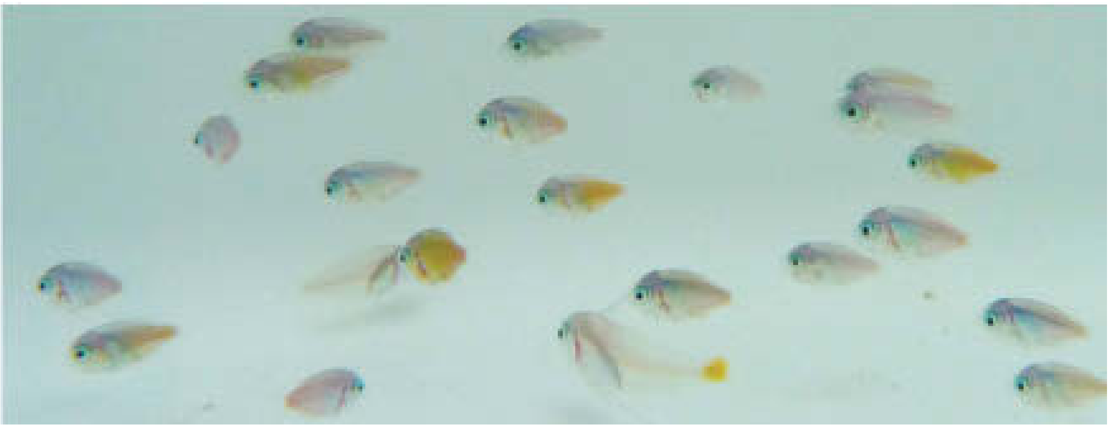
A long way home: orientation behaviour of fishes during the pelagic larval phase



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February 2016

Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

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Abstract

Determining the processes influencing how marine organisms disperse during the larval stage is a major challenge of marine ecology, yet is a task critical to understanding the scale and connectivity of marine populations. Sampling such tiny organisms in the expansive pelagic environment presents complex problems, making the pre-settlement stage a “black box” in our knowledge of the life history of marine organisms. In this thesis I aimed to address some of the main themes characterising this black box, namely when during the larval stage sensory responses to habitat cues develop, which sensory cues may be used for orientation in the pelagic environment and how the condition of the local environment affects settlement behaviour. The results of these experiments increase our understanding of behaviour and sensory abilities of larval fishes and provide new insights for predictive models of larval dispersal, integral tools for effective management of marine populations.

The ontogenetic development of olfactory responses in larvae of two fish species, which recruit to temperate estuaries, shows that chemotactic behaviour relevant to movement towards habitat develops shortly after tail flexion (Chapter 2). This point in ontogenetic growth when choice behaviour between estuarine and coastal water develops which is consistent over multiple cohorts, and correlates with existing data on ontogenetic increases in swimming endurance. The presence of seagrass cues was more important than changes in pH or salinity to this behaviour. This is the first evidence of a consistent size-based ontogeny of sensory response to natural water bodies across cohorts of temperate fish larvae.

In order to orient swimming when in the pelagic zone it is hypothesised that large-scale cue use, namely a celestial compass and/or a magnetic compass, would be required. Behavioural experiments to test the ability of coral reef fish larvae to use the sun's azimuth as a compass to orient swimming found significant differences in the mean orientation direction of larvae as individuals and among-individuals when exposed to different sun azimuths (Chapter 3). Cue-conflict experiments indicate that polarised light patterns also have an effect on orientation behaviour. This experimental data compliments field orientation data of other studies indicating the use of a sun compass as part of an orientation mechanism in larval fishes.

Orientation trials using magnets and a Helmholtz magnetic coil also indicate that coral reef fish larvae have the ability to detect changes in the local magnetic field, i.e. magnetoreception (Chapter 4). Individual larva responded predictably between control and treatment conditions, with a significant angular difference in mean bearings similar to the size of the shift in local magnetic field polarity within the magnetic coil. Larval orientation behaviour was affected differently by the presence of magnets of different strength and, as in Chapter 3, the presence of a polarised light pattern. This is first time magnetoreception has been shown in fish during the larval stage.

Choice experiments on coral reef fish larvae showed habitat cues with increased sediment concentrations changed behavioural response to habitat cues in both before and after settlement (Chapter 5). Pre-settlement stage larvae avoided olfactory cues of water infused with sediment at different concentrations. Settled larvae exposed to sediment at different concentrations for a period of five days changed their preference in comparison to larvae kept in “clean” water, choosing olfactory cues from dead coral over live coral. These results indicate that larvae may actively attempt to avoid settlement on degraded habitat, while those forced to settle on degraded habitat will have reduced fitness, linking increased sediment pollution to reduced recruitment success in fish larvae.

Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature:

Date: 05/02/16

Acknowledgements

When completing any four year mission, most of all a PhD in science, one looks back at all the highs and the lows on the road leading to the day of completion and remembers all of the good fortune (and bad fortune) and good people (and bad people) encountered along the way, without which reaching the finish line would have been next to impossible. As such, I must take this opportunity to give credit where credit is due, and acknowledge all those who have helped me on this journey.

Firstly, and most importantly, I want to thank my supervisors for giving me the opportunity to experience the life of a marine biologist, and for believing in me enough to give me this project and letting me run with it. To the creative spark behind this thesis, Jeff Leis, thank you for taking me on as your (possibly first ever male?) research assistant to Lizard Island, where this whole road began. That trip was a formative moment in my life in many ways. I have immense respect for you as a scientist and human being, and without your straight-shooting wisdom, dry humour and attention to good science I don't think I would have made it this far. To my long time supervisor Dave Booth, thank you keeping the ball rolling after our shark work and allowing me to take the thesis in many directions and to places all over the world. You trusted me to get the job done no matter where I went, you were always in my corner and there when I needed.

Of course I would also not have been able to finish this thesis without the help of my co-supervisor Steve Swearer, I can't thank you enough for giving me the opportunity to join your wonderful lab at the University of Melbourne. Working in the REEF lab has given me a different and invaluable perspective on research. You gave me all the support and opportunity I could ask for, when you had absolutely no obligation to do so. You're a great guy to work with, go for a beer with, and you have

a lab of great people working with you. To Matt Le Feuvre, James Shelley, Emily Fobert, Ben Cleveland, Luke Barrett, Sean Chia, Valeriya Komyakova, John Ford, Fletcher Warren-Myers, Rob Hale and Dean Chamberlain thank you for being such welcoming, friendly and knowledgeable workmates to the outsider and for keeping me sane.

While I don't see them as much these days as I used to, I will never forget my work mates from research's spiritual home, Sydney. To Team Red Soil (Hayden Beck and Gwen Cadiou), Davina Poulos, Ash Fowler, Cian Foster-Thorpe, Nikki Bromwell, Paloma Matis and Sam Goyen thanks for always being there for a chat or to go through the vagaries of research. To the lovely team at the Australian Museum, where this whole journey really started: Amanda Hay, Mark McGrouther, Sally Reader and John Paxton, your passion for fish and your indomitable spirit were a real inspiration to me as a young fish biologist, and I will always remember my time at Fish HQ fondly.

My thesis covered a range of new research areas which I had no previous experience with at the time, and I owe a lot to the researchers around the world which I met and managed to form collaborations with. Thank you to Rachel Muheim for your expertise in the heady world of animal orientation and circular statistics, Danielle Dixson for helping me get started in the equally heady world of aquatic olfaction, Uli Siebeck for your tough love and showing me the larval orientation ropes, Yohei Nakamura and David Lecchini for bringing me to Sesoko Island and sharing your enthusiasm for research, Mike Walker, Ian Birchall and Mirjana Bogeski for helping me in the quest for magnetite, Stew Fielder for giving me your expertise and the run of the fish hatchery at Port Stephens Fisheries Institute, Anne Hoggett and Lyle Vail for taking me under your wings and facilitating my fantastic field trips to

Lizard Island Research Station, and David Feary for your great help and guidance with ideas and strategy.

Of course no thesis acknowledgements would be complete without mentioning the hard working folk who put their hand up for the thankless task of helping me do my research in beautiful and exotic locations and environments. Big thanks to Chris Braun and Viktor Nilsson for your great help and companionship on LIRS (I think we made friends for life there), Ryuta Suzuki for his great help with behavioural experiments in Okinawa, Luke Pedini and Stephane Bertin for enduring the long days in the lab (and longer nights in the bunk house) at PSFI, the team at the PSFI hatchery – Luke Vandenberg, Luke Cheviot and Ben Morton – for being great mates and taking care of my (and the little larvae's) every need over three years, Luke, Emily, Dean and John for your time taking me in the water and helping me to collect hulafish from Port Phillip Bay for eventually unfruitful projects.

Lastly, but definitely not least I want to thank my family for their love and support over these four years and for many more before that, particularly my father, who always encouraged and supported me to pursue tertiary education. Also I'm thankful for the enduring support of my lovely fiancée Dr Rolanda Lange, for being a fantastic life mate and an inspirational researcher. Our partnership has forged a balance between life and work, which has helped me sail the stormy waters of this thesis without once looking like sinking.

This project was funded in part by an ARC Discovery Grant (ID: DP110100695), the Geddes Postgraduate Award (Australian Museum) and a UTS Research Excellence Scholarship.

Table of contents

List of figures

List of Tables

CHAPTER ONE

General Introduction: Sensory cues and orientation of pre-settlement stage larval fishes

Author observing a solitary larval fish (Monacanthidae) in open water, Great Barrier Reef Marine Park, QLD

General Introduction

In this thesis I aim to explore how larval fishes are able to orient their swimming towards habitat during the pelagic phase, by looking at the interplay between behaviour and sensory abilities from soon after hatch to settlement. Firstly, I aim to discover the beginning of a larva's behavioural influence on dispersal, by exploring the ontogenetic development of responses to sensory cues. Secondly, I aim to investigate the abilities of larval fish to use large-scale cues (celestial or geomagnetic) to direct their movement during the pre-settlement phase. Finally, I aim to detect the impact of pollution due to human activities on the behaviour and sensory cue use of larvae that have arrived at the settlement site.

Larval dispersal of marine organisms

Life histories of most marine environment organisms generally consist of two distinct phases. For many demersal (bottom-associated) animals this involves a period of development offshore (lasting days to months depending on the species), followed by a benthic phase in which larvae are required to find suitable habitat on which to settle and complete their life cycle (Leis et al. 2011). Demersal marine species, particularly coral reef species, are commonly site-attached as adults, often remaining within a certain geographic range of their initial settlement habitat (Feary et al. 2014). It is therefore the pelagic larval stage where the spatial scales of dispersal, metapopulation connectivity and recruitment in these fishes are predominantly determined (Swearer et al. 1999, Paris and Cowen 2004).

Driving population replenishment and persistence, dispersal of individuals within and among populations is a major factor in the population dynamics of marine species

(Cowen et al. 2006, Sponaugle 2015). However despite our increasing understanding of dispersal kernels, particularly in coral reef fishes, factors influencing successful dispersal are poorly understood (Buston and D'Aloia 2013, Jones 2015). When combined with factors influencing survival of the dispersing organisms, the exchange of individuals between geographically separated subpopulations of a metapopulation during the dispersal phase is referred to as population connectivity (Cowen and Sponaugle, 2009). Understanding the ecological processes affecting larval dispersal and connectivity is critical to management of economically important species, protection of endangered species (Almany et al. 2013, Myksvoll et al. 2014) and effective design of marine protected areas (Green et al. 2014, Le Port et al. 2014). However due to logistical limitations in tracking larvae *in situ* and the high interspecies variability a broad picture of dispersal remains a fundamental challenge to marine ecology (Leis et al. 2007, Lowe and Allendorf 2010, Selkoe and Toonen, 2011).

Successful recruitment of marine larvae to new or existing populations is dependant on a range of factors, including adult spawning, egg hatching and processes acting upon them during the pelagic dispersal phase (Hamilton et al. 2008, Shima and Swearer 2010). These processes can be extrinsic (environmental) or intrinsic (biotic) (Guichard and Steenweg 2008, Feary et al. 2014). Oceanographic features, such as currents and eddies, greatly influence the spatial scales of dispersal by facilitating either long-distance transport (Booth et al. 2007, Craig et al. 2007) or local retention (Swearer et al. 1999, Paris and Cowen 2004).

Traditionally, larval dispersal was considered a passive process, reliant mainly on hydrological processes (Roberts 1997). However a new paradigm is recognising biophysical models, incorporating the influence of intrinsic biological factors (e.g. larval duration, ontogenetic swimming ability and sensory ability) on successful dispersal (Shima and Findlay 2002, Paulay and Meyer 2006, Shanks 2009, Kough et al. 2013). This seminal shift was largely due to increasing data on recruitment which indicated that larval fish influence dispersal patterns independently of hydrological flows. For instance, research using tagging and microchemical properties of otoliths showed for the first time that fish populations were not strictly "open" systems, with a high proportion of coral reef fish larvae managing to be locally retained (Jones et al. 1999, Swearer et al. 1999). This self-recruitment can be remarkably consistent over time despite different local oceanic conditions (Berumen et al. 2012). It is becoming clear that the swimming abilities and behaviour of marine larvae play a larger role in their dispersal than could ever have been expected (Paris et al. 2011).

Behaviour of fishes during the larval phase

The new biophysical paradigm in larval dispersal has led to calls for research into how behaviour of individual larvae can influence population persistence and connectivity (Sale et al. 2005, Walther et al. 2015). At the same time, behaviour could play an important role in patterns of recruitment, especially where the dispersal kernel is smaller or larger than predicted by hydrology alone (Jones et al. 2009, Swearer and Shima 2010). Larval behaviour can be linked to biophysical interactions conducive to dispersal in both direct and indirect ways (Paris and Cowen 2004). Active vertical movement and changes in depth can give larvae access to favourable currents and different food regimes, indirectly affecting dispersal outcomes

(Armsworth 2001, Woodson and McManus 2007, Morgan and Fisher 2010). Larvae also show discrete choice behaviours to different habitat types and quality at a small scale (Lecchini et al. 2005). This behaviour is likely to directly influence the distribution and recruitment of fish populations, particularly in regards to degraded or polluted habitats (Dixson et al. 2014, O'Connor et al. 2016).

Furthermore, recent studies have found that larval fishes have the ability to orient their swimming direction in offshore areas during the pelagic phase (Leis et al. 2014). Coral reef fish larvae from different regions, observed with different methodologies, have displayed the same behaviour: individuals are able to swim with significant directionality over a kilometre from habitat, and this direction is generally consistent among conspecifics (Faillettaz et al. 2015, Leis et al. 2015). Such behaviours can increase the potential of encountering habitat in a patchy system such as coral reef (Zollner and Lima 1999) or enhance local retention (Leis 2015). In any case, it is likely that such orientation behaviours have evolved in fish under selective pressure (Staaterman and Paris 2014). This behaviour can be location-dependent or independent depending on the species, highlighting the need to investigate larval behaviour across a range of taxa (Leis and Carson-Ewart 2003).

Until the past few decades, most data on the swimming abilities of larvae was derived from temperate species of weak-swimming orders such as Clupeiformes and Gadiformes (Leis and Carson-Ewart 1997). However recent studies on coral reef fish larvae (predominantly Perciformes) showed swimming speeds and endurance which would enable them to influence their pelagic movement independently of oceanic currents, transitioning from plankton to nekton (Leis and Carson-Ewart 1997, Fisher and Bellwood 2002). This surprising ability is unlikely to influence dispersal however unless larvae are able to orient their movement using directional cues. Although research into the behavioural responses of larval fish to different sensory cues suggests that a hierarchy of different modalities facilitate movement of larvae from offshore to onshore habitat, there are still substantial gaps in our knowledge of the sensory abilities of pre-settlement larval fishes (Kingsford et al. 2002, Staaterman et al. 2012).

Sensory ability in larval fishes

By the end of their pelagic phase not only have many larval fishes developed formidable swimming capabilities, but also an array of sensitive sensory abilities (Leis et al. 2011a). This ability to use sensory cues as a method for directing movement towards favourable habitat helps to explain observed recruitment patterns of fish communities (Kough et al. 2013). Visual, chemical and acoustic cues influence the behaviour of larvae approaching settlement habitat (Barth et al. 2015), making the local environment at settlement an extrinsic factor influencing larval settlement (Feary et al. 2014). This is particularly important to consider in light of the changes to these environments occurring due to anthropogenic stressors (Siebeck et al. 2014).

Olfactory and auditory cues

Most of the research on sensory abilities of larval fishes to date, mainly on coral reef fishes, has related to olfactory and auditory cues. Fish larvae have been shown to perceive certain habitat-relevant sounds via particle motion detectors (hearing “generalists”) or acoustic pressure detectors (hearing “specialists”)

(Montgomery et al. 2006). Behavioural experiments show that auditory cues can be used for orientation to habitat in pre-settlement fish larvae (Simpson et al. 2004, Tolimieri et al. 2004), particularly in environments with high levels of biological noise such as temperate and tropical reef systems (Radford et al. 2008, Simpson et al. 2008). Comparisons of transmission distance of habitat sounds and auditory sensitivity of pre-settlement stage larvae indicates the effective range of auditory cues is likely to be less than one kilometre (Mann et al. 2007), making this modality less important for orientation behaviour further offshore.

Olfaction is an important sense for directing movement in animals across a wide range of taxa, including insects, birds and fish (Wolf and Wehner 2000, Wallraff 2015, Nosal et al. 2016). In the aquatic environment, chemosensory systems play an especially important role in how organisms interact with their surroundings (Brooker and Dixson 2016). As such, studies which have investigated how olfaction influences behaviour of larval fishes have been critical to our understanding of how habitat type and condition influences settlement dynamics. Habitats experiencing tidal fluctuations disperse odour particles symmetrically creating detectable “odour halos”, while unidirectional drift currents disperse odours in one direction, retaining a partial halo in the leeside area (Atema et al. 2015). These olfactory plumes may encourage long distance dispersal by orienting swimming into currents with preferred habitat odour, or retention in the odour halo of natal reefs (Atema et al. 2002, Gerlach et al. 2007). Generated by biota characteristic of the habitat type, olfactory cues may attract fish larvae at a scale of up to one kilometre (Lecchini et al. 2014).

Favourable habitat biota cues, although taxonomically and ontogenetically dependent, include odours from associated settlement habitat structure (e.g. coral and aquatic plant species) and conspecifics (Brooker and Dixson 2016). Olfactory cues can also provide information to approaching larvae on habitats which may decrease the chances of successful recruitment, such as degraded habitat or the presence of predators (McCormick et al. 2010, Vail and McCormick 2011). Olfaction operates in a hierarchy with auditory and visual cues to assist fish larvae in the vicinity of settlement habitat to orient their swimming (Kingsford et al. 2002). Very little is known about which sensory cues are important outside of the effective range of these cues.

Celestial and magnetic cues

Sensory cues, which can be used for orientation independently of distance and local weather conditions, will be advantageous for animals whose ecology requires that they travel large spatial scales (Bingman and Cheng 2005). The two main large-scale cues that have been identified as candidates for providing a “compass” mechanism for long distance movement in animals are celestial bodies (i.e. the sun, moon and stars) and the earth’s geomagnetic field (Åkesson et al. 2014). Studies on directional swimming in larvae of crab and tropical reef fish species suggest the potential use of a sun compass for orientation, which would be useful for orientation over large scales or when smaller-scale cues are unavailable (Shanks 1995, Leis and Carson-Ewart 2003).

Part of determining the ability of fishes to utilise orientation information from the sun is to understand how they respond to different light conditions in the marine environment. Migratory fishes have long been hypothesised to use the position of the sun as a compass reference as part of their orientation mechanisms (Quinn 1980). Fishes that are visually sensitive to polarised light can use the e-vector pattern of polarised light scattering from particles in air or water, giving directional information about the sun's azimuth when a direct view is not available (Horvath and Varju 2004). By using a biological clock to compensate for the spatial and temporal displacement of celestial reference points, fish larvae could maintain consistent directionality over time using such points as a compass (Merlin et al. 2009). Limited observations of fish larvae indicate that a celestial compass may be involved in orientation behaviour (Mouritsen et al. 2013), however whether they are responding to the sun position, polarised light or other cues remains unknown.

Earth's geomagnetic fields pervade all areas of the planet, also providing potential sensory cues for animal orientation, ranging from the broadest scales to smaller scale variations, resulting from changes in rock formations on the ocean floor (Kingsford et al. 2002). With both fixed spatial patterns and localised site-specific anomalies, magnetic fields could in theory be used to orient directional movement over large scales by assisting the determination of compass direction or navigation through local topography (Boles and Lohmann, 2003). Compass information enables the animal to maintain a consistent direction for oriented movement, while positional or map information allows the animal to assess its geographic position and adjust its movement towards habitat accordingly (Cain et al. 2005). Magnetic field intensity provides positional information due to a gradual change in intensity over the earth's surface, whereas field inclination can be used for navigation by detecting the continuous gradient between the equator and the poles in the inclination of the field line as it intersects the earth's surface (Hellinger et al. 2009). These features may combine as components of a navigational map, with the magnetic vector providing the compass orientation with directional information on north and south (Wiltschko and Wiltschko, 2005). In environments providing relatively little information for navigation such as the open ocean, the usefulness of a large scale, temporally stable navigation system based on magnetoreception is clear.

Despite ongoing debate about the physiological mechanisms for magnetoreception in animals, the ability to sense magnetic fields and use them for orientation has been shown in a wide range of phyla (Mann et al. 1988), with magnetic navigation long demonstrated in birds (Wiltschko et al. 2010) and bees (Walker and Bitterman, 1985), but also in rodents (Nemec et al. 2001), ants (Oliveira et al. 2009), deer (Begall et al. 2008) and newts (Phillips, 1986). Marine animals shown to respond to magnetic cues for orientation include sea turtles (Lohmann and Lohmann, 1993) and spiny lobsters (Boles and Lohmann, 2003), while within teleost fishes magnetoreception has been shown in salmon (Quinn and Dittman, 1990), tuna (Walker, 1984), rainbow trout (Walker et al. 1997) and zebrafish (Sherbakov et al. 2005), with zebrafish as young as 5 months old exhibiting a preference for particular magnetic directions (Takebe et al. 2012). Orientation using magnetoreception has been demonstrated in test animals that were exposed to magnetic fields characteristic of specific locations in their environment; when the fields were subsequently inverted or rotated, orientation behaviour was altered in birds, lobsters and turtles (Wiltschko and Wiltschko, 2002, Boles and Lohmann, 2003, Lohmann et al. 2004).

There are currently two main hypotheses for the magnetic orientation strategies in animals (1) a chemical compass based on a radical pair mechanism, and (2) biomagnetic magnetite particles (Wiltschko and Wiltschko, 2005). Based on the interaction of geomagnetic fields with photopigments that are likely to be located in the eye, radical pairs are created by the absorption of photons and transfer of electrons (Ritz et al. 2004). This alters their spin states, with a yield dependant on the receptor-molecule's alignment within the ambient magnetic field, enabling animals to detect the field direction (Muheim et al. 2006). Experimental evidence indicates a critical role of these receptor-molecules, believed to be photopigments known as cryptochromes, in magnetic navigation of birds and fruit flies (Rodgers et al. 2007, Geager et al. 2008). This model is light-dependant, relying on photons interacting with the cryptochromes that have been identified in the retina of various birds, such as warblers, robins and chickens (Mouristen et al. 2004, Nießner et al. 2011). Studies on migratory bird species show that when light conditions are changed orientation behaviour is altered, with compass navigation becoming less reliable (Wiltschko et al. 2010). This light-dependent effect has not been seen in other animals however, suggesting that the light-independent perception of two distinct magnetic field parameters in animals such as loggerhead turtles and rainbow trout may be related to magnetite-based magnetoreception (Putman et al. 2011, Hellinger and Hoffman, 2012).

The magnetite hypothesis revolves around biochemically precipitated ferromagnetic crystals, which transmit directional information on the ambient geomagnetic field in the form of torque into the nervous system through coupled mechanosensitive structures (Oliveira et al. 2009). These iron-rich crystals of single domain magnetite

(Fe_3O_4) are capable of detecting small changes in the geomagnetic field, with only a small number of crystals arranged in a chain thought to be required for a competent compass system (Kirschvink, 1983, Diebel et al. 2000, Eder et al. 2012). The potential for the presence of magnetite to provide a basis for orientation based on magnetoreception was discovered over 30 years ago in magnetotactic bacteria, where magnetite crystals aligned the organisms to magnetic fields lines allowing them to orient towards more favourable conditions (Blakemore, 1975). Since then magnetite has been found in vertebrates exhibiting potential magnetoreceptive capabilities, including fish, amphibians, reptiles, birds and mammals, where it appears to be located mostly in the ethmoid region in the anterior of the head (Gould, 2004, Wiltschko and Wiltschko, 2012). More importantly, magnetite particles in rainbow trout and homing pigeons have neural connections to the brain through magnetically responsive nerves associated with the olfactory epithelium of the olfactory organs (Semm and Beason, 1990, Walker et al. 1997, Kirschvink et al. 2001).

While the studies into the processes of how observed magnetoreception and its role in navigation operates in animal sensory systems, very little is known about the potential for this capability in organisms at the larval stage (Kingsford et al. 2002). In species where magnetoreception has long been studied however, there is evidence that the perception of magnetic cues is independent of developmental state, appearing at very early stages in both fish and turtle species (Cain et al. 2005, Hellinger and Hoffman, 2012). This opens the possibility that larval fish may be able to utilise this sensory strategy to perform the location-independent orientation behaviour noted in studies by Leis and Carson-Ewart (2003), however this ability remains to be verified.

Implications for management of marine populations

Achieving a realistic understanding of larval dispersal and connectivity in the marine environment is one of the major challenges in marine ecology (Cowen and Sponaugle 2009). More understanding is critical however for effective and efficient management of marine populations and areas (Harrison et al. 2012). Predictive modelling of larval dispersal attempts to quantify spatial and temporal scales of connectivity and the effect of contributing processes (North et al. 2008, Kanary et al. 2011). However models are still largely based on hydrological processes and a narrow suite of biological parameters, with behavioural and ontogenetic factors incorporated in simplistic ways due to a scarcity of empirical data (Largier 2003, Leis 2007).

Models are becoming increasingly sophisticated however as more discoveries on larval behaviours and abilities are reported (Wolanski and Kingsford 2014). Adding more empirical behavioural parameters to models of larval fish dispersal has lead to an improved correlation with observed recruitment patterns in some cases (Paris and Cowen 2004). The paradigm shift towards active rather than passive influence by larvae on their dispersal has also seen behaviour becoming an essential part of dispersal modelling (Fiksen et al. 2007). However evidence suggests the role and importance of larval behavioural traits can be species and location-specific, making it clear that data on behaviours from a broader range of species and locations are required (Le Port et al. 2014).

While some recent modelling studies on larval dispersal tout the importance of incorporating behaviour for improved accuracy, this remains difficult due to the paucity of empirical data available. Many models now incorporate vertical migration behaviour, however horizontal swimming behaviours are rarely included (Kough et al. 2013, Lacroix et al. 2013). Directional swimming parameters have shown to be critical for reproducing dispersal patterns observed in the field (Wolanski et al. 1997). Sensory cue use is also usually fairly simplistic regarding how it is included in dispersal models, often a presence/absence response to a "detection zone" around the habitat based on small-scale cue ranges (James et al. 2002). How and when large-scale orientation cues influence dispersal behaviours remains unknown (Staaterman and Paris 2014).

Another aspect to larval dispersal where empirical data are lacking is the ontogenetic development of sensory cue use and dispersal-relevant behaviours in larval fishes from various regions (Leis et al. 2013). The ontogeny of development for swimming and sensory abilities during the larval stage can have significant impacts on modelling dispersal outcomes in terms of the success and spatial extent (Le Port et al. 2014). Although various studies considered how larvae respond to sensory cues at the settlement stage, there are little data as to how these responses develop and change during the pre-settlement stage. What little research has been done on behavioural ontogeny of larvae has been on of a small number of tropical reef species (Dixson et al. 2011, Wright et al. 2011).

