

REPRODUCTIVE TIMING AND INVESTMENT DECISIONS OF A PROTOGYNOUS,  
HERMAPHRODITIC CORAL REEF FISH SPECIES

Pauline Mitterwallner

A thesis submitted to the Victoria University of Wellington in fulfilment of the requirements  
for the degree of Doctor of Philosophy

2020





## Abstract

Life-history theory suggests that an organism must balance its available energy between two competing physiological processes to maximize fitness: reproduction and somatic growth. Energetic trade-offs are a fundamental concept of life history theory and form the basis of intra- and inter-specific variation in life-history strategies. In fishes, reproduction-growth trade-offs are an essential component of life-history optimization. This is particularly true for species with protogynous sex-change (the most common reproductive mode among coral reef fish species), where reproductive success rapidly and disproportionally increases with body size/ corresponding social status. In such systems, lifetime fitness is inherently linked to patterns of growth and energy allocation strategies determined by an individual's size-specific rank within the dominance hierarchy. However, energy allocation strategies in a protogynous species may not only be a function of body size. Coral reef fish species are exposed to extremely variable environmental conditions and this can favour the evolution of strategies that utilize good times and avoid disadvantageous times for reproduction. Consequently, size- specific parental investment decisions may vary greatly in time and space according to environmental cues. My thesis focuses on the protogynous reef fish, *Thalassoma hardwicke* (the sixbar wrasse), which is extremely abundant on shallow coral reefs throughout the Indo-Pacific region. Specifically, I evaluate patterns of spawning and reproductive investment as a function of body size, social status, lunar phase and other environmental parameters. I address the question of whether females/males of differing size make different fitness-related decisions when away from spawning sites, and I evaluate context-dependency in these decisions. Finally, I will attempt to reconstruct the developmental histories (e.g., larval growth rates) of individuals from otoliths to evaluate potential relationships between developmental histories and fitness attributes.

In chapter 2, I investigated the key drivers mediating temporal and spatial variation in spawning patterns of the highly iteroparous sixbar wrasse, *Thalassoma hardwicke*. Periodic regression analysis indicated that temporal patterns of spawning (and population densities at the spawning site) were most strongly associated with the lunar cycle, and maximal around the new moon. Other recorded abiotic variables showed low or non-significant correlations with spawning intensity. Territory position seemed to strongly affect spatial variation in spawning intensity, with highest spawning frequencies in territories closest to the reef edge. My findings suggest that generalized responses to an easily detectable large-scale cue like the lunar state may provide specific selective advantages for resident species. Nevertheless, my study also suggests that certain local conditions may elicit behavioural

responses over very short time scales, as they may convey sufficient information in terms of initial offspring survival.

In chapter 3, I explored patterns of variation in distinct spawning strategies (pair spawning, group spawning, and sneaking tactics) across the lunar cycle, and in relation to proximity to the reef edge. I also explored variation in the sizes of individuals that engage in these different strategies. Frequencies of pair spawning, group spawning, and sneaking attempts all significantly increased around the new moon and were greatest in the most exposed territories (i.e., those located closest to the reef edge). Smaller individuals of both sexes curtailed reproduction during the full moon and third quarter moon, while larger individuals reproduced more consistently across the lunar month. Size-dependent reproductive decisions appeared to mediate frequencies of specific reproductive tactics (e.g., prevalence of pair spawning, group spawning and sneaking strategies). These results suggest that optimal tactics are shaped by size- and sex-dependent trade-offs between reproduction and growth in relation to an environmental context. My study also shows that intraspecific investment decisions determine the prevailing reproductive mode at a spawning site. Variation in female choice might thereby act as a mechanism for maintaining these two reproductive modes (pair spawning and group spawning) in a population.

In chapter 4, I investigated how the lunar cycle affects energy allocation decisions during non-spawning hours. Specifically, I predicted that activity budgets and mobility (i.e. feeding rates, social interactions and fine-scale movement patterns) during non-spawning hours should vary across the lunar cycle. Additionally, I evaluated whether these energy allocation patterns depended upon phenotypic status/body size. I found that foraging and reproductive activities varied across the lunar month, in a manner consistent with my predictions. Feeding rates significantly increased during full moon and declined close to new moon. Social activities (e.g., chases and group swimming) significantly increased at the new moon and declined to consistently low levels around first quarter and full moon. Individuals also exhibited significantly larger activity spaces and longer travel distances around the new moon period. Consistent with the 'size-advantage model', my findings indicate that fish of different social status and size allocate their time to foraging and reproduction-related activities in different ways. Terminal phase (TP) individuals fed notably less than initial phase (IP) individuals and disproportionally allocated their time and energy towards aggressive interactions. TP individuals also exploited a larger area for feeding than IP individuals and tended to display fine-scale movement patterns consistent with directional swimming.

In chapter 5, I explored size- and environmentally related variability in migration patterns and habitat use of the sixbar wrasse *Thalassoma hardwicke*. Specifically, I evaluated variation in home ranges,

foraging site fidelity and/or spawning site fidelity, and migration distances as a function of the lunar cycle. I also tested the hypothesis that these relationships are dependent on size and phenotypic status of an individual. My results suggest that an individual's phenotypic status, its size and sex all seem to significantly influence home range sizes, migration patterns and spawning site fidelity. Home range sizes and migration distances generally increased with body size and were significantly larger for TP individuals than for IP individuals. Smaller IP individuals showed a non-significant trend for higher affinity to specific spawning territories, while larger IPs tended to be more flexible in their choice of spawning territories. Conversely, smaller TP individuals visited several spawning territories throughout the study period, while larger TP individuals exhibited higher affinity to a single territory site. Additionally, migration patterns and spawning site choice suggested that individuals of all size classes undergo longer and more costly migrations during new moon.

In chapter 6, I reconstructed larval growth histories from otoliths to evaluate if "reproductive winners" (females or initial phase males that successfully transitioned into terminal phase males) are a subset of specific growth conditions during their early life history stages. Furthermore, I evaluated the question of whether adults exhibit size- specific trade-offs between gonadal investment and somatic growth to facilitate sex-change. My findings suggest that sex-change was not influenced by larval growth histories. A steep decline of gonadal investment relative to female and IP size indicated that individuals may disproportionately invest into somatic growth prior to sex-change.

Collectively, these results highlight the diversity of life history strategies present within a protogynous, hermaphroditic coral reef fish, and suggest that optimal tactics change with ontogeny, and across the lunar month. My findings indicate that adult sixbars exhibit great flexibility in the way they maximize fitness, by making informed decisions based on their body condition/phenotypic status and in relation to specific environmental cues. Overall, this thesis provides a more complete understanding about the underlying mechanisms of life-history variation of a protogynous species, by comprehensively assessing interrelationships of social status/body size, environmental variables and parental investment decisions.



## Acknowledgements

First, I would like to express my special thanks and gratitude to my primary supervisor Jeff Shima who gave me the opportunity to do this PhD project. Thank you for your valuable guidance, your encouragement and continuous support. I also want to thank my secondary supervisor, Stephen Swearer for his helpful input and ideas while conducting my fieldwork in French-Polynesia.

Katie Hillyer, I can't thank you enough for your assistance and support during our stay in French-Polynesia. Without your invaluable help, I would not have been able to do large parts of my PhD project. I could not have wished for better company during our long and sometimes stressful field season in Moorea.

My dear Harry, thank you so so much for your amazing persistence while fighting against the currents and chasing fish around all day long. I know it wasn't always easy. Thank you for making me laugh, for your support and incredible friendship. Love you lots my friend.

Special thanks also go to Phoebe. Thank you for your assistance, and great patience whenever things didn't go after plan. And also, a big thank to Lisa Woods for your invaluable statistical support and guidance.

Also a big thank you to the committee, for your time and effort in reviewing my PhD. Thank you for your helpful advices and constructive feedback.

A massive thanks also to my family, to my parents Lissy and Reiner, to my sister Hannah and my aunt Christel. Thank you for always supporting me throughout my life and scientific career, for going through all the ups and downs with me and for always being there for me.

Also a big thank you to the Burt family. For welcoming me so warmly in your home during the last couple of months of my PhD. Jo and Dave, I can't express how grateful I am for your incredible support and kindness. And a special thanks to you Jo, for patiently proofreading my PhD and your helpful suggestions.

Stacey Burt, you are absolutely the best thing that could have ever happened to me. I never would have thought to find such an amazing person at the other end of the world. Without you, I would have not been able to go through this process. Thank you for your patience, for your constant emotional support, for being my light in darker times, for making the past three years so much easier.





## Table of Contents

<b>List of figures .....</b>	<b>13</b>
<b>List of tables .....</b>	<b>17</b>
<b>General Introduction.....</b>	<b>23</b>
1.1 The evolution of reproductive strategies in coral reef fish .....	24
1.2 Protogynous hermaphroditism .....	25
1.3 Reproductive strategies of protogynous reef fish species .....	26
1.3.1 Reproductive strategies based on size.....	27
1.3.2 Behavioural strategies .....	28
1.3.3 Physiological strategies .....	30
1.3.4 Sex-allocation strategies.....	31
1.4 The impact of external conditions on reproductive investment strategies.....	33
1.4.1 Population density .....	33
1.4.2 Predation .....	34
1.4.3 Lunar periodicity.....	35
1.5 Open questions .....	36
1.5.1 Context-dependency .....	36
1.5.2 Size-dependency.....	36
1.5.3 Activities and interactions away from spawning aggregations .....	36
1.5.4 Sex-specific growth histories .....	36
1.6 Study system.....	37
1.7 Goals and overall approach.....	38
<b>The relative influence of environmental cues on reproductive allocation of a highly iteroparous coral reef fish species.....</b>	<b>41</b>
2.1 Introduction .....	42
2.2 Methods.....	45
2.2.1 Study site and species.....	45
2.2.2 Surveys of spawning activity .....	45
2.2.3 Data analysis.....	47
2.3 Results.....	49
2.3.1 Sources of variation in overall spawning frequency .....	49
2.3.2 Effect of interactions between main effects .....	51
2.3.3 Population density .....	53

2.4 Discussion .....	54
2.4.1 Lunar periodicity.....	54
2.4.2 Local environmental cues .....	55
2.4.3 Conclusion .....	57
<b>Influence of the lunar cycle and spatial gradients on size-dependent male and female reproductive investment decisions of a protogynous reef fish .....</b>	<b>59</b>
3.1 Introduction .....	60
3.2 Methods.....	63
3.2.1 Field surveys .....	63
3.2.2 Data analysis.....	64
3.3 Results.....	65
3.3.1 Pair and group spawning in relation to environmental parameters .....	65
3.3.2 Pair versus group spawning .....	69
3.3.3 Sneaking in relation to environmental parameters .....	71
3.4 Discussion .....	73
3.4.1 Temporal variation in female reproductive strategies.....	73
3.4.2 Temporal variation in male reproductive strategies .....	74
3.4.3 Spatial variation in female reproductive strategies .....	76
3.4.4 Conclusion .....	77
<b>Plasticity in energy allocation strategies of a protogynous coral reef fish species: the influence of size and environmental variations on activity budgets and movement patterns.....</b>	<b>79</b>
4.1 Introduction .....	80
4.2 Methods .....	82
4.2.1 Field surveys .....	82
4.2.2 Analysis of movement patterns .....	84
4.2.3 Data analysis.....	84
4.3 Results.....	85
4.3.1 Activity patterns .....	85
4.3.2 Movement patterns.....	88
4.4 Discussion .....	92
4.4.1 Variation across the lunar month .....	92
4.4.2 Phenotypic variation .....	94

4.4.3 Diurnal variation .....	96
4.4.4 Conclusion .....	97
<b>Patterns of migration and habitat use of a protogynous coral reef fish species in relation to size, sex and the lunar cycle .....</b>	<b>99</b>
5.1 Introduction .....	100
5.2 Methods .....	102
5.2.1 Study site and species.....	102
5.2.2 Tagging method .....	103
5.2.3 Sampling strategy .....	103
5.2.4 Data analysis.....	104
5.3 Results.....	105
5.3.1 Home range area .....	108
5.3.2 Spawning site choice and fidelity.....	110
5.3.3 Migration distances .....	113
5.4 Discussion .....	115
5.4.1 Foraging site fidelity and home range areas .....	115
5.4.2 Spawning territory fidelity .....	116
5.4.3 Migration distances and spawning site choice .....	117
5.4.4 Limitations and caveats .....	118
5.4.5 Conclusion .....	119
<b>The influence of early life history traits on the probability of sex-change.....</b>	<b>121</b>
6.1 Introduction .....	122
6.2 Methods .....	124
6.2.1 Study species .....	124
6.2.2 Gonadal allocation and larval life history traits .....	124
6.2.3 Data analysis.....	125
6.3 Results.....	126
6.3.1 Size-frequency distribution.....	126
6.3.2 Gonadal investment .....	127
6.3.3 Early life-history traits.....	128
6.3.4 Larval growth trajectories.....	129
6.4 Discussion .....	131
6.4.1 Do differences in larval growth histories predict sex-change? .....	131

6.4.2 Other potential mechanisms influencing sex-change .....	132
6.4.3 Future directions .....	133
<b>General discussion .....</b>	<b>135</b>
7.1 Summary of key findings.....	136
7.2 The lunar phase as primary environmental driver of parental investment decisions .....	137
7.3 Plasticity of energy allocation patterns based on size and reproductive phenotype .....	138
7.4 Context-dependency in size-structured activities .....	139
7.5 Potential drivers of sex-change .....	140
7.6 Future directions.....	140
7.7 Conclusion.....	141
<b>References.....</b>	<b>143</b>
<b>Appendices .....</b>	<b>157</b>

## List of figures

**Fig 1.1** The size advantage hypothesis. Sex-change from female to male is the most profitable strategy, if fecundity of a male is increasing more rapidly with size in comparison to the fecundity of an equally sized female. In theory, sex-change should occur where female and male fecundity curves intersect (see Warner 1984a; Munday 2006a).

**Fig 1.2** Terminal phase **(A)** and initial phase **(B)** of the sixbar wrasse (*Thalassoma hardwicke*)

**Fig 2.1** The two surveyed spawning sites (S1) and (S2) within a lagoon at the north shore of Moorea, French-Polynesia **(A)**. Within each site, I monitored focal territories (T1, T2, T3) of terminal phase males. These territories were distributed along a gradient from exposed (i.e., T1 closest to the reef edge) to sheltered conditions (i.e., T3 furthest from the reef edge).

**Fig 2.2** Average number of spawns per day (averaged over three territories) over 5 lunar cycles from February to June. Black circles indicate new moon phases, white circles indicate full moon phases. Error bars represent  $\pm 1SE$ .

**Fig 2.3** Sources of variation in overall sixbar spawning frequency through time. **(A)** Smoothed curve ( $\pm 95\%$  confidence interval) of the lunar effect on predicted spawning frequency during the 29.5 lunar cycle **(B)** Variation in spawning among territories (T1 is closest to reef edge, T3 furthest from reef edge) **(C)** Variation in spawning with current direction. Panels B and C give marginal means back-transformed to the original scale ( $\pm SE$ ) estimated with other fixed effects held at their mean values.

**Fig 2.4** Linear regression between population density at the local spawning site and number of spawns per 20 min. P values and regression coefficient indicate a significant positive relationship **(A)** Smoothed curve ( $\pm 95\%$  confidence interval) of the lunar effect on predicted spawning frequency during the 29.5 lunar cycle **(B)**

**Fig 3.1** Main sources of variation in spawning frequency (left column) and size of spawning females (right column). **(A)** Smoothed curve ( $\pm 95\%$  confidence interval) of the lunar effect on predicted pair and group spawning frequency during the 29.5 lunar cycle. **(B)** Smoothed curve ( $\pm 95\%$  confidence interval) of the lunar effect on predicted size of pair spawning females and group spawning females during the 29.5 lunar cycle. Variation in spawning frequency **(C)** and female size **(D)** among territories (T1 is closest to reef edge, T3 furthest from reef edge) Panel C and D give marginal means back-transformed to the original scale ( $\pm SE$ ) estimated with other fixed effects held at their mean values.

**Fig 3.2** Smoothed curve ( $\pm 95\%$  confidence interval) of the lunar effect on relative spawning frequency (in %) of small (7-11 cm) and larger individuals (12-16cm) during the 29.5 lunar cycle.

**Fig 3.3** Quantile regressions (tau= 50th and 95th quantile) for responses of pair spawning frequencies per territory and day (A) and female sizes of pair spawners per territory and day (B) to correspondent group spawning quantities per territory and day. Only the 95th quantile is significant for both graphs ( $p < 0.01$ )

**Fig 3.4** Linear regression for responses of pair spawning and group spawning activities (number of spawning events per territory and day) to quantities of females and males (number of individuals per 50 m<sup>2</sup>) at the spawning site. Only group spawning events were significantly correlated to densities at the spawning site ( $p < 0.0001$ ).

**Fig 3.5** Main sources of variation in frequency and size related to sneaking attempts (~ number of chases). Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted chasing frequency (A) and size of sneaker males (B) during the 29.5 lunar cycle.

**Fig. 4.1** Study sites across the north shore of Moorea, French-Polynesia. Activity and movement patterns of IP/TP sixbars were surveyed within 4 inshore and 4 offshore areas (O1, O2, O3, O4, I1, I2, I3, I4) of the lagoon.

**Fig 4.2** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted feeding (A), chasing (B) and group swimming frequencies (C) during the 29.5 lunar cycle. For illustrative purposes, predicted chasing frequencies were split by size (small individuals: 7-13 cm; large individuals: 13-19 cm), to visualize a significant interaction effect between size and lunar phase on chasing activities.

**Fig 4.3** Variation in feeding rates, chasing rates and group swimming rates dependent across the day (initial phase or terminal phase). Given are marginal means, back-transformed to the original scale ( $\pm$ SE), estimated with other fixed effects held at their mean values.

**Fig 4.4** Interaction plot of the predicted effect of time of the day on feeding (A) and chasing rates (B), dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly during spawning hours (13-15h), size had a strong effect on variation in feeding and chasing rates (i.e., feeding rates decreased significantly with size, and chasing rates significantly increased with size during spawning hours)

**Fig 4.5** Variation in feeding rates, chasing rates and group swimming rates dependent on colour phase (initial phase or terminal phase). Given are marginal means, back-transformed to the original scale ( $\pm$ SE), estimated with other fixed effects held at their mean values.

**Fig 4.6** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted MCP area (95% probability contour) (A), and total travel distance (B) during the 29.5 lunar cycle. For illustrative

purposes, predicted cumulative step lengths were split by size (small individuals: 7-13 cm; large individuals: 13-19 cm), to visualize a significant interaction effect between size and lunar phase on tracking distance.

**Fig 4.7** Interaction plot with the predicted effect of colour phase (IP=initial phase; TP=terminal phase) on MCP area (A), cumulative step length (tracking distance) (B) and turning angle (C), dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly for IP individuals, size had a strong effect on variations in movement variables (with a significantly positive increase of MCP area and cumulative step length with size, and a significantly negative decrease of mean turning angle with size for IP individuals).

**Fig 4.8** Variation in (A) activity space (90% mcp) and (B) travel distances dependent on reproductive phenotype (initial phase or terminal phase). Given are marginal means, back-transformed to the original scale (+SE), estimated with other fixed effects held at their mean values.

**Fig. 5.1** Study lagoon at the north shore of Moorea, French-Polynesia. Individuals were tagged across multiple locations (triangles) within different reef areas S1, I1, I2, O1 and O2 (dotted circles). The number of tagged individuals (n) are indicated in brackets. Spawning activities were monitored within 6 territories along the outer reef borders of S1, and 3 territories along the outer reef edges of I1 and I2 respectively (red dots).

**Fig. 5.2** Size frequency distribution (where size = TL) of all tagged (A) and relocated individuals (B). Black bars are initial phase individuals (IPs) and white bars are terminal phase individuals (TPs). A Kolmogorov Smirnov test indicated that resighting probability was not biased towards particular sizes.

**Fig. 5.3 A)** Tagged individuals in their feeding areas. Every single sighting of an individual is represented by a dot. Colours represent the original area of capture (within reef section S1, I1 or I2). Monitored area to relocate tagged individuals is indicated by dashed lines. **B), C), D):** Example plot of resightings of single individuals, including estimated home range area (95% MCP), mean position within feeding areas (star symbol), visited territories (red circles) and migration distances to visited territories (black dashed lines). Triangles represent the original area of capture.

**Fig. 5.4** Frequency-distributions of distances (m) between the initial tagging area and the mean location of an individual within its home range area, stratified by phenotype (initial phase and terminal phase).

**Fig 5.5** Interaction plot with the predicted effect of phenotype (IP=initial phase; TP=terminal phase) on MCP area dependent on individual size (cm). Plots show expected values (black line), confidence

interval (95%) of the expected value, and partial residuals (grey dots). Particularly for TP individuals, size had a strong effect on variation in MCP area. Subsequent analysis revealed, that both phenotypes exhibit a significant increase of MCP area with size (whereas only weakly for IP individuals).

**Fig 5.6** Interaction plot with the predicted effect of phenotype (IP=initial phase; TP=terminal phase) on spawning site fidelity, dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Although none of the predictors in the final model were significant, linear regressions between size and site fidelity for TP and IP individuals indicated a significant positive association of site fidelity and TP size.

**Fig 5.7** Interaction plot with the predicted effect of phenotype (IP=initial phase; TP=terminal phase) on MCP area dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly for TP individuals, size had a strong effect on variation in migration distance. Subsequent analysis revealed, that both phenotype exhibit a significant increase of migration distance with size.

**Fig 5.8** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted migration distance during the 29.5 lunar cycle (Fig. 5A). Fig 5B: For illustrative purposes, predicted migration distances were split by size (small individuals: 11-13 cm; large individuals: 13-19 cm) to visualize a significant interaction effect of size and lunar phase on migration distance.

**Fig. 6.1** Size frequency distribution (where size = TL) of female, IP and TP sixbars (n= 338).

**Fig.6.2 A)** Variation of gonadal investment (GSI) dependent on developmental stage (females, IP males and TP males). **B)** Smoothed curves of relative gonad weight (GSI) dependent on sixbar total length (TL) and developmental stage (females, IP males, TP males) with generalized additive model fits (GAM).

**Fig 6.3** Variation in larval age **(A)**, otolith radius at settlement **(B)** and otolith growth during larval stages **(C)** dependent on reproductive phenotype (female, IP male, TP male). Given are marginal means, back-transformed to the original scale ( $\pm$ SE), estimated with other fixed effects held at their mean values. None of the associations were significant (see table 3 and 4).

**Fig 6.4** Growth curves ( $\pm$  95% confidence interval) between larval age and predicted increment width (95% probability contour) dependent on reproductive phenotype (female, IP male, TP male). Parameters were fitted using polynomial regression (degree 2)



## List of tables

**Table 2.1** Measured environmental variables that potentially affect spawning activities. Given are the methods of estimation for each variable, and a qualitative description of the resulting data.

**Table 2.2** Parameter estimates and test statistics for best models explaining total spawning frequency of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). All models were fit using GLMER (Poisson distribution). Asterisk symbols reflect significance levels.

**Fig 2.3** Sources of variation in overall sixbar spawning frequency through time. **(A)** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted spawning frequency during the 29.5 lunar cycle **(B)** Variation in spawning among territories (T1 is closed to reef edge, T3 furthest from reef edge) **(C)** Variation in spawning with current direction. Panels B and C give marginal means back-transformed to the original scale ( $\pm$ SE) estimated with other fixed effects held at their mean values.

**Table 2.3** Parameter estimates and test statistics for best models explaining total spawning frequency of *Thalassoma hardwicke* at spawning sites if interactions between main effects are included. Best models given below were those with the lowest AICc score (among all permutations of the full model). All models were fit using GLMER (Poisson distribution). Asterisk symbols reflect significance levels.

**Table 2.4** Parameter estimates and test statistics for best models explaining population densities (average number of individuals per m<sup>2</sup>) of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). All models were fit using GLMER (Poisson distribution). Asterisk symbols reflect significance levels.

**Table 3.1** Descriptions of recorded spawning behaviours (pair-spawning, streaking, sneaking and group spawning) at the spawning site

**Table 3.2** Parameter estimates and test statistics for best models explaining (1) pair spawning frequency and (2) group spawning frequency, as well as size variations in pair and group spawning individuals of *Thalassoma hardwicke* at spawning sites. Models were fit using GLMER (Frequency, poisson distribution) and GLM (Size, gaussian distribution).

**Table 3.3** Parameter estimates and test statistics for GLM a model (poisson) explaining variations in spawning frequencies of small and large individuals dependent on moon phase of *Thalassoma hardwicke* at spawning sites.

**Table 3.4** Parameter estimates and associated significance for central and upper regression quantile models (0.5 and 0.95 quantile), for changes in pair spawning frequency and sizes of pair spawners according to rates of group spawning at the spawning site

**Table 3.5** Parameter estimates and test statistics for best models explaining (1) frequency of sneaking attempts as well as size variations of sneaker males (2) of *Thalassoma hardwicke*. All models were fit using GLMER (Frequency, poisson distribution) and GLM (Size, gaussian distribution)

**Table 4.1** Parameter estimates and test statistics for best models explaining (1) feeding (2) chasing and (3) group swimming frequencies of *Thalassoma hardwicke* at feeding sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). Models were fit using GLMER (Poisson distribution. Asterisk symbols reflect significance levels.

**Table 4.2** Parameter estimates and test statistics for best models explaining (1) activity space (95% minimum convex polygon) (2) tracking distance (cumulative step length) and (3) mean turning angle of *Thalassoma hardwicke* at feeding sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). Models were fit using GLMER (Gaussian distribution). MCP area and tracking distance were log transformed. Asterisk symbols reflect significance levels.

**Table 5.1** Parameter estimates and test statistics for best models explaining home range size (95% Minimum convex polygon) of *Thalassoma hardwicke*. Best model given was identified based on the lowest AICc score (among all permutations of the full model). Models were fit using GLM (Gaussian distribution). Asterisk symbols reflect significance levels.

**Table 5.2** Tagged individuals (52 in total) that occurred at territory locations across different areas within the reef (S1, I1 and I2) during spawning hours over the study period. Recorded individuals at spawning territories are stratified by area of capture (i.e. S1, I1 or I2). Since most individuals stayed close to the area where they were tagged, each area of capture closely represents their preferred feeding habitat (see also Fig. 5.3). Bold numbers indicate, if individuals migrated more than 100 m to a respective territory, asterisk symbols indicate, if these longer migration distances were associated with the new moon/end of the third quarter (lunar day 27-6).

**Table 5.3** Parameter estimates and test statistics for best models explaining site fidelity (%) of *Thalassoma hardwicke*. Best model given was identified based on the lowest AICc score (among all permutations of the full model). Models were fit using GLM (Gaussian distribution). In this case, none of the predictors were significant.

**Table 5.4** Parameter estimates and test statistics for best models explaining migration distances (m) of *Thalassoma hardwicke* to spawning sites. Best model given was identified based on the lowest AICc

score (among all permutations of the full model). Models were fit using GLM (Gaussian distribution). Asterisk symbols reflect significance levels.

**Table 6.1** Parameter estimates and test statistics describing variation in gonadal investment (GSI) dependent on weight, reproductive phenotype (female, IP male and TP male), and the interaction between weight and reproductive phenotype. Models were fit using simple linear regression (LM) in R. Asterisk symbols reflect significance levels.

**Table 6.2** Approximate significance of the smooth terms (generalized additive model fit) describing variation of GSI dependent on size for females, IP males and TP males. As indicated by the estimated degrees of freedom (edf), gonadal investment of females and IP males varies in a non-linear fashion with size. Gonadal investment of TP males describes a linear relationship with size (as indicated by an edf of 1).

**Table 6.3** ANOVA table of linear models describing (1) larval age (2) otolith radius at settlement and (3) otolith growth rate during larval stages of *Thalassoma hardwicke* adults, in relation to reproductive phenotype. Models were fit using simple linear regression (LM) in R. Asterisk symbols reflect significance levels.)

**Table 6.4** Parameter estimates and test statistics for models describing (1) larval age before settlement (2) otolith radius at settlement and (3) otolith growth rate during larval stages of *Thalassoma hardwicke* adults, in relation to reproductive phenotype (females, IP males and TP males). Models were fit using simple linear regression (LM) in R. Asterisk symbols reflect significance levels.

**Table 6.5** ANOVA table of linear and polynomial models describing otolith increment width dependent on reproductive phenotype\*larval age of *Thalassoma hardwicke* adults (categories: females, IP males and TP males). Asterisk symbols reflect significance levels.

**Table 6.6** Parameter estimates and test statistics describing variation in increment width dependent on reproductive phenotype\*larval age of *Thalassoma hardwicke* adults (categories: females, IP males and TP males). Models were fit using simple linear and polynomial regression (LMER) with fish id as random effect. in R. Asterisk symbols reflect significance levels.

**Table A.2.1** Model selection table of the 10 best models explaining variation in total spawning frequency of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cloud cover night-time (CCN), cloud cover day-time (CCD),  $\cos\theta$ , current direction (CD), current speed (CS), spawning site (S),  $\sin\theta$ , temperature (T), territory (Terr), visibility (VIS) and date (random factor) as independent variables. All models were fit using GLMER (Poisson distribution).

**Table A.2.2** Model selection table of the 10 best models explaining variation in population density (average number of individuals per m<sup>2</sup>) of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cloud cover night-time (CCN), cloud cover day-time (CCD), cos $\theta$ , current direction (CD), current speed (CS), spawning site (S), sin $\theta$ , temperature (T), visibility (VIS) and date as independent variables. All models were fit using GLMER (Poisson distribution).

**Table A.4.1** Model selection table of the 10 best models explaining variation in feeding frequency of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors and date and survey site as random factors. All models were fit using GLMER (poisson distribution).

**Table A.4.2** Model selection table of the 10 best models explaining variation in chasing frequency of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors and date and survey site as random factors. All models were fit using GLMER (poisson distribution).

**Table A.4.3** Model selection table of the 10 best models explaining variation in group swimming frequency of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors and date and survey site as random factors. All models were fit using GLMER (poisson distribution).

**Table A.4.4** Model selection table of the 10 best models explaining variation in activity space (log-transformed) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

**Table A.4.5** Model selection table of the 10 best models explaining variation in cumulative step length (log-transformed) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained  $\cos\theta$ , colour phase (CP),  $\sin\theta$ , size (S.rs), time of the day (TD), and interactions between  $\cos\theta$  and size (S.rs), colour phase (CP) and size (S.rs),  $\sin\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

**Table A.4.6** Model selection table of the 10 best models explaining variation in turning angles (degree) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained  $\cos\theta$ , colour phase (CP),  $\sin\theta$ , size (S.rs), time of the day (TD), and interactions between  $\cos\theta$  and size (S.rs), colour phase (CP) and size (S.rs),  $\sin\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

**Table A.5.1** Model selection table of the best models explaining variation in home range area ( $\text{m}^2$ ) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained colour phase (CP), size (S.rs), and interactions between colour phase (CP) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

**Table A.5.2** Model selection table of the best models explaining variation in spawning site fidelity of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained  $\cos\theta$ , colour phase (CP),  $\sin\theta$ , size (S.rs), and interactions between colour phase (CP) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

**Table A.5.3** Model selection table of the best 10 models explaining variation in migration distance of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained  $\cos\theta$ , colour phase (CP),  $\sin\theta$ , size (S.rs), and interactions between  $\cos\theta$  and size (S.rs),  $\sin\theta$  and size (S.rs), colour phase (CP) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).



## CHAPTER ONE

### GENERAL INTRODUCTION

### **1.1 The evolution of reproductive strategies in coral reef fish**

Life-history theory predicts that an individual can maximize its fitness by adopting behavioural traits in relation to specific external environmental cues and by taking into account its internal state (Stearns, 1992; Houston & McNamara, 1999; Clark & Mangel, 2000). Reproductive strategies are a strong determinant of fitness and often show tremendous variation across individuals, species, populations and habitats.

Coral reef fishes, in particular, evolved an extraordinary diversity of reproductive modes and life-history strategies, both within and among species (Thresher, 1984; Choat & Robertson, 2002). Some fish species exhibit reproductive patterns characterised by complete monogamy, demersal eggs and bi-parental care, while others may be highly promiscuous, with pelagic eggs, and no parental cares (Thresher, 1984; Johannes, 1987; Claydon, 2004). Intraspecific variability is no less impressive; reproductive strategies can vary between populations of the same species separated by just a few hundred metres (Thresher, 1984; Warner, 1984 ab; Henson & Warner, 1997; Gust, 2004). The tremendous diversity of reproductive strategies among coral reef fishes may have evolved as an adaptive response to the distinctive environmental characteristics governing coral reef ecosystems.

Predation pressure on coral reefs is generally considered to be high, due to high abundances of benthic and pelagic predators (Johannes, 1987). Consequently, coral reef fish species have developed a wide range of strategies to minimize egg predation. For example, most coral reef fish species reduce offspring mortality by producing high quantities of offspring, that disperse and develop in the open ocean (where predation risk is generally assumed to be lower), before settling back to the reef (Johannes, 1978; Thresher, 1984; Sponaugle, 2015). This complex, bipartite life cycle contributes to extreme spatial and temporal variation in recruitment rates, often leading to widely distributed adult populations spanning a variety of physical conditions (Doherty & Williams, 1988; Warner, 1997a; Shima, 2001a). Over relatively small geographical scales, environmental variables like food availability, predation pressure and hydrodynamics can differ dramatically between populations (Johannes, 1978, Ebersole, 1980; Victor, 1991; Warner, 1997a; Sancho et al., 2000; DeMartini et al., 2005; Almany & Webster, 2006). In addition, the sporadic nature of recruitment can result in extreme variations in population size structure. Body size of individuals can vary at settlement (e.g., as a result of their larval experience; Shima & Findlay 2002; Sponaugle & Grorud-Colvert, 2006; Shima et al., 2018), and density dependent growth rates (Shima et al., 2001b; Shima et al., 2008; Geange & Stier, 2009) can contribute to further variation in body size. Relative size influences an individual's access to important resources, including food and mating opportunities (Forrester, 1991; Schultz & Warner, 1991; Warner, 1997a; Walker et al., 2007).



Presumably as an adaptive response to extreme demographic and environmental variation, many coral reef fish display plasticity in traits that may help to maximise fitness. The remarkable diversity of life history strategies observed within reef fish communities is generally considered to be a reflection of this phenotypic plasticity (Warner, 1984a; Warner, 1997a; Taborsky, 1998).

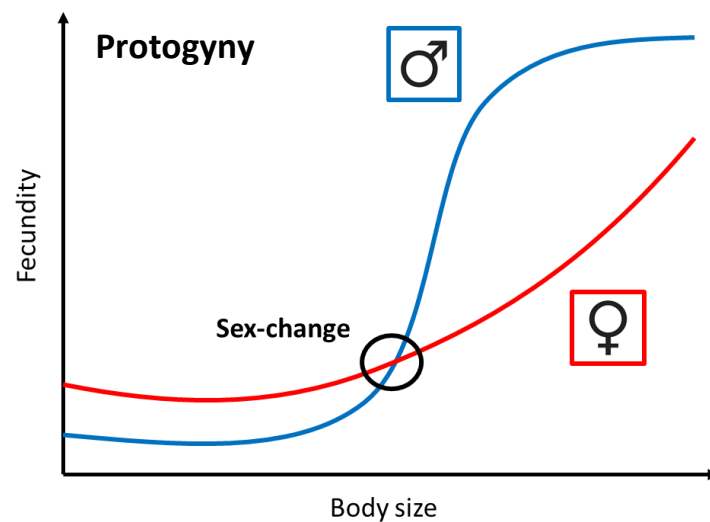
## **1.2 Protogynous hermaphroditism**

Some reef fish species add a further interesting element to the already very complex reproductive biology of reef fishes: the ability to change sex over their lifetime. Sexual flexibility, i.e. successional allocation of resources to male or female function dependent on an individual's life stage, is widespread among reef fish species (Warner 1984a; Munday et al., 2006 ab). While gonochromism (separate sexes over a lifetime) remains a common reproductive mode, some individuals can function as male or female at the same time (simultaneous hermaphroditism), whereas others can be one sex in their early stages and switch to another sex at some later stage (sequential hermaphroditism). Sequential hermaphroditism is particularly prevalent across diverse lineages of coral reef fishes. While protandry (sex-change from male to female) is relatively rare (sporadically occurring among six families), protogyny (female to male sex-change) is an extraordinarily common reproductive mode among coral reef fish families (occurring among 11 families; i.e. *Labridae*, *Serranidae*, *Pomacentridae*; see Choat & Robertson, 1975, Thresher, 1984; Warner, 1984a; Alonzo & Mangel, 2004; Munday et al., 2006a; Kobayashi et al., 2013)

The evolutionary significance of sex-change has been widely debated among scientists. Numerous hypotheses have been proposed, but to date, the 'size-advantage-model' remains the most parsimonious explanation for sequential hermaphroditism. This hypothesis proposes that sex-change might be favoured if fecundity changes between the sexes with size. In other words, sex change is adaptive when an individual reproduces more efficiently as one sex when young and small, and as the opposite sex when old or larger (Ghiselin, 1969; Warner, 1984a, Shapiro, 1987; Munoz & Warner, 2003, Munday et al., 2006a).

This model has been elaborated to incorporate specific habitat characteristics of reef ecosystems and helps to explain the disproportional occurrence of protogynous hermaphroditism within reef fish communities. Reef ecosystems can be spatially patchy and available resources such as food, shelter and spawning sites are rare and often unevenly distributed. Since most reef fish species live in close association with their benthic habitat (as it provides food, shelter, etc.), their mobility is relatively restricted - an ecological context that often results in monopolized mating as adaptive reproductive strategy (Warner, 1984a; Shapiro, 1987; Munday et al., 2006a). In this form of mating system, reproductive success is strongly dependent on size. Large, competitively superior males occupy and

defend spawning sites to procure easier access to female mates. Accordingly, larger males are disproportionately successful in producing a high number of offspring (since they have almost unlimited access to female mates), while smaller males (with lower size-specific competitive abilities) may have substantially lower reproductive success. In contrast, females of all size classes generally have unlimited access to mating opportunities, but their fertility is limited by the number of eggs they can produce since eggs are more costly to produce than sperm. Consequently, fecundity of an average male increases more rapidly with size than the fecundity of an equally sized female (see Fig 1.1). Thus, sex change from female to male, above a certain size threshold, is the most profitable sexual strategy in a hierarchical structured reef fish community (see “size-advantage hypothesis”, e.g. Warner, 1975, Warner, 1984a, Munoz & Warner, 2003, Munday et al., 2006a).



**Fig 1.1** The size advantage hypothesis. Sex-change from female to male is the most profitable strategy, if fecundity of a male is increasing more rapidly with size in comparison to the fecundity of an equally sized female. In theory, sex-change should occur where female and male fecundity curves intersect (see Warner 1984a; Munday 2006a).

### 1.3 Reproductive strategies of protogynous reef fish species

Protogynous reef fish species exhibit an extraordinary diversity of intra- and intersexual behavioural and physiological reproductive strategies to maximize their fitness (Johannes, 1978; Warner & Hoffman, 1980 ab; Warner 1984, ab; Cowen, 1990; Shapiro et al., 1991; Taborsky, 1998; Munday et al., 2006a). Habitat monopolization is common among protogynous reef fish, as exemplified by many wrasse and parrotfish species. If population density is relatively low, large mostly brightly coloured dominant males establish territories to gain privileged access to females. But as fertilization is carried out externally, spawning is not always exclusively restricted to single pairs (Warner, 1984b; Taborsky, 1998). Smaller, subordinate males without territories may adopt a range of adaptive strategies to

overcome their size-related competitive inabilities. Strategies vary from “sneaking”, where a small, subordinate male parasitizes single pair-spawning events, to group spawning, where large groups of small males quantitatively overwhelm territorial males and take over the spawning site (Warner and Hoffman 1980 ab; Colin and Clavijo, 1988; Warner, 1997b; Shapiro et al., 1997; Taborsky, 1998).

These general reproductive patterns in protogynous species are additionally modified by external parameters. Timing and intensity of spawning can vary greatly among and within protogynous species dependent on specific environmental conditions. Some individuals, for example, may choose to not spawn at all, or to intensify their spawning activities during certain times (Warner, 1984b; van Rooij et al., 1995; Claydon, 2004; Warner, 1998). Others switch between different tactics, like between pair and group spawning tactics, in relation to environmental fluctuations (Warner & Hoffman, 1980 ab; Suzuki et al., 2008).

A range of studies on reproductive patterns in protogynous reef fish have tried to assess and disentangle the specific processes promoting inter-individual differences in life history decisions and investment patterns. In the following paragraphs I outline the current scientific knowledge about reproductive strategies in protogynous reef fish, and the hypothesized underlying mechanisms that regulate reproductive investment decisions.

### **1.3.1 Reproductive strategies based on size**

Energetically, reproduction is one of the most demanding activities in the life of a reef fish (Roff, 1991; Mustonen et al., 2002; Mc Bride et al., 2012). In species that continue to grow after maturity (i.e. species with indeterminate growth), fecundity is a function of body size (Heino & Kaitala, 1995). Consequently, reproductive investment strategies are shaped by energetic trade-offs between growth and reproduction (Roff, 1983; Roff, 1991; Gunderson, 1997; Mc Bride et al., 2013). In protogynous species, reproductive success rapidly and disproportionally increases with body size (i.e., the size-advantage hypothesis; Warner, 1975; Muñoz & Warner, 2003; Munday et al., 2006a). Energy allocation strategies (growth vs. reproduction) are thus greatly dependent on size in a protogynous species (Schaffer, 1974; Hoffman et al., 1983; Schultz & Warner, 1991; Taborsky, 1998; Munday et al., 2006a; Brockmann & Taborsky, 2008). The size structure of adult reef fish populations can be highly variable due to a diversity of biological and physical processes affecting larval growth and recruitment:

The life cycle of a reef fish is typically very complex and consists of two main life stages - the pelagic stage in the open ocean and the benthic stage after larvae settle back to the reef. Throughout their development, newly hatched offspring are exposed to a series of pre-settlement (pelagic stage) and post-settlement (benthic stage) selection pressures. Pre-settlement survival and growth, for example,

is determined by variables like food supply and current dynamics (Searcy & Sponaugle, 2000; Shima & Swearer, 2019). Post-settlement survival and growth is mainly shaped by density-dependent processes and the intensity of predation (Shima, 2001 ab; Shima & Osenberg, 2003; McCormick & Hoey, 2004). Particularly settlement itself is extremely risky, and only a small fraction of arriving larvae cohorts survive this process (Doherty et al., 2004; Almany & Webster, 2006). However, survival potential substantially increases if settlement occurs during new moon phases, when peak tidal amplitudes enhance larval transport into the reef and darkness reduces predation pressure (Caley et al., 1996; Doherty et al., 2004, Forward & Tankersely, 2001; Sponaugle, 2015; Shima et al., 2018).

Many reef fish, including most protogynous reef fish species, exhibit extreme iteroparity as an evolutionary response to environmental uncertainty and spawn on a continuous, mostly daily basis, throughout the year (Wilbur & Rudolf, 2006, Warner, 1998, Claydon et al., 2014; Shima et al., 2018). Thus, parents generate offspring with a wide range of birthdates. The timing of birth and subsequent environmental selection pressures during settlement, as well as pre- and post-settlement development (e.g., food availability, settler density, habitat quality, lunar phase) can influence juvenile growth rates and survival potential (McCormick & Hoey, 2004; Sponaugle & Grorud-Colvert, 2006; Gagliano et al., 2007; Geange and Stier, 2009; Ford et al., 2016). For example, offspring spawned at certain times might experience more favourable conditions in terms of food availability, current regimes and predation pressure, and thus exhibit higher growth rates than offspring that were born at less advantageous times (Shima & Swearer, 2019). Consequently, the demographic structure of adult reef fish cohorts can be extremely heterogeneous, with substantial among-individual variation in demographic traits (Schultz & Warner, 1991; Brockmann & Taborsky, 2008; Noonberg et al., 2015; Shima et al., 2015; Shima & Swearer, 2019)

Consistent with life-history theory, that assumes size-specific trade-offs between reproduction and growth, the great size range of reproductive males and females in a protogynous reef fish population consequently sets the stage for diverse intraspecific reproductive strategies.

### **1.3.2 Behavioural strategies**

#### *During spawning hours*

As described above, an individual within a protogynous population must adopt the best strategy relative to its size to optimize its reproductive success. Therefore, individual reproductive behaviours can vary systematically with size, age, sex and maturity status (Warner, 1984 ab; Shapiro, 1991; Alonzo et al., 2000; Alonzo & Warner, 2000; Afonso et al., 2008).

