Title

Mahi-mahi (Coryphaena hippurus) life development: morphological, physiological, behavioral and molecular phenotypes

Journal

Developmental Dynamics, 248(5)

ISSN

1058-8388

Authors

Perrichon, Prescilla
Stieglitz, John D
Xu, Elvis Genbo
et al.

Publication Date

2019-05-01

DOI

10.1002/dvdy.27

Peer reviewed

Received: 3 December 2018 | Revised: 5 February 2019 | Accepted: 6 March 2019

DOI: 10.1002/dvdy.27

RESEARCH ARTICLE

Developmental Dynamics WILEY

Mahi-mahi (*Coryphaena hippurus*) life development: morphological, physiological, behavioral and molecular phenotypes

Prescilla Perrichon[1] | John D. Stieglitz[2] | Elvis Genbo Xu[3] | Jason T. Magnuson[3] | Christina Pasparakis[4] | Edward M. Mager[1] | Yadong Wang[4] | Daniel Schlenk[3] | Daniel D. Benetti[2] | Aaron P. Roberts[1] | Martin Grosell[4] | Warren W. Burggren[1]

[1]Department of Biological Sciences, University of North Texas, Denton, Texas

[2]Department of Marine Ecosystems and Society, University of Miami, Rosenstiel School of Marine and Atmospheric Science, Miami, Florida

[3]Department of Environmental Sciences, University of California Riverside, California

[4]Department of Marine Biology and Ecology, University of Miami, Rosenstiel School of Marine and Atmospheric Science, Miami, Florida

Correspondence
Prescilla Perrichon, Department of Biological Sciences, University of North Texas, Denton, TX 76203.
Email: prescilla.perrichon@gmail.com

Present address
Prescilla Perrichon, Institute of Marine Research, Austevoll Research Station, N-5392 Storebø, Norway.

Funding information
The Gulf of Mexico Research Initiative, Grant/Award Number: SA-1520; Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER). Data are publicly available through the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) at http://data.gulfresearchinitiative.org (doi<10.7266/n7-9h64-f119)

Abstract

Background: Mahi-mahi (*Coryphaena hippurus*) is a commercially and ecologically important fish species that is widely distributed in tropical and subtropical waters. Biological attributes and reproductive capacities of mahi-mahi make it a tractable model for experimental studies. In this study, life development of cultured mahi-mahi from the zygote stage to adult has been described.

Results: A comprehensive developmental table has been created reporting development as primarily detailed observations of morphology. Additionally, physiological, behavioral, and molecular landmarks have been described to significantly contribute in the understanding of mahi life development.

Conclusion: Remarkably, despite the vast difference in adult size, many developmental landmarks of mahi map quite closely onto the development and growth of Zebrafish and other warm-water, active Teleost fishes.

KEYWORDS

behavior, development, life span, mahi-mahi, molecular biology, physiology

1 | INTRODUCTION

Coryphaena hippurus, also known as the common dolphin fish or mahi-mahi,[1] is a highly migratory epipelagic fish distributed in the world's tropical and subtropical waters, where temperatures are typically between 25°C and 30°C.[2–4] Mahi-mahi (hereafter "mahi") is economically important for recreational and commercial fisheries

throughout this species' global range.[5] Global capture was estimated to be 115 658 tons in 2014.[6] The sustainable management, the circumglobal distribution, and the biology of mahi are attributes that make this species a viable choice for commercial aquaculture development.[5,7] Since the 1980s, technology for domestication of this species has been developed that allows mahi to be cultured successfully.[7–9] However, full commercial-scale productivity for aquaculture operations has yet to be demonstrated.

Aside from the interest in this species from a food-fish perspective, mahi has been identified as a promising candidate for research investigations. Indeed, during the last few decades, mahi has become an emergent model for examining population genetics,[10] developmental physiology,[11,12] metabolic responses,[13] nutritional physiology,[7] egg and larval performance over time,[14] and climate change effects.[15,16] Mahi have also been studied extensively regarding the impact of environmental toxicants.[17–30] The emergence of this model fish has been accelerated by the need to develop more sophisticated scientific approaches for understanding the impact of environmental stressors, especially the impacts of the *Deepwater Horizon* Oil spill in 2010. Immediate mortality or fitness declines have been shown in fin fish populations as a result of pollution exposure (see numerous chapters in Burggren and Dubansky[31]). It has become crucial for the scientific community to use resident species from the Gulf of Mexico for assessing economic feasibility operations, and mahi is ideal for this task.

