What Makes a Habitat a Home: Understanding Settlement and Recruitment Variation in European Sea Bass, *Dicentrarchus labrax*.

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ABSTRACT

Sea bass stocks in the UK are in decline as a result of increased fishing pressure and variable inter-annual recruitment. Recruitment variation is driven by survival in the early life stages; therefore, nursery habitats are thought to be able to stabilize recruitment through providing optimal growth conditions for juvenile fish. A thorough understanding of the factors that drive juvenile sea bass survival is needed, however, our understanding of what constitutes quality nursery habitat for juvenile sea bass is weak, with current knowledge based almost solely on saltmarshes. Juvenile sea bass were sampled using conventional seine and fyke nets across estuarine habitats, alongside dietary DNA metabarcoding to assess their distribution diet and condition, using measures of abundance, condition, stomach fullness, and diet. To determine whether the mechanism of larvae entering estuarine nurseries is an active or passive process the vertical distribution patterns of larval sea bass were compared across tidal cycles. Finally, over-winter survival was predicted based on energy budget modelling and temperature-dependent growth experiments, based on *in-situ* measurements of winter temperatures. Juvenile sea bass did not differentially select high tide habitats, but saltmarshes and sand provided increased foraging success. At low tide, however, sea bass were more abundant in complex habitat with lower foraging success. Diets mainly consisted of decapods and polychaete worms across habitats, but there was evidence of increased planktivory over mud. Larval sea bass did not show evidence of flood tide transport and likely rely on passive tidal forcing to migrate into estuaries, or they are trying to retain to deeper water. According to our models, winter thermal minima resulted in complete cohort loss in all scenarios on the East coast. The results of this study suggest that multiple habitats along the estuarine mosaic are important for juvenile sea bass at some point, and that a seascape approach to management is necessary, however, winter temperatures likely present a more extreme bottleneck to recruitment.

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CHAPTER ONE: GENERAL INTRODUCTION

FACTORS AFFECTING INTER-ANNUAL RECRUITMENT VARIATION IN JUVENILE MARINE FISH: IMPLICATIONS FOR EUROPEAN SEA BASS.

*Juvenile European sea bass, *Dicentrarchus labrax*

1. INTRODUCTION

In marine fish, the number of juveniles that survive to join the adult spawning population is arguably the most important factor in determining stock sizes between years, and is known as recruitment (Houde, 1987; Shulman & Ogden 1987; Beyer 1989; Caley *et al*., 1996; Caselle 1999; Armsworth 2002; Pepin, 2015). However, we know very little about the underlying drivers of recruitment variation and this has become a vital goal in fisheries biology. Many studies, particularly regarding coral reef assemblages, show a high degree of correlation between years of high recruitment and subsequent increases in year-class strength (Shulman & Ogden, 1987; Jones, 1990; Doherty & Fowler, 2003). A population whose growth is largely determined by recruitment, over other post settlement interactions, is said to be recruitment-limited (Hixon, 1998). There is a wealth of evidence of recruitment-limited populations, where in most cases fish catches are positively correlated with sites of higher recruitment, more so than other interacting factors (Karlson & Levitan, 1990; Forrester, 1995; Peterson *et al*., 1996; Caselle, 1999; Doherty & Fowler 2003). Therefore, measures of recruitment are useful in predicting stock resilience and have been included in many models used in fisheries management (Beraud *et al*., 2017; Walker *et al*., 2023).

Variable recruitment is thought to be largely influenced by mortality during the early life stages but, particularly egg and larval stages, where some species experience losses of up to 70% (Houde, 1987; Beyer 1989). Large degrees of cohort loss are experienced by many species during the pre-settlement periods, and this is largely thought to be driven the physical environmental conditions the larvae are exposed to, namely temperature and salinity, but also from biological factors through predation from planktivores (Houde, 1987; Beyer 1989; Meekan & Fortier, 1996; Garrido *et al*., 2015). Temperature is profoundly linked to physiological processes in fish, such as growth rates, metabolism and foraging efficiency which may all play an important role in the survival of larval fish (Letcher *et al*., 1996). However, these factors do not often act independently, for example, lower temperatures may result in reduced growth of larval fish, and in cases where there is an abundance of predators this may mean that larvae are unable to escape the window of predation, resulting in reduced recruitment (Quist *et al*., 2004). Temperature-dependent growth is also size-dependent, where larvae who are smaller larvae will grow at a slower rate than larger, therefore, increasing their predation window (Garrido *et al*., 2015). Food availability is another important factor in larval fish survival, where once the maternal yolk sac has been depleted there is a critical window within which to find the first meal or suffer large mortality events, resulting in reduced recruitment downstream (Houde, 2004, 2002).

The larval stage is often considered more important than the settled juvenile stages, due to the large amounts of mortality that restrict supply to regions, but this only really applies when settlement densities are not sufficient to create restrictions on resources (Steele, 1997). Steele (1997), however, argues that competition for resources is not the only driver of density dependent mortality, but other factors such as predation are important. These factors are often related to the habitat that the sedentary stage occupies, for instance densities of invertebrate prey that juvenile fish predate upon are linked to more habitat complexity (Bell *et al*., 2013; Hu *et al*., 2016; Wolters *et al*., 2018). Therefore, for these settled stages to persist there must exist habitat that supports the correct prey types to support food acquisition and subsequently growth, but also refuge from potential predation. Therefore, a better understanding of these habitat functions for recruitment limited species, where mortality is driven by density-dependent mortality in the sedentary stage, may allow us to target management efforts towards habitats that best support survival to adulthood and aid recruitment. For many important fish stocks, we do not understand whether a) larval fish inputs vary from region to region or b) whether variation in survival at the juvenile stage is responsible for differences in recruitment.

Crucially, we understand very little about how these complex environmental interactions drive juvenile fish survival and the implications on recruitment.

Nurseries are thought to provide habitat that supports predation refuge, foraging opportunities, and stable physical parameters. As a result, they are though to promote survival throughout the development of these early life stages, thus the potential to reduce recruitment variation between years in populations who are recruitment limited (Nash & Geffin, 2000; Nagelkerken *et al*., 2002; Pihl *et al*., 2005; Beraud *et al*., 2017). Beck *et al*., (2001) was the first paper that suggested a unified definition of what constitutes a nursery habitat, in what they termed the nursery role hypothesis. The nursery role hypothesis states that habitats that contribute disproportionately to recruitment of juvenile fish to the adult stock are considered nurseries (Beck *et al*., 2001). This works on the basis that some habitats may support growth and survival of a larger number of juvenile fish per unit area than other habitats where juveniles are found. However, Dhalgren *et al*., (2006) suggested that this definition of nursery habitats does not fully appreciate the major contribution to recruitment of habitats that support large numbers of juveniles, irrespective of area, which they term effective juvenile habitat (EJH). It is not suggested that these theories should oppose one another, but function in tandem to fully understand the habitats that contribute to high recruitment outputs. Nagelkerken *et al*., (2015) took this theory a step further, by unifying both theories under the seascape nursery approach. The seascape concept considers the multiple connected habitats that contribute to the growth, survival and recruitment of juvenile fish to the adult population (Nagelkerken *et al*., 2015), and this appears to be gaining traction within modern approaches to investigating estuarine habitat functions (Weinstein & Litvin, 2016; Amorim *et al*., 2018; Gilby *et al*., 2018; Henderson *et al*., 2021; Swadling *et al*., 2022). However, all cases operate under a similar framework for nursery identification, which is that a habitat should support increased abundance, survival and contribution of juvenile fish to the adult population. Survival in this instance is often measured through proxies, that provide a robust indicator of survival, such as growth rates and condition, but also habitat characteristics such as predation and food availability (Nagelkerken *et al*., 2015). Contribution, however, is far more difficult to assess, and requires an understanding of individual survival rates and the habitats used by that animal. To achieve this telemetry, population genomics and stable isotopes can be used to determine natal habitats and genetic connectivity between adult and juvenile populations. The early-life stages of many marine species utilize coastal nursery habitats, from both tropical mangroves to temperate estuaries, shallow bays, and seagrass beds. However, the ecosystem services that these habitats provide for juvenile fish, and how they influence survival is largely unknown. The prevailing theory is that they offer habitat that minimizes the trade-off between predation refuge and foraging potential (Sheaves *et al*., 2014; Nagelkerken *et al*., 2015).

European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758), herein referred to as sea bass, are large Perciformes that are predominantly marine, found in coastal and estuarine waters throughout their geographic range, spanning from Norway to Senegal and the Mediterranean (Pickett & Pawson, 1994; Sanchez & Munoz-Cueto, 2014; Cardoso *et al*., 2014). It is a member of *Moronidae*, a family that includes the North American striped bass (*Morone saxitillus*), white perch (*Morone americana*), and the South-East Atlantic spotted sea bass (*Dicentrarchus punctatus*). The majority of these species are either freshwater or brackish, found in riverine and estuarine systems across their range (*M. saxitillus* - Mohan *et al*., 2014; Baker *et al*., 2016; *M. americana* – Kerr & Secor, 2012; *D. punctatus* – Selleslagh *et al*., 2014). Superficially, sea bass are similar to the perch of freshwater systems, often garnering the common name ‘sea perch’ in many countries across Europe (Pickett & Pawson, 1994). The morphology of a typical sea bass is illustrated in Figure 1, though it should be noted that several morphological variations are displayed across their range (Pickett & Pawson, 1994).

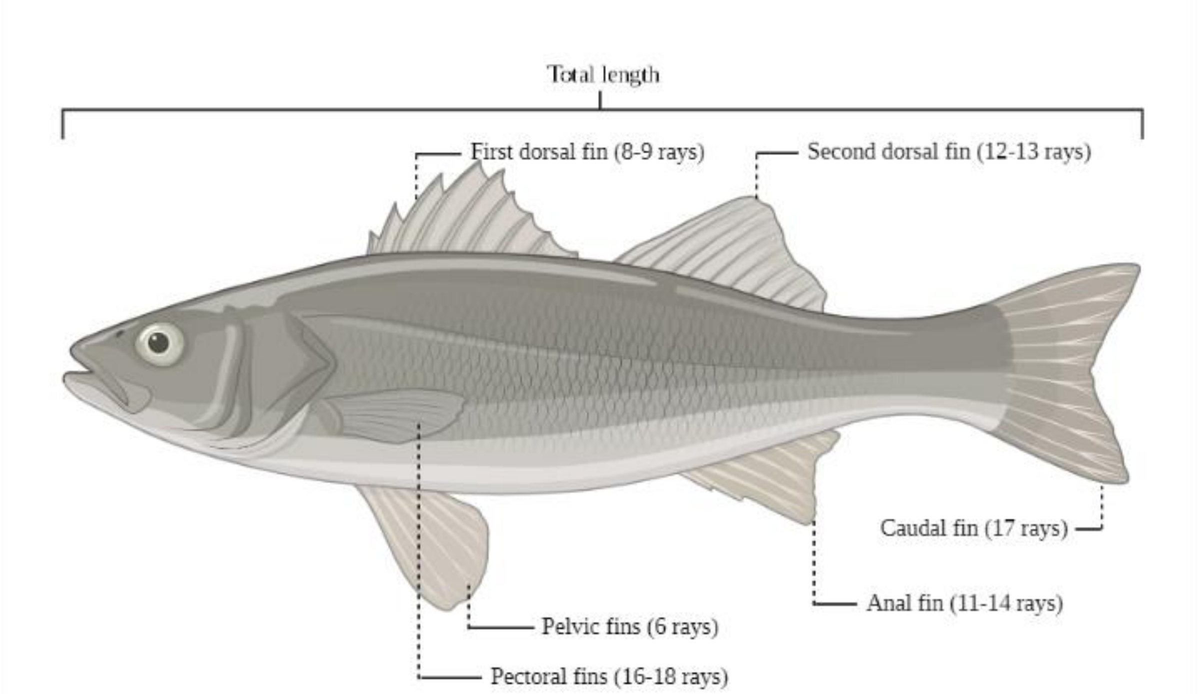

Figure 1. Diagram of sea bass morphology, including fin rays, based on Pickett and Pawson, 1994. Made in BioRender (2023).

European sea bass range across the North-East Atlantic throughout UK waters, to the North sea including Norway and most reccently the Dutch Wadden Sea (Cardoso *et al*., 2014), to the Mediterranean and Black Sea, and as far South as Senegal (Sanchez & Munoz-Cueto, 2014). In the UK, the sea bass fishery is divided into four subareas; i. Iberean Coast, ii. Bay of Biscay, iii. West of Scotland and South-West of Ireland, and iv. North Sea, English Channel, Celtic Sea and Irish Sea (ICES, 2012). There is considerable gene flow between the stocks present in subarea iv, such that they are considered a single population. However, the North Sea and Mediterranean stocks are considered genetically distinct populations (Tine *et al*., 2014; Souche *et al*., 2015), however, populations in Scotland and the North-West of England are not considered to be persistent, suggesting this is not a fully established population yet. Sea bass of the South East of England are thought to represent the northern range margin of the subarea iv stock, limited by the 9°C isotherm that is thought to constrain sea bass spawning and larval survival (Devauchelle & Coves, 1988; Pickett & Pawson, 1994). However, sea bass are believed to be experiencing a Northern range expansion in recent years, with sea bass becoming more abundant in Northern lattitudes with rising sea temperatures (Bagdonas *et al*., 2011; Brander *et al*., 2013; Cardoso *et al*., 2014; Bento *et al*., 2016). Over the past 20 years, sea bass have become progressively more abundant in the Dutch Wadden Sea, where they were only previously recorded as a transient visitor, to the point where they are now considered a resident species, as conditions have become optimal to support the growth and survival of juveniles (Cardoso *et al*., 2014).

Sea bass are a commercially important food species throughout the UK and Europe. Sea bass landings have seen dramatic rises since the turn of the millennium, effectively doubling from 2100 tonnes in 2000, to 4243 tonnes in 2013. Of this, United Kingdom landings accounted for 19% across Europe, second to France who accounted for 50% of landings (ICES, 2022). Since then, landings severely declined to 930 tonnes by 2018, with the UK now accounting for 50% of those landings. This is because of a decline in stock size over the past 20 years, owed mainly to increased fishing pressure and high levels of recruitment variation between years (ICES, 2015). The report from the ICES working group regarding sea bass in the UK (2015) suggests that high stock biomass was observed during the 1990s which resulted in increased fishing effort. This increased biomass was driven by a highly successful recruitment event in 1989, after poor recruitment throughout the 1980's. Following this boom in biomass there has been a steady decline following poor recruitment since (ICES, 2015). In 2018, the large reduction in commercial and recreational landings coincides with the introduction of emergency protection measures put in place as a precautionary approach to reduce the effects of overfishing that were forecasted (ICES, 2012; ICES, 2015; European Comission, 2015). These emergency protection measures severely limited commercial and recreational landings, with a blanket ban on pelagic trawling during the spawning window of

December through to April, as well as an increase in the minimum landing size to 42cm (European Comission, 2015). The fishery has since reopened, and the fishery is deemed to be operating above safe biological limits. However, UK stocks are still considered vulnerable to overexploitation, and this has attracted a lot of attention from research in the past 20 years. Sanchez and Munoz-Cueto (2014) noted that as of 2012 an average of 500 International Scientific Indexing publications pertaining to European sea bass ecology were published a year, which has likely increased in recent years.

Sea bass exhibit a biphasic life cycle, whereby an ontogenetic niche shift in habitat and diet occurs between the juvenile and adult phases (Pickett & Pawson, 1994), as illustrated in Figure 2. Juvenile fish are predominantly epi-benthic and feed on macro- and microinvertebrates but will also opportunistically predate surface insects and small fish (Pickett & Pawson, 1994; Green *et al*., 2009). Adults are pelagic and piscivorous (Spitz *et al*., 2013). Moreover, this change in diet entails a shift in habitat usage. While adults use deeper offshore waters (Pickett & Pawson, 1994), juveniles are thought to make use of estuarine and saltmarsh systems during their first three-four years of life (Kelley, 1988; Pickett & Pawson, 1994; Green *et al*., 2009; 2012). Older juveniles will migrate into deeper water of the estuary, sometimes making large migrations of hundreds of kilometers between neighboring estuarine systems, but generally show high fidelity to natal estuaries (Stamp *et al*., 2021).

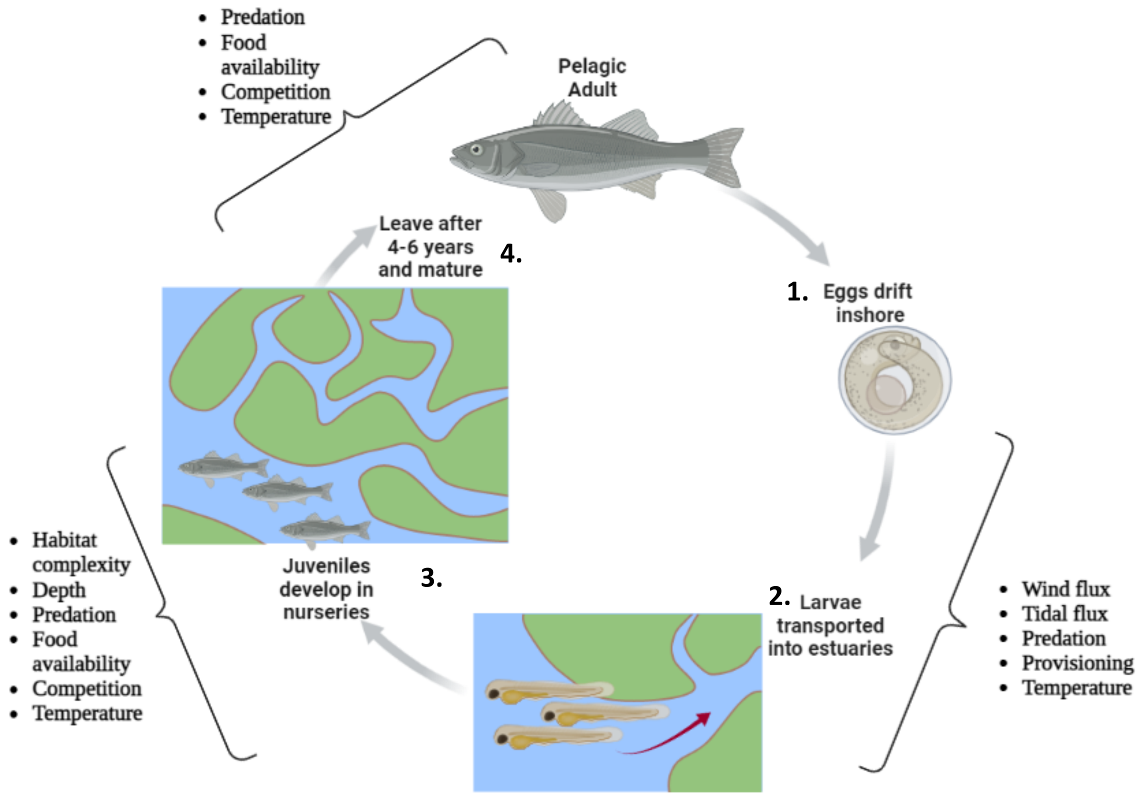

Figure 2. Diagram of sea bass life cycle and habitat, including potential drivers of survival, based on Pawson & Pickett, 1996. 1. Eggs drifting onshore, 2. Larvae entering estuaries, 3. Juvenile fish in estuarine nurseries, 4. Adult fish in offshore waters. Made in BioRender (2023).

Despite the importance of sea bass and the importance of estuarine nursery sites to recruitment, our understanding of factors that influence the selection of nursery habitats is relatively weak. Firstly, we do not fully understand what constitutes bass nursery habitat, as most research has focused on saltmarsh habitats (Kelley 1986, 1988b; Jennings & Pawson 1992; Cattrijsse *et al*., 1994; Pickett & Pawson, 1994; Laffaille *et al*., 2000; Patterson & Whitfield 2000; Cattrijsse & Hampel, 2006; Green *et al*., 2009, 2012; Jin *et al*., 2007; Dufour *et al*., 2009), and very few studies have investigated other estuarine habitats, such as sand, mudflats, and shingle edge habitats, or even outer estuary coastline. Secondly, very little data exists describing how biotic and abiotic habitat components drive selection and habitat usage, and the resultant distribution of juvenile bass within these estuarine nurseries, or whether habitat heterogeneity influences distributions at all. This applies both to the larval phase prior to settlement, and the post settlement juvenile phases. We do not currently understand over which stage recruitment bottlenecks occur for sea bass, or indeed many fish species. While large mortalities occur during the egg and larval stages, the estuarine habitats used by juvenile sea bass are under large reductions due to anthropogenic pressures, and there are large reductions in abundance of sea bass between 0-1 group fish who return to estuaries the following year (Green *et al*., 2009). Whether larval fish choose an estuarine system or habitat, and how do juveniles use this habitat, and why over others? Finally, we do not understand what the functions of these habitats are, and how they affect fish growth and survivability. Looking at other species of bass around the world, similar data deficiencies are seen in research pertaining to striped bass and white perch, where habitats are grouped coarsely, generally by stream position with little consideration for habitats across the estuarine mosaic (Kraus & Secor, 2005; Muffleman, 2006; Able *et al.*, 2011; Murphy & Stribling; Schloesser & Fabrizio, 2018). Conversely, research on the habitat use and function of Japanese sea bass (*Lateolabrax japonicus*) has revealed that riverine, eelgrass, and surf zone habitats tend to support increased foraging opportunities (Islam *et al.*, 2011). Understanding the habitat components that influence the desirability of nursery habitats is central to identifying and correctly managing these areas of critical habitat usage, and must take into acount the biological, physical, and physiochemical components that make up a habitat.

2. HABITAT TYPE

Habitat is a very broad definition that describes the biological, physical, and physiochemical environmental parameters experienced by an organism at a particular location. In this section I will be particularly referencing the biological and physical habitat components such as food, predation, and tri-dimmensional complexity. Nursery habitat is that which best promotes survival of the early-life stages, whether that be area-dependent or by total contribution to the adult stock (Beck *et al*., 2001; Dhalgren *et al*., 2006). It is thought that this is achieved through providing habitat that offers refuge from predation and increases foraging potential (Nagelkerken *et al*., 2015). Both of these habitat components are intimately linked to juvenile and larval fish mortality (Connell and Jones, 1991; Scharf *et al*., 2006; Pape and Bonhommeau, 2015). For example, predation of alefin Herring larvae (those with an intact yolk sac) accounts for up to 70% of cohort mortality (Litvak and Legget, 1992), however, once the yolk sac is expended it is thought that food availability underpins mortality rates (Buckley *et al*., 1984). In some cases, predation avoidance and foraging potential are not mutually exclusive, in that predation is size-selective (Tonn & Paszkowski, 1986; Post & Evans, 1989; Tonn *et al*., 1992; Scharf *et al*., 2000; Holmes & McCormick, 2012; Huss *et al*., 2010). Fish can essentially escape predation by outgrowing the gape width of their predators' mouths. Therefore, faster growth rates increase the chance of avoiding predation and surviving (Urban, 2007), which requires a food supply that supports a high enough growth rate to do so (Jones, 1986).

One aspect of a habitat that is well documented in its effect on predation and survival is habitat complexity (Levin *et* al., 1997; Horinouchi and Samo, 1999; Gratwicke *et* al., 2005; Kuffner *et al*., 2007; Canion and Heck, 2009; Harborne *et al*., 2011, 2012; Nanjo *et al*., 2011; Thiriet *et al*., 2014; Cheminée *et* al., 2016; Leslie *et al*., 2017; Paxton *et al*., 2017). The prevailing theory is that predation levels are lower in areas of increased habitat complexity, due to decreased visibility of prey species to predators (Savino and Stein, 1982; Almany, 2004; Horinouchi, 2007a). The converse argument, however, is that predators are also less visible to their prey, which would suggest an increased success rate for ambush predators (James and Heck, 1994). Tri-dimensional habitat complexity, provided by matrices of vertical structures, have been extensively studied with regards to the influence on predation and survival

(Horinouchi and Samo, 1999; Harborne *et al*., 2011, 2012; Thiriet *et al*., 2014; Cheminée *et* al., 2016; Leslie *et al*., 2017).

Perhaps the most well studied habitat relevant to estuarine systems is seagrass meadows. Typically, complexity in seagrass is based on emergent stem density and percentage cover (Levin *et al*., 1997), and in many cases a positive link between seagrass density and juvenile fish survival has been demonstrated (Adams *et* al., 2004; Shoji *et* al., 2007; Nanjo *et* al., 2011; Thiriet *et* al., 2014; Cheminee *et al*., 2016). Cheminée *et al*., (2016) demonstrated that survival, and subsequently abundance, of fish were significantly lower on sandy bottoms than those who utilised sea grass beds composed of *Caulerpa* and *Zostera*. Furthermore, the morphology of a fish affected the ability of macrophyte types to provide refuge, where species such as *S. ocellatus* showed lower survival rates than *C. julis* in *Caulerpa* habitat, which was attributed to a disadvantageous body shape meaning that they were not as well hidden. In pinfish (*Lagodon rhomboides*), while the presence of structure from seagrass meadows compared to sandy bottoms results in a 300% increase in abundance, the degree of complexity between seagrass patches does not appear to influence predation levels and survival (Levin *et al*., 1997). Similar results were described by Canion and Heck (2009), who also showed that survival rates were higher in seagrass compared to sand. It would appear in some cases, that complex habitat existing at all is more important than the degree of complexity. Another well studied habitat that provides vegetative cover, to a degree, is saltmarsh systems. Fish such as Cape snubnose (*Rhabdosargus holubi*) show higher abundance in more structurally complex vegetated habitat such as saltmarshes composed of *Zostera capensis* and *Spartina maritima*, over structurally simple sand banks, and show strong associations with *Zostera capensis* beds in particular (Leslie *et al*., 2017). Mangroves in tropical systems are also thought to provide structural complexity through the intricate root networks and fallen woody debris, however, several studies have failed to show that survival of juvenile fish is higher in mangroves than in other juvenile habitats, or even non-vegetated mudflats (Laegdsgaard & Johnson, 2001; Sheriden & Hays, 2003).

The vertical complexity provided by estuarine flora is not the only form of habitat complexity. This can also be provided by benthic complexity, where rugosity (roughness) is the most commonly employed measure (Gratwicke *et* al., 2005; Harborne *et al*., 2011, 2012; Kuffner *et al*., 2007; Paxton *et al*., 2017). Harborne *et al.* (2011) showed an increased abundance of fish in more complex coral-reef habitats than those that were simpler, but that different aspects of habitat complexity affected between species abundance. Increased abundance of smaller fish was more heavily associated with coral height, while increases in wrasse and surgeonfish abundance were more closely associated with increased coral densities. In estuarine systems, this benthic complexity is often provided by the presence of oyster reefs (Tolly *et al*., 2005; Dumbauld *et al*., 2015). Tolley *et al*., (2005) showed that fish abundance increased in all habitats where oyster shell, both living and dead, was present, while abundance was very low on sandy bottoms.

Depth is another aspect of an organism's habitat that influences predation (McIvor and Odum, 1985; Harvey 1991; Paterson & Whitfield, 2000). Harvey (1991) demonstrated that in the bluegill sunfish, predation from small littoral planktivorous fish would drive planktonic larval bluegills into deeper water, where largemouth bass (*Micropterus salmoides*) presence reduces that of small predatory fish. Once large enough to be considered prey by the size selective bass, they migrate into the littoral environment to avoid predation, where smaller littoral predators are no longer a threat, and the shallow water is inaccessible to larger bass (Harvey, 1991). In estuarine systems, edge habitats such as saltmarshes provide potential shallow water refuge from deeper estuary channels occupied by larger predatory fish (Patterson & Whitfield, 2000; Whitfield, 2017, 2020). Marsh fish are thought to use shallow creeks during low tide, which are less accessible to piscivorous predators (Harvey 1991; Paterson &

Whitfield, 2000; Whitfield, 2017; Whitfield, 2020) and are thought to provide increased foraging potential. In this instance, fish can minimise predation risk and maximise foraging potential. However, during high tide the predation risk increases as more habitat becomes accessible to larger fish (Rypel *et al*., 2007). At this point marsh fish should use shallow, less productive creeks on the marsh surface, trading off foraging potential for predation refuge. (McIvor and Odum, 1985). Saltmarshes themselves are formed of different habitats (Halpin, 2000; Green *et* al., 2012) that each carry their own relative predation risk and foraging potential within, and this too has an impact on how these systems are used. Halpin (2000) found that fish held in different saltmarshes that exhibited different habitat types within, would utilise different habitat based on availability and predation risk. If refuge habitat was present when predation risk was high, then this was utilised over other habitat types, however, if refuge was not present, habitat that maximised foraging potential was used (Halpin, 2000). In this instance a trade-off is thought to occur, in that refuge habitat may provide diminished foraging potential compared to other habitats (McIvor & Odum, 1985; Werner and Hall, 1988; Eklov, 1995; Tatrai & Herzig, 1995; 2006; Halpin, 2000; Macrae & Jackson, 2001; Fullerton & Lamberti, 2006). Therefore, in avoiding predation, fish are potentially sacrificing foraging efficiency and energetic gains (Lima and Dill, 1990; Peterson & Bronmark, 1993; Balaban-Feld *et al*., 2019). Predation here is thought to be influencing the relative time a fish will spend in a certain habitat type. In the absence of predation, fish will generally utilise optimal foraging patches, but when faced with predation, fish will act to use habitat that reduces the mortality risk over foraging (Gilliam & Fraser, 1987; Gotceitas, 1990; Peterson & Bronmark, 1993; Halpin, 2000).

Resource acquisition is a fundamental part of fish survivability. Lab-based studies suggest the effect of limiting food availability decreases feeding rates and, therefore, growth rates of fish (Ng *et* al., 2000; Sun *et al*., 2014; Luo *et al*., 2015; Xu *et al*., 2015). Food availability is in part associated with elements of an organism’s habitat, such as habitat complexity. Many fish species, during their juvenile stages, will feed on plankton (Gning *et al.*, 2008), macro- and microinvertebrates (Green *et al.*, 2009), and even other smaller fish species in some cases (Green *et al.*, 2009). The relationship between habitat and macroinvertebrate abundance has been explored well, showing a general increase in macroinvertebrate community richness with increased habitat complexity (Attril *et al*., 2000; Lloyd *et al*., 2006; Zbikowski and Kobak, 2007; Bazzanti *et al*., 2010; Cai *et al*., 2011, 2012; Ferreiro *et al*., 2011; Pan *et al*., 2012; Bell *et al*., 2013; Hu *et al*., 2016; Wolters *et al*., 2018). One thought is that the structural complexity provided by macrophyte communities reduces current flow significantly, such that more complex regions provide increased shelter (Wolters *et al*., 2018). However, in seagrass communities increased macroinvertebrate abundance is closely related to the biomass of seagrass as opposed to shoot density, as one might expect if refuge from currents was the sole driver (Attril *et al*., 2000). Complex mangrove habitats appear to be highly utilised by juvenile fish for foraging, likely due to the influence of the complex root structures on prey abundance (Whitfield, 2017). In a suboptimal site, not only might we see poor resource acquisition, resulting in reduced survival probability (Buckley *et al*., 1984), but also previous studies suggest that the trade-off between time spent foraging and predator avoidance are greatly affected by hunger, weight, and metabolism (Ludwig & Rowe, 1990; Killen *et al*., 2011). These factors may result in greater risks being taken by fish (Killen *et al*., 2011), thus further decreasing survival probability through a heightened risk of predation.

The tidal nature of the estuarine systems inhabited by juvenile sea bass means that habitats routinely cycle between available and unavailable, and the relative predation pressure changes for different habitats. The complex mosaic of structurally different habitats present poses the question of how this heterogeneity influences sea bass habitat usage. Habitats that offer tri-dimensional and benthic complexity exist alongside structurally simple habitats, that exist at the edge of deep-water habitat such as mudflats. Most work has focussed on shallow saltmarsh systems, with little regard for other habitats that sea bass may use, and so we are left with a large gap in our understanding of what habitat forms quality nursery habitat for juvenile sea bass and why. The majority of our understanding of juvenile sea bass habitat usage is derived from studies undertaken in the late 1980's and the 1990's (Kelley 1986, 1988b; Jennings & Pawson, 1992; Pickett & Pawson, 1994), with a handful of recent studies addressing the question (Green *et al.*, 2009, 2012; Stamp *et al.*, 2021). Studies undertaken in the Mediterranean suffer similar issues to those of the UK regarding the resolution of habitat designations, with work by Cabral & Costa (2000) generally focussing on shallow lagoons and deeper channels. Research in the UK, by Kelley (1986, 1988b) and Pickett and Pawson (1994), suggests that 0-group sea bass predominantly utilise shallow saltmarsh creeks and edge habitat, though the latter are coarsely defined as 'estuary'. Work by Cabral and Costa (2000) seems to corroborate these findings, showing that bass densities increased in shallow lagoon bays compared to deeper water. More recent work by Stamp *et al.*, (2021) in UK estuaries suggests that sub-adult sea bass show high site fidelity throughout much of the year to natal estuarine systems, despite large inter-estuary migrations of hundreds of kilometres. It is highly likely that a range of shallow habitats are desirable to sea bass beyond saltmarshes, but to what extent these shallow habitats are used, and what drives this habitat selection is still unknown.

3. PHYSIOCHEMICAL HABITAT

When discussing the physiochemical habitat that an organism occupies, this pertains to the environmental parameters such as temperature and salinity profiles. Being poikilothermic, temperature profoundly influences the physiology of fish, and is a key driver in distribution patterns (Murawski, 1993; Perry *et al.*, 2005; Buisson, *et al.*, 2007; Ferguson, 2011; Hollowed *et al.*, 2012; Hein *et al.*, 2013). This relationship occurs over both large and small spatial scales. Over large spatial scales, climate-based temperature differences result in significant segregation of species and whole communities of fish. We see distinct differences in distribution and species abundance between tropical and cold-water ecosystems. This is due to differences in the thermal niche of fish, the temperature range within which physiological processes perform optimally (Pörtner, 2010; Sunday *et al*., 2012; 2014). For example, Arctic cod (*Boreogadus saida*) show peak growth rate at 7.3^{o}C (Laurel *et* al., 2016), while Pearl gentian grouper from the southeast coast of China show this at much higher temperatures, around 34^{o}C (Zhang *et al*., 2018). Interestingly however, species occupying similar geographic regions can exhibit vastly different thermal niches, with walleye pollock (*Theragra chalcogramma*), who inhabit similar regions to Arctic cod, show best growth performance at temperatures around 13^{o}C (Laurel *et al*., 2016).

Perhaps more relevant are the influences of temperature on distribution and habitat usage patterns over smaller spatial scales. Organisms should use temperatures that both maximise growth, feeding and swimming potentials, as well as to mediate internal physiological processes. For example, catsharks utilise vertical thermal gradients to mediate their energetic expenditure, in a process known as thermoregulation (Sims *et al*., 2008). Catsharks will hunt in warmer surface waters to increase metabolic activity and use deeper cold water to rest to reduce energy expenditure, in what is referred to as the ‘hunt warm, rest cool’ theory (Sims *et al*., 2008). A similar process is also exhibited in salmonids, who use cool deep water during the day and feed at night in surface waters when they are cooler, resulting in a decreased metabolic expenditure during feeding (Brett, 1971). Thermoregulatory behaviour does not only benefit energetic efficiency. Adult Sole will utilise both vertical and horizontal thermal gradients to promote gonadal maturation, migrating into deeper offshore water, where water temperatures are slightly higher (Sims *et al*., 2006). This is due to the heat holding potential of larger bodies of water. In these warmer temperatures, gonadal development is thought to occur at a faster rate, so they are ready for early spring spawning (Sims *et al*., 2006).

This is due to the effect of temperature on metabolic activity and growth. Increases in temperature generally result in an increased metabolic activity up to a certain point, after which the rate will decrease (Clark and Johnston, 1999; Tirsgaard *et al*., 2015; Sandersfield *et al*., 2015; Ikeda, 2016). Generally, growth rates are linked to increases in metabolic activity (Clark and Johnston, 1999; Lankford and Targett, 1999; Wuenschel *et al*., 2004; Handleand *et al*., 2008; Sandersfield *et al*., 2015; Tirsgaard *et al*., 2015; Laurel *et al*., 2016, 2017; Kim *et al*., 2017; Phuong *et al*., 2017; Faulk *et al*., 2018; Fernandez-Montero *et al*., 2018; Zhang *et al*., 2018), but other aspects of a fish's physiology are also affected, such as activity levels and feeding rates (Swanson, 1998; Wuenschel *et al*., 2004; Laurel *et al*., 2016).

Seasonal temperature changes can also drive habitat usage and distribution patterns of fish throughout the year, often resulting in declines in species abundance and community diversity with decreasing temperatures moving into winter (Green *et al*., 2009; Castillo-Rivera *et al*., 2010). Many species will undertake offshore migrations into deeper, warmer habitat over winter to seek refuge from cold temperatures that may be experienced in shallow summer habitats (Swain *et al*., 1998; Hales & Able, 2001; Cote *et al*., 2004). For example, Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*), utilise inshore foraging habitat in summer, but move into deeper water over winter, showing a reduced habitat selectivity due to increased aggregation (Swain *et al*., 1998; Cote *et al*., 2004). The Black sea bass (*Centropristis striata*) will also undertake migrations away from estuarine environments used over summer, into deeper waters of the continental shelf, with adults occupying deeper water than juveniles (Hales & Able, 2001). However, other species, such as brown trout (*Salmo trutta*) in riverine environments, may migrate into shallow lotic habitat over winter, where they make use of rugose habitat as refuge (Maki-Ptyas *et al*., 1997). Seasonal temperature differentials can also limit the range expansion of species (McBride and Able, 1998; McCollum *et al*., 2003; Booth *et al*., 2007; Figueira *et al*., 2009; Wuenschel *et al*., 2012; Anderson &

Scharf, 2013). Fish are thought to enter a state of dormancy during winter, where physiological processes and activity are reduced (Hurst, 2007), in order to reduce energetic expenditure. Variability in winter thermal minima between years can result in starvation or chronic thermal stress, which can result in large scale winterkill events (Hurst, 2007; Reeve, 2020; Reeve & Lowry, 2022). This is best illustrated in tropical species at the edge of their range margins, whose larvae may drift with ocean currents and settle in temperate regions over summer, when conditions support optimal growth and survival (McBride and Able, 1998; Booth *et al*., 2007; Figueira *et al*., 2009; Wuenschel *et al*., 2012). However, over the winter, temperatures fall below the lethal limits of these species and either force migrations into lower latitudes (Vanderkoog and Muller, 2003) or result in large numbers of winter mortalities (McBride and Able, 1998; Booth *et al*., 2007; Figueira *et al*., 2009; Wuenschel *et al*., 2012).

Sea bass are thought to be relatively tolerant to a wide range of temperatures, showing the ability to acclimate to regional differences in thermal minima and maxima (Claireaux & Lagardere, 1999; Ruyet *et al*., 2004; Claireaux *et al*., 2006; Dulger *et al*., 2011; Yilmaz *et al*., 2020). For instance, sea bass reared at 15^{o}C showed a critical thermal minimum (CTmin) of 4.1^{o}C, compared to 6.7^{o}C in fish reared at 25^{o}C (Dulger *et al*., 2011). Therefore, the thermal performance curve (TPC) for sea bass may shift depending on the natal habitat. Generally, the optimum metabolic region of the TPC for sea bass is thought to fall within 24^{o}C to 27^{o}C, with an upper thermal limit of around 32^{o}C and a lower lethal limit of around 2^{o}C to 3^{o}C (Yilmaz *et al*., 2020). However, the majority of our understanding of sea bass thermal tolerances is derived from sea bass of the Mediterranean Sea and from aquaculture (Claireaux & Lagardere, 1999; Ruyet *et al*., 2004; Claireaux *et al*., 2006; Dulger *et al*., 2011; Yilmaz *et al*., 2020), who are genetically distinct from sea bass of the North Sea (Tine *et al*., 2014; Souche *et al*., 2015). Therefore, it is difficult to know how sea bass of the North Sea will respond to temperature differentials between seasons on the East Coast. Sea bass of the East Coast are thought to exist near the 9°C isotherm, which presents a boundary to spawning, below which female bass do not reach gonadal maturation (Devauchelle & Coves, 1988; Pickett & Pawson, 1994). As a result, the sea bass of the East Coast are thought to represent the Northern range margin for the species on the English Channel stock. Shifts in temperature may, therefore, greatly impact the survivability of early life-stages and so influence recruitment. In particularly cold years, survival of sea bass juveniles is thought to have been particularly low, with near whole cohort loss in the coldest years, recorded in the Celtic Sea population (Kelley, 1988a). Recruitment also appears to have been particularly high in the warmest years, suggesting that temperature may play a key part in the recruitment variation experienced by sea bass in the UK (Beraud *et al*., 2017). There is evidence that juvenile sea bass are not present in East Coast shallow estuarine habitats during the winter months (Pawson & Eaton, 2005; Green *et al*., 2009), but data on the whereabouts of this species and overwinter survival is non-existent in published materials. This is a particularly important factor in need of further investigation.

4. DISPERSAL

Dispersal is an important aspect of recruitment, so far as it determines the settlement patterns of organisms, and is intimately linked to oceanographic processes, as well as physiological and behavioural. The main stage where dispersal dynamics act upon is the planktonic larval stage, which is exhibited by almost all marine fish species. The duration of this stage is thought to be crucial in determining the dispersal potential of a larval organism, as this determines the length of time a propagule is subjected to transport from ocean currents (Leis, 1991; Morgan, 2001; Cowen *et al*., 2003; Palumbi, 2003; Leis, 2007; Munday *et al*., 2008, 2009). Therefore, recruitment patterns are thought to be largely underpinned by the interaction of an organism’s pelagic larval duration (PLD) and oceanographic processes, such that for the longest time recruitment models have treated larval fish as passive particles at the mercy of ocean currents (Macpherson & Raventos, 2006; Siegel *et al*., 2003; 2008; Kinlan *et* *al*., 2005; Edwards *et al*., 2007; Swearer *et* al., 2019; Leis *et al*., 2020). Planktonic larval duration is thought to firstly determine the distance with which a larval fish can be transported (Kinlan *et al*., 2005), and so the ability to reach settlement sites and survive, but also will determine where those settlement sites are. Planktonic larval duration can also influence the genetic connectivity between populations (Munday *et al*., 2008, 2009), and also facilitate range expansions (Munday *et al*., 2008; Figueira & Booth, 2010**;** Bashevkin *et al*., 2020).

The positive correlation between PLD and dispersal distance is well established in a wealth of literature (Zapata & Herron 2002; Kinlan and Gaines, 2003; Lester & Ruttenberg 2005 Macpherson and Raventos, 2006; O' Connor *et al*., 2007; Shanks, 2003, 2009). However, through a review of the PLD and dispersal distances of 44 species between two seminal papers, Shanks (2003, 2009) demonstrated that in many cases species with PLD's ranging from hours to days can exhibit similar dispersal distances. Shanks (2009) suggested that this would not be possible if only hydrodynamic processes governed transport of a larval fish and that the behaviour of a larvae must also be considered. The extent to which behaviour influences dispersal, compared to oceanographic processes, has been in debate for many years, but the prevailing theoretical transport model is one that utilises diel vertical migration and tidal currents, known as selective tidal-stream transport (STST). This relies on the concept of Ekman spiralling, that current velocity attenuates with increasing depth, due to the effect of the Earths rotational forces on the water column (Price & Sundermeyer, 1999). Surface currents in most cases, flow faster than currents in deeper water, especially that which runs near the benthos (Price & Sundermeyer, 1999). A larval organism utilising selective tidal-stream transport may ascend into the faster flowing surface currents on a given tide to facilitate movement in that direction, then descend into deeper currents near the bottom out of the tidal plume, to impede movement in the opposite direction (Walker *et* al., 1978; Forward *et al*., 1998). This results in a net movement in a given direction, often associated with inshore movements on the flood tide. Through this process, an organism can theoretically either increase the potential dispersal distance achievable, or retain to a desirable location (Forward *et al*., 1998).

Plaice larvae (*Pleuronectes platessa*) have been found to employ STST to migrate into estuarine nursery sites, increasing in abundance in the water column on the flood tide, and becoming closely associated with the bottom on ebb tides, resulting in a net inshore movement (De Veen, 1978). Similar behaviour has also been shown in flounder (*Platichthys flesus* – Jagger, 1999a, 1999b), sole (*Solea solea* – Champalbert and Koutsikopoulos, 2009), red drum (*Sciaenops ocellatus* – Holt *et al*., 1989) and pinfish (*Lagodon rhomboides* – Forward *et al*., 1998). Furthermore, this behaviour seems closely related to nocturnal tides in many species (Forward *et* al., 1998; Islam *et al*., 2007; Champalbert and Koutsikopoulos, 2009), possibly due to decreased predation risk in using surface currents, as a result of reduced visibility (James and Heck, 1995; Horppila *et al*., 2018). The main commonality is the use of the tidal plume on the flood tide to promote an inshore movement, and a decrease in abundance in the water column on the opposing ebb tide. Selective tidal-stream transport has also been demonstrated in adult fish (Jones *et* al., 1979; Metcalfe *et al*., 1990; Bennett & Burau, 2015; Fukuda *et al*., 2016; Verhelst *et al*., 2018) and a multitude of invertebrate species (Wooldridge & Erasmus, 1980; Tankersley *et al*., 1998; Forward *et al*., 2003; Knights *et al*., 2009).

In the case of sea bass, the estuarine nursery sites, where larval fish are thought to settle between April and June (Green *et al*., 2009, 2012), are highly tidal environments. The use of a tidal transport mechanism would be highly advantageous in promoting movement into desirable habitat. Again, in models of sea bass larval dispersal, movement into estuarine systems was not possible without the inclusion of tidal behaviour such as STST (Beraud *et al*., 2017). Despite this, no evidence of such behaviour exists, with relatively few studies actually addressing this issue. Understanding the use of this behaviour by larval sea bass may help identify a major driver of variable recruitment. A lack of tidal transport may indicate that the size of settlement to estuarine systems is random, while its employment may allow for a greater degree of predictability.

5. THESIS SUMMARY

It is evident that the distribution and habitat usage of fish is determined through complex interactions between multiple habitat variables. When concerning bass in estuaries we must assess firstly how these conditions are likely to differ between estuarine habitats. This raises some important questions within the context of sea bass selecting nursery habitat. As previously stated, there is a lack of information surrounding bass habitat usage and distributions within estuaries, even less so that consider the biotic and abiotic components that drive habitat selection, or whether habitat is neutral and distribution patterns are the result of stochastic processes (Chase *et al*., 2014).

Therefore, we must firstly ask; how do juvenile sea bass use different habitats along the estuarine mosaic, and what biotic and abiotic habitat components influence observed distribution patterns. I hypothesise that sea bass habitat usage is likely to differ between habitats, and be dependent on the presence of structure, and the relative foraging potential and predation risk those habitats present. Estuarine systems are highly heterogenous, with many structurally different habitat types, however, the majority of research to date has focussed either on estuaries as a whole or specifically on saltmarshes and their associated creeks and channels (Kelley, 1986, 1988b; Pickett & Pawson, 1994; Lafaille *et al*., 2000, 2001; Green *et al*., 2009, 2012). Little attention has been paid to differences in habitat use between edge habitats composed of mud, sand and shingle, and oyster reefs, in comparison to saltmarshes, including whether the habitat function differs between these habitats. Arguably, the shallow nature of all intertidal habitat could provide refuge from predation (Patterson & Whitfield, 2000), but then Halpin (2000) suggests that a fish should act to maximise foraging potential, often provided by increased structural complexity. Shifts in habitat complexity and turbidity in estuarine systems could influence the relative predation risk and foraging potential throughout the estuary and thus may influence sea bass habitat usage between them. Green *et al*., (2012) investigated such factors within the context of saltmarshes alone, demonstrating that sea bass abundance was more closely linked to the area of mudflat available adjacent to the marsh. This work suggested that bass abundance on a marsh was negatively correlated to the area of adjacent mudflat, implying that larger areas of mudflat provided sufficient amount of shallow habitat as refuge. This supports the theory proposed by McIvor and Odum (1985). However, again this study did not look directly at the presence of predators, food availability, habitat complexity, or even presence of the target species within these mudflats or other estuarine habitats. Therefore, Chapter 2 addresses the question of how the abundance and condition of juvenile sea bass differs between different estuarine habitats, and Chapter 3 specifically focusses on differences in diet and foraging behaviour between habitats using dietary DNA metabarcoding techniques to assess prey community composition and dietary differences, including how molecular techniques can be used to help inform us on fish distribution patterns.

