ResearchOnline@JCU

This file is part of the following reference:

Nadler, Lauren Elizabeth (2016) Behavioural and physiological effects of shoaling in a coral reef fish. PhD thesis, James Cook University.

Access to this file is available from:

http://researchonline.jcu.edu.au/46428/

The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owner of any third party copyright material included in this document. If you believe that this is not the case, please contact <u>ResearchOnline@jcu.edu.au</u> and quote <u>http://researchonline.jcu.edu.au/46428/</u>



Behavioural and physiological effects of shoaling in a coral reef fish

Thesis submitted by

Lauren Elizabeth Nadler, B.A., M.Res.

August 2016

For the degree of Doctor of Philosophy College of Science and Engineering James Cook University The research presented in this thesis was conducted in accordance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals, 8th Edition (2013) and the Queensland Animal Care and Protection Act (2001). This research was conducted under animal ethics approval from the James Cook University Animal Ethics Committee (A2103).

Statement of the Contribution of Others

This thesis includes collaborative work conducted with my supervisors, Prof. Mark McCormick, Prof. Phil Munday and Dr. Paolo Domenici, as well as Dr. Shaun Killen, Dr. Jacob Johansen, Ms. Eva McClure and Dr. Sue-Ann Watson. As part of this collaboration, I was responsible for project concept and design, data collection, data analysis, interpretation of results and manuscript writing. My co-authors provided intellectual guidance, editorial assistance, financial support and technical assistance.

Financial support for this work was provided by the Lizard Island Reef Research Foundation Doctoral Fellowship (L. Nadler), GBRMPA Science for Management Award (L. Nadler), James Cook University Graduate Research Scheme (L. Nadler), Australian Postgraduate Award (L. Nadler), International Postgraduate Research Scholarship (L. Nadler), Australian Research Council Discovery Project Scheme (M.I. McCormick) and the ARC Centre of Excellence for Coral Reef Studies (M.I. McCormick and P.L. Munday).

Acknowledgements

This thesis could not have been completed without the assistance, guidance and patient support of many people. First, thank you to my supervisors, Mark McCormick, Phil Munday and Paolo Domenici. Many thanks to Mark for giving me the freedom to explore my ideas, providing support in trouble-shooting unforeseen problems and offering general career advice to help me continue my future science journey. Thank you to Phil for teaching me about climate change research, strong experimental design and robust scientific writing practices throughout the publication process. Lastly, thank you to Paolo for giving up many hours of your time from afar over Skype (often at odd times due to the time change to Italy) to help me understand the vast literature on escape behaviour, to aid in devising a framework for quantifying schooling dynamics and to provide general career advice on how to progress past the PhD in an academic career.

I would also like to thank my collaborators on this thesis work. In particular, thank you so much to Shaun Killen for teaching me an incredible amount about how to run respirometry experiments and interpret the data as well as for being exceptionally generous with your equipment and time. Also, thanks to Jacob Johansen for providing advice while building my swim tunnel, which was used during data collection for two of my chapters. Many thanks to Sue-Ann Watson for providing limitless advice on how to design and run CO₂ experiments. Thank you as well to my amazing field assistants throughout my PhD, who all worked incredibly hard, including Eva McClure, Amy Cox, Carly Giosio, Laura Smith, Paloma Matis, Ana Guerra, Rahel Zemoi and Katherine Corkill. Thanks to Rhondda Jones for providing patient advice on statistics and R code throughout my candidature. Also, to my fellow lab-mates in the McCormick and Munday labs, thank you so much for providing support and fun throughout my PhD experience,

both in the field and at home. To my peer-editing crew, Paloma Matis, Maria Palacios and Steve Doo, thanks so much for all of your constructive and positive comments that helped enormously to build up my confidence in my scientific writing skills.

To everyone at the Lizard Island Research Station, thank you so much for allowing me to be apart of your beautiful and incredibly special community. Thank you to Anne Hoggett and Lyle Vail for your amazing dedication to LIRS and for providing endless support and enthusiasm for the science that is conducted at the station. Thank you also to the fantastic support staff that have helped me on many of my trips and helped make the social aspects of LIRS that much more fun, including Stuart, Kim, Bruce, Cassy, John and Marianne. Many thanks to the Lizard Island Reef Research Foundation for providing the resources to spend as much time as I did at LIRS and for helping to promote my work through social media and travel funds to international conferences.

Finally, thank you to my amazing family for supporting and encouraging me throughout this process. To my partner, Stephen, I am eternally grateful for everything you have done over the last nearly four years to help get me to this point, including moving to Australia, helping me build crazy contraptions for experiments, putting up with my frequent many-month absences for fieldwork/conferences and basically being my personal chef for the last 6 months or so of my PhD so that I didn't starve while writing up. Many thanks to my loving family, particularly my Mom, Dad and sister Meredith for always providing a supportive ear to bend and pushing me to follow my ambitions wherever they may take me (even if that happened to be half way around the world).

Animals must rapidly perceive, process and react to sensory information from their ambient environment in order to survive. For this reason, many animal species partake in cooperative group behaviours to enhance their survival, as having "many-eyes" increases an individual's chances of being informed of important stimuli. Fish shoals (social groups of fishes) are a classic example of a prolific, cooperative group behaviour found in nature. Approximately 50% of all fish species in the world's oceans shoal at some point during their lives. Therefore, effective execution of this behaviour is essential for the survival and success of many ecologically and economically important fish species. Shoaling provides benefits to a range of processes, including foraging, reproduction, social learning, predator avoidance and energetic demand. These benefits may vary depending on shoal composition and environmental conditions, but these effects remain poorly understood.

To better understand the costs and benefits of shoal membership, this thesis examined how factors external to and within the shoal affect the energy use by group members. These studies used the shoaling tropical damselfish *Chromis viridis* as a model species, as it thrives in the laboratory and is abundant near my study site at the Lizard Island Research Station (LIRS) in the northern Great Barrier Reef, Australia, making experimental laboratory manipulations possible.

Predator avoidance is one of the most important and well-studied benefits of grouping behaviour. In coordinated schools, predators have trouble focusing on specific prey to attack, creating a "confusion effect" that allows the group to benefit from higher survival. However, studies suggest that school cohesion and coordination may exhibit plasticity in response to environmental factors. In **Chapter 2**, I investigated the effect of water flow regime at a school's home reef on escape performance. Schools were collected from shallow reefs surrounding LIRS. The relative flow regimes for each collection site were measured over a three-week period. The school's escape response

vi

to an aerial mechanical stimulus was recorded in high-speed (240 fps) in a laminar flow swim tunnel. While school coordination and cohesion were unaffected by water flow regime, individual fast-start performance improved significantly in schools collected from high-flow regime reefs when compared to those from lower flow habitats.

The level of familiarity among the individuals of a group is also likely to impact school coordination and cohesion. Familiarity is a trait that develops following a prolonged period of social interaction among individuals and aids in fitness-enhancing processes such as foraging and social learning. Therefore, in **Chapter 3**, using the approach outlined for **Chapter 2**, I examined if familiarity of school members influenced the timing, maneuverability and propulsive performance of escape responses in fish schools. Members of familiar schools exhibited superior escape performance, with shorter latency times (higher reactivity), greater average turning rates (increased maneuverability) and longer distances covered (greater propulsive performance) than individuals from unfamiliar schools.

Group living may also induce a "calming effect" on individuals, reducing overall metabolic demand. This effect could occur by minimising the need for individual vigilance and reducing stress when allowed to associate with conspecifics. However, this effect has proved challenging to quantify due to the difficulty in isolating individuals for testing. In **Chapter 4**, I examined the effect of shoaling on metabolism and body condition. Using a novel respirometry methodology for social species, we found that the presence of visual and olfactory cues from shoal-mates led to a reduction in the estimated minimum metabolic rate of individuals. Fish held in isolation for one week also exhibited a reduction in body condition when compared to those held in shoals.

To better understand the results of my previous three chapters in the context of projected future global change, I examined the effect of elevated carbon dioxide on familiarity and metabolism in shoaling fish in **Chapter 5**. Shoals were acclimated to one of three CO₂ treatments: control (450 µatm), mid-CO₂ (750 µatm) or high-CO₂ (1000 µatm), with the latter representing projected CO₂ conditions for the year 2100. Familiarity was examined using a choice test. Under control conditions, individuals preferentially associated with familiar shoal-mates. However, this association was lost

vii

under both elevated CO₂ treatments. Yet, this loss of familiarity did not impact the calming effect of shoaling on metabolism (as measured using the methodology outlined in **Chapter 3**). Under all CO₂ treatments, individuals exhibited a significantly lower metabolic rate when measured in a shoal versus alone, highlighting the complexity of shoal dynamics and the processes that influence shoaling's benefits.

Understanding how organisms behave as a group is essential for assessing responses at the population and community level, particularly in socially and environmentally dynamic ecosystems such as coral reefs. My results suggest that individual behaviour and physiology is greatly influenced by both group living and habitat characteristics. Yet, these dynamics may be modulated by differences in shoal composition, through traits like familiarity, and projected future global change conditions. However, some benefits of group living, like the calming effect, may persist under climate change, potentially through sensory redundancy. These studies highlight the complexity of social behaviours on coral reefs and suggest that degree of sociality should be considered in studies on behaviour and physiology of coral reef fishes.

Table of Contents

DECLARATION OF ETHICS	ïI
STATEMENT OF THE CONTRIBUTION OF OTHERS	III
ACKNOWLEDGEMENTS	IV
GENERAL ABSTRACT	VI
LIST OF TABLES	XI
LIST OF FIGURES	XII
CHAPTER 1: GENERAL INTRODUCTION	1
1.1 SHOALING VERSUS SCHOOLING: DEFINITIONS	1
1.2 BENEFITS OF SHOALING	2
1.3 PLASTICITY IN SHOALING BEHAVIOUR	6
1.4 CLIMATE CHANGE AND SHOALING	7
1.5 SOCIAL BEHAVIOUR IN CORAL REEF FISHES	9
1.6 STUDY SPECIES	11
1.7 AIMS AND OBJECTIVES	11
CHAPTER 2: ROLE OF WATER FLOW REGIME IN THE SWIMMIN	<u>G BEHAVIOUR AND</u>
ESCAPE PERFORMANCE OF SCHOOLING FISH	13
2.1 SUMMARY	13
2.2 INTRODUCTION	14
2.3 MATERIALS AND METHODS	16
2.3 RESULTS	22
2.4 DISCUSSION	25
CHAPTER 3: FAMILIARITY IMPROVES FAST-START ESCAPE PE	RFORMANCE IN
SCHOOLING FISH	31
3.1 SUMMARY	31
3.2 INTRODUCTION	32
3.2 MATERIALS AND METHODS	33
3.3 RESULTS	38
3.4 DISCUSSION	42
CHAPTER 4: SHOALING REDUCES METABOLIC RATE IN A GREG	ARIOUS CORAL REEF
FISH SPECIES	45
4.1 SUMMARY	45
4.2 INTRODUCTION	45
4.3 MATERIALS AND METHODS	47
4.4 RESULTS AND DISCUSSION	51

CHAPTER 5: EFFECT OF ELEVATED CARBON DIOXIDE ON SHOAL FAMILIARITY AND	<u>)</u>
METABOLISM IN A CORAL REEF FISH	55
5.1 SUMMARY	55
5.2 INTRODUCTION	56
5.3 MATERIALS AND METHODS	59
5.4 RESULTS	67
5.5 DISCUSSION	70
CHAPTER 6: GENERAL DISCUSSION	75
6.1 PLASTICITY IN SCHOOL KINEMATICS	75
6.2 THE CALMING EFFECT OF SHOALING TO INDIVIDUALS	80
6.3 FAMILIARITY IN FISH SHOALS	84
6.4 ENVIRONMENTAL IMPACTS ON SHOALING FISH	86
6.5 CONCLUDING REMARKS	86
REFERENCES	87
ADDENDIV 1. EVIDENCE OF DUVELOI OCICAL ACCODTMENT AMONG WILD SCHOOLS	OF

<u>APPENDIX 1: EVIDENCE OF PHYSIOLOGICAL ASSORTMENT AMONG WILD SCI</u>	<u> 100ls uf</u>
A CORAL REEF FISH	121
A.1 SUMMARY	121
A.2 INTRODUCTION	122
A.3 MATERIALS AND METHODS	125
A.4 RESULTS	130
A.5 DISCUSSION	133
A.6 REFERENCES	137

List of Tables

TABLE 2.1. SUMMARY OF THE GENERALISED LINEAR MODEL ANALYSIS INVESTIGATING THE EFFECT OF WATER FLOW	l
RATE ON SCHOOL SWIMMING BEHAVIOUR, INCLUDING SCHOOL SHAPE, SCHOOL DENSITY, NEAREST NEIGHBOUF	ł
DISTANCE AND ALIGNMENT (N = 11 FOR ALL MODELS)	.23
TABLE 5.1. SUMMARY OF SEAWATER CHEMISTRY PARAMETERS IN CONTROL AND ELEVATED CARBON DIOXIDE	
TREATMENTS FOR EXPERIMENTS 1 and 2 . Estimated pCO $_2$ was calculated in the program CO2SYS	
USING THE OTHER MEASURED PARAMETERS. IN SITU PC O_2 was measured using a portable CO_2	
EQUILIBRATOR WITH NON-DISPERSIVE INFRARED (NDIR) SENSOR. ERROR IS S.E.M	.61

List of Figures

FIG. 1.1. VENN DIAGRAM ILLUSTRATING HOW COMMON FISH BEHAVIOURS ARE EXECUTED AS A SHOAL, SCHOOL OR INDIVIDUAL. FIGURE FROM PITCHER (1983)
FIG. 1.2. ILLUSTRATIONS OF SHOALS WITH (À) LOW COHESION AND ALIGNMENT, (B) HIGH COHESION AND LOW ALIGNMENT, (C) LOW COHESION AND HIGH ALIGNMENT AND (D) HIGH COHESION AND ALIGNMENT
FIG. 2.1. (A) MAP OF THE SEVEN COLLECTION SITES. (B) MEAN WATER FLOW RATE AT EACH OF THE COLLECTION SITES (± SEM). (C) COEFFICIENT OF VARIATION IN WATER FLOW AT EACH OF THE COLLECTION SITES
FIG. 2.2. SCHOOL PERFORMANCE THROUGHOUT THE ESCAPE RESPONSE, INCLUDING (A) NEAREST NEIGHBOUR DISTANCE (NND) AND (B) VARIABILITY IN INDIVIDUAL ALIGNMENT, AT 0, 20 AND 100 MS POST-STIMULUS.
DARS ARE MEAN \perp 5.E.M
INCLUDING (A) LATENCY (MS; LOG Y-AXIS), (B) AVERAGE TURNING RATE (°/S), (C) DISTANCE COVERED (MM) AND (D) DIRECTIONALITY (PROPORTION OF AWAY RESPONSES)
FIG. 3.1. TIME TO FAMILIARITY IN THE CORAL REFE DAMSELENCE CHROMIS VIRIOUS WHITE RARS INDICATE THE COUNT
OF INDIVIDUALS (OUT OF EIGHT TOTAL PER DAY) THAT PREFERRED THE FAMILIAR SCHOOL, WHILE THE GREY
BARS DENOTE THOSE INDIVIDUALS THAT PREFERRED THE UNFAMILIAR SCHOOL. PREFERENCE WAS DEFINED AS
THE SCHOOL THAT THE FOCAL INDIVIDUAL SPENT THE GREATEST PROPORTION OF TIME WITH DURING THE 15 -
MIN TRIAL. THE DOTTED LINE DENOTES THE NULL HYPOTHESIS OF AN EVEN $50{:}50$ SPLIT BETWEEN THE TWO
SCHOOLS ON EACH DAY (WHICH WOULD INDICATE NO PREFERENCE)
FIG. 3.2. EFFECT OF FAMILIARITY ON THE ESCAPE RESPONSE OF INDIVIDUALS IN FISH SCHOOLS (CHROMIS VIRIDIS) ON
(A) ESCAPE LATENCY (MS), (B) FREQUENCY DISTRIBUTION OF ESCAPE LATENCIES, (C) AVERAGE TURNING RATE
(°/S), (D) TYPICAL ESCAPE RESPONSES (LINES REPRESENT THE FISH MIDLINE AND ARROWS INDICATE THE
LOCATION OF THE HEAD IN SUCCESSIVE FRAMES AT 4.2 ms intervals) and E) distance covered. Fish
ILLUSTRATIONS (A, C AND E) REPRESENT THE ACTION TAKEN IN EACH STEP OF THE RESPONSE (GREY FISH
REPRESENT THE FISH'S POSITION IMMEDIATELY PRIOR TO THE STIMULUS AND THE BLACK FISH ILLUSTRATES THE MOVEMENT TAKEN). DATA IN A, C AND E ARE REPRESENTED BY MEAN ± S.E.M. ASTERISKS (**) INDICATE
STATISTICAL SIGNIFICANCE ($P < 0.05$)
FIG. 3.3. EFFECT OF FAMILIARITY ON ESCAPE PERFORMANCE OF FISH SCHOOLS (<i>CHROMIS VIRIDIS</i>). (A) NEAREST
NEIGHBOUR DISTANCE (NND) DENOTES THE DISTANCE TO THE CLOSEST NEIGHBOUR (CENTER OF MASS TO
CENTER OF MASS, MM J. (B) SCHOOL AREA INDICATES THE SCHOOL S HORIZONTAL SPREAD (CM ²). (C)
ALIGNMENT IS A MEASURE OF THE VARIATION IN THE URIENTATION OF ALL SCHOOL MEMBERS. THIS VARIABLE
IS CHARACTERISED BT THE LENGTH OF MEAN CIRCULAR VECTOR (R)
FIG. 4.1. THE EXPERIMENTAL SETUP WAS COMPOSED OF AN INNER RESPIREMENT CHAMBER (LENGTH $=$ 13.3 CM, INNER DIAMETER = 3.24 CM VOLUME OF CHAMBER AND ASSOCIATED CAS-IMDERMEARIETURING = 100 MI)
AND A LARGER OUTER SHOAL-MATE HOLDING CHAMBER (LENGTH = 120 cm inner diameter = 114 cm
VOLUME OF CHAMBER = 1.10 L) ARROWS INDICATE THE DIRECTION OF WATER FLOW THROUGH TUBING X'S
INDICATE WATER PUMPS USED FOR MIXING THE INNER CHAMBER AND FLUSHING BOTH CHAMBERS
Fig. 4.2. EFFECT OF HOLDING AND TESTING TREATMENT ON (A) MINIMUM METABOLIC RATE (MR_{MIN} , MG O ₂ H ⁻¹) AND
(B) ROUTINE METABOLIC RATE (RMR, MG O_2 H ⁻¹). (C) EFFECT OF HOLDING TREATMENT ON INDIVIDUAL BODY
CONDITION (FULTON'S K CONDITION FACTOR). (D) EFFECT OF HOLDING AND TESTING TREATMENT ON THE
INITIAL STRESS RESPONSE (ISR, MG O_2 H ⁻¹). METABOLIC RATE MEASURES WERE MASS-CORRECTED USING
RESIDUALS OF THE RELATIONSHIP BETWEEN LOG BODY MASS AND LOG METABOLIC RATE ADDED TO THE FITTED
VALUE FOR MASS = 1.84 G, the mean mass of all Fish used in the study. Error bars are s.e.m and N = 8
EQD ALL TREATMENTS: A STERIST (**) INDICATE STATISTICAL SIGNIERANCE ($n > 0.05$) 52
FOR ALL TREATMENTS. ASTERISKS () INDICATE STATISTICAL SIGNIFICANCE ($P < 0.05$)
FIG. 5.1. SCHEMATIC DIAGRAMS OF THE TWO EXPERIMENTAL SETUPS. (A) CHOICE TEST TANK USED IN EXPERIMENT 1
FIG. 5.1. SCHEMATIC DIAGRAMS OF THE TWO EXPERIMENTAL SETUPS. (A) CHOICE TEST TANK USED IN EXPERIMENT 1 (90 CM LENGTH X 30 CM WIDTH X 30 CM HEIGHT). THE DARK OVALS ON EITHER END OF THE TANK REPRESENT
FIG. 5.1. SCHEMATIC DIAGRAMS OF THE TWO EXPERIMENTAL SETUPS. (A) CHOICE TEST TANK USED IN EXPERIMENT 1 (90 CM LENGTH X 30 CM WIDTH X 30 CM HEIGHT). THE DARK OVALS ON EITHER END OF THE TANK REPRESENT THE SHOAL HOLDING CONTAINERS AND THE DARK OVAL IN THE CENTER OF THE TANK ILLUSTRATES THE
FIG. 5.1. SCHEMATIC DIAGRAMS OF THE TWO EXPERIMENTAL SETUPS. (A) CHOICE TEST TANK USED IN EXPERIMENT 1 (90 CM LENGTH X 30 CM WIDTH X 30 CM HEIGHT). THE DARK OVALS ON EITHER END OF THE TANK REPRESENT THE SHOAL HOLDING CONTAINERS AND THE DARK OVAL IN THE CENTER OF THE TANK ILLUSTRATES THE CONTAINER USED FOR THE FOCAL FISH DURING THE PRE-TRIAL ACCLIMATION PERIOD. (B) SIDE VIEW OF THE
FIG. 5.1. SCHEMATIC DIAGRAMS OF THE TWO EXPERIMENTAL SETUPS. (A) CHOICE TEST TANK USED IN EXPERIMENT 1 (90 CM LENGTH X 30 CM WIDTH X 30 CM HEIGHT). THE DARK OVALS ON EITHER END OF THE TANK REPRESENT THE SHOAL HOLDING CONTAINERS AND THE DARK OVAL IN THE CENTER OF THE TANK ILLUSTRATES THE CONTAINER USED FOR THE FOCAL FISH DURING THE PRE-TRIAL ACCLIMATION PERIOD. (B) SIDE VIEW OF THE RESPIROMETRY CHAMBER, WITH ARROWS INDICATING THE DIRECTION OF WATER FLOW THROUGH TUBING AND
FIG. 5.1. SCHEMATIC DIAGRAMS OF THE TWO EXPERIMENTAL SETUPS. (A) CHOICE TEST TANK USED IN EXPERIMENT 1 (90 CM LENGTH X 30 CM WIDTH X 30 CM HEIGHT). THE DARK OVALS ON EITHER END OF THE TANK REPRESENT THE SHOAL HOLDING CONTAINERS AND THE DARK OVAL IN THE CENTER OF THE TANK ILLUSTRATES THE CONTAINER USED FOR THE FOCAL FISH DURING THE PRE-TRIAL ACCLIMATION PERIOD. (B) SIDE VIEW OF THE RESPIROMETRY CHAMBER, WITH ARROWS INDICATING THE DIRECTION OF WATER FLOW THROUGH TUBING AND X'S DENOTING WATER PUMPS. ALL FOCAL INDIVIDUALS WERE TESTED IN BOTH AN ALONE TESTING TREATMENT

Group living is wide-spread among animal species and carries a number of benefits for foraging, social learning, reproduction, defense and energy expenditure (Krause and Ruxton 2002; Ward and Webster 2016). Animals must perceive, process, and react to sensory information from their ambient environment in order to survive. Cooperative group behaviours enhance survival, as having "many-eyes" increases an individual's chances of being informed of important stimuli (Krause and Ruxton 2002; Ward and Webster 2016). As individuals in the group receive this important information, they communicate it to the rest of the group using a variety of visual, auditory, chemical and mechanical cues (Brown and Smith 1994; John 1964; Larsson 2009; McCauley and Cato 2000; Partridge and Pitcher 1980). This information passes through the group in what is known as the "Trafalgar Effect," which describes the wave of information transmitted through the group as a result of localised interactions between individuals (Herbert-Read et al. 2015; Treherne and Foster 1981).

1.1 Shoaling versus schooling: Definitions

Fish shoals are a classic example of a prolific group behaviour found in nature (Delcourt and Poncin 2012; Pitcher 1983). Approximately 50% of all marine fishes shoal at some point in their life history, with approximately half of all shoaling species losing this trait following the juvenile phase (Shaw 1978). Colloquially, the terms fish "school" and fish "shoal" are used interchangeably in everyday language (Delcourt and Poncin 2012). However, scientifically, these two terms describe distinct social behaviours (Delcourt and Poncin 2012; Partridge 1982a; Pitcher 1983; Shaw 1978). A shoal describes any social group of 3 or more fish, while a school is a specific type of shoal that swims in a coordinated, synchronised and polarised (i.e., highly aligned) manner (Fig. 1.1; Delcourt and Poncin 2012; Partridge 1982a; Pitcher 1983). The Venn diagram

seen in Fig. 1.1 illustrates the relationship between shoaling, schooling and individual behaviours. The tradeoffs involved in executing shoaling versus schooling behaviour (and hence varying degrees of homogeneity in alignment) remain poorly understood. Shoaling fishes exhibit a high degree of intra- and inter-specific plasticity in cohesion and alignment in response to a variety of biotic and abiotic factors (Bode et al. 2010; Partridge 1982b; Partridge et al. 1980). Due to this variation, Delcourt & Poncin (2012) suggested that no social fish species fits exclusively under the definitions of either shoaling or schooling at all times. Each species instead lies somewhere along a continuous spectrum between schooling and shoaling, depending on the proportion of time spent at varying degrees of alignment. For the purpose of this thesis, I will refer to fish groups as schools in experiments where alignment was measured as a response variable. In all other instances, fish groups will be referred to by the more general term, shoal.





1.2 Benefits of shoaling

Fish gain a variety of benefits by partaking in shoaling behaviour (Krause and Ruxton 2002; Ward and Webster 2016). One key advantage is foraging in a group, as

shoaling increases access to information about the quality and quantity of different food resources (Baird et al. 1991; Lachlan et al. 1998; Laland and Williams 1997; Magurran and Pitcher 1983; Pereira and Ferreira 2013; Wolf 1987). Shoaling fish also have greater access to mates, a greater choice of who to mate with, the opportunity to mate with multiple partners and information on mate quality (Dugatkin and Godin 1993; Godin et al. 2005; Jennions and Petrie 1997; Webster and Laland 2013). In addition, individuals benefit from public information in the form of social learning, favouring processes such as recognition of threats and navigation (reviewed in Hoppitt and Laland 2013). Shoaling fish also gain benefits in individual fitness through improved defense against predation, as a result of more effective predator avoidance and dilution of individual risk (Domenici and Batty 1994; Domenici and Batty 1997; Foster and Treherne 1981; Herbert-Read et al. 2015; Karplus et al. 2006; Magurran 1990; Rieucau et al. 2014b). Lastly, individuals in fish shoals gain energetic benefits in the form of reduced metabolic demands, including increased hydrodynamic efficiency and reduced stress due to safety in numbers (Abrahams and Colgan 1985; Marras et al. 2015; Parker 1973; Schleuter et al. 2007). For my thesis research, I focused on these last two benefits, predator avoidance and energetic benefits.

Predator avoidance in shoals

Many fishes use group living as a mechanism for defense from predation, as shoaling renders individuals less vulnerable due a suite of factors (Brierley and Cox 2010; Croft et al. 2009; Marras et al. 2012; Rieucau et al. 2014a). One such factor is the dilution effect, in which the risk of being eaten decreases proportionally with increasing group size (Foster and Treherne 1981). In addition, a coordinated and polarised school benefits from a confusion effect, in which predators have trouble focusing on specific prey to attack, particularly when the group exhibits phenotypic homogeneity and executes rapid, coordinated escape maneuvers (Landeau and Terborgh 1986; Major 1978; Zheng et al. 2005).

One of the main forms of defense from predation in both schooling and solitary fishes is the fast-start escape response, which is a rapid, anaerobically-driven acceleration typically mediated by a pair of higher order command neurons called Mauthner cells (M cells) (Domenici 2010; Korn and Faber 2005). Fast-start escape responses occur on the order of milliseconds, which greatly enhances a prey's success in evading a predator's attack (Domenici 2010; Korn and Faber 2005; Tytell and Lauder 2008; Wakeling 2005). Studies suggest that schooling improves these responses, primarily through more accurate and effective escape trajectory (Domenici 2010; Domenici and Batty 1994; Domenici and Batty 1997; Domenici et al. 2011b). The trajectory of schooling fishes' acceleration is more likely to be oriented away from the predator than a solitary individual, helping to maximise the distance between the prey and predator (Domenici and Batty 1997). The tradeoff of this change in trajectory is that schooling fishes exhibit a longer average latency to respond to a threat stimulus (Domenici and Batty 1994; Domenici and Batty 1997). This additional time may be necessary to produce this more coordinated escape trajectory, which is a function of the speed of information transmission through the group (Domenici and Batty 1994; Domenici and Batty 1997; Herbert-Read et al. 2015).

The school's escape response also exhibits plasticity associated with traits of the group and the threat stimulus (Domenici 2010). First, schooling fishes' energy investment in escape responses is proportional to the perceived level of risk associated with the predator threat, with faster responses elicited by stronger and more imminent threats (Bode et al. 2010). The escape response also varies depending on the orientation of the threat to the school, with the most synchronous responses elicited by lateral stimulation $(60 - 120^\circ)$ and a higher proportion of split, uncoordinated responses resulting from frontal or posterior stimulation (Marras et al. 2012). Within groups, the speed and effectiveness of the response is improved when individuals exhibit defined roles of either leader or follower (Burns et al. 2012; Couzin et al. 2005; Marras and Domenici 2013). In this case, certain individuals will consistently lead the synchronous response away from an attacking predator, with this repeatable startle order aiding in

faster latency by removing the need for decision-making as to who will initiate the evasive maneuver (Marras and Domenici 2013). School density also affects the response, with high-density schools mounting a greater collective escape than lower density schools, potentially due to an increased rate of information transmission through the group (Rieucau et al. 2014b). Lastly, the sensory cues of the threat also modulate the strength of the response, with the fastest responses elicited by a combination of sensory cues (particularly combined visual and lateral line stimulation) (Rieucau et al. 2014a).

Energetic benefits of shoaling

Individuals within animal groups can reduce the energy needed for a variety of important tasks, including locomotion, parental care, defense and thermoregulation (Feeney et al. 2013; Hemelrijk et al. 2015; Jakob 1991; Scantlebury et al. 2006; Weimerskirch et al. 2001). Schooling fish gain energetic benefits due to hydrodynamic interactions with school-mates during swimming, with trailing fish exhibiting lower tail beat frequency and higher gait transition speed than leading fish (Fish et al. 1991; Herskin and Steffensen 1998; Killen et al. 2012; Marras et al. 2015). This benefit occurs as trailing fish are able to take advantage of vortices produced by the swimming patterns of leading fish in the school (Weihs 1973), with fish with lower aerobic performance tending to take advantage of these benefits most (Killen et al. 2012). The magnitude of these benefits is known to vary, however, depending on the configuration of the school (Hemelrijk et al. 2015).

Individuals from social species may also be able to reduce overall metabolic demand through group living in what is known as the calming effect (Martin et al., 1980; Parker, 1973; Trune and Slobodchikoff, 1976). In gregarious fish species, one factor that likely contributes to the calming effect is a reduced need for individual vigilance, as animal groups exhibit improved threat detection by having "many eyes" to scan for predators (Roberts, 1996; Ward et al., 2011). Individuals accustomed to a social

environment may also exhibit reduced stress when allowed to associate with conspecifics (Hennessy et al., 2009). However, in fish species, this effect has proved difficult to quantify due to difficulty in isolating individuals for testing while simultaneously realistically simulating shoaling conditions.