The biology of mahi provides some relevant benefits due to its fast growth rates, capacity of producing, high spawning frequency, and high reproductive energy allocation. Regarding numerous model fishes (eg, Zebrafish, medaka, trout), mahi shares several similar attributes including the ability for researchers to control the reproductive cycle to allow year-round egg production[32]; the high number of produced eggs; and rapid embryonic development and transparency of embryos. These attributes make mahi a tractable model for future experimental studies.

Key to expanding studies on the developmental effects of the environment on mahi is a set of tools that includes a detailed table of development. Although a general developmental table documenting the main embryonic stages has been provided by Palko et al,[4] it lacks numerous important details about morphological and physiological landmarks placed on a common timescale, and is mostly devoid of any behavioral or molecular data. Scattered observations currently exist in literature but have never been compiled into a comprehensive developmental table. Thus, in the present study, we have created a comprehensive developmental table for mahi based on the brood stock of the University of Miami Experimental Hatchery (UMEH). Rather than presenting a "conventional" (and somewhat tedious) table reporting development as primarily detailed observations of morphology, similar to that already available for numerous fishes,[33–40] in this study we have particularly stressed specific physiological, behavioral, and molecular traits from the zygote to adult stage, alongside the standard morphological changes.

2 | RESULTS AND DISCUSSION

Following fertilization, mahi eggs are approximately 1.2 to 1.6 mm in diameter (Figure 1A). The eggs contain a single oil globule measuring 257 to 307 μm in diameter, depending on the captivity time.[14] While no standardization exists in the embryogenesis staging of teleost fishes, major molecular and cellular processes that underlie early development are highly conserved across teleost fishes. Though many fish species share common features, not unexpectedly numerous differences also emerge among species, especially regarding staging timing and organ implementation or progression. Following fertilization, mahi grow rapidly[41] compared to other model fishes. The most striking feature is the range of size that a mahi can reach in the first year compared to other model fishes (Table 1). Mahi are capable of growing from a hatch length (standard length [SL]) of 3.7 to 3.8 mm at 40 hours postfertilization (hpf) to a reproductively mature fish of ~20 to 30 cm at 80 to140 days postfertilization (dpf), while the maximum size of adult fish (length: 1-2 m) is reached after 3 to 4 years. Longevity of mahi is particularly short compared to other pelagic species, with an average of 2 years and maximum of 4 to 5 years.[2,4]

As a whole, total embryogenesis from fertilization to the first exogenous feeding stage lasts for 104 hours at 26°C (Table 1 and Figure 1). Despite the size difference, this developmental duration for embryogenesis is somewhat comparable to that of Zebrafish (120 hpf at optimal temperature of 28°C). Other model fishes exhibit longer embryonic development: 9 to 11 days for *Oryzias latipes* (medaka; 6°C), 10 to 12 days for *Fundulus heteroclitus* (killifish; 20°C), 34 to 37 days for *Oncorhynchus mykiss* (rainbow trout; 10°C), and 15 days for *Perca fluviatilis* (Eurasian pikeperch; 13°C) (Table 2).[33,34,36,40] In mahi, the first period of cleavage occurs at 35 minutes postfertilization (mpf) and results in two blastomeres of equivalent size, as observed in most teleost fishes. Cell division continues (Figure 1B,C), and cell migration begins at 80 mpf (16 cells; Figure 1D). A blastula with a well formed blastodisc appears at 3 hpf (128 cells, seventh division; Figure 1F–H). At 6 to 7 hpf, the germ ring is well defined (Figure 1I) and the activation of zygotic gene transcription accompanied by extensive RNA transcription occurs. The first epiboly movements then begin. From 8 hpf, gastrulation takes place with the appearance of the embryonic shield (Figure 1J). Physiologically, urea and ammonia excretion have been measured during early gastrulation[42] (Table 1).