The second major question examines our understanding of how larval sea bass settle within estuarine systems, whether this is an active or passive process. Again, to date, very few published studies have been conducted on sea bass larvae, with most studies yielding very low catch rates, often focussing in other regions on the Western coast of the UK (Jennings & Pawson, 1992). The highest catch rates of sea bass larvae were recorded in a study from the Mediterranean, which focussed on larval distributions post settlement, finding high abundance in shallow coastal lagoons (Dufour *et al*., 2009). Most of our understanding of larval bass habitat selection is assumed based on distribution changes from larval planktonic fish drifting onshore in coastal waters and the abundance of juvenile stages found in estuaries (Pickett and Pawson, 1994). It is often assumed that bass will actively select estuaries as nurseries. A study by Beraud *et al*., (2017) suggested that bass would be unable to settle within estuarine systems on the East Coast of the UK without employing tidal behaviour such as STST, but to date there is no evidence that this behaviour is actually employed. Clearly whether this behaviour is employed is important for modelling movement of larval sea bass into coastal nursery systems, and therefore predicting settlement and recruitment patterns. Therefore, in Chapter four it must be considered whether larval sea bass exhibit such active tidal transport through changes in vertical distribution over different tidal phases. Based on Beraud *et al*., (2017), I propose that an increased abundance of sea bass larvae will be observed in the surface water during flood tides, and near the benthos during ebb tides.

Our final major question pertains to the survival and habitat use of juvenile sea bass over their first winter on the East Coast. Temperatures of the Essex coastline routinely within and below the lower lethal temperature range for young of year sea bass, yet we still find group-1 fish returning to the estuary the following summer, albeit in reduced numbers compared to group-0 (Green *et al*., 2009). To date relatively few studies have addressed the over-winter survival of group-0 sea bass, even less so regarding the habitat use over winter, with almost all studies focussing on the summer settlement period. Therefore, Chapter five will focus on predicting the growth and survival of group-0 sea bass entering their first winter using a combination of energy budget modelling (Sibley *et al*., 2013), adapting pre-existing models of sea bass energy consumption and expenditure (Watson *et al*., 2020), alongside lab-based temperature-dependant growth experiments. These approaches will use *in-situ* measurements of winter temperatures experienced within the estuarine habitats, used by sea bass over summer, and compare growth and survival to temperatures of the core range in the Mediterranean and Bay of Biscay.

CHAPTER TWO

HABITAT ASSOCIATIONS OF JUVENILE EUROPEAN SEA BASS IN ESTUARINE NURSERIES

*Seine netting in a bay of the Colne Estuary

1. INTRODUCTION

We have a poor understanding of what constitutes important nursery habitat for juvenile marine fish species (Litvin *et al*., 2018), such as European sea bass. Litvin *et al*., (2018) argue that many previous studies attempted to link nursery function purely to juvenile fish abundance and recruitment output between habitats, and that a better understanding of the underlying drivers of survival between habitats is required. European sea bass exhibit a biphasic lifecycle, during which they undergo a migration from an inshore/estuarine habitat in which they are found post settlement until aged two-three years when they live in a predominantly offshore marine habitat (Pawson and Pickett, 1994). This migration coincides with an ontogenetic niche shift and entails a change in diet from mostly benthic macro-invertebrates as juveniles (Aprahamian & Barr 1985, Ferrari & Chiegeregato 1981, Pickett & Pawson 1994, Green *et al*., 2009), to piscivory as adults (Pickett & Pawson 1994). Larval fish are thought to settle in coastal estuaries, following the main spawning event in March through to April (in Southern UK waters), where they remain for the first four years of their life (Pickett & Pawson, 1994). Estuaries are thought to provide nursery habitat for sea bass, and many other commercially important fish species such as plaice (*Pleuronectes platessa*; Nash & Geffin 2000, Phil *et al*., 2005) and haddock (*Melanogrammus aeglefinus*; Wright *et al*., 2010).

Most studies attempting to address nursery habitat usage of juvenile fish have predominantly focussed on single habitats, for example sampling in only saltmarsh systems (Kelley 1986, 1988; Jennings & Pawson 1992; Cattrijsse *et al*., 1994; Pickett & Pawson, 1994; Laffaille *et al*., 2000; Patterson & Whitfield 2000; Cattrijsse & Hampel, 2006; Green *et al*., 2009, 2012; Jin *et al*., 2007; Dufour *et al*., 2009; Green *et al*., 2009; Underwood *et al*., 2012). These are low lying wetland habitats characterised by a complex network of intertidal shallow creeks interspersed with large amounts of vegetation. Throughout the UK, saltmarshes fully drain at low tide, and flood at high tide. This shallow water complexity, coupled with the observed high numbers of juvenile fish in summer months (Green *et al*., 2009), has attracted much attention, particularly regarding habitat restoration and fisheries management (Doddy 2008, Litvin *et al*., 2018). At high tide saltmarshes are thought to provide refuge from predation, as shallow water is less accessible to predators (McIvor & Odum 2000) and vegetation is thought to reduce the efficiency of visual predators (Savino & Stein, 1982, Mattila 1992). They are also highly productive habitats in terms of carbon sequestration and macro-nutrient cycling (Beck *et al*., 2001; Beaumont *et al*., 2014), which may support high prey availability. The focus on saltmarshes appears to derive from vegetated habitats yielding large densities of juvenile fish, also seen in seagrass and mangrove habitats (McIvor and Odum 1988; Cattrijsse *et al*., 1994; Pickett & Pawson 1994; Laffaille *et al*., 2000; Minello, 2003; Green *et al*., 2009, 2012; Whitfield, 2017). However, there is a danger that by not also sampling in other habitats we are missing how multiple habitats can contribute to recruitment success of juvenile fishes.

Beck *et al*., (2001) and Dahlgren *et al*., (2006) suggest that our definition of nursery habitats is far too loose, and that any habitat must be compared to others where juveniles of the same species are found. Studies only sampling saltmarshes do not achieve this. The ‘nursery role hypothesis’ was originally put forward by Beck *et al*., (2001), where they suggested that too many studies investigating nursery habitats assumed that abundance correlated to survival and recruitment, which fails to account for habitats that disproportionately contribute to fish survival and recruitment relative to the area covered by that habitat. Therefore, Beck *et al*., (2001) suggest that a nursery should be defined as outputting a larger number of fish per unit area than other habitats where juveniles are found. Work by Minello *et al*., (2003) applied this theory to the role of saltmarshes in estuarine systems of the Mediterranean through a meta-analysis. They found that while growth did not differ between saltmarshes and other estuarine habitats such as open water, macroalgae beds, oyster reefs and seagrass, saltmarshes did exhibit increased survival of nekton. They concluded that saltmarshes provided a higher nursery value than other estuarine habitats, but lower than seagrass. However, Dahlgren *et al.*, (2006) argued that while the nursery role hypothesis provides a strong tool for assessing the relative value of a habitat to cohort strength it ignores those habitats that output larger numbers of fish overall, independent of area, which they term ‘effective juvenile habitat’ (EJH). Dahlgren *et al.*, (2006) illustrated the application of EJH through work by Kraus & Secor (2005), who demonstrated that brackish waters contributed considerably larger amounts of white perch (*Morone americana*) recruitment to the adult populations. Brackish habitat supplied 85% of recruits compared to 15% from freshwater habitats. However, due to the relative contribution per unit area of habitat available, freshwater habitats contributed disproportionate amounts of recruitment and so are considered nurseries according to Beck *et al.*, (2001). Effective juvenile habitat and those which provide a nursery role should not be considered as mutually exclusive, both have the potential to provide important measures of the value of a habitat to juvenile fish. Most recently, there has been a push to a seascape view of estuarine nurseries, accounting for the contribution of multiple connected habitats to the growth and survival of juvenile fish spatially, temporally, throughout ontogenetic shifts (Nagelkerken *et al.*, 2015). With little consideration for the vast amounts of habitat heterogeneity found in coastal estuaries it is difficult to suggest what provides “effective” nursery habitat for juvenile sea bass.

Other habitats are often only explored at low tide, or where open habitats are grouped coarsely as ‘estuarine’ or ‘channel’, if grouped at all (Cabral & Costa 2001, Dufour *et al.*, 2009, Martinho *et al.*, 2008). This is possibly based on the theory that juvenile fish should use the shallow surface of saltmarshes to avoid predation at high tide and should forage in the shallow mudflats at low tide (McIvor & Odum, 1988). However, an estuary mosaic is comprised of many structurally different habitats, from complex saltmarshes, oyster reefs, seagrass or other vegetated meadows and shingle beds, to simpler mudflats, sand banks and beach edge habitats consisting of different slopes and structures. Green *et al*., (2012) addressed this to a degree in trying to describe what characteristics of saltmarshes influenced fish abundance, suggesting that marsh usage was dependent on the area of mudflat available directly in front of the marsh. They reasoned that larger mudflats provide sufficient shallow habitat; such that neighbouring marshes are used to a lesser extent. However, the abundance of fish on mudflats was never measured directly. This still leaves us with the questions: how does estuarine habitat heterogeneity influence sea bass habitat associations and what constitutes important nursery habitat?

Identifying important nursery habitat is a crucial facet of fisheries management, specifically with regards to recruitment. Within this context, recruitment is the point at which a juvenile fish joins the adult spawning population (Caley *et al*., 1996), and so directly influences stock size (Houde, 1987, Shulman and Ogden 1987, Beyer 1989, Caley *et al*., 1996, Caselle 1999, Armsworth 2002, Pepin 2015). Inter-annual variation in recruitment, coupled with increasing fishing pressure in recent years, have resulted in a decline in sea bass stocks (ICES 2018, 2019). In 2018 heavy restrictions were implemented under emergency protection measures, that limited both commercial and recreational catch sizes, as well as the seasonal windows when catches were permissible, and the minimum landing size was increased to 42cm (European commission, 2015). These measures have since been lifted, but the vulnerable nature of sea bass stock dynamics still rightfully attracts attention as a commercially important species. Variation in recruitment seen in sea bass stocks is attributed to mortality in early life stages (ICES, 2015, 2018; Beraud *et al*., 2017; de Pontuel *et al*., 2019; Walker *et al*., 2020; zu Ermgassen *et al*., 2020; Tidbury *et al*., 2021). For example, the alevin stage (when the yolk sac is still intact) of capelin (*Mallotus villosus*) and jack mackerel (*Trachurus symmetricus*) can experience up to 80% mortality in a given year due to predation and negative changes in environmental conditions (Hewitt *et al*., 1985, Taggart & Legget 1987, Litvak & Leggett

1992). If a population is recruitment limited (Hixon *et al*., 1998) then reduced recruitment can lead to stock declines (Hixon *et al*., 1998), and a lower maximum sustainable yield (MSY) for many fisheries. As such, the juvenile stages of sea bass have been identified as the most important developmental stage for management, due to low survival rates and abundance of eggs, larvae, and juveniles. This has been attributed to the UK population existing on what is thought to be the Northern range margin of the species, restricted by the 9°C isotherm, that allows spawning to be viable (Devauchelle & Coves, 1988; Jennings & Pawson 1992, Pickett and Pawson 1994, ICES, 2015, 2018; Beraud *et al*., 2017; Walker *et al*., 2020).

Estuarine nurseries are thought to stabilise recruitment variation by providing habitat that promotes the development and survival of these early life stages (Nash and Geffin 2000). This may be achieved by providing habitat with increased foraging potential, predation refuge, or ideal growing conditions, but this is unclear for most species, including bass. The coastal estuarine systems exploited as nurseries by sea bass are subject to immense anthropogenic pressures, such as land reclamation and development (Amorin *et al*., 2017), commercial and recreational maritime activities and waste and agricultural pollution (Doddy 2008). For this reason, the management and conservation of these critical habitats is imperative. By identifying important nursery habitats, we can be more targeted when designating areas of management and conservation in these vulnerable habitats, for example under designation of Bass Nursey Areas (Hyder *et al*., 2018). Furthermore, a better understanding of critical habitat usage may help avoid disproportionate attention towards certain habitats with perceived benefits, which may mean we fail to identify and protect habitats which are equally as important (Beck *et al*., 2001; Brady *et al*., 2020).

This study examines how the distribution, age structure and condition of juvenile sea bass is influenced by estuarine habitat types, as a proxy for habitat utilisation, during the summer post-settlement period. I aim to better understand which habitats support post settlement habitat usage for group-0 sea bass. Specifically, (1) determine patterns of juvenile sea bass abundance among different estuarine habitats, and (2) assess the condition of sea bass among habitats and investigate whether condition provides evidence of differences in habitat quality.

2. MATERIALS & METHODS

2.1. Sample site

The Essex coastline is situated along the Southeast coast of the UK and forms part of the larger Thames Estuary basin. This is a relatively shallow stretch of the North Sea, typically not exceeding depths greater than 30m. The present study focussed specifically on the Colne and Blackwater estuaries (Figure 1) which form a single confluence where they meet at the sea. Both the Colne and Blackwater are commercially important for the inshore fish and shellfish industry, but also experience significant recreational fishing use. These estuaries were selected based on their accessibility and representative amounts of different estuarine habitats in an area thought to sit at the Northern range margin of *D. labrax* on the East coast of the UK. The Blackwater and Colne Estuaries are relatively shallow systems, though the Blackwater is the deeper of the two (15.4m depth at low tide). Both experience tidal shifts in habitat availability, where intertidal habitat is exposed at low tide, with the estuary creeks and intertidal completely draining on the lowest tides. Tidal flows also present changes in temperature and salinity with a stronger salinity gradient found in the Blackwater estuary compared to the Colne, however, both estuaries form freshwater rivers becoming hyposaline environments at the top of the estuary.

A total of 33 sites were sampled across both estuaries between the summer months July-October (Figure 1), when juvenile sea bass numbers reach their peak (Green *et al*., 2009). Sample sites were categorised based on tide, habitat type, and estuary position. Tide was included such that I could infer whether habitats that are important at high tide remain so at low tide, acknowledging that far less habitat by area and accessibility for sampling is available in this tidal state. Habitat type was determined based on the most prevalent and dominating structural components. Sites were assigned to saltmarsh, shingle bay/or beach, oyster reef, mud bay/beach, sand bay /beach, or non-estuarine coastal habitats (e.g. non-estuarine beach). Saltmarshes were only present at high tide, and oyster reefs only accessible at low tide. Further definitions of these habitats are provided in Table 1, along with details of the classification for each site. Coastal habitat was assessed to determine the strength of juvenile fish associations with estuaries themselves. Sample site position was either determined to be at the mouth, mid estuary, or upper estuary. A minimum of three sites per habitat in each estuary were sampled, except for oyster reefs which could only be accessed safely in the Blackwater estuary at two sites (Table 1).

Habitat type	Tide	Sites	Dates	Lat/ Long	Total sites	Description	Gear type	No. of Bass	Size range (mm)	Age range
Saltmarsh	High	1	29/07/2020	51.85174, 0.95558	6	Low lying, vegetated, network of intertidal creeks, mud sediment, high tide.	Fyke	37	24-44	0
		2	05/08/2020 24/09/2020	51.83491, 0.98748				583	23-200	0-1
		3	08/09/2020	51.78067, 1.04411				155	23-83	0
		4	31/08/2020	51.78561, 0.87273				54	33-62	0
		5	07/09/2020	51.71485, 0.85398				321	10-87	0-1
		6	01/09/2020 30/09/2020	51.69706, 0.77072				178	37-119	0-1
Shingle	High	8	20/08/2020	51.84306, 0.98127	6	Mud/clay sediment, large cobbles embedded into substrate, often macrophytes attached, high and low tide.	30m seine	63	30-173	0-2+
		9	16/07/2020	51.79444, 1.03149				38	28-155	0-1
		14	04/09/2020	51.73881, 0.83816				5	46-64	0
		17	16/09/2020	51.73904, 0.77446				35	34-89	0-1
		28	17/09/2020	51.73404, 0.72636				45	27-89	0-1
		30	24/08/2020	51.80638, 1.02111				81	32-140	0-1
	Low	7	27/07/2020	51.84800, 0.97890	4		25m seine	23	23-45	0
		10	16/07/2020	51.79485, 1.02893				17	105-153	1
		15	15/09/2020	51.73832, 0.84893				54	48-87	0-1
		16	11/09/2020	51.73678, 0.77692				437	43-174	0-2+
Sand	High	12	21/09/2020	51.71559, 0.83215	5	Mud/clay sediment, large cobbles embedded into substrate, often macrophytes attached, high and low tide.	30m Seine	352	30-101	0-1
		24	29/09/2020	51.77419, 0.94001				66	41-82	0-1
		26	18/08/2020	51.75213, 0.87350				0	-	-
		29	28/08/2020	51.704300, 0.7734				2	35-42	0
	Low	25	11/08/2020	51.74872, 0.92057	1		25m seine	16	21-57	0
Mud	High	18	31/07/2020	51.85201, 0.95387	3	Soft mud sediment, flat, structurally simple high and low tide.	30m seine	11	23-55	0
		22	03/08/2020	51.82582, 0.97573				4	28-41	0
		27	17/09/2020	51.73428, 0.72329				44	27-89	0-1
	Low	19	15/07/2020	51.85420, 0.95808	3			3	23-55	0
		20	14/07/2020	51.84063, 0.98457				0	-	-
		21	17/07/2020	51.83848, 0.98781				1	-	-
		23	31/07/2020	51.78367, 1.03039				5	117-161	1-2+
Oyster reef	Low	11	10/09/2020	51.74606, 0.89334	2	Live and dead oyster shell matrix embedded in mud sediment, high rugosity, low tide.	30m Seine	27	37-77	0
		13	10/09/2020	51.75528, 0.88403				66	49-91	0

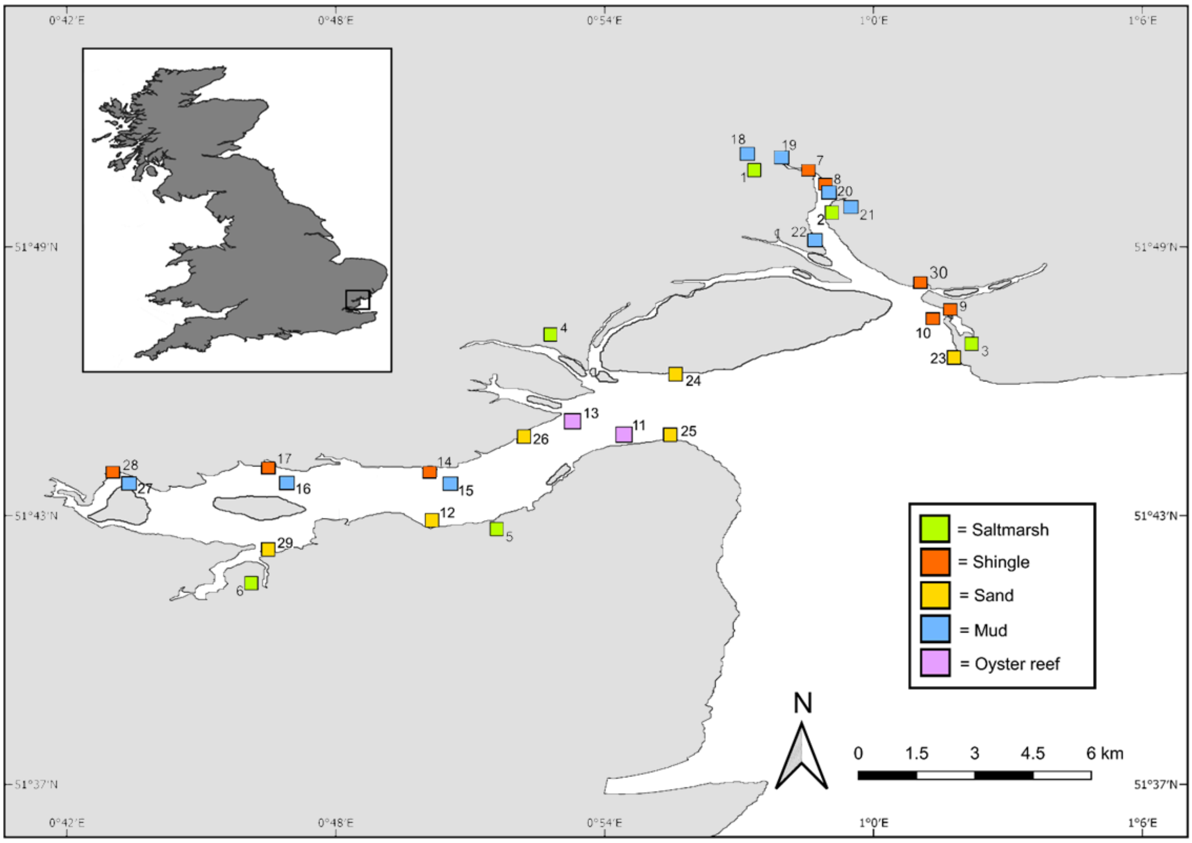

Figure 1. Map of sampling sites in the Colne and Blackwater estuaries, Essex, UK, between July-September 2020. Total sites = 30 (33 including coastal – not depicted). Made using QGIS, 3.2.3.

2.2. Sampling methods

A combination of beach seine and fyke netting was used to measure the abundance of juvenile sea bass present in each habitat. Beach seines were performed in relatively open habitat – shingle, oyster reef, seagrass, mud, sand. Seine nets are used to sample open habitats throughout the literature and are effective at capturing fish, where a habitat cannot be fully enclosed. Fyke nets are passive gear that can be used to capture fish in enclosed or open habitats.. Pilot studies conducted in the Blackwater and Colne estuaries using paired fyke nets with a "mesh wall" were regularly ineffective at capturing anything over open habitats. However, fyke style barrier nets have been used to great effectiveness for sampling saltmarsh creek habitats in numerous studies. While different, both barrier fyke nets and seines capture all fish under the area of a curve. The upstream area captured by a fyke is longitudinal (Green *et al*., 2009; Cattrijsse *et al*., 1994), while that of seines is determined by the arc of the net. In this sense, seine nets create a barrier to escape relative to the shore, while fyke nets create a barrier relative to the creek. In order to compare them the catch must be considered as a ratio of the area sampled in order to determine catch per unit effort. This makes effort calculations of effort difficult and less reliable than with beach seines, which are able to enclose an area that can be estimated and quantified. The only alternative methodology to these would be electrofishing, which is unfeasible at this scale. The combination of fyke and beach seine netting has been well utilised in other studies of fish in littoral and intertidal zones (Arthington *et al*., 2005; Clarke & Jackson, 2007)

Barrier fyke netting was used across saltmarsh creeks. Seines were not used on saltmarsh creeks as they are often too deep or too narrow to effectively deploy them by foot and are unlikely to be effective in sampling fish where the benthic topography is highly complex, which would likely result in large gaps in the net. Barrier fyke nets allow an entire creek to be barriered and so sample a large, fixed area of habitat that can be quantified, with little scope for escapes. Beach seines were performed at high and low tide with either a 25x1.5m or 30x3m seine net, with a 9mm mesh size. Three beach seines were performed per site, with the exception of two sites where the yield was either extremely high and therefore sampling was stopped on welfare grounds, or zero where sampling continued to confirm a zero value.

Seines were either performed in spatially different but nearby areas, for example further along the same shore. Alternatively, seines were undertaken in the same area after enough time had passed that the area could be deemed settled (15mins). Fyke nets were deployed at high tide, at the mouths or accessible points of saltmarsh creeks, similarly to the methods detailed in Cattrijsse *et al*., (1994). A minimum of three creeks per site were sampled. The fyke nets were composed of a 4-5mm mesh wall suspended between a buoyant top line and weighted bottom line of rope, with a cod end stitched into the centre. This cod end was constructed from a mesh cone with mesh valves that ensure fish are unable to swim back out again once in the net, e.g., part of a fyke net. The nets were left during the outgoing tide until the creeks had drained to ~10cm of water. It must be noted, however, that the differences in methodology must be considered when discussing abundance results. Fyke nets are able to sample entire creeks, which the fish have no escape from, while seine netting captures a relatively much smaller area, with higher potential to escape. The act of seining is also a more active process, compared to more passive fyke nets, and bias may be introduced in catch sizes as a result of completely netting off a habitat compared to only small subsamples of more open area. However, these are established methodologies with few alternatives when sampling across multiple habitat types.

Once nets had been brought in fish were immediately identified, counted and placed in an oxygenated recovery tank or where appropriate immediately returned to the sea. Length and weight were measured for all fish, except very large catches, where it was more prudent to take a subsample of 20 fish per seine for measurement and estimate the rest while removing from the net. A maximum of 20 juvenile bass were humanely euthanised per seine and retained for future assessment of stomach contents, in accordance with IFCA dispensations to retain and despatch juvenile sea bass (20200713AP) and under the Schedule 1 exemption for lethal sampling (ASPA 1986). A count or estimation of the total catch was always made, and all other fish were returned to the sea. This provided an estimate of abundance, age/size structure, body condition and samples for later diet analysis for each site. At each site temperature, salinity and pH, were measured using a YSI ProDSS multiparameter probe (Xylem, USA). Measurements were taken every three seconds over a 30s period and averaged for each site.

To standardise the estimate of catch per unit effort (CPUE), the effort for each sample was determined based on the area sampled by the net used. For saltmarsh samples using fyke nets, this was determined to be the area of creek available at high tide upstream of the net. For beach seines, the sampled area was assumed to be equal to the area under the curve of the net in a perfect arc. From this, catch per unit effort for each sample could be determined as the number of fish per metre^2.

Fulton's condition index (K) was determined to compare fish body condition between habitats. This is a metric of the weight to length ratio of a fish, obtained from the weight (kg) divided by the length^3 (m), as established through numerous works on salmonids (Springer *et al.*, 1990; Sutton *et al.*, 1998) and has become an accepted standard metric of fish body condition in the literature for juvenile sea bass (Bacon *et al.*, 2009; Kerambrun *et al.*, 2011, 2012; Cammilleri *et al.*, 2018). Length was measured as the total length, to the nearest 1mm and weight to the nearest 0.01g. Stomachs were not evacuated prior to measurement and so this does introduce issues with trying to disentangle whether increases in condition are the result of fuller stomachs or increased body mass. The age group a fish belonged to was based on its length and compared to *a priori* thresholds determined from well-established length at age curves (Pawson and Pickett, 1994).These are based on the lengths at which age crossovers began to appear, which were supported by length frequency distributions from my own data, which showed a primary mode between 0 to ~80mm, with a secondary mode between ~80-X. Fish were assigned to either 0-group (young of year; 0-79mm), 1-group (1st winter; 80-149mm), or 2-group (2nd winter; 150-180mm).

2.3. Statistical analysis

Abundance and condition data were assessed for normality through visual assessment of frequency distributions, and resembled a negative binomial distribution, exhibiting large degrees of kurtosis to the left. Abundance data was found to be homoscedastic and condition index data was found to be heteroscedastic using Levene's tests. Deviations from normality and homoscedasticity were considered during model construction.

Generalised linear mixed effects models (GLMM) were chosen as they account for the random effect introduced by technical replicates at each site and the negative binomial distribution of the data. These were built using the ‘glmmTMB’ package (Brooks *et al*., 2017) in the open-source statistical software R (v4.2.2., R Core Team, 2022). Model construction went through several iterations. The global model could include habitat, tide, temperature and salinity. Temperature and salinity did not vary with abundance of juvenile sea bass. This was assessed using visual inspection of scatterplots of abundance as a function of salinity and temperature separately, and no relationship was found. This is expected as all samples were collected during the summer months and I observed little variation in temperature and salinity between sites. Furthermore, we would not expect three or four way interactions between temperature, salinity and habitat variables to be either biologically relevant or statistically robust given my sample sizes – i.e. the model would produce poor parameter estimates. Therefore these variables were not included in my model. Repeated measurements of sites provided by multiple fyke nets and seines performed within the same site were treated as a random effect. Heteroscedasticity produced by differences in the number of replications of each habitat were accounted for by using habitat replication a dispersion parameter. I first planned a global model with tide status as a main effect, but due to different habitat types and different biological and hydrological mechanisms that may affect abundance of juvenile fishes at either high or low tide I later split thee analyses into two models, one for low tide abundance and another for high tide.

However, four way interactions between habitat, tide, temperature, and salinity are not biologically or statistically meaningful, and therefore the model was reduced based on biological relevance of variables. Temperature and salinity were not expected to have any meaningful impact on abundance of juvenile sea bass as all samples were collected during the summer months where there was little variation in temperature between months, outside of the relatively small differences between high and low tide. Similarly, the estuarine systems studied have very small salinity differentials between habitats. Salinity and temperature were expected to fall with low tide, and therefore were considered to be co-variates and were removed from the model. However, temperature and salinity data were collected to check whether a relationship exists. This was assessed using visual inspection of scatterplots of abundance as a function of salinity and temperature separately, and no meaningful difference was found.

Coastal habitats were dropped from all models during early data exploration, as all but one site yielded zero fish. Similarly, tidally separated GLMMs were built to assess how condition index differed between habitat types and accounted for the random effect of technical replication and individual fish within each replicate. Abundance and condition analyses were undertaken on 0-group fish only, due to the considerably lower catch rate of 1-group (1 winter) and 2-group bass (2 winters). Significant relationships were identified using likelihood ratio tests using the dropterm function in R. This is achieved through using single term deletions to compare a model including the fixed effect of habitat type against a null model using ANOVA. The resulting p-value is assigned to the fixed effect. Model diagnostics were performed through visual assessment of normal QQ-plots and residuals using the ‘DHARMa’ package in R (Hartig, 2017). To account for heteroscedasticity produced between habitats in the abundance model, dispersion functions were applied based on habitat, to account for heteroscedasticity resulting from differences in catch numbers, using the dispformula function in glmmTMB. Similarly, dispersion functions were applied using site and subsample in condition analyses to account for heteroscedasticity. Tukeys HSD *post hoc* tests were performed, using the ‘lsmeans’ package in R (Russel & Lenth, 2016), to determine where differences occurred between groups, for models of both abundance and condition. While I did not statistically analyse changes in abundance throughout the sampling period, as this we did not sufficiently repeat visits to each habitat type each month, and therefore was not built into the sampling design. However, I visually assessed whether abundance changed throughout the year using line graphs divided by habitat. All data has been uploaded to the Research data at Essex online repository.

3. RESULTS

3.1. Abundance and habitat

0-group sea bass were found at 30 of the 33 sites over both low and high tide. At high tide, no significant difference in bass abundance between habitats was found among habitats ($\chi^2_{(3, 16)} = 2.00$, $P < 0.05$). Mean abundance among habitats was similar, however, the largest individual CPUE was found on sand, at site 13, and saltmarshes (Figure 2). Site 13 exhibited a 587% increase in abundance compared to the mean for sand habitats, and 400% compared to the highest CPUE for saltmarshes and shingle. At low tide the relationship between habitat and abundance was found to be significant ($\chi^2_{(3, 10)} = 12.14$, $P < 0.01$). Significantly more fish were found in oyster reefs ($P = 0.04$) and shingle ($P = 0.04$) compared to sand. It is also worth noting that while no significant difference was found between oyster reefs and shingle compared to mud, considerable increases in mean CPUE were observed. Shingle exhibited the largest mean CPUE, increasing by 597%, while oyster reefs exhibited a 214% increase compared to mud. Size distributions were relatively similar across all habitats at both low and high tide, with the highest frequency appearing around the 50-60mm. However, at high tide, the largest individuals were found in saltmarshes and shingle, while at low tide they were found on shingle and mud. These larger individuals, above 90mm, were not found in any other habitats. Both saltmarsh, shingle and oyster reef also exhibit the largest range of sizes. While we did not statistically test changes in abundance throughout the year, Figure 4 shows that for the majority of habitats the abundance of 0-group and 1-group sea bass increased with month, especially in September.

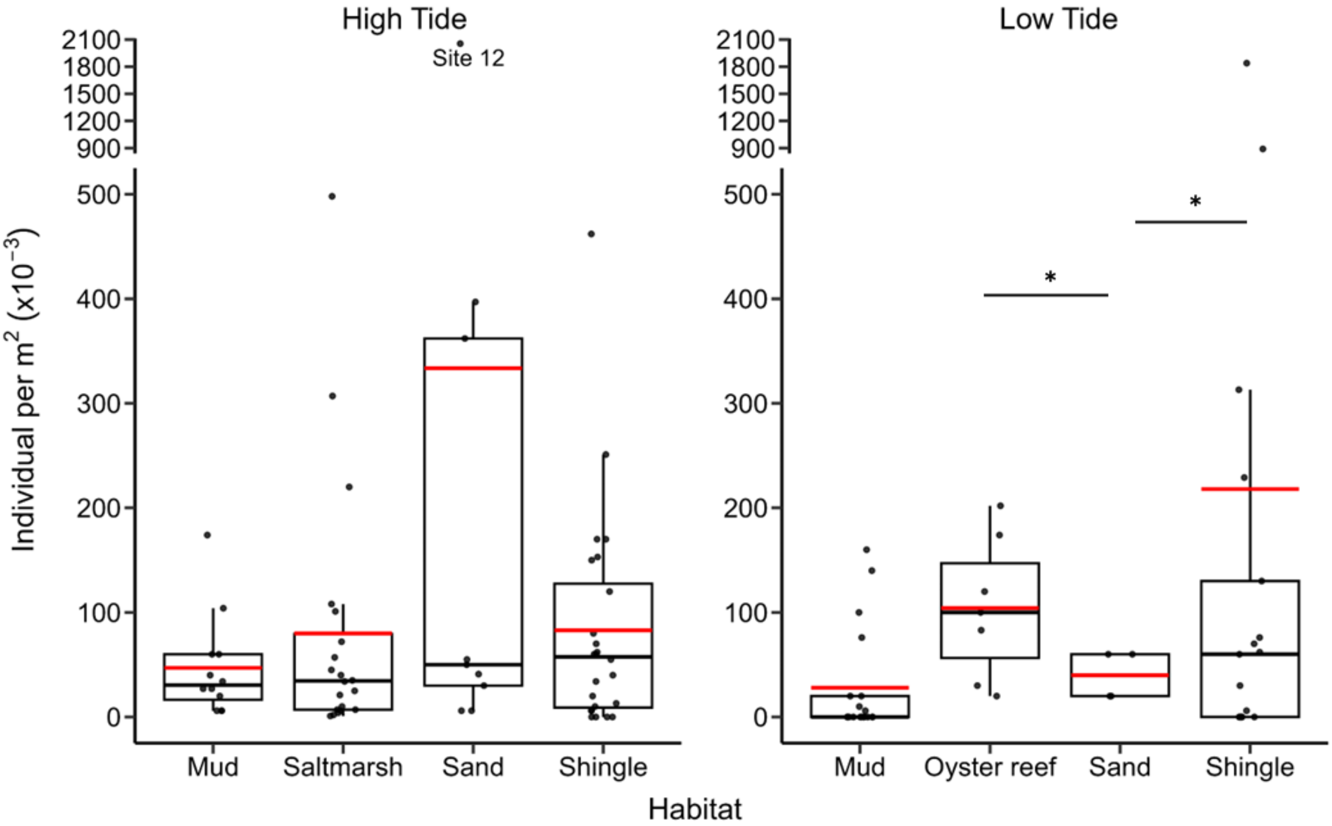

Figure 2. Boxplots for the average catch per unit effort (CPUE) of 0-group *D. labrax*, between estuarine habitats at high (left) and low (right) tide. Habitats are mud, saltmarsh, sand, shingle, and oyster reefs. CPUE is expressed as number of fish per m^2 x10^{-3}. Points represent individual data points for each site. Whiskers represent maximum data point within 1.5x the interquartile range. Red line indicates the mean, black line represents the median. Significant differences highlighted by *** when $p < 0.01$ and * when $p < 0.05$.

When looking at differences in abundance of other species present alongside sea bass, the mixed effects model for high tide herring abundance found no significant difference ($\chi^2_{(3, 16)} = 4.55$, $P > 0.05$). Their abundance was highest on sand habitats, with virtually no Herring found in other habitats (Figure, 5). Similarly for mullet, no significant difference was found ($\chi^2_{(3, 16)} = 7.55$, $P > 0.05$), but the largest abundance were exhibited on shingle habitat. The abundance of sand smelt did significantly differ between habitats ($\chi^2_{(3, 16)} = 13.73$, $P < 0.01$), with significantly more smelt present on sand ($P = 0.04$) and shingle ($P = 0.01$) compared to saltmarsh.

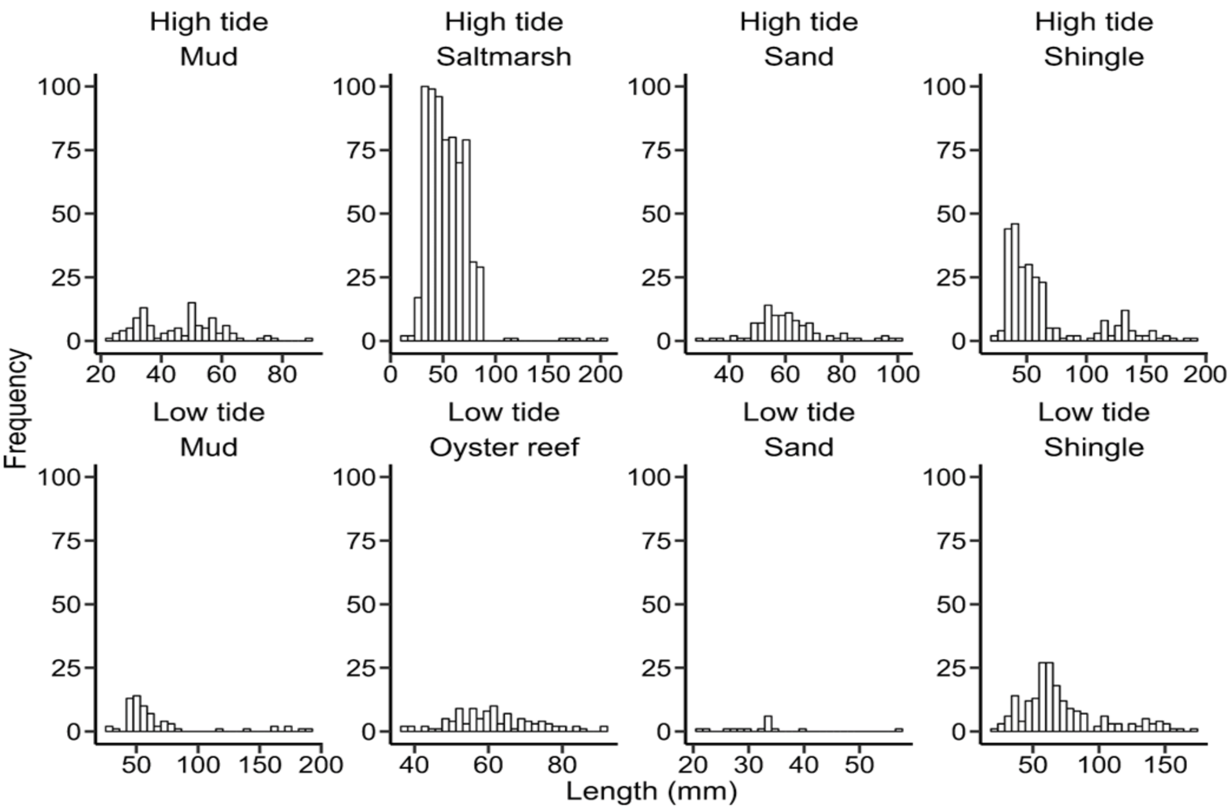

Figure 3. Size frequency distributions of 0-2+ group *D. labrax* length (mm), between estuarine habitats at high (top) and low (bottom) tide. Habitats are mud, saltmarsh, sand, shingle, and oyster reefs.

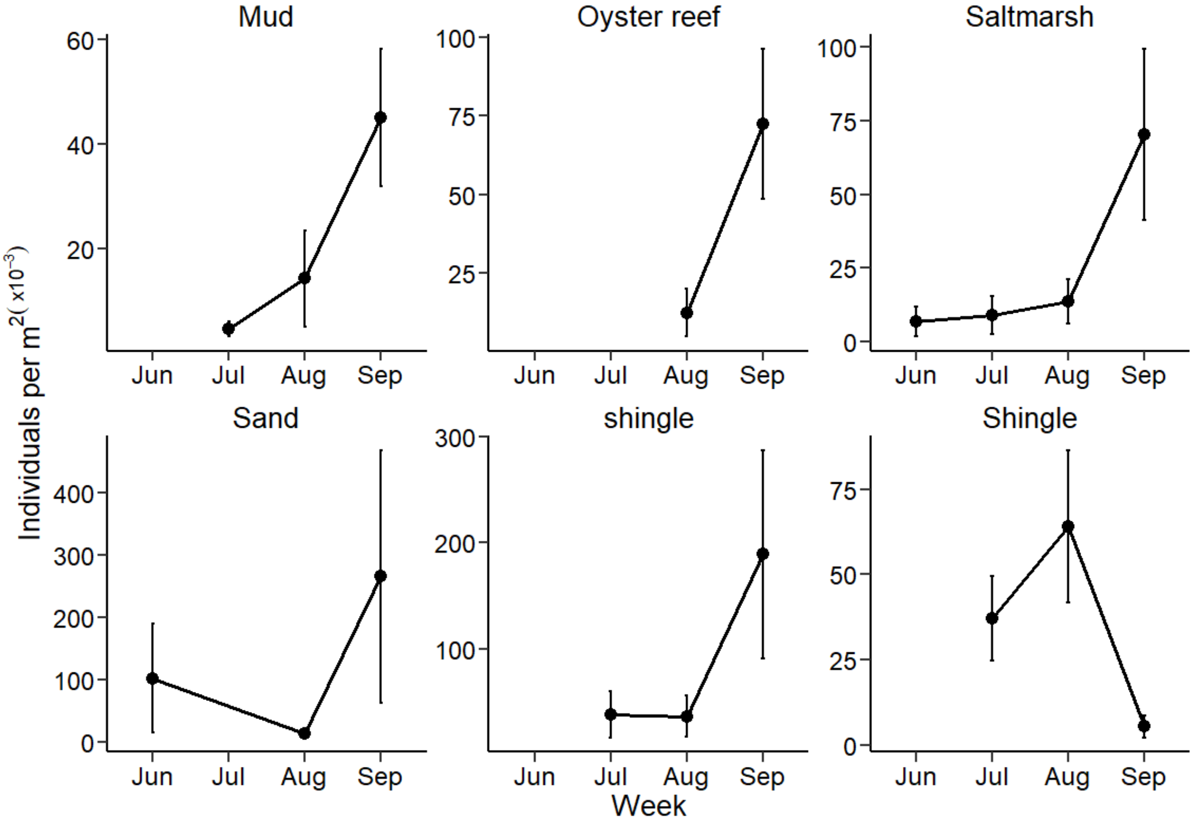

Figure 4. Abundance of 0-1-group *D. labrax* length (fish m^{-2}) , between estuarine habitats mud, saltmarsh, sand, shingle, and oyster reefs, with month of the year.

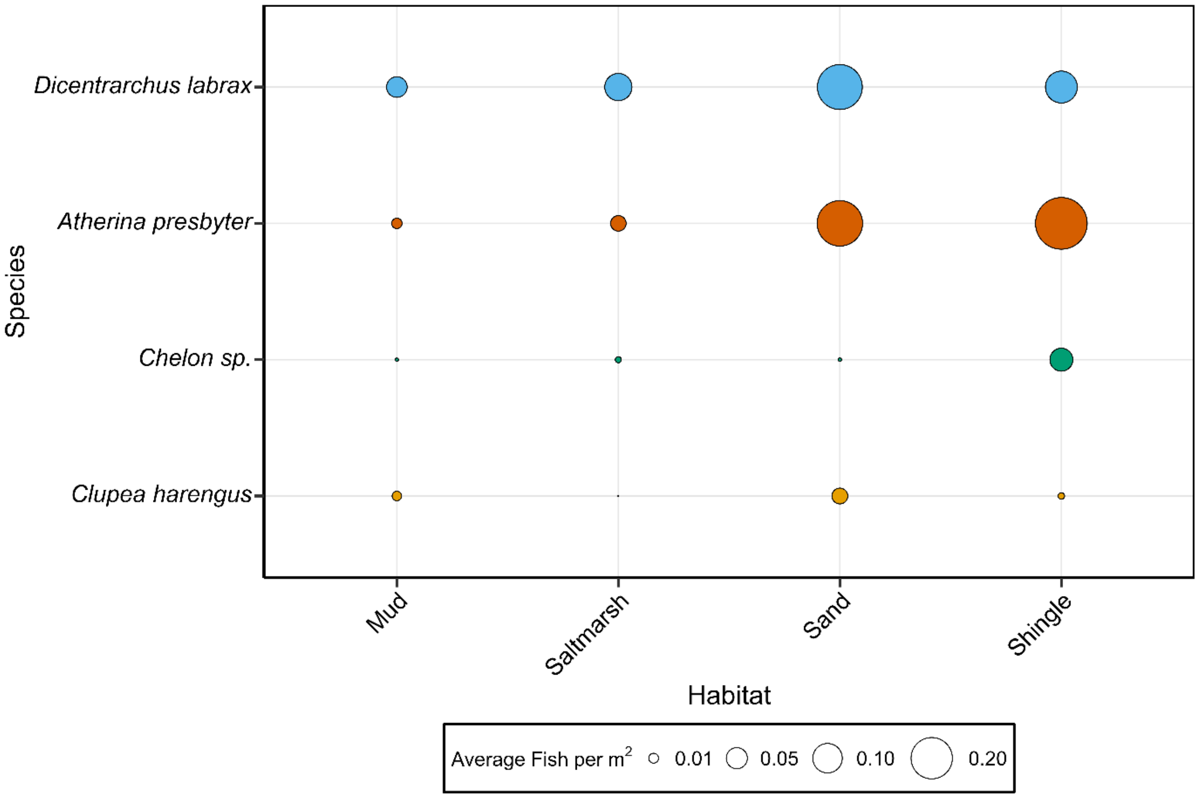

Figure 5. Bubble plot showing the average fish per m^2 of the most abundant species *D. labrax* (blue), *A. presbyter* (red), *Chelon sp*. (green), and *C. harengus* (yellow), between different estuarine habitats: Mud (n = 4), Saltmarsh (n = 6), Sand (n = 4), Shingle (n = 6).

3.2. Condition and habitat

No significant difference in condition was found between habitats at high tide ($\chi^2_{(3, 16)}$ = 4.93, P = 0.176). The mean fish condition was very similar across all habitat types, though the highest condition index were found in saltmarsh and sand (Figure 6). At low tide however, condition differed significantly between habitats ($\chi^2_{(3, 10)}$ = 27.34, P < 0.01), where condition was significantly lower on oyster reefs (P = 0.04) compared to mud, and shingle compared to sand and mud (P = 0.01), where bass abundance was highest. Condition on shingle was also significantly lower than on sand (P = 0.02). Mean condition increased by 22% on mud when compared to oyster reefs, and 25% compared to that of shingle. Similarly, sand exhibited an increase of 27% in mean condition from oyster reefs, and 30% from shingle. No significant difference was found between mud and sand, or sand and oyster reefs who exhibited similar mean condition.