To sum up, research effort into the pre-settlement processes affecting larval dispersal has increased over the last twenty years, leading to new insights into the abilities and behaviours of marine larvae and their potential importance to dispersal. This has led to a paradigm shift in our understanding of population connectivity (Leis et al.

2011b). There remain large gaps in our knowledge of the sensory abilities and their ontogenetic development during the larval stage, particularly in species from different locations (Staaterman and Paris 2014). Filling in these biological knowledge gaps will help to enable the development of more realistic contemporary models of population connectivity in the marine environment (Leis 2007). It is in this context that I undertook the work for this thesis.

Overview of the thesis

My overall aim was to investigate sensory cue use in larval fishes from soon after hatch until settlement on habitat, and to explore the behavioural mechanisms by which a larva can orientate to environmental cues and guide settlement, focussing on three main aspects. I first looked at the ontogenetic development of intrinsic responses to habitat-relevant sensory cues in multiple species. The second aspect involved how larvae, which had developed capable swimming and sensory abilities, managed to orient their swimming during the pre-settlement phase. For this I tested the intrinsic ability of fish larvae to detect large-scale cues, which would indicate the use of a celestial or geomagnetic compass mechanism. Lastly, since sensory abilities of larvae have been well studied during settlement and post-settlement stages, I built on this knowledge to investigate the effect of extrinsic factors on behaviour of larvae settling onto habitat. In addition, because anthropogenic pollution has been linked to reduced recruitment in degraded areas, I investigated the effect of increased sediment pollution on the sensory response and performance of settling coral reef fish.

Chapter 2 charts the ontogeny of responses of newly hatched reared larvae of two temperate fishes associated with a temperate, estuarine system to olfactory cues from different habitats. I tested choice behaviour in response to chemical cues from estuarine and coastal sections of the estuary, from flexion to settlement stage across two cohorts for each species from separate years. To determine which components of the chemical cues had more impact on chemotactic behaviour I tested the response to different levels of pH, salinity and organic matter. Results demonstrated for the first time a consistent ontogenetic point during the larval stage whereby species-specific olfactory responses emerged. The presence of seagrass appeared to be an important factor in this behaviour for these species.

Chapters 3 & 4 explore the ability of coral reef larvae to use celestial and magnetic cues to orient movement. I performed behavioural experiments at Lizard Island Research Station on the Great Barrier Reef to test the responses of damselfish (*Chromis atripectoralis*, Pomacentridae) caught offshore before settlement to various light conditions simulating a moving solar azimuth, and to various changes to the local magnetic field. The responses are discussed on both the within and among-individual level. Results show experimentally for the first time that large-scale cue use incorporating aspects of both a sun and magnetic compass are likely to facilitate orientation abilities in this species.

Chapter 5 investigates the role of extrinsic factors of the local environment on settlement behaviour in coral reef fish larvae. At Sesoko Island, Okinawa, where sediment pollution is causing degradation of coral reefs, I tested the responses of damselfish (*Chromis viridis*, Pomacentridae) to habitat cues in the presence of different levels of sediment. This involved manipulating light traps *in situ* with coral and sediment combined with choice chamber experiments in the laboratory. Taking things further, I then prolonged the time of exposure of larvae after settlement to test the longer-term effects. Results indicate larvae attempt to avoid degraded habitat, however if they are forced to settle there are negative impacts on physical and sensory performance.

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CHAPTER TWO

Emergence of chemotactic behaviour in larvae of estuarine-dependant fishes

*Presettlement-stage larva of the Australian Bass (*Macquaria novemaculeata*, top)*

*& Mulloway (*Argyrosomus japonicus*, bottom)*

Abstract

The spatial distribution and connectivity of most marine organisms and their populations are largely dependent on biophysical factors affecting dispersal during the larval phase. Behaviour and swimming ability of marine larvae is increasingly acknowledged to influence dispersal patterns, especially in fishes, but it is unclear which sensory cues are involved and critically when during the pre-settlement stage these abilities develop. To investigate this we studied the ontogeny of olfactory responses to habitat cues in reared larvae of two temperate estuarine-dependant fish species from notochord flexion to settlement stage. Since the movement of larval fishes from coastal waters to estuaries is a critical period, we compared olfactory cues from the coastal zone with those from the upper estuary to test preference behaviour. We found that fish larvae have dynamic responses to sensory cues in early life, which emerge at particular ontogenetic stages coinciding with increases in swimming ability. Chemotactic responses appear to be driven by innate species-specific preferences that were consistent across cohorts, suggesting different recruitment strategies between species. We also found that the presence of organic matter such as seagrass is an important component for chemical cues influencing swimming behaviour of pre-settlement larvae. These factors should be considered in modelling of larval connectivity and we recommend research into a greater range of taxa is needed to elucidate how larvae of species with different life histories behave during dispersal.

Introduction

The planktonic larval stage of many demersal marine organisms is a critical period in their life history, having profound impacts on influences on the distribution and magnitude of population replenishment, yet empirical data on their ecology remains limited (Kendall et al. 2013). This presents a challenge for effective marine environmental management that depends on accurate knowledge of the spatial dynamics of these populations, in particular the processes controlling population connectivity (Cowen et al. 2007, Siegel et al. 2008). Population connectivity, the extent to which populations within a species' range are linked by the exchange of individuals, plays a fundamental role at both evolutionary and ecological scales in how vulnerable local populations and metapopulations are to environmental disturbances (Hughes et al. 2003, Palumbi 2003).

For demersal marine organisms, connectivity and the spatial structure of populations are largely affected by the larval dispersal phase (Fogarty and Botsford 2007). Larval dispersal has traditionally been thought to be a purely physical process whereby larvae were transported by ocean currents towards settlement habitat as ostensibly passive particles (Stobutzki 2001). Research into larval capabilities since the 1990s however has resulted in a paradigm shift in our understanding towards a more active influence by larvae on their dispersal patterns (Leis 2015). However how and when this influence develops remains a gap in our understanding of larval biology, and is critical if we are to manage current and future environmental changes that may impact these processes (Saenz-Agudelo et al. 2011, Staaterman and Paris 2014).

By the time they are developmentally competent to settle at the end of their pelagic larval duration (PLD), larvae moving from the pelagic environment to demersal habitat have well-developed sensory abilities in various modalities allowing them to detect habitat-relevant cues at a range of spatial scales (Huijbers et al. 2012, Atema et al. 2015). These abilities can provide orientation to supplement the formidable locomotory capabilities of many species at this stage allowing larvae to influence their dispersal in ocean currents (Leis 2010, Kashef et al. 2014). Indeed, dispersal models that have incorporated biophysical processes such as larval behaviour and sensory abilities are often better correlated with patterns of recruitment observed in the field (Fiksen et al. 2007, Kough et al. 2013). Biophysical dispersal modelling however is hampered by a dearth of species-specific, empirical data on pre-settlement biology and behaviours of larvae which may influence dispersal trajectories, with these parameters often estimated or included as simply present or absent (Leis 2007, Treml et al. 2012). Furthermore, a few studies to date have shown that larval behaviour and locomotory capabilities are not static but change dynamically through ontogeny (Clark et al. 2005, Leis 2010). In order to effectively incorporate these dynamics into dispersal models we must first describe the patterns and understand their underlying causes.

Visual, auditory and chemical cues have long been hypothesised to play a role in a hierarchy of sensory information used for orientation towards favourable habitats by fishes during settlement (Sweatman 1988, Elliott et al. 1995, Lecchini et al. 2005). Of the senses required to utilise these cues olfaction has received the most study, with odour plumes and "halos" being a detectable indication of habitat for larvae at ranges up to kilometres from the source (Atema 2012). Fish larvae of tropical warm-water species demonstrate a variety of chemotactic responses and preference for cues such as discerning healthy coral reef vs. degraded coral reef, natal reef vs. neighbouring reef and the smell of conspecifics vs. predators (Gerlach et al. 2007, Dixson et al. 2010, Dixson et al. 2014). Studies on sensory cues of early stage larval fishes are limited however to a handful of species and most of these are tropical damselfishes. For instance, the only study to date on pre-settlement sensory ontogeny in temperate species found that responses to habitat-related auditory cues were undetectable in larval barramundi but for a three day window around metamorphosis (Rossi et al., 2015). This lack of information on the ontogeny of larval behaviour in a greater range of taxa, particularly in temperate species, must be addressed to improve the generality of dispersal models across different species and locations (Leis et al. 2013, Staaterman and Paris 2014).

Estuarine habitats are important nursery grounds for many species of fish and invertebrates (Beck et al. 2001). High productivity and lower predation risk are some of the many benefits provided by estuaries (Limburg et al. 1997, Manderson et al. 2004). However fish larvae recruiting to coastal areas must first locate them in order to take advantage of their benefits. Spatial patterns of fish larvae settling in estuaries and embayments show preferential recruitment to locations with cues indicating favourable habitat conditions for post-settlement success and chemotactic response to such cues in isolation, suggesting a behavioural influence on settlement in these systems (Hale et al. 2008, Radford et al. 2012). So far research into chemotactic effects has focused on settlement stage larvae, and little is known about how pre-settlement sensory ecology affects estuarine-dependant species. Determining these effects throughout the entire larval period is critical to understanding the role of behaviour on dispersal.

In order to gain insight into how sensory ontogeny may interact with larval ecology we selected two temperate estuarine-dependant species with contrasting life histories. The Australian bass, (*Macquaria novemaculeata,* Family: Percichthyidae), is found in streams and estuaries on the eastern coast of Australia (Trnski et al. 2005). A catadromous euryhaline fish important for recreational fishers and aquaculture, this species spends most of its life in fresh water, migrating down into estuaries during the winter months to spawn. During periods of heavy rainfall and flooding events less saline plumes of water can skirt the coastline, allowing adults and larvae to move out of the estuaries and into the coastal environment (Wolanski and Jones 1981). The presence of *M. novemaculeata* larvae, tolerant to marine salinities, moving on incoming tides into estuaries indicates the potential for oceanic dispersal among estuaries in this species (Trnski et al. 2005). This movement of adults and larvae between catchments allows for the development and maintenance of genetic population structure in this species (Jerry 1997), and makes this an interesting species for investigating the ontogenetic development of olfactory cue use in larvae. Percichthyid larvae are known to have well developed olfactory pits at a young age which may be used in some species to orient towards habitat cues (Gehrke 1990). Mulloway (*Argyrosomus japonicus,* Family*:* Sciaenidae) is another key species for recreational and commercial fishing and is also associated with estuaries throughout its life history. Small juveniles can be found in estuaries and adults often associate with the estuary – coastal boundary, however the timing, age and length at which mulloway recruit to estuaries after hatching in coastal waters is not well known (Fielder and Heasman 2011).

Studying wild early-stage larvae *in situ* can be fraught with logistical issues of successfully locating, sampling and observing animals of such small size and high fragility (Leis et al. 2006). Using reared larvae, as we have in this study, provides opportunities for observations of biological capabilities throughout the larval phase, and a glimpse into the black box of early-stage larval behaviour (Fisher et al. 2000). While there is a possibility that reared larvae may not have a "wild type" behaviour either due to selective breeding or rearing conditions, often cases comparisons of swimming ability and olfactory responses between reared and wild-caught larvae are congruous and suggest that the use of reared fish here may be useful to predict abilities of fish in the wild (Leis and Fisher 2006, Dixson et al. 2008, Faria et al. 2009, Berenshtein et al. 2014).

We tested olfactory responses of larval fish throughout the period from initial notochord flexion to settlement, comparing species from two families and multiple cohorts to address the following questions: (1) when do responses to olfactory cues develop in larval fishes, (2) are olfactory responses consistent throughout ontogeny both within and between cohorts, and (3) which characteristics of chemical cues may underlie any observed behavioural responses? As dissolved odour cues from estuarine habitats have been implicated in larval recruitment for various species (Boehlert and Mundy 1988, James et al. 2008), we hypothesised that responses to olfactory cues from coastal and estuary habitats would vary ontogenetically during the pre-settlement stage. The development of the ability to discern between water from coastal and estuarine areas may be important for orientation towards and within estuaries. As water taken from seagrass beds is a strong attractant in larval and post-larval fishes which settle to this habitat (Huijbers et al. 2008, Radford et al. 2012) we also hypothesised that seagrass scent itself could be a driver behind olfactory responses to estuary cues.

Materials & methods

Larval rearing

We reared larvae at Port Stephens Fisheries Institute (PSFI, NSW DPI) under controlled laboratory conditions in 2000L tanks following methods described in Fielder and Heasman (2011). Briefly, we supplied rearing tanks with treated estuary water which was filtered by a foam fractionator, ozonated to ~650 ORP (oxygen-reduction potential) and passed through sand, cartridge and activated carbon filters for effective disinfection and removal of trace residual oxidants. Broodstock for *M. novemaculeata* are wild-caught each year, while *A. japonicus* broodstock are second-generation hatchery-reared. We maintained a temperature of approximately 20°C, pH of 8.0 and salinity of 30ppt in the rearing tanks as per the optimal growth conditions for both species. We fed larvae rotifers (*Brachionus plicatilis*) from 4 days post hatching (dph) and brine shrimp (*Artemia*) nauplii from 15 dph.

Experimental conditions within the test chamber required active swimming by larvae in the presence of a minimal current, therefore we began behavioural trials once flexion of the notochord was completed (ca. 6mm SL for *M. novemaculeata* and ca. 6.5mm SL for *A. japonicus*). Every three days we randomly selected 20 individuals from the hatchery tanks for olfactory choice experiments over a period of 21 days. We herded selected individuals (without contact) by net into a plastic container and moved them to the laboratory to acclimate for a period between 1 and 2 hours depending on behavioural signs of stress (darting movement, affinity for the bottom).

All groups of test larvae sufficiently acclimated within this timeframe. Each fish was tested only once. Following testing, we euthanised larvae in an ice slurry then recorded individual standard lengths.

Choice experiments

We tested behavioural responses to olfactory cues in temperature-controlled laboratories onsite at PSFI, NSW. A two-channel Perspex choice flume of a similar design to Gerlach et al. (2007) was used to test preferences between olfactory cues in water sourced from different areas (Fig. 2.1). We introduced individuals into a piece of 25mm PVC pipe located in the centre of the downstream end of the flume to control for positional bias and them removed the pipe, after which they were free to explore the chamber and swim between the two adjacent flows for an acclimation period of two minutes. We discarded larvae that did not swim actively or explore both sides of the chamber during these two minutes from the trials. Three per cent of fish tested were discarded from both control and treatment trials. After the acclimation period we recorded the larva's position in the chamber at five-second intervals for a period of two minutes. Position of larvae in the flume (as swimming in one of the two sources of cue water) was recorded by an observer seated ~1m directly posterior to the flume from behind an opaque plastic blind. If larvae were located at the boundary between the two water flows during a time point position was recorded in terms of head position or swimming direction. In order to control for any side bias due to the experimental setup, we then used valves to switch over water sources to the opposite side of the chamber, giving a further 3 minutes (1 minute for the chamber to flush completely, 2 minutes acclimation) before observing the larva for a second 2-minute period. This provided two sets of 25 time points of choice behaviour for each individual. We calculated the percentage of time spent in each cue by dividing the number of observations in each cue by the total number of observations (50).

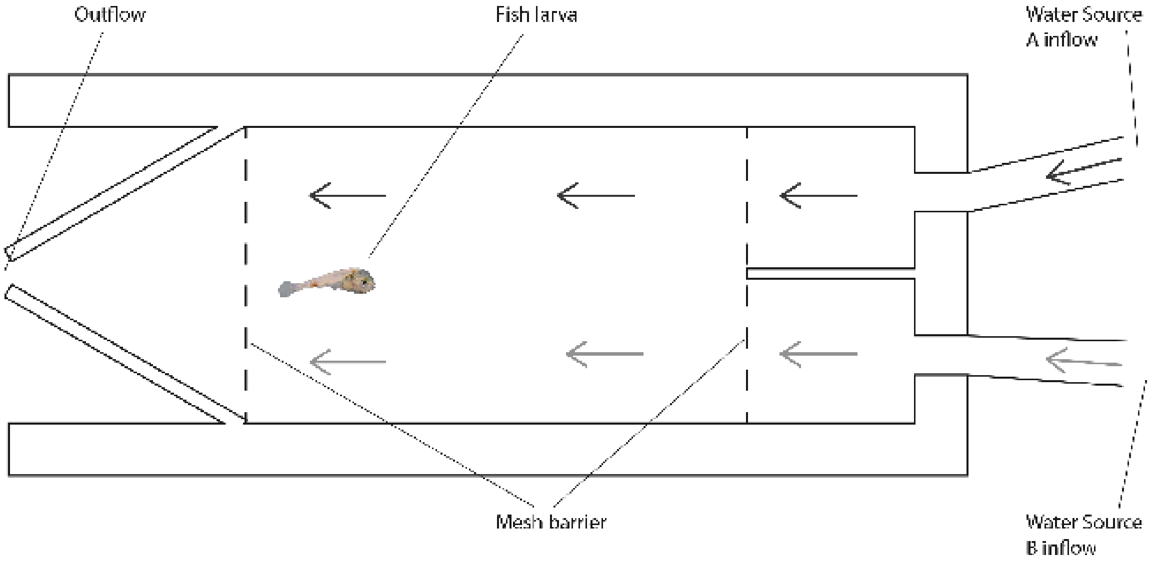

Fig. 2.1 Olfactory flume design (20cm x 5xm x 3cm).

We applied this procedure twice to each fish during a single trial. First, with a control treatment (where incoming water on both sides of the chamber was from the same source) and secondly with inflow switched to treatment conditions (testing preferences between water from two different sources), resulting in two comparable data sets for each fish. Although a pilot study indicated that the water source selected for the control treatment had no influence on the following choice behaviour during the experimental trial, the cue we used for control water (i.e., coastal or estuary water) was alternated for each experimental day. Flow rate was maintained using variable area flow meters (Dwyer Instruments, Unanderra NSW) at 200 mL min^{-1}, giving a depth of ~10mm in the test chamber. This flow of ca. 3.4 cm s^{-1} made possible by the small chamber size encouraged the larvae to swim without having to struggle against the current, ensuring movement was likely due to active choice (Dixson et al. 2011).

Dye tests were conducted in the chamber each day before testing to ensure a laminar flow on each side without eddies or mixing. This was particularly important during trials with water sources of different salinities. Through fine adjustments of the outflow aperture and chamber slope, the amount of stratified mixing crossing the centreline was kept to a minimum (Appendix, Fig. 1A). In addition, the shallow water in the chamber made the possibility of a larvae crossing sides of the chamber without detecting any olfactory change unlikely. Experiments were conducted in both 2013 and 2014 to test the repeatability of behaviours observed between different cohorts.

Olfactory cue preparation

As these species show movement within and between estuaries at different life history stages, we sourced water *in situ* from Port Stephens estuary to provide larvae with a choice between "coastal water" (taken on an incoming tide from the estuary mouth (32°42'34.7"S, 152°.11'04.1"E)) and "estuary water" (taken from the upper estuary at PSFI (32°44'41.8"S, 152°03'15.8"E))(Fig. 2.2). Collected cue water was run through a 1µm filter to remove particulate matter yet allow their related olfactory molecules to be retained before testing. Differences in turbidity and particulate matter between the two water sources needed to be minimised to reduce visual bias affecting choice behaviour.

Importantly however, in contrast to the rearing tank water, estuary cue water was not treated and filtered by foam fractionator or sand, cartridge and carbon filters or ozonation. Temperature was maintained to match that of the larval rearing water while being mixed and oxygenated by air stones. Monitoring of water quality parameters revealed the main differences between coastal water (COA) and estuary water (EST) were in salinity (mean = 35. 1ppt ±0.23SE and 29.8ppt ±0.43SE respectively) and pH (mean = 8.15 ±0.019SE and 8.02 ±0.015SE respectively), therefore we prepared artificial seawater (Ocean Nature Sea Salt, Aquasonic, Wauchope, NSW, Australia) to test behavioural responses between water sources with different salinity (low (28ppt) vs. high (35ppt)) or pH (low (7.8) vs. high (8.2)).

Fig. 2.2 Map of the Port Stephens estuary with collection sites for estuary water (1), seagrass samples (2) & coastal water (3) indicated by black circles. Research station located at point (1).

To test behavioural response to the presence of cues from organic matter we prepared batches of coastal water by soaking seagrass (*Zostera muelleri* subsp. *capricorni*) collected from Bagnall's Beach within Port Stephens estuary (Fig. 2.3 32°43'08.6"S, 152°07'37.0"E)). We rinsed the seagrass (300g wet weight) with freshwater to remove traces of epibionts and soil and soaked it for 2 hours in a 200L tank of coastal water to create seagrass-treated coastal water (SCOA), which was stored overnight in the laboratory under the same environmental conditions as the fish larvae. We then tested the larvae for preference behaviour to the seagrass cue against un-manipulated coastal water (i.e. SCOA vs. COA). To investigate whether the artificial seagrass cue was ecologically relevant we also tested seagrass soaked coastal water against un-manipulated estuary water (i.e. SCOA vs. EST).

Fig. 2.3 Author collecting seagrass from Port Stephens estuary

Data Analysis

To test for olfactory responses in larvae at age (days post-hatch) and size intervals (standard length (SL) rounded to the nearest 0.5mm) we used Wilcoxon signed-rank (WSR), a non-parametric paired test suitable for count data, to compare the difference among-individuals in the total time spent on each side of the test chamber between control and treatment conditions. We used the same test to compare behavioural responses to different conditions of salinity, pH and seagrass at the start (ca. 6mm SL for *M. novemaculeata* and ca. 6.5mm SL for *A. japonicus*) and the end of the experimental period (ca. 9.5mm SL for *M. novemaculeata* and ca. 15mm SL for *A. japonicus*) of the second cohort in 2014. In each case the null hypothesis was that there was no difference between the time larvae spent swimming in one side of the chamber during control conditions and when two different cues were presented.

To test whether age and size-based patterns of individuals making choices between cohorts differed between 2013 and 2014 we used analysis of covariance (ANCOVA) after assumptions were assessed to be satisfied with Shapiro-Wilk and Levene tests. We also used WSR tests to assess an individual as having made a choice by comparing time spent in COA (*A. japonicus*) or EST (*M. novemaculeata*) with the time spent on one side of the chamber during controls. We then used logistic regression to assess how the number of larvae making choices for habitat cues (EST for *M. novemaculeata* and COA for *A. japonicus*) changed with increasing age and size.

Results

Ontogeny of olfactory response

Age-based patterns

In *M. novemaculeata* larvae, significant responses to olfactory cues began from 30 dph in 2013 (WSR: $Z = -3.42$, $p < 0.001$, $r = 0.76$, $n = 20$) and 34 dph in 2014 (WSR: $Z = -3.14$, $p = 0.012$, $r = 0.62$, $n = 20$). The initial choice among individuals was for COA before switching to EST after 39dph in 2013 (WSR: $Z = -3.64$, $p < 0.001$, $r = 0.81$, $n = 20$) and after 37dph in 2014 (WSR: $Z = -3.14$, $p = 0.002$, $r = 0.70$, $n = 20$). This ontogenetic pattern of development in olfactory responses was consistent between years (Fig. 2.4). In *A. japonicas* larvae significant responses to olfactory cues began from 41dph in 2013 (WSR: $Z = -3.53$, $p = 0.0004$, $r = 0.79$, $n = 20$) and 28dph in 2014 (WSR: $Z = -3.31$, $p = 0.002$, $r = 0.70$, $n = 20$). Fish larvae spent significantly more time in COA after this preference developed in both years (Fig. 2.5).

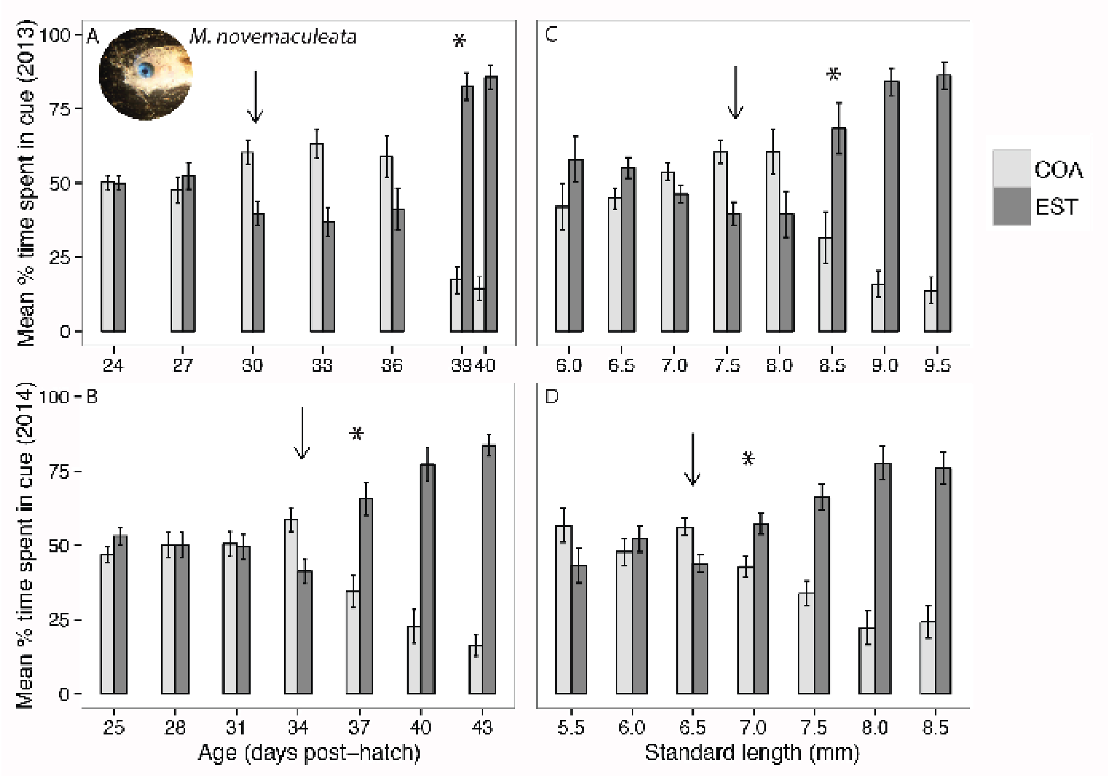

Fig. 2.4 Olfactory choice in relation to age (A,B) and size (C,D) of *M. novemaculeata* larvae when presented with cues from the coast (COA) and the upper estuary (EST), for cohorts reared in 2013 (A,C) and 2014 (B,D). Error bars indicate standard error. Arrows indicate developmental stage at which significant choice emerges (p < 0.001 (n = 20 per experimental day)), asterisk (*) indicates developmental stage of switch in response to olfactory cues.

Size-based patterns

Larvae of *M. novemaculeata* preferred COA over EST from 7.5mm SL in 2013 (WSR: Z = -2.34, p = 0.018, r = 0.42, n = 20) and 6.5mm SL in 2014 (WSR: Z = -2.04, p = 0.032, r = 0.372, n = 20). Olfactory preferences changed to EST from 8.5mm in 2013 (WSR: Z = -3.29, p = 0.001, r = 0.78, n = 20) and from 7mm in 2014 (WSR: Z = -2.57, p = 0.013, r = 0.49, n = 20) (Fig. 2.4). Larvae of *A. japonicus* preferred COA over EST from 9.5mm SL in 2013 (WSR: Z = -2.89, p = 0.016, r = 0.47, n = 20) and 9mm SL in 2014 (WSR: Z = -2.90, p = 0.004, r = 0.51, n = 20). Fish larvae spent significantly more time in COA from this size onwards for both years (Fig. 2.5).

