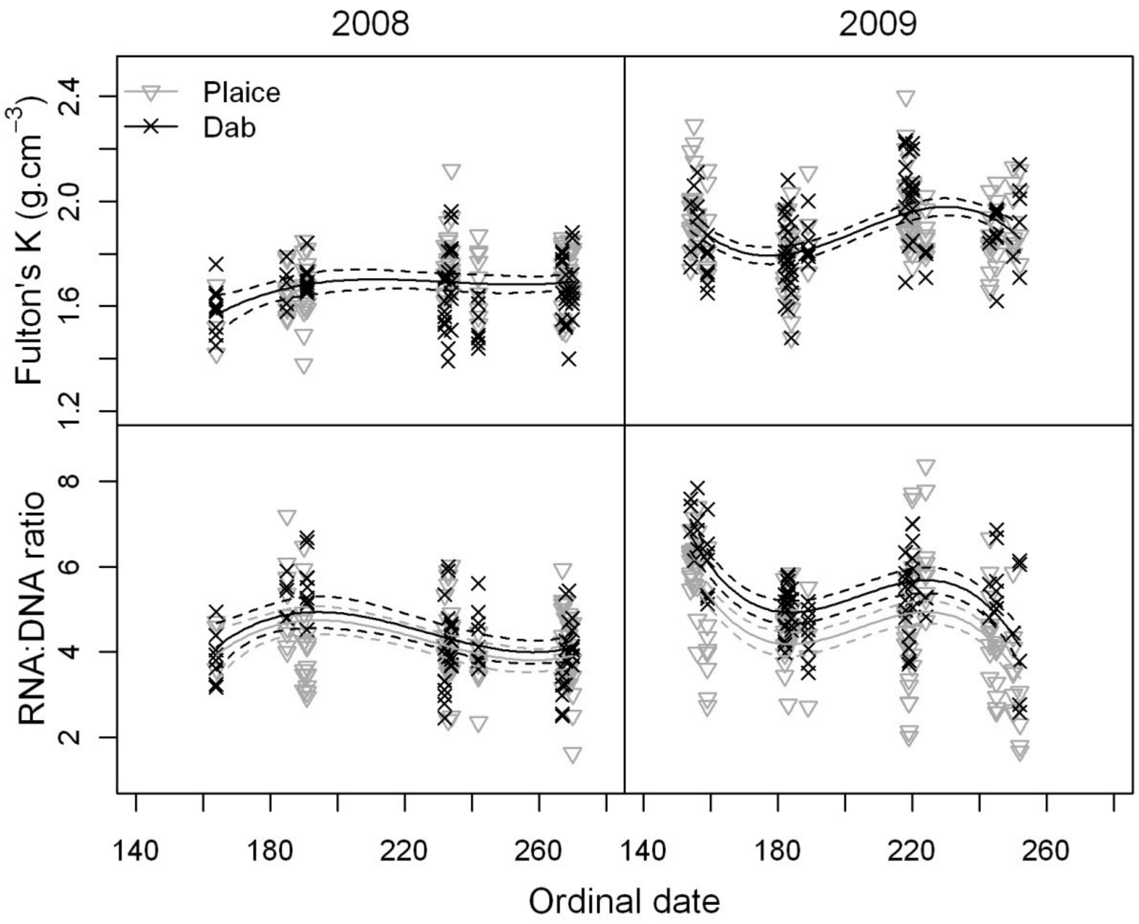

Fig. 3. Temporal and interspecific variability in Fulton's K and RNA:DNA ratio of plaice and dab (pooled from 4 different nursery grounds), collected monthly between June-September over two different years. Every dot represents a fish and the fitted lines with 95 % confidence intervals are the mean predicted values using Generalized Linear Modelling (GLM) with predictors; year, ordinal date (cubic relationship), species and the interactions between year with ordinal date and with species.

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).

Table 1: Spatial variability in condition indices of juvenile plaice and dab (corrected for fish length) between sites, accounting for temporal variation between and within years, using Generalized Linear Modelling (GLM). Sites; Bl: Ballyloughaun, Bv: Ballyvaughan, Si: Silverstrand, Tr: Traught. Levels of significance; ns: not significant, *: p <0.05, **: p <0.01, ***: p <0.001.

	Fulton's K plaice		**Fulton's K dab**		**RNA:DNA plaice**		**RNA:DNA dab**	
	F	pairwise test	F	pairwise test	F	pairwise test	F	pairwise test
Site	0.91 ^{ns}		7.32 ***	Si>Bl,Bv,Tr	2.85 *		26.47 ***	
Year	117.98 ***	2009>2008	125.79 ***	2009>2008	11.84 ***	2009>2008	73.49 ***	2009>2008
Ordinal Date (3rd order)	19.44 ***		1.40 ^{ns}		5.33 *		18.38 ***	
Ordinal Date : Year	8.69 **		23.83 ***		10.33 **		31.63 ***	
Site : Year	0.21 ^{ns}		1.48 ^{ns}		5.53 **	(In 2008: Bl,Si,Bv>Tr; In 2009: Bl,Bv,Tr>Si)	8.10 ***	(In 2008: Bv>Tr,Si,Bl & Tr,Si>Bl; In 2009: Bv,Tr>Si,Bl)

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 φ (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 φ (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.

Table 2. Goodness-of-fit statistics for the predictors of small-scale variability in Fulton's K and RNA:DNA ratio of juvenile dab and plaice, sampled over two years and in four distinct nursery grounds, using General Additive Modelling (GAM).

	Variable	edf	F value	p
Dab RNA:DNA				
	Sediment grain size	6.25	8.54	<0.001
	Crab density	6.00	2.60	0.017
	Temperature	8.75	12.39	<0.001
	Salinity	6.79	4.20	<0.001
Total deviance explained: 74.2 %				
Dab Fulton's K				
	Sediment grain size	2.83	6.39	<0.001
	Crab density	3.24	7.18	<0.001
	Temperature	4.28	3.31	0.008
	Salinity	6.82	6.18	<0.001
	Copepod abundance	2.79	7.32	<0.001
Total deviance explained: 62.40 %				
Plaice RNA:DNA				
	Sediment grain size	8.44	7.46	<0.001
	Crab density	7.25	2.46	0.156
	Depth	5.69	2.14	0.048
	Salinity	2.63	6.24	<0.001
Total deviance explained: 41.5 %				
Plaice Fulton's K				
	Sediment grain size	7.40	5.72	<0.001
	Crab density	6.65	7.29	<0.001
	Depth	4.08	5.56	<0.001
	Salinity	5.93	7.52	<0.001
	Gut fullness	1.00	10.19	<0.001
	Polychaete abundance	4.53	2.98	0.013
Total deviance explained: 51.4 %				