In principle, variation in reproductive tactics can occur in both sexes. But since females are usually not in competition for access to mates, their behavioural flexibility is comparatively limited (Brockmann & Taborsky, 2008). Variation in female reproductive strategies is mostly expressed in decisions with whom, where and when to spawn (Alonzo & Warner, 2000; Warner, 1998). Thus, alternative reproductive tactics are predicted to evolve more frequently in the male sex. One of the most common reproductive strategies in protogynous reef fish species is behavioural monopolization, where dominant males prevent subordinate males from gaining access to females (Hoffmann, 1983; Warner & Hoffman, 1980ab; Warner, 1984a; Henson & Warner, 1997; Taborsky, 1998; Mumby & Wabnitz, 2002). This strategy can only be adopted by comparatively large males, who have sufficient energy resources to occupy and defend a spawning site. Smaller males with less competitive abilities/energy resources may engage in “sneaking” behaviour as an alternative strategy. Sneaker males mimic the coloration patterns of females and hover around territories in order to parasitize territorial pair spawning events (Taborsky, 1994; Henson & Warner, 1997; Taborsky, 1998; Alonzo et al., 2000). Consequently, average standard lengths of territorial males and sneaker males are significantly different in a protogynous mating system, which may indicate an ontogenetic switch between the two behaviour types (see Taborsky et al., 1987). Because fecundity disproportionally increases with body size in a protogynous population (see size-advantage hypothesis), smaller sneaker males and females may prioritize growth over reproduction to increase their probability to reproduce at larger size. Consequently, smaller males (and females) may not engage in reproductive activities at certain times, or even throughout an entire spawning season, to devote a greater amount of their time and energy to growth (Warner, 1984b; Taborsky et al., 1987; Taborsky, 1994; Carter et al., 2014). Various examples of size-dependent intra and inter-sexual alternative behaviours in fish species exist in scientific literature.

In the bluehead wrasse (*Thalassoma bifasciatum*), for example, large, brightly coloured males control mating sites along the outer reef edges. These spawning sites typically attract females, as they facilitate faster transport of fertilized eggs away from benthic predators (Warner, 1995; Claydon et al., 2014). Females that enter the mating sites are intensively courted and stimulated to ascend to the surface to release their gametes. Smaller, peripheral “initial” phase males (i.e. smaller males who don’t have the characteristic morphological and colouration patterns of territory owners) parasitize the reproductive effort of dominant males by contributing their sperm to pair spawning events whenever a chance arises (Warner et al., 1975; Warner and Robertson, 1978; Warner and Hoffman, 1980ab).

Another prominent example is provided by the ocellated wrasse (*Symphodus ocellatus*). This species exhibits four distinct male reproductive phenotypes (Fiedler, 1964; Warner and Lejeune, 1985;

Taborsky et al., 1987). The largest males build and defend nests and provide parental care. Small-sized males sneak into nests and try to parasitize spawns (Fiedler, 1964; Taborsky et al., 1987; Taborsky, 1994). Intermediate “satellite” males at the outer edges of nests prevent other smaller males from sneaking into territories, but also try to parasitize spawns themselves. In the fourth phenotype, larger males may be non-territorial (“pirate” males), attempting to temporarily occupy a nest by force (Taborsky et al., 1987), but leave the brood to the former owner to provide ongoing parental care.

#### *During non-spawning hours*

During non-spawning hours, coral reef fish typically forage within particular home ranges. Their daily activity patterns might also be shaped by size and sex-specific trade-offs between reproduction and growth (Hoffman, 1983; Noordwijk & de Jong, 1986; Sano, 1993; van Rooij, 1995; Warner, 1995; Welsh et al., 2013). Smaller individuals, for example, might devote a greater proportion of their time into growth/food intake rather than reproduction. Conversely, larger individuals might have sufficient energetic reserves to exploit a larger feeding habitat and/or to assign a greater amount of their daily activities to reproduction (Hoffman, 1983; Shibuno, 1993; Welsh et al., 2013).

Many reef fish participate in costly migrations to spawning grounds, sometimes comprising more than 10% of their daily activity (Warner, 1995; Claydon et al., 2012). This energetic conflict might drive an individual’s decision about where and when to spawn. Small-bodied females might try to reduce their energetic costs related to reproduction by migrating to the closest spawning site (Shibuno, 1993). At certain times (e.g., during suboptimal environmental conditions for reproduction) they even might decide to not migrate at all, and instead, invest their resources into faster growth rates (Warner, 1984b). Larger males or females, in contrast, might spawn more frequently, be more selective in their choice of spawning locations and travel longer distances to reach certain habitats that are more beneficial for their offspring (Shibuno et al., 1993; Claydon et al., 2012; Howard et al., 2013; Welsh et al., 2013).

In conclusion, intra-specific variation in activity and movement patterns outside spawning periods might be conditioned by an individual’s assessment of its physiological status in relation to certain environmental parameters to optimize its future fitness.

### **1.3.3 Physiological strategies**

Ontogenetic shifts in behavioural strategies are often accompanied by morphological and physiological changes. In protogynous species, sexual selection also leads to variation in phenotypic appearance. After sex-change, territorial males invest into secondary sexual signals that convey their high quality to females (Warner & Robertson, 1978; Brockmann & Taborsky, 2008). In the bluehead

wrasse, *Thalassoma bifasciatum*, for example, a transitional change into the terminal phase is accompanied by a phenotypic change in coloration. Smaller, “initial phase” males, alternatively, mimic colouration patterns of females to be able to sneak into territories of dominant males (Warner & Robertson, 1978; Warner & Swearer, 1991).

A range of studies on size-dependent spermatogenesis in fish have documented a strong negative relationship between gonadal size and behavioural effort in protogynous species. As parasitic males experience high levels of sperm competition, they often increase their fertilization efficiency by investing into larger gonads and higher quantities of sperm (Warner & Robertson, 1978; Stockley et al., 1997; Taborsky, 1998). Thus, their gonadal allocation patterns closely resemble those of females that have to divert a similarly high proportion of their available energy into egg production. In contrast, dominant males are predicted to allocate most of their available energetic resources into territory defence to maintain their territorial status. Consequently, territorial males have relatively small testes in comparison to gonads of females and initial phase males (Hoffman, 1983; Taborsky, 1998; Schaerer & Robertson, 1999). The gonadosomatic index (GSI), a measure of gonadal weight relative to somatic weight ( $100 \times \text{gonad weight} / \text{total body weight}$ ), is a good indicator of sperm competition intensity: sneaker males and females have significantly larger gonads relative to their size in comparison to dominant, territorial males (Robertson & Warner 1978; Taborsky, 1994; Stockley et al. 1997; Taborsky, 1998; Molloy et al., 2007).

#### **1.3.4 Sex-allocation strategies**

Sex-allocation theory predicts that individuals should change sex when it increases their reproductive value (Munday et al., 2006a). Theoretically, sex-changing species are predicted to function as one sex when young and small, and to change sex when old or larger. Sex change should always occur at a single, optimal size, where male and female fertility curves intersect (Warner 1984a; Munday et al., 2006a). However, evidence has accumulated that the size-dependent timing of sex-change often deviates from theoretical expectations. Some individuals within a protogynous population might change sex early, some individuals late, and some individuals might opt to not change sex at all (Munoz & Warner, 2002; Munday et al, 2006a).

Relative size of an individual (Warner & Swearer, 1991; Buston, 2003), sex ratio (Shapiro, 1984; Warner & Swearer, 1991) and population density (Wright, 1989; Lutensky, 1994) have all been shown to strongly influence the timing of sex-change. In protogynous species, the largest female usually changes sex following the disappearance of a dominant male (Warner 1988a; Ross, 1990; Warner & Swearer, 1991). This is because her reproductive success substantially increases if she occupies a vacant territory and starts to reproduce with the remaining females in a group. Under certain circumstances,

however, the largest female may refrain from sex change, even if a dominant male position becomes vacant. If the largest female is significantly larger than other remaining females in the group, the aggregated fecundity of the other females within the harem might not exceed fecundity of the single largest female. Consequently, the largest female should remain female, as sex change would decrease her expected fitness; in such cases a smaller female within the group should change sex (Munoz & Warner, 2003; Munday, et al., 2006a).

If the group is composed of many small sized males, intense sperm competition might substantially lower expected reproductive success of a recently transitioned male individual. In this case sex change from female to male might also not be beneficial for larger females (Munoz & Warner, 2003).

In contrast, early sex-change might be favourable if territory availability is high, as there is a good chance that an individual male may successfully secure a territory. Under these circumstances, a small-sized female might change sex at an earlier life stage, as she has realistic chances to occupy a territory (Aldenhoven, 1986; Rogers, 2003). Early sex change may also be beneficial if local conditions facilitate higher growth rates after changing into the other sex. In this case small individuals trade growth against current reproduction to improve their chances to achieve the status of a territorial male (van Rooij, 1995). High future reproductive expectations are accordingly sufficient to compensate for a current decrease in reproductive success (van Rooij, 1995; Warner, 1998).

The relative amount of initial phase (sneaker) males within a sex-changing population might also be a result of environmental sex determination. In smaller populations with a higher proportion of territorial males, reproductive success of sneaker males might be limited by the presence of these dominant males. Hence, initial phase males are often quantitatively underrepresented, or even absent, in smaller populations. However, in larger populations, a relatively high proportion of juveniles may mature into initial phase males, because there may be a realistic chance of a higher reproductive success. This may be particularly common, if initial phase males exhibit reproductive tactics that majorly differ from those of territorial males, such as group spawning tactics (see Warner & Hoffman 1980ab; Taborsky, 1998; Munday et al, 2006ab).

In summary, a diversity of extrinsic cues is responsible for variations in the timing of sex change. Greatest variation should occur in high-density populations, where only a loosely organised harem system prevails. In such populations, a great overlap in size distribution between sexes should occur, with a relatively high proportion of larger-sized females, and a relatively high proportion of smaller-sized, initial phase males (Munday et al., 2006ab).



#### **1.4 The impact of external conditions on reproductive investment strategies**

Along with intrinsic parameters like individual condition, reproductive investment of a protogynous reef fish population may be extrinsically modified by environmental variations. Variables like population density, predation, hydrodynamics and lunar phase can lead to idiosyncratic variation in behavioural patterns, as well as overall spawning mode and intensity; in extreme cases, leading to large multi-male and -female group spawning assemblages.

##### **1.4.1 Population density**

The degree of habitat monopolization is typically dependent on the surrounding density of smaller, parasitizing males. If densities of sneaker males on a local spawning site exceed a certain threshold, large terminal phase males might be less able to defend a territory. The denser the distribution of smaller males on a local site, the more territorial males are subject to interference of sneaker males (Warner & Robertson, 1978; Warner & Hoffman 1980 ab). In extreme cases, territories may be entirely abandoned because the spawning site is swamped by dense conglomerates of sneaker males (Warner & Robertson 1978; Warner, 1982). Many protogynous fish species exhibit mixed strategies i.e., individuals may switch between pair and group spawning dependent on the local population size (Colin & Clavijo, 1988; Warner and Hoffman, 1980ab).

The stable co-existence of pair vs. group spawning arises from sexual conflict (Warner et al., 1995; Alonzo & Warner, 2000; Brockmann & Taborsky et al., 2008; Molloy et al., 2012). As female reproductive success increases with the number of males, females are predicted to prefer group spawning to maximise their fitness. For territorial males, reproductive success only maximises if they spawn in pairs. Group spawning would only yield limited reproductive success, due to the relatively small amount of sperm territorial males typically have available. If territorial males succeed in excluding sneaker males from the spawning site, the lack of smaller males will stimulate females to pair-spawn. However, if densities of non-territorial males at a spawning site increase, females may prefer to spawn in multi-male group spawning assemblages (Warner et al., 1995; Alonzo & Warner, 2000; Molloy et al., 2012).

The most intensely studied protogynous fish species that forms large spawning aggregations is the bluehead wrasse *Thalassoma bifasciatum*. In this species, spawning mode ranges between pair spawning and group spawning strategies within only a few kilometres. Reefs with low population densities are mostly controlled by large, territorial males. On reefs with high population densities however, mating is entirely taken over by small, initial phase males that form large group spawning

assemblages. On these sites, group spawning takes place year-round and often encompasses more than a thousand individuals (Warner & Hoffman 1980ab; Warner 1984ab; Warner et al., 1995).

#### **1.4.2 Predation**

Due to intense predation pressure in shallow, tropical marine habitats, estimated mortality rates of smaller reef fish species are substantially higher than those of more temperate species. Accordingly, reproductive tactics and investment decisions are often influenced by intense predation pressure operating on adults and their offspring in tropical marine environments (Johannes, 1987; Hixon & Beets, 1993).

In most mating systems, offspring survival potential is a critical element determining mating decisions. Any factor that increases current reproductive success, i.e. offspring survival until recruitment, appears to drive parental decisions about where, when and how often to spawn. Commonly, specific hydrodynamic regimes and/or habitat characteristics that facilitate rapid transport of the fertilized eggs off the reef and away from egg predators (Johannes, 1978; Lobel, 1978; Claydon et al., 2014), appear to drive reproductive decisions of coral reef fish species. Illustratively, spawning territories of protogynous reef fish species form prevalently at exposed sites along the outer edges of a reef (Colin, 1992; Sancho et al., 2000; Whaylen et al. 2006; Claydon et al., 2012; Claydon et al., 2014). Additionally, protogynous species often synchronize their spawning activities with outgoing tidal-driven currents during a particular window in daily and monthly cycles (Sancho et al., 2000; Claydon et al., 2014). Moreover, many protogynous species exhibit extreme iteroparity as an evolutionary response to high offspring mortality and reproduce continuously throughout the year. This “bet-hedging” strategy maximises their fitness, because the chances that at least some offspring survive until maturity may be increased (Goodman, 1984; Einum & Fleming, 2004; Wilbur & Rudolf, 2006; Shima et al., 2018).

Particularly in highly iteroparous species, reproductive decisions are additionally dependent on another important life-history component: parental survival (Warner, 1998). For species that spawn continuously over several reproductive seasons, a single mating event contributes only marginally to the lifetime reproductive success of an adult individual. So, highly iteroparous individuals should prioritize their own survival over slight increases in current reproductive output to be able to reproduce in subsequent periods. Since predation risk varies inversely with size, smaller males and females should be relatively risk averse and invest a higher proportion of their time and energy into growth instead of reproduction (Schaffer, 1974; Hoffman, 1983; Warner, 1984b; Stearns, 1992; Warner, 1998; Alonzo & Warner, 2000). For example, smaller females with higher size-related mortality risk may opt to skip reproduction at times and only reproduce if conditions benefit their own survival (Warner, 1998; Alonzo & Warner, 2000). Additionally, smaller females may trade offspring

against maternal survival potential and choose to reproduce in places and times that are less risky (Warner, 1998).

Spawning site choice might therefore be driven by female safety assurances, rather than by increased direct or indirect fitness benefits for the fertilized eggs. This may lead to predictable, size-structured female (and male) distributions in time and space as a function of predation pressure. Male courtship behaviour and colouration might in this context convey the current safety of a mating site rather than information about the male itself (Warner, 1998; Alonzo & Warner, 2000). And group spawning might, also in this context, offer another fitness benefit for females: seeking safety in numbers due to diluted predation rates on spawning adults (see Robertson, 1983; Warner, 1998; Claydon et al., 2004; Molloy et al., 2012).

### **1.4.3 Lunar periodicity**

The majority of coral reef fish species are known to have lunar spawning rhythms; studies have demonstrated that lunar cycles often mediate variation in reproductive investment and behavioural strategies (Robertson et al., 1990; Samoilys, 1997; Zeller, 1998; Takemura et al., 2004; Meyer et al., 2007; Claydon et al. 2014). Though scientists are still uncertain about the selective advantages underlying lunar periodicity, a variety of drivers have been hypothesized. Spawning relative to certain moon phases might, for example, simply represent a mechanism for synchronizing reproduction (Lobel, 1978, Colin and Clavijo, 1988; Colin and Bell, 1991; Claydon et al., 2014). Furthermore, as tidal amplitudes and tidal currents are often strongest during new moon and full moon, increased current velocities might facilitate enhanced dispersal of fertilized eggs (Domeier & Colin, 1997; Claydon et al., 2014).

If the developmental duration of pelagic larvae is invariant, the precise timing of spawning might determine the timing of settlement. Since most settlement pulses concentrate around the new moon (Sponaugle & Cowen, 1994; Robertson et al. 1999; Shima et al., 2018), reef fish might take the lunar state as an environmental cue to couple settlement with production (Robertson et al., 1988; Colin 2012). As an example, for the coral trout *Plectropomus leopardus* spawning mostly occurs around the new moon (Samoilys, 1997). Based on otolith increments (Doherty et al., 1994), larvae develop about 25 days in the pelagic phase, before settling back to the reef near the new moon, potentially indicating a strong coherence between the timing of reproduction and the optimal timing of settlement.

In summary, mating tactics and investment decisions are additionally conditioned by an amalgam of external parameters which, on the one hand, guarantee higher fertilization rates and higher offspring fitness and, on the other hand, also facilitate a higher survival potential of the individual itself.

## **1.5 Open questions**

Although a substantial body of literature about reproductive tactics in protogynous reef fish already exists, there are still gaps in our knowledge about the underlying drivers shaping reproductive decisions of a protogynous reef fish species.

### **1.5.1 Context-dependency**

How do individuals respond to specific environmental cues like time, predation pressure, lunar phase and population density? For example, do adults spawn continuously or more periodically according to lunar phases? Do adults intensify their reproductive effort according to other environmental parameters like current speed and direction that could enhance egg transport? What factors might determine the presence of alternative reproductive behaviours/reproductive phenotypes? Does population density influence spawning mode and effort? If population density of smaller initial phase males is high, do females prefer to spawn in groups? And how do territorial males cope? Are female distributions predictable in time and space as a function of predation pressure?

### **1.5.2 Size-dependency**

Do adults invest differently into reproduction as a function of size (as a trade-off between reproduction and growth)? How does size influence male/female reproductive phenotypes? Do larger, dominant individuals, for example, monopolize the best times and places to spawn? How do smaller subordinate individuals cope? Do they adopt alternative reproductive strategies by spawning at different times or places, or perhaps engaging in sneaking strategies? And how does size influence sex allocation strategies and investment into sperm production/gonadal size?

### **1.5.3 Activities and interactions away from spawning aggregations**

Do individuals allocate energetic resources differently as a function of size and sex when away from spawning sites? Smaller individuals, for example, might devote a greater proportion of time to feeding (as opposed to social interactions linked to positions in dominance hierarchies) in order to maximise growth. Larger individuals, on the other hand, might have sufficient energetic reserves to exploit larger areas for feeding (or they may exploit better foraging locations); this may enable larger individuals to allocate a greater proportion of their time and/or energetic budgets to reproduction (Claydon et al. 2012).

### **1.5.4 Sex-specific growth histories**

Are reproductive winners (here, defined by relative position in a social dominance hierarchy) a subset of faster growth rates during earlier life history stages? Or do individuals exhibit ontogenetic shifts in

their energy allocation patterns later in life, that facilitate accelerated growth shortly before sex-change?

For my PhD research, I used the sixbar wrasse (*Thalassoma hardwicke*) as a model system to address the questions posed above. Sixbars are a useful model because they are very abundant, reproduce daily and year-round in predictable locations, and have excellent otoliths and life-history traits that make them conducive to experimental investigation. I conducted field work from the University of California (Berkeley) Gump Research Station in Mo'orea, French Polynesia. The northern lagoon on this island provides tractable environmental conditions that enabled me to conduct the necessary field experiments and observational studies to answer my PhD research questions.

## **1.6 Study system**

The sixbar wrasse *Thalassoma hardwicke* is the most common protogynous labrid on Mo'orea, and is widely distributed along the shallow inshore habitats (Galzin, 1987). Adults are diurnally active and forage over particular home ranges (Shima, 1999a), targeting a variety of prey, including fish eggs, (Shima & Osenberg, 2003), benthic and planktonic crustaceans, small fishes and foraminiferans (Sano et al., 1984).

Reproduction appears to occur on a daily schedule, over much of the year, and mostly as part of multi-species spawning aggregations that form along the outer reef edges (densities of fish in these aggregations may range from five to 1000 individuals; Claydon et al., 2014). Bodies of terminal phase (TP) males are characterized by six vertical black bars which are progressively shorter posteriorly, and broad pink bands radiating from the eye (Sadovy & Cornish, 2000, Kuitert, 2006). TPs and 'initial phase' (IP) fish (females and 'sneaker' males that are morphologically indistinguishable from females) differ slightly in colour; generally speaking TP males are gaudier than IPs. The initial phase is light green in colour with six wedge-shaped, slightly diagonal, dark bars dorsally on the body (Sadovy & Cornish, 2000). Developing larvae spend 40-60 days in a pelagic larval stage before settling back to the reef (Victor, 1986).

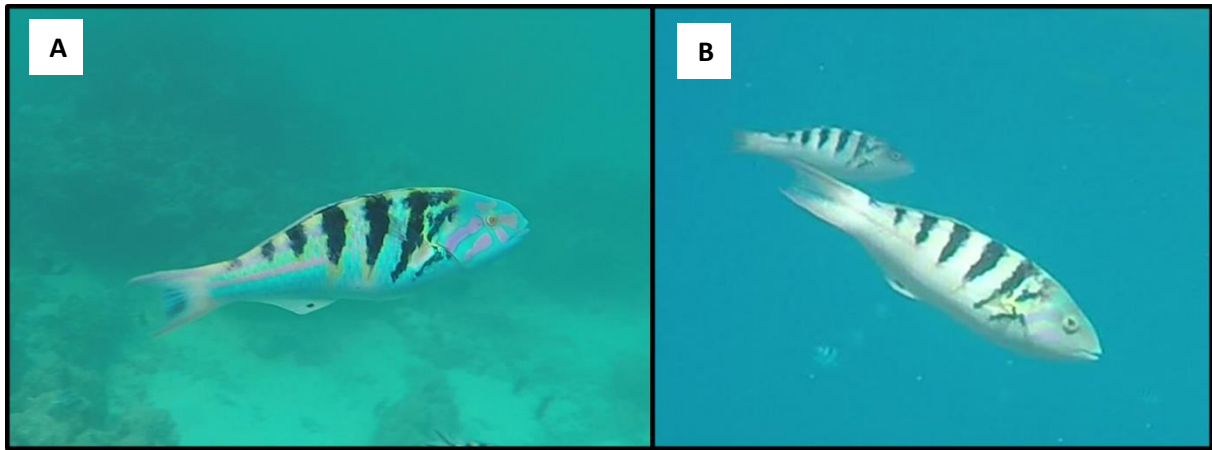


Fig 1.2 Terminal phase **(A)** and initial phase **(B)** of the sixbar wrasse (*Thalassoma hardwicke*)

My fieldwork will be conducted within the northern lagoon of Mo'orea (Fig.1; 17°30'S, 149°50'W). Mo'orea is an island of volcanic origin that is surrounded by a barrier reef, located one kilometre offshore, that encloses shallow lagoon habitats (1-3m deep) used by sixbars. Offshore of the barrier reef is a deeper outer reef slope that is not used by sixbars. The lagoon habitats are characterized by wide, flat areas of sand and rubble, disrupted by patches of coral colonies, predominantly composed of *Porites* spp., *Pocillopora* spp., *Montipora* spp. and *Acropora* spp., along with turf and extensive stands of macroalgae (*Sargassum*, *Turbinaria*, *Chnoospora*, *Dictyota*, *Padina*). Closer to the reef crest, coral cover and structural complexity increases. This is accompanied by a decrease in macroalgal cover and patch reefs are more densely distributed relative to the near shore regions of the lagoon.

### 1.7 Goals and overall approach

I investigated patterns of spawning and reproductive investment of the protogynous coral reef fish species *Thalassoma hardwicke* (the sixbar wrasse), as a function of size, lunar phase and other environmental parameters. I addressed the question of whether females/males of differing size make different fitness-related decisions when away from spawning sites (e.g., habitat use, foraging behaviour, social interactions, decision to migrate to a spawning site). In addition, I evaluated context-dependent variation in these decisions (i.e., do fitness-related decisions depend upon distance between foraging and spawning grounds). Finally, I reconstructed the developmental histories (e.g., larval growth rates, age at settlement) of individuals from otoliths to evaluate potential relationships between developmental histories and fitness attributes.

Answers to these questions will improve our knowledge about the mechanisms shaping the great diversity of life history strategies in a protogynous hermaphroditic reef fish species. By relating

parental investment decisions to phenotypic traits and environmental cues, a wider understanding of the proximate drivers of phenotypic variation may be attained. In a more theoretical context, my work, as it relates to potential ecological and evolutionary feedbacks, may shed light on the origin and maintenance of life-history strategies that help shape the diversity of life on our planet. From a more applied perspective, my work will provide basic information on the reproductive biology and population dynamics of protogynous reef fish. Given that such species are particularly vulnerable to human exploitation and other anthropogenic stressors (Jennings et al., 1999b, Armsworth, 2001, Alonzo & Mangel, 2004), my work may be able to inform management efforts.





## CHAPTER TWO

THE RELATIVE INFLUENCE OF ENVIRONMENTAL CUES ON REPRODUCTIVE  
ALLOCATION OF A HIGHLY ITEROPAROUS CORAL REEF FISH SPECIES

## 2.1 Introduction

Reproductive timing and investment employed by individuals of both sexes can be a strong determinant of fitness, and some reproductive strategies may outperform others (Lott, 1991, Shapiro, 1991). Reproductive success may be enhanced when individuals derive fitness benefits from specific environmental cues that indicate favourable conditions for reproduction (Gross, 1996; Warner, 1997a; Brockman & Taborsky, 2008). In marine systems, reproducing individuals commonly experience an extremely heterogeneous and rapidly changing physical environment. Such species often respond to specific environmental cues that enable them to optimize their reproductive success. For example, many temperate fish species initiate reproduction in response to changes in water temperature to improve food availability for their offspring (i.e. the ‘Match-Mismatch hypothesis’ of Cushing, 1975). However, in tropical marine environments with less seasonal variation, many coral reef fish species spawn continuously throughout the year (“extreme iteroparity”, Philippi & Seger, 1989; Warner, 1998; Wilbur & Rudolf, 2006; Shima et al., 2018). Some researchers have equated the existence of extreme iteroparity in tropical reef fish species as an evolutionary response to an extremely unpredictable marine environment for offspring survival (e.g. “bet hedging strategy”, see Goodman, 1984; Einum & Fleming, 2004; Hughes, 2017). Others have hypothesized, that adults may try to reduce the effect of density-dependent selection pressures on post-settlement offspring mortality, by spreading their reproductive effort through time (Shima et al., 2018).

In theory, extreme iteroparity, as a bet hedging strategy, implies that species that spawn on a fairly continuous (e.g., daily) basis, do not utilize external cues for reproductive investment decisions (Seger & Brockman, 1987). Indeed, reproductive investment of iteroparous, pelagic spawners appears to be relatively invariant in time and space (Warner, 1995; Sancho et al., 2000; Claydon et al. 2014). Spawning aggregations of many highly iteroparous species form on a daily basis, and often during a particular time of day (Robertson, 1983; Domeier & Colin, 1997; Sancho et al., 2000; Claydon et al. 2014). Spawning activities may also occur in particular locations and are often associated with specific geomorphological features such as the down-current edges of reef slopes (Randall and Randall, 1963; Colin & Bell, 1991; Warner, 1995; Heyman & Kjerfve, 2008; Claydon et al., 2014). Many protogynous reef fishes have harem structures and may spawn in pairs and/or smaller groups (Warner & Hoffman, 1980ab; Robertson, 1983; Warner, 1984a; Kuwamura et al. 2009). Although reproduction is spread along a temporal continuum, spawning intensity may still vary across space and time (Johannes, 1978; Robertson, 1983; Petersen et al., 1992; Gust, 2004; Claydon et al., 2014). The number of individuals at a spawning site can vary from a single spawning pair to thousand individuals over the course of a few

days (Warner and Hoffman 1980ab; Warner, 1995; Domeier & Colin, 1997; Claydon et al., 2014). Spawning frequencies can also vary substantially between sites that are separated by only a few meters (Warner & Hoffman 1980ab; Warner 1984a; Petersen et al., 1992). Consequently, even extremely iteroparous species may use environmental cues to make informed decisions about reproductive allocation in order to maximise their fitness.

A variety of hypothesis have been proposed to explain spatio-temporal variation in reproductive allocation of highly iteroparous reef fish. For example, fluctuations in spawning might be a plastic response to specific oceanographic conditions that promote survival of eggs and larvae until the recruitment stage (Johannes, 1978; Lobel, 1978; Robertson et al., 1990; Claydon, 2004). Such species may choose to spawn at times or places where local currents facilitate fertilization success, or rapidly transport fertilized eggs away from reef-associated benthic and pelagic predators (Johannes, 1978; Lobel, 1978; Sancho et al., 2000). In apparent support of this hypothesis, studies have documented a positive correlation between spawning intensity and tidal-driven, outflowing currents or increased water flux, that may facilitate a rapid dispersal of propagules into oceanic waters (Sancho et al., 2000, Claydon et al., 2014; Donahue et al., 2015).

Additionally, reproductive decisions may be influenced by risks associated to reproductive behaviour (Warner, 1998). Highly iteroparous individuals, in particular, should prioritize their own survival over slight increases in current reproductive output, because these benefits may be greatly outweighed by the fitness gains from future reproduction. Consequently, highly iteroparous species may be more risk averse and should intensify their spawning activities when conditions enhance their own survival, and conversely, avoid spawning in times or places of high predation risk. Environmental conditions that increase predation risk, like low light levels, reduced visibility, or extremely exposed spawning territories, should be avoided (Robertson & Hoffman, 1977; Shapiro et al., 1988; Warner et al., 1998; Claydon et al., 2014).

The consequences of local conditions on fitness may be difficult to predict as they are too variable in time and space. Consequently, spawning periodicity in iteroparous species may reflect a generalized adaptive response to an easily detectable, larger-scale environmental cue (Warner, 1997a). Environmental cues that prevail over a broad geographic range may not necessarily always maximise fitness; however, they may guarantee the highest *average* fitness for a population over a reproductive season. Many studies suggest that periodic spawning activities, as seen across a wide range of taxa, are correlated with the lunar cycle; potentially because the lunar cycle represents such an easily observable, large-scale cue (Robertson, 1983; Robertson et al., 1990; Zeller, 1998; Meyer et al., 2007; Claydon et al., 2014). Though scientists are still uncertain about the specific selective advantages

underlying lunar periodicity, a variety of drivers have been hypothesized. The lunar state could for example simply represent a convenient timing cue to synchronize reproduction (Lobel, 1978, Colin & Clavijo, 1988, Colin & Bell 1991; Claydon, 2004), or to couple reproduction with the optimal timing of settlement (Robertson et al., 1988; Doherty, 1994; Shima et al., 2018). Additionally, tidal fluctuations associated with full and new moon might enhance dispersal of fertilized eggs (Robertson et al., 1990; Claydon, 2004; Claydon et al., 2014).

In summary, reproductive timing and investment decisions may be shaped by an amalgam of local conditions and large-scale physical characteristics that may increase survival rates of spawning adults and/or their offspring. Although a substantial body of literature about reproductive tactics in reef fish already exists, there are still gaps in our knowledge about the relative strength of local versus large-scale cues on reproductive investment decisions in iteroparous reef fish species. Particularly the role of localised environmental conditions on spawning fishes remains poorly resolved. A more complete assessment of environmental conditions during spawning hours, in combination with highly resolved estimates of spawning intensity might shed new light on the key drivers shaping reproductive patterns in iteroparous reef fish species.

Here, I evaluate temporal and spatial variation in spawning patterns of the sixbar wrasse, *Thalassoma hardwicke*, and in relation to a set of environmental conditions. The sixbar wrasse is a small bodied, protogynous reef fish species. Spawning occurs almost every day, year-round, during a 2-hour period in the afternoon. Socially dominant males establish mating territories at particular locations on a daily basis. The predictability of this system enabled me to address the following questions: (1) Is spawning intensity constant or does it vary periodically through time? (2) What are the potential drivers of reproductive investment decisions? (3) To what extent are reproductive patterns associated with local cues versus larger-scale environmental cues?

## 2.2 Methods

### 2.2.1 Study site and species

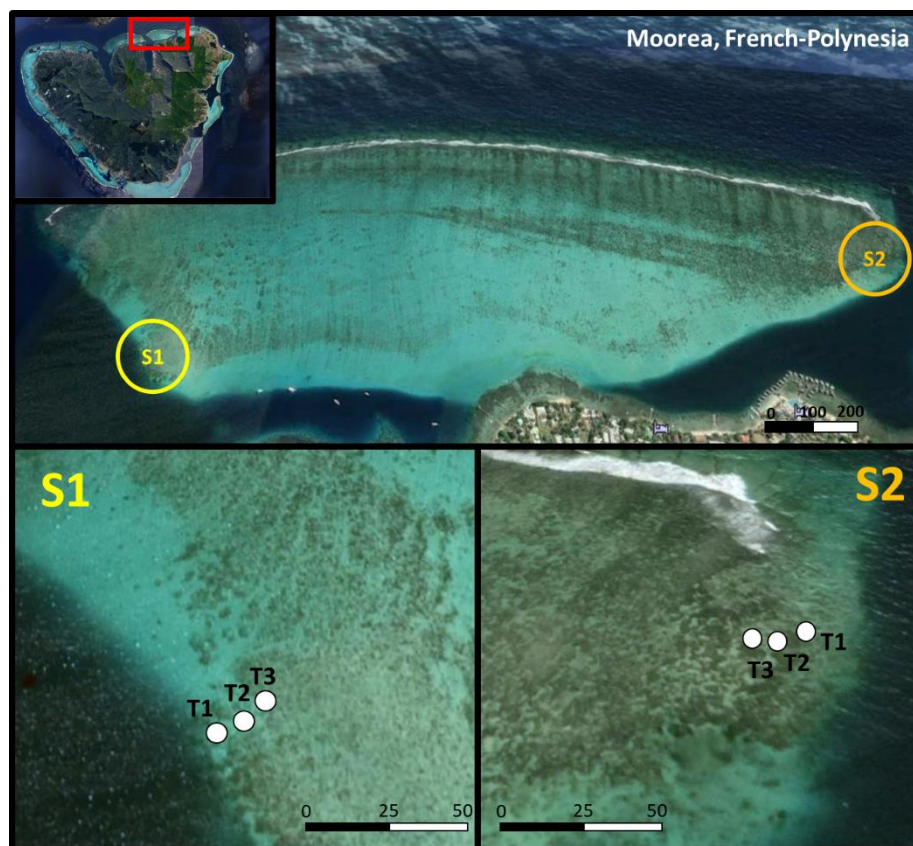
I surveyed spawning patterns of the sixbar wrasse (*Thalassoma hardwicke*) from February to June 2017, at two focal sites on the north shore of Moorea, French Polynesia. Sixbar wrasse are widely distributed throughout the Indo-Pacific region and they are one of the most conspicuous and abundant reef fishes within inshore reef habitats of French Polynesia (Galzin, 1987). Sixbar wrasses are protogynous hermaphrodites (Warner, 1984a). Local populations contain a mix of initial phase (IP) females and males which are morphologically indistinguishable from one another, and terminal phase (TP) males which are distinguishable by their brighter colour patterns and blunter heads. Sixbars are highly iteroparous, and they spawn throughout the lunar cycle and across much of the year (Claydon et al. 2014). Like other members of the genus (e.g., *Thalassoma bifasciatum*; Warner, 1984a; Warner, 1995), sixbars appear to migrate to particular areas (e.g., reef edges) to spawn. At these locations, TP males defend mating territories and actively court females. Spawners may engage in both pair spawning (i.e., one TP male and one female) and group spawning. IP males frequently attempt to “sneak” mating opportunities during bouts of pair spawning, and they appear to participate openly in group spawning. On Moorea, most sixbar spawning activity is concentrated from 2-4pm daily (P. Mitterwallner, *unpublished data*)

Moorea is surrounded by a barrier reef crest that delineates the outermost extent of the lagoon system (spanning a width of ~1km from reef crest to shore). Much of the lagoon is dominated by wide, flat areas (1-5m depth) of sand and rubble, interspersed by patches of coral colonies, algae turf and extensive stands of macroalgae (Galzin & Pointier, 1985). Water is forced into the lagoon by wave action, and generally flows shoreward from the reef crest, and then back out to sea through breaks (i.e., “passes”) in the reef crest (Galzin & Pointier, 1985). Many species including sixbars spawn at the reef edge near these passes (P. Mitterwallner, *unpublished data*).

### 2.2.2 Surveys of spawning activity

I conducted preliminary surveys to determine focal locations, durations, and times of day for subsequent spawning observations. Based on these initial surveys, I selected two focal sites (hereafter, S1 and S2) situated at the reef edge, near passes, on the northeastern side of the island (see Fig. 2.1). Within each site, I identified and mapped three focal territories (T1, T2, T3) of terminal phase males. These territories were distributed along a gradient from exposed (i.e., T1 closest to the reef edge) to sheltered conditions (i.e., T3 furthest from the reef edge; Fig 2.1).

I surveyed focal territories in each site on alternate days. Each site was surveyed between 2-4 pm, ~3-5 times per week over 5 lunar cycles (i.e., 20 weeks). During each survey, I recorded spawning activities within each focal TP territory (n=3) over a 20 min duration. ‘Spawning’ was evidenced by female(s) and focal male(s) rising towards the surface and releasing eggs and sperm simultaneously. I further categorized each observation of spawning based upon the participants : ‘pair spawning’ (involving the focal TP and a single female), ‘streaking’ (pair spawn event involving a non-territorial male in the focal male’s territory), ‘sneaking’ (involving an IP male that surreptitiously joins a pair spawning event), or ‘group spawning’ (a spawning event with more than three individuals, typically including multiple males and females). I also recorded a set of environmental variables during each observation period (detailed in Table 2.1.)



**Fig 2.1** The two surveyed spawning sites (S1) and (S2) within a lagoon at the north shore of Moorea, French-Polynesia (A). Within each site, I monitored focal territories (T1, T2, T3) of terminal phase males. These territories were distributed along a gradient from exposed (i.e., T1 closest to the reef edge) to sheltered conditions (i.e., T3 furthest from the reef edge). Location maps are produced from Google Earth Pro (2019).

**Table 2.1** Measured environmental variables that potentially affect spawning activities. Given are the methods of estimation for each variable, and a qualitative description of the resulting data.

Environmental variable	Measurement method	Variable type
Lunar day	internet data base ( <a href="http://www.timeanddate.com">www.timeanddate.com</a> )	continous variable, ranging from 1-29
Temperature	data logger	continous variable (°C)
Visibility	deployment of 50 m measurement tape at the spawning site - point of furthest visible distance from a standard object (coral boulder) as estimate of visibility	originally continous variable (m), but transformed into 2 categories: high (1) and low (2) visibility
Current speed	tennis ball, released on the surface along a measurement tape; time to drift a certain distance (3m) recorded as measure of current speed	originally continous variable (m/s), but transformed into 3 categories: high (1), moderate (2) and low (3) current speeds
Cloud cover during spawning hours	visual estimation	3 categories: sunny (1), mixed (2), cloudy (3)
Cloud cover during previous night	internet database ( <a href="https://giovanni.gsfc.nasa.gov/giovanni/">https://giovanni.gsfc.nasa.gov/giovanni/</a> ) average cloud cover between 11 pm to 5 am	originally continous variable (percent), but transformed into 3 categories: clear (1), mixed (2), cloudy (3)
Current direction	compass heading	3 categories: SE (1), SW (2), N (3)

### 2.2.3 Data analysis

Since the lunar calendar is fundamentally cyclical, I used a periodic regression (linear-circular regression) approach to evaluate primary sources of variation in sixbar spawning activity over time. Following methods of deBruyn & Meeuwig (2001), the calendar date of each observation was converted to a 'lunar day' (0-29.5; day 0 corresponds to the new moon, day 15 to the full moon, etc). Lunar days were then divided into 360° (or  $2\pi$  radians) to allocate each day an angular equivalent, theta ( $\theta$ ). The cyclical character of the lunar calendar was then expressed by sine and cosine transformations of theta. The cosine term describes a phase shift near 0° or 180° (full moon and new moon), and the sine term describes a phase shift between 90° or 270° (first and last quarter). A positive  $\cos\theta$  coefficient represents a peak at new moon, and a negative  $\cos\theta$  coefficient a peak at full moon. A significant positive  $\sin\theta$  coefficient corresponds to a peak during the first quarter, and a significant negative  $\sin$  coefficient corresponds to a peak during the last quarter. To simplify the statistical modelling process, I decided to not include  $\sin 2\theta$  and  $\cos 2\theta$  (describing potential two peaks per lunar month), since preliminary analysis indicated only singular activity peaks across the lunar cycle. The transformed data can then be analysed by using simple linear or linear mixed effect regression.

I used general linear mixed effect models (GLMERs) with a Poisson error distribution to evaluate the main sources of variation in sixbar spawning activity. Initially, I fitted a fully saturated model with all explanatory variables (but without including any interactions between these variables). I then used Aikaike's information criterion corrected for small sample sizes (AICc) to identify an appropriate reduced model (i.e., the candidate model with the lowest AICc; obtained via multi model selection methods developed by the MuMIn package in R, see Bartón, 2011). The fully saturated model included the explanatory variables as fixed effects: 'site' (e.g., S1 or S2), 'territory location (T1, T2, or T3)', 'sin $\theta$ ', 'cos $\theta$ ', 'temperature', 'daytime cloud cover', 'nighttime cloud cover' (previous night), 'visibility', 'current speed' and 'current direction' (see Table 1 for details). The full model also included 'Date' (coded numerically) as a random effect.

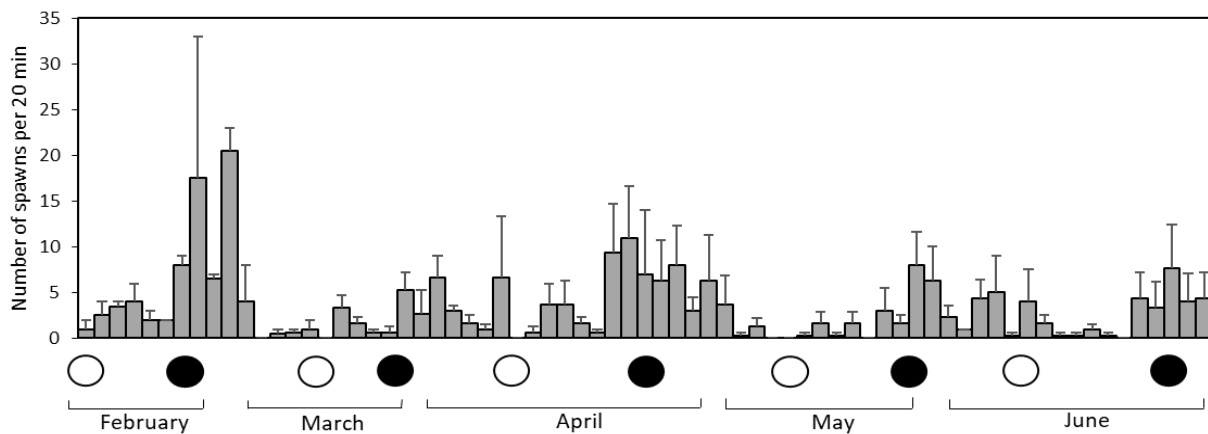
I conducted a secondary analysis to explore the potential influence of interactions between the main effects, and again I reduced model complexity based on AICc criteria (Judging from normalized quantile-plots of the residuals, the fit of the chosen models appeared to be all satisfactory).

All analyses were performed with the lme4 package (Bates et al., 2014) of the R 2.9.1 software (R Core Development Team 2019). To visualize the predicted effects of significant factors on the dependent variables, I additionally calculated estimated marginal means for each predictor, with covariates held at their mean value (function emmeans in the R package emmeans, see Lenth, 2018). I also calculated marginal  $R^2$ , an indicator of the variance explained by each of the fixed effects in general mixed effect models (as suggested by Nakagawa and Schielzeth, 2013) with the MuMIn package of R (Bartón, 2011).



## 2.3 Results

In total, I observed 649 bouts of spawning over 71 days of observation. On average, I recorded 3.2 spawning events per 20min observation period, and spawning frequency varied substantially through time (Fig 2.2).



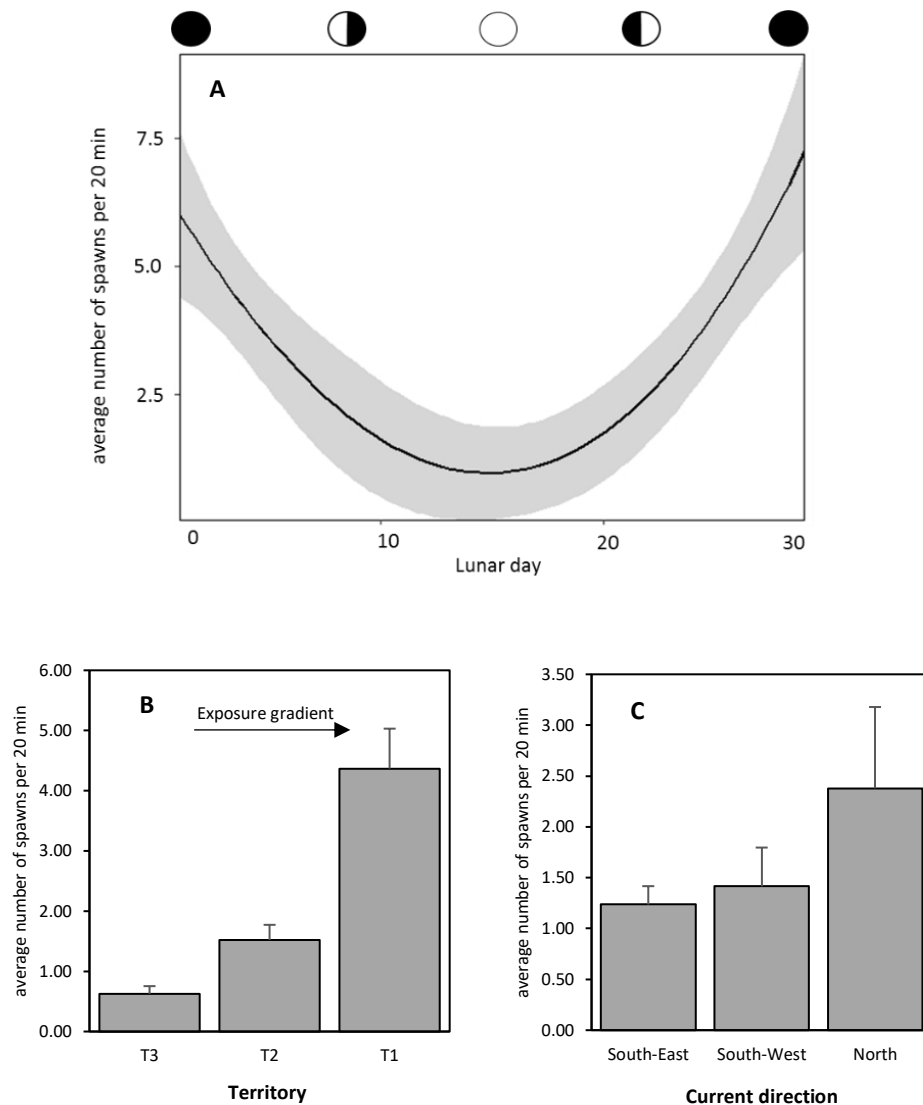
**Fig 2.2** Average number of spawns per day (averaged over three territories) over 5 lunar cycles from February to June. Black circles indicate new moon phases, white circles indicate full moon phases. Error bars represent  $\pm 1SE$ .