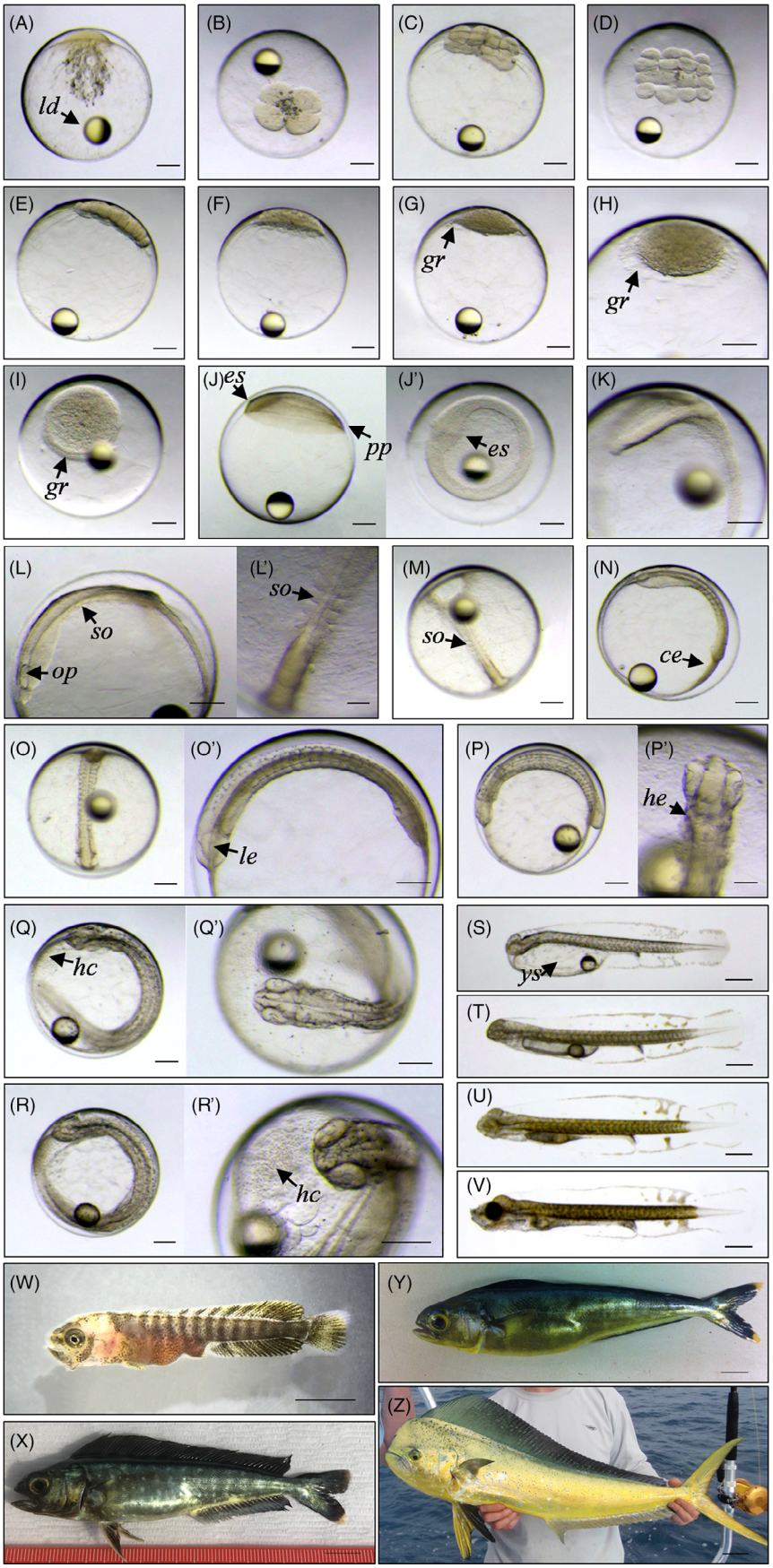

FIGURE 1 Embryo-larval development of mahi at 26°C. A-C, Cell cleavage. D-I, Morula/blastula. J-P, Gastrula/segmentation. Q–S, Pharyngula/hatching period. T–V, Post-hatching period/yolk sac larvae. W–Y, Juvenile period. Z, Adult phase. A, Zygote stage, 1 cell (pre-division, 0 hpf). B, 4 cells (second division, 50 mpf). C, 8 cells (third division, 70 mpf). D, 16 cells (fourth division, 80 mpf). E, 32 cells (fifth division, 120 mpf). F, 128 cells (seventh division, 3 hpf). G, 256 cells (eighth division, 4 hpf). H, 512 cells (ninth division, 5 hpf). I, Germ ring (6-7 hpf). J, 20% epiboly (8 hpf). K, 50%-60% epiboly (11 hpf). L, 80% epiboly, 3-4 somites (14 hpf). M, 90% epiboly, 5-6 somites (15 hpf). N, 100% epiboly, 8-9 somites (16 hpf). O, 12 somites (18 hpf). P, 26+ somites (22-23 hpf). Q, 35 hpf; R, Pre-hatching period (38-40 hpf). S, Hatched larvae (44 hpf). T, Yolk sac larvae (56 hpf). U, Protruding-mouth stage (80 hpf). V, Mouth-opening stage (104 hpf). W, Juvenile mahi (16 dph). X, 40 dph. Y, Transition to young adult phase (55 dph). Z, Adult male. ce, complete epiboly; dph, days post-hatching; es, embryonic shield; gr, germ ring; hc, cells from hatching gland; he, heart; ld, lipid droplet; le, lens; op, optic primordium; pp, posterior pole; so, somites; ys, yolk sac. Scale bars A-L,M-P,Q-R = 250 μm. Scale bars L′, P′, R′ = 100 μm. Scale bars S-V = 500 μm. Scale bars W-Y = 1 cm. Scale bar Z = 10 cm

A–V: Photo credit: P. Perrichon;
W–Z: Photo credit: J. D. Stieglitz

TABLE 1 Morphological, physiological, behavioral and molecular landmarks thought the life development of mahi

DEVELOPMENT			Phenotype							
			Morphology							