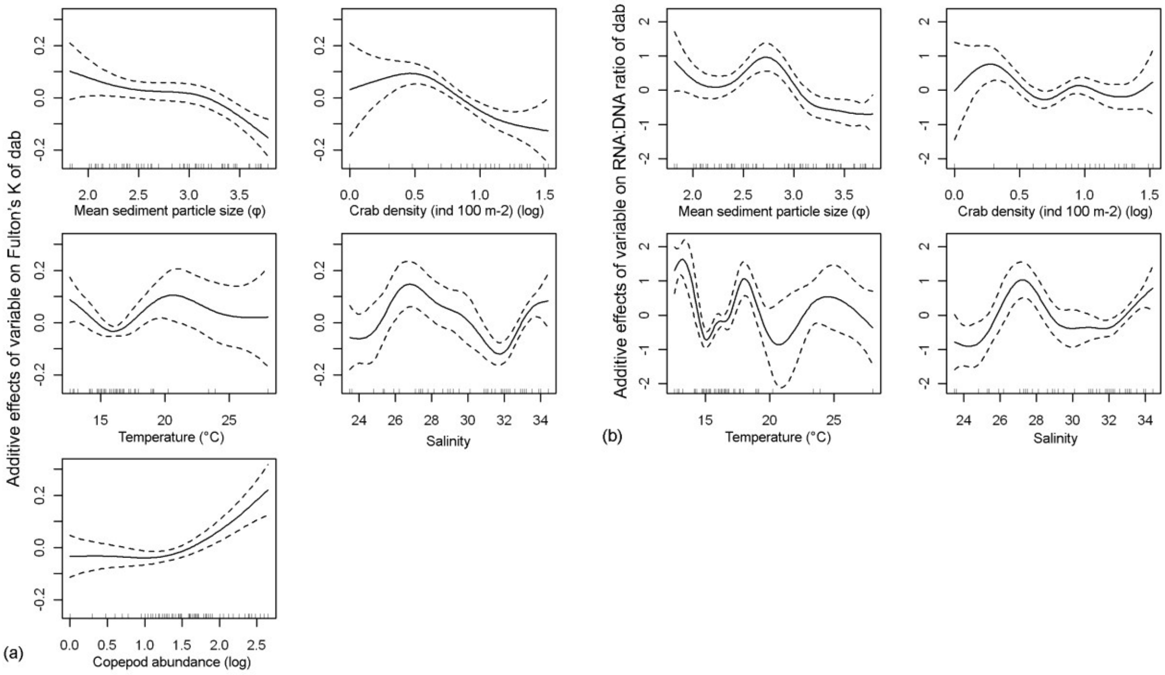

Fig. 4. GAM plots showing the additive effect of each variable on (a) Fulton's K and (b) RNA:DNA ratio of dab (n = 165).

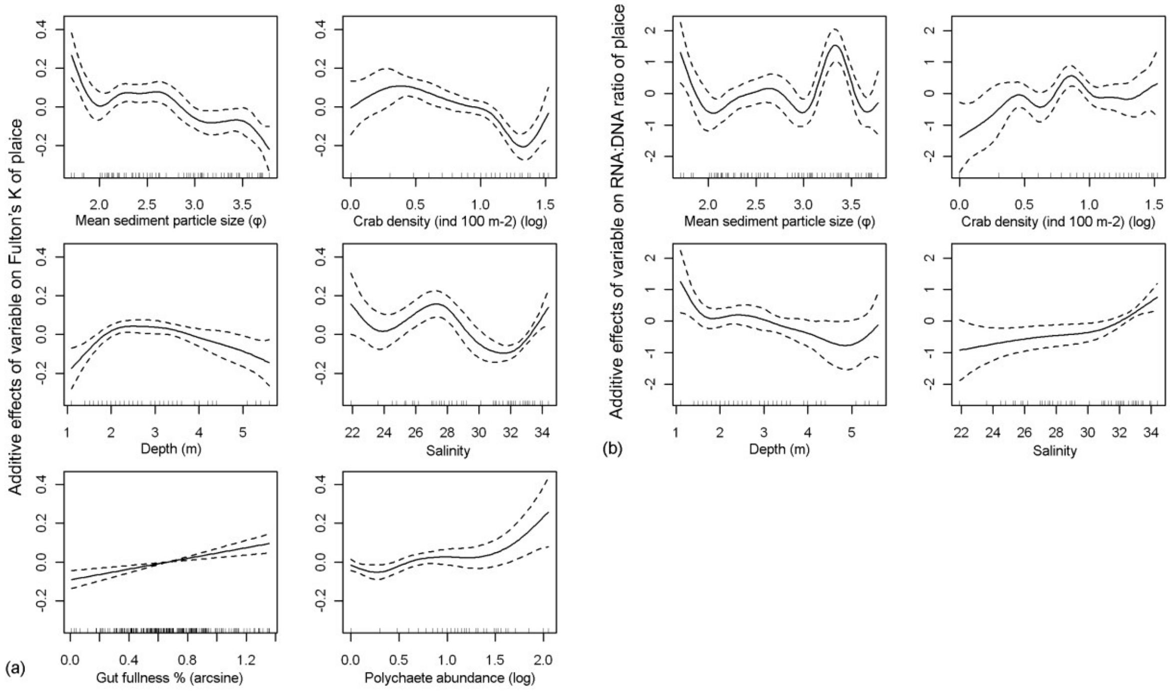

Fig. 5. GAM plots showing the additive effect of each variable on (a) Fulton's K and (b) RNA:DNA ratio of plaice (n = 222).

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) (Table 3). Since juvenile flatfish were sampled on average around high tide in both years, these differences are likely not reflecting chance variation in the timing of sampling relative to the tide. Compared to abiotic characteristics, diet features were highly variable between 2008 and 2009 (Table 4) indicating that annual differences in fish condition were driven by food availability. Dab had higher copepod and bivalve abundances and lower prey diversity in 2009 guts whereas polychaetes and bivalves were more abundant in plaice guts in 2009.

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.

	Year			
	2008	2009	H	Pairwise
Depth (m)	3.12 ± 1.23	2.53 ± 0.73	5.41 *	2008 > 2009
Salinity	30.61 ± 2.95	30.89 ± 3.30	5.95 *	2009 > 2008
Temperature (°C)	15.85 ± 0.93	16.32 ± 2.30	0.01 ^{ns}	
Sediment particle size (φ)	2.84 ± 0.51	2.73 ± 0.09	0.88 ^{ns}	
Crab density (ind 100^{-2}m)	7.92 ± 6.67	8.20 ± 7.11	0.01 ^{ns}	
n	38	51		

Table 4. Interannual variability in prey consumption and other diet characteristics of juvenile plaice and dab in Galway Bay, using a GLM with fish length included as a covariate. Levels of significance; ns: not significant, *: p <0.05, **: p <0.01, ***: p <0.001.

