This thesis contributes to a better understanding of the nursery-role hypothesis of non-reef habitats for coral reef fishes. Not only fish densities were studied in multiple habitats, but also factors which could drive ontogenetic habitat shifts by fishes, such as habitat structural complexity, food abundance, fish growth rates, predator abundance, survival from predation, fish condition and maturation. The existence of a complex, multi-stage habitat utilization pattern during the life-history of a common coral reef fish species was studied in detail, and compelling evidence is provided for the underlying mechanisms causing ontogenetic habitat shifts. An understanding of these mechanisms is of great importance and will provide for a more robust foundation to work toward effective management and conservation strategies.
CROSSING HABITAT BOUNDARIES

MECHANISMS UNDERLYING CROSS-HABITAT UTILIZATION BY REEF FISHES

Monique GG Groll
CROSSING HABITAT BOUNDARIES

MECHANISMS UNDERLYING CROSS-HABITAT UTILIZATION
BY REEF FISHES

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Crossing habitat boundaries

Mechanisms underlying cross-habitat utilization by reef fishes

Een wetenschappelijke proeve op het gebied van de Natuurwetenschappen, Wiskunde & Informatica

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Crossing habitat boundaries

Mechanisms underlying cross-habitat utilization by reef fishes

An academic essay in
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Success is not the key to happiness. 
Happiness is the key to success. 
If you love what you are doing, 
You will be successful.

--- Buddha ---
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GENERAL INTRODUCTION
Coral reefs are considered as the largest and most dynamic ecosystems world-wide and are under increasing pressure from myriads of natural and human impacts (Jackson et al. 2001, Knowlton 2001, Gardner et al. 2003, Hughes et al. 2003). Primary factors responsible are severe over-harvesting (Jackson et al. 2001, Myers & Worm 2003, Newton et al. 2007), pollution (Sandin et al. 2008), disease (Harvell et al. 2002), climate change (Gardner et al. 2003, Hughes et al. 2003, Munday et al. 2009), coastal development (Hughes et al. 2003), and heavy storms. Due to these threats, biodiversity and structure of coral reef communities including corals, invertebrates and fishes are declining (Bellwood et al. 2004, Mora 2008). Recent studies have focused on how disruption of connectivity among reefs and adjacent shallow-water ecosystems such as bays, lagoons and estuaries harbouring mangroves and seagrass beds may ultimately affect the health and function of coral reef ecosystems (Mumby & Hastings 2008, Verweij et al. 2008, Munday et al. 2009). For example, pelagic fish larvae which settle in mangroves and seagrass beds appear to be recruited from these habitats to coral reefs (Nakamura et al. 2008, Verweij et al. 2008), and thus affect the functioning of coral reef ecosystems (Adams et al. 2006, Mumby & Hastings 2008). It is therefore crucial to elucidate connectivity among components of the marine realm (Sale et al. 2005), and to isolate the underlying ecological mechanisms that give rise to it.

**Connectivity through life history**

The life history of many organisms is complex and throughout their life cycle individuals may shift from one to another habitat, strongly influenced by behavioural responses to their own changing needs or changes within the surrounding environment (Werner & Gilliam 1984, Dahlgren & Eggleston 2000, Adams et al. 2006). Many animals have a 2-phase life cycle with a dispersive larval phase and a demersal juvenile and adult phase. Certain species show ontogenetic habitat shifts during their life cycle between aquatic and terrestrial landscapes (e.g., amphibians [Werner 1986], insects [Schäfer et al. 2006], and crustaceans [Haywood & Kenyon 2009]), whilst others spent their whole lives in aquatic environments (e.g., freshwater fishes [Werner & Gilliam 1984], invertebrates [Haywood & Kenyon 2009]). Also nearshore marine organisms have a 2-phase life cycle with a larval dispersal phase that is often pelagic, and a sedentary demersal phase (Mora & Sale 2002, Haywood & Kenyon 2009). After a pelagic larval stage, many fish and invertebrate larvae settle directly onto live coral (Fig. 1: 2-phase life cycle; Mora & Sale 2002, Jones et al. 2004, Wilson et al. 2006). Therefore, degradation of coral reefs may restrict the size of future adult reef fish stocks (Halpern et al. 2005, Graham et al. 2007).

Larvae of many other tropical marine species do not settle directly in adult habitats such as coral reefs, but instead move across spatially-separated
ecosystems to settle in habitats that are distinct from those where the adults are found, e.g., shallow-water ecosystems such as mangroves and seagrass beds (Shulman & Ogden 1987, Acosta & Butler 1999, Dahlgren & Eggleston 2000, Pollux et al. 2007, Haywood & Kenyon 2009). High juvenile densities of commercially important species have been found in these shallow non-reef ecosystems (Nagelkerken et al. 2000b, Mumby et al. 2004), often referred to as ‘nurseries’ that are hypothesized to sustain coral reef populations (Adams et al. 2006). It has been assumed that juveniles spent at least several months in such nurseries and permanently migrate to their adult habitat, the coral reef (Fig. 1: 3-phase life cycle), at the onset of maturity (Cocheret de la Morinière et al. 2002, Nagelkerken & van der Velde 2003, Adams & Ebersole 2004). Therefore, maintaining the connectivity between coral reefs and/or nurseries is of great importance in management strategies with an emphasis on marine reserves (Roberts 1997, Mumby & Hastings 2008).

**Figure 1.** Two life history strategies used by many marine and freshwater fish and invertebrate species: 2-phase life cycle (dotted line) and 3-phase life cycle (solid line). Spawning takes place in offshore waters (a) after which larvae move towards the coast and settle on the coral reef (b1) or in non-reef nursery habitats such as mangroves and seagrass beds (b2). They migrate as (sub)adults to their permanent habitat, the coral reef (c), in order to reproduce (a).
NURSERY-ROLE HYPOTHESIS

For decades, shallow-water ecosystems such as mangroves and seagrass beds have been considered important nursery grounds for many marine fish and invertebrate species (Nagelkerken et al. 2000b, Haywood & Kenyon 2009, Nagelkerken 2009). However, these considerations are mainly based on the high juvenile densities found in the putative nurseries compared to the lower densities of the same species found on coral reefs (Nagelkerken et al. 2000b,c, Gillanders et al. 2003, Dorenbosch et al. 2004, 2005a,b, 2006, Eggleston et al. 2004). This does not necessarily mean that juvenile populations found in nurseries contribute to the adult population on the coral reef, and firm evidence has still to be provided (Beck et al. 2001, Heck et al. 2003, Sheridan & Hays 2003, Chittaro et al. 2004). Comparative research in bays with and without mangroves and/or seagrass beds showed that juveniles of nursery species were absent or found in very low densities in mangrove/seagrass-poor bays (Nagelkerken et al. 2001). In other studies, the value of nurseries was tested by comparing densities on reefs at a distance from, or in the absence of mangroves and seagrasses. The authors observed that some species were completely absent or occurred in very low densities on reefs far away or in the absence of the purported nurseries, and concluded that there are indeed some species relying on mangroves and seagrass beds as a juvenile habitat (Mumby et al. 2004, Dorenbosch et al. 2005a,b, Nagelkerken et al. 2007).

Beck et al. (2001) formulated the ‘nursery-role hypothesis’ and stated that a habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur. In these proposed nurseries, the greater proportion of recruitment to adult populations must be supported by a higher contribution by any of the following factors: (1) density, (2) growth rate, (3) survival rate, or (4) movement from the nurseries to adult habitats (Beck et al. 2001). Dahlgren et al. (2006) modified this hypothesis and introduced the term ‘effective juvenile habitat’ which describes juvenile habitats that, in terms of their overall contribution, are most important for maintaining adult populations. Thus, habitats should not be compared per unit area (Beck et al. 2001), but rather by the proportion of individuals of each habitat contributing to the adult population (Dahlgren et al. 2006).

So far, almost all studies in the last four decades have only investigated the factor density, often in a single habitat, regardless of fish size, and mostly using different techniques (reviewed by Heck et al. 2003). Hence, the importance of these habitats as nurseries is still ambiguous (Beck et al. 2001, Heck et al. 2003, Adams et al. 2006). In the last decade, studies have focussed more often on density and size distributions of reef fishes in multiple habitats (e.g., mangroves, seagrass beds, algal beds, rubble, sand flats, reef flats;
Nagelkerken et al. 2000b,c, Eggleston et al. 2004, Chittaro et al. 2005, Dorenbosch et al. 2005a,b). However, comparative research in mangrove, seagrass and coral reef habitats on the other three nursery-role factors (growth, survival and movement) is more difficult to perform than on abundance. These factors are often overlooked as has been mentioned in recent reviews (Beck et al. 2001, Heck et al. 2003, Sheridan & Hays 2003, Adams et al. 2006, Nagelkerken 2009). Thus, to provide a better insight into the function of non-reef habitats such as mangroves and seagrass beds as nursery grounds for juvenile coral reef fishes it is necessary to include growth, survival and movement in studies.

**Habitat complexity provides refuge & food**

The transition of larvae from pelagic waters to settlement habitats is time and energy consuming (Bennett & Marshall 2005, Stamps et al. 2005, Phillips et al. 2006) and newly settled fishes are especially vulnerable to predation (Johannes 1978, Leis & Carson-Ewart 1998, Acosta & Butler 1999, Hoey & McCormick 2004). For example, 90% of *Haemulon flavolineatum* will not survive the first month after settlement and 99.9% will die within the first year (Shulman & Ogden 1987, Sogard 1997). This mortality rate can vary markedly according to the characteristics of shelter and/or the absence or presence of competitors and/or predators in the settlement habitat (Jones 1988, Pechenik 1990, Doherty 2002, Holbrook & Schmitt 2002). Therefore, the preference of some fish species to settle and grow up in non-reef nurseries harbouring mangroves and seagrass beds, and the high abundance of juveniles in these putative nurseries may be related to the high structural complexity of these habitats which provide protection from predators and reduce predation pressure. In general, structurally complex habitats (e.g., mangroves, seagrass beds, macroalgae) harbour higher fish densities and species richness compared to less structured habitats (e.g., mudflats, unvegetated habitats; Orth et al. 1984, Jenkins & Wheatley 1998).

Highly structured habitats provide shelter against predators and negatively interfere with predator efficiency, both lowering the risk of being preyed upon (Orth et al. 1984, Werner & Gilliam 1984, Hixon & Beets 1993, Beukers & Jones 1997, Almany 2004). Additionally, nursery habitats are often located at a distance from the coral reef resulting in lower predator densities and higher survival from predation (Shulman 1985, Parish 1989). Due to the reduced water clarity, shallowness, or presence of shade in nurseries, predator efficiency might also be lower and thus reduce predation pressure (Cocheret de la Morinière et al. 2004, Manson et al. 2005, Verweij et al. 2006a).

Growth can be defined as an increase in the energy content of an organism as a result of food acquisition (Jobling 1994). It is assumed that in structurally
complex habitats such as mangroves and seagrass beds, the availability of food, which can be translated into growth, is higher than in less structured habitats (Orth et al. 1984, Laegdsgaard & Johnson 2001, Cocheret de la Morinière et al. 2003, Heck et al. 2003, Nakamura & Sano 2004, Verweij et al. 2006a). Secondly, if structured habitats are safer, less time is spent hiding from predators and more time can be spent on foraging and feeding, and thus increase growth (Sih 1992, Heck et al. 2003). Rapid growth enables newly settled juveniles to leave the most vulnerable size classes quickly, conferring a selective advantage over slow-growing species (Werner & Gilliam 1984, Jones 1991). Although daily food intake by fishes is used for growth in length and weight, the energy of ingested food can also be stored as energy reserves, for instance, around muscle or liver tissue. These reserves are of great importance and can be used in later stages of a fish’s life cycle, for example, gonadal development and maturation (Wootton 1998).

Habitats are dissimilar and vary greatly in their architecture and structural complexity, yet fishes selectively settle (Pollux et al. 2007, Haywood & Kenyon 2009). Some microhabitats are structurally more complex and may offer better advantages than others. Therefore, different characteristics of habitat complexity may play an important role in habitat selection and utilization. For example, fishes may be attracted to a high epibiont complexity on mangrove roots (MacDonald et al. 2008), shoot density in seagrass beds (Heck & Orth 1980), or number of shelter holes in corals (Almany 2004). Additionally, abiotic factors may also be important in habitat selection processes, for example, shade in mangroves (Cocheret de la Morinière et al. 2004). Despite this, many authors counted faunal densities in single habitats and did not differentiate between different complexity characteristics. Habitats were often manipulated by adding or removing pieces of substrate to create different levels of habitat complexity (low vs. high; Heck & Orth 1980, Gratwicke & Speight 2005) and/or were manipulated by adding or removing predators to study survivorship (Beukers & Jones 1997, Almany 2004). Our current understanding of habitat preference by coral reef fishes without the interference of biotic and abiotic factors remains rudimentary, and knowledge about the preference of fishes for habitat type and/or habitat structure, and the underlying mechanisms driving this process are needed.

In summary, species can maximize their fitness with increasing habitat complexity (Jones 1988, Sogard 1992). When fishes grow larger, changing requirements may cause habitat shifts according to their needs (Werner & Gilliam 1984, Dahlgren & Eggleston 2000). Changes in needs related to body size concern food resources (Werner & Gilliam 1984, Cocheret de la Morinière et al. 2003) and protection to predators (Shulman 1985, Laegdsgaard & Johnson 2001). When fishes become mature, they will need an environment that guarantees survival and distribution of their larvae (reviews by Johannes 1978, Roberts 1997, Acosta & Butler 1999, Nemeth 2009).
Habitat-selection behaviour is of great importance in ecological theory. Both net energy intake (growth) and the risk of predation (mortality) can influence habitat selection. However, a settlement habitat does not always offer the advantages of sufficient food and low predation risk simultaneously and therefore fishes are often faced with a cost-benefit analysis between obtaining food resources and remaining safe from predators, which can lead to a trade-off between food (growth) and predation (mortality). Body size is one of the primary factors regulating such trade-offs (Werner & Gilliam 1984). Small Nassau grouper, *Epinephelus striatus*, for example, faced a trade-off between living in the relatively safe algal habitat and achieving high growth rates in postalgal habitats. Larger individuals typically resided in the more risky postalgal habitats (Dahlgren & Eggleston 2000). To maximize fitness, an individual has to maximize its growth (g) and minimize its mortality (μ), or minimize the μ/g ratio. This can be accomplished by movements to another habitat, and thus by a cross-habitat distribution pattern (Werner & Gilliam 1984, Lima & Dill 1990, Utne et al. 1993, Dahlgren & Eggleston 2000).

Life history trade-offs between predator avoidance and foraging gains are common and evidence of such behavioural strategies to minimize the ratio of mortality to growth rate (minimize ‘μ/g hypothesis’) has been obtained for a diverse suite of taxa such as birds, marine mammals, sea turtles, freshwater and marine fishes, and krill (Lima & Dill 1990, Suhonen 1993, Dahlgren & Eggleston 2000, Heithaus et al. 2007, Urban 2007, Atkinson et al. 2008). However, our current understanding of the underlying mechanisms driving such across-habitat distribution patterns and linking adjacent marine habitats remains rudimentary. For example, ontogenetic habitat shifts between mangrove and seagrass nurseries, and the coral reef are a common pattern, but the mechanisms (e.g., food, predation, maturation) driving such shifts are poorly understood. Only a few studies have compared food abundances in some of these habitats (Orth et al. 1984, Cocheret de la Morinière et al. 2003, Nakamura & Sano 2004). Comparative in situ growth studies on fish are lacking for mangroves, seagrass beds and coral reefs, and it remains to be tested which habitat provides the most suitable shelter. Some studies have indicated, though, that predation pressure is lower in mangroves and/or seagrass beds than on coral reefs (Shulman 1985, Nakamura & Sano 2004, Chittaro et al. 2005).

To maintain connectivity among coastal ecosystems (Mumby 2006, Layman et al. 2007, Mumby & Hastings 2008) and identifying the mechanisms that lead to ecosystem-scale distribution patterns are of great importance and will provide for a more robust foundation to work toward effective management and conservation strategies.
INTRODUCTION

MODEL SPECIES: FRENCH GRUNT (HAEMULON FLAVOLINEATUM)

The French grunt (Haemulon flavolineatum (Desmarest 1823)), a common Caribbean coral reef fish species was used as model species throughout this study. After fertilization, larvae spend on average 15 days in pelagic waters after which they settle (Brothers & McFarland 1981, McFarland et al. 1985) in shallow coastal habitats such as embayments and lagoons rather than directly on coral reefs (Shulman & Ogden 1987, Adams & Ebersole 2004). In this early life, recruitment and mortality rates of H. flavolineatum are very high: per m² up to 44 recruits yr⁻¹ will settle, while only 10% survive the first year after settlement and only 0.1% will join the adult population (Shulman & Ogden 1987, Sogard 1997). Up to approximately 4–5 cm FL they are diurnal planktivores and feed on small copepods (Ogden & Ehrlich 1977, Verweij et al. 2006b) after which they shift to a nocturnal zoobenthivorous diet of tanaids and benthic worms (Randall 1967, Ogden & Ehrlich 1977, Cocheret de la Morinière et al. 2003). As (sub)adults they shift their diet towards small decapods (Cocheret de la Morinière et al. 2003).

Juveniles are highly abundant in mangroves and seagrass beds (Nagelkerken et al. 2000b,c, Eggleston et al. 2004, Chittaro et al. 2005), and probably at the onset of maturity, these fish migrate to coral reefs and join adult populations (Gaut & Munro 1983, Cocheret de la Morinière et al. 2002). At 14–18 cm FL fish become sexually mature, and maximum adult fish size is ~26 cm FL (Gaut & Munro 1983, Lindeman & Richards 2005).

THESIS OUTLINE

The central objective of this thesis is to understand the underlying mechanisms driving habitat selection and utilization through ontogeny by a coral reef fish. All studies were carried out on two southern Caribbean islands, Curaçao and Aruba. Although all studied embayments harbour mangroves, seagrass beds, boulder/notches, rubble, sand/mud flats and a fringing coral reef in front of the bay, they differ in their seascape. On Curaçao, research sites were situated in two large, inshore, non-estuarine, shallow-water bays with only one entrance to the bay, while on Aruba coral cays in front of the coast form a large lagoon that has several water inlets through which oceanic water can flow. In this thesis, studies on the underlying mechanisms are presented separately per island, but are discussed together to identify general patterns.

Early juveniles of many species show a stronger affinity for mangrove and seagrass habitats – the nurseries – than for coral reefs. It has been assumed that nursery habitats are preferred because they are structurally complex,
predation pressure is lower, and the abundance of food is greater. In chapter 2 the preference for a variety of microhabitats was tested in situ using artificial cages, in which the variables food and predation were excluded among treatments. In each corner of a cage, a mangrove, seagrass, rubble or coral microhabitat differing in structural complexity was created. Per microhabitat, the degree of the structural complexity characteristics differed (e.g., microhabitat height, rugosity, percent cover), and was measured using digitized photographs. The hypothesis tested was that fish would select for the most structurally complex microhabitat rather than a particular type of microhabitat or a structural characteristic.

Mangroves and seagrass beds harbour high densities of food for fish and invertebrate species, which is often related to the high structural complexity of these habitats. Thus, juveniles preferring structurally complex mangrove and seagrass nurseries are also suspected to achieve higher growth rates than on reefs. To test this hypothesis, I compared growth rates in situ between mangrove, seagrass and coral reef habitats (chapter 3). Planktonic and benthic food samples from inside and outside all cages were analysed to determine food availability and confirm that food was not deficient in the cages. Gut content analysis showed which food items were preferred by the enclosed fish.

Another important mechanism thought to drive habitat selection of fish is predation. Structurally complex mangrove and seagrass nurseries provide fish with more hiding places and lower predator efficiency. Additionally, mangroves and seagrass beds are located away from the coral reef, are shallow, and often turbid, all lowering the risk of predation. Taking all these factors into account, nursery habitats likely have a greater survival prospective compared to the coral reef. In chapter 4, I tested the assumption of reduced predation risk in mangrove and seagrass nurseries by comparing piscivore assemblages along a gradient from deep inside a mangrove and seagrass dominated bay to the fringing coral reef in front of the bay using underwater visual census surveys. In addition, I conducted predation experiments in all habitats with small, tethered juvenile fish.

According to the nursery-role hypothesis, the attractiveness to the putative mangrove and seagrass nurseries is related to the higher growth rates and lower mortality rates in these habitats. However, it is expected that fishes continuously have to make cost-benefit analyses to maximize their fitness, and therefore shift habitats to maximize growth and minimize mortality or minimize the $\mu/g$ ratio. To gain insight in the underlying mechanisms driving habitat shifts between the bay and the adjacent coral reef, I compared fish densities, growth rates obtained from otoliths, survival from predation in tethering experiments, and $\mu/g$ ratios for the whole size range of *H. flavolineatum* (chapter 5).

Larger fishes become less vulnerable to predation and it is assumed that at this time they shift to more profitable habitats (e.g., habitats facilitating higher
growth rates and/or reproduction). Our model species is an ontogenetic habitat shifter, settling on rubble from where after it migrates to seagrass beds, followed by mangroves, and finally to the coral reef where it joins the adult population. In chapter 6 the mechanisms causing this specific distribution pattern between multiple nursery habitats and the coral reef are examined in more detail.

In chapter 7, all findings in this thesis are combined, and general patterns identified. I conclude that *H. flavolineatum* during their life cycle shift habitats based on its changing needs, and often has to make cost-benefit analyses to meet their requirements. I further conclude that movement across habitat boundaries can have strong impacts on the dynamics and structure of communities, and that changes in one habitat can indirectly affect the community structure in a connected habitat. It represents a novel contribution to our understanding of processes regulating connectivity among marine fish populations, which are important criteria to consider in management and conservation initiatives in order to conserve our coral reef fish populations.

**REFERENCES**


INTRODUCTION


Chapter 1


INTRODUCTION


PREFERENCE BY A CORAL REEF FISH FOR DISTINCT SHALLOW-WATER MICROHABITATS IS NOT RELATED TO COMPLEXITY BUT TO HABITAT TYPE

GROL MGG, NAGELKERKEN I & BOSCH N

MARINE ECOLOGY PROGRESS SERIES (2010) IN REVIEW
HABITAT PREFERENCE BY REEF FISH

ABSTRACT

Coral reef populations of various fish species are replenished by individuals that utilize shallow-water coastal habitats such as mangroves and seagrass beds as settlement and juvenile habitats. These habitats vary greatly in their architecture and different characteristics of structure may play a role in their selection and utilization by fish. To solely investigate the role of structural complexity in microhabitat selection, *in situ* habitat preference of individual post-settlement stage fish was quantified for four structurally very different microhabitats (*i.e.*, mangrove, seagrass, rubble, and coral) that were offered simultaneously in each enclosure. Structural complexity of each microhabitat was randomly changed during each replicate experiment, and fish were expected to always select the structurally most complex microhabitat offered, independent of microhabitat type. Using digitized photographs, seven structural complexity characteristics (*e.g.*, microhabitat cover, rugosity, number of shelter holes) were measured. Fish did not have a preference for the structurally most complex microhabitat, and no relationship was found with any of the seven complexity characteristics. Fish were very consistent in their choice and always preferred seagrass and coral microhabitats even when offered at low cover, rugosity, or number of holes. A likely explanation for this unexpected result is that criteria such as absence/presence of structure, hanging vs. standing structure, and living vs. dead structure are more important in determining habitat preference by a demersal fish than the degree of structural complexity. In this light, the continuing degradation and loss of living benthic vegetation and biota as a result of human impacts is alarming. Marine protected areas are especially important in this context, as they can provide a framework for protection against habitat loss and destruction.
**INTRODUCTION**

Ecosystems are intricately linked by the flow of organisms across their boundaries. Many coral reef-associated species have pelagic larvae which settle selectively in spatially-segregated juvenile habitats, such as inshore mangroves and seagrass beds (Pollux *et al.* 2007, Haywood & Kenyon 2009), before contributing to adult populations on the coral reef (Verweij *et al.* 2008, Nagelkerken *et al.* 2009). Yet, there is little information about the underlying mechanisms causing such habitat shifts and the role that various habitat attributes may play in this.

Predation is one mechanism which causes habitat shifts. For example, juvenile *Haemulon flavolineatum* mainly utilized vegetated lagoonal habitats where predator densities were a magnitude of an order lower than on the coral reef and survival from predation was high (Dorenbosch *et al.* 2009). A second mechanism is through higher abundances of certain food items which enhance growth rates of juveniles in vegetated vs. unvegetated habitats (Orth *et al.* 1984, Laegdsgaard & Johnson 2001, Cocheret de la Morinière *et al.* 2004). Habitat complexity affects both mechanisms - predation risk and food abundance - and therefore structure-rich habitats are preferred as settlement and juvenile habitats by many fish and invertebrate species. Structurally complex habitats (*e.g.*, mangroves, seagrass beds, macroalgae) are often associated with higher fish density and species richness compared to less structured or unvegetated habitats (Orth *et al.* 1984, Jenkins & Wheatley 1998).

Predation is generally considered to be one of the key causes of mortality and driven by predation species may have evolved a preference for shelter-rich habitats. Such habitats provide prey with more shelter holes which reduces predation (Hixon & Beets 1993, Beukers & Jones 1997) and competition (Almany 2004, Schmitt *et al.* 2009) among species, both leading to enhanced survival. On the other hand, structurally complex habitats also reduce feeding efficiency for prey species as it is also harder for them to detect their prey and they cannot freely manoeuvre within the dense structure (Werner & Gilliam 1984, Beukers & Jones 1997, Heck & Orth 2006).

Habitats vary greatly in their architecture and different characteristics of structure may play a role in their selection and utilization by fish. In mangroves, for example, positive correlations have been found between fish abundance and pneumatophore density (Payne & Gillanders 2009) or epibiont complexity on mangrove roots (MacDonald *et al.* 2008). In seagrass beds, fish abundance is influenced by seagrass cover, height, and density (Orth *et al.* 1984, Bell & Westoby 1986, Gullstrom *et al.* 2008). On coral reefs, numbers of shelter holes, rugosity, and percent live coral have been identified as important aspects of complexity determining fish distribution (Luckhurst & Luckhurst 1978, Jones & Syms 1998, Gratwicke & Speight 2005).
Abiotic factors may also affect habitat selection by fishes and invertebrates, e.g., shade, water clarity, depth, temperature, and salinity (Blaber & Blaber 1980, Rypel et al. 2007, Gullstrom et al. 2008). Mangroves for example, may be selected by juvenile fish not only for their dense prop-root system that provides shelter and reduces predator manoeuvres, but because of the shade prey is also harder to detect by predators and thus reduces predation risk (Helfman 1981, Cocheret de la Morinière et al. 2004).

Reef fish species that settle and temporarily reside in shallow inshore areas as juveniles have a choice of many types of very dissimilar microhabitat types in terms of structural architecture (e.g., shelter holes in corals, shade in mangroves, flexible structure of seagrass leaves). Despite of this, many studies have focused on single habitats, taking only one or a few characteristics of complexity into account, and studied total fish abundance and species richness irrespective of species- and size-specific habitat preferences. This leaves us with little understanding of how structure contributes to selection and utilization of habitats by fish in a complex seascape, such as that often found in shallow back-reef, lagoonal or estuarine areas. In single habitat studies, Heck et al. (2003) found that the presence of structure per se appeared to be a more important determinant of the nursery value of a habitat than type of structure, and similarly Nagelkerken & Faunce (2007) suggested that the use of mangrove structure is not related to a predetermined preference by species but more likely to the presence of structure. Additionally, Jenkins & Wheatley (1998) concluded that while the presence of structure per se is sufficient for the recruitment of many species, some taxa will discriminate amongst habitats based on structural characteristics. Furthermore, it is possible that in cases where multiple microhabitats are readily available, preference is not related to presence of structure alone or to specific structural characteristics but rather to the type of microhabitat.

Here, the role of solely habitat complexity in microhabitat preference by post-settlement stage fish was tested experimentally, in isolation from other abiotic and biotic variables. In situ habitat preference was quantified for a juvenile fish species for four very different microhabitats. To investigate the role of various structural characteristics influencing microhabitat preference, digitized photographs were used and seven structural complexity characteristics for the four microhabitats were measured. The study organism was the French grunt, Haemulon flavolineatum, a common Caribbean coral reef fish species which preferentially utilizes mangroves and seagrass beds as juvenile habitats (Nagelkerken et al. 2000, Layman et al. 2004). As the use of structure is highly driven by predation risk, it was expected that fish in this study would always prefer the structurally most complex microhabitat within each enclosure rather than the same microhabitat or structural characteristic across experiments.
Materials & Methods

Study area and studied species – This study was carried out at two Caribbean islands, Curaçao and Aruba. Field experiments were conducted in front of Piscadera Bay on Curaçao (12°07'N, 68°51'W) and at a lagoon near Mangel Halto on Aruba (12°27'N, 69°58'W) on a sandy bottom (~2–4 m depth) away from other habitat types such as mangroves, seagrass beds, and coral reefs (Curaçao: >80 m, and Aruba: >300 m away). Larvae of the model species (Haemulon flavolineatum) recruit from the plankton in shallow-water coastal habitats such as lagoonal patch reefs, mangroves and seagrass beds (Nagelkerken et al. 2000, Layman et al. 2004) at a size of 7.9–11.5 mm fork length (FL) (Gaut & Munro 1983, Lindeman & Richards 2005). Fishes for the experiments (Curaçao: 3.7 ± 0.2 cm FL (mean ± SD), 0.8 ± 0.3 g total weight, and Aruba: mean 3.9 ± 0.3 cm FL, 1.0 ± 0.2 g) were collected in two different habitat types: on seagrass beds at Barcadera (12°28’N, 69°59’W) and a sandy/rubble zone near the lagoon entrance at Mangel Halto on Aruba, and on seagrass beds and rubble located in the channel area of Spanish Water Bay on Curaçao (12°04’N, 68°51’W) (see Grol et al. 2008).

Experimental design – Microhabitat preference by early juvenile Haemulon flavolineatum was studied in situ during January–February 2009 using experimental cages. The cages excluded predators but allowed inflow of planktonic food items. Their placement on a large shallow sandy area reduced the influence of factors such as strong ocean currents, nearby presence of other complex benthic habitats or aquatic vegetation, nearby presence of schools of other fish, and strong auditory or olfactory cues created by nearby fish or habitats. As a result, fish were forced to make a choice related to microhabitat structural complexity characteristics and choice was neither predator nor food nor environmentally induced.

The framework of the square experimental cages (1.5 x 1.5 x 0.7 m) was constructed using iron rods (Ø 8 mm) covered with galvanized wire (mesh size 6 mm), except for the bottom part which rested on the sandy bottom (Fig. 1). In total, six cages per island were placed at least 25 m apart from each other. Each cage was placed with one of its sides perpendicular to the direction of the waves and water current. In each of the four corners of a cage a different microhabitat (50 x 50 cm) was created using pieces collected freshly in the field: pieces of live soft and hard corals, coral rubble, mangrove prop roots (Rhizophora mangle), and seagrass plants (Thalassia testudinum). All microhabitats were placed on the sandy substratum, except mangrove roots. To mimic a hanging mangrove prop root habitat, the roots were attached to the top of the cage and the top of this corner was covered with a cloth to create shade. All substratum that was not covered by microhabitats consisted of bare...
sand and is referred to as ‘unstructured’ habitat (Fig. 1). During each experiment, the number of structures of each of the four microhabitats (viz. the number of coral pieces, rubble pieces, mangrove roots, and seagrass shoots) was randomly changed, as well as the mutual configuration of the four different microhabitats in the corners within a cage (Table 1). The latter was to ensure that the different microhabitats were continuously placed at different angles to, e.g., wave motion, currents, or the angle of sunlight so that the fish’ preference for a specific microhabitat could be isolated. In addition, changing the mutual placement of the microhabitats avoided habitat choice being influenced by non-random search behaviour of the fish in a cage, i.e., fish are more likely to move to another microhabitat along the edges of a cage than to swim across the sand to the opposite microhabitat. The iron gauze of the cages was scrubbed on the outside after each experiment to remove algal growth and to preserve a good flow of water through the cages.