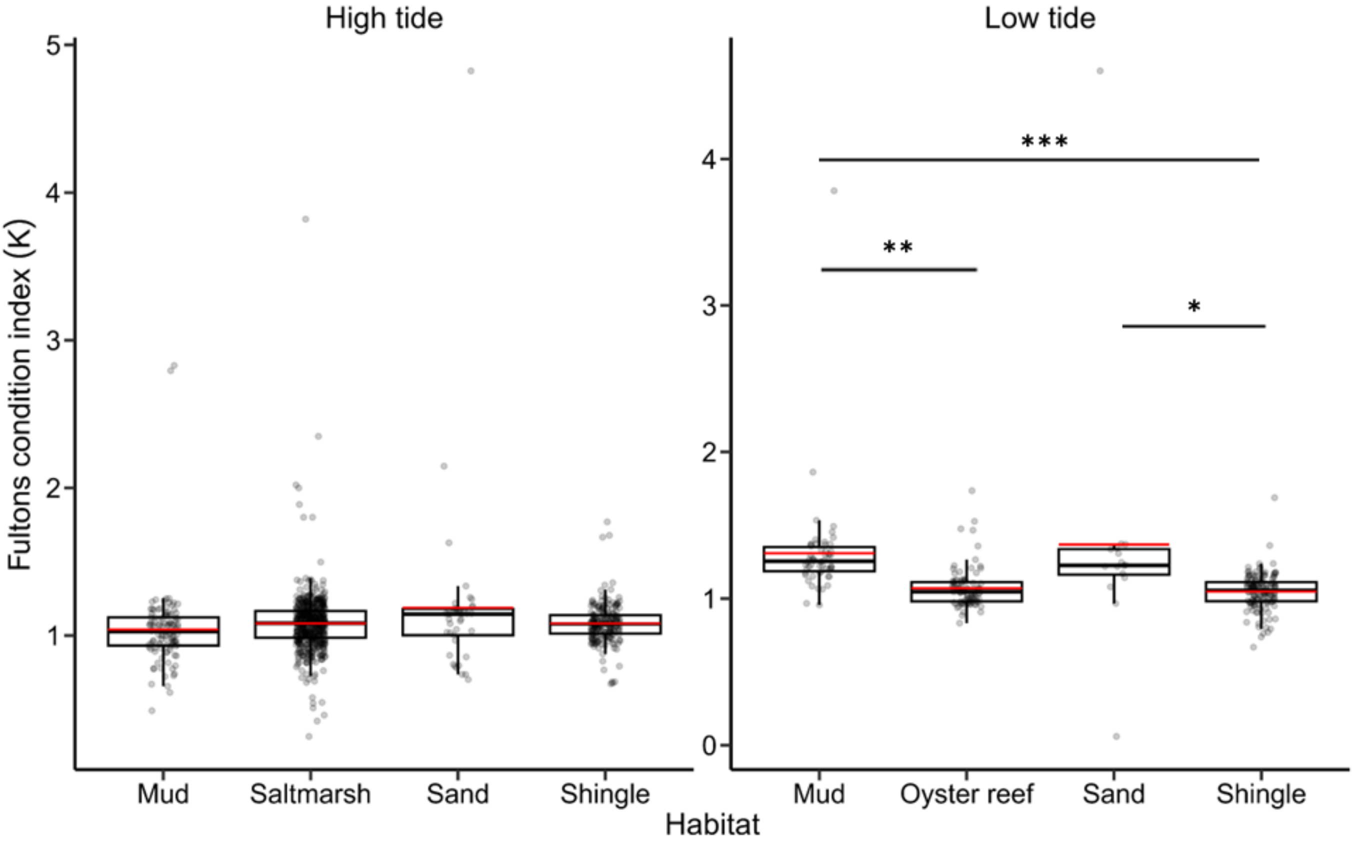

Figure 6. Boxplots for the average Fultons condition index (K) of 0-group *D. labrax*, between estuarine habitats at high (left) and low (right) tide. Habitats are mud, saltmarsh, sand, shingle, and oyster reefs. Condition index is the product of weight (mg) / length^3 (mm) x 100. Points represent individual data points for each fish. Whiskers represent maximum data point within 1.5x the interquartile range. Red line indicates the mean, black line represents the median. Significant differences highlighted by *** when $p < 0.01$ and * when $p < 0.05$.

Table 3. Results of mixed effects models assessing the effect of habitat and tide on juvenile sea bass abundance and condition

ANOVA results table							Model Summary				
Model	**Random effect**	**Dispersion parameter**	**Fixed effect**	**Adj. Chi^2**	**Pr**	**AIC**	**Coefficient**	**Est.**	**Std. Error**	**Z**	**Pr**
Abundance ~ Habitat + Tide	Site	Habitat	Habitat	5.387	0.250	1132.2	Oyster reef	2.42	1.26	1.92	0.055
							Saltmarsh	1.04	0.96	1.09	0.278
			Tide	1.571	0.210		Sand	1.48	0.93	1.57	0.117
							Shingle	1.24	0.77	1.60	0.110
							Low Tide	-0.88	0.71	-1.25	0.210
High tide abundance ~ Habitat	Site	Habitat	Habitat	2.00	0.572	704.6	Saltmarsh	0.59	0.75	0.78	0.434
							Shingle	1.28	0.94	1.36	0.174
							Sand	1.28	0.94	1.36	0.174
Low tide abundance ~ Habitat	Site	Habitat	Habitat	12.185	** >0.01	415.2	Oyster Reef	1.31	0.74	1.77	0.077
							Sand	0.36	0.74	0.48	0.632
							Shingle	2.05	0.92	2.23	0.025
Condition ~ Habitat + Tide	Site + Creek/Seine	Habitat	Habitat	19.77	0.527	4503.4	Oyster reef	-0.56	0.74	-0.76	0.447
							Saltmarsh	0.32	0.52	0.61	0.543
			Tide	17.402	>0.05		Sand	0.52	0.72	0.72	0.471
							Shingle	-0.33	0.45	-0.75	0.455
							Low Tide	0.92	0.42	2.19	0.028
High tide condition ~ Habitat	Site + Creek/Seine	Habitat	Habitat	2.81	0.422	3436.8	Saltmarsh	0.69	0.43	1.60	0.110
							Sand	0.38	0.78	0.49	0.626
							Sand	0.62	0.45	1.40	0.163
Low tide condition ~ Habitat	Site + Creek/Seine	Habitat	Habitat	26.863	***>0.01	1036.4	Oyster Reef	-1.84	0.58	-3.18	>0.01
							Sand	-0.47	0.76	0.61	0.544
							Shingle	-2.48	0.51	4.82	>0.01

4. DISCUSSION

An important criterion for establishing whether a habitat is a nursery is the comparison of juvenile fish output compared to other habitats where juvenile fish of the same species are found (Beck *et al*., 2001; Dahlgren *et al*., 2006). However, more recently increased focus has been placed on the contribution of multiple connected habitats within a system that contribute to juvenile survival and growth, in what is termed the seascape nursery (Sheaves *et al*., 2006; Sheaves, 2009; Nagelkerken *et al*., 2015; Amorim *et al*., 2017; Litvin *et al.*, 2018; Olson *et al*., 2019; Waltham *et al*., 2020). The present study provides new insights into habitat choice and utilisation of juvenile seabass across the tidal estuarine mosaic. Our results suggest that multiple habitats are important to juvenile sea bass distribution patterns and that a mosaic of habitats may support a nursery role for sea bass.

At high tide, juvenile sea bass showed similar abundance among all habitats investigated, suggesting no preference for saltmarshes or intertidal bay habitats fringing subtidal channels. Throughout the literature, temperate estuarine nurseries are discussed almost solely within the context of saltmarsh systems (Kelley 1986, 1988; Jennings & Pawson 1992; Cattrijsse *et al*., 1994; Pickett & Pawson, 1994; Laffaille *et al*., 2000; Patterson & Whitfield 2000; Cattrijsse & Hampel, 2006; Green *et al*., 2009, 2012; Jin *et al*., 2007; Dufour *et al*., 2009; Green *et al*., 2009; Underwood *et al*., 2012Whitfield, 2017, 2020), due to observations they support high numbers of juvenile fish (Pawson and Pickett, 1994; Cattrijsse *et al*., 1994; Laffaille *et al*., 2000). However, saltmarshes are rarely discussed within the context of surrounding connected intertidal habitats (Krumme *et al*., 2014). In other systems, seagrass meadows and mangroves are often discussed with relation to less structured but connected habitats and so offer better justification for nursery roles (Connolly, 1994; Jenkins, 1997; Hindell and Jenkins, 2004; Franco *et al*., 2006; Hosack *et al*., 2006; Levin *et al*., 2008). Indeed, saltmarshes appear to exhibit high abundance of juvenile bass in the present study, but no more so than other shallow intertidal habitats. Furthermore, an 11% subset of sand dominated habitats attributed to site 12, while not significant, exhibited a CPUE close to 600% higher than the mean of sand habitats, and over 400% higher than the maximum abundances of saltmarsh and shingle. Sand habitats were broadly characterised by the presence of a short sandy beach fringing intertidal flats composed of mud or mixed sediment. Site 12 was a highly vegetated sand dominated habitat that fringed a subtidal seagrass meadow, with associated drift algae that inundated the intertidal, creating a complex matrix of macroalgae. We defined habitat types based on the dominant structural characteristics observed at that site, primarily related to substrate type. However, this broad definition is limiting, in that it does not sufficiently consider structural differences within habitat types, which is assumed to be equal. For example, some shingle habitats support diverse macrophyte communities, while others exhibit more benthic complexity, but this is not sufficiently captured.

However, differences in sampling methodology between habitats must be considered. Fyke nets were used to sample saltmarsh systems, which net off an entire section of creek and, so, sample a much larger area of habitat when compared to beach seine nets. Furthermore, seine nets do not completely enclose off a habitat, and so do not capture all fish in that area, while fyke nets have very little scope for escape for a juvenile fish. Therefore, fyke nets may be more representative of what is in that habitat compared to seine nets. As such it is difficult to compare the CPUE between habitats due to the large differences in area sampled, however, in both instances juvenile sea bass are found in similarly high abundance, regardless of CPUE. For example, at site 12, which was a sandy bay associated with seagrass, 295 0-group sea bass were caught in a single seine, compared to 385 in a creek at site 2, while a shingle bay at site 16 produced 264 fish in a single seine. Therefore, while the effort may differ, high abundances of bass are still found between habitats. These results suggest that high abundancies of juvenile sea bass can be found in a multitude of habitats within estuarine nurseries, and suggest many habitats are desirable,, but that highly vegetated habitats have the potential to be preferable.

The use of shallow water by juvenile fish has been widely observed in many habitat types including saltmarshes (McIvor & Odum, 1988; Paterson & Whitfield, 2000; Rypel *et al*., 2007; Whitfield, 2017, 2020), mangroves (Vance *et al*., 1996; Hindell & Jenkins, 2004; Zayers *et al*., 2012; Gannon *et al*., 2015; Whitfield, 2017, 2020), and freshwater lakes and rivers (Whitfield, 2017). In South African saltmarshes juvenile fish occupy shallow creeks on the marsh surface, while larger piscivores occupy deeper channels (Patterson and Whitfield, 2000). In mangrove systems, juvenile fish travel further into the mangrove, into shallower water, while larger fish occupy the deeper fringes (Vance *et al*., 1996). Juvenile sunfish in freshwater lakes will inhabit the lotic zone where large bass predators are excluded (Harvey *et al*., 1991). Shallow water is thought to be less accessible to larger fish who prefer deeper water (Whitfield, 2017, 2020). A review on shallow littoral zones as predation refuge by Whitfield (2020) showed that in the majority of cases there is reduced presence of and predation from piscivores in shallow water. Use of shallow habitat at high tide, to a degree, agrees with suggestions from McIvor and Odum (1988) that at high tide juvenile fish should make use of shallow habitat on the marsh surface to avoid predation in the inundated subtidal channels. It is possible that juvenile fish are driven into shallow habitats at high tide due to the potential for increased predation potential in deep water channels of the main estuary. A rebuttal to Whitfield (2020) by Baker and Sheaves (2021) argues that predation from large fish is unlikely to be the single driver of high juvenile fish abundance in shallow habitats. Baker and Sheaves (2021) argue that predation on juvenile fish by other juvenile fish accounts for a high proportion of mortality experienced by newly settled fish, and that in fact shallow habitats may be a predation trap. While abundance in shallow habitats is high, they may not exhibit increased survival, and that in fact vegetative complexity may be a more important habitat component for increased survival than depth alone in littoral zones (Baker & Sheaves, 2021).

However, all habitats sampled in this study were of similar depth, due to limitations in accessibility to deeper habitats, and therefore, the use of shallow compared to deep habitat was not investigated here. It is, therefore, difficult to suggest with any certainty whether abundances of 0-group sea bass are different between shallow intertidal habitats and deeper subtidal channels, and whether these habitats are desirable because they are shallow or for some other reason. However, a study of juvenile bass connectivity between estuarine systems in Ireland noted that 0-group sea bass were reduced in the subtidal trawls undertaken, where mostly 1-group bass were found (Ryan, 2020). It is likely that similar behavioural patterns are exhibited in sea bass of this region as well. Whether the use of shallow estuary bays by juvenile sea bass is in direct response to predation pressure or a behavioural syndrome that has evolved across habitats is unknown, as predation was not directly tested in this study.

The structural complexity presented by seagrass meadows and other estuarine macrophytes is also thought to be a major driver in juvenile fish distribution patterns (Laegdsgaard & Johnson, 2001; Heck *et al*., 2003; Smith *et al*., 2008; Cheminée *et al*., 2016; Sheaves *et al*., 2016; Leslie *et al*., 2017; Whitfield *et al*., 2020; Baker & Sheaves, 2021). Most work on the role of habitat complexity has focussed on tropical seagrass (Heck *et al*., 2003; Minello *et al*., 2008; Cheminée *et al*., 2016; Leslie *et al*., 2017) and mangroves (Blaber, 2000; Kathiresan & Bingham, 2001; Shinnaka *et al*., 2007; MacDonald *et al*., 2008; Nagelkerken *et al*., 2008; Nanjo *et al*., 2011, 2014a, 2014b). Juvenile abundance and diversity has been demonstrated to increase in seagrass meadows compared to unstructured sand flats (Heck *et al*., 2003; Minello *et al*., 2008; Cheminée *et al*., 2016; Leslie *et al*., 2017), and the same is true for mangroves (Blaber, 2000; Kathiresan & Bingham, 2001; Shinnaka *et al*., 2007; MacDonald *et al*., 2007; Nagelkerken *et al*., 2008). This is understood to be because structured habitats provide refuge from predation, reducing the efficiency of visual predators by reducing detection and chase ability (Savino & Stein, 1982; Almany, 2004; Horinouchi, 2007a). Many studies suggest that survival is generally higher in structured habitats when compared to unstructured (Matilla, 1992; Minello *et al*., 2003; Almany, 2004; Nanjo *et al*., 2011, 2014a; Nakamara *et al*., 2012). Increased complexity is also thought to be connected to the abundance and diversity of invertebrate prey species as well. (Crooks, 2002; Hauser *et al*., 2006; Hosack *et al*., 2006; Thomaz *et al*., 2008; Bouma *et al*., 2009; Pinnel *et al*., 2021). Prey availability is also thought to drive fish distribution patterns (Nagelkerken *et al*., 2002; Horinouchi, 2007b; Horinouchi *et al*., 2013; Nakamura *et al*., 2012; Nanjo *et al*., 2014b). It is possible that the very high abundance on the single seagrass habitat investigated in this study is due to the increased predation refuge provided by shallow vegetated habitats. Saltmarshes are able to provide structural habitat complexity from halophilic flora along creek fringes, but these are only inundated on very high tides, with the highly vegetated marsh face only on the highest tides. However, arguments from Whitfield (2021) strongly suggest that differences in fish assemblages and location influence the relative importance of complex shallow littoral habitats. For example, many shallow habitats in Australia support numerous small-bodied piscivores that pose a potential high threat to juvenile fish, while the same is not seen in South Africa. In our study, we found that generally the occurrence of large bodied predatory fish was low, apart from small groups of transitory mullet and large bass, which did in fact enter shallow habitat. However, this was not directly investigated. Older juvenile sea bass are themselves opportunistic piscivores and share their habitat with other juvenile fish such as sand smelt (*Atherina presbyter*), mullet (*Chelon sp*.), and herring (*Clupea harengus*), none of which supported gape sizes capable of consuming other juvenile fish. The diet of many of these species' juvenile stages is primarily copepods, benthic invertebrates or surface insects (Green *et al*., 2009), and likely do not present a predation threat.

It seems that increased abundance across shallow habitats could represent a potentially problematic behavioural syndrome in the absence of complex habitat, but serve an important function in reducing predation, nonetheless. The increased use of seagrass associated sand habitats could indicate that complex shallow habitat is preferable. Work by Minello *et al*., (2003) has demonstrated that throughout the literature saltmarshes and seagrass habitats increase survival of juvenile fish. However, no direct measures of the influence of vegetative complexity or survival between habitats was possible for this species within the scope of this project. Therefore, it is difficult to determine whether the high abundance across estuary bays indicates that these habitats are as attractive as saltmarshes to a juvenile sea bass or an artefact of saltmarsh loss. Many bay habitats studied here likely once fringed saltmarshes that have since been reclaimed for agriculture, recreation, and maritime, which could be restricting fish to shallow bays closer to the deeper subtidal channels. Investigating the differences in both benthic and vegetative complexity between estuarine habitats and its effects on the survival of juvenile fish should be a focus of research moving forward to understand the relative benefits of connected habitats along the estuarine mosaic to sea bass nurseries.

Fish condition was also similar across habitats, suggesting that there was no segregation of high quality and lower quality fish between habitats. Condition is a measure of ‘fatness’, and so can be used as a proxy for the relative quality or fitness of a fish compared to others it coexists with (Dempster *et al*., 2011). Fish in better condition and higher quality are typically thought to be better at acquiring resources, owing to greater fat reserves (Skov *et al*., 2002; Dempster *et al*., 2011). Alternatively, the greater weight could be the result of greater stomach fullness, further supporting better resource holding ability. Fish of both high and low condition were found across all habitats and so suggests that lower quality fish are able to make use of habitat of similar quality to fish in better condition. This further supports that shallow habitats are important for juvenile sea bass, as all forms seem to utilise them, and are possibly valued similarly.

At high tide, we can be reasonably sure distribution patterns are a reflection of individual fish selection. On the flooding tide, habitats are inundated and become available over a 6-hour timeframe, which provides ample opportunity for selection to occur, whether that be a random/neutral selective process akin to a free distribution, or an active process of selecting shallow habitat. These habitats are, however, time-limited resources (Holt *et al*., 2008; Yang *et al*., 2008; Raposa *et al*., 2009; Calles *et al*., 2018), where temporal habitat availability with tide affects spatial availability to create ‘transient windows of connectivity’ (Ziegler and Fagan, 2014). At low tide, tidal forcing massively reduces the area of available habitat. The extent of shallow subtidal fringe habitat is very low during low tide, by comparison to high tide shallow intertidal, such that the element of selection is severely reduced (Rangeley & Kramer, 1995; Gibson, 2003; Childs *et al*., 2008; Raposa *et al*., 2009; Taylor *et al*., 2013; Gannon *et al*., 2015; Krumme *et al*., 2015). Therefore, due to this tidal-forcing it is very difficult to say if distribution patterns are the result of some form of diminished selection, or tidally forced aggregations into available shallow fringes. For example, juvenile pollock, on rising tides, will enter and disperse between shallow intertidal rocky shore habitat, where they remain throughout the high tide period using macroalgae for refuge and foraging (Rangeley & Kramer, 1995). On falling tides, juvenile pollock rapidly migrate out of shallow habitat and densely aggregate in subtidal water (Rangeley & Kramer, 1995). Similar tidal behaviour has been observed in highly mobile wading birds, that feed in the shallow water mudflats, which are only available to the birds at low tide, and these birds are physically excluded from these foraging sites on flooding tides, forcing them to use other habitats (Raposa *et al*., 2009). With this in mind, and despite restrictions in our ability to sample subtidal deep water, we assessed the distribution patterns of juvenile sea bass across low tide habitats due to the presence of complex and unstructured shallow habitat along the subtidal channel fringes. Our results suggest that juvenile bass show higher abundance in structured oyster reef and shingle, compared to unstructured sand and mud, where conversely condition was found to be higher.

As explored earlier, increased habitat structure, provided by oyster reefs and shingle beds, may be driving abundance distributions due to the relatively increased predation refuge and prey abundance. However, lower condition in these habitats may suggest that lower quality fish are excluded to these habitats, with higher quality fish using sand edges. This could represent a trade-off between predation refuge in more complex habitat, and foraging potential in less structured habitat. More structurally complex habitats, while exhibiting increased abundance and diversity in many systems, may also reduce the foraging efficiency of fish (Diehl and Eklov, 1995; Primavera, 1997; Nunn *et al.*, 2012). Furthermore, in many estuarine systems, structurally simple habitat, such as mudflats, may be of similar productivity to more complex habitats, such as mangroves and saltmarshes (Tse *et al.*, 2008; Sueiro *et al.*, 2010). These structurally simple habitats may entail a greater predation risk to juvenile fish of lower condition (Booth & Beretta, 2004). Booth & Beretta (2004) demonstrated that predation related mortality was significantly higher in young-of-year damselfish in low condition than those of a higher condition, such that in mixed groups all fish of lower condition fish were consumed by predators under experimental conditions. Furthermore, damselfish of higher condition were generally more despotic, consuming larger amounts of prey than fish in lower condition (Booth & Beretta, 2004). However, we believe there is considerably less scope for habitat selection in these low tide habitats, and so it is likely that the high abundance of lower condition bass in shingle and oyster reef could indicate that these habitats are generally of greater availability at low tide and generally more connected to high tide habitat. This could suggest that lower condition fish are tidally forced into aggregations in the most common habitats, while fish of higher condition are able to make use of potentially more profitable habitats. Therefore, low tide distribution patterns may represent a behavioural shift in lower quality fish to predation avoidance, or a lack of selective ability due to reduced habitat availability. It would be interesting in future work to assess whether the degree of connectivity between low and high tide habitats influences where we find juvenile fish aggregations, possibly through investigation of fish movements throughout the tidal cycle, possibly through the use of baited underwater video or temporal seine netting across ebbing and flooding tides. Furthermore, our work did not address the use of subtidal channels due to limitations in sampling equipment, and this remains an important question in considering what habitat is selected both at high and low tide. How abundance differs between shallow littoral zones and deep water of subtidal channels, or compared to shallow fringes at low tide is very important for understanding the behavioural mechanisms behind selection. Furthermore, no measurements of predation and survival of fish between shallow habitat and subtidal exists and should be a primary area of targeted research in future research.

However, there are various limitations to the interpretability of this study, and the implications for identifying important nursery habitat. Firstly, according to the framework set out by Beck *et al*., (2001), the designation of a nursery habitat is reliant on the contribution per unit area of juvenile fish to the adult spawning population. While we addressed this to a degree through proxies of habitat desirability (abundance) and survival probability (condition) we were unable to directly measure differences in growth rates and the contribution rates of different habitats. This is particularly difficult to achieve, as sea bass are highly transient and, based on the results presented in this study, likely utilise many habitats throughout estuarine environments. It is more likely that it is a combination of different habitats that contribute to sea bass survival and subsequent recruitment. To fully understand how the mosaic of estuarine habitats contributes to survival would require larger and more expensive studies utilising a combination of telemetry, population genomics and stable isotope microchemistry to monitor habitat use, growth, and interannual survival of individual fish, as well as more landscape level effects on genetic connectivity between populations, which was beyond the scope of this project. So it is not possible to assign a nursery function to any one habitat in this study. However, measurements of survival proxies can provide us with valuable insights into habitats that are of higher quality that might convey a survival advantage, and subsequently may support an increased contribution to adult stocks.

Secondly, we did not factor time of year into our survey design, as we intended to capture only the summer period, and therefore, we did not rigorously repeat measures of habitat types between months as the season progressed. Based on my findings, it is highly likely that there was a general increase in juvenile sea bass abundance as the summer progressed between June to September, and it is possible this may bias the results of abundance between habitats. Crucially low tide sand and oyster reefs had very low replication. Therefore, abundance estimates may be skewed towards those habitats that were more well represented towards August and September compared to June. However, we did attempt to replicate sampling temporally for some sites to confirm that abundance estimates were similar between months, and for these habitats, catch sizes were generally consistent.

In conclusion, our work suggests that juvenile sea bass make use of a broad range of shallow littoral habitats ranging from saltmarshes to bay habitats fringing the subtidal channels. However, the highest abundance was found in a single sand habitat fringing a seagrass meadow. This is possibly due to the potential refugia provided from shallow water and habitat complexity. Whether a mismatch between fish abundance and habitat that promotes highest survival is occurring is unknown, and alternatively these habitats could present predation traps. It is certainly a possibility that the use of many habitats suggests that many habitats are important to sea bass, or alternatively the use of bays could be an artefact of a behavioural syndrome, wherein fish would traverse into saltmarshes were they not reclaimed. Based on the evidence presented here, however, accepting that measurements of survival rates are required, and the difficulties in comparing CPUE between the large area sampled by a saltmarsh creek fyke compared to a beach seine, we believe that shallow littoral habitats may underpin the distribution patterns of juvenile sea bass found in estuarine nurseries. Attempting to assign saltmarsh systems alone as targeted management areas, effectively becoming a ‘saviour habitat’ is potentially dangerous and risks the loss of connected habitats that are equally as valuable. The high abundance of sea bass across all estuarine habitats at high tide suggests that it is important to consider the protection of a mosaic of interconnected habitats to support nursery functions, rather than focussing on individual habitat types. More work is needed to quantify the degrees of connectivity between these habitats and the functions they provide. A major question this chapter has not addressed is the actual function of these habitats. Chapter Three investigates whether differences in foraging occur between habitats along the estuarine mosaic, to investigate to what extent this may be driving distribution patterns observed in this study.

CHAPTER THREE

HABITAT DEPENDANT FORAGING BEHAVIOUR AND DIET OF JUVENILE EUROPEAN SEA BASS ACROSS THE ESTUARINE NURSERY MOSAIC: INSIGHTS FROM DIETARY DNA METABARCODING.

*Juvenile sea bass on a measuring board

1. INTRODUCTION

Understanding what drives habitat choice of juvenile fish is fundamental to ecosystem-based approaches to fisheries management (Litvin *et al*., 2019). Temperate coastal nursery systems utilised by the juveniles of many important species are formed of a mosaic of connected habitats, varying in structural complexity and composition, such as saltmarshes, tidal mudflats, and subtidal oyster reefs (Sheaves *et al*., 2006; Nagelkerken *et al*., 2015). Despite a push towards a seascape view of coastal nursery management (Sheaves *et al*., 2006; Nagelkerken *et al*., 2015; Litvin *et al*., 2019; Waltham *et al*., 2020), our understanding of the ecosystem services provided by each connected habitat is limited. Work by Beck *et al*., (2001), Dahlgren *et al*., (2006), and Sheaves *et al*., (2006) has helped to clarify our definition that the nursery role of a habitat is dependent on the ability of connected habitats to promote growth and survival to recruitment of juvenile fish to the adult population. Therefore, understanding the intrinsic value of a habitat to the nursery role of a system is of great importance to improve management of critical juvenile habitat, as it will support wild fish populations and fisheries. Neutral theory presents a null hypothesis that suggests the phenotype of a species and habitat heterogeneity are not responsible for differences in distribution, but merely the result of stochastic processes acting on population dynamics (Chase, 2014). Chase (2014) suggests, however, that the spatiotemporal scale over which observations of distributions are made resolves to what extent deterministic or stochastic processes drive distribution patterns. While there is evidence to support neutral theory over small spatial scales (Legendre *et* al., 2009; Dumbrell *et al*., 2010; Garzon-Lopez *et al*., 2014), the prevailing theories pertaining to the function of marine nursery habitats is that they provide productive foraging opportunities and refuge from predation in some form (Nagelkerken *et al*., 2015).

Juvenile fish conforming to optimal foraging theory should act to maximise energetic gains through balancing the ratio of prey energetic content to handling and pursuit times, while also minimising the risk of predation (Townsend, 1986; Lazzaro, 1987; McNamara & Houston, 1992; Nunn *et al*., 2012). However, there is often considerable mismatch between habitats that provide refuge and those that provide foraging potential (McIvor & Odum, 1985; Petterson & Bronmark, 1993; Hammerschlag *et al*., 2010; Ahrens *et al*., 2012). Fish engaging in predation avoidance tend to exhibit reduced activity and foraging to avoid detection (Peterson & Bronmark, 1993), and refuge habitat often exhibits lower productivity (Werner and Hall, 1988; Eklov, 1995; Tatrai & Herzig, 1995; Fullerton & Lamberti, 2006). This can incur a trade-off between the energetic gains of engaging in foraging and managing the risk of predation (Lima and Dill, 1990; Peterson & Bronmark, 1993; Balaban-Feld *et al*., 2019). The mechanism underpinning forage-predation trade-offs is thought to be state-dependant, where relative predation risk of a habitat and the hunger levels of the fish interact to determine the patterns of habitat usage (Peterson & Bronmark, 1993). For example, in laboratory experiments by Pettersson and Bronmark (1993), well fed and hungry crucian carp (*Carassius carassius*) forage benthic prey in open habitat when predatory pike are absent. However, in the presence of pike, activity and foraging levels were reduced, and only hungry crucians occupied open habitat close to refugia. In crucian carp, it is evident that predation risk is the stronger driver of habitat choice, overriding hunger, and appears to mediate foraging behaviour. Work by Halpin (2000) found that fish would occupy refuge when predation rates differed among marsh habitats, however, when predation risk was similar fish would choose refugia with the most productive foraging opportunities. Based on forage-predation trade-offs, McIvor and Odum (1985) suggested that, in order to minimise predation risk, juvenile fish in saltmarsh systems should occupy inundated shallow habitat on saltmarsh faces at high tide when the deeper subtidal channels pose an increased predation threat. Conversely, on low tides, fish should occupy the shallow subtidal channels to forage in more productive habitat. However, the foraging patterns of juvenile fish across estuarine nursery habitats, and the implications for forage-predation trade-offs, has been ill explored.

Many factors influence the relative predation risk and foraging potential exhibited by a habitat. Refuge from predation is thought to be provided by both habitat complexity (Canion and Heck, 2009; Thiriet *et al.*, 2014; Sheaves *et al.*, 2015; Cheminée *et al.*, 2016; Leslie *et al.*, 2017; Baker & Sheaves, 2021) and habitat depth (McIvor and Odum, 1985; Harvey 1991; Paterson & Whitfield, 2000, Whitfield, 2017; Whitfield, 2020). This is because increased habitat complexity is thought to reduce the success rate of visual predators, reducing a prey species visibility during the chase phase of the predation act (Savino & Stein, 1982; Almany, 2004; Horinouchi, 2007a). With regards to depth, shallow water supposedly provides refuge for small fish as it is less accessible to larger piscivorous fish (Harvey 1991; Paterson and Whitfield, 2000; Whitfield, 2017; Whitfield, 2020). Increased foraging potential is also thought to be correlated with habitat complexity, due to increased abundances of macroinvertebrate prey (Attril *et al.*, 2000; Hu *et al.*, 2016; Wolters *et al.*, 2018). However, in many cases juvenile fish may suffer from similar issues as their predators because of reduced visibility, such that foraging success may be higher in more structurally simple habitats (Eklov, 1995; Tatrai & Herzig, 1995; Fullerton & Lamberti, 2006; MacRae and Jackson, 2001).

Juvenile European sea bass in the UK make use of estuarine nursery habitat during the summer months, between June to November, following the settlement period from April through to August (Pickett and Pawson, 1994; Green *et al.*, 2009). Juvenile sea bass are thought to remain within estuaries for the first four years of life, after which they migrate into deeper waters at the mouth of the estuary (Pickett and Pawson, 1994; Stamp *et al.*, 2021). The shift in habitat is driven by an ontogenetic niche shift from a primarily epibenthic food source of invertebrates, such as bivalves and crustaceans, to a piscivorous diet (Pickett & Pawson, 1994; Vasquez & Munoz-Cueto, 2014; Cardoso *et al.*, 2014). Coastal estuaries are characterised by a mosaic of different habitat types, which can be broadly categorised as intertidal edge habitat or subtidal habitat. Edge habitats are those which exist on the shallow fringes of deeper estuary channels, which can take the form of structurally complex saltmarshes, oyster reefs, shingle beds, and seagrass meadows, or structurally simple sandy and soft sediment mud habitats. The use of saltmarshes by sea bass is well explored compared to other habitats in the estuarine mosaic, which are largely ignored or coarsely grouped (Green *et al.*, 2009, 2012). However, Chapter 2 of this thesis showed that sea bass were found across the full suite of available high tide habitats, while at low tide show increased abundance in subtidal shingle and oyster reef beds over less structurally complex mud and sand. Furthermore, those fish who were present on sand and mud at low tide exhibited a higher level of condition, suggesting some level of segregation. We suggested that the use of all shallow intertidal habitat was likely indicative of reduced predation risk compared to subtidal channels, however, no direct measures of predation or foraging were made. Therefore, it is still unclear what ecosystem function estuarine habitats provide, whether they all provide a similar or different value as foraging or refuge sources. A need to understand the relative quality of estuarine habitats is, therefore, necessary to understand the possible drivers of habitat usage, which underpins effective management of those habitats.

Due to forage-predation trade-offs it is often the case that fish will cease feeding activities or alter diets in response to increased predation risk levels, while making use of refuge habitat, and so measures of foraging or foraging success can be useful proxies for the exhibition of foraging and/or predation avoidance behaviour. One such metric is the relative stomach fullness. Stomach fullness is an index of the weight of stomach contents compared to the body length of the fish (Magnusson, 2011; Duguid *et al.*, 2021; Phan *et al.*, 2021). The maximum stomach fullness of a fish scales with its length, so the ratio of stomach contents mass to fish length is often used as a suitable proxy (Magnusson, 2011). A fish with a relatively high stomach fullness compared to other fish is said to have eaten more prey, and so is likely to be engaging in foraging. A full fish can either indicate a low predation risk or high food density, both being indicative of investment in foraging behaviour over predation avoidance. A lower stomach fullness is, therefore, said to suggest the opposite is true, that the fish is not feeding and is likely engaging in some other activity. Another valuable tool for understanding differences in foraging behaviour is to understand differences in diet composition and the diversity of prey within diets between habitats. This can be performed visually through the use of stereomicroscopy (Cabral and Costa, 2001; Laffaille *et al*., 2001; Szedlmayer & Lee, 2004) or through the use of dietary DNA (dDNA) metabarcoding (Berry *et al*., 2015; Jakubavičiūtė *et al*., 2017; Siegenthaler, *et al*., 2019; Nalley *et al*., 2021; Coker *et al*., 2022).

Juvenile sea bass generally exhibit an opportunistic diet that primarily consists of macroinvertebrates and some small fish species (Cabral & Costa, 2001; Laffaille *et al*., 2001; Green *et al*., 2009). *Green et* al., (2009) found that decapod crustaceans, amphipods, shrimp, and copepods tend to form the major part of the diet of juvenile sea bass in UK estuaries. Lafaille *et al*., (2001) found that diets were generally well conserved between fish using mudflats and saltmarsh systems. However, the abundance and diversity of macroinvertebrates is often correlated to increased habitat complexity (Bell *et al*., 2013; Hu *et al*., 2016; Wolters *et al*., 2018), therefore, more complex systems such as oyster reefs, shingle beds, and saltmarshes likely support higher prey diversity and abundance than mudflats and sand, and therefore may present enhanced foraging potential. Furthermore, studies of sea bass diet to date are based solely on visual analysis, which is susceptible to missing highly digested or rare taxa (Alonso *et al*., 2014; Berry *et al*., 20115). It is likely that our understanding of juvenile sea bass diets, while well documented, is likely not complete and requires an increased resolution. This can be achieved through dietary DNA metabarcoding (Berry *et al*., 2015).

Metabarcoding allows us to determine what prey taxa are present in the stomach of a fish that is otherwise imperceptible to the human eye and can give us a full catalogue of the species present, including rare species with little genetic material. Metabarcoding has proven to be a powerful tool in understanding differences in community structure within the marine environment (Siegenthaler *et al*., 2019; Sousa *et al*., 2019). Siegenthaler *et al*., (2019) utilised dDNA from the stomach contents of a known marine generalist forager, the brown shrimp (*Crangon crangon*), to quantify the metazoan community composition of saltmarshes. Siegenthaler *et al*., (2019) were able to demonstrate that dietary DNA samples are a powerful tool in cataloguing biodiversity, which identified twice as many species as classical towed net trawl surveys. Berry *et al*., (2015) demonstrated that, compared to classical morphological identification of stomach contents through stereomicroscopy, dDNA metabarcoding captured a similar breadth of species present in the diet, but to a higher taxonomic resolution. Through understanding dietary differences, we can better understand the relative quality of estuarine habitats for juvenile fish species, such as sea bass, regarding prey availability and diversity (Nagelkerken *et al*., 2015; Litvin *et al*., 2019).

In studies of biodiversity analysis using metabarcoding primers are selected top target hypervariable regions of genes that are highly conserved between species. Many studies have targeted the V9 region of the 18S gene in the identification eukaryote metazoans in eDNA and dDNA biodiversity studies (Albaina *et al*., 2016; López-Escardó *et al*., 2018; Waraniak *et al*., 2019). However, more recently, primers developed to target the 365bp region of the Cytochrome Oxidase subunit I gene have become widely used in marine metazoan biodiversity assessments using metabarcoding (Leray *et al*., 2013; Berry *et al*., 2015; Seigenthaler *et al*., 2019; Campbell *et al*., 2023; Coker *et al*., 2023). Often both regions are targeted in studies as they differ in effectiveness between certain species (Campbell *et al*., 2023; Coker *et al*., 2023), however, COI has been found to outperform 18S in the number of species identified (Leray *et* *al*., 2013, 2015). Therefore, while both sets of primers will be used in this study it is likely that COI will produce more comprehensive and reliable estimates of community diversity.

In the present study, I used measures of relative stomach fullness of juvenile European sea bass, between both high and low tide estuarine habitats, to determine whether differences in foraging activity are present in fish between group-0 to group-2 and discuss the implications for drivers of juvenile habitat usage patterns observed in chapter 2. I then also use dietary DNA metabarcoding to catalogue the relative contributions of prey communities to the diet of juvenile sea bass, between the habitats measured, to determine the relative quality and value of those habitats. We hypothesise, based on patterns observed in chapter 2, that juvenile fish in high tide habitats will exhibit increased stomach fullness than in that of low tide. We also hypothesise that dietary differences will occur between habitats and that more complex high tide habitats will yield increased prey diversity, while diversity will be reduced on low tide complex habitats, due to increased predation avoidance behaviour potentially exhibited (Chapter 2).

2. MATERIALS & METHODS

2.1. Sample site and methods

Group-0 to group-2+ juvenile sea bass were sampled from a total of 33 estuarine habitats of the Colne and Blackwater Estuary between June to September of 2020, Figure 1. Habitats were sampled over high and low tide. High tide habitats were classified as saltmarshes, and edge habitats that were either mud-dominated, sand-dominated, or shingle beds. Low tide habitats were classified as mud, sand, and shingle edge habitats and subtidal oyster reefs. For a detailed description of each habitat designation, and the sampling sites refer to Table 1. Fyke nets were used for saltmarsh creeks, while seine nets were used to sample open bays and edge habitats. A minimum of three replicate habitat types were sampled for both the Colne and

Blackwater estuary, and a minimum of three technical replicates were performed (three seines or three fykes) per site, which were either performed on the same day or different days. Fish were removed to aerated buckets and counts of sea bass between habitats were taken, and length and weight were measured, or estimated if catches were too large. Fish were then immediately returned to the estuary. A maximum of 20 juvenile sea bass were humanely euthanised per seine or fyke and retained for dietary analysis, in accordance with IFCA dispensations to retain and despatch juvenile sea bass (20200713AP) and under the Schedule 1 exemption for lethal sampling (ASPA 1986). For a detailed description of the sampling methods used to catch and measure morphometric data please refer to Chapter 2, section 2.1, and 2.2.

Habitat type	Tide	Sites	Dates	Lat/ Long	Total sites	Description	Gear type	No. of Bass	Size range (mm)	Age range
Saltmarsh	High	1	29/07/2020	51.85174, 0.95558	6	Low lying, vegetated, network of intertidal creeks, mud sediment, high tide.	Fyke	37	24-44	0
		2	05/08/2020 24/09/2020	51.83491, 0.98748				583	23-200	0-1
		3	08/09/2020	51.78067, 1.04411				155	23-83	0
		4	31/08/2020	51.78561, 0.87273				54	33-62	0
		5	07/09/2020	51.71485, 0.85398				321	10-87	0-1
		6	01/09/2020 30/09/2020	51.69706, 0.77072				178	37-119	0-1
Shingle	High	8	20/08/2020	51.84306, 0.98127	6	Mud/clay sediment, large cobbles embedded into substrate, often macrophytes attached, high and low tide.	30m seine	63	30-173	0-2+
		9	16/07/2020	51.79444, 1.03149				38	28-155	0-1
		14	04/09/2020	51.73881, 0.83816				5	46-64	0
		17	16/09/2020	51.73904, 0.77446				35	34-89	0-1
		28	17/09/2020	51.73404, 0.72636				45	27-89	0-1
		30	24/08/2020	51.80638, 1.02111				81	32-140	0-1
	Low	7	27/07/2020	51.84800, 0.97890	4		25m seine	23	23-45	0
		10	16/07/2020	51.79485, 1.02893				17	105-153	1
		15	15/09/2020	51.73832, 0.84893				54	48-87	0-1
		16	11/09/2020	51.73678, 0.77692				437	43-174	0-2+
Sand	High	12	21/09/2020	51.71559, 0.83215	5	Mud/clay sediment, large cobbles embedded into substrate, often macrophytes attached, high and low tide.	30m Seine	352	30-101	0-1
		24	29/09/2020	51.77419, 0.94001				66	41-82	0-1
		26	18/08/2020	51.75213, 0.87350				0	-	-
		29	28/08/2020	51.704300, 0.7734				2	35-42	0
	Low	25	11/08/2020	51.74872, 0.92057	1		25m seine	16	21-57	0
Mud	High	18	31/07/2020	51.85201, 0.95387	3	Soft mud sediment, flat, structurally simple high and low tide.	30m seine	11	23-55	0
		22	03/08/2020	51.82582, 0.97573				4	28-41	0
		27	17/09/2020	51.73428, 0.72329				44	27-89	0-1
	Low	19	15/07/2020	51.85420, 0.95808	3			3	23-55	0
		20	14/07/2020	51.84063, 0.98457				0	-	-
		21	17/07/2020	51.83848, 0.98781				1	-	-
		23	31/07/2020	51.78367, 1.03039				5	117-161	1-2+
Oyster reef	Low	11	10/09/2020	51.74606, 0.89334	2	Live and dead oyster shell matrix embedded in mud sediment, high rugosity, low tide.	30m Seine	27	37-77	0
		13	10/09/2020	51.75528, 0.88403				66	49-91	0

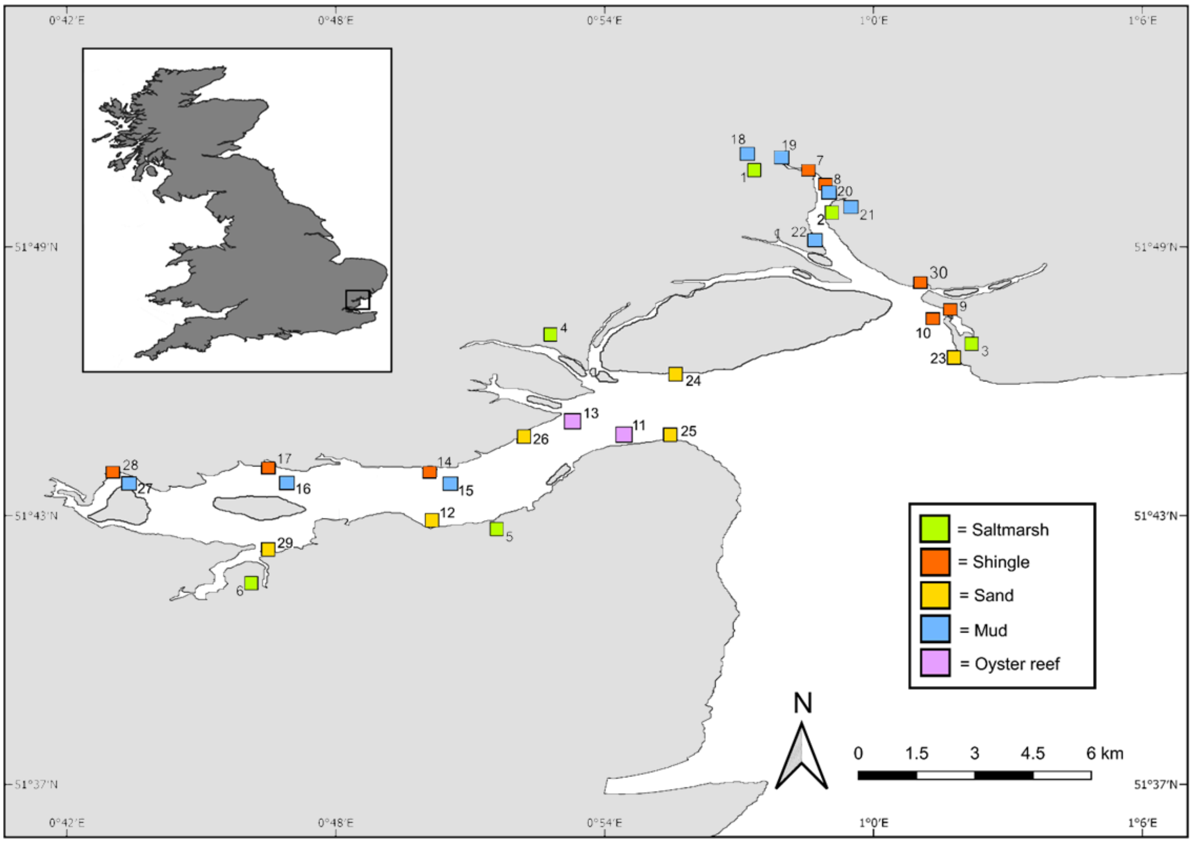

Figure 1. Map of sampling sites in the Colne and Blackwater estuaries, Essex, UK, between July-September 2020. Total sites = 30 (33 including coastal – not depicted). Made using QGIS, 3.2.3.

2.2. Dietary DNA extraction and stomach fullness index

Where possible, a maximum of 6 fish per age group were selected for each site where sea bass were caught, to be taken forward for measurements of stomach fullness index and dietary analysis using dietary DNA metabarcoding. Upon capture and euthanasia, fish were immediately placed on ice in a cold box for transportation to the laboratory where they were measurements of length and weight were taken, before they were frozen at -20°C. When needed, fish were thawed overnight in a fridge at 4°C to reduce DNA degradation and digestion of stomach contents. Prior to stomach dissections, and between samples, all surfaces and gloves were sterilised using a 70% ethanol solution, all equipment was sterilised by soaking in 99.6% ethanol. The skin of the fish to be dissected were cleaned using a 70% ethanol solution to remove estuarine contaminants on the fish's surface. Fish were cut along the ventral side from the anus to the gills. The stomach was then removed, and cut at the base of the oesophagus, and the base of the intestinal tract. Stomach digesta and lining were then squeezed out and removed to a sterile 2ml cryogenic vial, which were frozen at -20^{o}C for storage and preservation. Stomach samples were weighed separately to confirm enough material had been collected to take forward to extraction. This also provides a metric of stomach fullness.. The Stomach Fullness Index (SFI) was used to determine the degree to which fish had been foraging (Magnussen, 2011). The maximum stomach fullness of a fish is known to scale exponentially with the length of a fish, and therefore can be determined through the following equation:

$$SFI = \frac{Wst_f}{L_f^3} \times 100 \qquad \text{Eqn 1)}$$

Where L is the length of fish *f*. SFI was determined for each fish that was taken forward for dietary analysis.