	DAB				PLAICE			
	Year		Length		Year		Length	
	F	Pairwise	F	Slope	F	Pairwise	F	Slope
Stomach Fulness (%)	0.37 ^{ns}		3.40 ^{ns}		1.56 ^{ns}		21.88 ***	-
Shannon-Wiener prey diversity	24.02 ***	08 > '09	4.18 *	+	0.61 ^{ns}		1.95 ^{ns}	
Bivalve abundance	6.90 **	09 > '08	9.05 **	-	9.12 **	09 > '08	0.10 ^{ns}	
Malacostracan abundance	2.76 ^{ns}		40.58 ***	+	0.15 ^{ns}		35.16 ***	-
Copepod abundance	14.28 ***	09 > '08	10.33 **	-	0.01 ^{ns}		64.26 ***	-
Polychaete abundance	2.98 ^{ns}		4.60 *	+	29.77 ***	09 > '08	18.25 ***	+

Discussion

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 Raedemaecker *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 - 7.74) could not be compared to findings from other field studies due to the rare use of nucleic-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 (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 *Carcinus maenas* has been previously postulated as a regulating factor in juvenile flatfish densities (Edwards and Steele, 1968; Pihl, 1990; Amara and Paul, 2003). Processes like predator avoidance, reduced activity levels, increased burying behaviour and feeding delay can lead to impaired condition in juvenile flatish (Burrows and Gibson, 1995; Wennhage and Gibson, 1998; Gibson *et al.*, 2002). 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. Plaice 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; Imsland *et al.*, 2001; Imsland *et al.*, 2002). 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.*, in Progress). Since the osmotic concentration of plaice usually decreases with fish length, this suggests that juvenile fish are more hyposmotic to the surrounding sea (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. 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; Lankford and Targett, 1994; Mallekh *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 15º to 18 ºC) but within the temperature limit of 22 ºC, 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 acclimatised 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 are 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 Raedemaecker *et al.*, 2011b). Our results are consistent with findings from 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 characterising 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; Ramírez *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 standardised 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 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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CHAPTER 6

GENERAL DISCUSSION

Juvenile flatfish assemblages on coastal nurseries in west of Ireland

The overall aim of this thesis was to investigate ecological requirements and species interactions influencing the distribution, density and growth of juvenile plaice and dab in coastal nursery habitats on the west coast of Ireland. Flatfish assemblages, between June and September in 2008 and 2009, in Galway Bay were composed of six different species (Chapter 4); plaice (83 %), dab (15 %), common sole, turbot, flounder and brill. These species all contribute to commercial landings of flatfish in Ireland (Fig. 1; Chapter 1). Absence of the other abundant commercial flatfish species; megrim, lemon sole and witch flounder in Irish coastal nurseries can be explained by their offshore nursery location which overlap with adult population distributions (Powles and Kohler, 1970; ICES, 2009). The ubiquitous presence of plaice and dab juveniles in all sampled nurseries in Galway Bay is in accordance with other studies; these species are reported to be the dominant contributors of sandy beach fish assemblages throughout the north-east Atlantic (Edwards and Steele, 1968; Poxton et al., 1982; Pihl, 1989; Gibson et al., 1996; Beyst et al., 1999; Amara, 2004; Désaunay et al., 2006), with their relative contributions varying depending on the geographic area. Lower occurrences of turbot and brill in our surveys may indicate later settlement, more restrictive habitat requirements and smaller adult stock sizes compared to plaice and dab (Gibson, 1994; Nissling et al., 2007; Haynes et al., 2011). Beach seine surveys targeting turbot and brill on west of Ireland nursery grounds recorded highest densities on coarse sediment in September and October (Haynes et al., 2010). Flounder and sole start to appear in nursery grounds in April and show estuarine dependency during the juvenile phase (Coggan and Dando, 1988; Hutchinson and Hawkins, 1993; Cabral, 2000; Amezcua and Nash, 2001; Martinho et al., 2008). High densities of flounder have been reported in low saline areas in Galway Bay after upstream migrations (Keirse, 2008), which may explain their low densities in this coastal survey. Flatfish assemblages sampled in this study appear to be representative of the juvenile flatfish community in coastal sandy nursery habitats on the west coast of Ireland.

Spatial variability in juvenile plaice and dab densities

Plaice and dab settlement occurred consistently in all sampled nursery areas over two years. Based on a comparison of juvenile densities, all four sampled flatfish nurseries in Galway Bay provided equally important nursery habitat for plaice and dab. Dab did not show density fluctuations between distinct nurseries whereas Ballyloughaun and Traught maintained more plaice than did Ballyvaughan and Silverstrand (Chapter 4). These spatial density fluctuations between nursery areas in Galway Bay indicate that a wide range of nursery habitats within a geographic area need to be considered when evaluating variability in year-class strength. This is confirmed by observations for other flatfish species suggesting that variation in year-class strength may be controlled by factors operating at a local scale (Leggett and Frank, 1997; Philippart *et al.*, 1998; Pihl *et al.*, 2000; Wennhage and Pihl, 2001; Stoner *et al.*, 2007; Vinagre *et al.*, 2009). A literature review in table 1 & 2 gives a good overview of the range of flatfish densities at peak settlement recorded over a wide geographic range. Timing of peak settlement is known to vary substantially between years within regions and between regions. Environmental conditions during the pelagic period are known to affect spawning period and larval duration (Hyder and Nash, 1998; Bailey et al., 2005), with consequent implications for peak settlement timing and numbers of settling flatfish. Settlement pulses as a result of extended periods of batch-spawning are common for plaice and dab and it is suggested to increase their probability of survival for a certain sub-cohort when experiencing profitable environmental conditions (Al-Hossaini et al., 1989; Geffen et al., 2011). Any attempt to determine geographic trends in peak settlement and densities must therefore account for interannual and spatial variability in environmental factors. Spatial comparisons should also consider higher recruitment variability at the outer edges of a species' geographic range, as is shown for several groundfish species (Miller et al., 1991). Considering this, plaice and dab densities recorded on west of Ireland nursery grounds are within the range reported in the literature.

The accuracy of density estimates derived from coastal nursery monitoring programs is highly dependent on well designed sampling schemes, appropriate sampling gear and statistical solutions to account for gear variability. In this study, a beach seine was used for the collection of plaice over a wide area within distinct nursery grounds in south-west Ireland (Chapter 2). Integrating a continuum of water depth by covering a large swept area (1380 m^2 in this study) into one sample is a quick and cheap sampling technique for the collection of fish and invertebrates (Ruth and Berghahn, 1989). A drawback is the considerable variability in the towing speed and swept area, with disparities in efficiency and escape rates as a result, hence density estimates derived from beach seining were not used in this study.