Early juvenile *H. flavolineatum* were caught using nets and fish traps in seagrass and rubble habitats. On Curaçao, the collected fish were directly transported over land from Spanish Water Bay to the laboratory and were housed in two separate aquaria with flowing seawater. On Aruba, collected fish were directly transported to the experimental site near Mangel Halto and temporarily held in two small cages (40 x 40 x 50 cm) made of wire (mesh size 6 mm) and placed on the sand bottom as no aquaria with flowing seawater were available. Fish collected from seagrass and rubble habitats were kept separately and acclimatized for at least 24 h in the holding tanks before experiments started. Every other day new fish were collected from the field. Per island, six cages were used simultaneously. Replicates consisted of an experiment with a single fish which was used only once.

Fish were transported from the holding tanks to the experimental cages within 5 min in a semi-closed dark box through which fresh seawater could flow. Fish from seagrasses or rubble were selected randomly for each experiment (Table 1). One fish was introduced into each cage using a wire mesh cylinder (Ø 25 cm, 1.2 m in length) that was stuck through a closable window (25 x 25 cm) in the centre on top of the cage (Fig. 1a). To acclimatize to the environment in the cage and to provide the fish with the opportunity to see all four microhabitats before making a choice, fish were kept in the cylinder on the sandy substratum for 3 min. Hereafter, the cylinder was slowly removed and the experiment started. Each fish was observed for 15 consecutive minutes at a distance of at least 5 m using SCUBA on Curaçao and snorkelling equipment on Aruba. Every between-microhabitat movement within these 15 min was recorded by an observer, resulting in a time budget spent in each microhabitat.
Each experiment with the same fish lasted for almost 24 h. Repetitive observations were done between 14:30-15:30 and 17:00-18:00 (just before sunset) on day 1, and at 07:00-08:00 (just after sunrise) and 09:30-10:30 on day 2. Different time periods were chosen because it could affect microhabitat preference. Small fish feed continuously on zooplankton during daytime and do not shift habitats to feed (Verweij *et al.* 2006a, Grol *et al.* 2008), while larger individuals (approximately >5 cm FL) feed on zoobenthos and migrate in shoals at dusk and dawn to and from their benthic feeding areas (Ogden & Ehrlich 1977, Helfman *et al.* 1982). After the fourth and last observation, fish were released from the cages, cages were scrubbed, rearranged, and the experiments were repeated with new fish.

**Figure 1.** View of an experimental cage placed on sand - an unvegetated and unstructured substratum - from (a) the top and (b) the side. Per island, all six cages were placed perpendicular to the current and wave direction. Four different microhabitats (seagrass, coral, rubble and mangrove) with a randomly selected number of structures were randomly distributed at the four corners (1-4) of each cage. The dashed line in the central part of the cage is the place where *Haemulon flavolineatum* were released in a transparent wire mesh cylinder on the sand before the experiment started.
Table 1. Overview of the experimental design used to investigate microhabitat preference of early juvenile *Haemulon flavolineatum* on Aruba and Curaçao. In total, 24 fish were tested on each island, divided into four rounds with in each round six cages. The configuration of the four microhabitats (SG = seagrass, CR = coral, RB = rubble, and MG = mangrove) in each corner of each cage (1-4, see Fig. 1) as well as the number of structures per microhabitat (SG: number of shoots, CR: number of coral pieces, RB: number of rubble pieces, and MG: number of roots) were randomly selected. Catch location, fork length and weight are provided for each fish.

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**Photo analyses** – Each microhabitat in each cage for all replicate experiments was photographed at a distance of about 1.5-2.0 m to determine the degree of habitat complexity. Photos were taken in the horizontal (from above) and vertical (from the side) plane of each microhabitat, and reflected the approximate positions from which a fish could see the microhabitat. A measuring rod was placed in each microhabitat to scale the images. Photographs were printed and digitized, and using the measuring rod’s scale, pixels in each photo were convert to dimensions (cm) of the structural complexity characteristics using the program CPCe (Coral Point Count with Excel extensions; Kohler & Gill 2006). Automatic processing of the photographs was not possible due to low contrast. Therefore, the outlines of each microhabitat in every photo from above and from the side were drawn manually using the image analysis software GIMP version 2.6 (GNU Image Manipulation Program), an open source image editing software package (http://www.gimp.org/).

Per photo, six different structural complexity characteristics were measured within the manually-drawn habitat outlines: (1) top and (2) side percent cover of the microhabitat, (3) microhabitat rugosity, (4) maximum and (5) mean height of the microhabitat above the substratum, and (6) number of shelter holes. Total number of structures (7) was not determined from the photos as these were randomly selected for the experiments. For microhabitat cover, a self-written script using PHP (PHP hypertext pre-processor; http://www.php.net/) was used to count the total number of pixels of covered vs. uncovered microhabitat per photo, and the percent area covered was calculated for photos taken from above (top cover, where uncovered substratum consisted of sand) and from the side (side cover, where uncovered substratum consisted of the open water layer). For rugosity, the contour of the microhabitat was measured from photos taken from the side, and was calculated as the ratio of contour-following vs. straight distance between two end points of the microhabitat in the photo (Risk 1972). Maximum and mean heights of the microhabitats were also calculated from the photos taken from the side: per ‘pixel column’ the difference between the highest and lowest elevation of the microhabitat was determined to calculate the maximum microhabitat height per column in pixels. Mean microhabitat height was calculated by averaging the height of all pixel columns. Maximum/mean heights were converted from pixels to length in cm using CPCe. For number of shelter holes, the number of holes was counted from the photos that were taken from above the microhabitat.
Habitat preference by reef fish

Data and statistical analyses – Microhabitat preference was tested for a total of 24 fish on Curacao and 24 on Aruba. For each fish, total time spent in each microhabitat was expressed as a percentage of each 15-min test period, after which data from all four 15-min observations of each fish were averaged per microhabitat per island. Differences in microhabitat preference were tested using a repeated-measures ANOVA (GLM) for all fish pooled, and separately for fish caught on seagrass beds or rubble habitats. First a Mauchly’s test was used to test for homogeneity of variances. If variances were homogenous the assumption of sphericity was accepted, otherwise the Greenhouse-Geisser correction was used. Bonferroni post-hoc comparisons were used to identify differences in means among microhabitats. To test if microhabitat preference differed between fish caught on seagrass beds or rubble, and between islands, independent samples t-tests were performed. As no significant difference was found between seagrass and rubble fish, they were pooled for further analyses.

Temporal variation in microhabitat preference within cages was tested over the four 15-min observations per fish using a repeated-measures ANOVA (GLM), followed by Bonferroni post-hoc comparisons as described above.

Total time spent in the most preferred microhabitats by Haemulon flavolineatum (i.e., coral and seagrass) was categorized into four categories for each fish separately (0.0–24.9%, 25.0–49.9%, 50.0–74.9%, and 75.0–100.0% of their time). The frequency distribution of these categories was then plotted based on data of all fish, separately per island. Additionally, the number of fish which chose more than half of their time for a microhabitat were counted and plotted separately per island, divided into 50.0–74.9% and 75.0–100.0% of their time spent in a microhabitat. As most fish preferred coral and seagrass microhabitats and they only can spend time in one microhabitat at the time, these numbers were summed.

For each island separately as well as for both islands combined, linear and multiple linear regressions were used to explore relationships between H. flavolineatum microhabitat preference and the various microhabitat complexity characteristics: top cover, side cover, rugosity, mean and maximum height, number of shelter holes, and number of microhabitat structures as counted in situ. Rugosity and mean/maximum height were highly collinear ($R^2 \geq 0.632$, $P < 0.001$) as microhabitats with a higher mean/maximum height are also more rugose. As rugosity is more often used in other studies, mean/maximum heights were excluded from the multiple linear regressions.

As none of the above regressions showed significant results (see Result section), we hypothesized that fish might choose for the highest structural complexity within cages, independent of microhabitat type. Therefore, linear and multiple linear regressions across cages were also conducted irrespective of microhabitat type (‘pooled microhabitats’ in Table 2). Alternatively, microhabitat preference within each cage could be a reflection of the relative
structural complexity of the four microhabitats offered. Therefore, the theoretically expected percent time that fish would spend in each microhabitat based on the relative degree of structural complexity offered in each cage was calculated. For example, when only one highly complex microhabitat would be available fish would select this near to 100%; in the case of two highly complex microhabitats 50% of the time would be spent at each microhabitat, three habitats 33.3% of the time at each microhabitat, etc. This gets more complex when four microhabitats are present each with a different degree of relative structural complexity. Therefore, for each structural complexity characteristic separately, values for the four microhabitats were summed per cage. The ratio of each of the microhabitat’s complexity compared to that of the total cage reflected the expected ratio and thus percent time spent in each microhabitat. Linear regressions were then conducted for the expected preferences vs. the actual time spent at each microhabitat (observed preferences). To adjust for the difference in number of structures used to create different microhabitats, the number of structures was categorized in four abundance classes per microhabitat type to calculate the expected preference for this structural characteristic.

Mean values per structural characteristic per microhabitat were calculated for Aruba and Curaçao combined. To explore differences in habitat complexity characteristics among microhabitats, non-parametric Kruskal-Wallis tests followed by Games Howell post-hoc comparisons were used as data were not homogenous (Levene’s test).

All statistical analyses were performed using SPSS (version 16.0), and differences were significant if P-values were ≤0.050.

**RESULTS**

**Microhabitat preference** – Overall, early juvenile *Haemulon flavolineatum* showed highest preference, on average, for seagrass (Fig. 2), although significant differences in preference were only observed between seagrass and mangroves for all fish pooled (Bonferroni: Aruba $P = 0.004$, Curaçao $P = 0.026$), between seagrass and unstructured microhabitat on Curaçao (Bonferroni: all fish $P = 0.011$, rubble fish $P = 0.027$), and between seagrass and mangrove on Aruba (Bonferroni: seagrass fish $P = 0.040$). Coral was the next most preferred microhabitat, on average, especially on Aruba. Significant differences between coral and other microhabitats were only found for mangroves on (Bonferroni: all fish $P = 0.006$). The lack of strong significant differences between seagrass/coral and other microhabitats was principally caused by the large variation in choice among individuals (*i.e.*, single preference for either coral or seagrass), as shown further below. The above patterns were largely unaffected
by the origin of fishes (i.e., rubble vs. seagrass habitat). Preference for each of
the four microhabitats separately did not significantly differ between fish
captured from seagrass beds or rubble (independent samples \( t \)-tests; Aruba \( P \geq 0.328 \), Curaçao \( P \geq 0.200 \)). Therefore, these fish were pooled in all subsequent
analyses. Fish did not show significant differences in their preferences either
when each microhabitat was compared between islands (independent samples
\( t \)-tests; all fish \( P \geq 0.279 \), seagrass fish \( P \geq 0.319 \), rubble fish \( P \geq 0.223 \)).

**Figure 2.** Microhabitat preference (mean + SE) of early juvenile
*Haemulon flavolineatum* for various microhabitats on (a) Aruba
and (b) Curaçao, for all fish pooled (all fish), fish collected in
seagrass beds (SG fish), and fish collected on rubble (RB fish).
Different letters (A, B) represent significant differences among
microhabitats (Bonferroni post-hoc comparisons, \( P \leq 0.050 \)).
Even though *H. flavolineatum* show differential feeding behaviour with time of day (Ogden & Ehrlich 1977, Verweij *et al.* 2006a), no significant changes in microhabitat preference were evident among the different time periods (repeated-measures ANOVA; Aruba $P \geq 0.290$, Curaçao $P \geq 0.215$) (Fig. 3). During all time periods fish spent most time in seagrass and coral microhabitats at both islands, on average.

![Graph showing microhabitat preference](image)

**Figure 3.** Microhabitat preference (mean ± SE) of early juvenile *Haemulon flavolineatum* as a function of time on (a) Aruba and (b) Curaçao during the two observation days.
Although on average early *H. flavolineatum* showed a clear preference for seagrass and coral microhabitats, there was considerable variation among individuals as indicated by the relatively large error bars in Figs. 2 and 3. Individual fish did not show a strong preference for coral and seagrass at the same time. Instead, microhabitat choice showed a bimodal response at both islands (Fig. 4), with highest frequencies for the 0.0–24.9% and 75.0–100.0% time intervals for seagrass and coral microhabitats. As a fish can only be dominantly present in one microhabitat at the time, a high percentage of time spent on, for example, coral results automatically in a low percentage of time spent in a seagrass microhabitat. At the individual level this means that fish therefore preferentially choose for either coral or seagrass. Very few fish spent 25.0–74.9% of their time in these two habitats indicating that average preferences as shown in Figs. 2 and 3 are deceptive in this case.

**Figure 4.** Frequency distribution of microhabitat preference of early juvenile *Haemulon flavolineatum* ranked in four categories (0.0–24.9%, 25.0–49.9%, 50.0–74.9%, and 75.0–100.0%) on Aruba and Curaçao. Only the most preferred microhabitats (SG: seagrass and CR: coral) are shown. The sum of frequencies for the four categories on the x-axis form 100% (n = 24 fish) for each microhabitat at each island.
To further explore individual preference, a frequency distribution was drawn for microhabitat preference using only data of individuals that spent the majority of their total time observed in a single microhabitat (Fig. 5). This showed that all 24 fish per island (except one on Curaçao) stayed preferentially (≥50% of their time) in a single habitat, of which coral and seagrass microhabitats were the most frequently selected ones. Microhabitat preference for a single habitat was strong as indicated by the fact that the 75.0–100.0% group was more common than the 50.0–74.9% group.

**Figure 5.** Number of early juvenile *Haemulon flavolineatum* spending more than half of their observed time (50.0–74.9% and 75.0–100.0%) in the same microhabitat (SG = seagrass, CR = coral, RB = rubble, MG = mangrove, and UNS = unstructured) on Aruba and Curaçao.
Relationships with habitat complexity – Linear regressions of the observed microhabitat preference of early juvenile *Haemulon flavolineatum* as a function of various microhabitat complexity characteristics showed very low $R^2$-values for each of the four microhabitats separately as well as for pooled microhabitats (Table 2); only very few significant relationships but with low $R^2$-values were found. Furthermore, no consistent pattern was present as significant results included negative as well as positive relationships. Other curve estimations (i.e., logarithmic, inverse, and quadratic) in SPSS did not provide higher $R^2$-values or more significances (results not shown). Multiple linear regressions that combined complexity characteristics showed somewhat higher $R^2$-values, although mostly still insignificant. Linear regressions between the observed and expected microhabitat preference showed more significances; however, $R^2$-values were extremely low indicating that microhabitat preference was not related to the degree of structural complexity.

Table 2. R-squares of regression analyses representing the relationship between the degree of structural complexity and microhabitat preference of early juvenile *Haemulon flavolineatum* for Aruba, Curaçao, and for Aruba and Curaçao combined. Results are shown for individual microhabitats and for microhabitats pooled (‘pooled microhabitats’) for each of the seven different microhabitat complexity characteristics, as well as all characteristics combined (‘multiple linear regression’). Furthermore, regression results are shown for observed microhabitat preference as a function of the expected preference for each complexity characteristic. Negative values indicate negative relationships. *$P < 0.050$, **$P < 0.010$. 

<table>
<thead>
<tr>
<th>Island</th>
<th>Complexity characteristic</th>
<th>Seagrass</th>
<th>Coral</th>
<th>Rubble</th>
<th>Mangrove</th>
<th>All habitats</th>
<th>Expected preference</th>
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<td>0.00</td>
<td>0.03</td>
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<td>0.28</td>
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<td>-0.05**</td>
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<td>-0.00</td>
<td>-0.04**</td>
<td>-0.05**</td>
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<tr>
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<td>Number of shelter holes</td>
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<td>-0.02</td>
<td>0.06**</td>
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<tr>
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<td>0.12</td>
<td>0.09</td>
<td>0.11**</td>
<td>0.11**</td>
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</table>
Mean microhabitat preference was not reflective of mean values of any structural characteristic of the microhabitats either. Although values of each of the seven structural characteristics overlapped among microhabitats, their means differed significantly (Table 3). Top cover was significantly highest in coral and rubble habitats, but much lower for the preferred seagrass microhabitat. Side cover increased significantly from mangrove to seagrass to rubble to coral, while average microhabitat preference increased from mangrove to rubble to coral to seagrass. Mean values for rugosity, mean height, and maximum height were extremely high for mangrove, yet this microhabitat was hardly ever selected. Number of shelter holes was highest for seagrass, but lowest for coral which was the second most-preferred microhabitat. Finally, number of structures was highest for seagrass and rubble, but the latter habitat was much less preferred than the former.

Table 3. Mean (range) values of the seven complexity characteristics for each microhabitat, combined for Aruba and Curaçao. P-values show results of Kruskal-Wallis tests, while different letters (a–d) indicate significant differences in means among microhabitats (Games Howell post-hoc test).

<table>
<thead>
<tr>
<th>Island</th>
<th>Complexity characteristic</th>
<th>Seagrass</th>
<th>Coral</th>
<th>Rubble</th>
<th>Mangrove</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<td>Aruba &amp; Curaçao</td>
<td>Top cover</td>
<td>51.6° (27–71)</td>
<td>69.5° (49–87)</td>
<td>66.8° (56–81)</td>
<td>36.0° (22–48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Side cover</td>
<td>42.3° (24–59)</td>
<td>58.8° (47–72)</td>
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<td>33.1° (16–54)</td>
<td>&lt;0.001</td>
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<td>2.6° (2–6)</td>
<td>2.1° (2–3)</td>
<td>24.6° (7–60)</td>
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</tr>
<tr>
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<td>Mean height</td>
<td>18.4° (10–27)</td>
<td>13.9° (9–22)</td>
<td>12.1° (8–17)</td>
<td>42.2° (27–59)</td>
<td>&lt;0.001</td>
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<td>Maximum height</td>
<td>28.5° (16–37)</td>
<td>19.6° (13–36)</td>
<td>17.4° (12–25)</td>
<td>57.2° (37–70)</td>
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<td>Number of shelter holes</td>
<td>40.9° (11–121)</td>
<td>8.5° (4–17)</td>
<td>19.0° (12–31)</td>
<td>11.0° (1–28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Number of structures</td>
<td>35.3° (11–59)</td>
<td>10.3° (5–16)</td>
<td>33.1° (21–47)</td>
<td>7.5° (2–13)</td>
<td>&lt;0.001</td>
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</table>

**DISCUSSION**

In the present study, an *in situ* choice experiment was used to test the hypothesis that in isolation of other factors fish prefer the structurally most complex microhabitat that is available, independent of habitat type. Species often show a non-random distribution that is driven by biotic factors such as priority effects, competitive exclusion, predation risk, food quantity and quality (e.g., review by Adams & Ebersole 2009). Habitat complexity is one of the key influences that can affect these mechanisms positively (e.g., enhancing food abundance, reducing predation risk) and therefore species often show a strong preference for structure-rich habitats. However, the experiment in the present study that excluded interactions with other abiotic and biotic factors and solely investigated the importance of seven different habitat complexity characteristics across four different microhabitats showed lack of any
relationship between microhabitat preference and microhabitat structural complexity. Also, the observed microhabitat preference differed greatly from that of the predicted microhabitat preference as shown by a complete lack of a relationship between the two. There was quite a wide range in values for complexity characteristics of microhabitats within and among replicate experiments, so it is unlikely that these differences were too small to induce a response of fish to the highest structure. Therefore, the hypothesis of a preference for the most structure-rich habitat can be rejected for the microhabitats compared, and other habitat selection criteria seem to play a role in the present experiment. It is likely that due to the very contrasting architectures of the microhabitats (e.g., flexible vs. rigid, hanging vs. standing, living vs. dead) fish showed lack of the typically observed correlations between habitat preference and structural complexity in many of the studies that have focused earlier on single microhabitats.

One result that was in accordance with typical observations from other studies was the lack of preference for unstructured sandy microhabitat. After release of the test fish on the unvegetated sandy bottom in the experimental cages, 88% of the fish on Aruba and 79% on Curaçao moved within 30 seconds towards one of the four microhabitats. With a few exceptions, fish did not return to the sandy bottom after they had selected a microhabitat. Numerous studies have demonstrated higher preference for vegetated than for unvegetated habitats, irrespective of the type of bottom structure (Luckhurst & Luckhurst 1978, Orth et al. 1984). The importance of the presence of structure is further supported by the fact that the sandy bottom had a surface area that was ~5 times larger than that of each of the microhabitats, but was hardly selected even though the cages excluded nearby presence of predators. An important aspect of microhabitat preference by *Haemulon flavolineatum* is therefore the presence vs. absence of structure.

Vertical orientation of structure (i.e., hanging vs. standing) is another factor that can determine habitat preference. Although the hanging mangrove roots had, on average, the highest rugosity and maximum/mean heights of structure, and were potentially more attractive because they were also shaded (Cocheret de la Morinière et al. 2004), they were hardly ever selected by the test fish. An explanation for the latter is provided by a recent field experiment at Spanish Water Bay, Curaçao, using seven open experimental mangrove units (AMUs) with different combinations of root length in a hanging and/or standing orientation. The results showed that all demersal species (including *H. flavolineatum*) were equally attracted to AMUs with any type of standing roots, independent of root length and three-dimensional root structure (Nagelkerken et al. in revision); hanging roots were not preferred at all. In that study, the vertical orientation (hanging vs. standing) of mangrove roots was the primary explanatory factor for the observed differences in fish community variables. Although in the current experiment microhabitat type differed for
hanging and standing microhabitats, it is likely that fish did not select the
darker structure-rich mangrove microhabitat because hanging structure is less
effective shelter for demersal species.

Living structure may be more attractive as shelter to fishes than dead
structure. Although live coral and dead coral rubble showed much more
similarity in top and side cover, rugosity, and mean and maximum heights
compared to seagrass and mangrove microhabitats (see Table 3), fish hardly
ever chose rubble as primary microhabitat (see Fig. 5). Recent studies have
shown that fishes prefer live over dead coral (Graham et al. 2006, Feary et al.
2007), and live coral enhances fish abundance and diversity of species that are
dependent on live coral as settlement sites (Jones et al. 2004). The fact that fish
showed a similarly high preference for coral and seagrass microhabitats
despite their large differences in architecture, stiffness, and color, and the fact
that individual fish preferentially chose for either seagrass or coral
independent of their structural complexity (e.g., also preferred at low cover,
rugosity, density and height), supports the notion that habitat preference is
also driven by presence of living vs. dead structure.

The question remains why fish were never significantly associated with the
structurally most complex microhabitat within a cage and did not show a
relationship with any of the structural characteristics when analyzed across
cages. This is likely explained by the fact that fish were offered microhabitats
that had characteristics of structure that operated at a higher hierarchical level
than just the degree of complexity in terms of preference by fish. There are a
multitude of studies that have manipulated structural complexity for single
habitat types and have confirmed presence of a relationship with complexity
(e.g., reviews by Orth et al. 1984, Horinouchi 2007, Mellin et al. 2009). Had we
simply offered the same microhabitat but of different structural complexities
within the cages, we would probably have found similar results as the above
studies.

Combining the results of our study and that of previous single-habitat
studies, it seems likely that a theoretical hierarchical model such as presented
in Appendix 1 could explain the different results. Although testing this model
was not the objective of the current study, as testing the proposed hierarchical
levels was confounded by habitat type, the current study provides evidence
that such a model could apply to habitat preference by demersal species. The
fact that such a model has not previously been proposed is understandable
when the current literature is examined. By far the majority of studies has
simply compared preference by fish for low vs. high complexity for single
habitat types (e.g., Heck & Orth 1980, Gratwicke & Speight 2005, Horinouchi
2007). A second line of research has focused on choice for a specific habitat
complexity (mostly low vs. high too) while introducing or removing predators
(e.g., Bell & Westoby 1986, Beukers & Jones 1997, Almany 2004). In this case,
choice of a fish is forced by the presence of predators, and therefore does not necessarily reflect the initial choice or true preference for structure type.

Limitations of habitat choice experiments are diverse, and include presence of predators, differences in food abundance, environmental effects, time of the day, and duration of the experiment. These factors did not affect our experiment as the cages excluded predators, allowed similar water flow with planktonic food items through the microhabitats, were located at a distance from other habitat structures, and were continuously rotated while also changing the mutual positioning of the microhabitats to rule out environmental effects. Additionally, fish were observed multiple times of the day during almost 24 h, showing no consistent temporal changes in preference. Once fish selected a specific microhabitat, they did not significantly change their original preference among observations, meaning that their preference for living benthic microhabitats is fixed within their behaviour and was not a result of the experimental set-up.

Coral reefs, mangrove forests, as well as seagrass beds are extensively degraded worldwide as a result of continued human impacts to marine ecosystems (Valiela et al. 2001, Duarte 2002, Hughes et al. 2003), leading to loss of living benthic structure and loss of three-dimensional complexity (Graham et al. 2006, Wilson et al. 2006, Alvarez-Filip et al. 2009). This could dramatically affect the distribution and abundance of fishes recruiting, living, or feeding on coral reefs (Jones et al. 2004, Graham et al. 2006, Wilson et al. 2006, Paddack et al. 2009). In the light of the present study, this gives great concern as fish seem to prefer live seagrass and coral structure above dead coral structure and are not attracted to unvegetated habitats. Marine protected areas are especially important in this context, as they can provide a framework for protection against habitat loss and destruction, and thus preserving habitat structural complexity.

In conclusion, although it has been shown earlier that fish prefer structurally complex habitats, this study showed that in first instance it is not habitat complexity or the degree of structure per se, but microhabitat type which is an important determinant of habitat preference by fish. Fish consistently preferred coral and seagrass microhabitats – even when offered at low cover, rugosity, height, or number of shelter holes – above dead coral rubble, sandy substratum and mangrove microhabitats, which could be driven by preference for living benthic structure. The results of the present study contribute to the current understanding of habitat selection, which still remains rudimentary. More emphasis needs to be placed on the criteria involved in habitat selection to strengthen predictions about the causes of spatial and temporal variation in the abundance and diversity of coral reef fishes. This will provide a more robust foundation for successful conservation and management of our precious marine seascape.
Acknowledgements – This project was supported by the Netherlands Organization for Scientific Research (NWO) through a VIDI grant to I.N. Additional funding for fieldwork was received from the Department of Agriculture, Husbandry and Fisheries on Aruba (DLVV). We are grateful to the staff and personnel of the Carmabi Institute on Curaçao and DLVV for their hospitality and provision of research materials and work/laboratory space. This is Centre for Wetland Ecology publication No. 535.

References


Habitat preference by reef fish


HABITAT PREFERENCE BY REEF FISH


Appendix 1. Theoretical hierarchical model proposed for habitat preference by demersal fishes. The highest hierarchical level of microhabitat preference is determined by absence vs. presence of structure (a), the second level by vertical orientation of the structure (i.e., hanging vs. standing) (b), the third level by the presence of dead vs. living structure (c), and the fourth hierarchical level by structural complexity of individual habitat types (d). The bottom illustrations always represent the preferred habitat (thick arrows).
MANGROVES AND SEAGRASS BEDS DO NOT ENHANCE GROWTH OF EARLY JUVENILES OF A CORAL REEF FISH

GROL MGG, DORENBOSCH M, KOKKELMANS EMG & NAGELKERKEN I

MARINE ECOLOGY PROGRESS SERIES
ABSTRACT

Mangroves and seagrass beds have long been considered important nursery grounds for various species of juvenile reef fishes due to their higher abundances in these habitats compared to coral reefs. It is assumed that these putative nurseries provide juveniles with more shelter, higher food availability and higher growth and survival rates than on the reef. However, this nursery function is still ambiguous, and few experimental field studies have tested this hypothesis in these tropical habitats. In the present study, the growth rate of juvenile *Haemulon flavolineatum* and the availability of its preferred food were determined in seagrass, mangrove and coral reef habitats. It was hypothesized that somatic growth rates as well as the amount of preferred food are higher within these putative nurseries than on the reef (according to the nursery hypothesis). The growth of small juveniles (3.5 to 4.2 cm total length, TL) was studied at two Caribbean islands using *in situ* experimental growth cages. Gut content analysis of the caged fishes showed that Copepoda were by far the most consumed food items by juveniles in all three habitats. Copepoda in the plankton samples were more abundant on the reef than in the mangrove/seagrass habitats. Growth rates of fishes showed the same pattern: higher growth rates in length and weight (significant for Aruba, a trend for Curaçao), and a higher weight-length ratio on the reef compared to the mangrove/seagrass habitats. Based on these observations it appears that the coral reef would be a more suitable habitat for small juveniles, when not taking other factors such as predation risk into account. Nevertheless, the highest juvenile fish abundances are found in mangrove/seagrass nurseries where predation risk, but also growth rate, is lower. Therefore, it appears that a trade-off exists between food abundance/growth rate and predation pressure/mortality risk, where fish select habitats that minimize the ratio of mortality risk to growth rate.
INTRODUCTION

Shallow-water coastal ecosystems, such as mangroves and seagrass beds, are considered important habitats for various coral reef fish species and are thought to function as nurseries for their juveniles (Parrish 1989, Beck et al. 2001). Many field studies have shown that the highest juvenile densities of some coral reef fish species are found in back-reef shallow-water habitats, whereas the highest adult densities are observed on the coral reef (Nagelkerken et al. 2000a, Eggleston et al. 2004). It is assumed that juvenile fish use habitats like mangroves and seagrasses for several months and migrate to the coral reef at the onset of maturity (Cocheret de la Morinière et al. 2002). Nagelkerken et al. (2002) identified 17 Caribbean coral reef fish species which presumably use mangroves and seagrass beds as nurseries (‘nursery species’, sensu Nagelkerken et al. 2000a). Comparative research in bays with and without mangroves and/or seagrass beds showed that juveniles of these nursery species were absent or found at very low densities in mangrove/seagrass-poor bays (Nagelkerken et al. 2001). Studies in the Indo-Pacific (Dorenbosch et al. 2005) and in the Caribbean (Nagelkerken et al. 2002) on islands completely lacking mangrove and seagrass nurseries showed that adult fishes of several nursery species were completely absent or found at very low densities on the coral reef. Based on this it has been suggested that mangroves and seagrass beds contribute to the fish densities on the coral reef.

Based on the above concept, Beck et al. (2001) developed a nursery-role hypothesis in which they stated that a habitat is a nursery if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other juvenile habitats. In these nursery habitats, the greater contribution to adult recruitment must be supported by any combination of the factors: (1) density, (2) growth, (3) survival, or (4) movement from the nurseries to adult habitats (Beck et al. 2001). Dahlgren et al. (2006) suggested the same, but based on total productivity of a habitat type. So far, almost all studies in the last few decades have only investigated fish densities, often in just a single habitat, and mostly using different techniques (reviewed by Heck et al. 2003). Hence, the importance of these habitats as nurseries is still ambiguous (Beck et al. 2001, Heck et al. 2003). Only in the last decade have studies focused more often on density and size distributions of reef fish in multiple habitats (e.g., mangroves, seagrass beds, algal beds, sand flats and reef flats; Nagelkerken et al. 2000a,b, Christensen et al. 2003, Eggleston et al. 2004, Chittaro et al. 2005, Dorenbosch et al. 2005). However, comparative research among mangrove, seagrass and coral reef habitats on the other three nursery-role factors (growth, survival and movement) is practically nonexistent, as has been identified by all recent reviews (Beck et al. 2001, Heck et al. 2003). The few studies which have focused
Effects of mangroves & seagrass beds on coral reef fish growth

on nursery-role factors other than density were mainly done in estuaries and salt marshes and not in coastal habitats such as mangroves, seagrass beds and coral reefs (e.g., Gillanders et al. 2003). Furthermore, the majority of the studies in estuaries and salt marshes focused on invertebrate species (e.g., lobster, shrimp, crab and conch) and not on reef fish. Hence, quantitative data are largely lacking for coral reef fish to support the nursery hypothesis. In previous studies, we identified higher juvenile fish densities in mangrove and/or seagrass habitats than on the coral reef (Nagelkerken et al. 2000b, Dorenbosch et al. 2005). In the present study, we test the factor growth to further evaluate the nursery-role hypothesis.

Most coral reef fish begin their life as planktonic larvae that eventually settle onto the coral reef or in embayments/lagoons as early juveniles. During this first benthic life stage, mortality rates are very high, largely due to predation (Shulman & Ogden 1987). At this moment, rapid growth is of crucial importance as it enables a newly settled fish to outgrow the most vulnerable size classes quickly, leaving a selective advantage over slow-growing fishes. At a certain stage, they will outgrow their initial shelter habitat. Therefore, fishes may need to shift between habitats throughout their life cycle to avoid predation or because of feeding or reproduction needs (Sutherland 1996). Selection of habitats by juvenile fishes is based on the ‘minimize μ/g hypothesis’, where fishes shift habitats in a way that maximizes growth (g) and minimizes predation or mortality (μ) risk (Dahlgren & Eggleston 2000).