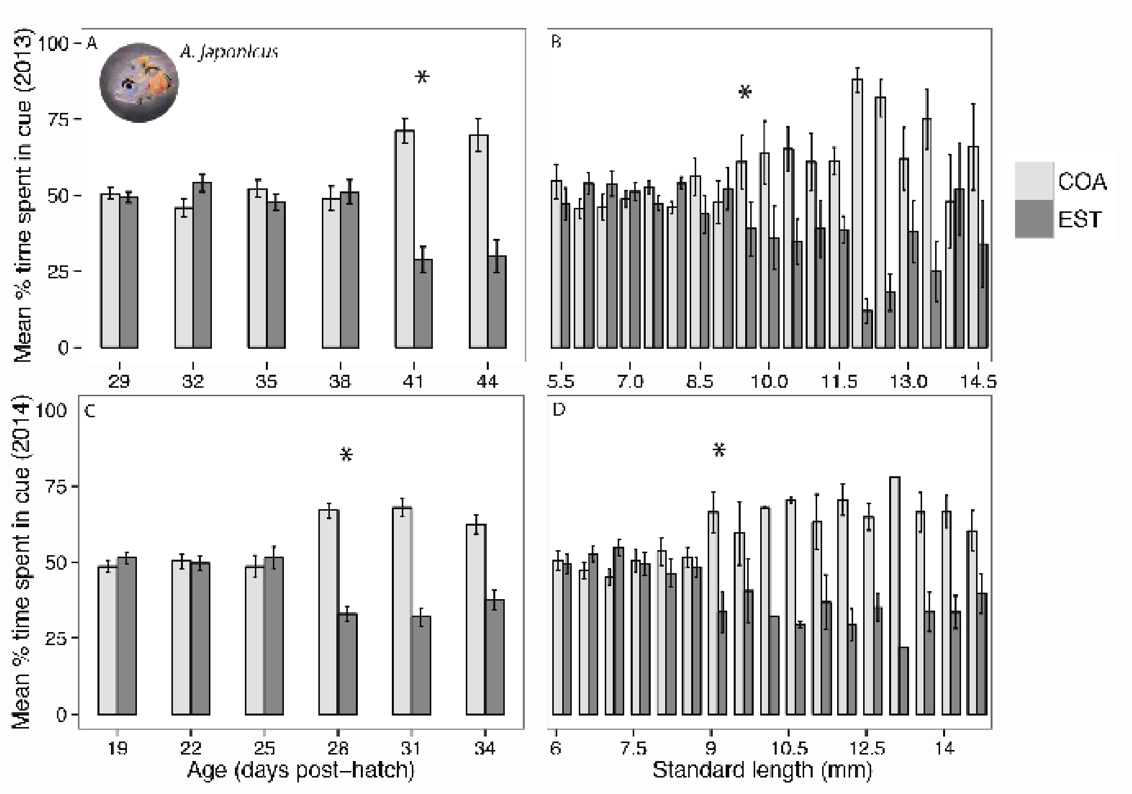

Fig. 2.5 Olfactory choice in relation to age (A,B) and size (C,D) of *A. japonicus* larvae when presented with cues from the coast (COA) and the upper estuary (EST), for cohorts reared in 2013 (A,C) and 2014 (B,D). Error bars indicate standard error. Arrows indicate developmental stage at which significant choice emerges ($p < 0.001$ (n = 20 per experimental day)).

Modelling the likelihood of an individual larva making a choice for EST in *M. novemaculeata* and for COA in *A. japonicus* (as opposed to no preference during a trial) showed both species were more likely to make olfactory-based choices with increasing age and size. Age-based patterns of choice behaviour differed between cohorts in *A. japonicus* ($F_{(1,237)} = 51.08$, p < 0001), but size-based patterns did not differ ($F_{(1,237)} = 0.45$, p = 0.50). In *M. novemaculeata* age-based patterns did not differ between cohorts ($F_{(1,277)} = 0.47$, p = 0.49), whereas size-based patterns did differ ($F_{(1,277)} = 14.89$, p = 0.0001). This suggests that the development of choice behaviour was consistent between the two cohorts, though interestingly the relative importance of age and size was different between species. The model with age as the predictor provided the best fit for *M. novemaculeata* ($R^2 = 0.19$), while size was a better predictor among models for *A. japonicus* ($R^2 = 0.15$) (Table 2.1). Size at age of *A. japonicus* larvae differed between cohorts due to an unintentional differentiation in the provision of food between 5 – 12 dph, however growth rates for both species remained similar during the experimental period (Appendix, Fig 2A).

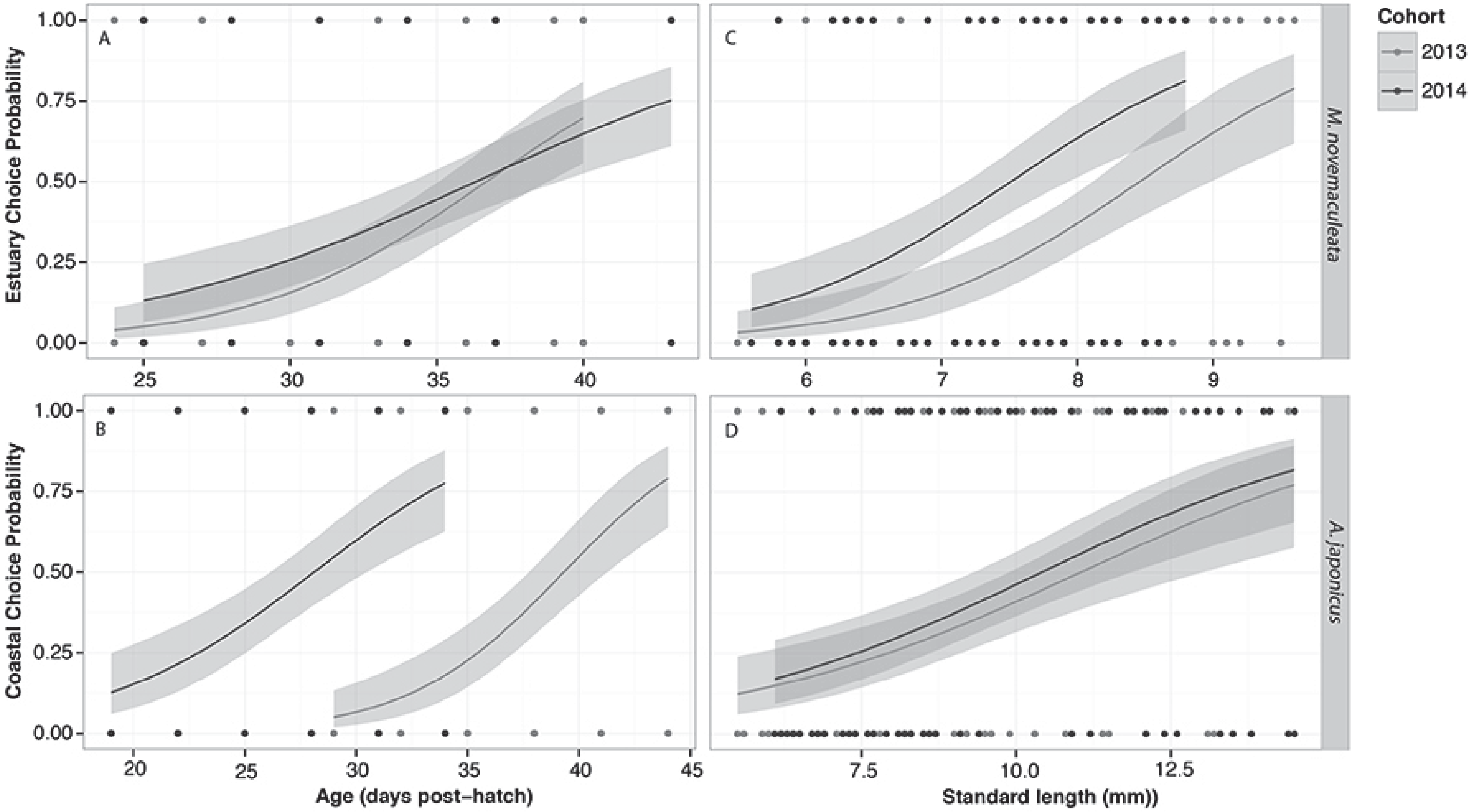

Fig. 2.6 Relationship of choice behaviour with increasing age and size for *M. novemaculeata* (EST choice, A,C) and *A. japonicus* (COA choice, B,D) larvae comparing two cohorts. Top and bottom circles indicate observations in 2013 (light) and 2014 (dark). Light line shows logistic regression for 2013, dark line shows 2014. Shading indicates predicted values for each model

Behavioural response to water properties

There was no significant choice behaviour in the smallest *M. novemaculeata* larvae (mean SL < 7mm) in response to differences in pH (WSR: $Z = 1.07$, p = 0.20, r = 0.24, n = 20) or salinity (WSR: $Z = 0.84$, p = 0.42, r = 0.19, n = 20). No significant preference was detected for seagrass cues at this size (WSR: $Z = 0.22$, p = 0.83, r = 0.05, n = 20). Likewise, no significant choice behaviour was seen in the smallest *A. japonicus* larvae (mean SL < 7mm) in response to pH (WSR: $Z = -0.32$, p = 0.85, r = 0.07, n = 20), salinity (WSR: $Z = 0.9168$, p = 0.37, r = 0.21, n = 20) or seagrass cues (WSR: $Z = -0.19$, p = 0.86, r = 0.04, n = 20).

Table 2.1 Comparison of logistic regression models of choice behaviour in larval fish with age and with growth, including R^2 and AIC values (n = 240 per species).

Model	Chi-square	df	p value	R^2	AIC
M. novemaculeata					
Age	69.23	1	1.11×10^{-16}	0.19	306.26
Size	47.65	1	5.09×10^{-12}	0.19	327.83
Age + Size	69.98	2	6.66×10^{-16}	0.13	307.50
A. japonicus					
Age	24.3	1	7.97×10^{-7}	0.08	302.7
Size	44.17	1	3.02×10^{-11}	0.15	282.9
Age + Size	46.77	2	6.99×10^{-11}	0.14	282.3

After choice behaviour had been detected (mean SL > 8 mm in both species) in response to COA and EST cues no significant preference for different pH levels was present (WSR: $Z = -0.56$, p = 0.57, r = 0.13, n = 20) in either *M. novemaculeata* or *A. japonicus* larvae (WSR: $Z = -1.53$, p = 0.11, r = 0.34, n = 20). At this developmental stage both species however had developed significant responses to differences in salinity and seagrass cues (Fig. 2.7). *M. novemaculeata* larvae > 8mm SL significantly preferred lower salinity of 28 ppt to 35 ppt (WSR: $Z = 2.72$, $p = 0.019$, $r = 0.61$, $n = 20$) and selected SCOA over COA (WSR: $Z = 3.18$, $p < 0.001$, $r = 0.71$, $n = 20$). Similarly, *A. japonicus* larvae > 8 mm also preferred 28 ppt salinity to 35ppt (WSR: $Z = 2.36$, $p = 0.020$, $r = 0.53$, $n = 20$). In contrast, *A. japonicus* larvae > 8mm SL significantly preferred COA to SCOA (WSR: $Z = 3.89$, $p < 0.0001$, $r = 0.87$, $n = 20$). *M. novemaculeata* larvae > 8mm SL significantly preferred EST to SCOA (WSR: $Z = -3.70$, $p < 0.0001$, $r = 0.83$, $n = 20$).

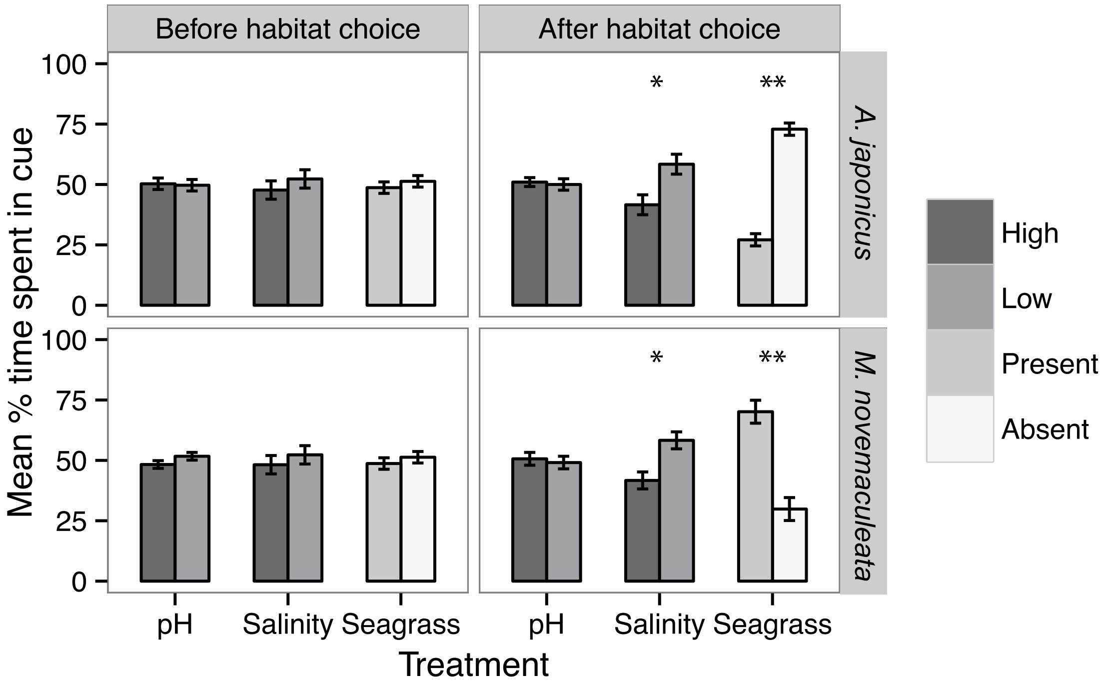

Fig. 2.7 Mean percentage of time (±SE) spent by larvae in water treatments when presented with different levels of pH, salinity and seagrass cues at developmental stages before and after significant choice behaviour was detected among individuals in the second cohort (2014) (ca. 6.5mm SL for *M. novemaculeata* and ca. 9mm SL for *A. japonicus*). Asterisks indicate significance levels (* < 0.05, ** < 0.001).

Discussion

Research on the biological factors influencing the ecology and dispersal of larval fishes is limited, particularly outside of the tropics. We found that larvae of two estuarine-dependant temperate species had particular points in their early life (ca. 6 – 15mm SL) where behavioural responses to olfactory stimuli emerged. These points coincide with evidence from other studies on these species reporting exponential increases in swimming endurance and changes in *in situ* swimming behaviour (Clark et al. 2005), suggesting a developmental milestone during the pelagic larval phase whereby swimming ability and behaviour combine and begin to influence movement towards habitat. From a lack of significant response at the time of flexion, preference emerged to olfactory cues from different habitats stimulating chemotactic behaviour. Interestingly, this olfactory cue choice was strongly species-specific and appeared to be an innate response influenced by the presence of organic matter such as seagrass.

The ontogenetic development of olfactory responses in larvae of these temperate estuary-dependant species indicates that the behavioural influence on movement is not static throughout the pelagic larval phase. This is consistent with one of the few other studies on sensory ontogeny in larval fish, whereby coral reef fish larvae (subfamily Amphiprioninae) were reported to change their olfactory preferences to cues from coral species between 5 and 7 days post-hatch (Dixson et al. 2011). Unlike these tropical species, which hatch from demersal eggs in an advanced state of development and display choice behaviour directly upon hatch, no preference behaviour was detected in the temperate species used for our study, which hatch from pelagic eggs, until at least a week after notochord flexion (> 28 days post-hatch). Larvae of many tropical fish species are generally larger and more developed upon hatch than temperate species, and these developmental idiosyncrasies should be taken into account when modelling larval dispersal of species in different environments, latitudes and spawning modes (Leis et al. 2013). In this way we can start to build more biologically realistic dispersal models.

Once choice behaviour emerged it persisted for the remainder of the test period, indicating distinct post-flexion processes in larval dispersal ontogeny: passive movement (before meaningful swimming and olfactory abilities have developed) and active movement guided by sensory cues. Correlations between studies on sensory and swimming ontogeny of larval fishes suggest that there is a common stage of development during the pelagic phase when both the ability for sustained swimming and the response to olfactory cues emerge. The transition between different responses to olfactory cues was noted in clownfish larvae by Dixson et al. (2011) as occurring approximately halfway through the larval period, coinciding with the development of sustained swimming ability in these species (Fisher et al. 2000). Although critical swimming speed in *M. novemaculeata* and *A. japonicus* measured in laboratory experiments increased linearly with growth during the larval phase, swimming endurance increased exponentially from approximately halfway through the pelagic larval period (ca. 7 mm SL in *M. novemaculeata* and ca. 8 mm SL in *A. japonicus*) (Clark et al. 2005). This ontogenetic stage was also suggested as the point from which swimming becomes an important factor in dispersal. This correlates with the size at which we found behavioural responses to habitat cues became significant in both of these species.

Prior to notochord flexion, critical swimming speeds in these species are > 5 cm s^{-1} in the best performers, which is still useful in the context of movement in coastal currents. However swimming endurance is considerably lower, meaning such speeds could not be maintained making them mostly relevant in the context of vertical migrations (Clark et al. 2005). Controlling depth through active swimming has been hypothesized to play an important role for marine larvae in cross-shelf waters for avoiding advection offshore in stratified currents, an optimal behaviour for maintaining position close to onshore habitat prior to recruitment (Shanks and Shearman 2009). This pattern has been shown in coastal areas both in field samples of crustaceans where vertical positioning of larvae helps them remain nearshore before moving shorewards to habitat as postlarvae (Morgan et al. 2009). However in modelling of dispersal in anchovy larvae, while active vertical migration contributed to maintaining nearshore position in early-stage larvae (< 20mm) it could not account for onshore movement of larger larvae (> 35mm) (Parada et al. 2008). This suggests the pelagic larval phase of many marine species could be characterised by a two-part ontogeny. Firstly, a pre-flexion processes involving vertical movement between preferred currents stratified in the water column during the first part of the PLD and secondly, by post-flexion processes in the second part of the PLD where increased swimming and sensory abilities induce horizontal movement towards onshore settlement habitat.

A larva's journey towards settlement in an estuary likely involves movement in adjacent coastal waters towards, or remaining in the vicinity of, the estuarine plume to facilitate entrainment in ambient tidal flux, which is used for passive or active tidal transport into the estuary (Grimes and Kingsford 1996, Boehlert and Munday 1998). Indeed, larvae of fishes which recruit to estuaries are often found concentrated in the estuarine plume and plume front compared to adjacent coastal waters (Kingsford and Suthers 1994). Chemotaxis using olfactory cues may be an important mechanism for this critical behaviour (Radford et al. 2012). This behavioural influence on movement may help to explain why some species recruiting to estuaries are only present in incoming flood tides at settlement competent sizes (Neira and Potter 1992, Trnski 2001). Selection of position in the water column by vertical movement also plays an important role in the use of tidal flux for recruitment into estuaries (Rijnsdorp et al 1985, Trancart et al. 2014). There are a myriad of physical factors involved in tidal flux which may serve as stimuli for larval behaviours relevant to movement towards habitat including current, salinity, temperature, turbidity and lunar cycle, which makes understanding how tidal flux plays a role in larval settlement a complex task (Boehlert and Munday 1998).

In *M. novemaculeata* and *A. japonicus* larvae seagrass cues elicited the strongest chemotactic response of the water characteristics we tested. As these responses were only present after choice behaviour to different habitat cues was detected and attraction or avoidance to seagrass cues were correlated with the different habitat preferences of each species, this indicates seagrass odour is important to orienting swimming behaviour in these species. Rich in phenolic acids, cues from seagrasses and other vegetation have been shown to elicit olfactory responses useful for locating habitat in settlement and post-settlement-stage fish from various taxa (Arnold and Targett 2002, Dixson et al. 2008, Huijbers et al. 2008, Radford et al. 2012). Indeed, Igulu et al. (2013) found that out of a range of potential olfactory cues present in estuary water, snapper post-larvae equally preferred cues from seagrass species to other cues from conspecifics heterospecifics and mangroves. Lignins from seagrass have been implicated in chemokinesis and chemotaxis related to settlement in red drum larvae (Havel and Fuiman 2015). Larvae of the blue crab (*Callinectes sapidus*) also demonstrate a chemotactic response to the presence of seagrass cues, after an ontogenetic shift from the intermolt to the premolt megalopae stage, which indicates that the contribution of seagrass odour to estuary plumes may be important for orientation in larvae of many estuary-dependant species (Forward et al. 2003). These olfactory signals could be transported by estuary plumes kilometres from the outflow source, making them useful for larvae coming to settle from deeper coastal waters (Chant et al. 2008). At this range certain olfactory cues may be useful in providing a signal to larvae to begin incorporating other sensory cues which may assist in orienting towards habitat rather than providing a directional gradient (Atema et al. 2015). This role should be considered when informing management decisions on seagrass habitats that are critical for use as post-settlement nurseries for many marine species (Dahlgren et al. 2006).

Olfactory tests on reared settlement-stage larvae (9–14mm SL) of the sparid *Pagrus auratus* show a preference for water taken near seagrass beds over water taken from the estuary mouth (Radford et al. 2012), which is similar to what we found in *M. novemaculeata* as they approached settlement-stage (9–10mm SL). This is perhaps expected as both species are known to settle in seagrass or otherwise vegetated environments (Trnski 2002, Trnski et al. 2005). In contrast, *A. japonicus* larvae avoided seagrass cues up to 14mm SL in this study. There is little empirical data on movement and habitat use by *A. japonicus in situ* at this growth stage, however recruits are thought to enter estuaries from 30-40mm (Taylor et al. 2006, Cowley et al. 2008). The preference for coastal water cues at this ontogenetic stage may be a between-estuary mechanism to either delay recruitment and enhance dispersal potential or a within-estuarine mechanism to avoid shallow vegetated areas and move towards the deeper holes and channels where juveniles are found (Fielder and Heasman 2011). In this case, where larvae are already inside the estuary seagrass cues may be used at a smaller scale as a way to avoid the shallow vegetated areas. *In situ* observations of reared *A. japonicus* larvae show an ontogenetic change in preference for deeper water as larvae grow, possibly to become epibenthic feeders around settlement size, a characteristic shared with other sciaenid species (Barnett et al. 1984, Leis et al. 2006). The size at settlement in *A. japonicus* is ambiguous, though it is estimated to be ca. 14mm SL (Clark et al. 2005), and so larvae may reveal further ontogenetic changes in olfactory responses in this species with continued growth.

Prior to a preference for estuary water cues, *M. novemaculeata* larvae also showed a preference for coastal water cues. In early stages of development *M. novemaculeata* grow faster in higher salinities (20 – 35ppt) (Van Der Wal 1985), so an initial chemotactic behaviour towards coastal cues in both study species could be a mechanism to locate optimal conditions for early growth. As spawning in *M. novemaculeata* occurs close to the mouth of the estuary in which the adults reside, an initial period of moving away from or delaying movement into the estuary may also increase the potential for genetic connectivity among estuaries (Bradbury et al. 2008, Shaddick et al. 2011). Such selectivity to different habitats resulting in delayed settlement and extended PLD has been shown in larvae of red drum, another estuarine-dependant species (Havel et al. 2015).

Once *M. novemaculeata* larvae neared settlement stage (< 7mm SL) and were making choices for EST over COA they also preferred SCOA to COA, however they preferred EST to SCOA. This is consistent with choice experiments on settlement-stage larvae from other fish species which suggest that while seagrass plays an important role in chemotaxis, other organic cues, such as those from prey items, conspecifics, predators or other vegetation types may contribute to the overall attractiveness of the estuary water habitat cue (Forward et al. 2003, Lecchini et al. 2007, Dixson et al. 2010, Radford et al. 2012). The choice for EST cues in *M. novemaculeata* is unlikely to be linked to the fact that rearing tank water was also sourced from the estuary for two reasons: i) water was ozonated with protein fractionation to disinfect and remove dissolved organic compounds (Fielder and Heasman 2011), and ii) *A. japonicus*, reared in the same water, developed an opposing preference towards coastal water.

A preference for lower salinity of 28ppt was also found in both species after choice behaviour in response to habitat cues emerged, indicating that the detection of salinity differences may play a role in olfactory choice behaviour of estuarine-dependant species. Indeed, salinity choice experiments on *Galaxias maculatus* larvae indicate that this species responds to reduced salinities near estuary mouths (Hale et al. 2008). However although preference for lower salinity levels is consistent with our observations of preference by *M. novemaculeata* larvae for estuarine water it does not correlate with the preference by *A. japonicus* larvae for the more saline coastal water, indicating that organic chemical cues may be more important than salinity for larval orientation in these species.

We aimed to fill a rarely studied gap in our knowledge of the development of behaviour in larval fish that could be relevant to their dispersal. We found that larvae of estuarine-dependant fishes have particular stages in their sensory ontogeny during the pelagic larval duration whereby swimming begins to be influenced by chemotactic responses. Such ontogenetic windows have also been identified for auditory habitat cues in larval barramundi, which show a phototactic response to habitat cues only around the metamorphic stage (Rossi et al. 2015). This has important implications for management of estuaries as anthropogenic pollution has been already been linked to alterations in sensory cue use required for these behaviours (Siebeck et al. 2014, O'Connor et al. 2015). Management of estuaries and estuarine species should also consider that seagrass is an important component of the chemical cues driving olfactory-based orientation in larvae of these estuarine-dependant fishes, which is consistent with studies on various other fish species (Arvedlund and Takemura 2006, Radford et al. 2012). More data on these ontogenetic milestones in sensory acuity and behaviour during the pelagic larval stage could greatly improve the realism of modelling of larval dispersal (Staaterman et al. 2012). Modelling of larval dispersal also indicates that ontogenetic shifts in behaviour increase the chances of survival throughout the larval period and further work on early behavioural ontogeny with a greater variety of species is needed to understand how such shifts contribute to connectivity in fish populations (Vikebø et al. 2007). Our results suggest that pre-settlement sensory ontogeny is more complex than previously thought and for the first time we suggest a particular ontogenetic point when these behaviours become relevant to dispersal.