The widespread beam trawling technique was used for the quantitative surveys in Galway Bay (Chapter 3-5) since it provides a good base for density studies, given the constant swept area and because it allows for the investigation of small-scale habitat preferences. However, accurate estimates of fish densities, growth and mortality rates are dependent on the assessment of gear efficiency which in turn is affected by size dependent net avoidance by fish. In this study, no estimates of true catch efficiency of the net were made, and no previous study has tackled this for a 1.2 m beam trawl. For a 2 m beam trawl, which is more frequently used in surveys of young flatfish, experimental enclosure studies established an efficiency of 25 % for plaice smaller than 30 mm, 33.3 % for plaice greater than 30 mm (Edwards and Steele, 1968) and a higher escape chance of larger fish (100-150 mm) with a gear efficiency of 22-45 % (Kuipers, 1975). There are, as yet, no comparable gear efficiency estimates for dab but an experimental field study showed comparable trawl efficiencies for both species (Wennhage et al., 1997) and due to similar morphological appearance, previous efficiency factors are applied for both plaice and dab (Poxton et al., 1982). Other studies suggest differences in catchability between both species may occur as a result of behavioural differences (Rogers and Lockwood, 1989) encouraging further research to elucidate the catch efficiency of a 1.2 m and 2 m beam trawl for plaice and dab. Simultaneous sampling with different fishing gear can provide a more accurate overall description of community structure (Hinz, 1989; Gibson et al., 1996). Other factors influencing the catchability of fish include temperature, wind, turbidity, topography, substrate and tidal state, which affect fish activity and thus catchability (Hinz, 1989; Kuipers et al., 1992; Bolle et al., 2001). Functioning of the net will thus depend on ambient environmental conditions with gear efficiencies possibly changing over time and between geographic regions (Kuipers, 1975). Comparison and interpretation of density data from trawl studies in varying environmental conditions is thus hampered by a lack of information on catch efficiencies and size-selectivity. Adjustments of plaice and dab catch estimates were not applied in this study since sampling conditions in Galway Bay remained fairly consistent throughout the study and density measures should therefore provide a good indication of relative abundance as well as spatial and temporal differences in habitat quality.

Table 1. Summary of field studies investigating temporal fluctuations of juvenile plaice densities in the post-settlement phase with the detection of peak densities in different geographic regions. Comparative results of the application of gear efficiency corrections are presented.

Area	Survey period	Sampling stations	Sampling depth range (m)	Timing peak density	Peak density (ind. 100 m^{-2})	Density July (ind. 100 m^{-2})	Correction gear efficiency	Reference
Galway Bay, west of Ireland	June -Sept 2008 + 2009	4 regions	1 - 4.5	?	?	9.2 ± 2.6	no	This thesis
Southern Bight of North Sea	May – July 1998	4 stations	5-10 Subtidal zone	May	3.6	14.7	no	Amara *et al.*, 2001; Amara, 2003
Canche Bay, English Channel	June-Sept 1997	7 stations in estuary	0-10 Subtidal zone	Late June	4.6 ± 1.9	1	no	Amara, 2004
Icelandic waters	May-Oct 1999	20 stations	Tidal flat	Late June	10	5	no	Hjörleifsson and Pálsson, 2001
West of Scotland	1995-1997	3 beaches	1 - 5	May	2-50	2-20	no	Gibson et al., 2002
Laholm Bay, Swedish west coast	May 1984 - May1987	3 areas	1.5-11	July	10-50		35 % (Edwards and Steele, 1968)	Pihl, 1989; van der Veer et al., 1990
Wadden Sea	March-Nov 1973-1982		Tidal flat	May	15-50	5-20	no	Zijlstra *et al.*, 1982; van der Veer and Bergman, 1986
Subarctic, Northern Norway	April-Oct 2005+2006	3 stations	?	July	35-65	35-65	25 -100 % (Kuipers, 1975)	Freitas et al., 2010
Loch Ewe, West of Scotland	June – September 1964-1967	2 parts	1 - 8	June	58.8 – 138.7	15.9 - 58.0	no	Edwards and Steele, 1968
Canche Bay, English Channel	April-Sept 2000	3 stations	Intertidal zone	Mid-April	280	8	25 – 100 % (Kuipers, 1975)	Amara and Paul, 2003
Gullmar Bay, Swedish west coast	May-June 1978-1988		0-0.7	Late May	20-280		35 % (Edwards and Steele, 1968)	Pihl, 1989; Pihl, 1990; van der Veer et al., 1990
Gullmar Bay, Swedish west coast	April-August 1991-1992		0-0.7	May	144-1000	41-225	no	Modin and Pihl, 1996

Table 2. Summary of field studies investigating temporal fluctuations of juvenile dab densities in the post-settlement phase with the detection of peak densities in different geographic regions. Comparative results of the application of gear efficiency corrections are presented.

Area	Survey period	Sampling stations	Sampling depth range (m)	Timing peak density	Peak density (ind. 100 m^{-2})	Density July (ind. 100 m^{-2})	Correction gear efficiency	Reference
Galway Bay, west of Ireland	June-Sept 2008 + 2009	4 stations in Bay	1 – 4.5 Subtidal	?	?	2.9 ± 1.2	no	This thesis
North Sea	1972-1980	461 hauls	1 - 70	October	0.5	0.02	no	Bolle et al., 1994
Southern Bight of North Sea	May 1998 – May 1999	6 stations	5-10 Subtidal zone	July	1.5	1.5	no	Amara, 2003
Southern Bight of North Sea	May – July 1998	4 stations	Around 5	July	1.5	1.5	no	Amara et al., 2001
Swedish west-coast, Kattegat	May 1984 - May 1987	3 areas in Bay	1.5 - 11	October	1.8	0	35 %	Pihl, 1989
Port Erin Bay, Irish Sea	April – December 1996-2001	5 transects	4 - 6	Start August	3.5	3.5	20 % prior to June, 30 % after (Nash et al., 1992)	Beggs and Nash, 2007
West of Scotland	1995-1997	3 beaches	1 - 5	July-August	2-12	1-8	no	Gibson et al., 2002
Wadden Sea	Sept 1971 – August 1972	2 stations	Around 1	October	12	0	no	Bolle et al., 1994
Canche Bay, English Channel	June-Sept 1997	7 stations in estuary	0-10 Subtidal zone	Late June	20.7 ± 15	10	no	Amara, 2004
Loch Ewe, West of Scotland	June – September 1964-1967	2 parts	1 - 8	June-July	7.1 - 57.5	2.0 - 9.6	no	Edwards and Steele, 1968
Dutch North Sea	1978-1982	4 stations	0.5-7	October	85	2.5	no	Bolle et al., 1994

Spatial, temporal and trophic resource partitioning

Coastal marine habitats are very productive and support high abundances and diversity of fish and invertebrates with similar niche requirements (Beck *et al.*, 2001). Competition between species simultaneously occupying these habitats can be reduced by differences in resource use between species (Schoener, 1974). Small-sized flatfish are particularly vulnerable to competition (Piet et al., 1998) and co-evolution of competitors may cause this resource partitioning (Connell, 1980). In this thesis, partitioning in time, space and diet was assessed for the dominant flatfish species in Galway Bay to investigate if competition in the post-settlement phase is a potential factor affecting year-class strength of plaice and/or dab.