Stage		Hours (unless otherwise indicated[4])	General Observations	Cell Number (Division)	Length (mm)	Epiboly (%)	Somite (#)	Physiology	Behavior	Molecular Biology
Embryo	Zygote	0	• Spawning and fertilization typically occur at 2 AM-5 AM[14] • Eggs positively buoyant	1 (pre-division)						
	Cleavage	35-40 min	• First cleavage	2 (1st division)						
		50-70 min	• Discoidal • Meroblastic cleavage in blastodisc • Greatest concentration of yolk at animal pole due to inhibition of cleavage at vegetable pole	4, 8 (2nd and 3rd divisions)						
	Morula/Blastula	80 min	• Blastomeres evident • Cell migration begins	16 (4th division)						
		120 min	• Cells irregular in shape and arrangement	32 (5th division)						
		150 min		64 (6th division)						
		3	• 7th cleavage creates blastula with well formed blastodisc • A primarily yolk-filled blastocoel	128 (7th division)						
		4	• Spreading of yolk syncytial layer	256 (8th division)						
		5	• Yolk sac syncytial layer evident	512 (9th division)						
		6	• Yolk syncytial layer • Enveloping layer and deep cell layer increasingly distinctive	1024 (10th division and beyond)						
		6-7	• Germ ring formation			0				• Zygotic gene transcription begins • Heavy RNA transcription
	Gastrula	8	• Onset of epiboly • Spreading of yolk syncytial layer and blastoderm over and across yolk sac • First appearance of embryonic shield			20				
		11	• Spread of embryonic shield • Invagination/involution to form mouth, anus, and digestive tube			50	0	• Urea and ammonia excretion measurable[42] • Urea excretion rises sharply[42]		
	Gastrula-Segmentation Transition	13 (12)	• Appearance of neural groove • Appearance of first somites		1.3-1.5	70	1-2			

(Continues)

TABLE 1 (Continued)