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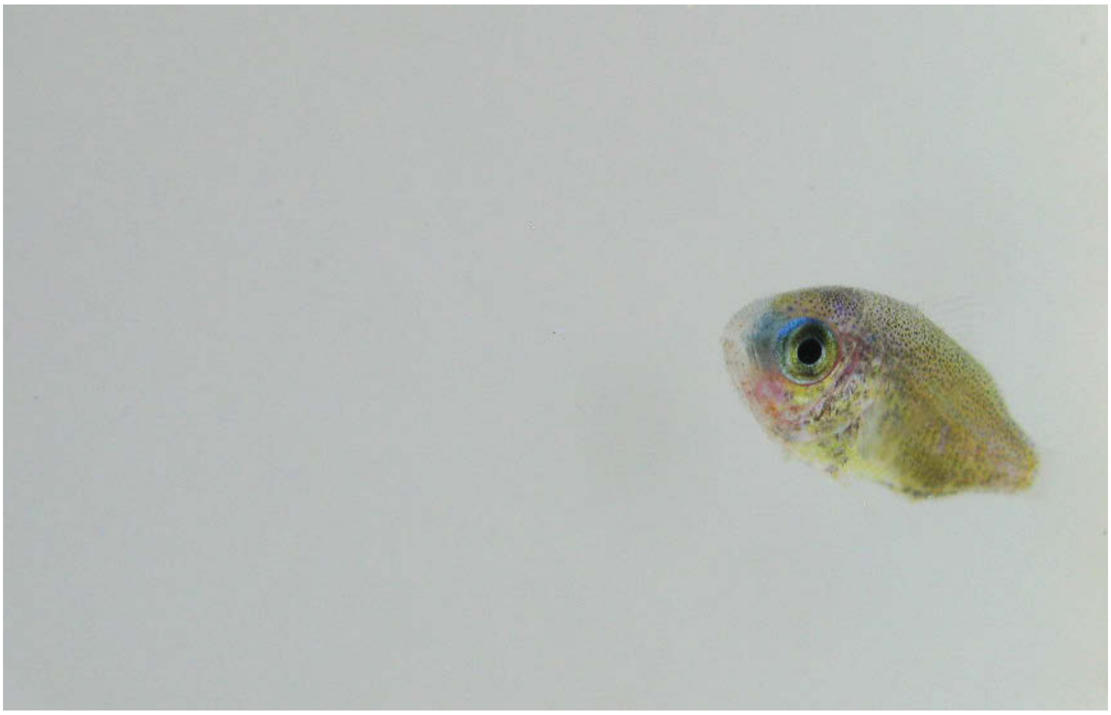
CHAPTER THREE

Sun position and the use of celestial cues in coral reef fish larvae



Chromis atripectoralis larva, Lizard Island Research Station

Abstract

Larval coral reef fish have shown the ability to orient their swimming to particular directions during the pelagic dispersal phase, a behaviour that has the potential to influence the connectivity and replenishment of their populations. A sun compass has been suggested as a potential mechanism, however it is not known how the sun azimuth and polarised light may be involved. Here we tested coral reef fish larvae at the end of the pelagic phase in orientation experiments to evaluate their response to changes in the sun's azimuth and polarised light patterns. In response to a shift in the apparent position of an artificial sun disk by 180°, larvae significantly changed their among-individual mean bearing by 123° and the angular distance between the two positions for each individual larvae was significantly similar among individuals with a mean of 158° clockwise. Individuals moved towards the side of the chamber with artificial sun in the morning, and to the opposite side in the afternoon. In cue-conflict experiments with changing artificial sun azimuth and polarised light pattern axis, larvae were only directional when the axis of polarisation was perpendicular to the sun's position. These results support the hypothesis that larval fishes use celestial cues to assist orientation behaviour. The sun compass likely uses the sun's azimuth to maintain direction, while other celestial cues may be required to calibrate the compass towards an innately preferred heading. This study provides a further step in our understanding of how larval behaviour may influence dispersal of marine populations.

Introduction

During their early life-history many demersal marine organisms move into offshore waters to develop and disperse. This larval phase is a time of considerable susceptibility to advection by ocean currents and vulnerability to predation and starvation meaning that abundance, demography, connectivity and spatial distribution of populations are heavily influenced by intrinsic and extrinsic processes which act upon larvae (Bergenius et al. 2002, Platt et al. 2003, Paris and Cowen 2004, Cowen and Sponaugle 2009). In the process of swimming towards and selecting settlement habitat we now suspect larvae influence their dispersal through intrinsic behaviours (Marshall and Keough 2003, Leis 2007). Studies on connectivity of fish populations suggest that behaviour of dispersing larvae can result in higher rates of self-recruitment than predicted by passive dispersal in ocean currents (Jones et al. 2009, Staaterman et al. 2012). Observations of larval fish in the field revealed an ability to maintain significantly oriented directions when swimming in the pelagic zone (Leis and Carson-Ewart 2003). However the mechanisms underlying these processes and behaviours remain unknown.

Celestial cues are a useful natural compass from which to infer directional information, as they are consistent over large scales and independent of local environmental conditions. The ability to use celestial cues to orient directional movement is found throughout the animal kingdom from ants and beetles to birds and lizards (Budzynski et al. 2000, Müller and Wehner 2007, Foà et al. 2009, Dacke et al. 2013). Precise directional orientation in many marine organisms suggests that this ability is prevalent in the marine animals also (Horton et al. 2011). Migratory fishes have long been hypothesised to use the position of the sun as a compass reference as part of their orientation mechanisms (Quinn 1980). Sensitivity to polarised light, which can also be used to determine the solar azimuth, has been shown in both adults and juveniles of fishes from different families (Waterman and Forward 1970, Parkyn et al. 2003, Berenshtein et al. 2014), but little is known about polarised light response in fishes during the larval phase. Experiments on larvae of coral reef fishes showed a change in their orientation behaviour after manipulating their internal clock, suggesting that they may use a sun compass to orient their swimming direction (Mouritsen et al. 2013). *In situ* observations of temperate fish larvae suggest that celestial cues are more of an influence on larval orientation than wind or current direction (Leis et al. 2014, Faillettaz et al. 2015). Evidence is accumulating that fish larvae are using a celestial compass for orientation by the time they have developed to settlement-stage, however it is still uncertain which celestial cues are being used to facilitate this ability.

A range of celestial cues have been investigated for their influence on animal orientation behaviour, including the sun's position, sky polarised light patterns, downwelling underwater polarised light patterns, the position of the moon and even the Milky Way (Ugolini et al. 2003, Reppert et al. 2004, Waterman 2006, Dacke et al. 2013, Faillettaz et al. 2015). For humans, the azimuth of the sun is the most obvious celestial cue from which to discern direction and its role in providing orientation information has been demonstrated for different species (Wehner and Müller 2006, Pfeiffer and Homberg 2007). Requiring an internal clock-mechanism to account for the diurnal movement, the sun's azimuth can be used by moving animals to provide geographical compass or to maintain a directional course (Guilford and Taylor 2014). However a direct view of the sun is not necessary to infer its azimuth if polarised light can be perceived. Fixed patterns of electric vectors (e-vectors) of partially polarised light scattered from particles in the atmosphere or water column vary predictably with sun's azimuth, giving a reliable cue for orientation (Åkesson et al. 2014). This mechanism can also be used as a calibrating mechanism for a compass relying on other sensory input (Phillips and Moore 1992, Muheim et al. 2006) and has the benefit of being visible even when the location of the sun is obstructed by cloud cover (Pomozi et al. 2001, Hegedüs et al. 2007).

In this study we observed the behaviour of settlement-stage coral reef fish larvae in response to the changing position of the sun's apparent azimuth. Previous studies have shown the ability of larvae at a similar growth stage to maintain oriented swimming (Leis et al. 2014, Leis et al. 2015). Here, we test the hypothesis that these larvae are able to use directional information from the sun's position for orientation by observing orientation behaviour in response to manipulations of the apparent azimuth. We also tested the hypothesis that e-vector patterns created by scattering polarised light may also be an important celestial-based cue for orientation by running cue-conflict experiments to test larval orientation in response of to both changing apparent sun position and polarised light pattern direction. If the ability of fish larvae to respond to large-scale cues can be shown it would provide a key piece of knowledge about intrinsic factors influencing larval dispersal, invaluable to improving attempts to realistically model larval dispersal and connectivity (Staaterman and Paris 2014, Treml et al. 2015).

Materials & methods

a) Field collection

We collected larvae of the common coral reef fish *Chromis atripectoralis* (Pomacentridae) at sunrise each day during seasonal settlement in November 2013 and February 2014 using light traps deployed at sunset the preceding day off the fringing reefs surrounding Lizard Island (14°40'42.2"S 145°25'43.4"E, Great Barrier Reef Marine Park, QLD, Australia). We caught larvae approaching coral reef habitat to settle at the end of their Pelagic Larval Duration (PLD)(pre-benthic-stage pigmentation (McCormick et al. 2002), 10-20 days post-hatch, 7-10mm SL (Thorrold and Milicich 1990)) and refer to them hereafter as settlement-stage larvae. We selected this species as it is abundant in light trap catches of the area, and more importantly shows the ability to orient swimming direction *in situ* (Leis and Carson-Ewart 2003, Leis et al. 2014).

b) Experimental protocol

Prior to behavioural testing we acclimated *C. atripectoralis* larvae to laboratory conditions in 10 L opaque holding aquaria situated in laboratories at Lizard Island Research Station with filtered seawater on a flow-through system for a minimum of two hours. For outdoor experiments we transported larvae in an opaque bucket with an air bubbler to minimise their exposure to the sky conditions. To begin a trial we introduced a larva into the centre of the test arena within a PVC cylinder which was then removed. For the first two minutes we allowed the larva to acclimate and explore the tank after which we recorded its movement for a period of ten minutes using a GoPro camera situated above the tank. After this we removed the larva to a holding tank and altered the test arena conditions for the next treatment before returning the same larva into the arena for the next treatment. Each larva was tested with only one set of treatments. Following trials we returned larvae to holding tanks in the laboratory before eventually releasing them back to the area where they were initially captured the night before.

c) Celestial cue manipulation experiments

We tested larvae individually (i.e. – one larvae in the test chamber at a time) in one of two experimental setups, each designed to investigate the behavioural responses of larvae to one of three cue types as follows:

i. Effect of artificial sun position

Orientation experiments with artificial sun movement were conducted inside temperature-controlled laboratories at the Lizard Island Research Station. Larvae were observed in a 45cm x 20cm arena within an ‚enclosed, semitranslucent plastic circular tank (65cm x 55cm). A blue polyethylene sheet was suspended 2m above the tank, providing a homogenous area minimising visual distractions and covering the visible field of view when looking up from within the test arena. A steel track suspended beneath this artificial “sky”, running along the East – West meridian provided an anchoring point for the artificial “sun” (a UV lamp (Radium - Sanolux HRC 300-280, 230V 300W)) deployed at three positions: i) directly above the centre of the tank; ii) due west at an elevation of 60° from the tank centre; and iii) due east at an elevation of 60° from the tank centre (Fig. 3.1). A diffusing filter was placed on top of the tank containing the test arena to further minimise distraction by unplanned visual cues and to remove any polarised light so the experiment could focus purely on the position of the artificial celestial cue. We tested 28 individuals over a period of 7 days, each given the three treatments consecutively in random order between 9am and 6pm. For this test we hypothesised that if larvae were using the sun position alone to determine a direction for orientation then we might detect changes of orientation direction in either the individual or group level of ca. 180° in response to artificial sun movement, and show reduced orientation when the artificial sun was directly overhead.

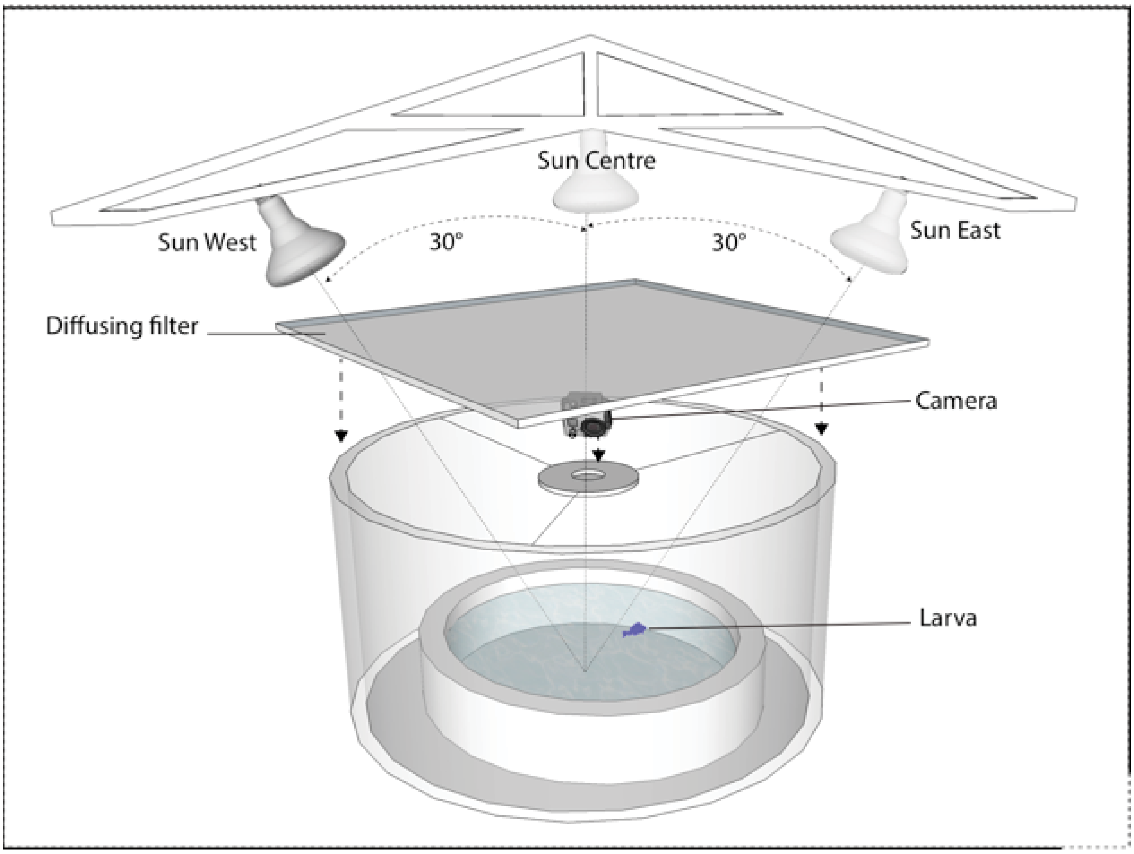


Fig. 3.1 Artificial sun position setup showing different position of the artificial sun during each of the three treatments presented to each individual larva: Sun East (60° zenith from arena centre, azimuth due magnetic east), Sun Centre (directly above arena centre) and Sun West (60° zenith from arena centre, azimuth due magnetic west). A diffusing filter was placed between light source and the chamber to eliminate any polarised light or external visual cues from above. The sun disk was visible to the larva from all sections of the tank, from ~45° zenith from the far side of the tank to a thin sliver of light directly above the tank edge closest to the east and west sun positions.

ii. Sun position vs. Polarised light pattern

Using protocols similar to the first experiment, this experiment was conducted in open air laboratories at Lizard Island Research Station using polyethylene sheeting and shade cloth to eliminate direct sunlight onto the test arena. Movement of artificial sun position was again presented to larvae, however for this experiment we also exposed larvae to polarised light patterns created by a UV linear polarizing filter (300-800 nm) at different orientations in order to examine the interaction between different visual cues. Due to structural limitations of the outdoor laboratory the artificial sun was placed in positions at the north and south sides of the chamber. For each sun position there were three conditions applied on top of the test arena: diffusing filter, polarising filter oriented parallel to magnetic north and polarising filter orientated perpendicular to magnetic north. This resulted in six treatments for each individual larvae:

i) Diffused light (Sun north)
ii) Parallel polarised light (Sun north)
iii) Perpendicular polarised light (Sun north)
iv) Perpendicular polarised light (Sun south)
v) Parallel polarised light (Sun south)
vi) Diffused light (Sun south)

Each set of treatments was run in order or reverse order alternatively. We introduced 29 larva (15 morning, 14 afternoon) to cue conflicts with the aim of teasing apart the relative effect of sun position and polarised light patterns on orientation behaviour. We hypothesised that if one cue was favoured by the larvae we would see increased and consistent orientation directionality in response to this cue despite variation of other cue types.

d) Data analysis

We extracted still images of larvae from the video observations every 10 seconds, creating an independent subset of 60 positions for each individual. We recorded the position of the larva in each image using ImageJ and analysed these data points to calculate mean direction (μ), directional precision (length of the mean vector, r) and directionality for each individual larva (first order analysis) using specifically designed scripts for the circular package in R (Irisson et al. 2009). We used a threshold value for r of > 0.2 to describe individuals as showing directional orientation. Pilot studies indicated that group orientation became more spread if individuals with r < 0.2 were included, and did not improve if only animals with r > 0.3 or higher were included. Thus, we concluded that r = 0.2 was the optimal threshold. To assess directionality among individuals (second order analysis) we used Oriana 4.0 (Kovach Computing Services) to calculate mean direction, median direction (including 95% CI) and directional precision (length of mean vector, *r*). We used Rayleigh tests to assess the uniformity of distributions, where significance indicates directionality of orientation among individuals for each treatment (Leis et al. 2014).

For among-individual orientation analysis we included only the mean bearings of significantly oriented individuals and analysed the distributions as both unimodal or bimodal to test which distribution type best explained the orientation behaviour, as the axiality of behaviour between individuals and groups can relate to different cue responses and motivations. As a rule we used axial orientation if r(axial) > r(uni), with the axis direction closest to unimodal direction used for the among-individual bearing. We used Watson's U^2 test to assess the difference in distribution of bearings between different treatments. We tested differences in angular dispersion (variance) in R using a two-sample Wallraff test and differences in precision (*r*) using a Kruskall-Wallis test, with all individuals included. In each test p values of < 0.05 were considered significant.

Results

i. Artificial sun position

Within-run orientation of *C. atripectoralis* larvae within the test arena was high, with 92% of larval tracks analysed showing significant directional orientation. 22 of 28 individuals (79%) were significantly oriented in all treatments. The majority (67%) of non-significantly orientated behavioural trials were recorded during the Sun Centre treatment. Among significantly oriented larvae, the direction of mean orientation in 69% of individuals moved between the east and west side of the chamber according to the relative sun position. The majority (78%), of these larvae chose the side of the chamber closest to the artificial sun in the morning whereas in the afternoon the majority chose the side of the tank opposite to the sun position (63%). All individuals had a significantly different distribution of bearings when the artificial sun was positioned in the east compared to the west position (Watson's U^2, $p < 0.001$). In the 14% of cases where distributions were not significantly different between two of the three treatments, each contained a comparison between with the central sun position and either the east or west position (Watson's U^2, $p < 0.05$).

Comparing the angular difference of each larva’s mean bearing (only significantly directional individuals included) between Sun East and Sun West treatments, we found that among individuals the difference from their initial orientation was significantly directional with a mean change of 158° clockwise (Fig. 3.2). The 95% C.I. encompasses the orientation deviation of 180° we would expect if larvae we adjusting their orientation to the position of the artificial sun (μ = 158° 95%CI 116 – 201°, r = 0.36, R test: p = 0.037, n = 25). The angular deviation of mean bearing for each individual larvae was not significant when comparing between the east and centre sun positions (μ = 99° 95%CI 32 – 167°, r = 0.24, R test: p = 0.26, n = 24) or west and centre (μ = 344° 95%CI 289 – 38°, r = 0.29, R test: p = 0.13, n = 24).

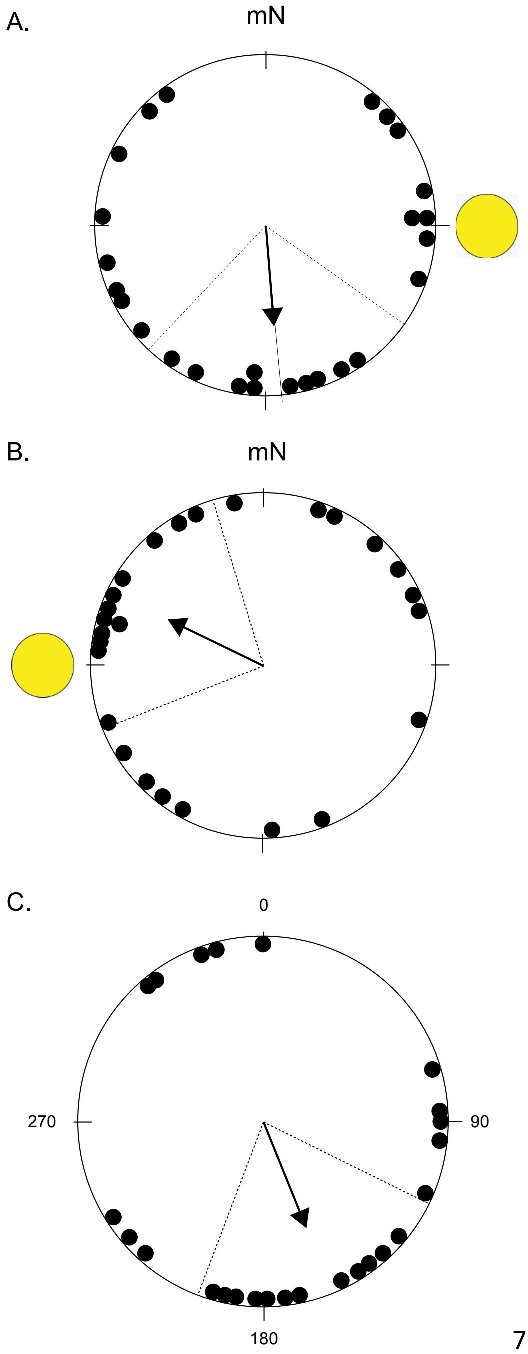


Fig. 3.2 Distribution of mean bearings for individual larvae approaching significant orientation with artificial sun positioned east (A) (p = 0.056) and west (B) (p = 0.066) of the test arena. Angular change (C) for each individual larva after the shift of sun position between the east and west sides of the test arena (initial position taken as 0°) shows a significant among-individual deviation (p = 0.037) close to 180°. Arrows indicate the direction of the among-individual mean bearing (arrow length proportional to

directional precision (r)), 95% C.I. bounded by radial dotted lines.

Larval orientation among-individuals differed between sun positions in the morning but not the afternoon. During morning trials the Sun West treatment was significantly directional with an orientation towards the northwest (μ = 294° 95%CI 260 – 329°, r = 0.52, R test: p = 0.009, n = 17). Orientation was more directional in the Sun East treatment (μ = 110° 95%CI 49 – 177°, r = 0.35, R test: p = 0.18, n = 14) than Sun Centre (μ = 10° 95%CI 255 – 124°, r = 0.17, R test: p = 0.63, n = 16), however neither treatment was significant. The angular difference between the mean bearing among individuals between Sun East and Sun West treatments was 176°, with significantly different distributions of bearings between the two treatments (Watson's $U^2 = 0.29$, $p < 0.01$) (Fig. 3.3). During the afternoon however Sun West was the only non-directional treatment (μ = 318°, r = 0.17, R test: p = 0.73, n = 12). Sun East (μ = 205° 95%CI 170 – 239°, r = 0.56, R test: p = 0.007, n = 15) and Sun Centre (μ = 273° 95%CI 238 – 308°, r = 0.59, R test: p = 0.009, n = 13) were both significantly directional, shifting their mean bearing more westerly compared to the morning trials. The distribution of bearings for these treatments was not significantly different (Watson's $U^2 = 0.13$, $p > 0.1$).

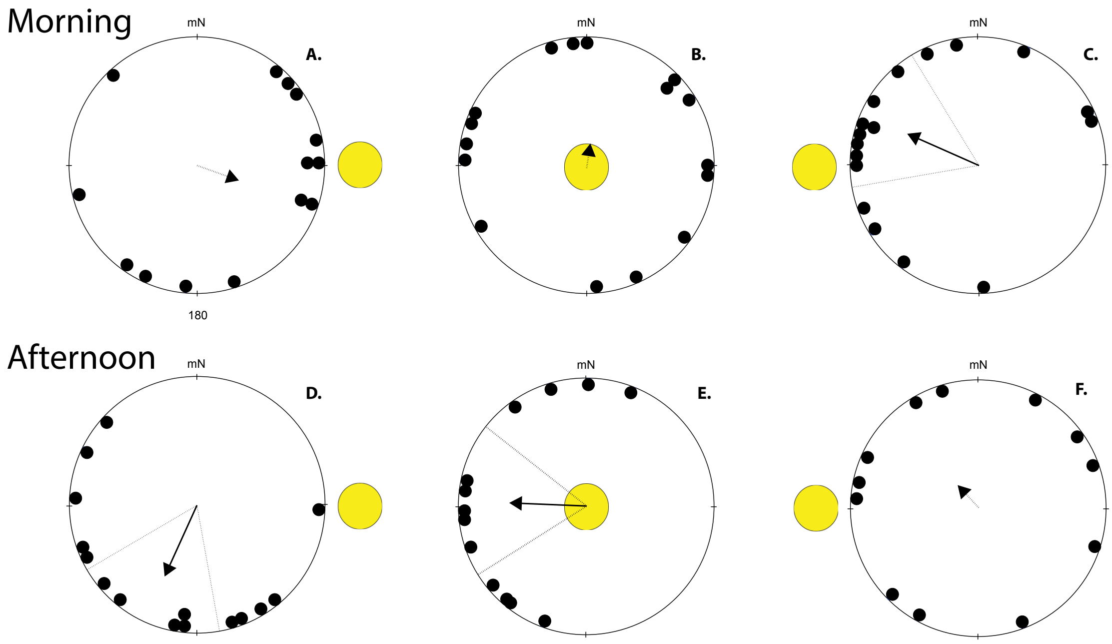


Fig. 3.3 Mean bearings of significantly directional larvae for each of the three artificial sun positions during morning and afternoon trials. Each individual was presented with three randomly ordered treatments: Sun East (A,D), Sun Centre (B,E) and Sun West (C,F). Arrows indicate the mean among-individual direction (arrow length proportional to directional precision (r)). Treatments with significant among-individual orientation are indicated with solid arrows and 95% C.I. bounded by radial dotted lines (mN indicates magnetic north).

When bearings from trials in morning and afternoon were pooled, no significant among-individual orientation was found in any of the three treatments. We found orientation approaching significance to the south for the Sun East position ($\mu = 179°$ 95%CI 133 – 224°, $r = 0.32$, R test: $p = 0.056$, $n = 27$) and to the northwest for Sun West ($\mu = 302°$ 95%CI 254 – 349°, $r = 0.31$, R test: $p = 0.066$, $n = 27$) with a difference of 123° between treatments. Larvae were comparatively less directional during the Sun Centre treatment ($\mu = 287°$ 95%CI 237 – 337°, $r = 0.29$, R test: $p = 0.09$, $n = 24$). Comparing the distribution of mean bearings between each treatment there was no significant difference between Sun Centre and either Sun East (Watson's $U^2 = 0.18$, $p > 0.05$) or Sun West treatments (Watson's $U^2 = 0.031$, $p > 0.5$). However the distribution of mean bearings of Sun East and Sun West were significantly different (Watson's $U^2 = 0.26$, $p < 0.02$). This difference was unlikely to be related to differences in angular dispersion, which was similar between the two treatments, and due to differences in mean direction of each treatment (Wallraff test: KW Chisq = 0.0071, $p = 0.93$). There was no significant difference in precision between the different sun position treatments (KW test, d*f* = 2, Chisq = 1.99, *p* = 0.37).

ii. Artificial sun position vs. polarised light pattern

C. atripectoralis larvae significantly oriented during 89% of cue-conflict trials. Precision of orientation for individuals did not differ significantly among treatments (KW Test, Chisq = 1.49, df = 5 , $p = 0.91$), with median r values ranging from 0.69 – 0.94. The majority (76%) of individual larvae had a significantly different direction under each of the six treatments during their trial (Watson's U^2: $n = 60$, $p < 0.005$). This did not translate into directional second order orientation among-individuals in all treatments however. Only two of the six treatments had a directional precision > 0.3, when light was polarised along the east-west axis, perpendicular to the sun positions in the north and south. Orientation was approaching significance to the northwest when sun position was in the north (μ = 319° 95%CI 273 – 5°, r = 0.34, R test: p = 0.059, n = 25) and significantly directional to the southwest when the sun position was to the south (μ = 262° 95%CI 221 – 303°, r = 0.36, R test: p = 0.028, n = 27). The mean bearings differed by 57° (Fig. 3.4), with the distribution of bearings significantly different from each other (Watson's U^2: df = 25, p < 0.005). Bearings had a significant bimodal distribution under the diffusing filter with the artificial sun in the south position (Table 3.1).