1.3 Plasticity in shoaling behaviour

Plasticity in shoal cohesion and coordination occurs in response to environmental stimuli (Fig. 1.2); for example, both traits are reduced in the presence of foraging opportunities and increase under predation threat (Domenici 2000a; Sogard and Olla 1997). Cohesion and coordination are mediated by three key forces: 1) near-range repulsion (to avoid collisions); 2) long-range attraction (to maintain cohesion); and 3) alignment (to enable rapid, synchronised movements) (Couzin 2009). The strictness of these three principles dictates the type of collective group behaviour that emerges and must be varied to suit ambient conditions (Couzin 2009). Cohesion can be measured through a number of variables, including school density and nearest neighbour distances (NND) (Ferno et al. 1998; Misund 1993; Pitcher and Partridge 1979), while coordination can be quantified using polarity and school shape (=length/width) as proxies (Ferno et al. 1998; Soria et al. 2007).

Another trait likely to affect group coordination and cohesion is the level of familiarity among the members of a shoal. This trait develops over time following a prolonged period of interaction between individuals (Ward and Hart 2003) and is achieved through a variety of sensory stimuli (Brown and Smith 1994; Morrell et al. 2007; Ward et al. 2002). Familiarity has been found to increase the efficiency of social learning (Swaney et al. 2001), antipredator behaviour (Chivers et al. 1995; Griffiths et al. 2004) and foraging (Atton et al. 2014), all of which have profound effects on individual fitness and survival. Many previous studies have illustrated fishes' preference to shoal with familiar individuals and groups (Barber and Wright 2001; Edenbrow and Croft 2012; Griffiths and Magurran 1999; Jacoby et al. 2012; Jordan et al. 2009; Lachlan et al. 1998;



Fig. 1.2. Illustrations of shoals with low cohesion and alignment, high cohesion and low alignment, low cohesion and high alignment and high cohesion and alignment. Alignment is along the x-axis and cohesion is along the y-axis.

Lee-Jenkins and Godin 2010; Magurran et al. 1994), with this preference even overriding the tendency to group with conspecifics (Ward et al. 2002). However, Griffiths and Magurran (1997b) found that as group size increased, the significance of this preference decreased, potentially indicating a maximum number of fish that can be recognised by an individual. In guppies, social recognition can be achieved in a matter of days and maintained following weeks-long separations (Bhat and Magurran 2006; Griffiths and Magurran 1997a); however, the level of species-specificity of these time frames has yet to be investigated.

1.4 Climate change and shoaling

Due to anthropogenic activity, carbon dioxide (CO₂) concentrations are rising in the atmosphere (Dlugokencky and Tans 2016; Luthi et al. 2008), leading to an increase in global mean temperatures (Collins et al. 2013). Global temperatures are expected to

rise by up to 4°C and CO₂ is projected to more than double to over 1000 μ atm by the year 2100 (Collins et al. 2013; Thuiller 2007). Sea surface temperatures (SST) in the world's oceans are rising at approximately 70% of the global average rate (Lough 2012), while ocean pCO₂ rises at the same rate as the atmosphere (Doney 2010). In addition, as the oceans absorb additional atmospheric CO₂, seawater chemistry (in particular pH) is altered, with progressive acidification expected to compromise the functioning of calcifying organisms (Kleypas et al. 1999; Sabine et al. 2004; Zeebe et al. 2008).

Ectotherms like marine fishes may be particularly affected by rising SSTs due to their inability to thermoregulate (Lefevre 2016). Tropical habitats, such as coral reefs, have relatively stable thermal regimes compared with higher latitude habitats (Lough 2012; Tewksbury et al. 2008). Consequently, tropical species are predicted to have narrower thermal tolerance ranges than many higher latitude species and may also be living on or near their thermal optima for fitness (Tewksbury et al. 2008). Rising temperature can lead to increased basic energetic needs in fish (Donelson and Munday 2012; Johansen and Jones 2011; McDonnell and Chapman 2016; Schulte 2015), resulting in higher food requirements (Johansen et al. 2015). This increased need for food may impact the tradeoffs of group living, between defense from predation and competition for food resources (Krause and Ruxton 2002; Ward and Webster 2016). Studies have shown that individuals exposed to elevated temperatures preferentially associate with smaller, less cohesive shoals (Bartolini et al. 2014; Hurst 2007; Weetman et al. 1999), potentially to maximise food intake and reduce competition in order to meet their greater energetic needs.

Rising CO₂ levels in the ocean are predicted to affect a range of behavioural (Briffa et al. 2012; Nagelkerken and Munday 2016) and physiological (Heuer and Grosell 2014; Portner et al. 2004) processes in marine organisms (Wittmann and Portner 2013). In fish, exposure to high CO₂ has been found to cause a range of behavioural effects, including reduced learning ability (Chivers et al. 2014; Jutfelt et al. 2013), altered activity levels (Ferrari et al. 2011a; Munday et al. 2010), higher anxiety (Hamilton et al. 2014) and reduced predator avoidance behaviour (Dixson et al. 2010; Ferrari et al. 2011b;

Munday et al. 2010). In addition, behavioural responses to visual (Ferrari et al. 2012b), olfactory (Munday et al. 2009b) and auditory (Rossi et al. 2016) cues are all impaired. These processes are essential to executing effective shoaling behaviour, indicating that the benefits of group living to individuals may become compromised in the future.

The effect of elevated pCO₂ and decreasing pH on other physiological characteristics of fishes are less clear. Though increased demands on regulatory mechanisms to prevent acidosis in a high CO₂ environment should theoretically increase overall energetic needs (Ishimatsu et al. 2008), most studies have found little evidence of any increases in basic energetic demand in fishes exposed to projected CO₂ conditions (Heuer and Grosell 2014; Lefevre 2016). However, another important consideration is that, although many studies have examined the effect of CO₂ on the metabolic rate of gregarious fish species (Miller et al. 2012; Munday et al. 2009a; Rummer et al. 2013), all have measured metabolic rate in solitary individuals, which can have effects on the measured metabolic rate due to the stress of isolation (Nadler et al. 2016). Therefore, how social context may modulate the effect of pCO₂ on metabolic traits remains unknown, with the potential for shoaling's benefits to increase under environmental stressors like rising CO₂.

1.5 Social behaviour in coral reef fishes

Social behaviours are prolific in coral reef fishes (Fishelson et al. 1974). Much of the research in this area has focused on pairing behaviour, dominance hierarchies and reproductive effects of sociality. Cooperative pair-formation is common in coral reef fish species and has a range of ecological benefits for reproduction, foraging and defense (reviewed in Brandl and Bellwood 2014). Dominance hierarchies are also prevalent in group-living coral reef fishes, as many species exhibit high site fidelity, group stability and competition for food resources (Krause et al. 2000; Ward et al. 2006). The studies in this area have helped uncover how dominance hierarchies are established and their influence on survival, sexual maturity, mating opportunities and foraging (e.g. Ang and

Manica 2010; Booth 1995; Buston and Cant 2006; Forrester 1991; Whiteman and Côté 2004; Wong 2011). Sociality also has a strong impact on reproductive strategies in coral reef fishes, due to the prevalence of sex changing species in this system (Munday et al. 2006a; Warner et al. 1975). In many species, sex change is controlled socially, occurring upon the death or removal of a group-mate of the opposite sex (e.g. McCormick 2016; Munday et al. 2006b; Robertson 1972; Ross et al. 1983; Warner and Swearer 1991)

Many coral reef fishes are also found living in cooperative, egalitarian groups with relaxed hierarchies, in which every member of the group exerts a similar influence on the group's phenotype (Demartini and Anderson 2007; Dugatkin 1997; Fishelson et al. 1974; Shaw 1978). Cooperative shoaling species on coral reefs are unique in comparison to shoals in other systems due to their higher temporal stability in composition, greater site fidelity and dependence on the benthic substratum and lower incidence of kin relationships (Krause et al. 2000). Due to the specialised nature of shoaling dynamics on coral reefs and the flexibility of shoal characteristics between species and social contexts (Mateo 2004), further research is needed to determine if the trends previously found in other habitats are applicable in shoaling coral reef fishes. The studies on shoaling on coral reefs to date have focused primarily on factors influencing motivation to shoal (Crook 1999a), assorting of characteristics between shoals (Crook 1999b), group foraging dynamics (Foster 1985; Pereira et al. 2012; Pereira and Ferreira 2013; Robertson et al. 1976; Welsh and Bellwood 2012; White and Warner 2007a), collective movement and navigation (Irisson et al. 2015; Ward et al. 2013; Welsh and Bellwood 2012), social transmission of information (Karplus et al. 2006; Mann et al. 2014) and mortality (White and Warner 2007b). There still remains a vast scope for further research on shoaling in coral reefs. In particular, no one has yet examined either the fast-start escape response or calming effect of shoaling in coral reef fishes. In addition, little work has been done in any system on the potential impacts of projected climate change on shoaling dynamics.

1.6 Study species

In this thesis, I used the gregarious tropical damselfish *Chromis viridis* as a model species. *C. viridis* is a live coral-associated reef species found throughout the Indo-Pacific region in groups ranging in size from three to hundreds of individuals (Nadler et al. 2014a; Ohman et al. 1998; Pratchett et al. 2012). This species was ideal for this set of experiments for a number of reasons. First, it thrives in a laboratory setting, adjusting to captivity and re-commencing active foraging and behaviour within days of capture from the reef (L. Nadler, pers. obs.). In addition, this species is ubiquitous in Indo-Pacific reefs, and highly abundant at my study site, Lizard Island, located in the northern Great Barrier Reef, Australia. As these experiments involved a range of laboratory manipulations, particularly of shoal composition, it was essential to have a sufficient number of distinct wild shoals from which to create experimental shoals.

In addition, the ecology and life history of *C. viridis* were ideal for answering the study questions. Unlike many other gregarious species on the reef, shoals of *C. viridis* exhibit highly cooperative behaviour, with limited evidence of dominance hierarchies observed during preliminary assessments and continuing experiments. This lack of hierarchy allowed focal individuals within shoals to be chosen randomly during experiments, without concern for confounding effects of position within the group's hierarchy. Lastly, *C. viridis* was ideal to investigate antipredator behaviour, as it is a common prey item for a range of predatory fish species, making its defensive capabilities a central process influencing individual fitness.

1.7 Aims and objectives

My thesis examined how shoal composition and environmental conditions influence the benefits of group living to individuals in a coral reef fish. I designed my research questions from two perspectives. First, I examined two different types of

shoals. Wild shoals consisted of individuals that were found naturally living together on the reef, in order to gain an understanding of natural shoal composition and how it influences behaviour and physiology. I also experimentally manipulated shoals, either by changing the composition of shoals or by changing the environmental conditions that shoals were acclimated to. Second, I investigated two of the primary benefits of shoaling: predator avoidance and energetic benefits. I tested the escape response of fish schools, in order to understand how different factors influence the coordination of the school's response and performance of individuals in response to a threat. Lastly, I examined how group living influences the metabolism of individuals in order to quantify the calming effect of shoaling. These ideas shaped the basis for each of my chapters. In Chapter 2, I examined how habitat influences the escape performance of wild fish schools. In Chapter 3, I tested the effect of familiarity on escape performance in schooling fish. In Chapter 4, I analysed the effect of shoaling on individual metabolism and body condition. And lastly, in **Chapter 5**, I placed the results of my previous chapters in the perspective of global change by measuring the effect of elevated CO_2 on familiarity and metabolism in shoaling fish. This work fills knowledge gaps on how shoal phenotypes may influence the spatial distribution of shoals on the reef and how these phenotypes may in turn be shaped by ambient environmental conditions.

Chapter 2: Role of water flow regime in the swimming behaviour and escape performance of schooling fish

This chapter was prepared for submission to Oecologia

Authors: LE Nadler, SS Killen, P Domenici and MI McCormick

2.1 Summary

Animals from many types of habitats have to contend with variable and rapidly changing environmental flow conditions, such as wind in terrestrial habitats and currents in aquatic systems. Animals must compensate for the resulting flow-induced positional drift to effectively forage and navigate. Many fishes use group living (e.g. schooling) as a mechanism to reduce energy costs associated with swimming against water flow. While previous evidence suggests that animals exhibit flow-induced plasticity in aerobic swimming performance, no one has yet examined whether similar plasticity is found in the anaerobic fast-start escape response. In this study, we collected 11 distinct wild schools of the tropical damselfish Chromis viridis from shallow reefs surrounding Lizard Island in the northern Great Barrier Reef, Australia. The flow regimes for each collection site were measured to ascertain differences in mean water flow rate and its temporal variability. Escape behaviour was tested in a laminar flow swim tunnel, in which the school's response to an aerial mechanical stimulus was recorded in high-speed (240 fps). Though each school's routine swimming behaviour and escape performance were not impacted by their local flow conditions, traits of individual fast-start performance (including latency, average turning rate and distance covered) were significantly

improved in individuals from high flow habitats. This improved performance could occur as a result of a variety of mechanisms, such as an in situ behavioural training effect or selection for faster performance phenotypes under high flow conditions.

2.2 Introduction

Animals from many types of habitats have to contend with variable and rapidly changing environmental flow conditions, such as wind in terrestrial habitats and currents in aquatic systems (Madin et al. 2006; McLaren et al. 2014). Flow of air or water adds a level of complexity to processes such as foraging and navigation, particularly for animals that utilise flying or swimming locomotion, like bumblebees, butterflies, birds and fish (Krupczynski and Schuster 2008; Riley et al. 1999; Srygley 2001; Thorup et al. 2003). This additional challenge is the result of drift, in which animals must compensate for downstream displacement in order to effectively engage in essential activities (McLaren et al. 2014).

In complex marine habitats, water flow patterns are influenced by wind, weather and tide conditions as well as the topography of the benthic landscape (Johansen 2014; Madin et al. 2006; Nikora 2010; Poff et al. 1997). In this era of rapidly changing climates, storm frequency and intensity is likely to increase in the future (Huntington 2006), potentially changing temporal and spatial water flow patterns. Given their mechanical force, storms can also break down structural complexity in marine ecosystems, degrading valuable habitat for the system's associated fish and invertebrates (Lilley and Schiel 2006; Madin and Connolly 2006). In delicate systems like coral reefs, changing climate conditions can also cause bleaching in reef-building corals, leading to further habitat degradation (Hoegh-Guldberg 1999). Acute high flow events resulting from rapidly changing topographic conditions could present problems for animal assemblages, as the behaviour and physiology of resident animals are likely suited to their habitat's native conditions (Fulton and Bellwood 2005; Munks et al. 2015; Nunes et al. 2013).

Many fishes use group living (e.g. schooling) as a mechanism to reduce energy costs associated with swimming against flow (e.g. Abrahams and Colgan 1985; Herskin and Steffensen 1998; Marras et al. 2015; Weihs 1973). Schooling is wide spread among fish species and carries benefits for individuals with respect to predator avoidance, foraging opportunities and optimising energy use (Krause and Ruxton 2002; Nadler et al. 2016; Shaw 1978). However, these benefits depend on how well members of the school can coordinate their behaviours (Handegard et al. 2012). To maximise the benefits of grouping, schools exhibit a high degree of behavioural plasticity in response to the individuals' needs and environmental stimuli, particularly in group cohesion, coordination, shape and positional preferences (Domenici et al. 2007; Hansen et al. 2015; Killen et al. 2012; Krause and Ruxton 2002; Sogard and Olla 1997; Ward and Webster 2016; Webster et al. 2007). While greater group cohesion and coordination aids in evading predators, looser and less coordinated configurations are advantageous while foraging (Partridge et al. 1980). School shape can be tailored to the behaviour of the attacking predator (Abrahams and Colgan 1985; Domenici et al. 2007). For position, leading fish benefit from greater access to food resources than followers, but are also the most vulnerable to predation (Bumann et al. 1997). In addition to these tradeoffs, environmental conditions such as water flow regime can influence behavioural and physiological phenotypes of both solitary and schooling fish (Appendix 1; Anwar et al. 2016; Binning et al. 2015; Langerhans 2008; Liao 2007; West-Eberhard 1989). Chicoli et al. (2014) found that individuals and schools exhibit a higher rate of reaction to a threat under an acute high flow treatment than a no flow treatment. In wild-caught fish, Binning et al. (2014) found that fish from wave exposed (and hence higher flow) sites exhibited greater swimming performance than individuals from sheltered (lower flow) sites.

A behavioural trait important for the survival of fishes is the fast-start escape response, which is one of the main forms of defense from predation. The fast-start escape response is a rapid, anaerobically-driven acceleration typically mediated by a pair of higher order command neurons called Mauthner cells (M cells) (Domenici 2010;

Korn and Faber 2005). This type of response typically occurs on the order of milliseconds and is generally divided into three stages for the purpose of comparative analysis: stage 1 (unilateral muscle contraction on the side of the body opposite to the stimulus, causing the fish to bend into a C shape), stage 2 (contralateral muscle contraction, causing the tail to flip around creating additional forward acceleration) and stage 3 (variable stage with fish either gliding or burst swimming) (Tytell and Lauder 2008; Wakeling 2005). Whether water flow rate may induce similar plasticity on fast-start escape responses of individual fish or fish schools, as was found for aerobic swimming performance (Binning et al. 2014; Binning et al. 2015), remains to be investigated.

Using schools of a gregarious coral reef fish as a model species, we investigated how native water flow regimes experienced at the school's home reef affected behavioural plasticity in school swimming behaviour, school escape response and individual escape performance. To the best of our knowledge, this study is the first to illustrate habitat-induced behavioural plasticity in fast-start performance resulting from local water flow conditions. We hypothesised that schools collected from higher flow habitats would exhibit more cohesive and coordinated swimming and escape behaviour than schools from lower flow regimes, as there is likely less room for error in rapidly moving environments.

2.3 Materials and methods

Fish collection and maintenance

Eleven schools of the tropical damselfish species *Chromis viridis* (SL: 3.45 ± 0.03 cm, BM: 1.72 ± 0.04 g, mean \pm SE) were collected from seven shallow reef sites (<4 m depth; Fig. 2.1A) in the Lizard Island Iagoon, northern Great Barrier Reef, Australia (14°40′08″S; 145°27′34″E). Schools were separated by a minimum of 50 m and sites were separated by 400 – 3000 m. *C. viridis* is an abundant, live coral-associated schooling species found on coral reefs throughout the Indo-Pacific region in groups

ranging in size from three to hundreds of individuals (Nadler et al. 2014a; Ohman et al. 1998; Pratchett et al. 2012). Fish were collected using hand nets and barrier nets and transported to the Lizard Island Research Station (LIRS). There, fish were placed into experimental schools of eight individuals and housed in replicate 20 L aquaria in a flowthrough seawater system. Fish were fed to satiation twice daily with INVE Aquaculture pellets and newly hatched *Artemia* sp.

Water flow measurement

Water flow rate was measured at each of the seven collection sites on five separate days under varying wind and weather conditions, in order to ascertain relative differences between sites. Measurements were always taken at high tide (± 1 hour). Flow rate was determined using a precision vane-wheel flow meter (Hontzsch Gmbh, Waiblingen, Germany) placed approximately 1.25 m below the water surface. Measures of flow speed (cm s⁻¹) were logged at 1 Hz for 180 s. An overall mean flow rate was then calculated for each site using data from all five days.

Swimming behaviour and escape response

Trials were conducted in a custom-built laminar flow swim tunnel (50 cm length x 40 cm width x 9 cm height). This device allowed schools to swim in non-turbulent conditions at a slow uniform swim speed of approximately one body length (L) per second (3.2 cm/s) for all trials, which mimics natural flow speed conditions at the seven collection sites on a calm day (Johansen 2014). Seawater in the system was maintained at the ambient temperature for the study period (27 – 29°C). Experimental schools were placed in the swim tunnel and allowed to acclimate for four hours. Afterwards, school swimming behaviour was video-recorded pre-stimulus for 15 mins (30 fps; Canon Powershot D10). Escape responses were elicited using a standardised threat protocol in which a mechanical stimulus is dropped from above (Domenici et al. 2015). This

stimulus was a black cylindrical object (2.5 cm diameter x 12 cm length, 37.0 g) with a tapered end (to minimise surface waves), suspended 137 cm over the surface of the water in the swim tunnel. To avoid visual cues prior to the stimulus reaching the water's surface, this object was dropped through a white PVC pipe ending immediately before it broke the water's surface (Domenici et al. 2008; Turesson and Domenici 2007). A thread connecting the stimulus to the release point prevented it from touching the bottom of the tank (Domenici et al. 2008; Turesson and Domenici 2007). As previous studies suggest that the school's alignment during an escape response is highest with lateral stimulation at an angle of $30 - 120^{\circ}$ (Marras et al. 2012), identical stimuli were placed 2 cm from the center of each of the lateral walls of the swim tunnel. To control for a stimulus side preference, the use of the left or right lateral stimulus was alternated between trials. These stimuli remained suspended above the swim tunnel for the duration of the acclimation period using an electromagnet. Following the acclimation period, the stimulus was released using a switch, once a minimum of 6 of the 8 fish were >3.5 cm from any wall of the swim tunnel and <4 L from the stimulus. This criterion aided in reducing the constraining effects that the walls of the swim tunnel may exert on individuals' escape response and controlled for differences in escape performance that can occur with varying distance and orientation to the stimulus (Eaton and Emberley 1991). Each school's escape response was video-recorded from below in high speed (240fps; Casio Exilim HS EX-ZR1000), using a mirror placed at a 45° angle. The swim tunnel was illuminated from above using two diffused 500-W spotlights.

Kinematic analysis

Videos were analysed using the ImageJ software (v 1.42). A number of prestimulus school swimming characteristics, school escape performance traits and individual fast-start performance attributes were examined as defined below.

School swimming behaviour

School swimming behaviour was characterised through school cohesion (nearest neighbour distance and total school area) and spatial organisation (variation in alignment of individuals and school shape). Five random frames from the 15-min prestimulus video recording were analysed for each of the characteristics outlined below and the mean for each characteristic was calculated for each experimental school. (1) Nearest neighbour distance (NND): distance to the closest neighbour within the school, as measured by the distance from each fish's CM.

(2) School area: the school's spread (cm²), as measured by the area between the fish at the edge of the school (defined as those fish whose head was at the vertex of the smallest convex polygon encompassing the group) (Marras and Domenici 2013).

(3) Alignment: the variation in the orientation of all school members to the horizontal (corresponding to the direction of flow; 180° = facing into the flow towards the front of the tank, 0° = oriented with the flow towards the back of the tank). As alignment angles spanned up to 360°, circular statistics were employed. This variable is characterised by the length of mean circular vector (r; high values denotes low variation in orientation) (Bachelet 1981), as calculated in the software Oriana 4.

(4) School shape: a measurement of symmetry in school shape, which is calculated as the ratio of school length (distance from the fish in the first position to the fish in the last position) to school width (the largest transverse distance between fish in the school) (Domenici et al. 2002).

School escape response

School escape response was assessed at three times post-stimulus (0, 20 and 100 ms post-stimulus) and was analysed based on the behaviour of each individual in the school. The stimulus onset was defined as the frame at which the stimulus first touched the water's surface. School cohesion through the escape response was measured using

NND as described above. The school's spatial organisation during escape was measured using alignment. However, in order to assess the alignment of each individual to their school-mates' orientation, alignment during escape was calculated as the difference between each individual's orientation and the mean circular vector of the group (as calculated in the software Oriana 4).

Individual fast-start performance

Individual escape performance was characterised through (1) latency, (2) average turning rate, (3) distance covered, and (4) directionality. Previous studies suggest that the (5) stimulus distance can influence latency, average turning rate and distance covered while (6) stimulus orientation can affect directionality (Domenici and Blake 1997), so these measures were included as a covariates in their respective statistical analyses.

(1) Latency: the time period between the stimulus onset (contact with the water surface) and the fish's first movement.

(2) Average turning rate: calculated by dividing the stage 1 angle (the angle between the lines intersecting the head and center of mass when stretched straight (CM) at the start and end of stage 1) by the duration of stage 1 (Domenici 2004). The location of the CM in video footage was measured as 0.35 of the fish's *L*, posterior of the snout, based on previous measurements of generalist fish species (Webb 1978). Stage 1 is the stage immediately post-stimulus, in which fish contract the muscles on the side of the body opposite to the stimulus, causing the fish to bend into a C shape.

(4) Distance covered: distance that the fish's CM travelled within the first 10 frames (i.e. 42 ms) of their reaction. This time frame was determined by calculating the average duration of stage 1 and stage 2 in the escape response for 24 individuals (one random fish per trial). This short time frame was used as a proxy for swimming speed in order to avoid issues with wall effects. Individuals less than 2 *L* from any wall of the swim tunnel

at the time of their response were excluded from this analysis (10% of total; Eaton and Emberley 1991).

(5) Stimulus distance: distance from the stimulus to the fish's CM.

(6) Directionality: the direction of the escape response for each individual in the school, as categorised by "away" and "towards" responses, referring to the direction that the head moved in stage 1 (Domenici et al. 2011b).

(7) Stimulus orientation: the orientation of the fish to the stimulus at the time of stimulation. This angle was determined using the lines between the head and CM and the CM and stimulus.

Statistical analysis

All statistical analysis was conducted in the R Statistical Environment v3.2.4 (R Devloment Core Team 2015), using the packages "nlme", "Ime4" and "car" (Bates and Maechler 2009; Pinheiro et al. 2016). Residual and guantile-guantile plots were assessed for each model in order to ensure that all assumptions were met. To meet the assumptions of the statistical tests, water flow rate and latency were log-transformed. Differences in water flow rate between sites were assessed using a linear mixed-effects model (LMM), with sampling date as a random effect to account for differences in wind and weather conditions between measuring days. Tukey's HSD post-hoc tests were used to further investigate significant differences between sites detected by the LMM. As mean water flow rates were not normally distributed among sites, sites were categorised for all other analysis as high (mean flow > 20 cm/s) or low (mean flow < 11cm/s). The influence of water flow rate on school swimming behaviour was assessed using a generalised linear model (GLM). The relationship between water flow rate and school escape performance was tested using a LMM with water flow rate and time poststimulus as fixed effects and school number as a random effect (which nested each individual within their school for the purpose of analysis). Individual fast-start performance (latency, average turning rate and distance covered) was examined using a

LMM with flow as a fixed effect and school number as a random effect. Stimulus distance was included as a covariate in this analysis. Analysis of directionality was conducted using a binomially distributed GLM with a logit link-function, weighted for total number of individuals per school in three categories of stimulus orientation (head-on: $0 - 60^{\circ}$, perpendicular: $60 - 120^{\circ}$, tail-on: $120 - 180^{\circ}$). Previous work (Domenici and Batty 1997; Domenici et al. 2011b; Domenici and Blake 1993) has shown that stimulation from a perpendicular orientation to the fish's axis elicits a non-random directional response, with significantly more away than towards responses. Tail-on or head-on stimulation, conversely, elicits responses with a random distribution of away and towards responses. Therefore, perpendicular orientations ($60 - 120^{\circ}$) were analysed separately from head-on/tail-on oriented ($0 - 60^{\circ}$ and $120 - 180^{\circ}$) stimulations.

2.3 Results

Water flow rate was significantly different between sites (Fig. 2.1B; LMM: $F_{6,24}$ = 6.50, p = 0.0004). In particular, sites 4 and 5 exhibited a significantly higher water flow rate than sites 1, 2, 3, 6 and 7 (Tukey's test: p < 0.05 for all comparisons between 4/5 and 1/2/3/6/7). In addition, the coefficient of variation was also higher in sites 4 and 5 (Fig. 2.1C).

School swimming behaviour did not vary between habitats with different water flow regimes. Water flow regime at a school's home reef did not influence any of the parameters measured, including school shape, density, NND or alignment pre-stimulus (p > 0.05; Table 2.1).

School escape response was not affected by the water flow regime at the school's home reef, including both NND and alignment (NND LMM: $F_{1,9} = 0.01$, p = 0.9937; Alignment LMM: $F_{1,9} = 0.22$, p = 0.6451). However, a significant effect of time post-stimulus on school escape response was found. There was a significant effect of time post-stimulus on NND (Fig. 2.2A; LMM: $F_{2,172} = 13.06$, p < 0.0001), with schools


Fig. 2.1. (A) Map of the seven collection sites. (B) Mean water flow rate at each of the collection sites (\pm s.e.m). (C) Coefficient of variation in water flow at each of the collection sites.

exhibiting an increase in NND 100 ms post-stimulus (Tukey's test: $p_{0-100} < 0.0001$, $p_{20-100} < 0.0001$, $p_{0-20} > 0.05$). Time post-stimulus also had a significant effect on alignment throughout the response (Fig. 2.2B; LMM: $F_{2,172} = 3.11$, p = 0.0469). Post-hoc tests indicated trends in reduced alignment at 20 and 100 ms post-stimulus, when compared to immediately prior to the stimulus (Tukey's test: $p_{20-100} = 0.0625$, $p_{0-20} = 0.0995$, $p_{0-100} > 0.10$).

Variable School shape	F 0.07	d.f.P-value	
		9	0.7940
School density	0.14	9	0.7128
Nearest neighbor distance	0.88	9	0.3716
Alignment	0.91	9	0.3759

Table 2.1. Summary of the generalised linear model analysis investigating the effect of water flow rate on school swimming behaviour, including school shape, school density, nearest neighbour distance and alignment (n = 11 for all models).



Fig. 2.2. School performance throughout the escape response, including (A) nearest neighbour distance (NND) and (B) variability in individual alignment, at 0, 20 and 100 ms post-stimulus. Bars are mean \pm s.e.m.

Individual escape performance was greater in individuals from high flow regime reefs, when compared with those collected from lower flow habitats. Individuals from high flow exhibited a trend for shorter latency (Fig. 2.3A; LMM: $F_{1,9} = 4.62$, p = 0.0601). In addition, there was a significant interaction between stimulus distance and water flow regime for latency, as the effect of water flow regime on latency decreased with

increasing stimulus distance (LMM: $F_{1,74} = 7.54$, p = 0.0076). Average turning rate was also significantly higher in individuals from high flow reefs (Fig. 2.3B; LMM: $F_{1,9} = 8.96$, p = 0.0151), with a significant interaction again indicated between water flow regime and stimulus distance (LMM: $F_{1,9} = 8.96$, p = 0.0370). For distance covered, trends were indicated for water flow regime (Fig. 2.3C; LMM: $F_{1,9} = 4.37$, p = 0.0661) and the interaction between water flow regime and stimulus distance (LMM: $F_{1,61} = 3.14$, p = 0.0813), with individuals from high-flow regime habitats swimming a further distance with their escape reaction than individuals from lower flow reefs. Stimulus distance alone did not exert a significant effect on latency (LMM: $F_{1,74} = 1.88$, p = 0.1743), average turning rate (LMM: $F_{1,72} = 1.14$, p = 0.2885) or distance covered (LMM: $F_{1,61} = 2.11$, p = 0.1512). In addition, individuals oriented 60 – 120° from the stimulus were significantly more likely to turn away from the stimulus if they were from a high flow than a low flow habitat (Fig. 2.3D; LMM: $\chi^2 = 10.671$, p = 0.0011). No significant differences were found in response directionality for any other stimulus orientations (GLM: p > 0.05 for $0 - 60^\circ$ and $120 - 180^\circ$).