### 2.3.1 Sources of variation in overall spawning frequency

The best fitting model ( $AIC_c=858.3$ ) that accounted for variation in the spawning frequency (i.e., the total number of spawning events observed per 20min observation period) retained moon phase ( $\sin\theta$  as well as the  $\cos\theta$  term), current direction, current speed and territory location as explanatory variables (see table 2.2 and appendix 2.1). Parameter estimates as well as predicted marginal R square values suggested that moon phase and territory location (i.e., proximity to the reef edge) are particularly important sources of variation in overall spawning frequency (Fig 2.3 A and 2.3 B). A positive  $\cos\theta$  regression coefficient in the final model indicated significantly higher spawning activities around new moon in comparison to full moon periods (GLMER:  $p < 0.001$ ). A slightly significant negative  $\sin\theta$  regression coefficient suggested elevated activities of spawning during the third quarter (GLMER:  $p=0.08$ ). Together these results suggest a steady increase of spawning activities from first quarter/full moon towards third quarter/new moon, with greatest activity peaks around the new moon period (roughly 6 times higher at new moon relative to full moon; Fig 2.3 A).

Spawning intensity was also strongly influenced by territory position: exposed territories close to the reef edge exhibited significantly higher spawning frequencies relative to more sheltered territories (GLMER:  $p < 0.001$ , 30-50% increase in T1 relative to T2 and T3, Fig 2.3 B).

Although temporal variation in spawning was most strongly associated with moon phase, the reduced model suggests that current direction also had a marginal effect on spawning intensity. Currents during the observation period flowed predominantly from northwest to southeast. Regression coefficients as well as estimated marginal means (which considers only fixed effects in the model) indicated, that a shift of water movements to the North (offshore flow) doubled the number of spawning events in a 20 min period (GLMER:  $p=0.03$ ; Fig 2.3 C).



**Fig 2.3** Sources of variation in overall sixbar spawning frequency through time. **(A)** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted spawning frequency during the 29.5 lunar cycle **(B)** Variation in spawning among territories (T1 is closed to reef edge, T3 furthest from reef edge) **(C)** Variation in spawning with current direction. Panels B and C give marginal means back-transformed to the original scale ( $\pm$ SE) estimated with other fixed effects held at their mean values.

**Table 2.2** Parameter estimates and test statistics for best models explaining total spawning frequency of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). All models were fit using GLMER (Poisson distribution). Asterisk symbols reflect significance levels.

<i>Model</i>	<i>marg R2</i>	<i>levels</i>	<i>Estimate</i>	<i>SE</i>	<i>z value</i>	<i>p value</i>		<i>AICc</i>
<b>total number of spawns~</b>								
Territory	44.0	Territory 2	-1.05	0.09	-11.31	<0.001	***	
		Territory 3	-1.94	0.15	-12.77	<0.001	***	
Moon phase: COS ( $\theta$ )	40.4	-	0.89	0.16	5.65	<0.001	***	
Current direction	9.5	North East	0.05	0.31	0.16	0.87		
		South	0.80	0.36	2.20	0.03	*	
Current speed	4.6	medium	0.23	0.27	0.87	0.38		
		high	-0.42	0.30	-1.43	0.15		
Moon phase: SIN ( $\theta$ )	1.5	-	-0.30	0.17	-1.77	0.08	.	858.3

### 2.3.2 Effect of interactions between main effects

With statistical interactions included, the most supported model contained a range of significant interactions as explanatory variables. The AICc value for this model with interactions was 831.3, which is lower than the most supported model without interactions. Models with significant interactions suggest that synergistic effects may additionally mediate spawning frequency. For example, daytime cloud cover, in combination with other environmental variables, had a notable effect on spawning intensity. The best model with interactions suggests that the positive effect of new moon and third quarter on spawning activities might additionally be amplified during sunny days. Spawning frequency may also increase if sunny conditions are accompanied by intermediate current speeds or northwards directed currents. Higher cloud cover during the preceding night may also increase spawning frequencies around the third quarter moon. During new moon, high current velocities may increase spawning frequencies (relative to days with medium to low current velocities). High current velocities in more sheltered territories might, on the other hand, reduce spawning frequencies (see table 2.3 for supporting statistics).

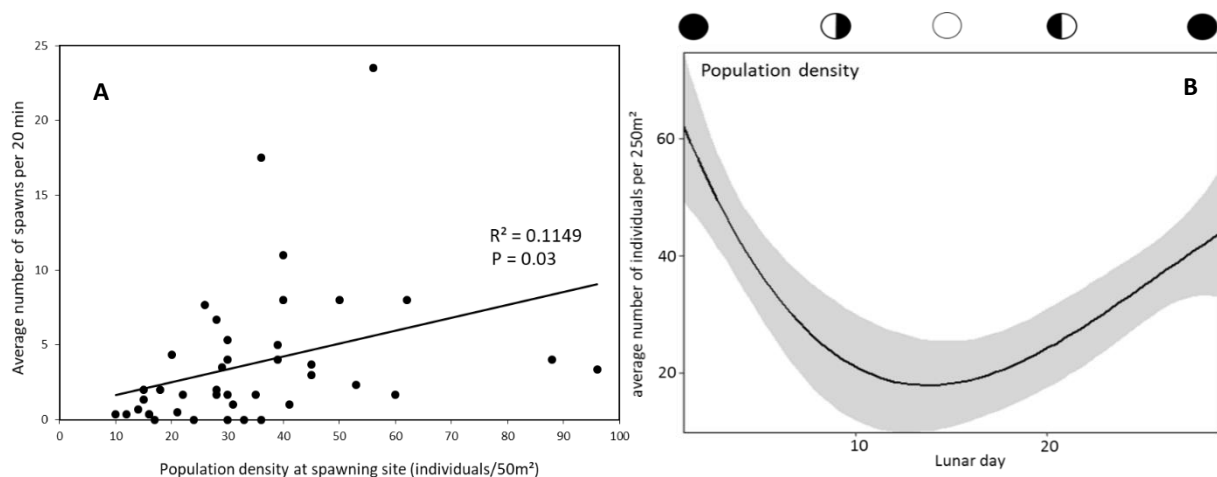
**Table 2.3** Parameter estimates and test statistics for best models explaining total spawning frequency of *Thalassoma hardwicke* at spawning sites if interactions between main effects are included. Best models given below were those with the lowest AICc score (among all permutations of the full model). All models were fit using GLMER (Poisson distribution). Asterisk symbols reflect significance levels.

Model	levels		Estimate	SE	z value	p value	AIC
<b>total number of spawns~</b>							
COS	-		-0.23	0.35	-0.65	0.52	
SIN	-		1.41	0.45	3.16	<0.001	**
current speed	medium		-3.23	0.97	-3.34	<0.001	***
	high		-0.57	0.56	-1.03	0.30	
current direction	South West		-0.49	0.47	-1.05	0.29	
	North		0.64	0.63	1.02	0.31	
Cloud cover	sunny		-1.96	0.61	-3.20	<0.001	**
	mixed		-0.37	0.51	-0.73	0.47	
Cloud cover (nighttime)	mixed		-0.43	0.25	-1.71	0.09	.
	cloudy		-0.42	0.27	-1.60	0.11	
Territory	territory 2		-0.68	0.15	-4.44	<0.001	***
	territory 3		-2.03	0.30	-6.86	<0.001	***
COS*Territory	*territory 2	20.3	0.37	0.16	2.33	0.02	*
	*territory 3		-0.15	0.23	-0.68	0.49	
COS*Cloud cover	*sunny	18.0	1.55	0.46	3.39	<0.001	***
	*mixed		0.97	0.36	2.71	0.01	**
COS*current speed	*medium	12.7	-0.17	0.35	-0.48	0.63	
	*high		0.93	0.36	2.58	0.01	**
SIN*Cloud cover	*sunny	4.1	-1.03	0.44	-2.37	0.02	*
	*mixed		-1.32	0.42	-3.13	<0.001	**
SIN*Cloud cover (nighttime)	*mixed	8.0	-0.28	0.41	-0.67	0.51	
	*cloudy		-1.18	0.37	-3.15	<0.001	**
Current speed*Cloud cover	current speed(medium)*cloud cover (high)	9.4	4.64	1.06	4.38	<0.001	***
	current speed(high)*cloud cover (high)		0.90	0.71	1.27	0.20	
	current speed(medium)*cloud cover (medium)		3.33	1.02	3.26	<0.001	**
	current speed(high)*cloud cover (medium)		-0.85	0.68	-1.25	0.21	
Current direction*Cloud cover	current direction (SW)*cloud cover (sunny)	13.1	1.83	0.68	2.70	0.01	**
	current direction (N)*cloud cover (sunny)		2.71	0.96	2.83	<0.001	**
	current direction (SW)*cloud cover (medium)		0.62	0.65	0.95	0.34	
	current direction (N)*cloud cover (medium)		-0.35	0.71	-0.49	0.63	
Current speed*Territory	current speed(medium)*territory 2	14.5	-1.20	0.22	-5.33	<0.001	***
	current speed(high)*territory 2		-0.79	0.26	-3.08	<0.001	**
	current speed(medium)*territory 3		0.24	0.36	0.67	0.50	
	current speed(high)*territory 3		-0.13	0.46	-0.28	0.78	
							831.3

### 2.3.3 Population density

The number of sixbars at the spawning site ranged from 10 to 96 individuals per observation period. Linear regression analysis revealed that spawning activities were significantly positively correlated to population densities at the spawning site ( $p=0.03$ , see Fig 2.4 A).

Densities of sixbars aggregated at the spawning sites were strongly related to the  $\cos\theta$  term of the final model, with highest population densities at new moon (GLMER:  $p<0.001$ , see table 2.4 and Fig 2.4 B). Site also seemed to influence population densities, with higher population densities at the second spawning site, S2 (GLMER:  $p=0.07$ ). The final GLMER model indicated, that changes in population densities were not influenced by any of the other physical variables (see table 2.4 and appendix 2.2).



**Fig 2.4** Linear regression between population density at the local spawning site and number of spawns per 20 min. P values and regression coefficient indicate a significant positive relationship **(A)** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted spawning frequency during the 29.5 lunar cycle **(B)**

**Table 2.4** Parameter estimates and test statistics for best models explaining population densities (average number of individuals per  $m^2$ ) of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). All models were fit using GLMER (Poisson distribution). Asterisk symbols reflect significance levels.

Model	variance (%)	levels	Estimate	SE	z value	p value	AICc
<b>Spawning population (frequency)~</b>							
Site	80.8	-	0.27	0.15	1.83	0.07 .	
Moon phase: COS ( $\theta$ )	19.2	-	0.41	0.09	4.38	<0.001 ***	332.2

## 2.4 Discussion

The sixbar wrasse displayed highly iteroparous spawning behaviour. Spawning activities were observed almost every day in the afternoon hours throughout a 5-month observation period. Nevertheless, spawning intensity and densities of the sixbar wrasse exhibited pronounced temporal and spatial variability. Temporal patterns of spawning, as well as population densities at the spawning site, were most strongly associated with the lunar cycle, and maximal around the new moon. Other recorded abiotic variables showed low or non-significant correlations with spawning intensity. Territory position seemed to strongly affect spatial variation in spawning intensity, with highest spawning frequencies in territories closest to the reef edge.

### 2.4.1 Lunar periodicity

As shown by previous studies, a broad range of tropical reef fish species exhibit lunar spawning patterns, with spawning occurring particularly around the new and full moon (Johannes, 1978; Domeier and Colin, 1997; Takemura et al., 2004; Nemeth et al., 2007; Claydon et al., 2014). In a rapidly changing environment, the degree of dispersal after egg release presumably may not be predictable by adults that spend most of their life in enclosed lagoon habitats. Generalized responses to an easily detectable large-scale cue like the lunar state may therefore provide several selective advantages for resident species (Warner, 1997a).

Studies have suggested that favourable conditions for larval dispersal, like outgoing tides and high current velocities are often associated with new or full moon phases (Johannes, 1978, Robertson, 1983; Robertson et al., 1990; Domeier & Colin, 1997, Claydon et al., 2014). However, current regimes in shallow lagoon habitats are often driven by local wind conditions or shear flow generated eddies (Warner, 1997a; Heyman & Kjerfve, 2008; Karnauskas et al., 2011; Donahue et al., 2015). Correspondingly, this study couldn't detect any lunar associated fluctuations in local current velocity or direction. The lunar state might therefore be utilized as an easily measurable indicator (in an otherwise turbulent environment) for large-scale oceanographic processes that facilitate a faster entrainment of propagules into deeper waters (Robertson et al., 1990; Warner, 1997a; Claydon, 2004; Claydon et al., 2014). However, lunar illumination itself might convey fitness benefits for dispersing eggs. Many juvenile reef fish settle onto the reef during new moon, presumably to avoid benthic predation pressure (Sponaugle & Cowen, 1994; Robertson et al., 1999; Shima et al. 2018). Similarly, the degree of lunar illumination at night might drive investment decisions of adults. Since a majority

of iteroparous, protogynous species spawn in the afternoon (Colin & Clavijo, 1988; Sancho et al. 2000; Claydon et al., 2014), dark nights during the new moon might reduce predation pressure on fertilized eggs that are still in close vicinity to the reef.

Several studies have emphasized that the lunar cycle might not provide any selective advantage, other than representing a timing cue for reproduction (Lobel, 1978, Colin & Bell, 1991; Domeier & Colin, 1997, Claydon et al., 2014). In this study, smaller initial phase males and females regularly swarmed the spawning site, forming dense aggregations during new moon. Though the sixbar wrasse reproduces daily, periodic, lunar initiated mass migrations to a spawning site may provide several fitness benefits for particularly smaller individuals: synchronized reproduction in large groups may improve fertilization rates, minimize intraspecific competition, and dilute predation on adults and eggs (Robertson, 1983; Warner et al., 1995; Marconato et al., 1997; Molloy et al., 2012). Additionally, constraining the portion of the month over which spawning occurs may enable smaller individuals to allocate more time to feeding and growth instead of to costly reproductive behaviours (i.e. sneaking, courtship and mate choice tactics; Hoffman, 1983; Colin & Clavijo, 1988; Molloy et al., 2012).

If the developmental duration of pelagic larvae is invariant, the precise timing of spawning might dictate the timing of larval recruitment to the benthos. Reef fish might use the lunar phase as an environmental cue to couple settlement with production (Robertson et al., 1988; Shima et al. 2018). For example, spawning of the coral trout *Plectropomus leopardus* mostly occurs around the new moon (Samoilys, 1997). Based on otolith increments (Doherty et al., 1994), larvae develop about 25 days in the pelagic phase, before settling back to the reef near new moon, which is generally considered to be the optimal time for larval settlement. However, available evidence suggests this may not necessarily be the case for sixbars (Shima et al., 2018). The pelagic larval duration for sixbars is 47 days (Victor, 1986). Consequently, if most spawning occurs near the new moon, most larvae should settle back to the reef closer to the full moon (47 d later). However, most settlement appears to happen on the new moon (Shima et al., 2018), raising some interesting questions about plasticity in larval developmental times (which are beyond the scope of this study).

#### **2.4.2 Local environmental cues**

Although my findings suggest that spawning intensity is most strongly shaped by a large-scale environmental cue (i.e., the lunar cycle), model predictions also indicate important, albeit weaker, effects of local environmental variables. Offshore currents appear to increase spawning activities by a

factor of 2. Many studies already described a correlation of spawning activities with outgoing current flow for a variety of coral reef species in the Indo-Pacific region (Robertson, 1983, Moyer, 1989, Colin & Bell, 1991, Sancho et al., 2000). As directional water flow can reduce exposure of the fertilized eggs to benthic predators (Randall & Randall, 1963; Johannes, 1978; Lobel, 1978), instant behavioural responses to changes in current directions might selectively be beneficial (Johannes, 1978, Barlow, 1981; Sancho et al., 2000). So, despite a strong effect of the lunar cycle, highly iteroparous species may still utilize local, short-term environmental cues to optimize their fitness (Moran, 1991; Warner, 1997a).

Additional analyses that included statistical interactions suggest an important role of environmental interdependencies. When specific environmental conditions (i.e., sunny conditions, or high current speeds) coincide with a new moon and/or a third quarter moon, spawning events increase disproportionately. The influence of cloud cover on spawning activities is rarely considered. Higher light intensity during spawning hours might facilitate visual detection of predators (Hobson, 1974, Danilowicz & Sale, 1999). Furthermore, positively buoyant, transparent eggs in light-saturated surface waters might be less visible for egg predators. Another interesting finding concerns the influence of nocturnal cloud cover on spawning activities: During the third quarter, spawning activities increased, if the previous night was covered by clouds. This may suggest that sixbars rely on nocturnal illumination just prior to sunrise as a cue to trigger spawning on a given day. Because the timing of moonrise and moonset also varies with lunar phase, cloud cover during the third cover may be indistinguishable from a new moon (because in both instances, the hours just prior to sunrise may be equally dark). Consequently, overcast nights around third quarter might result in increased spawning activity because individuals are mistaking these days for a new moon phase. Though this is an intriguing idea, I note that it is highly speculative, because the pattern is relatively weak, and data on nocturnal cloud cover was extracted from external sources (i.e., satellite data made at a larger spatial scale, which might not be a true reflection of local nocturnal illumination).

Spawning intensity was strongly structured along a spatial gradient. Throughout all lunar phases, spawning intensity was greatest in extremely exposed territories along the outer reef edges. In fact, my analyses suggest that the male territories closest to the reef edge (i.e., T1) had ~6-fold more spawning events than relatively sheltered territories (T3). This effect size is similar to the magnitude of increase in spawning intensity from full moon to new moon. Despite intense courtship efforts of territorial males in sheltered territories, most females tended to pass through those territories to mate with males that occupied the more exposed territories closest to the reef edge. Potentially, exposed



territories might provide an advantage immediately following the release of eggs (Hensley et al., 1994): shear flows along outer reef edges produce hydrological turbulences that may rapidly entrain the fertilized eggs into larger current systems, and out of reach from benthic and pelagic predators (Colin & Clavijo, 1988; Claydon, 2004; Hamner & Largier, 2011; Claydon et al., 2014; Donahue et al. 2015). These immediate consequences might be easier to assess for adults than long-term consequences on larval dispersal (Warner, 1997a). Thus, territory selection might be mediated by a site-specific trade-off between predation pressure on adults, and the initial survival potential of released eggs (Robertson & Hoffman, 1977; Sancho et al., 2000; Claydon, 2004). If these fitness trade-offs are additionally structured by size i.e. if predator prone, small-sized individuals tend to favour more sheltered territories, has yet to be evaluated.

### **2.4.3 Conclusion**

In conclusion, temporal variation in spawning intensity is shaped by a large-scale, global cue: the lunar cycle. In a temporarily variable environment, purely conditional adjustments may be evolutionary detrimental, since individuals are not able to predict long-term consequences on offspring dispersal (Lloyd, 1984; Seger & Brockman, 1987; Moran, 1992; Warner 1997a). Consequently, selection may favour fixed responses to a global cue (the lunar cycle), as a mechanism that guarantees intermediate fitness across a range of environmental states (Moran, 1992; Warner, 1997a). Nevertheless, my study also suggests that individuals can fine-tune their reproductive effort to specific local environmental conditions. Current direction and specific locations of spawning territories appear to convey sufficient information in terms of initial offspring survival, to stimulate behavioural responses over short time scales (Warner, 1997a, Sancho et al., 2000).

By incorporating an exceptionally wide variety of environmental variables, my study improves our understanding of the relative strengths of large-scale versus local cues on reproductive dynamics of a highly iteroparous reef fish species. Lunar periodicity is well documented in some reef fish, but further studies are needed to evaluate the specific selective forces underlying lunar mediated reproduction. Further knowledge on dispersal trajectories for example, and the early life history stages of pelagic larvae, may shed more light on the effects of lunar-periodic reproduction on recruitment.



## **CHAPTER THREE**

INFLUENCE OF THE LUNAR CYCLE AND SPATIAL GRADIENTS ON SIZE-DEPENDENT  
MALE AND FEMALE REPRODUCTIVE INVESTMENT DECISIONS OF A PROTOGYNOUS  
REEF FISH

### 3.1 Introduction

Life history theory predicts that parents should adopt reproductive tactics that maximise fitness (Stearns, 1992; Warner, 1998; Taborsky, 1998; Roff, 2002; Munday et al., 2006a). Reef fishes exhibit an extraordinary diversity of reproductive tactics, both within and among species (Aldenhoven, 1986; Johannes, 1978; Warner & Hoffman, 1980ab; Warner, 1984a; Shapiro, 1991; Munday et al., 2006a). Reproductive tactics may reflect adaptive responses to specific environmental conditions, to enhance fertilization success of adult individuals and/or reduce juvenile mortality (Johannes, 1978; Robertson et al., 1990; Warner, 1997a; Sancho et al., 2000; Claydon et al., 2014). Highly iteroparous species that can spawn continuously throughout the year may be better equipped to reproduce in particular times or during specific environmental conditions that reduce mortality to their offspring (Sancho et al., 2000; Claydon et al., 2014; McBride et al., 2015).

Many tropical reef fishes appear to use lunar phase as external cue for reproductive investment decisions (Johannes, 1978; Robertson et al., 1990; Claydon et al., 2014). A number of studies have hypothesized that lunar-related hydrodynamic conditions (i.e., tides) may facilitate faster transport of fertilized eggs away from higher concentrations of reef-associated predators (Johannes, 1978; Lobel, 1978; Claydon, 2004; Whaylen et al. 2006; Claydon et al., 2014). Alternatively, lunar cycles may simply provide a useful cue to synchronize reproduction across individuals to maximize fertilization rates, or to minimize predation rates on eggs (Lobel, 1978, Colin and Clavijo, 1988; Robertson et al., 1990; Domeier & Colin, 1997; Nemeth et al, 2007). More recent research (Hernandez-Leon, 2008, Shima & Swearer, 2019) suggests that larvae born around a certain lunar phase may experience better feeding conditions during pelagic larval development.

Parental survival is another important component of lifetime fitness, particularly for highly iteroparous species (Warner, 1998). For individuals that can spawn frequently, and over many reproductive seasons, any single mating event contributes only marginally to lifetime reproductive success. Highly iteroparous individuals should therefore prioritize their own survival over slight increases in current reproductive output (i.e., selection should favour risk-averse spawning when individuals have future opportunities to reproduce). Such tactics may be particularly prevalent in species with protogynous hermaphroditism (i.e., sex change from female to male). Many iteroparous reef fish have this pattern of sex change, in which lifetime reproductive success can be heavily influenced by an individual's size- and sex-dependent status within the dominance hierarchy (Munday et al., 2006a). In these systems, lifetime fitness can increase substantially for individuals that survive long enough to become territorial males, because they can then monopolize mating opportunities

with many females (Warner, 1984a; Munoz & Warner, 2003). Because fecundity disproportionately increases with size in a protogynous species, individuals of different sizes may adopt different tactics or at least weigh up risks differently. For example, smaller individuals may allocate a greater proportion of their energetic reserves to growth, instead of reproduction (Hoffman, 1983; Stearns, 1992; Warner 1984b; Roff, 2002; Warner et al., 1998; Rideout et al., 2005; McBride et al., 2015). In addition, smaller individuals may choose to spawn in places or times that are less risky (Shibuno et al., 1993; Warner, 1998; Claydon et al., 2014).

Drivers of selection for size-specific reproductive tactics may differ for males and females. Alternative reproductive tactics appear to be a common feature for males of many species (Henson & Warner, 1997; Alonzo & Warner, 2000). Smaller males with limited energetic resources or capacity to compete openly for access to females may engage in “sneaking” behaviour, whereby they mimic phenotypes and behaviour patterns of females in order to parasitize territorial pair spawning (Fiedler, 1964; Taborsky et al., 1987; Warner & Hoffman, 1980ab). Such males are often referred to as ‘initial phase’ (IP) males. In this way, smaller males can circumvent energetic costs associated with courtship or competitive interactions, and potentially redirect a higher proportion of their energetic reserves to growth (Hoffman, 1983; Warner, 1984b; Taborsky, 1998). The effectiveness of male reproductive tactics may be context-dependent. For example, if small males become locally common, larger territorial males may be less able to defend a territory. Above a certain threshold density of small males, territoriality may become ineffective to the point that most matings go to smaller (initial phase) males in large group spawning assemblages (Warner & Hoffman, 1980ab; Warner & Robertson, 1987). Some of the best examples of size- and context-dependent reproductive tactics come from studies on the protogynous bluehead wrasse, *Thalassoma bifasciatum*. On reefs with high densities of *T. bifasciatum*, large groups of females and small (i.e., sneaker) males readily form, and group spawning supersedes pair spawning in this context. In contrast, on reefs where *T. bifasciatum* are present in lower densities, territoriality and pair spawning is often more common (Warner & Hoffman, 1980ab).

Competition among females for access to males appears to be less common in protogynous hermaphrodites (Warner, 1998; Brockman & Taborsky, 2008), and consequently their behavioural flexibility seems comparatively limited. However, reproductive tactics among females are likely still important, as females must make energetic trades offs, most often expressed as decisions about where, when, and with whom to spawn (Petersen, 1992; Warner, 1998; Alonzo & Warner, 2000). Small females may be more vulnerable to predators during and immediately after spawning, and therefore they may be more selective about when and where they spawn (Robertson & Hoffman, 1977; Warner,

1998; Rideout et al., 2005; McBride et al., 2015). In turn, larger females might allocate a larger proportion of their energetic reserves to current reproduction, and may spawn more consistently through time. Generally speaking, the risk of predation decreases with size for reef fishes (Hixon & Beets, 1993; DeMartini et al., 2005; Welsh et al., 2013), and this may lead to size-structured spatial distributions in spawning activities. Larger females might preferentially spawn at more exposed locations that promote rapid advection of offspring off the reef and away from high concentrations of egg predators. Small females may spawn in less risky locations by prioritizing their own survival over the survival potential of their offspring. Small males might show the same level of reluctance in employing their sperm allocation strategies (Warner, 1998), because they too have the potential to develop into highly successful territorial males.

In short, size structured populations are likely to be comprised of individuals that vary in their reproductive tactics, as a function of both size and sex. Moreover, the optimal tactic for an individual of a given size and sex may further depend upon environmental context. Few studies have attempted to evaluate context-dependence in size-structured reproductive tactics.

In this chapter, I explore size-structured, context dependent reproductive tactics of the sixbar wrasse, *Thalassoma hardwicke*. The sixbar wrasse is a small bodied reef fish that is common on shallow coral reefs across the Indo-Pacific. It is a protogynous hermaphrodite, and highly iteroparous (chapter 2). All individuals first develop as small initial phase males or females, and at a certain size, some of these individuals may become brightly coloured terminal phase males (for initial phase females, this requires sex change). Terminal phase males defend and attempt to control mating territories, but their success may depend upon local densities of smaller initial phase males, which may vary in space and time. In chapter 2, I found that overall spawning activity varies strongly with the lunar cycle (with the most spawning activity occurring during the new moon) and with proximity to the reef edge (where advection of eggs, but also predation risk to adults is presumed to be greatest).

Here, I explore patterns of variation in distinct spawning strategies (pair spawning, group spawning, and sneaking tactics) across the lunar cycle, and in relation to proximity to the reef edge. I also explore variation in the sizes of individuals that engage in these different strategies. Specifically, I address the following questions: (1) Do smaller sized females (or males) constrain spawning activities to certain times or locations? (2) Do females of different sizes more readily engage in group spawning than pair spawning?

## 3.2 Methods

### 3.2.1 Field surveys

I surveyed spawning patterns of the sixbar wrasse (*Thalassoma hardwicke*) from February to June 2017, at two focal sites on the north shore of Moorea, French Polynesia (see chapter 2 for details of study system and methodology). Briefly, I sampled spawning-related activities of the sixbar wrasse at two sites, and three focal territories within each site, 3-5 times per week for 20 weeks (for details, see chapter 2). During each survey, I scored the following behaviour patterns: (i) pair spawning, (ii) group spawning, (iii) sneaking attempts (non-territorial males attempting to enter territories, but chased away by the territory holder), (iv) successful sneaking (sneaker males successfully participating in spawning) and (v) streaking events (for more detailed descriptions of spawning behaviours, see table 3.1). I estimated the sizes (total lengths, TL) and phenotypes (initial phase [IP] or terminal phase [TP]) of all individuals involved in a behaviour pattern. Visual size estimates were continuously calibrated against objects of known size (i.e., PVC pipe, cut to length, and suspended horizontally in the water column near areas of fish activity) to ensure accuracy.

**Table 3.1** Descriptions of recorded spawning behaviours (pair-spawning, streaking, sneaking and group spawning) at the spawning site

behaviour	
Pair-spawn	As soon as a female enters a territory, the large and brightly coloured territorial male starts to display courtship behaviour. Courtship behaviour is characterized by rapid vibrations of the pectoral fins and swimming in fast, tight circles above the female. If interested, the female ascends, positions herself under the TP male and the pair rushes to the surface to release a gamete cloud.
Streaking	If a TP male is involved in chasing activities, his territory may be occasionally unoccupied. Under these circumstances, a passing non-territorial TP male may pair-spawn with a female within the focal male's territory.
Sneaking	Initial phase 'sneaker' males (same coloration and morphological traits as females) that hover around territories in order to parasitize territorial pair spawning events. Mostly, these sneaker males are recognized by the territory owner due to olfactory stimuli (testicular glands of the sneaker male) and chased away. However, occasionally a sneaker male remains undetected and is able to successfully sneak into a territory. If a chance arises, the sneaker male surreptitiously joins a pair spawning event to contribute his sperm at the moment of mating. After gamete release, the TP male typically notices that a third individual was involved and aggressively chases it off his territory.

Group-spawn	A spawning event with more than three individuals, normally including multiple IP males and females. Group spawning typically develops when several initial phase males merge to form larger groups. These groups move erratically around within TP territories and are gradually joined by multiple females. At a certain point, the group, or subdivisions of the group, rushes upwards to release a large gamete cloud.
-------------	--

---

## Reproductive behaviour

### Description

Pair-spawn	As soon as a female enters a territory, the large and brightly coloured territorial male starts to display courtship behaviour. Courtship behaviour is characterized by rapid vibrations of the pectoral fins and swimming in fast, tight circles above the female. If interested, the female ascends, positions herself under the TP male and the pair rushes to the surface to release a gamete cloud.
Streaking	If a TP male is involved in chasing activities, his territory may be occasionally unoccupied. Under these circumstances, a passing non-territorial TP male may pair-spawn with a female within the focal male's territory.
Sneaking	Initial phase 'sneaker' males (same coloration and morphological traits as females) that hover around territories in order to parasitize territorial pair spawning events. Mostly, these sneaker males are recognized by the territory owner due to olfactory stimuli (testicular glands of the sneaker male) and chased away. However, occasionally a sneaker male remains undetected and is able to successfully sneak into a territory. If a chance arises, the sneaker male surreptitiously joins a pair spawning event to contribute his sperm at the moment of mating. After gamete release, the TP male typically notices that a third individual was involved and aggressively chases it off his territory.
Group-spawn	A spawning event with more than three individuals, normally including multiple IP males and females. Group spawning typically develops when several initial phase males merge to form larger groups. These groups move erratically around within TP territories and are gradually joined by multiple females. At a certain point, the group, or subdivisions of the group, rushes upwards to release a large gamete cloud.

---



### 3.2.2 Data analysis

#### *Sources of variation in (1) frequency of spawning, and (2) size of spawners*

A primary objective of this chapter is to understand how individual size and frequency of intraspecific reproductive strategies vary with the lunar cycle and across spatial gradients. I used periodic regression GLMERs and GLMs to evaluate cyclical lunar effects on reproductive patterns. Specifically, I modelled dependent variables (detailed below) as a function of a  $\sin\theta$  and  $\cos\theta$  term (to capture the cyclical components of the first/last quarter moons and new/full moons respectively) and spatial gradients (territory position and site). I used an underlying poisson error distribution to evaluate frequency of spawning, and a gaussian error distribution to evaluate size of spawners. I included date as a random effect into the analysis of spawning frequencies (GLMER), but dropped it for evaluations of size distributions, since the effect of date was not significant ( $p=0.21$ ). The full model for all response variables was:

$$\text{Frequency} \sim \text{site} + \text{territory position} + \sin\theta + \cos\theta + \text{date (random effect)} \quad (\text{eq. 1})$$

$$\text{Sizes} \sim \text{site} + \text{territory position} + \sin\theta + \cos\theta \quad (\text{eq. 2})$$

Specifically, I used this general model structure to evaluate (in separate models) sources of variation of following reproductive activities (1) pair spawning (frequency, and size of involved females); (2) group spawning (frequency, and sizes of involved males and females; using mean TL for each group); (3) sneaking attempts (frequency, and size of involved males trying to enter territories but being chased away) and successful sneaking events (frequency). I did not consider interaction terms for these models since preliminary analysis indicated that their inclusion resulted in only marginal improvements to the fit of the model. Inspection of normalized quantile-plots indicated a satisfactory

fit to the data for all models. All analyses were performed with the lme4 (Bates et al., 2012) in R (R Core development team 2019).

To facilitate visualization of model parameters, I calculated the predicted response of the dependent variables to the lunar cycle with the 'predict' function of the lme4 package. For the effect of categorical variables on spawning patterns, I estimated marginal means for each predictor, with covariates held at their mean value (using function emmeans in the R package emmeans, see Lenth, 2018). The relative importance of predictors in the best-fit model was assessed by partitioning  $R^2$  (relative variance contribution) with the "relaimpo" R package (Grömping, 2006), which utilizes the Lindeman, Merenda and Gold method (Lindeman et al., 1980).

#### *Relationships between pair and group spawning activities*

I conducted quantile regressions on 201 observations to investigate potential correlations between (1) pair spawning frequency (total number of spawning events per territory and day) and correspondent group spawning frequency (total number of spawning events per territory and day); and (2) body sizes of pair spawning individuals (average per territory and day) and group spawning frequency (total number of spawning events per territory and day). Quantile regression was chosen because preliminary analyses suggested wedge shaped patterns evident in this relationship. Quantile regressions enabled me to evaluate whether relationships were stronger for the upper boundaries. Wedge shaped distributions in a dataset imply that there may be only a weak or no relationship between the mean of the response variable distribution and the measured predictor variable, but a potentially stronger relationship at the upper boundaries of data distributions. Therefore, I described rates of change in quantiles at maximal response (95% quantiles), in comparison to quantiles at medium response (50% quantiles) with the quantreg package in R (Koenker, 2011).

### **3.3 Results**

I observed 649 bouts of spawning over 71 days of observation. 59% of these were pair spawning, 37% were group spawning, 3.5% were sneak spawning, and 0.7% were streak spawning.

#### **3.3.1 Pair and group spawning in relation to environmental parameters**

##### *Predictors of spawning frequency*

Pair spawning frequency was strongly influenced by the  $\cos\theta$  term describing moon phase. This parameter predicts highest spawning frequencies close to the new moon (frequency of spawning on new moon increases by  $\sim 80\%$  relative to full moon; GLMER:  $p=0.001$ ). Spawning frequency remained low from the first quarter to the full moon (Fig 3.1 A). Group spawning frequency was also strongly influenced by the  $\cos\theta$  term (GLMER,  $p < 0.001$ ; see Fig. 3.1 A), but spawning frequencies ceased entirely at the full moon and then rapidly increased ( $\sim$ six-fold) in the lead up to the new moon (Fig 3.1 A, see Table 3.1).

Pair spawning and group spawning frequencies markedly differed across territories that varied in their proximity to the reef edge. Territories located closest to the reef edge had greater frequencies of pair and group spawning relative to territories located further from the reef edge, and this disparity was most pronounced for pair spawning (pair spawn: GLMER,  $p<0.001$ ; see Fig 3.1 C and Table 3.1). Group spawning activities were significantly higher (almost 90%) at the second spawning site (S2), an area at the extreme down-current edge of the reef (GLMER,  $p<0.001$ , see Table 3.1). In contrast, pair spawning activities were significantly higher at the first spawning site (an area located further away from the channel entrance; GLMER,  $p<0.001$ , see Table 3.1).

#### *Predictors of spawner sizes*

Females of a wide range of sizes engaged in both pair spawning and group spawning. Pair spawning females ranged from 7 to 17 cm TL (mean  $\pm$  1SE:  $12.1 \pm 1.3$  cm). Group spawning rushes consisted of 5 to 30 recorded individuals, with sizes varying from 9-16 cm ( $12.6 \pm 1.5$  cm).

Variation in size of female pair spawners was strongly influenced by the lunar cycle. The stronger influence of the  $\sin\theta$  component (with a negative coefficient; GLM:  $p<0.001$ , Table 3.2) indicates that the mean size of pair spawning females was greatest on the third quarter moon (potentially indicating that a disproportionate number of large females were choosing to spawn at this time, and/or small females were opting out; Fig 3.1 B). Mean size of female spawners was lowest just after the new moon (potentially indicating more smaller females and/or fewer larger females spawn at this phase of the moon; Fig 3.1 B).

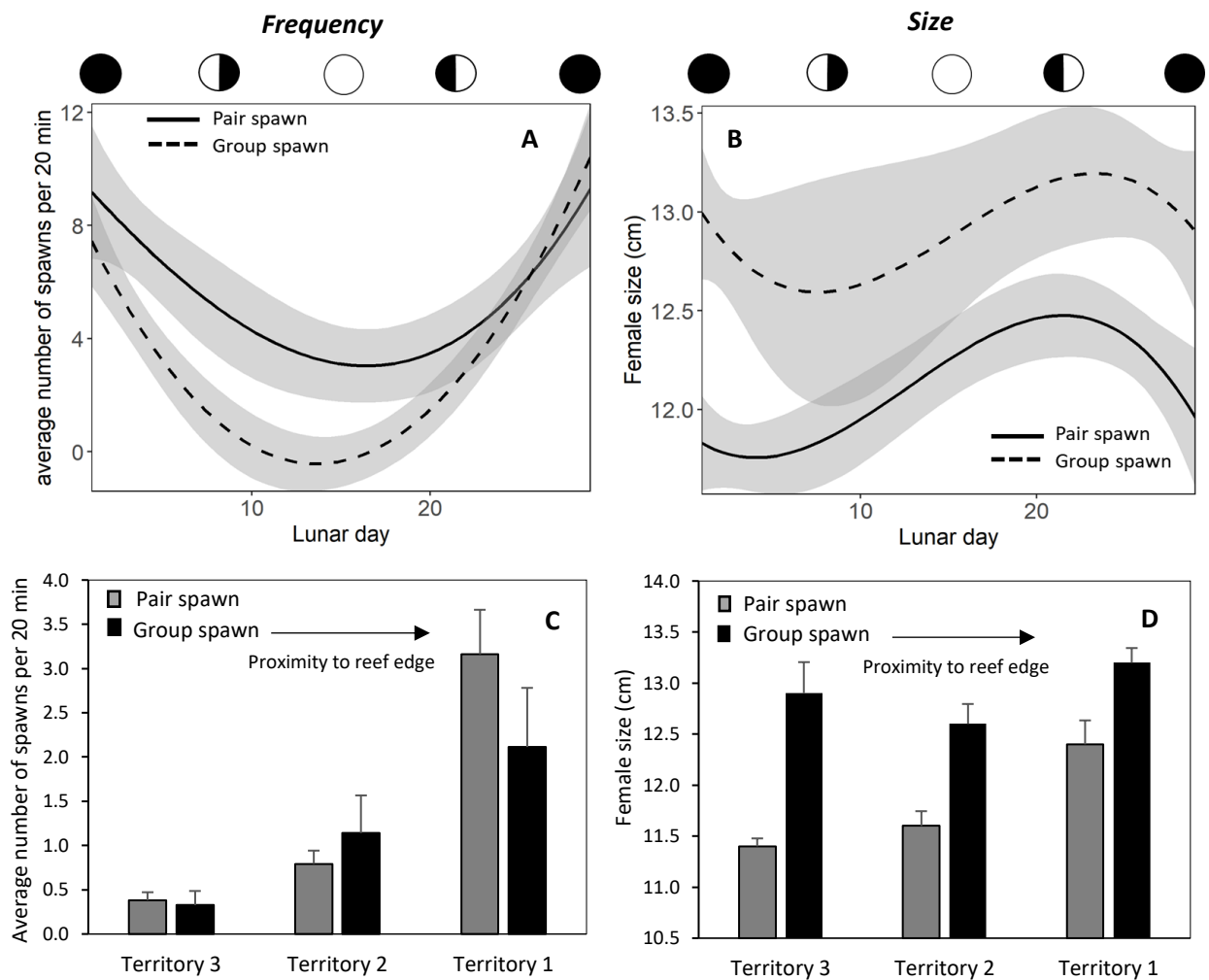
The mean size of group spawners tended to be larger, but also more variable, relative to pair spawners. However, average sizes of group spawners didn't exhibit any significant correlation with the lunar cycle, although showing a qualitatively similar pattern of variation with respect to moon phase (see Table 3.2, Fig 3.1 B). Size distributions of female group spawners appear to be skewed

toward larger size classes due to two main observational difficulties that I faced during surveys: (1) Since IP males and females are phenotypically indistinguishable from another I was not able to differentiate between sexes in a group spawning rush (and IP males are typically slightly larger than females). (2) Group spawning events typically happened in rapid succession and consisted of a sudden upwards rush of several subgroups, encompassing 4 to sometimes 30 females and IP individuals, which made it difficult to visually assess size ranges. Due to these methodological complications, presented mean sizes of group spawning females are probably slightly overestimated and should be treated with caution.

Though sizes of spawners varied with moon phase, spawning locations had a much greater effect on sizes of pair spawners (i.e., territory location accounted for 48% of the variance in pair spawner size). Mean sizes of females involved in pair spawning significantly decreased by ~8% (territory 3) along a gradient from exposed to sheltered (GLM:  $p < 0.001$ ; see Fig 3.1 C). The model also indicated, that average sizes of pair spawning females were significantly higher in a spawning site close to the reef crest (S2) relative to a spawning site located in the middle section of the lagoon (GLM:  $p < 0.001$ ; see Table 3.2). Sizes of group spawning individuals didn't vary significantly among territories (GLM:  $p = 0.09$ ; see Fig 3.1 D) or spawning site ( $p = 0.49$ ).

**Table 3.2** Parameter estimates and test statistics for best models explaining (1) pair spawning frequency and (2) group spawning frequency, as well as size variations in pair and group spawning individuals of *Thalassoma hardwicke* at spawning sites. Models were fit using GLMER (Frequency, poisson distribution) and GLM (Size, gaussian distribution).