The high abundance of juvenile fish and the preference of larvae to settle in mangroves and seagrass beds is likely based on the minimize μ/g hypothesis: these habitats may have lower predation risk because they provide excellent shelter against predators, a lower abundance of predators due to the distance away from the coral reef and a low predator efficiency due to relatively turbid waters in these habitats, while mangroves and seagrass beds possibly maximize growth due to a high food availability (Blaber 2000). The latter remains to be tested, however.

The aim of the present study was to test the hypothesis that juvenile coral reef fishes grow faster in mangrove and seagrass habitats than on the coral reef. This was done using experimental growth cages and studying in situ growth rates in length and weight of early juveniles of the common reef fish Haemulon flavolineatum for several months on two Caribbean islands (Curaçao and Aruba). Predation was excluded so that the factor growth could be evaluated independently. Gut contents of the fish and food abundance in the three habitat types were analysed to test whether the abundance of preferred food could be responsible for the observed juvenile growth rates.
CHAPTER 3

MATERIALS & METHODS

Study areas – The present study was carried out at two islands in the southern Caribbean Sea: at Spanish Water Bay on Curaçao, Netherlands Antilles, and along the south-western coast of Aruba (Fig. 1). Spanish Water Bay is ca. 3 km² in surface area and is situated on the south-western side of Curaçao. The embayment is relatively shallow (depth <6 m) and harbours seagrass *Thalassia testudinum* beds and fringing mangroves *Rhizophora mangle*. The fringing coral reef in front of the embayment runs along the entire southwest coast of Curaçao. It starts from the shore with a narrow submarine terrace of 50-100 m width and turns into a drop-off at 7-12 m depth that slopes steeply to 50-60 m (Bak 1975). In contrast to Curaçao, Aruba has no embayments with mangroves and seagrass beds like Spanish Water Bay, and no steep coral reef slopes are found either. Instead, Aruba has a large continental shelf with small coral cays along the south-western coast. The chain of cays forms a large lagoon with seagrass *T. testudinum* beds and mangroves *R. mangle*, and protects the coast against strong wave action and ocean currents (Fig. 1). The fringing coral reef is located south of the cays and is characterized by a shallow terrace (<8 m deep, >100 m wide) and a drop-off at 8 to 12 m from where the reef gradually slopes to a depth of 25 to 30 m, where a sandy plain starts.

The average daily tidal range on both islands is 0.3 m with a maximum of 0.55 m (de Haan & Zaneveld 1959). Horizontal water clarity ranged on average between 4.0 and 7.8 m in the mangroves and seagrass beds, and between 17.2 and 29.5 m on the coral reef (Table 1), and was measured twice a month using a Secchi disk. Study sites at Spanish Water Bay and along the south-western coast of Aruba have no freshwater input other than rain. Temperature and salinity were measured weekly using a handheld conductivity-temperature-TDS meter (YSI 30 and CyberScan CON 410 for Curaçao and Aruba, respectively) and ranged on Curaçao from 30.1 to 30.5°C and from 33.2 to 33.5, respectively, and on Aruba from 27.6 to 28.4°C and from 34.0 to 34.2, respectively, among the three habitats (Table 1).

Studied species – This study focussed on the common reef fish species French grunt *Haemulon flavolineatum*. Juveniles of this species are highly abundant in Caribbean mangroves and seagrass beds and the adults are found on the coral reef (Nagelkerken *et al.* 2000a, Christensen *et al.* 2003, Eggleston *et al.* 2004, Chittaro *et al.* 2005). This species also recruits on the coral reef, although densities are much lower than in the mangroves and seagrass beds (Shulman & Ogden 1987, Nagelkerken *et al.* 2000b). In the present study, relatively recently settled juveniles were used in the growth experiments. They feed on plankton during day time, but shift to nocturnal feeding on
Effects of mangroves & seagrass beds on coral reef fish growth

zoobenthos when they grow larger (Ogden & Ehrlich 1977, Cocheret de la Morinière et al. 2003). Studies on feeding behaviour confirmed this for juvenile H. flavolineatum in the mangroves and seagrass beds of Spanish Water Bay and showed that H. flavolineatum juveniles up to ca. 5 cm in length still feed in the water column during day time (Verweij et al. 2006).

Figure 1. (a) Research areas, locations of placement of the experimental growth cages and catch sites of juvenile Haemulon flavolineatum on (b) Curaçao and (c) Aruba. (d,e) Number of cages per site.
Table 1. Mean (± SD) water salinities, temperatures and water clarities for the three habitat types (mangroves, seagrass beds and coral reef) where experimental growth cages for juvenile *Haemulon flavolineatum* were placed at Spanish Water Bay on Curaçao (2005) and along the south-western coast of Aruba (2006), and the mean length, weight and weight–length (WL) ratio of the fish at the start of the experiment.

<table>
<thead>
<tr>
<th>Island</th>
<th>Habitat</th>
<th>Salinity (‰)</th>
<th>Temperature (°C)</th>
<th>Water (m)</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
<th>WL ratio (g cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curaçao</td>
<td>Mangroves</td>
<td>33.2 ± 0.9</td>
<td>30.5 ± 0.8</td>
<td>4.2 ± 0.6</td>
<td>4.2 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Seagrass beds</td>
<td>33.5 ± 1.0</td>
<td>30.5 ± 0.8</td>
<td>4.6 ± 1.8</td>
<td>4.0 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Coral reef</td>
<td>33.3 ± 0.7</td>
<td>30.1 ± 0.7</td>
<td>29.5 ± 3.5</td>
<td>4.0 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Aruba</td>
<td>Mangroves</td>
<td>34.2 ± 0.6</td>
<td>28.4 ± 1.1</td>
<td>4.0 ± 1.6</td>
<td>3.8 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Seagrass beds</td>
<td>34.1 ± 0.8</td>
<td>28.4 ± 1.0</td>
<td>7.8 ± 3.6</td>
<td>3.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Coral reef</td>
<td>34.0 ± 0.8</td>
<td>27.6 ± 0.8</td>
<td>17.2 ± 3.7</td>
<td>3.7 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
</tbody>
</table>

**Growth study** – Growth of juvenile *Haemulon flavolineatum* was studied in three habitat types (mangroves, seagrass beds and the coral reef) using *in situ* experimental growth cages. Predators were excluded from the cages to avoid interaction with the factor growth. Growth experiments were conducted in a total of 24 cages, with 12 at each island. In each habitat type, 8 cages (4 per island) were placed. The location of each cage depended on the availability of habitat type and its configuration (Fig. 1). At sites where 2 cages were placed (see Fig. 1), they were separated by at least 25 m. Size of a cage was $1.5 \times 1.5 \times 0.7$ m. The frameworks were constructed using iron rods ($8$ and $10$ mm in diameter for seagrass/mangrove and coral reef habitats, respectively) and covered with iron gauze with a mesh size of $6$ mm. The bottom was uncovered and exposed to the natural substratum. Closable windows ($30 \times 30$ cm) were constructed at 2 opposite sides to introduce and remove fish from the cages. At both islands, experimental cages were placed on the natural substratum in each habitat type at a depth between 1.2 and 5.8 m. The iron gauze of the cages was scrubbed twice a week to remove algal growth and to preserve a good flow of water and plankton through the cages.

Juvenile *H. flavolineatum* were caught on sandy/rubble areas located in-between the reef and the mangrove/seagrass beds (Fig. 1). Only fish with a minimum length of $3.5$ cm and a maximum of $4.2$ cm were used in the experiment. Total length (TL, to the nearest mm) and total wet weight (to the nearest mg) of each fish were measured in the field before introduction into the cages. In each cage, 20 small juveniles differing no more than $3$ mm in length were introduced, and densities within the cages were thus $8.9$ ind. m⁻². No data on typical *in situ* densities of early juveniles were available to determine fish density in the cages. However, on Aruba as well as on Curaçao early juveniles are found in large schools of up to several hundreds of individuals (authors’ personal observation, chapter 5).
juvenile *H. flavolineatum* (up to 3 cm in length) were observed by Ogden & Ehrlich (1977) on patch reefs surrounded by seagrass beds. Juvenile fish remained in the cages for on average 8 wk on Curaçao (September to October 2005) and 6 wk on Aruba (July to August 2006). At the end of the experiment, fish were recovered from the cages, re-measured in length and weight and their gut removed and frozen directly.

**Food availability** – Since the studied juvenile *Haemulon flavolineatum* were still diurnal planktivores, densities of zooplankton were quantified in the water column at each site where cages were placed in the mangroves, seagrass beds and coral reef at both islands during the two study periods. The water layer just above the substratum was sampled twice inside and twice outside each of the 24 cages by hauling a plankton net (diameter mouth 25 cm, mesh size 80 to 120 μm) 6 times along a 1 m long transect. Samples were taken inside as well as outside each cage to exclude the possibility that food availability in the cages was limiting for growth, and all samples were taken during day time when these small juvenile grunts feed. All plankton samples were preserved in 70% alcohol and dyed with Bengal Rose before the total number and type of planktonic invertebrates were quantified using a stereomicroscope. All zooplankton was categorized to taxon level. To determine the diet of the fish, the content of the entire digestive tract of at least 13 juvenile fish per habitat type per island was quantified using the same taxonomic levels as for the food samples.

**Data and statistical analysis** – For each individual fish, the mean growth rate d⁻¹ in length (GL) was calculated as:

\[
GL = \frac{(L_f - L_i)}{t} \quad \text{d}^{-1}
\]

where \(L_f\) is the length of each individual fish at the end of the experiment, \(L_i\) is mean length of fish per cage at the start of the experiment calculated by averaging the fork lengths of all introduced juvenile fish per cage, and \(t\) is duration of the experiment in days for each individual fish. \(L_i\) was based on mean values since fish were not tagged individually and their individual initial weight or length could therefore not be identified at the end of the experiment. The growth rate d⁻¹ in weight (GW) was calculated similarly using weight data. In addition, the weight–length (WL) ratio of each fish removed from the cages was calculated. The WL relationship of captured fish is often used to assess growth (e.g., Keys 1928). Mean growth in length and weight and mean WL ratio per habitat were calculated by pooling all individual fish per habitat for each island and testing for statistical differences among habitats using 1-way ANOVAs. Homogeneity of variances was tested with a Levene’s test. Multiple comparisons of means among the three different
h{}abitats were done using a Hochberg post-hoc test. One cage on the reef of Curacao and 2 cages on the reef of Aruba were not included in the analysis, because these cages were destroyed and all juveniles escaped.

The total number of individuals of all taxa found in the plankton samples was pooled per habitat and expressed per m$^3$. Independent $t$-tests were used to test whether there was a significant difference in the number of Copepoda (the main food source of juvenile *Haemulon flavolineatum*) in the plankton samples between the inside and outside of the cages for the three habitats separately per island. Copepoda densities in the plankton were compared among the three habitats (separately for inside and outside the cages) using a 1-way ANOVA after log ($x + 1$) data transformation. Homogeneity of variances was tested with a Levene’s test, and multiple comparisons in densities between habitats were analysed using a Tukey HSD post-hoc test.

The number of food items of all taxa found in the entire digestive tract of a juvenile were quantified by eye and calculated as percentages of the total number found per taxa.

Data were analysed separately for each island, and all statistical analyses were performed using SPSS (version 14.5). Differences were significant if $P$-values were $\leq0.050$.

**RESULTS**

On Aruba, a significantly higher mean GL and GW of *Haemulon flavolineatum* was found on the coral reef compared to the mangrove/seagrass habitats (Figs. 2a,b, Tables 2 & 3). The mean GL and GW of fish on Curacao were highest on the reef, although no significant difference was found among the three habitat types. At both islands, the mean WL ratio was significantly higher on the coral reef than on the seagrass beds (Fig. 2c, Tables 2 & 3). In addition, WL ratios were significantly higher on the reef than in the mangroves on Aruba. GL, GW and WL ratios did not differ significantly between mangroves and seagrass beds at either of the islands.

Juvenile *H. flavolineatum* growth rates ranged from -0.2 to 0.7 mm d$^{-1}$ and from -10.0 to 60.0 mg d$^{-1}$, while the WL ratio at the end of the experiment ranged from 5.1 to 50.0 mg mm$^{-1}$ (Fig. 3). On Aruba, the mode of the frequency distribution of all three variables on the reef was skewed to the larger growth classes compared to that of mangroves/seagrass beds, whereas on Curacao the mode was more similar among habitats. On Curacao, the three variables all showed a small peak for the larger growth classes.
Figure 2. (a) Mean (+ SE) growth in length (GL), (b) mean (+ SE) growth in weight (GW) and (c) mean (+ SE) weight-length (WL) ratio of juvenile *Haemulon flavolineatum* kept in experimental cages in mangroves (MG), seagrass beds (SG) and coral reefs (CR) on Curacao and Aruba. Different letters (A, B) represent significant differences among the 3 habitat types per island.
**Table 2.** Results of 1-way ANOVA on mean growth in length (GL), mean growth in weight (GW) and mean weight-length (WL) ratio of juvenile *Haemulon flavolineatum* comparing the three habitat types mangroves, seagrass beds and the coral reef for Curaçao and Aruba.

<table>
<thead>
<tr>
<th>Island</th>
<th>Variable</th>
<th>P-value</th>
<th>df</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curaçao</td>
<td>GL</td>
<td>0.179</td>
<td>2</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>0.062</td>
<td>2</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>WL ratio</td>
<td>0.018</td>
<td>2</td>
<td>4.20</td>
</tr>
<tr>
<td>Aruba</td>
<td>GL</td>
<td>&lt;0.001</td>
<td>2</td>
<td>11.21</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>&lt;0.001</td>
<td>2</td>
<td>32.95</td>
</tr>
<tr>
<td></td>
<td>WL ratio</td>
<td>&lt;0.001</td>
<td>2</td>
<td>30.93</td>
</tr>
</tbody>
</table>

**Table 3.** P-values of multiple post-hoc comparisons (Hochberg’s test) among the three habitat types mangroves, seagrass beds and coral reef, separately for growth in length (GL), growth in weight (GW) and weight-length (WL) ratio of *Haemulon flavolineatum*, for Curaçao and Aruba.

<table>
<thead>
<tr>
<th>Island</th>
<th>Variable</th>
<th>Habitat</th>
<th>Mangroves</th>
<th>Seagrass beds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curaçao</td>
<td>GL</td>
<td>Seagrass beds</td>
<td>0.511</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>0.864</td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>Seagrass beds</td>
<td>0.557</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>0.397</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>WL ratio</td>
<td>Seagrass beds</td>
<td>0.471</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>0.232</td>
<td>0.014</td>
</tr>
<tr>
<td>Aruba</td>
<td>GL</td>
<td>Seagrass beds</td>
<td>0.151</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>Seagrass beds</td>
<td>0.172</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>WL ratio</td>
<td>Seagrass beds</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 3. Frequency distributions of (a) growth rate in length, (b) growth rate in weight and (c) weight-length ratio classes for *Haemulon flavolineatum* juveniles recovered from the experimental growth cages in mangroves (MG), seagrass beds (SG) and on the coral reef (CR) at the end of the experiment on Curaçao and Aruba.
In total, 80 juvenile *H. flavolineatum* were introduced in the experimental growth cages per habitat type per island. In Curaçao, 43% of the fishes were recovered from the mangrove cages, 50% from the seagrass cages and 24% from the reef cages, while on Aruba 38, 54 and 19% were recovered from the mangrove, seagrass and reef cages, respectively.

Gut content analysis showed that the diet of juvenile *H. flavolineatum* mainly consisted of Copepoda (mean range 48 to 82%, Table 4). Tanaidacea (0 to 25%) and Ostracoda (2 to 21%) formed a less important component of their diet. On the coral reef at both islands, juveniles consumed more Copepoda (>75%) than in the mangroves and seagrass beds, while the contribution of Tanaidacea and Ostracoda to the diet was larger in the latter two habitats than on the coral reef.

**Table 4.** Gut contents of juvenile *Haemulon flavolineatum* recovered from experimental growth cages in mangroves, seagrass beds and coral reef on Curaçao and Aruba. The mean (± SD) length and weight of the recovered fishes (n) and the mean percentage of Copepoda, Tanaidacea, Ostracoda and Rest group (all other less-common food species pooled) in the entire digestive tract.

<table>
<thead>
<tr>
<th>Island</th>
<th>Habitat</th>
<th>n</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
<th>Copepoda (%)</th>
<th>Tanaidacea (%)</th>
<th>Ostracoda (%)</th>
<th>Rest group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curaçao</td>
<td>Mangroves</td>
<td>13</td>
<td>4.9 ± 0.6</td>
<td>0.9 ± 0.3</td>
<td>63 ± 19</td>
<td>16 ± 14</td>
<td>13 ± 11</td>
<td>8 ± 7</td>
</tr>
<tr>
<td></td>
<td>Seagrass beds</td>
<td>13</td>
<td>4.5 ± 0.5</td>
<td>0.8 ± 0.5</td>
<td>48 ± 16</td>
<td>25 ± 27</td>
<td>18 ± 21</td>
<td>10 ± 9</td>
</tr>
<tr>
<td></td>
<td>Coral reef</td>
<td>13</td>
<td>4.3 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>76 ± 19</td>
<td>0 ± 0</td>
<td>2 ± 5</td>
<td>23 ± 20</td>
</tr>
<tr>
<td>Aruba</td>
<td>Mangroves</td>
<td>13</td>
<td>4.3 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>50 ± 20</td>
<td>17 ± 14</td>
<td>11 ± 15</td>
<td>21 ± 21</td>
</tr>
<tr>
<td></td>
<td>Seagrass beds</td>
<td>13</td>
<td>4.5 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>53 ± 30</td>
<td>19 ± 18</td>
<td>21 ± 23</td>
<td>7 ± 8</td>
</tr>
<tr>
<td></td>
<td>Coral reef</td>
<td>15</td>
<td>4.9 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>82 ± 13</td>
<td>4 ± 5</td>
<td>4 ± 7</td>
<td>10 ± 8</td>
</tr>
</tbody>
</table>

Copepoda, the main food source of juvenile *H. flavolineatum*, showed no significant difference in density between the plankton samples collected inside and outside the cages in any of the three habitat types of either island, except for the mangrove cages on Curaçao where a significantly higher number of Copepoda was found outside the experimental growth cages (Table 5). In Curaçao, the total density of Copepoda was always significantly higher on the coral reef compared to mangroves and seagrass beds, inside as well as outside the cages (Fig. 4, Tables 5 & 6). On Aruba, Copepoda densities did not differ significantly among habitats inside the cages (although there was a trend of higher densities on the reef), and outside the cages the reef only harboured a significantly higher Copepoda density than the mangroves.
Figure 4. Copepoda density (+ SE) in the plankton inside and outside the experimental growth cages in the mangroves (MG), seagrass beds (SG) and coral reef (CR) on Curaçao and Aruba. Different letters (A, B) represent significant differences among the three habitat types, per island, and separately for inside and outside the cages.

Table 5. Results of independent t-tests on total Copepoda density in the plankton comparing the density inside and outside the experimental growth cages (within habitats), and results of 1-way ANOVAs on total Copepoda density in the plankton comparing the three habitat types mangroves, seagrass beds and coral reef for Curaçao and Aruba, inside (in) and outside (out) the experimental growth cages.

<table>
<thead>
<tr>
<th>Island</th>
<th>Habitat</th>
<th>P-value t-test</th>
<th>df</th>
<th>F</th>
<th>P-value ANOVA</th>
<th>df</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curaçao</td>
<td>Mangroves</td>
<td>0.012</td>
<td>14</td>
<td>5.50</td>
<td>&lt;0.001</td>
<td>2</td>
<td>16.54</td>
</tr>
<tr>
<td></td>
<td>Seagrass beds</td>
<td>0.573</td>
<td>14</td>
<td>1.08</td>
<td>0.002</td>
<td>2</td>
<td>8.08</td>
</tr>
<tr>
<td></td>
<td>Coral reef</td>
<td>0.234</td>
<td>14</td>
<td>2.61</td>
<td>0.096</td>
<td>2</td>
<td>2.64</td>
</tr>
<tr>
<td>Aruba</td>
<td>Mangroves</td>
<td>0.510</td>
<td>14</td>
<td>0.05</td>
<td>0.096</td>
<td>2</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>Seagrass beds</td>
<td>0.128</td>
<td>14</td>
<td>0.38</td>
<td>0.055</td>
<td>2</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>Coral reef</td>
<td>0.338</td>
<td>13</td>
<td>4.67</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. *P*-values of multiple post-hoc comparisons (Tukey HSD) among the three habitat types mangroves, seagrass beds and coral reef, separately for Copepoda densities in the plankton inside (in) and outside (out) the experimental growth cages, for Curaçao and Aruba.

<table>
<thead>
<tr>
<th>Island</th>
<th>in/out cage</th>
<th>Habitat</th>
<th>Mangroves</th>
<th>Seagrass beds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curaçao</td>
<td>in</td>
<td>Seagrass beds</td>
<td>0.042</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>&lt;0.001</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>out</td>
<td>Seagrass beds</td>
<td>0.912</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>0.010</td>
<td>0.004</td>
</tr>
<tr>
<td>Aruba</td>
<td>in</td>
<td>Seagrass beds</td>
<td>0.227</td>
<td>0.862</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>0.101</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>out</td>
<td>Seagrass beds</td>
<td>0.676</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coral reef</td>
<td>0.048</td>
<td>0.232</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results from the present study show that the mean growth of early juvenile *Haemulon flavolineatum* is not significantly higher within mangrove and seagrass nurseries as hypothesized earlier, but that on Aruba, instead, the fastest GL and GW, and highest WL ratios were found on the coral reef. A trend of a higher GW and GL on the reef was visible on Curaçao, while the WL ratio was significantly higher on the reef than on the seagrass beds. Individual growth of fish depends on several factors, such as food availability, food consumption, fish condition, predator pressure, competition and abiotic variables such as water temperature and salinity (e.g., Baltz et al. 1998, Connell 1998, McCormick 1998, Boeuf & Payan 2001). The most likely factor explaining the differences in growth rates among habitat types in this experiment is food abundance; most other factors can be excluded here as discussed farther below.

Recently settled larvae and juvenile *H. flavolineatum* are diurnal planktivores (Ogden & Ehrlich 1977) up to ca. 5 cm in length (Verweij *et al.* 2006). At the end of the experiment, caged fishes in our experiment averaged between 4.3 and 4.9 cm in length among habitats on both islands, and they were still diurnal planktivores (authors’ personal observation). Gut content analysis of the caged fishes showed that they primarily consumed Copepoda, with some additional feeding on Tanaidacea and Ostracoda in mangroves and seagrass beds. The diet composition of uncaged juvenile *H. flavolineatum* between 2.5 and 5.0 cm in length at Spanish Water Bay showed a similar result, in which mainly Copepoda (>83%) and some Tanaidacea (12%) were
found in their digestive tracts (Cocheret de la Morinière et al. 2003). Also in an embayment in Bermuda fish of the genus Haemulon, including H. flavolineatum, showed a strong selection for harpacticoids, an order of the subclass Copepoda (Alheit & Scheibel 1982). Hence, it is clear that small-sized juvenile H. flavolineatum typically feed on Copepoda under natural conditions.

Cage experiments were used in the present study to follow growth of juvenile fish in the field under practically natural environmental and habitat conditions. Cages did not affect the food availability, since the commonly consumed food type (Copepoda) in the plankton inside and outside the cages did not show a significant difference in density in the three habitat types of the two islands, except for the mangrove cages on Curaçao where somewhat more food was available outside the cages. In all plankton samples in the three habitat types Copepoda showed the highest abundance, while all other taxa were less common or completely absent. A higher number of Copepoda was found on the coral reef compared to the seagrass beds and mangroves. Also growth rates for small juvenile H. flavolineatum were highest on the coral reef of Aruba compared to the mangrove and seagrass nurseries, while such a trend was also present for Curaçao. This suggests that if all other factors are excluded, a higher availability of food on the coral reef could have resulted in the higher growth rates of small juvenile H. flavolineatum on the reef of Aruba and possibly also on Curaçao. This would only be true, however, if food is limiting in these two bay habitats. Nevertheless, many studies have found much higher juvenile fish densities within the mangrove and seagrass beds compared to the coral reef, including for H. flavolineatum (Nagelkerken et al. 2000a, Eggleston et al. 2004). This indicates that not only food availability is an important factor for juvenile fish when selecting a settlement habitat and raises the question: Why do recruits not settle massively on the coral reef where food availability can be higher?

Habitat selection and use by fishes are based on a strategy which maximizes feeding and growth rates and minimizes mortality risk by avoiding predation (Werner et al. 1983, Werner & Hall 1988, Dahlgren & Eggleston 2000). Therefore, it is assumed that fish shift habitats so that the ratio of mortality risk to growth rate is minimized. In vegetated habitats an increased amount of food, which can indirectly increase growth rates, and an increased refuge from predators are advantages for juvenile fishes (Sogard 1992). However, often a habitat which contains more food is also a habitat in which fishes experience a higher predation risk (Werner et al. 1983, Werner & Hall 1988).

In the present study, a higher amount of food and higher growth rates (significant for Aruba, a trend on Curaçao) were found on the reefs of both islands compared to the mangrove and seagrass beds, but according to other studies a higher predation risk is also found on the reef compared to back-reef habitats (Shulman 1985, Chittaro et al. 2005). This was also true for predation
on juvenile *H. flavolineatum* (ranging from 3.1 to 4.5 cm TL) in a tethering experiment in the same three habitat types at Spanish Water Bay during the same time period, where survival after 90 min of tethering was 0% on the coral reef versus 10 to 80% in mangroves and seagrass beds (depending on the distance to the reef) (Dorenbosch *et al.* 2009). It thus appears that for minimization of mortality rates, mangrove and seagrass beds are more attractive than the coral reef, while for maximization of growth rates the reef is often more suitable. Juveniles therefore possibly have to make a decision between living on the reef, with higher food abundances but more predation risk, or living in safer lagoonal environments with less food; *i.e.*, there appears to be a trade-off between food/growth rates and predation/mortality risk. The higher abundances of early juvenile *H. flavolineatum* in mangroves or seagrass beds than on the reef thus suggests that a habitat with reduced predation risk is apparently more attractive than a habitat with higher food abundances.

Besides food availability and predation risk, various other factors can influence growth rates of fish, but they are assumed to be negligible in the present study. Mortality of caged juveniles during the experiments could hypothetically have affected growth rates by reducing the total number of fish per cage and leaving more space and food for the surviving fish. However, most juveniles disappeared late in the second half of the experiment, which suggests that mortality did not affect growth. Other studies also demonstrated that growth rates of juveniles were not significantly related to the density of fishes in a cage (Sogard 1992). Water depth of the cages, water temperature and salinity were equal among the habitats and could thus not have affected growth. Water clarity could potentially play an indirect role in fish growth, since a high water turbidity could result in a lower visual foraging efficiency and reduce feeding success, which could decrease growth rates (Grecay & Targett 1996). The mangroves and seagrass beds in the present study indeed showed a much lower water clarity compared to the reef, which could indicate that juveniles on the reef fed more efficiently and therefore grew faster there compared to the mangrove/seagrass nurseries. However, all fishes were caged and could only forage within the boundaries of the cages, which were much smaller than the minimum water clarity. Therefore, water clarity did probably not contribute to differences in growth rates among habitats.

The present study shows that caged juvenile *H. flavolineatum* at two Caribbean islands on average grew faster on the coral reef on Aruba compared to the putative mangrove and seagrass nursery habitats, while such a trend was also visible for Curaçao. A lower growth rate in these potential nurseries is possibly the result of a lower Copepoda abundance in the water layer, the food source which is most consumed by small juvenile *H. flavolineatum* according to the analysed gut contents and other published studies (Alheit & Scheibel 1982, Cocheret de la Morinière *et al.* 2003). These results suggest that the coral reef serves as a more suitable habitat in some areas for small juvenile
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*H. flavolineatum*, in terms of growth and food availability. This contrasts with the nursery-role hypothesis as formulated by Beck *et al.* (2001). Probably due to the high predation pressure on the reef, juvenile densities are higher in mangroves and seagrass beds. It should be noted, however, that in the present study small juveniles were used and that these trade-offs may change with ontogeny of the fishes, where mangroves and seagrass beds may become more important than the reef in terms of benthic food abundance for large juvenile *H. flavolineatum*, which turn to nocturnal benthic feeding.

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**References**


Effects of mangroves & seagrass beds on coral reef fish growth


Piscivore assemblages and predation pressure affect relative safety of some back-reef habitats for juvenile fish in a Caribbean bay

Dorenbosch M, Grol MGG, de Groene A, van der Velde G & Nagelkerken I

Marine Ecology Progress Series
PISCIVORES & PREDATION IN BACK-ReEF HABITATS

ABSTRACT

An important process thought to drive habitat selection during (post-) settlement of coral reef fish is predation. It is assumed that in back-reef habitats such as seagrass beds and mangroves predation is lower than on coral reefs. However, recent studies have suggested that significant piscivore assemblages are present in back-reef habitats. The assumption of reduced predation pressure in back-reef habitats can therefore be debated. We compared piscivore assemblages along the coast of a Caribbean island using underwater visual census surveys in a spatial gradient of coral reefs, seagrass beds and mangroves. We also performed predation experiments in combination with video observations in these habitats using tethered, recently-settled Haemulon flavolineatum. High piscivore densities and reduced survival showed the presence of significant piscivore assemblages in both reef and back-reef habitats. Both reef-associated piscivores and piscivores that use back-reef habitats as a juvenile habitat contributed to the piscivore assemblage in the investigated gradient. Habitat type had a significant effect on the composition of the piscivore assemblage, density, and prey survival. Piscivore density was highest on the reef, mangroves and notches, all of which are habitats with a high structural complexity. On the contrary, seagrass beds showed lowest piscivore density. Tethering experiments showed lowest survival on the reef but also reduced survival on back-reef habitats located close to the coral reef. Seagrass beds and mangroves located farther away from the reef into the bay showed highest survival. The present study shows that the concept of reduced predation in back-reef habitats used by juvenile fish does not apply to all habitats, since these habitats can harbour significant piscivore assemblages. However, depending on the spatial setting in the seascape (distance to the reef and presence of other habitat types) some Caribbean seagrass beds show low densities of piscivores and increased survival when compared to other habitats.
INTRODUCTION

Predation on early recruits of marine fish is considered an important mechanism that structures species assemblages in tropical shallow-water habitats by reducing densities and influencing size distributions as well as affecting competition (Hoey & McCormick 2004, McCormick & Hoey 2004, Hixon & Jones 2005, Almany & Webster 2006). Many species of coral reef fishes may reduce post-settlement mortality from predation by 2 strategies. (1) Recently-settled recruits on coral reefs may have an adaptive growth strategy. Individuals with higher growth rates or compensatory growth show higher survival because predation on coral reefs is concentrated on the smaller recruits (Vigliola & Meekan 2002, Hoey & McCormick 2004, Holmes & McCormick 2006, Gagliano & McCormick 2007). (2) Recruits may avoid predation on coral reefs by direct settlement into back-reef habitats located away from the coral reef, such as seagrass beds and mangroves (Watson et al. 2002, Arvedlund & Takemura 2006, Pollux et al. 2007). Back-reef habitats are thought to have a lower predation pressure and thus a higher post-settlement survival of small juvenile fish (Beets 1997, Dahlgren & Eggleston 2000). In combination with other factors that increase growth and survival, such as high food availability, high water temperature and refuge from physical disturbance (Blaber 2000, Beck et al. 2001), shallow-water habitats such as seagrass beds and mangroves are attractive habitats for juvenile coral reef fishes. After spending their juvenile life phase in these habitats, these fishes may replenish the adult population on a coral reef via ontogenetic migration (Verweij et al. 2008). When the per capita production of juveniles from these habitats to the adult stock population is higher than from other habitats, these habitats function as nurseries (Beck et al. 2001).