2.4 Discussion

Water flow regime is a major driver in the distribution and abundance of behavioural, physiological and functional traits in fish assemblages in a range of systems (Binning et al. 2015; Fulton and Bellwood 2005; McGuigan et al. 2003; Sinclair et al. 2014). While previous studies have characterised aerobic swimming performance under varying flow conditions (Binning et al. 2014; Johansen et al. 2007; Peake et al. 1997), this study is the first investigation of how native water flow regimes influence anaerobic performance. The results suggest that while the routine swimming behaviour and escape response of fish schools are maintained across a range of localised water flow conditions, higher and more variable relative water flow rates may drive differences in individual fast-start performance. These findings indicate that individual performance could be tailored to the prevailing conditions at their home reef.





Water flow rate could influence fast-start performance as a result of a variety of mechanisms, stemming from the greater environmental pressures placed on individuals living in high flow habitats. Under high flow conditions, animals must maintain a faster pace and hence may have less room for error, particularly while escaping predators, than conspecifics from lower flow habitats. Behavioural plasticity in response to environmental cues has been demonstrated previously in response to water flow rate. Sinclair et al. (2014) observed increased boldness and aggression in the mosquitofish *Gambusia holbrooki* acclimated to a high flow environment when compared to those accustomed to low flow conditions. Any factor that increases the intensity and frequency of exercise in resident fishes may create a training effect that leads to improved physiological and behavioural performance (Anttila et al. 2011; Davison 1997;

Killen et al. 2016a). A number of controlled laboratory studies have measured a training effect of water flow rate on aerobic metabolism and swimming performance, with higher maximum metabolic rate, gait transition speed and critical swimming speed found with increasing water flow (Binning et al. 2015; Sinclair et al. 2014). The results here indicate that anaerobic performance is likely subject to a similar training effect under high flow conditions. Further investigation of differences in physiological characteristics would help to determine the absolute capacity for plasticity in anaerobic processes.

Fast-start escape performance could also vary due to differences in selective pressure between high and low flow regimes (Higham et al. 2015). Previous studies have illustrated differential survival between fishes with varying locomotor performance (Swain 1992). However, behavioural phenotypes may not experience a uniform degree of selective pressure across habitat types. For instance, slower performing individuals may experience a higher degree of mortality, and hence be selected against, in high flow habitats than low flow regimes. This could account for the lower proportion of slow faststart reactions in schools collected from high flow regime reefs. In a study by Fu (2015), gingbo carp Spinibarbus sinensis exhibited a lower mortality rate when they had been acclimated to a high flow environment as compared to those acclimated to a still-water environment. Studies also suggest that water flow may reduce the ability of the lateral line to detect perturbations in the water created by attacking predators (Anwar et al. 2016; Feitl et al. 2010; Liao 2006). This suggests that fish accustomed to a high flow rate may become accustomed to its disruptive effect on lateral line sensitivity, potentially compensating through sensory redundancy (e.g. visual cues). Further studies on how flow impacts predator strike performance and success would aid in understanding the contribution of selection to the distribution of fast-start phenotypes between habitat types.

Different water flow regimes may also provide varying levels of food availability and foraging effort as well as modifying foraging tactics in fish communities. Greater water current speeds increase the abundance of zooplankton species on coral reefs

(Hamner et al. 1988). For planktivores like *C. viridis*, this greater provisioning could contribute to improving escape performance in coral reef fishes. This greater abundance of prey can also influence foraging effort, with some species of coral reef fish exhibiting increased foraging effort under high water rates (Clarke et al. 2009). To save energy on locomotion under these higher flow conditions, fishes can also modify their foraging tactics, from active searching under low flow conditions to ambush predation under high flow conditions (Allouche and Goudin 2001). By modifying foraging effort and tactic, fishes may be able to extract more energy per unit foraging effort under high flow conditions than low flow conditions. These mechanisms (plasticity, selective pressure and food provisioning) are likely not exclusive, with some combination dictating prevailing escape performance phenotypes under different water flow conditions.

Theory typically suggests that fishes exhibit a tradeoff between aerobic and anaerobic locomotor performance, as the traits that create high performance in one locomotory mode may reduce the capacity for performance in the other (Langerhans 2008). Continuous aerobic swimming may be prioritised under high flow conditions due to its importance in essential activities, like foraging, that promote growth and fitness (Taylor and McPhail 1986). As an abundance of evidence illustrates the benefits of high flow to aerobic swimming capacity (Binning et al. 2014; Binning et al. 2015; Peake et al. 1997), we may have expected to see diminished fast-start performance, as it is fueled anaerobically. However, the benefits of strong aerobic swimming capacity in fishes from complex habitats like coral reefs may be modified due to the intricate habitat structure. Studies show that coral reef fish exhibit refuging behaviour in the coral structure, where flow is reduced by 75-100%, allowing individuals with lower aerobic swimming capacity to live in higher flow habitats than they would otherwise (Johansen et al. 2008; Johansen et al. 2007). This reduced need for strong aerobic swimming abilities may allow individuals to invest in functional traits that promote anaerobic swimming performance, like fast-start behaviour, which could account for the results found in this study. In addition to varying by habitat, the tradeoff between aerobic and anaerobic locomotor traits may be species specific. Marras et al. (2013) found no evidence of a

tradeoff between aerobic and anaerobic locomotor modes in the European seabass, *Dicentrarchus labrax*, suggesting that the dynamics between morphological, physiological and behavioural traits may be more complex than previously thought.

Individuals from higher flow habitats were significantly more likely to turn away from a predator stimulus than individuals collected from lower flow reefs. A number of previous studies have illustrated this tendency for away responses following lateral stimulation (Domenici and Batty 1994; Domenici et al. 2011b). In contrast, individuals from lower flow habitats exhibited an even split between away and towards responses under all stimulus orientations. As a higher proportion of away responses following lateral stimulation is expected based on previous work (Domenici and Batty 1994; Domenici et al. 2011b), these results suggest that the individuals from high flow environments conform to expectations while individuals from low flow differ for this trait. Despite the potential perils of swimming towards a predator, the directional response may be a strategy employed to confuse the predator. One benefit reaped from exhibiting a random directional response (e.g. 50:50 away:towards response proportion) is that predators are unable to learn and predict the escape trajectory of their prey (Domenici et al. 2011b). This difference in strategy could stem from the effect of flow on predator attack behaviour. Under high flow, predators need to execute more rapid acceleration during attacks in order to account for downstream displacement, which leads to reduced attack accuracy. Conversely, under lower flow conditions, predators execute slower but more accurate attack orientations, potentially making confusion a necessary tactic for escape (Higham et al. 2015).

Unlike fast-start performance, there was no influence of water flow regime on routine school swimming behaviour or school escape response. Previous studies have illustrated the plasticity of school cohesion and coordination in response to biotic and abiotic cues (Chivers et al. 1995; Cook et al. 2014; Sogard and Olla 1997; Webster et al. 2007; Weetman et al. 1998). Under an acute high flow treatment, Chicoli et al. (2014) found that schools of the giant danio *Devario aequipinnatus* increased alignment, orienting upstream into the flow. However, the results of this study suggest that this

acute effect of flow on school structure may not translate into longer lasting effects on the school's behaviour.

Determining root causes of phenotypic divergence in wild-caught animals can be complicated by the difficulty in characterising all factors that may possibly be influencing results. Although we found differences in the relative water flow conditions between our study reefs, we were unable to determine exactly which factors were driving the escape phenotypes measured. It is possible that additional factors that are correlated with water flow (e.g. predator density or behaviour, food availability) could be influencing the observed trends (Fu et al. 2013; Yan et al. 2015), rather than water flow rate being the causative factor. Despite these potential disadvantages of characterising phenotypic plasticity between animals from diverse natural environments, these types of studies are essential to place laboratory-based results in an ecological context and understand real-world processes.

To our knowledge, this is the first study to show that the native water flow regime is correlated with fast-start escape responses in teleosts, with links to the timing (latency), motor output (average turning rate and distance covered) and trajectory (directionality) of this performance trait. While behavioural phenotypes are likely shaped by day-to-day environmental conditions in complex habitats like coral reefs, changes in topography due to disturbances such as storms can drastically alter flow conditions (Lilley and Schiel 2006; Madin and Connolly 2006). The results presented here suggest that fishes' defensive behaviour is tailored to ambient environmental conditions, suggesting that acute high flow events could present problems for animal assemblages into the future (Fulton and Bellwood 2005; Munks et al. 2015; Nunes et al. 2013).

Chapter 3: Familiarity improves fast-start escape performance in schooling fish

This chapter was submitted to *Current Biology* (correspondence format) and reformatted for readability

Authors: LE Nadler, P Domenici, JL Johansen, MI McCormick

3.1 Summary

In social groups, a learned familiarity can be attained following a prolonged period of interaction among individuals and aids in fitness-enhancing processes such as foraging and social learning. In fish species, one of the greatest benefits of living in a group (e.g. schooling) is enhanced predator avoidance and dilution of individual risk. One of the main forms of defense from predation in fish species is the fast-start escape response, which is a rapid, anaerobically-driven acceleration typically mediated by a pair of higher order command neurons called Mauthner cells (M cells). Although environmental and mechanical factors have previously been shown to influence M cells' stimulus threshold and timing, no previous study has examined if familiarity can influence the motor output of the fast-start escape response. In this study, we employed a novel approach to examine if familiarity influences the timing, maneuverability and propulsive performance of escape responses in schools of the tropical damselfish Chromis viridis (Pomacentridae). Familiar schools exhibited superior individual escape performance, with shorter latency times (higher reactivity), greater average turning rates (increased maneuverability) and longer distances covered (greater propulsive performance) than unfamiliar schools. These results indicate that M cell firing may be inhibited in unfamiliar schools, potentially due to an increase in stress or reduced reliability of

information. The faster startle response in familiar schools would likely lead to increased survival of predator attacks in the wild and hence familiarity may be selectively advantageous in social species.

3.2 Introduction

Many animals live in groups due to the potential benefits associated with defense and foraging (Krause and Ruxton 2002). Throughout the animal kingdom, cognitive processes are central to facilitate group living (Couzin 2009). In fishes, for instance, a learned familiarity can be attained following a prolonged period of interaction between social individuals (reviewed in Ward and Hart 2003), increasing the probability of reciprocal cooperation between members of an animal group (Granroth-Wilding and Magurran 2013). This greater cooperation can have benefits for a range of fitness-enhancing processes, including foraging, social learning, body condition and survival (Atton et al. 2014; Seppä et al. 2001; Swaney et al. 2001). In fish, a number of studies have illustrated individuals' preference to group (i.e. school) with familiar school-mates over unfamiliar conspecifics, with individual identification achieved primarily through olfactory stimuli (Brown and Smith 1994; Partridge and Pitcher 1980; Ward et al. 2002).

One of the main forms of defense from predation in both schooling and solitary fishes is the fast-start escape response, which is a rapid, anaerobically-driven acceleration typically mediated by a pair of higher order command neurons called Mauthner cells (M cells) (Domenici 2010; Korn and Faber 2005). A fast-start escape response in fish is generally divided into three stages for analysis: stage 1 (unilateral muscle contraction on one side of the body, causing initial acceleration as the fish bends into a C shape), stage 2 (contralateral muscle contraction, causing additional forward acceleration as the tail flips around to the opposite side of the body) and stage 3 (variable stage with fish either gliding or burst swimming) (Domenici 2010; Tytell and Lauder 2008). Although little is known about how social context mediates escape

performance, previous studies have illustrated the plasticity of M cell activity in response to differences in environmental and mechanical factors (Korn and Faber 2005; Mirjany and Faber 2011; Preuss and Faber 2003).

One of the greatest benefits of schooling to individual fitness is more effective predator avoidance and dilution of individual risk (Krause and Ruxton 2002). Previous work suggests that familiar schools exhibit more effective antipredator behaviour than unfamiliar schools, with quicker reaction times to threats, potentially due to a reduced need for conspecific inspection (Dukas 2002; Griffiths et al. 2004). However, no one has yet examined how familiarity may influence the motor output of the fast-start escape response. Here, we employed a novel approach to examine how familiarity influences the reactivity, maneuverability and propulsive performance of fast-start escape responses in schools of the tropical damselfish *Chromis viridis* (Pomacentridae).

3.2 Materials and methods

Fish collection and maintenance

This experiment was conducted at the Lizard Island Research Station (LIRS) in the northern Great Barrier Reef, Australia ($14^{\circ}40'08''S$; $145^{\circ}27'34''E$). Distinct schools of the tropical damselfish *C. viridis* (standard length: 3.33 ± 0.02 cm, mean \pm S.E.M.) were collected from reefs in the lagoon adjacent to LIRS using hand nets, a dilute anaesthetic solution of clove oil (Munday and Wilson 1997) and barrier nets. *C. viridis* are obligate live coral dwellers that exhibit a high degree of site fidelity to individual coral colonies and live in schools ranging in size from three to hundreds of individuals (Nadler et al. 2014a; Ohman et al. 1998; Pratchett et al. 2012). Once collected, all wild schools were maintained in sensory isolation from one another (both visual and olfactory isolation) in a flow-through aquaria system at a density of approximately 1 fish per 2.5L. Fish were fed to satiation twice daily with INVE Aquaculture pellets and newly hatched *Artemia* sp.

Time to familiarity: Experimental procedure

Prior to conducting the escape response experiment, the length of time necessary for C. viridis to establish familiarity was determined. To do this, we created eight experimental schools composed of 15 fish each that were equally unfamiliar with each other, by joining 15 individuals from 15 geographically distinct wild schools (schools separated by a minimum of 50 m). The development of familiarity over time was determined through a choice test methodology, based on the protocols used in Griffiths and Magurran (1997a). In this protocol, a focal fish was placed in a translucent, porous barrier in the center of a testing tank (90 cm length x 30 cm width x 30 cm height). At either end of the testing tank, a school composed of seven fish of either familiar individuals (i.e. from a school the focal fish has been housed with) or unfamiliar individuals were placed in translucent, porous bottles (24 cm height x 10 cm diameter). The focal fish and the two schools were allowed to acclimate for 30 mins before the barrier of the focal fish was removed using a pulley system (which prevented the focal fish from seeing the observer). Trials lasted 15 mins each and the amount of time that the focal fish spent schooling with each group was recorded. The location of the familiar and unfamiliar schools (right or left container) was randomised between trials, to eliminate a potential side preference effect. The focal fish was considered to be schooling when it swam within two body lengths (L) of either school. One individual was tested from each experimental school every 2 days for 21 days and a different individual was used for each trial. School preference was defined as the school that the focal fish schooled with for the greatest proportion of time.

Escape response: Experimental procedure

Escape responses were examined in schools composed of eight fish each. All individuals in familiar experimental schools were allowed to familiarise for a period of three weeks (which was adequate to firmly establish familiarity, see Fig. 3.1). Similarly, unfamiliar schools were housed for three weeks in groups of eight before being reassembled with unfamiliar individuals immediately prior to experimentation.

Trials were conducted in a custom-built laminar flow swim tunnel (50 cm length x 40 cm width x 9 cm height). This device allowed schools to swim in non-turbulent conditions at a slow uniform swim speed of approximately one *L* per second (3.2 cm/s), which mimics natural flow speed conditions on the LIRS reefs on a calm day (Johansen 2014). Seawater in the system was maintained at the ambient temperature for the study period ($27 - 29^{\circ}C$).

Experimental schools were placed in the swim tunnel and allowed to acclimate for a period of four hours. Escape responses were then elicited using a standardised threat protocol in which a mechanical stimulus was dropped from above (Chapter 2; Domenici et al. 2015). This stimulus was a black cylindrical object (2.5 cm diameter x 12 cm length, 37.0 g) with a tapered end (to minimise surface waves), suspended 137 cm over the surface of the water in the swim tunnel. To avoid visual cues prior to the stimulus reaching the water's surface, this object was dropped through a white PVC pipe ending immediately before it broke the water's surface (Domenici et al. 2008; Turesson and Domenici 2007). A thread connecting the stimulus to the release point prevented it from touching the bottom of the tank (Domenici et al. 2008; Turesson and Domenici 2007). As previous studies suggest that the school's alignment during an escape response is highest with lateral stimulation at an angle of $30 - 120^{\circ}$ (Marras et al. 2012), identical stimuli were placed 2 cm from the center of each of the lateral walls of the swim tunnel. To control for a stimulus side preference, the use of the left or right lateral stimulus was alternated between trials. These stimuli remained suspended above the swim tunnel for the duration of the acclimation period using an electromagnet. Following the acclimation period, the stimulus was released using a switch, once a minimum of 6 of the 8 fish were >3.5 cm from any wall of the swim tunnel and within 4 L of the stimulus. This criterion aided in reducing the constraining effects that the walls of the swim tunnel may exert on individuals' escape response and variable effects that stimulus distance can have on the motor output of the escape response (Eaton and

Emberley 1991). Each school's escape response was video-recorded from below in high speed (240fps; Casio Exilim HS EX-ZR1000), using a mirror placed at a 45° angle. The swim tunnel was illuminated from above using two 500-W spotlights.

Kinematic analysis

Videos were analysed using ImageJ software. The stimulus onset was defined as the frame at which the stimulus first touched the water's surface. A number of individual performance and school escape response characteristics were examined as defined below.

Individual fast-start performance

Individual fast-start performance was characterised through (1) latency, (2) average turning rate and (3) distance covered. Previous studies suggest that the stimulus distance can influence these individual traits (Domenici and Blake 1997), so this measure was included as a covariate in these analyses.

(1) Latency: the time period between the stimulus onset and the fish's first movement. (2) Average turning rate: calculated by dividing the stage 1 angle (the angle between the lines intersecting the head and center of mass when stretched straight (CM) at the start and end of stage 1) by the duration of stage 1. The location of the CM in video footage was measured as 0.35 *L* posterior of the snout, based on previous measurements of generalist fish species (Webb 1978). Stage 1 is the stage immediately following the stimulus, in which fish contract the muscles on the side of the body opposite to the stimulus, causing the fish to bend into a C shape.

(3) Distance covered: distance that the fish's CM travelled within the first 10 frames (e.g. 42 ms) of their reaction. This time frame was determined by calculating the average duration of stage 1 and stage 2 in the escape response for 24 individuals (one random fish per trial). This short time frame was used as a proxy for swimming speed in order to

avoid issues with wall effects. Individuals less than 2 *L* from any wall of the swim tunnel at the time of their response were excluded from this analysis (10% of total; Eaton and Emberley 1991).

(4) Stimulus distance: distance from the stimulus to the fish's CM.

School escape response

School escape response was characterised through school cohesion (nearest neighbour distance and total school area) and spatial organisation (alignment of individuals). These school characteristics were measured at set time points throughout the escape response (0, 20 and 100 ms following the stimulus) (Marras et al. 2012). (1) Nearest neighbour distance (NND): distance to the closest neighbour within the school, as measured by the distance from each fish's CM.

(2) School area: the school's horizontal spread (cm²), as measured by the area between the fish at the edge of the school (defined as those fish whose head was at the vertex of the smallest convex polygon encompassing the group; Marras and Domenici 2013).
(3) Alignment: the variation in the orientation of all school members to the horizontal (corresponding to the direction of flow; 0° = facing into the flow towards the front of the tank, 180° = oriented with the flow towards the back of the tank). As alignment angles spanned up to 360°, circular statistics were employed. This variable is characterised by the length of mean circular vector (r) (Bachelet 1981), as calculated in the software Oriana 4.

Statistical analyses

All statistical analysis was conducted in the R Statistical Environment (v3.2.4, R Development Core Team 2015), using linear mixed-effects models (LMM) in the packages "nlme", "Ime4" and "car" (Bates and Maechler 2009; Pinheiro et al. 2016). Residual and quantile-quantile plots were assessed for each model in order to ensure that all assumptions were met. Time to familiarity was analysed using a LMM with a binomial distribution, comparing preference for the familiar school (true or false) with day as a fixed effect and school as a random effect. For individual performance traits, latency was log-transformed in order to normalise its distribution, while average turning rate and distance covered did not require transformation. These traits were all analysed with school type as a fixed effect (familiar or unfamiliar), stimulus distance as a covariate and school as a random effect. For school performance traits, no transformations were necessary. Differences in NND, school area and alignment were assessed with treatment and time as fixed effects with school as a random effect. For NND, individual was also included as a random effect (as this variable was assessed on an individual basis). No autocorrelation correction was deemed necessary for these repeated measures, based on the Akaike information criterion (AIC) for models with and without this correction. Tukey's HSD post-hoc tests were used to further investigate differences detected by the LMM.

3.3 Results

Time to familiarity

Time of exposure had a significant effect on school preference (Fig. 3.1; LMM: χ^2 = 4.311, p = 0.038). A majority of individuals first begin exhibiting a preference for the familiar school on day 15 (63%). This trend continued through days 17 (75%), 19 (88%) and 21 (75%), indicating that familiarity is firmly established within three weeks.



Fig. 3.1. Time to familiarity in the coral reef damselfish *Chromis viridis*. White bars indicate the count of individuals (out of eight total per day) that preferred the familiar school, while the grey bars denote those individuals that preferred the unfamiliar school. Preference was defined as the school that the focal individual spent the greatest proportion of time with during the 15 min trial. The dotted line denotes the null hypothesis of an even 50:50 split between the two schools on each day (which would indicate no preference).

Individual fast-start performance

Individual escape performance improved significantly with familiarity. In familiar schools, latency was nearly 500% shorter on average (Fig. 3.2A; LME: $F_{22} = 10.5$, p=0.004), indicating that individuals in familiar schools were reacting more quickly to the stimulus than those in unfamiliar schools. Individuals from unfamiliar schools had a greater proportion of slow latency times, many greater than 120 ms (Fig. 3.2B). In addition, average turning rate was approximately 50% higher (Fig. 3.2C; LME: $F_{22} = 5.7$, p = 0.026) in fast-start responses from familiar schools than unfamiliar schools. This



Fig. 3.2. Effect of familiarity on the escape response of individuals in fish schools (*Chromis viridis*), including (A) escape latency (ms), (B) frequency distribution of escape latencies, (C) average turning rate (°/s), (D) typical escape responses (lines represent the

fish midline and arrows indicate the location of the head in successive frames at 4.2 ms intervals) and E) distance covered (mm). Fish illustrations (A, C and E) represent the action taken in each step of the response (grey fish represent the fish's position immediately prior to the stimulus and the black fish illustrates the movement taken). Data in A, C and E are represented by mean ± S.E.M. Asterisks (**) indicate statistical significance (p < 0.05).

slower turning rate is illustrated in Fig. 3.2D, which displays typical escape responses found in familiar and unfamiliar schools. Unfamiliar fish exhibit a slower turning rate, as evidences by the higher number of midlines (i.e. longer time) to accomplish a similar turning angle. Lastly, distance covered was more than 60% longer in familiar schools (Fig. 3.2E; LME: F₂₂ = 9.0, p = 0.007), when compared to individuals from unfamiliar schools.

School escape response

Unfamiliar schools exhibited significantly greater cohesion and alignment than familiar schools, in terms of cohesion and spatial organisation. A significant treatment*time interaction was found for both measures of cohesion: NND (Fig. 3.3A; LME: $F_{379} = 3.041$, p = 0.011) and school area (Fig. 3.3B; LME: $F_{43} = 5.520$, p = 0.001). NND increased in familiar schools during the escape response, but remained consistently low for unfamiliar schools (Tukey's Test: $p_{fam0<fam100} = 0.018$; for all other comparisons, p > 0.05). School area increased in familiar schools throughout the escape response and remained consistently low in unfamiliar schools (Tukey's Test: $p_{fam0<fam100} < 0.001$, $p_{fam20<fam100} < 0.001$, $p_{unfam100<fam100} = 0.016$; for all other comparisons, p > 0.05). Alignment decreased in familiar schools at 20 ms following the stimulus (Fig. 3.3C; Tukey's test: $p_{fam0>fam20} = 0.002$), but recovered to pre-stimulus levels by 100 ms after stimulation (Tukey's test: $p_{fam20<fam100} = 0.013$; for all other comparisons, p > 0.05).



Fig. 3.3. Effect of familiarity on escape performance of fish schools (*Chromis viridis*). (A) Nearest neighbour distance (NND) denotes the distance to the closest neighbour (center of mass to center of mass, mm). (B) School area indicates the school's spread (cm²). (C) Alignment is a measure of the variation in the orientation of all school members. This variable is characterised by the length of mean circular vector (r).

3.4 Discussion

Familiar schools exhibited superior individual escape performance, with shorter latency times (higher reactivity), greater average turning rates (increased

maneuverability) and longer distances covered (greater propulsive performance) than unfamiliar schools. Latencies among familiar individuals are consistent with M cell driven escape responses. Previous work (using microelectrodes to measure M cell action potentials under a comparable temperature and mechanical stimulus) found an average latency of 16 ms in M cell fast-start responses (Eaton et al. 1981). In the present study, nearly twice as many fish in familiar schools exhibited latencies \leq 16 ms than in unfamiliar schools (47 vs. 24 individuals, in familiar and unfamiliar schools respectively; n = 96 individuals treatment⁻¹; Fig. 3.2B), suggesting that M cell initiation may be delayed or inhibited in unfamiliar schools.

Previous studies have shown that fish exposed to cues of an unfamiliar school exhibit an increase in the plasma concentration of stress hormones like cortisol (Yue et al. 2006). Stress typically increases brain serotonin (5-hydroxytryptamine, 5-HT) neurotransmission (Winberg and Nilsson 1993). This may in turn modify the stimulus threshold necessary for M cell activation (Whitaker et al. 2011), indicating that a stress response could have played a role in the reduced responsiveness and escape performance in unfamiliar schools. Indeed, individuals in unfamiliar schools all showed longer reaction times, reduced angular velocity and diminished propulsion capacity, suggesting that fast start escape responses occurred in the absence of M cell firing (Domenici 2010; Eaton et al. 1981; Korn and Faber 2005). However, familiar schools exhibited lower cohesion and alignment during the school's escape response than unfamiliar schools. This result could indicate that familiar schools are able to reap the same benefits of group living to defense under higher NND conditions as unfamiliar schools with lower NND. This greater NND may aid in minimising the risk of collisions with school-mates while swimming at rapid speeds, thereby permitting a stronger M cell response (Domenici 2010).

To our knowledge, this is the first study to show that familiarity modulates faststart escape responses, aiding in both the timing of the response (latency) as well as its motor output (average turning rate and distance covered). While previous work has illustrated that altered biophysical conditions can impact escape behaviour (Mirjany and

Faber 2011; Preuss and Faber 2003), the present findings demonstrate a prominent role of social context in the fast-start escape responses of gregarious species. As the chances of surviving a predator attack increase with faster escape performance (Walker et al. 2005), familiarity is likely to be selectively advantageous for schooling fish.

Chapter 4: Shoaling reduces metabolic rate in a gregarious coral reef fish species

This chapter was published in Journal of Experimental Biology (Letter format)

Authors: LE Nadler, SS Killen, EC McClure, PL Munday and MI McCormick

4.1 Summary

Many animals live in groups due to the potential benefits associated with defense and foraging. Group living may also induce a "calming effect" on individuals, reducing overall metabolic demand. This effect could occur by minimising the need for individual vigilance and reducing stress through social buffering. However, this effect has proved difficult to quantify. We examined the effect of shoaling on metabolism and body condition in the gregarious damselfish *Chromis viridis*. Using a novel respirometry methodology for social species, we found that the presence of shoal-mate visual and olfactory cues led to a reduction in the minimum metabolic rate of individuals. Fish held in isolation for one week also exhibited a reduction in body condition when compared to those held in shoals. These results indicate that social isolation due to environmental disturbance could have physiological consequences for gregarious species.

4.2 Introduction

Group living is wide-spread among animal species and carries a number of benefits (Krause and Ruxton 2002). It has been shown, for example, that individuals within groups can reduce their energy expenditure in a variety of situations, including the costs of flight in birds (Weimerskirch et al. 2001), swimming in fish (Hemelrijk et al.

2015), web-building in spiders (Jakob 1991) and thermoregulation in mice (Scantlebury et al. 2006). Individuals may also be able to reduce overall metabolic demand through group living in what is known as the "calming effect" (Martin et al. 1980; Parker 1973; Trune and Slobodchikoff 1976). One factor that likely contributes to this effect is a reduced need for individual vigilance, as animal groups exhibit improved threat detection by having "many eyes" to scan for predators (Roberts 1996; Ward et al. 2011). Individuals accustomed to a social environment may also exhibit reduced stress when allowed to associate with conspecifics (Hennessy et al. 2009).

A number of methods have thus far been employed to estimate the magnitude of the calming effect in a range of gregarious fish species. First, the ventilation rate of grouped versus solitary individuals has been recorded to estimate metabolic rate. A second method uses respirometry (where oxygen uptake is measured as a proxy for aerobic metabolism) to compare the sum of each individual's metabolic rate when measured alone with the metabolic rate of the group measured together (Parker 1973; Schleuter et al. 2007). While these methods have provided supporting evidence for the calming effect, they do not directly measure social influences on individual physiology. Lastly, a third method has been employed, in which cues of conspecifics are presented to a solitary individual either by allowing conspecifics to freely move around the respirometry chamber (Plath et al. 2013) or by placing individuals in neighbouring respirometry chambers (Herskin 1999). However, this method has not detected evidence of a calming effect, suggesting that this methodology may fail to sufficiently simulate shoaling conditions to elicit one. A calming effect may not be detected if the shoal-mates move too far away from the focal individual or olfactory cues of shoalmates are too weak to allow social recognition (Brown and Smith 1994; Herskin 1999; Plath et al. 2013; Ward et al. 2002).

In this study, we developed a novel method to measure the calming effect's influence on body condition and metabolic rate in a gregarious coral reef fish. Fish were held for two weeks either alone or in a shoal before measurement of metabolic rate. Metabolic rate of solitary versus shoaling individuals was then tested using custom

respirometry chambers that were designed to provide visual and olfactory cues of shoalmates to a focal individual. We hypothesised that individuals housed in groups and tested with shoal-mates would exhibit the greatest body condition, lowest minimum metabolic rate and reduced physiological reaction to stress compared to individuals in solitary treatments.

4.3 Materials and methods

Fish collection and maintenance

This experiment was conducted at the Lizard Island Research Station (LIRS, a facility of the Australian Museum) in the northern Great Barrier Reef ($14^{\circ}40'08''S$; $145^{\circ}27'34''E$), using a gregarious damselfish species, the blue-green puller *Chromis viridis* (Cuvier, 1830). Wild shoals of juvenile *C. viridis* (standard length = 3.69 ± 0.03 cm, wet mass = 1.84 ± 0.04 g; average \pm S.E.) were collected from reefs adjacent to LIRS using hand nets and barrier nets. Fish were placed into either groups of 10 individuals (group holding treatment, n = 8) or held in isolation (alone holding treatment, n = 8) at a stocking density of 1 fish/L. Fish were fed a body-mass specific diet twice daily with INVE Aquaculture pellets and newly hatched *Artemia* sp.

Body condition measurement

Focal individuals were chosen at random and tagged with visible implant elastomer (Northwest Marine Technology Inc., Tumwater, WA, USA) so they were identifiable over time (Hoey and McCormick 2006). Each holding treatment (alone, group) was maintained for two weeks under a natural light cycle (14h light: 10h dark). At three time points during this period (weeks 0, 1 and 2), focal fish were measured for wet weight ($W \pm 0.0001$ g) and standard length ($L \pm 0.01$ cm), from which Fulton's K condition factor ($K = 100 * (W/L^3)$) was calculated.