Model	variance	levels	Estimate	SE	z value	p value	
<b>pair spawn (frequency)~</b>							
Territory	64.1	Territory 2	-1.38	0.13	-10.64	<0.001	***
		Territory 3	-2.11	0.19	-10.96	<0.001	***
Moon phase: COS ( $\theta$ )	22.8	-	0.54	0.17	3.18	0.001	**
Site	13.0	S2	-0.78	0.24	-3.20	0.001	**
Moon phase: SIN ( $\theta$ )	0.1	-	-0.06	0.18	-0.34	0.74	
<b>group spawn (frequency)~</b>							
Site	54.3	S2	3.43	0.80	4.30	<0.001	***
Moon phase: COS ( $\theta$ )	33.9	-	2.03	0.50	4.03	<0.001	***
Territory	6.5	Territory 2	-0.62	0.14	-4.47	<0.001	***
		Territory 3	-1.62	0.25	-6.52	<0.001	***
Moon phase: SIN ( $\theta$ )	5.2	-	-0.93	0.54	-1.73	0.08	.
<b>pair spawn (female size)~</b>							
Territory	48.6	Territory 2	-0.83	0.16	-5.07	<0.001	***
		Territory 3	-0.96	0.24	-3.94	<0.001	***
Site	28.0	S2	0.63	0.13	4.69	<0.001	***
Moon phase: SIN ( $\theta$ )	13.6	-	-0.30	0.10	-3.12	<0.001	**
Moon phase: COS ( $\theta$ )	9.8	-	-0.18	0.09	-2.01	0.05	*
<b>group spawn (average size)~</b>							
Territory	55.3	Territory 2	-0.53	0.30	-1.75	0.09	.
		Territory 3	-0.33	0.31	-1.05	0.30	
Moon phase: COS ( $\theta$ )	18.0	-	-0.11	0.20	-0.52	0.60	



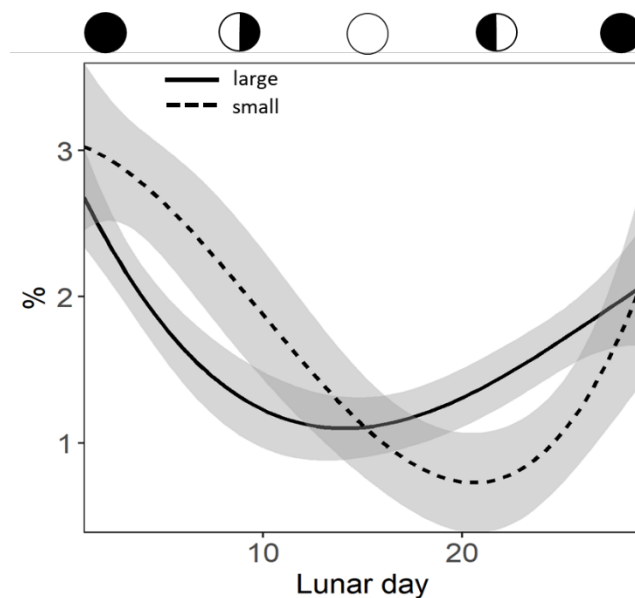
**Fig 3.1** Main sources of variation in spawning frequency (left column) and size of spawning females (right column). **(A)** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted pair and group spawning frequency during the 29.5 lunar cycle. **(B)** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted size of pair spawning females and group spawning females during the 29.5 lunar cycle. Variation in spawning frequency **(C)** and female size **(D)** among territories (T1 is closest to reef edge, T3 furthest from reef edge) Panel C and D give marginal means back-transformed to the original scale ( $\pm$ SE) estimated with other fixed effects held at their mean values.

Because the patterns of variation in mean size of female pair spawners might be the result of small or large females opting in or opting out of spawning at different locations or time, I conducted an additional analysis to attempt to resolve size-dependent spawning patterns in my data. Specifically, I

chose to categorise female pair spawners into two size classes: small individuals (7-11cm) and large individuals (12-16 cm). I then tried to assess variations in pair spawning frequencies of small and large females relative to the lunar cycle (e.g. pair spawning frequency  $\sim$  size category (small/large)\*  $\sin\theta$  + size category (small/large)\*  $\cos\theta$ ) with a GLM model (poisson error distribution). For illustrative purposes, predicted quantities of large and small individuals were transformed into relative numbers. Overall, spawning frequencies of small and large individuals were highest around new moon (as indicated by a significant positive cos term) and lowest around third quarter (see Table 3.3). However, as indicated by the model, a significantly higher proportion of large individuals was spawning around third quarter in comparison to smaller individuals (significant negative  $\sin\theta$  for large individuals; see Fig 3.2 and Table 3.3).

**Table 3.3** Parameter estimates and test statistics for GLM a model (poisson) explaining variations in spawning frequencies of small and large individuals dependent on moon phase of *Thalassoma hardwicke* at spawning sites.

Model	levels	Estimate	SE	z value	p value	
<b>total number of spawns~</b>						
category	large	1.18	0.14	8.57	<0.001	***
SIN	-	0.35	0.16	2.20	0.03	*
COS	-	0.60	0.16	3.81	<0.001	***
size category*SIN ( $\theta$ )	large	-0.40	0.18	-2.18	0.03	*
size category*COS ( $\theta$ )	large	-0.20	0.18	-1.12	0.26	



**Fig 3.2** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on relative spawning frequency (in %) of small (7-11 cm) and larger individuals (12-16cm) during the 29.5 lunar cycle.

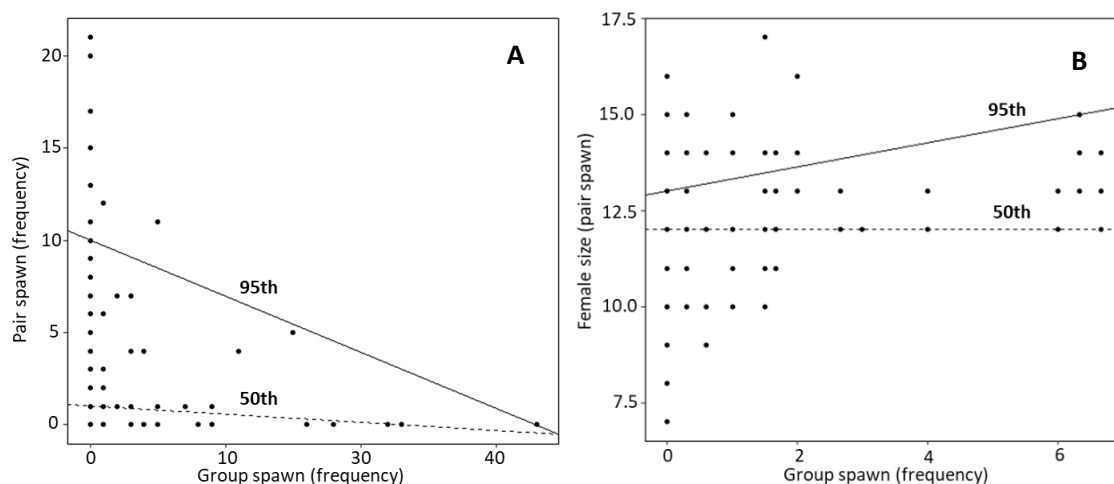
### 3.3.2 Pair versus group spawning

Quantile regression suggests that the frequency of pair spawning declines with increasing frequency of group spawning, at particularly the upper boundaries of the data distribution (see Table 3.4). The regression line of the 95% quantile significantly decreased ( $p < 0.01$ ) in comparison to the 50% regression line, with a change of slopes from -0.04 (50%) to -0.30 (95%). Furthermore, quantile regression indicated that sizes of female pair spawners were increasing with increasing group spawning frequency. The 95% regression line of group spawning vs female size of pair spawners significantly increased ( $p < 0.01$ ) at the 95% level (upper boundaries of the data distribution), with a change of slopes from 0 (50%) to 0.3 (95%). As indicative for a dataset with a limiting factor (e.g. group spawning frequency), the 95% quantile therefore gave the better estimate in changes of pair spawning frequency and sizes of pair spawners in relation to group spawning (see Table 3.4 and Fig 3.3 A+B).

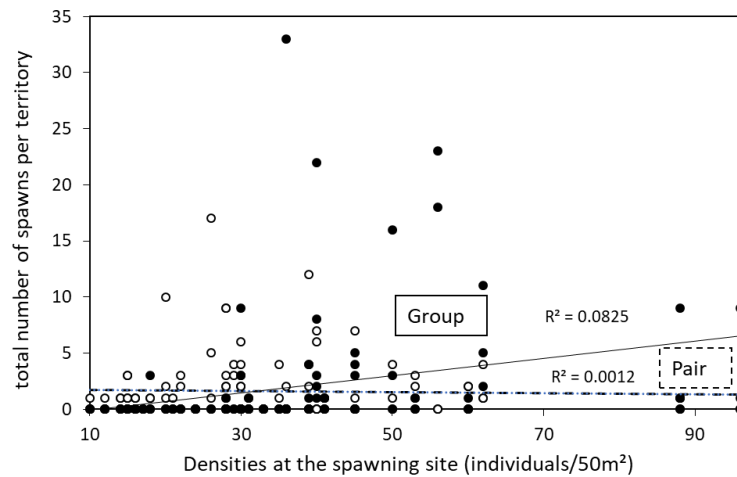
Linear regression analysis suggested that group spawning activities were significantly positively correlated to increasing densities of sixbar individuals (individuals per 50 m<sup>2</sup>) at the spawning site ( $p = 0.002$ ), whereas pair spawning activities didn't show any significant associations with spawning site density ( $p = 0.71$ , see Fig 3.4).

**Table 3.4** Parameter estimates and associated significance for central and upper regression quantile models (0.5 and 0.95 quantile), for changes in pair spawning frequency and sizes of pair spawners according to rates of group spawning at the spawning site

	pair spawn (frequency) ~ group spawn		pair spawn (female size) ~ group spawn	
Quantile	tau = 0.5	tau = 0.95	tau = 0.5	tau = 0.95
Intercept	1.00	10	12	13
Slope	-0.04	-0.3	0	0.32
p value	0.19	<0.001	1	<0.001



**Fig 3.3** Quantile regressions (tau= 50<sup>th</sup> and 95<sup>th</sup> quantile) for responses of pair spawning frequencies per territory and day **(A)** and female sizes of pair spawners per territory and day **(B)** to correspondent group spawning quantities per territory and day. Only the 95<sup>th</sup> quantile is significant for both graphs ( $p < 0.01$ )



**Fig 3.4** Linear regression for responses of pair spawning and group spawning activities (number of spawning events per territory and day) to quantities of females and males (number of individuals per 50 m<sup>2</sup>) at the spawning site. Only group spawning events were significantly correlated to densities at the spawning site ( $p < 0.0001$ ).

### 3.3.3 Sneaking in relation to environmental parameters

#### *Frequencies*

GLMER analysis revealed that sneaking attempts (non-territorial males trying to enter territories but being chased away by territory owners) were again predominantly influenced by moon phase (see Table 3.5). The final model indicated, that activity peaks of sneaker males were strongly correlated with the last quarter and new moon period, while frequency levels markedly dropped around first quarter/full moon (Fig 3.5 A). Furthermore, sneaking attempts exhibited significantly lower frequencies in more sheltered territories (GLMER,  $p < 0.001$ , see Table 3.4). Because of the extreme rare occurrence of successful sneaking or streaking events (e.g. only 3.5 and 0.7 percent respectively out of 649 bouts of spawning), statistical evaluations were not possible. However, a qualitative evaluation of the data suggest that sneaker males may have been slightly more successful around new moon.

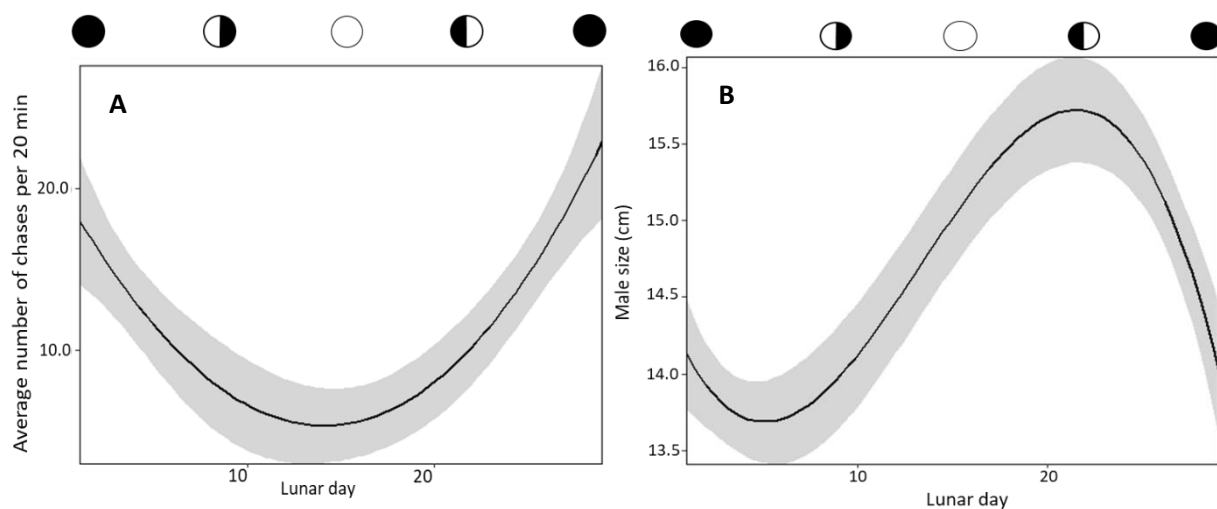


## Sizes

The sizes of unsuccessful sneaking males (i.e., males that attempted a sneak spawn but were chased away by the territory owner) were strongly related to the lunar cycle. Average size of unsuccessful sneakers increased around full moon/third quarter, and significantly decreased around new moon/first quarter (see Fig 3.5 B and Table 3.5). Site and proximity to the reef edge didn't show any significant effect on sizes of sneaker males (see Table 3.5).

**Table 3.5** Parameter estimates and test statistics for best models explaining (1) frequency of sneaking attempts as well as size variations of sneaker males (2) of *Thalassoma hardwicke*. All models were fit using GLMER (Frequency, poisson distribution) and GLM (Size, gaussian distribution)

<i>Model</i>	<i>variance</i>	<i>levels</i>	<i>Estimate</i>	<i>SE</i>	<i>z value</i>	<i>p value</i>	
<b>sneaking attempts (frequency)~</b>							
Moon phase: COS ( $\theta$ )	44.2	-	0.57	0.14	4.07	<0.001	***
Site	32.8	S2	0.66	0.20	3.27	<0.001	**
Moon phase: SIN ( $\theta$ )	16.2	-	-0.39	0.15	-2.56	0.01	*
Territory	6.8	Territory 2	-0.09	0.05	-2.01	0.04	*
		Territory 3	-0.37	0.06	-6.53	<0.001	***
<b>sneaking attempts (male size)~</b>							
Moon phase: SIN ( $\theta$ )	50.3	-	-0.61	0.16	-3.91	0.00	***
Moon phase: COS ( $\theta$ )	28.6	-	-0.45	0.17	-2.66	0.01	**
Site	17.8	S2	-0.40	0.22	-1.83	0.07	.
Territory	3.4	Territory 2	-0.20	0.23	-0.84	0.40	
		Territory 3	-0.30	0.26	-1.16	0.25	



**Fig 3.5** Main sources of variation in frequency and size related to sneaking attempts (~ number of chases). Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted chasing frequency (**A**) and size of sneaker males (**B**) during the 29.5 lunar cycle.

### **3.4 Discussion**

The sixbar wrasse exhibited substantial variability of reproductive investment decisions that depend upon the lunar cycle, location, body size, and reproductive status of individuals. Frequencies of pair spawning, group spawning, and sneaking attempts all significantly increased around the new moon. Frequencies of these activities were also greatest in the most exposed territories (i.e., those located closest to the reef edge). Smaller individuals of both sexes curtailed reproduction during the full moon and third quarter moon, while larger individuals reproduced more consistently across the lunar month. Size-dependent reproductive decisions appear to mediate frequencies of specific reproductive tactics such as pair spawning, group spawning and sneaking strategies.

#### **3.4.1 Temporal variation in female reproductive strategies**

Temporal patterns of pair spawning indicate that larger females spawn throughout much of the lunar month (with elevated activities around new moon), whereas smaller individuals tend to concentrate their reproductive investment around the new moon period. Intraspecific variation in spawning activities may reflect size-dependent trade-offs between reproduction and growth (Warner & Henson, 1997; Warner, 1998; Heino & Kaitala, 1999; Alonzo & Warner, 2000; Clark & Mangel, 2000). Typically, reproductive activities can entail substantial expenses in energy (Nielsen et al., 2012; McBride et al., 2015), as adults must divert their available resources to gametogenesis, migration and reproductive behaviour (Roff, 1991; Shapiro et al., 1994; Warner, 1995; McBride et al., 2015). Consequently, energy investment into reproduction can come at the cost of reduced somatic growth and associated survival potential.

As fecundity and survival potential significantly increases with size in iteroparous protogynous species (Warner, 1984a; Schultz & Warner, 1991; Munday et al., 2006a), smaller females may prioritize growth over reproduction (van Rooij et al., 1995; Warner, 1998) to increase their probability of surviving to spawn at a larger size. Environmental conditions at the new moon may be sufficiently beneficial to

warrant spawning even at a small size. Larger females may have more excess energy available for investment into gametogenesis and migratory behaviour, and thus may be able to afford more frequent reproduction (Warner, 1998). Additionally, larger females may be more willing to engage in reproductive activities, because expected fitness rewards may exceed relative risks associated with reproduction (mortality risk decreases with size, see Warner, 1984b; Hixon & Beets, 1993; Warner, 1998; DeMartini et al., 2005; Welsh et al., 2013).

Increased reproductive activities during new moon suggest that this time may provide a selective advantage for reproduction (e.g. synchronized reproduction, higher adult or offspring survival, see Johannes, 1978; Robertson et al., 1990; Claydon et al., 2014; Shima et al., 2018). So why does selection favour continuous reproduction outside of favourable environmental conditions for larger individuals? Larval survival in oceanic environments varies substantially due to a variety of unpredictable environmental selection pressures (Warner, 1997a; Searcy & Sponaugle, 2000; Shima et al., 2018). Consequently, larger females with sufficient energy resources may increase their fitness, by spreading their reproductive effort over an extended period, to bet-hedge against unpredictable variation in larval survivorship (Wilbur & Rudolf, 2006). This strategy may maximise fitness because the chances that at least some offspring surviving to recruitment may be increased; particularly if pelagic larvae with an inauspicious birthdate can actively alter their developmental trajectories (as indicated by Shima et al., 2018).

### **3.4.2 Temporal variation in male reproductive strategies**

#### *Initial phase males*

Initial phase males (IP males) increased their reproductive effort during the new moon but showed limited reproductive activities during the rest of the lunar month. These findings align with my hypothesis that reproductive investment patterns of small initial phase males should mirror the investment strategies of smaller females (Warner, 1975; Warner, 1998). Because small males are essentially always operating in conditions of sperm competition, they must invest a large proportion of their excess energy into sperm production (testes weights of IP males are much bigger than those of TP males; see Shapiro et al., 1994; Taborsky, 1998; Molloy et al., 2007). Thus, current reproduction is limited by energy, and this implies that IP males might not be able to gather and reallocate enough energetic resources to reproduce over the entire month (Hoffman, 1983; Warner, 1984b). Additionally, initial phase males face many future reproductive events and have the potential to

become territorial males with exclusive access to females. Hence, competitively inferior males should direct a significant portion of their time and energy to foraging activities, at the cost of continuous reproduction, in order to facilitate growth and associated survival to a competitive size (Hoffman, 1983; Warner, 1984b; Warner, 1998).

Given that 95% of all sneaking attempts were unsuccessful, sneaking alone appears to be an extremely ineffective reproductive strategy. So why do initial phase males (i.e., presumably, an alternative reproductive strategy) exist, if at first glance, their probability of successful fertilization of eggs appears to be vanishingly small relative to females? The answer might lie in their behavioural flexibility to switch between alternative tactics during new moon. Territory defence efficiency sometimes dramatically declined with increasing abundances of IP males at the spawning site during new moon. At a certain density threshold, initial phase males quantitatively outcompeted territory owners, and most matings went to IP males in group spawning assemblages. By periodically employing group spawning strategies, IP males might therefore attain a similarly high reproductive success relative to females (Warner, 1975; Warner & Hoffman, 1980ab; Henson & Warner, 1997; Warner, 1998).

Group spawning as an alternative tactic may be evolutionarily maintained in a population if females are either not discriminating between male reproductive phenotypes, or actively choose between male alternatives if direct fitness benefits occur (Warner & Hoffman, 1980ab; Alonzo & Warner, 2000; Brockman & Taborsky, 2008). My analyses indicate that different sized females vary in their choice of male alternatives: smaller females appeared to prefer multi-male group spawning assemblages, while larger females showed a tendency towards pair spawning activities. These findings coincide with behavioural observations on the closely related bluehead wrasse *Thalassoma bifasciatum*. Female blueheads tend to consistently reproduce at group spawning sites when small but shift to pair spawning activities when reaching larger sizes (Warner & Robertson, 1978). It is still not exactly known why females choose one male phenotype over the other dependent on their physiological condition. Potentially, spawning in large groups may reduce mortality risk for smaller females via dilution effects and earlier predator detection (Robertson, 1983), while larger females with lower size-related mortality risk may accrue greater fitness by reproducing with a high-quality male (Henson & Warner, 1997; Alonzo & Warner, 2000). Irrespective of the proximate drivers, size-dependent variation in female choice might maintain a stable co-existence of the two male reproductive phenotypes (initial phase males and terminal phase males) in a sex-changing population (Henson & Warner, 1997; Alonzo & Warner, 2000; Brockman & Taborsky et al., 2008).

### *Territorial males*

Because of their size-related ability to continuously occupy and control a mating site over an extended time, territorial males accrue a substantially higher lifetime reproductive success relative to females and IP males (Warner, 1984a; Warner et al., 1995). However, their fitness only maximizes if they succeed to exclude initial phase males from the mating site. Allocation patterns of terminal phase males thus fundamentally differ from females and initial phase males (Hoffman, 1983; Warner and Hoffman, 1980a; Taborsky, 1998). As their mating success depends on mating site control, territorial males expend most of their energy on aggressive behaviour (their rate of aggression is much higher than in any other reproductive phenotype, see also Warner and Hoffman, 1980a). Consequently, TP males show the lowest growth rates and have relatively small testes in comparison to gonads of females and initial phase males (Hoffman, 1983; Warner and Hoffman, 1980ab; Taborsky, 1998). During the new moon, mating success of territorial males sometimes dramatically declined with increasing numbers of initial phase males at the spawning site. At higher densities, territorial males shifted most of their time towards territory defence, which detracted from time available for mating activities. Even after group spawning assemblages predominated the spawning site, territorial males avoided any participation in group spawning events, and continued to vigorously defend their mating territory. Group spawning, with chronic sperm competition, would yield minimal reproductive success for terminal phase males due to their relatively small testis size (Warner et al., 1995). Thus, territorial males are predicted to prioritize territory defence, because loss of a territory would equal almost complete loss of fertilization success (Hoffman, 1983; Warner et al., 1995). Despite reduced mating success during the new moon, continuous access to female mates outside the new moon period probably overcompensates for any short-term losses in reproduction.

### **3.4.3 Spatial variation in female reproductive strategies**

Highly iteroparous females tend to spawn in locations that maximize immediate survivorship of offspring (Johannes, 1978; Colin, 1992; Whaylen et al. 2006; Claydon et al., 2014). Hence, site quality rather than male quality is the object of female choice (Warner et al., 1998). Preferred mating territories are mostly located in relatively exposed areas that promote dispersal of fertilized eggs away from benthic predators (Johannes, 1978; Lobel, 1978; Sancho et al., 2000; Claydon et al., 2014). The findings of this study align with the general trend (highest pair spawning activity in exposed sites), however mating site preferences exhibited great among-individual variation: smaller females preferred to spawn in sheltered territories, while larger females showed a higher affinity for exposed territories. As females have to ascend to the surface to release their gametes, any spawning event

critically exposes them to pelagic predators. Adults that spawn in territories close to the reef shelf are at particularly high risk to attacks from piscivores that hunt along the reef edge (Johannes, 1978; Robertson & Hoffman, 1977; Robertson, 1983; Moyer, 1987; Sancho et al., 2000). In a few instances, I observed predation attempts (mostly from trevallies) on pair spawning females within exposed territories. Highly iteroparous females in a sex-changing population are predicted to prioritize growth and their own survival over any potential short-term gain in fitness (Warner, 1998). Intraspecific variation in spawning site choice may thus be mediated by size-dependent trade-offs between offspring and maternal survival potential (Shibuno et al., 1993; Warner, 1998). Since predation risk varies inversely with size, smaller females may choose territories in more sheltered areas of the reef to increase their own probability of survival at the cost of increased offspring survival. In contrast, larger females with reduced predation risk may be more willing to reproduce in exposed territories that facilitate offspring dispersal.

#### **3.4.4 Conclusion**

Collectively, these results highlight the diversity of life history strategies present within a species of coral reef fish, and suggest that optimal tactics change with ontogeny, and across the lunar month. Smaller males and females may substantially increase their fitness if they direct most of their energy towards growth and target their reproductive effort using external clues that may indicate the most profitable times (e.g., around the new moon period) and places (e.g., proximity to the reef edge). The relative benefits of this targeted strategy may change as individuals grow larger, because they have more energy available for reproductive investment and a higher, size-related survival potential. My study also shows that intraspecific investment decisions in relation to an environmental context determine the prevailing reproductive mode at a spawning site; if smaller males and females reduce their reproductive effort, pair spawning and sneaking strategies predominate. If they shift resource allocations towards reproductive activities in response to environmental cues, higher abundances at the spawning site lead to group spawning activities. Variation in female choice might thereby act as a mechanism for maintaining these two reproductive modes (pair spawning and group spawning) in a population.



## **CHAPTER FOUR**

PLASTICITY IN ENERGY ALLOCATION STRATEGIES OF A PROTOGYNOUS CORAL REEF  
FISH SPECIES: THE INFLUENCE OF SIZE AND ENVIRONMENTAL VARIATION ON  
ACTIVITY BUDGETS AND MOVEMENT PATTERNS



## 4.1 Introduction

Life-history theory suggests that an organism must balance its available energy between two competing physiological processes to maximize fitness: reproduction and somatic growth. Energetic trade-offs are a fundamental concept of life history theory, and form the basis of intra- and inter-specific variation in life-history strategies (Roff, 1983; Stearns, 1992). In fishes, reproduction-growth trade-offs are an essential component of life-history optimization (Roff, 1983). Since most fishes continue to grow after maturity (i.e., indeterminate growth, see Heino & Kaitala, 1995), individual fecundity typically increases with body size (Bagenal, 1978; Wootton, 1998). Consequently, energy allocation strategies may vary among individuals of a given species, in part, because investment in current reproduction may come at a cost to future reproductive success (Reznick, 1985; Warner, 1998; van Rooij et al., 1995). Trade-off strategies can vary from skipped spawning (Warner, 1984b; Jorgensen et al., 2006; Rideout & Tomkiewicz, 2011), where individuals refrain from reproduction (to promote faster growth, and therefore survival), to income-breeder strategies, where acquired energy is immediately transferred into reproductive effort (i.e. extreme iteroparity, see Warner, 1998; Wilbur & Rudolf, 2006; Shima et al., 2018).

Trade-offs between current and future reproduction are likely to play a particularly important role in life history trajectories of sex-changing fishes. In mating systems with protogynous sex-change (the most common form of sex-change in coral reef fish species), mating opportunities are often monopolized by a few large territorial males (Warner & Hoffman, 1980ab; Warner, 1988a). As a result, reproductive success rapidly and disproportionately increases with body size and corresponding social status (Warner, 1984a; Warner, 1988a). Thus, lifetime fitness of a sex-changing species is inherently linked to patterns of growth, and energy allocation strategies determined by an individual's size-specific rank within the dominance hierarchy (Hoffman, 1983; van Rooij et al., 1995; Wong et al., 2012). In response to this mating system, low-ranking females and males may invest disproportionately in somatic growth, e.g., by increasing foraging activities (Warner, 1984b). In other words, such individuals may be more likely to forego current reproduction to increase probability of attaining a future size-related competitive advantage (i.e., as a territory-holding male). Alternatively, competitively superior territorial males may opt to invest greater energy to current reproduction, as their fitness is maximized by non-foraging activities, such as mating behaviour and territoriality (Warner & Hoffman, 1980 ab; Hoffman, 1983; van Rooij et al., 1995). In such mating systems, smaller males and females are predicted to allocate more resources to foraging to maximise growth and future reproductive potential, while larger territorial males should allocate more resources to current

reproductive activities (Schoener, 1971; Hoffman, 1983; Sano, 1993). If described sex-specific trade-offs between time spent foraging and time spent reproducing exist, initial phase males and females may exhibit significantly higher feeding rates throughout the day relative to large, territorial males. Conversely, territorial males may be more engaged in aggressive interactions, and show reduced foraging activities.

Size-dependent variation in the relative investment to growth and reproduction appears to be common in sex-changing, iteroparous species. For example, patterns of somatic growth in parrotfish (*Sparisoma viridis*; van Rooij et al., 1995) suggest that enhanced reproductive effort leads to lower growth rates in territorial males. Analyses of otolith microstructure of the wrasse *Halichoeres miniatus* suggests that successful territorial males are comprised of a subset of individuals that exhibited disproportionately fast growth rates during their earlier life stages (McCormick et al., 2010).

Size- and sex-specific allocation strategies might further vary through time. Many iteroparous reef fish species tend to synchronize their reproductive activities to lunar or tidal cycles. Spawning periodicity in relation to external cues may provide several reproductive benefits for adults and their offspring. Synchronized reproduction might enhance reproductive success due to diluted predation rates on adults and eggs, increase mate-encounter rates, improve mate choice opportunities and enhance fertilization success (Johannes, 1978; Lobel, 1978; Shapiro et al., 1988, Robertson et al., 1990). Additionally, larval survival in marine environments is highly variable and dependent on hydrodynamic regimes that facilitate fast dispersal of fertilized eggs (Johannes, 1978; Robertson et al., 1990; Zeller, 1998; Sancho et al., 2000; Takemura et al., 2010). Hence, production of a high number of offspring during conditions that benefit larval survival such as lunar-associated tidal regimes, significantly improves the net gain in adult fitness (Robertson et al., 1988; Claydon et al., 2004; Claydon et al., 2014; Shima et al. 2018). Consequently, energy allocation strategies and activity budgets may not only be a function of body size, but also be shaped by environmental cues that enhance adult and offspring fitness. For example, in highly iteroparous species with lunar reproductive cycles, social activities might significantly increase at specific lunar phases (e.g. full or new moon), while foraging activities may decrease considerably. These temporal variations in activity budgets might additionally differ between smaller and larger individuals, with larger individuals allocating relatively more of their time into social activities during certain lunar phases relative to smaller individuals, potentially at the cost of foraging activities.

Here, I evaluate the hypothesis that the lunar cycle affects energy allocation decisions during non-spawning hours. Specifically, I predict that activity budgets and mobility, such as feeding rates, social interactions and fine-scale movement patterns during non-spawning hours, should vary across the lunar cycle. Additionally, I evaluate whether these energy allocation patterns depend upon phenotypic status/body size. As in previous chapters, my work focuses on the protogynous coral reef fish species, the sixbar wrasse *Thalassoma hardwicke*. My results from chapters 2 and 3 indicate that spawning frequency of the sixbar wrasse is strongly linked to the lunar cycle (with a significant increase of spawning frequencies during new moon), and that reproductive investment also depends upon body size. Insight into intraspecific variation of energetic trade-offs relative to an environmental context will help to improve our understanding of the complex behavioural patterns of an iteroparous, protogynous reef fish species.

## **4.2 Methods**

### **4.2.1 Field surveys**

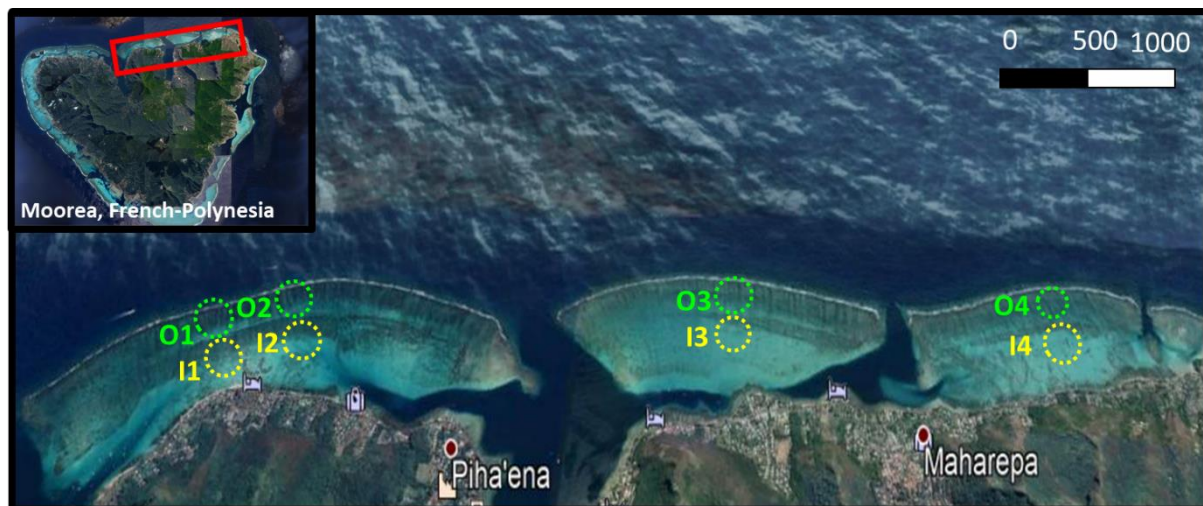
I surveyed daily activity patterns of the sixbar wrasse (*Thalassoma hardwicke*) from February to June 2017, at focal sites on the north shore of Moorea, French Polynesia. The sixbar wrasse is one of the most abundant reef fish species throughout the Indo-Pacific. Adults are diurnally active and forage over particular home ranges (Shima 1999a), targeting a variety of prey, including fish eggs (Shima & Osenberg, 2003), benthic and planktonic crustaceans, small fishes, foraminiferans (Sano et al., 1984) and fish larvae (Holbrook et al., 2003; see chapter 2 for more details of study system and species).

Study sites were evenly distributed across the north shore lagoon and included 4 offshore and 4 inshore sites (Fig 4.1). Habitat structure across sites was fairly consistent and characterized by moderate to relatively low coral cover, extensive patches of sand and coral rubble, as well as dense stands of macroalgae.

To investigate size-specific movement and behavioural patterns of the sixbar wrasse, I conducted an extensive observational study over 5 lunar cycles. At each site, I selected four focal individuals that were easily distinguishable from each other based on size and colour phase (e.g., a small and a large IP; a small and a large TP) or other distinctive features (e.g., little injuries or prominent coloration patterns) to avoid repeated measurements of the same individual per site and day. After selecting a focal individual, I visually estimated its size (total length, TL, to the nearest cm) and colour phase (initial phase, IP; terminal phase, TP). I then followed each focal individual for 15 min, scoring key behaviour patterns. The following behaviour patterns were recorded: (i) feeding (=biting the substrate or

chewing), (ii) chases or being chased by conspecifics, (iii) group swimming, (iv) any other intra- or inter-specific social interactions. I defined group swimming as an activity, where an individual continuously swam in company of other conspecifics for more than 30 seconds. I conducted surveys for about 2-4 hours a day, 3-5 times per week, over several day times and lunar cycles. I attempted to remain >3m from focal individuals to avoid disturbing natural behaviour patterns. I varied the time of day of observations (stratified across lunar phases and sites) so that each focal area was sampled at a range of different times (time periods: 9-11h, 11-13h, 13-15h, 15-17h). I then evaluated spatial and temporal variation in behaviour patterns as a function of lunar phase, time of day, colour phase (IP or TP), and size of an individual.

To supplement my observations of behaviour patterns (described above), I also estimated movement patterns of each focal individual using a towed GPS unit (a handheld unit in a waterproof case, towed in a float behind me). The start and end time of every tracking period was noted with a waterproof watch, and the approximate location of each individual was recorded every 30 seconds with the GPS. These GPS tracks allowed me to evaluate statistical properties of short-term movement patterns, including an estimate of total distance, step length, turning angle and m<sup>2</sup> area covered. I evaluated variation in these metrics as a function of fish size, putative sex (as indicated by specific colour patterns), time of day, and lunar phase.



**Fig. 4.1** Study sites across the north shore of Moorea, French-Polynesia. Activity and movement patterns of IP/TP sixbars were surveyed within 4 inshore and 4 offshore areas (O1, O2, O3, O4, I1, I2, I3, I4) of the lagoon. Location maps are produced from Google Earth Pro (2019).

#### 4.2.2 Analysis of movement patterns

I used the *adehabitat* package of R (Calenge, 2006) to evaluate the activity space (in m<sup>2</sup>) a focal individual covered during the 15 min of observation. Activity spaces were estimated from 95% Minimum convex polygons (MCP) for each focal individual. MCP estimates are generated in the *adehabitat* package by constructing the smallest convex polygon that encompasses 95% of all locational fixes (Powell, 2000).

Additionally, I characterised fine-scale movement patterns by calculating mean step length (distance in m between consecutive points), total travel distance (cumulative step length) and mean turning angle (degree), with the *moveHMM* package in R (Michelot et al., 2016). Large distances, high step lengths, and low turning angles are typically characterized by highly directional swimming. Small distances, low step lengths and high turning angles represent more undirected and clustered movements (as typical for foraging individuals, Michelot et al., 2016).

#### 4.2.3 Data analysis

A primary objective of this chapter is to understand if/how activity and movement patterns vary with moon phase, time of the day, sex and size of an individual. Since moon phase is a cyclical variable, I characterised lunar day with Sine and Cosine terms to capture the cyclical components of the first/last quarter moons and new/full moons respectively, and I explored variation as a function of moon phase using periodic regression (see chapter 2 and 3).

I removed collinearity between body size (TL) and colour phase (TP individuals are generally larger than IP individuals), by standardizing each individual's TL relative to the mean TL of each colour phase. More specifically, *standardized TL* represents the residuals from a one-way ANOVA of differences in TL between colour phases. This allows me to include fish size as well as colour phase (TP or IP) as independent variables into one model.

I used general mixed models with random effects (GLMERs) with poisson distributions to investigate the effect of lunar phase (expressed in  $\cos\theta$  and  $\sin\theta$  terms), colour phase, time of the day and size on following activity patterns: (1) feeding rates (2) chasing activities (chased and being chased combined) and (3) swimming with other conspecifics (group swimming). I also included location and date into the model as random effects. General linear models with a gaussian error distribution were used to evaluate the effect of above-mentioned independent variables on movement patterns: (4) activity space (MCP), (5) total distance (cumulative step length) and (6) turning angle. Because the random

effects 'date' and 'location' accounted for <1% of the unexplained variation for all models containing movement parameters, they were not included into statistical evaluations of movement patterns. Additionally, all movement variables (apart from turning angle) were log-transformed due to excessive deviations from normality.

Initially, I fitted the following model with all main effects, and a subset of interactions that I considered to be potentially important:

Dependent variable  $\sim \sin\theta + \cos\theta + \text{Size residuals} + \text{time of day} + \text{colour phase} + \sin\theta * \text{Size residuals} + \cos\theta * \text{Size residuals} + \text{Time of the day} * \text{Size residuals} + \text{Colour phase} * \text{Size residuals}$

I then used model selection criteria (AICc; Burnham and Anderson 2002) to identify a reduced model, (using the subset of main effects and interactions that yielded the lowest AICc; calculated with lme4 and MuMIN packages in R, see Barton, 2015). To facilitate visualization of model parameters, I calculated the predicted responses of the dependent variables with the 'predict' and 'cbind' function of the lme4 package (Bates et al., 2012), as well as with the visreg package in R (Breheny & Burchett, 2017). For the effect of categorical variables on spawning patterns, I estimated marginal means for each predictor, with covariates held at their mean value (using function emmeans in the R package emmeans; Lenth, 2018).

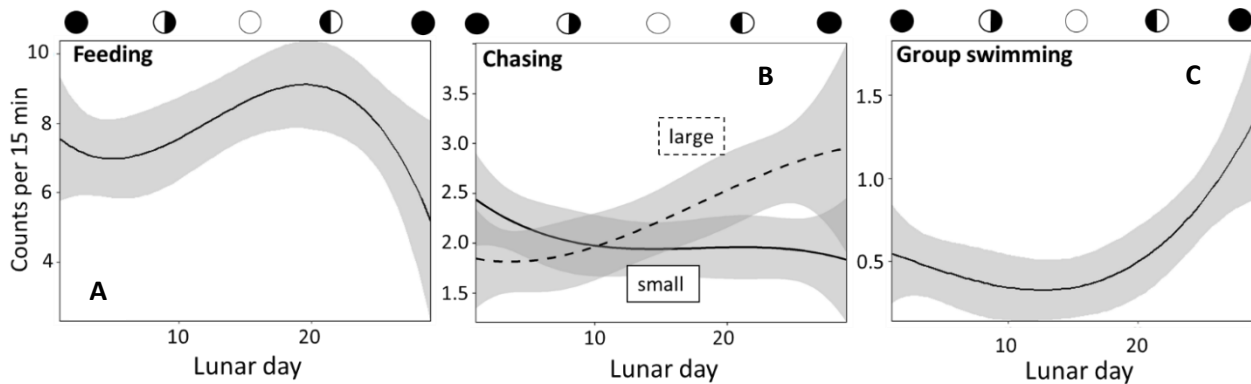
## 4.3 Results

### 4.3.1 Activity patterns

In total, I observed 255 individuals over 51 days of observation. Sizes of recorded individuals varied between 7 and 19 cm total length (TL). 66.6 percent of all observed individuals were initial phase (IP), and 33.4 percent terminal phase (TP) individuals. 74% of all documented activities were feeding activities (45% biting the substrate and 29% planktivorous feeding), 14.5% chasing activities (10% chased and 4.5 % being chased by conspecifics), 6 % group swimming and 6 % being chased by pomacentrids.

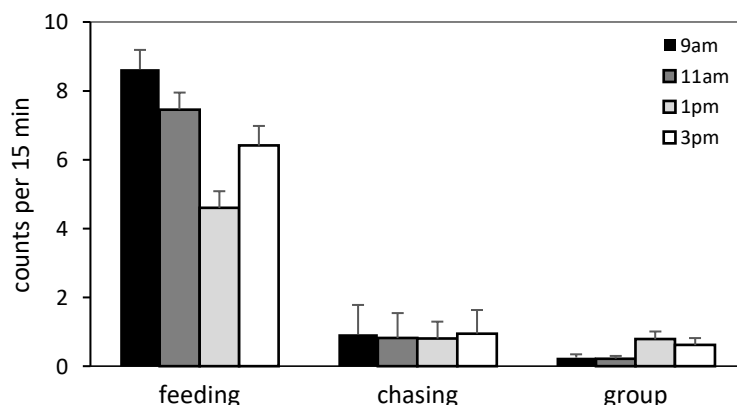
The best fitting GLMER models (see Table 3, appendix 4.1.4.) indicated, that the lunar cycle ( $\cos\theta$  term) was a significant determinant of feeding activities as well as social interactions (see Table 1). A negative  $\cos\theta$  indicated, that overall bite rates were significantly enhanced around full moon (GLMER:  $p=0.01$ ), and notably dropped around new moon (almost 50% decline in comparison to full moon

periods, see Fig 4.2 A). Social interactions (chasing activities and swimming with other conspecifics) generally increased around third quarter and new moon (see Fig 4.2 B and C, Table 4.1), although only larger individuals were engaged in higher chasing activities towards the end of the lunar month (as indicated by significant interaction terms between individual size and the  $\sin\theta$  as well as  $\cos\theta$  predictors, GLMER:  $p<0.001$ , see Fig 4.2 B, Table 4.1).

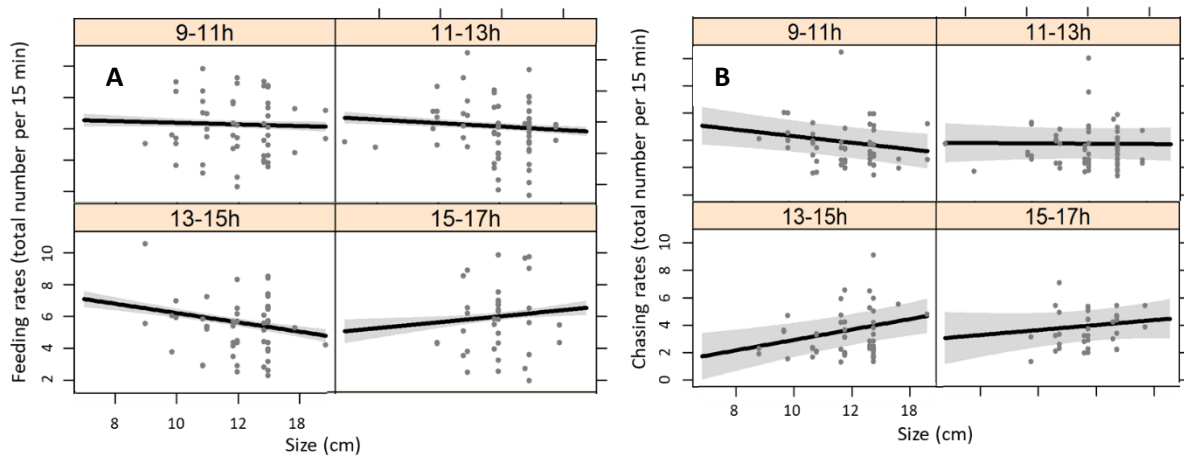


**Fig 4.2** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted feeding (A), chasing (B) and group swimming frequencies (C) during the 29.5 lunar cycle. For illustrative purposes, predicted chasing frequencies were split by size (small individuals: 7-13 cm; large individuals: 13-19 cm), to visualize a significant interaction effect between size and lunar phase on chasing activities.

Bite rates and group swimming activities varied with time of the day and size of an individual (see Fig 4.3 and 4.4 A/B). In general, feeding rates were highest during the morning hours, and significantly decreased shortly before spawning hours (see Fig 4.3 and Table 4.1). A significant positive correlation between individual size and feeding rates during spawning hours (1 - 3 pm) implied that larger individuals notably lowered their food intake around this time of the day, and increased agonistic interactions (GLMER:  $p<0.001$ , see Table 4.1). In contrast, feeding rates markedly increased shortly after spawning hours (3-5pm) for larger individuals (GLMER:  $p<0.001$ ). Group swimming activities also significantly increased around spawning hours but didn't show any size dependent patterns (see Table 4.1).