Decreased predation pressure is frequently mentioned as an explanation for the high juvenile fish densities in back-reef habitats (e.g., seagrass beds and mangroves). However, there is no consensus with respect to this assumption. Various studies that have indicated lower predation pressure in seagrass beds and/or mangroves also showed substantial variation in predation, suggesting predation may be confounded with other effects such as complexity and spatial configuration of the habitat (Shulman 1985, Nakamura & Sano 2004, Chittaro et al. 2005). Eggleston et al. (1997) and Almany (2004) demonstrated that predation of small fish on coral reefs may be reduced by the protective capacity provided by the structural complexity of the reef. Relatively high densities of piscivores can occur in shallow estuaries, suggesting that predation in back-reef habitats may be considerably higher than generally assumed (Nakamura & Sano 2004, Baker & Sheaves 2005, 2006, 2007, Kulbicki et al. 2005). However, no studies have assessed the assemblage and density of predators in a coral reef-seagrass-mangrove seascape in combination with
experimental assessment of survival. Therefore, in this study we combined measurements of predator assemblages and densities in multiple habitats with experiments of tethered fish that assessed relative survival across a gradient of back-reef to nearshore reef habitats.

Although high selective mortality of juvenile fish from predation has predominantly been reported from coral reefs, we hypothesize that predation pressure on small juvenile fish in back-reef habitats may be significant, and possibly similar to predation pressure on the coral reef. Back-reef habitats may border a coral reef, which facilitates entry of reef-associated piscivores to these non-reef habitats for feeding (Baker & Sheaves 2007, Dorenbosch et al. 2007, Valentine et al. 2007). Aside from this, back-reef habitats located farther away from the coral reef may harbour resident populations of piscivores (Baker & Sheaves 2005), including larger juvenile piscivorous fish (Eggleston et al. 2004). Within these back-reef habitats, structurally complex habitats, such as rocks and mangroves, may concentrate fish, including piscivores. This could result in differences in predation risk on small juvenile fish among back-reef habitats, and subsequently lead to habitat segregation of size classes (Mittelbach & Chesson 1987). To investigate the existence of differences in predation pressure between shallow-water habitat types in the field, the following questions were addressed: (1) Is piscivore density and predation risk for small juvenile coral reef fishes after settlement influenced by shallow-water habitat type and distance to the coral reef? (2) How does the composition of piscivore species assemblages and size structure distribution change in a gradient of back-reef to coral reef habitats?

**Materials & Methods**

**Study area and study sites** – The present study was conducted in the southern Caribbean Sea on the island of Curaçao, Netherlands Antilles (Fig. 1). Twelve study sites were selected on the leeward side of the island at Spanish Water Bay and on the adjacent coral reef (Fig. 1). Spanish Water Bay is a sheltered marine bay connected to a fringing coral reef by a narrow entrance channel (water depth ~15 m) that opens into a wide and shallow bay area. Within the bay, seagrass beds are present together with stands of mangroves. In areas where the shore consists of a fossilized limestone plateau, rocks, notches and crevices characterize the shoreline of the bay. The entrance of the bay consists of a large sandy plateau (water depth ~6 m), lined by degraded reef and rubble. In front of the bay is a continuous fringing coral reef consisting of a reef flat (water depth ~5 m) that steeply drops off at a water depth of ~8 m.
Figure 1. Island of Curaçao and the 12 study sites at Spanish Water Bay. Study sites are located in a 2500 m distance gradient with respect to the reef (see ‘Materials and Methods’): bay (sites 1–2), channel (sites 3–5), entrance (sites 6–10) and fringing coral reef (sites 11–12). Arrows indicate sites where predation experiments were conducted. Site:replicates show each site (number) and the number of replicate transects for predator density counts.
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Tidal range in the area is ~30 cm and only weak tidal currents are present; all habitats stay inundated during the tidal cycles with a minimum water depth of ~50 cm during low tide (mangroves and notches). Influence of the tidal cycles on the results of the present study is therefore considered very low. Mean water temperature in the study period (August to November 2005) was 28.7°C on the reef and 30.9°C in the bay, mean salinity was 33.9 on the reef and 34.5 in the bay, underwater visibility ranged between 15 and 33 m (horizontal Secchi disk distance) on the reef (mean 28.3 m) and between 3 and 14 m in the bay (mean 6.7 m).

In a 2500 m gradient stretching from the fringing coral reef to the centre of the bay, 12 study sites were selected, representing all major shallow-water habitat types of the study area (Fig. 1): seagrass beds (n = 3), mangroves (n = 3), notches, crevices and rocks (hereafter referred to as notches, n = 2), and coral reefs (n = 4). Seagrass beds consisted of monospecific beds of *Thalassia testudinum* with 100% canopy cover whereas mangroves stands consisted of *Rhizophora mangle* with a dense prop root system. Water depth of seagrass beds, mangroves and notches was ~1 m. Two reef sites (depth ~3 m) were located in the entrance of the bay on a sandy slope and were characterized by low live coral cover (average 5%) and dominated by rubble and degraded hard corals. The 2 other reef sites (depth ~5 to 8 m) were located outside the bay and were connected to the continuous fringing coral reef along the coast of Curaçao; live coral coverage at these 2 sites (average 41%) was considerably higher than at the 2 reef sites in the entrance of the bay. Water depth of reef sites ranged between 3 and 5 m.

The sites selected are located in an area of Spanish Water Bay in which previous work has shown high densities of juvenile fish in seagrass beds, mangroves, notches and the shallow coral reef (Nagelkerken *et al.* 2000a, Cocheret de la Morinière *et al.* 2002, Pollux *et al.* 2007). Depending on the species, densities of small juvenile fish (<7.5 cm) were high in either the coral reef in front of the bay, habitats in the entrance of the bay or the centre of the bay. It is assumed that shallow-water habitats in the bay function as an important habitat for juveniles of some fish species that use the coral reef as an adult habitat.

**Visual census of piscivore assemblages** – To identify the fish species assemblages, densities and sizes of potential piscivores, we conducted underwater visual census surveys at all study sites using belt transects as used by Nagelkerken *et al.* (2000a). All fish species for which fish is part of the diet (see references in ‘Data analyses and statistics’) and other potential predators (*e.g.*, squid, octopus) were recorded during the surveys. Visual census surveys were conducted by 2 independent observers using SCUBA at sites with a water depth >1 m, whereas snorkelling gear was used at shallower sites (seagrass beds, mangroves, notches). Observers were well trained with respect
to visual census methodology (i.e., species identification and estimation of numbers and sizes of fish) a priori to surveys. Transect size was $25 \times 4$ m at all sites with the exception of notches ($25 \times 3$ m) and mangroves ($10 \times 2$ m), where spatial complexity allowed only smaller transects. The submersed prop root system of the mangroves was on average 2 m wide. Due to relatively clear water (underwater visibility >3 m), fishes hiding in the root system of the mangroves could be well detected during the visual census surveys. Permanent transects were placed randomly at each site and the number of replicate transects per site varied between 4 and 8, depending on the surface area of the habitat. The number of transects per site is listed in Fig. 1. The sequence of transects that were surveyed each time at each site was random. Minimum distance between replicate transects was 12.5 m. Five independent visual census surveys were performed in each transect from August to November 2005, between 09:00 and 13:00 h. Time between surveys ranged from 3 to 12 days. Since predation may also be significant at night (Danilowicz & Sale 1999) and the piscivore assemblage may be underestimated by merely conducting surveys during the day (Unsworth et al. 2007), all transects were also surveyed once at night to obtain information on the structure of the piscivore assemblage at night. Surveys performed at night started 1 h after sunset, when the period of twilight migrations of fishes ended. At night, transects were carefully searched with a high intensity underwater torch according to Nagelkerken et al. (2000fr).

All potential piscivores were identified underwater and size (total length, TL) of each fish was estimated to the nearest cm. Underwater estimation of fish size was intensively trained simultaneously by the observers prior to the surveys.

**Predation experiments** - In addition to the visual census surveys of the piscivore assemblages of the study sites, we conducted predation experiments at 8 sites along the gradient to study differences in predation pressure among habitats (Fig. 1): seagrass beds (n = 3), mangroves (n = 3), and reefs (n = 2). Experiments were conducted by examining removal of 203 tethered juvenile *Haemulon flavolineatum* by predators in 90-min time periods. In total, 74 *H. flavolineatum* were tethered on seagrass beds, 80 in mangroves, and 49 on the reefs in the entrance and outside the bay (see Table 2). Removal experiments of tethered fish are regularly used to measure mortality by predation of coral reef associated fish (Shulman 1985, Danilowicz & Sale 1999, Dahlgren & Eggleston 2000, Chittaro et al. 2005). Because *H. flavolineatum* occurs in high densities in the bay habitats (Nagelkerken et al. 2000a) and also uses the reef as a juvenile habitat (Dorenbosch et al. 2004), it is suitable as a prey fish model species.

For the tethering experiments we used the smallest available juveniles of recently-settled *H. flavolineatum*. Selected fish ranged between 3.1 and 4.5 cm (TL, mean 4.2 cm) and were all characterized by early juvenile body coloration
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Consequently, fish used in the experiments all represented the early juvenile life stage after settlement. All juveniles were caught by a stationary trap net at a single location in the entrance of the bay. The time between capture of juveniles and the start of tethering experiments was on average 1 h. By using a thin needle, each fish was attached through its lower jaw to a 50 cm monofilament line (diameter 0.2 mm) anchored to the substratum by an iron pole. Removal of tethered fish by piscivores or other predators was visually checked at 10-min intervals by an observer (with snorkelling gear in seagrass and mangrove habitats, SCUBA in reef habitats) for a period of 90 min. During the 90-min period, the observer waited at a distance of ~50 m from the experimental location and approached each tethered fish very briefly with a minimum distance of ~5 m after each 10-min interval to verify whether the fish had been consumed, was dead, or was alive and still active. Although this methodology may cause disturbance to predators, the procedure could be standardized very well for all sites. Since water clarity was high on the coral reef, disturbance may have been stronger there compared with habitats located in the bay; however, prior to the experiments, the method was extensively practised and evaluated during pilot studies on the coral reef (n = 20) and seagrass beds (n = 20). These pilots showed that tethered fishes were not able to break the tether line. A broken tether line was therefore considered as removal by a predator. Although the presence of an observer resulted in some disturbance of the fish community, most piscivores (such as Aulostomus maculatus, Epinephelus striatus, Lutjanus apodus and Sphyraena barracuda) returned rapidly (within 1 min) and behaved naturally. During the pilot studies, the behaviour of the tethered fishes was observed carefully. Tethered fishes did not behave naturally (e.g., resting in schools) but showed a continuous active swimming behaviour. However, this behaviour was similar among individuals and habitats and swimming continued during the entire 90-min period. Experiments were conducted at water depths >1 m. Structural complexity within each habitat type (i.e., with respect to shelter for tethered fishes) was comparable among sites and within a site; tethering experiment locations were located randomly at each site.

A prey removal experiment was considered successful when the fish behaved actively (i.e., showing swimming behaviour) during the entire 90-min period or until the moment of removal by a predator. When tethered fish were dead or inactive close to the substrate, experiments were not included in the data set. In total, 253 fish were tethered, of which 203 trials were considered successful experiments. The number of successful experiments varied per site and is listed in Table 2. Various experiments were conducted simultaneously at each site with a maximum of 8 individuals in one 90-min period. Minimum distance between experiments was 10 m. Predation experiments were run within the same timeframe as the visual census surveys (between 09:00 and 13:00 h).
In addition to the visual observations, 40% of the performed predation experiments were recorded continuously (90 min) by an underwater video camera on a tripod at a distance of ~3 m from the tethered fish to verify and identify removal of tethered fish by predators. Video observations were performed in all habitats. In total, 33 removals recorded by the video camera could be used for this purpose.

**Data analyses and statistics** – Foraging strategies differ greatly among piscivorous fish species, resulting in different diets. Some species feed entirely on fish; for other species fish is only part of their diet. Various species show an ontogenetic shift from zoobenthos and/or zooplankton feeding to fish feeding throughout their life cycle. We therefore investigated 3 diet classes for the piscivore assemblage: highly specialized piscivores (high piscivores), moderately specialized piscivores (moderate piscivores), and lowly specialized piscivores (low piscivores, Appendix 1). High piscivores depend entirely on fish feeding during their entire lives (75 to 100% of diet). Moderate piscivores frequently have fish in their diet (25 to 75% of diet), but also feed on other prey items such as zooplankton and zoobenthos. Low piscivores normally feed on other prey items, but occasionally also feed on fish (in general <25% of diet). Diet data was derived from the following studies: Rooker 1995, Duarte & Garcia 1999, St. John 1999, Nagelkerken et al. 2000b, Marnane & Bellwood 2002, Cocheret de la Morinière et al. 2003a,b, Kamukuru & Mgaya 2004, Baker & Sheaves 2005, Froese & Pauly 2005, Kulbicki et al. 2005. For various species of piscivores, a clear ontogenetic diet shift is known to occur at specific lengths (Appendix 1). Individuals observed in the present study with a TL below the size for which fish have been found to be part of the diet were considered non-piscivores and omitted from piscivore data analyses.

Optimal predator-to-prey size varies with species and predator size. In general, as predators grow, they prefer larger prey. However, some predator species shift their prey preference towards smaller prey as they grow (Floeter & Temming 2005). Scharf et al. (2000) and Kulbicki et al. (2005) demonstrated that larger piscivores can prey more efficiently on small juvenile fish than can smaller piscivores. Therefore, both small and large piscivores may be effective predators on small juvenile fish. In addition to the effect of diet type, we also investigated the effect of predator size on the composition of the total piscivore assemblage. The piscivore assemblage at each site was therefore divided into 4 size classes representing increasing predator-to-prey size ratios: 5 to 15 cm (predator-to-prey size ratio from 1.2 to 3.6), 16 to 30 cm (3.8 to 7.1), 31 to 45 cm (7.4 to 10.7), >45 cm (>11.0). These predator-to-prey size ratios were calculated *a priori* to data analysis and were based on the mean length (4.2 cm) of tethered juvenile *Haemulon flavolineatum*. The smallest observed piscivore was 5 cm.
For each piscivore species, the mean density per transect was calculated based on the 5 surveys conducted during day time. For each transect, a mean species richness was calculated based on the total species counts during the 5 surveys. For night time, data were only available for 1 survey. Subsequently, for each habitat type, mean total piscivore density and mean species richness was determined for the entire piscivore assemblage (all species pooled, transects as replicates). Additionally, mean total piscivore density was calculated for the 3 diet classes (all species pooled per diet class, transects as replicates) and the 4 size classes (all species pooled per size class, transects as replicates).

Similarity in the piscivore assemblage structure among the investigated sites in the gradient was calculated using cluster analysis of piscivore species densities (per site, all size classes pooled, transects as replicates), using the BioDiversity Pro computer program (McAleece 1997) on basis of Bray-Curtis distances and using group average linkage with log_{10} transformed mean species densities.

The effect of habitat type and location of a site in the investigated gradient (hereafter referred to as site) on mean total piscivore density and mean total species richness per habitat type was tested with separate 2-way ANOVAs for day and night data. An additional 2-way ANOVA was performed to simultaneously investigate the effect of habitat type and site on mean total piscivore density during day and night. In the latter ANOVA, densities during day and night were set as the 2 dependent variables. For all analyses, site was set as a random factor that was nested within habitat. The 4 habitat types were set as a fixed factor and transects were treated as independent replicates (see Table 1).

The effect of habitat type and site on total piscivore density of the 3 diet classes and 4 size classes was investigated using 1-way analyses of covariance (ANCOVAs). These analyses were only performed for the day time data. When using single transects as replicates for the different diet and size classes, many transects had 0 counts, which violated parametric assumptions. Therefore transects were averaged per site. The 4 habitat types were set as a fixed factor and site averages were treated as independent replicates. The effect of the distance of a site towards the coral reef on the piscivore assemblage of each site was determined by using the distance (m) of each site to the coral reef (the shortest distance fishes had to swim to reach the start of the fringing coral reef) as a covariable. ANCOVAs are summarized in Table 1.

For all analyses, assumptions for normality were checked by residual analysis and spread versus level plots, homogeneity of variance was checked by means of a Levene’s test. Data for all ANOVAs and ANCOVAs was log_{10} transformed to increase normality. Post-hoc comparisons were performed using Hochberg’s GT2 tests for 2-way ANOVAs and Sidak’s tests for 1-way ANCOVAs.
Independent linear regressions were used to further analyse the relationship between distance between a site and the coral reef, and mean total piscivore density during day and night, mean total species richness, and mean total density of the 3 diet classes and 4 size classes, respectively. For each regression either one of the variables listed above was set as the dependent variable, whereas distance of each site to the coral reef was set as the independent variable.

Prey removal data from the predation experiments was analyzed with a Kaplan-Meier survival analysis. This procedure compares survival and removal times of tethered fish among habitats based on censored and complete data. Removal of a tethered fish is considered as a complete observation, while a censored observation refers to a fish that survives the 90-min experiment time. Both overall comparison and pair-wise comparisons among the 8 investigated sites were made using the Breslow procedure, based on a generalized Wilcoxon test.

For 8 sites both survival data and data on mean total density of the piscivore assemblage were available. The effect of habitat type and distance of a site to the coral reef on prey removal efficiency and mean total piscivore density was analyzed using a 2-way ANCOVA (summarized in Table 1). Mean total piscivore density and survival (based on the tethering experiments at a site) were set as the 2 dependent variables. The 8 sites were used as replicates. Mean total piscivore density was calculated based on all transects per site. Habitat was set as a fixed factor. Distance of each site to the coral reef was set as a covariable. Data for the 2-way ANCOVA was log_{10} transformed to increase normality. All analyses described above were performed using SPSS version 14.0.

**RESULTS**

**Piscivore assemblages** – During the visual census surveys, 32 fish species were classified as piscivores (Appendix 1). Although other potential predators, such as Cephalopoda, occur on the reefs of Curacao, these were not observed during the surveys. Based on diet, 8 fish species were distinguished as high piscivores, 13 as moderate piscivores, and 11 species as low piscivores (Appendix 1). This piscivore assemblage showed a spatial distribution along the studied gradient in which 3 species groups could be distinguished (Appendix 1). Eight species occurred exclusively in habitats in the bay or both in the bay and entrance, 10 species occurred exclusively on the reef, in the entrance or in both, while 14 species occurred along the entire gradient.

Cluster analysis of piscivore assemblages showed a distinction primarily among assemblages based on habitat type (Fig. 2). Notches and mangroves
Piscivores & Predation in Back-reef Habitats

(characterized by high densities of piscivores), and seagrass beds in the entrance and channel (characterized by low densities of piscivores) showed separated clusters. Also the 2 fringing coral reefs in front of the bay clustered together, whereas the reef in the entrance formed a separate cluster. The cluster containing the seagrass bed in the centre of the bay and the reef located further in the entrance of the bay formed an exception. Piscivore assemblages of these 2 sites were most similar to each other predominantly based on comparable relative densities of *Caranx latus*, *Haemulon parra*, *Lutjanus apodus*, *L. griseus*, and *Ocyurus chrysurus* (Appendix 1).

Figure 2. Cluster analysis of the piscivore assemblage (32 species) from the 12 sites in the gradient. Bray-Curtis similarity and the group average linkage method were used to cluster log10 transformed mean species densities. For each site, mean total piscivore density during day time is shown; numbers in parentheses indicate site numbers listed in Fig. 1. Cr: coral reef, Sg: seagrass beds, Mg: mangroves, Notch: notches, cha: channel, ent: entrance.

Piscivore densities and habitat types – The variables habitat type and site (nested within habitat type) showed highly significant effects for the mean total piscivore density and species richness during day time (2-way ANOVA, Table 1). During the day, total piscivore density (Fig. 3a) and species richness (Fig. 3b) were significantly highest in the mangroves and lowest on the seagrass beds. Comparable to day time, habitat type and site showed highly significant effects for the mean total piscivore density and species richness at
night (2-way ANOVA, Table 1). During the night seagrass beds, as well as mangroves, showed significantly lowest piscivore densities (Fig. 3a). Piscivore species richness was significantly lowest in seagrass beds, but no difference was found among mangroves, notches and reefs (Fig. 3b). When total piscivore densities during day and night were compared simultaneously in an additional 2-way ANOVA, both habitat type and site also showed highly significant effects for total piscivore density during day and night (Table 1).

**Figure 3.** Mean (+ SE) total piscivore fish density (a) and species richness (b) of habitat types during day and night. Effect of habitat type and site (nested within habitat type) on fish density or species richness (see ‘Materials and Methods’) was tested on day and night data separately by 2-way ANOVAs (Table 1). Within each graph, different letters (day time; A-D) or numbers (night time; 1, 2) among habitat types indicate significant differences (Hochberg’s GT2 post-hoc comparison, $P < 0.050$).
Table 1. Overview and results (P-values) of ANOVAs and analyses of covariance (ANCOVAs) conducted in the present study. Habitat: habitat type; site: site location in gradient; distance: distance of site to coral reef. NS: not significant. aVariable nested within the main factor. bVariable was set as a covariable.

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>Main factor</th>
<th>Nested factor/ covariable</th>
<th>Dependent variable(s)</th>
<th>Data</th>
<th>Results (P-values)</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Main factor</td>
</tr>
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<td>2-way ANOVA</td>
<td>Habitat</td>
<td>Site (^a)</td>
<td>Total density piscivores</td>
<td>Day</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Species richness piscivores</td>
<td>Day</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-way ANOVA</td>
<td>Habitat</td>
<td>Site (^a)</td>
<td>Total density piscivores</td>
<td>Night</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Species richness piscivores</td>
<td>Night</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-way ANOVA</td>
<td>Habitat</td>
<td>Site (^a)</td>
<td>Total density piscivores</td>
<td>Day</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Total density piscivores</td>
<td>Night</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>Total density high piscivores</td>
<td>Day</td>
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</tr>
<tr>
<td>1-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Total density moderate piscivores</td>
<td>Day</td>
<td>0.011</td>
</tr>
<tr>
<td>1-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Total density low piscivores</td>
<td>Day</td>
<td>NS</td>
</tr>
<tr>
<td>1-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Total density piscivores 0–15 cm</td>
<td>Day</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Total density piscivores 15–30 cm</td>
<td>Day</td>
<td>0.050</td>
</tr>
<tr>
<td>1-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Total density piscivores 30–45 cm</td>
<td>Day</td>
<td>0.015</td>
</tr>
<tr>
<td>1-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Total density piscivores &gt;45 cm</td>
<td>Day</td>
<td>0.028</td>
</tr>
<tr>
<td>2-way ANCOVA</td>
<td>Habitat</td>
<td>Distance (^b)</td>
<td>Survival tethered fishes</td>
<td>Tethering</td>
<td>0.011</td>
</tr>
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<td></td>
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</tbody>
</table>

Notes: p < 0.05 is considered statistically significant.
As for total piscivore densities, mean densities of high, moderate, and low piscivores were lowest on seagrass beds (Fig. 4). However, significant effects of habitat type were only observed for high and moderate piscivores (1-way ANCOVA, Table 1). For both high and moderate piscivores, seagrass beds showed significantly lower densities than mangroves (Fig. 4). Notches and reefs did not differ significantly from seagrass beds and mangroves with respect to densities of these 2 groups of piscivores. The covariable distance indicated no significant effect (Table 1). Densities of low piscivores did not differ significantly among any habitat.

**Figure 4.** Mean (+ SE) density distribution of the piscivore assemblage in the 4 habitat types during day time classified into 3 diet classes (high, moderate, and low piscivores, see 'Materials and Methods' and Appendix 1). The effect of habitat type (main factor) and distance to the coral reef (covariable) on piscivore density was tested for each diet class separately by 1-way analysis of covariance (Table 1). Different letters (moderate piscivores; A, B) and numbers (high piscivores; 1, 2) among habitat types indicate significant different fish densities (Sidak’s post-hoc comparison, $P < 0.050$), mean fish densities of low piscivores did not significantly differ.
Significant effects of habitat type were also observed with respect to different size classes within the piscivore assemblage. For piscivores >15 cm, densities in seagrass beds were considerably lower than in the other habitat types (Fig. 5). Although there were only significant differences between densities of piscivores of 31 to 45 cm on mangroves and seagrass beds, (1-way ANCOVA, Table 1), densities were high on the reef, mangroves and notches, whereas lowest densities were observed on seagrass beds (Fig. 5). Likewise, densities of piscivores >45 cm were only significantly different between the coral reef and seagrass beds (1-way ANCOVA, \( P = 0.015 \)), with lowest densities observed on seagrass beds. The covariable distance had a significant effect on densities of both the >45 cm and 31 to 45 cm size classes (Table 1). Small piscivores (≤15 cm) showed a pattern that differed from these 2 larger size classes (Fig. 5). Densities of these small piscivores were significantly higher in notches and mangroves than on the reef and seagrass beds (1-way ANCOVA, Table 1). The covariable distance did not have a significant effect on densities of these piscivores, and no significant effect was found for densities of 16 to 30 cm piscivores.

**Distance to the coral reef** – To analyze the effect of distance to the coral reef on the mean density or species richness of the piscivore assemblage at all sites in the gradient (including all habitat types), we performed linear regression analyses on the total piscivore density (day and night), the 3 diet classes and the 4 size classes of piscivores. Only two of these regressions showed significant relationships. Both mean density of piscivores >45 cm during day time \((P = 0.035, R^2 = 0.37, \beta = -0.61)\) and night time \((P = 0.019, R^2 = 0.44, \beta = -0.66)\), showed a negative relationship with increasing distance from the coral reef. However, both \(R^2\)-values were low and explained only a small part of the variation.

**Video observations and predation experiments** – Besides visual observations in 10-min time intervals, video observations of removals of 33 tethered fishes could be used to visually identify the predator (Table 2). Prey removals were recorded on the reef \((n = 18)\), seagrass beds \((n = 9)\) and mangroves \((n = 6)\). Most prey removals were performed by piscivorous fish \((n = 24,73\%, \text{Table 2})\). The remaining prey removals \((n = 9,27\%)\) were performed by larger Malacostraca \((n = 3,9\%)\) or non-piscivorous fish \((n = 6,18\%)\). All prey removals on the reef were by non-piscivorous fish were observed on the reef, whereas all observed non-piscivore prey removals on seagrass beds \((n = 2)\) and mangroves \((n = 1)\) were performed by larger Malacostraca. _Aulostomus maculatus_ (high piscivore, \(n = 6\)), _Lutjanus apodus_ (moderate piscivore, \(n = 5\)), _Ocyurus chrysurus_ (moderate piscivore, \(n = 5\)), _Sphoeroides spengleri_ (low piscivore, \(n = 4\)) were responsible for most prey removals \((n = 20,83\%)\) of all observed piscivorous fish species.
Figure 5. Mean (± SE) density distribution of the piscivore assemblage in the 4 habitat types during day time classified into 4 size classes (0–15 cm, 16–30 cm, 31–45 cm, >45 cm). The effect of habitat type (main factor) and distance to the coral reef (covariable) on piscivore density was tested for each size class separately by 1-way analysis of covariance (Table 1). In each graph, different letters (A, B) among habitat types indicate significantly different fish densities (Sidak’s post-hoc comparison, \( P < 0.050 \)). Mean fish densities of 16–30 cm piscivores did not significantly differ.
Table 2. Results of predator identification of successfully recorded prey removals during underwater video recording of predation experiments (n = 33). Predator size is expressed as large juvenile, subadult or adult, estimated based on fish coloration pattern and/or estimated in relation to known landmarks in the surrounding (stones, corals, prop roots, seagrass leaves). Values in heading show the location of the site in the investigated gradient (Fig. 1). The first column shows the identified predator species and whether the species is considered a piscivorous fish or a non-piscivorous fish or Malacostraca (see also Appendix 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Predator size indication</th>
<th>No. of recorded prey removals per site</th>
<th>Total no. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Piscivorous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aulostomus maculatus</td>
<td>Trumpetfish</td>
<td>Adult</td>
<td>5 1</td>
<td>6</td>
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<tr>
<td>Cephalopholis fulva</td>
<td>Coney</td>
<td>Subadult</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lutjanus apodus</td>
<td>Schoolmaster</td>
<td>Large juvenile</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=3), Adult (n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutjanus griseus</td>
<td>Gray snapper</td>
<td>Adult</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ocyurus chrysurus</td>
<td>Yellowtail snapper</td>
<td>Large juvenile</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sphoeroides spengleri</td>
<td>Bandtail puffer</td>
<td>Adult</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sphyraena barracuda</td>
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<td>Subadult</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Tylosurus crocodilus</td>
<td>Houndfish</td>
<td>Adult</td>
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<td>1</td>
</tr>
<tr>
<td><strong>Non-piscivorous</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Brachyura</td>
<td>Crab</td>
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<td>Stomatopoda</td>
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<td>Halichoeres garnoti</td>
<td>Yellowhead wrasse</td>
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<td>Scaridae</td>
<td>Parrotfish</td>
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<tr>
<td>Sparisoma aurofrenatum</td>
<td>Redband parrotfish</td>
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<tr>
<td><strong>Total no. of tethered fish (n_{tot} = 203)</strong></td>
<td></td>
<td></td>
<td><strong>21</strong></td>
<td><strong>28</strong></td>
</tr>
</tbody>
</table>
Overall comparisons of cumulative survival of tethered fish among sites showed significant differences (generalized Wilcoxon test, $\chi^2_{df=1} = 9.80, P = 0.002$). Pairwise comparisons showed that survival was significantly highest in the mangroves in the channel (Fig. 6) and lowest on the fringing coral reef site outside of the bay (#12, Fig. 1). At the end of the 90-min experiments, survival was 0 on the fringing coral reef outside the bay. The seagrass bed and mangrove in the entrance of the bay also showed a relatively low survival after the 90-min period (<20%). In contrast to the seagrass bed in the entrance of the bay, survival after the 90-min period was relatively high in the seagrass bed in the centre of the bay. For the fringing coral reef outside the bay, the mangrove in the entrance and the seagrass bed in the channel, survival dropped very fast and was below 50% after 20 min. On the contrary, cumulative survival in the seagrass bed in the centre of the bay dropped below 50% only after 70 min. Survival at the mangrove site in the channel showed a pattern different from the other sites, remaining high (75%) and not changing anymore after 40 min. For 8 sites data for predation experiments as well as data for total piscivore density were available. ANCOVA that investigated the effect of habitat type and distance to the coral reef (as a covariable) on survival of tethered fish and mean total piscivore density, showed only a significant effect of habitat type on mean total piscivore fish density (2-way ANCOVA, Table 1).

**DISCUSSION**

Observations in the present study showed that high densities of piscivore assemblages occur on both the coral reef and in back-reef habitats. Although species assemblages differed greatly, these habitats harboured species for which fish is an important part of their diet (in the present study defined as highly and moderately specialized piscivores). Back-reef habitats were characterized by high densities of piscivore species that most likely used Spanish Water Bay during their settlement and early juvenile life phase (e.g., *Lutjanus griseus, L. apodus, Sphyraena barracuda*, defined by Nagelkerken et al. 2000a), complemented with reef associated visiting piscivores (e.g., *Carangoides ruber, Caranx latus, Scorpaena plumieri*). Conversely, the reef was characterized by high densities of typical reef-associated piscivores (e.g., *Aulostomus maculatus, Cephalopholis cruentatus, C. fulva*), complemented with (sub)adults of piscivores that most likely used the bay as a juvenile habitat and had migrated to the coral reef (e.g., *Lutjanus apodus, Ocyurus chrysurus*).

Predation experiments illustrate that the presence of piscivores in both reef and back-reef habitats results in reduced survival of small juvenile fish. Low survival was observed on the coral reef as well as in various habitats located in
Figure 6. Relative survival of tethered, recently settled juvenile *Haemulon flavolineatum* in predation experiments in 4 habitat types spread over 8 sites (see ‘Materials and Methods’ and Fig. 1). Different letters (A–E) indicate statistical differences obtained by survival analysis (generalized Wilcoxon post-hoc comparisons, *P* < 0.050).
the bay. Although predation experiments by means of tethered fish do not resemble a natural situation, it is reasonable to assume that the observed piscivores in reality contribute to reduced survival of juvenile fish (see also next paragraph). These observations therefore support the hypothesis that back-reef habitats harbour important piscivore assemblages (Baker & Sheaves 2005, 2006, 2007, Chittaro et al. 2005).

Despite the presence of considerable piscivore assemblages in both reef and back-reef habitats, there was a clear influence of habitat type on the structure and density of piscivore assemblages in the studied habitats. Total piscivore densities were highest in habitats with a high structural complexity. This was most evident in the mangroves, which are characterized by a dense system of submerged prop roots, providing shelter places for smaller fishes. Nagelkerken et al. (2000a) showed high fish densities in structurally complex habitats (mangroves and boulders in notches) at Spanish Water Bay, indicating that fishes concentrate in these habitats. Likewise, the present study also shows a concentration of piscivorous fishes in these habitats, indicating the habitats also provide a shelter function for piscivores.