Respirometry

Metabolic rate was measured for each focal fish using custom respirometry chambers composed of two cylindrical glass tubes (inner respirometry chamber and larger, outer shoal-mate holding chamber) with acrylic end caps and immersed in separate, temperature-controlled water baths $(29^{\circ}C \pm 0.5^{\circ}C)$ (Fig. 4.1). All individuals from both treatments were retrieved from holding tanks using plastic tubs to minimise capture time and to eliminate air exposure. Focal fish were then placed in 1 L plastic bags filled with seawater for ~10 mins prior to transfer to the inner respirometry chamber, in order to allow focal fish to recover from the capture protocol. The inner respirometry chamber was connected to a recirculating pump to mix water in the respirometer, and a flushing pump, to flush the chamber with oxygen-saturated water for 3 min between each 9 min measurement period. The timing of this flushing and measurement cycle ensured that oxygen saturation in the inner chamber remained above 80% air saturation at all times (Hughes 1973). The outer chamber was affixed to the exterior of the inner chamber, to provide visual and olfactory cues of shoal-mates to the focal individual; this chamber was aerated with a continuously running flush pump and the water leaving the outflow port was attached to the in-flow vent for the inner chamber's flush pump, in order to provide the shoal-mates' olfactory cues to the focal individual. Water mixing from the two chambers was confirmed with preliminary tests using food coloring. To ensure that the inner chamber was being flushed with equally oxygenated water in both testing treatments, the flush pump utilised a mixture of the outflow water from the outer chamber and ambient water from the surrounding aquarium. The diameter of this outer chamber prevented shoal-mates from swimming >1.5 body lengths from the focal individual.

Dissolved oxygen concentration in the inner chamber was measured every 2s using a Fire-Sting fibre-optic oxygen meter (Pyroscience, Germany) connected to a computer. The oxygen-sensing optode was mounted in the recirculation loop in a flow-



Fig. 4.1. The experimental setup was composed of an inner respirometry chamber (length = 13.5 cm, inner diameter = 3.24 cm, volume of chamber and associated gasimpermeable tubing = 100 ml) and a larger, outer shoal-mate holding chamber (length = 12.0 cm, inner diameter = 11.4 cm, volume of chamber = 1.10 L). Arrows indicate the direction of water flow through tubing. X's indicate water pumps used for mixing the inner chamber and flushing both chambers.

through cell (Svendsen et al. 2016). Focal fish were starved for 24-25 hours prior to experimentation to ensure that they were in a post-absorptive state (Niimi and Beamish 1974) and were left undisturbed in the respirometers for 11 – 12 hours overnight, as *C. viridis* is quiescent at night. Preliminary studies of *C. viridis* run for 36 hours indicated that oxygen consumption stabilised within ~5 hours, with the lowest levels achieved overnight (Killen et al. unpublished data). This was consistent with the data presented here (average time to stabilise = 4.6 hours, and was not significantly different between treatments, p > 0.05). A dim light remained on through the night in the laboratory, allowing the focal fish to see their shoal-mates in group-testing trials. Slopes (*s*) were calculated from plots of oxygen concentration versus time using linear least squares regression (LabChart v6 software) and converted to rate of oxygen uptake (\dot{M}_{O2} , mg O₂ h⁻¹), excluding the first and last minute of each measurement period to allow the oxygen concentration in the recirculation loop to stabilise following the flushing period. All r² values were greater than 0.97. Bacterial respiration was measured in empty chambers for three measurement periods before and after trials and was then subtracted from all fish respiration measurements, assuming a linear increase in bacterial respiration over time (Rodgers et al. 2016).

The metabolic rate of each focal fish was recorded in an alone (no shoal-mates in the outer chamber) and in a group testing treatment (6 shoal-mates in the outer chamber). Three measures of metabolic rate were analysed. First, minimum measured metabolic rate in fish exposed to each treatment (MR_{min}) was estimated using the protocol typically employed to measure standard metabolic rate (the metabolic rate of a resting ectotherm) in the literature. This was accomplished by taking MR_{min} as the lowest 10th percentile of \dot{M}_{02} measurements (Chabot et al. 2016; Killen 2014) and comparisons were drawn between individuals tested alone and with a group. Second, routine metabolic rate (RMR, the metabolic rate of an undisturbed animal including costs of random activity) was calculated as the mean $\dot{M}_{\rm O2}$ excluding the first 5 hours in the respirometer and differences between fish tested alone (RMR_{alone}) and fish tested in groups (RMR_{group}) were assessed (Killen et al. 2011). Third, the initial stress response (ISR) was taken as the difference between the initial metabolic rate (first slope following transfer to the respirometer) and MR_{min}. \dot{M}_{O2} is commonly used as an indicator of stress and reaction to threats like predation, due to the previously established link between oxygen uptake and stress hormones including cortisol and epinephrine (e.g. Brown et al. 1982; Morgan and Iwama 1996). In this study, the stressor was the handling stress induced during transfer to the respirometer and any stress of being in isolation.

Statistical Analyses

All statistical analyses were conducted in R v.3.2.4, using package "nlme" (Pinheiro et al. 2015; R Development Core Team 2015). Differences in body condition over time were assessed using a linear mixed-effects model (LMM) corrected for autocorrelation, with holding treatment (group or alone) and time (weeks 0, 1, 2) as fixed effects and individual as a random effect. Differences in the MR_{min}, RMR and ISR

were analysed using a LMM with holding pattern (group or alone) and testing pattern (shoaling or solitary) as fixed effects, body mass as a covariate (to account for differences in body size between individuals) and individual as a random effect.

4.4 Results and discussion

The results show that the minimal estimated metabolic rate of gregarious species may be higher when individuals are measured alone versus when they are measured in a group, potentially due to an increase in energy spent on vigilance or an autonomic stress response to social isolation (Barreto and Volpato 2011; Hennessy et al. 2009; Roberts 1996). MR_{min} tested in a group was significantly lower than MR_{min} tested alone, with an average reduction of 25.9% (5 – 60% range; Fig. 4.2A; LMM: F_{1,14} = 27.27, p = 0.0004). Similar results were also found for RMR, with RMR_{group} significantly lower than RMR_{alone} (Fig. 4.2B; LMM: F_{1,14} = 17.34, p = 0.0019). Respirometry treatment had a comparable effect on individuals from both the alone and group holding treatments (MR_{min} LMM: F_{1,14} = 1.26, p = 0.2812; RMR LMM: F_{1,14} = 1.14, p = 0.3033).

Holding treatment did have a significant impact on body condition. Individuals that were kept alone in their holding tanks exhibited a reduction in condition factor from week 0 to 1 (Fig. 4.2C; LMM: $F_{1,30} = 9.16$, p = 0.0050). The measured increase in MR_{min} in the alone testing treatment likely contributed to this reduction in body condition. As feeding rate can decrease immediately following social isolation in gregarious species (Barreto and Volpato 2011), reduced food intake in the alone holding treatment may have compounded this effect. Individuals accustomed to the group holding treatment exhibited a stronger physiological reaction to handling stress during transfer to the respirometer than those acclimated to an isolated condition. ISR was more than double in focal individuals that had been held in groups as compared to



Fig. 4.2. Effect of holding and testing treatment on (A) minimum metabolic rate (MR_{min} , mg $O_2 h^{-1}$) and (B) routine metabolic rate (RMR, mg $O_2 h^{-1}$). (C) Effect of holding

treatment on individual body condition (Fulton's K condition factor). (D) Effect of holding and testing treatment on the initial stress response (ISR, mg O_2 h⁻¹). Metabolic rate measures were mass-corrected using residuals of the relationship between log body mass and log metabolic rate added to the fitted value for mass = 1.84 g, the mean mass of all fish used in the study. Error bars are s.e.m and n = 8 for all treatments. Asterisks (**) indicate statistical significance (p < 0.05).

individuals held alone (Fig. 4.2C; $F_{1,14} = 9.62$, p = 0.0078), regardless of whether fish were measured for MR_{min} in a shoal or in isolation (Fig. 4.2D; $F_{1,14} = 0.21$, p = 0.6559). Plath et al. (2013) found a similar trend in shoaling minnows, in which oxygen consumption rate increased immediately following exposure to a group testing treatment. Individuals that were held in groups but measured for metabolic rate in isolation exhibited elevated ISR likely due to the stress of acute social isolation, which can increase circulating glucocorticoids in gregarious species (Galhardo and Oliveira 2014; Lyons et al. 1993). Fish in the alone holding treatment may have grown accustomed to being alone, relying less on the presence of shoal-mates for risk assessment and stress reduction. As aloneheld fish would not have had shoal-mates to aid in vigilance, they may have increased the threshold of threat at which they instigate a stress response, which could explain their lower ISR. However, further studies quantifying the role of individual vigilance in the calming effect would be essential to tease apart this mechanism (Roberts, 1996).

A variety of factors likely influence the magnitude of the calming effect in social species, such as the degree of social organisation, ontogenetic stage and novelty of the environment (Hennessy et al. 2009). Therefore, further studies should investigate if the calming effect is maintained under different conditions. In highly territorial species, the presence of conspecifics can increase metabolic demand and aggressive behaviours (Killen et al. 2014; Sloman et al. 2000), highlighting the importance of behavioural traits in physiological responses to conspecifics. In addition, many of the benefits of group living increase up to an optimal shoal size, including vigilance and foraging (Fischer et al. 2015; Goldenberg et al. 2014; Magurran and Pitcher 1983). Therefore, the magnitude of

the calming effect is likely to vary depending on the group size presented during testing. Lastly, as recent studies indicate evidence of intraspecific variation in sociability (Hennessy et al. 2009; Killen et al. 2016), the adaptive value of group living to fishes may vary among individuals due to differences in physiological and behavioural characteristics.

In an ecological context, disturbances such as storms and flooding can lead to group disruption and forced social isolation in animal communities (e.g. Lassig 1983; Yoon et al. 2011). The results of this study suggest that solitary members of gregarious species may experience increased physiological reactions to stress and energy expenditure. This suggests that isolation could be energetically costly in species for whom group living is the natural state. An anxiogenic, autonomic stress response due to social isolation could have a range of additional repercussions for social species, with implications for overall fitness (Hennessy et al. 2009).

Chapter 5: Effect of elevated carbon dioxide on shoal familiarity and metabolism in a coral reef fish

This chapter is in revision at Conservation Physiology

Authors: LE Nadler, SS Killen, MI McCormick, S-A Watson and PL Munday

5.1 Summary

Atmospheric carbon dioxide (CO_2) is expected to more than double by the end of the century. The resulting changes in ocean chemistry will affect the behaviour, sensory systems and physiology of a range of fish species. Though a number of past studies have examined CO2 effects in gregarious fishes, most have assessed individuals in social isolation, which can alter individual behaviour and increase metabolism in species accustomed to a social environment. Within social groups, a learned familiarity can develop following a prolonged period of interaction between individuals, with fishes preferentially associating with familiar conspecifics due to benefits such as improved social learning and greater foraging opportunities. However, social recognition occurs through detection of shoal-mate cues and hence may be disrupted by near-future CO_2 conditions. In the present studies, we examined the influence of elevated CO₂ on shoal familiarity and the metabolic benefits of group living in the gregarious damselfish species the blue-green puller (Chromis viridis). Shoals were acclimated to one of three CO_2 treatments: control (450 µatm), mid- CO_2 (750 µatm) or high- CO_2 (1000 µatm). Familiarity was examined using a choice test, in which individuals were given the choice to associate with familiar shoal-mates or unfamiliar conspecifics. Under control conditions, individuals preferentially associated with familiar shoal-mates. However, this association was lost under both elevated CO₂ treatments, suggesting that the tools for

social recognition were disrupted, the preference to associate with familiar shoal-mates was lost or the memory of familiar conspecifics was diminished. However, elevated CO₂ did not impact the calming effect of shoaling on metabolism, as measured using an intermittent flow respirometry methodology for social species. Under all CO₂ treatments, individuals exhibited a significantly lower metabolic rate when measured in a shoal versus alone, highlighting the complexity of shoal dynamics and the processes that influence shoaling's benefits.

5.2 Introduction

Atmospheric carbon dioxide (CO₂) has risen to over 400 ppm (Dlugokencky and Tans 2016) due to human activity, higher than any time in the last 800,000 years (Luthi et al. 2008). The partial pressure of CO₂ (pCO₂) in the world's oceans is rising at approximately the same rate as the atmosphere (Doney et al. 2009; Le Quéré et al. 2013). If current anthropogenic CO₂ emissions continue unabated, average CO₂ levels in the atmosphere and surface ocean will more than double from current-day levels by the year 2100 (Collins et al. 2013; Fabry et al. 2008). Furthermore, new models indicate that seasonal cycles in ocean pCO₂ will be amplified in the future, meaning that marine organisms will experience extended periods of ocean pCO₂ in excess of 1000 µatm by the end of this century (McNeil and Sasse 2016). Rising CO₂ levels are predicted to affect a range of behavioural (Briffa et al. 2012; Nagelkerken and Munday 2016) and physiological (Heuer and Grosell 2014; Portner et al. 2004) processes in marine organisms, with potentially far-reaching effects on marine ecosystems (Wittmann and Portner 2013).

Higher environmental CO₂ levels can be a problem for marine organisms because they act to acidify the blood and tissues and thus affect pH dependent physiological processes (Portner et al. 2004). Fish defend against acidosis in a high CO₂ environment by actively regulating acid-base relevant ions in their blood and tissues (Heuer and Grosell 2014). Consequently, they are able to maintain a pH suitable for cellular

processes, even at very high ambient CO₂ levels (Ishimatsu et al. 2008). However, this acid-base regulation leads to changes in extracellular ion concentrations that may interfere with the function of neurotransmitter receptors (Nilsson et al. 2012). These neurological changes can lead to altered behaviour and impaired sensory systems. Behavioural effects of exposure to high CO₂ include reduced learning ability (Chivers et al. 2014; Jutfelt et al. 2013), altered activity levels (Ferrari et al. 2011a; Munday et al. 2010), higher anxiety (Hamilton et al. 2014), disrupted behavioural lateralisation (Domenici et al. 2011a; Jutfelt et al. 2013) and reduced predator avoidance behaviour (Dixson et al. 2010; Ferrari et al. 2011b; Munday et al. 2010). Behavioural responses to visual (Ferrari et al. 2012b), olfactory (Munday et al. 2009b) and auditory (Rossi et al. 2016) cues are all affected, although not always to the same extent, with responses to visual cues less affected than olfactory preferences at projected near-future CO₂ levels (Lönnstedt et al. 2013).

The effect of elevated pCO₂ and decreasing pH on other physiological characteristics are less clear. Theoretically, the energetic cost of increased regulatory mechanisms (such as acid-base balance regulation) should manifest in higher overall energetic needs (Ishimatsu et al. 2008). However, studies measuring standard metabolic rate (SMR; the metabolic rate of a resting, fasting and non-stressed individual, a measure of basic energetic needs) of fishes under elevated pCO₂ have found highly variable results, reporting increases, decreases and no effects of pCO_2 on SMR, suggesting that the effects may be species or context specific (Heuer and Grosell, 2014; Lefevre, 2016). However, another important consideration is that, although many studies have examined the effect of pCO_2 on the metabolic rate of gregarious fish species (Miller et al. 2012; Munday et al. 2009a; Rummer et al. 2013), all have measured metabolic rate in solitary individuals, which can have effects on the measured metabolic rate due to the stress of isolation (Chapter 3). Therefore, how social context may modulate the effect of pCO₂ on metabolic traits, such as SMR, remains unknown. Recent work found that the immediate social environment could have a significant impact on metabolic rate, with individuals tested in the presence of shoal-mate cues exhibiting a

significantly lower minimum metabolic rate than individuals tested in social isolation (**Chapter 3**). One factor that likely contributes to this calming effect is a reduced need for individual vigilance, as animal groups exhibit improved threat detection by having "many eyes" to scan for predators (Roberts 1996; Ward et al. 2011). Individuals accustomed to a social environment may also exhibit reduced stress when allowed to associate with conspecifics (Hennessy et al. 2009). The importance of these benefits could increase under environmental stressors like rising pCO₂.

Group living is wide-spread among fish species and carries benefits for individuals with respect to predator avoidance, foraging opportunities and energy use (Krause and Ruxton 2002; Shaw 1978). A learned familiarity can be attained following a prolonged period of interaction between social individuals (reviewed in Ward and Hart 2003), increasing the probability of reciprocal cooperation between members of an animal group (Granroth-Wilding and Magurran 2013). This greater cooperation can have benefits for a range of fitness-enhancing processes and characteristics, including foraging, social learning, body condition and survival (Atton et al. 2014; Seppä et al. 2001; Swaney et al. 2001). As a result, fish prefer to shoal with familiar conspecifics (e.g. Bhat and Magurran 2006; Edenbrow and Croft 2012; Griffiths and Magurran 1997a; Magurran et al. 1994), with individual identification achieved primarily through olfactory stimuli (Brown and Smith 1994; Partridge and Pitcher 1980; Ward et al. 2002). As elevated pCO₂ is known to impact behavioural traits and sensory abilities necessary for social recognition, the ability to recognise familiar shoal-mates may be compromised under future environmental conditions.

Elevated pCO₂ may affect the calming effect and the ability of fish to recognise conspecifics due to its effects on behaviour, sensory abilities or physiology. In the present study, we examined the effect of elevated pCO₂ on familiarity and the calming effect in the blue-green puller, *Chromis viridis* (Cuvier, 1830), a common species of shoaling damselfish. Shoals were acclimated to one of three CO₂ treatments: control (450 µatm), mid-CO₂ (750 µatm) or high-CO₂ (1000 µatm). Our aims were: (i) to determine if elevated pCO₂ modulates familiarity and (ii) to explore whether the calming
effect was altered by environmental pCO_2 . We hypothesised that familiarity will be disrupted under elevated pCO_2 . Given the known benefits of familiarity to shoaling fish (Atton et al. 2014; Seppä et al. 2001; Swaney et al. 2001), we further predicted that the calming effect on the minimal measured metabolic rate would be reduced if familiarity were disrupted at elevated pCO_2 .

5.3 Materials and methods

Fish collection and maintenance

Experiments were conducted at the Lizard Island Research Station (LIRS) in the northern Great Barrier Reef, Australia ($14^{\circ}40'08''S$; $145^{\circ}27'34''E$). Shoals of *C. viridis* (SL: 3.22 ± 0.03 cm, BM: 1.29 ± 0.04 g, mean \pm SE) were collected from reefs in the lagoon adjacent to LIRS using hand nets and barrier nets. *C. viridis* is an abundant, live coral-associated shoaling species found on coral reefs throughout the Indo-Pacific region in groups ranging in size from a few to hundreds of individuals (Randall et al. 1997). Fish were placed into groups composed of eight individuals and housed in replicate 30 L aquaria in a flow-through seawater system. All experimental shoals were held together for a minimum of 15 days to ensure that they exhibited a uniform degree of familiarity (Ward et al. 2003). Fish were fed to satiation twice daily with INVE Aquaculture pellets and newly hatched *Artemia* sp.

CO2 treatments and administration

Shoals were acclimated to one of three CO_2 treatments: 450 µatm (ambient control), 750 µatm or 1000 µatm (seawater chemistry summarised in Table 5.1). These elevated CO_2 treatments were chosen based on the range of CO_2 levels projected for the year 2100 (Collins et al. 2013; McNeil and Sasse 2016). Seawater was pumped directly from the ocean into 60L header tanks. Elevated- CO_2 seawater treatments were achieved

by dosing CO₂ to a set pH, using a pump placed into each sump through which CO₂ was diffused. This pump aided in rapid dissolution of CO₂ and vigorous stirring of water in the sump. This additional uptake of CO₂ causes changes in the carbon chemistry of seawater (increased pCO₂, decreased pH and altered relative abundance of carbonate and bicarbonate ions) but does not directly impact other biochemical cycles (e.g. the nitrogen cycle) (Doney 2010). A pH-controller (Aqua Medic, Germany) attached to each CO₂ treatment header tank maintained pH at the desired level. In control header tanks, air was diffused through sump pumps. Equilibrated seawater was then pumped at a rate of ~700 ml/min to each of the replicate 30 L experimental tanks. For each of these replicate tanks, seawater pH_{NBS} (Mettler Toledo SevenGo Pro) and temperature (Comark C22) were recorded daily. Seawater CO_2 was confirmed with in situ CO_2 measurements, using a portable CO₂ equilibrator and non-dispersive infrared (NDIR) sensor (Vaisala GMP343) (Hari et al. 2008; Munday et al. 2014b). For experiment 1, in situ CO₂ measurements were conducted once weekly in the control and 1000 µatm treatments to confirm pH measurements. During experiment 2, these measurements were conducted on each treatment at least three times weekly, during which CO₂ measures were recorded. These measurements are detailed in Table 1, and confirm our calculated pCO₂. Salinity was measured by an automated float in the Lizard Island lagoon (Bainbridge 2015). Water samples were taken twice weekly and analysed for total alkalinity by Gran titration (888 Titrando, Metrohm, Switzerland) to within 1% of certified reference material (Prof. A. Dickson, Scripps Institution of Oceanography). Average pCO_2 was calculated with the program CO2SYS, from measured pH_{NBS} , temperature, salinity and total alkalinity, using constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and Dickson for KHSO₄.

Treatment	Experiment #	Temperature (°C)	Salinity (psu)	^{pH} NBS	Total Alkalinity (μmol kg ¹ SW)	pCO ₂ (calculated, µatm)	pCO ₂ (in situ, µatm)
Control CO ₂	1	28.8 (±0.2)	35.5 (±0.01)	8.15 (±0.010)	2284 (±1)	442 (±9)	465 (±13)
	2	28.9 (±0.3)	35.0 (±0.03)	8.13 (±0.002)	2309 (±8)	461 (±2)	449 (±11)
Mid CO ₂	1	29.1 (±0.2)	35.5 (±0.01)	7.96 (±0.001)	2285 (±12)	734 (±5)	-
	2	29.0 (±0.3)	35.0 (±0.03)	7.96 (±0.001)	2296 (±8)	753 (±2)	766 (±11)
High CO ₂	1	28.8 (±0.2)	35.5 (±0.01)	7.86 (±0.001)	2296 (±2)	963 (±7)	981 (±17)
	2	28.8 (±0.3)	35.0 (±0.03)	7.87 (±0.001)	2297 (±11)	952 (±3)	983 (±15)

Table 5.1. Summary of seawater chemistry parameters in control and elevated carbon dioxide treatments for experiments 1 and 2. Estimated pCO₂ was calculated in the program CO2SYS using the other measured parameters. In situ pCO₂ was measured using a portable CO₂ equilibrator with non-dispersive infrared (NDIR) sensor. Error is s.e.m.

Experiment 1: Effect of elevated CO₂ on familiarity

Nine experimental shoals of C. viridis were acclimated to each CO_2 treatment for a period of 4 - 7 days prior to experimentation. This time period is sufficient for elevated CO₂ to induce behavioural changes in reef fishes and previous studies indicate that longer acclimation periods do not change results (Munday et al. 2014a; Munday et al. 2013a; Welch et al. 2014). Two individuals per group were chosen randomly for testing for shoal association preferences (n=18 individuals/treatment). These individuals were distinguished from each other and their shoal-mates using unique visible implant elastomer (VIE) tags (Hoey and McCormick 2006). VIE tags were administered 24-48 hours prior to placement in CO₂ treatment. Shoaling preference was established using a choice test, using the methodology adapted from Griffiths et al. (1997a). An elongate testing tank (Fig. 5.1A) was filled to a depth of 20 cm with seawater at the same CO_2 level as the relevant treatment. Identical 2L plastic containers (height: 24 cm x diameter: 10 cm) were placed at each end of the tank, 6 cm from the sidewall. The plastic containers were transparent and made porous to olfactory cues by holes drilled around the circumference. Shoals composed of 7 fish of either the familiar or an unfamiliar group was placed in these bottles. The location of the familiar shoal (right or left bottle) was randomised in order to control for any side preference or undetected differences between the optical properties of the plastic in the containers. The shoal used as unfamiliar was also randomised, to ensure that each shoal within a treatment was used as the unfamiliar shoal a uniform number of times and that a different unfamiliar shoal was used when testing each of the two focal fish from a shoal. The focal

fish was placed in a clear, porous container in the centre of the tank. This container sat over a small coral shelter and the bottom 3 cm of the container was opaque to allow the fish to take shelter. All fish were left to acclimate in this container for 15 mins. The container surrounding the focal fish was then lifted using a pulley system so that the focal fish would not be disturbed by visual cues of the observer. Trials lasted 15 mins and were video-recorded.

Videos were analysed for: 1) the proportion of time spent shoaling with each group, 2) initial shoal choice following removal of the barrier and 3) total shoal visits (a proxy for activity, which indicates the number of times that the focal fish traversed the experimental tank). Individuals were said to be shoaling when they were swimming within two body lengths of the shoal (Pitcher and Parrish 1993). To ensure that focal fish were making an informed choice (e.g. had experienced the sensory cues of both stimulus shoals), they had to visit both shoal preference zones within a trial or they were re-tested the following day.

Experiment 2: Effect of elevated CO₂ on the calming effect

Ten experimental shoals were acclimated to each CO₂ treatment for a period of 17 - 20 days. This longer acclimation period was used for this experiment, as studies show that metabolism requires a longer period of acclimation to adjust to elevated CO₂ treatments (Enzor et al. 2013). One individual per group was chosen randomly for testing (n = 10 individuals/treatment) and was identified using VIE tags (Hoey and McCormick 2006). VIE tags were administered 24-48 hours prior to placement in CO₂ treatment. The calming effect was measured using a previously described intermittent-flow respirometry methodology for social species (**Chapter 3**; Fig. 5.1B). Each respirometry chamber was composed of two cylindrical glass tubes: an inner tube (length =13.5 cm, inner diameter = 3.24 cm, total volume of chamber and associated gas-impermeable tubing = 100 ml) and an outer tube (length = 12.0 cm; inner diameter = 11.4 cm, total volume of chamber minus volume occupied by inner chamber = 1.10 L). The outer

chamber was affixed to the exterior of the inner chamber and was used to provide visual and olfactory cues of shoal-mates to the focal individual. This larger chamber was aerated with a continuously running flush pump. To provide olfactory cues of shoal-mates to the focal individual, the water leaving the outflow port was attached to the inflow vent for the inner chamber's flush pump. The inner chamber was connected to a recirculating pump (to mix water in the respirometer) and a flushing pump that flushed the chamber with oxygen-saturated water for 3 min between each 9 min measurement period. The water used to flush the chamber between measurement periods was maintained at the same pH and pCO_2 as the focal fishes' treatment. Chambers were immersed in separate, temperature-controlled water baths (29 °C ± 0.5 °C). The metabolic rate of each focal fish was recorded in an alone testing treatment (no shoal-mates in the outer chamber) and a group testing treatment (6 shoal-mates in the outer chamber).

Dissolved oxygen concentration in the inner, focal chamber was measured every 2 s and logged using a Fire-Sting fibre-optic oxygen meter (Pyroscience, Germany), connected to a computer. The oxygen-sensing optode was mounted in the recirculation loop in a flow-through cell, to ensure that flow was sufficient for a fast response time of the sensor (Svendsen et al. 2016). Focal fish were fasted for a minimum of 24 hours prior to experimentation to ensure that they were in a post-absorptive state and were left undisturbed in the respirometers for 17 – 19 hours overnight, as C. viridis is quiescent at night. A dim light remained on through the night in the laboratory to simulate moonlight, allowing the focal fish to see their shoal-mates in group testing trials. Activity was recorded during daylight hours using a webcam (H264 Webcam software) and was measured by counting the number of 180° turns for 10 minutes/hour of testing (from which turns/min was calculated). Slopes (s) were calculated from plots of oxygen concentration versus time using linear least squares regression (LabChart v6) and converted to rate of oxygen uptake (\dot{M}_{02} , mg O₂ h⁻¹). For all trials, background respiration was measured in empty chambers for three measurement periods both before and after trials. Bacterial respiration was then subtracted from all fish respiration





measurements, assuming a linear increase in bacterial respiration over time (Rodgers et al. 2016).

Once focal individuals had completed both the alone- and group-testing trials, maximal metabolic rate (MMR) was measured, so that each individual's aerobic scope

(AS) could be calculated. AS is an individual's aerobic metabolic capacity, which indicates the available energy that an individual has for all aerobic processes beyond basic maintenance (Farrell 2016). MMR was measured using the chase protocol, in which individuals are exercised to exhaustion through manual chasing (Roche et al. 2013). Fish were considered exhausted when they no longer responded to chasing by burst swimming. Fish were then air-exposed for 30 s to further ensure that they had depleted all endogenous oxygen stores. Individuals were then transferred to their respective respirometry chambers and oxygen uptake was measured for 8 – 10 mins (this time frame was used to ensure that oxygen saturation in the water remained > 80% air saturation). This method elicits anaerobic exercise in individuals and maximal rates of oxygen uptake were measured during subsequent recovery. MMR was measured for all fish in an alone testing treatment. These oxygen uptake slopes were measured at 3-min intervals, with the greatest oxygen uptake during this period taken as MMR.

Three measures of metabolic rate were analysed. First, minimum measured metabolic rate in fish exposed to each treatment (MR_{min}) was estimated using the protocol typically employed to measure SMR in the literature. This was accomplished by taking MR_{min} as the lowest 10th percentile of \dot{M}_{O2} measurements (Chabot et al. 2016; Killen 2014) and comparisons were drawn between individuals tested alone and with a group. Second, routine metabolic rate (RMR, the metabolic rate of an undisturbed animal including costs of random activity) was calculated as the mean \dot{M}_{O2} excluding the first 5 hours in the respirometer and differences between fish tested alone (RMR_{alone}) and fish tested in groups (RMR_{group}) were assessed (Killen et al., 2011). Third, individuals' response to stress was also determined by using the first slope (FS) of each trial, following transfer to the respirometer. The stress response was calculated in the context of AS (AS = MMR – MR_{min}), in order to determine the proportion of AS that fish were using in response to stress. The initial stress response (ISR) was therefore calculated using the following equation:

 $ISR = (FS - MR_{min})/AS$

 $\dot{M}_{\rm O2}$ is commonly used as an indicator of stress and reaction to threats like predation, due to the previously established link between oxygen uptake and stress hormones including cortisol and epinephrine (e.g. Brown et al. 1982; Morgan and Iwama 1996). In this study, the stressor was the handling stress induced during transfer to the respirometer and any stress of being in isolation.

Statistical analysis

Statistical analysis was conducted in the R Statistical Environment (v. 3.2.4) using the packages "nlme", "multcomp", "Ime4" and "car" (Bates and Maechler 2009; Pinheiro et al. 2016; R Development Core Team 2015). For experiment 1, preference for the familiar shoal within each treatment (as measured by the proportion of time spent with the familiar shoal) was tested against a $H_o = 0.5$ using linear mixed effects models (LMM), with shoal number as a random effect (so that each individual was nested within their experimental shoal). Initial shoal choice was tested using a LMM with a binomial distribution, with CO₂ treatment as a fixed effect and shoal number as a random effect. Differences in activity (total shoal visits) between treatments were tested using a LMM with CO₂ treatment as a fixed effect and shoal number as a random effect.