Dietary DNA extractions were performed with the DNEasy Blood and Tissue kit (Qiagen), following the manufacturer's instructions. Samples were allowed to defrost completely and return to room temperature. Samples were first lysed in a mixture of SDS (sodium dodecyl sulphate) buffer solution and proteinase K, for 3 hours, incubated at 56^{o}C. During this time, samples were vortexed every hour to aid breakdown. After incubation, 200ul of 30-50% guanine hydrochloride and maleic acid were added to the samples, to be immediately mixed with 200ul of ethanol through vortexing. The resulting mixture was then removed to a sterile 1.5ml DNEasy spin column and centrifuged at 8000rpm for 1 minute. Following this, spin columns were placed into a new collection tube, and the old one discarded with the flow through. The first wash buffer was then added, after which the column was spun down again at 8000rpm for 1min. After addition of the second wash buffer samples were spun down at 13,400rpm for 3 minutes to dry the spin column membrane. To maximise DNA during the elution step, the addition of 100ul of elution buffer with centrifugation at 8000rpm for 1 minute was performed twice to make up 200ul of yield. Samples were stored at -20^{o}C until further processing.

2.3. PCR and high throughput sequencing

To determine the dietary constituents of juvenile *D. labrax*, extracted stomach contents were amplified using two primer sets. Leray XT primers target the 313-bp region of the cytochrome c. oxidase subunit I (herein referred to as COI) gene (Leray *et al*., 2013). COI is highly conserved between most metazoans and eukaryotes, yet variation is sufficient for identification to genus level. Leray XT was selected as it has been shown to be able to capture a wide variety of invertebrate species, including many of the expected prey species that have previously been found in the stomachs of *D. labrax* (Green *et al*., 2009). The second set targets the hypervariable V9 region of small subunit (SSU) 18S ribosomal RNA genes (here in referred to as 18S), which again targets a broad range of eukaryotes (Amarel-Zettler *et al*., 2009).

The Leray XT primer set uses the mICOIintF 5'-(GGWACWRGWTGRACWITITAYCCYCC-3') forward primer (Leray *et al*., 2013) and the jgHCO2198 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3') reverse primer (Geller *et al*., 2013). The 18S primer set uses the 1391f forward primer, and the EukBr reverse primer (Amarel-Zettler *et al*., 2009). DNA extracts were diluted 1:10 in Milli-Q water to reduce PCR inhibition. PCR amplification occurred in 25 µl reactions consisting of 2.5 µl of diluted DNA, 12.5 µl of Taq, 7 µl of H_2O, 1 µl of forward primers and 1 µl of reverse primers (10 µM). Primers were modified to contain Illumina-specific overhang adapters. Reverse primers also contained a 12 base Golay barcode to distinguish samples during multiplex sequencing. Thermal cycling for COI amplification consisted of an initial annealing stage of 95°C for 3mins; 16 cycles of 95°C for 10secs, a touch-down of 62°C to 58°C for 30secs, and 72°C for

1min; and a final 25 cycles of 95°C for 10secs, 46°C for 30secs and 72°C for 1min, with a final extension step held at 72°C for 7mins.Thermal cycling for 18S amplification consisted of an initial annealing stage of 95°C for 3mins, followed by 30 cycles of 95°C for 30secs, 57°C for 45secs, and 72°C for 45secs, followed by a final stage of 72°C for 7mins.

Following the first PCR, samples were purified using Ampure XP beads (Beckman Coulter) following manufacturer’s instructions. After purification, a short secondary PCR reaction was performed to attach Nextera XT indices for compatibility with illumine sequencing machines, which consisted of 2.5 µl of purified PCR, 2.5 µl of Nextera i5 and i7 index, 12.5 µl of Taq and 5 µl of H_2O. Thermal cycling conditions consisted of an initial denaturation step of 3 min at 95°C followed by 8 cycles each of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C. PCR products were purified again using the Ampure XP beads and DNA was quantified using PicoGreen® dsDNA quantification assays (Thermo Fisher Scientific) on a POLAR star Omega (BMG Labtech) plate reader, as per manufacturer’s instructions. Indexed amplicons were then pooled in equimolar concentration. The Illumina-overhang adapted DNA concentration was determined with the NEBNext Library Quant Kit for Illumina (New England BioLabs). DNA libraries were sequenced on an Illumina NovaSeq 6000 with 2x250bp paired-end chemistry at the Earlham Institute.

2.4. Sequencing and Bioinformatics

Sequence reads were processed as detailed in Dumbrell *et al*., (2016). First quality filtering was carried out with Sickle (Joshi and Fass, 2011). Sequence reads were trimmed when the average Q score, a measure of read accuracy, dropped under Q20 across a moving 35 base pair window. Error correction was carried out in SPAdes (Bankevich *et al*., 2012), which uses the BayesHammer algorithm to correct for misidentified bases during sequencing (Nikolenko *et al*., 2013). Paired-end reads were merged into single contigs in the program

PANDAseq (Masella *et al*., 2012) using the PEAR algorithm (Zhang *et al*., 2014). Further quality filtering was carried out in the open-source bioinformatics package MOTHUR (Schloss *et al*., 2009) to remove homopolymer inserts longer than 12. Sequences were dereplicated using VSEARCH, which removes singleton sequences, sorts by abundance, and clusters sequences around an Operational taxonomic unit (OTU) centroid at a 97% similarity threshold (Rognes *et al*., 2016). Taxonomy was assigned to OTU centroids using the ‘classify-consensus-vsearch’ method of the q2-feature classifier in QIIME2 (Boylen *et al*., 2019) against 18S and COI databases generated from NCBI with RESCRIPt (Robeson *et al*., 2021). OTU tables were decontaminated using the ‘microDecon’ package (McKnight *et al*., 2019) in R (v4.2.2., R Core Team, 2022), which removes a representative number of reads from contaminant species OTU’s present in negative controls. OTU counts were normalised using the cumulative sum scaling (CSS) method in the ‘metagenomeSeq’ package (Paulson *et al*., 2013), to account for differences in sequencing depth between samples. The ‘phyloseq’ package (McMurdie and Holmes, 2013) was used to transform counts into relative abundances, analyse the taxonomic composition of communities, calculate differences in alpha and beta diversity, and perform redundancy analysis (RDA).

2.5. Visual Identification of stomach contents

To confirm the accuracy of metabarcoding, the stomach contents were visually identified for a random subsample of fish which were put forward for sequencing. A minimum of 3 fish per age group were selected from a minimum of 3 examples of each high and low tide habitat. This gave a total subsample of 229 fish. Based on an *a priori* decision to only assess what had recently been eaten and to maximise the ability to correctly identify less digested prey items in the visual comparison, stomach samples were only removed from the fore gut, ignoring the hind gut and intestines, as this will give the best estimate of what juvenile sea bass had been eating while occupying the sampling site. Stomach samples were removed following the protocol outlined for dietary DNA extraction in section 2.3. These samples were then moved to a sterile measuring boat, and the total gut content weight was recorded. Stomach samples were then removed to sterile petri dishes and were identified to the lowest taxonomic group possible using light microscopy. Counts of individuals within each taxon were performed, and individual weights of each taxon were taken using sterile weighing boats. Samples were then removed to Eppendorf tubes to be frozen at -20^{o}C. The relative percentage contribution of each taxon to a fish's diet was determined by equation 1.

$$\%M_t = \left(\frac{Mt_f}{Mst_f}\right) \times 100 \qquad \text{Eqn 2)}$$

Where *Mt* is the mass of individual taxa for fish *f* and *Mst* is the mass of the total stomach contents for fish *f*. All surfaces were sterilised between each sample.

2.6. Statistical analysis

Following initial visual assessment of normality distributions, to determine the effect of habitat type on the stomach fullness of juvenile sea bass, generalised linear mixed effects models were employed, using the 'glmmTMB' package (Brooks *et al*., 2017) in the open-source statistical software R (v.4.2.2., R Core Team, 2022). Mixed effects models were selected to account for the random effect introduced by technical replicates in sampling performed at each site, and the nested random effect of using multiple individuals from the same subsample. The package 'glmmTMB' allows the use of mixed effects models using a very wide range of distributions such as negative binomial and zero-inflation. Models were separated by tide, due to fundamental differences in habitat structure and availability over high and low tide. For each habitat type replication was provided by visiting multiple sites that were representative of a habitat type. We also took technical replicates spatially and temporally from the same site by either performing multiple seines at the location, or surveying multiple creeks on a saltmarsh, and we considered these to be repeat measures. Therefore, these repeat measures were considered to be random effects in the subsequent mixed effects model. Furthermore, each individual fish used from a single seine or creek is considered to be a psuedoreplicate of that seine or creek and, therefore, was also accounted for in the random effect structure. Habitat type was considered to be the fixed effect. Similar mixed effects models were used to determine whether differences in OTU richness and Shannon diversity were driven by habitat type. Richness and diversity models were also separated by tide. The least complex model was determined using least likelihood single term deletions through the 'dropterm' function in R. This essentially compares the model to a null model and returns a p-value that allows you to determine the significance of that term in the model. Pairwise comparisons were performed using Tukey's *post hoc* test using the 'lsmeans' (Russell & Lenth, 2016) package in R.

To determine drivers of community composition differences a redundancy analysis (RDA) was performed using the 'phyloseq' package in R. The RDA performed multiple regressions of the effect of habitat and tide on the community composition of the fish diets between sites. The resultant fitted distance matrix is then used to construct an ordination biplot. A permutation test was then performed to understand whether a significant relationship exists between the response variables (community composition) and the environmental variables (habitat and tide) exists. Permutation tests were used as they can account for a wide range of non-normal distributions, through random 1000 permutations of the data to create a reference distribution of the F statistic, that can be compared to the 'true distribution' of the data. A p-value is then determined based on the proportion of similarity between the permuted values and the 'true' values (Borcard *et al.*, 2011). The effect of habitat and tide on community dissimilarity was then also assessed using a PERMANOVA based on Bray-Curtis dissimilarity index (Bray and Curtis, 1957), generated through non-multidimensional scaling using the ‘vegan’ package in R (Oksanen *et al.*, 2022). Due to the relatively small sample size, to determine the effects of habitat on % contribution of prey species to sea bass gut contents, based on visual identification, individual Kruskal Wallis tests were used for each species identified, over low and high tide separately. All analyses were performed across 0-1 group fish. Size was not accounted for in the models as the diet constituents characterised by Green *et al.*, (2009) is well conserved between 0 and 1-group sea bass. All data has been uploaded to the Research data at Essex online repository.

3. RESULTS

3.1. Stomach fullness

At high tide differences in stomach fullness between habitats were found to be significant $\chi^2_{(3, 14)} = 14.06$, ($P < 0.01$). Fish exhibited significantly lower SFI in muddy habitats compared to saltmarshes ($P = 0.01$) and sandy habitats ($P < 0.01$). Mean SFI was highest on saltmarsh and sand compared to mud but were comparable to shingle (Figure 2). The largest individual SFI values were also found on saltmarsh systems and shingle. Mud exhibited the largest variability in SFI, and the smallest SFIs were found here. There are also significant differences in SFI between low tide habitats ($\chi^2_{(3, 11)} = 52.36$, $P < 0.01$). For example, SFI was significantly larger on sand compared to mud ($P = 0.01$), oyster reef ($P < 0.01$), and shingle ($P < 0.01$), but generally similar between shingle, oyster reef, and mud. Shingle showed the largest amount of variability, where the lowest SFIs was found.

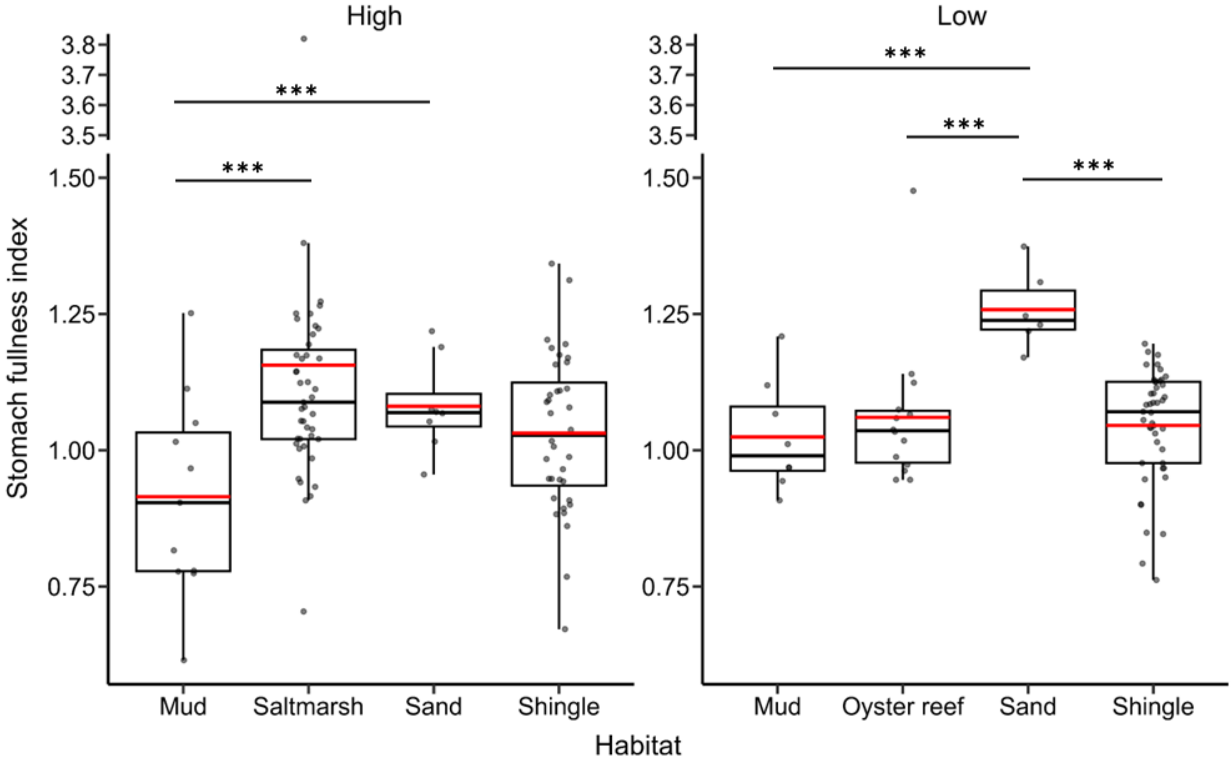

Figure 2. Boxplots for the average stomach fullness (SFI mgmm^{-3}) of 0-group *D. labrax*, between estuarine habitats at high tide (left) and low (right) tide. Habitats are mud, saltmarsh, sand, shingle, and oyster reefs. Whiskers represent maximum data point within 1.5x the Interquartile range, red bars represent means, and black bars represent medians. Thick black line represents the median, red line indicates mean stomach fullness. Significant differences highlighted by *** when $p < 0.01$ and * when $p < 0.05$).

3.2. Relative abundance

For both 18S and COI sequences, the relative abundance of the most dominant genera changed between habitats, Figure 6. For mud-dominated habitats, 18S sequences revealed the dominant prey taxa were *Acartia* copepods, which accounted for 86% of reads, while *Gammarus* amphipods were the only other taxa present at 13.6%. COI, however, found considerably more dominant genera, with planktonic genera accounting for the largest proportion of dietary constituents. Copepods of the genus *Acartia*, *Mesopodpsis*, as well as neomysis accounted for 46.2% of the diet, while *Carcinus* decapods accounted for 15.0%.

Finally, *Hediste* worms contributed 7.1% to the diet. Interestingly, 13.8% of the diet constituted of terrestrial *Plagiozopelma*. On Oyster reefs, 18S sequences identified considerably fewer planktonic *Acartia* copeods (2.6%), but *Gammarus* amphipods contributed 72.8% to the dietary constituents. *Idoteid* isopods also accounted for 24.0%. Similarly, COI sequences revealed *Ampelisca* amphipods formed 32.9% of the diet, with *Carcinus* decapods accounting for 17.2%. *Hippolyte* and *Dexamine* copepods contributed 27.1% to the diet. 18S sequences identified very similar diets for saltmarsh and sand, which were primarily comprised of *Acartia* (saltmarsh: 71.4%, sand: 45.3%) and *Gammarus* (saltmarsh: 24.3%, sand: 51.7%). However, COI sequences found that *Hediste* worms contributed the largest proportion of the diet at 59.4%, which was far larger than any other habitat, followed by *Carcinus* decapods (17.9%), *Idotea* (4.9%) and *Nephtys* worms (3.7%). Similarly, sand habitats exhibited 33.8% of *Hediste* to the diet, followed by 15.6% for *Carcinus*, and 10.4% for *Nephtys*. Finally, on shingle, 18S sequences again found that the dominant taxa were *Acartia* (29.5%) and *Gammarus* (32.7%), however, these were followed by *Palaemon* decapods (15.9%). *Saccostrea* were also found in the diet on shingle (12.3%); however, this is unlikely to be the case for this region and is likely a misclassification of the Pacific Rock Oyster (*Crassostrea gigas*). COI, however, found that *Carcinus* decapods accounted for the largest relative abundance (51.2%), considerably more than any other habitat. *Hediste* only accounted for 3.4% of taxa present, the lowest of all habitats, alongside *Idotea* (2.6%), *Neomysis* (3.6%), *Neptis* (5%), and a relatively large representation of *Solea* (10.9%). For the visual analysis, Kruskall-Wallis tests revealed only the % contribution of copepods to the total weight of stomach contents significantly differed between habitats, and only at low tide ($\chi^2_{(3,\ 15)} = 9.33$, P = 0.025), where they were most abundant on sand compared to all other habitats, Figure 7. Diets were primarily composed of decapods across all high tide habitats, while at low tide, sand was more characterised by the presence of copepods. Polychaetes were only found in saltmarsh diets, and isopods on shingle at high tide, and oyster reefs at low tide.

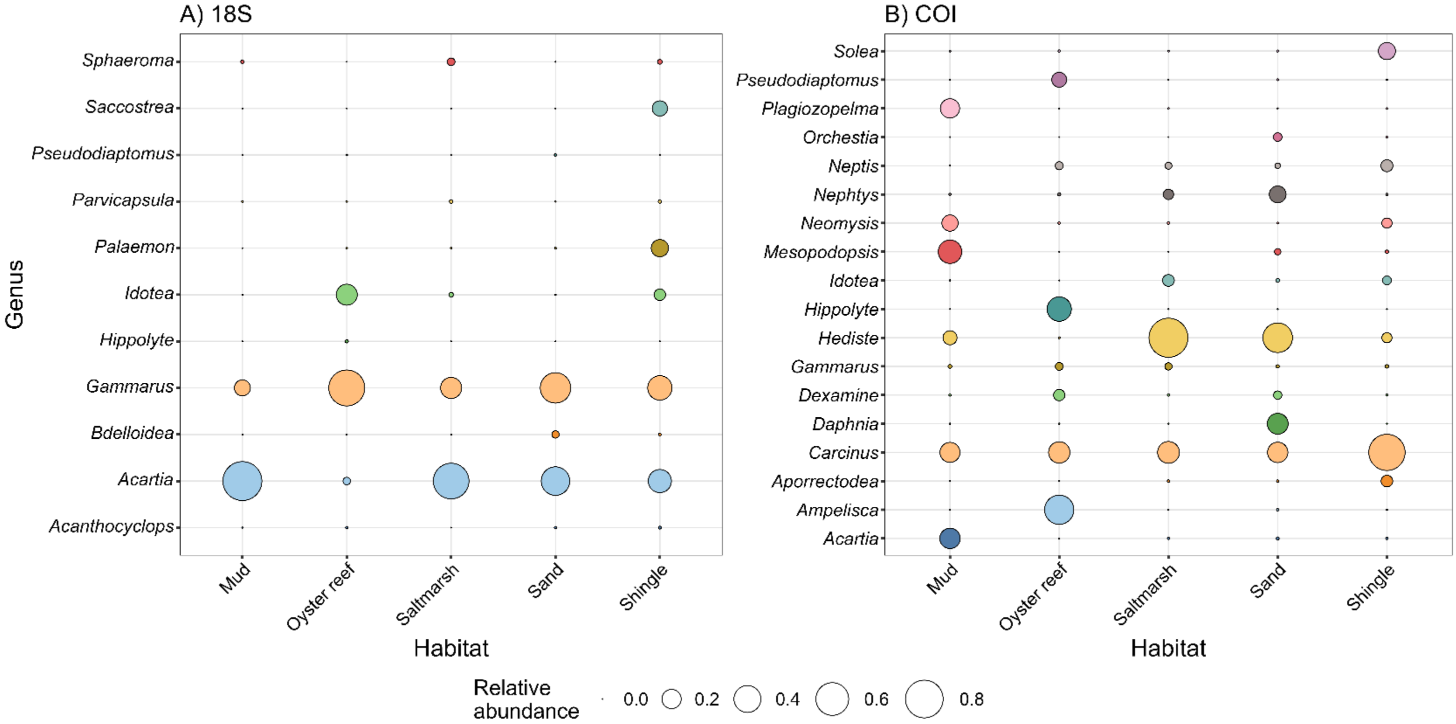

Figure 6. Bubble plots showing the relative abundance (depicted by size) of the most abundant (top 5) genera across different estuarine habitats: mud, oyster reef, saltmarsh, sand, and shingle based on A) 18S sequences, and B) COI sequences, caught in and shingle, identified to genus level.

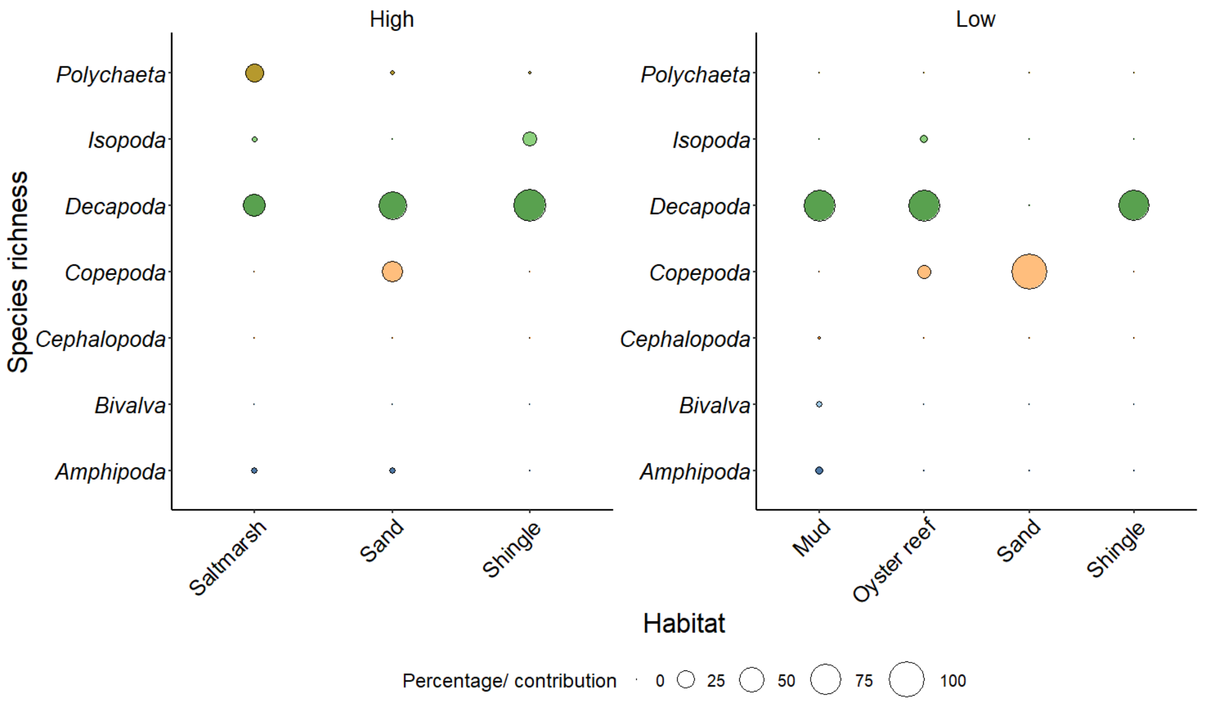

Figure 7. Bubble plots showing the percentage contribution to stomach contents weight (depicted by size) of visually identified genera across different estuarine habitats: mud, oyster reef, saltmarsh, sand, and shingle based on A) high, and B) low tide,

3.3. Redundancy analysis and PERMANOVA

For 18S sequences, considerable overlap between diets occurred (Figure 5A). The redundancy analysis (RDA) model significantly explained 5.78% ($F_{(6, 222)} = 3.33$, $P < 0.01$) of the variation in diet community composition. Mud and sand had the largest effect on community dissimilarity, while Sand and shingle had similarly large but opposing forces on community dissimilarity. High and low tide acted in equal opposing directions, while low tide appeared to interact with shingle habitat to drive community composition. Mud habitat appeared to drive large amounts of variation in community composition, while saltmarshes and oyster reefs appeared to drive similar community differences, opposing that of mud. This suggests that the strongest drivers of community dissimilarity were shingle, mud and sand habitats.

For COI sequences, generally there was considerable overlap in the diets of sea bass among habitat types and tide (Figure 5B). The redundancy analysis model significantly explained 2.31% ($F_{(6,\ 222)} = 1.09$, $P < 0.01$) of the variation in diet community composition. Sand, oyster reef, and mud-dominated habitats had little effect on differences between community structure between fish diets, but opposed the differences driven by saltmarshes and shingle beds. Shingle and saltmarshes had much larger effects, which again had opposing effects on community structure, indicating that saltmarshes exhibit decreased abundance of prey species found in shingle beds. Low tide had a similar, but lesser, effect on community composition compared to shingle habitats, resulting in an opposite effect to high tide. High tide had little effect on differences in community composition by comparison. This suggests that the strongest differences in diet composition were driven by saltmarsh and shingle habitats.

The PERMANOVA revealed that for COI sequences, habitat did have a significant effect on the Bray-Curtis community dissimilarity ($F_{(6,\ 222)} = 1.69$, $P < 0.01$), but tide did not ($F_{(1,\ 222)} = 1.24$, $P = 0.113$). Similarly for 18S, habitat had a significant effect ($F_{(4,\ 222)} = 2.46$, $P < 0.01$), while tide did not ($F_{(1,\ 222)} = 1.69$, $P < 0.05$).

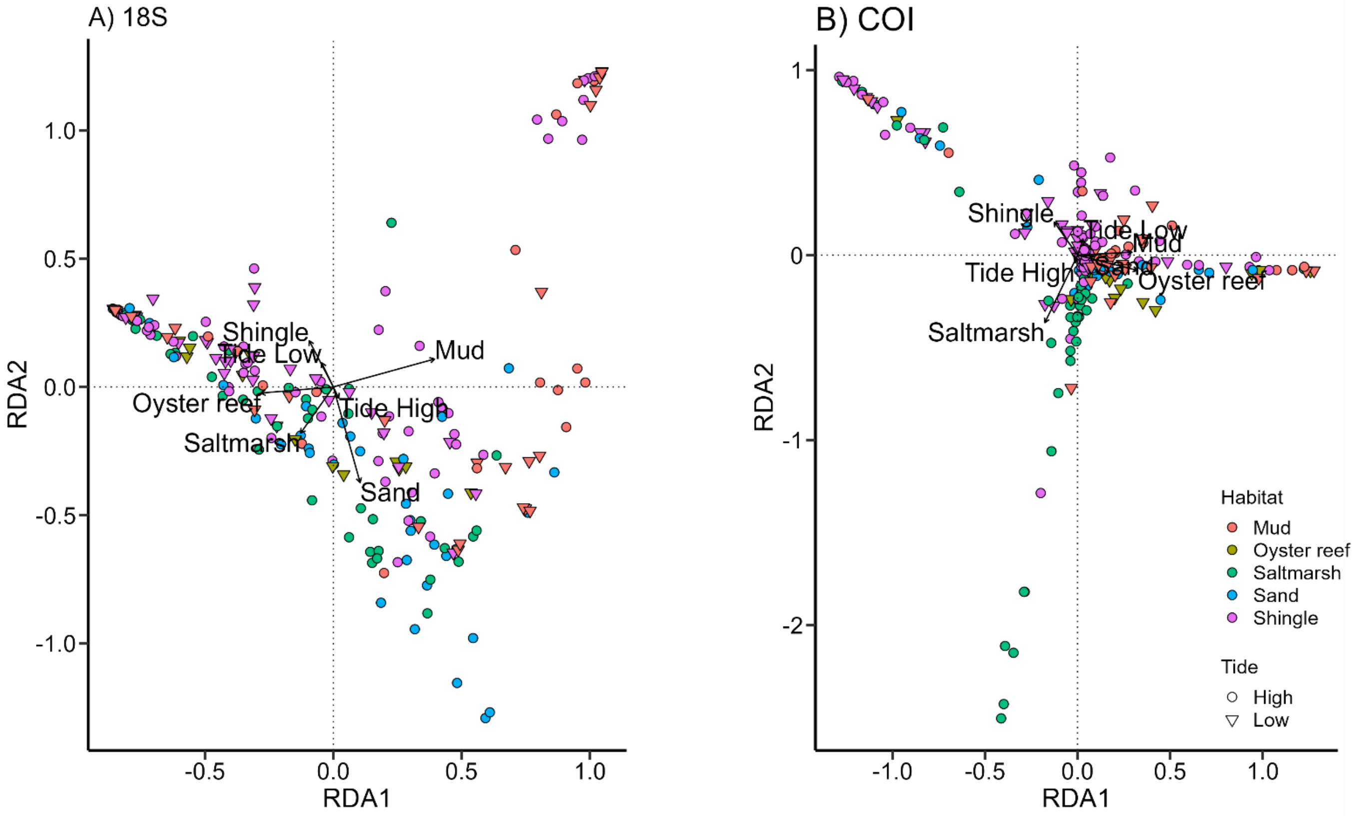

Figure 5. RDA biplots for juvenile *D. labrax* diets, amplified by A) 18S and B) COI, constrained by habitats: mud (red), oyster reefs (yellow), saltmarsh (green), sand (sand), and shingle (purple), and tides: high (dots) and low (triangles). Scaling = 1.

3.4. Alpha diversity

3.4.1. 18S

Differences in 18S operational taxonomic unit (OTU) richness of juvenile sea bass diets between habitats at high tide were found to be significant ($\chi^2_{(3, 157)} = 13.24$, $P < 0.01$). Median diet richness was significantly higher on shingle ($P < 0.01$) compared to mud and to sand ($P < 0.05$), Figure 3A. Median OTU richness was highest on shingle, while the largest individual richness was found on saltmarshes and shingle (Figure 3B). No significant difference was found in Shannon diversity between high tide habitats ($\chi^2_{(3, 157)} = 1.32$, $P > 0.05$), Figure 3B. No significant difference was found in OTU richness ($\chi^2_{(2, 74)} = 3.16$, $P = 0.206$) or diversity

($\chi^2_{(2, 74)}$ = 2.48, P = 0.29) between low tide habitats. The largest median OTU richness was found on oyster reefs, while the largest individual richness value was found on shingle.

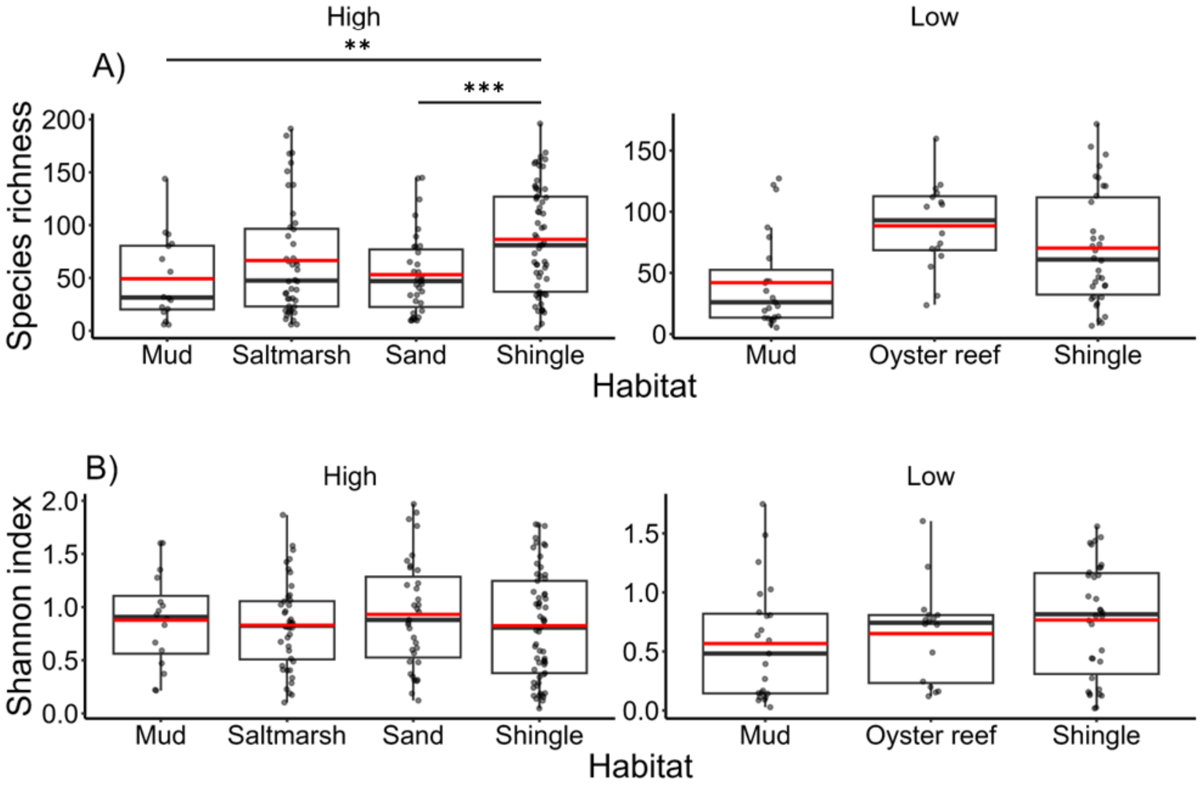

Figure 3. Boxplots for A) average 18S OTU richness and B) average Shannon diversity of 0-group *D. labrax* diets, between estuarine habitats mud, oyster reefs, saltmarsh, sand, and shingle. Whiskers represent maximum data point within 1.5x the Interquartile range, red bars represent means, and black bars represent medians. Thick black line represents the median, red line indicates mean stomach fullness. Significant differences highlighted by *** when $p < 0.01$ and * when $p < 0.05$).

3.4.2. COI

For COI sequences, there were no significant differences in either the OTU richness ($\chi^2_{(3, 157)}$ = 0.29, P = 0.962) or Shannon diversity ($\chi^2_{(3, 157)}$ = 1.87, P = 0.599) at high tide. However, mud habitats exhibited the lowest mean richness (Figure 4A) and diversity (Figure 4B). Similar to the 18S analysis, the largest individual richness values were found on saltmarshes and shingle habitats, and the largest individual diversity values were found on sand alone. There was no significant difference in either OTU richness ($\chi^2_{(2, 74)} = 4.85$, P = 0.089) or Shannon diversity ($\chi^2_{(2, 74)} = 1.91$, P = 0.386) at low tide as well.

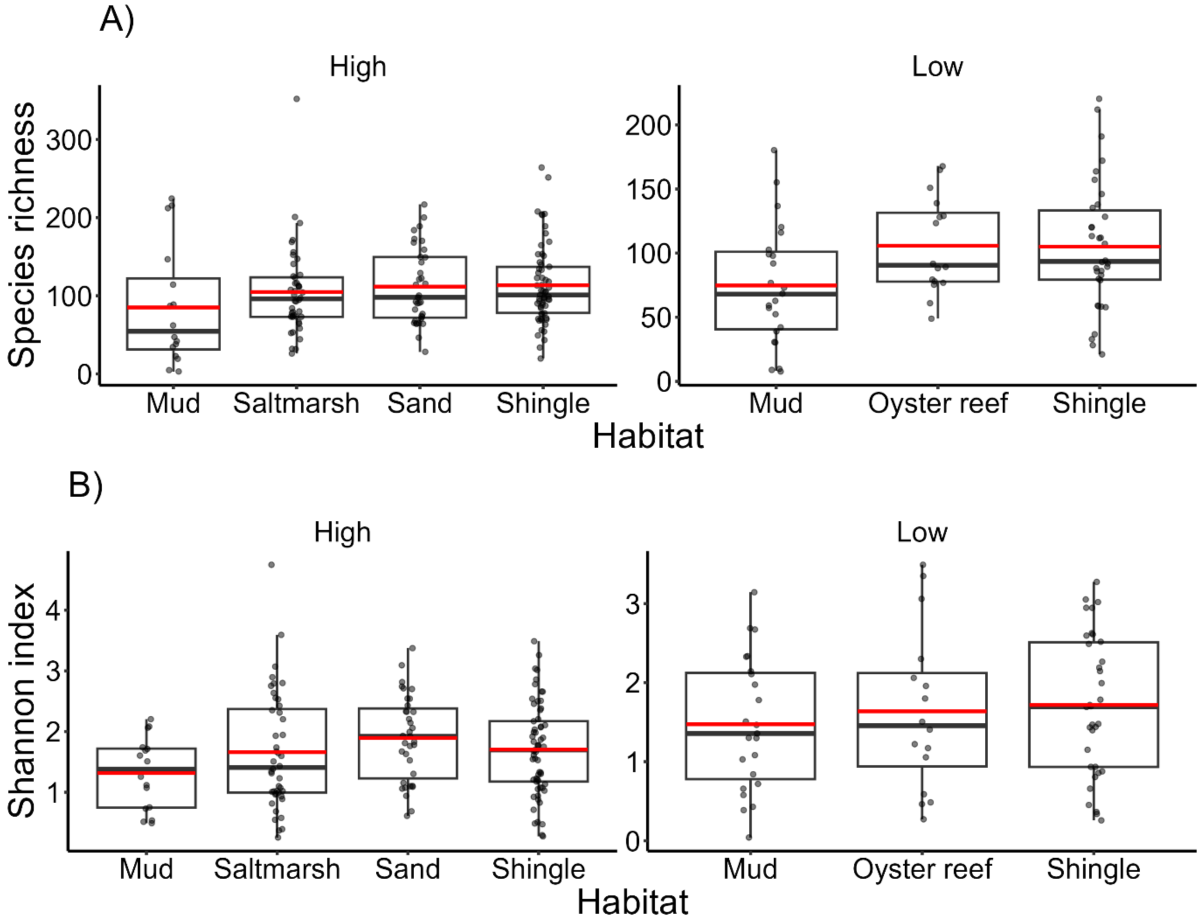

Figure 4. Boxplots for average COI OTU A) richness and B) Shannon diversity of 0-group *D. labrax* diets, between estuarine habitats mud, oyster reefs, saltmarsh, sand, and shingle. Whiskers represent maximum data point within 1.5x the Interquartile range, red bars represent means, and black bars represent medians. Thick black line represents the median, red line indicates mean stomach fullness.

4. DISCUSSION

While in recent years great efforts have been made to better understand the role of connected habitats in juvenile fish growth, survival, and recruitment, we still lack a thorough understanding of the ecosystem function these habitats provide to enable seascape level management of nurseries. In temperate estuarine systems, an almost exclusive focus on saltmarsh habitats over the past 30 years of nursery research in the UK has hindered our understanding of the roles of other habitats in the estuarine mosaic. The results presented in

Chapter two clearly indicate that many habitats across the mosaic are as well utilised as saltmarshes, but the value of these habitats for growth and survival cannot simply be conveyed by abundance alone. For example, while we may find juvenile fish in these habitats in high abundance, if they are of poor quality they may not contribute to recruitment (Lipcius *et al.*, 1997, McBride and Able 1998; Beck *et al.*, 2001; Heck *et al.*, 2003). Litvin *et al.*, (2019) suggested that more focus should be placed on the ecosystem functions provided by connected habitats that promote survival, such as foraging success that is what I have done here. I found that, while juvenile sea bass abundance is similar between high tide habitats (Chapter two), saltmarshes and sand dominated habitats may provide better foraging potential. Across low tide habitats, higher SFI was only found in those less complex habitat types where I found fish of higher body condition. To my knowledge, the results of the present study offer the first insights into the outcomes of the diet composition and foraging success of group-0 to group-2 juvenile sea bass across habitats along the estuarine mosaic in the UK. This study provides new evidence on the relative value of different habitats for juvenile sea bass, suggesting that while habitats do not appear to be differentially selected (Chapter 2), some may convey a higher foraging success than others.

18S and COI sequencing revealed different estimates of OTU richness and diversity. For 18S sequences, OTU richness was found to be highest in both shingle and oyster reef habitats compared to mud and sand but were generally similar to saltmarshes. Shannon diversity that incorporates both the number of OTUs present and the abundance of each, however, did not differ between habitats. This result suggests that shingle and oyster reef provide a greater number of different species of prey organisms than sand and mud, but not high abundances of all taxa. However, COI sequences revealed both richness and diversity were similar among all habitat types. The disparity presented here between 18S and COI could be attributable to their differing abilities to identify metazoans, with COI outperforming 18S regarding the number of taxa identified (Atienza *et al*., 2020). The ability to identify taxa correctly is also dependant on the reliability of the reference sequences in the database. It is possible that the increased resolution provided by COI fills in the gaps in the diet that are not captured by 18S. The reduced resolution of 18S could result in a larger perceived response to habitat changes and ultimately false results. Alternatively, it is possible that the taxa identified by 18S are more susceptible to changes in habitat type than those amplified by COI. However, the COI results presented here appear to give a more reliable estimate of the range of taxa that constitutes the diet of juvenile sea bass (decapods, amphipods, polychaetes, copepods, insects – Laffaille *et al*., 2001; Green *et al*., 2009) and so shall take precedence in the following discussion. The lack of difference in richness and diversity between habitat types is surprising considering the structural differences exhibited by oyster reefs and shingle compared to sand and mudflats. Increased habitat complexity from structures such as oyster reef matrices, seagrass meadows, and kelp forests is often associated with increased invertebrate community richness and diversity (Hosack *et al*., 2006; Tomaz *et al*., 2008; Crooks, 2002; Hauser *et al*., 2006; Hosack *et al*., 2006; Thomaz *et al*., 2008; Bouma *et al*., 2009; Lown *et al*., 2021; Pinnel *et al*., 2021). This is thought to be driven by increased food provisioning, predation refuge, and niche availability for those invertebrate prey species (Connor and McCoy, 2001).

Many juvenile fish species are considered to be opportunistic foragers, whose dietary constituents reflect the availability of different prey taxa at a site (Cabral 2000; Laffaille *et al*., 2001; Maes and Ollevier 2002; Sá *et al*., 2006; Roberts *et al*., 2011; Selleslagh & Amara, 2014), however, the degree to which fish prey selectivity underpins differences in diet is poorly understood, and varies by species, size, and life stage. Relatively few studies directly measure and compare the relative prey availability at a site and that of a fish's diet (Rezende *et al*., 2011; Selleslagh & Amara, 2014). Where prey selection has been studied there is often evidence of a degree of selectivity, however, dominant prey taxa available at a site are overwhelmingly reflected in the relative diet composition of fish (Rezende *et al*., 2011). Therefore, a similar level of diet diversity between structurally complex saltmarshes, oyster reefs and shingle beds compared to structurally simple sand and mud is at first glance surprising. However, in estuarine systems, soft sediment habitats such as mud and sand can be highly productive (Tse *et al*., 2008; Sueiro *et al*., 2010). Tse *et al*., (2008) determined that prey diversity was similar between complex mangrove habitats and the adjacent mudflats in estuarine systems of Hong Kong. They found that the diet diversity of fish using both habitats was similar, despite fish generally spending a larger portion of time foraging in the mangroves. Sueiro *et al*., (2010) compared the macroinvertebrate communities of multiple habitats, of differing structural complexity, in estuarine systems. They found that with increased complexity there was an increase in diversity, however, overall abundance of those species decreased. As prey diversity and richness of sea bass diets in the present study is maintained between habitats, this suggests that juvenile sea bass exhibit a relatively opportunistic generalist diet, maintaining a wide dietary breadth (Salleslagh *et al*., 2015).

Increased stomach fullness in sea bass found in saltmarsh systems compared to mud bays at high tide suggests an increased foraging potential in these habitats. Furthermore, the diversity of benthic taxa was highest in fish using saltmarshes compared to sand and mud bays. Many studies have shown that saltmarshes support a high abundance and diversity of macroinvertebrate taxa (Rader, 1984; Schalles *et al*., 2013; zu Ermgassen *et al*., 2021) and many juvenile fish species display increased foraging rates when using marshes compared to other neighboring estuarine habitats (West and Zedler, 2000; Laffaille *et al*., 2001; Deegan *et al*., 2002; Hampel *et al*, 2005). However, mudflats have been found to support a greater abundance and diversity of macroinvertebrates when compared to saltmarshes in multiple studies (Saldago *et al*., 2007; Bloomsfield & Gillanders, 2005; Sueiro *et al*., 2010). This could suggest that fewer types of macroinvertebrate species that are found in mud bays are desirable prey species for sea bass compared to saltmarshes and sand bays, however, given their generalist diet, this is unlikely to be the case. Mud forms a dense matrix that provides refugia for benthic fauna, which might make foraging harder for juvenile sea bass. Therefore, if foraging is easier within vegetated areas of saltmarsh habitats and loose sand substrates, then fish might be able to acquire more food and the prey species present might be better represented in the diet.

The diet of juvenile bass using mud bays shows an increased relative abundance of zooplankton with few benthic species present besides *Carcinus* crabs (most likely *Carcinus maenus*). It is possible that over mud bays, where it might be difficult to forage on benthic species that can burrow, sea bass undertake foraging in the water column, which may result in a more zooplanktivorous diet (Szedlmayer & Lee, 2004). *Carcinus* crabs, however, may still be an accessible and highly abundant benthic prey species over mud habitats. *Carcinus maenas* were found in high abundance across all habitats during sampling and were a dominant dietary component in all habitats. Therefore, *C. maenus* may form an important dietary constituent, which is seen in other studies of sea bass diets (Cabral & Costa, 2001; Green *et al*., 2009). In saltmarsh and sand habitats *Hediste* worms (most likely *Hediste divesicolor*) formed the largest part of the diet compared to all other habitats, and this suggests these are also an important prey species, which is also identified in the literature (Lafaille *et al*., 2001; Green *et al*., 2009). The coinciding of greater stomach fullness with a greater presence of *Hediste* in the diet suggests that habitats where *Hediste* are easier to forage on, such as saltmarshes and sand bays, may provide important habitat to juvenile sea bass that supports a foraging function. If that increased foraging potential supports faster growth rates and escape from predation it is possible that these habitats present a higher quality nursery role than mudflats, where food may be harder to forage. However, we did not account for body size in the models tested and, therefore, it is possible that, while the diet of sea bass has been found to be well conserved between age groups

(Green *et al*., 2009), differences in the relative abundance of benthic and planktonic taxa in the diet may be due to gape size limitations. The abundance of plankton in the diet may be a bias produced by an abundance of very small 0-group fish in those habitats. However, chapter 2 revealed that 0-group sea bass were the dominant age group across all habitats, so these results are likely reflective of what this age class is eating.