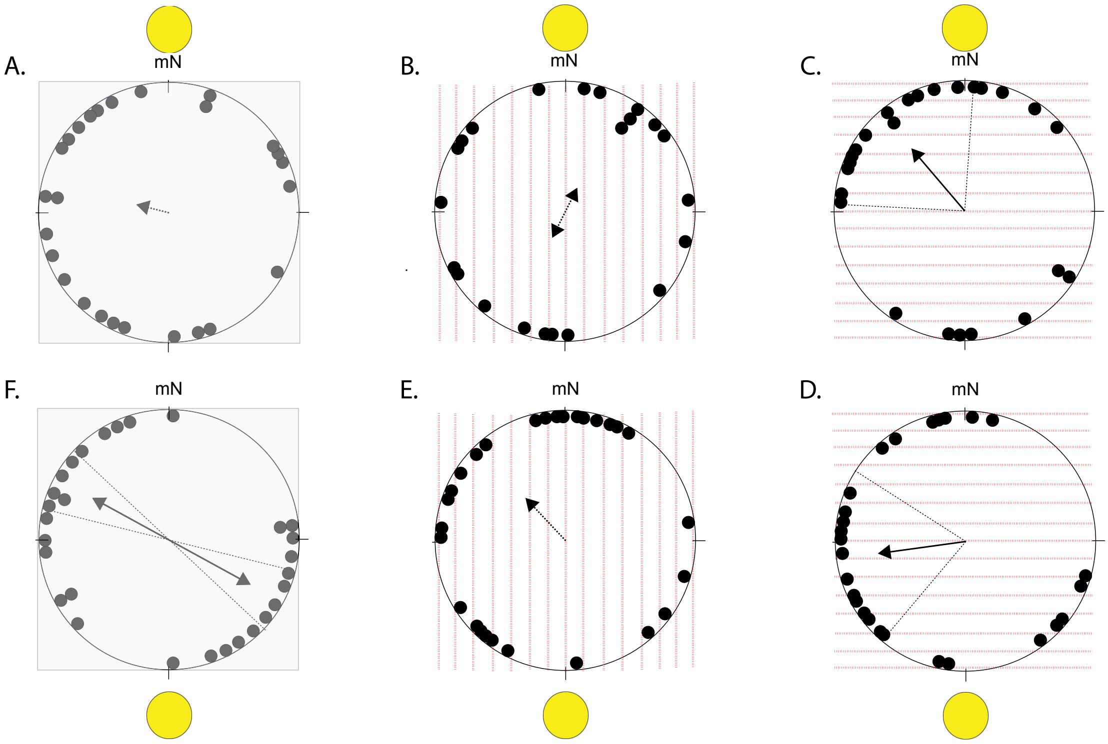


Fig. 3.4 Distribution of mean bearings for individual larvae during six orientation trials under different sun positions (due north and south) and light filters, including diffusing filter (A,F), parallel polarised light pattern (B,E) and perpendicular polarised light pattern (C,D). Red lines illustrate the polarising plane, whereas arrows indicate direction of the mean among-individual bearing. Significant among-individual orientation in treatments indicated by solid arrows (arrow length proportional to directional precision (r)) and 95% C.I. indicated by dotted radial lines.

Larval orientation among individuals was more directional in the morning than the afternoon in the cue-conflict experiment, with 5 out of 6 treatments having a directional precision > 0.4 compared to 1 out of 6 in the afternoon (Fig. 3.5). Again, when the axis of the polarised light pattern was perpendicular to the sun position during morning trials orientation had relatively high directional precision (r) to the northwest when sun position was in the north ($\mu = 331°$, 95%CI 273 – 5°, $r = 0.47$, R test: $p = 0.065$, $n = 12$) and to the west when the sun position was to the south ($\mu = 273°$ 95%CI 216 – 330°, $r = 0.41$, R test: $p = 0.11$, $n = 13$).

Table 3.1. Orientation of *Chromis atripectoralis* larvae in six treatments presenting different sun positions and polarised light treatments. Number of larvae analysed (n) includes only individuals that were significantly directional. Mean bearings (μ) with asterisks (**) indicate bimodal distribution. R test p-values in bold if orientation was significant ($p < 0.05$) or approaching significant ($p < 0.06$) in a given treatment.

Sun Position	**North**			**South**		
Treatment	**Diffuser**	**Polarised N - S**	**Polarised E - W**	**Diffuser**	**Polarised N - S**	**Polarised E - W**
n (significant)	26	23	25	29	28	27
Mean Bearing	284°	27°**	319°	118°**	317°	262°
95% CI	206° - 2°	345 - 69°	273 - 5°	104 - 133°	262 - 11°	221 - 303°
Precision (r)	0.20	0.11	0.34	0.47	0.27	0.36
R test *p*-value	0.37	0.42	**0.059**	**0.001**	0.13	**0.028**

When the polarised light pattern was parallel to the sun position during morning trials orientation was significantly and axially distributed to the northeast when sun position was in the north ($\mu = 16°$, 95%CI 358 - 35°, $r = 0.58$, R test: $p = 0.015$, $n = 12$) and significantly unidirectional to the west when the sun position was to the south ($\mu = 309°$ 95%CI 265 – 353°, $r = 0.48$, R test: $p = 0.035$, $n = 14$). Under the diffusing filter larvae showed non-significant unidirectional orientation to the northwest (μ = 328°, 95%CI 246 - 50°, r = 0.3, R test: p = 0.26, n = 15) when the sun was positioned northerly and significant axial orientation to the southeast (μ = 115°, 95%CI 96 - 134°, r = 0.55, R test: p = 0.015, n = 13) when the sun was positioned southerly. In the afternoon larvae on a group level had a generally non-significant orientation towards west-southwest (Table 3.2).

Table 3.2. Orientation of *Chromis atripectoralis* larvae analysed by time of day (before or after midday) including only individuals that were significantly directional. Mean bearings (μ) with asterisks (**) indicate bimodal distribution. R test p-values in bold if orientation reported as significant ($p < 0.05$) or approaching significant ($p < 0.066$) in a given treatment (missing 95% C.I. could not be calculated for some treatments).

Morning	**North**			**South**		
Treatment	**Diffuser**	**Polarised N - S**	**Polarised E - W**	**Diffuser**	**Polarised N - S**	**Polarised E - W**
n (significant)	15	12	12	13	14	13
Mean Bearing	328°	16°**	331°	115°**	310°	273°
95% CI	246 - 50°	358 - 35°	282 – 20°	96 - 134°	265 - 353°	216 - 330°
Precision (r)	0.3	0.58	0.47	0.55	0.48	0.41
R test *p*-value	0.26	**0.015**	**0.065**	**0.015**	**0.035**	0.11
Afternoon	**North**			**South**		
Treatment	**Diffuser**	**Polarised N - S**	**Polarised E - W**	**Diffuser**	**Polarised N - S**	**Polarised E - W**
n significant	11	12	12	14	14	13
Mean Bearing	**225°**	100°**	164°**	126°**	**4°**	**256°**
95% CI	137 - 313°	-	125 – 202°	95 – 156°	-	200 - 312°
Precision (r)	0.33	0.21	0.35	0.033	0.088	0.42
R test *p*-value	0.31	0.58	0.24	0.14	0.9	0.1

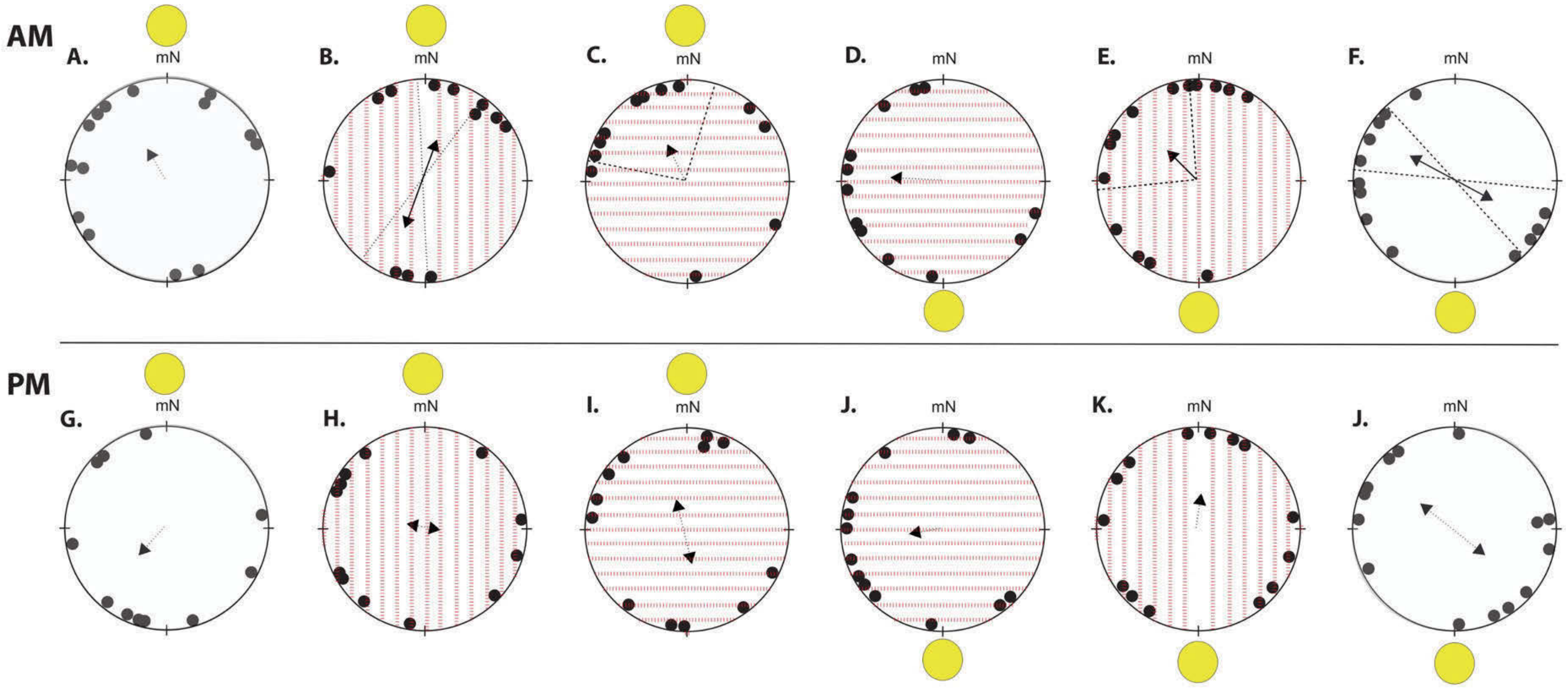


Fig. 3.5 Orientation of *C. atripectoralis* larvae tested at Lizard Island Research Station during summer recruitment of 2013 – 2014 during the morning (AM) and afternoon (PM). Each individual was presented with six treatments in order and reverse order alternatively: Sun north + diffusing filter (A), Sun north + parallel polarised light (B), Sun north + perpendicular polarised light (C), Sun south + perpendicular polarised light (D), Sun south + parallel polarised light (E) and Sun south + diffusing filter (F). Each data point in the circular diagrams shows the mean orientation for each significantly directional larva. Arrows indicate the mean among-individual direction (arrow length proportional to directional precision (r)). Double-headed arrows indicate axis of bimodally distributed samples. Dotted radial lines give the 95% confidence interval for the mean direction in treatments where larvae showed significant ($p < 0.05$) or approaching significant among-individual orientation.

Discussion

In this study on coral reef fish larvae we investigated the relative effects of artificial celestial cues on orientation behaviour to tease apart the potential drivers of directional swimming abilities observed in the field. Larvae were highly directional during our orientation experiments using artificial celestial cues, with similar directional precision values as reported for previous *in situ* observations of the same species in the same area (Leis et al. 2015). Directional behaviour in larvae appears to be a strong tendency at settlement stage, adhered to despite being subjected to novel experimental conditions. With pelagic larvae of marine, demersal fishes increasingly considered nekton as opposed to plankton during later larval stages (Leis 2015), modelling of larval fish dispersal indicates that active behavioural influence optimises dispersal and replenishment of populations (Fiksen et al. 2007, Staaterman et al. 2012).

For the individual, being able to maintain a straight direction increases the chances of encountering habitat in a patchy environment such as a coral reef (Goodwin et al. 1999). The direction in which larvae oriented at both a first order (individual) and second order (among-individual) level was affected in a consistent way in response to a changing artificial sun disk position. This provides some evidence for the first time of the mechanism behind orientation behaviours observed in larval fishes linked to a sun compass (Mouritsen et al. 2013, Faillettaz et al. 2015). There is also an indication from our cue-conflict experiment that polarised light patterns may influence orientation behaviour and play an important role in the directionality of orientation as part of a celestial compass requiring multiple cues.

When presented with an artificial sun azimuth as the only orientation cue, among-individual orientation of larvae followed the side of the chamber towards the artificial sun in the morning. Orientation was directional towards the southeast with the artificial sun in the east, and towards the northwest when artificial sun was positioned to the west. If the azimuth was being used to orient direction, then the larvae were orientated towards apparent southeast. This is a similar direction to that reported for *C. atripectoralis* larvae during *in situ* observations in the same region (Leis et al. 2014). Significant orientation treating the morning and afternoon data separately seems even more striking when considering the smaller number of replicates available for each daily phase. Directionality was less oriented on a group level when the artificial sun was directly overhead in the morning treatments. This dynamic in orientation of larval fish in relation to changing sun position in the laboratory is also reflected by *in situ* observations of Leis et al. (2014), and was in part the basis for the hypothesis of sun compass use in larval fish movement.

In contrast larval orientation in the artificial sun position experiment was directed on a group level to the side of the chamber away from the sun position in the afternoon. This indicated an adjustment in swimming direction in anticipation of the dynamic azimuth of the sun from one side of the sky to the other across the day. Whereas orientation was towards the artificial sun in the morning, providing a southeast direction, in the afternoon orientation was away from the sun towards apparent northeast. *In situ* observations of *C. atripectoralis* at Lizard Island show a change in among-individual orientation during the course of the day, although this shift is usually from southeast in the morning to southwest in the afternoon (Leis et al. 2014). A potential explanation for the orientation shift from southeast in the morning to northeast in the afternoon under laboratory conditions is that individuals tested in the afternoon were kept indoors in holding aquaria after catch, and had potentially lost the ability to calibrate their sun compass from natural sky cues. The use of compass orientation in animals is hypothesised to require two aspects: a mechanism to determine the desired cardinal direction, and a reference by which to maintain movement in this direction. For example, migratory birds have the ability to use a magnetic compass which requires calibration with sky polarisation cues (Able and Able 1993, Cochran et al. 2004, Muheim et al. 2006). If larvae were interpreting the sun's position in accordance with their last natural view of the sky in the morning, then the intended direction during the sun east treatment in the afternoon trials would be towards apparent southwest, more in line with *in situ* observations. Although larvae tested by Leis (2003) *in situ* were also kept from a view of the sky before observations, they had access to all available natural cues during trials. Other orientation experiments show evidence for a rapid recalibration of test animals to natural cues after periods of manipulated deprivation (Budzynski et al. 2000).

In any case, there was a significant difference in the orientation of larvae when presented with an artificial celestial cue at different azimuths. In addition, looking at the angular changes in direction for each individual between the different sun positions showed there was a significant shift among individuals in proportion to the 180° shift in azimuth. This indicates the possibility of coral reef fish larvae to derive directional information from the sun's position alone and to use this cue to maintain a fixed direction, supporting the hypothesis of a particular orientation for coral reef fish larvae around Lizard Island towards the southeast assisted by the sun's position as a directional cue.

In the cue conflict experiment we found that the only significant or close to significant unidirectional orientation among individuals was in treatments where the axis of polarised light was perpendicular to the location of the artificial sun. This is the arrangement most closely resembling natural conditions, where the maximum skylight polarisation pattern is at an angle near 90° to the azimuth of the sun (Cronin and Marshall 2011). This orientation changed significantly by 57° from northwest to southwest between the two different sun positions, again suggesting an influence of the artificial sun position on the orientation behaviour of coral reef fish larvae, but also an influence of the polarised light pattern on orientation. A study by Muheim et al. (2016) found zebra finches were able to orient when polarised light axis and magnetic field lines were parallel, but appeared to lose that ability when the cues were shifted perpendicular to each other. An attempt to integrate both polarised light and sun azimuth cues may help to explain why unimodal orientation occurred here in the cue-conflict experiment only when the combination of sun position and polarised light resembled natural conditions. It is likely that sky polarisation patterns alone are insufficient as an orientation compass (Muheim et al. 2016).

Post-larvae of another coral reef Pomacentrid (*Premnas biaculeatus*) were found to change their orientation in response to a changing axis of polarized light, indicating a role in pelagic orientation (Berenshtein et al. 2014) and there is evidence that the adults of the congeneric coral reef fish *Chromis viridis* have the ability to discriminate changes in a polarised light pattern orientation (Mussi et al. 2005). If pre-settlement larvae have the same ability, polarised light may be used as part of a hierarchy of cues related to orientation behaviour. *In situ* observations of coral reef fish larvae show a decrease in orientation precision under cloudy conditions, under which polarised light patterns would still be available, suggesting a view of the sky is an important factor (Hegedüs et al. 2007). This is also the case for orientation behaviour of birds in some cases, where directionality was lost when visibility of the sun was obscured by cloud cover. (Budzynski et al. 2000). Future work should look at the retinal structure of coral reef fish larvae to ensure that the receptor mosaic supports the behavioural results indicating the ability for polarised vision (Shand et al. 1999).

Orientation in the cue-conflict experiment was more directional in the morning than the afternoon. This is in line with *in situ* observations by Faillettaz et al. (2015) who reported that orientation precision in larvae of the congeneric *Chromis chromis* in the Mediterranean Sea declined in a linear fashion throughout the day. *In situ* observations of *C. atripectoralis* around Lizard Island by Leis et al. (2014) show that directionality of orientation decreased towards the middle of the day, but increased again in the late afternoon. Our cue-conflict experiments were usually completed by the mid-afternoon, so no replicates for the late afternoon were available although sun positions and light conditions were consistent for all trials.

In the morning, orientation during cue-conflict trials was either generally towards the actual sun position when the sun was positioned at the north side of the tank (or towards apparent east), or towards west-southwest when the sun was positioned towards the south (or apparent south). This generally supports the results of the artificial sun position experiment, however unlike in that experiment unimodal orientation under the diffusing filter was not significant during cue-conflict trials. The artificial sun was positioned at azimuths perpendicular to natural conditions during the cue-conflict experiment. As larval orientation was less directional under the diffusing filter compared to the artificial sun position experiment (where artificial sun azimuths were closer to natural cardinal directions), this may suggest the availability of another unanticipated directional cue resulting in increased confusion in response to the unnatural sun azimuth.

Curiously, the only treatment in the afternoon that returned significant orientation was sun south under the diffuser, an obvious axial distribution running along the northwest-southeast axis. This orientation was the same regardless of the time of day, however interestingly was not present under the diffusing filter with the sun positioned to the north. A bimodal distribution suggests orientation using a linear cue, perhaps geomagnetic or a polarized light cue (Muheim et al. 2009). With polarised light eliminated by the diffusing filter and the artificial sun position in an unfamiliar cardinal direction (south) this may indicate cue-switching to another available modality (Able 1991, Kingsford et al. 2002, Igulu et al. 2013). Experiments by (Wiltschko et al. 1981) showed that when deprived of the opportunity to calibrate a sun compass using natural sky conditions in the morning, homing pigeons switched to a magnetic compass to determine a bearing home. Experiments on other sensory modalities such as magnetoreception are required to ascertain the range of cues which may facilitate different aspects of an orientation mechanism.

Although it is generally thought that most larval recruitment to coral reef habitat occurs at night, when there is a cover of darkness which assists in successfully negotiating the predator gauntlet, larvae may also recruit during daylight or crepuscular hours (Sweatman 1988, Dixson et al. 2010). Daytime movement may expose pelagic larvae to a greater risk of predation, however once growth has reached the settlement stage larvae may have a limited window of time in which to successfully locate habitat (McCormick 1999, Booth and Parkinson 2011). Patterns of self-recruitment being reported in many tropical fish populations give evidence for existing adaptations allowing larvae to be close to habitat when growth reaches the settlement stage (Swearer et al. 1999, Jones et al. 2009). Indeed, many coral reef fish larvae are observed in situ within the first 5m of the water column, suggesting that at least some species spend part of the day closer to the waters surface, where celestial cues are more easily visible and planktonic food is also likely to be more plentiful (Leis 1996). Due to depth effects celestial cues viewed through Snell's window are mainly useful for orientation also at these shallow depths (Leis et al. 2011). It is likely that large-scale orientation cues provide a mechanism for mitigating the dispersing effects of ocean currents for a proportion of the population (Leis et al. 2015), while smaller-scale cues such as auditory and olfactory habitat cues are used if a larvae is able to approach or stay within range of habitat (Wright et al. 2005).

Little is known about larval orientation at night, although fish larvae may utilise non-visual cues (auditory or olfactory) at night when in range (Stobutzki and Bellwood 1998, Tolimieri et al. 2004). Considering larger scales, larvae are unlikely to use currents as a directional cue as a larva embedded within a pelagic current may not be able to detect its direction without an external frame of reference (Leis 2007). Observing larvae during this time is an enduring challenge of larval ecology, however some amazing experiments have shown the ability to orient using the milky way and the moon in other taxa which also use a sun compass, suggesting the possibility for diurnal use of celestial cues in larval fish (Ugolini et al. 2003, Dacke et al. 2013).

During crepuscular periods however there is a strong directional signal from both the sun and its associated polarised light pattern (Cronin et al. 2006). Techniques should be developed to investigate the ability of larval fishes to use celestial cues diurnally.

Various studies have reported the ability for coral reef fish larvae to orient their movement with surprising precision, with a sun compass suggested as the likely mechanism (Mouritsen et al. 2013, Faillettaz et al. 2015, Irisson et al. 2015, Leis et al. 2015). However this is the first study to use manipulated experiments to investigate how larval fish may interpret and respond to different aspects of celestial compass information. We found that larval fish responded to the movement of an artificial sun disk on both an individual and a group level in ways that suggest they were using the sun's azimuth for directional information. When sun position and polarised light cues were presented together in different alignments orientation was only significant when the cues resembled natural conditions. This suggests polarised light is not only visible to coral reef fish larvae, but may be part of the compass mechanism in a hierarchy of available cues (Kingsford et al. 2002). Indeed, most animals using a sun compass for an orientation mechanism due not use single cues in isolation unless there is no other option, and this combination of cues may be necessary to calibrate a compass direction and to maintain a straight path of most efficient movement (Able 1991, Goddard and Forward 1991, Lebhardt and Ronacher 2015). While this study gives evidence for the orientation of fish larvae following the movement of the sun more work is required to elucidate the range of sensory abilities some larval fishes are using to facilitate their extraordinary behaviours, which in turn may influence the population dynamics of fish communities of coral reefs around the world.

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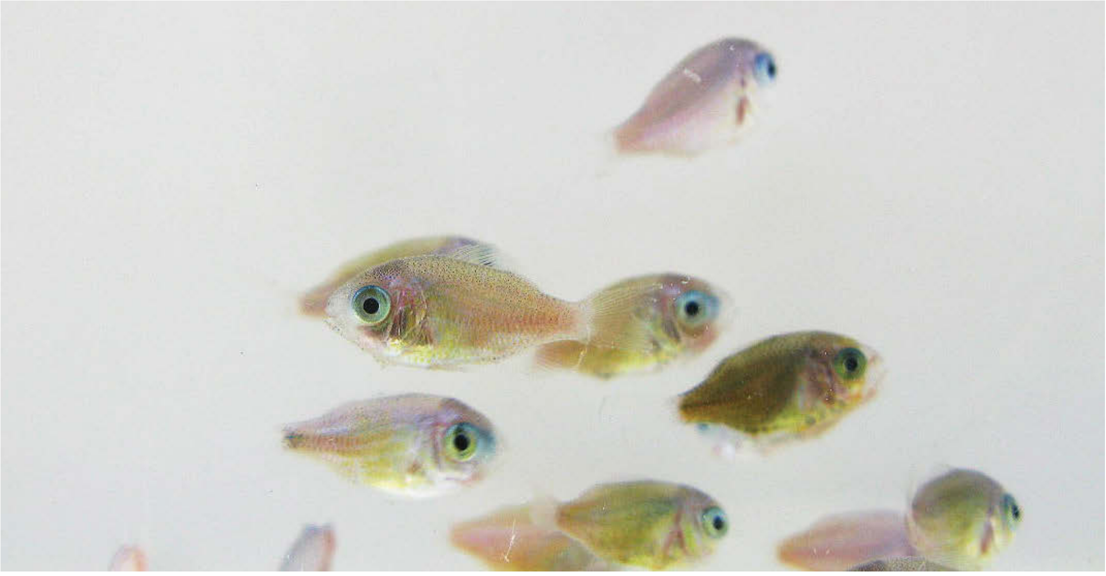
CHAPTER FOUR

Responses of coral reef fish larvae to changes in the local magnetic field and polarised light plane



One Chromis atripectoralis larva going against the flow, Lizard Island Research Station

Abstract

Observations of coral reef fish larvae have revealed remarkably consistent orientation behaviour while swimming offshore with large-scale cues hypothesised to play a role. However the mechanisms underlying this behaviour are still being investigated. Analysing the orientation behaviour of the coral reef fish *Chromis atripectoralis* (Pomacentridae) we found that larvae responded to changes in the local magnetic field, showing for the first time that magnetoreception may be part of a sensory hierarchy used to guide swimming during the pre-settlement phase. Individuals had a significantly directional response to a 90° shifts in local magnetic field polarity compared to control conditions. Changes in non-significant among-individual orientation under different magnetic treatments were also consistent with a detection of changes in the magnetic field. In the absence of other natural cues this magnetic sense appears to have been used for fixed-direction orientation compass to maintain a bearing rather than a magnetic map sense. Under a static polarised light pattern individual larvae had a higher median orientation precision. Unlike the under opaque cover, larvae had significant among-individual orientation in one of the two treatments and maintained a southeast direction despite the 90° magnetic polarity shift under polarised light conditions. These responses provide the first evidence of the ability of coral reef fish larvae to use cues from magnetic fields and polarised light to orient movement in the pre-settlement phase. This has implications for how behaviour is incorporated into modelling of larval dispersal.

Introduction

The ability of animals to orient their movement during large-scale transitions between different habitats is one of the most studied phenomena in the natural world (Gould and Gould 2012). Research into sensory cues which can elicit ecologically relevant behavioural responses has revealed a wide array of abilities for animals to derive directional information, including the use of olfactory, auditory and visual cues (Von Frisch 1974, Wehner and Lanfranconi 1981, Hedwig and Poulet 2004, Dacke et al. 2013, Gagliardo et al. 2013). Of particular interest in the case of animals moving across large spatial scales are cues, which can provide consistency and availability regardless of distance travelled and local weather conditions, including celestial cues and geomagnetic fields (Wehner 2001, Ritz et al. 2002, Merlin et al. 2009).

Species from an increasing number of taxa are found to orient movement according to changes in the local geomagnetic field including insects, amphibians, birds, mammals, crustaceans, reptiles and fishes (Wiltschko and Wiltschko 1995, Boles and Lohmann 2003, Mouritsen and Ritz 2005, Muheim et al. 2006a, Hellinger and Hoffmann 2009, Phillips et al. 2010, Lohmann et al. 2012, Sandoval et al. 2012). Such a widespread ability has given rise to the idea that this evolutionary development is likely to be shared across many more organisms (Åkesson et al. 2014), however there remain many species and life stages that remain to be investigated. Here we investigated the ability of larval coral reef fishes to detect magnetic fields, which would help to explain their extraordinary ability to orient their swimming in the pelagic environment (Leis et al. 2014).

Recent studies of marine species show that the ability to orient using magnetic fields is present across different life history stages, however exactly when during ontogeny this ability emerges remains unknown. For juveniles of semelparous fishes evidence suggests the use of inherited magnetic maps directing animals back towards their breeding grounds (Putman et al. 2014a, Putman et al. 2014b). For fishes with a pelagic larval dispersal phase, directed swimming facilitating efficient movement in the expanse of the open ocean increases the likelihood of encountering settlement habitat and may facilitate higher levels of self-recruitment (Caldwell and Nams 2006, Leis et al. 2011).