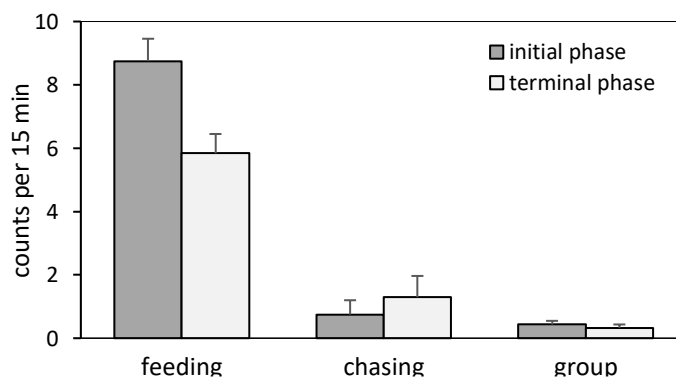


**Fig 4.3** Variation in feeding rates, chasing rates and group swimming rates dependent on time of the day. Given are marginal means, back-transformed to the original scale ( $\pm$ SE), estimated with other fixed effects held at their mean values.



**Fig 4.4** Interaction plot of the predicted effect of time of the day on feeding **(A)** and chasing rates **(B)**, dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly during spawning hours (13-15h), size had a strong effect on variation in feeding and chasing rates (i.e., feeding rates decreased significantly with size, and chasing rates significantly increased with size during spawning hours)

The best-supported reduced models (see Table 4.1 and appendix 4.1) also indicated that feeding rates of IP individuals were significantly higher than for TP individuals (see Table 4.1, GLMER:  $p < 0.001$ ; post-hoc Tukey test:  $p < 0.001$ ). Colour phase was also a significant predictor of chasing activities, with a higher proportion of TP individuals being involved in chasing activities (GLMER:  $p = 0.02$ , see Fig 4.5 and Table 4.1, appendix 4.2). Although colour phase was not a significant predictor of group swimming activities, the final candidate model indicated that group swimming activities were positively correlated with individual size (significant influence of size residuals on rates of group swimming, GLMER:  $p < 0.001$ , see Table 4.1 and appendix 4.3).



**Fig 4.5** Variation in feeding rates, chasing rates and group swimming rates dependent on colour phase (initial phase or terminal phase). Given are marginal means, back-transformed to the original scale (+SE), estimated with other fixed effects held at their mean values.



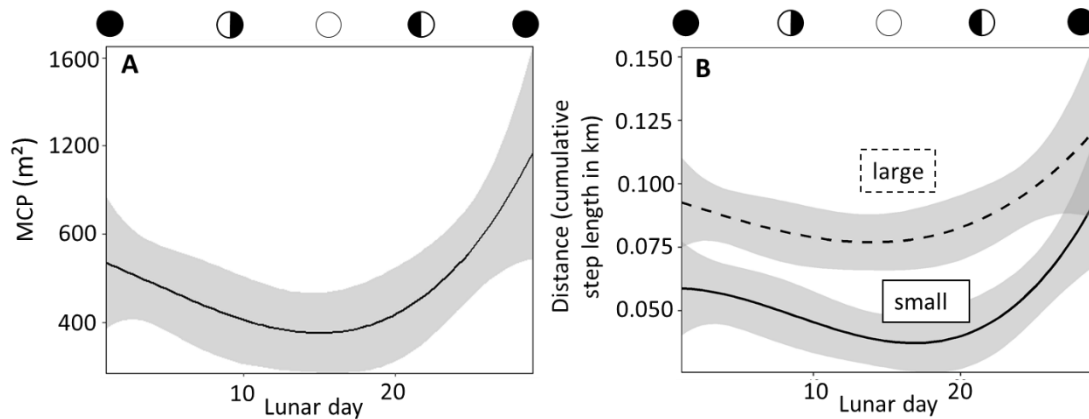
**Table 4.1** Parameter estimates and test statistics for best models explaining (1) feeding (2) chasing and (3) group swimming frequencies of *Thalassoma hardwicke* at feeding sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). Models were fit using GLMER (Poisson distribution. Asterisk symbols reflect significance levels.

<i>Model</i>	<i>levels</i>	<i>Estimate</i>	<i>SE</i>	<i>z value</i>	<i>p value</i>		<i>AICc</i>
<b>feeding ~</b>							
Moon phase: COS ( $\theta$ )	-	-0.09	0.04	-2.16	0.03	*	
Size residuals	-	-0.05	0.04	-1.36	0.18		
Colour phase	TP	-0.34	0.06	-5.82	<0.001	***	
Time of day	11-13 h	-0.13	0.07	-1.93	0.05	.	
	13-15 h	-0.65	0.09	-7.68	<0.001	***	
	15-17 h	-0.27	0.08	-3.32	<0.001	***	
Size residuals*Time of the day	*11-13 h	-0.06	0.05	-1.17	0.24		
	*13-15 h	-0.24	0.06	-3.90	<0.001	***	
	*15-17 h	0.24	0.08	3.05	<0.001	**	1482
<b>chasing ~</b>							
Moon phase: COS ( $\theta$ )	-	0.02	0.10	0.24	0.81		
Moon phase: SIN ( $\theta$ )	-	-0.23	0.09	-2.54	0.01	*	
Size residuals	-	-0.22	0.09	-2.39	0.02	*	
Colour phase	TP	0.22	0.12	1.82	0.02	*	
Time of day	11-13 h	-0.07	0.17	-0.44	0.66		
	13-15 h	-0.25	0.19	-1.29	0.20		
	15-17 h	0.14	0.20	0.70	0.48		
Size residuals*Time of the day	*11-13 h	0.23	0.13	1.82	0.07	.	
	*13-15 h	0.60	0.15	3.97	<0.001	***	
	*15-17 h	0.44	0.18	2.49	0.01	*	
COS ( $\theta$ )*Size residuals	-	0.26	0.08	3.25	<0.001	**	
SIN ( $\theta$ )*Size residuals	-	-0.19	0.07	-2.69	0.01	**	860
<b>group swimming~</b>							
Moon phase: COS ( $\theta$ )	-	0.50	0.17	2.86	<0.001	**	
Moon phase: SIN ( $\theta$ )	-	-0.47	0.16	-2.86	<0.001	**	
Size residuals	-	0.39	0.09	4.22	<0.001	***	
Time of day	11-13 h	-0.06	0.34	-0.17	0.87		
	13-15 h	1.21	0.34	3.51	<0.001	***	
	15-17 h	0.95	0.37	2.55	0.01	*	398

#### 4.3.2 Movement patterns

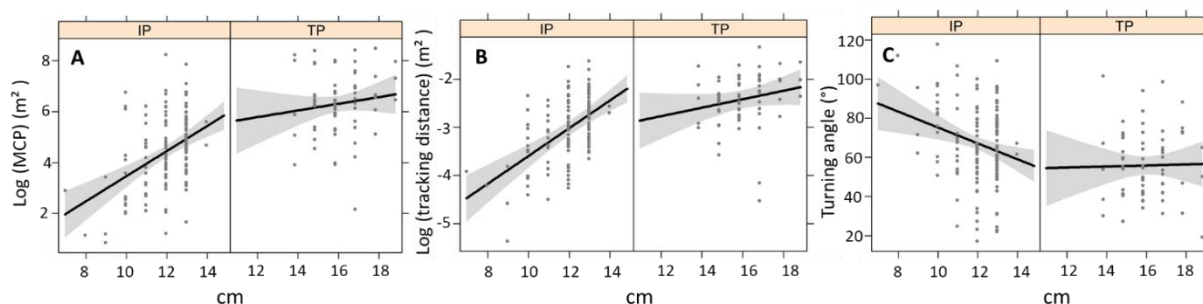
The mean distance individuals travelled in a 30 s time step (consecutive GPS locations were recorded at 30s intervals) varied between 60 cm and 10 meters. Cumulative step length (i.e., total distance in m within a 15 min time period) ranged from 8 to 300 m. Activity spaces, as estimated by the 95% probability contour of a minimum convex polygon, varied between 2 and 6418 m<sup>2</sup>.

A negative  $\sin\theta$  regression coefficient in the final model (see Table 4.2, appendix 4.4) suggested, that activity area (95% MCP) as well as mean cumulative step length (m) were significantly higher during third quarter/beginning of new moon (GLM:  $p=0.04$  and  $p=0.03$  respectively, see Table 4.2). Mean MCP area increased from an average of about 400 m<sup>2</sup> around full moon, to 1200 m<sup>2</sup> around the last quarter of the lunar cycle. Mean cumulative step length increased almost 2-fold from about 60 m around full moon, to about 110 m around the last quarter (see Fig 4.6 A and B). A significant positive parameter estimate for the interaction between size and  $\sin\theta$  suggests that smaller individuals reduce their travel distances significantly more than larger individuals during the first quarter of the lunar month (GLM:  $p=0.03$ , see Fig 4.6 B and Table 4.2). Model selection suggested (see appendix 4.6), that the lunar cycle didn't affect variation in turning angles (though preliminary analysis indicated a decrease of mean turning angle around new moon).



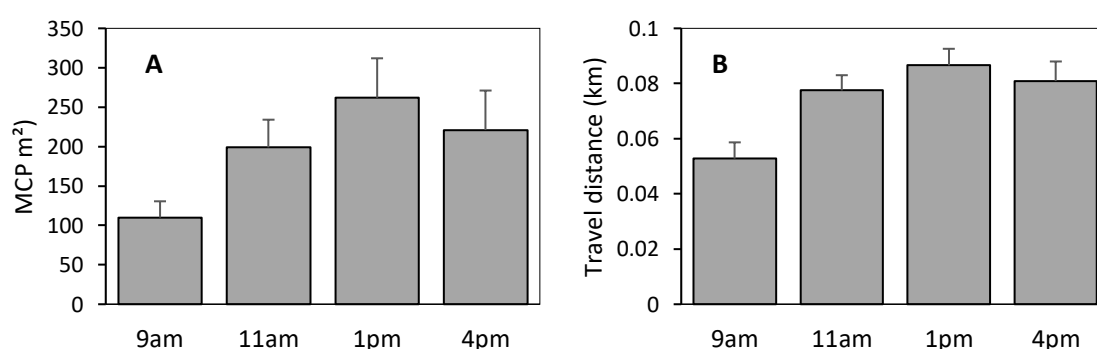
**Fig 4.6** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted MCP area (95% probability contour) **(A)**, and total travel distance **(B)** during the 29.5 lunar cycle. For illustrative purposes, predicted cumulative step lengths were split by size (small individuals: 7-13 cm; large individuals: 13-19 cm), to visualize a significant interaction effect between size and lunar phase on tracking distance.

Mean MCP area, cumulative distance and turning angle significantly varied with size and colour phase. Mean MCPs and travel distances tended to be larger for TP individuals than for IP individuals. In general, mean MCP and cumulative step length increased with body size (GLM:  $p<0.001$ ). However, an increase of activity space/cumulative step length was more strongly size-dependent for IP individuals than for TP individuals (see table 4.2, Fig 4.7 A and B). IP individuals tended to have significantly higher turning angles than TP individuals, and turning angles generally decreased with body size (see Table 4.2). However, correlation coefficients suggest that variation in turning angle may be dependent on body size, and this relationship differed between IP and TP individuals: While turning angles rapidly decreased with IP size, TP individuals didn't show any significant correlations between turning angle and size (GLM:  $p=0.06$ , see table 4.2 and Fig 4.7 C).



**Fig 4.7** Interaction plot with the predicted effect of colour phase (IP=initial phase; TP=terminal phase) on MCP area (A), cumulative step length (tracking distance) (B) and turning angle (C), dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly for IP individuals, size had a strong effect on variations in movement variables (with a significantly positive increase of MCP area and cumulative step length with size, and a significantly negative decrease of mean turning angle with size for IP individuals).

Mean MCP and cumulative step length also exhibited strong diurnal variation. Both variables were significantly higher during spawning hours between 1-3 pm (GLM:  $p < 0.001$ ), in comparison to morning and late afternoon hours (see Fig 7A and B). Turning angles on the other hand didn't show any significant variation throughout the day (whereas preliminary analysis indicated a decrease in mean turning angle during spawning hours). Best fitting models for all variables suggested, that variation across the day was not structured by size (i.e. interaction terms between size and time of the day were not significant, and therefore excluded from the best fitting models, see Table 4.4, 4.5 and 4.6 appendix).



**Fig 4.8** Variation in (A) activity space (90% mcp) and (B) travel distances dependent on reproductive phenotype (initial phase or terminal phase). Given are marginal means, back-transformed to the original scale (+SE), estimated with other fixed effects held at their mean values.

**Table 4.2** Parameter estimates and test statistics for best models explaining (1) activity space (95% minimum convex polygon) (2) tracking distance (cumulative step length) and (3) mean turning angle of *Thalassoma hardwicke* at feeding sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). Models were fit using GLMER (Gaussian distribution). MCP area and tracking distance were log transformed. Asterisk symbols reflect significance levels.

<i>Model</i>	<i>levels</i>	<i>Estimate</i>	<i>SE</i>	<i>z value</i>	<i>p value</i>		<i>AICc</i>
<b>Log(MCP)~</b>							
Moon phase: SIN ( $\theta$ )	-	-0.26	0.13	-2.07	0.04	*	
Size residuals	-	0.48	0.09	5.44	<0.001	***	
Colour phase	TP	1.88	0.19	9.69	<0.001	***	
Time of day	11-13 h	0.55	0.24	2.27	0.02	*	
	13-15 h	0.84	0.25	3.35	<0.001	***	
	15-17 h	0.62	0.28	2.22	0.03	*	
Size residuals*colour phase	*TP	-0.34	0.16	-2.22	0.03	*	686
<b>Log(cumulative step length)~</b>							
Moon phase: SIN ( $\theta$ )	-	-0.12	0.06	-2.17	0.03	*	
Size residuals	-	0.27	0.04	6.48	<0.001	***	
Colour phase	TP	0.61	0.09	7.09	<0.001	***	
Time of day	11-13 h	0.30	0.11	2.80	0.01	**	
	13-15 h	0.44	0.11	3.91	<0.001	***	
	15-17 h	0.37	0.12	2.98	<0.001	**	
Size residuals*colour phase	*TP	-0.21	0.07	-2.86	<0.001	**	
SIN ( $\theta$ )*Size residuals		0.11	0.05	2.17	0.03	*	355
<b>turning angle~</b>							
Size residuals	-	-4.05	1.33	-3.04	<0.001	**	
Colour phase	TP	-11.30	2.85	-3.96	<0.001	***	
Size residuals*colour phase	*TP	4.31	2.32	1.86	0.06	.	1788

Together these results suggest that individuals tend to move faster, and more directionally during the last quarters of the lunar cycle and around spawning hours. Movement characteristics also indicate colour phase-specific tendencies, with smaller IP individuals exhibiting more clustered, and non-directional movements in comparison to larger TP individuals.

## 4.4 Discussion

The present study confirms my initial hypothesis that fish of different social status and size allocate their time to foraging and reproduction-related activities in different ways. Consistent with initial predictions, allocations to foraging versus reproductive activities also vary across the lunar month and are dependent on individual size. Additionally, my results confirm that the lunar phase and social status influence investment decisions that ultimately relate to foraging behaviour or reproductive activities, like habitat use and fine-scale movement patterns.

### 4.4.1 Variation across the lunar month

#### *Behavioural activities*

Spawning periodicity of the sixbar wrasse *T. hardwicke* is closely linked to the lunar cycle (with highest spawning frequencies around new moon, see chapter 2 and 3). This pattern may indicate that spawning at specific lunar phases is adaptive. Results from the present study suggest that the lunar cycle also influences time budgets and activity patterns of a protogynous coral reef fish species outside of spawning hours. Time budgets and activity patterns of the sixbar wrasse exhibited distinct variation across the lunar month, with increased feeding rates during full moon and a steep decline of feeding rates close to new moon. Social activities like chases and group swimming significantly increased at new moon and declined to consistently low levels around first quarter and full moon.

These patterns indicate that individuals invest relatively more energy into spawning-related patterns, like chasing activities and group swimming, during conditions of enhanced reproductive effort (as indicated by increased reproductive activities during new moon, see chapter 2 and 3). Increased aggressive interactions and group swimming around the new moon phase might be important mechanisms to assess potential competitors and to consolidate positions within the dominance hierarchy. Since females tend to arrive in smaller groups at spawning sites during new moon (based on personal observations, but also reported for a closely related species, the bluehead wrasse *Thalassoma bifasciatum*, see Warner 1988a, 1995), increased group swimming might represent early initiations of migratory behaviour. Reduced investment into reproduction-related activities prior to new moon may naturally increase the time available for feeding activities. However, a rapid increase of feeding activities just before the third quarter might also indicate that individuals strategically accumulate energy resources during specific portions of the lunar month, to optimize their reproductive potential during better times of the month. Enhanced food consumption prior to a

reproductive period can determine the number and quality of eggs an individual is able to produce, and it may also be required for energetically costly migrations to a spawning site (e.g. Slotte, 1999; Donelson et al. 2008; Friedland et al., 2009; Chapman et al., 2011; Brooker et al., 2013; Carter et al., 2014). For example, Pecquerie et al. 2009 predicted that batch fecundity of the European anchovy qualitatively varied dependent on feeding conditions prior to a spawning episode. Warner, 1995, demonstrated that higher food intake energetically compensated for metabolically demanding, long-distance migrations in the bluehead wrasse *Thalassoma bifasciatum*. Elevated food consumption shortly before a period of high reproductive effort may suggest that the sixbar wrasse fuels spawning during new moon (which, collectively, requires enhanced gonadal production, long-distant migration and increased behavioural reproductive activities) through stored energy obtained during increased foraging around the full moon. These strategies are commonly also described as mixed breeding strategies, where a typical income breeder supplements available energy for gonad production and other accessory activities, with energy that was accumulated during an earlier feeding period (Stephens et al., 2009; Villegas-Rios et al., 2014; McBride et al., 2015).

### *Movement patterns*

Movement patterns also suggest variation across the lunar month, with significantly larger activity spaces and longer travel distances around the new moon period. These patterns are most likely associated with increased group swimming and chasing behaviours that may signal the onset of spawning, and mediate a shift towards directional movement characteristics. Large-scale spawning migrations during the new moon may further account for longer travel distances and larger activity spaces. Just before spawning hours (12 am -2 pm) at the new moon, IP and TP individuals regularly abandoned any social behaviour or foraging activities and started to move extremely fast and unidirectionally. Collectively, these patterns suggest that the new moon period seems to initiate early, long-distance mass migrations to spawning sites, leading to the formation of large group spawning assemblages at mating sites (see chapter 2 and 3). I hypothesise that these patterns are determined by cost-benefit ratios, in that the benefits associated with spawning at a certain place and time (the new moon period) outweigh the energetic costs of long-distance migrations to a spawning site (Warner, 1995; Chapman et al., 2000; Claydon et al., 2012).

#### 4.4.2 Phenotypic variation

##### *Behavioural activities*

The study also revealed an ontogenetic basis in the plasticity of energy allocation patterns. Terminal phase individuals fed notably less than initial phase individuals and disproportionately allocated their time and energy towards aggressive interactions. These general tendencies align with Schoener (1971), Hixon (1982) and Hoffman (1983), who predicted that energy allocation strategies in a sex-changing population are highly sex- and size-dependent. Females are expected to maximize their foraging time at a cost to reproductive activities for a number of reasons. First, lifetime reproductive success in a protogynous mating system rapidly increases with size (Warner, 1984a; Munday et al., 2006a). The faster a female grows, and the more she avoids risky reproductive behaviours, the higher are her prospects to attain territorial male status. Hence, females tend to trade off current reproduction against future reproduction by directing more excess energy to growth. This may increase probability of survival to sex-change and its associated fitness gains (Schoener, 1971; Hoffman, 1983; Sano, 1993, van Rooij et al., 1995; Warner, 1998; Villegas-Rios et al., 2014). Second, eggs are energetically more costly to produce than sperm (Trivers, 1972; Taborsky, 1998). Consequently, *current* female reproductive success largely depends on energy investment into gamete production (Hoffman, 1983; Schultz & Warner, 1991; Villegas-Rios et al., 2014). Taken together, females tend to maximize their foraging time, because current reproductive success is constrained by energetic demands for gonadal production, and future reproductive success is dependent on rapid growth and survival. I note that the fundamental inequality in male and female gamete size (anisogamy) also predicts reduced intrasexual aggression among females (Trivers, 1972; Hoffman, 1983; Taborsky, 1998), and this is consistent with my observations. Energy allocation strategies of smaller males should closely resemble female allocation strategies, since their reproductive success is also primarily dependent on fast growth and sperm production (smaller IP males have similarly large gonads to females due to intense intrasexual sperm competition, see Schaerer and Robertson, 1999).

Higher aggressive interactions and lower feeding rates suggest, that reproductive success of TP males depends proportionally more on reproduction-related activities, than on growth or gonadal production. As soon as a terminal phase male gains territorial status, any further growth will not substantially change his fitness (Hoffman, 1983; Warner, 1984a; Warner, 1988a). Territorial males should therefore minimize their foraging time and allocate a higher proportion of their time and

energy into territoriality/social dominance, since loss of a territory is equivalent to almost a complete loss of mating success. (Warner & Hoffman, 1980a; Hoffman, 1983; van Rooij et al., 1995). Additionally, territorial males do not require large gonads for successful reproduction, so energetic costs to produce sperm are lower than for females or IP males (Taborsky, 1998, Shapiro et al., 1994; Villegas-Rios et al., 2014).

Similar sex- and size-specific energy allocation patterns in a protogynous species have been described for a wide range of families, including hogfishes in the genus *Bodianus* (Hoffman, 1983), the parrotfish *Sparisoma viride* (van Rooij et al., 1995), the sandperch *Parapercis polyophtalma* (Sano, 1993) or the ballan wrasse *Labrus bergylta* (Villegas-Rios et al., 2014).

### *Movement patterns*

Protogynous sex change and sex-specific variation in size may also manifest as ontogenetic shifts in short-term movement patterns (e.g. space use, travel distance and turning angle). In general, TP individuals exploited a larger area for feeding than IP individuals and tended to display fine-scale movement patterns consistent with directional swimming. Activity spaces of IP individuals rapidly increased with body size, while they didn't substantially change with TP body size. Size- and/or sex-specific nutritional requirements, predation risk, and social behaviour may influence differences in movement patterns across sizes and between colour phases.

Larger feeding areas may facilitate faster growth (if the underlying distribution of resources is homogeneous), because the probability to encounter high-quality food resources substantially increases (Welsh et al., 2013; Nash et al., 2015). However, in environments with high predation risk, smaller foragers may be disproportionately vulnerable to predation (Hixon & Beets, 1993; van Rooij, 1995; DeMartini et al., 2005; Holmes & McCormick, 2010). Thus, smaller individuals may trade foraging space for shelter opportunities to reduce predation risk. Their activity spaces may also be restricted to areas of high complexity, such as coral boulders, that offer shelter against potential predators (Fox & Bellwood, 2007; Welsh et al. 2013). As body size of foragers increases, predation risk is likely to decrease, and larger individuals may increase their foraging area to acquire sufficient energy resources that meet their metabolic demands (Haskell et al., 2002; Welsh et al. 2013; Nash et al., 2015). Activity area expansion reaches its maximum with sex change due to improved size-specific locomotory abilities and increased social behaviour like competitive interactions and territoriality (Jones, 2005; Afonso et al. 2008; Fulton & Bellwood, 2002; Welsh & Bellwood, 2012; Welsh et al.,



2013). Activity spaces didn't increase as quickly with size after sex-change; potentially because terminal phase males must invest a larger proportion of their available energy into territory defence during spawning hours, which constrains habitat exploration during non-spawning hours. Similar size-related increases of activity spaces in a wrasse species have been found for *Notolabrus tetricus* (Shepherd & Clarkson, 2001) and *Halichoeres* sp. (Shibuno et al., 1993; Jones, 2005).

Consistent with activity patterns, variation in fine-scale movement patterns mirrors differential energy allocation strategies dependent on colour phase. Because IP individuals invest a larger proportion of their time into foraging activities to achieve higher growth rates, their fine-scale movement patterns naturally resemble intense searching behaviour, as characterized by short step lengths and high turning angles (Michelot et al., 2016). In turn, TP individuals tend to invest more of their time into social interactions, as indicated by more directional and non-random movement patterns. Statistical analysis also suggested that larger IP individuals tended to move more directionally with size than smaller IP individuals. Greater directional swimming with increasing IP size might be attributable to ontogenetic shifts in prey preferences. While smaller individuals may target whatever comes into reach, higher size-related mobility and larger gape size might enable larger individuals to target less abundant prey with higher energy content (Green et al., 1984; Welsh et al., 2013; Nash et al., 2015). Therefore, increasingly selective preferences may, in part, mediate a shift towards more directional, non-random swimming behaviour in larger IP individuals. Additionally, greater directional swimming of larger IP individuals might be a result of enhanced migration and social behaviour.

#### **4.4.3 Diurnal variation**

Activity patterns of the sixbar wrasse also exhibited strong diurnal variation. Feeding activities peaked in the morning and declined shortly before spawning hours, and then increased after spawning hours. Conversely, social activities like group swimming significantly increased prior to spawning hours, along with travel distances and activity spaces. A range of iteroparous species synchronize their reproductive activities with oceanographic conditions like high tides that facilitate offspring survival (Johannes, 1978, Sancho et al., 2000; Claydon et al. 2014). So hydrostatic pressure in the form of increasing tide levels around midday might be the external cue stimulating elevated social interactions and space use close to spawning hours (Takemura et al., 2010; Kuwamura et al., 2016). Why feeding rates are highest in the early morning remains speculative. Higher energy intake in the morning might partly fund costly reproductive activities in the afternoon hours. Or individuals might simply shift their feeding activities to the morning hours so that feeding doesn't conflict with reproduction in the afternoon. But other

additional drivers, like food availability/quality and prey distributions might shape diurnal variations of foraging activities (Shepherd & Clarkson, 2001).

As shown by size-specific diel activity patterns, larger individuals exhibited reduced feeding rates and displayed increased aggressive behaviour in the afternoon hours. Again, these dynamics align with the general prediction that every individual tries to allocate its energy in a way that it optimizes its fitness (Roff, 1983; Stearns, 1992; McBride et al., 2015). During pre-spawning hours, larger individuals tend to prioritize activities that ultimately relate to reproduction, while smaller individuals reduce their foraging activities for brief periods only to not impede somatic growth (Hoffman, 1983; Green et al., 1984; van Rooij et al., 1995).

To decrease model complexity, three-way interactions between lunar phase, time of the day and size were not considered. Hence, this study can't confirm, if feeding activities around spawning hours were significantly more reduced during new moon (where spawning activities are generally elevated) in comparison to the other lunar phases (and if these patterns are additionally structured by size).

#### **4.4.4 Conclusion**

I demonstrate that energy allocation patterns (i.e. behavioural activities and movement patterns) of a protogynous, iteroparous species vary substantially with colour phase, size and lunar phase. My results are consistent with the size-advantage model (Ghiselin, 1969; Warner, 1975; Muñoz & Warner, 2003; Munday et al. 2006a), where time budgets and energy allocation strategies are expected to vary with both size and sex, to maximize an individual's lifetime fitness. Terminal phase males maximize their fitness by investing a large amount of time into aggressive interactions. IP individuals maximize their fitness by directing most of their energy towards growth to increase their chances of achieving future reproductive gains. My time budget data also demonstrates a strong lunar periodicity in energy allocation strategies, which may further influence reproductive success.



## **CHAPTER FIVE**

PATTERNS OF MIGRATION AND HABITAT USE OF A PROTOGYNOUS CORAL REEF  
FISH SPECIES IN RELATION TO SIZE, SEX AND THE LUNAR CYCLE

## 5.1 Introduction

In patchy and uncertain environments, well-established home ranges may enable organisms to maintain spatial maps of food resources, shelter sites, and potential mating opportunities (Chapman & Kramer, 2000; Welsh et al., 2013; Green et al., 2015). However, the spatial extent of an animal's home range can vary systematically with size, sex and reproductive status (Afonso et al., 2009; Welsh & Bellwood, 2012; Howard et al., 2013; Welsh et al., 2016). Home ranges often increase with body size, due to ontogenetic shifts in diet, predation risk, and social behaviour (Mumby and Wabnitz, 2002; Jones, 2005; Dahlgren et al., 2008; Marhsell et al., 2011). Many fishes that inhabit coral reefs routinely extend their movement activities beyond their regular foraging habitat, to migrate to specific sites for the purpose of reproduction (Robertson, 1983; Shibuno et al., 1993; Warner, 1995; Zeller, 1998; Claydon et al., 2014).

Many reef fishes that migrate to spawning sites congregate at particular times, and form large spawning in aggregations (Claydon et al., 2014). Spawning in aggregations may increase reproductive success via diluted predation rates on adults and eggs, increased mate-encounter rates, improved mate choice opportunities and enhanced fertilization success (Johannes, 1978; Shapiro et al., 1988; Claydon et al., 2014). Spawning at specific locations may also increase reproductive success, facilitated by the physical characteristics at the spawning site. For example, many spawning sites are located down-current of major reef areas, and on promontories or along reef edges; such locations are likely to facilitate rapid transport of fertilized eggs away from reef-based egg predators (Robertson, 1983; Domeier & Colin, 1997; Claydon, 2004). However, migration to these reproductively beneficial sites can be costly to adults (Roff, 1991). First, extensive spawning migrations are metabolically demanding and often entail additional energy expenditure, at the cost of growth or gametogenesis (Roff, 1991; Warner, 1995, McBride et al., 2015). Second, the time spent migrating may reduce the time available for feeding; again, this may reduce growth potential. Third, migratory behaviour critically exposes individuals to predators, due to limited shelter opportunities, unfamiliar territory and the conspicuous nature of movement (Robertson, 1983; Shibuno et al., 1993; Claydon et al., 2012).

The relative costs associated with migration are likely to be determined by an individual's size and sex. Smaller individuals often invest proportionally more energy to growth, and long-distance movements may come at a greater energetic cost. Smaller individuals are also more vulnerable to predators. Consequently, smaller reproductive individuals are predicted to minimize migration costs by undertaking shorter migrations, or alternatively by spawning within their usual home range area. In contrast, larger individuals with greater energy stores may show a higher flexibility in their choice of

spawning sites and may migrate greater distances to reach more distant sites that maximise adult fitness (Fitch & Shapiro, 1990; Shibuno et al., 1993; Zeller, 1998; Claydon et al., 2012).

Size-dependence in the relative costs and benefits of migration may be particularly important for reef fishes that change sex over their lifetime (e.g., the size-advantage model; (Ghiselin, 1969; Warner, 1975; Muñoz & Warner, 2003; Munday et al., 2006a). Protogynous hermaphroditic species are common on coral reefs (Warner, 1984a). Many of these species spawn daily and throughout the year (extreme iteroparity) at predictable sites, to which females and males migrate from their feeding areas (Robertson & Hoffmann, 1977; Colin & Bell, 1991; Shibuno et al., 1993; Warner, 1995; Claydon et al., 2014). Large, dominant males typically establish mating territories at these sites to court and pair-spawn with females (Warner & Hoffman, 1980a; Fitch & Shapiro, 1990). Because only the largest, most dominant males obtain mating territories, and because they can monopolise mating opportunities with many females, reproductive success substantially increases with size in a protogynous mating system (Warner & Hoffman, 1980a; Warner, 1984ab). Smaller females and males are expected to allocate a disproportionate amount of their surplus energy to growth to enhance their future reproductive potential (i.e. to develop into territorial males with exclusive access to mating sites and females). As a corollary to the size-advantage model, protogynous species thus hypothetically exhibit a high degree of intraspecific variability in habitat use, migration patterns, foraging site and spawning site fidelity. However, studies that evaluated this hypothesis remain limited (but: see Shibuno et al., 1993).

Spawning activities of protogynous reef fish species often exhibit lunar-related rhythms, with elevated activities during full or new moon (Johannes, 1978, Claydon et al., 2014). Some researchers have suggested that the lunar cycle may influence the costs of migration (Robertson et al., 1990). However, few studies have examined variation in migratory distances as a function of the lunar cycle. Thus, the question of whether migration distances and spawning site choice are influenced by the lunar cycle remains open. And additionally, are potentially lunar-cyclic migration patterns also dependent upon the size and phenotypic status of an individual?

In this chapter I explore size- and environmentally related variability in migration patterns and habitat use of a highly iteroparous, protogynous coral reef fish, the sixbar wrasse *Thalassoma hardwicke*. The sixbar wrasse is one of the most abundant labrid species throughout the Indo-Pacific, and is known to display lunar-related reproductive patterns (Chapter 2, 3). I hypothesise that migration distances and habitat use vary significantly with social status, size and the lunar cycle. Specifically, I address the

following questions: (1) Do home ranges vary with size and phenotypic status of an individual? (2) Does foraging site fidelity and/or spawning site fidelity vary with size, phenotypic status and lunar phase? (3) Do migration distances vary with size, phenotypic status and the lunar cycle? (4) What is the spatial scale of spawning movements, and do habitat breaks function as natural barriers to migratory movements?

## 5.2 Methods

### 5.2.1 Study site and species

I studied movement patterns of the sixbar wrasse (*Thalassoma hardwicke*) in the northern lagoon of Moorea, French-Polynesia, from April to May 2018. More detailed descriptions of the study species can be found in previous chapters. Briefly, the sixbar wrasse is a sequential protogynous hermaphrodite and exhibits two distinct colour phases: initial phase (IP) fish (females and smaller males that resemble the female phenotype) and terminal phase (TP) fish (large, dominant males). Initial observations suggest that adult sixbars migrate from their feeding areas to specific spawning sites between 2pm and 4pm to reproduce. Reproduction occurs daily, year-round and along the outer edges of a reef. During spawning hours, large solitary TP males occupy and defend territories to pair spawn with females; spawning frequencies are most strongly associated with the lunar cycle, and maximal around the new moon (see chapter 2 and 3).

The focal study site for this chapter was an inshore-reef on the north shore of Moorea, French-Polynesia (see Fig. 5.1). Initial studies suggested that mating territories of the sixbar wrasse are distributed all along the outer reef margins of the lagoon. Probably due to its physical characteristics and close proximity to a break in the barrier reef crest, an area of reef along the south western borders of the lagoon appears to be an extraordinarily popular spawning site for sixbars (as well as multiple other species like parrotfish and surgeonfish species; *personal observations*). During spawning hours, the outer reef margins of this site (here referred to as “S1”) are generally densely occupied by numerous mating territories. Large group spawning assemblages regularly form around the last quarter of the lunar month. Whereas S1 varies remarkably from similar locations in terms of size and number of aggregated fish, other mating sites are loosely scattered along the southern margins of the reef.

### 5.2.2 Tagging method

To evaluate migration distances, spawning site fidelity and home range size of the sixbar wrasse *T. hardwicke*, I tagged a total of 378 individuals in April 2018. Specifically, I captured individuals with barrier nets and hand nets, across a range of locations within each of five main sampling areas S1, I1, I2, O1 and O2 (see Fig. 5.1). Tagging sites were successively further to the east of a well-known mass mating site (S1, see Fig 5.1). Captured individuals were transported to a nearby boat, where they were lightly anesthetized with clove oil, and their phenotypes (IP or TP), standard lengths (SL) and total lengths (TL) were recorded. Individuals were then tagged through the dorsal musculature with unique colour-coded t-anchor tags. After a recovery period of 30 min, tagged individuals were released at their site of capture. All work was conducted in accordance with VUW Animal Ethics Permit number 22038.

### 5.2.3 Sampling strategy

#### *Surveys during non-spawning hours*

Surveys to relocate tagged individuals during non-spawning hours were conducted across the three main sampling areas S1, I1 and I2 from April 29 to May 22. For spawning surveys, I identified 6 focal spawning territories along the outer reef edges of S1, and three spawning territories along the outer reef edges of I1 and I2 respectively (12 in total, see Fig. 5.1).

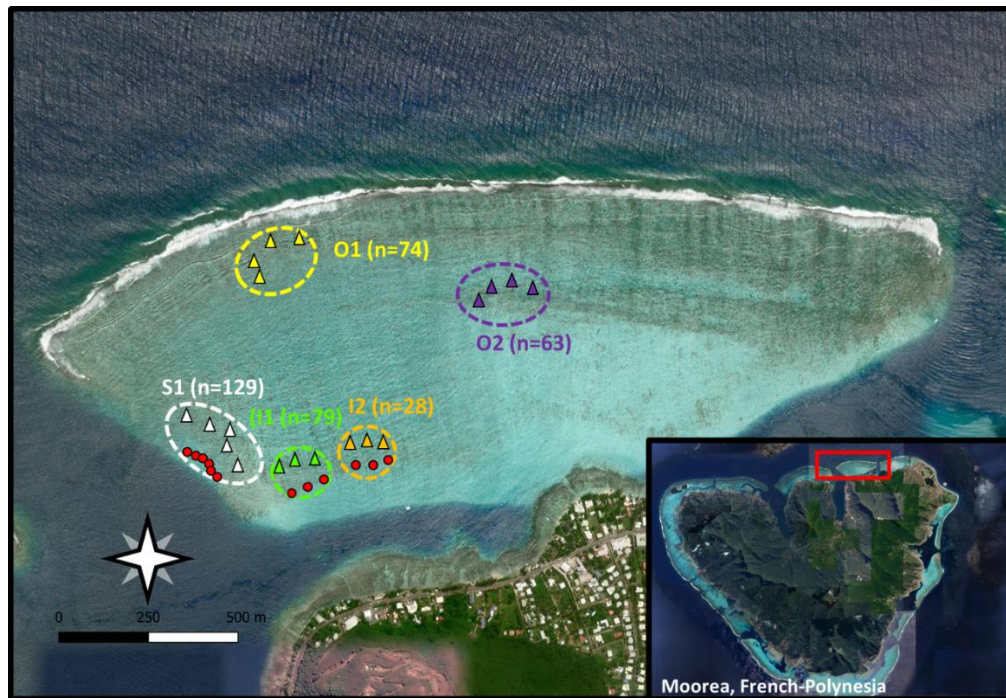
Although I tagged some individuals (138 in total) ~700 m further offshore, and about ~950 m towards the midsection of the reef (O1 and O2, see Fig. 5.1), I decided to not conduct any further surveys in those areas due to time limitations. Nevertheless, tagged individuals from O1 and O2 were useful to assess potential longer-distance migrations of sixbar individuals.

*Surveys during non-spawning hours*

I surveyed each tagging area every day on a rotating schedule, during feeding hours, between 9am and 1.30 pm (for example, day 1 - S1: 9 am -10.30 am, I1: 10.30 am -12 pm, I2: 12 pm - 1.30 pm; day 2 – I2: 9 am - 10.30 am, S1: 10.30 am – 12 pm, I1: 12 pm-1.30 pm, etc.). Aided by an assistant, I searched S1, I1 and I2 for tagged fish, by swimming tight parallel transect lines from the outer reef edges towards the midsection of the reef. The inner and outer transect borders were characterized by the point where coral-dominated habitat transitioned to sand and rubble-dominated habitat (my preliminary surveys suggest that sand and rubble are generally avoided by sixbars). This method allowed us to cover approximately 30000-40000 m<sup>2</sup> per area a day (see Fig. 5.3 in the result section for a more detailed overview of transect area and size). Whenever a tagged fish was resighted, its GPS location was recorded (via handheld GPS), along with its tag identification (i.e., a specific colour code).



Daily, repetitive transect surveys across the month allowed us to establish a record of multiple GPS positions for every relocated individual.



**Fig. 5.1** Study lagoon at the north shore of Moorea, French-Polynesia. Individuals were tagged across multiple locations (triangles) within different reef areas S1, I1, I2, O1 and O2 (dotted circles). The number of tagged individuals (n) are indicated in brackets. Spawning activities were monitored within 6 territories along the outer reef borders of S1, and 3 territories along the outer reef edges of I1 and I2 respectively (red dots). Location maps are produced from Google Earth Pro (2019).

#### *Surveys during spawning hours*

I monitored the 12 focal territories daily during spawning hours (2-4 pm) for 20 min (again on a rotating time schedule: I1+I2 territories from 2-3 pm, S1 territories from 3-4 pm and vice versa). Whenever a tagged individual entered the spawning site, I recorded its tag identification and location, and documented potential reproductive activities like spawning, courtship or chases.

#### **5.2.4 Data analysis**

The main objective of this study was to investigate if spawning site fidelity, home range size and migration distances vary with phenotype (IP vs TP) and size of an individual, and whether any of these patterns also varied with respect to the lunar phase. I estimated home range sizes in  $m^2$  by

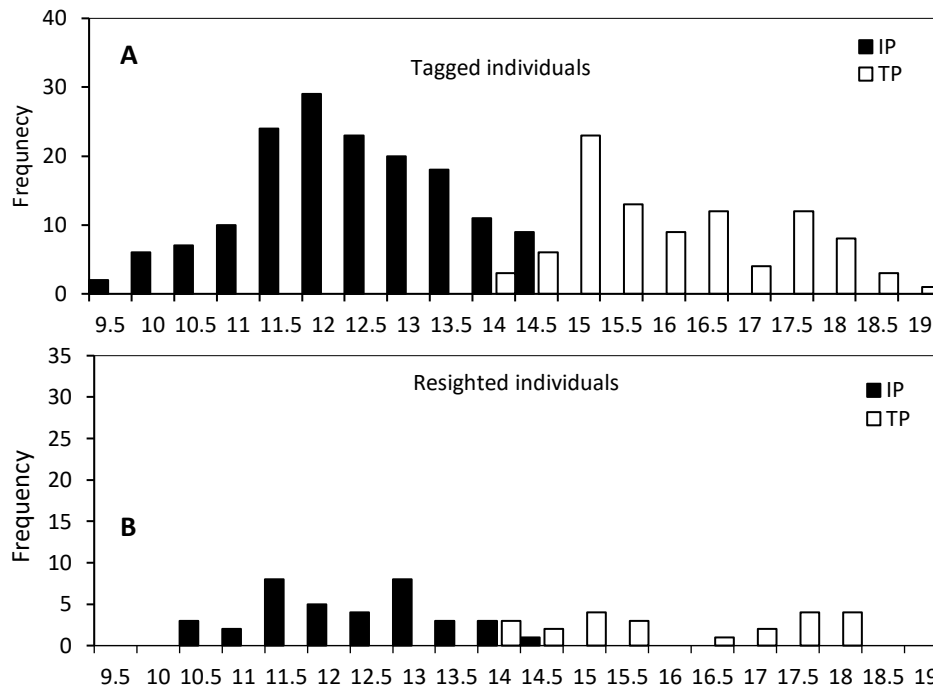
constructing the smallest convex polygon (MCP) around 95% of all GPS positions of an individual (adehabitat package in R, see Calenge, 2006). I only calculated home range estimates for individuals that were detected at more than 5 locations during non-spawning hours. For every individual, I additionally calculated mean positions (arithmetic mean of all GPS locations) within feeding areas to estimate daily migration distances to spawning sites. Migration distance was estimated as a straight line from an individual's mean position within its feeding area, to its location (i.e. territory position) within a spawning site (geosphere package in R, see Hijmans, 2011). Additionally, spawning site fidelity was estimated by dividing the number of days a fish was detected at a spawning territory, by the total number of days between its first and last detection (i.e. the proportion of time a fish visited a territory relative to the period a fish was monitored). Values around zero indicate low site fidelity, values close to one indicate a high degree of site fidelity.

In order to assess variation in migration distances as a function of lunar periodicity, I used periodic regression GLMs with a gaussian error structure. For this analysis, I only was able to include individuals for which I had sufficient data (i.e., observed at least once at a spawning site, and more than 5 times within a feeding area; this constrained my data to a subset of 52 individuals in total). Specifically, I modelled migration distances of these 52 tagged individuals as a function of lunar phase (characterised by Sine and Cosine terms to capture the cyclical components of the first/last quarter moons and new/full moons respectively), size (total length) and colour phase (IP or TP phenotype) of an individual. Initially, I fitted a full model containing interaction terms between size (total length) and  $\cos\theta/\sin\theta$ /colour phase respectively. The model with the smallest AICc weight was then chosen as the final model (based on model selection methods with the MuMIN package in R, see Bartón, 2011). To analyse variation in site fidelity and home range size as a function of size and phenotype, I used GLM models with a gaussian error distribution. I also included an interaction term between size and colour phase for both predictors. Due to collinearity issues between TL (total length) and phenotype, I standardized mean TL relative to the mean TL of each phenotype. In a separate analysis, I additionally conducted simple linear regressions for both colour phases (IP and TP) between size and the predictor variable. Based on model results, I generated prediction plots with the visreg package in R (Breheny & Burchett, 2017). Additionally, I conducted two-sample Kolmogorov-Smirnov tests to investigate if resighting probability of TP and IP individuals is size-dependent.