Other studies have found that in group-0 sea bass, foraging in saltmarsh systems of France, the primary dietary constituents are Gammarus amphipods, copepods, and mysid shrimps (Laffaille *et al*., 2001). *Hediste* polychaetes and *Carcinus* crabs were present, but in considerably lower numbers and contributed a lower total biomass. However, work by Cabral and Costa (2001), in the Tagus estuary, Portugal, revealed that decapods, mysids, and isopods formed the dominant dietary constituents of group-0 sea bass. Given this, it is likely that the diet of juvenile sea bass is relatively adaptable to prey availability of the habitat they occupy. The shift in dominant dietary components from *Hediste* and decapods, to zooplanktivorey between high tide habitats further supports this.

Sea bass caught in oyster reef and shingle habitats exhibited an increased dietary OTU diversity of benthic taxa compared to sand and mud suggesting that a wide range of benthic prey taxa are available to sea bass using these habitats. Oyster reefs and shingle bays exhibit increased benthic habitat complexity provided by the tri-dimensional structure of shell, cobble, rock, and macrophytes, which has been linked to increased diversity of macroinvertebrate communities (Attril *et al*., 2000; Lloyd *et al*., 2006; Zbikowski and Kobak, 2007; Bazzanti *et al*., 2010; Cai *et al*., 2011, 2012; Ferreiro *et al*., 2011; Pan *et al*., 2012; Bell *et al*., 2013; Hu *et al*., 2016; Wolters *et al*., 2018) and supports fish foraging, particularly where live oyster shell is found (Posey *et al*., 1999). Indeed, Posey *et al*., (1999) found that oyster reefs with live shell supported large amounts of fish foraging. However, the results of this study suggest that stomach fullness was lower on oyster reef and shingle compared to sand but was comparable to mud.

There are several possible explanations for why stomach fullness might be lower on oyster reef and shingle habitats compared to sand. Firstly, the increased habitat complexity found on oyster reefs and shingle, while supporting large and diverse communities of macroinvertebrates, may also provide refuge for these species. Structurally complex habitats are thought to provide refuge from predation by obstructing the prey finding and pursuit ability of visual predators (Savino & Stein, 1982; Almany, 2004; Horinouchi, 2007a). Again, in this instance foraging may be more difficult for juvenile sea bass, such that they provide a reduced foraging potentially compared to sand. The lack of structure on sand may reduce refuge for benthic fauna and make them easier to predate upon (Winfield, 1986; Diehl, 1988; Diehl and Eklov, 1995; Primavera, 1997; Nunn *et al.*, 2012; Murray *et al.*, 2016). Primavera (1997) showed that the foraging success of sea bass was lower in complex mangrove habitat when compared to bare sand, despite high levels of hunting activity on both.

Alternatively, it is possible that juvenile sea bass are seeking refuge in the increased benthic complexity of oyster reefs and shingle habitat themselves, as explored in chapter 2. Fish engaging in predation avoidance often exhibit reduced foraging activity (Peterson & Bronmark, 1993), such that a trade-off likely occurs between utilising complex habitat with a lower predation risk and foraging potential, or habitat that exhibits increased foraging opportunities with an increased risk of predation. These trade-offs are common for juvenile fish in nursery habitat, such that fish should seek to minimise this trade-off by using habitats that maximise foraging potential and minimise predation risk (Halpin, 2000). The use of shingle and oyster reef habitats could represent the best balance between foraging potential and predator avoidance. Chapter 2 also revealed that fish in poorer condition used oyster reefs and shingle compared to those using mud and sandy bays. This could suggest that fish of higher condition are better able to make use of riskier habitat that might provide more accessible foraging opportunities with less refuge for prey. It is also possible that stomach fullness is density dependant. Stomach fullness could be lower on oyster reef and shingle habitat because of the increased abundance of sea bass resulting in increased competition for resources, while higher condition fish foraging over sand are fewer resulting in reduced competition. This could explain why stomach fullness is lower, yet benthic diet diversity remains high in oyster reefs and shingle.

There are notable limitations to the conclusions drawn from the results put forward in this study, that should be addressed to direct future research in this field. Direct measurements of predation risk and survival between habitats were beyond the scope of this project, which relies on reduced foraging activity as a proxy for predation avoidance. This is due to the aforementioned trade-off that occurs between habitats that support this behaviour and would require longer term telemetry studies that examine the survival outcomes for fish in the youngest age classes that utilise given habitats over the summer period. Without direct measurements of predator abundance, predation rates, and survival between habitats it is very difficult to say to what extent shallow habitats such as shingle bays, oyster reefs, and saltmarshes provide either a primarily foraging function or predation refuge. This is an area that needs addressing in future studies of juvenile fish habitat usage within estuarine systems of the UK.

Secondly, we did not measure the community diversity present within the habitat itself, so this hinders our ability to determine whether the diversity and relative abundances of prey within the diet of sea bass is reflective of the actual habitat community, or whether it is a product of prey selection by the sea bass themselves. There is some evidence that taxa such as decapods and polychaetes are selected for sea bass diets, but very little work has actively compared community diversity alongside dietary diversity (Rezende *et al.*, 2011). This is another important area of future research, as this would help to further disentangle whether sea bass are truly opportunistic generalists or whether there is a degree of diet specialisation that occurs. This could have interesting management implications regarding habitats that best support those species. Finally, the extent to which metabarcoding data can be considered quantitative i.e., the number of sequence reads correlates to biomass present, is still debated heavily within the field (reviewed by Lamb *et al.*, 2019). A review by Lamb *et al.*, (2019) of the relationship between sequence reads and the original biomass of material present in the gut suggested a weak correlation between the two, with an r2 of 0.52, with large amounts of variation. Therefore, relative abundance estimates based on sequence reads should be taken with caution, as they are not representative of absolute abundance. However, relative abundance is a widely used metric when discussing what is present in a sample (Kowalczyk *et al.*, 2011; Soininen *et al.*, 2015; Sousa *et al.*, 2016; Vaz *et al.*, 2017; Lamb *et al.*, 2019).

Finally, high stomach fullness found in juvenile sea bass using sand habitats could indicate that our data is not overly representative of recent foraging, and prey may have been sourced from other habitats, possibly at high tide. We attempted to address this by only assessing the stomach contents of the oesophagus and stomach, discarding that of the hind gut and intestines. This method provides the data on the most recent meal eaten by the fish, but it is difficult to be certain of where the prey has come from, and to the authors knowledge there are no methods that could provide suitable resolution in this regard due to the relatively short periods of a tide relative to the fish digestion/gut clearance rates (He *et al.*, 1993; Preston *et al.*, 2017). Measurements of connectivity between habitats and their prey communities is also necessary to understand how transient the youngest sea bass are between habitat types over a tidal cycle, possibly through telemetry studies. It is also the case that low tide sand habitat was very uncommon, such that only a single site was present at low tide, so it is very difficult to draw general conclusions regarding sand habitat use at low tide. The inclusion of this single sand habitat could potentially bias results if this habitat is more productive than other sand habitats. In either case, these results illustrate that complex habitats provide an important habitat for juvenile sea bass at low tide, but sand may provide better foraging habitat for higher condition fish.

In conclusion, the diet of juvenile sea bass is similarly diverse across all habitat types, but the dominant constituents shift. *Hediste* worms and *Carcinus* crabs appear to be the most important species found in the diet across all habitats, particularly sand and saltmarshes, while a shift towards a more planktivorous diet is apparent on mud dominant habitats. Foraging success appears to be highest on saltmarsh and sand at high tide, where *Hediste* worms were primarily found, while at low tide in the absence of saltmarshes foraging success is highest on sand. This indicates that while the diet of juvenile sea bass is relatively adaptable to different habitat types, those habitats where *Hediste* is accessible appear to be the most profitable. Reduced foraging in shingle and oyster reef habitats, which are used by lower quality fish with less selective ability, at low tide suggests that sea bass are likely engaging in refugia here, while those higher quality fish are better able to make use of more profitable but less complex and risky sand habitats. Therefore, at high tide, while all shallow edge habitat is desirable to sea bass, sand and saltmarsh may be more profitable if utilised, while at low tide oyster reef and shingle may provide more important refuge habitat while sand is more profitable for foraging. This further suggests that multiple habitats are important to sea bass across the estuary mosaic beyond saltmarshes and that more attention is required to fully understand the function and benefits they provide, especially with regards to predation pressure, refugia, and connectivity. In systems that are under immense anthropogenic threat, where saltmarsh reduction has restricted the dominant shallow water habitats to bays and edge habitats, it is vitally important to consider how the culmination of multiple habitat types contributes to the survival of sea bass and other members of the estuary community. Attempts at habitat restoration and conservation can only work when considering the seascape of connected habitats that provide a nursery, beyond single habitats such as sea bass. While this study does not directly identify differences in survival or contribution rates of different habitats to recruitment, it does provide valuable insight into habitats that provide support for foraging and predation refuge, which may in turn support better growth and survival. This means a nursery role cannot be inferred; however, this study does provide evidence of important juvenile habitats, that need further investigation. Our study supports this paradigm shift and suggests that shallow habitats throughout the estuary play an important role in nursery provisioning for juvenile sea bass, and that consideration of the connectivity of estuarine habitats is vital for better understanding the recruitment dynamics of estuarine nurseries for this species.

CHAPTER FOUR

TIMING IS EVERYTHING: THE INFLUENCE OF TIDAL PHASE ON THE VERTICAL DISTRIBUTION OF LARVAL EUROPEAN SEA BASS ENTERING ESTUARINE NURSERY HABITAT.

*Microscopy image of a typical plankton sample containing larval sea bass

1. INTRODUCTION

Many economically important fish species across temperate regions, such as the UK, exhibit a planktonic larval stage (Moser *et al*., 1984, Leis 1991, Fuiman & Werner 2002) which, in most cases, is the most important life-stage in determining dispersal and distribution patterns of adult fish populations (Cowen, 2002; Sale, 2004; Kinlan *et al*., 2005; Shanks, 2009). Larval dispersal mechanisms can underpin species distribution patterns through influencing genetic connectivity between populations (Leis, 1991; Morgan, 2001; Cowen *et al*., 2003; Palumbi, 2003; Leis, 2007; Munday *et al*., 2008, 2009), and range expansions as a result of increasing water temperatures (Munday *et al*., 2008; Figueira & Booth, 2010**;** Bashevkin *et al*., 2020). For example, a meta-analysis of autralian fish distributional changes, revealed that the most likely drivers of range shifts were water temperature, changes in currents, and freshwater flow (Booth *et al*., 2011). Similarly, in a meta-analysis of multiple species across a range of marine genera, fish distributional changes were found to be consistent with temperature increases due to global ocean warming (Lenoir *et al*., 2020). This is firstly due to the vulnerability of larval stages to mortality via starvation, as a result of food availability (Pepin, 1991) and predation (Litvak and Leggett, 1992), where in some cases mortality rates of up to 70% can be experienced in species such as capelin (Litvak and Legget, 1992). Secondly, and perhaps more relevant to this study, is the influence of planktonic larval duration (PLD) on dispersal distance and settlement location (Kinlan and Gaines, 2003; Macpherson and Raventos, 2006; O' Connor *et al*., 2007; Shanks, 2003, 2009).

The planktonic larval duration can vary on the scale of hours, days, or weeks between species, but it can also vary within species depending on maternal investment (Kerrigan, 1997) and temperature (Robitzch, 2016). For example, the Ambon damselfish (*Pomacentrus amboinensis*) have a PLD of 9-12 days (Jones *et al*., 1999; Shanks, 2009), while the Black rockfish (*Sebastes melanops*) have a duration of 83-174 days (Miller & Shanks, 2004b; Shanks, 2009), and this directly influences the amount of time a larva is exposed to hydrodynamic processes, such as tides and currents, that may carry the larvae along (Kinlan *et al.*, 2005). Therefore, historically it has been suggested that an increased PLD may result in an increased dispersal distance (Macpherson & Raventos, 2006). While positive correlations have been found in many species (Zapata & Herron 2002, Lester & Ruttenberg 2005; Shanks, 2009), there are a considerable number of exceptions where this is not the case (Shanks, 2003, 2009, Gary *et al.*, 2020). A review of 44 marine species by Shanks (2009) found that some species with longer PLDs of one day showed dispersal distances of ~1km, which was comparable to dispersal distances of those with considerably shorter PLD's of 1hr. Furthermore, some species with PLDs close to one week were able to disperse up to 100km, while some who exhibited PLDs closer to one month dispersed only 1km. This suggests that though PLD can be a reasonable predictor of dispersal potential and differences in hydrodynamics may play a crucial role, larval behaviour (e.g., swimming ability) and physiology (e.g., buoyancy) must also be considered.

Historical models used to predict species dispersal and recruitment patterns considered larvae as passive particles at the mercy of oceanographic processes (Siegel *et al.*, 2003; 2008; Kinlan *et al.*, 2005; Edwards *et al.*, 2007; Beraud *et al.*, 2017), and indeed in modern models where there are data deficiencies in tidal behaviour, larvae as passive particles are still used (reviewed by Swearer *et al.*, 2019). These models often fail to correctly predict the size and location of settlement and recruitment events (Shanks, 2009; Staaterman *et al.*, 2012; Faillettaz *et al.*, 2018a, b; Leis, 2020), leading to the increasing inclusion of larval behaviour in such models (Fiksen *et* al., 2007; Leis, 2007, 2021; Sundelöf and Jonssen, 2012; Beraud *et al.*, 2017; Swearer *et al.*, 2019; Gary *et al.*, 2020). However, the converse is also true, where tidal behaviours are implemented into dispersal models without the evidence to support the employment of that behaviour in the target species (Beraud *et al.*, 2017). Though larval fish are thought to employ many swimming strategies to influence dispersal, the most well documented is selective tidal-stream transport (STST). This is the process where a larval organism mediates their vertical position in the water column to take advantage of faster flowing surface currents or slower bottom currents, to aid swimming and facilitate movement in a given direction (Walker *et* al., 1978; Forward *et al*., 1998). For example, a larva trying to travel inshore might remain in surface waters on the flood tide, then descend into deeper water on the corresponding ebb tide, to result in a net shoreward movement.

Selective tidal-stream transport has been observed in the larvae of many fish species (Matsumiya *et* al., 1982; Holt *et* al., 1989; Rowe and Epifanio, 1994; Forward *et* al., 1998; Schultz *et* al., 2000; Edwards *et* al., 2008; George *et al*., 2011; Islam *et* al., 2011; Patrick and Strydom, 2014; Deschepper *et al*., 2019), with the majority of work focussed on plaice (*Plueronectes platessa* – De Veen, 1978; Rijnsdorp *et* al., 1985; Bolle *et al*., 2009), sole (*Solea solea* – Champalbert and Koutsikopoulos, 2009) and flounder (*Platichthys flesus* – Jager, 1999a, 1999b) in the UK. Walker *et al*., (1978) and De Veen (1978) were among the first to establish that larval plaice promote shoreward movement into coastal nurseries by making use of surface currents on nocturnal flood tides. Furthermore, STST is a prominent behaviour found in the larval and adult stages of many invertebrate species, including ovigorous blue crabs moving to spawning locations (Tankersley *et al*., 1998; Forward *et al*., 2003), and barnacle larvae moving to settlement sites (Knights *et al*., 2006). Copepods are also able to occupy different areas around estuary inlets through mediating their vertical position (Wooldridge & Erasmus, 1980). During the day larval plaice are more strongly associated near the bottom, most likely to avoid predation (Rijnsdorp *et al*., 1985). Most interestingly though, is that general abundance is higher regardless of depth on flood tides. The general theory here is that the risk of predation in the water column is higher during the day for planktonic organisms (Loose *et al*., 1994; Hays, 2003; Gibson *et al*., 2016) and so larval plaice will become more associated with the benthos, thus reducing exposure to strong currents in the tidal plume, promoting retention. However, when predation risk is reduced at night, larval plaice are utilising the tidal plume on flood tides to promote shoreward transport. It seems, however, that in most cases tidal transport in larval fish is achieved by simply entering and exiting the tidal plume on opposing tides (Forward *et al*., 1998).

Recently sea bass populations have been in decline, largely due to fluctuations in inter-annual recruitment success (ICES, 2015). Sea bass from the English Channel and North Sea stocks are thought to migrate into spawning grounds in the Eastern English Channel between the months of February and April (Jennings and Pawson, 1992). The eggs and larvae are then thought to drift with oceanic currents into settlement sites along the coast of the North Sea where larvae are thought to begin migration into shallow estuarine environments, prior to settlement. Beraud *et* al., (2017) suggests that larval sea bass should be able to mediate their vertical position at 60 days, at a length of 15mm, however, these estimates are based on development of striped sea bass (*Morone saxitillus*). This would suggest that larval fish may have the potential to exhibit vertical migrations, and therefore employ STST, to enter estuaries of the Essex coastline between May and June, following spawning in February-March. It is thought that the North Sea stock represents the northernmost settlement sites of the English Channel population, due to the 9^{o}C isotherm boundary area, beyond which temperatures do not support maturation of female gonads (Devauchelle & Coves, 1988; Pickett & Pawson, 1994). Given the fragility of this isotherm in a changing climate, and the large degrees of recruitment variation observed (ICES, 2022), there is an urgent need to understand how larval sea bass behaviour influences their own settlement patterns. Beraud *et al*., (2017) modelled factors that influence settlement success of larval sea bass across settlement sites across the UK fishery management subareas. They found that settlement was predicted to be highest in warmer years, and where tidal behaviour, such as STST, was incorporated during the later larval stages.

However, to date no direct observation has been made of tidal behaviour in larval sea bass, with very few studies even attempting to address the question. This creates two problems: 1. There is no ground truth to the inclusion of STST in settlement models, and 2. There is no understanding of how sea bass may implement such a behaviour, i.e., do they attempt to promote retention, dispersal or both, and when? However, Beraud *et al*., (2017) highlights the need to better understand whether sea bass do undergo selective tidal-stream transport as they approach estuaries in mid-summer, given its potential importance in determining settlement success.

Here I present the results of an initial assessment of the vertical positioning of larval sea bass across two different stages of a tidal cycle in a southern North-Sea estuary, the Blackwater estuary, Essex, UK. Using vertical position data, I evaluate whether larval sea bass are likely to demonstrate directed movement. I test the hypothesis that sea bass larval numbers will not be equally distributed through the water column on flood and ebb tides, and that sea bass will show highest abundance on flood tides in the surface water to aid shoreward movement into estuaries. To test this hypothesis, I compared variations in the abundance of sea bass larvae with depth over both the flood and ebb tide. It is hoped that these findings will help to establish behavioural mechanisms that facilitate the colonisation of estuarine habitat and provide a baseline for further understanding the nature of such a behaviour, if indeed one exists.

2. METHODS & MATERIALS

2.1. Site Selection

Between the months of June to July 2021, ichthyoplankton samples were collected from the mouth of the Blackwater estuary, which flows into the Essex coastline (UK) of the North Sea (Figure 1). Due to a delay in the onset of warmer waters, sampling was offset to June and

July to account for this. The Blackwater forms a confluence with the Colne estuary that makes up part of the Thames Estuary basin. On the flood tide water is driven up from the south into the Thames basin, and along the coast into the Blackwater and Colne estuaries. Both the Blackwater and Colne estuaries provide nursery habitats where sea bass larvae may settle, in the form of shallow edge habitat. The Blackwater is the larger of the two estuaries, in area , volume , and depth , and this is largely due to dredging and the presence of a shipping channel running through the middle. Samples were collected from the deepest part of the channel, located in the confluence of the Colne and Blackwater at (51.758384, 0.936995), with a depth of 17m at low tide to 20m at high tide. This area was selected to provide the largest amount of stratification of tidal flow possible, and to provide distinct sampling depths. The benthos at this site is composed of rocky substrata underneath soft mud sediment. A description of the sampling session undertaken can be found in Table 1.

Table 1. Description of data collection times in the Blackwater estuary

Week	Dates Sampled	Tide	No. larval sea bass	Length range (mm)	Lat/Long
1	02/06/2021	Flood	6	0.46-3.85	51.758272, 0.931301
2	08/06/2021	Flood	27	1.50-4.33	
	10/06/2021	Ebb	18	1.55-2.94	
3	14/06/2021	Flood	6	1.90-2.50	
	15/06/2021	Ebb	30		
4	21/06/2021	Ebb	23	1.22-2.37	
	25/06/2021	Flood	29	1.81-6.34	
5	30/06/2021	Ebb	11	1.51-3.31	

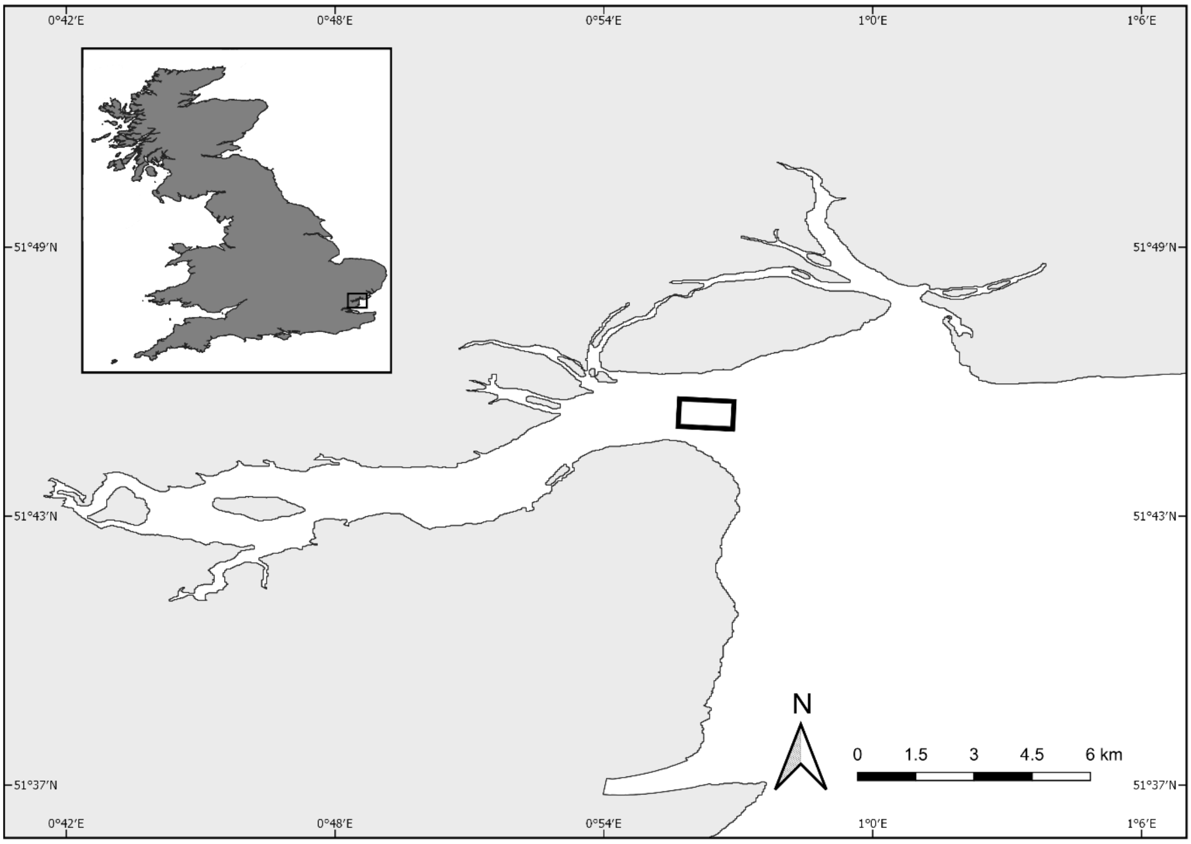

Figure 1. Map of sampling area in the Blackwater estuary, Essex, UK, throughout June 2021. Made using QGIS, 3.2.3.

2.2. *Sampling*

Samples of ichthyoplankton were collected using two specifically designed continuous plankton pumps, which pumped seawater from two depth regimes, deep (56m-47m) and surface (6m), simultaneously, deployed from the rear of a large boat (Figure 2). Deep samples were collected from 2m above the benthos, while surface samples were collected from 2m below the surface. Continuous plankton pumps were selected over the use of classic towed nets, firstly to allow increased distcrimination between depth stratifications, to minimise cross contamination of surface water plankton with deep water, which is inevitable when recovering towed nets. Secondly, plankton pumps have been shown to be equally as efficient in sampling ichtyoplankton when compared to towed nets, even factoring in damage to samples (Taggart & Leggett, 1984).

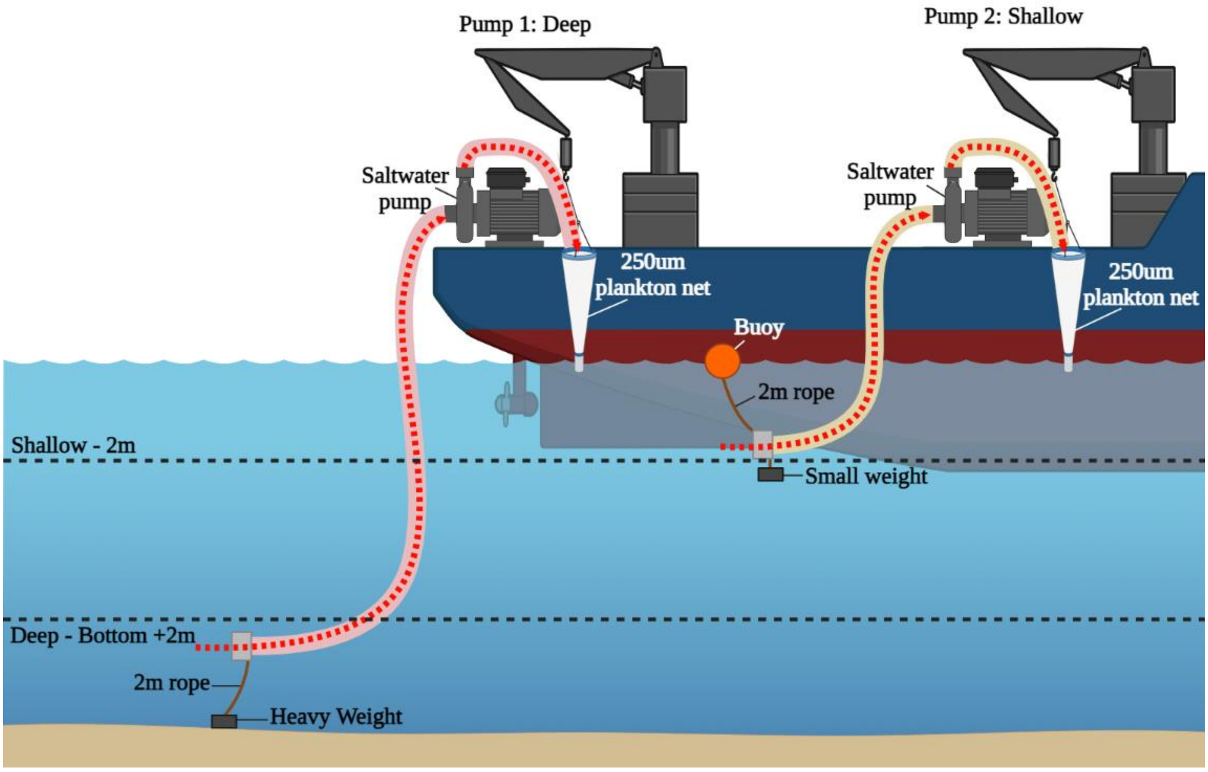

Figure 2. Diagram of Continuous plankton pump setup and sampling depth regimes, deep (red tube) and shallow (yellow tube). Red dotted arrows indicate direction of water flow. Made using Biorender (2023).

The system was designed to be relatively inexpensive and easy to use and assemble, using readily available parts. A pair of Hyundai DHYC50E diesel wastewater pumps were used, able to withstand water containing particulate matter and will prevent damage to samples. These feature an isolated cast iron impellor that drives water through an intake and outlet pipe at a maximum flow rate of 533L/min at 3600rpm. Flow rate was reduced using the throttle on the pump to reduce damage to samples and was determined by measuring the length of time taken to fill a container of known volume (18L). Following preliminary sampling and given the generally low densities of sea bass larvae reported in previous studies, pumps ran for 30 minutes to maximise catch rates.

Sampling from depth was achieved using 30m of rigid, spiral supported plastic tubing, protected by an intake guard. A 50kg weight was attached above the intake guard by 2m of rope. To allow clearance from the bottom and reduce the sediment load taken through the pump. This also set the sampling depth to be 2m above the maximum water depth, which was determined using the boats SONAR system. To sample the surface depth stratification a 10m length of rigid, spiral supported plastic tubing with intake guard, was deployed. A 5kg weight was attached directly above the intake guard to weigh the unit down, but to set the sampling depth the intake was coupled to a 2m length of rope tied to a buoy.

Samples were collected on ebb and flood tides. On each tide, a deep and surface sample was collected simultaneously at three one-hour intervals: one hour before the peak flow period (BPF), at the peak flow period (PF), and one hour after the peak flow period (APF). This provided replication for each tide measured, but also provides information on how larval densities and behaviour might change over the tidal cycle. The peak flow periods were determined to be 3hrs before and after high tide, when water flow was at its maximum. A total of four ebb and four flood tides were sampled, yielding 48 samples in total. Measurements of temperature, salinity, and pH were taken for each depth prior to sampling on a given tide, however, these were not considered further in analyses, as all parameters were generally similar between depths and tides. Water was pumped from depth, and measurements obtained using a YSI ProDSS multimeter probe (Xylem, USA).

Pump outflows were directed into separate 250um mesh plankton nets, which were washed through into separate 300ml Nalgene bottles using a solution of 70% ethanol and seawater, within which they were stored for identification back in the laboratory. Seawater was used to maintain the salt balance within the specimens and minimise damage. Prior to identification samples were filtered through a 450um mesh sieve and stored in 70% ethanol solution. Sea bass larvae were identified, counted, and length measured using imaging microscopy (Leica DMI6000 B, Leica Microsystems, Germany), based on Russell (1976). Images of all individual larva were taken to provide confirmation of identification using the Leica DMI6000 B imaging microscope (Leica Microsystems, Germany).

2.3. *Statistical analysis*

Prior to analysis, counts of larval sea bass were normalised to fish density per m^3, using the total volume of water pumped for each sample, based on estimated flow rate and time the pump was ran for. Density data was found to be heteroscedastic using Levene's tests. Deviations from normality and homoscedasticity were considered during model construction.

Due to the relatively low sample size of this preliminary study, Kruskal-Wallis tests were used in the open-source statistical software R (v4.2.2., R Core Team, 2022). Kruskal-Wallis tests were chosen as these allow for multiple factor levels. Individual models were composed, to assess differences in larval density: 1. Overall Between tides, irrespective of depth, to understand whether tidal selection occurs, 2. Between depths, for flood and ebb tides separately, to understand whether larval sea bass select tidal streams, 3. Between tidal phases, for flood and ebb tides separately, to understand whether the rate of flow impacts the behaviour, 4. Between weeks sampled to understand whether behavioural changes occurred as the settlement period progressed. Differences in the length of larval sea bass between depths on flood and ebb tides separately was also assessed, to understand whether tidal selection has a length dependant component. All data has been uploaded to the Research data at Essex online repository.

3. RESULTS

While no significant difference in overall density was found between tides ($\chi^2_{(1,48)} = 0.86$, $P = 0.354$), Figure 3, significantly more larval sea bass were found in the surface waters compared to deep waters on ebb tides ($\chi^2_{(1,24)} = 5.95$, $P = 0.01$), Figure 4. However, no significant difference in sea bass density was found between depths on flood tides ($\chi^2_{(1,24)} = 0.04$, $P = 0.836$). The largest abundances for both flood and ebb tides were almost identical.

Median density was slightly larger on ebb tides than floods, though this was not statistically significant.

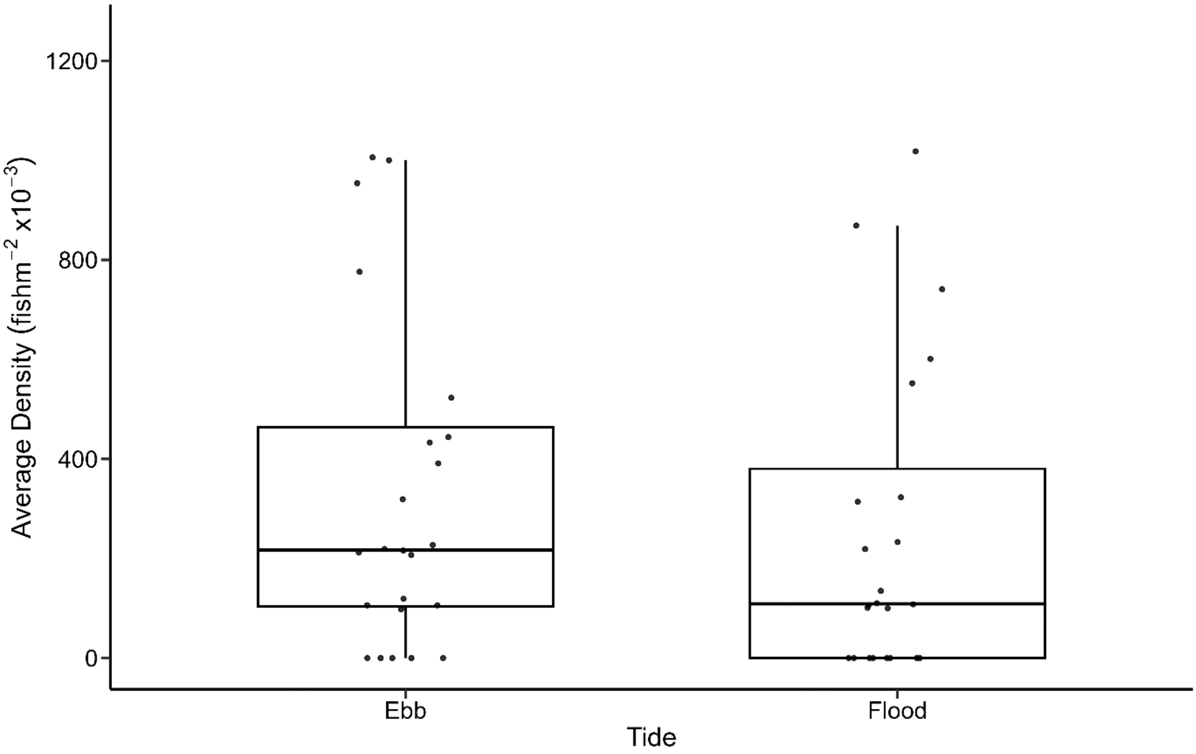

Figure 3. Boxplots for the average density (fish m^{-3}) of larval 0-group *D. labrax*, between ebb and flood tides when both sample depths are combined. Density is expressed as number of fish m^{-3} x10^{-3}. Whiskers represent maximum data point within 1.5x the Interquartile range. Points are raw count data from individual samples.

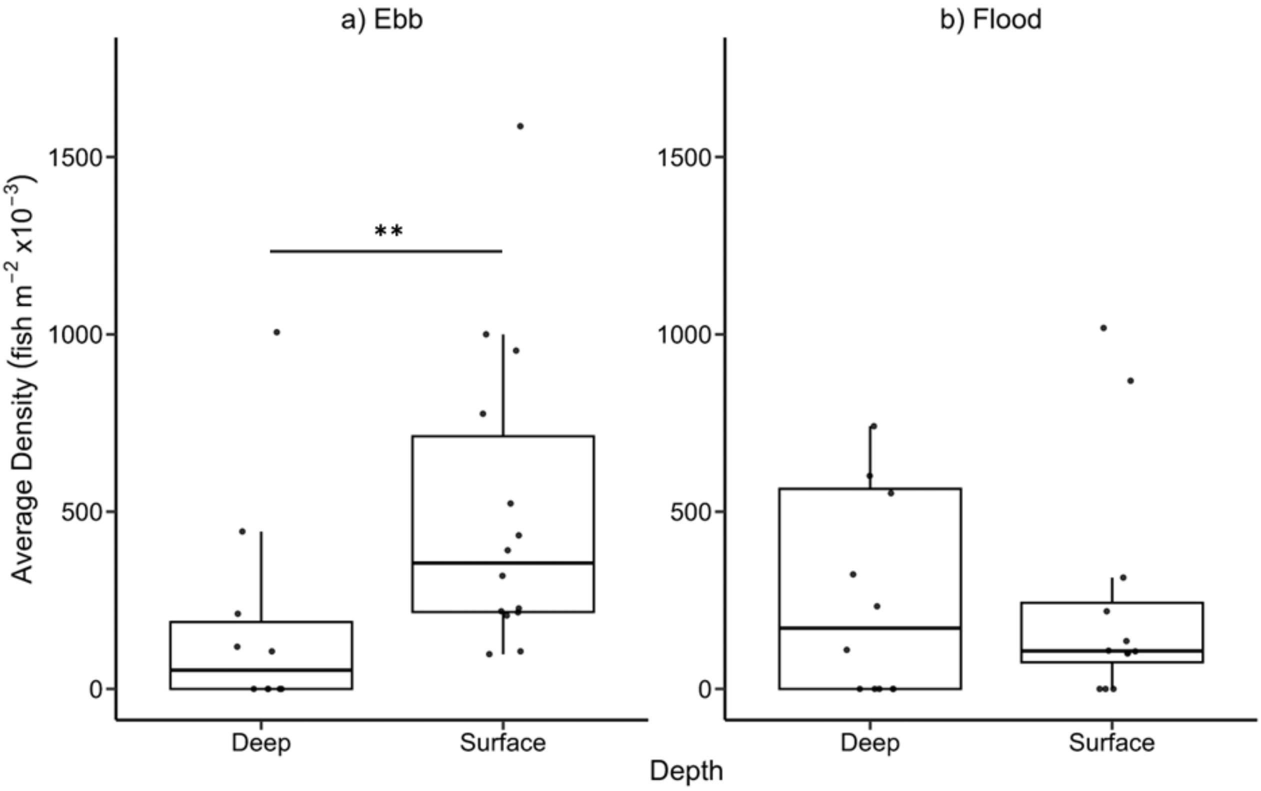

Figure 4. Boxplots for the average density (fish m^{-3}) of larval 0-group *D. labrax*, between depth regimes during ebb (left) and flood (right) tides. Depth regimes are Deep and Surface. Density is expressed as number of fish m^{-3} x10^{-3}. Whiskers represent maximum data point within 1.5x the Interquartile range. Points are raw count data from individual samples. Significant differences highlighted by *** when $p < 0.01$ and * when $p < 0.05$.

The effect of tidal phase, BPF (1hour before peak tidal flow period), PF (at peak flow period), and APF (1 hour after peak flow period), on the density of larval sea bass was not found to be significant on flood ($\chi^2_{(2,24)} = 1.62$, P = 0.44) or ebb tides ($\chi^2_{(2,24)} = 2.44$, P = 0.29). However, while not statistically significant, Figure 5 revealed that the largest individual density of sea bass during the ebb tide was found during PF, with generally lower densities either side on BPF and APF. On the flood tide, the median sea bass density was highest on BPF.

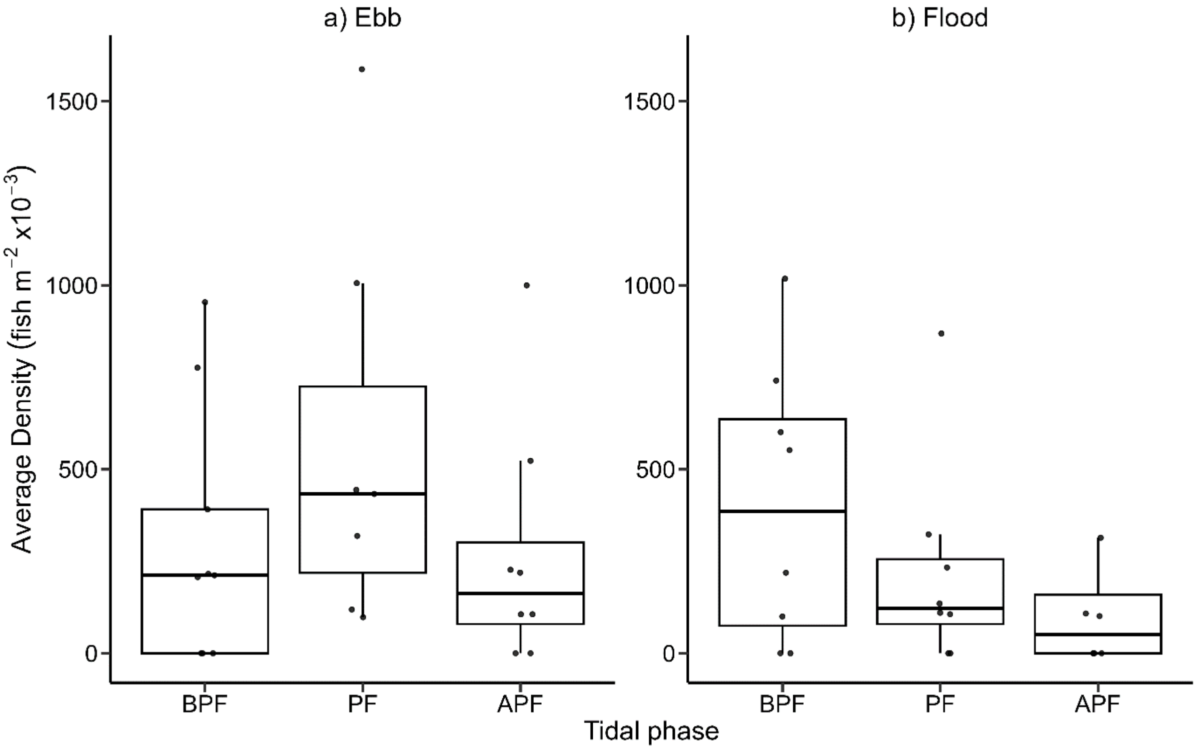

Figure 5. Boxplots for the average density (fish m^{-3}) of larval 0-group *D. labrax*, between tidal phases during a) ebb and b) flood tides. Tidal phases are BPF - 1 hour before peak flow period, PF - during peak flow period, APF - 1 hour after peak flow period (green). Density is expressed as number of fish per m^3 x10^{-3}. Whiskers represent maximum data point within 1.5x the Interquartile range.

Abundance also did not significantly differ with week for both the ebb ($\chi^2_{(3,24)} = 1.64$, $P = 0.65$) and flood tide ($\chi^2_{(3,24)} = 2.79$, $P = 0.42$), Figure 6. However, Figure 5 shows that average density of sea bass larvae increased between weeks 1 and 4 overall, while for flood tides the density of sea bass decreased between weeks 1 and 4.

The average length of sea bass did not significantly differ between depths on ebb tides ($\chi^2_{(1,96)} = 1.74$, $P = 0.187$). Average lengths between depth regimes are very similar, with very similar levels of variation exhibited as well, though the largest individuals were found in deep water. However, on flood tides the average length was significantly larger in deeper water than in surface waters ($\chi^2_{(1,58)} = 7.15$, $P < 0.01$), Figure 7.

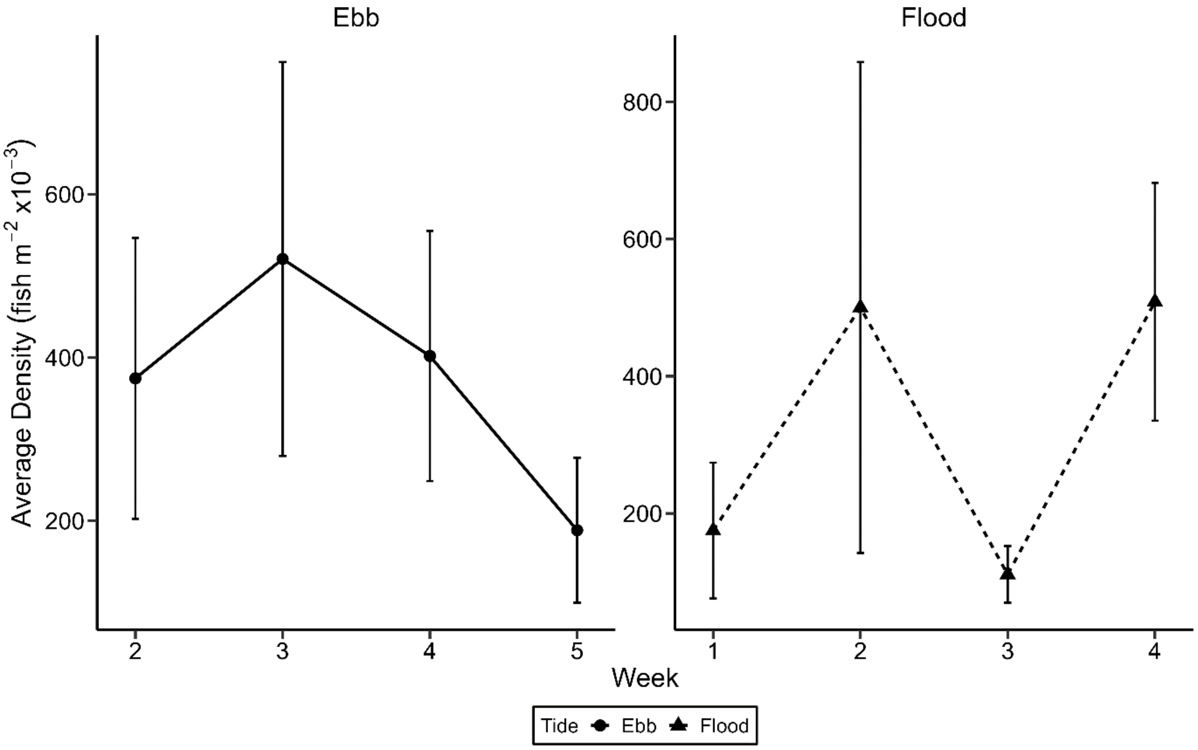

Figure 6. Line plots for the average density (fish per m^3) of larval *D. labrax*, between weeks for ebb (left) and flood tides (right). Density is expressed as number of fish m^{-3} x10^{-3}. Error bars represent 1 standard error.

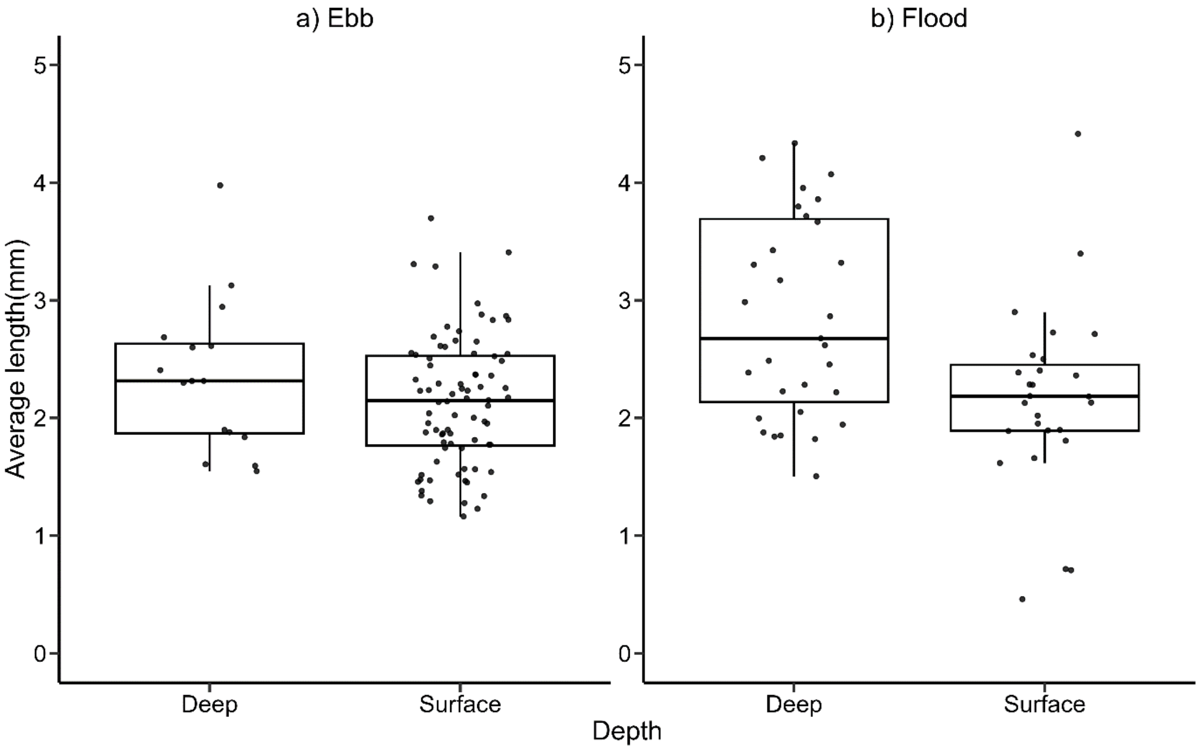

Figure 7. Boxplots for the average length (mm) of larval 0-group *D. labrax*, between depth regimes during a) ebb and flood b) tides. Depth regimes are Deep and Surface. Whiskers represent maximum data point within 1.5x Interquartile range.