As opposed to the coral reef, notches and mangroves, the lowest total density of piscivores was observed on seagrass beds. When only focusing on total piscivore densities, this suggests that seagrass beds are the safest habitat for small juvenile fish. However, there was no consistent pattern of higher survival of tethered fish on seagrass beds compared to the other habitats. Only survival of tethered fish at the seagrass bed located deep inside the bay was relatively high, as opposed to those located in the channel and at the bay entrance. The low density of piscivores on seagrass beds is therefore likely to be confounded by site effects (e.g., the distance to the coral reef or the adjacent habitat type) and significant predation on small juvenile fish may therefore still occur in seagrass beds. Predation pressure on some of the seagrass beds was partly illustrated by video-recorded removals of tethered fish by Ocyurus chrysurus.

It is not merely total piscivore density that determines predation risk. The diet and size of the piscivores may influence predation risk in a habitat as well. Larger predators and predators with a strict piscivore diet are likely to be very efficient predators on small juvenile fish (Scharf et al. 2000, Kulbicki et al. 2005). However, all studied habitats were dominated by moderately specialized piscivores and showed a comparable density distribution of the distinguished diet types, in which all seagrass beds were characterized by low overall densities. It is therefore unlikely that habitat type had a strong influence on the structure of the piscivore assemblages based on diet.

On the contrary, a clear pattern could be distinguished in the distribution of size classes of the piscivore assemblage in each habitat. The piscivore assemblages in seagrass beds, notches and mangroves were characterized by high densities of small piscivores (0 to 15 cm). As has been suggested above, a
PISCIVORES & PREDATION IN BACK-REEF HABITATS

large part of the piscivore assemblage in the bay is likely to consist of larger juveniles and subadults of fish species that used habitats at Spanish Water Bay as a juvenile habitat. Various species that are considered as piscivores in the present study have also been categorized as nursery species before (i.e., reef fish species that predominantly use seagrass beds and mangroves as a juvenile habitat; Nagelkerken et al. 2000a). Although these individuals are relatively small, several of these species are likely to feed on small, recently settled juvenile fish. *Lutjanus apodus* and *Ocyurus chrysurus* <15 cm feed partly on fish (Cocheret de la Morinière et al. 2003a), and video analyses in the present study showed that larger juveniles (~10 to 20 cm TL) of these species actually removed tethered fish. Also, smaller *Sphyraena barracuda* (<15 cm TL), a piscivore that occurs in relatively high densities in the bay, feed predominantly on fish (Lugendo et al. 2006). A large part of the piscivore assemblage in back-reef habitats may therefore consist of species that use these habitats originally as settlement and early juvenile habitats and eventually contribute to predation pressure in these habitats in later life stages (Baker & Sheaves 2005).

There was no clear influence of distance to the coral reef/entrance of Spanish Water Bay on piscivore density, species richness and prey fish survival. However, the species structure of the piscivore assemblage greatly changed along the gradient. Various larger reef-associated predator were observed in the entrance of the bay (e.g., *Carangoides crysos*, *C. ruber*, *Caranx latus*, *Cephalopholis cruentatus*, *Synodus intermedius*, *Oligoplites saurus*). Some of these species occasionally visited habitats located in the centre of Spanish Water Bay (i.e., *Caranx latus*, *Oligoplites saurus*, Appendix 1). Additionally, a video observation showed that the reef-associated *Aulostomus maculatus* consumed a tethered fish on the seagrass bed in the entrance of the bay (Table 2). This indicates that visiting piscivores enter the entrance area and join the local piscivore assemblage (Dorenbosch et al. 2007, Valentine et al. 2007). Larger piscivores are assumed to be more efficient predators on small juvenile fish than smaller predators (Scharf et al. 2000, Kulbicki et al. 2005). Since most of the visiting piscivores are larger (i.e., >15 cm) and have a strict fish diet, it can be inferred that they contribute significantly to prey removal in the seagrass bed and mangroves in the entrance area of the bay, hereby resembling the pattern of prey removal on the fringing coral reef.

**Limitations of the study** – *A priori* classification of the piscivore assemblage into diet classes was based on data in the literature and was not evaluated in the study area for most species. For fish species that have fish only as a minor part of their diet (in this study defined as low piscivores), their contribution to predation pressure in a specific habitat can therefore be debated. However, for some species that do not entirely depend on fish in their diet, such as *Sphoeroides spengleri* and larger juveniles of *Lutjanus apodus* and *Ocyurus*...
Chapter 4

Chrysurus, video recording confirmed that these species prey on small juvenile fish as part of their diet. Settlement of coral reef fishes occurs in clear peaks that subsequently result in temporarily high densities of small juveniles. It is therefore likely that fish species with a broad diet that includes fish, to some extent, actually prey on small juvenile fish when this is a widely available food source, for example, after settlement peaks.

Although video observations showed that most successful prey removals could actually be ascribed to piscivorous fishes (n = 24), there were also some prey removals (n = 9) by Malacostraca and fishes that were not considered as real piscivores (such as Scaridae). It is therefore likely that prey removals by non-piscivorous fishes resulted in some overestimation of predation pressure. Since the observed prey removals by non-piscivorous fish were considerably higher on the reef, this overestimation was most likely strongest on the coral reef. With respect to this overestimation of predation pressure, the following can be concluded. (1) Prey removal of tethered fish by piscivorous fish was demonstrated unambiguously in all habitats by video observations. Based on these observations it is very likely that piscivorous fish contributed most to removal of tethered fish in all habitats. (2) Overestimation of predation pressure would most likely affect observed predation pressure on the coral reef, which may be lower than the data suggest. This hypothesized lower predation pressure on the coral reef would only decrease the observed difference between predation pressure on the coral reef and back-reef habitats by piscivorous fish. In our opinion, a possible overestimation of predation pressure in habitats, in particular on the coral reef, therefore does not strongly undermine the major finding of the present study: piscivore assemblages in various back-reef habitats contribute to significant mortality of small juvenile fish in some of these habitats.

Various studies suggest predation may be high during dusk, dawn or at night (McFarland 1991, Danilowicz & Sale 1999) and that piscivore fish assemblages change greatly during night time (Unsworth et al. 2007). Consequently, this could result in different piscivore assemblages between day and night in the habitats and may undermine the day time observations of the present study. However, the survey conducted at night also showed a significant effect of habitat type on the piscivore assemblage. The coral reef showed high densities of piscivores comparable to the densities observed during the day. Mangroves showed considerably lower densities at night, indicating these fishes leave this habitat during night time. Nagelkerken et al. (2000b) showed a similar observation and suggested fishes that shelter in mangroves during day feed in adjacent habitats at Spanish Water Bay at night. Data in the present study suggest this is also valid for the piscivore assemblage in mangroves. Since the total surface of mangroves at Spanish Water Bay is only small in relation to the total surface of seagrass beds (Nagelkerken et al. 2000a), the number of piscivores that shelter in mangroves
Piscivores & Predation in Back-Reef Habitats

during day is distributed over the extensive seagrass beds (the most adjacent habitat), which subsequently results in a low piscivore density. As a result, it can be concluded that total piscivore density in mangroves greatly declines at night, but that the low piscivore density observed during the day on seagrass beds does not greatly change during the night.

Piscivore assemblages and nursery function – It is assumed that back-reef habitats are important nurseries for coral reef fishes, as a result of increased food availability and/or reduced predation pressure. Dahlgren & Eggleston (2000) suggested that for juvenile fish in these back-reef habitats a trade-off exists between minimizing predation risk and maximizing growth. The observations in the present study show that back-reef habitats used by juvenile fish have significant piscivore assemblages that may result in a high predation pressure, possibly even comparable with that on the reef. In line with results of other studies (Baker & Sheaves 2005, 2006, 2007, Chittaro et al. 2005), these piscivore assemblages in back-reef habitats may be a determining factor for the importance of the nursery function of these habitats.

Mangroves are in general considered an important nursery habitat because the structural complexity provides efficient shelter for small juvenile fish (Laegdsgaard & Johnson 2001). The present study shows that this is not always the case. Mangroves with a high structural complexity concentrated piscivorous fishes, which at 2 sites could clearly be related to reduced survival of tethered fish. However, the present study also showed significant variability in piscivore assemblages in mangroves. There was a clear spatial effect present for total piscivore density at the various mangrove sites at Spanish Water Bay, while tethering experiments in the channel mangroves showed considerably higher survival in comparison with the other 2 investigated mangrove sites. A similar pattern of variability was found in the seagrass beds. In general, the data of the present study showed low total piscivore densities in all sampled seagrass beds, but high survival of tethered fishes was only observed in the seagrass bed located in the centre of Spanish Water Bay. This contradicts observations of relatively low survival of tethered fish in seagrass beds in the entrance and channel of the bay. These observations suggest that survival as a result of predation in back-reef habitats is confounded by site specific effects, e.g., the distance to the coral reef or the type of adjacent habitat.

Still, of various back-reef habitats, total piscivore densities were only low in seagrass beds, and at least 1 seagrass bed located away from the reef showed relatively high survival. Depending on the configuration of habitats within the seascape, some seagrass beds may therefore be an attractive habitat for recently settled small juvenile fish to spend their early juvenile life stage, which is in line with observations of directed settlement on specific seagrass sites in a gradient of habitats (Watson et al. 2002, Pollux et al. 2007).
Furthermore, it should be considered that the total surface area of seagrass beds is much larger than that of mangroves, notches, and reefs at Spanish Water Bay (see Nagelkerken et al. 2000a), while the bay area, with its safer seagrass habitats, has a much larger surface area than the channel and entrance of the embayment. Therefore, for large areas at Spanish Water Bay predation risk is expected to be relatively low.

The present study shows the presence of significant piscivore assemblages on both the coral reef and in back-reef habitats in the investigated gradient. Both areas encompass high densities of piscivores that result in a relatively high predation risk, as indicated by predation experiments. Immigration of visiting piscivores from the coral reef and high densities of larger resident piscivorous nursery species present in structure-rich habitats in the bay may explain this observation. However, of all habitat types in the investigated gradient, seagrass beds showed the lowest piscivore densities, while 1 seagrass bed away from the coral reef also indicated higher survival of tethered fish than in other habitats. Depending on the seascape configuration of habitats (i.e., distance to the coral reef and the presence of other habitats that concentrate predators), this suggests that in a Caribbean shallow-water seascape (such as the sheltered marine bay in the present study) some seagrass beds may be safe habitats for small juvenile coral reef fishes.

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REFERENCES


Appendix 1. Piscivore assemblages and mean densities in the studied gradient during day. Species was categorized *a priori* into 3 diet classes based on their degree of specialisation on feeding on fish: high, moderate and low specialised (see text). Bold densities show for each species the habitat with the highest mean density; numbers between parentheses indicate site locations as listed in Fig. 1; ≥ refers to the fish size (cm) at which an ontogenetic diet shift is reported (see text), individuals with a TL < the given size are omitted from analysis; ■ present study confirmed species preyed on tethered fish by underwater video recording; ♦ shows the gradient zone in which the species were observed.

<table>
<thead>
<tr>
<th>Species occurring in bay and/or entrance (n = 8)</th>
<th>Common name</th>
<th>Diet class</th>
<th>Recorded on video</th>
<th>Occurrence in gradient</th>
<th>Notches</th>
<th>Seagrass beds</th>
<th>Mangroves</th>
<th>Coral reef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caranx latus</td>
<td>Horse-eye trevally</td>
<td>High</td>
<td>♦</td>
<td>Bay (6) Channel (3)   Entrance (2) Coral reef (1)</td>
<td>0.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Snook</td>
<td>High</td>
<td>♦</td>
<td>Bay (2)</td>
<td>-</td>
<td>0.33</td>
<td>-</td>
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<td>♦</td>
<td>Bay (7)</td>
<td>-</td>
<td>13.83</td>
<td>9.83</td>
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<td>♦</td>
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<td>♦</td>
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<td>0.36</td>
<td>0.25</td>
<td>0.67</td>
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<table>
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<th>Species occurring on the reef and/or entrance (n = 10)</th>
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<th>Recorded on video</th>
<th>Occurrence in gradient</th>
<th>Notches</th>
<th>Seagrass beds</th>
<th>Mangroves</th>
<th>Coral reef</th>
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<td>Synodus intermedius</td>
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<td>-</td>
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<tr>
<td>Aulostomus maculatus</td>
<td>Trumpetfish</td>
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Species occurrence on the reef and/or entrance (n = 10): Synodus intermedius, Gymnathorax moringa, Aulostomus maculatus, Cephalopholis cryos, Cephalopholis fulva, Cephalopholis cruentatus, Bothus lunatus.
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<th>Species</th>
<th>Common name</th>
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<th>Occurrence in gradient</th>
<th>Mean density (100 m$^{-2}$)</th>
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SIMPLE ECOLOGICAL TRADE-OFFS GIVE RISE TO EMERGENT CROSS-ECOSYSTEM DISTRIBUTIONS OF A CORAL REEF FISH

GROL MGG, NAGELKERKEN I, RYPEL AL & LAYMAN CA

OECOLOGIA
(2010) IN REVIEW
ABSTRACT

Ecosystems are intricately linked by the flow of organisms across their boundaries, and such connectivity can be essential to the structure and function of the linked ecosystems. For example, many coral reef fish populations are purportedly supported by the movement of individuals from spatially-segregated juvenile habitats (i.e., nurseries), such as mangroves and seagrass beds, yet it is often difficult to elucidate the underlying mechanisms that drive across-system movements. Here we investigate trade-offs between growth, predation risk, and maturation that give rise to distribution patterns of a common Caribbean coral reef fish species, *Haemulon flavolineatum* (French grunt), through ontogeny. As suggested, adults were primarily found on coral reefs, whereas juvenile fish only occurred in non-reef habitats. Analysis of length-at-age revealed that growth rates were highest on reefs. Survival rates in tethering trials were 0% for recently-settled fish on reefs, while as fish grew, rates increased up to a point where survival approached those in non-reef habitats. Predation seems to be the primary factor responsible for driving across-ecosystem distributions of juvenile fish, and thus the primary reason why mangrove and seagrass habitats function as nursery grounds. Continued human impacts (e.g., over-exploitation of predators and habitat destruction) may alter core ecological and evolutionary mechanisms that give rise to similar cross-ecosystem patterns in many coral reef fishes. Identifying the mechanisms that lead to ecosystem-scale distribution is critical to developing appropriate conservation initiatives.
INTRODUCTION

Trade-offs between maximizing growth and minimizing predation risk are one of the unifying themes in ecological research (Stephens & Krebs 1986). Evidence of behavioural strategies to minimize the ratio of mortality to growth rate exists for a diverse suite of taxa (Lima & Dill 1990, Suhonen 1993, Heithaus et al. 2007, Urban 2007, Atkinson et al. 2008). Body size is one of the primary factors regulating such trade-offs (Werner & Gilliam 1984). Through ontogeny, foraging strategies and vulnerability to predators can change, and thus the optimal habitat or ecosystem to maximize an individual’s fitness may shift dramatically. This well-developed body of ecological research focuses primarily on within-ecosystem shifts among localized habitat types, e.g., the foundational example of a fish shifting between littoral and pelagic habitats through ontogeny (Werner & Hall 1988).

Larger-scale, across-ecosystem, ontogenetic niche shifts are intriguing to consider in this conceptual framework. Ecosystems are intricately linked by the flow of organisms across their boundaries (Polis et al. 1997, Post et al. 2006, Schreiber & Rudolf 2008), yet it is often difficult to elucidate the mechanisms behind across-ecosystem movements. For instance, many coral reef fish populations are purportedly supported by the movement of individuals from spatially-segregated juvenile habitats (such as mangroves and seagrass beds, e.g., Verweij et al. 2008), and such connectivity can be essential to the structure and function of the linked ecosystems (Mumby et al. 2004, Mumby & Hastings 2008). As with other examples of large-scale, across-ecosystem movements by individuals through ontogeny, the underlying mechanisms driving these patterns are difficult to isolate. But this conceptual framework ultimately could provide a link between some core tenets of behavioural ecology (such as the growth/predation trade-off) and emergent, large-scale, patterns of community structure and ecosystem function.

‘Nursery’, is a term with a central place in the marine literature, and generally refers to an ecosystem (or habitat) that supplies a large proportion of individuals to a spatially-separated adult population (Beck et al. 2001, Adams et al. 2006). Although definitions and applications of the concept vary, and are still emerging (Faunce & Layman 2009), the interplay between growth and mortality comprises a foundation for assigning particular habitats as nurseries. Cross-ecosystem linkages between purported nurseries and coral reefs typically are inferred from distributional patterns of constituent organisms. For example, Mumby et al. (2004) provided compelling evidence of connectivity by comparing fish densities on coral reefs with and without adjacent mangrove ecosystems. Other studies likewise provided strong inferential evidence of such linkages in marine ecosystems (Nagelkerken et al. 2000, 2002, Dorenbosch et al. 2005, 2006, 2007).
In the present study, we examined the underlying trade-offs that give rise to distribution patterns of a coral reef fish through ontogeny. Because of frequent \(i.e.,\) daily small-scale movements of fishes among non-reef habitats (Verweij & Nagelkerken 2007), it is often hard to isolate predation risk, growth rates, and maturation of individuals separately in mangroves and seagrasses. Our study system provides an opportunity to compare these variables in two spatially-segregated ecosystems: (i) an inland bay harbouring adjacent and inter-connected non-reef habitats, such as mangroves and seagrasses, and (ii) the coral reef located outside of the bay proper. We hypothesized that fish species utilize non-reef habitats to maximize growth rates and minimize mortality rates as compared to the coral reef. Our model study organism is the French grunt, \textit{Haemulon flavolineatum}, a common Caribbean coral reef fish species whose juveniles inhabit shallow coastal habitats and adults populate the coral reef (reviewed by Nagelkerken 2009). Our approach included extensive surveys of \textit{H. flavolineatum}, quantification of growth rates and maturity stages, and \textit{in situ} estimation of relative mortality rates through ontogeny. The end product is a detailed explanation of the trade-offs between growth, predation, and maturation that helps to explain a pervasive distribution pattern for many coral reef fish species.

**Materials & Methods**

**Study area** – At Spanish Water Bay \(12^\circ 04'\ N, 68^\circ 51'\ W;\) Fig. 1) on the southern Caribbean island of Curacao, we studied fish density, growth, predation risk, and sexual maturation of \textit{Haemulon flavolineatum} across non-reef habitats in a marine embayment and on the adjacent coral reef. At Spanish Water Bay, a spatial distinction was made between habitats located deep inside the bay (referred to as ‘bay’) and habitats located in the channel and entrance of the bay closer to the coral reef (referred to as ‘channel’) (see Fig. 1). Seagrass beds \(\textit{Thalassia testudinum}\) and stands of fringing mangroves \(\textit{Rhizophora mangle}\) are found along the shorelines of both the bay and channel. Habitats consisting of hard bottom substratum are only found in the channel. For example, rocks and boulders in front of crevices within the shoreline fossil limestone plateau (boulder/notch) are found throughout the whole channel, while small pieces of dead coral on the sandy substratum (rubble) are only found in the channel near the entrance of Spanish Water Bay. The fringing coral reef is located directly in front of Spanish Water Bay and runs along the entire south-west coast of the island (see Nagelkerken \textit{et al.} 2000 for further details).
Distribution patterns – Abundance and total length (TL) of *Haemulon flavolineatum* were estimated along permanent belt transects in four different habitats throughout the bay and channel, and on the coral reef (July–November 2005) using SCUBA and snorkelling gear (see Nagelkerken *et al.* 2000 for details on census methodology). Transects were separated by at least 12.5 m, and each transect was 25 m long x 4 m wide, where possible. Per habitat type, 2–3 sites were selected, and per site, 2–8 permanent transects were placed depending on the size of the habitats. Transects were surveyed 6–8 times during the study period, at least once every ten days. Fish counts of *H. flavolineatum* were then grouped for the bay, channel and reef area and averaged over time, number of transects, and habitats, and normalized to a 100 m$^2$ census area per 4-cm size class (*i.e.*, 0.0–3.9, 4.0–7.9, 8.0–11.9, 12.0–15.9, and 16.0–19.9 cm TL). In total, 95 bay, 336 channel, and 76 reef transects were surveyed.

Growth rates – *Haemulon flavolineatum* were captured using hook and line, fish traps, and beach seine nets in the three primary areas. Fork length (FL) was measured, otolith sagittae were removed and the left otolith used for age determination under a dissecting microscope utilizing reflected light (DeVries & Frie 1996). In total, 72 bay, 124 channel, and 108 reef fish otoliths were analyzed. Ages were determined blindly (*i.e.*, with no knowledge of the sample number or fish size) twice by an experienced reader, and disagreements between reads one and two (4% of all samples) were settled using another experienced reader. Fish growth was modelled using the Von Bertalanffy Growth Function (1938), $L_t = L_\infty [1 - e^{-k(t-t_0)}]$, where $L_t =$ length at time $t$, $L_\infty =$ the theoretical maximum length, $k =$ a growth coefficient (the rate at which length approaches $L_\infty$), $t =$ fish age in years, and $t_0 =$ theoretical time at age 0. Likelihood ratio tests were used to evaluate differences in Von Bertalanffy growth curves among the bay, channel and coral reef (Kimura 1980).

Best-fitting Von Bertalanffy growth curves were calculated separately for bay, channel and reef using mean length at each age class. Then, a best fitting curve was calculated by combining all individuals from the three areas being compared (*i.e.*, coincident curves). Thus, Von Bertalanffy growth curve fits (analyzed by the residual sum of squares) were sequentially compared across bay, channel and reef. If the improvement in fit for each comparison was significant (relative to the chi-squared distribution) then growth was considered to be significantly different between bay, channel and reef. To test for differences in size-at-age among bay, channel and reef, a 1-way ANOVA followed by a Hochberg’s GT2 post-hoc comparison test was performed per year class when data were homogenous. Independent samples $t$-tests were performed when only two areas were compared (*i.e.*, year 0 channel and coral reef).
**Survival rates** – Relative predation rate on tethered *Haemulon flavolineatum* was estimated in the bay, channel and reef area during the day. Tethering trials were conducted for three life stages: recently-settled fish (2.4–4.5 cm FL), approximate size at which fish start migrating to reefs (8.1–12.0 cm FL), and adult fish commonly found on the reef (13.8–16.9 cm FL). Fishes were individually attached with thin monofilament line (50–80 cm length) and a small hook to an iron pole (~40 cm) that was pushed into the substratum. Each fish was able to swim and hide within any present vegetation. Tethering was conducted along the same transect sites as those for the visual surveys, and lasted 90 min. The sequence of tethering was done randomly across the different sites, habitats and size classes. Only fish that were alive or that had clearly been attacked or eaten (*i.e.*, hook and/or part of the line missing) at the end were considered successful trials and were included in the analyses; dead and possibly detached fish (*i.e.*, line and hook undamaged) were excluded. The total number of successful trials ranged from 10–17 fish per site per habitat for each of the two smaller size classes and 6–7 fish per site per habitat for the largest size class. Percent survival at the end of the experiment (at 90 min) was averaged across replicates per habitat and site, separately for bay and channel, and the coral reef. Differences in survival among the three areas was compared using logistic regression and post-hoc chi-square tests with survival (0 or 1) as a dependant variable, and size class as an independent variable. In total, 65 bay, 186 channel, and 56 reef trials were included in the analysis.

Mortality/growth ratios are commonly used in ecological studies to yield insight on how organisms maximize their fitness (Werner & Anholt 1993, Dahlgren & Eggleston 2000). In this study, we calculated mortality/growth ratios for bay, channel, and reef fish. Growth rates were defined as the slopes of the length–log_{10}(age) regressions for bay, channel, and reef (Rypel & Layman 2008). Mortality rates were defined as the percent fish which did not survive the 90-min tethering trials. All growth and mortality rates were transformed to fractions (0–1) to obtain a single unit of measure. Mortality/growth ratios were calculated by dividing mortality over growth and averaging per area and size class. Ratios were compared among the three areas using a 1-way ANOVA followed by a Gabriel’s post-hoc comparison when data were homogenous (Levene’s test, $P < 0.050$), otherwise non-parametric Kruskal-Wallis tests followed by Games Howell post-hoc comparisons were used.
Maturation — Reproductive status was assessed for every fish caught in each of the three areas (i.e., bay, channel and reef) by external macroscopic sexual characteristics of the gonads, including size, colour, shape and texture using modified standard protocols (Ntiba & Jaccarini 1990, Kulmiye et al. 2002). In total, maturation stage of 77 bay, 324 channel and 200 reef fish was estimated, classified in six different maturation stages, subdivided as immature (stage 1 and 2) and mature (stages 3 to 6). Fishes are ready to spawn when gonads reach stage 5. Mean maturation stage was calculated per 4-cm size class, separately per area. Data were not homogenous (Levene’s test, \( P < 0.050 \)) and therefore non-parametric Kruskal-Wallis tests followed by Games Howell post-hoc comparisons were used to test for significant differences by 4-cm size class among the three main areas.

Statistical analyses were performed using SPSS (version 15.0), and differences were significant if \( P \)-values were \( \leq 0.050 \).

Figure 1. Distribution of *Haemulon flavolineatum* in the bay and channel of Spanish Water Bay, and on the coral reef on Curaçao. Grey indicates land, white indicates sea. Relative fish densities are represented in 4-cm size classes (TL) in pie charts.
RESULTS

At Spanish Water Bay, smaller fish inhabited the bay and channel, whereas larger fish typically inhabited the coral reef (Fig. 1). In the channel, the fish population was dominated by recently-settled (0.0–3.9 cm TL) and small *Haemulon flavolineatum* (4.0–7.9 cm TL), while inside the bay the population was composed of small to sub-adult individuals (4.0–11.9 cm TL).

Otolith ageing of *H. flavolineatum* showed that fish growth was significantly faster on the coral reef than in the bay or channel (Fig. 2, likelihood ratio test of coincident curves, both P-values <0.0001). Furthermore, growth was significantly faster in the bay than in the channel (likelihood ratio test of coincident curves, $P < 0.0001$). Isolating the smaller fish, year 0 and year 1 fish were larger on the reef compared to the bay and/or channel (for example year 0 fish in channel and bay, independent samples $t$-test: $P = 0.031$; year 1 fish among bay, channel and reef, Hochberg’s GT2: both P-values <0.0001).

![Figure 2. Von Bertalanffy growth curves for Haemulon flavolineatum in the bay and channel of Spanish Water Bay, and on the coral reef on Curaçao. Von Bertalanffy parameters for *H. flavolineatum* were: $L_\infty = 14.0$, $t_0 = -0.53$, and $K = 0.69$ for the bay; $L_\infty = 19.6$, $t_0 = -1.56$, and $K = 0.18$ for the channel; and $L_\infty = 18.7$, $t_0 = -0.64$, and $K = 0.52$ for the coral reef.](image-url)
In tethering trials, a significantly lower survival from predation was found for recently-settled *H. flavolineatum* on the coral reef compared to the bay (*P* = 0.009; Fig. 3a). Relative predation risk was high on the reef: no fish survived the 90-min tethering trials. Predation risk decreased with size, and for fish 14–16 cm FL survival rates approached those of non-reef habitats, with rates in channel and reef being equal (*P* = 0.127). For the mortality/growth ratios, a similar pattern was demonstrated: the ratio of mortality over growth decreased with size and was equal between areas for fish 14–16 cm FL (*P* = 0.124; Fig. 3b).

**Figure 3.** (a) Survival from predation in tethering trials (mean + SE) and (b) mortality/growth ratios (mean + SE) of *Haemulon flavolineatum* for three different life stages in the bay and channel of Spanish Water Bay, and on the coral reef on Curacao. Letters (A, B) indicate significant differences (*P* ≤ 0.050). ‘0’ for size class 2.4–4.5 cm in 3a indicates a survival rate of 0% and ‘0’ for size class 13.8–16.9 cm in 3b indicates a mortality/growth ratio of 0%.
Across all areas, fish <12 cm FL did not reach a mean maturation stage of 3, indicating all these fish were still immature (Fig. 4). Thus, no significant differences were found for mean maturation stage among bay, channel, and reef for fishes <12 cm FL. However, there were significant differences in mean maturation stage for fishes ≥12.0 cm FL (Games Howell: bay-reef $P = 0.001$, channel-reef $P < 0.0001$). Fishes from the coral reef had well-developed gonads, including many individuals at stage 5 (prepared to spawn) as well as post-spawning stage 6. Fishes ≥12.0 cm FL from the bay and channel only reached the initial stages of maturation (stage 3 and a few individuals stage 4) and became increasingly more abundant on the reef with increasing size (see Fig. 1), suggesting that they left the bay before fully maturing.

**Figure 4.** Maturation stage (mean + SE) of *Haemulon flavolineatum* per 4-cm size class FL in the bay and channel of Spanish Water Bay, and on the coral reef on Curaçao. Fish gonads categorized in stages 1 and 2 are immature, while stages 3 to 6 are mature. Letters (A, B) indicate significant differences ($P \leq 0.050$).
DISCUSSION

Coral reefs are under increasing pressure from myriad human impacts (Gardner et al. 2003, Hughes et al. 2003, Bellwood et al. 2004). Recent studies have focused on how disruption of connectivity among reefs and adjacent ecosystems may ultimately affect the health and function of coral reef ecosystems (Mumby & Hastings 2008, Munday et al. 2009). For example, pelagic fish larvae which have settled in mangroves and seagrass beds appear to recruit from these nurseries to reefs (Nakamura et al. 2008, Verweij et al. 2008), and thus affect the function of coral reef ecosystems (Adams et al. 2006, Mumby & Hastings 2008). Isolating the mechanisms that give rise to cross-ecosystem distributions in marine environments is critical to developing appropriate conservation initiatives (Sale et al. 2005).

It has become an accepted paradigm that mangroves and seagrass beds provide either higher food availability (and thus purportedly faster growth) or lower predation risk for juveniles of many coral reef fishes (Beck et al. 2001, Adams et al. 2006). Yet, empirical studies providing support for one alternative or the other are rare. In this study, risk of predation was clearly the underlying mechanism giving rise to the distributional pattern of recently-settled Haemulon flavolineatum. Juvenile grunts (<8 cm TL) had the highest abundance in the bay and channel where survival from predation was significantly higher and mortality/growth ratios lower than on the reef. The trade-off was a reduced growth rate, body size, and thus slower sexual maturation. These data provide strong support that predation intensity, not increased growth potential, ultimately gives rise to the apparent nursery function of mangroves and seagrass beds in bay and channel habitats.

Large-scale, across-ecosystem, movements are common for many taxa, including a wide diversity of fish species. Salmon migrations between freshwaters and the ocean are perhaps the most well-known example. As with mangroves and seagrass nurseries in the case of coral reef fishes, freshwater rivers are assumed to provide abundant food or reduced predation threat to salmon parr. Yet the relative balance of these two factors is difficult to resolve because of the massive spatial scales involved and the myriad factors which differ among systems (Gross et al. 1988, Gibson 1993, Klemetsen et al. 2003). Curaçao provided a unique opportunity to isolate specific mechanisms, largely because of the proximity of the distinct ecosystem types. Replicating such studies across multiple species and locations is necessary to evaluate the generality of this pattern, and explore whether reduced predation threat typically is the most important factor explaining the value of nursery habitats for coral reef fishes.
Larger *H. flavolineatum* shifted from the bay and channel to the reef at ~8.0–12.0 cm TL, which could be related to ontogenetic changes in vulnerability to predation (Werner & Gilliam 1984, Laegdsgaard & Johnson 2001). Our data indeed showed that predation risk no longer differed between channel and reef for large *H. flavolineatum*. Mumby *et al.* (2004) showed that biomass of haemulid predators followed the same distribution pattern as mean haemulid size, *i.e.*, lowest in seagrass beds and highest on forereefs. Larger fishes are less vulnerable to predation (Hixon 1991) and escape from smaller predators which are gape-limited (Urban 2007). Despite higher abundance of potential predators on the reef, predator gape-limitations may render *H. flavolineatum* less susceptible to predation (Mumby *et al.* 2006). This is especially true for overfished predator populations (as is the case on Curaçao) where large, top predatory fishes have been removed from food webs over time (Hoetjes *et al.* 2002).