For experiment 2, differences in the MR_{min} , ISR and activity were analysed using a LMM with CO_2 treatment and testing treatment (alone or group) as fixed effects, body mass as a covariate (to account for differences in size between individuals) and individual as a random effect. In figures, metabolic rate measures were mass-corrected by plotting the residual values for each measure from the relationship between log body mass and log metabolic rate (each residual was added to the fitted value for mass = 1.29 g, the mean mass of all fish used in the study). Significant differences in CO_2 treatments (which had 3 levels) discovered using LMM were further investigated using Tukey multiple comparisons post-hoc tests. Differences in MMR and AS with CO_2 treatment were examined using a general linear model (GLM), with body mass as a covariate.

5.4 Results

Experiment 1: Effect of elevated CO₂ on social recognition

Individuals exhibited a significant preference for the familiar shoal under control conditions, but this preference was lost under both elevated CO₂ treatments (Fig. 5.2A; 450 µatm: $F_{1,10} = 6.10 \text{ p} = 0.033$; 750 µatm: $F_{1,10} = 0.660 \text{ p} = 0.438$, 1000 µatm: $F_{1,10} = 0.001 \text{ p} = 0.991$). Trends in initial shoal choice matched those found for shoal preference, but the effect of CO₂ treatment on initial shoal choice was not statistically significant (Fig. 5.2B; $\chi^2 = 0.8103$, p = 0.368). Total shoal visits were not significantly different between the CO₂ treatments (Fig. 5.2C; $F_{2,25} = 0.1138 \text{ p} = 0.893$), with individuals exhibiting an overall mean of 44.7 shoal visits per trial (range: 2 to 133 shoal visits per trial).

Experiment 2: Effect of elevated CO₂ on the calming effect

MR_{min} tested in a group was significantly lower than MR_{min} tested alone (Fig. 5.3A; $F_{1,26} = 29.01$, p < 0.0001), regardless of CO₂ treatment (Fig. 5.3A; $F_{2,27} = 0.37$, p = 0.698), with 26 out of 30 fish tested exhibiting an average reduction in MR_{min} of 22.8% (the remaining 4 fish exhibited an average increase in MR_{min} of 10.5% when tested in a group). The interaction between testing and CO₂ treatment was not significant ($F_{2,26} = 0.71$, p = 0.5008); however, the magnitude of the calming effect was higher in both elevated CO₂ treatments than it was under control conditions (450 µatm: 13.9 ± 5.6%; 750 µatm: 21.4 ± 4.2%; 1000 µatm: 19.8 ± 7.3%; Fig. 5.3A). Elevated CO₂ treatments produced a trend towards higher ISR (Fig. 5.3B; $F_{2,27} = 2.94$, p = 0.069), with differences due to a significant increase in ISR from the control to the high CO₂ treatment (450 µatm vs 1000 µatm: p = 0.028; for all other comparisons, p > 0.05). ISR was not affected by testing treatment ($F_{1,26} = 0.27$, p = 0.606).

Results for RMR were consistent with those for MR_{min} . RMR_{group} was significantly lower than RMR_{alone} (Fig. 5.4A; $F_{1,26}$ = 33.84, p < 0.0001). Respirometry treatment had a



Fig. 5.2. Effect of CO_2 on shoal preference and activity. (A) Proportion of time spent with each shoal (familiar and unfamiliar). (B) Initial shoal choice following removal of the barrier. (c) Mean activity per trial (number of shoal visits). Error bars are s.e.m. and n = 18 for all treatments. Asterisks (**) indicate statistical significance (p < 0.05).

comparable effect on RMR in individuals from all CO₂ treatments ($F_{2,27}$ = 0.73, p = 0.4897) and there was no significant interaction between testing and CO₂ treatment

(F_{2,26} = 0.43, p = 0.7137). Neither testing (F_{1,26} = 0.31, p = 0.583) nor CO₂ treatment (F_{2,27} = 0.32, p = 0.732) exerted a significant effect on activity (Fig. 5.4B), with individuals exhibiting an overall mean of 9.5 turns per min during daylight hours (range = 0.5 - 55.7 turns per min). MMR (F_{1,27} = 1.15, p = 0.294) and AS (F_{1,27} = 1.93, p = 0.176) were not significantly different between CO₂ treatments.



Fig. 5.3. Effect of CO₂ and testing treatment on the (A) minimum metabolic rate (MR_{min} , mg O₂ h⁻¹) and (B) initial stress response (ISR, mg O₂ h⁻¹). Metabolic rate measures were mass-corrected using residuals of the relationship between log body mass and log metabolic rate added to the fitted value for mass = 1.29 g, the mean mass of all fish used in the study. Error bars are s.e.m. and n = 10 for all treatments. Asterisks (**) indicate statistical significance (p < 0.05).

5.5 Discussion

Elevated CO₂ disrupted familiarity, but not the calming effect, in *C. viridis*. As familiarity is important for a range of processes in shoaling fish (Ward and Hart 2003), many of the benefits of group living may be altered under changing environmental conditions. However, the calming benefit of shoaling on metabolic rate was maintained under high CO₂ conditions, indicating that the benefits of group living on overall metabolic demand will likely persist under projected future pCO₂.

The loss of familiarity under elevated CO₂ could have occurred due to a number of possible mechanisms. First, social recognition may have been disrupted if fish lost the sensory abilities necessary for identifying individuals, particularly by olfactory cues (Brown and Smith 1994; Chung et al. 2014; Partridge and Pitcher 1980; Ward et al. 2002; Welch et al. 2014). The changes in shoal-mate association found with rising CO₂ in this study are consistent with previous work that tested for preferences between conspecifics from different reefs (home versus foreign reef site) in the cardinalfish Cheilodipterus quinquelineatus (Devine et al. 2012). In that study, fish lost the association for conspecifics from their home reef under elevated CO₂, suggesting that association preferences generally may be altered. Alternatively, individuals may still be able to recognise familiar shoal-mates, but may simply have lost the preference to shoal with familiar over unfamiliar individuals. Many previous studies have established that shoaling fish prefer to group with familiar conspecifics (e.g. Bhat and Magurran 2006; Edenbrow and Croft 2012; Griffiths and Magurran 1997a; Magurran et al. 1994), but few have investigated what factors may cause this preference to be lost (Granroth-Wilding and Magurran 2013). Neural circuitry likely contributes to the development of social behaviour and preferences in fish species (Dreosti et al. 2015). As neurotransmitter function may be impaired by elevated pCO₂ conditions (Heuer and Grosell 2014; Nilsson et al. 2012), this effect may account for the loss of preferential association with familiar shoal-mates. In addition, memory and learning play an integral role in familiarity, by allowing individuals to learn about their shoal-mates and remember their identity. While it is known that learning is interrupted by elevated CO_2 (Chivers et al. 2014; Ferrari et al.

2012a), no studies have yet examined effects on fish memory. Nevertheless, a disruption to memory could account for the loss of association preference found here in the high CO_2 treatments.



Fig. 5.4. Effect of CO_2 and testing treatment on the (A) routine metabolic rate (RMR, mg O_2 h⁻¹) and (B) activity (number of 180° turns per min). Metabolic rate measures were mass-corrected using residuals of the relationship between log body mass and log metabolic rate added to the fitted value for mass = 1.29 g, the mean mass of all fish used in the study. Error bars are s.e.m. and n = 10 for all treatments. Asterisks (**) indicate statistical significance (p < 0.05).

These mechanisms of familiarity disruption could have a number of ecological implications. If social recognition is disrupted, either due to a loss of the sensory abilities or a loss of memory, a number of important processes may be affected. First, social

learning may be impaired as individuals are unable to distinguish between informed and naïve shoal-mates (Swaney et al. 2001). Second, personality traits like exploratory behaviour and boldness may decrease, which has previously been found in studies on unfamiliar shoals (Galhardo et al. 2012). Third, defensive behaviours may become less effective, as unfamiliar shoals are slower to react to a predator threat and exhibit reduced motor output during escape responses than familiar shoals (**Chapter 3**; Griffiths et al. 2004). Alternatively, if only the preference for the familiar shoal is lost, a range of traits related to shoaling dynamics could be impacted. First, shoal fidelity may decrease, because, without the preference for the familiar shoal, the tradeoffs of staying with the familiar shoal versus migrating to a more suitable, unfamiliar shoal may shift (Muleta and Schausberger 2013). Second, cooperation between shoal-mates may decrease, as individuals' perception of shoal-mates could shift from that of a collaborator to a competitor in this different social context, as the reliability of reciprocal cooperation may be compromised (Engelmann and Herrmann 2016; Granroth-Wilding and Magurran 2013).

Unlike familiarity, the calming effect was maintained under high CO₂. Given the benefits of familiarity to a range of important shoaling processes including foraging and social learning (Atton et al. 2014; Seppä et al. 2001; Swaney et al. 2001), we expected the magnitude of the calming effect to suffer under elevated CO₂. This surprising result implies that familiarity and the calming effect may rely on different mechanisms. Previous studies highlight the central role of olfactory sensing abilities in social recognition of familiar shoal-mates (Brown and Smith 1994; Partridge and Pitcher 1980; Ward et al. 2002), which are more vulnerable to the effects of elevated CO₂ than the visual system (Lönnstedt et al. 2013). Therefore, unlike familiarity, the calming effect may be able to compensate for olfactory impairments using visual cues, as has previously been found for anti-predator behaviours (Lönnstedt et al. 2013). In addition, no effect of CO₂ was found on any of the metabolic traits measured (including MR_{min}, RMR, MMR and AS). While some studies have indicated an effect of CO₂ on metabolism,

most have not, indicating that the results presented here are consistent with many of the studies in the literature (Lefevre 2016).

The initial physiological reaction to stress increased with high CO₂. This result is consistent with previous behavioural research that found greater incidences of anxious behaviour in fish exposed to elevated CO_2 (Hamilton et al. 2014). In social species like C. viridis, this amplified stress response could stem from the mechanisms presented above for familiarity. If social recognition or memory were lost, individuals may have perceived their shoal-mates to be unfamiliar, due to the inability to distinguish between individuals. Stress hormones like cortisol increase when individuals are exposed to an unfamiliar shoal (Yue et al. 2006), which could account for the greater acute stress response that was measured with high CO2. Conversely, the increased metabolic stress response may have contributed to the loss of preference for familiar shoal-mates. Shoaling motivation increases with stress and predation risk (Croft et al. 2009; Stier et al. 2013); therefore, the desire to shoal may outweigh the strategic choice to shoal with familiar fish under elevated CO₂ conditions. No matter what the underlying mechanism is, these results indicate that shoaling may become even more important under altered environmental conditions, with the potential to be used as a behavioural compensatory mechanism (Connell and Ghedini 2015).

Overall activity (total shoal visits and number of 180° turns) did not vary in either experiment in response to CO₂ treatment, indicating that differences in activity cannot explain the results found. Previous studies have reported a range of findings on the effect of CO₂ on activity. For instance, Munday et al. (2014a) reported an increase in the activity of reef fish species, Regan et al. (2016) found a reduction in the activity of a river catfish species and Munday et al. (2016) measured no effect in larvae of a pelagic kingfish species. These trends imply that CO₂ may have variable effects on activity depending on a range of traits such as the natural mobility of the study organism, ontogenetic stage and environmental conditions.

As with all ocean acidification research, these results must be viewed in the context in which the study was conducted. This type of study must be conducted in the

laboratory in order to expose fish to controlled, elevated CO₂ conditions. Although every effort is made to make these conditions as realistic as possible, the laboratory setting may impart unknown effects on our results. Results could also change under varying durations of CO₂ acclimation period. In reality, fishes will incrementally reach these projected conditions over a period of many decades, so there may be the potential for acclimation or adaptation over this time period (Munday et al. 2013b). Parental exposure to elevated CO₂ does not ameliorate impairments to a number of relevant behavioural and sensory systems (Welch et al. 2014), but whether adaptation could reduce the behavioural effects of high CO₂ over longer time frames is unknown.

Future research should work to tease apart which mechanism (social recognition, preference for familiarity or memory) is more likely to be causing the effect of CO₂ on familiarity. Familiarity is important for many aspects of shoaling dynamics (Griffiths et al. 2004; Swaney et al. 2001), so its disruption may create further carry-over effects on a range of processes. The maintenance of the calming effect under high CO₂, however, highlights the complexity of shoal dynamics, and illustrates that many processes in addition to familiarity influence the benefits of shoaling.

Cognitive processes are central to facilitate group living in animal species (Couzin 2009). On an individual and group level, effective social behaviour relies on acquiring and integrating information, whether it be developing familiarity with group-mates, establishing social niches (i.e., leader vs. follower) in defensive manoeuvres or recognising and integrating cues of conspecifics into energy budgets (Griffiths and Magurran 1997a; Laskowski et al. 2016; Marras and Domenici 2013). In social species from dynamic habitats like coral reefs, differences in environmental conditions can also induce an added layer of complexity in social groups (i.e., shoaling fishes), who must also incorporate information from their ambient environment in order to tailor their behaviour to prevailing conditions (Anwar et al. 2016; Binning et al. 2015; Langerhans 2008; Liao 2007; West-Eberhard 1989). This thesis provides an in-depth investigation of plasticity in shoaling dynamics on coral reefs, by detailing how group-living influences behavioural and physiological characteristics. These studies illustrate that escape responses in schooling fish are influenced by both abiotic (water flow regime, Chapter 2) and biotic (shoal composition, Chapter 3) factors. In addition, shoaling fish benefit from a reduction in basic energetic needs (Chapter 4) that will persist even under climatechange induced alterations in ambient environmental conditions (Chapter 5). However, some aspects of shoaling behaviour may be susceptible to rising CO_2 , particularly familiarity (Chapter 5), which could create a range of further carry-over effects that may impact the tradeoffs of group-living in gregarious coral reef fishes.

6.1 Plasticity in school kinematics

Fishes exhibit a high degree of plasticity in their kinematics (or locomotory traits), including routine swimming behaviour, escape response and fast-start escape performance, in response to both biotic and abiotic cues (Domenici 2010). However,

few studies to date have investigated how this individual plasticity translates through to impact the group's kinematic phenotype. The studies presented in this thesis represent some of the first experimental investigations of how a school's escape performance changes in response to external (water flow) and internal (familiarity) characteristics. Adaptive plasticity in response to ambient conditions may aid in maximising fitness, by increasing antipredator behaviour when predation risk is high and maximising food intake when food resources are available (Luttbeg and Sih 2010; Stamps 2007).

School routine swimming behaviour

The coordination and cohesion of routine school swimming behaviour can vary in response to a variety of stimuli. This flexible school structure is maintained through plasticity in attraction, repulsion and alignment, largely as a result of localised interactions between pairs of fish in the school (Couzin 2009; Herbert-Read et al. 2011; Katz et al. 2011). A range of biotic and abiotic factors have previously been found to influence school structure, including hypoxia (Cook et al. 2014; Domenici 2000b), foraging (Hansen et al. 2016b; Ryer and Olla 1997; Sogard and Olla 1997) and predation risk (Felipe et al. 2009; Ryer and Olla 1997; Sogard and Olla 1997). However, the research presented in this thesis suggests that these traits can also be highly consistent, and maintained across a range of environmental conditions and school compositions (Chapters 1 and 2).

This consistency of the school's routine swimming phenotype suggests that *C. viridis* behaviour is dictated by the interaction and feedback between the two levels of organisation in collective animal groups: the individual versus the shoal. First, these results could indicate a limited capacity for behavioural plasticity on an individual level, which can vary depending on a range of factors. Plasticity in school traits, including alignment and nearest neighbour distance (NND), is highly species specific, likely depending on the degree of sociality of the species and the type of habitat the species is found in (Kim 2016; Soria et al. 2007). In addition, level of behavioural plasticity can vary

both between populations and within populations on an individual level (Dingemanse and Wolf 2013; Nussey et al. 2007), as a result of differences in environmental conditions, variability in genetic make-up and the interaction of these two factors (Piersma and Drent 2003). Therefore, this consistency in routine swimming behaviour of schools could be indicative of *Chromis viridis*, populations at Lizard Island or more generally on coral reefs.

Variability in individual plasticity may also favour more stable group phenotypes. In order to maintain group coordination and cohesion, individuals with high capacity for plasticity will likely match their phenotype to individuals with a lower level of plasticity (Dingemanse and Wolf 2013; Wolf et al. 2011). This conformity is common in a range of social animal species in order to maximise the benefits of group living, particularly to stay together while moving (Conradt and Roper 2005; Conradt and Roper 2010; Sumpter et al. 2008). Therefore, the consistency in routine school swimming behaviour may not have resulted from a lack of capacity for plasticity, but instead phenotypic matching by flexible individuals for the traits of more behaviourally consistent school-mates.

This stability in group phenotype may also be related to a kinematic benefit of this school configuration, rather than any limitations on plasticity. Schooling fish gain energetic benefits due to hydrodynamic interactions with school-mates during swimming, with followers exhibiting lower tail beat frequency and higher gait transition speed than leading fish (Fish et al. 1991; Herskin and Steffensen 1998; Killen et al. 2012; Marras et al. 2015). This benefit occurs as trailing fish are able to take advantage of vortices produced by the swimming patterns of leading fish in the school (Weihs 1973). Fish with lower aerobic performance tend to take advantage of these benefits most (Killen et al. 2012). The magnitude of these benefits is known to vary, however, depending on the configuration of the school (Hemelrijk et al. 2015). Therefore, the configuration measured in these studies (**Chapters 1 and 2**) may be maintained across treatments in order to maximise these benefits.

School escape response

Although the school's escape response generally remained consistent between experimental treatments, the school's cohesion and alignment decreased following the threat stimulus in both Chapters 1 and 2. As described for school routine swimming behaviour, this consistency of school phenotype between treatments throughout the escape response could be the result of a limited capacity for behavioural plasticity, intraspecific variability in flexibility leading to consensus decision-making or a kinematic benefit of these configurations (Dingemanse and Wolf 2013; Hemelrijk et al. 2015; Sumpter et al. 2008). The decline in school cohesion and alignment as individuals mount fast-start escape responses indicates that schooling fishes may prioritise individual speed and performance over maintaining the school's preferred configuration, likely to decrease incidences of collision with school-mates moving at rapid swimming speeds (Domenici 2010; Domenici and Batty 1997). Marras et al. (2012) found similar evidence of a reduction in school cohesion and alignment following stimulation in the Atlantic herring *Clupea harengus*, but only when stimulation came from a frontal orientation. Therefore, the ability to maintain school phenotype while swimming and maneuvering at rapid speeds may depend on a range of traits related to both the schooling species tested and the stimulus.

Fast-start escape performance

Fast-start defensive maneuvers are typically mediated by the pair of M Cell higher order command neurons (Domenici 2010; Korn and Faber 2005). The findings presented in this thesis (**Chapters 1 and 2**) indicate that the escape response, which had in the past been considered 'hard-wired' (Giles 1984; Song et al. 2015), exhibits a high capacity for plasticity in response to acute and longer-term conditions (Medan and Preuss 2014). Any reductions in escape performance could have drastic effects on individual survival to predator attacks (Walker et al. 2005).

One source of individual plasticity could be related to characteristics of the stimulus. Individuals may perceive the strength of the threat differently if they are alerted to it by a school-mate through social transmission of information (SToI), rather than the stimulus itself (Gerlotto et al. 2006). SToI is one of the greatest benefits of group-living, as it increases the chance of being informed of important stimuli, like imminent threats, and aids in the accuracy of the response (Clément et al. 2015; Strandburg-Peshkin et al. 2013). Information spreads locally through the group through detection of neighbours' sensory cues and movement behaviour (Clément et al. 2015; Strandburg-Peshkin et al. 2013). However, responses resulting from SToI generally exhibit delayed latency times, as they inherently occur following the latency period of neighbouring fish (Domenici 2010; Marras et al. 2012). Therefore, if individuals are distracted by conspecific inspection due to a lack of familiarity (**Chapter 2**), they may be more likely to respond to SToI than directly to the stimulus itself. Although responses due to SToI cannot be distinguished from responses to the stimulus in these studies, these ideas would form interesting avenues for further research.

Although little is known about how social context mediates escape performance, previous studies have illustrated the plasticity of M cell activity under varying environmental conditions (Korn and Faber 2005). The behavioural switch from routine swimming to escape occurs through the inhibition of slow motor neurons controlling swimming activity and the excitation of fast, escape motor neurons (Song et al. 2015). Responsiveness of motor neuron circuitry is modified by the primordial neuromodulator serotonin (5-hydroxytryptamine, 5-HT; Hultborn and Kiehn 1992; Marquez et al. 2013), a signaling system which in turn is highly sensitive to social context (Winberg and Thörnqvist 2016). Therefore, neuromodulators directly promote startle behaviour and the strength of the escape response itself (Song et al. 2015; Yeh et al. 1996). Stress typically increases 5-HT neurotransmission (Overli et al. 1999; Winberg and Nilsson 1993). This neurochemical change may in turn modify the stimulus threshold necessary for M cell initiation (Whitaker et al. 2011), but serotonergic modulation of the fish

startle response remains controversial (Griffiths et al. 2012; Medan and Preuss 2014; Mintz and Korn 1991).

Social regulation of escape neural circuitry has previously been illustrated in the African cichlid fish *Astatotilapia burtoni* due to differences in position within a dominance hierarchy (Neumeister et al. 2010). Socially dominant fish exhibited a higher startle rate and a larger M cell synaptic response to a threat stimulus than subordinate individuals. This effect was mediated by 5-HT, with differences in behaviour and M Cell excitability between dominant and subordinate fish ameliorated by pharmacological modulation of 5-HT signaling (Whitaker et al. 2011). Whether the effects of familiarity and water flow regime on escape response are also modulated by the same serotonergic pathways and if it could be reinstated by pharmacological inhibition of 5-HT remains unknown. Future studies should investigate how neurophysiological mechanisms vary between schools from different environments and containing varying compositions to determine their role in the effects observed in **Chapters 1 and 2**.

6.2 The calming effect of shoaling to individuals

Although the literature on social behaviour of fishes indicates that individuals benefit from both hydrodynamic and stress-related reductions in energetic needs (Hemelrijk et al. 2015; Hennessy et al. 2009), investigations of the energetic benefits of shoaling have primarily focused on the hydrodynamic impacts of locomotion (Fish et al. 1991; Herskin and Steffensen 1998; Killen et al. 2012; Marras et al. 2015). Investigations of the so-called "calming effect" have thus far focused on either proxies for metabolic rate (e.g. gill ventilation rate) or examined changes in metabolism on a group level (Lefrancois et al. 2009; Schleuter et al. 2007). The data presented in this thesis in **Chapters 3 and 4** represent the first evidence of a calming effect at an individual level. As studies indicate that metabolic traits can vary greatly even between individuals of the same species (Burton et al. 2011; Killen et al. 2010), understanding this effect on an

individual level is important in order ascertain the degree of intraspecific variability in its magnitude.

Minimum metabolic rate

MR_{min} represents the basic energetic needs of individuals. By testing individuals both with and without cues of shoal-mates, **Chapters 3 and 4** were able to quantify the impact of group living on overall energetic demand in a gregarious fish species. This reduction in energetic needs means that individuals require less food, and hence a lower foraging effort, to maintain basic bodily processes or can allocate this energy to other important, fitness-enhancing tasks like growth and reproduction. The mean proportional reduction in MR_{min} matched quite closely between the studies in **Chapters 3 and 4 (Chapter 3**: 26%, **Chapter 4**: 23%), indicating that this effect is consistent and repeatable through time.

Although these studies provide strong evidence for a calming effect of shoaling in gregarious species, the underlying mechanisms responsible for this effect and how its magnitude may change under varying conditions remain unknown. Therefore, there remains a vast scope for further studies on this topic. First, intraspecific variability in the degree of sociability is likely to impact the magnitude of the calming effect (Hennessy et al. 2009; Killen et al. 2016b), with less sociable individuals likely exhibiting a lower proportional reduction in metabolic rate in response to shoal-mates. In addition, the calming effect may vary depending on the type of shoal-mates presented. For instance, as shoaling fish prefer to group with familiar shoal-mates over unfamiliar conspecifics (Ward and Hart 2003), the calming effect may not be elicited by cues from unfamiliar conspecifics. The sensory cues necessary to elicit a calming effect also remain unclear. Previous studies indicate that social recognition and individual identification occur primarily through olfactory cues (Brown and Smith 1994; Muleta and Schausberger 2013; Ward et al. 2003), and may in turn be most important for producing a calming effect of shoaling. In addition, the major tradeoff of group living is competition for

resources like food (Stier et al. 2013). Therefore, the magnitude of the calming effect is likely to vary depending on the nutritional state of the individuals, with a hungry individual potentially prioritising foraging over the safety of being in a group (Hansen et al. 2016b; Killen et al. 2016b).

Environmental conditions are also likely to impact the magnitude of the calming effect. Although CO₂ did not impact metabolic benefits (**Chapter 4**), climate change will also create a concurrent increase in ambient temperatures (Collins et al. 2013). As temperature increases metabolic rate, and hence food requirements (Claireaux and Lefrancois 2007; McDonnell and Chapman 2016; Schulte 2015), the tradeoffs of safety with food requirements may increase the competition for limited food resources and consequently decrease individual sociability (Hansen et al. 2016b; Killen et al. 2016b). Lastly, predator density, alternatively, may increase the importance of safety due to the dilution of risk acquired from group living and hence individuals from a predator rich environment may exhibit a higher proportional calming effect than fish from habitats with low predator density (Croft et al. 2009; Hager and Helfman 1991; Magurran et al. 1993; Stier et al. 2013). Future studies will need to investigate how differences in environmental conditions influence the magnitude of the calming effect to individuals.

As aerobic metabolic traits clearly vary depending on the social context (Chapters 3 and 4), isolation is also likely to influence other energetically costly behaviours, like the fast-start escape response. Although fast-starts are anaerobically fueled (Domenici 2010; Korn and Faber 2005), recent evidence illustrated the relationship between aerobic metabolic traits and anaerobically fueled behaviours (Killen et al. 2015). Therefore, future studies utilising the methods outlined in Chapters 1 and 2 would benefit from comparing fast-starts in schools from different treatments to fast-starts in fishes in isolation. This additional isolation treatment would help to account for variation in aerobic metabolism due to the acute social context.

Initial stress response

ISR is an indicator of an individual's acute response to stress or threat (Chapters 3 and 4). The results presented in Chapters 3 and 4 illustrated that acute social context does not change the metabolic response to stress. However, both the longer term holding pattern and the CO₂ treatment altered the proportional initial physiological response to stress. These results taken together suggest that individuals exhibit plasticity in response to social context and environmental conditions in both the threshold of the threat required to initiate a physiological stress response and in the strength of the stress response itself. However, these changes in threshold and strength of the response are not instantaneous, requiring a period of acclimation to become apparent. Plasticity in behavioural responses to threats has previously been illustrated (Dingemanse and Wolf 2013; Luttbeg and Sih 2010; Stamps 2007) but this thesis presents physiological evidence to underpin previous behavioural studies.

ISR was lower in individuals that had been held in isolation for a period of two weeks, when compared to fish held in groups (**Chapter 2**). Shoaling fishes integrate cues from a range of modalities when determining the risk associated with various threats, in order to balance antipredator behaviour with important, fitness-enhancing activities like foraging and reproduction (Brown et al. 2006a; Brown et al. 2006b; Rieucau et al. 2014a). The threshold of threat required to stimulate a defensive behaviour or physiological stress response is likely to be higher under conditions of greater threat frequency, due to what is known as the "sensory habituation" scenario (Ferrari et al. 2010). When specific threatening cues are experienced frequently, prey may progressively invest less energy in defense against it than if exposed to a novel risk (Hamilton and Heithaus 2001; Pecor and Hazlett 2003; Rodriguez-Prieto et al. 2009). This trend could explain the reduced ISR value in individuals held alone, as solitary individuals would not have shoal-mates to rely on for predator vigilance and hence would have been constantly bombarded with threat cues (Roberts 1996).

ISR was also higher under elevated CO₂ conditions, following two to three weeks of acclimation (**Chapter 4**). This result is consistent with previous behavioural research that found greater incidences of anxious behaviour in fish exposed to elevated CO₂ (Hamilton *et al.*, 2014). This amplified stress response could also result from a loss of familiarity in high CO₂ treatments, which was illustrated in **Chapter 4**. If social recognition or memory of shoal-mates were lost, individuals may have perceived all conspecifics to be unfamiliar, which can increase circulating glucocorticoid stress hormones like cortisol (Yue *et al.*, 2006) and could account for the greater acute stress response that was measured with high CO₂.

6.3 Familiarity in fish shoals

In fishes, a learned familiarity can be attained following a prolonged period of interaction between social individuals (reviewed in Ward and Hart 2003). A study by Griffiths and Magurran (1997a) indicated that this process of familiarity acquisition took approximately 12 days in the Trinidadian guppy *Poecilia reticulata*. However, this time frame is likely to vary depending on the species, system or ontogenetic stage. In juvenile *C. viridis*, this process took longer, with a consistent preference for familiar shoal-mates beginning on day 15 (**Chapter 2**). Once acquired, this familiarity benefits a range of fitness-enhancing processes including foraging, social learning, body condition and survival (Atton et al. 2014; Seppä et al. 2001; Swaney et al. 2001). A number of studies have illustrated fishes' preference to group with familiar shoal-mates over unfamiliar conspecifics, with individual identification achieved primarily through olfactory stimuli (Brown and Smith 1994; Partridge and Pitcher 1980; Ward et al. 2002).

Chapter 2 found that fish schools composed of unfamiliar individuals exhibit significantly diminished escape performance in response to a threat, when compared to schools containing individuals that are familiar with each other. This result suggests that M cell initiation may be delayed or inhibited in unfamiliar schools. Inhibition in unfamiliar schools could be elevated due to what is known as the limited attention

theory, which dictates that information processing capacity is lower than the rate of environmental information encountered (Dukas 2002). Therefore, the brain has mechanisms to prioritise the most vital tasks, which can delay escape behaviours due to reduced predator vigilance (Domenici 2010; Dukas 2002). Therefore, if the need for school-mate inspection is removed by prior knowledge of individuals' behaviour under similar circumstances, then greater cognitive attention and neural processing capacity may be prioritised to defense (Dukas 2002; Griffiths et al. 2004).

C. viridis on coral reefs could encounter unfamiliar shoal-mates under a number of circumstances. First, individuals may migrate to nearby shoals that exhibit phenotypes better matched to their own, that host spawning aggregations or that live on a coral head with preferred characteristics. This has been found in *C. viridis*, with individual migrations of up to 80 m measured on the Great Barrier Reef (Nadler, Killen, Cox and McCormick, unpublished data). In addition, disturbances such as storms and flooding can lead to group disruption and forced association with unfamiliar shoals due to the sheer force of resulting water currents (T. Hempson, pers. comm.; Lassig 1983; Yoon et al. 2011). **Chapter 4** also indicates that familiarity may be disrupted by projected climate change conditions. Based on the escape performance results presented in **Chapter 2**, any disruption to familiarity is likely to have a profound influence on survival in shoaling fishes (Walker et al. 2005).

These results indicate the importance of group composition in the success and survival of individual members of animal groups. Further studies should examine how group characteristics other than familiarity influence escape responses and the calming effect in gregarious coral reef fishes. Varying compositions of personality, body length, coloration, sex, parasite infection and species are likely to influence the resulting phenotype measured and will present interesting avenues for future research (Croft et al. 2009; Crook 1999a; Dyer et al. 2008; Griffiths and Magurran 1998; Laskowski et al. 2016; Spagnoli et al. 2016; Ward et al. 2002).