DEVELOPMENT		Phenotype							
		Morphology							
Stage	Hours (unless otherwise indicated[4])	General Observations	Cell Number (Division)	Length (mm)	Epiboly (%)	Somite (#)	Physiology	Behavior	Molecular Biology
	14	• Otic placode evident • Optic primordium appears without pigmentation		1.5	80	3-4			
	15	• Kupffer's vesicle formation at tail bud end • Optic primordium evident		1.6-1.7	90	5-6			
	16	• Blastopore closes to complete epiboly and signal full transition to segmentation phase • Tail bud appears • Early body pigmentation		1.7-1.8	100	8-9			
Segmentation	17 (*17-18*)	• Yolk sac pigmentation appears, followed by start of spread to body surface		1.9-2.0		10			• Urea transporter gene starts to express
	18	• Cardiac precursors and myocytes visible • Myotomes begin to form in previously formed somite • Embryo trunk curved ~50% of egg capsule diameter		1.9-2.0		12			
	185 (*17*)	• Somite number continues to increase		2.1-2.5		13-14			
	19 (*18*)	• Cardiomyocytes visible • Retina present • Otic vesicle forms from otic placode • Pronephric duct		2.2-2.6		16-17	• No peristaltic heartbeat		
	22	• Heart tube rudiment in anterior position, close to eye • Otoliths evident • Neural tube forming		2.6-2.8		26+	• Heartbeat onset as irregular peristaltic movements • Skeletal muscle contraction initiated		
Pharyngula	23 (*26*)	• Maximum somite numbers achieved • Heart tube elongating		2.8-3.0		30-34	• Rudimentary sensory reflexes	• Coordinated body movement begins • Spontaneous weak embryo twitches within egg capsule	
	24	• Embryo trunk curved 50%-60% of diameter of egg capsule • Melanophores numerous on body • Single dorsal/caudal fins begin development, but tail still attached to yolk sac, which is large and conspicuous					• Heart rate at 90-95 beats/min^{-1}	• Intensification of body movements • Reflex movements from touch	• Expression of genes involved in general developmental biology (eg, cellular development, tissue development, organ development)[30]
	26-27 (*26*)	• Embryo trunk curved 60%-70% of diameter of egg capsule • Heart chambers not yet clearly delineated but discernable in videos of contracting heart atrium anteriorly located		3.0-3.2			• Regular heart rate at 120-130 beats/min^{-1}		

(Continues)

TABLE 1 (Continued)

DEVELOPMENT			Phenotype							
			Morphology							
Stage		Hours (unless otherwise indicated[4])	General Observations	Cell Number (Division)	Length (mm)	Epiboly (%)	Somite (#)	Physiology	Behavior	Molecular Biology
			• Elongated caudal fin starts to migrate off yolk sac							
		32	• Heart still in embryonic configuration but greater delimitation of cardiac chambers • Embryo position changing within yolk sac		3.4-3.5			• Vigorous cardiac contraction • Heart rate[12] increases to 160-165 beats/min^{-1}		
		34	• Cardiomyocytes dividing to create thickening heart walls		3.7-3.8			• Oxygen consumption[24] ~30-35 PMol/ind^{-1}/min^{-1}		
		37-38	• Early pigmentation in eye, which remains largely transparent • Caudal fin pigmentation starts (peripheric pigmentation)		3.7-3.8			• Urea excretion continues to increase[42] • Oxygen consumption increases sharply[24]	• Frequent embryonic movement within egg • 60%-75% of eggs are negatively buoyant	• Urea excretion rate and urea transporter gene expression reaches peak level around 36 hpf and steadily decreases thereafter[42]
		39	• Prominent cells (granules) of hatching gland, present on pericardium over the anterior yolk sac • Chorion begins to weaken • No blood circulation						• 95%-100% eggs are negatively buoyant	
Hatching Embryo		41	• Earliest embryos hatch • Pigmentation abundant in dorsal part • Peripheral pigmentation in caudal, dorsal, and ventral • Large pericardium and advanced in anterior position • Pectoral fins rudiment visible		3.7-3.8			• 2%-5% of eggs hatched	• Swimming reflex	
		42	• Major veins apparent, no blood circulation		3.9-4.0			• 10%-20% of eggs hatched		• Ammonia transporter gene (*rhag* and *rhbg*) expression significantly increase[42]
		43	• Erythropoiesis initiation evident from appearance of transparent circulating blood cells • Central circulation apparent as modest flow • Major arteries and veins evident		3.9-4.0			• 40%-45% of eggs hatched • Peristaltic blood flow through cardiac chambers well established • Complete constriction between atrium and ventricle during cardiac cycle		
		44	• Circulating in major arteries and veins • Very low blood cells circulation		4.0-4.1			• 80%-86% of eggs hatched		
		45	• Last embryos hatch		40-41			• >97% of eggs hatched		
Free-swimming Larva	Yolk Sac	42-46	• Peripheral fin pigmentation • Pectoral bud starts to develop (shallow dome) • Large pericardium • Atrium larger than ventricle • Body curvature still apparent							• Ammonia excretion rate increases significantly

(Continues)

TABLE 1 (Continued)