### 5.3 Results

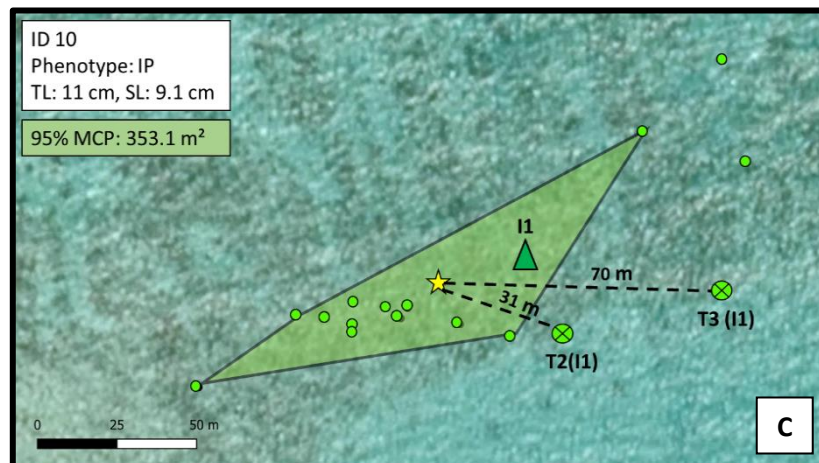
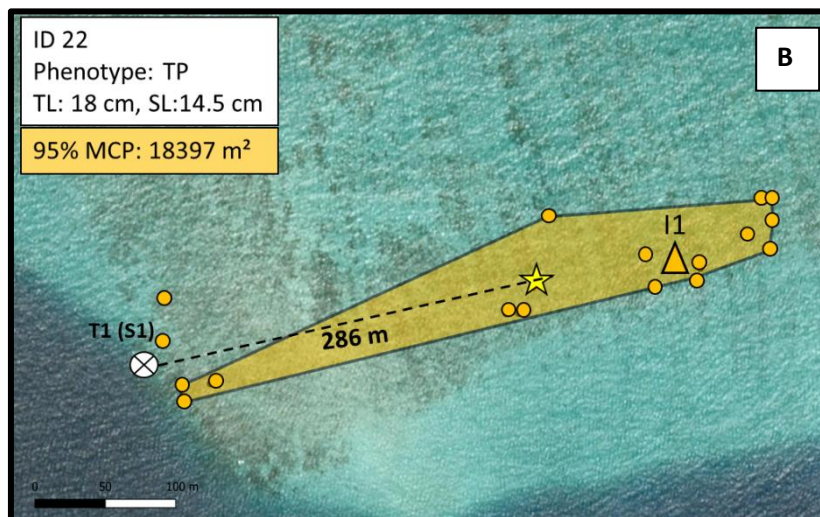
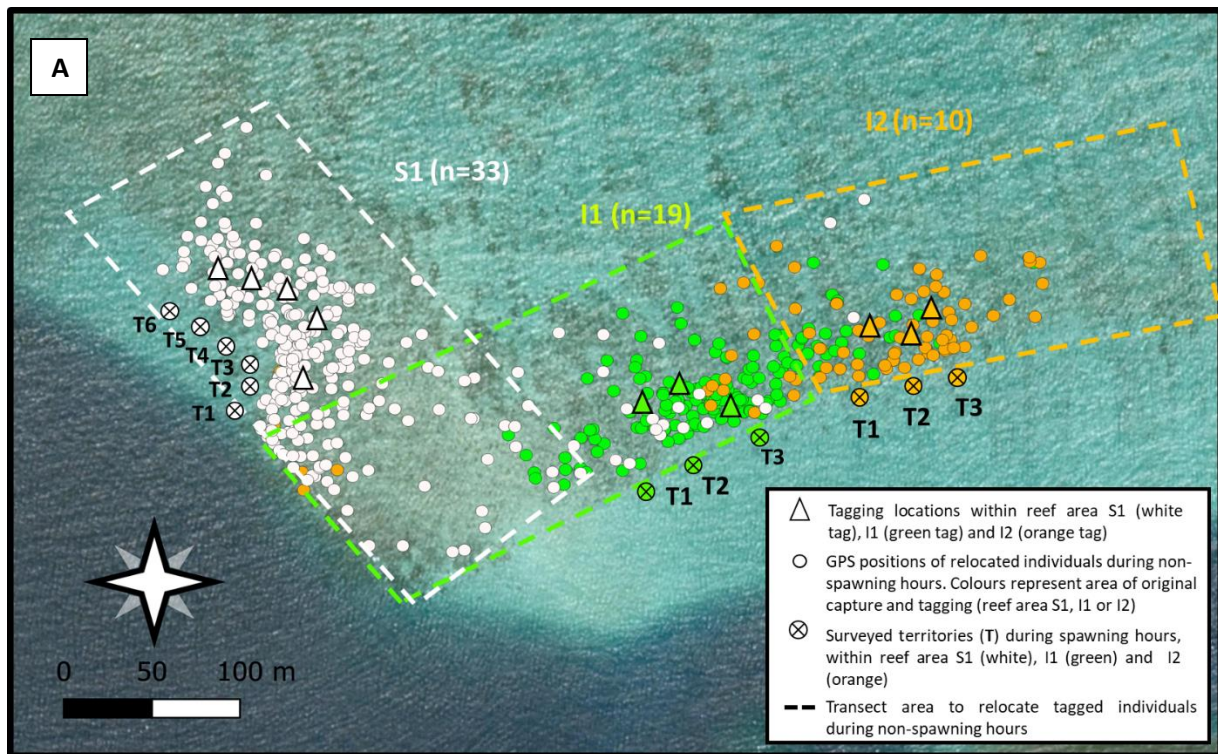
In total, 241 individuals were tagged across the 3 different tagging areas S1, I1 and I2. Over the course of 18 daily surveys, a total of 65 individuals, 33 from site S1, 19 from I1, and 10 from I2, were relocated

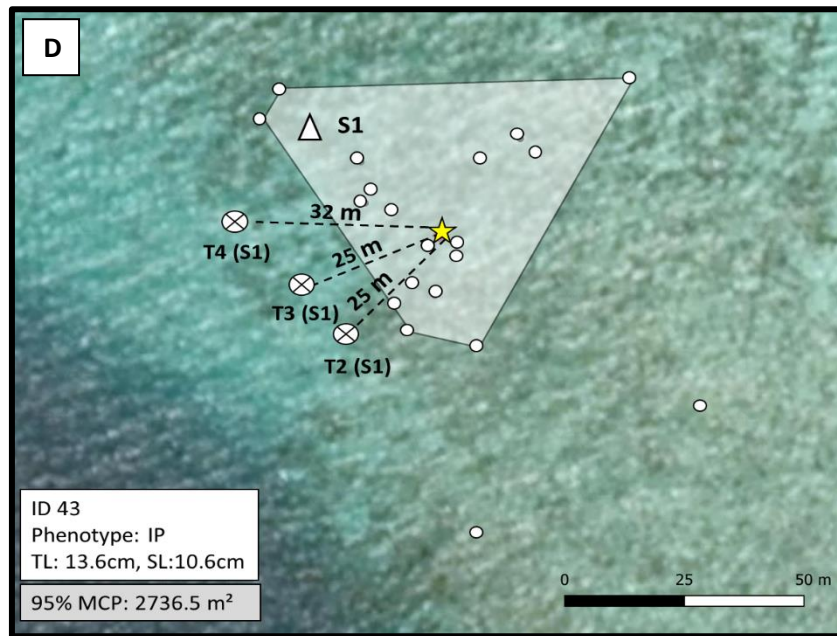
and identified at least once. Of these, 62% were IP individuals (TL range: 10.5-14.5 cm) and 38% TP individuals (TL range: 14-18 cm; (see Fig. 5.2 A). The initial size frequency distribution of all tagged fish (mean = 13.7 cm, SD = 2.16) was indistinguishable from the size frequency distribution of re-sighted individuals (mean = 13.9, SD = 2.18), suggesting no differential mortality or tag loss with respect to size (Kolmogorov–Smirnov two-sample test:  $D(241,65) = 0.07$ ,  $p = 0.97$ ; Fig. 5.2).



**Fig. 5.2** Size frequency distribution (where size = TL) of all tagged (**A**) and relocated individuals (**B**). Black bars are initial phase individuals (IPs) and white bars are terminal phase individuals (TPs). A Kolmogorov Smirnov test indicated that resighting probability was not biased towards particular sizes.





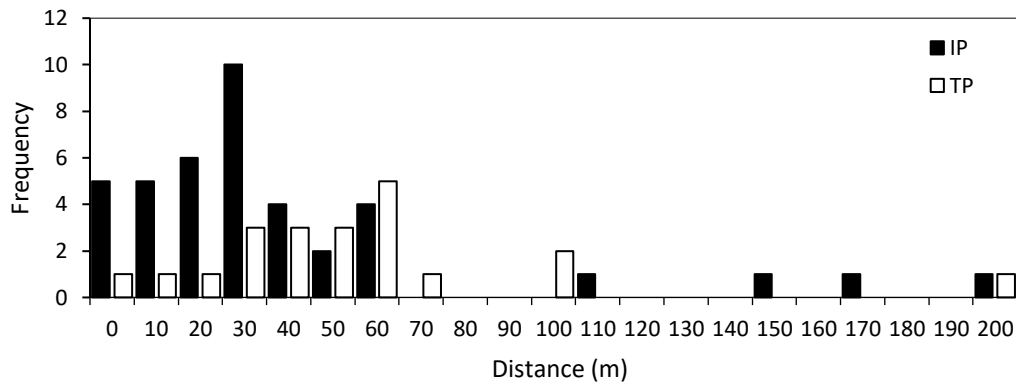


**Fig. 5.3 A)** GPS positions of all tagged and resighted individuals within their feeding areas. Every single sighting of an individual during non-spawning hours (8am-12pm) is represented by a coloured dot. Colours represent the original area of capture and tagging (reef section S1: white, reef section I1: green and reef section I2: orange). Transect area to relocate tagged individuals during non-spawning hours is indicated by dashed lines. **B), C), D):** Example plot of location points, home range area and migration distances of single individuals (i.e., individual 22, individual 10 and individual 43). Coloured dots represent GPS positions of resighted individuals during non-spawning hours. Coloured polygons represent estimated home range area around 95% of all location points (95% MCP). Star symbols represent the mean position within a home range area, coloured crosses represent visited territories during spawning-hours, and black, dashed lines represent migration distances to visited territories. Triangles represent the original area of capture and tagging. Location maps are produced from Google Earth Pro (2019).

### 5.3.1 Home range area

Home range areas (95% minimum convex polygon) varied between 11 and 2700 m<sup>2</sup> for IP individuals, and between 160 and 18400 m<sup>2</sup> for TP individuals. Apart from a few exceptions, most individuals were resighted in close proximity to their tagging location (see Fig. 5.4), and there was no extensive overlap of location points between the three tagging areas (see Fig. 5.3). Only three large, terminal phase males were observed foraging in all three tagging areas over the course of a month (with home ranges varying between 16000 and 18000 m<sup>2</sup>). Linear regression analysis between IP size and home range area, as well as TP size and home range area suggested, that home range area was positively correlated to size for both phenotypes (LM:  $p=0.06$  for IPs;  $p=0.03$  for TPs). The best-fitting GLM model with the lowest AICc (1129) indicated that home range area increased with size, and TP individuals exhibited significantly larger home range sizes than IP individuals (see Table 5.1 and appendix 5.1). The model also contained a significant interactive effect of phenotype (IP or TP) and total length,

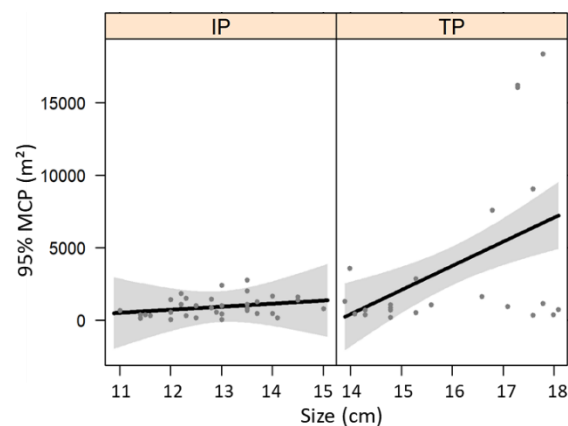
suggesting that variation in home range size was more associated to an increase in TP size (GLM:  $p=0.046$ , see Table 5.1 and Fig. 5.5).



**Fig. 5.4** Frequency-distributions of distances (m) between the initial tagging area and the mean location of an individual within its home range area, stratified by phenotype (IP/TP - initial phase and terminal phase).

**Table 5.1** Parameter estimates and test statistics for best models explaining home range size (95% Minimum convex polygon) of *Thalassoma hardwicke*. Best model given was identified based on the lowest AICc score (among all permutations of the full model). Models were fit using GLM (Gaussian distribution). Asterisk symbols reflect significance levels.

Model	levels	Estimate	SE	z value	p value	Significance level	AICc
<b>MCP (95%)~</b>							
Size residuals	-	209.8	546.8	0.384	0.702		
Colour phase	TP	2818.9	870.6	3.238	0.002	**	
Colour phase*Size residuals	TP	1457.7	714.4	2.041	0.046	*	1129



**Fig 5.5** Interaction plot with the predicted effect of phenotype (IP=initial phase; TP=terminal phase) on MCP area dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly for TP individuals, size had a strong effect on variation in MCP area. Subsequent analysis revealed, that both phenotypes exhibit a significant increase of MCP area with size (whereas only weakly for IP individuals).

### **5.3.2 Spawning site choice and fidelity**

In total, I recorded 51 tagged individuals at focal spawning territories during spawning hours. Out of these, 79% travelled to spawning sites closest to their mean coordinates within feeding areas. Over the course of a month, 6 TP and 5 IP individuals migrated to spawning sites that were substantially further away from their activity centre (see Table 5.2). Most of these longer migrations were recorded during new moon/the end of the third quarter, and happened between feeding areas in the southern parts of the reef (tagging site I1 and I2) and spawning site S1 (south western part of the reef). Although actual pair and group spawning events were rarely observed, many tagged individuals resighted within focal spawning territories were actively participating in spawning-related activities (e.g. courtship or chases). No individuals tagged at O1 and O2 were observed at the monitored spawning sites.

**Table 5.2** Tagged individuals (52 in total) that occurred at territory locations across different areas within the reef (S1, I1 and I2) during spawning hours over the study period. Recorded individuals at spawning territories are stratified by area of capture (i.e. S1, I1 or I2). Since most individuals stayed close to the area where they were tagged, each area of capture closely represents their preferred feeding habitat (see also Fig. 5.3). Bold numbers indicate, if individuals migrated more than 100 m to a respective territory, asterisk symbols indicate, if these longer migration distances were associated with the new moon/end of the third quarter (lunar day 27-6).

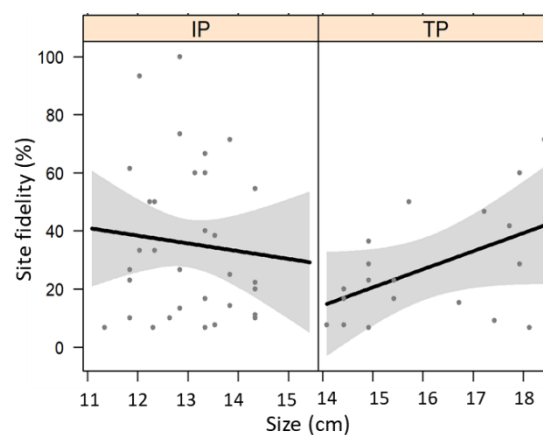
Reef area			S1						I1			I2		
Mating territory			1	2	3	4	5	6	1	2	3	1	2	3
Fish ID	Phenotype	Area of capture												
1	IP	I1									1			
2	IP									4	2			
4	IP									6	1			
5	TP								6	2				
6	IP										6			
7	IP								1	6	1			
8	TP		<b>5*</b>								1			
10	IP									1			1	
11	TP								1					
13	TP		<b>1</b>	<b>1</b>	<b>1*</b>									
14	IP								2					
16	TP		<b>1*</b>						7					
19	IP								1		4	1	2	
64	IP			<b>1*</b>					5					
21	TP	I2									1			
22	TP		<b>8*,1</b>											
27	IP			<b>1*</b>									1	1
28	IP												1	
29	TP											1	1	1
69	TP			<b>1*</b>										
70	IP				<b>1*</b>									
71	IP			<b>1*</b>										
30	IP	S1		5										
31	IP			5	2									
32	TP		1											
33	IP			4	6									
34	IP		<b>1*</b>	<b>2*</b>		<b>2*</b>								
35	IP						1							
36	IP		1	3										
37	IP		5	1										
38	IP					<b>3*</b>								
39	IP		8											
40	IP				3									
41	TP						1							
42	TP		1	3										
43	IP			1	4	9								
45	TP				2									
46	IP					3	4	10						
48	TP								1	1	1			
49	TP						1							
50	IP		1											
52	IP					1	1							
53	IP					3	3							
56	TP			12										
57	IP				14									
58	IP						1							
60	IP		2		2									
61	IP					11								
62	TP					6	8	4						
65	TP			1	2									
66	TP				1	4	1							
68	TP								2					



Neither phenotype nor size were significant predictors of overall variation in territory site fidelity (GLM analysis, see Table 5.3 and appendix 5.2). However, additional linear regression analysis indicated a significant positive relationship between TP size and territory site fidelity (LM:  $p=0.03$ ). In contrast, linear regression did not suggest any significant relationship between IP size and territory site fidelity (although smaller IP individuals tended to have higher site fidelity than larger IP individuals, see Fig. 5.6). A handful of individuals were visiting/occupying the exact same territory whenever occurring at a spawning site. These individuals were 8 IP individuals (11.5 - 14 cm TL, consistently visiting the same territory site) and 3 large territorial males (17 -18 cm TL, consistently occupying the same territory site).

**Table 5.3** Parameter estimates and test statistics for best models explaining site fidelity (%) of *Thalassoma hardwicke*. Best model given was identified based on the lowest AICc score (among all permutations of the full model). Models were fit using GLM (Gaussian distribution). In this case, none of the predictors were significant.

Model	levels	Estimate	SE	z value	p value	Significance level	AICc
<b>Site fidelity~</b>							
Size residuals	-	-2.667	4.822	-0.553	0.583		
Colour phase	TP	-8.913	6.903	-1.291	0.203		
Colour phase*Size residuals	TP	8.905	6.084	1.464	0.15		476.2



**Fig 5.6** Interaction plot with the predicted effect of phenotype (IP=initial phase; TP=terminal phase) on spawning site fidelity, dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Although none of the predictors in the final model were significant, linear regressions between size and site fidelity for TP and IP individuals indicated a significant positive association of site fidelity and TP size.

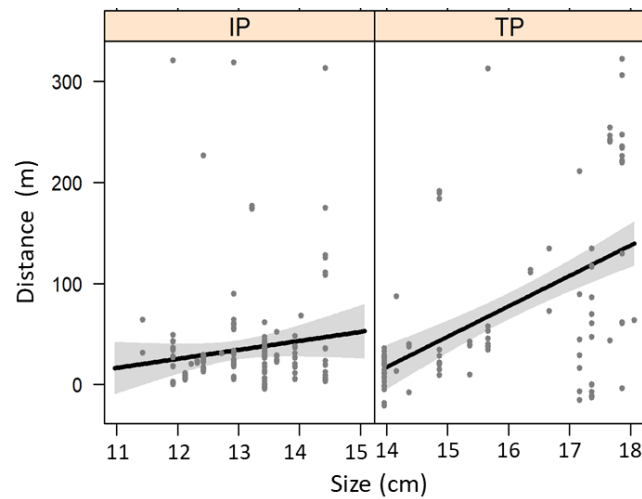
### 5.3.3 Migration distances

Migration distances varied between 0.8 and 322 m (average:  $49.7 \pm 72.6$  SD). Of the 52 individuals observed entering a spawning site, 11 individuals migrated more than 100 m to a respective territory (see Table 5.4).  $\cos\theta$ , size, phenotype and the interaction between size and  $\cos\theta$ , and size and phenotype, were retained as significant predictors in the best-fitting model (see Table 5.4, see appendix 5.3). On average, TP individuals migrated significantly longer distances than IP individuals (IPs: 0.8 -322m; TPs: 8-321m, see Table 5.4 and Fig. 5.7). For both phenotypes, travel distances significantly increased with increasing total length (LM:  $p=0.04$  for IPs and  $p<0.01$  for TPs). However, a significant interaction term between size and phenotype in the final GLM model indicated that an increase of migration distance was more strongly size-dependent for TP individuals than for IP individuals (GLM:  $p<0.001$ , see Table 5.4).

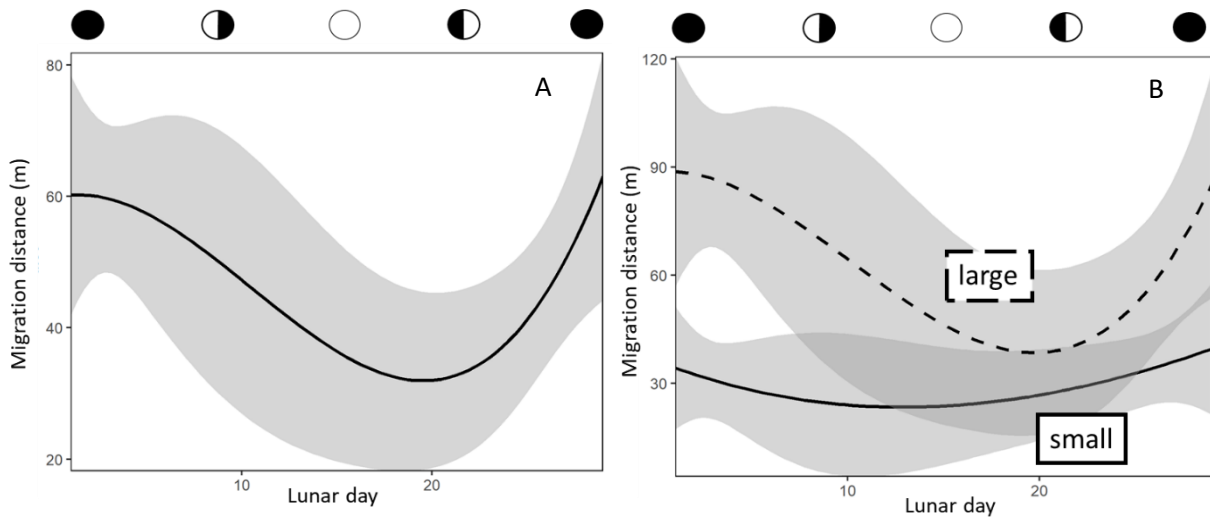
In general, temporal variation in migration distance was strongly associated with the lunar cycle, with migration distances significantly increasing from an average of 35 m during full moon to 60 m around new moon (GLM:  $p<0.001$ , see Table 5.4 and Fig. 5.8 A). A significant interaction term between  $\cos\theta$  and body size indicated, that larger individuals travelled significantly longer distances during new moon relative to smaller individuals (GLM:  $p<0.001$ , see Fig. 5.8 B).

**Table 5.4** Parameter estimates and test statistics for best models explaining migration distances (m) of *Thalassoma hardwicke* to spawning sites. Best model given was identified based on the lowest AICc score (among all permutations of the full model). Models were fit using GLM (Gaussian distribution). Asterisk symbols reflect significance levels.

Model	levels	Estimate	SE	z value	p value	Significance level	AICc
<b>migration distance~</b>							
Moon phase: COS ( $\theta$ )	-	20.88	6.68	3.13	<0.001	**	
Size residuals	-	-5.22	6.96	-0.75	0.45		
Colour phase	TP	44.83	8.06	5.57	<0.001	***	
COS ( $\theta$ )*Size residuals	-	23.09	6.09	3.79	<0.001	***	
Colour phase*Size residuals	TP	25.26	7.46	3.39	<0.001	***	2934.7



**Fig 5.7** Interaction plot with the predicted effect of phenotype (IP=initial phase; TP=terminal phase) on MCP area dependent on individual size (cm). Plots show expected values (black line), confidence interval (95%) of the expected value, and partial residuals (grey dots). Particularly for TP individuals, size had a strong effect on variation in migration distance. Subsequent analysis revealed, that both phenotype exhibit a significant increase of migration distance with size.



**Fig 5.8** Smoothed curve ( $\pm$  95% confidence interval) of the lunar effect on predicted migration distance during the 29.5 lunar cycle (A). Fig 5B: For illustrative purposes, predicted migration distances were split by size (small individuals: 11-13 cm; large individuals: 13-19 cm) to visualize a significant interaction effect of size and lunar phase on migration distance.

## 5.4 Discussion

My findings confirm that the sixbar wrasse displays a high degree of intraspecific variability in migration patterns, habitat use and spawning site choice. As expected, an individual's phenotypic status, its size and sex all seemed to influence home range sizes, migration patterns and spawning site fidelity. Additionally, temporal variation in migration patterns and spawning site choice was significantly correlated to the lunar cycle.

### 5.4.1 Foraging site fidelity and home range areas

A high proportion of tagged individuals were resighted close to their original area of capture and tagging. This suggests well-delineated home ranges, commonly described for small-bodied reef fish species (Chapman & Kramer, 2000; Jones, 2005; Claisse et al., 2011; Marshall et al., 2011; Welsh & Bellwood, 2011). Home range size increased with body size, supporting my hypothesis that smaller individuals should restrict their movements, and that they are relatively more site-attached. Coral reefs typically comprise high abundances of benthic and pelagic predators, so spatially constrained home ranges may facilitate higher survival probabilities due to consequent knowledge of potential shelter sites (Chapman & Kramer, 2000; Claisse et al., 2011). Furthermore, site-attachment provides several other fitness benefits, including locational knowledge of sleeping sites, food resources and mating opportunities (Wootton, 1990; Barrett, 1995; Claisse et al., 2011; Claydon et al., 2012). Significant correlations between fish size and home range size have been found in several other protogynous coral reef fish species, like in the wrasse *Halichoeres* sp. (Green, 2005), and a variety of parrotfish species (*Scarus rivulatus*, *Scarus rubroviolaceus*, *Scarus frenatus*, *Chlorurus sordidus*, see Mumby and Wabnitz, 2002; Welsh et al., 2013; Howard et al., 2013). Enhanced swimming abilities, reduced predation pressure and shifted resource requirements have been hypothesized as the primary factors influencing size-specific home range expansion (Chapman & Kramer, 2000; Dahlgren et al., 2008; Welsh et al., 2013; Green et al., 2015).

Home range sizes were significantly larger for TP individuals than for IP individuals. High ranking territorial TP males exhibited disproportionately large home ranges, and often occurred in all three reef sections throughout the study period. Improved swimming efficiency, reduced predation risk, and the negligible costs of spermatogenesis may enable large TPs to search for high-quality food resources over a more extensive foraging habitat (Schaerer & Robertson, 1999; Howard et al., 2013; Welsh & Bellwood, 2013). Furthermore, expanded home range areas may be a response to ontogenetic shifts in behavioural traits: IP individuals are expected to be relatively risk averse and more reluctant to leave familiar habitat, because a slightly higher risk in mortality reduces future reproductive success.

Reproductively highly successful TP males on the other hand are commonly relatively risk prone, and therefore more willing to relocate to non-familiar foraging habitat (Warner & Hoffman 1980ab; Warner, 1998; Chapman & Kramer, 2000).

#### **5.4.2 Spawning territory fidelity**

Smaller IP individuals showed a non-significant trend for higher affinity to specific spawning territories, while larger IPs tended to be more flexible in their choice of spawning territories. Conversely, smaller TP individuals visited several spawning territories throughout the study period, while larger TP individuals exhibited higher affinity to a territory site. While these patterns were not statistically significant, the interaction between phenotype and size indicated a statistical trend ( $p=0.15$  in the most-supported model) that is consistent with typical characteristics of a harem mating system.

Large TP males generally have sufficient energy resources to occupy and defend mating sites, in order to gain primary access to female mates. These mating sites are generally in fixed locations along the outer reef edges of a reef section and occupied by the same territorial male on a daily basis (Warner & Robertson, 1978; Warner & Hoffman, 1980a; Warner & Schultz, 1992). In this study, the three largest terminal phase males indeed consistently occupied and defended the same localised territories during spawning hours. Smaller terminal phase males do not have the size-specific competitive abilities to successfully attain territorial status (Warner, 1984a; Muñoz & Warner, 2003a; Munday et al. 2006 ab). Thus, they devote a high proportion of their energy to growth (to achieve higher competitive abilities) and are often sexually inactive (Warner & Schultz, 1992; van Rooij et al., 1995). However, visiting multiple territories during spawning hours might be an important mechanism to assess potential competitors and to contend for mating site acquisition if a territory becomes vacant (Warner & Schultz, 1992). Some of these tagged, non-territorial terminal phase males were observed to be actively participating in group spawning activities during new moon.

Smaller IP individuals tended to be resighted at the same spawning territory, suggesting that they may only utilize one. Smaller females should be more risk averse (e.g., Warner 1998); so affiliation with a single spawning territory, and associated use of familiar migration routes may substantially limit mortality risk (Warner, 1990, 1995, 1998; Mazeroll & Montgomery, 1998). Furthermore, higher flexibility in territory choice with increasing size may enable larger females to exercise mate choice to maximise their fitness, and/or this may facilitate the acquisition of information to maximise future fitness (e.g., in the attainment of territorial status after sex-change; Warner, 1986; Shibuno, 1993).

### 5.4.3 Migration distances and spawning site choice

Migration distance (mean distance to spawning sites) increased with body size for both IP and TP individuals, and TP individuals travelled greater distances than IP individuals on average.

These findings support my hypothesis that variation in migration distances may be determined by size- and sex dependent trade-offs between reproduction and growth (Hoffman, 1983; Roff, 1990; Warner, 1998). A significant increase of migration distances with size demonstrates that smaller individuals may prioritize growth and survival by avoiding energy expenditure and risks imposed by long-distance migrations. Larger individuals migrated longer distances; this is consistent with my prediction that the amount of energy available to reproduction-related activities increases with size (Hoffman, 1983; Roff, 1990; Shibuno, 1993). Of particular note, two large territorial males continuously migrated up to 300 meters from their preferred area of feeding, to a site with the highest mating stock density (where they established mating territories).

Evidence of this hypothesis is also provided by Roff 1988, 1990, who predicted a positive correlation of size and migration distance based on bioenergetic calculations. Similar patterns of intraspecific variations in migration distances have also previously been reported for another tropical wrasse species, *Halichoeres miniatus* (Shibuno, 1993).

While migration distances of TP individuals were, on average, considerably larger than those for IP individuals, the two longest migration distances were measured for two IP individuals. These two individuals (12 cm TL) migrated more than 300 meters from their home range area to spawning site S1 during new moon. Although mainly larger individuals exhibited a significant tendency of travelling longer distances, these observations collectively suggest that individuals of all size classes undergo longer and more costly migrations during new moon. Presumably, the new moon might represent an external cue that stimulates adults to migrate to particular sites (in this case S1) that maximise their fitness (spawning at particular sites, e.g., further down current is more worthwhile closer to the new moon). Due to its proximity to a break in the barrier reef crest, S1 might provide optimal hydrodynamic conditions during new moon for larval dispersal (Domeier & Colin, 1997; Sancho et al., 2000; Claydon et al., 2004, 2014). Alternatively, the new moon period might facilitate migrations to specific sites to synchronize reproductive activities. Synchronized spawning in dense colonies might incur important selective advantages for adults and their offspring, like increased fertilization rates, and lower predation rates on adults and eggs (Robertson et al., 1990; Claydon et al., 2004, 2014).

Irrespective of the proximate drivers, individuals seem to be aware of alternative sites, but only migrate there during specific environmental conditions that potentially increase fitness. These findings contradict Warner 1990, 1995 and Claydon et al., 2012, who speculated that iteroparous

individuals repeatedly migrate to a single site, even if alternative sites may provide a higher reproductive output. Continued use of a spawning site may be traditional and learned or determined by its proximity to a feeding location (Warner 1988b; Claydon et al., 2012). However, flexibility of individual migration behaviour, as found in the present study, may entail several selective advantages: foremost, individuals are able to continuously optimize their reproductive investment by assessing the costs of migrating to a specific location relative to the benefits of spawning there (cost-benefit optimisation). For example, individuals may prefer to reproduce at a nearby spawning site if the risks of migrating to another site exceed potential reproductive benefits. If environmental cues signal a site-specific increase of fertilization rates or offspring/adult survival, individuals may be more willing to accept greater risks in travelling longer distances. Further size-related, physiological trade-offs might act in addition to this, with smaller individuals potentially not migrating at all, or not as frequently, to more distant spawning sites during new moon (Rideout et al., 2005; Jorgensen et al., 2008; McBride et al., 2014).

The study also revealed the relative spatial scale of spawning migrations. Individuals inhabiting the mid- and offshore reef sections never appeared at a seemingly popular mating site like S1. This may suggest that wide patches of sand could act as barriers to fish movements, and/or that the costs of migrating to a site like S1 may simply exceed any potential gain in adult fitness (Robertson, 1988; Chapman & Kramer, 2000; Meyer et al., 2010).

#### **5.4.4 Limitations and caveats**

Low resighting rates revealed limitations associated to the t-bar tagging method. Most likely, the low percentage of relocated individuals derives from disproportionally high tag loss. I frequently noticed tagged adults trying to remove their tags after release, by physically grinding their sides against rocks and coral boulders (a natural behaviour to remove parasites). Furthermore, conspecifics often seemed to confuse the brightly coloured tags with some sort of food item (most likely a parasite- I have previously observed similar behavioural displays on passing convict surgeonfish swarms) and started to aggressively hunt tagged individuals after release. Probably due to the above listed reasons, I frequently spotted individuals with only half of a tag, or a typical, tagging-related cut-in point. Furthermore, biofouling covered some of the tags within only two weeks, so that original colour patterns on the tags could no longer be identified.

Other tagging methods like acoustic telemetry, applied over a longer time period, might provide substantially higher sample sizes per individual. This would probably permit a greater spatial

resolution of size- and lunar-associated migration patterns and site fidelity. Acoustic monitoring systems would for example reveal, if larger individuals commute more frequently to a spawning site than smaller individuals, or if some individuals don't spawn at all throughout the month.

Due to the described limitations of the t-bar tagging method, I was not able to statistically evaluate home range size via kernel utilization distribution (KUD) estimates. MCP calculations are strongly influenced by outliers and sample size, so MCP home range estimates might not precisely describe actual home range size (Jennrich & Turner, 1969; Powell, 2000; Jones, 2005). Moreover, daily activities were most likely concentrated in small core areas within home ranges. Previous investigations on habitat use of sixbars have shown that females and males move within small activity centres, approximately spanning only a small proportion of their total home range area (see chapter 3). Evidence of these core activity areas have been demonstrated in other protogynous coral reef fish, like several wrasse and parrotfish species (Jones 2005, 2007; Welsh & Bellwood, 2012; Howard et al., 2013).

#### **5.4.5 Conclusion**

Spatial and temporal variation in spawning migrations and habitat use are shaped by interactions between reproductive phenotype, size and lunar phase. Adult sixbars appear to make informed decisions about where and when to spawn based on environmental cues, and they exhibit a high degree of behavioural flexibility in their choice of a spawning location. This study provides valuable insight into the spatial dynamics of a protogynous, highly iteroparous reef fish species, and emphasizes the potential impact of the lunar phase on individual variability in migration patterns and spawning site choice.





## **CHAPTER SIX**

### **THE INFLUENCE OF EARLY LIFE HISTORY TRAITS ON THE PROBABILITY OF SEX- CHANGE**

## 6.1 Introduction

A fundamental objective of life history theory is to understand the processes that lead to variation in reproductive output among individuals (Roff, 2002; Stearns, 1992). From the moment of fertilization, individuals vary in development and growth rates, and this can lead to variation in performance in their later life stages (Sponaugle & Cowen, 1994; Shima & Findlay, 2002; Vigliola & Meekan, 2002; McCormick & Hoey 2004).

In organisms with complex life cycles like reef fishes, variance in developmental trajectories can lead to distinct variations in phenotypic traits (e.g. size) at the point of maturity (Schultz & Warner, 1991; Brockmann & Taborsky, 2008; Noonberg et al., 2015; Shima et al., 2015). Differential growth histories may result from maternal carry-over effects (McCormick, 2006), and these effects may be further modified by the timing of birth and subsequent environmental selection pressures like food availability, lunar phase, settlement habitat and predator densities (see for example McCormick & Hoey 2004; Walker & McCormick, 2004; Shima & Swearer, 2019).

Many coral reef fish species exhibit hierarchical mating systems, whereby a large, dominant male monopolizes females or environmental resources to gain exclusive access to mating opportunities (Ghiselin, 1969; Warner, 1984a). In these systems, large males with high, size-related, competitive abilities are disproportionately successful. Consequently, individuals optimize their fitness by maturing as females first, and changing sex when they reach competitive size in later life stages (Ghiselin, 1969, Warner, 1984a, 1988a; Munday et al., 2006a). Since only larger individuals change sex, reproductive success in a protogynous system is strongly dependent on patterns of growth (Warner, 1988a; Munday et al., 2004; Munday et al., 2006a). Individuals from the same cohort may change sex at different ages and sizes due to differential growth histories during their ontogeny (Walker & McCormick, 2004; Munday et al., 2006a; Walker et al., 2007). Several underlying mechanisms might determine which individual within a group will successfully transition into a territorial male, and monopolize mating opportunities.

Studies have found, that sex-change may be a result of accelerated growth prior to sexual transition (Ross, 1987; Walker & McCormick, 2004; Walker et al., 2007; McCormick et al., 2010). Shortly before and during sex-change, individuals may disproportionately divert their energy into somatic tissue, mostly at the cost of a higher reproductive output. For example, Walker & McCormick, 2004 demonstrated that large males of the protogynous sandperch *Parapercis cylindrica* exhibited a growth spurt (based on otolith increment widths as a proxy of growth rates) shortly before sex-change. Sex-

change associated growth acceleration may come at the cost of reduced investment into gonadal tissue (as a trade-off between reproduction and growth; Taborsky, 1998; Villegas-Rios et al., 2014).

Size-at-age differences may also be a result of faster growth during earlier life stages. Variable environmental conditions during the pelagic stage, such as food availability and hydrodynamics, may lead to differences in larval growth performance, with some individuals growing faster than others (Sponaugle & Cowen, 1994; Vigliola & Meekan, 2002; Shima & Swearer, 2019). Higher growth rates prior to settlement may markedly influence subsequent juvenile growth and survival and, ultimately, the probability of sexual transition (Walker & McCormick, 2004; Walker et al., 2007; McCormick et al., 2010; Sponaugle, 2010). For example, larger settlers may be able to secure social dominance within a cohort and maintain it throughout their juvenile development. An early, size-specific dominance rank may facilitate faster growth rates, and thus a larger size at maturity, due to improved access to limited resources (Forrester, 1991; Schultz & Warner, 1991; McCormick & Hoey, 2004; Walker et al., 2007).

However, original size advantages at settlement may be modified by a variety of processes governing juvenile development (Holbrook & Schmitt, 1999; Steele & Forrester, 2002; Sponaugle et al., 2006; Gagliano et al., 2007). Juvenile growth rates may vary greatly dependent on the initial habitat individuals settle into, as it determines predator densities, densities of conspecifics and the distribution of resources (Booth, 1995; Shima & Osenberg 2003; McCormick & Hoey, 2004; Shima et al. 2008; Geange & Stier 2009). Consequently, size-differences at maturity might be a manifestation of environmental selection pressures affecting juvenile growth rates after settlement (Sponaugle & Grorud-Colvert, 2006; Holmes & McCormick, 2010). Additionally, smaller sized settlers may compensate for their lower growth rates during larval development, by a period of rapid growth following settlement (McCormick & Hoey, 2004; Gagliano & McCormick, 2007).

The aim of this study was to evaluate the relationship between individual growth history and sex-change (based on otolith increments and gonadal allocation patterns) of a protogynous hermaphroditic species. Specifically, I hypothesized, that “reproductive winners”, i.e. females or initial phase males that successfully transitioned into terminal phase males, are a subset of more favourable growth conditions during their early life history stages (i.e. higher growth rates during larval development, larger sizes at settlement). Furthermore, I evaluated the question of whether adults exhibit size- specific trade-offs between gonadal investment and somatic growth to facilitate sex-change (as potentially reflected by accelerated growth prior to sex-change).

## 6.2 Methods

### 6.2.1 Study species

I estimated variation in growth histories and energy allocation strategies of adult sixbars (*Thalassoma hardwicke*), sampled from the northern lagoon of Moorea, French-Polynesia. Sixbars are one of the most common protogynous coral reef fish species throughout the Indo-Pacific. Local populations contain initial phase (IP) individuals (females and sneaker males, indistinguishable from another) and terminal phase (TP) males (distinguishable from the initial phase by their larger size and brighter coloration). Reproductive investment of adult sixbars is strongly lunar cyclic, with highest investment rates around the new moon (see previous chapters). Larvae develop for an average of 47d in the open ocean and settle back to patch reefs within the lagoons of Moorea (Victor, 1986; Shima et al., 2001a). Settlement occurs at night (Dufour & Galzin, 1993) and is largely dependent on the lunar cycle (with greatest settlement rates around the new moon). Larvae may delay or shorten their pelagic larval duration to settle during darker periods, when predation risk is limited (Acosta and Butler, 1999). Post-settlement growth and survival is strongly density dependent (Shima, 2001b, Shima & Osenberg, 2003), and additionally determined by inter-specific competition and predator densities (Shima, 2001a, Shima et al. 2006, Shima et al. 2008, Geange & Stier, 2009)

### 6.2.2 Gonadal allocation and larval life history traits

I obtained 20 sixbar individuals (a mixture of females and males, spanning a range of sizes) at weekly intervals from February to June 2017 (338 individual in total). All fish were caught via hook and line by a local fisher woman, from an area of fringing reef on the north shore of the island. All collections were made on the same day of the week and in the morning hours between 6-10 am. On the day of collection, I recorded total length (TL), weight, developmental phase (initial phase male, female or terminal phase male) and gonadal weight of all individuals. Developmental phase was determined by an individuals' phenotypic appearance (colour pattern), as well as by macroscopic examination of its gonads under the dissection microscope. Female ovaries are commonly characterized by their orange-yellow coloration and rough surface texture. Male testes are typically characterized by their whitish coloration and smoother surface. Based on these estimates, I evaluated the gonadosomatic index ( $GSI = \text{gonad weight} * 100 * \text{somatic weight}^{-1}$ ) as an indicator of size-specific reproductive investment.

Additionally, I removed sagittal otoliths from each fish and stored them dry until further processing. A subset of otolith from 164 individuals were then sent to the Barcelona Otolith Reading Service to estimate larval growth histories from otolith microstructure. Each otolith was mounted sulcus-side down, and then polished along the sagittal plane to expose daily growth increments (validated in Shima, 1999) along the postrostral axis. Daily increment widths from the otolith core to the

conspicuous settlement mark were tagged and measured along this axis using the 'caliper tool' of ImagePro Premier.

Several studies have validated a strong association between otolith growth and somatic growth in wrasse larvae. It is generally assumed that otolith increments are deposited daily (Victor, 1982), and that the width of each increment provides a relative estimate of somatic growth (Searcy & Sponaugle, 2000). The timing of settlement can generally be identified by a conspicuous settlement-mark. Thus, the microstructure of the extracted otolith provides a reliable estimate of larval growth rates, age and approximate size at settlement.

Age at settlement was estimated by counting the number of daily growth increments from the core to the settlement mark plus two (to account for the lag between spawning and the initiation of otolith increments: Victor, 1986). Additionally, the width between two successive increments (as a proxy of daily growth rates during the larval phase) was estimated to the nearest  $\mu\text{m}$ . Radius width at settlement, as a proxy for larval size at settlement, was validated by measuring the distance between the settlement core to the settlement mark. Total otolith radius was estimated by measuring the distance between the otolith core to the distal end of the otolith.

### **6.2.3 Data analysis**

I used Kolmogorov-Smirnov two-sample tests to evaluate differences in size-frequency distributions between females, initial phase males and terminal phase males.

Exploratory analysis indicated that the relationship between GSI and TL (total length), dependent on developmental phase, was non-linear for some levels of developmental phase. Therefore, I used generalized additive models (GAM) to assess variation in GSI (as a proxy of gonadal investment) dependent on a smooth interaction of the variables TL and developmental phase (females, initial phase males and terminal phase males). Estimated degrees of freedom for each category level indicate the degree of nonlinearity for each function. The lower the estimated degrees of freedom (edf), the more linear the estimated relationship, with an edf of 1 corresponding to a linear fit. This analysis allowed me to test for significant differences in gonadal investment between sexes, and between male strategies. I compared the fit of the GAM model with the fit of a simple linear model based on likelihood ratio tests (ANOVA). Due to collinearity issues between TL (total length) and phenotype, I standardized mean TL relative to the mean TL of each phenotype. GAM and linear models were performed with the mgcv package (Wood, 2011) in R (R Core Development Team 2019).

To investigate patterns of variation in larval life-history traits as a function of sex (or male strategies), I modelled pelagic larval duration (total number of increments until settlement) and otolith radius at

settlement, as a function of developmental phase (using two separate general linear models). Significant differences could suggest that larval developmental histories shape future life history strategies.

To evaluate potential differences in average larval growth rates between reproductive phenotypes, I computed a linear model for each individual, with larval age as independent variable, and radius width (cumulative increment width dependent on larval age) as dependent variable. After fitting each model, I obtained a parameter estimate that described patterns of growth for every individual. I then analysed these parameters using linear regressions, with the parameter estimate as dependent and reproductive phenotype as independent variable. I visually checked assumptions of linearity based on residual plots and data distributions.

Additionally, I assessed if rates of growth throughout larval development varied as a function of reproductive phenotype. Because exploratory analysis indicated non-linearity, I conducted a polynomial regression analysis, with increment width (a proxy of daily growth) as dependent variable and an interaction between larval age and reproductive phenotype as dependent variable. All analyses were performed with the R 2.9.1 software (R Core Developmental team 2019).