The movement by larger fish from the bay and channel to the reef may have been driven by other specific life history traits. For example, examination of gonads revealed that larger fish that remained in the bay and channel were rarely sexually mature, whereas fish on the reef nearly always were. Fishes likely maximize fitness by releasing their gametes in environments with strong water currents that favour larval dispersal away from predator-rich coral reefs (Johannes 1978, Roberts 1997). Habitats confined in embayments and lagoons do not favour such dispersal. Thus, non-reef habitats such as mangroves and seagrasses temporarily alleviate juvenile fish from high predation risk, and serve as ‘waiting rooms’ until they are at the onset of maturity and can immigrate to reef habitat.

These data provide further evidence for the importance of ecosystem-based management initiatives that best preserve natural ecological and evolutionary systems (Pikitch *et al.* 2004). Our data also emphasize the need to maintain connectivity among coastal ecosystems (Mumby 2006, Layman *et al.* 2007, Mumby & Hastings 2008). Either a shift in habitat availability (*e.g.*, mangrove or seagrass) or predation risk could affect ontogenetic shifts in *H. flavolineatum*. Marine protected areas are especially important in this context, as they can provide a framework for protection against both overfishing and habitat loss. Our current understanding of organism movements that link adjacent marine ecosystems remains rudimentary in many ways, and knowledge of the mechanisms that lead to such movements is even less well-understood. Continued efforts to identify causal mechanisms of organism movement and ecosystem distribution patterns will provide a more robust foundation for successful conservation and management of marine resources.
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REFERENCES


CROSS-ECOSYSTEM CONNECTIVITY BY REEF FISH


Chapter 5


Cross-ecosystem connectivity by reef fish


FOOD, PREDATION RISK AND MATURATION DRIVE ONTOGENETIC HABITAT SHIFTS IN A CORAL REEF FISH

GROL MGG & NAGELKERKEN I

(2010) READY TO SUBMIT
Mechanisms driving ontogenetic habitat shifts

Abstract

Animals have complex life cycles and utilize various habitats during different life stages. Many marine fish species have juvenile and adult life stages that occupy spatially separated habitats. Juveniles often recruit to nearshore shallow-water habitats such as mangroves and seagrass beds (i.e., nurseries), whereas adults dominate coral reefs. Through ontogeny, shifts in habitat use commonly arise from changes in resource needs, yet the underlying mechanisms driving across-habitat distribution patterns for each particular life stage remain debated. Here we investigated the mechanisms causing a 5-phase, and possibly even a 6-phase life cycle pattern of a common Caribbean coral reef fish species, Haemulon flavolineatum (French grunt), across multiple tropical shallow-water coastal habitats. In each specific life stage, fish had to make a cost-benefit analysis based on their own needs to maximize fitness while selecting an optimal habitat. Pelagic larvae settled on rubble near bay entrances where they reached an optimum between predation risk (survival) and food abundance (growth). From here, fish shifted to seagrass beds probably driven by resource acquisition, followed by a shift to mangroves for optimal shelter. Boulder/notch habitats could function as an intermediate habitat between the mangroves and the uni-directional movement from bays to coral reefs which appeared to be driven by the need for reproduction. The current study shows a strong linkage among a suite of different habitats, which ultimately support the viability of populations on coral reefs. With the ongoing loss and degradation of nearshore coastal ecosystems, across-habitat migrations may be altered to such a degree that it affects trophic interactions and successful movement of fish among habitats which may ultimately lead to a decrease in ecosystem productivity and resilience. To develop appropriate conservation initiatives, it is necessary to identify the mechanisms that lead to cross-habitat distribution patterns.
Many animals have complex life cycles consisting of multiple stages in which larvae and adults occupy different environments. The 2-phase life cycle with a dispersive larval phase and a demersal juvenile and adult phase is most common; however, some animals have more than two phases. To the extreme end, certain species show ontogenetic habitat shifts between aquatic and terrestrial landscapes during their life cycle (e.g., review by Werner & Gilliam 1984, amphibians [Werner 1986], insects [Schäfer et al. 2006], and crustaceans [Haywood and Kenyon 2009]). The strategy of selecting different habitats through ontogeny is expected to provide advantages for each particular life stage (e.g., a higher density of food which could enhance growth, or more shelter against predators), and thus maximize survival. Eventually, after metamorphoses, growing larger, and/or maturing, these animals migrate towards other aquatic or terrestrial areas where they spend the rest of their life span. These shifts between different habitats can be linked to changes in body size (Werner & Gilliam 1984) and are dangerous transitions which cost energy but are needed to reduce vulnerability to predators by decreasing the exposure time to them (Kaufman et al. 1992, Zollner & Lima 2005).

Also nearshore marine organisms have a 2-phase life cycle with a larval dispersal phase that is often pelagic, and a sedimentary demersal phase (Leis 1991, Mora & Sale 2002, Haywood & Kenyon 2009). Several spawning strategies have evolved in these animals to reduce the intense predation pressure on pelagic eggs and larvae, e.g., spawning at (semi)lunar cycles or at night when currents are stronger and there is less light which lessens exposure to predators (Acosta & Butler 1999). Offshore dispersal of eggs and larvae minimizes the risk of predation by benthic and demersal predators which are present in adult habitats such as tropical coral reefs. Nevertheless, most pelagic larvae return to coastal waters and eventually settle onto the benthos to complete their life cycles (reviews by Pechenik 1999, Mora & Sale 2002). Hence, habitat selection behaviour during the early life phase is of great importance to maximize an individual’s fitness and survival rate (Stephens & Krebs 1986, Sutherland 1996).

Pelagic larvae of many tropical marine species do not recruit directly to adult habitats such as coral reefs, but instead move across spatially-separated ecosystems to settle in habitats that are distinct from those where the adults are found, e.g., bay habitats such as mangroves and seagrass beds (Shulman & Ogden 1987, Acosta & Butler 1999, Dahlgren & Eggleson 2000, Pollux et al. 2007, Haywood & Kenyon 2009). Selection of such specific settlement habitats could maximize net benefits, based on habitat characteristics such as a complex structure providing shelter against predators or increased living space for prey organisms. Growing larger, shifts in habitat use commonly arise...
MECHANISMS DRIVING ONTOGENETIC HABITAT SHIFTS

from changes in resource needs, such as a change in diet (Werner & Gilliam 1984, Cocheret de la Morinière et al. 2003), changing predation risk (Shulman 1985, Laegdsgaard & Johnson 2001), or possibly the need to reproduce in a habitat where larval survival is enhanced and larvae are better dispersed (reviews by Johannes 1978, Nemeth 2009).

Compared to direct settlement on a coral reef, the transition from offshore waters across coral reefs towards potentially more profitable bay habitats will confront larvae with higher levels of predation during longer time periods (Johannes 1978, Leis & Carson-Ewart 1998, Acosta & Butler 1999). The longer dispersal period is also energetically very costly and will reduce energy resources dramatically (Bennett & Marshall 2005, Stamps et al. 2005, Phillips et al. 2006). This may cause larvae to die before reaching these specific settlement habitats or be in a poor condition at settlement, and thus being more vulnerable to predation (Booth & Beretta 2004, Hoey & McCormick 2004, Figueira et al. 2008). To compensate this high risk, juveniles must receive greater benefits from such bay habitats (review by Beck et al. 2001). Thus, when selecting an optimal habitat, animals have to make a cost-benefit analysis based on their needs in each specific life phase. This analysis appears to reflect avoidance of predation (minimizing mortality risk), the acquisition of resources (maximizing growth rate), or reduction of the ratio of mortality over growth (Lima & Dill 1990, Dahlgren & Eggleston 2000, Haywood & Kenyon 2009). The outcome of such cost-benefit analyses likely changes with body size (Werner & Gilliam 1984).

A recent study by Grol et al. (chapter 5) showed that a coral reef fish that spent its juvenile life phase in an embayment benefited from enhanced survival rates, but to the detriment of growth rates which were lower than on the reef. Several descriptive studies have shown, however, that during their residency in bays, fish show ontogenetic movements among bay habitats. For instance, shifts of juvenile fish from seagrass beds to mangroves (Rooker & Dennis 1991, Nagelkerken et al. 2000c, Burke et al. 2009) or between post algal and algal microhabitats in a tidal creek (Dahlgren & Eggleston 2000). It is still unclear which mechanisms drive such ontogenetic movements of fish among major bay habitats in a tropical marine seascape. This has proved difficult as such habitats are also connected through short-term tidal and diel migrations, making it challenging to isolate the advantages that individual habitats offer.

In this study we evaluate whether the life cycle distribution pattern of a tropical marine fish species, Haemulon flavolineatum (French grunt), during its residency in different bay habitats is driven by factors such as resource acquisition, risk of mortality from predators, and reproduction. We show that there is a strong linkage among a suite of different habitats, which ultimately support the viability of populations on coral reefs. Approaches used here were investigating H. flavolineatum distribution patterns across multiple bay habitats, and quantification of gut contents, food abundance, condition, and
reproduction indices. The results of this study provide an itemized description of life history traits that act as the underlying mechanisms leading to differential distributions of marine organisms across tropical coastal habitats during various stages of their non-adult life cycle. Understanding these mechanisms is of great concern for fisheries management and to develop appropriate conservation initiatives. We hypothesize that due to an overall lower predation risk in bay habitats (Dorenbosch et al. 2009, chapter 5), other traits such as acquisition of food and refuges play a larger role in explaining ontogenetic shifts among bay habitats.

**MATERIALS & METHODS**

**Model species** – Nagelkerken et al. (2000b) described *Haemulon flavolineatum* as a ‘nursery species’, whose recruits settle within 15 days after fertilization (Brothers & McFarland 1981, McFarland et al. 1985) in bay habitats, such as mangroves and seagrass beds (Pollux et al. 2007), at a size of 7.9–11.5 mm fork length (FL) (Gaut & Munro 1983, Lindeman & Richards 2005). At the onset of maturity, fish migrate back to the coral reef and join adult populations (Gaut & Munro 1983, Cocheret de la Morinière et al. 2002, chapter 5). Maximum adult fish length is 26.4 cm FL and at 14.0 to 17.6 cm FL they become sexually mature (Gaut & Munro 1983, Lindeman & Richards 2005). Until approximately 4 to 5 cm FL they are diurnal planktivores and mainly feed on Copepoda (Ogden & Ehrlich 1977, Verweij et al. 2006b, Grol et al. 2008). Through ontogeny their feeding pattern shifts towards a nocturnal zoobenthivoric diet of benthic worms and larger crustaceans (Randall 1967, Ogden & Ehrlich 1977, Cocheret de la Morinière et al. 2003).

**Study area** – The present study was conducted in the southern Caribbean at the islands of Curaçao and Aruba. The locations Spanish Water Bay (12°04’ N, 68°51’ W) and Fuik Bay (12°02’ N, 68°49’ W) are two largely shallow (<5 m depth) sheltered bays connected to a fringing coral reef through a narrow entrance, and are situated at the south-western coast of Curaçao (Appendix 1). In both bays, seagrass beds (*Thalassia testudinum*) and stands of fringing mangroves (*Rhizophora mangle*) are found along the shoreline, as well as hard bottom substratum, such as rocks and boulders in front of crevices in the shoreline fossil limestone plateau (hereafter referred to as ‘boulder/notch’ habitat) and smaller pieces of dead coral on a sandy substratum (hereafter referred to as ‘rubble’ habitat). The latter was only found in the channel of Spanish Water Bay near and in the mouth of the bay. The coral reef consists of a narrow reef flat (~100 m wide) steeply dropping off at about 6–8 m, is located in front of the embayments, and runs along the entire south-west coast.
MECHANISMS DRIVING ONTOGENETIC HABITAT SHIFTS

of Curaçao. Aruba has no embayments like Curaçao; instead, Aruba has a large, relatively shallow continental shelf with small coral cays in front of the south-western coast which form a single large lagoon (12°28’ N, 69°59’ W; Appendix 1). Throughout the lagoon, seagrass beds (T. testudinum), stands of fringing mangroves (R. mangle), boulder/notch and rubble habitats are found. Like on Curaçao, boulder/notch habitats are found in front of crevices in the shoreline fossil limestone plateau, while rubble habitats were present within the lagoon close to the inlets between the coral cays, both habitats found only nearby Mangel Halto. The fringing coral reef runs south of the cays along the south-west coast of Aruba. For a brief description of the surrounding environmental and habitat characteristics of the three locations on Curaçao and on Aruba see Nagelkerken et al. (2000a,b) and Grol et al. (2008).

To study condition, reproduction and gut contents, specimens of Haemulon flavolineatum were collected at the western part of Spanish Water Bay and Fuik Bay on Curaçao (October 2007 – January 2008) and at the lagoon on Aruba (August–November 2007) (Appendix 1). Fishes were caught in mangroves, seagrass beds, boulder/notch and rubble habitats, except for Fuik Bay where no rubble zone exists, and on the coral reefs in front of the three study locations. H. flavolineatum were caught using a beach seine net, a self-constructed V-shaped net, Antillean fish traps, or hook and line. After being caught, fishes were directly put on ice and transferred to the laboratory for further analyses (see Appendix 2 for an overview of the number of fishes used in the different analyses described below).

Distribution of Haemulon flavolineatum – Abundance and total length (TL) of H. flavolineatum were estimated at Spanish Water Bay on Curaçao (July–November 2005) and at the lagoon on Aruba (July–December 2007) using a visual census technique. Permanent belt transects were surveyed using SCUBA or snorkelling equipment in the four shallow bay habitats and on the coral reef (see Nagelkerken et al. 2000b for details on the census methodology). Per island, 2-5 sites per habitat type were selected and at each site, 2–9 permanent transects were placed, depending on the size of the habitats. Transects were separated at least 12.5 m apart from each other, and each transect was 25 m long and 4 m wide (100 m²), except for mangrove transects which were shorter because of space limitation. Surveys were repeated at least once every ten days at each site, and each transect was censused at least six times during the study period on Curaçao and 3–4 times on Aruba. Densities of H. flavolineatum were calculated per 100 m² census area per island and represented as the relative total abundance per size class (i.e., 0.0–1.9, 2.0–3.9, 4.0–7.9, 8.0–11.9, 12.0–15.9, 16.0–19.9, and >20.0 cm TL) present in the bay (all habitats pooled) or on the coral reef (Figs. 1a,b). For the individual bay habitats (Figs. 1c,d), the density per size class was multiplied by the respective total habitat surface area and expressed as relative total fish abundance per
bay habitat per size class. On the reef, densities were averaged per 100 m² census area.

**Condition and maturation of Haemulon flavolineatum** – Fish condition was determined on basis of the relationship between weight and length of each fish (WL ratio; based on eviscerated wet body weight in mg and FL in mm) and liver-lipid content of each fish liver. To estimate the total lipid content, wet weight of livers was determined with an accuracy of 0.00001 g, after which total lipids were extracted from each liver by homogenizing the tissue with a chloroform : methanol : water solvent (1:1:1) according to the protocol used by Bathgate et al. (2006). Extracted lipids were dried and weighed with an accuracy of 0.00001 g, and expressed as a percentage of the wet liver weight.

Reproductive status of each fish caught was assessed on basis of external macroscopic sexual characteristics of the gonads, such as size, colour, shape and texture. Each individual was classified into immature (stage 1 and 2) and mature (stage 3–6) based on six different maturation stages using a modified protocol of Ntiba & Jaccarini (1990) and Kulmiye et al. (2002) in which we combined the stages 2a and 2b.

Wet weight of both fish gonads was weighed with an accuracy of 0.001 g and was used to calculate the gonadosomatic index (GSI): GSI = (GW / (W - GW)) * 100, where GW is the wet weight of both gonads and W the eviscerated wet body weight (Ntiba & Jaccarini 1990, Kulmiye et al. 2002).

**Diet of Haemulon flavolineatum** – Gut content of each fish was identified and quantified to taxon level using a stereomicroscope following the procedures of Cocheret de la Morinière et al. (2003). Plant material and sand were not taken into account and guts filled with fish bait or of which the contents were 100% unidentifiable were excluded from the analyses. The relative volumetric abundance of the food items was estimated by eye, where the total volume of contents of the digestive tract was set at 100%. In this paper, we only use data for the most common prey groups (i.e., Copepoda, Tanaidacea, benthic worms and Decapoda).

Densities of zooplankton in the water column and of infauna in the top layer of the substratum were determined in mangrove, seagrass, rubble, and coral reef habitats at Spanish Water Bay in 2005 and on Aruba in 2006. Sediment samples were collected only from the top layer of the substratum (3–4 cm deep) while zooplankton samples were taken in the water layer just above the substratum (using the same methodology as used by Grol et al. 2008). In total, 103 sediment samples and 52 plankton samples were collected at Spanish Water Bay, and 108 sediment and 53 plankton samples on Aruba. Identification and quantification of the planktonic and benthic invertebrates in each sample was done using the same method as for gut contents of the fish (see above).
**Mechanisms driving ontogenetic habitat shifts**

**Statistical analyses** – Means for condition factors, GSI, maturation stages, and diet composition of the fishes (see Appendix 2 for the total number of fish used) were calculated per 4-cm size classes (i.e., 0.0–3.9, 4.0–7.9, 8.0–11.9, 12.0–15.9, and 16.0–19.9 cm TL) per habitat per location per island. Associations between diet components and *Haemulon flavolineatum* of different size classes from different habitat types were investigated using a $\chi^2$-test for each location. To explore differences in WL ratio, liver-lipid content, GSI, mean maturation stage, and the abundance of food among habitats per size class for each location per island, 1-way ANOVAs followed by Hochberg’s GT2 post-hoc comparisons, or Tukey HSD comparisons for the abundance of food, were performed when data had homogeneity variances (Levene’s test). Otherwise a non-parametric Kruskal-Wallis test followed by a Games Howell post-hoc comparison was used. Data were transformed using a log $(x + 1)$ or square root $(x + 0.5)$ transformation if needed. When only two habitat types per 4-cm size class could be compared, an independent samples t-test was used.

Statistical analyses were conducted with SPSS version 15.0, and $P$-values ≤0.050 were considered statistically significant.

**Results**

**Distribution across habitats** – The smallest individuals of *Haemulon flavolineatum* were only observed in bays on Curaçao and Aruba, and never on reefs (Figs. 1a,b). In contrast, the largest-sized fishes were almost only found on coral reefs. Within the bay environments, fishes showed multiple ontogenetic habitat shifts. Settlement of *H. flavolineatum* (0.0–1.9 cm TL) mainly occurred in rubble habitats (Figs. 1c,d). The subsequent size class of 2.0–3.9 cm TL showed a sudden increase in total abundance on seagrass beds on Curaçao (Fig. 1c), where abundance remained highest up to the 8.0–11.9 cm size class. On Aruba, the 2.0–3.9 cm size class also showed a sudden increase on seagrass beds, but not such a dramatic drop in relative abundance on rubble as on Curaçao (Fig. 1d). The subsequent size class of 4.0–7.9 cm TL showed a further increase on seagrass beds and decrease on rubble (Fig. 1d). Total abundance increased gradually in mangroves and was highest of all habitats for fishes of 12.0–15.9 cm TL on Curaçao and 8.0–15.9 cm TL on Aruba (Figs. 1c,d). The transition from bay towards reef largely took place in the 8.0–11.9 and 12.0–15.9 cm size classes on Curaçao (Fig. 1a) and in the 12.0–15.9 cm size class on Aruba (Fig. 1b). The largest-sized fishes (16.0–19.9 cm TL) on Curaçao showed an additional peak in abundance in boulder/notch habitats (Fig. 1c). On Aruba, this size class was not observed within the lagoon.
Figure 1. Relative density of *Haemulon flavolineatum* in the bay (all habitats pooled) and on the coral reef for (a) Curaçao and (b) Aruba, and relative total abundance for individual bay habitats for (c) Curaçao and (d) Aruba, per 4-cm size class. Note that the size class 0.0–3.9 cm TL is separated into 0.0–1.9 cm and 2.0–3.9 cm TL, and that on Aruba the boulder/notch habitat was not included in the study.
Mechanisms driving ontogenetic habitat shifts

Table 1. Relative abundance (± SD) of Copepoda, Tanaidacea, benthic worms and Decapoda found in the digestive tracts of Haemulon flavolineatum collected in mangroves (MG), seagrass beds (SG), rubble habitats (RB), boulder/notch habitats (BN) and on coral reefs (CR) per 4-cm size class per location per island (Aruba and Curaçao). The number (n) of analyzed digestive tracts per habitat per size class per location per island are represented. Positive associations between diet components and H. flavolineatum of different size classes from different habitat types per location per island are highlighted in grey (Spanish Water Bay $\chi^2_{33} = 719.27$, Fuik Bay $\chi^2_{24} = 425.07$, Lagoon $\chi^2_{36} = 600.92; P < 0.010$).

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Diet and food abundance – Gut content analyses showed that juvenile *Haemulon flavolineatum* (<12.0 cm FL) consumed primarily Copepoda (up to, on average, 79% of the total stomach content) and secondarily Tanaidacea (up to 52%) (Table 1) in bay and reef habitats. As fish size increased (≥12.0 cm FL), a switch in diet was noticeable from Copepoda and Tanaidacea towards larger-sized decapod crustaceans (up to 76%). Fishes ≥8.0 cm FL also consumed considerable amounts of benthic worms (up to 45% on average); however, this pattern was only visible for fishes collected from hard substrata (i.e., rubble habitat, boulder/notch habitat, coral reef).

Planktonic Copepoda – most preferred food items by juvenile *H. flavolineatum* (<12.0 cm FL) - occurred in significantly higher densities in the water layers above the substratum of coral reefs than in various bay habitats, and did not differ among mangroves and seagrass beds (Figs. 2a,b, Table 2). Tanaidacea – which were the second most important food items for fish <12.0 cm FL – showed higher densities on seagrass beds (water layer for Curaçao, water layer and substratum for Aruba) than in most other habitat types (Fig. 2, Table 2). Benthic worms, a favoured food item of fish ≥8.0 cm FL, were significantly more abundant in the sediment on reefs than in bay habitats, except for the seagrass beds on Aruba (Figs. 2c,d, Table 2).

### Table 2. P-values of statistical tests comparing the abundance of preferred food of *Haemulon flavolineatum* among habitats in the sediment and water layer, separately per island. One-way ANOVA’s (⁎) and non-parametric Kruskal-Wallis tests (⁎) were performed and data were log- or square root-transformed if needed. Significant differences are highlighted in grey (P ≤ 0.050).

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<td>0.003  b</td>
<td>0.000  a</td>
</tr>
<tr>
<td>Sediment</td>
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<td>0.000  b</td>
<td>0.000  a</td>
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Figure 2. Densities (+ SE) of Copepoda, Tanaidacea and benthic worms found in the water layer (a,b) and sediment (c,d) samples collected in mangrove, seagrass, rubble, and coral reef habitats on Curaçao (a,c) and on Aruba (b,d). Different letters (A–C) represent significant differences among habitat types, per food item per island (Tukey HSD and Games Howell post-hoc comparisons, P ≤ 0.050).
**Condition and growth** – The two condition indices, weight-length (WL) ratio and liver-lipid content, showed a clear distinction between small- and large-sized fish (Fig. 3, Table 3). The smallest-sized fish (0.0–3.9 cm FL) did not show a clear difference in WL ratio among bay habitats or compared to the reefs. Likewise, fish in consecutive size classes (Spanish Water Bay 4.0–11.9 cm FL; Fuik Bay and Aruba 4.0–7.9 cm FL) did not show a clear difference in WL ratio or fat content among habitats. Larger-sized fish (Spanish Water Bay 12.0–15.9 cm FL; Fuik Bay and Aruba 8.0–11.9 cm FL) did not show strong differences among bay habitats for WL ratio and fat content, but did show a significantly higher WL ratio and significantly lower fat content on reefs. These patterns were also discernable for the largest-sized fish (Spanish Water Bay ≥16.0 cm FL; Fuik Bay and Aruba ≥12.0 cm FL).

**Gonadal development** – As was the case for the condition indices, the GSI and mean maturation stages showed different results for small- vs. large-sized fish. Smaller fish (<12.0 cm FL for all three locations) generally did not show a significant difference for GSI and mean maturation stage among habitats (Fig. 4, Table 3), and, on average, never reached maturation stage 3 indicating they were still immature (Figs. 4d,e,f). For larger fish (Spanish Water Bay and Fuik Bay ≥12.0 cm FL; Aruba ≥16.0 cm FL), GSI and mean maturation stage were significantly higher on the coral reef than in all bay habitats. Between bay habitats and between Spanish Water Bay, Fuik Bay and Aruba, values for GSI and mean maturation stage were comparable (Fig. 4, Table 3). Larger individuals on the coral reef even reached maturation stage 5, reflecting readiness to reproduce (Figs. 4d,e,f).
Figure 3. Mean (+ SE) weight-length ratio (WL ratio) and fat percentage of liver tissue (Fat) of *Haemulon flavolineatum* in mangrove, seagrass, rubble, boulder/notch, and coral reef habitats pooled per 4-cm size class for (a,d) Spanish Water Bay and (b,e) Fuik Bay on Curaçao, and (c,f) the lagoon on Aruba. Different letters (A–C) represent significant differences among habitat types, per size class per location per island (Hochberg’s GT2 and Games Howell post-hoc comparisons, \( P < 0.050 \)). Note that at the lagoon on Aruba no significant difference in WL ratio was found for fishes of 0.0–3.9 cm FL (Table 3, \( P = 0.181 \)), and at Fuik Bay on Curaçao no significant difference was found in fat content for fishes of 8.0–11.9 cm FL (Table 3, \( P = 0.193 \)), however, a post-hoc comparisons did show significant differences between habitats.
Figure 4. Mean (+ SE) gonadosomatic index (GSI) and maturation stage of *Haemulon flavolineatum* in mangrove, seagrass, rubble, boulder/notch, and coral reef habitats pooled per 4-cm size class for (a,d) Spanish Water Bay and (b,e) Fuik Bay on Curaçao, and (c,f) the lagoon on Aruba. Different letters (A–C) represent significant differences among habitat types, per size class per location per island (Hochberg’s GT2 and Games Howell post-hoc comparisons, *P* ≤ 0.050). Note that a significant difference among habitats was found for the mean maturation stage for fishes of 4.0–7.9 cm FL at Spanish Water Bay (Table 3, *P* = 0.021), however, a post-hoc comparison did not show any significant difference between habitats.
The current study shows the existence of a complex, multi-stage habitat-use pattern through the ontogeny of a common coral reef fish, and provides compelling evidence for the underlying mechanisms. While many species have a 2- or 3-phase life cycle (Roughgarden *et al.* 1988, Leis 1991, Haywood & Kenyon 2009), the fish species studied here (*Haemulon flavolineatum*) shows a 5-phase life cycle and possibly even a 6-phase life cycle across multiple tropical shallow-water coastal habitats. After a pelagic dispersal phase (phase 1), settlement occurs preferentially in rubble habitats (phase 2) located near the mouth of embayments and lagoons or water inlets between coral cays as seen on Aruba (<2.0 cm total length, TL), which often is the first habitat that larvae come across when entering a bay environment. After settlement, juveniles (2.0–7.9 cm TL) appear to move deeper into the bay/lagoon onto seagrass beds (phase 3), especially on Curaçao. Growing larger (8.0–15.9 cm TL) their abundance decreases on seagrass beds, while increasing in mangroves (phase 4), especially on Aruba. Fishes >16.0 cm TL within bays were predominantly distributed over hard substratum on Curaçao (possibly phase 5); while on Aruba this size class was absent from all surveyed bay habitats, and boulder/notch habitats were not included in the study. Finally, fish disappeared from the bays and were only observed on coral reefs (phase 6).

Each of these phases involves a shift in habitat which appears to be characterized by different cost-benefit analyses that likely maximize an individuals’ fitness as discussed below.

Settlement in bay environments (phase 2) seems to be driven by a cost-benefit analysis between resource acquisition and predation risk. With respect to predation, Grol *et al.* (chapter 5) showed that juveniles which settled in an embayment increased their survival from predation, but at the detriment of growth (otolith increment study). In this life stage, fishes are diurnally-active planktonic feeders (Ogden & Ehrlich 1977, Verweij *et al.* 2006b), predominantly feeding on copepods. With respect to resource acquisition, the current study shows that food abundance, in terms of pelagic copepods, was higher on reefs than within bay environments. Not only prey standing stock, but likely also prey turnover rate is higher on reefs through continuous replenishment by along-shore oceanic currents. In contrast, consumed prey in shallow and semi-enclosed embayments is only partly (through local productivity) or temporarily (through inflowing tides with oceanic waters) replenished. Thus by settling just a short distance away from the coral reef (i.e., rubble in the bay mouth), fishes increase their survival rate considerably but still benefit from oceanic currents replenishing planktonic prey items. Although a low abundance of copepods was found on rubble, we suggest that fish do benefit from these along-shore oceanic currents. Settlement thus seems highly
spatially-driven for this specific type of seascape where an optimum between predation risk (affecting mortality) and resource acquisition (affecting growth) is the likely underlying mechanism. Similar results in which individuals shift habitats to minimize the ratio of growth to mortality exist in a wide range of taxa (Werner 1986, Lima & Dill 1990, Dahlgren & Eggleston 2000, Urban 2007, Haywood & Kenyon 2009).

An ontogenetic habitat shift from settlement sites in bay mouths to seagrass beds (phase 3) is likely driven primarily by resource acquisition. Dorenbosch et al. (2009) showed a similar degree of predation risk of early juveniles among bay habitats along a spatial gradient. Therefore, predation risk is unlikely to act as the primary or sole mechanism driving ontogenetic habitat shifts within the bay at this life stage. In this size class (2.0–7.9 cm FL), *H. flavolineatum* are known to gradually change from pelagic to benthic feeding, and from diurnal to nocturnal feeding (Ogden & Ehrlich 1977, Helfman et al. 1982, Verweij et al. 2006b). The former is exemplified in the present study by an ontogenetic shift in diet from pelagic Copepoda to benthic Tanaidacea. Our data indicated that among bay habitats, Tanaidacea showed highest densities in seagrass beds, in the water column just above the vegetation as well as in the substratum. Furthermore, the open seagrass habitat facilitates the still partially pelagic feeding on copepods compared to the mangrove prop-root habitat (Verweij et al. 2006b). In this life stage, fish thus benefit from abundant benthic food sources on seagrass beds. However, this did not result in higher WL ratios, fat content or gonadal development compared to the rubble habitat. An additional reason why early juvenile fish move to seagrass beds is that these areas provide a better protection against predators for this specific size class than rubble (i.e., more effective shelter among seagrass blades than among small-sized coral rubble pieces).

In phase 4 (8.0–15.9 cm TL), *H. flavolineatum* in the bay appeared to undergo an ontogenetic habitat shift from seagrass beds to mangroves. This shift is probably driven by the need for optimal shelter. Fishes of this size class are nocturnal feeders and may have outgrown their day-time refuges between seagrass leaves. In this case, resource acquisition is not the underlying mechanism for an ontogenetic habitat shift as fish still feed on seagrass beds at night (Ogden & Ehrlich 1977, Helfman et al. 1982, Nagelkerken et al. 2000a, Nagelkerken & van der Velde 2004, Verweij et al. 2006b), where food resources are more abundant. If sufficient or suitable shelter would have been provided by seagrass beds, then fishes would presumably not risk moving into mangroves during day-time because movement increases mortality risk through increased detection by predators (Crowl 1989). In contrast, the dark, structure-rich mangrove prop-roots form ideal day-time shelter environments for larger fishes (Laegdsgaard & Johnson 2001, Cocheret de la Morinière et al. 2004, Verweij et al. 2006a). Verweij et al. (2006a) showed that during the day, large-sized *H. flavolineatum* are largely inactive and show schooling behaviour
Mechanisms driving ontogenetic habitat shifts

in mangroves. In the absence of predators, fish were observed just in front of mangroves, but moved into mangroves to seek shelter when predators were nearby (Laegdsgaard & Johnson 2001). Optimal feeding cannot be accomplished in the relatively small fringing mangroves due to (1) low benthic prey densities in the substratum (this study), and (2) the individualistic feeding behaviour of *H. flavolineatum* (Ogden & Ehrlich 1977, Verweij et al. 2006a) which forces fish to select habitats with larger surface areas (such as seagrass beds) to avoid competition while feeding. Various studies (Ogden & Ehrlich 1977, Helfman et al. 1982) have shown that schools of *H. flavolineatum* leave their day-time shelter in groups at dusk, after which they quickly disperse onto seagrass beds in a dendrytic pattern and feed solitary or in small groups during the night, and return at dawn. In addition, Verweij et al. (2006b) showed that *H. flavolineatum* of the same size class spent a higher percent of their time budget on feeding and took more bites per time unit in seagrass beds compared to mangroves. Stable isotope studies further supported that *H. flavolineatum* sheltering in mangroves of Spanish Water Bay during day-time primarily depended on seagrass food sources (Nagelkerken & van der Velde 2004). Hence, diurnally inactive fishes in phase 4 select optimal mangrove shelter habitat, and migrate at night to seagrass beds harbouring highest densities of favored benthic prey items. WL ratios, fat content and gonadal development did not increase due to the ontogenetic shift from seagrass beds to mangroves, showing no increased benefits in terms of fitness.