DEVELOPMENT		Phenotype							
		Morphology							
Stage	Hours (unless otherwise indicated[4])	General Observations	Cell Number (Division)	Length (mm)	Epiboly (%)	Somite (#)	Physiology	Behavior	Molecular Biology
	48-49	• Trunk and peripheral fin pigmentation increasing • Pectoral bud increases in size (height dome and bud curve) • Ventral fin well developed • Intensification of body pigmentation and eye • Brain area and tail end still relatively transparent • Reduction of pericardium volume • Head and body are aligned • Retinal pigmentation appearing		4.2-4.3			• Heart rate at 175-180 beats/min^{-1}	• Larvae are vertically oriented in water	• Expression of genes involved in RNA binding, ATP binding, neurogenesis, and development of cardiovascular, visual, and muscular systems[30]
	50-51	• Cardiac chambers well differentiated • Heart begins S-folding • Oil droplet persistent • Pectoral fins continue to develop		4.3-4.5			• Cardiac chambers (including bulbus arteriosus) beat in a coordinated sequence • Arterial branchial and systemic circulation established	• Sporadic, "twitch-like" swimming	
	56	• Atrium, still larger than ventricle, positioned dorsal to ventral • Bulbus cordis and sinus venosus become differentiated • Eye pigmentation clearly observed but still light • Pectoral fin well developed but still "attached" to the body • Stereocilia of anterior neuromast well developed • Faster blood flow through body but blood cells still discernable		4.5-4.6			• Rudimentary valving action evident between contracting and relaxing heart chambers • Heart rate[11,26] at 190-195 beats/min^{-1} • Cardiac output[11,26] at 27-29 nL/min^{-1} • Oxygen consumption[24] at ~250-275 PMol/ind^{-1}/min^{-1}		• *Rhag* and *Rhbg* expressed in gills[42] • *Rhag* expressed in yolk sac[42] • *NHE3* expressed in heart and intestines[42]
	80	• Protruding mouth • Pectoral fins continue elongation • Movement of pectoral fins start • Cardiac ventricle growing in size relative to atrium		4.8-4.9				• Larvae are not vertically oriented in water anymore • Swimming behavior increases, but fin movement limited	
	104	• Mouth open • Eye pigmentation intensifies • Yolk sac absorption nearly complete • Branchial structures (gill arches and operculum) largely formed		4.9-5.0				• Active pectoral fins movement • First exogenous feeding • Vision-dependent behavior [JTM, unpublished data]	• Rhch2 expressed in skin[42] • Expression of genes involved in metabolism functions (eg, lipid metabolism, carbohydrate metabolism), RNA binding, ATP binding, cellular catabolic process, metabolism, neurogenesis, development of cardiovascular, visual and muscular systems[30]
Post-yolk Sac Absorption	120-128	• Retina, lens, ganglion, epithelium, plexiform, and photoreceptor layer anatomically distinct		4.9-5.0			• Irregular buccal and opercular pumping creating gill ventilation at ~70-90		

(Continues)

TABLE 1 (Continued)

DEVELOPMENT		Phenotype							
		Morphology							
Stage	Hours (unless otherwise indicated[4])	General Observations	Cell Number (Division)	Length (mm)	Epiboly (%)	Somite (#)	Physiology	Behavior	Molecular Biology
							movements/min^{-1} [PP, unpublished data]		
	152			4.9-5.0			• Regular buccal and opercular ventilation at 100-110 movements/min^{-1} [PP, unpublished data]		
	176	• Dorsal, caudal, and anal fins still a single elongated fin • Yolk sac resorbed		5.1-5.2					
Juvenile	10 days	• Flexion stage[26]						• Larva responsive to changes in surrounding visual field [JTM, unpublished data]	
	11-13 days	• Postflexion[26]						• Visual field sensitivity increases [JTM, unpublished data]	
	15 days	• Adult fin configuration • Body pigmentation and coloration of alternating dark and light bars • Blunt snout characteristic of adults appearing • Eyes and mouth fully developed • Musculature evident		15			• Olfactory response to environmental components evident	• Negative chemotaxis	
	18 days	• Transition from pale brown/yellow coloration to darker brown/black coloration (fish gain ability to manipulate color phase at this point, particularly evident during feeding events)							
	20 days	• Caudal fin shape transition begins from rounded (pre-20 days) to emarginate (21- ~55 days) and then eventually to forked at ~55+ days							
	26 days	• Body mass 200-300 mg		33-35					
	32 days	• Body mass 600-700 mg • Lateral banding on body less distinctive than earlier juvenile states • Caudal fin begins to develop deep fork characteristic of adults		42-46			• Standard O_2 consumption[28] at 1.0 mg $O_2/g^{-1}/h^{-1}$ • U_{crit} at 4.8-5.0 body length/sec^{-1} [21,28]	• Cannibalism continues throughout juvenile and adult life	
	45 days	• Body coloration transitions from primarily darker phase (dark brown/black) to a more silvery/reflective phase • Upon transition to the juvenile stage with silvery appearance, whereby stressed individuals will express the "classic" yellow-green coloration						• Classic mahi-mahi yellow/green coloration when stressed	