## 6.3 Results

### 6.3.1 Size-frequency distribution

Total lengths of dissected females ranged between 9.2 and 16.2 cm (mean:  $12.5 \pm 1.4$  SD). Total lengths of IP males largely overlapped with female total lengths and varied between 10.0 and 16.0 cm (mean:  $13.6 \pm 1.4$  SD). TP total lengths encompassed sizes from 15 to 19.2 cm (mean:  $17.0 \pm 0.97$  SD). Kolgomorov-Smirnov tests indicated, that size distributions of females, IP males and TP males significantly varied from each other (Fig. 6.1).

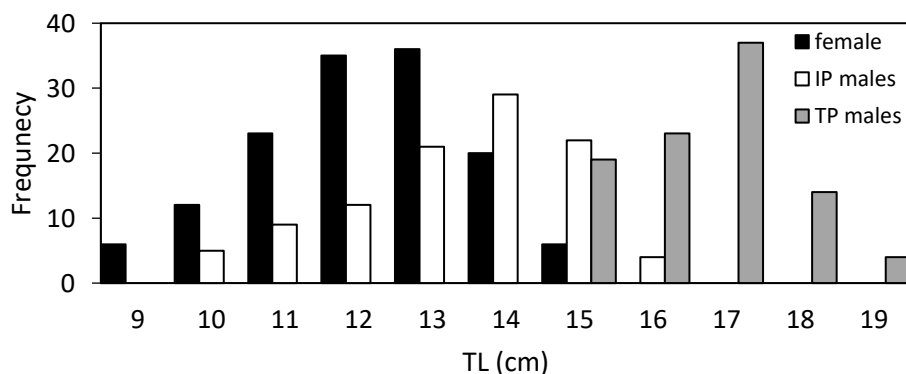
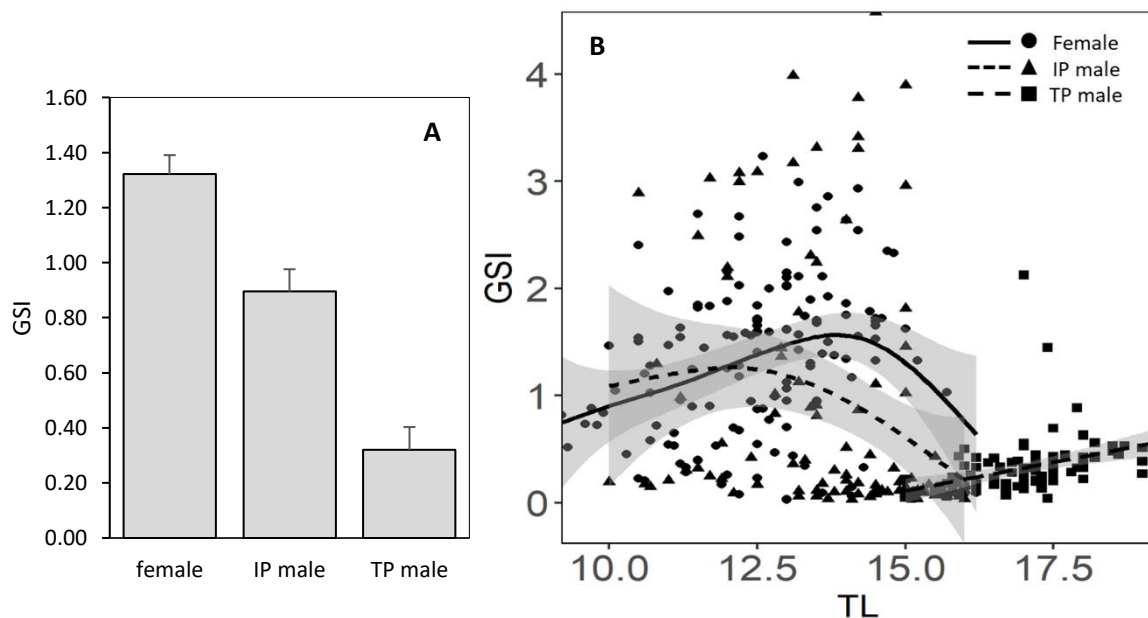


Fig. 6.1 Size frequency distribution (where size = TL) of female, IP and TP sixbars (n= 338).

### 6.3.2 Gonadal investment

Parameter estimates and test statistics indicated that average GSIs significantly varied between females, IP males and TP males. Linear models suggested that females invest significantly more into gonadal size in comparison to IP and TP males. TP males exhibited by far the lowest average GSI, with an average of  $0.32 \pm 0.08$  SE in comparison to  $0.9 \pm 0.08$  SE for IP males and  $1.3 \pm 0.06$  SE for females (see Fig. 6.2 A and Table 6.1).

The non-linear pattern of the GAM model performed significantly better than a linear fit between GSI and total length dependent on developmental stage ( $p < 0.01$ ). The degrees of freedom (edf) associated to each smooth function in the GAM model suggest that gonadal investment of females and IP males varied in a non-linear fashion with size (see Table 6.2). An edf of 1 for TP males indicated that the relationship between GSI and TP size was linear. The smoothed curve of the fitted GAM suggests that, after a linear increase, the rate of gonadal investment rapidly decreases for larger females and IP males (see Fig. 6.2 B). By increasing almost linearly with size, size-dependent gonadal investment rates of TP males significantly differed from those of females and IPs (see Fig. 6.2 B and Table 6.2).



**Fig.6.2 A)** Variation of gonadal investment (GSI) dependent on developmental stage (females, IP males and TP males). **B)** Smoothed curves of relative gonad weight (GSI) dependent on sixbar total length (TL) and developmental stage (females, IP males, TP males) with generalized additive model fits (GAM).



**Table 6.1** Parameter estimates and test statistics describing variation in gonadal investment (GSI) dependent on weight, reproductive phenotype (female, IP male and TP male), and the interaction between weight and reproductive phenotype. Models were fit using simple linear regression (LM) in R. Asterisk symbols reflect significance levels.

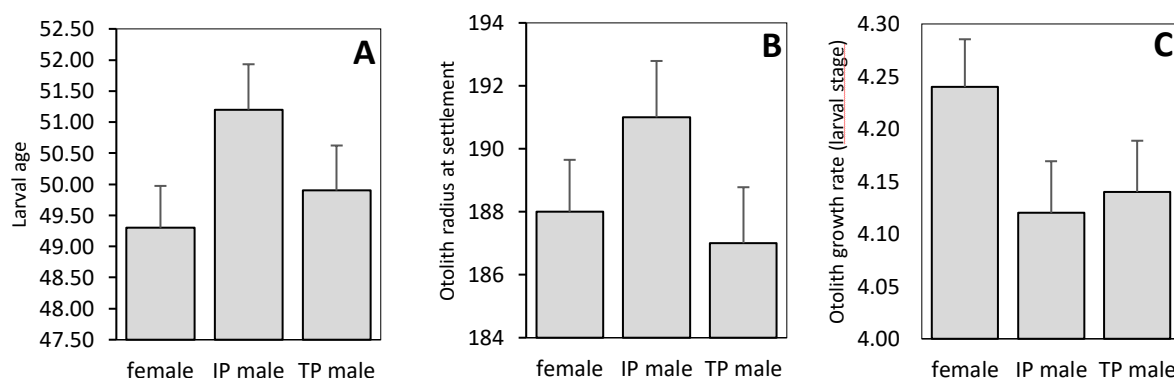
Model	levels	Estimate	SE	t value	p value	
<b>GSI~</b>						
<b>sex</b>	IP male	-0.406	0.104	-3.912	<0.001	***
	TP male	-1.014	0.104	-9.723	<0.001	***

**Table 6.2** Approximate significance of the smooth terms (generalized additive model fit) describing variation of GSI dependent on size for females, IP males and TP males. As indicated by the estimated degrees of freedom (edf), gonadal investment of females and IP males varies in a non-linear fashion with size. Gonadal investment of TP males describes a linear relationship with size (as indicated by an edf of 1).

Model	edf	Ref.df	F	p value	
<b>GSI~</b>					
s(Size res.)*female	2.995	3.776	3.376	0.016	*
s(Size res)*IP	2.616	3.286	5.905	<0.001	***
s(Size res)*TP	1.000	1.001	1.673	0.197	

### 6.3.3 Early life-history traits

There were no significant differences in early life history traits between females, IP males and TP males (see Table 3). However, IP males showed a slight trend of settling at an older age and at a larger size (as indicated by otolith radius at settlement) in comparison to females and TPs (see Fig. 6.3 and Table 4). Additionally, females appeared to exhibit slightly faster larval growth rates relative to IP and TP males (see Fig. 6.3 C).



**Fig 6.3** Variation in larval age (**A**), otolith radius at settlement (**B**) and otolith growth during larval stages (**C**) dependent on reproductive phenotype (female, IP male, TP male). Given are marginal means, back-transformed to the original scale (+SE), estimated with other fixed effects held at their mean values. None of the associations were significant (see table 6.3 and 6.4).

**Table 6.3** ANOVA table of linear models describing (1) larval age (2) otolith radius at settlement and (3) otolith growth rate during larval stages of *Thalassoma hardwicke* adults, in relation to reproductive phenotype. Models were fit using simple linear regression (LM) in R. Asterisk symbols reflect significance levels.

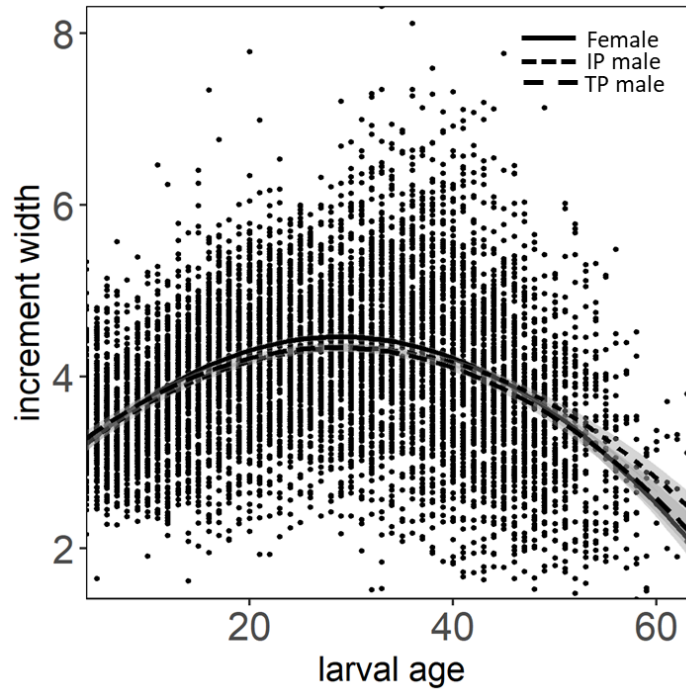
		Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Total larval age~</b>	sex	2	98.5	49.248	1.8053	0.1678
<b>Otolith radius at settlement~</b>	sex	2	462.8	231.41	1.4094	0.2473
<b>Otolith growth (larval stage)~</b>	sex	2	0.5007	0.2503	2.0276	0.135

**Table 6.4** Parameter estimates and test statistics for models describing (1) larval age before settlement (2) otolith radius at settlement and (3) otolith growth rate during larval stages of *Thalassoma hardwicke* adults, in relation to reproductive phenotype (females, IP males and TP males). Models were fit using simple linear regression (LM) in R. Asterisk symbols reflect significance levels.

Model	levels	Estimate	SE	t value	p value
<b>total larval age~</b>					
sex	IP male	1.874	0.995	1.883	0.062 .
	TP male	0.640	0.990	0.646	0.519
<b>Otolith radius at settlement~</b>					
sex	IP male	2.74	2.44	1.12	0.26
	TP male	-1.43	2.43	-0.59	0.56
<b>Otolith growth rate (larval stage)~</b>					
sex	IP male	-0.12	0.07	-1.83	0.07 .
	TP male	-0.11	0.07	-1.59	0.11

#### 6.3.4 Larval growth trajectories

Larval growth curves of females, IP and TP males indicated accelerated growth halfway through larval development (around day 30), and a steep decline of larval growth rates shortly before settlement (see Fig. 6.4). A direct comparison of larval growth curves (larval age in relation to increment width) among the three reproductive phenotypes indicated significant differences between developmental stages ( $p=0.003$ , see Table 6.5). The polynomial regression model suggested that IP males exhibited slightly higher growth rates (LMER:  $p<0.001$ ) towards the end of their larval development (~during the last ten days before settlement) relative to females and TP males (see Table 6.6).



**Fig 6.4** Growth curves ( $\pm$  95% confidence interval) between larval age and predicted increment width (95% probability contour) dependent on reproductive phenotype (female, IP male, TP male). Parameters were fitted using polynomial regression (degree 2)

**Table 6.5** ANOVA table of linear and polynomial models describing otolith increment width dependent on reproductive phenotype\*larval age of *Thalassoma hardwicke* adults (categories: females, IP males and TP males). Asterisk symbols reflect significance levels.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
sex	1.05	0.53	2	1705.7	0.81	0.445	
larval age	849.08	849.08	1	7836.8	1306.36	< 0.001	***
l(larval.age^2)	793.43	793.43	1	7874.0	1220.74	< 0.001	***
sex*larval age	5.76	2.88	2	7836.7	4.43	0.012	*
sex*l(larval.age^2)	7.50	3.75	2	7874.0	5.77	0.003	**

**Table 6.6** Parameter estimates and test statistics describing variation in increment width dependent on reproductive phenotype\*larval age of *Thalassoma hardwicke* adults (categories: females, IP males and TP males). Models were fit using simple linear and polynomial regression (LMER) with fish id as random effect. in R. Asterisk symbols reflect significance levels.

Model	levels	Estimate	SE	df	t value	p value	
<b>increment width~</b>							
sex	IP male	2.2E-02	2.4E-02	1.9E+03	0.92	0.356	
	TP male	3.1E-02	2.5E-02	1.9E+03	1.23	0.219	
larval age		2.8E-02	1.2E-03	7.9E+03	23.74	< 0.001	***
l(larval.age^2)		-5.0E-04	2.1E-05	7.9E+03	-23.48	< 0.001	***
sex*larval age	*IP male	-5.0E-03	1.7E-03	7.8E+03	-2.97	0.003	**
	*TP male	-3.7E-03	1.8E-03	7.8E+03	-2.08	0.038	*
sex*l(larval.age^2)	*IP male	1.0E-04	2.9E-05	7.9E+03	3.59	< 0.001	***
	*TP male	5.6E-05	3.1E-05	7.9E+03	1.79	0.073	.

## 6.4 Discussion

The main objective of this study was to investigate a potential relationship between life-history traits and sex-change. My findings suggest that larval life-histories did not determine which individuals became territorial males in later life stages. There was only a non-significant tendency of initial phase males settling at a larger size and at an older age in comparison to females and TP males.

As typically found in high-density populations, IP males almost comprised a third of the sample population (Warner & Hoffman, 1980b; Munday et al., 2006b). The high proportion of an alternative male phenotype suggests that this reproductive strategy may be relatively successful in the sampled sixbar population. Since IP males experience high levels of sperm competition, their relative testes weights were significantly higher in comparison to TP males (Taborsky, 1998; Schaerer & Robertson, 1999). However, GAM correlation curves between size and gonadal investment indicated, that IP males (and females) notably reduced their gonadal investment at larger sizes.

### 6.4.1 Do differences in larval growth histories predict sex-change?

A study of Walker et al., 2007 on potential linkages between larval life-histories and sex-change illustrated, that successfully transitioned males of the protogynous species *Parapercis synderi* exhibited higher growth rates during their larval phase in comparison to females. They speculated that initial size advantages at settlement may carry-over to the adult stage because larger recruits establish immediate dominance within a cohort. And this dominance and its associated fitness benefits is maintained throughout juvenile development until maturity.

This study couldn't verify a significant relationship between larval life history traits (e.g. size and age at settlement) and sex-change. It is possible that larger sizes at settlement may facilitate initial survival probabilities, as successful recruitment may require a minimum of energy reserves (Searcy & Sponaugle, 2000). However, the great spatio-temporal variability of environmental regimes (e.g., topographic complexity, social environment and predator densities) that recruits are exposed to, may negate any initial size advantages after settlement (McCormick & Hoey, 2004).

For example, size-specific mortality might substantially vary dependent on the composition and density of predator assemblages, that juveniles are exposed to during their post-settlement phase. Illustratively, an aquarium-based predator choice experiment of Holmes & McCormick, 2010 demonstrated, that some coral reef predators selectively removed larger settlers from an experimental reef, while others only preyed on smaller settlers. Other studies (McCormick & Hoey, 2004; Gagliano et al., 2007) demonstrated that smaller settlers compensated for their size disadvantages, by displaying accelerated growth during juvenile development. Additionally, other factors such as topographic complexity, density-dependent processes and interspecific competition

may lead to marked variation in juvenile growth performances, independent of the initial size at settlement (Shima, 2002; Shima & Osenberg, 2003; McCormick & Hoey, 2004; Geange & Stier, 2009).

The insignificant trend of IP males settling at larger sizes raises interesting questions concerning the mechanisms of primary sexual differentiation. Munday et al., 2006b suggested that the differentiation into primary males is determined by local social conditions such as population density and sex ratio, and individual genetic sensitivity to those conditions. In other words, some individuals are genetically programmed to take the initial phase male pathway if population densities exceed a certain threshold. Most likely, slightly larger sizes-at-settlement for IP males, as found in this study, are an artefact of a relatively small sample size. However, would a larger sample size yield significant results? If so, this could imply that a size threshold represents the developmental switch that triggers differentiation into primary males at higher population densities. This assumption may be supported by the fact that initial phase males were on average significantly larger than females in this study.

#### **6.4.2 Other potential mechanisms influencing sex-change**

Potentially, sex-change may be a consequence of faster juvenile growth rates that lead to larger sizes-at-age during adulthood. As a corollary to the size-advantage model, a larger size at a given age may facilitate sex-change due to higher competitive abilities in defending and occupying a territory (Francis & Barlow, 1993; Adams & Williams, 2001).

However, a variety of studies (Walker & McCormick, 2004; Walker et al., 2007; McCormick et al., 2010) hypothesized that rapid growth during juvenile development may not necessarily provide a selective advantage in a strictly hierarchically structured mating system. When individuals enter an adult population, it may be even more advantageous for larger individuals to initially limit growth in order to avoid energetically expensive conflicts with higher ranking individuals (Wong et al, 2007; McCormick et al., 2010). Mature females and primary males with divergent juvenile growth trajectories might therefore be of similar size at a given age due to growth inhibition.

Sexual allocation studies have shown that sex-change is strongly dependent on sex ratio and population densities. Consequently, relative size and not absolute size might determine the timing of sex-change. For example, sex-change may not be beneficial for a larger individual if aggregated fecundity of other females within a harem might not exceed fecundity of the single largest female. A larger individual may also not change sex, if the population is composed of many initial phase males, since intense sperm competition might substantially lower expected reproductive success (Munoz & Warner, 2003, 2004; Munday, et al., 2006a).

Studies on growth trajectories of successfully transitioned individuals suggest that sex-change may more likely be a result of accelerated growth just before and during sex-change (Ross, 1987; Walker & McCormick, 2004; Walker et al., 2007; McCormick et al., 2010). In all these cases juvenile growth did not determine which individual changed sex and at what size and age sexual transition was achieved. A relatively steep decrease in gonadal investment rates with increasing IP male and female size, as evident in this study, might indirectly indicate that larger individuals disproportionately reallocate energy from reproductive tissue to somatic tissue to facilitate sex-change (Taborsky, 1998; Walker & McCormick, 2004).

#### **6.4.3 Future directions**

I note that the juvenile growth hypothesis, as well as the adult growth-spurt hypothesis are both possible mechanisms influencing sex-change. More detailed assessments are necessary to evaluate the relative contribution of juvenile and adult growth to the initiation of sex-change in the sixbar wrasse. For example, a complete record of daily increment widths, from the settlement mark up to the sex-change mark (a typical characteristic in otoliths of sex-changing species), in combination with gonadal histology, may elucidate the specific mechanisms underlying sex-change in the sixbar wrasse (Walker & McCormick, 2004).

Another interesting question, not explored in this study due to time restrictions, is the influence of the timing of birth on sex-change. Since sixbars significantly enhance their reproductive effort at new moon, the question arises if reproduction during the new moon may provide a selective advantage for offspring. Shima et al., 2019 indicated that offspring born around the new moon may indeed display faster growth rates while in the pelagic phase. This stimulates the intriguing question if a significantly higher proportion of successfully transitioned individuals was potentially born at a specific time, like for example around the new moon period. Such analysis would shed more light on the specific selective advantages underlying lunar-cyclic reproduction. However, it would necessitate an exact count of daily increments from the moment of birth up to the sex-change mark.



## CHAPTER SEVEN

### GENERAL DISCUSSION



## 7.1 Summary of key findings

The main goal of this thesis was to gain a better understanding of the key factors influencing intra-specific variation in life-history strategies of a protogynous, highly iteroparous coral reef fish species. By focusing on a single species as model organism, my research has provided valuable insight into the processes mediating reproductive investment decisions and energy allocation strategies. Since protogynous sex-change is the most common reproductive mode among tropical reef fishes around the world, my findings may have broader implications for coral reef fish in general. My key findings were:

- (1) In chapter two I established that overall reproductive activities of the sixbar wrasse are disproportionally influenced by the lunar cycle and spatial gradients, with highest activities around the new moon and in exposed territories
- (2) In chapter three I evaluated that individuals of different sizes and sexes invest differently into reproduction dependent on the lunar cycle and spatial gradients, with profound implications to the prevailing mating system at a spawning site
- (3) In chapter four I found that energy allocation strategies during non-spawning hours (foraging, social activities and movement patterns) significantly varied across the lunar month and depending on social status
- (4) In chapter five I showed that energy investment into migration and spawning site choice was strongly size-dependent, and significantly influenced by the lunar cycle
- (5) In chapter six I established that sex-change may not be influenced by larval growth histories, but rather by other processes such as growth acceleration prior to sex-change

My thesis revealed the complexity of processes that may influence life-history variation in a protogynous coral reef fish species. In the following chapter I try to outline how my findings improve our understanding about the key drivers that shape investment decisions and life history traits of a sex-changing coral reef fish species.

## 7.2 The lunar phase as primary environmental driver of parental investment decisions

Delineating the lunar component of reproductive investment decisions is of special importance, since the lunar cycle has always been a crucial factor influencing important life-history events in reef fish species. In this study, the lunar cycle seemed to disproportionally shape parental reproductive investment decisions of the sixbar wrasse, a common, highly iteroparous reef fish species.

The specific selective mechanisms underlying lunar periodicity remain speculative. However, it is reasonable to assume that lunar-cyclic spawning may increase adult fitness. For species with a dispersive larval stage, fixed responses to a large-scale environmental cue may be favoured if the influences of local conditions on reproductive success are relatively unpredictable (because they are too variable in time and space). Several potential mechanisms may be responsible for the selection of lunar-associated spawning: (1) maximal dispersal of propagules due to higher peak tidal amplitudes during new moon, (2) synchronized spawning to maximise fertilization rates and minimize predation on adults and eggs, (3) enhanced survival probability of larvae until the recruitment stage (Robertson et al., 1990; Domeier & Colin, 1997; Claydon, 2004; Shima et al., 2018). I note these factors may not be mutually exclusive, and further research will be required to disentangle the specific selective processes driving lunar periodicity in spawning. Reproduction during new moon may not necessarily always maximize fitness, but it may guarantee the highest average fitness for adults and/or offspring. However, sixbars may also utilize detectable local cues for reproduction. Territory location and (to a lesser extent) current direction seem to convey enough information to elicit behavioural responses over very short time scales.

Based on my initial findings, I tried to explore if the lunar cycle also affects energy allocation strategies during non-spawning hours. My findings suggest that activities like feeding, social interactions and movement patterns indeed significantly varied with the lunar cycle. In accordance with life-history theory, that suggests adults should make energetic trade-offs between reproduction and growth to maximize fitness (Stearns, 1992), 'better' times for reproduction (the new moon period) might stimulate activities that, ultimately, relate to reproduction (e.g. social activities). Conversely, individuals may disproportionally invest into growth, as reflected in higher feeding rates and more clustered movement patterns, during portions of the month that are less advantageous for reproductive investment. A peak of feeding activities during the full moon and just before the third quarter, raises the question whether adult sixbars strategically accumulate energy resources during specific portions of the lunar month, to optimize their reproductive potential during the new moon (i.e., also described as "mixed income strategies"). Taken together, my findings provide valuable

insight into how an environmental cue (i.e., the lunar cycle) for reproduction may also drive temporal variation in activity patterns during non-spawning hours.

In conclusion, my study provides comprehensive information about the influence of environmental fluctuations on reproductive investment and time budget patterns of a common reef fish species. Lunar associated spawning periodicity has been observed in other locations (Claydon et al., 2014), and it appears to be a common phenomenon among coral reef fish species. However, by incorporating a more complete assessment of local abiotic conditions at a spawning site, as well as extending behavioural studies to non-spawning hours, my findings highlight the disproportionate influence of the lunar cycle on life history events of a protogynous reef fish species.

### **7.3 Plasticity of energy allocation patterns based on size and reproductive phenotype**

My study also revealed an ontogenetic basis in the plasticity of energy allocation patterns. Smaller females and males appeared to curtail their reproductive activities during the full moon and third quarter moon, while larger individuals reproduced more consistently throughout the month. Outside of spawning hours IP males and females invested a higher proportion of their time into feeding activities, while territorial males allocated more of their time and energy towards aggressive interactions. Moreover, TP individuals exploited a larger area for feeding than IP individuals, tended to display fine-scale movement patterns consistent with directional swimming and migrated longer distances to reach specific spawning sites.

The general finding that smaller/low-ranking individuals allocate a higher proportion of their time to non-reproductive activities, is consistent with the size-advantage model (Munoz & Warner, 2003) suggesting that smaller individuals are predicted to invest more into future reproductive success, if fitness rapidly increases with size and age. In a mating system, where large males monopolize mating opportunities, smaller males and females are expected to prioritize growth over reproduction to increase their probability of survival to sex-change (and its associated fitness gains). Consequently, smaller individuals should be more risk averse and attempt to avoid any activities that entail greater energy expenditure or mortality risk, like competitive interactions, reproductive behaviour, migratory behaviour and habitat exploration. The net reproductive rewards likely increase with size, because larger individuals may have more excess energy available for gametogenesis, competitive behaviour, habitat exploration and migration. Additionally, they may be more willing to engage in these activities, since mortality risk typically decreases with increasing body size.

Collectively, my findings align with the general consensus that size and associated dominance rank are significant factors explaining among-individual variation in life-history strategies of a protogynous species (Hoffman, 1983; Warner, 1984 ab; Warner, 1998). More specifically, in a mating system where reproductive potential rapidly increases with dominance rank, parental investment decisions are primarily shaped by rank-specific trade-offs between current and future reproduction. My study provides further valuable insight about how a mating system governed by territoriality can lead to profound ontogenetic shifts in the way individual fitness is maximized.

#### **7.4 Context-dependency in size-structured activities**

Context-dependency in size-structured reproductive strategies has rarely been considered in literature. My study took a novel approach by investigating the influence of lunar illumination on temporal variation in size-specific, male and female energy allocation strategies (e.g., reproductive activities, migration and spawning site choice). Smaller males and females may substantially increase their fitness by constraining their reproductive effort to portions of the month that might indicate the most profitable times for reproduction (i.e., the new moon period). The relative benefits of this targeted strategy may change with ontogeny; larger individuals, with sufficient energy reserves and lower mortality risk, may maximise their fitness by reproducing more consistently throughout the month, to bet-hedge against unpredictable variation in offspring survivorship.

My study also sheds new light on the proximate drivers shaping mating dynamics at a spawning site. Lunar cyclic variation in size-dependent parental investment decisions may mediate relative abundances of smaller females and sneaker males at the spawning site, which in turn has an overriding effect on the resulting mating system (i.e., monogamous pair spawning or promiscuous group spawning). Most research on protogynous mating dynamics linked the prevailing social system (hierarchical structured versus more loosely organised) to the overall population size at a site, as exemplified by the closely related bluehead wrasse *Thalassoma bifasciatum*. In this species, territoriality prevails on loosely populated reefs, while group spawning predominates on reefs with high population densities (Warner & Hoffman, 1980b). This study reveals that both extremes, i.e. strict territoriality and promiscuous group spawning, can occur in a fluctuating fashion at the same spawning site, with the lunar cycle as regulatory element. My study suggests that size-dependent variation in female choice might maintain a stable co-existence of the two mating tactics (pair spawning and group spawning) in a sex-changing population.

My results also indicated that size-dependent investment strategies were additionally structured around a spatial cue: proximity to the reef edge. This unique finding aligns with the general prediction that adult mortality risk exerts a strong selective force on parental investment decisions of a highly iteroparous and protogynous species (Warner, 1998). Intraspecific variation in spawning site choice may thus be a result of size-dependent trade-offs between offspring and maternal survival potential. Smaller females may choose territories in more sheltered areas of the reef to increase their own probability of survival, while larger females with reduced predation risk may be more willing to reproduce in exposed territories that facilitate offspring dispersal.

In conclusion, this study highlights how an individual's investment decisions are shaped by interactions between its social status and its physical environment. My findings demonstrate the general necessity to incorporate exogenous factors into individual-based models of behavioural plasticity, to gain a better understanding of the mechanisms driving intraspecific variation in life-history strategies and associated population dynamics in a protogynous reef fish species.

### **7.5 Potential drivers of sex-change**

My findings could not reveal any influence of early larval life history traits on sex-change, indicating that other processes might determine sex-change (i.e., juvenile growth or adult growth histories). However, the lack of statistical significance may be a result of low statistical power or small effect sizes, so these findings yield no definitive proof. Size-specific gonadal investment rates indicated that larger IPs and females disproportionately invested into somatic growth, at the cost of reproductive investment. This could suggest that sex-change may be facilitated by a 'growth-spurt' prior to sex-change. However, my approach lacked the ability to explore fine-scaled growth patterns of adult individuals, making this prediction highly speculative. Only a detailed reconstruction of otolith-based adult growth histories, i.e. a complete record of increment widths after settlement until sex-change for a set of TP individuals, would enable a more powerful test of this assumption. The slight trend of IP males settling at larger sizes and ages, raises intriguing questions if primary sex differentiation may be mediated by a size-dependent developmental switch, i.e. only individuals that pass a certain size-threshold may differentiate into initial phase males.

### **7.6 Future directions**

Although well studied, the adaptive significance of lunar-associated spawning remains speculative. Further work is needed to unveil the selective advantages underlying lunar periodicity. For example,

evaluations of egg dispersal trajectories may clarify if specific oceanographic features during the new moon facilitate faster dispersal/a higher survival potential of fertilized eggs directly after release. While more recent studies provide important knowledge about the effect of lunar periodicity on early life history traits, such as larval growth and size at settlement (see Shima et al., 2018; Shima & Swearer, 2019), qualitative linkages between the timing of birth and developmental stage at maturity might provide valuable information about the origins of life-history strategies and associated eco-evolutionary feedbacks. For example, a complete record of daily increments of large terminal phase males may elucidate if 'reproductive winners' (i.e. territorial males) are a subset of well-timed births (i.e., born during the new moon phase) that enabled them to grow faster or to circumvent specific pre- and post-settlement population bottlenecks like predation pressure or the timing of settlement. Also relevant is a better understanding about how the lunar cycle affects certain aspects of an individuals' physiology. For example, further research may clarify whether the new moon period influences the size, number and quality of female eggs. Furthermore, precise assessments of fertilization rates across the lunar cycle may provide further insight about the specific fitness benefits associated to lunar-mediated group spawning. Additionally, more advanced tagging methods like acoustic telemetry may permit a greater spatial resolution of size- and lunar-associated migration patterns and site fidelity. This would extend our understanding of size-specific energetic trade-offs, such as the magnitude of reproductive investment dependent on size (i.e., do larger individuals reproduce daily, or even several times a day, etc.).

Additional surveys, over broader geographical scales, may provide valuable information about how varying population densities affect intraspecific variation in mating tactics of *Thalassoma hardwicke*. For example, do different population densities and associated competition intensity influence time budgets and energy allocation strategies of smaller individuals? In other words, do smaller males and females spend more time in reproduction, if abundances of territorial males are lower relative to other populations? Also, how does this affect population dynamics and the mating system at a site?

## **7.7 Conclusion**

Understanding the ecological and evolutionary drivers shaping energy investment decisions is an important focus of life-history theory. Most research has focused on isolated aspects of life history variation in a protogynous coral reef fish species. My thesis took a more comprehensive approach by assessing key linkages between physical environments, phenotypic traits and parental investment decisions. By focusing on a highly abundant and unexploited model organism, whose activities can be

easily tracked and recorded over an extended time, this study provides detailed information on the ecological and evolutionary drivers of phenotypic plasticity and population dynamics. Although lunar-cyclic reproduction has been addressed in various studies, my findings particularly highlight the disproportionate influence of the lunar cycle on energy allocation strategies of a protogynous coral reef wrasse. Given that this species is part of an extremely diverse and functionally important group (the labrid group), my work may provide further scientific knowledge needed to reinforce management plans to protect such species.

## References

- Adams, S., & Williams, A. J. (2001). A preliminary test of the transitional growth spurt hypothesis using the protogynous coral trout *Plectropomus maculatus*. *Journal of Fish Biology*, 59(1), 183–185.
- Afonso, P., Fontes, J., Holland, K. N., & Santos, R. S. (2008). Social status determines behaviour and habitat usage in a temperate parrotfish: Implications for marine reserve design. *Marine Ecology Progress Series*, 359, 215–227.
- Aldenhoven, J.M. (1986). Different reproductive strategies in a sex changing coral reef fish *Centropyge bicolor* (Pomacanthidae). *Aust. J. Mar. Freshwater Res.* 37, 353–360.
- Almany, G. R., & Webster, M. S. (2006). The predation gauntlet: Early post-settlement mortality in reef fishes. *Coral Reefs*, 25(1), 19–22.
- Alonzo, S. H., Taborsky, M., & Wirtz, P. (2000). Male alternative reproductive behaviours in a mediterranean wrasse, *Symphodus ocellatus*: Evidence from otoliths for multiple life-history pathways. *Evolutionary Ecology Research*, 2(8), 997–1007.
- Alonzo, S. H., & Warner, R. R. (2000). Female choice, conflict between the sexes and the evolution of male alternative reproductive behaviours. *Evolutionary Ecology Research*, 2(2), 149–170.
- Alonzo, S.H. & Mangel, M. (2004). The effects of size-selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. *Fishery Bulletin*, 102, 1-13.
- Alonzo, S. H., & Mangel, M. (2005). Sex-change rules, stock dynamics, and the performance of spawning-per-recruit measures in protogynous stocks. *Fishery Bulletin*, 103(2), 229–245.
- Armsworth, P.R. (2001). Effects of fishing on a protogynous hermaphrodite. *Canadian Journal of Fisheries and Aquatic Science*, 58, 568–578.
- Bagenal, T.B. (1978). Aspects of fish fecundity. in S. D. Gerking, ed. Ecology of freshwater fish production. *Blackwell Scientific, Oxford*, 75–101.
- Barlow, G.W. (1981). Patterns of parental investment, dispersal and size among coral-reef fishes. *Environmental Biology of Fishes*, 6, 65–85.
- Barrett, N.S. (1995). Short- and long-term movement patterns of six temperate reef fishes (Families Labridae and Monacanthidae). *Marine and Freshwater Research* 46, 853–860.
- Barton, K. (2015). MuMIn: Multi-Model Inference. R package version 1.13.4. See <https://CRAN.R-project.org/package=MuMIn>.
- Bates, D., Maechler & M., Bolker B. (2012). lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0. Available: <http://cran.r-project.org/web/packages/lme4/lme4.pdf>. Accessed 2013 Jan 11.
- Booth, D. J. (1995). Juvenile groups in a coral-reef damselfish: Density-dependent effects on individual fitness and population demography. *Ecology*, 76(1), 91–106.
- Breheny, P., & Burchett, W. (2017). Visualization of regression models using visreg. *R Journal*, 9(2), 56–71.



- Brockmann, H. J., & Taborsky, M. (2008). Alternative reproductive tactics and the evolution of alternative allocation phenotypes. *Alternative Reproductive Tactics: An Integrative Approach*, 25–51.
- Brooker, R. M., Jones, G. P., & Munday, P. L. (2013). Prey selectivity affects reproductive success of a corallivorous reef fish. *Oecologia*, 172(2), 409–416.
- Buston, P. (2003). Size and growth modification in clownfish. *Nature* 424, 145–146.
- Calenge, C. (2006). The package “adehabitat” for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling*, 197(3–4), 516–519.
- Caley, M.J., Carr, M.H., Hixon, M.A., Hughes, T.P., Jones, G.P. & Menge, B.A. (1996). Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics* 27, 477–500.
- Carter A. B., Russ G. R. , Tobin A. J., Williams A.J., Davies C.R., Mapstone B.D. (2014) Spatial variation in the effects of size and age on reproductive dynamics of common coral trout *Plectropomus leopardus*. *Journal of Fish Biology*, 84,1074–1098
- Carter, A. B., Carton, A. G., McCormick, M. I., Tobin, A. J. & Williams, A. J. (2015). Maternal size, not age, influences egg quality of a wild, protogynous coral reef fish *Plectropomus leopardus*. *Marine Ecology Progress Series*, 529, 249–263.
- Chapman, M. R., & Kramer, D. L. (2000). Movements of fishes within and among fringing coral reefs in Barbados. *Environmental Biology of Fishes*, 57(1), 11–24.
- Chapman, E. W., Jørgensen, C., & Lutcavage, M. E. (2011). Atlantic bluefin tuna (*Thunnus thynnus*): A statedependent energy allocation model for growth, maturation, and reproductive investment. *Canadian Journal of Fisheries and Aquatic Sciences*, 68(11), 1934–1951.
- Choat, J.H. & Robertson, D.R. (1957). Protogynous hermaphroditism in fishes of the family Scaridae: intersexuality in the animal kingdom. *Springer*, 263–283.
- Choat, J.H. & Robertson, D.R. (2002). Age-based studies on coral reef fishes. In: Sale PF (ed) *Coral Reef Fishes: dynamics and diversity in a complex ecosystem*. *Academic Press, San Diego*, 57–80.
- Claissie, J. T., Clark, T. B., Schumacher, B. D., McTee, S. A., Bushnell, M. E., Callan, C. K., Laidley, C. W., & Parrish, J. D. (2011). Conventional tagging and acoustic telemetry of a small surgeonfish, *Zebrasoma flavescens*, in a structurally complex coral reef environment. *Environmental Biology of Fishes*, 91(2), 185–201.
- Clark, C.W., & Mangel, M. (2000). *Dynamic state variable models in ecology: methods and applications*. Oxford University Press, New York.
- Claydon, J. A. B., McCormick, M. I., & Jones, G. P. (2012). Patterns of migration between feeding and spawning sites in a coral reef surgeonfish. *Coral Reefs*, 31(1), 77–87.
- Claydon, J. A. B., McCormick, M. I., & Jones, G. P. (2014). Multispecies spawning sites for fishes on a low-latitude coral reef: Spatial and temporal patterns. *Journal of Fish Biology*, 84(4), 1136–1163.
- Colin, P.L. & Clavijo, I.E. (1988). Spawning activity of fishes producing pelagic eggs on a shelf edge coral reef, southwestern Puerto Rico. *Bulletin of Marine Science* 43, 149–179982.

- Colin, P.L. & Bell, L.J. (1991). Aspects of the spawning of labrid and scarid fishes (Pisces: Labroidae) at Enewetak Atoll, Marshall Islands with notes on other families. *Environmental Biology of Fishes*, 31, 229–260.
- Colin, P.R. (1992). Reproduction of the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae) and its relationship to environmental condition. *Environmental Biology of Fishes*, 34, 357–377.
- Cowen, R. K. (1990). Sex -change and life history patterns of the labrid, *Semicossyphus pulcher*, across an environmental gradient. *Copeia* 1990,787–795.
- Cushing, D. H. (1975). Marine ecology and fisheries. *Cambridge University Press, Cambridge, UK*.
- Dahlgren, C. P. & Eggleston, D. B. (2000). Ecological processes underlying ontogenetic habitat shifts in a coral reef fish. *Ecology* 81, 2227–2240.
- Danilowicz, B.S. & Sale, P.F. (1999). Relative intensity of predation on the French grunt, *Haemulon flavolineatum*, during diurnal, dusk, and nocturnal periods on a coral reef. *Marine Biology*, 133, 337–343.
- deBruyn, A.M.H. & Meeuwig, J.J. (2001). Detecting lunar cycles in marine ecology: periodic regression versus categorical ANOVA. *Marine Ecology Progress Series* 214, 307–310.
- DeMartini, E. E., Friedlander, A. M., & Holzwarth, S. R. (2005). Size at sex change in protogynous labroids, prey body size distributions, and apex predator densities at NW Hawaiian atolls. *Marine Ecology Progress Series*, 297, 259–271.
- Doherty, P.J. & Williams, D.McB. (1988). The replenishment of coral reef fish populations. *Oceanography and Marine Biology, Annual Review*, 26, 487–551.
- Doherty, P.J., Fowler, A.J., Samoilys, M.A. & Harris, D.A. (1994). Monitoring the replenishment of coral trout (Pisces: Serranidae) populations. *Bulletin of Marine Science*, 54(1), 343–355.
- Doherty, P.J., Dufour, V., Galzin, R., Hixon, M.A. & Planes, S. (2004). High mortality during settlement is a population bottleneck for a tropical surgeonfish. *Ecology*, 85, 2422–2428.
- Domeier, M. L., & Colin, P. L. (1997). Tropical reef fish spawning aggregations defined and reviewed. *Bulletin of Marine Science*, 60(3), 698–726.
- Donahue, M. J., Karnauskas, M., Toews, C., & Paris, C. B. (2015). Location isn't everything: Timing of spawning aggregations optimizes larval replenishment. *PLoS ONE*, 10(6), 1–14.
- Donelson, J. M., McCormick, M. I., & Munday, P. L. (2008). Parental condition affects early life-history of a coral reef fish. *Journal of Experimental Marine Biology and Ecology*, 360(2), 109–116.
- Dufour, V. (1991). Variations of fish larvae abundance in reefs: effect of light on the colonization of the reefs by fish larvae. *Comptes Rendus de l'Académie des Sciences (Paris)*, 313, 187–194.
- Ebersole J. P. (1980) Food density and territory size: an alternative model and a test on the reef fish *Eupomacentrus leucostictus*. *American Naturalist*, 115,492—509
- Einum, S., & Fleming, I. A. (2004). Environmental unpredictability and offspring size: Conservative versus diversified bet-hedging. *Evolutionary Ecology Research*, 6(3), 443–455.

- Fiedler, K. (1964). Verhaltensstudien an Lippfischen der Gattung *Crenilabrus* (Labridae, Perciformes). *Z. Tierpsych.*, 21, 521–591.
- Fitch, W. T. S. & Shapiro, D. Y. (1990). Spatial dispersion and nonmigratory spawning in the bluehead wrasse (*Thalassoma bifasciatum*). *Ethology*, 85, 199–211.
- Ford, J. R., Shima, J. S., & Swearer, S. E. (2016). Interactive effects of shelter and conspecific density shape mortality, growth, and condition in juvenile reef fish. *Ecology*, 97(6), 1373–1380.
- Forrester, G. E. (1991). Social rank, individual size and group composition as determinants of food consumption by humbug damselfish, *Dascyllus aruanus*. *Animal Behaviour*, 42(5), 701–711.
- Forward, R.B. Jr & Tankersley, R.A. (2001). Selective tidal- stream transport of marine animals. *Oceanography and Marine Biology Annual Review*, 39, 305–353.
- Fox, R.J. & Bellwood, D. R. (2011). Unconstrained by the clock? Plasticity of diel activity rhythm in a tropical reef fish, *Siganus lineatus*. *Functional Ecology*, 25, 1096 – 1105
- Francis, R. C., & Barlow, G. W. (1993). *Social control of primary sex differentiation in the Midas cichlid*. 90(November), 10673–10675.
- Friedland, K.D., Hare, J.A. & Wood, G.B. (2009). Reply to the comment by Payne et al. on “Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition? *Canadian Journal of Fisheries and Aquatic Sciences*, 66, 873–877.
- Fulton, C. J., & Bellwood, D. R. (2002). Ontogenetic habitat use in labrid fishes: An ecomorphological perspective. *Marine Ecology Progress Series*, 236, 255–262.
- Gagliano, M., & McCormick, M. I. (2007). Compensating in the wild : is flexible growth the key to early juvenile survival? *Oikos*, 116, 111–120.
- Gagliano, M., McCormick, M. I., & Meekan, M. G. (2007). Survival against the odds: Ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. *Proceedings of the Royal Society B: Biological Sciences*, 274(1618), 1575–1582.
- Galzin, R. & Pointier, J.P. (1985). Moorea Island, Society Archipelago. Pages in B. Delesalle, R. Galzin, & B. Salvat, editors. *Fifth International Coral Reef Congress, Tahiti, French Polynesia*, 1, 75–101.
- Galzin, R. (1987). Structure of Fish Communities of French Polynesian Coral Reefs. II. Temporal Scales. *Marine Ecology Progress Series*, 41, 137–145.
- Geange, S. W., & Stier, A. C. (2009). Order of arrival affects competition in two reef fishes. *Ecology*, 90(10), 2868–2878.
- Ghiselin, M.T. (1969). The evolution of hermaphroditism among animals. *Quarterly Review of Biology* 44, 189–208
- Goodman, D. (1984). Risk spreading as an adaptive strategy in iteroparous life histories. *Theoretical Population Biology*, 25, 1–20.
- Google, 2019. Google Earth Pro.
- Green, J., Martel, G. & Martin, D. (1984). Comparisons of the feeding activity and diets of male and female cunners *Tautoglabrus adspersus* (Pisces: Labridae). *Marine Biology*, 84, 7–11.