Temporal resource partitioning between plaice and dab was revealed from monthly length-frequency distributions (Fig. 2; Chapter 4); length ranges of dab in June were generally smaller than those of plaice and settlement of plaice in June had ceased whereas dab settlement continued up to July. This earlier settlement of plaice compared to dab is supported by studies in Scottish Bays and the English Channel (Steele and Edwards, 1970; Amara, 2004). The exact timing of peak settlement was not identified since the sampling period (from June until September) started after plaice settlement was completed and towards the end of the settlement period for dab. Settlement peaks of plaice have been previously recorded in west of Ireland nurseries between early March and late April (Allen et al., 2008) but further otolith increment analyses are needed to investigate timing of peak settlement for dab.

Spatial resource partitioning between plaice and dab in Galway Bay was suggested by their distinct patterns of distribution. Depth-stratified sampling from <1 m to 5 m revealed a higher prevalence of plaice in the intertidal zone between 1 and 3 m depth, whereas dab displayed highest densities at the deepest sampling locations and were rarely caught in the intertidal area (Fig. 5; Chapter 4). Our random sampling with regards to tidal heights did not allow for investigations of plaice movements relative to tidal height but it is known that tidal rhythmicity is a behavioral adaptation of plaice which decreases spatial overlap with other species like dab that only display diel rhythmicity (Gibson, 1973; Nash et al., 1994; Gibson et al., 1996). Depth has also been identified as an important resource partitioning factor between juvenile flatfish in Alaskan inlets (Norcross et al., 1997), in British waters (Riley et al., 1981) and within Pacific coast estuaries (Rooper et al., 2006). Since depth incorporates information of various habitat variables, it may be the complex interaction between i.a. sediment composition, prey availability, predator densities and physicochemical conditions that determines the spatial separation between plaice and dab. Depth distributions of settling dab have not been previously studied on the west coast of Ireland but high records of 0-group dab in the annual Irish groundfish surveys suggests that settlement also occurs in deeper waters (David Stokes, Pers. Comm.). This is in agreement with reports from the North Sea, Irish Sea, west of Scotland and south-eastern Kattegat where peak densities of dab are found within 10 m and where settlement extends in offshore waters up to 70 m depth (Edwards and Steele, 1968; Pihl, 1989; Bolle et al., 1994; Dickey-Collas et al., 1997; Beggs and Nash, 2007). Early life history of plaice and dab are thus potentially influenced by different environmental conditions. Impacts on shallow nursery grounds (e.g. by beach erosion or rising seawater temperature) or on deeper nursery grounds (e.g. by decreased prey availability due to pollution) may result in habitat loss for plaice and dab respectively. Increased competition is thus expected when species are forced to occupy overlapping distributions or exploit more of the same resources.

Trophic resource partitioning between morphologically similar plaice and dab in Galway Bay was detected for fish >50 mm whereas a high diet similarity was found for the smaller fish feeding on meiofaunal prey (Fig. 2; Chapter 3). The lack of evidence for inter- and intra-specific competition negatively influencing the density or condition of either species implied that flatfish densities did not exceed the carrying capacity of the nursery grounds in Galway Bay. Contrasting results were reported in nursery areas in the Irish Sea and the Wadden Sea where density-related processes on juvenile plaice occurred (van der Veer, 1986; Nash and Geffen, 2000), possibly due to higher fish densities. Considering that intensity of competition is dependent on the density of predators and prey (Evans, 1983), the absence of competition between plaice and dab in Galway Bay is not a static finding and should be evaluated on the long term using an ecosystem based approach. The application of the self-thinning rule by Nash et al. (2007) revealed evidence of occasional density dependent effects in British plaice nursery grounds in years supporting high population levels (14 indiv. m^{-2}). A difference in productivity between single nursery grounds was reflected in the elevation of their dynamic thinning slope, demonstrating the interplay of benthic biomass and flatfish densities as a regulating factor in supporting the settlement of large flatfish cohorts (Nash et al., 2007). Simultaneous resource separation in time and space may add to the minimized niche overlap between juvenile plaice and dab, in accordance with partitioning of flatfish communities in other areas (Cabral et al., 2002; Attrill and Power, 2004; Vinagre et al., 2005; Rooper et al., 2006).

Variability in juvenile growth and survival

Habitats with higher juvenile densities are likely to make a greater contribution to the adult stock. However, examining a single factor, such as fish density, in various habitats does not provide a conclusive measure of the nursery value of a habitat (Beck et al., 2003). Small annual differences in growth and mortality rates in the early life stages of fishes can generate large fluctuations in year-class strength and subsequent recruitment (Houde, 1987). Faster growing fish that reach a larger size-at-age will generally gain a survival advantage over fish with a smaller body length (Suthers, 1998). Mechanisms supporting the increased survival probability of larger and faster growing individuals involve the reduced susceptibility to predators (Gibson et al., 1995; Wennhage, 2000), a shortened stage duration of the most vulnerable period to predation and starvation (Chambers and Leggett, 1992; Houde, 1997), better foraging success (Dower et al., 2009) and better tolerance to environmental extremes and diseases (Sogard, 1997).

The different metrics used in this thesis as proxies for growth rate did not display very consistent trends in relative habitat quality between nursery areas and/or years. Over short periods, marginal increment widths proved to be a reliable indicator of somatic growth with a strong link to feeding conditions (Chapter 2), consistent with other studies (Karakiri et al., 1989; Suthers et al., 1992; Amara and Galois, 2004; López-Rasgado and Herzka, 2009). Estimates of habitat-related growth rates based on marginal increment widths of fish varying in size or age requires intercalibration and complicates the application of this growth proxy, as demonstrated by the study of Gunnarsson et al. (2010). The morphometric condition factor (Fulton's K) detected similar spatial patterns in habitat quality as well as additional interannual variability. Longer-term variability in habitat quality is thus integrated in this growth proxy which is more easily implemented in several surveys (Claireaux et al., 2004). Similar interannual differences in growth rates were observed using RNA:DNA ratios and Fulton's K condition, even when only three fish per haul were studied (Chapter 3 and 5) or when only the subtidal area was assessed (Chapter 5). The biochemical and morphometric condition indices were not correlated and displayed considerable spatio-temporal variability. RNA:DNA ratios appeared to be more sensitive to short-term environmental fluctuations since between-site variability in RNA:DNA ratios were not consistent across years. In contrast site-related patterns in Fulton's K for plaice and dab showed no inter-annual variation. Fulton's K proved to be advantageous for routine use whereas RNA:DNA ratios may provide a powerful tool to detect periods of slow growth in environments affected by anthropogenic impacts or intense natural fluctuations (Dahlhoff, 2004; Amara et al., 2009). Low correlations between different growth proxies derived from the same individual are widely detected in other studies (Gilliers et al., 2004; Vinagre et al., 2008; Vasconcelos et al., 2009) and may be due to 'latency', the difference in temporal responses of the indices to environmental factors (Suthers, 1998). It was concluded from this study that both condition indices represent different ecological information and should be used in a complementary fashion to give an adequate description of habitat quality.