6.4 Environmental impacts on shoaling fish

Taken together, the results presented in **Chapters 1 and 4** indicate that environmental conditions are drivers of plasticity in shoaling dynamics. **Chapter 1** illustrated plasticity in fast-start escape performance in schooling fish in response to differences in water flow regime, while **Chapter 4** indicated that familiarity may be altered under future CO₂ conditions. Familiarity, the calming effect and the fast-start escape response therefore represent ideal indicators of the capacity for plasticity in physiological and behavioural traits of gregarious fishes. Future studies should examine how other environmental factors influence the dynamics of shoaling fishes on coral reefs, including temperature, habitat degradation, food availability, predator density and richness, turbidity, habitat complexity and tidal cycle (Ajemian et al. 2014; Croft et al. 2009; Ford and Swearer 2012; Hansen et al. 2016a; Hansen et al. 2016b; McCormick and Lonnstedt 2016; Webster et al. 2013; Weetman et al. 1998).

6.5 Concluding remarks

By examining the physiological and behavioural traits of a model species, this thesis contributes to the broader understanding of the physiological mechanisms underpinning complex behaviours in wild animal groups and their impacts on individual fitness and survival. This work also illustrates that testing solitary individuals of social species may not provide an accurate interpretation of the organism's behaviour or physiology in their natural environment. Finally, this work highlights the breadth of questions still unanswered on the ecology and physiology of social species, particularly on coral reefs. Future work will be able to build on these findings to create a more holistic understanding on the tradeoffs of group living and how they may be affected by the rapid pace of environmental change caused by human activities.

References

Abrahams MV, Colgan PW (1985) Risk of predation, hydrodynamic efficiency and their influence on school structure. Environmental Biology of Fishes 13:195-202

Ajemian MJ, Sohel S, Mattila J (2014) Effects of turbidity and habitat complexity on antipredator behavior of three-spined sticklebacks (*Gasterosteus aculeatus*). Environmental Biology of Fishes

- Allouche S, Gaudin P (2001) Effects of avian predation threat, water flow and cover on growth and habitat use by Chub, *Leuciscus cephalus*, in an experimental stream. Oikos 94:481-492
- Álvarez DN, A.G. (2005) Is metabolic rate a reliable predictor of growth and survival of brown trout (*Salmo trutta*) in the wild? Canadian Journal of Fisheries and Aquatic Sciences 62:643-649
- Ang TZ, Manica A (2010) Unavoidable limits on group size in a body size-based linear hierarchy. Behavioral Ecology 21:819-825
- Anttila K, Jokikokko E, Erkinaro J, Jarvilehto M, Manttari S (2011) Effects of training on functional variables of muscles in reared Atlantic salmon *Salmo salar* smolts: connection to downstream migration pattern. Journal of Fish Biology 78:552-566
- Anwar SB et al. (2016) The effects of steady swimming on fish escape performance. Journal of Comparative Physiology A 202:425-433
- Arnott SA, Chiba S, Conover DO (2006) Evolution of intrinsic growth rate: metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. Evolution 60:1269-1278
- Atton N, Galef BJ, Hoppitt W, Webster MM, Laland KN (2014) Familiarity affects social network structure and discovery of prey patch locations in foraging stickleback shoals. Proceedings of the Royal Society B: Biological Sciences 281:20140579
 Bachelet E (1981) Circular statistics in biology. Academic Press, London

Bainbridge S (2015) Lizard Island Sensor Float 2 Salinity @10.9m. In: (IMOS) IMOS (ed) IMOS FAIMMS Sensor Network data,

http://data.aims.gov.au/metadataviewer/faces/view.xhtml?uuid=9be0f96ce3ee-4e71-9dcf-4199446bf37f

- Baird T, Ryer CH, Olla BL (1991) Social enhancement of foraging on an ephemeral food source in juvenile walleye pollock, *Theragra chalcogramma*. Environmental Biology of Fishes 31:307-311
- Barber I, Wright HA (2001) How strong are familiarity preferences in shoaling fish? Animal Behaviour 61:975-979
- Barreto RE, Volpato GL (2011) Ventilation rates indicate stress-coping styles in Nile tilapia. Journal of Biosciences 36:851-855
- Bartolini T, Butail S, Porfiri M (2014) Temperature influences sociality and activity of freshwater fish. Environmental Biology of Fishes 98:825-832
- Bates D, Maechler M (2009) Ime4: Linear mixed-effects models using S4 classes.
- Bhat A, Magurran AE (2006) Benefits of familiarity persist after prolonged isolation in guppies. Journal of Fish Biology 68:759-766
- Binning SA, Roche DG, Fulton CJ (2014) Localised intraspecific variation in the swimming phenotype of a coral reef fish across different wave exposures. Oecologia 174:623-630
- Binning SA, Ros AFH, Nusbaumer D, Roche DG (2015) Physiological plasticity to water flow habitat in the amselfish, *Acanthochromis polyacanthus*: Linking phenotype to performance. PLoS ONE 10:e0121983
- Biro PA, Stamps JA (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology & Evolution 25:653-659
- Block HE, Steele MA (2014) Spatial variation in selective mortality on larval traits in the coral reef fish *Chromis viridis*. Marine Ecology Progress Series 509:303-308

- Bode NW, Faria JJ, Franks DW, Krause J, Wood AJ (2010) How perceived threat increases synchronization in collectively moving animal groups. Proceedings of the Royal Society B: Biological Sciences 277:3065-3070
- Booth DJ (1995) Juvenile groups in a coral-reef damselfish density-dependent effects on individual fitness and population demography. Ecology 76:91-106
- Brandl S, Bellwood D (2014) Pair-formation in coral reef fishes: An ecological perspective. 52:1-80
- Brierley AS, Cox MJ (2010) Shapes of krill swarms and fish schools emerge as aggregation members avoid predators and access oxygen. Current Biology 20:1758-1762
- Briffa M, de la Haye K, Munday PL (2012) High CO₂ and marine animal behaviour: potential mechanisms and ecological consequences. Marine Pollution Bulletin 64:1519-1528
- Brown GE, Bongiorno T, DiCapua DM, Ivan LI, Roh E (2006a) Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. Canadian Journal of Zoology 84:1-8
- Brown GE, Rive AC, Ferrari MCO, Chivers DP (2006b) The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. Behavioral Ecology and Sociobiology 61:9-16
- Brown GE, Smith RJF (1994) Fathead minnows use chemical cues to discriminate natural shoalmates from unfamiliar conspecifics. Journal of Chemical Ecology 20:3051-3061
- Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789
- Brown MR, Fisher LA, Rivier J, Spiess J, Rivier C, Vale W (1982) Corticotropin-releasing factor: Effects on the sympathetic nervous system and oxygen consumption. Life Sciences 30:207-210
- Bumann D, Krause J, Rubenstein D (1997) Mortality risk of spatial positions in animal groups: The danger of being in front. Behaviour 134:1063-1076

- Burns ALJ, Herbert-Read JE, Morrell LJ, Ward AJW (2012) Consistency of leadership in shoals of mosquitofish (*Gambusia holbrooki*) in novel and in familiar environments. PLoS ONE 7:e36567
- Burton T, Killen SS, Armstrong JD, Metcalfe NB (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society B: Biological Sciences 278:3465-3473
- Buston PM, Cant MA (2006) A new perspective on size hierarchies in nature: patterns, causes, and consequences. Oecologia 149:362-372
- Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. Journal of Fish Biology 88:81-121
- Chicoli A, Butail S, Lun Y, Bak-Coleman J, Coombs S, Paley DA (2014) The effects of flow on schooling *Devario aequipinnatus*: school structure, startle response and information transmission. Journal of Fish Biology 84:1401-1421
- Chivers DP, Brown GE, Smith JFR (1995) Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for antipredator behavior. Canadian Journal of Zoology 73:955-960
- Chivers DP et al. (2014) Impaired learning of predators and lower prey survival under elevated CO₂: a consequence of neurotransmitter interference. Global Change Biology 20:515-522
- Chung WS, Marshall NJ, Watson SA, Munday PL, Nilsson GE (2014) Ocean acidification slows retinal function in a damselfish through interference with GABA-A receptors. Journal of Experimental Biology 217:323-326
- Claireaux G, Lefrancois C (2007) Linking environmental variability and fish performance: integration through the concept of scope for activity. Philosophical Transactions of the Royal Society B: Biological Sciences 362:2031-2041
- Clarke RD, Finelli CM, Buskey EJ (2009) Water flow controls distribution and feeding behavior of two co-occurring coral reef fishes: II. Laboratory experiments. Coral Reefs 28:475-488

References

- Clément RJG, Wolf M, Snijders L, Krause J, Kurvers RHJM (2015) Information transmission via movement behaviour improves decision accuracy in human groups. Animal Behaviour 105:85-93
- Collins M et al. (2013) Long-term Climate Change: Projections, Commitments and Irreversibility. In: Stocker TF et al. (eds) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA
- Connell SD, Ghedini G (2015) Resisting regime-shifts: the stabilising effect of compensatory processes. Trends in Ecology & Evolution 30:513-515
- Conradt L, Roper TJ (2005) Consensus decision making in animals. Trends in Ecology & Evolution 20:449-456
- Conradt L, Roper TJ (2010) Deciding group movements: where and when to go. Behavioural Processes 84:675-677
- Cook DG, Brown EJ, Lefevre S, Domenici P, Steffensen JF (2014) The response of striped surfperch *Embiotoca lateralis* to progressive hypoxia: Swimming activity, shoal structure, and estimated metabolic expenditure. Journal of Experimental Marine Biology and Ecology 460:162-169
- Couzin ID (2009) Collective cognition in animal groups. Trends in Cognitive Sciences 13:36-43
- Couzin ID, Krause J, Franks NR, Levin SA (2005) Effective leadership and decision-making in animal groups on the move. Nature 433:513-516
- Croft DP et al. (2003) Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. Oikos 100:429-438
- Croft DP, Darden SK, Ruxton GD (2009) Predation risk as a driving force for phenotypic assortment: a cross-population comparison. Proceedings of the Royal Society B: Biological Sciences 276:1899-1904
- Croft DP, James R, Ward AJW, Botham MS, Mawdsley D, Krause J (2005) Assortative interactions and social networks in fish. Oecologia 143:211-219

- Crook AC (1999a) A quantitative analysis of the relationship between interspecific encounters, schooling behaviour and colouration in juvenile parrotfish (family Scaridae). Marine and Freshwater Behaviour and Physiology 33:1-19
- Crook AC (1999b) Quantitative evidence for assortative schooling in a coral reef fish. Marine Ecology Progress Series 176:17-23
- Davison W (1997) The effects of exercise training on teleost fish, a review of recent literature. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 117:67-75
- Delcourt J, Poncin P (2012) Shoals and schools: back to the heuristic definitions and quantitative references. Reviews in Fish Biology and Fisheries 22:595-619
- Demartini E, Anderson TW (2007) Habitat associations and aggregation of recruit fishes on Hawaiian coral reefs. Bulletin of Marine Science 81:139-152
- Devine BM, Munday PL, Jones GP (2012) Homing ability of adult cardinalfish is affected by elevated carbon dioxide. Oecologia 168:269-276
- Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A Oceanographic Research Papers 34:1733-1743
- Dingemanse NJ, Wolf M (2013) Between-individual differences in behavioural plasticity within populations: causes and consequences. Animal Behaviour 85:1031-1039
- Dixson DL, Munday PL, Jones GP (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecology Letters 13:68-75

Dlugokencky E, Tans P (2016) Trends in atmospheric carbon dioxide. In: NOAA/ESRL (ed)

- Domenici P (2000a) Spacing of wild schooling herring while encircled by killer whales. Journal of Fish Biology 57:831-836
- Domenici P (2000b) The effect of progressive hypoxia on swimming activity and schooling in Atlantic herring. Journal of Fish Biology 57:1526-1538
- Domenici P (2004) Escape manoeuvres in the spiny dogfish (*Squalus acanthias*). Journal of Experimental Biology 207:2339-2349

Domenici P (2010) Context-dependent variability in the components of fish escape response: integrating locomotor performance and behavior. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 313A:59-79

Domenici P, Allan B, McCormick MI, Munday PL (2011a) Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. Biology Letters 8:78-81

- Domenici P, Batty RS (1994) Escape maneoeuvres of schooling *Clupea harengus*. Journal of Fish Biology 45:97-110
- Domenici P, Batty RS (1997) Escape behaviour of solitary herring (*Clupea harengus*) and comparisons with schooling individuals. Marine Biology 128:29-38
- Domenici P, Blagburn JM, Bacon JP (2011b) Animal escapology I: theoretical issues and emerging trends in escape trajectories. Journal of Experimental Biology 214:2463-2473
- Domenici P, Blake RW (1993) Escape trajectories in angelfish (*Pterophyllum eimekei*). Journal of Experimental Biology 177:253-272
- Domenici P, Blake RW (1997) The kinematics and performance of fish fast-start swimming. Journal of Experimental Biology 200:1165-1178
- Domenici P et al. (2015) Fast-starting after a breath: air-breathing motions are kinematically similar to escape responses in the catfish *Hoplosternum littorale*. Biology Open 4:79-85
- Domenici P, Lefrancois C, Shingles A (2007) Hypoxia and the antipredator behaviours of fishes. Philosophical Transactions of the Royal Society B 362:2105-2121
- Domenici P, Silvana Ferrari R, Steffensen JF, Batty RS (2002) The effect of progressive hypoxia on school structure and dynamics in Atlantic herring *Clupea harengus*. Proceedings of the Royal Society B: Biological Sciences 269:2103-2111
- Domenici P, Turesson H, Brodersen J, Bronmark C (2008) Predator-induced morphology enhances escape locomotion in crucian carp. Proceedings of the Royal Society B: Biological Sciences 275:195-201
- Donelson JM, Munday PL (2012) Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. Journal of Animal Ecology 81:1126-1131

References

- Doney SC (2010) The growing human footprint on coastal and open-ocean biogeochemistry. Science 328:1512-1516
- Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: The other CO₂ problem. Annual Review of Marine Science 1:169-192
- Dreosti E, Lopes G, Kampff AR, Wilson SW (2015) Development of social behavior in young zebrafish. Frontiers in Neural Circuits 9:39
- Dugatkin LA (1997) Cooperation among animals: An evolutionary perspective. Oxford University Press, New York
- Dugatkin LA, Godin JG (1993) Female mate copying in the guppy (*Poecilia reticulata*): age-dependent effects. Behavioral Ecology 4:289-292
- Dukas R (2002) Behavioural and ecological consequences of limited attention. Philosophical Transactions of the Royal Society B: Biological Sciences 357:1539-1547
- Dupont-Prinet A, Chatain B, Grima L, Vandeputte M, Claireaux G, McKenzie DJ (2010) Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed deprivation in the European sea bass (*Dicentrarchus labrax*). Journal of Experimental Biology 213:1143-1152
- Dyer JRG, Croft DP, Morrell LJ, Krause J (2008) Shoal composition determines foraging success in the guppy. Behavioral Ecology 20:165-171
- Eaton R, Emberley D (1991) How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish. Journal of Experimental Biology 161:469-487
- Eaton RC, Lavender WA, Wieland CM (1981) Identification of Mauthner-Initiated response patterns in goldfish: Evidence from simultaneous cinematography and electrophysiology. Journal of Comparative Physiology A 144:521-531
- Edenbrow M, Croft DP (2012) Kin and familiarity influence association preferences and aggression in the mangrove killifish *Kryptolebias marmoratus*. Journal of Fish Biology 80:503-518
Engelmann JM, Herrmann E (2016) Chimpanzees trust their friends. Current Biology 26:252-256

- Enzor LA, Zippay ML, Place SP (2013) High latitude fish in a high CO₂ world: Synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164:154-161
- Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science 65:414-432
- Farrell AP (2016) Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. Journal of Fish Biology 88:322-343
- Feeney WE et al. (2013) Brood parasitism and the evolution of cooperative breeding in birds. Science 342:1506-1508
- Feitl KE, Ngo V, McHenry MJ (2010) Are fish less responsive to a flow stimulus when swimming? Journal of Experimental Biology 213:3131-3137
- Felipe TRA, Suarez YR, Junior WFA (2009) The social organization of fish schools antipredator responses of *Moenkhausia sanctaefilomenae* (Characidae, Tetragonopterinae) under simulated predation in the laboratory. Sociobiology 54:2009
- Ferno A et al. (1998) The challenge of herring in the Norwegian Sea: Making optimal collective spatial decisions. Sarsia 83:149-167
- Ferrari MC et al. (2012a) Effects of ocean acidification on learning in coral reef fishes. PLoS One 7:e31478
- Ferrari MCO et al. (2011a) Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. Global Change Biology 17:2980-2986
- Ferrari MCO, Elvidge CK, Jackson CD, Chivers DP, Brown GE (2010) The responses of prey fish to temporal variation in predation risk: sensory habituation or risk assessment? Behavioral Ecology 21:532-536

- Ferrari MCO et al. (2012b) Effects of ocean acidification on visual risk assessment in coral reef fishes. Functional Ecology 26:553-558
- Ferrari MCO et al. (2011b) Putting prey and predator into the CO₂ equation qualitative and quantitative effects of ocean acidification on predator-prey interactions. Ecology Letters 14:1143-1148
- Fischer S, Bessert-Nettelbeck M, Kotrschal A, Taborsky B (2015) Rearing-group size determines social competence and brain structure in a cooperatively breeding cichlid. The American Naturalist 186:123-140
- Fish F, Fegely J, Xanthopoulos C (1991) Burst-and-coast swimming in schooling fish (*Notemigonus crysoleucas*) with implications for energy economy. Comparative Biochemistry and Physiology 100A:633-637
- Fishelson L, Popper D, Avidor A (1974) Biosociology and ecology of Pomacentrid fishes around the Sinai Peninsula (northern Red Sea). Journal of Fish Biology 6:119-133
- Ford JR, Swearer SE (2012) Shoaling behaviour enhances risk of predation from multiple predator guilds in a marine fish. Oecologia 172:387-397
- Forrester GE (1991) Social rank, individual size and group composition as determinants of food consumption by humbug damselfish, *Dascyllus aruanus*. Animal Behaviour 42:701-711
- Foster SA (1985) Group foraging by a coral reef fish: a mechanism for gaining access to defended resources. Animal Behaviour 33:782-792
- Foster WA, Treherne JE (1981) Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. Nature 293:466-467
- Fraser MR, McCormick MI (2014) Gender-specific benefits of eating eggs at resident reef fish spawning aggregation sites. Marine Ecology Progress Series 517:209-216
- Fry FEJ (1971) The Effect of Environmental Factors on the Physiology of Fish. Fish Physiology, Volume 6. Academic Press, pp 1-98
- Fu SJ (2015) Flow and stress acclimation both enhance predator avoidance in a common cyprinid fish. Aquatic Biology 24:1-8

- Fu SJ, Cao ZD, Yan GJ, Fu C, Pang X (2013) Integrating environmental variation, predation pressure, phenotypic plasticity and locomotor performance. Oecologia 173:343-354
- Fulton CJ, Bellwood DR (2005) Wave-induced water motion and the functional implications for coral reef fish assemblages. Limnology and Oceanography 50:255-264
- Galhardo L, Oliveira RF (2014) The effects of social isolation on steroid hormone levels are modulated by previous social status and context in a cichlid fish. Hormones and Behaviour 65:1-5
- Galhardo L, Vitorino A, Oliveira RF (2012) Social familiarity modulates personality trait in a cichlid fish. Biology Letters 8:936-938
- Gerlotto F, Bertrand S, Bez N, Gutierrez M (2006) Waves of agitation inside anchovy schools observed with multibeam sonar: a way to transmit information in response to predation. ICES Journal of Marine Science 63:1405-1417
- Giles N (1984) Development of the overhead fright response in wild and predator-naive three-spined sticklebacks, *Gasterosteus aculeatus* L. Animal Behaviour 32:276-279
- Glazier DS (2005) Beyond the '3/4-power law': variation in the intra-and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662
- Godin J-GJ, Herdman EJE, Dugatkin LA (2005) Social influences on female mate choice in the guppy, *Poecilia reticulata*: generalized and repeatable trait-copying behaviour. Animal Behaviour 69:999-1005
- Goldenberg SU, Borcherding J, Heynen M (2014) Balancing the response to predation the effects of shoal size, predation risk and habituation on behaviour of juvenile perch. Behavioral Ecology and Sociobiology 68:989-998
- Goldshmid R, Holzman R, Weihs D, Genin A (2004) Aeration of corals by sleep swimming fish. Limnology and Oceanography 49:1832-1839
- Granroth-Wilding HM, Magurran AE (2013) Asymmetry in pay-off predicts how familiar individuals respond to one another. Biology Letters 9:20130025

- Griffiths BB, Schoonheim PJ, Ziv L, Voelker L, Baier H, Gahtan E (2012) A zebrafish model of glucocorticoid resistance shows serotonergic modulation of the stress response. Frontiers in Behavioural Neuroscience 6:68
- Griffiths SW, Brockmark S, Hojesjo J, Johnsson JI (2004) Coping with divided attention: the advantage of familiarity. Proceedings of the Royal Society B: Biological Sciences 271:695-699
- Griffiths SW, Magurran AE (1997a) Familiarity in schooling fish: how long does it take to acquire? Animal Behaviour 53:945-949
- Griffiths SW, Magurran AE (1997b) Schooling preferences for familiar fish vary with group size in a wild guppy population. Proceedings of the Royal Society B: Biological Sciences 264:547-551
- Griffiths SW, Magurran AE (1998) Sex and schooling behaviour in the Trinidadian guppy. Animal Behaviour 56:689-693
- Griffiths SW, Magurran AE (1999) Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. Behavioral Ecology and Sociobiology 45:437-443
- Hager MC, Helfman GS (1991) Safety in numbers: Shoal size choice by minnows under predatory threat. Behavioral Ecology and Sociobiology 29:271-276
- Hamilton IM, Heithaus MR (2001) The effects of temporal variation in predation risk on anti-predator behaviour: an empirical test using marine snails. Proceedings of the Royal Society B: Biological Sciences 268:2585-2588
- Hamilton TJ, Holcombe A, Tresguerres M (2014) CO2-induced ocean acidification increases anxiety in rockfish via alteration of GABA-A receptor functioning. Proceedings of the Royal Society B: Biological Sciences 281:20132509
- Hamner WM, Jones MS, Carleton JH, Hauri IR, Williams DM (1988) Zooplankton, planktivoroush fish, and water currents on a windward reef face: Great Barrier Reef, Australia. Bulletin of Marine Science 42:459-479

- Handegard NO, Boswell KM, Ioannou CC, Leblanc SP, Tjostheim DB, Couzin ID (2012) The dynamics of coordinated group hunting and collective information transfer among schooling prey. Current Biology 22:1213-1217
- Hansen MJ, Morrell LJ, Ward AJW (2016a) The effect of temporally variable environmental stimuli and group size on emergence behavior. Behavioral Ecology doi:10.1093/beheco/arv237
- Hansen MJ, Schaerf TM, Simpson SJ, Ward AJW (2016b) Group foraging decisions in nutritionally differentiated environments. Functional Ecology 10.1111/1365-2435.12646
- Hansen MJ, Schaerf TM, Ward AJW (2015) The influence of nutritional state on individual and group movement behaviour in shoals of crimson-spotted rainbowfish (*Melanotaenia duboulayi*). Behavioral Ecology and Sociobiology
- Hari P et al. (2008) High-frequency measurements of productivity of planktonic algae using rugged nondispersive infrared carbon dioxide probes. Limnology and Oceanography: Methods 6:347-354
- Harrison HB et al. (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. Current Biology 22:1023-1028
- Hart PBJ (1997) Foraging Tactics. In: Godin JGJ (ed) Behavioural Ecology of Teleost Fishes. Oxford University Press, New York, pp 104-133
- Hemelrijk CK, Reid DAP, Hildenbrandt H, Padding JT (2015) The increased efficiency of fish swimming in a school. Fish and Fisheries 16:511-521
- Hennessy MB, Kaiser S, Sachser N (2009) Social buffering of the stress response: diversity, mechanisms, and functions. Frontiers in Neuroendocrinology 30:470-482
- Herbert-Read JE, Buhl J, Hu F, Ward AJ, Sumpter DJ (2015) Initiation and spread of escape waves within animal groups. Royal Society Open Science 2:140355
- Herbert-Read JE, Perna A, Mann RP, Schaerf TM, Sumpter DJT, Ward AJW (2011) Inferring the rules of interaction of shoaling fish. Proceedings of the National Academy of Sciences 108:18726-18731

- Herskin J (1999) Effects of social and visual contact on the oxygen consumption of juvenile sea bass measured by computerized intermittent respirometry. Journal of Fish Biology 55:1075-1085
- Herskin J, Steffensen JF (1998) Energy savings in sea bass swimming in a school: measurements of tail beat frequency and oxygen consumption at different swimming speeds. Journal of Fish Biology 53:366-376
- Heuer RM, Grosell M (2014) Physiological impacts of elevated carbon dioxide and ocean acidification on fish. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 307:R1061-1084
- Higham TE, Stewart WJ, Wainwright PC (2015) Turbulence, temperature, and turbidity: The ecomechanics of predator-prey Interactions in fishes. Integrative and Comparative Biology 55:6-20
- Hoare DJ, Krause J (2003) Social organisation, shoal structure and information transfer. Fish and Fisheries 4:269-279
- Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research 50:839-866
- Hoey AS, McCormick MI (2006) Effects of subcutaneous fluorescent tags on the growth and survival of a newly settled coral reef fish, *Pomacentrus amboinensis* (Pomacentridae). Proceedings of the 10th International Coral Reefs Symposium 2006:420-425
- Hoppitt W, Laland KN (2013) Social Learning: An Introduction to Mechanisms, Methods, and Models. Princeton: Princeton University Press
- Hughes GM (1973) Respiratory responses to hypoxia in fish. American Zoologist 13:475-489
- Hultborn H, Kiehn O (1992) Neuromodulation of vertebrate motor neuron membrane properties. Current Opinion in Neurobiology 2:770-775
- Huntingford FA et al. (2010) Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*. Journal of Fish Biology 76:1576-1591

- Huntington TG (2006) Evidence for intensification of the global water cycle: Review and synthesis. Journal of Hydrology 319:83-95
- Hurst TP (2007) Thermal effects on behavior of juvenile walleye pollock (*Theragra chalcogramma*): Implications for energetics and food web models. Canadian Journal of Fisheries and Aquatic Sciences 64:449-457
- Irisson JO, Paris CB, Leis JM, Yerman MN (2015) With a little help from my friends: Group orientation by larvae of a coral reef fish. PLoS One 10:e0144060
- Ishimatsu A, Hayashi M, Kikkawa T (2008) Fishes in high-CO₂, acidified oceans. Marine Ecology Progress Series 373:295-302
- Jacoby DMP, Sims DW, Croft DP (2012) The effect of familiarity on aggregation and social behaviour in juvenile small spotted catsharks *Scyliorhinus canicula*. Journal of Fish Biology 81:1596-1610
- Jakob EM (1991) Costs and benefits of group living for pholcid spiderlings: losing food, saving silk. Animal Behaviour 41:711-722
- Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: A review of causes and consequences. Biological Reviews 72:283-327
- Johansen JL (2014) Quantifying water flow within aquatic ecosystems using load cell sensors: a profile of currents experienced by coral reef organisms around Lizard Island, Great Barrier Reef, Australia. PLoS One 9:e83240
- Johansen JL, Bellwood DR, Fulton CJ (2008) Coral reef fishes exploit flow refuges in highflow habitats. Marine Ecology Progress Series 360:219-226
- Johansen JL, Fulton CJ, Bellwood DR (2007) Avoiding the flow: refuges expand the swimming potential of coral reef fishes. Coral Reefs 26:577-583
- Johansen JL, Jones GP (2011) Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. Global Change Biology 17:2971-2979
- Johansen JL, Pratchett MS, Messmer V, Coker DJ, Tobin AJ, Hoey AS (2015) Large predatory coral trout species unlikely to meet increasing energetic demands in a warming ocean. Scientific Reports 5:13830

- John KR (1964) Illumination, vision, and schooling of *Astyanax mexicanus*. Journal of the Fisheries Research Board of Canada 21:1453-1473
- Jones KA, Croft DP, Ramnarine IW, Godin J-GJ (2010) Size-assortative shoaling in the guppy (*Poecilia reticulata*): The role of active choice. Ethology 116:147-154
- Jordan LA, Wong MYL, Balshine SS (2009) The effects of familiarity and social hierarchy on group membership decisions in a social fish. Biology Letters 6:301-303
- Jutfelt F, Bresolin de Souza K, Vuylsteke A, Sturve J (2013) Behavioural disturbances in a temperate fish exposed to sustained high-CO₂ levels. PLoS One 8:e65825
- Karplus I, Katzenstein R, Goren M (2006) Predator recognition and social facilitation of predator avoidance in coral reef fish *Dascyllus marginatus* juveniles. Marine Ecology Progress Series 319:215-223
- Katz Y, Tunstrom K, Ioannou CC, Huepe C, Couzin ID (2011) Inferring the structure and dynamics of interactions in schooling fish. Proceedings of the National Academy of Sciences 108:18720-18725
- Killen SS (2014) Growth trajectory influences temperature preference in fish through an effect on metabolic rate. Journal of Animal Ecology 83:1513-1522
- Killen SS, Atkinson D, Glazier DS (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology Letters 13:184-193
- Killen SS, Croft DP, Salin K, Darden SK, Higham T (2016a) Male sexually coercive behaviour drives increased swimming efficiency in female guppies. Functional Ecology 30:576-583
- Killen SS, Fu C, Wu Q, Wang Y-X, Fu S-J (2016b) The relationship between metabolic rate and sociability is altered by food-deprivation. Functional Ecology DOI: 10.1111/1365-2435.12634
- Killen SS, Marras S, McKenzie DJ (2011) Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology 80:1024-1033

- Killen SS, Marras S, Metcalfe NB, McKenzie DJ, Domenici P (2013) Environmental stressors alter relationships between physiology and behaviour. Trends in Ecology & Evolution 28:651-658
- Killen SS, Marras S, Steffensen JF, McKenzie DJ (2012) Aerobic capacity influences the spatial position of individuals within fish schools. Proceedings of the Royal Society B: Biological Sciences 279:357-364
- Killen SS et al. (2014) Aerobic scope predicts dominance during early life in a tropical damselfish. Functional Ecology 28:1367-1376
- Killen SS, Reid D, Marras S, Domenici P (2015) The interplay between aerobic metabolism and antipredator performance: vigilance is related to recovery rate after exercise. Frontiers in Physiology 6:111
- Killen SS, McKenzie DJ (2011) Fuel, fasting, fear: Routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology 80:1024-1033
- Kim S-Y (2016) Fixed behavioural plasticity in response to predation risk in the threespined stickleback. Animal Behaviour 112:147-152
- Kleypas JA, Buddemeier RW, Archer D, Gattuso JP, Langdon C, Opdyke B (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science 284:118-120
- Korn H, Faber DS (2005) The Mauthner cell half a century later: a neurobiological model for decision-making? Neuron 47:13-28
- Krause J, Butlin RK, Peuhkuri N, Pritchard VL (2000) The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field. Biological Reviews 75:477-501
- Krause J, Godin J-GJ (1994) Shoal Choice in the Banded Killifish (Fundulus diaphanus, Teleostei, Cyprinodontidae): Effects of Predation Risk, Fish Size, Species Composition and Size of Shoals. Ethology 98:128-136
- Krause J, Ruxton GD (2002) Living in groups. Oxford University Press