Data on larval fish dispersal over the past decades have resulted in a new paradigm whereby dispersal in the ocean is no longer considered a purely physical process, but a bio-physical one; in other words, these tiny larvae are now regarded as having swimming and sensory abilities enabling them to influence the spatial extent of their dispersal (Kingsford et al. 2002, Jones et al. 2009). Fish larvae respond to sensory stimuli giving directional information on potential habitat at a scale of less than a kilometre, such as visual, auditory and olfactory cues, from an early stage in development (Tolimieri et al. 2004, Dixson et al. 2011, Igulu et al. 2011). However, this is the first study investigating how magnetoreception in larval fishes may influence orientation. Understanding the mechanisms that animals use to conduct movement patterns is important for elucidating the potential anthropogenic impacts on their population ecology (Engels et al. 2014).

Although the mechanisms underlying offshore orientation in larvae of marine demersal fishes remain to be explained, there is clear evidence of their ability to do so. Observations of coral reef fish larvae *in situ* reveal that multiple species swam in a similar directional orientation independent of location in respect to coral reef habitat up to 1 km away (Leis and Carson-Ewart 2003). This behaviour is consistent regardless of spatial or temporal differences and persists in the pelagic zone outside of the effective range of the known habitat sensory cues (visual, auditory and olfactory), suggesting the use of other larger-scale cues (Leis et al. 2014). Such abilities in larvae of coral reef fishes, many of which have formidable swimming abilities from early growth stages, would have major implications for the way larval dispersal is regarded and modelled (Fisher et al. 2000, Staaterman et al. 2012). Empirical studies of larval recruitment patterns indicate an active influence of larvae on population connectivity, and although dispersal models are increasingly incorporating more complex pre-settlement behaviours the response to sensory cues in these models is still regarded as a range-limited effect (Armsworth 2000, Atema et al. 2015). If large-scale cues are used by larval fish to orient their swimming, then this has drastic implications for dispersal modelling.

Two main mechanisms are hypothesised to facilitate the ability to detect the geomagnetic field in vertebrates (Wiltschko and Wiltschko 1995, Muheim et al. 2014, Muheim and Liedvogel 2015): a biochemical mechanism based on cryptochrome photopigments of the eye (Ritz et al. 2000), and a ferromineral mechanism based on biogenic magnetically-reactive intracellular particles (Shaw et al. 2015). These two magnetoreception mechanisms have been shown to occur as complementary systems in the same organism (e.g., in amphibians and birds) providing input to either the magnetic compass (ferromagnetic) or a magnetic map sense (cryptochromes) (Phillips 1986, Munro et al. 1997). Although sensors enabling magnetoreception remain enigmatic, it is likely that specialisation of orientation subsystems has evolved following phylogenetic patterns (Gould 2008). For example, behavioural experiments have shown that light is a key prerequisite to magnetic orientation in birds and amphibians, which implies the use of a magnetic compass based on chemical reactions in cryptochromes, whereas some marine species such as lobsters and fish have the ability for light-independent orientation involving the polarity of the geomagnetic field suggesting reliance on ferromagnetic-based orientation (Lohmann et al. 1995, Hellinger and Hoffmann 2012).

For this study we tested the effect of manipulations of the local geomagnetic field on the behaviour of coral reef fish larvae of the same species (*Chromis atripectoralis*, a pomacentrid damselfish), developmental stage and location as have previously demonstrated orientation abilities *in situ* (Leis and Carson-Ewart 2003, Leis et al. 2014). We hypothesised that if larvae could detect changes in the local magnetic field, predictable changes in orientation behaviour would result. This is the first step in determining if a magnetic sense plays a role in how larval fish influence their dispersal.

Materials & methods

(a) Field collection

We collected larvae of the coral reef fish *Chromis atripectoralis* at sunrise each day during seasonal recruitment in November 2013 and February 2014 using light traps deployed at sunset the preceding day off the fringing reefs surrounding Lizard Island (14°40'42.2"S 145°25'43.4"E, Great Barrier Reef Marine Park, QLD). We caught larvae (7-10 mm SL) approaching coral reef habitat at the end of their pelagic phase. Larvae were yet to undergo metamorphosis and we refer to them hereafter as settlement-stage larvae. We selected this species as it is abundant in light trap catches of the area, and more importantly, has demonstrated the ability for directional swimming *in situ* (Leis and Carson-Ewart 2003, Leis et al. 2014). Each larva we obtained was tested on its day of catch under one of the experimental treatments outlined below. While awaiting testing we kept larvae in a temperature controlled laboratory in aerated 10 l aquaria separated by opaque barriers and supplied with filtered seawater on a flow-through system.

(b) Experiment 1 - Magnetic field disruption

For magnetic field disruption trials we tested larvae in an open-air laboratory at Lizard Island Research Station in a plastic circular arena placed a minimum of two metres from any metal structure to minimise magnetic field interference. We used a 45 cm diameter arena within a larger, enclosed, plastic circular tank (65 cm x 55 cm) to exclude external stimuli from visual environmental factors (Fig. 4.1). Filtered seawater, replaced between each fish larvae tested, was maintained at 15 cm depth in the arena. We tested the orientation of 30 individuals over a period of 7 days. Each orientation test consisted of four consecutive trials with the following treatments:

(i) Control 1 (no magnet)

(ii) Magnet with north pole placed against arena wall at an orientation of 315°

(iii) Magnet with north pole placed against arena wall at an orientation of 45°

(iv) Control 2 (no magnet)

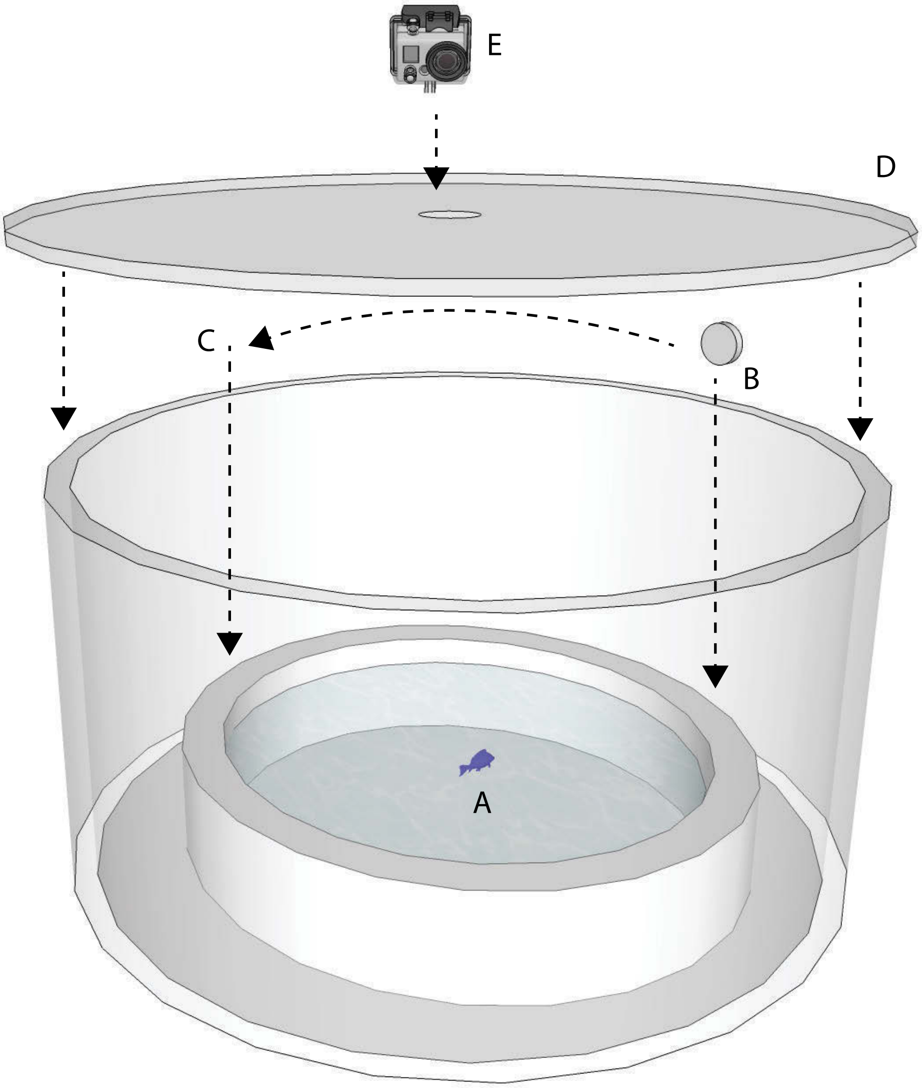


Fig. 4.1 Experiment 1 - Experimental setup for magnetic field disruption trials. Larvae of *C. atripectoralis* are placed the circular arena (A) and movement is recorded from above with a HD camera (E) mounted in the tank cover (D). Under treatment conditions a magnet is placed on the outer arena wall at 45°(B) or 315° orientation (C).

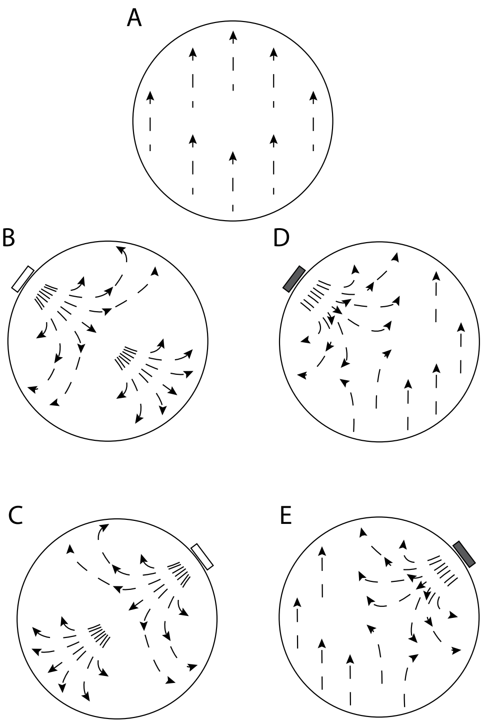


Fig. 4.2 Experiment 1 - Compass-mapped magnetic field lines under control conditions (A), north pole of neodymium magnet placed against arena at 315° (B) and 45° orientation (C), north pole of ferric magnet against arena at 315° (D) and 45° orientation (E). Arrows indicate direction of magnetic North in the

In order to minimise order effects treatments were run in alternating order (i – iv, iv –i) bookended by the two control treatments. We selected magnet positions for maximum disruption of the natural magnetic polarity in the test arena. Each treatment used either a ferric (weak - 650 Gauss) or neodymium / rare-earth (strong, 3,300 Gauss) axially magnetised 15 mm x 5 mm magnet. Each magnet type affected the local magnetic field in different ways proportional to strength (Fig. 4.2). Two control treatments were used to investigate the consistency of orientation under unmanipulated conditions. To begin each trial we introduced a single larva into the centre of the circular arena within an opaque cylinder of 4 cm diameter that was subsequently removed to allow the larva to explore the arena without positional bias. We allowed each larva to acclimate to the arena for a two minute period before recording movement and position for a 10 minute period using a GoPro HD camera mounted centrally in the tank's cover. Following this observation period we scooped the larva into a holding beaker for ~2 minutes to minimise handling stress and allow for recovery. During this time we prepared the next treatment and subsequently re-introduced the larvae back into the test chamber.

(c) Experiment 2 - Magnetic field manipulation

We used a single Helmholtz coil design (Kirschvink 1992) to manipulate magnetic field polarity in the test arena (Fig. 4.3). In order to shift magnetic north within the coil by 90° clock-wise, the coil was orientated at 135° to natural magnetic north. Use of 2.8 V (1.17 A) supplied by a Powertech DC regulated power supply at 2.7 Ω resistance in the activated coil shifted the magnetic field polarity by 90° compared to control conditions (power supply switched off, but grounded). Magnetic field intensity and inclination were not altered under treatment conditions. We monitored magnetic field parameters within the coil using a high-speed 3-axis digital fluxgate magnetometer (Applied Physics Systems, Model 693, Mountain View, CA, USA). To attempt to minimise the amount of ambient magnetic field interference due to man-made electro-magnetic fields, we conducted orientation experiments in the magnetic coil ~50 m from the research station, undercover of a wooden gazebo to block direct sunlight.

Using a similar protocol to experiment 1, we introduced single larvae into the centre of the circular test arena (55 cm diameter, water depth 20 cm) using a PVC cylinder. After we removed the cylinder, the larva had a 2 minute acclimation period before its movement was recorded by a HD video camera mounted centrally above the coil for a period of 10 minutes. Between treatments we removed larvae to a holding tank before placing them back into the centre of the test arena once treatment conditions had been switched. We tested 31 individuals over a period of 7 days, each given two consecutive trials with the following treatments presented in random order:

(i) Control (coil switched off and grounded)

(ii) Magnetic north shifted 90° clock-wise

To test if light conditions inside the test arena influenced magnetic orientation a further 20 individuals were tested with the same procedures, but with the opaque cover replaced by a UV linear polarising filter created by a linear polarizing polyvinyl alcohol-iodine filter with the e-vector of polarised light oriented along the magnetic north–south plane.

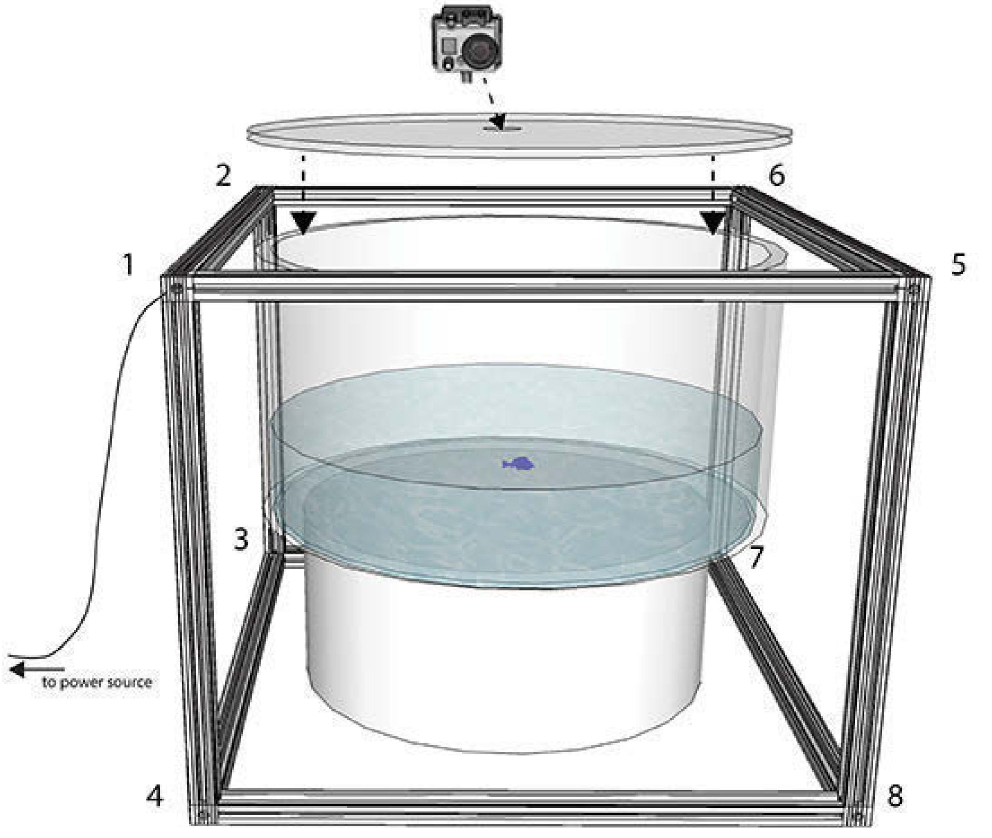


Fig. 4.3 Experiment 2 - Helmholtz coil setup for manipulation of local magnetic field polarity. Parallel coils formed by wraps of enamelled copper wire around on each side of the coil frame (coil 1 around corners 1,2,3,4, coil 2 around corners 5,6,7,8). Larva (blue) tested in circular arena in the centre of the frame.

(d) Data analysis

From the video recordings we automatically sampled the position of larvae in the arena at 15 frames per second (9000 data points per treatment) with a custom-made video tracking program written in MATLAB® 2013b (The MathWorks Inc., Natick, MA, USA). The program provided a complete track of the movements of each individual larva (Fig. 4.4) and calculated the mean direction (μ) and directional precision (length of the mean vector, r) using vector addition (Batschelet 1981). For each individual (first order analysis), we calculated the mean orientation for both the unimodal and axial distributions (Batschelet 1981), as the axiality of behaviour between individuals and groups can relate to different cue responses and motivations. If the axial distribution was more directed, i.e. if the mean vector length of the axial distribution was larger than for the unimodal distribution, r(axial) > r(uni), we used the axial direction, with the axis direction closest to the unimodal direction as the primary direction. For second order analysis (among-individuals) we included only the mean bearings of individuals with mean vector lengths $r > 0.2$. Pilot studies indicated that group orientation became more spread if individuals with $r < 0.2$ were included, and did not improve if only animals with $r > 0.3$ or higher were included. Thus, we concluded that $r = 0.2$ was the optimal threshold.

To assess group orientation we used Oriana 4.0 (Kovach Computing Services) to calculate median direction (including 95% CI) and the length of mean vector r as a measure of group scatter for both the unimodal and axial distributions. We calculated individual responses between two treatments for a larva by setting mean bearing during the first treatment to 0° and determining the size of the angle moved to the mean bearing during the second treatment. We then used these angle changes to determine a 95% C.I. across the group and compared with the expected response between two treatment conditions. As we had a hypothesised angle change for the magnetic coil experiment (a 90° shift proportional to the 90° change in the magnetic field polarity within the coil under treatment conditions), we used a V-test to compare the observed mean angle change among individuals with the expected mean (Mahan 1991). We used Watson's U^2 tests to assess the difference in distributions between different treatments and tested differences in angular dispersion (variance) using a two-sample Wallraff test and differences in precision (r) using a Kruskall-Wallis test, with all individuals included.

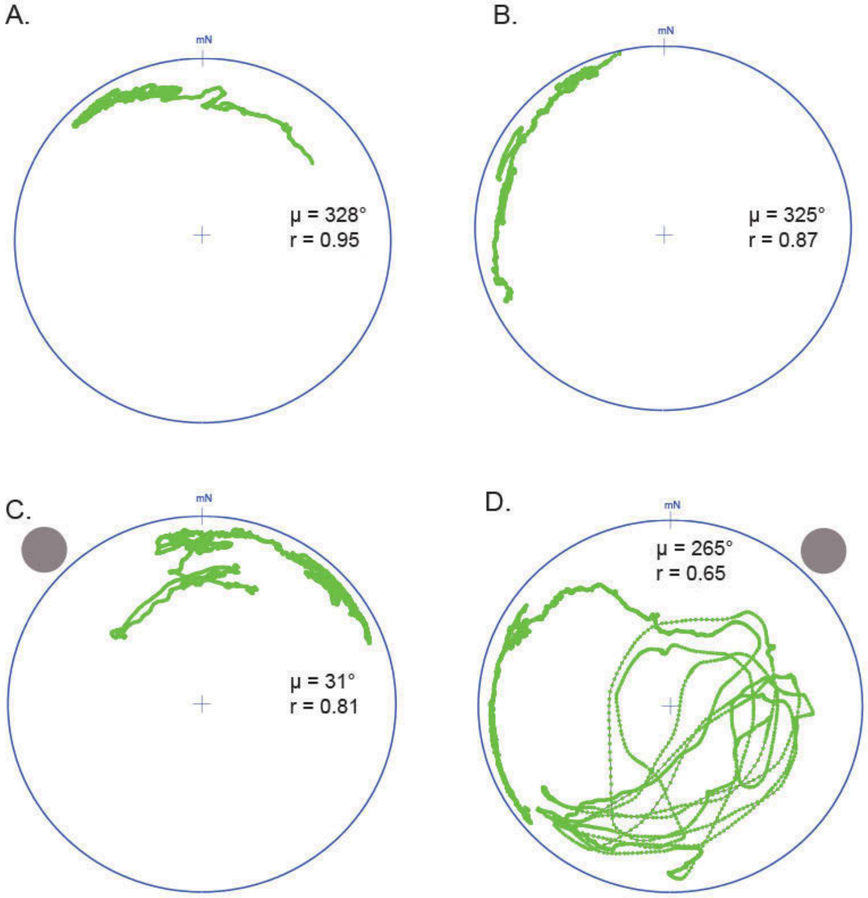


Fig. 4.4 Experiment 1 - Example of 10 minute tracks obtained through video analysis of movement within test arena for one individual *C. atripectoralis* larva during control (A & B) and magnet (C & D) treatments. Grey circle indicates magnet position. Green line was constructed by position of larva at 15pps (points per second). Mean bearing (μ), directional precision (r) and Rayleigh test p-value reported for each treatment. mN indicates magnetic north.

Results

a) Experiment 1 - Magnetic field disruption

First order orientation of *C. atripectoralis* larvae within the test arena was generally high, with 83% of trials being directional (median r = 0.7). Second order orientation was towards northwest in the test chamber, significantly in Control 1 (μ = 335°, 95%CI 294 – 9°, r = 0.35, R test: p = 0.03, n = 29) and non-significantly in Control 2 (μ = 315°, 95%CI 276 – 2°, r = 0.29, R test: p = 0.1, n = 27). When a magnet was placed against the chamber, the groups of larvae were non-significantly directed, but more northerly compared to the control treatments (magnet in the northwest (315°): μ = 352°, 95%CI 305 – 39°, r = 0.28, p = 0.14, n = 25; magnet in the northeast (45°): μ = 14°, 95%CI 270 – 119°, r = 0.15, p = 0.58, n = 27) (Fig. 4.5). There was no significant difference in the distribution of mean bearings between the four treatments (Watson's $U^2 = 0.12$, $p > 0.2$). Precision of mean bearings between pooled control (median = 0.66, n = 60) and magnet (median = 0.73, n = 60) treatments was not significantly different (Wilcoxon's signed-rank test: Z = -1.30, p = 0.20).

Larvae behaved differently in response to the type of magnet used to disrupt the local magnetic field, with significant orientation in the presence of the weaker ferric magnets and non-significant orientation with the stronger neodymium magnets. Larvae were directional to the north with a ferric magnet placed at 315° (μ = 2°, 95%CI 323 – 40°, r = 0.28, p = 0.02, n = 19). When the magnet was moved to 45° larvae were bimodally distributed on either side of the magnet location (μ = 156°, 95%CI 137 – 174°, r = 0.48, p = 0.018, n = 17) (Fig. 4.6). These distributions were significantly different from each other (Watson's $U^2 = 0.36$, $p < 0.002$).

Individual larvae responded to the ferric magnet at 315° with a significantly directional fashion, shifting either 35° clockwise or 145° counter clockwise from their position in the Control 1 treatment (μ = 35°/215°, 95%CI 16 – 54°, r = 0.47, p = 0.016, n = 18). The response of larvae to the ferric magnet at 45° treatment, while not as significant, shifted 36° counter-clockwise relative to their position in the Control 2 treatment (μ = 324°, 95%CI 256 – 37°, r = 0.39, p = 0.097, n = 15). The response of larvae between the two ferric magnet positions was not significantly directional (μ = 24° 95%CI 308 – 101°, r = 0.16, R test: p = 0.64, n = 17).

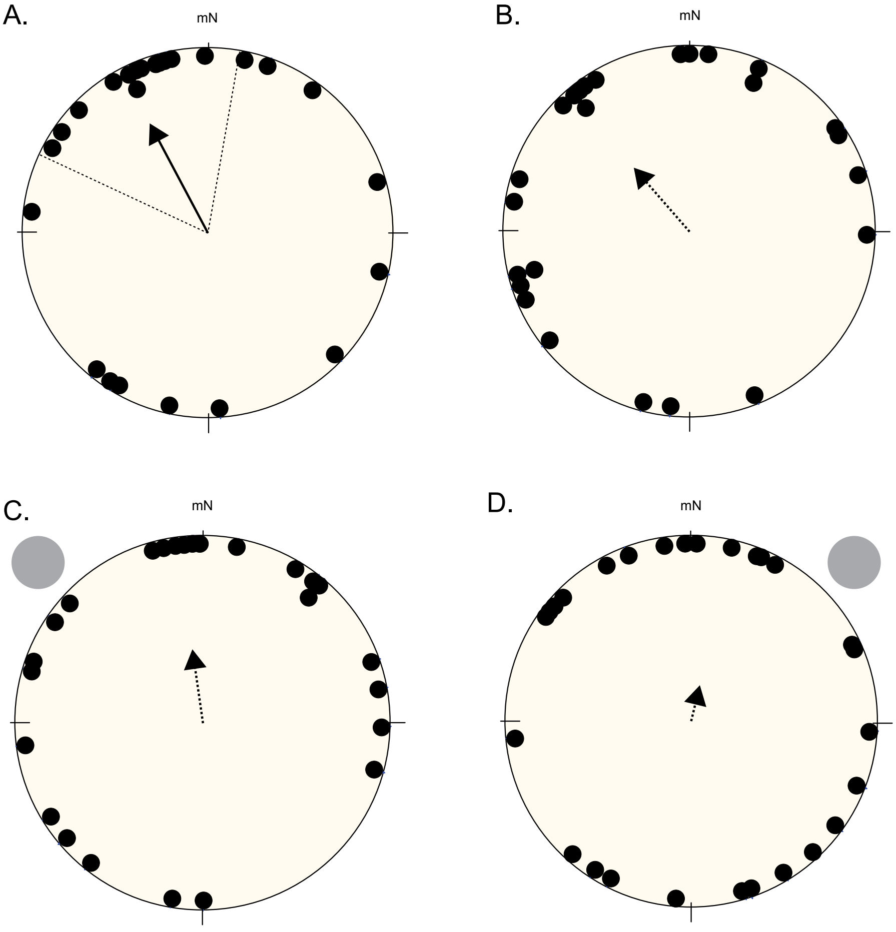


Fig. 4.5 Experiment 1 - Distribution of mean bearings for individual larvae during control (A & B) and magnet (C & D) treatments. Grey circle indicates magnet position (magnet types pooled). Arrows indicate the mean among-individual direction, length proportional to the directional precision (r).

Significant orientation indicated by solid arrow bounded by dotted radial lines indicating the 95% confidence interval.

In treatments using neodymium rare earth magnets, among-individual orientation was not significant when a magnet was placed at 315° (μ = 316° 95%CI 144 – 128°, r = 0.27, R test: p = 0.53, n = 9) or at 45° (axial: μ = 33°/213° 95%CI 354 – 73°, r = 0.37, R test: p = 0.26, n = 10). This may also be a consequence of lower replicate numbers for the rare earth treatments, as both Control 1 (μ = 357° 95%CI 218 – 136°, r = 0.29, R test: p = 0.47, n = 10) and Control 2 (axial: μ = 171°/351° 95%CI 132 – 210°, r = 0.39, R test: p = 0.29, n = 9) were also not significantly directional. Looking at the angular difference of individuals between the treatments with a neodymium magnet at 315° and 45°, there was a response approaching significance of 94° (95%CI 47 - 141°, r = 0.55, R test: p = 0.061, n = 9). This is consistent with the predicted shift of 90° expected if the magnet position was being used by individuals for fixed-direction orientation (Fig. 4.7). Taking the angular difference of each individual larva from their orientation in the Control treatment 1 and the neodymium magnet at 315° treatment, the response was significantly directional and bimodally distributed at 170°/350° (95%CI 149 – 192°, r = 0.56, p = 0.038, n = 10). The angular deviation between the neodymium magnet at 45° treatment and the Control 2 treatment the deviation was non-directional with bimodal distribution better describing the data at 54° (95%CI 9 – 92°, r = 0.36, p = 0.32, n = 9).