At a population level, growth and mortality rates are traditionally calculated from cross-sectional sampling data and the comparison of catches over several time intervals. The slope of the regression of the natural logarithm of catch abundances against age or length can be calculated as a measure of instantaneous mortality whereas the growth rate can be estimated as the difference in average length from one time period to the next. Demographic analyses of growth of 0-group plaice and dab populations was carried out in isolated inshore nursery areas in Galway Bay (Chapter 4). Spatial variability in these population estimates was not examined in greater detail since strict assumptions limit the usefulness of these catch-curve analyses (Miller et al., 1997). Growth and mortality must be constant throughout the duration of the study to allow accurate estimates of growth and mortality rates. However, fish mortality generally decreases through successive life history stages. A strong dependence of mortality on developmental stage was demonstrated in the literature for plaice, with highest mortalities in the egg and larval stages and a decline in the demersal 0-group fish in summer and autumn (Zijlstra et al., 1982). Although the change in mortality rate is most dramatic with a change from one to another life history stage (Anderson, 1988), it is suggested that size-dependent mortality also occurs for several species within the egg (Rijnsdorp and Jaworski, 1990; Rijnsdorp and Vingerhoed, 1994), larval (Pepin, 1993; Hare and Cowen, 1997) and juvenile (Steele and Edwards, 1970; Post and Prankevicius, 1987) stage. Ongoing work by the author (not presented in this thesis) promises to provide more insight into the population dynamics of juvenile plaice and dab after peak settlement using length-based cohort modelling which integrates both growth and mortality rates by progression of cohorts (DeLong et al., 2001). These models allow growth and mortality to decrease with increasing length and inference can be drawn to make prediction of which individuals are likely to survive the early stages where mortality rates are extremely high and variable.

Spatial variability in juvenile movement

To examine the consequences of the detected spatial variation in juvenile densities and growth rates between nursery areas in Galway Bay on the recruitment to the adult stock, it is crucial to investigate the connectivity amongst geographically separated juvenile and adult populations. This novel approach may simultaneously identify essential nursery habitat for juvenile fish; where an above average number of juveniles (in terms of overall contribution or contribution per unit area) contributes to the adult stock (Beck et al., 2001; Dahlgren et al., 2006). Several techniques could be used to identify the unknown fish movements between west of Ireland nursery grounds and different feeding and spawning grounds; including chemical, genetic and parasitic tags (Gillanders et al., 2003). Naturally occurring elemental signatures in otoliths have shown great promise as a tool to discriminate coastal and estuarine juvenile fishes over wide geographic ranges (Campana et al., 1995; Gillanders, 2005; Brown, 2006; Elsdon et al., 2008). These chemical habitat tags in otoliths have identified effective juvenile habitat for various fish species based on their proportional contribution to the adult stock (Thorisson et al.; Gillanders, 2002; Rooker et al., 2008; Vasconcelos et al., 2011). The continuation of population dynamics studies in the west of Ireland is therefore encouraged with the aim to determine the quantitative contribution of recruiting fish from separate nursery areas to the adult stocks.

Regulating processes of year-class strength in post-settlement period

It is accepted that population regulation in flatfish operates mainly on the pelagic life stages (Bannister *et al.*, 1974; Leggett and Deblois, 1994; van der Veer *et al.*, 1998; Fox *et al.*, 2000b; Wennhage *et al.*, 2007; Oshima *et al.*, 2010). Further variability-generating processes in the demersal stage can determine year class strength (Pihl, 1990; Nash and Geffen, 2000). Despite the detected difference in year-class strength of dab in Galway Bay, longer time series are required to evaluate settlement variability and allow inference on factors (playing at the pelagic and juvenile phase) affecting interannual differences in year class strength. This could lead to important observations such as the exceptionally strong year classes for North Sea plaice in 1963, 1985 and 1996 (Fig. 2; Chapter 1) which had considerable effects on the spawning stock biomass and the production of consequent strong year classes (van der Veer *et al.*, 2000; Secor, 2007). The complex relationships between biotic and abiotic habitat components were studied to gain insight in the factors influencing growth of, and habitat quality for, plaice and dab juveniles. Four major processes (feeding success, predation, burial behavior and metabolic activity) were identified to be responsible for variability in habitat quality within and between distinct nursery areas along the west coast of Ireland.

Feeding success

Food availability is one important determinant of habitat quality that can affect condition and growth, and thus survival, of flatfish (Gibson, 1994). The level of intraspecific variation in plaice diet that occurs at small-scales is relatively unknown despite the many dietary studies of this ecological and economic important flatfish species (Edwards and Steele, 1968; Braber and de Groot, 1973a; Kuipers, 1977; Whyche and Shackley, 1986; Beyst *et al.*, 1999; Amara *et al.*, 2001; Rijnsdorp and Vingerhoed, 2001). Diet composition in plaice guts differed at two different scales in the south-west of Ireland: among beaches and hauls, most likely as a result of different food availability (Chapter 2). Differences in food availability in other studied areas have also caused growth variability of plaice (Karakiri et al., 1989; van der Veer and Witte, 1993; Berghahn et al., 1995) and other flatfish species (Amara, 2003; Sparrevohn and Støttrup, 2008), emphasizing that food quantity and quality should be of major interest during the assessment of nursery habitat quality. The negative correlation between prey diversity in the guts and plaice growth (expressed by both marginal otolith increment width and Fulton's K condition factor), suggests that this is a valuable indicator for habitat quality on a comparative basis. The link between diet and growth through the quantity and quality of available macrobenthic prey in the sediment was confirmed in Chapter 3. These results highlight the importance of considering small scale variation when attempting to link habitat quality to feeding, growth and condition of juvenile flatfish. New insights into intraspecific differences in mouth morphology of plaice were revealed in Chapter 2 and a possible link to predation on different prey type was suggested. This topic invites further experimental and field research to assess the degree of morphological adaptability to changing environments and the possible use in stock identification studies.