Although boulder/notch habitats were not surveyed on Aruba, the shift from mangroves to boulder/notch habitats (phase 5) was clearly visible on Curaçao. Studies have shown that adult *H. flavolineatum* are typically associated with hard substrata (Kendall 2003, Lindeman & Richards 2005) and a shift to this habitat is possibly associated with this preference of larger-sized fish.

The uni-directional movement from bays to coral reefs (phase 6) appears to be driven by the need for reproduction. Fish in bay environments remained immature, independent of their size and habitat type occupied; even fishes of 16.0–19.9 cm FL in bays reached only the initial stages of maturation, while smaller fishes on reefs had already matured. There is considerable evidence that a large size, fast growth rate, and high lipid content are important criteria for initiation of sexual development (Rowe et al. 1991, Silverstein et al. 1997). Our results respond to this as on reefs fish achieved high growth rates and WL ratios, had a large size and probably invested their energy in gonadal development, as shown by the much lower liver-fat contents and higher gonadal somatic index compared to fish in bay habitats. Continuing residence within bay habitats hence does not appear to facilitate maturation and thus inhibits fitness. Reproduction on coral reefs by this pelagic spawner will be more successful than in semi-enclosed bays, explaining the need to ultimately move to reef habitats, which is the typical residence habitat for large adults. It
is unlikely that predation is the driving factor to shift habitats for this size class as larger fishes become less vulnerable to predation (Lima & Dill 1990, Hixon 1991, Dahlgren & Eggleston 2000, chapter 5).

Most organisms have to deal with cost-benefit analyses during their life cycle and may therefore inhabit different habitat types during different life stages, which indirectly result in connectivity among these habitats. Movement across habitat boundaries can have strong impacts on the dynamics and structure of communities (Polis et al. 1997), where changes in one habitat can indirectly affect the community structure in a connected habitat (Schreiber & Rudolf 2008). The current study shows a strong linkage among a suite of different habitats, which ultimately support the viability of populations on coral reefs. With the ongoing loss and fragmentation of nearshore coastal ecosystems (Alongi 2002, Duarte 2002, Lotze et al. 2006), ontogenetic and diel feeding migrations across habitats may be altered to such a degree that it affects trophic interactions and successful movement of animals among habitats. This may ultimately lead to a decrease in ecosystem productivity and resilience (Bellwood et al. 2004, Hughes et al. 2005).

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REFERENCES


Mechanisms driving ontogenetic habitat shifts


Mechanisms driving ontogenetic habitat shifts


Appendix 1. Map showing the study locations at the two Caribbean islands Aruba and Curaçao; the lagoon on Aruba, and Fuik Bay and Spanish Water Bay on Curaçao. The locations of mangroves, seagrass beds and coral reefs are shown. Rubble habitats (indicated by *) are located in and near the mouth of Spanish Water Bay and in front of the water inlets at the eastern part of the lagoon on Aruba. Boulder/notch habitats (not shown on the map) are located in front of crevices in the shoreline fossil limestone plateau of all three locations.
Appendix 2. Number of *Haemulon flavolineatum* collected for quantifying fish condition indices weight-length ratio (n WL) and liver-lipid content (n Fat), the number of gonad pairs used to calculate the gonadosomatic index (n GSI) as well as to determine maturation stages (n Mat), and the number of guts used for diet analyzes (n gut), per island per location per habitat. MG = mangroves, SG = seagrass beds, RB = rubble habitats, BN = boulder/notch habitats, and CR = coral reef.

<table>
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<th>n GSI</th>
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SYNTHESIS
During the last few decades, shallow-water coastal habitats such as mangroves and seagrass beds have received much attention and have been considered as important nursery habitats for many juvenile coral reef fish and invertebrate species as they replenish coral reef populations (Beck et al. 2001, Verweij et al. 2007, 2008, Haywood & Kenyon 2009, Nagelkerken 2009). However, the importance of these nurseries has mainly been based on studies showing high juvenile fish densities in mangroves and seagrass beds of which the adults are found on coral reefs (Nagelkerken et al. 2000a,b, Gillanders et al. 2003, Sheridan & Hays 2003, Dorenbosch et al. 2004, 2005a,b, 2006a, Eggleston et al. 2004). This does not necessarily purport that juveniles indeed contribute to adult populations, and the question why many juvenile coral reef fish species are attracted to mangrove and seagrass nurseries is poorly understood. Although recently authors provided evidence for movement of fishes between these putative nurseries and coral reefs (Chittaro et al. 2004, Nakamura et al. 2008, Verweij et al. 2008, Mateo et al. 2010), the underlying mechanisms of such movements still remain unclear. There is an ongoing debate that mangrove and seagrass nurseries are attractive because they are structurally complex which enhances the abundance of food (growth) and lowers the risk of predation (survival) (Orth et al. 1984, Hixon & Beets 1993, Laegdsgaard & Johnson 2001), both of which are mechanisms that could drive across-habitat distribution patterns.

Shallow coastal waters such as our study systems on Curaçao and Aruba are unique in a way that many different habitat types occur close to each other (e.g., mangroves, seagrass beds, rubble, mud/sand flats, boulders and notches) and fishes can shift easily between them. Yet, there is not an equal distribution of fishes among these habitats (chapters 4-6). Fishes do have a preference for one habitat above the other (chapter 2), and while selecting a settlement habitat or migrating towards other habitats through ontogeny in order to meet their needs, they are confronted with multiple factors at the same time (e.g., different levels of habitat structural complexity, predation risk, food availability; chapters 2-6). The existence of a complex, multi-stage habitat utilization pattern during the life-history of a common coral reef fish is shown in this thesis, and it provides compelling evidence for the underlying mechanisms causing habitat shifts. This thesis contributes to a better understanding of the nursery-role hypothesis for coral reef fishes as not only fish densities were studied in multiple non-reef and reef habitats, but also other important aspects which could drive ontogenetic habitat shifts (e.g., food abundance, fish growth rates, predator abundance, survival from predation, fish condition, and maturation; chapters 2-6). Understanding these mechanisms is of great concern for fisheries management and conservation. It is essential to strengthen predictions about the causes of spatial and temporal distribution patterns of coral reef fishes, and for evaluating the consequences of habitat degradation as a result of, e.g., climate change and anthropogenic
stressors such as destructive fishing and pollution on the diversity and function of the tropical seascape.

**EVALUATION OF THE NURSERY-ROLE HYPOTHESIS**

After a pelagic larval phase, fishes have a whole range of habitats to choose from, yet they selectively settle (Jones et al. 2004, Dorenbosch et al. 2005a, 2006b, Pollux et al. 2007, Huijbers et al. 2008a, Haywood & Kenyon 2009). Many coral reef fish species settle directly on the coral reef (Mora & Sale 2002, Jones et al. 2004, Dorenbosch et al. 2005a, Huijbers et al. 2008a), whilst others settle in spatially-segregated nursery habitats, such as inshore mangroves and seagrass beds (chapters 5 & 6, Nagelkerken et al. 2000b, Dorenbosch et al. 2004, 2006b, Mumby et al. 2004). According to the framework proposed by Beck et al. (2001), the greater proportion of recruitment to adult populations from these proposed nurseries is the result of any combination of (1) higher juvenile densities, (2) higher growth rates, (3) higher survival rates, and (4) movement from the nurseries to adult habitats (i.e., connectivity). Results from the present thesis evaluated the nursery function for different bays on Curaçao and Aruba by testing these factors separately (chapters 3–6).

**Fish distribution patterns** – Juvenile *Haemulon flavolineatum* were distributed in much higher densities in bays than on adjacent coral reefs, while (sub)adults were almost exclusively found on reefs (chapters 5 & 6) which is in line with the nursery-role hypothesis.

Juveniles that had recently recruited from the plankton were most abundant close to the entrance of the bays, especially at the rubble zone (chapters 5 & 6). This was often the first habitat, although with a very small surface area, that larvae came across when entering bay environments. Settlement close the reef is not a rare event and has been observed elsewhere by Shulman (1985) who found settlement of some species to peak on rubble/sand areas just 20–30 m into the lagoon from the reef crest. Furthermore, settlement peaks of nursery species on rubble were also reported by Adams & Ebersole (2002) and Tupper (2007). Within bays, clear across-habitat distribution patterns were visible as fishes grew in size. Fishes moved deeper into the bay (chapter 5) and shifted from rubble to seagrass beds to mangroves to boulder/notch habitats (chapter 6). The latter habitat was occupied by the largest specimens in the bay and was located at a short distance from coral reefs. This habitat could be a final stop within the bay before fish move to the reef for permanent residence. Although higher juvenile densities were found in nursery habitats compared to reefs, little information
Supporting the nursery function of shallow-water coastal habitats can be obtained from these data.

Structurally complex habitats are especially preferred by juvenile fish as they provide more food and shelter (Orth et al. 1984, Laegdsgaard & Johnson 2001, Verweij et al. 2006a, Nagelkerken & Faunce 2008). Various habitats provide such complex structure, but they have very contrasting three-dimensional orientation and architecture, and differ in their degree of structural complexity. Although fish were expected to always select the structurally most complex microhabitat offered, independent of microhabitat type, they did not have a preference for the structurally most complex habitat, and no relationship was found with any of the measured complexity characteristics. Based on these characteristics a theoretical hierarchical model was proposed in chapter 2, which proposes that early juvenile *H. flavolineatum* show a strong preference for live, benthic seagrass and coral microhabitats. Remarkably, size-specific density distributions of *H. flavolineatum* (chapters 5 & 6) did not show any juveniles on coral reefs, only in shallow non-reef habitats located in the bay. This indicates that although early juveniles included coral as their initial choice of preferences (chapter 2), under natural circumstances they will not select or survive in this habitat (chapters 4–6). Therefore, ultimate habitat distribution appears to be driven by other mechanisms than solely habitat preference in case of coral structure (chapters 3–6).

**Food, growth & condition** – Until approximately 4 to 5 cm fork length (FL) *Haemulon flavolineatum* are diurnal planktivores and they mainly feed during the day on Copepoda (chapters 3 & 6, Randall 1967, Ogden & Ehrlich 1977, Verweij et al. 2006b), supplemented with Tanaidacea (chapters 3 & 6, Cocheret de la Morinière et al. 2003). Larger specimens shifted towards a zoobenthivoric diet consisting of benthic worms and larger crustaceans (i.e., Decapoda; chapter 6). It was therefore suspected that higher densities of preferred food items would occur in the habitats where fish densities were highest. However, the opposite was true and the abundance of preferred food items was higher on reefs (chapters 3 & 6), and so were growth rates (chapters 3 & 5). Lower *in situ* growth rates were found for early juveniles in nurseries than on reefs (chapter 3), while the abundance of food was not deficient in the experimental cages used as revealed from planktonic and benthic food samples (chapter 3). Using experimental growth cages (chapter 3) many factors which could influence growth rates (e.g., predation, competition) were excluded and therefore in this study the most likely factor explaining the higher growth rates on coral reefs was the higher amount of preferred food on reefs. Additionally, growth rates obtained from otolith increments of wild-caught specimens (0–20 cm FL) showed similar results (chapter 5). Fish with the same age had greater lengths on reefs compared to fish from nursery habitats, and
Chapter 7

thus grew faster (chapter 5). Growth rates did not differ between different nursery habitats (chapters 3 & 5). This concludes that juveniles were not attracted to nursery habitats because of higher growth rates as a possible result of higher food abundances. This is opposite of what was expected from the nursery-role hypothesis which stated that within the nurseries higher growth rates are achieved compared to adult habitats (Beck et al. 2001).

Higher growth rates of fishes and invertebrates at locations where the amount of preferred food is highest is not an uncommon theme. For example, growth rates of juvenile blue crabs (Callinectes sapidus) in enclosures in the York river, Virginia, and the availability of food were both greatest at upriver sand (Seitz et al. 2005). But studies comparing growth rates across mangrove and seagrass nurseries, and coral reefs are lacking, and growth studies conducted in nursery and adult habitats hardly ever compared fishes of the same size class or studied the whole size range of a species (i.e., juveniles and adults). Dahlgren & Eggleston (2000) compared in situ growth rates of three juvenile size classes of the Nassau grouper (Epinephelus striatus) in a tidal creek and also found higher growth rates in the most profitable microhabitats harbouring coral structures compared to algal nursery habitats. However, like the results in chapters 3, 5 & 6, the smallest-sized fishes did not reside in the most beneficial habitat in terms of growth rates.

Survival – Mangrove and seagrass nurseries are also assumed to provide fishes and invertebrates with more hiding places as they are structurally complex and thus lower predation risk (Orth et al. 1984, Beck et al. 2001, Laegdsgaard & Johnson 2001). Additionally, mangroves and seagrasses are located away from coral reefs, are shallow and often turbid, all lowering the risk of predation (chapters 4 & 5, Shulman 1985, Cocheret de la Morinière et al. 2004). Taking all these factors into account, nursery habitats likely have a greater survival prospective compared to the coral reef. Indeed, survival rates of tethered, early juveniles were higher in the putative nurseries in bays than on coral reefs, and increased with an increasing distance from the mouth of the bay (chapters 4 & 5, Valentine et al. 2007, Vanderklift et al. 2007). Piscivore densities on the other hand, were not only high on the coral reef, but also in mangroves in the bay (chapter 4), and predation may be considerably higher there than generally assumed (Nakamura & Sano 2004, Baker & Sheaves 2005, 2006, 2009). The high piscivore density in mangroves is most likely caused by the fact that also nursery species act as predatory fish (e.g., Lutjanus griseus, L. apodus, Sphyraena barracuda), complemented with reef-associated visiting piscivores (e.g., Carangoides ruber, Caranx latus). Secondly, mangroves have a very small surface area compared to the other habitats. Correcting for surface area, piscivore density was a magnitude lower in bay habitats than on the reef as suspected (unpublished data). Although survival rates of early juveniles were much higher in nursery habitats than on reefs (chapters 4 & 5), they did
show a preference for coral microhabitats in the absence of predators (chapter 2). This suggests that the risk to be preyed upon plays an important role in the nursery function of bay habitats and could be the underlying mechanism causing high settlement and juvenile densities in bays.

**Movement to adult population** – Despite the fact that fish distribution patterns showed that adult *Haemulon flavolineatum* densities on reefs were much higher than in nurseries (chapters 5 & 6), this does not prove that a greater proportion of recruitment to adult populations is driven by nurseries as compared to other habitats (Beck *et al.* 2001). The final migration from nurseries to reefs is poorly understood and only recently true evidence has been provided for this final shift for a few species (Brown 2006, Nakamura *et al.* 2008, Verweij *et al.* 2008).

Mark and recapture studies can be a method to show that (sub)adult *H. flavolineatum* indeed migrate from bays towards coral reefs and thus contribute to coral reef fish populations (Gillanders *et al.* 2003). Therefore, >5000 fish were marked using coded wire tags (CWTs) in the embayment on Aruba between Mangel Halto and Barcadera (2005–2007) and >5000 fish at Spanish Water Bay, Curaçao (2004–2007). During 2006–2010 all reef and bay *H. flavolineatum* caught on both islands were scanned for the presence/absence of a CWT. On Aruba, 13 fish were recaptured (i.e., a CWT was present) of >7000 caught. Only one fish had migrated to the reef, while the other 12 fish had not moved (n = 9) or only a few meters away (n = 3) from the same location in the embayment as where they were tagged in previous years (i.e., different boulder/notch habitats; unpublished data). On Curaçao, 92 fish were recaptured in the bay (85 at the same location and 7 at a different location) and 3 fish on the fringing coral reef (unpublished data). The number of recaptures was extremely low (Aruba 0.3% and Curaçao 1.9% of the tagged fish) and especially for recaptures on reefs (Aruba 0.02%, Curaçao 0.06%). Verweij & Nagelkerken (2007) failed to recapture CWT tagged fish on the reef, but did recapture 4.6% of the tagged fish (n = 1114) in the bay of which 4% showed a reef-directed movement. In general, offshore recaptures of artificially tagged animals are very low and a distinction between individual juvenile habitats such as mangroves and seagrass beds was seldom made (reviewed by Nagelkerken 2009). An exception to this was a study by Bouwmeester (2005) who recaptured a large amount of CWT tagged fish which had moved across the inner shelf. However, specific habitat types or characteristics were not defined. In total, 85% small, recaptured juveniles (17.2% recaptured of total fish tagged [n = 168]; 7–12 cm FL) and ~40% large, recaptured juveniles (2.1% [n = 1311]; ≥10 cm FL) had moved. Note that these fish had been released less than a year before and moved no further than ~200 m, while fish in our study were recaptured over a time span of 6 years in a much larger area (>3 km²).
The low number of recaptures and fish that had moved in our study could be a result of the fact that during the study years fish had died or had been fished. Fishing pressure in the bay and on the reef near the bay entrance can be very high, especially on Curaçao (Hoetjes et al. 2002). Another reason could be that fish migrated further away and/or deeper than we sampled (i.e., reef areas were sampled close to the bay entrances [<300 m] and the shallower parts in bays and on reefs [<8 m depth]). Further research and/or different mark and recapture techniques (e.g., stable isotopes or microelements in otoliths or muscle tissue, acoustic telemetry) are necessary to provide evidence for migration from nurseries to reefs for this species.

THE NURSERY-ROLE HYPOTHESIS IN A BROADER PERSPECTIVE

Defining the functional role of the purported nurseries in supporting fish and invertebrate populations has received great attention, but true evidence has never been provided as studies testing all four nursery-role factors across multiple habitats and species are lacking (Beck et al. 2001, 2003, Heck et al. 2003, Sheridan & Hays 2003, Nagelkerken 2009). Although in this thesis all four factors were investigated (chapters 2-7), no conclusive proof could be provided for the replenishment of adult populations on reefs for Haemulon flavolineatum.

Alternative juvenile habitats – No juveniles were observed on coral reefs (chapters 5 & 6) where growth rates were faster (chapters 3 & 5) but survival lower (chapters 4 & 5). Other studies, however, have observed juvenile Haemulon flavolineatum, as well as other nursery species, on shallow coral structures and patch reefs (Chittaro et al. 2004, Dorenbosch et al. 2004, 2005a, 2006b, Nagelkerken 2007, Haywood & Kenyon 2009). This indicates that alternative juvenile habitats could also contribute to the adult stock. For example, Huijbers et al. (2008a) showed that if mangroves and seagrass beds are scarce, several species can use alternative nearshore habitats as juveniles. The juvenile subpopulation in such alternative habitats may be greater than in other habitats. However, it is possible that due to an elevated local mortality in these habitats, the on average population growth will be lowered (Fodrie et al. 2009). Furthermore, the use of alternate habitats is often dependent on the configuration and presence of different habitats in the seascape and is species-specific. Therefore, it is difficult to consider a particular habitat type as a true nursery.
Cost-benefit analysis – In general, juvenile survival rates have been shown to be higher in nurseries than in adult habitats for species of fish and invertebrates (chapters 4 & 5, Shulman 1985, Acosta & Butler 1999, Chittaro et al. 2005, Tupper 2007, Haywood & Kenyon 2009), while for growth rates this is debatable. Studies comparing growth rates between mangrove/seagrass nurseries and reefs are very scarce and like in this study, habitats offering high survival rates may not always provide juveniles with higher growth rates which is common for a diverse suite of taxa (chapters 3–5, Werner et al. 1983, Lima & Dill 1990, Suohonen 1993, Dahlgren & Eggleston 2000, Heithaus et al. 2007, Urban 2007, Atkinson et al. 2008). Searching for a suitable habitat is likely driven by a cost-benefit analysis and different strategies may have been evolved in which individuals maximize growth while minimizing mortality in order to survive.

In this thesis, Haemulon flavolineatum settled in safer embayments away from reefs at the detriment of growth (chapters 3–6). But species settling directly onto reefs may initially be in a better condition as they did not traverse such a long distance which is time- and energy consuming (Bennett & Marshall 2005, Stamps et al. 2005, Phillips et al. 2006), have more food available and thus achieve higher or compensatory growth rates (Skalski et al. 2005, Gagliano & McCormick 2007). Due to this, they probably outgrow the most vulnerable size class very quickly and in this way reduce the high risk of predation on newly settled fish on reefs (chapters 4 & 5, Johannes 1978, Webster 2002, Hoey & McCormick 2004). Between species, mortality rates are very different (Shulman & Ogden 1987) and reef-settling species may have developed other advantageous strategies such as larger size at settlement, shoaling behaviour, settlement at specific locations on the reef, predator avoidance tactics (e.g., camouflaging), or parental care to better cope with predators (Hixon 1991, Wellington 1992, Ohman et al. 1998, McCormick & Meekan 2007). At the end, all different strategies have one common theme: maximizing fitness.

Trade-offs among shifting requirements will drive fishes to use different habitats at different sizes, with either gradual or rapid transitions (chapters 3–6, Werner & Gilliam 1984, McCormick & Makey 1997). The studied species in this thesis showed a 5-phase life cycle and possibly even a 6-phase life cycle across multiple tropical coastal habitats (Fig. 1), and each habitat shift reflects a different cost-benefit analysis as discussed in chapter 6. The presence of multiple shifts between different juvenile habitats makes it nearly impossible to designate a single habitat as a nursery that contributes to adult populations on reefs.
Chapter 7

**Importance of nursery species** – Another point which needs to be taken into account when evaluating the nursery-role theory is that, although the number of species utilizing non-reef nurseries is low, and not only in the Caribbean, their importance should not be underestimated as many of these species are among the most common species in terms of density (e.g., Haemulidae, Lutjanidae), are often of great commercial importance (e.g., Sphyraena barracuda, Lutjanidae), are endangered species (e.g., Scarus guacamaia, Cheilinus undulates), and include top predators regulating food web structures on reefs (e.g., Sphyraena barracuda, Lutjanidae).

**Factors initiating movement to reefs**

It remains unclear what the underlying mechanism is that causes the shift from nurseries to coral reefs. Large fishes are less vulnerable to predation (chapter 5, Hixon 1991) and can escape from smaller predators which are gape-limited (Urban 2007). Thus for large fishes residing in nurseries it is less risky to migrate to the structural complex reef.

Food availability is another factor that can drive ontogenetic habitat shifts (chapter 6, Dahlgren & Eggleston 2000, Cocheret de la Morinière et al. 2003). If food availability would become limiting in the bay, this could cause this habitat shift. However, large *Haemulon flavolineatum* feeding on larger crustaceans such as Decapoda (chapter 6) share the same diet as, e.g., *Lutjanus griseus*, a species of which adult specimens utilize mangroves and seagrass beds (Randall 1967, Serafy et al. 2003, Faunce & Serafy 2007). Thus, it is not likely that food will be limiting and thus cause a habitat shift to the reef.

Several studies proposed that the reef-ward migration takes place at the onset of maturity (chapters 5 & 6, Beck et al. 2001, Cocheret de la Morinière et al. 2002, Nagelkerken & van der Velde 2003, Adams & Ebersole 2004). In this thesis we included analysis of the gonads to evaluate whether species indeed migrate to reefs in order to reproduce as reefs may favour larval dispersal (chapters 5 & 6, Johannes 1978, Roberts 1997). We therefore expected to find mature fishes in the bay close to the reef which had their gonads further or fully developed compared to individuals deeper inside the bay. However, fishes residing in bay environments were still immature, independent of their size and habitat type occupied. Even the largest fishes in the bay reached only the initial stages of maturation, while smaller fishes on reefs had already matured (chapters 5 & 6). This suggests that continuing residence within bay habitats does not appear to facilitate maturation and thus inhibits fitness. It also suggests that fish move out of the bay before they fully mature. The question ‘what is the trigger that causes the shift from the bay towards the
reef’ cannot be answered unambiguously, but the need to further develop their gonads and reproduce is one of the likely explanations.

**IMPLICATIONS FOR MANAGEMENT AND FUTURE RESEARCH**

It is now widely accepted that climate change is occurring, and at a faster rate in the world’s oceans than anywhere else. Coral reef ecosystems are highly valued, but threatened by regional human population growth and are under increasing pressure from myriad human impacts (Gardner *et al.* 2003, Hughes *et al.* 2003, Bellwood *et al.* 2004). Habitat connectivity is of great importance and the loss of settlement and juvenile habitats can be catastrophic. Identifying the nursery value of different habitats and isolating the mechanisms that give rise to cross-ecosystem distributions in marine environments is critical for developing appropriate conservation and management initiatives.

Much of our current understanding of marine biodiversity is based on species richness and densities. However, to provide a better insight into the function of non-reef habitats as nursery grounds for juvenile coral reef fishes and invertebrates it is necessary to include growth, survival and movement in studies on these animals. Especially movement to adult habitats is difficult to measure and still poorly investigated. Fortunately, nowadays there is a multitude of different techniques (e.g., stable isotopes, genetic markers, telemetry tags, otolith microchemistry or marking, acoustic tagging) that can be used to track migration patterns between nurseries and adult habitats (review by Gillanders 2009).

To determine the nursery value of putative nursery habitats, all life-stage habitats used by juveniles should be compared and not only the most familiar habitats (e.g., mangroves and seagrass beds), habitats with the largest surface area or habitats which harbour highest fish densities. In addition to this, habitat preference is species-specific and thus different habitats within the seascape can have a different nursery value for different species. Habitats in which a species does not reside but only benefits from (e.g., juveniles residing in mangroves, but foraging on seagrass beds [chapter 6]) should also be included. Biotic and abiotic factors (e.g., food availability, competition, risk of predation, water temperature, depth and salinity) and habitat heterogeneity (i.e., configuration, availability, and accessibility of different habitat types) also need to be considered as they vary over geographical and temporal scales. Furthermore, recruitment occurs in pulses throughout the year and results between years may differ drastically due to e.g., hurricanes and storms, overfishing, habitat destruction, climate change. Studies need to be replicated
Figure 1. Life cycle of *Haemulon flavolineatum*: a 5- or possible 6-stage life cycle across multiple tropical coastal habitats, driven by different mechanisms. Spawning takes place in offshore waters away from coral reefs where strong ocean currents flow (a). Pelagic larvae move towards the coast and settle on rubble: the first safe habitat they come across entering the bay and where planktonic food is replenished by along-shore oceanic currents (b). Early juveniles undergo a diet shift from planktonic to benthic feeding and move to more profitable seagrass beds (c). Growing larger, juveniles seek shelter in dark, structure-rich mangroves during the day, while still feeding on seagrass beds at night (d). They possibly migrate as (sub)adults to hard-bottom boulder/notch habitats (e), just before they shift to their permanent adult habitat, the coral reef (f), where they reproduce (a).
over multiple years (i.e., long-term monitoring) and over a spatial gradient because of this potential variability.

With the emphasis on establishing marine protected areas, identification, quantification and subsequent management of nursery habitats must be considered. The optimal design of marine protected areas must include all habitats essential for fishes to complete their life cycles (i.e., from larval settlement to adulthood). Thus not only coral reefs, but also non-reef ecosystems which function as important settlement and juvenile habitats must be included, as well as spawning grounds to preserve and conserve connectivity among populations in order to support offshore fisheries and ensuring species survival into the future.

Research has focussed on post-settlement larvae due to the ubiquitous problem of studying identifiable pre-settlement larvae. This means that at the moment, we know far more about the ecology of reef fishes after settlement than prior to that. Despite of this, it is necessary to study pre-settlement processes as they can influence post-settlement growth, survival and habitat selection. For example, settling larvae with a suppressed growth as a result of deficient food during their pre-settlement phase can have a poor condition and thus be more vulnerable to predation and decrease their survival chance (Booth & Hixon 1999, McCormick & Hoey 2004). But also questions related to where these larvae come from and where adults spawn need attention. Spawning aggregations of *Epinephelus striatus* and Lutjanidae have been well studied (Colin 1992, Claro & Lindeman 2003), but data on when and where Haemulidae spawn are lacking, especially for our model species.

In addition, more research needs to be done on the orientation mechanisms that fish use. Although this area is currently a hot topic for larvae and recently-settled fish (Tolimieri et al. 2000, Atema et al. 2002, Lecchini et al. 2005, Leis 2006, Huijbers et al. 2008b) there are still a lot of research opportunities and questions to be asked. For example, do larvae and early juveniles orientate to specific habitat odours (e.g., chemical compounds from plant material) as has recently been suggested or do they orientate towards odours released by juveniles of the same species in the putative nurseries? For adult salmon returning to their home river there are different hypotheses drawn such as that they are attracted by pheromones emitted by juvenile fishes or that they imprint to certain distinctive odours of the home stream (Hara 1993). How do fish orientate while shifting between habitats driven by predation, food availability or maturation? How do they know that at a certain location predation risk is high or habitat quality is good? How do they recognize the presence of predators? Do they use sight or odours? And what triggers an immature (sub)adult in the bay to mature and/or to shift to the coral reef: hormones/pheromones or sounds/calls released by others?

At the moment, a central theme in marine ecology is to achieve an understanding of the mechanisms regulating abundance and distribution
patterns of marine populations. These mechanisms are species- and size-specific and can be used to develop a conceptual basis for ecosystem-based management. However, one of the critical components is to quantify rates of connectivity among populations of marine organisms via larval dispersal, which is one of the main criteria when designing a network of marine reserves. The use of computational power in modelling the marine environment can greatly facilitate marine reserve design. Based on the coupling of basic life-history information with that of oceanographic information, predictions of the location of high recruitment areas can be made. Mapping dispersal routes of pelagic larvae will enable the identification of beneficial management and for marine reserves to be effective they need to be established in dense networks in which international cooperation constitutes an important basis. Thus, scientists and management teams need to collaborate for providing the basis for ecosystem-based management, conservation and sustainable fisheries.

REFERENCES


Chapter 7


Ecosystems are intricately linked by the flow of organisms across their boundaries. Individuals shift from one to another habitat during their complex life cycles, and are strongly influenced by behavioural responses to their own changing needs or changes within the surrounding environment. Many marine fish species have juvenile and adult life stages that occupy spatially distinct habitats, separated by a few metres to hundreds of kilometres. Therefore, populations can be linked by the exchange of larvae, juveniles, and/or adults. Furthermore, juveniles often inhabit more than one habitat type, for example, mangroves and seagrass beds. These habitats are considered as important nurseries and are among the world’s most productive ecosystems, but are under serious threats along with coral reefs. Primary factors responsible are heavy over-harvesting, climate change, pollution, disease and coastal development. This results in habitat loss and degradation, reduced ecosystem functions, and a declining biodiversity and population connectivity. Improving our understanding of habitat connectivity and continued efforts to identify the mechanisms that lead to ecosystem-scale distribution patterns are critical for ecological and management-based initiatives and will provide for a more robust foundation to work toward effective management and conservation strategies. This thesis shows the existence of a complex, multi-stage habitat-use pattern during the ontogeny of a common coral reef fish, and provides compelling evidence for the underlying mechanisms.

After a pelagic larval phase, fishes have a whole range of habitats to choose from, yet they selectively settle in specific habitats. Some species settle directly onto coral reefs, whilst others recruit to nearshore shallow-water habitats such as mangroves and seagrass beds where they reside for months to years before migrating to offshore habitats to join the adult population. These nearshore habitats are the so-called ‘nursery’ habitats and are likely to vary in quality. According to the nursery-role hypothesis, nurseries harbour high juvenile densities and are assumed to offer great advantages above the coral reef. They are structurally complex which provides juveniles with good refuge possibilities lowering the risk to be preyed upon and harbour high food abundances enhancing growth rates. However, the present thesis shows that these assumptions are not always true and that fishes often have to make cost-benefit analyses and shift habitats in order to maximize their fitness. As a fish grows it continuously has to adjust its cost-benefit analysis and different mechanisms drive cross-habitat shifts during different life phases.