4. DISCUSSION

An initial assessment of the vertical distribution patterns of larval sea bass across tidal cycles did not provide evidence for selective tidal-stream transport. The evidence presented in this study suggested larval sea bass may use ebb tides to retain to deeper water of the estuary mouth at this ontogenetic stage. The density of larval sea bass present in the water column did not differ between flood and ebb tides, suggesting larval sea bass showed no selective preference for tidal phase. However, depth distribution did differ between tides, with sea bass homogenously dispersed between surface and epibenthic depths during flood tides, while a higher density of sea bass were associated with surface waters of ebb tides. The homogenous use of flooding tidal-streams exposes sea bass to the full range of the tidal plume in a shoreward direction, however, use of surface currents on ebb tides would favour a primarily seaward movement, away from shallow intertidal habitats where group-0 sea bass are typically found in high densities during the summer months (Cattrijsse *et al*., 1994; Pawson & Pickett, 1994; Laffaille *et al*., 2000, 2001; Cabral & Costa, 2001; Green *et al*., 2009, 2012; Chapter 2). This is likely to result in a net-zero change in position within the estuary, possibly even favouring seaward movement (Fortier & Leggett, 1983; Schultz *et al*. 2003, Miller and Shanks 2004, Islam *et al*.2007).

The vertical distribution patterns of sea bass in this study are relatively atypical compared with those seen in many other estuarine dependant fish species, where the larval stages utilise flood tide transport (FTT) to disperse throughout shallow habitat of the estuary (Holt *et al*., 1989; Forward *et al*., 1998; Jager 1999, 2001; Epifanio and Garvine 2001; Vargas *et al*., 2003; Hare *et al*. 2005; Rooper *et al*. 2006; Islam *et al*., 2007).The lack of typical tidal transport behaviour could suggest one of multiple behavioural mechanisms are being employed. Firstly, the lack of consistent structure in depth distribution and tidal usage in this study could indicate that at this ontogenetic stage larval sea bass do not exhibit tidal behaviours.

Our hypotheses and assumptions that larval sea bass would be arriving and ready to settle in intertidal nursery habitat were based on data of larval sea bass abundance from power station surveys in the region, as well as estimations of size and timing of vertical swimming ability from Beraud *et al*., (2017), who themselves based estimates on striped sea bass (*Morone saxitillus*). Finally, we also based our assumptions on readiness to settle on data from chapter 2, which showed group-0 juvenile sea bass did not appear in estuarine samples until early July. The large abundance of larval sea bass found in the present study suggests that our sampling design successfully captures the peak abundance of larval sea bass within the estuary, but perhaps not peak settlement. However, the high abundance of larval sea bass at this developmental stage within the estuary could be indicative of a desire to settle. One possibility is that our sampling captures a period when it is too dangerous to settle in shallow intertidal habitats, which could in fact present a predation trap. In Chapter 2 the smallest average size of group-0 sea bass found in shallow habitats was 56mm in July, with the smallest individual size being 10mm in August, while the average length of larval sea bass found in the present study was 2.3mm (sd = 0.78). In June, when our sampling took place, the shallow intertidal bays and edge habitats were dominated by group-1+ juvenile fish, which still exhibit planktivory (Laffaille *et al*., 2001; Green *et al*., 2009). It is possible that for both scenarios, deep water retention or no tidal behaviour, that at this developmental stage, tidal behaviour is not employed and that our sampling was performed too early to capture such behaviour. Ontogenetic shifts in tidal behaviour are common in estuarine dependant species. In many cases, tidal behaviour is not exhibited until the larvae are large (Fortier and Leggett, 1983; Holt *et al*., 1989; Rowe & Epifanio, 1994a, b; Hare *et al*., 2005; Hale & Targett, 2016). Fortier and Leggett (1983) determined that the rate and size of vertical migrations of larval capelin increased with increasing size, and so too did the strength of longitudinal tidal transport. Similarly, while yolk sac larvae of weakfish show a more passive transport mechanism, older larval weakfish demonstrate an increased velocity compared to tidal currents, favouring a shoreward movement (Rowe & Epifanio, 1994$_b$). The phenology of tidal behaviours can also change throughout ontogeny, such is the case with Atlantic menhaden and Atlantic croaker entering estuarine nurseries of Chesapeake Bay (Hare *et al*., 2005; Hale & Targett, 2016; Teodosio *et al*., 2016). The early larval stages of menhaden and croaker use wind fluxes near the surface of the water column to promote inshore transport, later moving to the use of residual shoreward bottom currents, which are the product of typical estuarine circulation dynamics (Dyer, 1997), before finally moving to tidal-stream transport in the later larval stages (Hare *et al*., 2005; Hale & Targett, 2016). Holt *et al*. (1989) presented similar results, where larger larval fish were better able to mediate their vertical position and were predominantly found in the surface layers of flood tides and the deeper layers of ebb tides.

Shallow intertidal habitats may present significant hazards for larval fish, most notably through predation (Jacobsen *et al*., 1997; Compte *et al*., 2010; 2011). Shallow systems typically exhibit a high abundance of planktivorous fish (Harvey, 1991; Patterson & Whitfield, 2000; Green *et al*., 2009; Chapter 3), while deeper water supports higher abundances of piscivores (Patterson & Whitfield, 2000; Whitfield, 2017; Whitfield, 2020). For example, the shallow lotic zone of riverine systems support large abundances of planktivorous adult sunfish (*Lepomis* spp.). In these systems, larval sunfish and minnows (*Campostoma anomalum*) will avoid shallow water and retain to deeper water, where piscivorous largemouth sea bass (*Micropterus salmoides*) exclude the smaller adult planktivorous sunfish. Once the larvae reach a suitable size to escape predation from adult sunfish, but become desirable to largemouth sea bass, they then enter lotic areas (Harvey, 1991). This represents what is termed a window of vulnerability (Huss *et al*., 2010). Predation is size-selective (Mittelbach and Persson 1998, Scharf *et al*., 2000; Holmes & McCormick, 2012; Huss *et al*., 2010), where once larvae reach a threshold size, they can effectively escape predation from predation. For example, young-of-year perch larvae are subject to large amounts of cannibalism from older perch, the degree of which is inversely correlated with larvae length (Huss *et al*., 2010). Huss *et al*., (2010) found that initially larval perch grew into a window of increased predation pressure as they reached a desirable size for older perch cannibalism that coincided with peak older perch abundance. As the larval perch increased in size, the level of predation decreased (Huss *et al*., 2010). It is possible that the larval stage investigated in the present study were in a size range that represented a window of vulnerability to predation from hyper abundant planktivorous species such as juvenile sand smelt (*Atherina presbyter*), mullet (*Chelon labrosus*), and other sea bass (Green *et al*., 2009; Chapters 2, 3), that would exclude them from shallow habitats. Therefore, it is possible that larval sea bass arriving in June are not yet ready to undertake tidal transport into potentially dangerous shallow habitats.

In this scenario, up-estuary transport and dispersal would be reliant on passive physical processes associated with tidal forcing, upwelling and wind flux (Miller & Shanks, 2004a; Hare *et al*., 2005; Quieroga *et al*., 2007). The increased advective forces of strong flood tides, such as spring tides (Schultz et al., 2003), have the potential to increase vertical mixing (Zimmerman, 1986), breaking down the stratification of tidal-streams, therefore resulting in a more uniform current velocity between depths. These advective forces also have the effect of dispersing larvae more homogenously through the water column and severely reducing, if not eliminating, the potential for vertical depth selection (Chant et al., 2000; Hale & Targett, 2016). This could explain the homogenous distribution of sea bass on flood tides, and may suggest that horizontal transport is achieved passively and that vertical behaviour has little to no impact on transport on flood tides. This phenomenon has not been reported for ebb tides to my knowledge, and without the requisite current velocity data it is difficult to suggest whether tidal currents are stratified or well mixed. However, the presence of a significant difference between abundance in the surface and deeper water on ebbing tides suggests that larval sea bass may have a greater potential for vertical current selection, and so behaviour may have a greater impact on transport on ebb tides. Hale and Targett (2016) suggested that for Atlantic croaker (*Micropogonias undulatus*), a similar mechanism of advection on flood and ebb tides would result in retention of the larvae to the estuary, and that the larvae likely rely on larger flood and smaller ebb tides to disperse into shallow nursery habitat in a passive manner, where dispersal is reliant on hydrodynamic forcing, as opposed to active selection of tidal plumes,. Similarly, for weakfish larvae (*Cynoscion regalis*) in Delaware bay, yolksac larvae entering the estuary could achieve upstream migration through passive use of tidal currents, showing no difference between larval velocity and that of tidal currents.

Alternatively, the net-zero movement produced by use of both flood and particularly surface ebb tidal currents could suggest larval sea bass are trying to retain to the deeper water of the estuary mouth. This may be indicative of a form of station holding, whereby fish are trying to avoid up-estuary dispersal into shallow habitat. Similar behaviour is exhibited in other species that favour retention to deeper estuary waters, such as herring larvae (*Clupea harengus*), who actively ascend into surface waters on flooding tides and descend on ebbing tides in a manner that, when modelled alongside hydrodynamics of the region, mediated a position within the main estuary channel (Fortier and Leggett, 1983). This could indicate that larval sea bass are avoiding shallow habitat, which may present a trap at this developmental stage. Therefore, it is important to consider the importance of the ontogenetic phenology of settlement behaviour.

Tidal current velocities were not measured and, therefore, our hypothesis is based on the assumption that bottom currents flow at a reduced rate to surface currents. However, this assumption may be incorrect, and the region may exhibit unexpected flow regimes. For example, if there is very little or no difference in current velocities between surface and deep water, then we may not expect larval fish to exhibit STST.

However, there are multiple limitations to the interpretability of the results put forward in this study, owed to a constrained sampling design. Therefore, caution should be advised when considering the behavioural implications of the vertical distribution patterns reported here. Primarily our design did not account for diel or lunar cycles. Lunar cycles affect tidal amplitudes throughout the month, resulting in spring and neap tides. This can affect the tidal behaviour of marine species, such as *Solea senegalensis* and *Sardina pilchardus* which utilises flood tide surface currents on spring tides to enter estuarine nurseries of the Mondego in Portugal (Primo *et al*., 2012). Many studies have also demonstrated a significant diel effect on tidal behaviour (Rowe and Epifanio 1994a; Forward *et* al., 1998; Jager 1999; Islam *et al*., 2007; Primo *et al*., 2012). This is observed in Japanese sea bass entering Ariake Bay, Japan (Islam *et al*., 2007), whereby sea bass were more abundant on nocturnal floods, and lower in abundance across all other tides. This is possibly a result of increased predation risk during daylight hours, which drives diel vertical migrations in many plankton. It is possible there is a diel component to European sea bass tidal migrations, which may have been missed by this study. When looking at the weekly differences in flood and ebb tide usage by larval sea bass, abundance fluctuated dramatically between weeks. While not significant, fluctuating weekly tidal abundances could demonstrate that there are likely some other factors, such as diel and lunar cycles, that is influencing the tidal behaviour of sea bass (Lozano & Houde, 2013; Hale & Targett, 2016). It is possible that the limitations of our sampling design masked the actual behaviour being exhibited. Furthermore, sampling was constrained to the estuary mouth, which exhibits reduced stratification compared to offshore waters, which may reduce the scope for tidal behaviours to be exhibited. We also did not investigate the full tidal cycle or depth profile, and so we do not know how fish use slack water compared to peak flow periods, or the middle depth bands. Future work should seek to address the vertical distribution of sea bass over full tidal cycles with respect to diel and lunar cycles through nocturnal sampling.

In conclusion, while limited in interpretability due to constraints on sampling design, the present study provides valuable first insights into the early settlement behaviour of larval sea bass. Our results suggest that it is unlikely during this developmental stage that larval sea bass exhibit flood tide transport. They likely rely on passive tidal dispersal or actively try to retain to deeper water to avoid potentially dangerous shallow habitat during a potential window of vulnerability. This further supports the need to understand the ontogenetic phenology of the timing of tidal behaviours in larval fish if they are to be incorporated into models of dispersal, settlement, and recruitment. More research is clearly needed to elucidate the full range of tidal behaviours exhibited by juvenile fish, such as sea bass, and how that might affect the recruitment and distribution patterns of juvenile fish.

CHAPTER FIVE

WINTER THERMAL MINIMA: A BARRIER TO RECRUITMENT FOR EUROPEAN SEA BASS ON NORTHERN RANGE MARGIN OF UK?

*Saltmarsh in winter

1. INTRODUCTION

The effect of winter temperatures on the growth and survival of juvenile fish is thought to be a key driver of inter-annual recruitment variation (Ellis *et al.*, 2017; Younes *et al.*, 2020). However, our understanding of the relationship between temperature and survival for many commercially important species, such as European sea bass (*Dicentrarchus labrax*), is weak. In Western Europe *D. labrax* post-larvae settle to estuarine nurseries in June (Beraud *et al.*, 2017; Chapter 4), where juveniles continue to reside and develop over the summer months between July and October (Jennings & Pawson, 1999; Picket and Pawson, 1994; Beyst *et al.*, 1999; Cabral and Costa, 2001; Dufour *et al.*, 2008; Green *et al.*, 2009; Chapter 2). Throughout their range juvenile sea bass are understood to reside in estuaries for the first four years of their life, migrating to the estuary mouth as they increase in size (Pickett & Pawson, 1994; Stamp *et al.*, 2021). However, along the Kent and Essex coastline and estuaries of the Western UK coastline, juvenile sea bass are absent from estuarine communities during the winter months (Kelley *et al.*, 1986; Pawson & Eaton, 2005; Green *et al.*, 2009; Chapter 2). Very few studies focus on habitat usage and survival of juvenile *D. labrax* during these winter months or attempt to ask why this species abandons what are apparently suitable habitats throughout much of the rest of the year. A brief review of literature suggests most studies primarily focus on summer nursery habitats, particularly saltmarshes, and this applied whether that is the study of European sea bass (Cabral & Costa, 2001; Dufour *et al.*, 2009; Green *et al.*, 2009, 2012; Day *et al.*, 2021), salmon and trout species (*Onchorynchus spp.* - Huusko *et al.*, 2007; Brady *et al.*, 2020), Atlantic cod (*Gadus morhua* - Warren *et al.*, 2010; Lilley *et al.*, 2014), and plaice (*Pleuronectes platessa* - Gibson *et al.*, 1998; Wennhage *et al.*, 2007; Salleslagh *et al.*, 2013; Cariou *et al.*, 2021; Day *et al.*, 2021). A similar bias is also present in terrestrial species, including birds, amphibians, reptiles and mammals, where the breeding season is disproportionately surveyed compared to other points of the annual cycle (Marra *et al.*, 2015). A lack of winter focussed studies is in part due to the difficulties and discomfort of sampling juvenile fish in winter, though a bias may exist due to the perceived potential of temperate summer nurseries to stabilise recruitment variation (Nash and Geffen 2000; Beck, 2001). Summer nurseries are thought to provide ideal conditions for growth and survival through the provision of foraging opportunities, predation refuge, and optimal temperatures, and therefore may disproportionally contribute to recruitment to adult populations compared to other juvenile habitats, (Beck, 2001), which likely drives this bias. However, suitable conditions are required in both summer and winter habitats to contribute to the positive output of recruits to the adult population, with Anderson & Scharf, (2013), suggesting that year-class strength cannot be determined until after the first winter due to the disparity in abundance between young-of-year (YoY), otherwise referred to as group 0, in autumn and group 1 fishes the following spring. Therefore, summer habitat cannot be considered in exclusion of winter when considering drivers of recruitment (Marra *et al.*, 2015; Brady *et al.*, 2020).

Fish are poikilotherms, and as such their internal physiological processes are controlled by external temperatures (Houde, 1989; Imsland *et al.*, 1996; Wuenschel *et al.*, 2004; Handleand *et al.*, 2008; Sandersfeld *et al.*, 2017). Therefore, temperature is strongly linked to survival across all life-stages of fish, through its influence on rates of growth (Jobling, 1997; Jonassen *et al.*, 1999; Ruyet *et al.*, 2004; Wuenschel *et al.*, 2004; Handleand *et al.*, 2008; Yilmaz *et al.*, 2020), foraging **(**Ruyet *et al.*, 2004; Handeland *et al.*, 2008; Volkoff *et al.*, 2020), and metabolism (Clarke *et al.*, 1999; Ruyet *et al.*, 2004; Sandersfeld *et al.*, 2017). These physiological rates will increase with temperature up to a maximum, after which point rates will plateau before decreasing again, in what is referred to as a thermal performance curve (TPC) (Schulte *et al.*, 2011; Dell *et al.*, 2011;2013). The maximum region is known as the thermal optimum (Schulte *et al.*, 2011; Schulte, 2015), while the upper and lower extremes at either side of TPC's are the critical thermal maximum (CTmax) and minimum (CTmin), which define the points at which fish will lose equilibrium resulting in death (Claireaux & Lagardere, 1999; Pörtner, 2010; Dülger *et* al., 2011; Schulte, 2015; Laurel *et* al., 2016; Zhang *et al*., 2018; Zhou *et al*., 2021). The shape of the relationship between temperature and physiological rates changes between species and will define the thermal niche of that species (Pörtner, 2010; Sunday *et al*., 2012; 2014). As a result, fish distributions are often constrained by range margins controlled by climatic conditions, which may expand in years of elevated temperatures, while in years of reduced temperatures they may recede (Johnson & Evans, 1990; Pickett & Pawson, 1994; Perry *et al*., 2005; Munday *et al*., 2008; Figueira & Booth, 2010; Able *et al*., 2014; Cure *et al*., 2018). Increasing temperatures as a result of climate change have seen poleward shifts in range margins across marine (Perry *et al*., 2005; Last *et al*., 2011; Spies *et al*., 2020; King *et al*., 2021), freshwater (Comte & Grenouillet, 2013) and terrestrial populations including insects (Thomas *et al*., 2001; Hickling *et al*., 2006), mammals (Hickling *et* al., 2006; Anderson *et al*., 2009; Ancillotto *et al*., 2019), birds (Hickling *et al*., 2006; Hitch & Leberg, 2007; Maclean *et al*., 2008; Rushing *et al*., 2020), and also evidenced in trees (Pederson *et al*., 2004; Lenoir & Svenning, 2014). However, some populations show resilience to changing climatic conditions through phenotypic plasticity in thermal tolerance (Sunday *et al*., 2013; 2014), while others are unable to shift because of a lack of suitable settlement habitat (Petitgas *et al*., 2013; Gibson-Reinemer *et al*., 2017; McLean *et al*., 2019; Stuart-Smith *et al*., 2021), or due to anthropogenic disturbances (Gibson-Reinemer *et al*., 2017; McLean *et al*., 2019; Stuart-Smith *et al*., 2021). Increasing temperatures can also affect dispersal distances, shortening the duration of the pelagic larval phase in species who exhibit one (Raventos *et al*., 2021), therefore shortening the window for utilizing ocean currents for transport to settlement sites (Munday *et al*., 2008). For populations that exist on their range margins, interannual temperature fluctuations can result in large variation in recruitment between years (Myers, 1998), such is the case for European sea bass on the East Coast of the UK (ICES 2018, 2019).

During winter months in temperate regions, temperatures will fall well below the optimum range for many species, and below the lethal limit in particularly cold years. In response, many fish species will enter a stage of the life cycle known as overwintering, where rates of activity, growth, and metabolism are reduced to minimise energetic costs (Hurst, 2007). In some species, feeding stops altogether below a threshold temperature, and the fish survives on internal fat reserves (Hurst, 2007; Anderson & Scharf, 2013). Some species alter their habitat usage in response to temperature, by migrating into deeper water with a higher heat holding capacity, as a form of thermoregulation (Able & Fahay, 1998; Hales & Able, 2001; Cote *et al*., 2004; Sims, *et al*., 2004, 2006; Miller *et al*., 2016). Young-of-year fish entering their first winter may not have sufficient fat reserves to meet energetic costs, which may result in death. Winter mortality is often attributed to either starvation from total expenditure of fat reserves or increased thermal stress causing maintenance costs that are too high to sustain, resulting in a breakdown of physiological equilibrium (Hurst, 2007). Particularly cold years can result in large scale deaths termed ‘winterkills’ (McCollum *et al*., 2003; Hurst, 2007), which is also observed in bird populations undertaking winter migrations (Clark *et al*., 2004; Newton, 2007). In the Celtic Sea of the UK, near total cohort loss has been reported for group-0 *D. labrax* when temperatures fell below a threshold of 6^{o}C (Kelley *et al*., 1988), and results from Chapter 2 reveal that there is generally a significant reduction in the number of juvenile fish returning to shallow estuarine habitat in subsequent years (group-1 and group-2^{+}). It is thought that when multiple cold winters are experienced, larval sea bass may settle later in the season, and in fewer numbers, which may result in reduced persistence over winter (ICES, 2012, 2014). Winterkills have the potential to contribute to this low annual survival of group-0 fish and therefore create persistent bottlenecks for fishery or population recruitment if multiple and simultaneous cold years are encountered.

Studies have shown that the optimum temperature range for *D. labrax* is between 24-27^{o}C for metabolism (Yilmaz *et al*., 2020), 26^{o}C for growth and feeding (Ruyet *et al*., 2004), but tolerance around this may vary depending on the acclimation and rearing temperatures (Dulger *et al*., 2011). For example, a juvenile hatched in the Mediterranean may be able to tolerate higher temperatures than those of the UK. The minimum metabolic rate (Tcrit) for sea bass is reached at 6^{o}C (Dulger *et al*., 2011; Yilmaz *et al*., 2020), while temperatures of 2^{o}C to 3^{o}C are considered lethal (Dulger *et al*., 2011). The North Sea subarea of the sea bass fishery on the East coast of the UK is thought to remain within the 9^{o}C isotherm, which may limit their spring spawning. The North Sea population is thought to be undergoing a Northern range expansion due to rising sea temperatures in the North Atlantic (Quero, 1998; O'Brien *et al*., 2000; Stebbing *et al*., 2002; Claireaux *et al*., 2006). Preston *et al*. (2008) suggested that range expansions can only occur if adequate habitat and resources are available at all life stages, the settler can survive predation and competition within the community, and that climatic conditions are within the settler's physiological tolerances. Temperatures on the east coast of the UK routinely fluctuate around the Tcrit for European sea bass, and this is partially thought to underpin recruitment variation, alongside summer nursery habitat availability, which presents two possible outcomes; that group-0 sea bass exhibit near complete die-off over winter, or they migrate into warmer habitat with potentially higher predation risk. However, to date, the effect of winter thermal minima and maxima on the habitat usage and survival of juvenile sea bass has been ill explored, where currently no data exists on winter distribution patterns for the region.

In cases where data deficiency is limiting the ability to understand behavior, habitat usage modelling can be a powerful tool in generating testable hypotheses to take forward and apply to real world situations. Energy budget models (EBM) which utilise calculations of energetic intakes and costs based on the size of an organism and temperature, using taxon or species-specific constants, and normalizations which can be used to predict growth, mortality, and effects on condition under different scenarios. Watson *et al*., (2020) developed an energy budget that could be used to investigate the effects of sublethal stressors encountered by a fish, through the effects on energy expended escaping a stressor. The model was further applied to determine the long-term implications of escapes, beyond immediate energy loss, to determine loss of growth and fecundity within a year (Watson *et al*., 2020). While the model presented in Watson *et al*., (2020) was applied specifically to fish escaping angling fights, it is easily applicable to numerous stressors. However, the Watson *et al*., (2020) model does not account for the effect of changing temperatures on the physiological rates in sea bass and specifically targets the adult stage.

Energy budget models have been developed to specifically investigate the thermoenergetic effects of temperature on juvenile fish over winter. Such models can be adapted across a broad range of species, by applying the ecological theory of metabolism, which relies on a highly conserved relationship of metabolic rates with body size and temperature (Sibley *et al*., 2013). Many EBMs utilize these equations to which model mortality from starvation as the point at which maintenance costs cannot be met by energy intake, which would lead to the catabolism of energy reserves and skeletal muscle (Broekhuizen *et al*., 1994; Jones *et al*., 2002; Hedge *et al*., 2013; Van Kooten *et al*., 2004, 2009; Butcher *et al*., 2005; Johnston *et al*., 2013; Pethybridge *et al*., 2013; Raab *et al*., 2013; Sibley *et al*., 2013; MacDonald *et al*., 2018; Dambrine *et al*., 2020; Watson *et al*., 2020). All of these models are based on the concepts first put forward by Koojiman (1986), Starvation as a result of maintenance costs relative to energy intake is thought to be an important mechanism of winter mortality, therefore, an energy budget model is well suited to modelling mortality as a result of temperature dependent mortality. However, we do not know how the mechanics of low temperature mortality operate through reserve depletion, with no studies directly addressing energetic costs of maintenance at low temperatures, however, here I assume that this mechanism is underpinned by maintenance costs. This presents a major knowledge gap that this study attempts to answer. For example, MacDonald *et al*., (2018) applied an EBM to assess the effects of winter thermal minima on the energetic expenditure and survival of sandeels. Through modelling temperature dependent foraging and seasonal copepod prey availability they were able to determine that overwinter mortality was largely the result of acute thermal stress as opposed to starvation. Similar models have been adapted for salmon (Broekhuizen *et al*., 1994; Jones *et al*., 2002; Hedge *et al*., 2013) and anchovies (Pethybridge *et al*., 2013; Raab *et al*., 2013). Regarding sea bass, previous models of juvenile survival have focused on Mediterranean populations (Lika *et al*., 2014; Stavrakidis-Zachou *et al*., 2018), with Waston *et al*., (2020) and Dambrine *et al*., (2020) being notable exceptions. Dambrine *et al*., (2020) were able to use dynamic energy budget theory (Koojiman, 2010) to develop a thermoenergetic model of growth in juvenile and larval stages, to explore the effects of different temperatures and levels of food availability on the growth and survival. Koojimann (2010) was able to successfully predict the growth and survival rates captured under experimental conditions. However, to my knowledge no study has attempted to determine the survival of YoY sea bass using real world winter temperatures.

The present study explores the effect of winter temperatures under different habitat defined scenarios on cohort growth and mortality for group-0 *D. labrax* using an EBM. To achieve this, an existing EBM (Watson *et al*., 2020) was extended and developed to investigate the impact of winter temperature on growth and survival of sea bass. It is intended that this model will serve as a preliminary investigation tool for later inclusion in a larger individual-based model of recruitment that considers ontogenies and associated behaviours beyond group-1 sea bass. The model will assist in identifying potential sea bass winter nursery habitat and recruitment bottlenecks. Using the EBM I quantify cohort mortality of juvenile bass in the southern North Sea and investigate the likelihood of complete cohort failure leading to high interannual variation in bass recruitment.

2. MATERIALS AND METHODS

2.1. Temperature dependent growth experiments

To determine how well outputs from the energy budget model reflect how juvenile sea bass growth rates respond to winter temperatures we investigated the effects of winter temperatures on sea bass growth rate by measuring weekly changes in length and weight of juvenile sea bass held at three experimental temperature treatments. Two experiments were undertaken, a preliminary experiment to determine whether changes in growth rate could be detected, and a repeated secondary experiment using a similar but larger set up. In both cases fish were exposed to three temperature treatments 27°C, 10°C, and 6°C. The CTmin of juvenile sea bass is 2-4°C (Dulger *et al.*, 2011), at which point fish lose equilibrium and die, but metabolism is thought to reach a minimum at 6°C (Dulger *et al.*, 2011). While we aimed to explore observed winter temperatures in local Essex and Suffolk estuaries, following consultation with the UK home office ASPA consulting service the minimum experimental temperature selected was 6°C. Based on our reading of likely outcomes, the home office ASPA veterinarian agreed with our assessment that exposure to lower temperatures would expose fish to undue stress and morbidity and therefore require a Home Office Institutional, Project and Person license. In addition, we agreed to stop the experiment should excessive mortality or poor condition be observed in fishes. A second experimental temperature of 10°C was selected as this approximated the ambient range of temperatures recorded during the collection of sea bass entering winter (measured between 8-12°C in field using YSI ProDSS multiparameter probe). A final treatment of 27°C was selected as this represents the thermal optimum of juvenile sea bass (Ruyet *et al*., 2004; Dulger *et al*., 2011) and the ambient laboratory conditions within which the fish were held.

2.1.1. Fish collection and husbandry

Juvenile sea bass were collected from the lower Colne Estuary complex (Figure 1) in September 2020, using beach seine nets. Collections were undertaken twice, each time performing three seines at high tide, following methods described in chapter 2. A total of 40 YoY fish between 50-80mm in length were retained and transported to the laboratory using 18L aerated buckets. Upon arrival at the laboratory length and weight were measured. Fish lengths were measured to the nearest 1mm using a measuring board. To reduce stress all surfaces and hands were wetted. To measure weight, fish were placed individually into a pot containing a known volume of water and weighed using a field balance to the nearest 0.01g. Importantly gut contents were not flushed prior to weighing as this would likely induce large amounts of stress and result in mortality, however, fish were not fed for 24 hours prior to weighing, During which time we assumed gut contents had been passed. Fish were then placed into a holding tank held at 10^{o}C for 17 days to acclimate to ambient estuary temperatures. Fish were kept in darkness and not fed for 24hrs to reduce stress and ammonia spikes, after which fish were fed a diet of frozen mysid shrimp at a ration of 3% total tank biomass once per day. Fish conversion ratios were assumed to be 100% for daily ration calculations, however, excess food was frequently found on the tank floors and removed during partial water changes. Between experiments fish were re-acclimated to laboratory holding tanks held at 12^{o}C, at a rate of 2^{o}C per day over 4 days, where they were allowed to recover for 2 months.

Prior to experimentation, filters were seeded using API QuickStart bacterial seed, and a feed source of ammonium nitrate to start the nitrogen cycle. Filters were allowed to cycle for 3 weeks. Water changes were performed bi-weekly, and water parameters were recorded daily. If conditions were determined to be below or approaching thresholds, a sufficient water change was performed immediately. Water changes were performed using settled and filtered sea water supplied from the collection site area. The acclimation period also allowed for quarantine and observation to determine if any low condition or morbidity was present. At the first sign of health deterioration fish were quarantined and monitored. If morbidity was suspected or the fish was considered moribund, then fish were euthanised. Holding tanks were filtered using sump filter systems, and 90L experimental tanks used internal Eheim Aquaball 130 filters, with a turnover rate of 180L per hour. Following conclusion of the experiments fish were re-acclimated to ambient estuary temperatures (20°C) at a rate of 2°C per day, before release at the collection site.

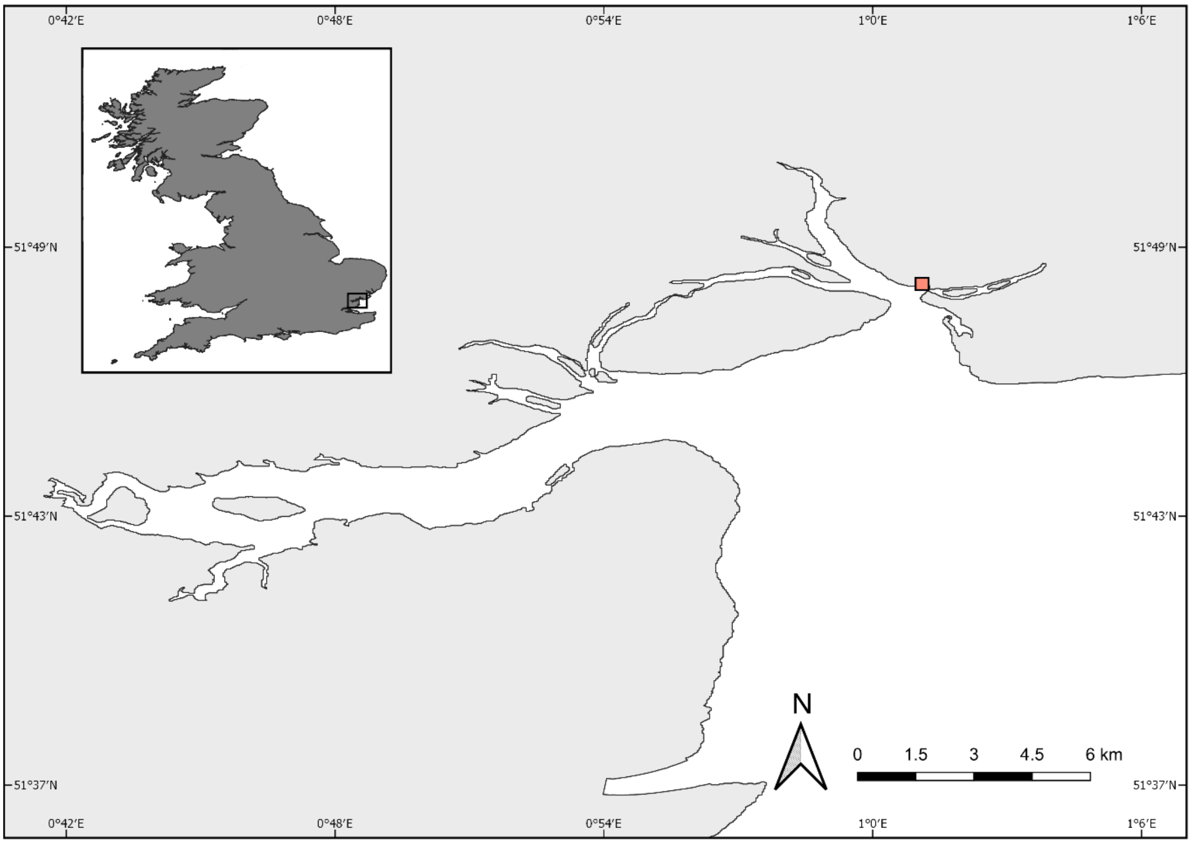

Figure 1. Map of the sampling site (red) within the Colne Estuary, Essex, UK. A total of 40 fish were retained in September 2020. Made in QGIS 3.2.3.

2.1.2. *Experimental conditions*

Both the pilot study and subsequent repeated experiment followed the same experimental design and protocols. Following acclimation fish were placed into experimental tanks and allowed to acclimate to experimental temperatures at a rate of 2^{o}C per day over 3 days. Fish were exposed to three temperature treatments, 6oC, 10oC, and for 28 days (4 weeks), and weekly measures of length and weight for each fish were taken to measure changes in growth rate. Length was measured using a wetted measuring board, and weight using a field balance and a pot containing water to reduce stress. Again, fish were not fed 24hrs prior to weighing to allow stomach contents to be excreted. Fish were housed in triplicate 90L tanks, each containing four fish to give a total N = 36, and an n = 12 per treatment, and fish were length paired to provide a similar mean length between tanks and treatments. We were not permitted to mark or tag individual fish, which would require a Home Office Institutional, Project and Person license. Therefore, monitoring the growth of individual fish was not possible. This would have increased the statistical power of the study. It was also not possible to separate fish into individual tanks, as juvenile sea bass prefer to be kept in small groups where possible to reduce stress (Dulger *et al.*, 2011). Therefore, measurements of length and weight were averaged for each tank. Throughout the experiment fish were fed a diet of 3% total tank biomass once per day. Feed conversion was assumed to be 100% for subsequent daily ration calculations, and rations were adjusted following weekly weight measurements.

2.1.3. Statistical analysis

Length (mm) and mass (grams) measurements for all fish in each tank were averaged. As a result, a mixed effects approach could not be adopted, however, were marking possible in the future this would be the preferred approach for this setup. Normality was determined visually using histograms of frequency distributions and, combined with the low sample size, it was decided that non-parametric Kruskall-Wallis tests were to be performed to determine: 1. Differences in instantaneous growth rates between treatments; and 2. Differences in weekly growth rates. Instantaneous growth rate was determined based on the change in ln(mass) per unit time (28 days). All analyses were performed in R statistical software (v4.2.2., R Core Team, 2022).

2.2. *The Energy Budget model*

The present study developed an energy budget model that extended work by Watson *et al. (*2020) to investigate the effects of temperature on the growth, metabolism, and survival of YoY European sea bass throughout the year, following settlement in June. The model produced by Watson *et al*., (2020) uses calculations of energetic intake and expenditures on metabolism, growth, and reproduction to determine energy consumption, growth, and fecundity in a year for an adult bass. They use this to model the effects of non-lethal stressors, specifically hooked fish resisting capture in recreational angling, on energy expenditure to determine the loss of growth and fecundity in a year as a result of a fight. Our model uses the basic the energetic calculations put forward in Watson *et al*., (2020), that we adapted to work with juvenile fish, as well as including a thermal stress cost on metabolism, and a temperature dependant ingestion rate. In our model, individual YoY sea bass are modelled in terms of reserve energy (KJ), which represents a fishes lipid reserves based on the estimate of 10% total body weight. Energy is ingested on a daily rate as a function of body length and temperature, and this energy is allocated to vital processes by importance (Figure 2). Metabolic costs are often perceived as the most vital process, underpinning the function of the organism, and so this is assigned first, based on body mass and temperature (Sibley *et al*., 2020). Metabolic costs are often measured in oxygen consumption per unit time as a proxy (mgO_2 per day). Secondly, as the model focuses on YoY fish we are not interested in gonadal maturation as a cost, therefore, growth is considered the next most important cost, again underpinned by body length and temperature.

Fish grow daily at the maximum rate allowed by physiological and temperature constraints as per Sibley *et al*., (2013). If energy remains after metabolic and growth costs are considered then this is allocated to fat reserves, up to a maximum value determined as a function of body mass. Once the maximum reserves have been met, any excess is lost as waste. If the energy ingested is not sufficient to meet metabolic and growth costs, resulting in a negative residual energy value, then the energy debt is removed from the reserves (Sibley *et al*., 2013).

When reserves reach zero the fish is assumed to be dead (MacDonald *et* al., 2009), following which the daily mass is assigned to zero for the remainder of the model runs and no longer contributes to measures of cohort biomass. Unlike Dynamic energy budgets, which assume fractions of available energy are allocated to vital processes, our model assumes that: 1. Energy is allocated to metabolism and growth to meet the maximum cost; 2. Fish will metabolise and grow until the point of death; and 3. Metabolism and growth rates are determined by body mass and temperature and are not mediated by available energy (i.e. less energy intake does not reduce the ratio of energy available to metabolic activity or growth). Below I shall describe the various sub-models used to inform the DEB output.

2.3. Model parameterisation

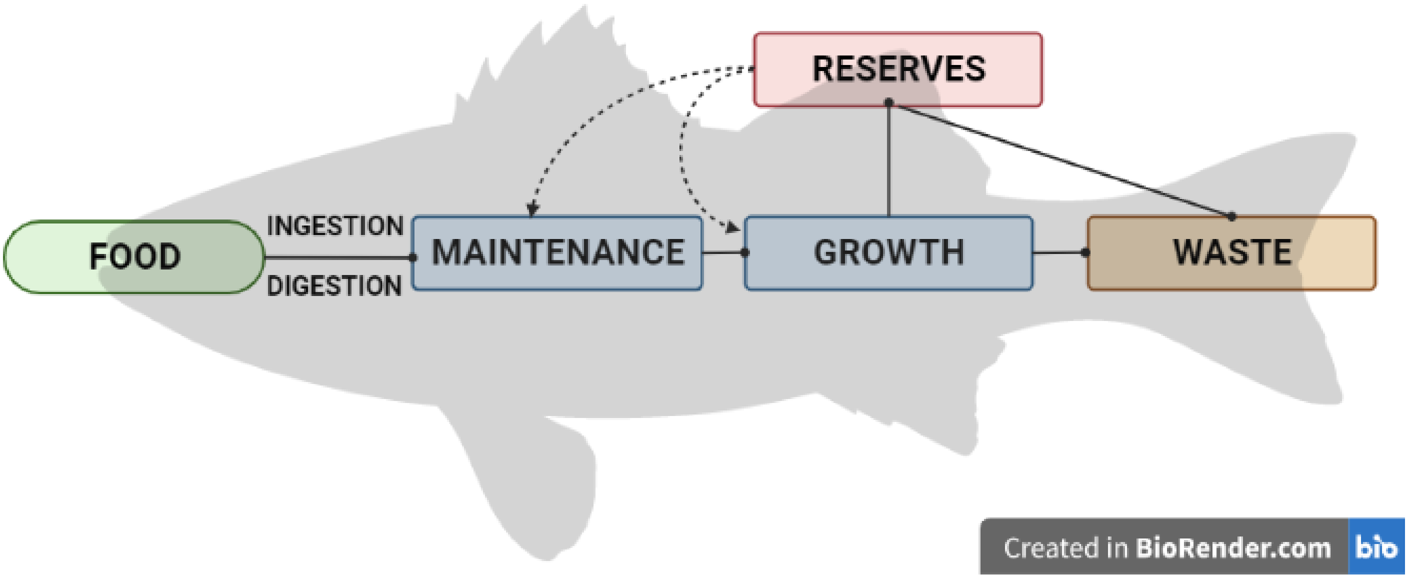

Figure 2. Schematic of the flow of energy through the Dynamic Energy Budget model in order of priority, after Sibley *et al*., 2013. Energy is ingested from food and allocated to maintenance and growth. Any left over is assigned to reserves or waste. If energy is not sufficient to meet requirements, then energy is removed from the reserves until they reach 0 and the fish dies. Diagram created in Biorender.

Estimations of the parameters were achieved hierarchically (Table 1). Firstly, if the parameter had previously been estimated experimentally or utilised in similar published models then the parameter could be applied in our model. Secondly, if no such parameter could be identified in the literature, then the parameter was estimated using data from similar species or data repositories and non-modelling publications (e.g., Russel *et al*., 1996; Ruyet *et al*., 2004). Finally, if no such data existed, then the parameter was considered a fitted value, and was estimated using the model functions.

Table 1. List of dynamic energy budget model parameters, symbols, values, units, with references and data sources.

Symbol	Parameter	Value	Unit	Reference
B0	Species specific normalisation constant	0.076	--	Peters, 1986
TrefM	Reference temperature for metabolism	287.15	K	Watson *et al.* (2020)
TrefG	Reference temperature for growth	285.15	K	Watson *et al.* (2020)
El	Energy stored in 1g of lipid	39.3	KJ	
IGmax	Mean ingestion rate	0.5	g/day	Lanari, D'Agaro, and Ballestrazzi (2002)
Mm	Maximum mass	10	Kg	Watson *et al.* (2020)
z	Cost to grow 1g of flesh	10.6	KJ	Watson *et al.* (2020)
Pf	Fraction of food that is flesh	0.5	--	Watson *et al.* (2020)
Ae	Assimilation efficiency	0.75	--	Watson *et al.* (2020)
Ky	Yearly growth constant		g/year	Watson *et al.* (2020)

2.3.1. Ingestion

Ingestion rates scale with body length and temperature, where larger fish are able to consume more prey, and higher temperatures increase the rate of feeding. The amount of energy available from ingested prey is also dependant on the amount of flesh contained within prey, and the assimilation efficiency of the fish. The equation for ingestion was derived from Watson *et al. (*2020), which is dependent on body length.

$$\mathrm{IG}_e = \mathrm{IG}_{max} \times P_f \times z \qquad \text{Eqn. 1a)}$$

Where IG_{max} is the species-specific maximum mass of ingested material, P_f is the percentage of ingested material that is flesh, z and is the energy (KJ) contained within 1g of flesh. Equation 1a was adapted to include a temperature dependant feeding rate function. Equation 1a was estimated based on data of foraging rates for juvenile sea bass from Russel *et al. (*1996) and Ruyet *et al. (*2004). The function was originally developed as a polynomial equation based on

Ruyet *et al.* *(*2004), however, this function is specific to the Mediterranean temperatures assessed within their study, and do not go as low as those experienced in this study. Use of the Ruyet *et al.* *(*2004) polynomial would result in maximum feeding rates occurring at 0^{o}C and 28^{o}C. Therefore, data based on sea bass of the Celtic Sea (UK) from Russel *et al.* *(*1996) was included alongside data from Ruyet *et al*., (2004), and used to produce a new curve to better reflect UK winter temperatures. A negative sigmoid relationship was used to model the effect of temperature (T) on feeding rate (f_r), which asymptotes towards zero below 6^{o}C (Russel *et al*., 1996), Figure 3, to give the following equation:

$$f_r = 1.45 \times (1 - e^{-0.0002T^3}) \qquad \text{Eqn. 1b)}$$

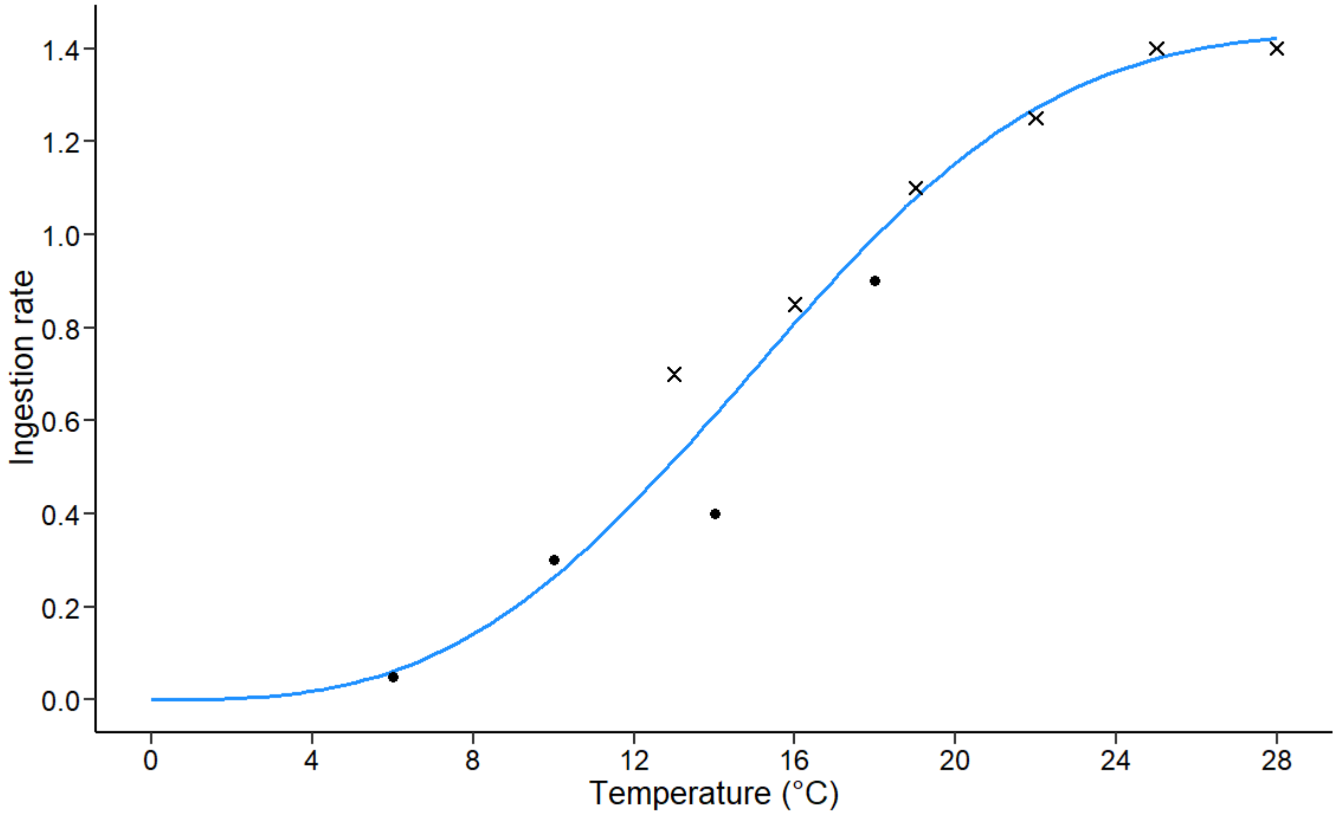

Figure 3. Fitted curve for the relationship between relationship between ingestion rate and temperature (°C). Curve fitted to data from Ruyet *et al.*, 2004 (×) and Russel *et al.*, 1996 (•)

2.3.2. Metabolism

The total cost of metabolism is the sum of all metabolic processes as part of an organism's physiology, excluding growth, reproduction, and maturation. There are many metrics of metabolism, but by far the most common is the standard metabolic rate (SMR). Our study is concerned with the daily cost of metabolism, and so SMR in this instance is measured in $mgO_2.day^{-1}$. Standard metabolic rate scales with body mass as a power law, and exponentially with temperature to give the following equation:

$$\mathrm{B} = \mathrm{B}_0 \mathrm{M}^{\gamma} e^{-E/kT} \qquad \text{Eqn. 2a)}$$

Where B is metabolic cost, B_0 is a taxon-specific normalisation constant, M is the body mass, γ is a species-specific allometric exponent derived from experimental data, E is the activation energy for thermic reactions, k is Boltzmann's constant (Gillooly *et al.*, 2011;

Sibley *et al.*, 2013), and T is temperature (K). The Arrhenius equation $e^{-E/kT}$ can be further expanded to include a reference temperature (T_{ref}), measured in Kelvin, used to determine B_0 to give the equation:

$$\mathrm{B} = \mathrm{B}_0 \mathrm{M}^{\gamma} e^{-E/k(1/T-1/T_{ref})} \qquad \text{Eqn. 2b)}$$

2.3.3. *Growth*

The somatic growth rate of a poikilothermic organism is primarily underpinned by body mass and external temperature. Energy is allocated to growth sequentially after metabolic costs are met. The maximum growth rate of an organism is presented by the von Bertalanffy equation (von Bertalanffy, 1957):

$$\Delta\mathrm{M} = \mathrm{M}_m^{1/3}\mathrm{M}^{2/3} - \mathrm{M} \qquad \text{Eqn. 3a)}$$

Where M_m is the species-specific maximum attainable mass. Growth rate also scales exponentially with temperature, using the Arrhenius equation, to give the following temperature dependant growth rate:

$$\Delta\mathrm{M} = r_B e^{\left(-\frac{E}{k}\right)\left(\left(\frac{1}{T}\right)-\left(\frac{1}{T_{ref}}\right)\right)}(\mathrm{M}_m^{1/3}\mathrm{M}^{2/3} - \mathrm{M}) \qquad \text{Eqn. 3b)}$$

Where r_B is the species-specific Bertalanffy growth constant. The daily growth rate can then be multiplied by the energy required to grow 1g of flesh to derive the cost of growth.