Predation

Predation is regarded as the major cause of flatfish mortality in the demersal stage (van der Veer and Bergman, 1987; van der Veer et al., 1990; Bailey, 1994) and probability of predation decreases with size for many flatfish species (Cushing, 1974; Seikai et al., 1993; Witting and Able, 1993). Field studies quantifying size-selective mortality in juvenile flatfish are scarce. Experimental studies with juvenile plaice established a clear decrease in predation rate by shrimps and crab when reaching 30 mm and 50 mm respectively (van der Veer and Bergman, 1987). The approach adopted in this thesis allowed for a simultaneous evaluation of predation pressure from crustaceans on fish densities and condition. This revealed that crab densities were negatively correlated with condition of plaice and dab whereas shrimp densities were negatively associated with densities of small-sized juveniles in spring. A longer time series could elucidate how interannual variability in the impact of shrimps is affected by local temperature conditions. High spring temperatures in combination with variable winter temperatures in Swedish bays have resulted in fluctuating shrimp densities between years with an increased variability in plaice recruitment as a result (Pihl, 1990; van der Veer et al., 1990). Density-dependent mortality has been postulated to decrease recruitment variability in the Wadden Sea (van der Veer, 1986). Given the importance of predation, long-term quantitative field sampling in Galway Bay starting at the timing of metamorphosis is suggested to investigate if density-dependent mortality occurs at a certain life-stage and if this determines year-class strength of plaice and dab. The postulated negative impact of crab densities on fish growth in Galway Bay is an important finding given the difficulties associated with extracting information on the effect of one variable in complex natural environments. Due to consistent results for plaice and dab and the relatively high percentage explained variability in both Fulton's K and RNA:DNA ratio (Chapter 4 and 5), predation was concluded to be the most important factor affecting growth of juvenile plaice and dab. Reduced activity levels, increased burying behaviour and feeding delays are the major mechanisms accounting for growth reductions imposed by predation risk for a variety of flatfish species (Burrows and Gibson, 1995; Wennhage and Gibson, 1998; Gibson et al., 2002).

Burial behaviour

Substrate-mediated burial success was suggested to indirectly increase growth rates due to the high correlation of larger sediment size particles (and negative association of muddy areas) with increased Fulton's K and RNA:DNA ratio of both plaice and dab (Chapter 4 and 5). 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 are known to help reduce metabolic rates and conserve energy (Howell and Canario, 1987). Burial behavior studies showed that newly metamorphosed plaice are unable to bury in grain sizes larger than 500 µm (Riley et al., 1981) and avoid sediment with more than 10 % mud fraction (Jager et al., 1993), so it is likely that particle size fractions towards these extremes negatively affect fish growth. Since sediment structure co-varied with other substrate characteristics (particle size, prey abundance and organic content), additional experimental research is suggested to confirm the direct growth effect from sediment structure.

Metabolic activity

Despite the importance of temperature as a controlling factor of fish growth by regulating the metabolism (Fry, 1971; Fonds et al., 1992; Yamashita et al., 2001), the results of this thesis reveal no consistent temperature effects (Chapter 4 and 5). A direct link between temperature and juvenile densities and Fulton's K condition was not detected in Chapter 4 where GLMs were applied to investigate linear relationships with habitat variables. Using a GAM approach was more successful to detect non-linear relationships between temperature and two different proxies of growth for dab (Chapter 5). The lack of any relationship with temperature in density or condition of plaice may reflect interspecific differences in growth-related temperature optima (Bolle et al., 1994). In addition, tidally mediated movements in plaice confer a higher degree of plasticity in their response to temperature fluctuations. These findings also highlight the potential of GAMs to establish non-linear relationships between condition indices and the additive effects of habitat variables to aid in understanding ecological processes regulating fish growth. Effects of temperature on the growth rate of juveniles have implications for the length at which maturation is attained because fast-growing fish not only mature at an earlier age but are also longer at maturity than slow-growing fish (Gibson, 1994). As previously mentioned, temperature extremes may also have an indirect effect on fish survival through the effect on predator densities, as was demonstrated in the Wadden Sea where a strong year class of plaice was attributed to abnormally low temperatures, reducing predators to a minimum (Zijlstra and Witte, 1985).

Metabolic loss due to osmoregulation is an important factor in spatial and temporal growth variability of juvenile flatfish (Yamashita et al., 2001). Flatfishes tolerate high variations in salinity but the optimum ranges for growth is species and stage specific (Jager et al., 1993). Like many marine teleosts, flatfish have an internal osmotic pressure corresponding with intermediate salinities (8-16), at which energetic costs are minimized and optimal growth of juvenile flatfish is expected (Boeuf and Payan, 2001; Imsland et al., 2001; Imsland et al., 2002). Higher abundances of dab in less saline areas in Galway Bay (Chapter 4) may reflect an adaptation to reduce osmoregulation and improve growth. This phenomenon has been observed for a variety of species (Marshall and Elliott, 1998). Direct associations between salinity and juvenile growth were, similar to the effect of temperature, not detected when linear relationships were considered (Chapter 4). However, a peak in condition, generally occurring at salinities between 26 and 28, was presented in Chapter 5. Different mechanisms possibly accounting for the discrepancy between expected and observed peaks in salinity were discussed (selection of areas with more stable salinity, co-variance between salinity and another habitat variable, trade-off between salinity preference and optimal levels of other habitat features) and emphasized the limited knowledge of physiological responses to salinity and the interplay with other environmental factors throughout ontogenetic development.

Assessing juvenile growth performances at an individual or population level is complicated by the dynamic nature of estuarine and coastal zones. Modeling approaches have been developed to increase our understanding of physiological responses in environmental dynamic conditions. Application of the dynamic energy budget (DEB) theory may add insight in growth parameters linking various physiological processes in a mathematical framework to capture the energy budget of organisms (Kooijman, 2000; van der meer, 2006). Another ecophysiological framework to estimate fish growth based on the effect of temporally fluctuating environments on fish metabolism and bioenergetics (Neill et al., 2004) has been a successful approach to assess habitat quality for juvenile sole (Fonseca et al., 2010). The established mechanisms of individual plaice and dab response to environmental conditions may aid in the application of this modeling approach to the flatfish populations in west of Ireland nursery grounds.

Threats to sandy beach ecosystems

Increased human activities along rivers, estuaries and in coastal areas affect ecosystem structure and functioning (Blaber et al., 2000; Diaz and Rosenberg, 2008; Halpern et al., 2008). Habitat loss and degradation are the most serious threats affecting the nursery function for various flatfish species due to activities such as coastal development, hardening of shorelines and dredging activities (Ahn and Choi, 1998; Courrat *et al.*, 2009; Defeo *et al.*, 2009; Rochette *et al.*, 2010). Economic complications of coastal deprivation can be valuated by combining ecological and economic models to calculate decreased profits in fisheries or recreational activities (Paulsen, 2007). This thesis identifies ecological processes shaping flatfish assemblages on nursery grounds which can be used to predict how human and climate induced changes to the environment may affect flatfish communities. Given the designation of Galway Bay as a candidate special area of conservation (SAC), the described structure and functioning of Galway Bay nurseries and the potential impacts from anthropogenic sources may facilitate the development of coastal zone management plans to ensure a sustainable use of coastal resources.