- Green, A. L., Maypa, A. P., Almany, G. R., Rhodes, K. L., Weeks, R., Abesamis, R. A., Gleason, M. G., Mumby, P. J., & White, A. T. (2015). Larval dispersal and movement patterns of coral reef fishes and implications for marine reserve network design. *Biological Reviews*, 90, 1215–1247.
- Grömping, U. (2006). Relative importance for linear regression in R: The package relaimpo. *Journal of Statistical Software*, 17, 1–27.
- Gross, M. R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *TREE*, 11(2), 92–98.
- Gunderson, D.R. (1997). Trade-off between reproductive effort and adult survival in oviparous and viviparous fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 990–998.
- Gust, N. (2004). Variation in the population biology of protogynous coral reef fishes over tens of kilometres. *Canadian Journal of Fisheries and Aquatic Sciences*, 61(2), 205–218.
- Hamner, W.H. & Largier, J.L. (2011). Oceanography of the planktonic stages of aggregation spawning reef fishes. In: Reef fish spawning aggregations: biology, research and management (eds Y.S. de Mitcheson, P.L. Colin), 159–190. *Dordrecht, The Netherlands: Springer*.
- Haskell, J.P., Ritchie, M.E. & Olff, H. (2002). Fractal geometry predicts varying body size scaling relationships from mammal and bird home ranges. *Nature*, 418, 527–530.
- Heino, M., & Kaitala, V. (1999). Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. *Journal of Evolutionary Biology*, 12(3), 423–429.
- Henson, S. A., & Warner, R. R. (1997). Male and female alternative reproductive behaviors in fishes: A new approach using intersexual dynamics. *Annual Review of Ecology and Systematics*, 28, 571–592.
- Hernández-León, S. (2008). Natural variability of fisheries and lunar illumination: a hypothesis. *Fish and Fisheries*, 9, 138–154.
- Heyman, W.D. & Kjerfve, B. (2008). Characterization of multi-species reef fish spawning aggregations at Gladden Spit, Belize. *Bulletin of Marine Science*, 83(3), 531–551.
- Hijmans, R. J. (2011). *Introduction to the "geosphere" package ( Version 1 . 2-19 )*. 1–19.
- Hixon, M.A. (1982). Energy maximizers and time minimizers: theory and reality. *American Naturalist*, 119, 596 – 599.
- Hixon, M.A. & Beets, J.P. (1993). Predation, prey refuges, and the structure of coral-reef fish assemblages. *Ecological Monographs*, 63, 77–101.
- Hobson, E.S. (1974). Feeding relationships of teleostean fishes on coral reefs in Kona, Hawaii. *Fish Bulletin NOAA*, 72, 915–1031.
- Holbrook, S. J., & Schmitt, R. J. (2003). Spatial and temporal variation in mortality of newly settled damselfish: Patterns, causes and co-variation with settlement. *Oecologia*, 135(4), 532–541.
- Holmes, T. H., & McCormick, M. I. (2010). Size-selectivity of predatory reef fish on juvenile prey. *Marine Ecology Progress Series*, 399, 273–283.
- Hoffman, S.G. (1983). Sex-related foraging behaviour in sequentially hermaphroditic hogfishes

- (*Bodianus* spp.). *Ecology*, 64, 798–808.
- Houston, A. I. & McNamara, J.M. (1999). Models of adaptive behaviour: an approach based on state. *Cambridge University Press, New York*.
- Howard, K. G., Claissie, J. T., Clark, T. B., Boyle, K., & Parrish, J. D. (2013). Home range and movement patterns of the Redlip Parrotfish (*Scarus rubroviolaceus*) in Hawaii. *Marine Biology*, 160(7), 1583–1595.
- Hughes, P. W. (2017). Between semelparity and iteroparity : Empirical evidence for a continuum of modes of parity. *Ecology and Evolution*, 7, 8232–8261.
- Jennings, S., Greenstreet, S. P. R. & Reynolds, J.D. (1999). Structural changes in an exploited fish community: a consequence of differential fishing effects on species with contrasting life histories. *Journal of Animal Ecology*, 68, 617–627.
- Johannes, R. E. (1978). Reproductive strategies of coastal marine fishes in the tropics. *Environmental Biology of Fishes*, 3(1), 65–84.
- Jones, K. M. M. (2005). Home range areas and activity centres in six species of Caribbean wrasses (Labridae). *Journal of Fish Biology*, 66(1), 150–166.
- Jørgensen, C., Ernande, B., Fiksen, Ø., & Dieckmann, U. (2006). The logic of skipped spawning in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 63(1), 200–211.
- Karnauskas, M., Cherubin, L.M. & Paris, C.B. (2011). Adaptive Significance of the Formation of Multi-Species Fish Spawning Aggregations near Submerged Capes. *PLoS ONE*, 6,e22067.
- Kobayashi Y., Nagahama Y., Nakamura M. (2013). Diversity and plasticity of sex determination and differentiation in fishes. *Sex Dev*, 2013, 7: 115–125
- Koenker, R. (2011). quantreg: Quantile Regression. R Package Version 4.62.<http://CRAN.R-project.org/package=quantreg>
- Kuiter, R.H. (2006). Guide to sea fishes of Australia. A comprehensive reference for divers and fishermen. *New Holland Publishers (Australia) Pty Ltd., Sydney, Australia*.
- Kuwamura, T., Suzuki, S., & Kadota, T. (2009). Interspecific variation in the spawning time of labrid fish on a fringing reef at Iriomote Island, Okinawa. *Ichthyological Research*, 63(4), 460–469.
- Leggett, W.C. & DeBlois, E. (1994). Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages. *Netherlands Journal of Sea Research*, 32, 119–134.
- Lenth, R.V. (2018). emmeans: Estimates Marginal Means, aka Least-Squares Means. Retrieved from <https://CRAN.R-project.org/package=emmeans>.
- Lindeman, R. H., Merenda P., & Gold R. (1980). Introduction to Bivariate and Multivariate Analysis. Scott, Foresman, Glenview, IL.
- Lloyd, D.G. (1984). Variation strategies of plants in heterogeneous environments. *Biological Journal of the Linnean Society*, 21, 357–385.
- Lobel, P.S. (1978). Diel, lunar, and seasonal periodicity in the reproductive behavior of the pomacanthid fish, *Centropyge potteri*, and some other reef fishes in Hawaii. *Pacific Science*, 32,

- Lott, D.F. (1991). Intraspecific variation in the social systems of wild vertebrates. Cambridge University Press, Cambridge. 238 pp.
- Lutenesky, M.M.F. (1994). Density-dependent protogynous sex change in territorial-haremic fishes: models and evidence. *Behavioural Ecology*, 5, 375–383.
- Marconato, A., Shapiro, D. Y., Petersen, C. W., Warner, R. R., & Yoshikawa, T. (1997). Methodological analysis of fertilization rate in the bluehead wrasse *Thalassoma bifasciatum*: Pair versus group spawns. *Marine Ecology Progress Series*, 161, 61–70.
- Marshall, A., Mills, J. S., Rhodes, K. L., & McIlwain, J. (2011). Passive acoustic telemetry reveals highly variable home range and movement patterns among unicornfish within a marine reserve. *Coral Reefs*, 30(3), 631–642.
- Mcbride, R. S., Somarakis, S., Fitzhugh, G. R., Albert, A., Yaragina, N. A., Wuenschel, M. J., Alonso-Fernández, A., & Basilone, G. (2015). Energy acquisition and allocation to egg production in relation to fish reproductive strategies. *Fish and Fisheries*, 16(1), 23–57.
- McCormick, M. I., & Hoey, A. S. (2004). Larval growth history determines juvenile growth and survival in a tropical marine fish. *Oikos*, 106(2), 225–242.
- Mccormick, M. I., & Mccormick, I. (2009). Mothers matter : crowding leads to stressed mothers and smaller offspring in marine fish. *Ecological Society of America*, 87(5), 1104–1109.
- McCormick, M. I., Ryen, C. A., Munday, P. L., & Walker, S. P. W. (2010). Differing mechanisms underlie sexual size-dimorphism in two populations of a sex-changing fish. *PLoS ONE*, 5(5).
- Meyer, C. G., Papastamatiou, Y. P., & Clark, T. B. (2010). Differential movement patterns and site fidelity among trophic groups of reef fishes in a Hawaiian marine protected area. *Marine Biology*, 157(7), 1499–1511.
- Molloy, P. P., Goodwin, N. B., Côté, I. M., Reynolds, J. D., & Gage, M. J. G. (2007). Sperm competition and sex change: A comparative analysis across fishes. *Evolution*, 61(3), 640–652.
- Molloy, P.P., Cote, I.M. & Reynolds, J.D. (2012). Why spawn in aggregations? In: Reef Fish Spawning Aggregations: Biology, Research and Management. (eds Y. Sadovyde Mitcheson and P.L. Colin). Springer, New York, pp.57–83.
- Moyer, J.T. (1987) Quantitative observations of predation during spawning rushes of the labrid fish *Thalassoma cupido* at Miyake-jima, Japan. *Japanese Journal of Ichthyology*, 34, 76–81
- Moyer J. T. (1989) Reef channels as spawning sites for fishes on the Shiraho coral reef, Ishigaki Island, Japan. *Japanese Journal of Ichthyology*, 36, 371–375
- Moran, N.A. (1992). The evolutionary maintenance of alternative phenotypes. *American Naturalist*, 139, 971-989.
- Mumby, P. J., & Wabnitz, C. C. C. (2002). Spatial patterns of aggression, territory size, and harem size in five sympatric Caribbean parrotfish species. *Environmental Biology of Fishes*, 63(3), 265–279.
- Munday, P. L., Buston, P. M., & Warner, R. R. (2006a). Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology and Evolution*, 21(2), 89–95.
- Munday, P. L., White, J. W., & Warner, R. R. (2006b). A social basis for the development of primary males in a sex-changing fish. *Proceedings of the Royal Society B: Biological Sciences*, 273(1603),

2845–2851.

- Muñoz, R. C., & Warner, R. R. (2003). A new version of the size-advantage hypothesis for sex change: Incorporating sperm competition and size-fecundity skew. *American Naturalist*, 161(5), 749–761.
- Munoz, R.C. & Warner, R.R. (2004). Testing a new version of the size- advantage hypothesis for sex change: sperm competition and size- skew effects in the bucktooth parrotfish, *Sparisoma radians*. *Behavioural Ecology*, 15, 129–136
- Mustonen, A.M., Nieminen & P., Hyvarinen, H. (2002). Leptin, ghrelin, and energy metabolism of the spawning burbot (*Lota lota*, L.). *Journal of Experimental Zoology*, 293, 119–126.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining  $R^2$  from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 2, 133–142.
- Nash K. L., Welsh J. Q. , Graham N. A. J., Bellwood D. (2015) Home-range allometry in coral reef fishes: comparison to other vertebrates, methodological issues and management implications. *Oecologia*, 177, 73–83
- Nielsen, J.R., Lambert, G., Bastardie, F., Sparholt, H. & Vinther, M. (2012). Do Norway pout (*Trisopterus markii*) die from spawning stress? Mortality of Norway pout in relation to growth, sexual maturity, and density in the North Sea, Skagerrak, and Kattegat. *ICES Journal of Marine Science*, 69, 197–207.
- Nemeth, R. S., Blondeau, J., Herzlieb, S., & Kadison, E. (2007). Spatial and temporal patterns of movement and migration at spawning aggregations of red hind, *Epinephelus guttatus*, in the U.S. Virgin Islands. *Environmental Biology of Fishes*, 78(4), 365–381.
- Noonburg E. G., Chen A., Shima J. S., Swearer S. E. (2015) Demographic heterogeneity and the dynamics of open populations. *Ecology*, 96, 1159–1165
- Pecquerie, L., Petitgas, P., & Kooijman, S. A. L. M. (2009). Modeling fish growth and reproduction in the context of the Dynamic Energy Budget theory to predict environmental impact on anchovy spawning duration. *Journal of Sea Research*, 62(2–3), 93–105.
- Petersen C. W., Warner R. R., Cohen S., Hess H. C., Sewell A. T. (1992). Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating. *Ecology* 73, 391–401
- Philippi, T. & Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends in Ecology & Evolution*, 4, 41–44.
- Powell, R.A. (2000). Animal home ranges and territories and home range estimators. In: Boitani L, Fuller TK (eds) Research techniques in animal ecology: controversies and consequences. *Columbia University Press, New York*, 65–110.
- Randall, J. E. & Randall, H. A. (1963). The spawning and early development of the Atlantic parrotfish, *Sparisoma nubilipinne*, with notes on other scarid and labrid fishes. *Zoologica*, 48, 49–60.
- Rankin, T. L. & Sponaugle, S. (2014). Characteristics of settling coral reef fish are related to recruitment timing and success. *PLOS One* 9, e108871.
- R Core Team (2019). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. URL <https://www.R-project.org/>.

- Reznick D. (1985). Costs of reproduction: an evaluation of the empirical evidence. *Oikos*, 44, 257-267.
- Rideout, R.M., Rose, G.A., & Burton, M.P. (2005). Skipped spawning in female iteroparous fishes. *Fish and Fisheries*, 6, 50–72.
- Rideout, R. M., & Tomkiewicz, J. (2011). Skipped spawning in fishes: More common than you might think. *Marine and Coastal Fisheries*, 3(1), 176–189.
- Robertson, D.R. & Hoffman, S.G. (1977). The roles of female mate choice and predation in the mating systems of some tropical labroid fishes. *Zeitschrift für Tierpsychologie*, 45, 298–320.
- Robertson, D. R. (1983). On the spawning behavior and spawning cycles of eight surgeonfishes (Acanthuridae) from the Indo-Pacific. *Environmental Biology of Fishes*, 9, 193-223.
- Robertson, D.R., Green, D. G., & Victor, B.C. (1988). Temporal coupling of production and recruitment. *Ecology*, 69, 370–381.
- Robertson, D.R., Petersen C.W. & Brawn J. D. (1990). Lunar reproductive cycles of benthic brooding reef fishes: reflections of larval biology or adult biology. *Ecological Monographs*, 60, 311-329.
- Robertson, D.R., Swearer, S.E., Kaufmann K. & Brothers, E. B. (1999). Settlement vs. environmental dynamics in a pelagic-spawning reef fish at Caribbean Panama. *Ecological Monographs*, 69, 195–218.
- Robertson, D. R. (2011). Temporal Coupling of Production and Recruitment of Larvae of a Caribbean Reef Fish Author ( s ): D . Ross Robertson , David G . Green , Benjamin C . Victor Published by : Ecological Society of America Stable URL : <http://www.jstor.org/stable/1940435> . TEM. *America*, 69(2), 370–381.
- Roff, D. A. (1983). An allocation model of growth and reproduction in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 40(9), 1395–1404.
- Roff, Derek A. (1991). Life history consequences of bioenergetic and biomechanical constraints on migration. *Integrative and Comparative Biology*, 31(1), 205–216.
- Roff, D.A. (2002). Life history evolution. *Sinauer, Sunderland, Massachusetts*.
- Rogers, L. (2003). Odds-playing and the timing of sex change in uncertain environments: you bet your wrasse. *Behavioural Ecology*, 14, 447–450.
- Ross, R.M. (1987). Sex-change linked growth acceleration in a coral-reef fish, *Thalassoma duperrey*. *Journal of Experimental Zoology*, 244, 455–461.
- Ross, R. M. (1990). The evolution of sex-change mechanisms in fishes. *Environmental Biology of Fishes*, 29, 81–93.
- Ross, R.M., Hourigan T.F., Lutensky & M.M.F., & Singh, I. (1990). Multiple simultaneous sex changes in social groups of a coral reef fish. *Copeia*, 427-433.
- Sadovy, Y. & Cornish, A.S. (2000). Reef fishes of Hong Kong. *Hong Kong University Press, Hong Kong*.
- Sadovy De Mitcheson, Y., & Colin, P. L. (2012). Reef fish spawning aggregations: Biology, research and management. In *Reef Fish Spawning Aggregations: Biology, Research and Management*.



- Samoilys, M.A. (1997). Periodicity of spawning aggregations of coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the northern Great Barrier Reef. *Marine Ecology Progress Series*, 160, 149–159.
- Sancho, G., Solow, A. R., & Lobel, P. S. (2000). Environmental influences on the diel timing of spawning in coral reef fishes. *Marine Ecology Progress Series*, 206, 193–212.
- Sano, M., Shimizu, M. & Nose, Y. (1984). Food habits of teleostean reef fishes in Okinawa Island, southern Japan. *University of Tokyo Press, Tokyo, Japan*.
- Sano, M. (1993). Foraging activities and diets of males and females in a harem sandperch (Pisces: *Pinguipedidae*). *Marine Ecology Progress Series*, 98, 55–59.
- Schaffer, W. M. (1974). Selection for optimal life histories: the effects of age structure. *Ecology*, 5, 291–303.
- Schärer, L., & Robertson, D. R. (1999). Sperm and milt characteristics and male v. female gametic investment in the Caribbean reef fish, *Thalassoma bifasciatum*. *Journal of Fish Biology*, 55(2), 329–343.
- Schoener, T.W. (1971). Theory of feeding strategies. *Annual Review of Ecological Systems*, 2, 369–404.
- Schultz, E. T., & Warner, R. R. (1991). Phenotypic plasticity in life-history traits of female *Thalassoma bifasciatum* (Pisces: Labridae): 2. Correlation of fecundity and growth rate in comparative studies. *Environmental Biology of Fishes*, 30(3), 333–344.
- Searcy, S. P., & Sponaugle, S. (2000). Variable larval growth in a coral reef fish. *Marine Ecology Progress Series*, 206, 213–226.
- Seeger, J. & Brockman, H.J. (1987). What is bet-hedging? *Oxford Surveys in Evolutionary Biology*, 4, 181–211.
- Shapiro, D.Y. (1984). Sex reversal and socio demographic processes in coral reef fishes. In *Fish Reproduction: Strategies and Tactics* (Potts, G.W. and Wootton, R.J., eds), pp.103–118, *Academic Press*.
- Shapiro, D.Y. (1987). Differentiation and evolution of sex change in fishes. *Bioscience*, 37, 490–497.
- Shapiro, D., Hensley, D. & Appeldoorn, R. (1988). Pelagic spawning and egg transport in coral-reef fishes: a skeptical overview. *Environmental Biology of Fishes*, 22(1), 3–14.
- Shapiro, D.Y. (1991). Intraspecific variability in social systems of coral reef fishes. pp. 331–355. In: P. Sale (ed.) *The Ecology of Fishes on Coral Reefs*, *Academic Press, San Diego*.
- Shapiro, D.Y., Marconato, A. & Yoshikawa, T. (1994). Sperm economy in a coral reef fish, *Thalassoma bifasciatum*, *Ecology*, 75, 1334–1344.
- Shepherd, S.A. & Clarkson, P.S. (2001). Diet, feeding behaviour, activity and predation of the temperate blue-throated wrasse, *Notolabrus tetricus*. *Marine and Freshwater Research*, 52, 311–322.
- Shibuno, T., Gushima, K. & Kakuda, S. (1993). Female spawning migrations of the protogynous wrasse, *Halichoeres marginatus*. *Japanese Journal of Ichthyology*, 39, 357–362.

- Shima, J.S. (1999). Variability in relative importance of determinants of reef fish recruitment. *Ecology Letters*, 2, 304–310.
- Shima, J.S. (2001a). Recruitment of a coral reef fish: Roles of settlement, habitat, and postsettlement losses. *Ecology*, 82(8), 2190–2199.
- Shima, J.S. (2001b). Regulation of local populations of a coral reef fish via joint effects of density- and number-dependent mortality. *Oecologia*, 126(1), 58–65.
- Shima, J.S. (2002). Mechanisms of density- and number-dependent population regulation of a coral-reef fish. *Marine and Freshwater Research*, 53(2), 175–179.
- Shima, J.S., & Findlay, A. M. (2002). Pelagic larval growth rate impacts benthic settlement and survival of a temperate reef fish. *Marine Ecology Progress Series*, 235, 303–309.
- Shima, J.S., & Osenberg, C. W. (2003). Cryptic density dependence: Effects of covariation between density and site quality in reef fish. *Ecology*, 84(1), 46–52.
- Shima, J.S., Osenberg, C. W., St. Mary, C. M., & Rogers, L. (2006). Implication of changing coral communities: do larval traits or habitat features drive variation in density-dependent mortality and recruitment of juvenile reef fish? *Proceedings of the 10th International Coral Reef Symposium*, 226–231.
- Shima, J.S., Osenberg, C. W., & St. Mary, C. M. (2008). Quantifying site quality in a heterogeneous landscape: Recruitment of a reef fish. *Ecology*, 89(1), 86–94.
- Shima, J. S., & Swearer, S. E. (2009). Spatially variable larval histories may shape recruitment rates of a temperate reef fish. *Marine Ecology Progress Series*, 394, 223–229.
- Shima, J.S., Noonburg, E. G., & Swearer, S. E. (2015). Consequences of variable larval dispersal pathways and resulting phenotypic mixtures to the dynamics of marine metapopulations. *Biology Letters*, 11(2), 2014–2017.
- Shima, J. S., Noonburg, E. G., Swearer, S. E., Alonzo, S. H., & Osenberg, C. W. (2018). Born at the right time? A conceptual framework linking reproduction, development, and settlement in reef fish. *Ecology*, 99(1), 116–126.
- Shima, J. S., & Swearer, S. E. (2019). Moonlight enhances growth in larval fish. *Ecology*, 100, e02563. <https://doi.org/10.1002/ecy.2563>.
- Slotte, A. (1999). Differential utilization of energy during wintering and spawning migration in Norwegian spring-spawning herring. *Journal of Fish Biology*, 54, 338–355.
- Sponaugle, S., & Cowen, R. K. (1994). Larval durations and recruitment patterns of two Caribbean gobies (Gobiidae): contrasting early life histories in demersal spawners. *Marine Biology*, 120(1), 133–143.
- Sponaugle, S. (2010). Otolith microstructure reveals ecological and oceanographic processes important to ecosystem-based management. *Environmental Biology of Fishes*, 89(3), 221–238.
- Sponaugle, S., & Grorud-Colvert, K. (2006). Environmental variability, early life-history traits, and survival of new coral reef fish recruits. *Integrative and Comparative Biology*, 46(5), 623–633.
- Sponaugle, S., Grorud-Colvert, K., & Pinkard, D. (2006). Temperature-mediated variation in early life history traits and recruitment success of the coral reef fish *Thalassoma bifasciatum* in the Florida Keys. *Marine Ecology Progress Series*, 308, 1–15.

- Sponaugle, S. (2015). Recruitment of coral reef fishes: Linkages across stages. *Ecology of Fishes on Coral Reefs*, 28–33.
- Stearns, S.C. (1992). The Evolution of Life Histories. *Oxford University Press, New York*.
- Steele, M.A. & Forrester G.E. (2002). Variation in the relative importance of sublethal effects of predators and competitors on growth of a temperate reef fish. *Marine Ecology Progress Series*, 237, 233–245.
- Stephens, P.A., Boyd, I.L., McNamara, J.M. & Houston, A.I. (2009). Capital breeding and income breeding: their meaning, measurement, and worth. *Ecology*, 90, 2057–2067.
- Stockley, P., M., Gage J. G., Parker, G. A., & Møller A. P. (1997). Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *American Naturalist*, 149, 933–954.
- Suzuki, S., Toguchi, K., Makino, Y., Kuwamura, T., Nakashima, Y., & Karino, K. (2008). Group spawning results from the streaking of small males into a sneaking pair: Male alternative reproductive tactics in the threespot wrasse *Halichoeres trimaculatus*. *Journal of Ethology*, 26(3), 397–404.
- Taborsky, M., Hudde, B., Wirtz, P. (1987). Reproductive behaviour and ecology of *Symphodus (Crenilabrus) ocellatus*, a European wrasse with four types of male behaviour. *Behaviour*, 102, 82–118.
- Taborsky, M. (1994). Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction, *Advances in the Study of Behaviour*, 23, 1–100.
- Taborsky, M. (1998). ‘Bourgeois’ Males and Parasitic Spawning. *Science*, 13(6), 222–227.
- Takemura, Akihiro, Rahman, M. S., Nakamura, S., Young, J. P., & Takano, K. (2004). Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes. *Fish and Fisheries*, 5(4), 317–328.
- Takemura, A., Rahman, M. S., & Park, Y. J. (2010). External and internal controls of lunar-related reproductive rhythms in fishes. *Journal of Fish Biology*, 76(1), 7–26.
- Taylor, B. M., & Choat, J. H. (2014). Comparative demography of commercially important parrotfish species from Micronesia. *Journal of Fish Biology*, 84(2), 383–402.
- Thresher, R.E. (1984). Reproduction in reef fishes. *T. E H. Publications, Neptune City*.
- Trivers, R. (1972). Parental investment and sexual selection. pp. 139–179 In: B. Campbell ed. Sexual selection and the descent of man 1871-1971. *Aldine Press, Chicago*.
- Van Rooij, J. M., Bruggemann, J. H., Videler, J. J., & Breeman, A. M. (1995). Plastic growth of the herbivorous reef fish *Sparisoma viride*: Field evidence for a trade-off between growth and reproduction. *Marine Ecology Progress Series*, 122(1–3), 93–106.
- van Noordwijk, A. J. & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, 128, 137–142.
- Victor, B.C. (1982). Daily otolith increments and recruitment in two coral-reef wrasses, *Thalassoma bifasciatum* and *Halichoeres bivittatus*. *Marine Biology*, 71, 203–208.
- Victor, B.C. (1986). Duration of the planktonic larval stage of one hundred species of Pacific and

- Atlantic wrasses (family Labridae). *Marine Biology*, 90(3), 317-326.
- Victor, B.C. (1991). Settlement strategies and biogeography of reef fishes. In: Sale PJ (ed) The ecology of fishes on coral reefs. *Academic Press, New York*, pp 231-260.
- Vigliola, L., & Meekan, M. G. (2002). Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia*, 131(1), 89–93.
- Villegas-Ríos, D., Alonso-Fernández, A., Domínguez-Petit, R., & Saborido-Rey, F. (2014). Energy allocation and reproductive investment in a temperate protogynous hermaphrodite, the ballan wrasse *Labrus bergylta*. *Journal of Sea Research*, 86, 76–85.
- Walker, Stefan P.W., & McCormick, M. I. (2004). Otolith-check formation and accelerated growth associated with sex change in an annual protogynous tropical fish. *Marine Ecology Progress Series*, 266, 201–212.
- Walker, S. P.W., Ryen, C. A., & McCormick, M. I. (2007). Rapid larval growth predisposes sex change and sexual size dimorphism in a protogynous hermaphrodite, *Parapercis snnyderi* Jordan & Starks 1905. *Journal of Fish Biology*, 71(5), 1347–1357.
- Warner, R. R. (1975). The adaptive significance of sequential hermaphroditism in animals. *American Naturalist*, 109(965), 61–82.
- Warner, R.R. & Robertson, D.R. (1978). Sexual patterns in the labroid fishes of the western Caribbean, In: the Wresses (Labroidae). *Smithson Contrib. Zool.*, 254, 1–27.
- Warner, R.R. & Hoffman, S.G. (1980a). Population density and the economics of territoriality in a coral reef fish. *Ecology*, 61, 772–780.
- Warner, R. R., & Hoffman, S. G. (1980b). Local Population Size as a Determinant of Mating System and Sexual Composition in Two Tropical Marine Fishes (*Thalassoma* Spp.). *Evolution*, 34(3), 508.
- Warner, R.R. (1982). Mating systems, sex change, and sexual demography in the rainbow wrasse, *Thalassoma lucasanum*. *Copeia*, 1982, 653-61.
- Warner, R. R. (1984a). Mating Behavior and Hermaphroditism in Coral Reef Fishes: The diverse forms of sexuality. *American Scientist*, 72(2), 128–136.
- Warner, R. R. (1984b). Deferred Reproduction as a Response to Sexual Selection in a Coral Reef Fish: A Test of the Life Historical Consequences. *Evolution*, 38(1), 148.
- Warner, R.R. & Lejeune, P. (1985). Sex change limited by parental care: a test using four Mediterranean labrid fishes, genus *Symphodus*. *Marine Biology*, 87, 89–99.
- Warner, R.R. (1988a). Sex change in fishes: hypotheses, evidence, and objections. *Environmental Biology of Fishes*, 22, 81–90.
- Warner, R.R. (1988b). Traditionality of mating-site preferences in a coral reef fish. *Nature*, 335, 719–721.
- Warner, R. R., & Swearer, S. E. (1991). Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *Biological Bulletin*, 181(2), 199–204.
- Warner, R.R. & Schultz, E.T. (1992). Sexual selection and male characteristics in the bluehead wrasse, *Thalassoma bifasciatum* – mating site acquisition, mating site defense, and female choice.

*Evolution*, 46, 1421–1442.

- Warner, R. R. (1995). Large mating aggregations and daily long-distance spawning migrations in the bluehead wrasse, *Thalassoma bifasciatum*. *Environmental Biology of Fishes*, 44(4), 337–345.
- Warner, R. R., Shapiro, D. Y., Marcanato, A., & Petersen, C. W. (1995). Sexual conflict: males with highest mating success convey the lowest fertilization benefits to females. *Proceedings of the Royal Society B: Biological Sciences*, 262(1364), 135–139.
- Warner, R. R. (1997a). Evolutionary ecology: How to reconcile pelagic dispersal with local adaptation. *Coral Reefs*, 16(SUPPL. 1), 115–120.
- Warner, R. R. (1997b). Sperm allocation in coral reef fishes - Strategies for coping with demands on sperm production. *Bioscience*, 47(9), 561–564.
- Warner, R. R. (1998). The role of extreme iteroparity and risk avoidance in the evolution of mating systems. *Journal of Fish Biology*, 53(sA), 82–93.
- Welsh, J. Q., & Bellwood, D. R. (2012). Spatial ecology of the steephead parrotfish (*Chlorurus microrhinos*): An evaluation using acoustic telemetry. *Coral Reefs*, 31(1), 55–65.
- Welsh, J. Q., Goatley, C. H. R., & Bellwood, D. R. (2013). The ontogeny of home ranges: Evidence from coral reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, 280(1773), 1–7.
- Whaylen, L., Pattengill-Semmens, C.V., Semmens, B.X., Bush, P.G. & Boardman, M.R. (2004). Observations of a Nassau grouper, *Epinephelus striatus*, spawning aggregation site in Little Cayman, Cayman Islands, including multi-species spawning information. *Environmental Biology of Fishes*, 70, 305–313.
- Wilbur, H. M., & Rudolf, V. H. W. (2006). Life-history evolution in uncertain environments: Bet hedging in time. *American Naturalist*, 168(3), 398–411.
- Wong, M. Y L, Fauvelot, C., Planes, S., & Buston, P. M. (2012). Discrete and continuous reproductive tactics in a hermaphroditic society. *Animal Behaviour*, 84(4), 897–906.
- Wong, Marian Y L, Buston, P. M., Munday, P. L., Jones, G. P., Superior, C., & Cienti, D. I. (2007). The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. *Proceedings in Biological Science*, 274, 1093–1099.
- Wootton, R.J. (1998). Ecology of teleost fishes. *Dordrecht: Kluwer*. 396 pp.
- Wright, W.G. (1989). Intraspecific density mediates sex-change in the territorial patellacean limpet *Lottia gigantea*. *Marine Biology*, 100, 353–364.
- Zeller, D. C. (1998). Spawning aggregations: Patterns of movement of the coral trout *Plectropomus leopardus* (Serranidae) as determined by ultrasonic telemetry. *Marine Ecology Progress Series*, 162, 253–263.

## Appendices

### Appendix Two

**Table A.2.1** Model selection table of the 10 best models explaining variation in total spawning frequency of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cloud cover night-time (CCN), cloud cover day-time (CCD),  $\cos\theta$ , current direction (CD), current speed (CS), spawning site (S),  $\sin\theta$ , temperature (T), territory (Terr), visibility (VIS) and date (random factor) as independent variables. All models were fit using GLMER (Poisson distribution).

Model	(Int)	CCN	CCD	$\cos\theta$	CD	CS	S	$\sin\theta$	Tmp	Terr	VIS	df	logLik	AICc	delta	weight
13	2.18			0.89	+	+		-0.300		-1.00		9	-419.70	858.3	0	0.048
9	2.33			0.87		+		-0.298		-1.00		7	-422.11	858.8	0.47	0.038
1	2.24			0.88				-0.269		-1.00		5	-424.41	859.1	0.78	0.033
5	2.15			0.90	+			-0.265		-1.00		7	-422.29	859.2	0.81	0.032
33	2.65			0.92				-0.287	0.31	-1.00		6	-423.42	859.3	0.93	0.030
37	2.57			0.93	+			-0.282	0.31	-1.00		8	-421.29	859.3	0.99	0.029
35	2.39		+	0.92				-0.305	0.36	-1.01		8	-421.31	859.4	1.02	0.029
45	2.43			0.93	+	+		-0.313	0.26	-1.00		10	-419.15	859.5	1.13	0.028
99	2.50		+	0.90				-0.298	0.35	-1.00	+	9	-420.29	859.5	1.18	0.027
11	2.14		+	0.88		+		-0.295		-1.01		9	-420.40	859.7	1.4	0.024

**Table A.2.2** Model selection table of the 10 best models explaining variation in population density (average number of individuals per m<sup>2</sup>) of *Thalassoma hardwicke* at spawning sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cloud cover night-time (CCN), cloud cover day-time (CCD),  $\cos\theta$ , current direction (CD), current speed (CS), spawning site (S),  $\sin\theta$ , temperature (T), visibility (VIS) and date as independent variables. All models were fit using GLMER (Poisson distribution).

Model	(Int)	CCN	CCD	$\cos\theta$	CD	CS	S	$\sin\theta$	Tmp	VIS	df	logLik	AICc	delta	weight
17	3.15			0.41			+				4	-161.57	332.2	0.00	0.063
81	2.88			0.40			+		-0.17		5	-160.46	332.6	0.38	0.052
1	3.35			0.42							3	-163.18	333.0	0.77	0.043
145	3.24			0.43			+			+	5	-160.67	333.0	0.80	0.042
49	3.12			0.43			+	-0.1176			5	-160.83	333.4	1.12	0.036
129	3.43			0.44						+	4	-162.15	333.4	1.17	0.035
209	2.97			0.42			+		-0.17	+	6	-159.52	333.5	1.26	0.034
130	3.68	+		0.43						+	6	-159.75	334.0	1.73	0.027
21	3.11			0.36	+		+				6	-159.90	334.3	2.02	0.023
65	3.17			0.42					-0.13		4	-162.61	334.3	2.08	0.022

## Appendix Four

**Table A.4.1** Model selection table of the 10 best models explaining variation in feeding frequency of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors and date and survey site as random factors. All models were fit using GLMER (poisson distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	TD	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	TD*S.rs	df	logLik	AICc	delta	weight
284	2.297	-0.086	+		-0.050	+				+	12	-728.22	1482.1	0	0.242
316	2.292	-0.089	+		-0.050	+	-0.037			+	13	-727.47	1482.9	0.80	0.163
283	2.294		+		-0.046	+				+	11	-730.39	1484.2	2.10	0.085
348	2.301	-0.086	+		-0.043	+		+		+	13	-728.15	1484.2	2.15	0.083
288	2.299	-0.086	+	-0.006	-0.050	+				+	13	-728.21	1484.3	2.26	0.078
380	2.295	-0.090	+		-0.042	+	-0.037	+		+	14	-727.41	1485.0	2.97	0.055
416	2.304	-0.087	+	-0.011	-0.044	+			-0.038	+	14	-727.44	1485.1	3.03	0.053
320	2.294	-0.090	+	-0.009	-0.049	+	-0.038			+	14	-727.45	1485.1	3.05	0.053
448	2.299	-0.090	+	-0.013	-0.045	+	-0.033		-0.033	+	15	-726.88	1486.3	4.25	0.029
347	2.296		+		-0.041	+		+		+	12	-730.36	1486.4	4.29	0.028

**Table A.4.2** Model selection table of the 10 best models explaining variation in chasing frequency of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors and date and survey site as random factors. All models were fit using GLMER (poisson distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	TD	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	TD*S.rs	df	logLik	AICc	delta	weight
448	-0.184	0.023	+	-0.230	-0.221	+	0.260		-0.186	+	15	-414	860.5	0.00	0.370
446	-0.087	0.036		-0.208	-0.222	+	0.260		-0.186	+	14	-415.6	861.4	0.89	0.237
512	-0.182	0.018	+	-0.236	-0.272	+	0.240	+	-0.205	+	16	-413.3	861.6	1.11	0.212
320	-0.183	-0.004	+	-0.220	-0.241	+	0.239			+	14	-417.6	865.4	4.91	0.032
479	-0.219		+	-0.233	-0.323	+		+	-0.202	+	14	-417.7	865.6	5.08	0.029
318	-0.084	0.010		-0.198	-0.241	+	0.240			+	13	-419.3	866.5	6.02	0.018
415	-0.202		+	-0.219	-0.247	+			-0.168	+	13	-419.3	866.6	6.13	0.017
413	-0.107			-0.202	-0.244	+			-0.168	+	12	-420.9	867.5	7.04	0.011
384	-0.184	-0.006	+	-0.220	-0.258	+	0.232	+		+	15	-417.5	867.6	7.13	0.010
480	-0.221	0.010	+	-0.232	-0.323	+		+	-0.202	+	15	-417.7	867.9	7.40	0.009

**Table A.4.3** Model selection table of the 10 best models explaining variation in group swimming frequency of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors and date and survey site as random factors. All models were fit using GLMER (poisson distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	TD	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	TD*S.rs	df	logLik	AICc	delta	weight
30	-1.495	0.499		-0.468	0.388	+					9	-189.9	398.7	0.00	0.313
158	-1.501	0.491		-0.499	0.399	+			0.068		10	-189.8	400.7	1.98	0.116
62	-1.504	0.512		-0.470	0.392	+	-0.026				10	-189.9	400.9	2.17	0.106
32	-1.486	0.499	+	-0.466	0.387	+					10	-189.9	400.9	2.20	0.104
190	-1.513	0.507		-0.504	0.405	+	-0.033		0.072		11	-189.7	402.8	4.15	0.039
160	-1.488	0.492	+	-0.497	0.399	+			0.069		11	-189.8	402.9	4.20	0.038
96	-1.494	0.506	+	-0.472	0.357	+		+			11	-189.8	403.0	4.28	0.037
64	-1.494	0.513	+	-0.468	0.392	+	-0.027				11	-189.9	403.1	4.40	0.035
286	-1.405	0.499		-0.451	0.177	+				+	12	-188.8	403.1	4.46	0.034
26	-1.596	0.565			0.356	+					8	-193.6	404.0	5.32	0.022

**Table A.4.4** Model selection table of the 10 best models explaining variation in activity space (log-transformed) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	TD	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	TD*S.rs	df	logLik	AICc	delta	weight
95	4.076		+	-0.259	0.485	+		+			9	-333.6	686.2	0.00	0.216
223	4.079		+	-0.255	0.522	+		+	0.147		10	-332.7	686.6	0.34	0.182
96	4.080	0.072	+	-0.257	0.485	+		+			10	-333.5	688.1	1.90	0.083
91	4.077		+		0.470	+		+			8	-335.9	688.5	2.23	0.071
224	4.083	0.074	+	-0.253	0.522	+		+	0.148		11	-332.6	688.5	2.25	0.070
31	4.031		+	-0.260	0.371	+					8	-336.2	689.1	2.86	0.052
128	4.070	0.074	+	-0.256	0.483	+	-0.068	+			11	-333.3	689.9	3.69	0.034
256	4.070	0.076	+	-0.251	0.523	+	-0.083	+	0.157		12	-332.2	690.1	3.83	0.032
92	4.081	0.082	+		0.470	+		+			9	-335.7	690.2	4.02	0.029
32	4.037	0.088	+	-0.257	0.373	+					9	-336	690.8	4.60	0.022



**Table A.4.5** Model selection table of the 10 best models explaining variation in cumulative step length (log-transformed) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	TD	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	TD*S.rs	df	logLik	AICc	delta	weight
223	-3.292		+	-0.120	0.268	+		+	0.106		10	-167.210	355.6	0.000	0.293
224	-3.288	0.080	+	-0.117	0.268	+		+	0.107		11	-166.224	355.8	0.260	0.257
256	-3.293	0.081	+	-0.117	0.268	+	-0.032	+	0.110		12	-165.968	357.6	2.010	0.107
95	-3.294		+	-0.123	0.241	+		+			9	-169.641	358.2	2.650	0.078
96	-3.290	0.079	+	-0.120	0.241	+		+			10	-168.705	358.6	2.990	0.066
128	-3.293	0.080	+	-0.120	0.241	+	-0.021	+			11	-168.597	360.6	5.010	0.024
91	-3.294		+		0.234	+		+			8	-172.127	361.0	5.430	0.019
92	-3.289	0.084	+		0.234	+		+			9	-171.098	361.1	5.560	0.018
31	-3.315		+	-0.123	0.188	+					8	-172.380	361.5	5.940	0.015
32	-3.310	0.086	+	-0.120	0.190	+					9	-171.293	361.5	5.950	0.015

**Table A.4.6** Model selection table of the 10 best models explaining variation in turning angles (degree) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), time of the day (TD), and interactions between cos $\theta$  and size (S.rs), colour phase (CP) and size (S.rs), sin $\theta$  and size (S.rs) and time of the day (TD) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	TD	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	TD*S.rs	df	logLik	AICc	delta	weight
75	67.15		+		-4.050			+			5	-888.7	1788	0	0.181
11	67.15		+		-2.624						4	-890.5	1789.2	1.40	0.090
79	67.15		+	-0.974	-3.994			+			6	-888.6	1789.6	1.85	0.072
143	66.90		+	-0.892	-2.246				2.933		6	-888.6	1789.6	1.87	0.071
207	66.95		+	-0.913	-3.429			+	2.279		7	-887.6	1789.8	2.03	0.066
76	67.14	0.394	+		-4.051			+			6	-888.7	1789.8	2.08	0.064
91	69.20		+		-3.947	+		+			8	-887.1	1790.9	3.10	0.038
15	67.15		+	-0.968	-2.568						5	-890.3	1791.0	3.24	0.036
108	67.14	0.322	+		-4.036		1.335	+			7	-888.3	1791.2	3.46	0.032

## Appendix Five

**Table A.5.1** Model selection table of the best models explaining variation in home range area (m<sup>2</sup>) of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained colour phase (CP), size (S.rs), and interactions between colour phase (CP) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

Model	(Int)	CP	S.rs	CP*S.rs	df	logLik	AICc	delta	weight
8	929.6	+	209.8	+	5	-558.932	1129.0	0	0.707
4	961.1	+	1064		4	-561.085	1130.9	1.91	0.272
2	921.8	+			3	-565.323	1137.1	8.09	0.012
3	2045		1075		3	-565.777	1138.0	8.99	0.008
1	2016				2	-569.502	1143.2	14.22	0.001

**Table A.5.2** Model selection table of the best models explaining variation in spawning site fidelity of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), and interactions between colour phase (CP) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

Model	(Int)	CP	S.rs	CP*S.rs	df	logLik	AICc	delta	weight
1	32.66				2	-234.93	474.1	0	0.344
2	35.71	+			3	-234.24	475.0	0.88	0.221
3	32.59		3.105		3	-234.380	475.3	1.17	0.192
4	35.69	+	3.183		4	-233.65	476.2	2.07	0.122
8	35.72	+	-2.667	+	5	-232.43	476.2	2.09	0.121

**Table A.5.3** Model selection table of the best 10 models explaining variation in migration distance of *Thalassoma hardwicke* at survey sites. Best models given below were those with the lowest AICc score (among all permutations of the full model). The fully saturated model contained cos $\theta$ , colour phase (CP), sin $\theta$ , size (S.rs), and interactions between cos $\theta$  and size (S.rs), sin $\theta$  and size (S.rs), colour phase (CP) and size (S.rs) as fixed factors. All models were fit using GLM (gaussian distribution).

Model	(Int)	cos $\theta$	CP	sin $\theta$	S.rs	cos $\theta$ *S.rs	CP*S.rs	sin $\theta$ *S.rs	df	logLik	AICc	delta	weight
60	21.69	16.09	+		-2.453	24.69	+		7	-1460.1	2934.7	0.00	0.558
64	23.16	14.51	+	3.966	-2.499	24.64	+		8	-1459.9	2936.3	1.61	0.250
128	23.08	14.49	+	3.791	-0.415	22.13	+	5.90	9	-1459.1	2936.9	2.24	0.182
28	22.65	14.65	+		14.160	21.24			6	-1465.9	2944.1	9.48	0.005
32	24.23	12.96	+	4.267	14.050	21.21			7	-1465.6	2945.7	11.01	0.002
96	24.14	12.95	+	4.075	16.120	18.53		6.34	8	-1464.8	2946.1	11.47	0.002
112	26.00	11.15	+	3.824	12.990		+	12.56	8	-1466.4	2949.4	14.69	0
111	32.29		+	7.309	13.900		+	12.15	7	-1467.9	2950.3	15.60	0
44	25.43	11.96	+		11.710		+		6	-1470.3	2953	18.35	0
80	26.48	10.33	+	4.052	24.740			12.04	7	-1469.9	2954.2	19.58	0