- Krupczynski P, Schuster S (2008) Fruit-catching fish tune their fast starts to compensate for drift. Current Biology 18:1961-1965
- Lachlan R, Crooks L, Laland KN (1998) Who follows whom? Shoaling preferences and social learning of foraging information in guppies. Animal Behaviour 56:181-190
- Laland KN, Williams K (1997) Shoaling generates social learning of foraging information in guppies. Animal Behaviour 53:1161-1169
- Landeau L, Terborgh J (1986) Oddity and the 'confusion effect' in predation. Animal Behaviour 34:1372-1380
- Langerhans RB (2008) Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology 48:750-768
- Larsson M (2009) Possible functions of the octavolateralis system in fish schooling. Fish and Fisheries 10:344-353
- Laskowski KL, Bell AM (2014) Strong personalities, not social niches, drive individual differences in social behaviours in sticklebacks. Animal Behaviour 90:287-295
- Laskowski KL, Montiglio PO, Pruitt JN (2016) Individual and group performance suffers from social niche disruption. American Naturalist 187:776-785
- Laskowski KL, Pruitt JN (2014) Evidence of social niche construction: persistent and repeated social interactions generate stronger personalities in a social spider. Proceedings of the Royal Society of London B: Biological Sciences 281:20133166
- Lassig BR (1983) The effects of a cyclonic storm on coral reef fish assemblages. Environmental Biology of Fishes 9:55-63
- Le Quéré C et al. (2013) Global carbon budget 2013. Earth System Science Data Discussions 6:689-760
- Lee-Jenkins SSY, Godin JGJ (2010) Social familiarity and shoal formation in juvenile fishes. Journal of Fish Biology 76:580-590
- Lefevre S (2016) Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO₂ and their interaction. Conservation Physiology 4:cow009

- Lefrancois C, Ferrari RS, Moreira da Silva J, Domenici P (2009) The effect of progressive hypoxia on spontaneous activity in single and shoaling golden grey mullet *Liza aurata*. Journal of Fish Biology 75:1615-1625
- Leis JM (2015) Is dispersal of larval reef fishes passive? In: Camilo Mora (ed) Ecology of Fishes on Coral Reefs. Cambridge University Press, Cambridge, pp 223-226
- Liao JC (2006) The role of the lateral line and vision on body kinematics and hydrodynamic preference of rainbow trout in turbulent flow. Journal of Experimental Biology 209:4077-4090
- Liao JC (2007) A review of fish swimming mechanics and behaviour in altered flows. Philosophical Transactions of the Royal Society B: Biological Sciences 362:1973-1993
- Lilley SA, Schiel DR (2006) Community effects following the deletion of a habitat-forming alga from rocky marine shores. Oecologia 148:672-681
- Lönnstedt OM, Munday PL, McCormick MI, Ferrari MCO, Chivers DP (2013) Ocean acidification and responses to predators: can sensory redundancy reduce the apparent impacts of elevated CO₂ on fish? Ecology and Evolution 3:3565-3575
- Lough JM (2012) Small change, big difference: Sea surface temperature distributions for tropical coral reef ecosystems, 1950-2011. Journal of Geophysical Research: Oceans 117:C09018
- Luthi D et al. (2008) High-resolution carbon dioxide concentration record 650,000-800,000 years before present. Nature 453:379-382
- Luttbeg B, Sih A (2010) Risk, resources and state-dependent adaptive behavioural syndromes. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3977-3990
- Lyons DM, Price EO, Moberg GP (1993) Social grouping tendencies and separationinduced distress in juvenile sheep and goats. Developmental Psychobiology 265:251-259
- Madin JS, Black KP, Connolly SR (2006) Scaling water motion on coral reefs: From regional to organismal scales. Coral Reefs 25:635-644

Madin JS, Connolly SR (2006) Ecological consequences of major hydrodynamic disturbances on coral reefs. Nature 444:477-480

Magurran AE (1990) The adaptive significance of schooling as an anti-predator defence in fish. Annales Zoologici Fennici 27:51-66

Magurran AE, Pitcher TJ (1983) Foraging, timidity and shoal size in minnows and goldfish. Behavioral Ecology and Sociobiology 12:147-152

Magurran AE, Seghers BH, Carvalho GR, Shaw PW (1993) Evolution of adaptive variation in antipredator behaviour. Marine Behaviour and Physiology 23:29-44

- Magurran AE, Seghers BH, Shaw PW, Carvalho GR (1994) Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. Journal of Fish Biology 45:401-406
- Major PF (1978) Predator-prey interactions in two schooling fishes, *Caranx ignobilis* and *Stolephorus purpureus*. Animal Behaviour 26:760-777

Mann RP, Herbert-Read JE, Ma Q, Jordan LA, Sumpter DJ, Ward AJ (2014) A model comparison reveals dynamic social information drives the movements of humbug damselfish (*Dascyllus aruanus*). Journal of the Royal Society Interface 11:20130794

- Marquez BT, Krahe R, Chacron MJ (2013) Neuromodulation of early electrosensory processing in gymnotiform weakly electric fish. Journal of Experimental Biology 216:2442-2450
- Marras S, Batty RS, Domenici P (2012) Information transfer and antipredator maneuvers in schooling herring. Adaptive Behavior 20:44-56
- Marras S, Domenici P (2013) Schooling fish under attack are not all equal: Some lead, others follow. PLoS ONE 8:e65784
- Marras S, Killen SS, Domenici P, Claireaux G, McKenzie DJ (2013) Relationships among traits of aerobic and anaerobic swimming performance in individual European sea bass *Dicentrarchus labrax*. PLoS One 8:e72815
- Marras S, Killen SS, Lindström J, McKenzie DJ, Steffensen JF, Domenici P (2015) Fish swimming in schools save energy regardless of their spatial position. Behavioral Ecology and Sociobiology 69:219-226

Martin RA, Fiorentini M, Connors F (1980) Social facilitation of reduced oxygen consumption in *Mus musculus* and *Meriones unguiculatus*. Comp Biochem Physiol A Mol Integr Physiol 65:519-522

Mateo JM (2004) Recognition systems and biological organization: the perception component of social recognition. Annales Zoologici Fennici 41:729-745

- McCauley RD, Cato DH (2000) Patterns of fish calling in a nearshore environment in the Great Barrier Reef. Philos Trans R Soc Lond B Biol Sci 355:1289-1293
- McCormick MI (1999) Delayed metamorphosis of a tropical reef fish (Acanthurus triostegus): a field experiment. Marine Ecology Progress Series 176:25-38
- McCormick MI (2016) Protogyny in a tropical damselfish: females queue for future benefit. PeerJ 4:e2198
- McCormick MI, Lonnstedt OM (2016) Disrupted learning: habitat degradation impairs crucial antipredator responses in naive prey. Proc Biol Sci 283
- McDonnell LH, Chapman LJ (2016) Effects of thermal increase on aerobic capacity and swim performance in a tropical inland fish. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 199:62-70
- McGuigan K, Franklin CE, Moritz C, Blows MW (2003) Adaptation of rainbow fish to lake and stream habitats. Evolution 57:104-118
- McLaren JD, Shamoun-Baranes J, Dokter AM, Klaassen RH, Bouten W (2014) Optimal orientation in flows: providing a benchmark for animal movement strategies. Journal of the Royal Society Interface 11:20140588
- McNeil BI, Sasse TP (2016) Future ocean hypercapnia driven by anthropogenic amplification of the natural CO₂ cycle. Nature 529:383-386
- Medan V, Preuss T (2014) The Mauthner-cell circuit of fish as a model system for startle plasticity. Journal of Physiology Paris 108:129-140
- Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurements of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography 18:897-907

- Metcalfe NB, Thomson BC (1995) Fish recognize and prefer to shoal with poor competitors. Proceedings of the Royal Society of London B: Biological Sciences 259:207-210
- Miller GM, Watson S, Donelson JM, McCormick MI, Munday PL (2012) Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nature Climate Change 2:858-861
- Mintz I, Korn H (1991) Serotonergic facilitation of quantal release at central inhibitory synapses. The Journal of Neuroscience 11:3359-3370
- Mirjany M, Faber DS (2011) Characteristics of the anterior lateral line nerve input to the Mauthner cell. Journal of Experimental Biology 214:3368-3377
- Misund OA (1993) Dynamics of moving masses: variability in packing density, shape, and size among herring, sprat, and saithe schools. ICES Journal of Marine Science 50:145-160
- Morgan JD, Iwama GK (1996) Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki clarki*) parr. Fish Physiology and Biochemistry 15:385-394
- Morrell LJ, Hunt KL, Croft DP, Krause J (2007) Diet, familiarity and shoaling decisions in guppies. Animal Behaviour 74:311-319
- Muleta MG, Schausberger P (2013) Smells familiar: group-joining decisions of predatory mites are mediated by olfactory cues of social familiarity. Animal Behaviour 86:507-512
- Munday P, Wilson S (1997) Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. Journal of Fish Biology 51:931-938
- Munday PL, Buston PM, Warner RR (2006a) Diversity and flexibility of sex-change strategies in animals. Trends in Ecology & Evolution 21:89-95
- Munday PL, Cheal AJ, Dixson DL, Rummer JL, Fabricius KE (2014a) Behavioural impairment in reef fishes caused by ocean acidification at CO₂ seeps. Nature Climate Change 4:487-492

- Munday PL, Crawley NE, Nilsson GE (2009a) Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. Marine Ecology Progress Series 388:235-242
- Munday PL et al. (2009b) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proceedings of the National Academy of Sciences 106:1848-1852
- Munday PL, Dixson DL, McCormick MI, Meekan M, Ferrari MCO, Chivers DP (2010) Replenishment of fish populations is threatened by ocean acidification. Proceedings of the National Academy of Sciences 107:12930-12934
- Munday PL et al. (2013a) Elevated CO_2 affects the behavior of an ecologically and economically important coral reef fish. Marine Biology 160:2137-2144
- Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ (2013b) Predicting evolutionary responses to climate change in the sea. Ecology Letters 16:1488-1500
- Munday PL, Watson S-A, Chung W-S, Marshall NJ, Nilsson GE (2014b) Response to 'The importance of accurate CO2 dosing and measurement in ocean acidification studies'. Journal of Experimental Biology 217:1828-1829
- Munday PL et al. (2016) Effects of elevated CO₂ on early life history development of the yellowtail kingfish, *Seriola lalandi*, a large pelagic fish. ICES Journal of Marine Science 73:641-649
- Munday PL, Wilson White J, Warner RR (2006b) A social basis for the development of primary males in a sex-changing fish. Proceedings of the Royal Society of London B: Biological Sciences 273:2845-2851
- Munks LS, Harvey ES, Saunders BJ (2015) Storm-induced changes in environmental conditions are correlated with shifts in temperate reef fish abundance and diversity. Journal of Experimental Marine Biology and Ecology 472:77-88
- Nadler LE, Killen SS, McClure EC, Munday PL, McCormick MI (2016) Shoaling reduces metabolic rate in a gregarious coral reef fish species. Journal of Experimental Biology 219:2802-2805

- Nadler LE, McNeill DC, Alwany MA, Bailey DM (2014a) Effect of habitat characteristics on the distribution and abundance of damselfish within a Red Sea reef. Environmental Biology of Fishes 97:1265-1277
- Nadler LE, McNeill DC, Alwany MA, Bailey DM (2014b) Effect of habitat characteristics on the distribution and abundance of damselfish within a Red Sea reef. Environmental Biology of Fishes 97:1265-1277
- Nagelkerken I, Munday PL (2016) Animal behaviour shapes the ecological effects of ocean acidification and warming: Moving from individual to community-level responses. Global Change Biology 22:974-989
- Neumeister H, Whitaker KW, Hofmann HA, Preuss T (2010) Social and ecological regulation of a decision-making circuit. Journal of Neurophysiology 104:3180-3188
- Niimi AJ, Beamish FWH (1974) Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. Canadian Journal of Zoology 52:447-456
- Nikora V (2010) Hydrodynamics of aquatic ecosystems: An interface between ecology, biomechanics and environmental fluid mechanics. River Research and Applications 26:367-384
- Nilsson GE et al. (2012) Near-future carbon dioxide levels alter fish behavior by interfering with neurotransmitter function. Nature Climate Change 2:201-204
- Nunes JdACC, Sampaio CLS, Barros F (2013) How wave exposure, group size and habitat complexity influence foraging and population densities in fishes of the genus Halichoeres (Perciformes: Labridae) on tropical rocky shores. Marine Biology 160:2383-2394
- Nussey DH, Wilson AJ, Brommer JE (2007) The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology 20:831-844

- Ohman MC, Munday PL, Jones GP, Caley MJ (1998) Settlement strategies and distribution patterns of coral-reef fishes. Journal of Experimental Marine Biology and Ecology 225:219-238
- Overli O, Harris CA, Winberg S (1999) Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. Brain Behavior and Evolution 54:263-275
- Parker FR (1973) Reduced metabolic rates in fishes as a result of induced schooling. Transactions of the American Fisheries Society 102:125-131
- Partridge BL (1982a) Rigid definitions of schooling behavior are inadequate. Animal Behaviour 30:298-299
- Partridge BL (1982b) Structure and function of fish schools. Scientific American 245:114-123
- Partridge BL, Pitcher TJ (1980) The sensory basis of fish schools: Relative roles of lateral line and vision. Journal of Comparative Physiology A 135:315-325
- Partridge BL, Pitcher TJ, Cullen JM, Wilson J (1980) The three-dimensional structure of fish schools. Behavioral Ecology and Sociobiology 6:277-288
- Peake S, McKinley RS, Scruton DA (1997) Swimming performance of various freshwater Newfoundland salmonids relative to habitat selection and fishway design. Journal of Fish Biology 51:710-723
- Pecor KW, Hazlett BA (2003) Frequency of encounter with risk and the tradeoff between pursuit and antipredator behaviors in crayfish: A test of the risk allocation hypothesis. Ethology 109:97-106

Pereira PHC, Feitosa JLL, Medeiros DV, Ferreira BP (2012) Reef fishes foraging facilitation behavior: Increasing the access to a food resource. Acta Ethologica 16:53-56

Pereira PHC, Ferreira BP (2013) Effects of life phase and schooling patterns on the foraging behaviour of coral-reef fishes from the genus Haemulon. Journal of Fish Biology 82:1226-1238

- Piersma T, Drent J (2003) Phenotypic flexibility and the evolution of organismal design. Trends in Ecology & Evolution 18:228-233
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2016) nlme: Linear and Nonlinear Mixed Effects Models R package version 3.1-122
- Pitcher TJ (1983) Heuristic definition of shoaling behaviour. Animal Behaviour 31:611-613
- Pitcher TJ, Partridge BL (1979) Fish school density and volume. Marine Biology 54:383-394
- Plath M, Sarbu A, Erkoc K, Bierbach D, Jourdan J, Schleucher E (2013) Energetic costs of group-living? A reversed "group effect" in shoaling minnows (*Phoxinus phoxinus*). Bulletin of Fish Biology 14:1-10

Poff NL et al. (1997) The natural flow regime. BioScience 47:769-784

- Pörtner HO, Farrell AP (2008) Physiology and climate change. Science 322:690-692
- Portner HO, Langenbuch M, Reipschlager A (2004) Biological impact of elevated ocean CO₂ concentrations: Lessons from animal physiology and earth history. Journal of Oceanography 60:705-718
- Pratchett MS, Coker DJ, Jones GP, Munday PL (2012) Specialization in habitat use by coral reef damselfishes and their susceptibility to habitat loss. Ecology and Evolution 2:2168-2180
- Preuss T, Faber DS (2003) Central cellular mechanisms underlying temperaturedependent changes in the goldfish startle-escape behavior. The Journal of Neuroscience 23:5617-5626
- Priede IG (1985) Metabolic scope in fishes. In: Calow PTP (ed) Fish Energetics: New Perspectives. Croom-Helm, London
- Pruitt JN, Keiser CN (2014) The personality types of key catalytic individuals shape colonies' collective behaviour and success. Animal Behaviour 93:87-95
- Randall JE, Allen GR, Steene RC (1997) Fishes of the Great Barrier Reef and Coral Sea. University of Hawaii Press

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biological Reviews 82:291-318

Regan MD et al. (2016) Ambient CO₂, fish behaviour and altered GABAergic neurotransmission: Exploring the mechanism of CO₂-altered behaviour by taking a hypercapnia dweller down to low CO₂ levels. Journal of Experimental Biology 219:109-118

Rieucau G, Boswell KM, De Robertis A, Macaulay GJ, Handegard NO (2014a) Experimental evidence of threat-sensitive collective avoidance responses in a large wild-caught herring school. PLoS One 9:e86726

Rieucau G, De Robertis A, Boswell KM, Handegard NO (2014b) School density affects the strength of collective avoidance responses in wild-caught Atlantic herring *Clupea harengus*: a simulated predator encounter experiment. Journal of Fish Biology 85:1650-1664

Riley JR et al. (1999) Compensation for wind drift by bumble-bees. Nature 400:126

Roberts G (1996) Why individual vigilance declines as group size increases. Animal Behaviour 51:1077-1086

- Robertson DR (1972) Social control of sex reversal in a coral-reef fish. Science 177:1007-1009
- Robertson DR, Sweatman HPA, Fletcher EA, Cleland MG (1976) Schooling as a mechanism for circumventing the territoriality of competitors. Ecology 57:1208-1220
- Roche DG, Binning SA, Bosiger Y, Johansen JL, Rummer JL (2013) Finding the best estimates of metabolic rates in a coral reef fish. Journal of Experimental Biology 216:2103-2110
- Rodgers GG, Tenzing P, Clark TD (2016) Experimental methods in aquatic respirometry: the importance of mixing devices and accounting for background respiration. Journal of Fish Biology 88:65-80

- Rodriguez-Prieto I, Fernandez-Juricic E, Martin J, Regis Y (2009) Antipredator behavior in blackbirds: habituation complements risk allocation. Behavioral Ecology 20:371-377
- Ross RM, Losey GS, Diamond M (1983) Sex change in a coral-reef fish: Dependence of stimulation and inhibition on relative size. Science 221:574-475
- Rossi T, Nagelkerken I, Pistevos JC, Connell SD (2016) Lost at sea: Ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification. Biology Letters 12:20150937
- Rummer JL, Stecyk JAW, Couturier CS, Watson SA, Nilsson GE, Munday PL (2013) Elevated CO₂ enhances aerobic scope of a coral reef fish. Conservation Physiology 1:cot023
- Ryer CH, Olla BL (1997) Altered search speed and growth: social versus independent foraging in two pelagic juvenile fishes. Marine Ecology Progress Series 153:273-281

Sabine CL et al. (2004) The oceanic sink for anthropogenic CO₂. Science 305:367-371

- Scantlebury M, Bennett NC, Speakman JR, Pillay N, Schradin C (2006) Huddling in groups leads to daily energy savings in free-living African Four-Striped Grass Mice, *Rhabdomys pumilio*. Functional Ecology 20:166-173
- Schleuter D, Haertel-Borer S, Fischer P, Eckmann R (2007) Respiration rates of Eurasian perch *Perca fluviatilis* and Ruffe: Lower energy costs in groups. Transactions of the American Fisheries Society 136:43-55
- Schulte PM (2015) The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. Journal of Experimental Biology 218:1856-1866
- Seppä T, Laurila A, Peuhkuri N, Piironen J, Lower N (2001) Early familiarity has fitness consequences for Arctic charr (*Salvelinus alpinus*) juveniles. Canadian Journal of Fisheries and Aquatic Sciences 58:1380-1385

Shaw E (1978) Schooling fishes. American Scientist 66:166-175

- Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378
- Sinclair ELE, de Souza CRN, Ward AJW, Seebacher F, Herrel A (2014) Exercise changes behaviour. Functional Ecology 28:652-659
- Sloman KA, Motherwell G, O'Connor KI, Taylor AC (2000) The effect of social stress on the standard metabolic rate (SMR) of brown trout, *Salmo trutta*. Fish Physiology and Biochemistry 23:49-53
- Sogard SM, Olla BL (1997) The influence of hunger and predation risk on group cohesion in a pelagic fish, walleye pollock *Theragra chalcogramma*. Environmental Biology of Fishes 50:405-413
- Song J, Ampatzis K, Ausborn J, El Manira A (2015) A hardwired circuit supplemented with endocannabinoids encodes behavioral choice in zebrafish. Current Biology 25:2610-2620
- Soria M, Freon P, Chabanet P (2007) Schooling properties of an obligate and a facultative fish species. Journal of Fish Biology 71:1257-1269
- Spagnoli S, Sanders J, Kent ML (2016) The common neural parasite *Pseudoloma neurophilia* causes altered shoaling behaviour in adult laboratory zebrafish (*Danio rerio*) and its implications for neurobehavioural research. Journal of Fish Diseases DOI: 10.1111/jfd.12512
- Srygley RB (2001) Compensation for fluctuations in crosswind drift without stationary landmarks in butterflies migrating over seas. Animal Behaviour 61:191-203
- Stamps JA (2007) Growth-mortality tradeoffs and 'personality traits' in animals. Ecology Letters 10:355-363
- Steffensen J (1989) Some errors in respirometry of aquatic breathers: How to avoid and correct for them. Fish Physiology and Biochemistry 6:49-59
- Stier AC, Geange SW, Bolker BM (2013) Predator density and competition modify the benefits of group formation in a shoaling reef fish. Oikos 122:171-178
- Strandburg-Peshkin A et al. (2013) Visual sensory networks and effective information transfer in animal groups. Current Biology 23:R709-711

- Sumpter DJ, Krause J, James R, Couzin ID, Ward AJ (2008) Consensus decision making by fish. Current Biology 18:1773-1777
- Svendsen MB, Bushnell PG, Steffensen JF (2016) Design and setup of intermittent-flow respirometry system for aquatic organisms. Journal of Fish Biology 88:26-50
- Swain DP (1992) The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. Evolution 46:987-997
- Swaney W, Kendal J, Capon H, Brown C, Laland KN (2001) Familiarity facilitates social learning of foraging behaviour in the guppy. Animal Behaviour 62:591-598
- Taylor EB, McPhail JD (1986) Prolonged and burst swimming in anadromous and freshwater threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 64:416-420
- R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Tewksbury JJ, Huey RB, Deutsch CA (2008) Ecology: Putting the heat on tropical animals. Science 320:1296-1297
- Thorup K, Alerstam T, Hake M, Kjellen N (2003) Bird orientation: Compensation for wind drift in migrating raptors is age dependent. Proceedings of the Royal Society of London B: Biological Sciences 270 Suppl 1:S8-11

Thuiller W (2007) Biodiversity: Climate change and the ecologist. Nature 448:550-552

- Treherne JE, Foster WA (1981) Group transmission of predator avoidance behaviour in a marine insect: The Trafalgar Effect. Animal Behaviour 29:911-917
- Trune DR, Slobodchikoff CN (1976) Social effects of roosting on the metabolism of the Pallid Bat (*Antrozous pallidus*). Journal of Mammalogy 57:656-663
- Turesson H, Domenici P (2007) Escape latency is size independent in grey mullet. Journal of Fish Biology 71:253-259

Tytell ED, Lauder GV (2008) Hydrodynamics of the escape response in bluegill sunfish, Lepomis macrochirus. Journal of Experimental Biology 211:3359-3369

Wakeling JM (2005) Fast-start Mechanics. In: Shadwick RE, Lauder GV (eds) Fish Physiology, vol 23. Elsevier Academic Press Inc., San Diego, CA, pp 333-368

- Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznick DN (2005) Do faster starts increase the probability of evading predators? Functional Ecology 19:808-815
- Ward A, Webster M (2016) Sociality: The Behaviour of Group-Living Animals. Springer, Switzerland
- Ward A, Webster MM, Hart PJB (2006) Intraspecific food competition in fishes. Fish and Fisheries 7:231-261
- Ward AJW, Axford S, Krause J (2002) Mixed-species shoaling in fish: The sensory mechanisms and costs of shoal choice. Behavioral Ecology and Sociobiology 52:182-187
- Ward AJW, Axford S, Krause J (2003) Cross-species familiarity in shoaling fishes. Proceedings of the Royal Society of London B: Biological Sciences 270:1157-1161
- Ward AJW, Hart PJB (2003) The effects of kin and familiarity on interactions between fish. Fish and Fisheries 4:348-358
- Ward AJW et al. (2013) Initiators, leaders, and recruitment mechanisms in the collective movements of damselfish. The American Naturalist 181:748-760
- Ward AJW, Herbert-Read JE, Sumpter DJT, Krause J (2011) Fast and accurate decisions through collective vigilance in fish shoals. Proceedings of the National Academy of Sciences 108:2312-2315
- Warner RR, Robertson DR, Leigh EG (1975) Sex change and sexual selection. Science 190:633-638
- Warner RR, Swearer SE (1991) Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatusm* (Pisces: Labridae). Biological Bulletin 181:199-204
- Webb PW (1978) Fast-start performance and body form in seven species of teleost fish. Journal of Experimental Biology 74:211-226
- Webster MM, Atton N, Hoppitt WJE, Laland KN (2013) Environmental complexity influences association network structure and network-based diffusion of foraging information in fish shoals. The American Naturalist 181:235-244

- Webster MM, Goldsmith J, Ward AJW, Hart PJB (2007) Habitat-specific chemical cues influence association preferences and shoal cohesion in fish. Behavioral Ecology and Sociobiology 62:273-280
- Webster MM, Laland KN (2013) Local enhancement via eavesdropping on courtship displays in male guppies, *Poecilia reticulata*. Animal Behaviour 86:75-83
- Webster MM, Ward AJ (2011) Personality and social context. Biological Reviews 86:759-773
- Weetman D, Atkinson D, Chubb JC (1998) Effects of temperature on anti-predator behaviour in the guppy *Poecilia reticulata*. Animal Behaviour 55:1361-1372
- Weetman D, Atkinson D, Chubb JC (1999) Water temperature influences the shoaling decisions of guppies *Poecilia reticulata* under predation threat. Animal Behaviour 58:735-741

Weihs D (1973) Hydrodynamics of fish schooling. Nature 241:290-291

- Weimerskirch H, Martin J, Clerquin Y, Alexandre P, Jiraskova S (2001) Energy saving in flight formation. Nature 413:697-698
- Welch MJ, Watson S, Welsh JQ, McCormick MI, Munday PL (2014) Effects of elevated CO₂ on fish behaviour undiminished by transgenerational acclimation. Nature Climate Change 4:1086-1089
- Welsh JQ, Bellwood DR (2012) How far do schools of roving herbivores rove? A case study using *Scarus rivulatus*. Coral Reefs 31:991-1003
- West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20:249-278
- Whitaker KW, Neumeister H, Huffman LS, Kidd CE, Preuss T, Hofmann HA (2011) Serotonergic modulation of startle-escape plasticity in an African cichlid fish: a single-cell molecular and physiological analysis of a vital neural circuit. Journal of Neurophysiology 106:127-137
- White JW, Warner RR (2007a) Behavioral and energetic costs of group membership in a coral reef fish. Oecologia 154:423-433

White JW, Warner RR (2007b) Safety in numbers and the spatial scaling of densitydependent mortality in a coral reef fish. Ecology 88:3044-3054

Whiteman EA, Côté IM (2004) Dominance hierarchies in group-living cleaning gobies: causes and foraging consequences. Animal Behaviour 67:239-247

Winberg S, Nilsson GE (1993) Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 106:597-614

Winberg S, Thörnqvist P-O (2016) Role of brain serotonin in modulating fish behavior. Current Zoology 62:317-323

Wittmann AC, Portner HO (2013) Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change 3:995-1001

Wolf M, Van Doorn GS, Weissing FJ (2011) On the coevolution of social responsiveness and behavioural consistency. Proceedings of the Royal Society of London B: Biological Sciences 278:440-448

Wolf NG (1987) Schooling tendency and foraging benefit in the ocean surgeonfish. Behavioral Ecology and Sociobiology 21:59-63

Wong MYL (2011) Group size in animal societies: The potential role of social and ecological limitations in the group-living fish, *Paragobiodon xanthosomus*. Ethology 117:638-644

Yan GJ, He XK, Cao ZD, Fu SJ (2015) Effects of fasting and feeding on the fast-start swimming performance of southern catfish *Silurus meridionalis*. Journal of Fish Biology 86:605-614

Yeh S, Fricke RA, Edwards DH (1996) The effect of social experience on serotonergic modulation of the escape circuit of crayfish. Science 271:366-369

Yoon J-D, Jang M-H, Joo G-J (2011) Effect of flooding on fish assemblages in small streams in South Korea. Limnology 12:197-203

- Yue S, Duncan IJH, Moccia RD (2006) Do differences in conspecific body size induce social stress in domestic rainbow trout? Environmental Biology of Fishes 76:425-431
- Zeebe RE, Zachos JC, Caldeira K, Tyrrell T (2008) Oceans: Carbon emissions and acidification. Science 321:51-52
- Zheng M, Kashimori Y, Hoshino O, Fujita K, Kambara T (2005) Behavior pattern (innate action) of individuals in fish schools generating efficient collective evasion from predation. Journal of Theoretical Biology 235:153-167

Appendix 1: Evidence of physiological assortment among wild schools of a coral reef fish

This appendix is in revision at Oecologia

Authors: SS Killen[†], LE Nadler[†], MI McCormick

[†]These authors contributed equally to this manuscript

A.1 Summary

Group living occurs in most animal taxa and carries a variety of tradeoffs. The exact fitness benefits for any group member are largely dependent on the characteristics of its group-mates. In order to maximise these benefits, groups may assort according to a variety of phenotypic traits, with individuals preferentially associating with conspecifics to which they are related or morphologically similar. Although assorting according to morphological and behavioural traits has been investigated in a range of studies, no one has yet examined whether social groups also sort according to physiological characteristics like metabolism. In this study, we examined whether wild schools of the gregarious coral reef damselfish Chromis viridis displays inter-group differences in whole-animal physiological traits and whether such variation is associated with local habitat characteristics (temperature, depth and flow rate). Mean maximal metabolic rate and aerobic scope varied among schools found at different sites, with individuals from schools in areas with higher current flow rates exhibiting a greater maximal metabolic rate and aerobic scope. Within a given site, maximal metabolic rate and aerobic scope did not vary between schools but standard and routine metabolic rates often showed considerable inter-school variance. In addition, schools found at greater depths were observed to have a higher mean routine metabolic rate. Overall, these

results suggest that in large-scale coral reef habitats on the order of hundreds to thousands of meters, a combination of passive sorting, phenotypic plasticity and/or selective mortality of individuals in social groups likely generate variation in physiological traits among social groupings. At a more localised scale, over tens of meters along continuous reef sections, there may also be an active component to assortment, resulting in differences in metabolic demand among groups of conspecifics. Whether occurring by active or passive means, physiological assortment could be an important factor affecting not only social group dynamics but also the underlying intraspecific biodiversity within habitats.

A.2 Introduction

Group living can be found in every major animal taxa and carries a number of benefits for individual group members, including increased foraging success, reduced risk of predation and increased efficiency of movement (Krause and Ruxton 2002). There are potential costs, however, such as competition for resources, agonistic encounters and exposure to disease or parasites. The composition of the group greatly influences the extent to which any individual within a group experiences these trade-offs (Metcalfe and Thomson 1995; Pruitt and Keiser 2014), thus highlighting a potential link between the composition of animal groups and the fitness of individual group members.