2.3.4. *Reserves*

Reserves in juvenile fish take the form of stored fat. A juvenile's maximum reserve size is constrained to a fraction of that animal's body mass (P_{fat}). Therefore, the maximum reserve energy available to a fish at any given time is provided by the following equation:

$$\mathrm{R}_{max} = \left(\mathrm{M}_{f} \times \mathrm{P}_{fat}\right) \times E_{l} \qquad \text{Eqn. 4)}$$

Where R_{max} is the maximum energy that can be allocated to reserves (KJ), M_{f} is the mass of an individual fish and E_{l} is the energy per gram of lipid (KJ).

2.3.5. *Thermal stress*

The temperature dependant growth experiments and other studies (Kelley, 1986) indicate that at temperatures of 6°C we see decreases in growth rate and condition, such that chronic exposure or further decreases in temperature would result in mortality, and that some energetic cost must be acting upon the fish. However, in our initial model incorporating only temperature dependant ingestion, metabolism, and growth, without the inclusion of a thermal stress term, juvenile sea bass experience no mortality at low temperatures, which does not reflect mortality rates in Kelley (1986). Therefore, our model includes a proposed stress cost associated with maintenance outside of the optimum thermal range of an organism. For juvenile sea bass the optimal metabolic temperature range is considered to be between 22-28°C. At extremely low temperatures, close to CTmin and Tcrit thermal shock may occur, which causes oxidative and osmotic stress on cellular metabolic function (Donaldson *et al.*, 2008; Reid *et al.*, 2022), and therefore the fish should inherit some form of energetic cost as a result of stress and recovery (Bevilheimer & Bennet, 2000). Bevilheimer & Bennet (2000) developed a model for thermal stress accumulation approaching CTmax and its effect on fish in a bioenergetics model, but they do not quantify the energetic costs of said stress. Thermal stress is thought to act primarily on fish neuroendocrine responses, secondarily on metabolic, osmoregulation and cardiology, and thirdly on whole organism, growth and survival (Donaldson *et al*., 2008; Reid *et al*., 2022). However, to date no data exists on the energetic costs of thermal stress in fish. Therefore, thermal stress is considered a fitted variable within the model. Thermal stress in our model scales the energetic cost of metabolsim at a given temperature, and increases as temperatures approach CTmin, and with increasing body length. Sea bass should experience little to no thermal stress within the optimum range, but at the CTmin (4°C) the energetic costs of metabolism and growth should be equal to the ingested energy and reserves combined to result in death. Consequently, the thermal stress scaling factor on metabolism was modelled as a function of temperature by determining the necessary increase in metabolism to deplete reserves, for a fish of a given size, and was found to increase as a power law with decreasing temperatures:

$$\mathrm{TFI} = \ 310.34T^{-3.291} \qquad \text{Eqn 5a)}$$

However, equation 5a alone had lower effects than expected on fish of larger sizes, and larger effects than expected on fish of smaller sizes. Therefore, how the cost should change with body length was also investigated and found to increase as a power law with increased body mass, Figure 4. Combining the two functions produced the following equation of thermal stress scaling with temperature and body length. The equations and subsequent curve were fitted based on estimates of energy ingestion, growth costs, and metabolic costs for fish of a range of lengths (30, 32, 34, 36, 38, 40mm). Thermal stress costs are size dependant, as mortality is unlikely to be size dependant, as 0-group fish are likely to respond to low temperatures in a similar way. However, the time to mortality might be size dependant, which I have attempted to capture here.

$$\mathrm{TFI} = 310.34T^{-3.291} \times 0.685L^{-1.14} \qquad \text{Eqn 5b)}$$

The results of the laboratory experiments, regarding rates of growth and condition loss at 6°C and 10°C were used to calibrate the strength of the thermal stress cost to better reflect mortality rates.

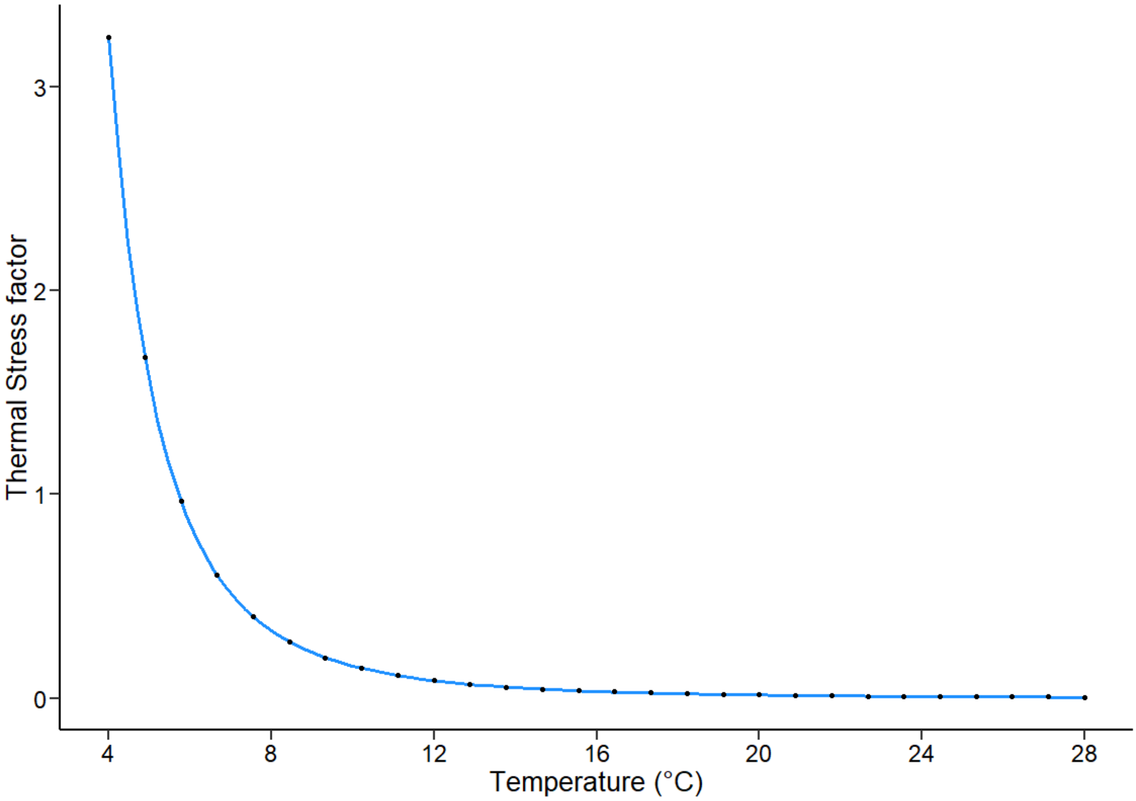

Figure 4. Fitted curve for the relationship between temperature (°C) and thermal stress factor.

2.4. Model experiments and sensitivity analysis

The objective of the energy budget model proposed in this study was to simulate the growth and mortality of individual YoY sea bass from settlement in June throughout their first winter, based on the temperatures experienced in estuarine environments across their northern range, and use this to estimate the total cohort post-winter biomass of juvenile bass reaching group-1. The final biomass output after one year was compared between model runs using input temperatures from different habitats using temperature data measured from estuarine channels from the Colne and Blackwater estuaries in Essex, UK, L'Elorn estuary in France, and the Tagus in Portugal. In each estuary location winter temperatures were measured at 10-minute intervals *in-situ* from intertidal saltmarsh creeks and adjacent deeper creeks, subtidal main channel habitat and outer estuary channel habitat, using HOBO temperature data loggers (Onset) from December 2020 to May 2021. A description of the sampling locations can be found in Table 1. Temperatures were averaged as daily temperatures, excluding low tides periods from intertidal habitats where necessary. Where logger data for a given creek type was not available for a given location, it was estimated from online data repositories, e.g., Tagus, Portugal, which was estimated by data obtained from geoportal between March 2020 to March 2021, which represented the most complete temperature data set. The model was also run for a smoothed temperature profile, using a seven-day moving average temperature. Summer temperatures for each location were fixed in the models and were estimated from data obtained online from average monthly temperatures measured from the Colne estuary, Essex, UK, where necessary for averages recorded for a given month of a year. The model uses a nursery habitat settlement input of 1000 post-larval fish, with randomly selected lengths from a normally distributed pool of 20-40mm, with a mean of 30mm and standard deviation of 10mm.

Sensitivity analyses were performed to understand how changes in model parameters; that is stress cost, temperature, settlement length, and feeding rate influenced growth and survival of fish within the model. To do so the model was run against 10% increases and decreases in: 1. Daily temperatures; 2. Settlement length; 3. Thermal stress scaling factor; and 4. Feeding rate. The new scenarios were then run with the new parameters and the results compared to the baseline scenarios. Large deviations in biomass and growth would suggest a sensitivity to changes in any of the test parameters. All data has been uploaded to the Research data at Essex online repository.

Table 1. Description of temperature logger deployment sites and data source

Region	Habitat type	Sites	Deployment date	Lat/ Long	Total sites	Logger type	Data source
Essex	Saltmarsh	1	17/12/2020	51.836685, 0.987776	2	Onset HOBO light + temp	-
		2	17/12/2020	51.776639, 1.046167			
	Creek	3	19/12/2020	51.791924, 0.868262	2		-
		4	19/12/2020	51.843264, 0.980455			
	Channel	1	21/12/2020	51.781989, 1.021603	1	Onset HOBO temp	-
	Outer	1	21/12/2020	51.766997, 1.031783	1		-
Bay of Biscay	Channel	1	01/02/2020	48.342609, -4.290666	1	Onset HOBO temp	
Tagus	Channel	1	-	38.763000, -9.087000	1	-	Geoportal

3. RESULTS

3.1. *Temperature dependant growth experiments*

Instantaneous growth rate was not significantly different between the 6°C and 10°C temperature treatments ($\chi^2_{(2,\ 3)}$ = 0.8, P = 0.670). Median growth rates of all temperature treatments were similar, though lower at 6°C than 10°C (Figure 5). Daily growth rates did not differ significantly between weeks at 6°C ($\chi^2_{(3,\ 4)}$ = 5.15, P = 0.161) and 27°C ($\chi^2_{(3,\ 4)}$ = 3.21, P = 0.361), but there was a significant decrease in daily growth rate over weeks at 10°C ($\chi^2_{(3,\ 4)}$ = 9.67, P = 0.022). Figure 6 shows that daily growth rates are generally lower at 6°C than at 10°C between weeks 1 to 3, while at week 4 daily growth rate was higher at 6°C than 10°C.

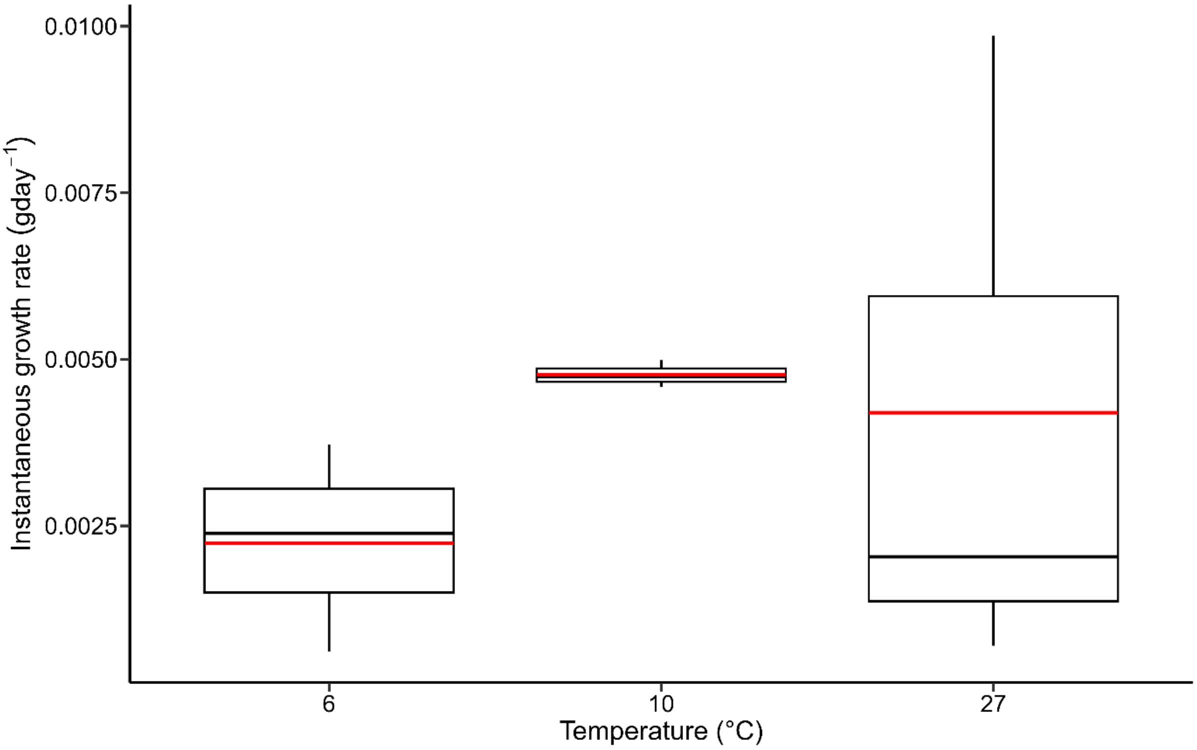

Figure 5. Boxplots for the average instantaneous growth rate (gday^{-1}) of juvenile *D. labrax*, between experimental temperatures 6°C, 10°C, 27°C. Central black line represents median, red lines represent means. Whiskers represent maximum data point within 1.5x the Interquartile range.

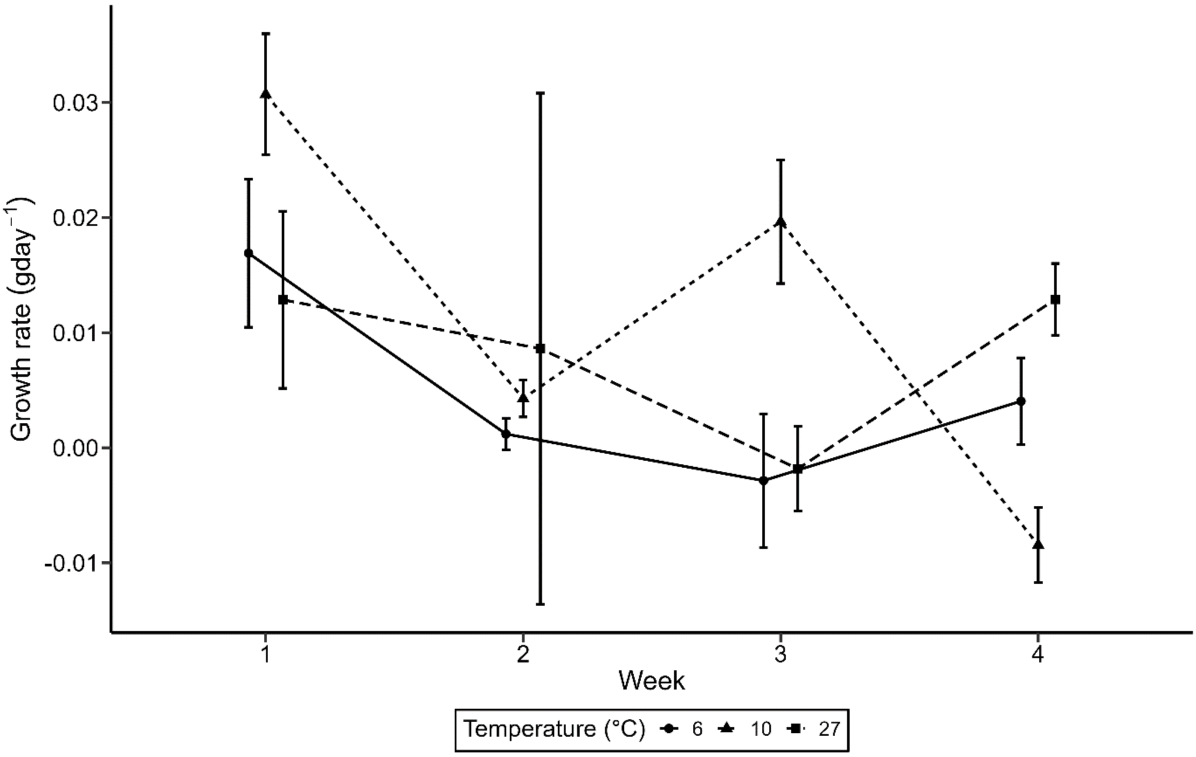

Figure 6. Line plots for the average daily growth rate (gday^{-1}) of juvenile *D. labrax*, between weeks for experimental temperatures 6°C (dot/solid), 10°C (triangle/dotted), 27°C (square/dashed). Error bars represent 1 standard error.

3.2. Model predictions

3.2.1. Essex estuarine habitats

The effects of winter thermal minima on juvenile sea bass biomass throughout a year following settlement are presented for multiple different simulated estuarine habitats in Figure 7. As expected, in all cases over the summer months, with temperatures between 15^{o}C to 23^{o}C biomass increases, as ingested energy exceeds costs due to growth and metabolism. As fish get larger, the rate of biomass increase also increases. However, in all Essex scenarios total loss of biomass is experienced when temperatures fall below 4^{o}C, indicating complete cohort mortality. In our model, the earliest complete biomass loss occurs in saltmarsh and adjacent intertidal/subtidal habitat at 212 days, when the temperature falls to 2.5^{o}C, after a 125% increase in biomass. In deeper subtidal creeks total mortality occurs at 217 days, when temperatures reach 3.6^{o}C, after an 127% increase in biomass.

Complete cohort loss in deeper main estuary channels of Essex estuaries and outer estuary habitat does occur, but later than in saltmarshes and creeks following an initial loss of biomass and recovery in both habitats. In main channel habitat, after a 139% increase in biomass there is an initial 38% loss of biomass at 233 days when temperatures fluctuate between 3.5-4.7^{o}C. Following this loss, a 3% recovery is made, resulting in a net 52% biomass increase before complete loss at 257 days when temperatures reach 1.86^{o}C corresponding to what is observed in February in these systems. For outer estuary habitat the initial loss of biomass is less severe. Biomass increases by 133% percentage until day 226, when temperatures reach 4.2^{o}C, following a drop to 3.7^{o}C, resulting in a 3.1% reduction in biomass. Following this loss, a recovery of 3.5% biomass is made, resulting in an overall 134% increase in biomass, before complete cohort loss at 256 days, when temperatures fall to 2.1^{o}C. Smoothing the temperature data with a seven-day moving average had no effect on the final output biomass in all Essex scenarios (Figure 8). In all cases complete cohort loss occurred at similar timings to those of the daily average temperatures.

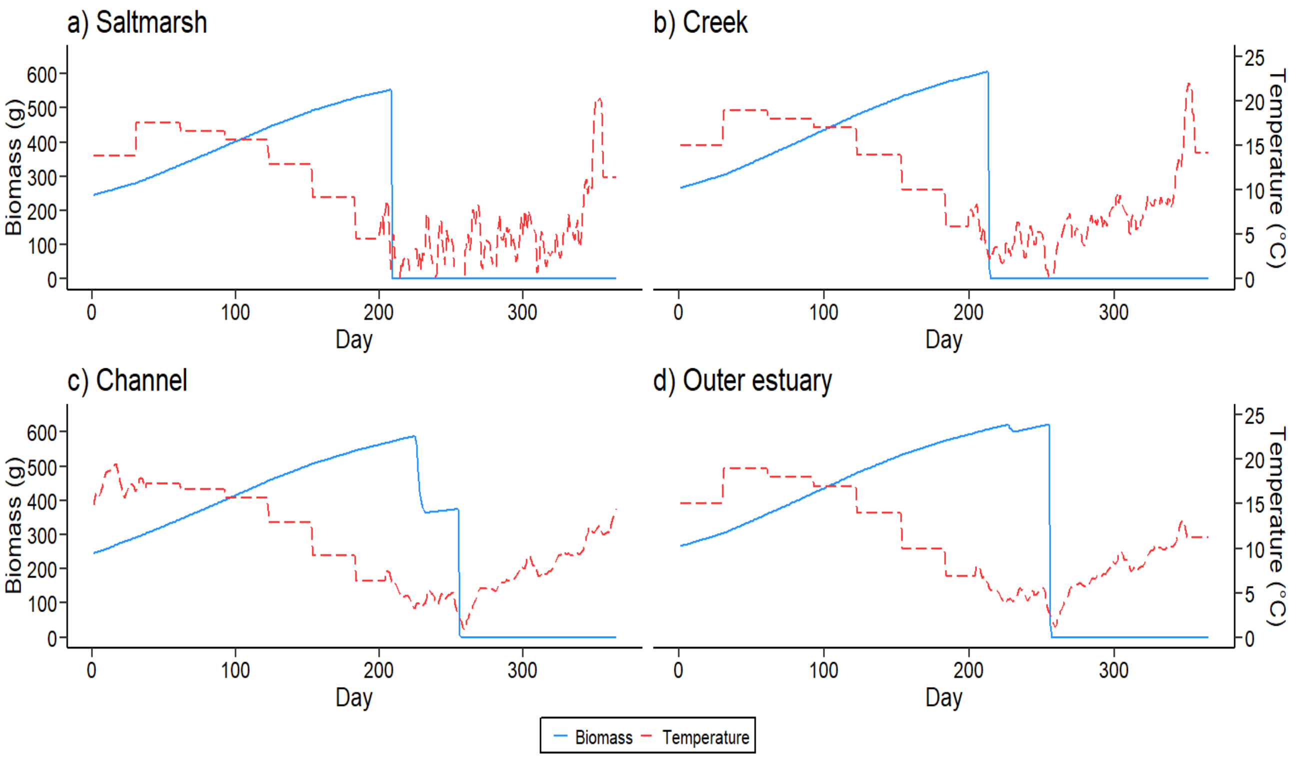

Figure 7. Effects of temperature (ºC - red/dashed) on the biomass (g - blue/solid) of juvenile *D. labrax* over the course of 1 year, following settlement in June (day 0), ending in May (day 365), in different simulated estuarine habitats of the Colne Estuary, Essex, UK. Effects are determined for a cohort of 1000 fish of random length between 20-30mm for a) saltmarshes, b) creeks, c) channels, and d) outer estuary habitat.

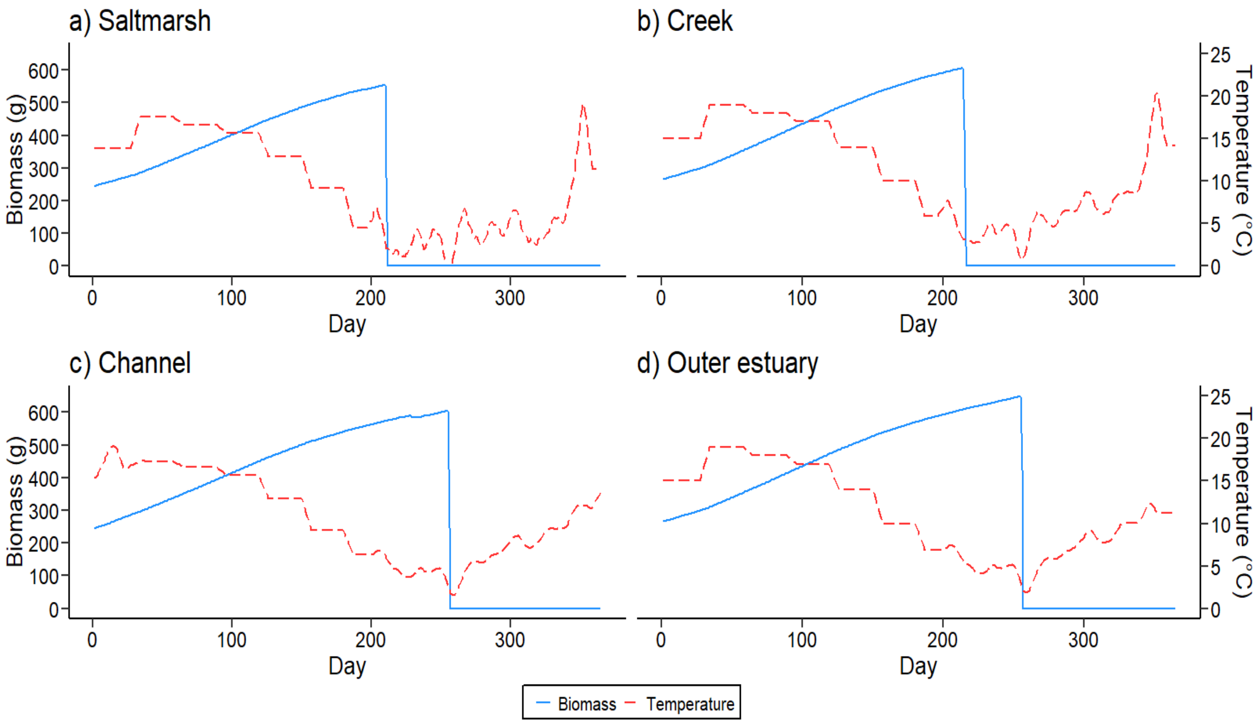

Figure 8. Effects of smoothed temperature as a 7-day moving average (ºC - red/dashed) on the biomass (g - blue/solid) of juvenile *D. labrax* over the course of 1 year, following settlement in June (day 0), ending in May (day 365), in different estuarine habitats of the Colne Estuary, Essex, UK. Effects are determined for a cohort of 1000 fish of random length between 20-30mm for a) saltmarshes, b) creeks, c) channels, and d) outer estuary habitat.

3.2.2. Core range

The effects of winter thermal minima on juvenile sea bass biomass after a year following settlement are presented for channel habitat in two key areas of the core range of sea bass, the L'Elorn estuary in France, and the Tagus estuary in Portugal, Figure 9. Biomass generally increases during the summer months, with the rate increasing both with increased temperature and individual fish mass. Generally, temperatures were greater in Portugal than in France over the summer period, which seemed to influence the increased growth rate. The final overall increase in biomass was 149% in France, and 153% in Portugal, such that biomass increase was 3.6% higher in the Tagus channel compared to the L'Elorn channel, and 34.3% more than in the Essex channel.

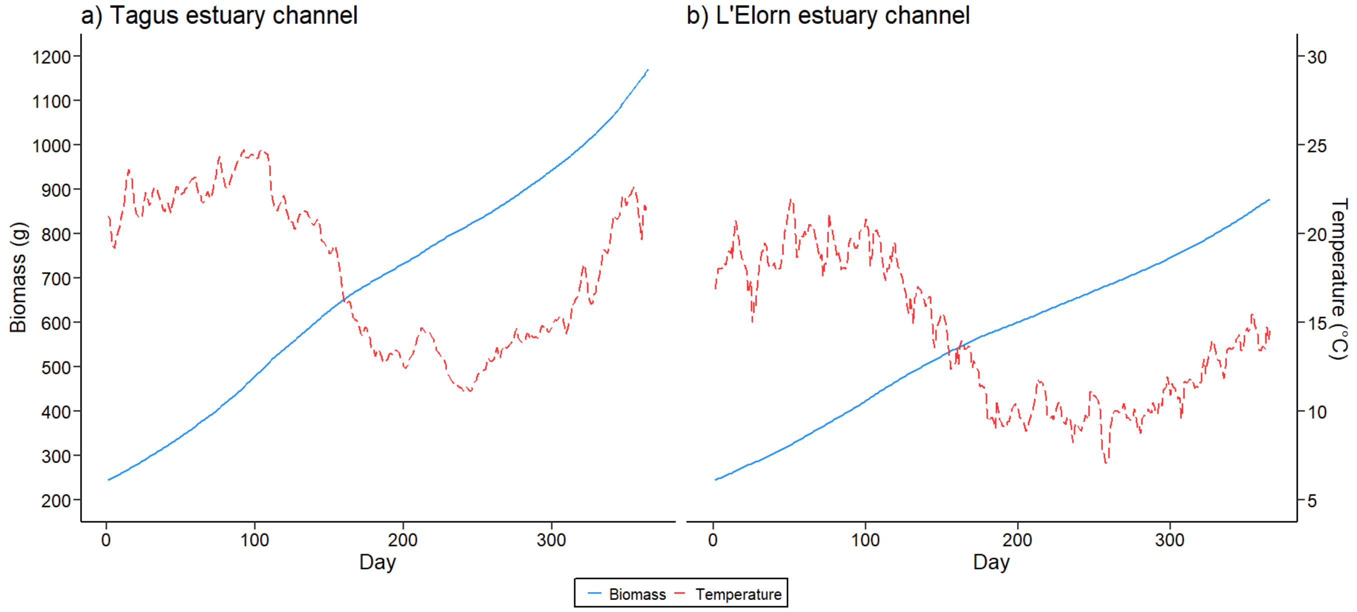

Figure 9. Effects of temperature (ºC - red/dashed) on the biomass (g - blue/solid) of juvenile *D. labrax* over the course of 1 year, following settlement in June (day 0), ending in May (day 365), in different estuarine habitats of the Colne Estuary, Essex, UK. Effects are determined for a cohort of 100 fish of random length between 20-30mm for a) saltmarsh, b) creek, c) channel, and d) outer estuary habitat.

3.3. Sensitivity analysis

Increasing and decreasing the parameters; temperature, starting length, thermal stress cost, and feeding rate by 10%, compared to baselines of sea bass in outer estuary habitat, had no effect on the overall biomass after 365 days, Figure 10. When temperatures are increased by 10%, compared to baseline temperatures in outer estuary habitat, the point of complete cohort loss does not change, despite a 12.8% increase in total biomass, while no initial loss is experienced. However, a 10% decrease in temperature results in a 7.5% reduction in overall biomass, and the magnitude of the initial loss of biomass increases to 84%, compared to 18%. Both mortality events occur at the same time points as with baseline temperatures. A 10% increase in starting length, and therefore mass, results in a larger increase in biomass of 26.5%, and similarly a 10% reduction yields a 26.5% reduction in biomass. When the thermal stress cost is increased by 10% the magnitude of the initial loss in biomass increases in magnitude from 3% to 72%, while a reduction in the cost negates any initial loss. A 10% increase and decrease in the feeding rate have little effect on the biomass increase and point of mortality.

The date of extinction differs very little between increases or decreases in parameters. Increasing the thermal stress cost and decreasing temperature appears to affect magnitude of initial cohort loss the most. Decreasing temperatures results in an 8.1% increase in initial biomass loss compared to increasing the thermal stress cost. When comparing the maximum biomass attained prior to total extinction, length appears to have the largest affect, with a 10.8% increase compared to changes in temperature, 25.9% compared to feeding rate, and 27.2% compared to thermal stress cost. Therefore, the effect of increasing and decreasing the starting length appears to have the largest overall impact on the increase in biomass before the point of total cohort loss, and therefore the amount of biomass loss.

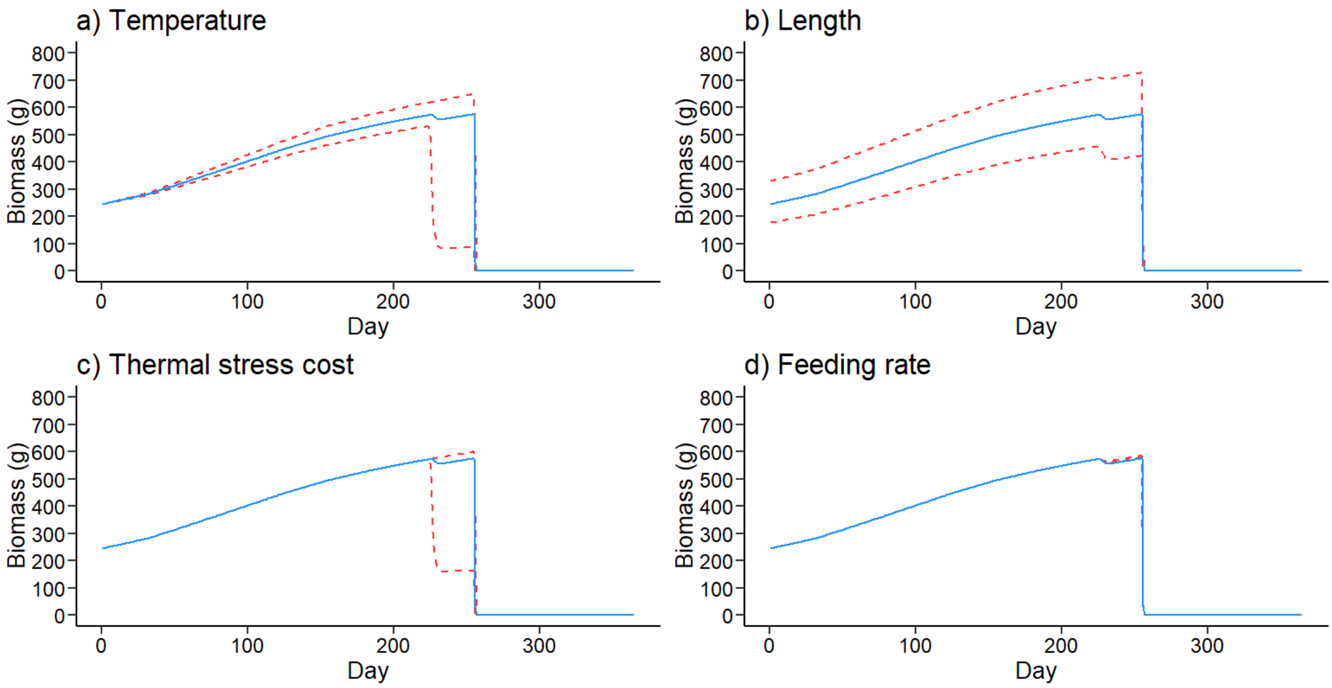

Figure 10. Effects of 10% increases and decreases in a) temperature, b) sea bass starting length, c) thermal stress cost, d) feeding rate on the biomass (g - red/dotted lines) of juvenile *D. labrax* over the course of 1 year, following settlement in June (day 0), ending in May (day 365), compared to a baseline biomass output (g - blue/solid line) for outer estuary habitat of the Colne Estuary, Essex, UK. Effects are determined for a cohort of 1000 fish of random length between 20-30mm.

4. DISCUSSION

Growth and survival in the early life stages of fish are thought to drive recruitment patterns to adult stocks. During the summer months estuarine nursery systems are thought to provide habitat that best promotes survival, and this likely drives a bias in sampling that is potentially influencing our perception of these habitats as ‘saviour’ habitats. However, during the winter months temperatures can fall below the lethal limits of commercially important species and lead to large scale winterkill events, which are one potential driver of recruitment limitation, especially for YoY fish. Therefore, bias towards summer habitat use, and a focus towards management of summer nurseries, is potentially dangerous and may not be adequately tackling sources of bottlenecks for species such as sea bass. Sea bass in the North Sea are thought to be undergoing a Northern range expansion along the East Coast of the UK, driven by rising sea temperatures (Quero, 1998; O’Brien *et al*., 2000; Stebbing *et al*., 2002; Brander *et al*., 2003; Claireaux *et al*., 2006). Despite the importance of temperature for juvenile fish, the effect of winter thermal minima on the physiology, behaviour and survival of juvenile sea bass has never been investigated. Through energy budget modelling, this study predicts that while summer nursery habitat conditions in the southern North Sea may support growth and survival of juvenile sea bass (e.g. green e.g. underwood, winter temperatures fall to below the lethal limit in both shallow and deep estuarine habitats of the Essex coastline, which results in complete cohort die-off. Consistent cohort loss in all scenarios suggests that despite an apparent Northern expansion along the East Coast, winter thermal minima may still present a bottleneck to survival and recruitment of YoY sea bass on the East coast of the UK. The results presented in our study were achieved through adapting existing models of sea bass energy budgets for use with juvenile fish, and accounting for temperature related starvation and thermal stress on maintenance costs.

4.1. Model Predictions

In the model, complete cohort loss was experienced among all Essex estuarine habitats measured, however, the point of complete collapse occurred at different times in different habitats. In shallow intertidal saltmarshes and associated tidal creeks, complete cohort loss was experienced when temperatures breached the lower lethal limit of 2^{o}C to 3^{o}C, which occurred at 210-220 days post settlement in June. Complete mortality occurred at 230-260 days for subtidal channels and the outer estuary habitat. Similar results were found when compared to a 7-day moving average temperature, suggesting that lethal temperatures are experienced for prolonged durations, sufficient to deplete what little fat reserves juvenile fish may accrue over summer. The complete loss of cohorts at 4^{o}C suggests a threshold effect, however, while there may be a threshold response in nature, this is not known. We have assumed in our model that there is not an explicit threshold at 4^{o}C where mortality occurs, but that as temperatures approach 4^{o}C bass struggle to maintain bodily functions. Therefore, we have placed the cost of maintenance as a function of temperature, such that as temperature approaches 4oC they are more likely to die. Therefore, the threshold becomes an emergent property of the model, rather than a predefined one. As expected from the model's construction, mortality was not size dependant. Future work needs to look to understand whether the model can capture any size dependency, however, mid-winter East Coast estuaries experience temperatures below the 4oC lethal temperature limit, which is relatively typical for the region. This is likely to result in mortality regardless of body size. Temperature dependant winter mortality is consistent with observations of other species seen both in the field and through modelling, such as red drum (*Sciaenops ocellatus*), which shows complete mortality of all size groups below 5^{o}C as a result of thermal shock (Anderson & Scharf, 2013). The effects of thermal shock are seen to override the effects of reserve depletion, as a result of reduced ingestion, for species at the limits of their ranges (McCollum *et al*., 2003; Anderson & Scharf, 2013). For sea bass on the Essex coastline, at the range limit of the Channel population on the East coast, temperature induced mortality could well be the case. However, our model does not consider varying prey availability or quality. MacDonald *et al*. (2018) found that thermal stress was less of an influence on overwinter mortality than copepod density and size in sandeels in the North Sea. There has been no such work to quantify differences in summer and winter prey availability and diet for juvenile sea bass. It is likely that populations of invertebrate prey such as decapods and bivalves that sea bass feed on in summer decrease in winter, however, this assumes no seasonal dietary shift occurs.

Juvenile sea bass are thought to remain within shallow estuarine systems throughout the first 4-5 years of life, moving to the estuary mouth as adolescents, though recent work by Stamp *et al*., (2021) suggests these larger sea bass frequently move between estuarine systems, but still exhibit high fidelity to their estuary of origin. However, we measured the temperatures of these deeper habitats in and around estuary mouths, which are well mixed systems, and found temperatures generally reached similar minimums. Our results suggest that shallow estuarine habitats along the Essex Coastline, that provided summer nursery roles for YoY sea bass become thermal traps in winter – they are largely sink habitats. Historical work has shown that in years where temperatures fall below 6°C, near 100% cohort loss can occur for juvenile sea bass in the Celtic Sea (Kelley, 1988). On the East coast of the UK, in the Medway Estuary, Pawson and Eaton (2005) showed that in creek channels over winter YoY sea bass were present in significantly reduced numbers. My own work concurs with that of Green *et al*., (2009) that found YoY sea bass left shallow creek and saltmarsh habitats in October and November, while group 1 sea bass were present in low numbers throughout much of the year. Mismatch between thermal tolerance and temperature is typically observed in populations of fish that exist at the limits of their species ranges, where environment-recruitment interactions are thought to be strongest (Myers, 1998). Most notably so in tropical and subtropical species settling in temperate waters, which often occurs when the pelagic larvae are transported in oceanic currents, as seen in Australia via the East Australian current (McBride and Able, 1998). Larvae are transported to temperate areas in the summer, where temperatures can support their growth and survival. However, at the onset of winter newly settled YoY will perish due to temperatures well below their physiological limits, seen in many tropical fish species in Australia (McBride and Able, 1998; Booth *et al*., 2007 Figueira *et al*., 2009; Wuenschel *et al*., 2012), most notably in grey snapper (*Lutjanus griseus*). The adult and juvenile stages of grey snapper are non-migratory, and show high site fidelity, therefore distributions are underpinned by larval transport. Wuenschel *et al. (*2012) found that the distribution of adults closely aligned with regions within the thermal tolerance of the larval stages, suggesting that temperature is a limiting factor to range expansion. Similarly, it is believed that sea bass eggs drift with tidal currents from Southern spawning grounds in the warmer English Channel into estuarine systems of the Essex coastline (Jennings & Pawson, 1992; Pickett & Pawson, 1994; Beraud *et al*., 2017), where larvae metamorphose and settle during the spring and summer. It is possible that a mismatch is occurring between the thermal tolerance of sea bass juveniles and the winter temperatures experienced along the East Coast of the UK. It is worth noting that it is currently thought that two cold winters can dramatically reduce and delay the supply of YoY sea bass to nurseries in the summer. Delayed and reduced recruitment, compounded with lethal temperatures on nursery areas could have seriously detrimental effects on the survival and recruitment of YoY cohorts. That being said, larger sub-adult and adult bass are regularly found along the Essex, Suffolk and Norfolk coasts, beyond where our model predicts YoY cohort failure. Whether these are recruits from nursery habitats much further to the south or from pulsed intermittent recruitment from more local estuaries experiencing milder winters is currently unknown. Based on the criterion suggested by Preston *et al*. (2008) for a range expansion to occur, this could represent a false range expansion, where recruitment has occurred at some point under favourable conditions and supplied adult fish to the region, that are better able to tolerate low temperatures and evade them, however complete expansion has not yet occurred due to increased recruitment variation on a longer timescale.

Juvenile sea bass would be expected to act to mediate the threat of adverse temperatures, which are presented as three behavioural changes they could exhibit. Firstly, sea bass may reduce activity and attempt to overwinter with what little fat reserves they have developed, which is likely to result in high mortality, as seen in our model and Kelley (1988). There is the potential for mesohaline environments, with salinities close to that of blood levels, to alleviate the osmotic stress induced by the effect of temperature on ion channel function (Hurst & Connover, 2002; Hurst, 2007), and so prolong survivability at CTmin. Striped sea bass (*Morone saxatilis*) exhibit highest survivability at low temperatures when exposed to salinities of 25ppt (Hurst & Connover, 2002), and might explain the salinity-driven distributions of black sea bass (*Centropristis striata*) during winter (Miller *et al.*, 2016). Sea bass metabolic cost has been found to be lowest when exposed to salinities of 5ppt at low temperatures, however, survival has not been directly measured (Claireaux & Lagardère, 1999). Given the reduced metabolic costs at low salinities we might expect to find sea bass further up the estuary closer to freshwater. However, observations from Pawson and Eaton (2005) and our own surveys in the upper Colne estuary have shown that YoY Sea bass are all but absent from upper estuaries in winter, so this is unlikely. An alternative strategy would be to migrate into deeper water at the onset of adverse temperatures. Deeper water is thought to have a higher heat holding capacity and so maintain relatively stable and warmer conditions than high energy coastal habitats during winter. Offshore winter migrations are exhibited in many juvenile fish (Able & Fahay, 1998; Hales & Able, 2001; Cote *et al*., 2004), such as Black sea bass off New Jersey (Hales & Able, 2001). Juveniles enter estuarine systems in summer, then become absent in winter. Young-of-year are thought to migrate into shallow continental shelf waters of the mid Atlantic, while older juveniles and adults occupy the mid shelf region (Hales & Able, 2001). Migration to the continental shelf is seen as essential, based on 100% mortality rates when exposed to winter thermal minima of the region in laboratory experiments (Hales & Able, 2001). Similar behaviour is exhibited in Atlantic cod juveniles, who migrate offshore at the onset of the breakdown of the thermocline (Cote *et al*., 2004). The decision of whether to migrate is thought to be determined by the development of sufficient fat reserves to allow migration, as fish that migrated were generally found to be in better condition. Those without may not migrate and default to option one, which will ultimately result in death. Indeed, YoY Atlantic cod, who may not possess adequate fat reserves, remain in shallow coastal habitats. With this in mind, migration itself can be considered a potentially risky strategy.