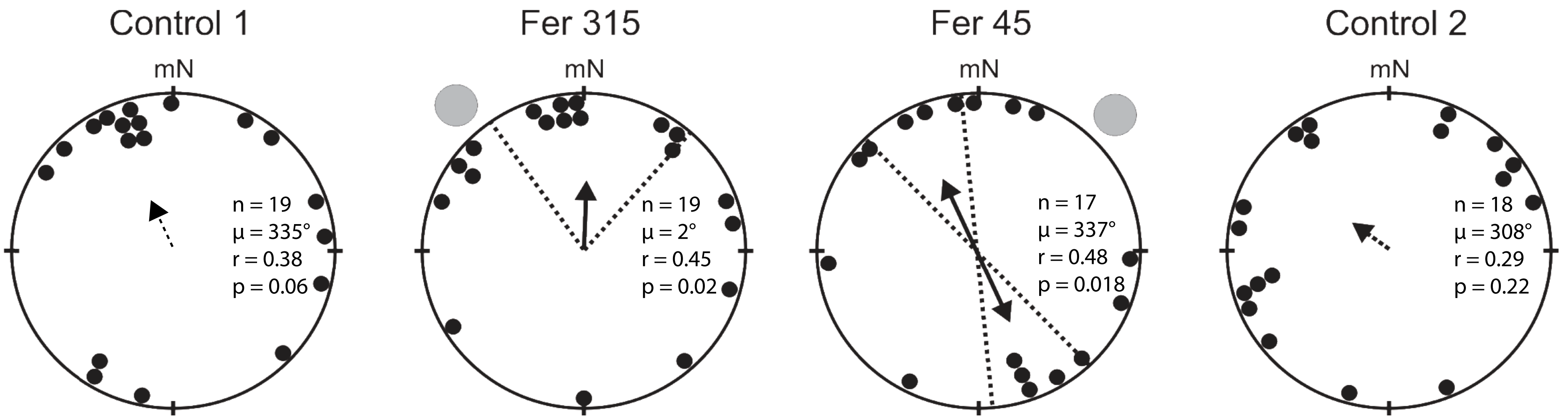


Fig. 4.6 Orientation of *C. atripectoralis* larvae in the presence of ferric magnets only. Each individual was presented with four treatments: (i) Control 1, (ii) magnet placed outside test chamber at 315° bearing (indicated by grey circle), (iii) magnet placed outside test chamber at 45° bearing and (iv) Control 2. Each data point in the circular diagrams represents the mean orientation for an individual larva (only significantly oriented individuals included). Arrows indicate the mean among-individual direction. Double-headed arrows indicate axially distributed samples. Significant orientation in a treatment indicated by solid arrow and dotted radial lines showing the 95% confidence interval.

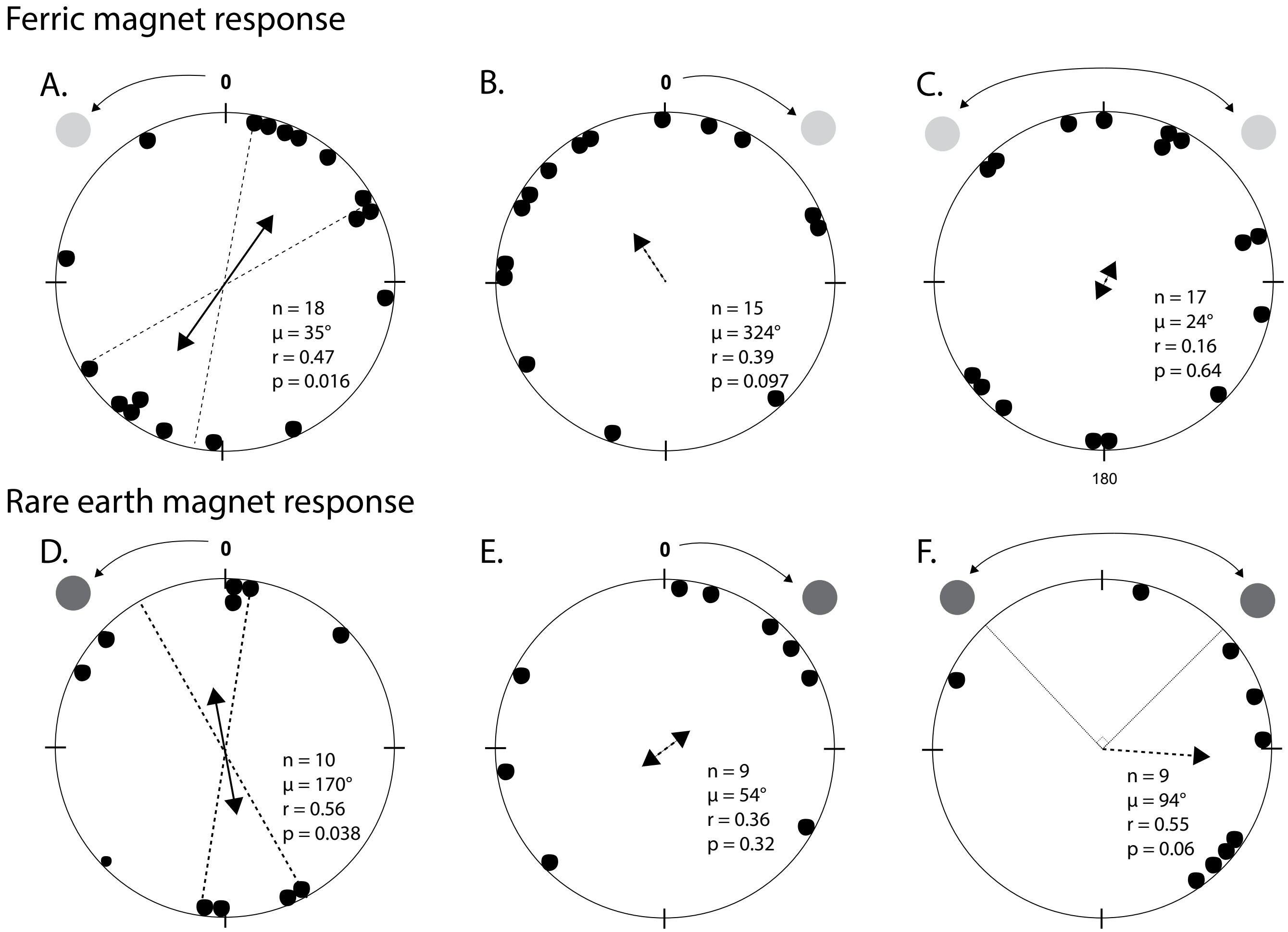


Fig. 4.7 Angular difference of *C. atripectoralis* larvae between different magnet treatments. Each data point indicates angular difference of a larva's mean bearing between two different treatments (initial bearing set to 0°), including: A) control to ferric magnet at 315; D) control to rare earth magnet at 315°; B) control to ferric magnet at 45 E) control to rare earth magnet at 45° ;C) between the two positions of the ferric magnet and F) between the two positions of the rare earth magnet. Grey circles indicate presence and position of magnets. Arrows indicate the mean among-individual direction. Double-headed arrows indicate axially distributed samples. Dotted radial lines give the 95% confidence interval for the mean direction in significantly oriented groups.

b) Experiment 2 - Magnetic field manipulation

(i) Opaque cover

As with Experiment 1, *C. atripectoralis* larvae were generally directional in 87% of trials at an individual level (median r = 0.64). Among individuals *C. atripectoralis* larvae showed non-significant orientation to the southeast under control conditions ($\mu = 104°$, 95%CI 47°-161°, r = 0.26, R test: p = 0.16, n = 27). When magnetic north was shifted 90° clockwise the mean orientation on the group level within the magnetic coil to the southwest was not significant ($\mu = 199°$, 95%CI 135°-263°, r = 0.24, R test: p = 0.22, n = 27), although the angular difference of 95° clockwise from the control treatment was as expected (Fig. 4.8). The distribution of bearings between the two treatments was not significantly different however (Watson's $U^2 = 0.1$, $p > 0.2$), nor was the precision of bearings significantly higher when the coil was switched off (median r = 0.75, n = 31) than when it was activated (median r = 0.69, n = 31) (Wilcoxon's signed-rank test: Z = 0.12, p = 0.92).

Comparing the angular difference of each larva's mean bearing between control and magnetic field shifted treatments, we found that among individuals the deviation from their initial orientation was significantly directional with a mean deviation of 125° (Fig. 8). The 95% C.I. encompasses the 90° angular difference we hypothesised if the magnetic field polarity was being used for orientation (95%CI 87 – 162°, r = 0.39, R test: p = 0.015, n = 27). The V-test confirmed the observed mean angle change was significantly similar to the expected mean angle change ($V_{\text{exp } 90°} = 0.32$, p = 0.009).

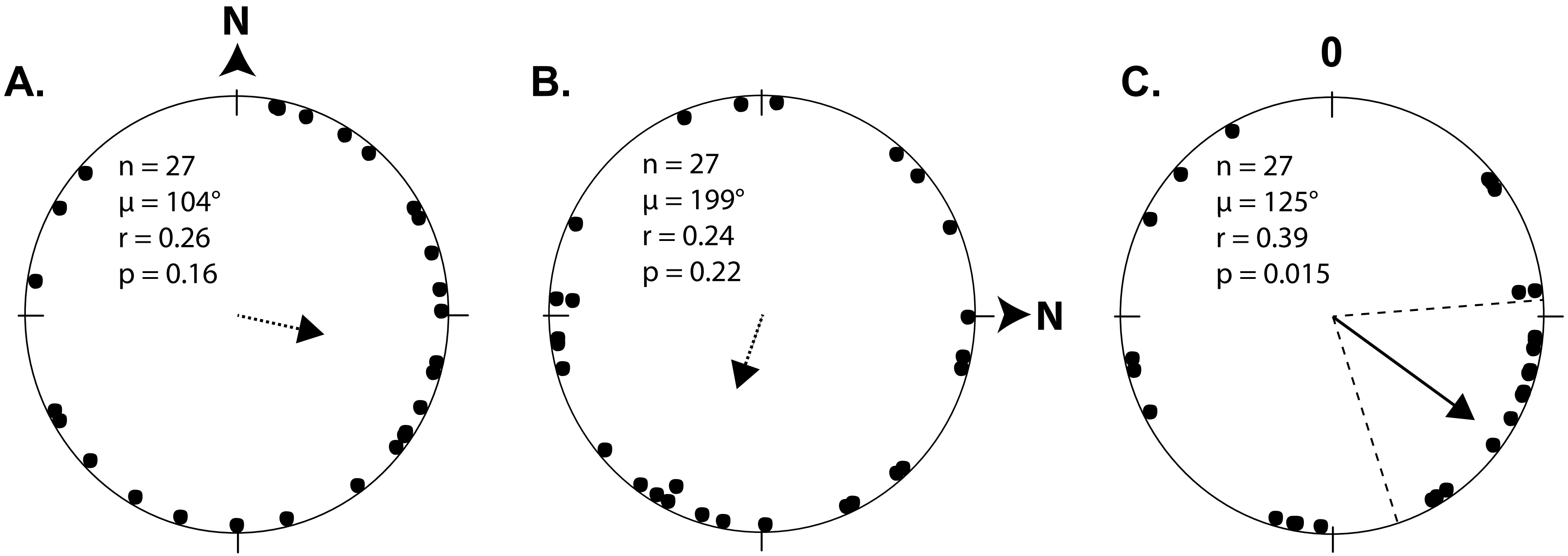


Fig. 4.8 Distribution of mean bearings for individual larvae during the control (A) and with magnetic north shifted 90°(B). C indicates the angular difference between the position of each larva during the control (set to 0) and the polarity shifted treatment. Arrows indicate the mean among-individual direction, length proportional to the directional precision (r). Significant orientation in treatments indicated by solid arrow bounded by dotted radial lines indicating the 95% confidence interval.

(ii) Polarised light pattern

Under the polarizing filter 80% of trials were directional on an individual level (median r = 0.8). Among-individual orientation of *C. atripectoralis* larvae approached significance in a bimodal orientation along the southeast-northwest axis under control conditions (μ = 119°, 95%CI 95°-144°, r = 0.39, R test: p = 0.085, n = 16). When magnetic north was shifted, larvae showed significant group-level orientation towards the southeast (μ = 160°, 95%CI 117°-204°, r = 0.44, R test: p = 0.045, n = 16)(Fig. 9). The distribution of bearings between the control and shifted treatments was significantly different in this case (Watson's $U^2 = 0.22$, $p < 0.05$). The precision of bearings was not significantly different when the coil was switched off (median r = 0.56, n = 20) than when it was activated (median r = 0.46, n = 20)(Wilcoxon's signed-rank test: Z = 1.08, p = 0.29).

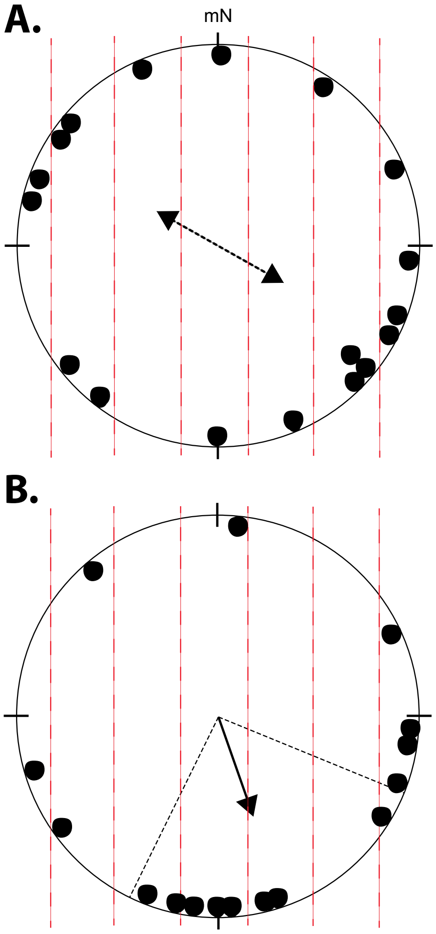


Fig. 4.9 Distribution of mean bearings for individual larvae during the control (A) and with magnetic north shifted 90°(B) under a polarised light pattern. Axis of red dotted lines indicates plane of polarisation. Arrows indicate the mean among-individual direction. Double-headed arrows indicate axially distributed samples. Significant orientation in a treatment indicated by solid arrow and dotted radial lines showing the 95% confidence interval.

Discussion

Larvae of some coral reef fishes observed in the field have shown remarkably consistent location-independent orientation behaviour at local and meso-scales, however the mechanisms underlying this ability are still unknown (Leis et al. 2015). Under laboratory conditions, responses of *C. atripectoralis* larvae revealed changes in orientation behaviour consistent with different local magnetic field conditions, showing that sensitivity to geomagnetic fields is a potential mechanism to guide swimming in the pre-settlement phase. In the absence of other natural cues this ability appears to have been used for fixed-direction orientation to maintain a compass bearing as opposed to a magnetic map sense. Individuals shifted their position between control and treatment conditions in the magnetic coil in a significantly orientated way, consistent with the expected deviation in response to our manipulations of the local magnetic field. On a group level orientation was not significant in all treatments, although behaviour suggests that the presence of polarised light, the strength of the magnetic field disruption and the geographic location influenced orientation behaviour differently.

Interestingly, magnets of different strengths had a strikingly different effect on the orientation of the larvae. Larvae were still directional among individuals in the presence of weaker ferric magnets and had significantly different distributions between the two magnet positions. This is reflected in the within-individual shifts in orientation between the control and ferric magnet treatments, where larvae shifted their orientation by approximately 30° in response to a ferric magnet relative to their position in the control treatments. This response indicates that the presence of the magnet affected the orientation of the larvae as expected. Among-individual orientation in the 45° ferric treatment also shows a bimodal distribution perpendicular to location of the magnet, a response seen in magnetic field experiments on birds attributed to the axial characteristics of the cue itself (Muheim et al. 2009). This is in contrast to the unidirectional distribution of mean bearings in the 315° ferric magnet treatment. These significantly different distributions suggest that the larvae were able to sense the artificial magnetic field and responded with altered orientation, but it is unclear why their response was axial in one magnet treatment and unimodal in the other. Unlike the rare earth magnets, the effect of the ferric magnet on the local magnetic field was strongest at close proximity and may have had reduced effects on the far side of the tank from the ferric magnet (Molteno and Kennedy 2009), which may explain why the direction of orientation was affected, but with shifts less than 90° with these weaker magnets.

In the rare earth magnet treatments among-individual orientation was not significantly directional in either the control or magnet treatments. This may have been due to the greater strength of the magnetic field disruption by the neodymium magnets (compared to the ferric magnets) overwhelming the sensory sensitivity of the larvae, or because these larvae were not orienting for other reasons as the control treatment orientation was also non-significant. The lower number of replicates contributed to reduced analytical power in the rare earth magnet treatments, however it is interesting to note that the non-significant distribution of mean bearings was axial in line with the rare earth magnet in each treatment. As a result, despite the low number of replicates, the angular difference of individual larvae between the two magnet treatments was close to significant and in proportion to the angular difference between the two magnet positions. The strength of the effect caused by magnets in animal orientation also likely depends on the method of magnetoreception being used (Mouritsen et al. 2003), and experiments on birds have shown that even strong magnets have been unable to disrupt orientation in some cases. This is perhaps due to the ability of birds to cue-switch to celestial cues to compensate for local magnetic disruption (Wang et al. 2006), an option unavailable to larvae in our arena-based experiments.

Larvae oriented differently during the control treatments of the enclosed magnet test arena (Experiment 1) compared to within the magnetic coil (Experiment 2). Significant orientation under control conditions is in itself curious given that no other obvious directional cues were available for orientation purposes. Although the among-individual orientation in the magnetic coil control treatment was non-significant to the southeast, the significant northwest orientation observed among individuals in the magnet arena control is in the opposite direction to the consistent southerly direction seen in individuals of *C. atripectoralis* observed *in situ* in the vicinity of Lizard island (Leis and Carson-Ewart 2003), the same area from which we sampled larvae for this laboratory-based study.

A comparison of *C. atripectoralis* larvae sampled from different regions > 600km apart has revealed that although there may be differences in the mean bearing of larval cohorts among different locations (Leis et al. 2015), the mean bearing of individuals found in the same locality is fairly stable over time (Leis et al. 2014). We would therefore predict that if larvae in the laboratory on Lizard Island were behaving naturally, they would also orientate to the south under control conditions. It is likely that the ability to sense changes in the magnetic field is adaptive (Kirschvink et al. 2001), however for orientation to be calibrated for movement in a preferred direction, the magnetic sense may require the synchronous availability of other cues (Phillips et al. 2002). In the absence of other cues larvae may have used the available magnetic field for a “fixed direction” response, as has been seen in species of birds when cues other than magnetic ones were unavailable (Stapput et al. 2008).

There are two potential explanations for this difference in orientation direction, assuming that a magnetic sense is part of the mechanism allowing southerly *in situ* orientation in larvae caught around Lizard Island. Firstly, despite the remote island location of the research station the proximity of our experimental apparatus to the research station infrastructure may have interfered with the local magnetic field (Kirschvink et al. 2010, Putman et al. 2014a). Indeed, there is evidence that anthropogenic electromagnetic radiation in the radio frequency may alter the orientation behaviour of animals to the earth’s natural magnetic field (Ritz et al. 2004, Landler et al. 2015). This may explain why the larvae oriented in directions closer to those observed in the field within the coil compared to the magnet arena. The local magnetic field in the coil may have experienced less interference than that of the magnet arena, because it was further away from the research station and had properties similar to a Faraday cage when grounded. In order to minimize the potential for radiofrequency interference, magnetic orientation experiments on fish larvae should use electrically-grounded aluminium screening to attenuate background electromagnetic noise and be located as far from infrastructure as possible (Engels et al. 2014).

Secondly, larvae were captured in light traps placed predominantly in an area ~1 km northwest of the test location, a similar direction to their mean bearing during control treatments. If orientation of fish larvae is based on true navigation, then the directional discrepancy between laboratory and *in situ* orientation may relate to larvae orienting themselves towards the last known familiar environment where reef habitat was in close proximity. Various taxa have demonstrated a compensatory ability for spatial displacement (Phillips 1986, Boles and Lohmann 2003, Thorup et al. 2011), however true navigation remains debatable in many species (Papi 2006, Mouritsen et al. 2013b). This explanation seems less likely, as displacement to various locations around Lizard Island did not result in different orientation directions for *C. atripectoralis in situ* (Leis and Carson-Ewart 2003). It also does not address why orientation directions were different between the research station-based magnet arena and the more distant magnetic coil.

Finally, fish larvae are known to have dynamic ontogenies in relation to sensory responses in their early life history (see Chapter 2). It is hypothesised that fish larvae may cue switch between different modalities within a sensory hierarchy depending on spatial scale and growth stage (Kingsford et al. 2002). As the larvae used in this experiment were at settlement stage after a period of pelagic movement and development, and were caught in close proximity to reef habitat, they may have switched from large scale cues to finer scale cues. As they may have been pre-exposed to olfactory and auditory cues from the reef it is possible that this behavioural shift between prioritised sensory modalities contributed to the variability observed in orientation data in within the test arena.

Aside from biological applications, if a magnetic sense plays an important role in orientation of larval fishes, then it has ecological implications for anthropogenic development of marine areas. Increasing interest in renewable energy sources of hydrokinetic energy and offshore wind power has led to research into the potential effects on marine organisms of electromagnetic field alteration due to submarine electrical infrastructure (Öhman et al. 2007, Inger et al. 2009, Bevelhimer et al. 2013). Electromagnetic field disturbance has the potential to disrupt the natural orientation of animals that use a magnetic sense for orientation (Engels et al. 2014). In the case of individuals that move long distances such as coral reef fish larvae, alterations to the local geomagnetic field could have unpredictable influences on spatial patterns of dispersal.

C. atripectoralis larvae did not have strong among-individual orientation within the magnetic coil. Although there was an easterly mean bearing during the control and a southerly mean bearing when the magnetic field polarity was shifted by 90°, neither treatment was significantly oriented. However, the individual responses of the larvae between control and shifted treatments revealed a change in orientation significantly proportional to the shift in magnetic field polarity. This response indicates the ability to sense and interpret directional information from the local magnetic field. We investigated only effects of changes in magnetic field polarity in the magnetic coil. Although the use of a polarity-based magnetic compass is hypothesized for fishes and lobsters (Lohmann et al. 1995, Hellinger and Hoffmann 2012), a compass based on the inclination of geomagnetic fields is used by other animals such as birds, amphibians and reptiles (Phillips 1986, Light et al. 1993, Wiltschko and Wiltschko 2005, Lohmann et al. 2008). Future work should investigate behavioural responses to manipulation of other aspects of the magnetic field such as inclination.

Orientation behaviour of larvae in the magnetic coil was different when a polarized light pattern was present than when a diffused light environment was present. Larvae tested in the presence of polarised light showed orientation that was significant or approaching significance towards the southeast in both the control and the shifted treatments, however the distributions of mean bearings between the two treatments were significantly different. As in treatments without the polariser, larvae were oriented more easterly in the control treatment, moving to a more southerly distribution with the shift in the magnetic field. Under the polarizer, however, control treatments were bimodally distributed with a higher directional precision, despite a considerably lower number of replicates available for analysis. The implications of this are two-fold: (i) coral reef fish larvae may be able to detect polarized light patterns, and (ii) polarized light cues are likely to be an important factor in the interpretation of orientation cues. Recent research on larval fish orientation indicates that celestial cues may influence larval orientation (Mouritsen et al. 2013a, Faillettaz et al. 2015), while the ability to sense polarized light has been shown in juveniles of a congener of *C. atripectoralis* (Hawryshyn et al. 2003). Larvae were significantly directional in their orientation under only one of the two polarities in the magnetic coil however, suggesting that the combination of alignments of the polarized light pattern and magnetic field polarities in the coil may have confused larvae attempting to integrate both cues.

Polarised light in itself may play an important role as a large-scale cue for orientation of movement in animals (Reppert et al. 2004). In the majority of cases, animals that demonstrate orientation to polarized light cues are also sensitive to magnetic cues, incorporating the different modalities as part of a sensory hierarchy (Avens and

Lohmann 2003, Banks and Srygley 2003), or as a calibration mechanism for a magnetic compass (Cochran et al. 2004, Muheim et al. 2006b). However a higher directional precision by larval fish in the presence of polarized light in the magnetic coil may relate to a magnetoreception mechanism that requires light to function properly. Such a mechanism, relying on certain wavelengths of light to create a chemical reaction sensitive to natural magnetic fields in photoreceptive photopigments known as cryptochromes, is thought to be critical for interpretation of magnetic compass information in many species (Muheim and Liedvogel 2015). A light-dependent component in magnetic orientation is not necessarily independent of a ferromagnetically-based mechanism however (Phillips et al. 2010). Indeed, the latest mechanism postulated for magnetoreception in *Drosophila* consists of a cluster protein combining the properties of magnetite and cryptochromes (Qin et al. 2015).

The majority of individual larvae showed significant directionality during trials in the circular arena, with a similar median precision to that reported for *C. atripectoralis* larvae *in situ* (Leis and Carson-Ewart 2003)). Significant directionality has now been reported in larvae of multiple coral reef fish species using various observational methodologies (Mouritsen et al. 2013a, Leis et al. 2014, Faillettaz et al. 2015). Maintaining high levels of individual directionality despite being tested in an unfamiliar location may indicate that coral-ref fish larvae use available orientation cues to maintain a straight heading, increasing the chances of finding habitat in a patchy environment, such as a coral reef (Zollner and Lima 1999, Caldwell and Nams 2006), and the capacity for common orientation directions among individuals may be an adaptive response to prevailing current conditions, providing a mechanism to minimise dispersion and transport of fish larvae (Mouritsen et al. 2013a, Leis et al.

2015). This highlights the potential importance of magnetoreceptive abilities to dispersal-relevant behaviours in pre-settlement stage coral reef fishes.

In any case orientating significantly in the absence of visual cues and the orientation response of individuals between control and magnetic treatment conditions provides new evidence of a mechanism to detect the local magnetic field by fishes in the larval stage. This ability to sense magnetic fields in larval fish is likely part of a sensory hierarchy which allows location-independent orientation of swimming, a behaviour that will influence dispersal outcomes and connectivity of fish populations (Mouritsen et al. 2013a). Larvae were not strongly oriented to the same direction observed *in situ* under all control treatments, however orientation of individuals adjusted proportionately to shifts in the local magnetic field, which suggests the need to calibrate the orientation to other sensory cues to provide overall group direction. Orientation behaviour was altered by the introduction of a polarized light pattern, suggesting this as another potential cue used in conjunction with magnetoreception by coral reef fish larvae in the pelagic zone. Further research building on these results should incorporate strategies to mitigate the effects of radio frequency interference and proximity to electrical components, and investigate the role of different light conditions on magnetoreception in larval fishes.

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CHAPTER FIVE

Sediment pollution impacts sensory ability and performance of settling coral reef fish

Newly settled fishes Chromis viridis and Pomacentrus amboinensis (Pomacentridae), Okinawa, Japan

O'Connor JJ, Lecchini D, Beck HJ, Cadiou G, Lecellier G, Booth DJ, Nakamura Y (2016). Sediment pollution impacts sensory ability and performance of settling coral reef fish. *Oecologia*, 180(1), 11-21.