In this dissertation, the functional role of the purported nurseries in supporting fish and invertebrate populations was investigated across habitats on the Caribbean islands of Aruba and Curacao. Research on Curacao was conducted at two large inshore non-estuarine shallow-water bays with only one bay entrance. On Aruba research took place in a large lagoon (hereafter referred to as bay) with multiple connections to the open sea. All three bays
harbour stands of fringing mangroves along the shorelines and extensive seagrass beds. Furthermore, habitats consisting of hard bottom substratum such as rocks and boulders in front of crevices within the shoreline fossil limestone plateau (boulder/notch) are found in the channel of the bays not far from the reef, while small pieces of dead coral on sandy substratum (rubble) are only found near the entrances. The fringing coral reef is located directly outside the bays and runs along the entire south-west coast of both islands. The target species in this thesis is the French grunt, *Haemulon flavolineatum*, a common coral reef fish species on Aruba and Curaçao.

Habitat preference of juveniles is often positively related to habitat structural complexity. However, different habitats differ greatly in their architecture, e.g., flexible vs. rigid, hanging vs. standing, and the degree of structural complexity *per se* seemed to be less important than the type of habitat (chapter 2). Using experimental cages which excluded interfering factors such as food quantity and quality, and predation, habitat preference for four very different inshore microhabitats (*i.e.*, seagrass, mangrove, rubble, and coral) was tested for individual fish. In the cages, fish always moved towards structured microhabitats instead of residing on bare sand (*i.e.*, unstructured). Furthermore, fish did not have a preference for the structurally most complex microhabitat, and no relationship was found with any of the seven different complexity characteristics measured. Instead, fish were very consistent and always preferred seagrass and coral microhabitats even when offered at low cover, rugosity, or number of holes. Of all four structured microhabitats, benthic structures (*i.e.*, seagrass, coral and rubble) were preferred above hanging structures (*i.e.*, mangroves), and fish preferred live (*i.e.*, seagrass and coral) over dead microhabitats (*i.e.*, rubble). Therefore, the presence of structured, benthic, living habitats may be a more important criteria determining habitat preference by a demersal fish than the degree of structural complexity. On the basis of these results, chapter 2 proposes a theoretical hierarchical model which could apply to habitat preference by various demersal species.

Under natural circumstances, juveniles hardly ever settled on coral habitats. Instead, extensive underwater visual census surveys showed that pelagic larvae settled massively on small rubble areas near the entrances of the bays (chapters 5 & 6). This is the first relatively safe habitat they come across when entering the bays (chapters 4 & 5) and where sufficient planktonic food is available that is presumably replenished continuously by along-shore oceanic currents. Up to 4–5 cm in length, *H. flavolineatum* are diurnally-active planktonic feeders (chapters 3 & 6) and more preferred food items are available on rubble near the reef compared to habitats deeper inside bays which favours growth (chapters 3 & 6). This despite the fact that on Aruba the abundance of planktonic food was relatively low (chapter 6). An optimum between predation risk (affecting mortality [$\mu$]) and resource acquisition
(affecting growth [g]) is the likely underlying mechanism for settlement close to coral reefs, but just inside the bays. This concentration of recently-settled juveniles within this particular small area of rubble in the mouth of the bays could represent a bottleneck for the recruitment of grunts to adult populations and needs to be included in conservation and management plans.

Predation may be considerably higher in shallow nearshore habitats than generally assumed. Extensive underwater visual surveys showed that significant piscivore assemblages were not only present on coral reefs, but also in mangroves (chapter 4). Mangroves also provide refuge to nursery species which act as predatory fish and bays are also visited by reef-associated piscivores. Nevertheless, survival rates of tethered fish were, on average, much higher in nursery habitats than on reefs, and increased with an increasing distance from the mouth of the bay (chapters 4 & 5). Survival rates were lower at sites with high piscivore densities (chapter 4), and in the absence of predators, fish showed a preference for coral microhabitats (chapter 2). This suggests that the risk to be preyed upon plays an important role in the nursery function of bay habitats and could be the underlying mechanism causing high settlement and juvenile densities in bays.

Within bays, juvenile *H. flavolineatum* had the highest abundance, benefitted from higher survival rates and minimized $\mu/g$ ratios compared to coral reefs (chapters 4-6). The trade-off was a reduced growth rate, body size, and slower sexual maturation (chapters 3, 5 & 6). Mechanisms such as resource acquisition and shelter availability caused across-habitat distribution patterns within embayments before joining adult populations on coral reefs (chapters 3-6). After a planktonic feeding life stage, early juveniles shifted to a nocturnal zoobenthivoric diet and shifted from rubble to more profitable seagrass beds deeper inside bays where more benthic food was available (chapters 3, 5 & 6). After this, an ontogenetic shift followed towards dark, structure-rich mangroves (chapter 6) which provide good hiding places during the day as fish may have outgrown the shelter provided by seagrass leaves. At night, however, fish left the mangroves and still fed on the zoobenthos-rich seagrass beds (chapter 6). The largest fish in bays were observed in hard-bottom boulder/notch habitats located at a short distance from coral reefs (chapters 5 & 6). This habitat could be a final stop within the bay before fish move to the reef for permanent residence (chapters 5 & 6). Ontogenetic changes in vulnerability to predation could cause this ultimate shift to reefs as predation risk no longer differed between the channel part of the bay and the reef for large *H. flavolineatum* (chapter 5). The same applies to $\mu/g$ ratios: no difference was found between bay, channel and reef for large fish (chapter 5). The uni-directional movement from bays to coral reefs could also be driven by the need for further development of the gonads as fish in bays were rarely sexually mature, whereas fish on reefs nearly always were and had a higher biomass of the gonads (chapters 5 & 6). Continuing
residence within bays does not appear to facilitate maturation and thus inhibits fitness. Reproduction on reefs by this pelagic spawner will be more successful than in semi-enclosed bays, explaining the need to ultimately move to the reef habitat, which is the typical residence habitat for large adults (chapters 5 & 6).

The results of this thesis provide a better understanding of the value of different habitats as nurseries for juvenile fishes during their ontogeny, although no conclusive proof could be provided for the replenishment of adult populations on reefs. During ontogeny, the search for a suitable habitat is driven by different mechanisms and fishes often have to make cost-benefit analyses which can be species- and/or site-specific. Therefore, it is difficult to indicate a single habitat type as a nursery. In addition, habitat heterogeneity (e.g., configuration, accessibility) and the presence of different habitats in the seascape play important roles in habitat utilization patterns. Maintaining habitat connectivity is therefore of great importance and the loss of settlement and juvenile habitats can be catastrophic for population viability. Due to the strong ecosystem linkages that exist in the coastal seascape, many current marine reserves may be ineffective in supporting and protecting reef populations of some economically and ecologically important species. The optimal design of marine protected areas must include all habitats essential for fishes to complete their life cycles, i.e., from larval settlement to adulthood – including spawning grounds. In addition, scientists and managers need to collaborate for providing a basis for ecosystem-based management, conservation and sustainable fisheries.
SAMENVATTING
(DUTCH SUMMARY)
Ecosystemen zijn onlosmakelijk met elkaar verbonden door migrerende dieren die de grenzen tussen deze systemen doorkruisen. Hierbij verschuiven individuen gedurende hun complexe levenscyclus van het ene naar het andere habitat (leefgebied) wat sterk wordt beïnvloed door hun gedragsmatige reacties op hun eigen veranderende behoeften of veranderingen binnen de directe omgeving. Vele mariene vissoorten hebben een complexe levenscyclus met een jonge, onvolwassen (juveniele) en een volwassen (adulde) levensfase in welk ze elk een verschillend habitattype bezetten, gescheiden door een paar meter tot wel honderden kilometers. Als gevolg van de uitwisseling van juvenielen en/of adulten tussen de verschillende habitats worden populaties aan elkaar gekoppeld. Juvenielen bezetten bovendien vaak meer dan één type habitat gedurende deze juveniele levensfase, bijvoorbeeld mangroven en zeegrasvelden. Deze laatste twee habitattypes worden beschouwd als belangrijke kraamkamers voor vele juveniele vissoorten en behoren tot de meest productieve ecosystemen ter wereld. Echter, samen met de koraalriffen worden ze ernstig bedreigd. Dit resulteert in de degradatie en het verlies van habitats, vermindering de ecosysteem functies, en een afnemende biodiversiteit en populatie connectiviteit. Primaire factoren die hier verantwoordelijk voor zijn, zijn overbevissing, klimaatverandering, vervuiling, ziektes en kustontwikkeling. Het is noodzakelijk meer inzicht te verkrijgen in de connectiviteit tussen habitats en het identificeren van de onderliggende mechanismen die leiden tot ecosystem brede verspreidingspatronen van koraalriffvissen. Dit is van cruciaal belang voor ecologische en management gerichte initiatieven welke bijdragen aan een solide basis om aan effectieve beheer- en beschermingsmaatregelen te werken. Deze dissertatie laat het bestaan zien van een complex verspreidingspatroon tijdens de ontogenie van een veelvoorkomende koraalriffvissoort waarbij meerdere habitattypes worden bezet, en levert een overtuigend bewijs voor de onderliggende mechanismen.

Adulte rifvissen paren op het rif of verder uit de kust. Na een pelagische larvale fase vestigen de kleine juvenielen zich in de kustwateren waar ze een groot scala aan habitats hebben om tussen te kiezen, echter, ze selecteren specifieke habitats om zich te vestigen. Sommige soorten vestigen zich direct op het koraalrif, terwijl anderen zich vestigen in ondiepe habitats dichterbij de kust zoals mangroven en zeegrasvelden. Hier verblijven ze enkele maanden tot zelfs jaren voordat ze migreren naar de koraalriffen waar ze de adulte populaties aanvullen. Mangroven en zeegrasvelden zijn de zogenaamde ‘kraamkamer’ habitats en variëren in kwaliteit. Volgens het ‘kraamkamerconcept’ zijn de juveniele visdichtheden in deze habitats hoger en wordt er aangenomen dat ze grote voordelen bieden boven het koraalrif. Zo wordt er gedacht dat de structurele complexiteit van deze kraamkamer habitats juvenielen voorziet van goede schuilmogelijkheden wat het risico om belaagd te worden verlaagd (grote overlevingskans) en dat er veel voedsel aanwezig is wat de groei bevordert. Echter, deze dissertatie laat zien dat deze
uitgangspunten niet altijd waar zijn en dat vissen voortdurend te maken hebben met kosten-batenanalyses en tussen habitats migreren om hun ‘fitness’ (potentie voor het doorgeven van genen) te maximaliseren. Tijdens de groei van een vis moet deze zijn kosten-batenanalyse continu aanpassen en gedreven door bepaalde mechanismen vinden er gedurende de verschillende levensfases meerdere verschuivingen tussen habitats plaats.

In deze dissertatie wordt de functionele rol van kraamkamers voor vispopulaties onderzocht rondom de Caribische eilanden Aruba en Curaçao. Onderzoek op Curaçao is uitgevoerd in twee ondiepe, niet estuariene baaien met slechts één doorgang naar de open zee. Op Aruba vond onderzoek plaats in een uitgestrekte lagune (verder in de tekst ook baai genoemd) met vele verbindingen naar de open zee. Langs de kustlijn in alle drie de baaien staan mangroven en zijn er uitgestrekte zeegrasvelden aanwezig. Verder zijn er habitats aanwezig die bestaan uit harde bodem substraat zoals rotsen en keien voor de kustlijn van het fossiele kalksteen plateau in het kanaal van de baaien niet ver van het rif, terwijl kleine stukjes dood koraal op zand (rubble) alleen te vinden zijn dichtbij de ingangen van de baaien. Het koraalrif bevindt zich direct buiten de baaien en loopt langs de gehele zuid-west kust van beide eilanden. De doelsoort in deze dissertatie is het roodbekje (*Haemulon flavolineatum*), een veelvoorkomend vissoort op Aruba en Curaçao.

De voorkeur van juveniele vissen voor een specifiek habitat is vaak positief gerelateerd aan de structurele complexiteit van het habitat. Alhoewel, verschillende habitats variëren in hun architectuur (bv. flexibele versus stijve of hangende versus staande structuren) en het is gebleken dat de mate van structurele complexiteit minder belangrijk is dan het habitattype zelf (hoofdstuk 2). Met behulp van experimentele kooien is de voorkeur voor vier heel verschillende microhabitats (te weten zeegras, mangroven, rubble en koraal) gegetest voor afzonderlijke vissen. Door het gebruik van kooien werden storende factoren zoals voedsel kwantiteit en kwaliteit en predatie uitgesloten. Gedurende het experiment verplaatsten vissen zich altijd in de richting van één van de vier gestructureerde microhabitats in plaats van te blijven hangen boven de kale, ongestructureerde zand bodem. Verder lieten de vissen geen specifieke voorkeur zien voor het meest structureel complexe microhabitat en er werd geen relatie gevonden tussen de voorkeur van een vis en één van de zeven gemeten complexiteits kenmerken. In plaats daarvan waren vissen heel consistent in hun microhabitat keuze en lieten ze ten alle tijde een voorkeur zien voor zeegras en koraal microhabitats, zelfs wanneer deze werden aangeboden met een lagere bedekking, geplooidheid, of aantal gaten (schuilplaatsten). Van alle vier de gestructureerde microhabitats hadden benthische structuren (zeegras, koraal en rubble) de voorkeur boven de hangende structuren (mangroven) en levende (zeegras en koraal) boven dode microhabitats (rubble). Daarom is de aanwezigheid van benthische, levende en gestructureerde habitats waarschijnlijk een belangrijker criteria voor het
bepalen van de habitat voorkeur van demersale vissoorten dan de mate van structurele complexiteit. Op basis van deze resultaten wordt in **hoofdstuk 2** een theoretisch hiërarchisch model voorgesteld dat van kracht kan zijn bij het kiezen van een habitat naar voorkeur voor verschillende demersale vissoorten.

Onder natuurlijke omstandigheden vestigen kleine juvenielen zich zelden op het koraalrif. Uit intensieve onderwater vistellingen is gebleken dat pelagische larven zich massaal vestigden op rubble dichtbij de mondingen van de baaien (**hoofdstukken 5 & 6**). Dit is het eerste relatief veilige habitat dat pelagische larven tegenkomen bij het binnen zwemmen van de baaien (**hoofdstukken 4 & 5**) en hier is hoogstwaarschijnlijk voldoende planktonisch voedsel beschikbaar doordat dit continu wordt aangevuld door oceanische stromingen langs de kust. Tot 4–5 cm in lengte foerageren *H. flavolineatum* overdag op voedsel partikels in de waterkolom (plankton) en door de continue aanvoer van plankton op rubble is hier mogelijk meer voedsel beschikbaar wat de groei bevordert ten opzichte van habitats dieper gelegen in de baaien (**hoofdstukken 3 & 6**). Dit ondanks het feit dat op Aruba de hoeveelheid planktonisch voedsel relatief laag was in watermonsters (**hoofdstuk 6**). Een optimum tussen predatie risico (welke invloed heeft op de mortaliteit [μ]) en het verkrijgen van voedsel (met gevolgen voor groei [g]) is hoogstwaarschijnlijk het onderliggende mechanisme voor de vestiging van uit het plankton afkomstige larven op rubble in de buurt van koraalriffen. De concentratie van pas gevestigde kleine juvenielen in dit kleinschalige, heel specifieke rubble habitat zou wel eens een ‘bottleneck’ (knelpunt) kunnen zijn voor de aanvulling van adulte populaties op het koraalrif. Het is daarom noodzakelijk dat ook dit habitat wordt opgenomen in de behoud- en beheersplannen van deze baaien.

Predatie kan aanzienlijk hoger zijn in ondiepe, dichtbij de kust gelegen habitats (bv. mangroven en zeegrassen) dan algemeen wordt aangenomen. Uitgebreide onderwater vistellingen hebben aangetoond dat niet alleen op het koraalrif grote roofvis aantallen aanwezig zijn, maar ook in de mangroven (**hoofdstuk 4**). Mangroven bieden onderdak aan vele kraamkamersoorten, inclusief soorten die ook optreden als roofvissoorten. Daarbij, mangroven en zeegrassvelden worden ook regelmatig bezocht door roofvissen afkomstig van het koraalrif. Ondanks dit was in veldexperimenten met aangelijnde kleine juveniele vissen de overlevingskans gemiddeld veel hoger in de mangroven en zeegrassvelden dan op de koraalriffen en deze nam toe met een toenemende afstand van de mondingen van de baaien (**hoofdstukken 4 & 5**). De overlevingskans was lager op locaties waar roofvisdichtheden hoog waren (**hoofdstuk 4**) en in de afwezigheid van roofvissen lieten kleine juvenielen een voorkeur zien voor rif microhabitats (**hoofdstuk 2**). Dit duidt aan dat het risico om opgegeten te worden een belangrijke rol speelt in de kraamkamer functie van baaien en kan het onderliggende mechanisme zijn voor de hoge
Dutch Summary

dichtheden van (kleine) juveniele vissen in baaien in vergelijking tot het koraalrif.

In baaien waar juveniele H. flavolineatum abundant zijn, profiteerden juvenielen van de hogere overlevingskansen en minimaliseerden ze de verhouding tussen mortaliteit en groei (µ/g) ten opzichte van koraalriffen (hoofdstukken 4–6). De ‘trade-off’ (afweging) echter, was een afname in groei en lichaamsgroutte en een langzamere ontwikkeling van de voortplantingsorganen (hoofdstukken 3, 5 & 6). Gedurgende het verblijf in de baaien verschuiven vissen tussen de verschillende habitats en mogelijke mechanismen die deze verspreidingspatronen drijven zijn het verkrijgen van voedsel en de beschikbaarheid van schuilplaatsen (hoofdstukken 3–6). Na een planktonische foerageerfase waarbij de kleine juvenielen overdag eten gaan ze over op een bentisch dieet waarbij ze ‘s nachts op zoek gaan naar voedsel in het sediment (hoofdstuk 6). Tegelijkertijd vind er een verschuiving in visdichtheid plaats van het rubble habitat naar zeegrasvelden dieper in de baaien waar meer bentisch voedsel beschikbaar is (hoofdstukken 3–6). Hierna volgt een ontogenetische verschuiving naar de donkere, structuurrijke mangroven (hoofdstuk 6) welke hoogstwaarschijnlijk een betere bescherming tegen roofvissen bieden dan de zeegrasbladeren die de juvenielen op deze lengte ontgroeid zijn. Echter, in de nacht migreren vissen vanuit de mangroven terug naar de voedselrijkere zeegrasvelden om te foerageren (hoofdstuk 6). De grootste vissen in de baaien werden geobserveerd op het harde bodem substraat (rotsen en keien) welke in de kanalen dichtbij de mondingen van de baaien zijn gelokaliseerd (hoofdstukken 5 & 6). Dit habitat kan functioneren als een laatste stop in de baai voordat de vissen definitief naar het koraalrif migreren waar ze de adulte populaties aanvullen (hoofdstukken 5 & 6). De uiteindelijke migratie naar het koraalrif kan het gevolg zijn van ontogenetische veranderingen in de kwetsbaarheid voor roofvissen. Grote H. flavolineatum lieten niet langer meer een verschil zien in het risico op predatie tussen het kanaal in de baai en het koraalrif (hoofdstuk 5). Hetzelfde geldt voor de verhouding tussen mortaliteit en groei (µ/g); geen verschil meer tussen de baai, het kanaal en het rif voor grote vissen (hoofdstuk 5). De uni-directionele verschuiving van baaien naar koraalriffen kan ook gedreven worden door de noodzaak om de voorplantingsorganen (gonaden) verder te ontwikkelen. Dit omdat vissen die in de baai verbleven zelden geslachtsrijp waren, terwijl vissen op het koraalrif dit bijna altijd waren en de biomassa van hun geslachtsorganen hoger was (hoofdstukken 5 & 6). Voortzetting van het verblijf in baaien lijkt niet ten goede te komen van de geslachtsrijping en zal dus de fitness remmen. Voortplanten op koraalriffen zal voor deze soort succesvoller zijn dan in semi-gesloten baaien en dit verklaart ondermeer de noodzaak voor grote viseen om uiteindelijk naar het koraalrif te verplaatsen (hoofdstukken 5 & 6).
De resultaten van deze dissertatie dragen bij aan het verkrijgen van een beter inzicht in de waarde van verschillende habitattypes als kraamkamers voor juveniele vissen gedurende de ontogenie. Echter, er kan geen bewijs worden geleverd dat juveniele vissen uit de baaien ook daadwerkelijk de adulte populaties op de koraalriffen aanvullen. Gedurende de ontogenie wordt de zoektocht naar een geschikt habitat gedreven door verschillende mechanismen en vissen moeten vaak kosten-batenanalyses maken welke soort- en/of locatie-specifiek zijn. Daarom is het onmogelijk om één enkel habitat type als kraamkamer te benoemen. Bovendien, habitat heterogeniteit (bv. configuratie, toegankelijkheid) en de aanwezigheid van verschillende habitats in het zeeaangezicht spelen een belangrijke rol in het habitat gebruik van vissen. Het behoud van de connectiviteit tussen verschillende habitats is daarom van groot belang en het verlies van vestigings and juveniele habitats kan katastrofaal zijn voor de overleving van een populatie. Doordat ecosystemen sterk met elkaar verbonden zijn, zijn mogelijk veel van de huidige mariene reservaten ineffectief in hun ondersteuning en bescherming van koraalrifvispopulaties van enkele economisch en ecologisch belangrijke soorten. Het optimale ontwerp voor een marien reservaat zou alle habitats moeten omvatten die van essentieel belang zijn voor vissen om hun complete levencyclus te voltooien, dat wil zeggen, vanaf het moment van vestiging vanuit het pelagische water tot en met de volwassen levensfase - met inbegrip van de paaigebieden. Hieraan toegevoegd, wetenschappers en managers moeten gaan samenwerken en op deze manier een goede basis vormen voor ecosysteem gericht management, beheer en behoud en duurzame visserij.
ACKNOWLEDGEMENTS
It all started on an afternoon in 2004 in Ivan’s office when I had to answer the question if I wanted to do a PhD at the Department of Animal Ecology and Ecophysiology at the Radboud University Nijmegen. Of course not, that’s nothing for me, but... Most people don’t hesitate when answering this question and they would have answered it before they had even thought about it. But me, I had to think about it! It was a great opportunity, a big challenge and a possibility to go back to my roots, the Caribbean. Therefore I accepted the job, and as I began this thesis with, if you are happy and love what you are doing, you will be successful. This dissertation is my proof of being successful and I am proud of it! However, it was not a one woman job and I have had help of a lot of people, organizations and governments who I would like to thank.

I am very grateful to Dr. Ivan Nagelkerken who has asked me the above question 6 years ago when I was still a master student writing my thesis about fish distribution patterns in Tanzania and the Comoros. During my PhD, Ivan was a great supervisor (and co-promotor) and I have enjoyed working with him. Together we travelled throughout the Caribbean and collected a lot of underwater visual census data from hundreds of transects at different islands next to our daily work on Curaçao and Aruba. But also in the Netherlands he was a fine colleague who has helped me out when I was struggling with a manuscript, gave valuable suggestions and corrections on the manuscripts I thought I had finished and with whom you could have a nice chat about daily live. I also would like to thank Prof. Dr. Sjoerd Wendelaar-Bonga for being my promoter and for his comments on this thesis.

The first year of my PhD, I spent 8 months on the beautiful island of Curaçao. Here I worked together with Arjan de Groene, Susanne Wartenbergh and Dr. Martijn Dorenbosch who was my supervisor during my master internship in Tanzania and the Comoros. During this internship and attributable to Martijn, I got really passionate about marine research which was encouraged by the fact that we dived almost 7 days a week, each time on a different, spectacular reef in the waters around the Tanzanian islands. The first year in Curaçao was pretty tough for all of us as we worked on different projects sharing
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One boat (bathtub) and one pickup. While working many hours a day we learned how to get around on the island, where to buy the cheapest research materials (and beers), and how to find our way on- as well as underwater. Unfortunately, we missed the daily happy hours at Hookse Hut because we were so busy and had to pay the full price for the best frappachino’s in the Caribbean. Nevertheless, we managed to collect data that until today still is used in our manuscripts. I also like to thank the staff and personnel of the Carmabi Foundation for providing office space and the use of laboratory facilities. And, I will never forget those stupid experimental cages made of wire which we used to measure growth rates and predation risk. Constructing them on the beach was fun, but once out on the water they were a nightmare. After this fieldwork season I promised myself never to work with cages again, however, the opposite was true... Until my last visit to the Caribbean I was confronted with them and used them in multiple, almost all, experiments!

Knowing that your parents live so close to Curaçao, the temptation was there to regularly take a weekend off and fly to Aruba. As a result, I spent 6 months of my second year as a PhD on Aruba together with four master students, Eva Kokkelmans, Rik van der Kant, Jasper de Bie and Jeroen Meijer. They were a great help in the field as well as in the lab, and I still think of all those nice cocktails we had at Havanna after work and the parties at my parents place. On Aruba, we worked closely with the Department of Agriculture, Husbandry & Fisheries (DLVV), in particular the department of fisheries. They provided us with an office space which we used for all different purposes (e.g., laboratory, storage, and dive equipment room), pickup trucks, research materials, a ‘real’ boat to go out at sea and a boatcaptain. I am very thankful to Patty Portier and Teophilo Damian working at DLVV for all their help, support and advices. And not to forget, I would like to thank all our boatcaptains throughout the years. The first year on Aruba we worked with Francisco who knew the ocean very well. After that we worked with Randy and his best friend, his mobile phone. Gibby was our ‘spare’ captain with whom we got very drunk one day at Zeerover.

These first two years of my PhD, Ivan, Martijn and I also conducted some separated monthly projects on other Caribbean Islands in collaboration with different organizations and/or governments. I would like to thank all the people that made this great experience possible. We went to (and worked closely with) Bonaire (Stichting Nationale Parken Bonaire - STINAPA), Aruba (DLVV), Cayman Islands (Department of Environment), Bahamas (Bimini
Acknowledgements

Biological Field Station or SharkLab), Bermuda (Bermuda Biological Station for Research), Jamaica (Discovery Bay Marine Laboratory – DBML) and Curaçao (CARMABI).

In 2007, I went back to Aruba for another 8 months. This time I was accompanied by four other master students, Sarah Smith, Susanne van Herwijnen, Eva van Engelen and Nick Rossen who helped me out with all the lab- and fieldwork. Nick and Eva also went along with me on one of the two monthly fieldtrips I made to Curaçao this year. Here we worked together with my fellow PhD Chantal Huijbers and her crew of master students. Everyday we went fishing and collected over 2000 French grunts from all sizes and in the evenings we sat in the lab and dissected the fish. This trip was very tiring, but not only because of the hard work... We did work many hours a day including some weekends, but above all, Nick and Eva knew how to party all night long. Fortunately, I was already accustomed to this as this wasn’t any different than on Aruba. I remember one day out on the ocean. Everybody partied the night before and I decided to go out on the reef to conduct some work there. If I had though this through, I would know that this would be a disaster...

This year we also volunteered in some local projects, e.g., we chased after sterns on the coral cays in front of the Lago (oil refinery), joined the yearly reef clean up, and every morning someone had to wake up very early (05:30 am) to drive to the beach and walk for an hour to check for turtle nests (Tortugaruba). I also joined the Coastal Zone Management (CZM) team on Aruba and volunteered for two weeks on the Cayman Islands where we monitored the spawning aggregation of Nassau groupers. I would like to thank the Cayman Department of Environment for this opportunity, and in particular Croy McCoy who invited me.

The following years I went back to Aruba and Curaçao several times and stayed there for 10 days up to 3 months. I could not have collected so many data without the great help of colleagues and students. Niek Bosch who accompanied me in 2008 to work on a habitat preference experiment on both islands, Sanne van Delft and Rob Fraaije who joined me in 2009 to conduct tethering experiments on Aruba, and Chantal Huijbers, Laura Govers, Jan de Brouwer and Ines Schulten with whom I conducted more tethering experiments in 2010 on Curaçao. In addition, in 2009 I went to Florida International University, Department of Biological Sciences, in North Miami and finished one of my manuscripts with the great help of Dr. Craig Layman.
During these wonderful years as a PhD in the Caribbean more people and companies assisted me in my work and I also would like to thank them for their great help, support, advices, use of facilities and materials, and so on. Without them I would not be so successful and I am very grateful to them: Sea-aquarium Curacao, especially Frank Isabella, Oscar and Carlos from CARMABI, the dive companies Hook’s Diving and Silent Immersion on Curacao, Jads Dive Centre and Fly ‘n Dive on Aruba and Duikschool Oost in Nijmegen, the Bislick family who runs Zeerover on Aruba, the Veterinary Department Aruba, the Technical Inspection Service (DTI) Aruba, Landslaboratorium Aruba, the Coastguard, Jeff Mac Mootry (commander of the Marines in Savaneta on Aruba), Andrew Rypel, and all the people that I did not list here and might have forgotten...

And last but not least, I would like to thank my family and friends who supported me all those years. My parents, Willem and Diny, were always happy to see me when I came back to Aruba. I loved to stay at their place, which was home. Here I was surrounded by family and all our pets, including Paluca who hated me for leaving him 15 years ago. Everday when I got home dinner was ready, I had enough space to store my research materials, do some lab-work and clean my dive gear, they allowed me to use their pickup, and I enjoyed the pool after a day of hard work. In 2009 I was very lucky. My sister Nicole and I were on Aruba when my youngest sister Daniëlle gave birth to a wonderful son, Olivier. Hopefully he will be a good diver some day...

I also want to give Alex Dubero a big hug. He’s my best friend on the island for 20 years and I always miss him when I have to go back to Holland. My grandparents, Rie and Wytze, always missed me so much when I was abroad, but the waiting was worth this final moment. For my best friends in Holland I can understand it was tough sometimes and I am very grateful to them. Imagine your best friend is happy and smiling as she informs you that she is leaving again for a few months to conduct more fieldwork in the Caribbean. Marit and Eva, I am very thankful to both of you for understanding that this was my passion and that I was doing what I love most. Everytime when I came back to Holland you always were there, like nothing had have happened. A bottle of wine, a nice dinner, and we started were we had left off a few months ago. This is what you call true friendship!
LIST OF PUBLICATIONS
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**SCIENTIFIC PUBLICATIONS IN PEER-REVIEWED JOURNALS**


Grol M.G.G., Nagelkerken I. & Bosch N. Preference by a coral reef fish for distinct shallow-water microhabitats is not related to complexity but to habitat type. In review *Mar Ecol Prog Ser*.


Grol M.G.G. & Nagelkerken I. Food, predation and maturation drive ontogenetic habitat shifts by a coral reef fish. Ready to submit.
CURRICULUM VITAE
Monique Grol was born on the 16th of April 1977 in Nijmegen, the Netherlands. The first 14 years of her life she lived in the small village of Mook, south of Nijmegen, where not much happened besides going to school and doing her homework. During summer holidays she went to Italy and Yugoslavia with her parents were she was introduced to the fascinating underwater world. At the age of 12 she did her first SCUBA course on the Italian island of Elba and fell in love with the ocean. In 1991 she moved with her parents to the Caribbean island of Aruba. Here the author’s true adventure started. Every afternoon, she walked from school to the beach and spent her free time at ‘Hallo Aruba Dive Shop’. Here she became an advanced diver. After 5 years, a few hundred dives and her secondary school diploma she moved back to Holland, to Utrecht. For the next two years she worked in hotels, bars and restaurants and moved from city to city. As it was not enough, she joined the Royal Dutch Army as a Sergeant Logistics for a couple of years and moved around Europe. She even served the NATO in 1999 for 6 months in Kosovo. Finally, after several years of moving around she was back where it all started: back in Nijmegen to study Biology at the Radboud University Nijmegen. After following classes for three years, it was time to travel again. For her first master internship, the author spent 7 months in Tanzania and the Comoros and studied habitat connectivity through fish distribution patterns across multiple different habitats in the Indo-Pacific Ocean. The data collected were unique as little was known from this region, which resulted in co-authoring several scientific papers. Although she truly fell in love with Africa and the tropical marine environment, her second internship was completely different. She moved to Bolivia, a country without an ocean, for 6 months and studied plant-animal interactions in a semi-dry tropical rainforest. Following her heart, she quickly finished her masters in May 2005 and started to work as a PhD in fish ecology at the department of Animal Ecology and Ecophysiology, Radboud University Nijmegen. As a PhD she travelled multiple times with great joy to and around the Caribbean to study the underlying mechanisms that drive across-habitat utilization patterns in coral reef fishes, of which the product is this thesis. During these days she supervised several master students, and her work was published in various peer reviewed journals and presented at several international symposia.