Fish undertaking winter migrations may encounter a greater predation risk (Hurst, 2007). Firstly, deeper water often supports larger numbers of piscivorous fish (Able & Fahay, 1998; Paterson and Whitfield, 2000), including larger juvenile and adult sea bass (Pawson *et al*., 1987; Pickett and Pawson, 1994; Quayle *et al*., 2009), which are thought to be cannibalistic (Henderson & Corps, 1997). Higher predator abundance could increase the incidence rate between piscivorous schooling and adult sea bass, and YoY sea bass. Furthermore, at low temperatures the activity and swimming ability of fish is known to decrease, which is underpinned by decreases in metabolic activity with temperature (Koumoundouros *et al*., 2002; Claireaux *et al*., 2006). For juvenile sea bass at 7^{o}C the maximum swimming speed is approximately halved compared to the maximum swimming speed at 30^{o}C (Claireaux *et al*., 2006). Reduced swimming speed and activity is thought to reduce the ability of a fish to escape pursuit predation, from that such as adult sea bass (Temple & Johnston, 1998; Ojanguren & Brana, 2000). An increased predator incidence rate coupled with a reduced escape ability could result in a considerably higher predation risk to YoY sea bass migrating into deeper water than over summer. The effect of reduced swimming ability is potentially compounded further when considering the presence of predatory species of different thermal guilds who may be more well adapted to colder temperatures, such as cod, haddock and pollock, the latter of which is known to predate juvenile sea bass (Pickett and Pawson, 1994). For the YoY of cold-freshwater species such as largemouth bass (*Micropterus salmoides*), bluegills (*Lepomis macrochirus*), and walleye (*Sander vitreus*), predation is a major driver of mortality during the first winter (Garvey *et al*., 1998, 2004; Pratt & Fox, 2002; Santucci & Whal, 2003). For example, in YoY largemouth sea bass, who continue to feed throughout winter to maintain reserves, survival rates are between 10-97% when exposed to predators compared to 77-93% in ponds without them (Garvey *et al*., 2004). The act of migration itself incurs an energetic cost from swimming activity. Reduced swimming speed as a result of low temperatures could result in an increased time for migration and exposure to potential threats. However, while swim speed decreases with temperature, the cost of transport does not (Claireaux *et al.*, 2006) which could mean that during a period of reduced foraging and ingestion rates, when fish should reduce activity, the energetic cost of migrating into deeper water could impact the reserves available to overwinter. Migration into deeper water may provide an escape from thermal shock but may also result in increased predation levels and energetic costs, potentially accelerating cohort mortality in sea bass.

Southward migration into warmer waters is a subset of the movement strategy to avoid winterkills, which is employed by Spotted Trout juvenile who settle at the Northern range margin of the species (Vanderkoog and Muller, 2003). Were this the case, it is uncertain whether sea bass migrating south would then return to natal habitats the following summer. It would seem unlikely juvenile YoY 70mm sea bass are leaving Essex for the most southern English or northern French coast over winter.

An alternative strategy, that is often overlooked is the use of warm water effluent streams from power stations associated with shallow coastal habitats. To my knowledge, only one such study exists pertaining to juvenile sea bass, on the effect of coastal power stations on winter survival, by Pawson and Eaton (2005), which only assessed a single station associated with the Medway estuary, which forms part of the greater Thames estuary basin. Pawson and Eaton (2005) found that, while abundance of YoY sea bass was very low in the Medway estuary channels during winter, they were in high abundance in a channel which received thermal effluent from the associated power station. Furthermore, the sea bass caught near the Medway power station were of larger body size, beyond the postulated threshold size for overwinter survival of 6cm (Kelley, 1988; Pawson & Eaton, 2005). Young-of-year sea bass were otherwise absent from the estuary. Pawson and Eaton (2005) suggested that the Medway power station outflow provided refuge for juvenile sea bass from thermal shock resulting from lethal winter thermal minima, and so YoY sea bass would aggregate within this area to maximise growth and survival. The authors argue that, in the absence of thermal effluent runoff the estuary would be too cold to support sea bass survival and that seaward migrations should occur or risk large scale mortality. Power station thermal effluent such as that of Pawson and Eaton (2005) could provide bastions of thermal refuge for YoY sea bass in an otherwise inhospitable environment. Prior to 2002, a nuclear power station was active within the Blackwater estuary where this study was focussed. The nuclear station first began generating electricity in 1962 and was decommissioned in 2002, no longer producing warm water outflow into the estuary. This may have once provided shallow water thermal refuge for overwintering bass, akin to the Medway estuary, unfortunately however, no empirical evidence exists within this estuary. It would be interesting to investigate the effect of other sources of thermal effluent within the estuary, such as non-nuclear power stations that are still active, on the distribution of YoY sea bass within the Blackwater over winter.

4.2. Model limitations and future work

The predictions of our model are only as robust as the underlying functions that determine them. Generally, the functions used to model metabolism and growth with body length and temperature are robust, as these relationships are highly conserved between species, and based on models applied to many fish species including salmonids (Jones *et al*., 2002), sand eels (MacDonald *et al*., 2013), and indeed sea bass (Watson *et al*., 2020). Sensitivity analysis revealed that model outcomes were generally robust to small increases and decreases in model parameters; temperature, starting length, rate of change in feeding rate, and rate of change in thermal stress cost. In all scenarios total cohort loss was exhibited in a typical Essex winter, however, the magnitude of initial cohort loss was most sensitive to changes in thermal stress cost and temperature, and biomass increase was most sensitive to settlement length.

Increases in settlement length resulted in a greater increase in biomass at the point of total mortality, while decreases resulted in lower biomass. Complete cohort loss in all scenarios indicates that the model is reasonably robust to small fluctuations in yearly temperatures, cohort settlement length, and individual variations in thermal stress cost and feeding rates. However, large increases in temperature, such as those seen in the L'Elorn and Tagus estuary result in no die off as a result of starvation or thermal stress, further highlighting that winter temperatures appear to present a significant bottleneck to sea bass survival. As sea temperatures increase in the North Atlantic (Quero, 1998; O'Brien *et al*., 2000; Stebbing *et al*., 2002; Claireaux *et al*., 2006), under current warming scenarios it is possible that in future years YoY sea bass could survive their first winter without the need for offshore migrations. Multiple years of high temperatures could allow juvenile nurseries to become more established between years, which is observed in many tropical species that travel into warming temperate waters that are able to survive their first winters and become established (Stebbing *et al*., 2002).

While generally robust to change, previous work by Green *et al*., 2009 and my own work in chapter 2 shows that small numbers of group 1 and 2 fish are present in the estuary over summer, implying that some fish do survive their first winter despite large amounts of mortality. This directly contradicts the predictions of this model. This is likely because our model constrains habitat use to relatively well-mixed habitats within the estuarine system, and does not account for offshore migrations into deeper, potentially warmer habitats. This is because, to date, no empirical data on sea bass winter habitat usage and survival exists across the range of *D. labrax*, with previous work only indicating evidence of absence in shallow estuarine systems over winter. While bass are likely to remain in deeper estuarine systems occupied in the summer, such as those of the Southwest coast of the UK (Kelley, 1986) they are unlikely to do so in shallower systems such as those of Northern Essex and the West Coast of the UK (Kelley *et al*., 1986, Green *et al*., 2009). In recent years, too great a focus on summer habitats has become an emerging complaint, not just for the habitat usage of juvenile fish (Litvin *et al*., 2019; Brady *et al*., 2020), but across disciplines including saltmarsh and lotic habitat productivity (Crotty *et al*., 2018; Lõhmus *et al*., 2019; Lynum *et al*., 2020). The focus on summer habitat is leading to claims that investment in habitat restoration of perceived biological hotspots will deliver improved recruitment of fish species such as sea bass (Geist & Hawkins, 2016; Whitfield, 2017; Baker *et al*., 2020; Billah *et al*., 2021). However, this is a potentially dangerous mindset, as saltmarshes are primarily summer habitats and are not utilised over winter (Kelley *et al*., 1986; Pawson & Eaton, 2005; Green *et al*., 2009). Winter temperatures may pose a greater bottleneck to YoY survival and recruitment than summer habitat availability, at least for fish settling to range margins, such that critical winter habitat may be of greater value to stabilising recruitment variation. Therefore, we suggest that winter habitat usage of juvenile sea bass should be a primary target of future research and hypothesise that YoY sea bass should migrate into deeper offshore waters at the onset of winter. To an extent the absence of wintering sea bass in high shore nursery habitats, where they are found in great numbers over summer, supports this theory (Green *et al*., 2009; Chapter 2). An alternative hypothesis would be that sea bass of the Essex coastline remain in the estuary until temperatures become lethal, and YoY experience large scale winterkill events in most years. Yearly winterkill events may explain the significant reduction in abundance of group 1+ fish compared to group 0 that return to estuaries the following summer (Chapter 2).

Furthermore, thermal tolerances of fish, including sea bass, are significantly affected by the acclimation temperature the fish was reared at (Dulger *et al*., 2012), and this was not accounted for in our model, which is primarily derived from data obtained in Mediterranean and Celtic sea individuals. Most work on sea bass physiological responses to temperature have been derived from farmed or wild Mediterranean populations (Claireaux & Lagardere, 1996; Ruyet *et al*., 2004; Claireaux *et al*., 2006; Dulger *et al*., 2011; Lika *et al*,. 2014; Stavrakidis-

Zachou *et al*,. 2018), which have been found to genetically differ from their Atlantic counterparts (Tine *et al*., 2014). For juvenile sea bass reared at different temperatures, a decrease in acclimation temperature from 25^{o}C to 15^{o}C showed a decrease in CTmin from 6.77^{o}C to 4.10^{o}C. How acclimation influences the energetic performance of sea bass of the Essex coastline, who develop at lower temperatures than Mediterranean and channel counterparts, is unknown. We also don't understand whether plasticity in the thermal tolerance of sea bass is purely developmental or reversible, occurring only within a single generation, or whether it is transgenerational, where the thermal environment of previous generations interacts with that of the current to influence the phenotype (Angilletta, 2009; Munday *et al*., 2013, 2014; Donnelson *et al*., 2022). There is a growing body of evidence, across both terrestrial and marine species, that suggests phenotypic plasticity, in response to increasing temperatures, can act over multiple generations to result in local adaptations in thermal tolerance over a long enough timescale (Massamba-N'Siala *et al*., 2014; Sandblom *et al*., 2016; Gu *et al*., 2019; Pilakouta *et al*., 2021; Potts *et al*., 2021; Bernall *et al*., 2022). Combined with potential for some warmer habitats over winter this could explain why we usually find evidence of some YoY cohort survival when sampling – with some small numbers of group-1 and group 2 fish encountered regularly across many sites and years.

Secondly, thermal stress cost is a fitted variable, where the cost was derived based on energy requirements predicted by the model's growth, metabolism, ingestion, and maximum reserves functions, and so is not based on existing data. To date there has been no effort to quantify the energetic cost of thermal stress on the maintenance of a fish due to osmotic and oxidative stress. Our understanding of the effects of thermal shock on juvenile sea bass is scarce and needs to be investigated further. The complete loss of fish when temperatures approach 4^{o}C appears to present a threshold effect that may not be an energy budget concern. It is certainly true that if mortality predictions are based on mortality purely as a result of temperatures falling to and below 4°C, the this is not likely to be best modelled using energy budgets and is a biological effect. However, in cases where low temperature results in the loss of body condition, in this case in the form of loss of fat reserves to the point of starvation, or where mortality is experienced through the cumulative costs of maintaining body condition within sub-lethal temperature ranges, then this is likely to be an energy budget concern. The temperature cost curve that I developed is able to capture costs below temperatures of 10°C, in the sublethal range for sea bass, however, these costs do not cumulatively lead to mortality until the temperature approaches 4°C. This is likely unrealistic and future work will have to better parameterise the costs of low temperature metabolism within the sublethal range in this and other species, for which there is no published literature.

Our model also only accounts for a single year of temperatures. This does not allow us to fully account for inter-annual variation in temperatures between seasons, and how well our data represents a typical year of temperatures of the Essex coastline. Our measurements may have captured a particularly cold year and so biased our results towards a negative outcome. We attempted to account for this by using a smoothed temperature profile, to reduce the effect of high and low temperature spikes, however, this still resulted in complete cohort loss. A better understanding of inter-annual temperature variations in shallow water habitats of the East Coast is needed to better predict how sea bass recruitment will be affected between years. Climate change has been shown to result in either increases in ocean temperature (Polyaakov *et al*., 2010; Cheung *et al*., 2013; Cheng *et al*., 2019; 2021) or increased variability in the temperature (Niedrist *et al*., 2018), as well as the frequency and timing of extreme weather events (Stott, 2016; Wang *et al*., 2021). This may result in multiple years that may present beneficial temperatures for survival, followed by large mortality events in bad years. How many good years are required for a population to become established is unclear, but year-class strength is thought to only be predictable after the first winter post settlement (Anderson & Scharf, 2013).

Therefore, we suggest that future models should account for multiple cohorts and multiple years of temperature data to predict long term trends in survival and recruitment. We hypothesise that, under current trends in warming sea surface temperatures for the North Sea (Belkin, 2009), temperatures will provide more suitable winter conditions to support juvenile sea bass survival at range margins.

Finally, our model assumes food ingestion is based on temperature and body length alone and does not account for differences in prey availability and quality. Densities of prey are likely to change between summer and winter, with the latter being considerably lower for invertebrate prey that sea bass predate in summer, but this also assumes that dietary needs of sea bass do not shift between seasons, which is seen in many fish transitioning between life stages (Duguid *et al*., 2021; Holt *et al*., 2019; Sanchez-Hernandez *et al*., 2019, 2022; Sewall *et al*., 2019). A better understanding of seasonal dietary shifts in juvenile sea bass is required, as well as seasonal changes in food availability between shallow and coastal habitats. It is likely that YoY fish, who do not possess adequate fat reserves, may need to continue foraging throughout winter, which is seen in juvenile black sea bass, who in laboratory experiments continued feeding until the point of mortality when exposed to lethal temperatures (Miller *et al*., 2016). However, it is worth noting that in our growth experiments that feeding rates, while not measured, were considerably lower in the 6°C treatment tanks, who frequently had large amounts of food left on the tank bottom after 10 minutes. Russel *et al*., (1996) showed that for juvenile sea bass, reductions in ration size significantly reduced the growth rate of bass, particularly at higher temperatures. Similar results have been presented for Asian sea bass (*Lates calcarifer*; Wijayanto *et al*., 2022) and cuneate drum (*Nibea miichthioides*; Wang *et al*., 2007). At temperatures around 6°C, however, Russell *et al*., (1996) found that sea bass feed intake rates were at a minimum, and would not accept excess food, such that weight loss was exhibited, indicating an energetic deficiency. Given that juvenile sea bass appear to continue feeding at low temperatures, we hypothesise that reduced food density over winter will contribute to energetic deficiencies and may result in energetically detrimental foraging activity, that may also expose them to increased predation risks.

In conclusion, due to the hostility of coastal systems in winter on the Essex coast, and the increased risk of mortality involved with offshore migrations for YoY fish, our model provides evidence that winter thermal minima presents a bottleneck to regular survival and recruitment to young-of-year and even group 2 sea bass from estuarine nursery habitats. Inshore habitats become traps that may push juveniles into riskier habitat and reduce survivability, which is likely to significantly reduce the abundance of group 1 fish that return to the estuary the following summer. Future work should focus on identifying areas of critical winter habitat usage of YoY sea bass. Many other unknowns also need to be addressed regarding seasonality in foraging patterns, diet and food density, the influence of acclimation temperatures on the thermal tolerances of stress, and also the cost of thermal stress on maintenance processes within fish. Our model highly suggests that under current climatic conditions, winter temperatures still remain a bottleneck to recruitment of sea bass to the North Sea stock, and likely presents a barrier to further range expansion along the East coastline of the UK.

CHAPTER 6: GENERAL DISCUSSION

WHAT MAKES A HABITAT A HOME?

*The diversity of structures present on a shingle bay.

1. Sea bass and recruitment

Recruitment, the point at which juvenile fish mature and join the adult spawning population, underpins the resilience and sustainability of wild capture fisheries, yet our understanding of the drivers of inter-annual recruitment variation is still weak. Many commercially important fish stocks, the world over, are in decline, largely owed to mismanagement (O'Leary *et al*., 2011; Ye & Gutierrez, 2017; Palomares *et al*., 2020; Dadswell *et al*., 2021; Warren & Steenbergen, 2021; Canovas-Melina & Garcia-Frapolli, 2022). This is highly concerning given that global production of wild capture fisheries in 2020 was estimated at 78.8 million tonnes, valued at USD $141 Billion (FAO, 2022), with many countries depending on them for livelihoods and as a primary source of protein (FAO, 2022). There has been a shift in recent years towards an ecosystem approach to fisheries management, which relies on an understanding of the interactions between species and their environment that drive positive effects on recruitment (Link, 2002; Hall & Mainprize, 2004; Townsend *et al*., 2019; Link *et al*., 2020). Recruitment is predominantly influenced by mortality in the early-life stages (Houde, 1987; Shulman & Ogden 1987; Beyer 1989; Cushing, 1995; Caley *et al*., 1996; Caselle 1999; Armsworth 2002; Stige *et al*., 2013; Pepin, 2015), therefore, a better understanding of the conditions that support growth and development of juvenile fish is required (Martinho *et al*., 2012). Juvenile habitats that support a disproportionately high amount of survival to recruitment compared to other habitats where juveniles are found are termed nurseries (Beck *et al*., 2001). However, this concept has expanded to include the value of the multiple connected habitats that contribute to juvenile survival, in what are termed seascape nurseries (Nagelkerken *et al*., 2016). Coastal estuarine systems are thought to provide nursery habitats for a plethora of commercially important species in the UK, including European sea bass.

Sea bass are a commercially high value species throughout their range, including the UK and Mediterranean (ICES, 2022). However, sea bass stocks have been declining in the UK, as a result of increased fishing pressure and high variation in inter-annual recruitment (ICES, 2015). Therefore, a thorough understanding of what constitutes quality nursery for juvenile sea bass is urgently needed to aid an ecosystem approach to sustainable fisheries management. While the movement to seascape level management of marine systems puts focus on the network of habitats used by juvenile fish, to date research on sea bass nurseries has focused almost solely on saltmarshes and summer habitat (Kelley 1986, 1988b; Jennings & Pawson 1992; Cattrijsse *et al*., 1994; Pickett & Pawson, 1994; Cabral & Costa, 2000; Laffaille *et al*., 2000, 2001; Cattrijsse & Hampel, 2006; Green *et al*., 2009, 2012; Jin *et al*., 2007; Dufour *et al*., 2009). A substantial body of evidence suggests that sea bass in UK estuaries are highly abundant in saltmarshes throughout the summer months, across much of the UK (Kelley *et al*., 1986, 1988b; Pawson & Jennings, 1992; Pickett & Pawson, 1994; Laffaille *et al*., 2000; Green *et al*., 2009, 2012), while in the Mediterranean they appear to be present in shallow coastal lagoons (Cabral & Costa, 2001; Dufour *et al*., 2009). However, the plethora of other habitats across the estuarine mosaic remain largely unexplored.

We lack a thorough understanding of the types of estuarine habitat used by juvenile sea bass, and what function these habitats provide. We also don't understand the mechanism by which sea bass arrive at estuarine sites, with much of our knowledge being speculative at best. Finally, currently no data exists for survival of young-of-year (YoY) sea bass over their first winter on the East coast, which is thought to exist near the Northern range margin of the Channel population. Therefore, we lack a basic understanding of where bottlenecks in sea bass recruitment are likely to occur. To address these knowledge gaps I (**1**) looked at distribution patterns of juvenile sea bass along the estuarine mosaic, (**2**) determined differences in foraging behavior and diet composition between habitats, (**3**) investigated the vertical distribution patterns of larval sea bass across tidal cycles during the settlement period to establish whether tidal behavior exists, (**4**) used energy budget modelling and temperature-dependent growth experiments to predict YoY survival over the first winter and discuss the implications for habitat use.

2. Settlement behaviour

While larval settlement behaviour was addressed in my fourth chapter, logically it follows that the earliest life stages should be discussed first. Current models that predict dispersal and settlement events in larval sea bass are reliant on the assumption that tidal behavior is implemented to allow movement into estuarine nurseries (Beraud *et al.,* 2017). The assumption is reasonable, as tidal behaviours such as selective tidal-stream transport (STST) are employed by the larval stages of many marine fish (Matsumiya *et* al., 1982; Holt *et* al., 1989; Rowe and Epifanio, 1994; Forward *et* al., 1998; Schultz *et* al., 2000; Edwards *et* al., 2008; George *et al*., 2011; Islam *et* al., 2011; Patrick and Strydom, 2014; Deschepper *et al*., 2019). However, to date no evidence in published literature exists that confirms whether sea bass larvae exhibit such behaviours. Understanding the use and timing of tidal behaviours is vital for accurately predicting species distribution patterns, settlement events, and recruitment (North *et al*., 2009; Swearer *et al*., 2019; Leis, 2020). Therefore, in order to determine whether directed, tidal, or passive transport strategies are exhibited by larval sea bass entering the Blackwater and Colne estuary complex, I sampled the vertical distribution of larval sea bass across tidal cycles at the estuary mouth. To do this I used continuous plankton pumps to sample from the surface and above the benthos during what we predicted was the first onset of larval sea bass.

Our results showed that densities of larval sea bass were similar between flood and ebb tides. This contradicts the behaviours exhibited by the larva of many estuarine-dependent species as they enter nursery settlement sites, who show an increased abundance on flood tides, and settle out of the water column on ebb tides (Holt *et al*., 1989; Forward *et al*., 1998; Jager

1999, 2001; Epifanio and Garvine 2001; Vargas *et al*., 2003; Hare *et al*. 2005; Rooper *et al*. 2006; Islam *et al*., 2007). Larval sea bass were also homogenously dispersed throughout the water column on flooding tides, while on ebb tides they were more abundant in the surface layer. This distribution pattern may result in net-zero movement or be the result of tidal advective forces suspending larvae into the water column. These results suggested that larval sea bass, at this developmental stage, do not exhibit flood tide migration behaviour, but may employ a passive transport mechanism by use of flood tide advective forces, seen in Atlantic croaker (Hale & Leggett, 2016), or be attempting to retain to deeper water of the estuary, which is seen in herring larvae (Fortier and Leggett, 1983). I suggest it is likely that our sampling window was able to capture the peak abundance of larval sea bass on the Essex coast, but not peak settlement, and at this developmental stage larval sea bass are not attempting to enter up-estuary settlement sites, due to a possible window of vulnerability to predation from planktivores (Harvey, 1991; Huss *et al*., 2010). These findings highlight that tidal behaviour in juvenile fish is species specific and its employment likely changes with ontogeny and windows of vulnerability. The phenology of settlement behaviour with ontogeny needs to be incorporated into settlement models on a species-specific basis to accurately predict species distribution patterns.

This study provides valuable new insights into the settlement behavior of an enigmatic larval stage, however there are several notable limitations that should be considered. Firstly, our sampling window was limited to a single month of settlement, which I selected based on the assumption that larval sea bass would be arriving at the estuary and able to exhibit tidal behaviours in June. This was derived from larval abundance data from power station surveys in the region, and estimates of development in sea bass swimming ability from Beraud *et al*., (2017). Tidal behaviors develop and change throughout ontogeny (Holt *et al*., 1989; Hare *et al*., 2005; Hale & Targett, 2016; Teodosio *et al*., 2016), therefore, it is highly likely that our hypothesis was false, and that tidal behaviours may be exhibited prior to arrival within the estuary complex, or at a later stage, when larvae are ready to settle to nursery habitats. Secondly, both the possibility of net-zero movement within the estuary and passive tidal transport are predicated on the assumption that there is sufficient tidal-stream stratification with depth to result in faster surface currents compared to bottom currents, however, we did not measure tidal flow velocities. Without this, it is difficult to disentangle to what extent the distribution patterns observed are a reflection of larval depth selection or tidal forcing. Other studies have modelled the net transport patterns of larval fish based on measurements of vertical distribution of larvae and tidal flow velocities, to estimate the overall velocity of the larval fish (Fortier & Leggett, 1983). Through this, Fortier and Leggett (1983) were able to demonstrate that the use of flood surface currents and ebb bottom currents achieved a net-zero movement, based on the unique hydrodynamics of the region. Finally, we did not account for diel and lunar rhythms that may interact with tidal rhythms. Nocturnal tides have been found to be of great importance to tidal migration behaviour, where larval fish will ascend into surface waters or show increased abundance on nocturnal flood tides to promote shoreward transport (Rowe and Epifanio 1994a; Forward *et* al., 1998; Jager 1999; Islam *et al*., 2007; Primo *et al*., 2012). Similarly, some species will utilize spring tides to enter estuarine nursery habitats (Lozano & Houde, 2013; Hale & Targett, 2016). Indeed, larger tides in the present study generally resulted in increased current velocity, to the extent that on the strongest ebb tides some samples were discounted due to shearing of the sampling hose used to sample fish from depth. It is likely that our relatively constrained sampling design is masking the true behaviour exhibited by larval sea bass, and a more rigorous follow up study is necessary.

3. Habitat use and function

Chapters two and three of this thesis both addressed different facets of the overarching question; what constitutes quality nursery habitat for juvenile sea bass? Therefore, chapters two and three shall be discussed together. Studies pertaining to the habitat use and feeding ecology of juvenile sea bass in estuarine nurseries have almost exclusively focused on saltmarshes (Kelley 1986, 1988b; Jennings & Pawson 1992; Cattrijsse *et al*., 1994; Pickett & Pawson, 1994; Laffaille *et al*., 2000, 2001; Cattrijsse & Hampel, 2006; Green *et al*., 2009, 2012; Jin *et al*., 2007). Juvenile sea bass become dominant members of the saltmarsh community during the summer months of June to October, following settlement (Green *et al*., 2009, 2012; Laffaille *et al*., 2000). However, under the nursery role hypothesis, measures of abundance in saltmarshes alone does not confirm a nursery role (Beck *et al*., 2001), which requires a comparison with other habitats, and an understanding of the factors that might drive survival, such as predation dynamics and foraging opportunities (Litvin *et al*., 2019). Furthermore, the emergent concept of seascape nurseries, which is gaining traction in its application to the restoration of marine systems, is not compatible with the study of a single habitat (Nagelkerken *et al*., 2016). Regarding sea bass, very little attention has been paid to the plethora of habitats that compose the estuarine mosaic, such as structurally complex habitats like sub-tidal oyster reefs and shingle edge habitats, but also structurally simple habitats such as sand and mud edge habitats. Further still, we don’t fully understand what ecosystem services these habitats provide to support sea bass, if any at all. Therefore, as part of my second and third chapters, firstly, I investigated how the distribution and condition of juvenile sea bass was influenced across high tide and low tide habitats of the estuarine mosaic of the Colne and Blackwater estuaries. To do this, I used a combination of fyke and seine netting to measure abundance, and condition between saltmarshes, shingle, mud and sand edge habitats, and oyster reefs, over high and low tide. Secondly, to address habitat function, I investigated differences in stomach fullness and diet composition between habitats to gain insight into how juvenile sea bass use different habitats. To do this, I measured the weights of stomach contents relative to body length (stomach fullness index – SFI) alongside dietary DNA metabarcoding.

My results showed that juvenile sea bass abundance and condition were similar across all high tide habitat. This corroborates research by Kelley (1986, 1988b), who found sea bass across marshes and shallow estuarine habitat. This suggests that all shallow habitat is desirable at high tide, and this is likely because shallow water provides predation refuge from larger piscivorous fish (Patterson & Whitfield, 2000; Whitfield, 2017, 2020), though it does not completely exclude them in all cases (Baker & Sheaves, 2021). This is also supported by Cabral and Costa (2001), who found sea bass were more abundant in shallow coastal lagoons compared to deeper water. Interestingly, abundance was near 600% higher on the single sand habitat that exhibited high vegetative complexity from associated seagrass beds, which suggests that vegetative complexity likely also plays a role in habitat selection, likely predation refuge (Baker & Sheaves, 2021), that was unable to be captured sufficiently by our sample design. Stomach fullness, however, was highest on saltmarsh and sand habitats, with a diet primarily composed of ragworms (*Hediste diversicolor*) and shore crabs (*Carcinus maenas*). This agrees with work by Laffaille *et al*., (2001) who showed *H. diversicolor* and *C. maenas* formed predominant dietary constituents, though notably amphipod abundance was considerably lower in my study. This suggests that ragworm and shore crabs form a key dietary component, and that saltmarsh and sand are more productive in prey availability, or that these prey items are more accessible. Therefore, I suggest that, while juvenile bass do not differentially select high tide habitats, saltmarsh and sand habitats may provide a higher foraging advantage and represent the best trade-off between predation refuge (shallow water) and food availability (high stomach fullness).

At low tide, however, juvenile bass were more abundant in shingle and oyster reef compared to sand and mud. Oyster reef and shingle provide structural complexity, while sand and mud do not, therefore, oyster reef and shingle may present more effective predation refuge at low tide (Savino & Stein, 1982; Almany, 2004; Horinouchi, 2007a). Condition and stomach fullness were highest on sand habitats, which suggests that, while lower quality fish predominantly use refuge at low tide, higher quality fish make use of potentially higher foraging potential, but riskier habitat, over sand. However, habitat choice is severely reduced at low tide and limits the scope for habitat selection (Rangeley & Kramer, 1995; Gibson, 2003; Childs *et al*., 2008; Raposa *et al*., 2009; Taylor *et al*., 2013; Gannon *et al*., 2015; Krumme *et al*., 2015), therefore distribution patterns at low tide could simply reflect where sea bass were at high tide, or the effect of hydrodynamic forcing with the ebb tide. The findings of this study suggest that during the summer settlement period multiple shallow estuarine habitats contribute a nursery function for juvenile sea bass, and likely changes with hydrodynamics and body condition, supporting that consideration of the importance of a mosaic of estuarine habitats is needed to correctly manage this species. These findings confirm that the breadth of habitats used by juvenile fish must be considered when investigating critical habitat use or risk the dangers of wrongfully attributing positive bias towards particular habitats in management and fail to protect others of similar value (Beck *et al*., 2001; Brady *et al*., 2020). The abundance and similar condition of juvenile sea bass throughout all shallow habitat persisted throughout the summer period and suggests that summer habitat availability likely does not present a substantial bottleneck for the species on the East coast.

However, the limitations of this study should also be considered when interpreting these results. Firstly, our sampling design was not homogenous between habitats, which may potentially create bias and mask underlying behaviours. For example, at low tide sand habitats were considerably scarcer within the estuary, such that we were only able to sample one. This under-represents the habitat in the subsequent analysis. Similarly, oyster reefs were only accessible at low tide at two sites due to accessibility issues, and so again these are under-represented. Finally, seagrass was only present as an associated habitat at one high tide site and so we could not sufficiently factor this habitat into our sampling design. This follows on to the second limitation, that the considerably increased abundance over the seagrass associated sand habitat likely suggests that our habitat designations were not sufficient to capture the effect of vegetative complexity. Compared to previous studies I designated habitats at a higher spatial resolution, but even so it appears that an increased resolution is necessary. Habitat complexity is thought to be a primary driver of habitat use patterns (Laegdsgaard & Johnson, 2001; Heck *et al.*, 2003; Smith *et al.*, 2008; Cheminée *et al.*, 2016; Sheaves *et al.*, 2016; Leslie *et al.*, 2017; Whitfield *et al.*, 2020; Baker & Sheaves, 2021). Finally, direct measurements of predation and survival on juvenile sea bass between habitats were not possible within the scope of this project. We currently do not possess a license that would allow the assessment of predation through tethering experiments, which are questionable in their applicability to the real world – they remove the ability of fish to escape from predators and potentially bias towards predation success (Aronson *et al.*, 2001).

Regarding dietary work, perhaps the largest limitation is that we did not quantify the species composition and relative abundance of prey species within the habitat as well as sea bass guts. Therefore, our estimates of prey availability are reliant on what the sea bass have eaten. This does not tell us, for example, whether worms or crabs are in higher abundance in different habitats or whether sea bass are actively selecting those prey species (Rezende *et al.*, 2011; Selleslagh & Amara, 2014). While the assumption that the diet of fish is relatively representative of the prey available is reasonable, it is an assumption, nonetheless. Finally, our theories and hypotheses surrounding stomach fullness and foraging behaviour are built on the assumption that stomach contents are indicative of feeding on that habitat at the time of capture.

This is very difficult to actually quantify, given the relative motility of fish, and the digestion rates of prey species (He *et al.*, 1993; Preston *et al.*, 2017), further clouded by the timescales of tidal movements. However, it is a necessary assumption that is applied throughout the field of feeding ecology in fish (Primavera, 1997; Andersen *et al.*, 2005; Grønkjaer *et al.*, 2007; Tse *et al.*, 2008; Saldego *et al.*, 2010; Cardozo *et al.*, 2020). This doesn't necessarily mean this assumption is acceptable, but it means our results are comparable. Overcoming this limitation would require sampling of invertebrate populations between habitats, coupled with telemetry studies of bass habitat fidelity over tidal cycles to estimate the connectivity of foraging habitats, and therefore the likely sources of diets (Ceriani *et al.*, 2012; Rooker *et al.*, 2018; Brownscombe *et al.*, 2022). There is scope for the use of stable isotopes to answer this question, if suitable differences in isotope composition exist between prey guilds at a suitable resolution (Brownscombe *et al.*, 2022). Mesocosm experiments could also help to elucidate diet sources, however, the highly tidal environment of estuaries makes their employment challenging.

4. Winter survival

While the previous chapters contribute to our understanding of what constitutes important summer habitat for sea bass, this only addresses one part of the larger question of the relative habitat value and importance to recruitment variation. Group-0 sea bass are hyperabundant throughout all estuarine habitats during the summer, however, we do not find any over winter (Pawson & Eaton, 2005; Green *et al.*, 2009), and a reduced number of group-1 fish return the following year. It is entirely possible that winter conditions present a greater bottleneck to year-class strength than summer habitat availability, however, very few studies address the matter, generally only finding evidence of absence in shallow estuarine systems (Pawson & Eaton, 2005; Green *et al.*, 2009), with no data for winter survival existing for the East coast. Sea bass of the East Coast are thought to live close to the Northern range margin of the Channel population, and so are potentially sensitive to changes in thermal minimums between years (Myers, 1998; Hurst, 2007). The early-life stages of many populations present at range margins may experience successful growth over summer and are then subject to large scale winter-kills due to lethal temperatures (McBride and Able, 1998; Booth *et al.*, 2007 Figueira *et al.*, 2009; Wuenschel *et al.*, 2012). On the Essex coastline, temperatures of summer habitats routinely fall close to and below the lethal limit of 2ºC to 3ºC for juvenile sea bass (Dulger *et al.*, 2011; Yilmaz *et al.*, 2020) over winter, and yet still no data of winter survival or habitat use exists. Therefore, in chapter four I adapted established energy budget models (EBMs – Sibley *et al.*, 2013) for adult sea bass (Watson *et al.*, 2020), for use with the juvenile stage, incorporating temperature-dependent ingestion, growth, and metabolism, alongside a hypothetical thermal stress cost to predict survival over the first winter for YoY sea bass. To do this I used time-series measurements of winter temperatures across estuarine habitats used by sea bass over summer, alongside temperature-dependant growth experiments to assess how winter temperatures affect growth and condition. The results were then compared with temperatures measured in estuaries of the Bay of Biscay (Elorn) and Portugal (Tagus).

The results of this study showed that 100% cohort mortality was experienced by simulated YoY sea bass across East coast estuarine habitats, primarily associated with chronic thermal stress over starvation. This suggests that, while estuarine habitats of the East Coast are productive habitats that support YoY sea bass over summer, they become thermal traps over winter that present a potentially high mortality risk. Therefore, YoY seabass entering their first winter either migrate out of shallow estuaries of the East Coast into deeper water over winter, which may provide thermal refuge, or YoY sea bass on the East Coast suffer large scale mortality events over their first winter. Winter migrations are seen in several species as a form of thermoregulation (Able & Fahay, 1998; Hales & Able, 2001; Cote *et al.*, 2004), but large winter-kill events are also common for species undergoing range expansions (McBride and

Able, 1998; Booth *et al*., 2007 Figueira *et al*., 2009; Wuenschel *et al*., 2012). The absence of juvenile sea bass in estuarine habitats in other studies (Pawson & Eaton, 2005; Green *et al*., 2009), and the return of reduced group-1 sea bass the following summer seems to support the former hypothesis. However, migration into deeper water for early life stages also presents a high mortality risk, from energy expenditure (Claireaux *et al*., 2006) and increased predation (Able & Fahay, 1998; Paterson and Whitfield, 2000; Hurst, 2007). Therefore, I suggest, that winter temperatures likely present a more urgent bottleneck for YoY sea bass survival, and therefore recruitment to the adult stock, than availability of summer nursery habitat. This study highlights the importance of accounting for the entire annual cycle when considering areas of critical habitat for juvenile fish (Brady *et al*., 2020), and that fish seascapes must be considered in both a spatial and temporal context. Identifying areas of winter habitat use that support survival of juvenile sea bass and quantifying survival over their first winter is perhaps the most urgent area of future research.

However, the exploratory nature of this study entails several limitations to its interpretability, and highlights that more work is needed to fully understand winter dynamics for sea bass. Firstly, our model does not account for temperature acclimation that may occur in sea bass of the East Coast. Sea bass are able to acclimate to a wide range of temperatures (Dulger *et al*., 2012), however, no data exists on thermal tolerance of sea bass of the East Coast, who are genetically distinct from those of the Mediterranean (Tine *et al*., 2014; Souche *et al*., 2015), where the majority of research has been undertaken (Claireaux & Lagardere, 1996; Ruyet *et al*., 2004; Claireaux *et al*., 2006; Dulger *et al*., 2011; Lika *et al*,. 2014; Stavrakidis-Zachou *et al*., 2018; Yilmaz *et al*., 2020). My temperature-dependent growth studies suggested that condition declines at 6^{o}C, but we were unable to investigate what the lower thermal limit for sea bass of the East Coast is, and therefore to what extent this may influence overwinter survival. Furthermore, we don’t know whether phenotypic plasticity occurs during the juvenile stage, as an acclimation response, or whether it is a cross-generational adaptation (Angilletta, 2009; Munday *et al*., 2013, 2014; Donnelson *et al*., 2022). This is another important area of future research. Secondly, we modelled the cost of thermal stress as a hypothetical ‘fitted’ variable that increases the energetic cost to metabolise at a given temperature outside the optimum range. Currently, our understanding of thermal stress in fish is that it negatively affects the functioning of osmotic gradients and ion channels, as well as causing oxidative stress (Hurst, 2007; Donaldson *et al*., 2008; Reid *et al*., 2022). However, there is to my knowledge no work that quantifies the cumulative energetic cost of stress as a result of acute or chronic thermal shock. The general consensus is that fish undergo a period of dormancy over-winter, where energetic costs are reduced (Hurst, 2007). It is largely unknown what an energetic thermal stress cost looks like in a juvenile fish or how to properly model it, and so this model is relatively hypothetical in nature. This is an urgent goal for future research to better model winter survival. Finally, the model is not spatial in the classic sense and does not account for the potential movement out of estuaries. This is primarily because there is currently no published data on the distribution patterns of YoY sea bass on the East Coast during winter. This seriously limits the ability of the model to accurately predict sea bass survival as a result of estuarine temperatures, because it cannot account for sea bass acting to escape lethal temperatures. This again is an area that needs to be addressed urgently.

5. Future work

This body of work presents brand new insights into the early-life ecology of a commercially and culturally important species, of which very little is understood. Chapter two and three has helped shed light on the fine-scale habitat usage patterns and the potential function they provide. Chapter four has shown that tidal behaviour in sea bass is a complex process that needs a better appreciation of ontogenetic processes. Finally, chapter five has shown that, while summer habitat usage is an important aspect of the life-cycle, winter conditions are likely to present a more urgent bottleneck to the species on the Northern range margin. However, as is often the case with research, answering the questions we initially set out to answer has brought up yet more questions. There is a considerable amount of scope with which to take this work forward, partly to address some of the limitations identified throughout, but more so to expand upon the greater question of what is influencing recruitment variation. For the remainder of this discussion, I shall outline directions in which I believe this work could be taken forward, in an ideal world scenario, not considering budgetary or personnel restraints.

Firstly, while habitat use is relatively consistent across high tide habitats, vegetative complexity seems to be an important habitat component that our study did not sufficiently address. Saltmarshes and sand seemed to support more foraging, while oyster reefs and shingle might provide predation refuge, but how representative of recent foraging is stomach fullness alone, and how do survival and predation rates actually differ between habitats? To address these questions, habitats must be classified at a higher resolution. Therefore, alongside continued monitoring of juvenile sea bass abundance, using classical seine and fyke netting, measures of both vegetative complexity (i.e., emergent stem density – saltmarsh creeks, shoot density – seagrass and macrophytes) and benthic complexity (i.e., rugosity), must be assessed and for a greater number of oyster reef and seagrass associated habitats. The connectivity of habitats should also be addressed in greater detail, with measurements of distance between all high tide and low tide sites. This can be used to construct distance matrices, which can be used to assess whether the proximity of different habitat types influences the use of them – i.e., whether low tide oyster reefs are more well used if they are closer to saltmarshes or high tide mud edge habitats. This can be further complimented alongside mark recapture or acoustic telemetry studies on YoY sea bass. A better understanding of the habitat fidelity over tidal cycles would greatly improve our understanding of how these habitats are used. Are the youngest juveniles limited in their ability to move once they have entered a habitat at high tide, or can they move freely between them? Finally, mesocosm experiments may help to better elucidate predation and foraging differences between habitats. Experiments of this design should be applied to multiple regions of the East coast and for multiple species that sea bass may contend with, to better understand the effects of habitat availability and competition with resident species as sea bass continue to undergo Northern range expansions in the UK.

Secondly, and perhaps most pressing, our models predict 100% cohort mortality, but cannot account for movement out of estuaries, thermal acclimation, or thermal stress costs when we don't know what these look like in juvenile sea bass of the East Coast. To address these urgent knowledge gaps acoustic telemetry could be utilised to understand the seasonal changes in habitat use with ontogeny, whether they leave the estuary over winter, and if not, where in the estuary are they using? However, acoustic telemetry would not address where they go if they do leave the estuary, which would require extensive sampling using trawls or potentially baited video cameras in deeper water. If diets change significantly over the winter period this may be traceable in the stable isotope content of otoliths and muscle, but again we don't know what the diet of juvenile sea bass is over winter. To better understand acclimation and thermal stress in sea bass of the east coast, alongside deep-water trawls, *in-situ* cold water shock experiments could be employed, alongside lab-based experiments that assess the CTmin of juvenile sea bass along the East Coast. While these experiments would allow us to understand whether temperature sensitivity influences overwinter survival on the East coast, it would not inform us of whether this is an acclimation in the juvenile stage or a heritable cross-generational adaptation (dependent on maternal experience). For this, an understanding of the genetic structure of East coast juvenile sea bass would need to be undertaken, to compare to adult populations and an understanding of the temperatures they were exposed to.

6. Conclusions

The aim of this body of work was to better understand the factors that may drive recruitment variation in juvenile sea bass. I believe that this study presents new and valuable insights that goes some way to addressing this question. Larval settlement behaviour is likely dependent on ontogeny, where movement into shallow habitat is potentially dangerous at the early larval stages, such that they try to station hold in deeper water. Juvenile sea bass use a multitude of habitats over the estuarine mosaic, which provide various important functions. Saltmarshes and sand habitats may provide better foraging opportunities, while oyster reefs and sand may provide more of a refuge function. This ultimately suggests that the nursery habitat of juvenile sea bass is not solely reliant on saltmarshes, but a connected mosaic of structurally different habitats that differ with fitness, ontogeny, and hydrodynamics. However, winter temperatures will likely result in large scale winterkill events if sea bass remain in summer nursery habitats over the first winter, but movement out of estuaries is also a very risky strategy. Therefore, winter thermal minima likely present a greater bottleneck to recruitment on the East Coast than summer habitat use and may limit northern range expansion.

Yet, there are many unknowns left to answer that future work should focus on, including the effects of habitat connectedness, vegetative complexity and relative predation risk on habitat use and survival, winter habitat use, temperature sensitivity, and overwinter survival. Finally, it is important to consider the effects of diel and lunar cycles on tidal behaviour, as well as ontogeny. In the face of rising sea temperatures (Polyaakov *et al*., 2010; Cheung *et al*., 2013; Cheng *et al*., 2019; 2021), rapid degradation of coastal habitats (Foster *et al*., 2013; Burden *et al*., 2020; McAffee & Connell, 2020; Green *et al*., 2021), and the push towards wholescale management of marine habitats (Nagelkerken *et al*., 2016; Link *et al*., 2020), while the contributions of this work are valuable, it is urgent that the questions generated in this study are answered in future work.

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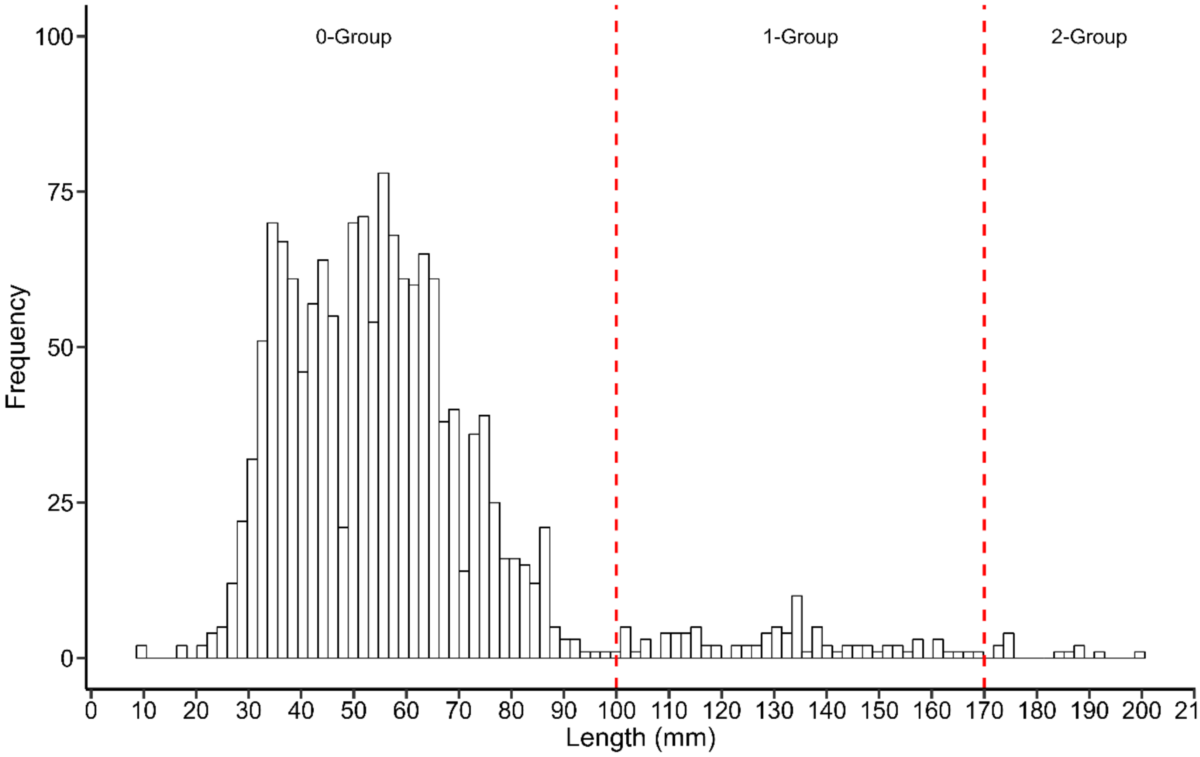
APPENDICES

Appendix 1.

Size frequency distribution of juvenile sea bass caught across all sample sites in the Colne and Blackwater estuary between June and September 2020. Red lines indicates the decided threshold for between 0-group (10-100mm), 1-group (100-170mm), and 2+ group (170$^+$mm) sea bass, determined based on bimodal distribution tails and peaks.



Appendix 2.

Scatter plots of 0-group sea bass abundance (fish m^{-2} x10^{3}) against; A, temperature (^{o}C) and B, salinity (psu) measured across all sample sites in the Colne and Blackwater estuaries between June and September 2020.