

**Establishing the mangrove killifish, *Kryptolebias marmoratus*, as a
model species for developmental biology**

Submitted by

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to the University of Exeter as a thesis for the degree of Doctor of Philosophy in
Biological Sciences in November 2012

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Acknowledgements

First and foremost I thank my supervisor, Dr Tetsuhiro Kudoh, for his mentoring and guidance throughout this project.

I also thank NERC for the funding provided to conduct this research.

I thank those who helped throughout the whole process. You know who you are.

Most importantly, I thank my family for their encouragement and support.

Abstract

The mangrove killifish, *Kryptolebias marmoratus*, has the potential of becoming a strong model organism for a range of biological disciplines thanks to its ability to self-fertilise, a process only known to occur in invertebrate animals until its discovery. Selfing, a natural occurrence in this species, has led to the formation of clonal lineages composed of highly homozygous individuals. The aim of this thesis was to further establish *K. marmoratus* in the field of developmental biology by providing an information infrastructure to help advance research on this peculiar animal and further promote its place in the pantheon of model organisms.

To do so, I first set out to standardise *K. marmoratus* embryology by providing defined developmental stages with clear visual representations of key embryonic structures. This staging series is an essential tool that will ensure repeatability and consistency within and across different laboratories. Secondly, I examined several techniques for embryonic manipulation and for imaging that can be used in an array of experimental designs. Using these techniques I demonstrated microinjection of embryos by monitoring the yolk syncytial layer and its nuclei, and time-lapse analyses of the yolk surface during embryonic development. Finally, I applied the knowledge gained from my first two studies and examined Bmp signalling in *K. marmoratus* embryos and its influence on body patterning. By inhibiting this pathway, I found a new phenotype characterised by an extremely short and split body axis. These data highlighted the importance of studying known signalling pathways in unknown organisms as species-specific differences may improve our understanding of fundamental developmental processes.

This thesis demonstrates that with its easily obtainable and manipulated embryos, *K. marmoratus* can be used for embryological research in the same light as other model organisms such as zebrafish or medaka. The rising amount of information on mangrove

killifish will help further take advantage of this unique and intriguing species, and supports the use of this hermaphroditic vertebrate as a strong comparative model in developmental biology.

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Author's declaration

The experimental chapters in this thesis are presented as a series of publications. The contribution made by the first author and writer of this thesis (SM) are declared below.

Chapter 2: Mourabit, S., Edenbrow, M., Croft, D.P., and Kudoh, T. (2011). Embryonic development of the self-fertilizing mangrove killifish *Kryptolebias marmoratus*. *Developmental Dynamics* 240, 1694-1704.

SM planned and carried out the experiments, analysed and interpreted the data, and wrote the manuscript.

Chapter 3: Mourabit, S., and Kudoh, T. (2012). Manipulation and imaging of *Kryptolebias marmoratus* embryos. *Integrative and Comparative Biology* 52, 761-768

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SM planned and carried out the experiments, analysed and interpreted the data, and wrote the manuscript.

List of abbreviations

11-KT	11-ketotestosterone
17 α 20 β -P	17 α -20 β -dihydroxy-4-pregnen-3-one
ab	air bladder
af	anal fin
AP	anterior-posterior
bd	blastodisc
bm	blastomeres
Bmp	bone morphogenetic proteins
cer	cerebellum
cf	caudal fin
ch	chorion
df	dorsal fin
di	diencephalon
dl	dorsal lip
DM	dorsomorphin
DMSO	demethyl sulfoxide
dpf	days post-fertilisation
DV	dorsal ventral
E2	17 β -oestradiol
em	embryo
EVL	enveloping layer
ey	eye
fb	forebrain
Fgf	fibroblast growth factor
fmb	forebrain–midbrain boundary

fr	fin ray
gb	gallbladder
gt	gut
h	heart
H ₂ S	hydrogen sulphide
hb	hindbrain
HE	hatching enzyme
hpf	hours post-fertilisation
hv	hindbrain ventricle
l	lens
lj	lower-jaw
lv	liver
mb	midbrain
MeOH	methanol
mhb	midbrain–hindbrain boundary
MT	17 α -methyltestosterone
n	needle
nd	nephric duct
no	notochord
od	oil droplet
op	olfactory pit
ot	otolith
ov	otic vesicle
p	melanophore
pf	pectoral fin
PFA	paraformaldehyde

ps	perivitelline space
psu	practical salinity units
PTU	1-phenyl 2-thiourea
RA	retinoic acid
s	somite
sc	spinal cord
t	testosterone
tec	optic tectum
tel	telencephalon
uj	upper-jaw
vv	vitelline vessels
y	yolk
YSL	yolk syncytial layer

List of species namesSpecies NameCommon Name*Artemia*

Brine shrimp

Danio rerio

Zebrafish

Drosophila

Fruit fly

*Escherichia coli**E. coli**Fugu rubripes*

Puffer fish

Fundulus heteroclitus

Mummichog

Kryptolebias marmoratus (previously *Rivulus marmoratus*)

Mangrove killifish

*Kryptolebias ocellatus**Mus musculus*

House mouse

Oryzias latipes

Medaka

Saccharomyces cerevisiae

Budding yeast

Xenopus