There has been a recent surge in research examining the factors that generate and maintain variation in behavioural, morphological and physiological traits within species. Most of this work has focused on variation among individuals (Killen et al. 2013; Réale et al. 2007; Sih et al. 2004), or among populations that inhabit different geographical regions (Arnott et al. 2006). In comparison, little is known about trait assortment or differentiation among social groups of the same species that live within close proximity. Among work examining this topic, it has been shown that animals may preferentially assort in groups with individuals that are morphologically similar to themselves (e.g. similar in size, sex or colour; Croft et al. 2003; Croft et al. 2009; Jones et

al. 2010). This phenotypic assortment may help reduce the oddity effect, whereby an individual that appears different from the rest of the group is more obvious and is preferentially targeted by predators (Landeau and Terborgh 1986; Peuhkuri 1997). Further, joining a group of similar individuals may minimise compromises made when conforming to the behaviour of the group. Within most species, there is consistent variation in behaviour and physiological traits (Biro and Stamps 2010; Killen et al. 2013; Laskowski and Bell 2014). Despite this variability, animal groups such as bird flocks, fish schools and insect swarms are remarkable for their synchronous behaviour. In fish schools, for example, individuals swim approximately the same speed and exhibit simultaneous group responses to changes in environmental factors such as hypoxia (Abrahams and Colgan 1985; Domenici et al. 2002). This suggests that school members shift their individual behavioural responses toward a collective common-ground (Webster and Ward 2011). Joining a group of alike individuals would lessen the phenotypic adjustments made by each group member.

There is little known about how other factors, besides behaviour or morphology, may affect phenotypic assortment of animal groups. For example, the possibility that individuals may sort into groups according to individual physiological characteristics has thus far remained unexplored. Body size and behaviour are both correlated with numerous whole-animal physiological traits, particularly those related to energy metabolism (Brown et al. 2004; Glazier 2005; Killen et al. 2010). Thus, similarity in appearance, body size or behaviour could act as proxies for similarity in physiological traits or performance capacity. Resting metabolic rate (standard metabolic rate, SMR, in ectotherms; basal metabolic rate in endotherms), for example, has been linked to food requirements and risk-taking. Variation in maximal aerobic metabolic rate (MMR) or aerobic scope (AS, the difference between SMR and MMR) are related to differences in the capacity for locomotion among individuals and potentially the degree to which individuals can tolerate environmental stressors (Claireaux and Lefrancois 2007; Killen et al. 2013; Pörtner and Farrell 2008).

Such whole-animal physiological traits could also have a direct impact on group cohesiveness and synchronicity. For instance, within fish schools swimming at high speeds, individuals tend to position themselves according to AS, with those fishes with a higher AS leading at the front of the school and those with a lower AS more often found located toward the back of the group (Killen et al. 2012). It would therefore be disadvantageous for a fish to join a school consisting of individuals with a much higher or lower capacity for aerobic swimming compared to itself – faster fish could leave the individual behind during a predator attack or exposure to fast current speeds, whereas slower fish may limit group performance if group cohesion is to be maintained. It would also be beneficial to associate with conspecifics with similar tolerances to environmental stressors. Metabolic rate and aerobic scope are linked to individual temperature sensitivity and hypoxia tolerance in ectotherms, including fish (Claireaux and Lefrancois 2007; Fry 1971), and it would not be advantageous for an individual to join a group comprised of animals with a tolerance for thermal extremes that exceeds its own.

It is also possible that physiological assortment could occur by more passive means. Links between metabolic traits and habitat preference, behavioural patterns and environmental tolerances may cause individuals with specific phenotypes to experience spatial and temporal overlap and thus coexist within the same group (Croft et al. 2003). Within fish species, for example, individuals with a higher aerobic scope may be more able to occupy areas with increased flow rates where they may access more planktonic prey. Importantly, differentiation among groups could also arise through either phenotypic plasticity or selective mortality, in response to localised variation in environmental conditions. Whether occurring by active or passive mechanisms, differences in physiological phenotype among groups of the same species could affect the spatial distribution of individuals with specific physiological traits, therefore structuring populations according to phenotype and affecting the underlying intraspecific biodiversity within habitats or regions. This is particularly important when specific locations may be better for offspring survival, such as marine protected areas or spawning aggregation sites (Fraser and McCormick 2014; Harrison et al. 2012), which

means the phenotypes inhabiting those areas may contribute more to the next generation.

To date, the possibility of physiological differences among groups of a species in the wild has never been investigated. We studied this issue in wild schools of a gregarious coral reef damselfish *Chromis viridis*. Our objectives were to: 1) establish whether there were differences in metabolic traits (standard, routine and maximal metabolic rates, and aerobic scope) among wild schools, and among schools inhabiting different sites within a larger reef habitat; and 2) investigate links between the physiological traits of schools and habitat characteristics (temperature, depth and flow rate). We observed that MMR and AS varied among sites, but that within a given site schools at that location differed in terms of SMR and RMR. It was also found that site with higher flow rates tended to have fish with a higher MMR and AS.

A.3 Materials and methods

Study site and specimen collection

This study was conducted at the Lizard Island Research Station (LIRS) in the northern Great Barrier Reef, Australia (14°40′S, 145°28′E), from November to December 2013. Wild schools of the damselfish *Chromis viridis* were collected from shallow reef sites (< 4 m, Fig. A.1) surrounding LIRS using a barrier net, hand nets and a dilute anaesthetic solution composed of clove oil, ethanol, and seawater (Munday and Wilson 1997). *C. viridis* are a gregarious schooling species found living in close-association with live branching coral, particularly *Pocillopora* and *Acropora* spp. (Fishelson et al. 1974; Goldshmid et al. 2004; Nadler et al. 2014b). In the vicinity of Lizard Island, schools of *C. viridis* can contain anywhere from a few individuals to several hundred (L. Nadler, pers. obs.). Eight individuals were collected from each of 11 schools from seven sites and





transported back to LIRS. At three sites one school was collected and at the other four sites two separate groups of 8 fish were collected. Schools within a site were collected on the same day. Within a given site, schools were separated by 50 m, while sites were separated by 400 – 3000 m (Fig. A.1). Sites were also considered separate when they occurred on non-continuous sections of reef (i.e., fish would need to cross a section of sand substrate to move between sites but not between two schools at the same site). Each of the sites where multiple schools were collected did not vary in depth along the continuous reefs and exhibited consistent topography and flow regimes between the locations where schools were collected. Prior to measurement of metabolic traits, schools were housed in 15 L flow-through aquaria under natural light conditions and

ambient temperature. Fish were fed to satiation twice daily with INVE Aquaculture pellets and newly hatched *Artemia* sp.

Respirometry

Fish were held in the lab for 3-5 days before determination of metabolic rate. Metabolic rates of individual fish were estimated by intermittent-flow respirometry as rate of oxygen uptake (Steffensen 1989). To determine maximal metabolic rate (MMR), fish that had been fasted for 24 h were individually exercised to exhaustion by manual chasing in a small (40 cm diameter) container filled to a depth of approximately 20 cm. Fish were considered exhausted when they would no longer respond to chasing by burst swimming. This method elicits anaerobic exercise in individuals, and maximal rates of oxygen uptake are measured during subsequent recovery. After exhaustion, fish were air exposed for 30 s to further ensure that they had utilised all endogenous oxygen stores. They were then transferred to individual cylindrical glass respirometers with acrylic end-caps (total volume of chamber plus associated tubing = 75 ml). Respirometers were immersed in a water bath, which was supplied with water from a header tank in which temperature was controlled to approximately 28.5°C. Fish were left undisturbed in respirometers overnight and in complete darkness for the next 14 h. Initial attempts were made to quantify individual activity by observing movement within the chambers using indirect lighting, but fish were invariably dormant within the chambers after darkness and were completely inactive aside from maintaining upright posture. Opaque dividers were placed between adjacent respirometers to prevent any possible visual contact between fish. Water flow from the temperature controlled header tank through the respirometers was driven by an external pump in an adjacent header tank set to alternatingly turn on (2 min) and off (7 min) throughout the measurement period. This allowed decreases in water oxygen content to be measured every 2 s for 7 min while the respirometer was in the closed state, and then the respirometer was flushed with aerated water for 2 min. An exception was the first

closed phased after exhaustive exercise, during which the chamber was closed for at least 10 min to ensure that the immediate post-exercise phase was measured for oxygen uptake. Water mixing within each respirometer was achieved with a peristaltic pump that moved water through the chamber and round an external circuit of gasimpermeable tubing. Also located within the circuit for each respirometer was a flowthrough cell, which housed an oxygen-sensing optode attached to an oxygen sensor (Firesting 4-Channel oxygen meters; Pyroscience, Germany) and computer. Slopes were calculated from the plots of oxygen concentration versus time using linear least squares regression, and then converted to determine the rate of oxygen uptake (mg O_2 h⁻¹). The first minute of each closed phase was excluded from analyses to ensure adequate mixing within the chamber and external circuit. SMR was taken as the lowest 10th percentile of measurements excluding the first 2 h after exercise. Maximal metabolic rate (MMR) was calculated by analysing the first slope after exhaustive exercise in successive three-minute intervals, and then taking the highest measure recorded during this time as MMR. Aerobic scope (AS) was calculated as MMR - SMR. To correct for background bacterial oxygen consumption, all respirometers were run empty at the beginning and end of each working day, and measurements carried out during the day were corrected as needed assuming a linear increase in bacterial oxygen consumption with time. Each morning all respirometers, flow-through cells and tubing were thoroughly cleansed with soap, bleach and hot water. To examine the extent to which estimates of SMR, RMR, MMR and AS in C. viridis are repeatable, fish from two schools (schools 2 and 4 in Table A.1; i.e. n = 16 fish in total) were measured for SMR, RMR, MMR, and AS twice, with five days between measurements. Measures of RMR, MMR, and AS were highly repeatable among individuals over the course of five days (intraclass correlation coefficients: RMR: r = 0.54, p = 0.011, MMR, r = 0.86, p < 0.001; AS, r = 0.82, p < 0.001), while SMR showed more moderate levels of repeatability (SMR: r = 0.27, p =0.142).

Environmental Variables

Water temperature and mean flow rate were measured at each site on five separate days throughout December 2013. Surface temperatures were taken with a thermometer while flow rate at each location was determined using a precision vane-wheel flow meter (Hontzsch Gmbh, Waiblingen, Germany) that was placed into the flow approximately 1.25 m below the surface. Measures of flow speed (cm s⁻¹) were logged at 1 Hz for 180 s, with the value for each day being taken as the average value over this time period. An overall mean temperature and flow rate were then calculated for each site using data for all five days. Depth was measured at each collection site via a dive computer (Suunto Vyper, Suunto, Vantaa, Finland).

Data and Statistical Analysis

All analyses were performed with SPSS statistics v20.0 (SPSS Inc. and IBM, Chicago, IL, USA). The level of significance for all tests was $\alpha = 0.05$. When required, normality, linearity and homogeneity of residuals were verified by inspection of residual-fit plots. The variation among collection sites was compared using the intraclass correlation coefficient (ICC), calculated according to the methods of Lessels and Boag (1987). In this analysis, the statistic *r* represents the proportion of variation within the dataset that is attributable to differences in traits among sites, as opposed to within sites. Data for the calculation of ICCs for SMR, RMR, MMR, and AS were standardised to a body mass of 1.72 g (the mean mass of all fish used in the study). This was performed by constructing linear relationships of either log SMR, log RMR, log MMR or log AS vs. log body mass, calculating the residual values for each individual, then adding these residuals to the fitted value for a 1.72 g animal in each regression. The effects of school and collection site on metabolic traits were also examined using general linear models (GLM), with raw SMR, RMR, MMR or AS (all mg O₂ h⁻¹) as the dependent variable, and body mass (g) and school nested within collection site as explanatory variables. Separate GLMs were used to analyse the effect of habitat characteristics on metabolic traits, with flow rate, depth and body mass as explanatory variables. Flow rate was designated as a categorical variable, with flow > 20 cm s⁻¹ designated as 'high flow' and all other flows (< 11 cm s⁻¹) designated as 'low flow'. Collinearity prevented these environmental variables from being included in the models examining the effects of school and home range site. Mean temperature showed almost no variation among sites and so was not included in the analysis.

A.4 Results

There was variation in metabolic traits among schools and sites (Table A.1). The highest mean SMR measured for a school (collected at Horseshoe) was approximately 22% greater than that of the lowest (collected at Bird Islet); the highest RMR was 32% (Horseshoe) greater than the lowest (South Island); the highest MMR (Loomis Beach) was 34% greater than in the lowest (South Island); and the highest AS (Loomis Beach) was 40% higher than in the lowest (South Island).

There were significant differences among sites for MMR (GLM, effect of site, $F_{6, 87} = 3.43$, p = 0.005; ICC = 0.16, p = 0.005) and AS (GLM, effect of site, $F_{6, 87} = 3.09$, p = 0.009; ICC = 0.15, p = 0.008), but both SMR (GLM, effect of site, $F_{6, 87} = 1.38$, p = 0.233; ICC = 0.00, p = 0.466) and RMR (GLM, effect of site, $F_{6, 87} = 2.08$, p = 0.065; ICC = 0.07, p = 0.075) showed extremely low variance among sites.

When comparing between schools within sites, however, there were significant differences between schools for SMR (GLM, effect of school nested within site, $F_{10, 75} = 2.53$, p = 0.011) and RMR ($F_{10, 75} = 2.62$, p = 0.0009), with mean differences between schools of between 16.4% and 19.5%, respectively. Mean differences between schools at a site for MMR ($F_{10, 75} = 2.02$, p = 0.043) and AS ($F_{10, 75} = 1.86$, p = 0.065) were less substantial, only differing by as much as 6.8% and 4.7%, respectively. Schools that were collected from a greater depth had a lower mean RMR (Fig. A.2; GLM, effect of
		map		flow	temperature	depth	mass (g)	SMR	RMR	MMR	AS
site	school	label	coordinates	(cm s ⁻¹)	(°C)	(m)	Sumar	$(mg O_2 h^{-1})$	$(mg O_2 h^{-1})$	$(mg O_2 h^{-1})$	$(mg O_2 h^{-1})$
		1	S 14° 41.052',				1.206±0.073	0.725±0.049	0.811±0.026	3.376±0.177	2.647±0.177
Channel	1		E 145° 26.938'	21.8±8.3	28.6±0.2	1.8					
	2	1				2	1.559±0.086	0.635±0.066	0.740±0.039	3.478±0.233	2.843±0.246
Loomis		2	S 14° 41.125',				1.666±0.064	0.687±0.072	0.804±0.034	3.980±0.236	3.292±0.233
Beach	3	-	E 145° 27.020'	21.8±8.4	28.5±0.3	2.3					
	4	2				2.3	1.781±0.067	0.680±0.019	0.767±0.016	4.032±0.196	3.352±0.191
		3	S 14° 41.363',				1.751±0.087	0.613±0.048	0.742±0.075	3.516±0.162	2.903±0.199
Lagoon	5		E 145° 27.363'	8.4±1.57	28.1±0.3	2.5					
	6	3				2.5	1.853±0.195	0.710±0.043	0.865±0.055	3.499±0.284	2.726±0.276
	. 0	4	S 14° 41.885',				1.816±0.09	0.600±0.106	0.687±0.045	3.196±0.202	2.597±0.206
Bird Islet	7		E 145° 27.651'	10.0±1.8	28.1±0.2	4					
	8	4				3.6	2.047±0.085	0.698±0.022	0.820±0.036	3.416±0.263	2.719±0.262
		5	S 14° 41.387',				1.576±0.076	0.730±0.031	0.912±0.071	3.167±0.229	2.435±0.224
Horseshoe	9		E 145° 26.689'	10.0±2.2	28.5±0.2	2.2					
Station		6	S 14° 40.845',				1.677±0.089	0.663±0.038	0.752±0.046	3.639±0.282	2.976±0.265
Beach	10		E 145° 26.746'	8.2±1.2	28.2±0.2	2.0					
South		7	S 14° 42.022',				1.955±0.108	0.626±0.023	0.692±0.023	3.016±0.089	2.391±0.089
Island	11		E 145° 27.173'	9.0±1.3	28.1±0.2	3.4					

Table 1. Characteristics of the sites sampled in the current study, with the mean metabolic traits measured for fish within schools ateach site (standardised to a common mass of 1.72 g). The column 'map label' refers to the corresponding label number in Fig. A.1.Error = s.e.m. SMR = standard metabolic rate; RMR = routine metabolic rate; MMR = maximal metabolic rate; = AS = aerobic scope.



Fig. A.2. Links between environmental factors and metabolic traits of individuals within schools of *Chromis viridis*. A) Depth vs. RMR; each point represents the mean RMR for individuals within a given school. B) Metabolic traits and location with high and low flow rates (dark = high flow; white = low flow). Bars in panel B represent means for all

individuals sampled under a given flow regime n = 32 for high flow and 56 for low flow. Error bars = s.e.m.

depth, F = 5.12, p = 0.026). MMR and AS were both higher for fish in schools exposed to high flow rates (Fig. A.2; MMR: GLM, effect of flow rate, $F_{1, 87}$ = 5.01, p = 0.028; AS: GLM, effect of flow rate, $F_{1, 87}$ = 5.16, p = 0.026). The mean SMR of fish within a school was not related to either depth or flow rate (GLM, p > 0.05 in all cases).

A.5 Discussion

These results provide the first evidence of differences in physiological traits among social groups of the same species in the wild. For the damselfish *C. viridis*, there were strong differences in mean MMR and AS for schools collected at sites with differing environmental characteristics. In particular, sites with a higher flow contained groups of fish with a higher MMR and AS. Yet within a specific site, schools differed in mean SMR and RMR. Overall, different habitats within a geographical zone may be heterogeneous in the physiological phenotypes they contain. In addition, these findings suggest that, in addition to sex, morphology and relatedness (Croft et al. 2003; Croft et al. 2005; Hoare and Krause 2003; Jones et al. 2010; Krause and Godin 1994; Krause et al. 2000; Morrell et al. 2007), group composition may also be related to physiological traits.

There are four non-mutually exclusive processes that could explain the variation in AS and MMR among sites with flow regime: 1) passive assortment, in which individuals prioritise selection of a site that suits their individual phenotype, which then causes spatial overlap with conspecifics of a similar phenotype; 2) active assortment, in which individuals select group mates with a similar phenotype, taking up residency at sites that are well-suited to their physiological and behavioural traits; 3) phenotypic plasticity, in which individuals within groups change their phenotype (e.g., aerobic scope) so that they can cope with environmental conditions within a site; and 4) selective mortality, in which individuals within a specific phenotypic range remain. The life-

history of C. viridis suggests that the observed variation in MMR and AS among sites probably results from a combination of all of these factors. After hatching, C. viridis exist as pelagic larvae, but then settle at sites on coral reefs as they enter the juvenile stage. The site of settlement is determined by both the prevailing currents and the larvae themselves, as they are relatively strong swimmers and are guided to the reef by auditory and olfactory cues (Leis 2015). To some extent, however, coral reef fishes may also be able to delay their entry into metamorphosis (McCormick 1999), which could allow them to exercise some degree of selectivity over their settlement site. Throughout early development, or even during the adult stage, plasticity could then enhance differences in phenotypes across broad differences in habitat (e.g. flow regimes). Coral reef fish held in the laboratory under high flow conditions can develop an increased capacity for aerobic swimming, including increased MMR and AS (Binning et al. 2015). Even if fish are not pushed to their maximal capacity for aerobic swimming at sites with a current, an increased AS would provide more physiological capacity for additional tasks while performing even a moderate amount of swimming (Priede 1985). For example, individuals with an increased AS could maintain position in sites with stronger currents while still having the capacity to forage and digest captured prey. There could also be a loss of metabolically unsuited individuals at a given site via selective mortality. Traits such as growth rate, size at settlement and post-larval duration influence postsettlement survival in C. viridis, but the strength of selection on these traits varies among sites (Block and Steele 2014). Growth rate and swim performance are often related to AS and MMR in fishes (Claireaux and Lefrancois 2007), and so any selection on these traits could produce correlated selection on metabolic traits. More work is needed to disentangle the relative importance of each of these processes in the phenotypic variation observed among sites.

While schools within a site showed similar mean levels of MMR and AS, they generally showed large differences in SMR and RMR. Furthermore, there was no evidence that either SMR or RMR were related to habitat characteristics across sites. Flow regimes and topography were similar within a given site and so differences in traits

between schools at the same site are less likely to be due to plasticity or selective mortality, and suggests that there may be an active component to the physiological assortment observed among schools within a given site. SMR can be related to food demand and growth rate, and so individuals may sort according to food availability or foraging behaviour. Similarly, RMR may be related to activity level and so perhaps related to similarities in behaviour among individuals within a school. Resting metabolic rate is believed to be linked to trade-offs between predator avoidance and foraging (Biro and Stamps 2010; Huntingford et al. 2010; Killen 2011), and consequently growth rate (Alvarez 2005; Burton et al. 2011), and so sorting of groups based on SMR and RMR may be related to habitat factors not measured here including predator abundance, food availability or habitat complexity. More work is needed to establish the degree to which active versus passive assortment is occurring, over what spatial scales and which factors are involved. However, the general picture emerging from the current study is that, in coral reef environments, passive sorting is more likely to occur at scales over hundreds of meters or across sites on non-continuous reefs. Phenotypic differences among sites may then be augmented by phenotypic plasticity or selective mortality. Active sorting, conversely, may occur at a more localised scale (over tens of meters along continuous reef sections), resulting in differences in metabolic demand (SMR and RMR) among groups of conspecifics living in close proximity.

Associating with similar conspecifics may carry a number of benefits, including decreased predation risk (Hart 1997; Krause and Ruxton 2002) and competition for resources (Metcalfe and Thomson 1995). For example, joining a group of individuals with a similar physiology may be advantageous because group mates will have similar capacities for locomotory performance. In fish schools, slower individuals would be more vulnerable during a predator attack if they are unable to match the performance of their group mates and are left behind as the group flees (Killen et al. 2012). Similarities in metabolic traits such as AS may also confer similarities in tolerances to environmental stressors such as variation in temperature or oxygen availability (Claireaux and Lefrancois 2007), and so belonging to a group of physiologically similar

animals will lessen the likelihood of being lead into an undesirable area. It has been shown, for example, that individuals of the same fish species can vary substantially in their temperature preference in a manner that is directly tied to their SMR (Killen 2014b). Associating with conspecifics with similar environmental tolerances or preferences would therefore decrease the compromises made by each individual within the group. It should be noted, however, that there may also be drawbacks associated with grouping with physiologically similar individuals. Metabolic rate may be tied to an demand for food (Dupont-Prinet et al. 2010; Killen 2011), for example, and so it is conceivable that groups comprised of individuals with a high metabolic demand may experience increased competition for available resources. This is especially likely given that traits such as SMR and AS may also be linked to aggression among individuals (Biro and Stamps 2010; Killen et al. 2014). Variation in metabolic traits may also facilitate the establishment of stable hierarchies within social groups (Laskowski and Pruitt 2014). The possible trade-offs involved in group composition, and the various environmental factors that may influence such trade-offs, will be an important avenue for future research.

It is important to note that although there were differences in the mean traits measured among sites and schools, there was also variation within each school. This indicates that individuals may still be constrained to some extent by the behaviours, tolerances and preferences of group mates. This variation could also affect the spatial distribution of phenotypes within groups (Killen et al. 2012). For example, in fish schools swimming at relatively high speeds, individuals with a higher AS are located toward the front of the moving group. In site-attached coral reef species, there could also be vertical spatial structuring, with different phenotypes occupying differing distances from the reef within the water column in accordance with localised flow regimes and food availability. Individuals with a higher MMR or AS may be more able to tolerate increased flow away from the reef where they can have greater access to planktonic food. In contrast, it is also possible that social structures can reinforce intra-specific phenotypic differences, perhaps by niche construction within social groups (Laskowski and Pruitt

2014). Over time, different individuals may begin to fulfill specific roles or become established with a social hierarchy, which may in turn influence physiological traits such as metabolic rate (Burton et al. 2011; Killen et al. 2014). Links between social dominance and physiology could amplify phenotypic differences among individuals. Among fishes, this is likely to be an important difference between site-attached social species and those that form pelagic schools, an area which needs further study. Overall, the current study suggests that although individuals display a degree of physiological assortment among schools, the amount of variation present within groups could still affect intragroup dynamics.

In summary, the current study provides evidence of physiological assortment among fish schools in the wild. There has been a recent research focus on the ecological and evolutionary importance of intraspecific differences in behavioural and physiological traits in animals, but given that most animals live in groups, it is likely that individual traits interact with those of conspecifics in its social environment to influence the net ecological effect of individual differences (Webster and Ward 2011). The physiological performance of group mates will play an important role in modulating these outcomes, particularly during events which have the greatest impact on individual fitness, such as during predator attacks or exposure to stressful environments. Additional work is needed to understand the mechanisms responsible for physiological assortment and its importance for structuring intra-specific diversity within regions and the selective pressures experienced by different phenotypes.

A.6 References

Álvarez DN, Nicieza AG (2005) Is metabolic rate a reliable predictor of growth and survival of brown trout (*Salmo trutta*) in the wild? Canadian Journal of Fisheries and Aquatic Sciences 62:643-649

- Arnott SA, Chiba S, Conover DO (2006) Evolution of intrinsic growth rate: metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. Evolution 60:1269-1278
- Binning SA, Ros AFH, Nusbaumer D, Roche DG (2015) Physiological plasticity to water flow habitat in the damselfish, *Acanthochromis polyacanthus*: Linking phenotype to performance. PLoS ONE 10:e0121983
- Biro PA, Stamps JA (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology & Evolution 25:653-659
- Block HE, Steele MA (2014) Spatial variation in selective mortality on larval traits in the coral reef fish *Chromis viridis*. Marine Ecology Progress Series 509:303-308
- Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789
- Burton T, Killen SS, Armstrong JD, Metcalfe NB (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society B: Biological Sciences 278:3465-3473
- Claireaux G, Lefrancois C (2007) Linking environmental variability and fish performance: integration through the concept of scope for activity. Philosophical Transactions of the Royal Society B: Biological Sciences 362:2031-2041
- Croft DP et al. (2003) Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. Oikos 100:429-438
- Croft DP, Darden SK, Ruxton GD (2009) Predation risk as a driving force for phenotypic assortment: a cross-population comparison. Proceedings of the Royal Society B: Biological Sciences 276:1899-1904
- Croft DP, James R, Ward AJW, Botham MS, Mawdsley D, Krause J (2005) Assortative interactions and social networks in fish. Oecologia 143:211-219
- Dupont-Prinet A, Chatain B, Grima L, Vandeputte M, Claireaux G, McKenzie DJ (2010) Physiological mechanisms underlying a trade-off between growth rate and

tolerance of feed deprivation in the European sea bass (*Dicentrarchus labrax*). Journal of Experimental Biology 213:1143-1152

- Fishelson L, Popper D, Avidor A (1974) Biosociology and ecology of pomacentrid fishes around the Sinai Peninsula (northern Red Sea). Journal of Fish Biology 6:119-133
- Fraser MR, McCormick MI (2014) Gender-specific benefits of eating eggs at resident reef fish spawning aggregation sites. Marine Ecology Progress Series 517:209-216
- Fry FEJ (1971) The Effect of Environmental Factors on the Physiology of Fish. Fish Physiology, Volume 6. Academic Press, pp 1-98
- Glazier DS (2005) Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662
- Goldshmid R, Holzman R, Weihs D, Genin A (2004) Aeration of corals by sleep-swimming fish. Limnology and Oceanography 49:1832-1839
- Harrison HB et al. (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. Current Biology 22:1023-1028
- Hart PBJ (1997) Foraging Tactics. In: Godin JGJ (ed) Behavioural Ecology of Teloest Fishes. Oxford University Press, New York, pp 104-133
- Hoare DJ, Krause J (2003) Social organisation, shoal structure and information transfer. Fish and Fisheries 4:269-279
- Huntingford FA et al. (2010) Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*. Journal of Fish Biology 76:1576-1591
- Jones KA, Croft DP, Ramnarine IW, Godin J-GJ (2010) Size-assortative shoaling in the guppy (*Poecilia reticulata*): The role of active choice. Ethology 116:147-154
- Killen SS (2014) Growth trajectory influences temperature preference in fish through an effect on metabolic rate. Journal of Animal Ecology 83:1513-1522
- Killen SS, Atkinson D, Glazier DS (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology Letters 13:184-193

- Killen SS, Marras S, Metcalfe NB, McKenzie DJ, Domenici P (2013) Environmental stressors alter relationships between physiology and behaviour. Trends in Ecology & Evolution 28:651-658
- Killen SS, Marras S, Steffensen JF, McKenzie DJ (2012) Aerobic capacity influences the spatial position of individuals within fish schools. Proceedings of the Royal Society B: Biological Sciences 279:357-364
- Killen SS et al. (2014) Aerobic scope predicts dominance during early life in a tropical damselfish. Functional Ecology 28:1367-1376
- Killen SS, McKenzie DJ (2011) Fuel, fasting, fear: Routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology 80:1024-1033
- Krause J, Godin J-G (1994) Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): Effects of predation risk, fish size, species composition and size of shoals. Ethology 98:128-136
- Krause J et al. (2000) Fish shoal composition: mechanisms and constraints. Proceedings of the Royal Society of London. Series B: Biological Sciences 267:2011-2017

Krause J, Ruxton GD (2002) Living in groups. Oxford University Press, New York.

Laskowski KL, Bell AM (2014) Strong personalities, not social niches, drive individual differences in social behaviours in sticklebacks. Animal Behaviour 90:287-295

Laskowski KL, Pruitt JN (2014) Evidence of social niche construction: persistent and repeated social interactions generate stronger personalities in a social spider. Proceedings of the Royal Society of London B: Biological Sciences 281

Leis JM (2015) Is dispersal of larval reef fishes passive? In: Camilo Mora (ed) Ecology of Fishes on Coral Reefs. Cambridge University Press, Cambridge, pp 223-226

McCormick MI (1999) Delayed metamorphosis of a tropical reef fish (*Acanthurus triostegus*): a field experiment. Marine Ecology Progress Series 176:25-38

Metcalfe NB, Thomson BC (1995) Fish recognize and prefer to shoal with poor competitors. Proceedings of the Royal Society of London B: Biological Sciences 259:207-210

- Morrell LJ, Hunt KL, Croft DP, Krause J (2007) Diet, familiarity and shoaling decisions in guppies. Animal Behaviour 74:311-319
- Munday P, Wilson S (1997) Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. Journal of Fish Biology 51:931-938
- Nadler LE, McNeill DC, Alwany MA, Bailey DM (2014) Effect of habitat characteristics on the distribution and abundance of damselfish within a Red Sea reef. Environmental Biology of Fishes 97:1265-1277

Pörtner HO, Farrell AP (2008) Physiology and Climate Change. Science 322:690-692

- Priede IG (1985) Metabolic scope in fishes. In: Calow PTP (ed) Fish Energetics: New Perspectives. Croom-Helm, London
- Pruitt JN, Keiser CN (2014) The personality types of key catalytic individuals shape colonies' collective behaviour and success. Animal Behaviour 93:87-95
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biological Reviews 82:291-318
- Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378
- Steffensen JF (1989) Some errors in respirometry of aquatic breathers: How to avoid and correct for them. Fish Physiology and Biochemistry 6:49-59
- Webster MM, Ward AJW (2011) Personality and social context. Biological Reviews 86:759-773