Organic enrichment of coastal sediments caused by eutrophication encourages microbial activity and the consumption of dissolved oxygen in bottom waters (Diaz and Rosenberg, 2008). This increases events of oxygen depletion, which may be persistent in areas prone to stratification, and can have reverse effects on benthic faunal abundance and diversity (Pearson and Rosenberg, 1978). Although dissolved oxygen levels were not measured in this study, associations of high organic enrichment with low benthic diversity, plaice density and dab condition (Chapter 3 and 4) were detected in Ballyloughaun. This area is located in the vicinity of the outfall of Mutton Island treatment plant, which could explain the higher sediment organic matter as a result of the flocculation out of suspended solids present in the effluent (Hall et al., 1997). A high exposure to reduced oxygen, potentially following increased organic matter, can directly influence fish metabolism (Lefrançois and Claireaux, 2003). Laboratory experiments have revealed that low levels of oxygen deficiency may affect predation efficiency of juvenile flounder (Tallqvist et al., 1999), reduce food intake and conversion efficiency of juvenile turbot (Pichavant et al., 2001; Person-Le Ruyet et al., 2003). These mechanisms may translate in subsequent reduction of fish growth at continuous or fluctuating exposure to reduced oxygen (Bejda et al., 1992; Petersen and Pihl, 1995; Taylor and Miller, 2001; Yamashita et al., 2001; Stierhoff et al., 2009). Tolerance limits to low oxygen concentrations are species specific and avoidance responses have been detected several flatfish species (Marshall and Elliott, 1998; Tallqvist et al., 1999; Brady and Targett, 2010). It is expected that decreased fish growth as a result of lower fish metabolism and movements from patches of anoxic sediment to less optimal habitat in terms of other habitat variables may affect recruitment levels (Gibson, 1994).

Opportunistic macroalgae species such as mat-forming *Ulva* spp. and filamentous *Cladophora* spp. and *Enteromorpha* spp. are natural components of coastal ecosystems (Grønkjær et al., 2007). As a consequence of increased eutrophication in shallow waters, the formation of algal mats on nursery grounds has been a regular phenomenon in recent years (Pihl et al., 1996; Pihl et al., 2005). Compared to these previous reports, macroalgae cover was relatively less severe in the areas surveyed in Galway Bay but a negative association was detected with small-scale differences in plaice densities (Chapter 4). In other nursery areas, this trend may have lead to a concentration of juveniles in the remaining sand habitats and an increased density dependent predation by shrimps (Wennhage, 2002). Other impacts associated with persistent algal mats: reduced habitat complexity, reduced foraging efficiency and oxygen deficiency (Everett, 1994; Norkko and Bonsdorff, 1996; Aarnio and Mattila, 2000; Nordström and Booth, 2007; Carl et al., 2008) provide evidence of the severity of this phenomenon with resulting reduced growth and recruitment for fish dependent on these shallow sandy beaches.

Management actions are therefore crucial to prevent alterations in nursery habitat structure with increased organic enrichment and the presence of algal mats. Pollution as a result of increased anthropogenic activities is also an increasing global problem with significant effects on flatfish in several estuarine and coastal systems (Le Pape *et al.*, 2003b). An inverse relationship between exposure to contaminants and growth rates, condition and survival has been widely observed for a variety of fishes (Burke et al., 1993; Smith and Suthers, 1999; Rowe, 2003; Alquezar et al., 2006). The benthic association of flatfish and dependence on infaunal invertebrates as prey makes them especially vulnerable to sediment contamination. Concentrations of sediment contaminants, including heavy metals and polycyclic aromatic hydrocarbons (PAH), were the most important contributor of the growth variability of juvenile sole from seven nursery sites in the Southern Bight of the North Sea (Amara et al., 2007). Growth reductions in several other flatfish species were experimentally observed after exposure to petroleum oil (Moles and

Norcross, 1998; Claireaux et al., 2004). Chemical data on these other potentially environmentally damaging compounds should be monitored in nursery areas to establish temporal pollution trends in Galway Bay and used in coastal zone management.

Changes in the nursery functioning of nearshore marine ecosystems have been related to global warming. The expected rise in sea level, coupled with an increase and/or intensity of storms is likely to lead to escalating beach erosion and consequent loss of habitat (Brown and McLachlan, 2002). Extreme droughts and floods may alter fish community composition in coastal and estuarine habitats (Martinho et al., 2007; Diouf et al., 2009) or directly eliminate parts of populations (Scheffer et al., 2001). Temperature increases at shallow coastal beaches may lead to an offshore movement of juveniles as a reflection of the optimal growing temperature which is reported to be between 15-18 ºC for dab and between 18-20 ºC for plaice (Attrill and Power, 2004). A movement of young plaice into deeper water, most likely as a response to higher winter temperatures, has been observed in the North Sea (van Keeken et al., 2007) but the impact of higher predation rates and different prey assemblages at greater depths is unknown. Northward shifts of various flatfish species have already been identified in synchrony with winter warming over the last decades (Cabral et al., 2001; Désaunay et al., 2006; Hermant et al., 2010; van Hal et al., 2010). In years when sea water temperature was warmer during the pelagic stage, lower abundances of juvenile flatfish have been recorded at nursery sites (Haynes et al., 2010). A simultaneous increase in the abundance of predators at the time of settlement (Pihl, 1990; van der Veer et al., 1990) may result in a two-fold negative effect on warming seawater for the year-class strength of flatfish. Given the high influence of temperature and hydrodynamics during the pelagic phase on year-class strength (Wilderbuer *et al.*, 2002; Nash and Geffen, 2004; Vinagre *et al.*, 2007; Bolle *et al.*, 2009), factors influencing all life stages is necessary to predict the effects of climate change on flatfish populations (Rijnsdorp and Van der Veer, 2008). More research is needed to predict the complex interrelated impacts of the predicted future changes in sea temperature, salinity, productivity, and hydrodynamics on flatfish populations, especially for the early life stages which will be most negatively affected by climate change (Rijnsdorp et al., 2009).

Conclusions

Management of flatfish populations is in general impaired by limited knowledge of different aspects of their biology and the effect of natural environmental variability on year-class strength. Comparisons of nursery habitat quality for juvenile plaice and dab in Galway Bay improved the understanding of ecological processes and mechanisms structuring flatfish assemblages in this vulnerable early life stage. Influences of biotic and abiotic habitat characteristics on the distribution, abundance and growth of juvenile plaice and dab were assessed and the resulting species-habitat associations may aid prediction of the possible consequences of changing coastal nurseries in altering recruitment. Characterization of the structure and functioning of coastal nurseries in Galway Bay allows effective monitoring and management to protect these vulnerable ecosystems against further degradation and loss of habitat.

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