(Author Contributions: JO, YN and DB conceived and designed the experiments. JO, HB, GW and DL performed the experiments. JO and GL analysed the data. JO wrote the manuscript; other authors provided editorial advice)

Abstract

Marine organisms are under threat globally from a suite of anthropogenic sources, but the current emphasis on global climate change has deflected the focus from local impacts. While the effect of increased sedimentation on settlement of coral species is well studied there is little known about the impact on larval fishes. Here, the effect of a laterite "red soil" sediment pollutant on settlement behaviour and post-settlement performance of reef fish was tested. In aquarium tests that isolated sensory cues we found significant olfaction-based avoidance behaviour and disruption of visual cue use in settlement-stage larval fish at $50mgL^{-1}$, a concentration regularly exceeded *in situ* during rain events. *In situ* light trap catches showed lower abundance and species richness in the presence of red soil but were not significantly different due to high variance in the data. Prolonged exposure to red soil produced altered olfactory cue responses, whereby fish in red soil made a likely maladaptive choice for dead coral compared to controls where fish chose live coral. Other significant effects of prolonged exposure included decreased feeding rates and body condition. These effects on fish larvae reared over five days occurred in the presence of a minor drop in pH and may be due to the chemical influence of the sediment. Our results show that sediment pollution of coral reefs may have more complex effects on the ability of larval fish to successfully locate suitable habitat than previously thought, as well as impacting on their post-settlement performance and ultimately recruitment success.

O'Connor JJ, Lecchini D, Beck HJ, Cadiou G, Lecellier G, Booth DJ, Nakamura Y (2016). Sediment pollution impacts sensory ability and performance of settling coral reef fish. *Oecologia*, 180(1), 11-21.

CHAPTER SIX

General Discussion

*Releasing new recruits (*Dascyllus aruanus*) back to coral reef habitat*

General Discussion

In this thesis I present a comprehensive empirical study of sensory abilities influencing orientation behaviour of larval fishes. The new insights into pre-settlement sensory ontogeny and abilities make this thesis an important step forward in our knowledge of larval fish biology, and of the intrinsic and extrinsic factors influencing larval dispersal and settlement. These insights will be used to inform and refine predictive modelling of connectivity in marine populations.

The data presented here confirm that active directional swimming of larvae during the pelagic larval phase is an intrinsic behaviour shared by fish species from different families and geographical regions. Firstly, I characterised for the first time in temperate fish larvae the pre-settlement ontogeny of chemotactic responses to different habitat cues, adding to the few studies done on coral reef species (Dixson et al. 2011). Secondly, I was able to record compelling empirical evidence for the role of behavioural responses to large-scale cues, namely the sun's azimuth and the earth's magnetic field, in orientation behaviour of fish larvae that has previously been observed in the pelagic environment (Leis et al. 2015). Thirdly, I documented the effect of extrinsic factors of benthic habitat on sensory cue use and performance of larval fish, showing that larvae may avoid habitat polluted with increased sediment at settlement. Such habitat also had negative impacts on post-settlement biology, as has been described in previous studies on other settlement-stage Pomacentrids (Wenger et al. 2011, Wenger et al. 2012). Sensory abilities, behavioural ontogeny and habitat effects revealed in this study may help us understand the factors underpinning the connectivity evidence indicating a behavioural influence on larval dispersal (Green et al. 2014, Jones 2015) and will be used in a comparative modelling study to investigate how predictions of larval fish dispersal are affected.

For two temperate fishes that recruit to estuarine habitats, active choice behaviour in response to olfactory cues from estuarine and coastal water began in larvae shortly after tail flexion. The ontogeny of this chemotactic response was consistent across multiple cohorts in both species, and interestingly was species-specific, with each species preferring opposing olfactory cues. In comparison with ontogenetic data on swimming ability in these two species, the size at which larvae begin actively using olfactory preferences to direct their swimming correlates to the size at which swimming endurance drastically increases in these species (Clark et al. 2005). This correlation of behavioural ontogenies indicates for the first time a quantifiable "ontogenetic event" in the larval phase of these fishes when chemotaxis and habitat cues begin to influence dispersal (Fuiman et al. 1998). This new dynamic theory of behavioural ontogeny of fish larvae needs more consideration in contemporary dispersal models (Leis 2007).

Behavioural ontogeny of fish larvae has rarely been investigated at as fine a temporal scale (21 days across three annual cohorts including the pilot study) as used in this thesis, often comparing responses before and after settlement (Wright et al. 2005, Lecchini et al. 2007). The few studies at a finer temporal scale have been primarily on tropical species (Dixson et al. 2011, Wright et al. 2011). The ontogeny of swimming abilities has been more widely studied (Leis et al. 2006, Leis et al. 2007, Guan et al. 2008, Faria et al. 2009, Leis et al. 2012), with species across different families showing similar ontogenetic increases in swimming endurance as *M.*

novemaculeata and *A. japonicus* (Houde 1969, Fisher et al. 2000, Leis et al. 2009). Studies on behavioural ontogeny in larvae across a wider range of taxa are needed to test the theory of the behavioural-physiological ontogenetic event marking the start of behavioural influence during the larval phase.

Studies on larval orientation in the pelagic environment hypothesised a sun compass as a likely mechanism due to reduced directional precision during the middle of the day and under cloud cover (Leis and Carson-Ewart 2003, Leis et al. 2014, Faillettaz et al. 2015). Clock-shifting experiments also indicated sun compass orientation in Apogonid larvae (Mouritsen et al. 2013). Here, orientation behaviour of coral reef fish larvae exposed to artificial celestial cues in an experimental chamber supports the sun compass mechanism theory. Furthermore, orientation behaviour was affected by altering local magnetic field conditions, showing the capacity for magnetoreception in coral reef fish larvae. These sensory abilities are surprising in such young, tiny animals, however previous studies on other modalities have revealed sensitive olfactory, visual and auditory abilities of fish larvae by the settlement stage (Leis et al. 2011). Being able to orient horizontal swimming using the sun and geomagnetic fields changes the way spatial scales at which larvae influence their dispersal are approached in marine population ecology.

Perhaps unsurprisingly in light of these results, polarised light patterns influenced orientation behaviour in both the sun compass and magnetoreception experiments. In animals known to navigate over large spatial scales, celestial cues and magnetic cues work together to calibrate and operate an orientation compass (Quinn 1980, Cochran et al. 2004, Muheim et al. 2006, Phillips et al. 2010). It appears there is a similar mechanism in pelagic orientation by larval fishes. The ability to use large-scale cues to orient swimming may assist with the behavioural influence on the non-random patterns of larval dispersal, the mechanisms of which have long been mysterious (Kough et al. 2014, Jones 2015). Now that we have empirical evidence of the ability and inclination of larval fish to use large scale cues for orientation, predictive modelling of dispersal will need to consider enhancing the complexity of the behavioural components, and the scale at which sensory cues become relevant.

Conditions of the benthic habitat influenced settlement behaviour of coral reef fish larvae, with the presence of sediment affecting responses to sensory cues from habitat. This thesis contributes to empirical data on the negative effects of increased sediment pollution on settlement of larval fishes which include increased predation, prolonged pelagic larval duration and disrupted ability to locate favourable habitat (Wenger et al. 2013, Wenger and McCormick 2013, Wenger et al. 2014). The presence of sediment with habitat cues disrupted the ability of settlement-stage larvae to perform natural choice behaviours by using olfactory and visual cues, which are critical for selecting favourable habitat so crucial for both short and long-term survival (Brooker and Dixson 2016). This may be responsible for the altered patterns of larval habitat choice in the presence of sediment recorded in previous studies (Wenger et al. 2011, Wenger and McCormick 2013). Management of pollution in marine and coastal areas should consider the potential effect of sediment at the levels described here on recruitment in fish populations.

The benthic habitat also played a role in sensory responses of estuarine-associated fish larvae, with seagrass cues influencing chemotaxis between coastal and estuarine water. This thesis supports previous work showing the effect of seagrass chemical cues on larval settlement behaviour (Hadfield and Paul 2001, Arvedlund and Kavanagh 2009, Radford et al. 2012). Again, in light of global fish stock decline and anthropogenic pollution of the marine environment, the importance of these cues to larval replenishment of fish populations must not be understated (Carpenter et al. 2008, Worm and Branch 2012). Aside from providing essential nursery habitat for juvenile fishes, orientation cues for larvae in coastal waters provided by seagrass beds may be an important contributing factor for recruitment. This should be taken into account in management and conservation of seagrass habitats, which are also in global decline (Short et al. 2014).

Increasing our understanding of ecological factors influencing larval dispersal assists our ability to anticipate how changing climate and environmental conditions will affect marine populations in the future. For organisms that rely so much on detecting and interpreting chemical cues for successful dispersal during this life history stage, changes in the marine environment may have serious consequences (Dixson et al. 2014). On top of this, if behavioural ontogeny has a widespread influence on larval dispersal it is a factor that may be susceptible to predicted sea temperature rises, as warmer temperatures can alter the timing of ontogenetic events (Fuiman et al. 1998). In this case olfaction ontogeny of larval fishes may be a process vulnerable to anthropogenic impacts in the marine environment. This has the potential to affect the spatial distribution of populations, as ontogenetic changes in sensory responses often coincide with habitat shifts during the larval phase (Higgs and Fuiman 1998). With biodiversity on coral reefs already in a global decline, it is critical to determine how the changing environment will affect the sensory ontogeny of dispersing larvae on which the resilience of fish populations rely (Hays et al. 2005, Brooker and Dixson 2016).

The behavioural studies in this thesis were largely laboratory-based, such is the nature of attempting to isolate, manipulate and simulate realistic orientation cues for very discerning fish larvae. Orientation behaviour of fish larvae under laboratory conditions has to do with directional tendency, which is treated here as a proxy for intended straight line swimming direction. Orientation direction in a circular chamber has been shown in *in situ* experiments however to be statistically similar to that of free-swimming larvae observed by divers on scuba (Leis et al. 2014). Another encouraging factor supporting the validity of orientation behaviour observed in my laboratory experiments is that larvae oriented in a similar direction to those of previous studies of the same species and locality under both control and manipulated conditions (Chpts. 3 & 4). While the laboratory experiments allowed me to manipulate and quantify particular cues in a way near impossible to do in natural conditions, the cues and environment larvae were exposed to were artificial and isolated. The directionality of orientation behaviour may be underestimated as swimming individuals may move back and forth along the perimeter of the chamber rather than stay still in one location. This tendency appeared to be a behavioural trait with individual variation, as would be the stress response to experimental conditions.

This thesis opens up the following important options for future research: (i) development of new dispersal models and validating them using spatial patterns of larval recruitment in the field across a wider range of taxa, (ii) further observations of larval orientation in the pelagic environment using new tracking techniques such as fluorescent nanoparticles (Ekvall et al. 2013) or remotely operated vehicles (Yim et al. 2013), (iii) expanding manipulation of orientation cues to *in situ* conditions using techniques such as the Drifting In Situ Chamber or DISC (Paris et al. 2013) and developing polarising and magnetic additions, (iv) investigation of both behavioural and physiological mechanisms of magnetoreception in fish larvae (histology of olfactory organs in *C. atripectoralis* gave inconclusive results for the presence of magnetite – Fig. 7A), (v) mapping the ontogenetic development of large-scale cue use. Each of these options is likely to require the development of new sampling techniques and experimental apparatus.

Synthesising the chapters of this thesis, we now better understand which sensory cues fishes use during their early life history and how they develop with ontogeny. It has been theorised that larval fishes are able to use a suite of cues at different spatial scales to allow them to orient swimming towards habitat (Kingsford et al. 2002), however this is the first empirical data we have on the effect of larger scale celestial and magnetic cues on larval fish behaviour. It now seems apparent that sun position and magnetic fields are able to be utilised by coral reef fish larvae to orient when out of range of olfactory and auditory cues, as has been observed *in situ*, as a way of limiting advection away from natal sites by ocean currents (Leis and Carson-Ewart 2003, Leis et al. 2014). Olfactory cues are still important in the pre-settlement stage, and were useful in demonstrating that sensory modalities are not used uniformly by fish larvae throughout the pelagic phase.

It is likely that this sensory hierarchy functions by using larger scale cues to either keep fish larvae in a spatial position to begin recruitment once a certain growth stage has been reached or to guide larvae in a direction favourable for optimal dispersal strategies. Once a species-specific growth stage is reached fish larvae can then switch to finer scale cues to choose settlement habitat. Due to the different dynamics in development between tropical and temperate species there are likely to be differences in how these sensory hierarchies are used, however it is also likely there are inter-specific differences between species sharing similar habitats. This use of a sensory hierarchy to find habitats may help to explain the high levels of recruitment reported in some fish populations, however we also need to understand how these sensory abilities assist dispersal and connectivity to other populations. In any case, this thesis calls for a refinement of dispersal modelling to incorporate location independent swimming and species-specific activation of chemotaxis with ontogeny. The location-dependant effect of environmental pollution on this sensory hierarchy must also be taken into account to generate more realistic predictions of connectivity.

Final conclusion

In my thesis I have looked at behavioural aspects of marine fish dispersal during the larval phase from tail flexion through to post-settlement and found that larval behavioural phenotypes must not be viewed as static, but dynamic. Sensory responses are not simply present or absent, but also change over time and may be linked to intrinsic biological ontogenetic events or extrinsic environmental factors. Quantifying these events and factors is a new direction in linking biology and behaviour of marine larvae. The sensory abilities of marine larvae continue to astound and challenge our expectations of what animals are capable of regardless of their size, and here I have quantified for the first time the ability of larvae to use large-scale cues for directional information. I have been fortunate to be part of the vanguard of researchers discovering these abilities, however there is much more research required to solidify our understanding of the mechanisms and ecological significance of these abilities. Just like a larval fish finding itself in deep offshore waters, we are still a long way from home.

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APPENDIX

Further experimental data and descriptions

Author and supervisor (Jeff Leis) working with larvae in situ, Lizard Island, Great Barrier Reef, QLD

Fig. 1A Dye test example, with water of equal salinity. Dotted line indicates the maximum intrusion by stratified dye when testing water of different salinities.

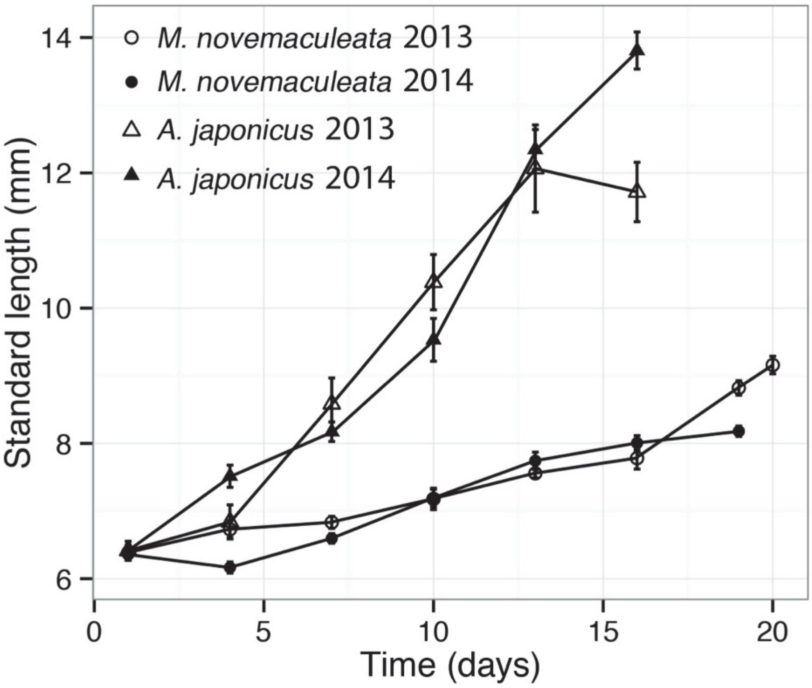

Fig. 2A Mean standard length (±SE) on each day of the experimental period (not days post-hatch) for *M. novemaculeata* and *A. japonicus* cohorts in 2013 and 2014.

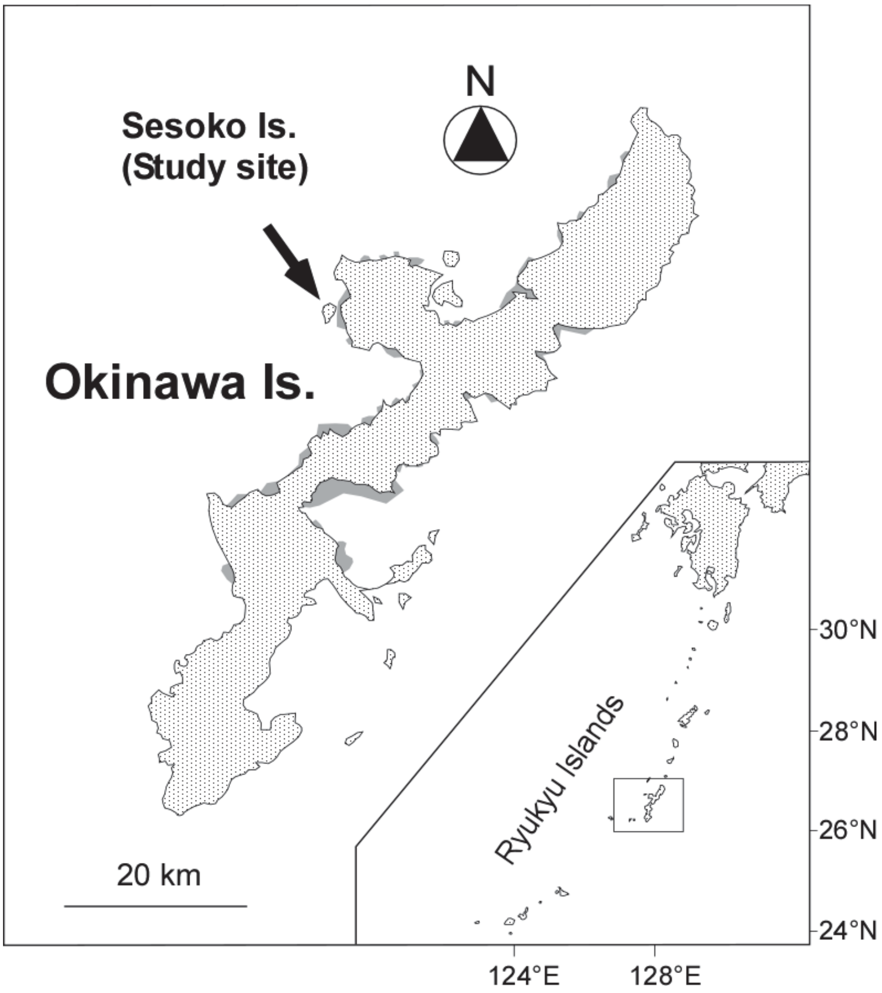

Fig. 3A Map showing location of the study site and red soil pollution areas around Okinawa Island in 1992-1993 (dark coloured areas in the map)

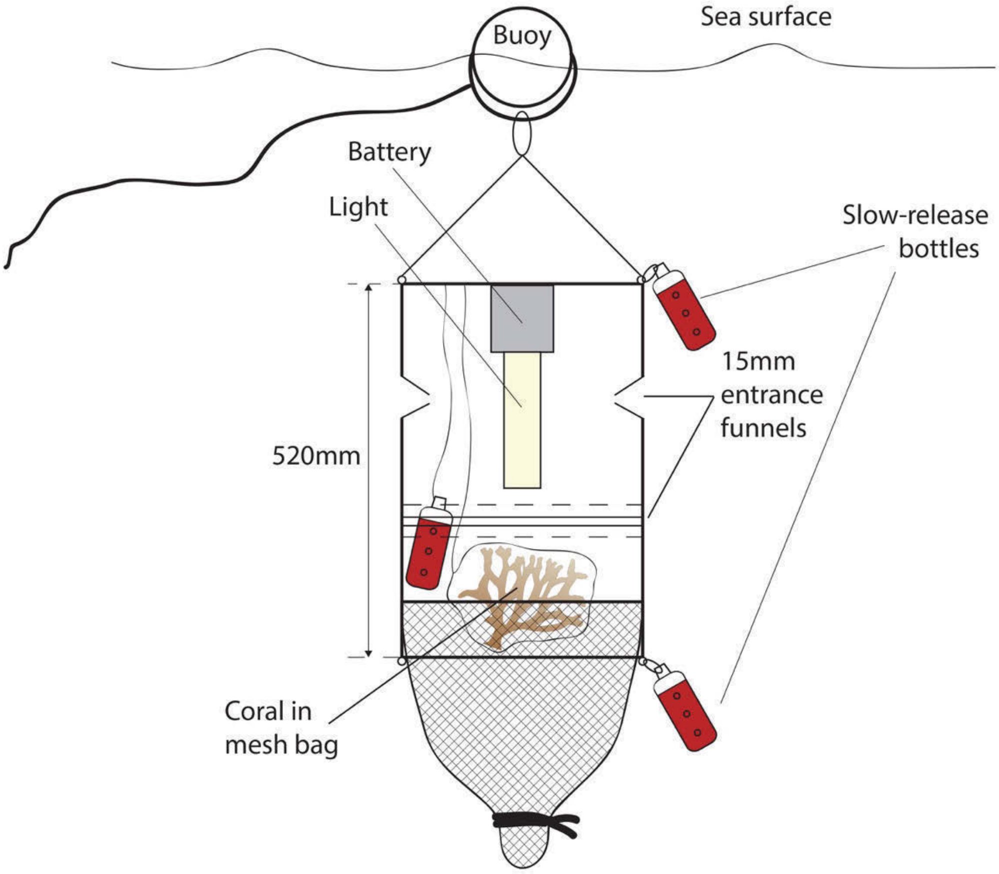

Fig. 4A Diagram of light trap setup showing placement of slow release bottles and live coral. Treatments without red soil or coral had empty bottles and/or mesh bag attached

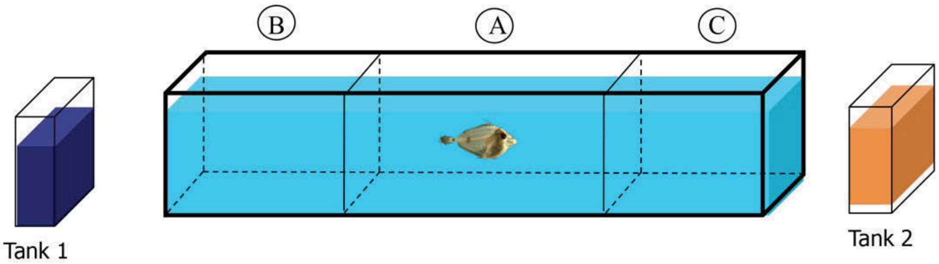

Fig. 5A Test chamber used for two visual cues placed in Tank1 and Tank 2. Larvae is released into the central compartment (A) and time is recorded spent in each compartment, indicating a choice for cues from Tank 1 (B), Tank 2 (C) or no choice (A)

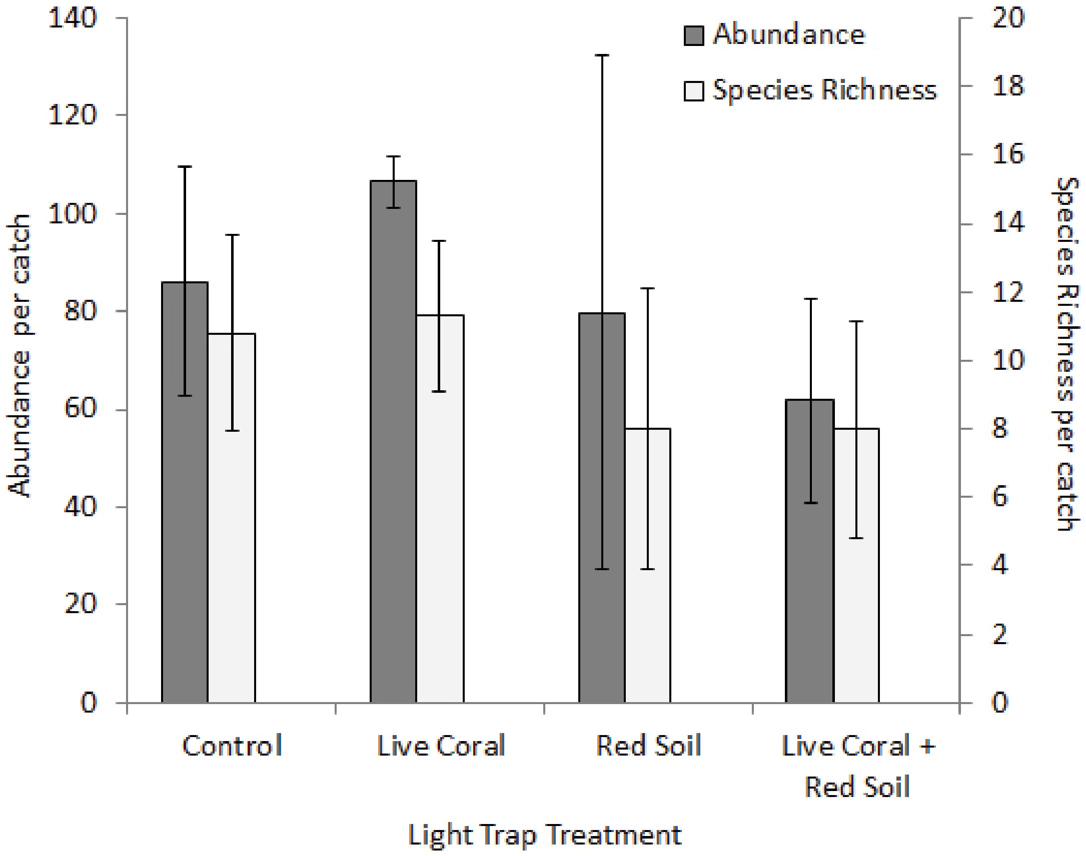

Fig. 6A Mean abundance (n_{total} = 3343) and species richness (47 coral reef species recorded) of larval fish caught by different light trap treatments per day (±SE)

Fig. 7A *C. atripectoralis* larvae collected via light trap and not used in behavioural experiments were fixed for histology by immersion for 1 week in 10%NBF. Standard histology processing protocols were followed and each sample was embedded in Paraplast+ tissue embedding media. The embedded samples were sectioned transversely at a thickness of 5 micron with a Leica RM 2235 microtome. To demonstrate the presence of intracellular materials containing Fe^{II} and Fe^{III}, we stained the sections with Perl's stain using the DAB intensification method (Meguro et al. 2007)(B, white scale bar = 200 μm). Trout olfactory rosettes were used as a control for magnetite crystal staining, following Diebel et al. (2000). Inconclusive stained cells in olfactory epithelium circled (A, scale bar = 100 μm). The olfactory rosette was the focus of attention in larval samples in this study, however no obvious positively stained cells visible (C, 40 μm).

Table 1A General Linear Model of larval fish abundance in three light trap positions. Coefficients are significant ($p < 0.001$), with treatments listed in ascending order of predicted effect on light trap catch abundance of larval fish.

Treatment	Estimate	Std. Error	*Z* value	p
Control + Red Soil	**0.76913**	0.06376	12.06	< 0.001
Live Coral + Red Soil	**1.15986**	0.04062	28.55	< 0.001
Control	**1.29586**	0.04536	28.57	< 0.001
Live Coral	**1.61244**	0.03872	41.64	< 0.001

Table 2A Mean pH levels for water from control and two red soil concentrations from 0-2 hours post-mixing and 3-7 days post-mixing

Treatment	Time	pH
Control	0h	8.10
50mgL^{-1}	0h	8.09
200mgL^{-1}	0h	8.08
Control	2h	8.10
50mgL^{-1}	2h	8.09
200mgL^{-1}	2h	8.07
Control	3days	8.10
50mgL^{-1}	3days	8.09
200mgL^{-1}	3days	8.06
Control	7days	8.10
50mgL^{-1}	7days	8.08
200mgL^{-1}	7days	8.04

References

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