(Continues)

TABLE 1 (Continued)

DEVELOPMENT		Phenotype							
		Morphology							
Stage	Hours (unless otherwise indicated[4])	General Observations	Cell Number (Division)	Length (mm)	Epiboly (%)	Somite (#)	Physiology	Behavior	Molecular Biology
		typically associated with mahi-mahi, the fish will begin schooling behavior							
	55 days	• Tail fully forked							
Adult	80-90 days	• Sexual maturity reached by earliest maturing individuals under optimal rearing conditions		20-30 cm					
	120-140 days	• Body mass 255-301 g • Sexual maturity reached by majority of population[32,42]		~30 cm			• Heart rate[23] at 140-164 beats/min^{-1} • Cardiac output[23] at 50-60 mL/min^{-1}/kg^{-1} • Standard O_2 consumption at 490-500 mg O_2/kg^{-1}/h^{-1} • U_{crit} at 4.0-4.2 body length/sec^{-1},21		
	4-5 years	• Maximum age and size acquired • Up to 40 kg (IGFA record)		1-2 m					

hpf, hours postfertilization.

One notable feature during mahi embryonic development is that the segmentation process/somitogenesis overlaps with the epiboly process (Figure 1J–N). The cellular front reaches 50% epiboly at 11 hpf (Figure 1K) and 70% of the yolk surface by 12 to 13 hpf, corresponding to the formation of the first somites. At the beginning of the somitogenesis, mahi embryos are 1.3 to 1.5 mm in length. Complete epiboly is reached at 16 hpf (8-9 somites; SL = 1.7-1.8 mm; Figure 1N). The somites progressively increase in number during the segmentation process (17-22 hpf; Figure 1O,P). The epiboly process also overlaps with somitogenesis in rainbow trout (3-9 dpf, until 29 formed somites) (Table 2).[40] While the epiboly and segmentation steps progressively succeed one another in Zebrafish, the timing of morphogenesis and cardiogenesis is very similar in both Zebrafish and mahi (Tables 1 and 2).[37]

At the beginning of the segmentation period in mahi, early body pigmentation and individual cardiomyocytes are visible. Urea transporter genes also begin their expression.[42] At 22 hpf (SL = 2.6-2.8 mm; 26 somites; Figure 1P), the onset of the heartbeat begins with irregular peristaltic movements, which closely resemble those of the Zebrafish embryo, where heartbeat occurs at ~24 hpf. At this stage, expression of genes associated with cellular, tissue, and organ development is also accelerating.[30] The first muscle contractions of mahi embryos are also observed at this time in development, and embryonic movements sharply increase with a touch reflex apparent from 26 hpf. Regular heart rate (120-130 beats/minute^{-1}) is established by 26 hpf in mahi, whereas heart chambers are discernable but not yet delineated. Heart rate frequency then increases with further development.[11,12] Urea transporter gene expression peaks around 36 hpf.[42] Erythropoiesis is initiated from transparent circulating blood cells, and circulation in the central vasculature appears as a modest flow during hatching (43 hpf) (SL = 3.9-4.0 mm). At this point in development, ammonia transporter (Rhag and Rhbg) gene expression increases as urea transporter expression decreases.[42] Complete morphological constriction between the atrium and ventricle is seen during the hatching period, and the heart (including bulbus arteriosus) initiates its S-folding configuration at 50 hpf (SL = 4.3-4.5 mm). Concurrently, arterial branchial and systemic circulation are established. Oxygen consumption (cutaneous respiration or simple diffusion) is measurable in early stages from 34 hpf (Table 1).

Mahi eggs are positively buoyant in the laboratory and are assumed to float near the surface of the water column in the field. Prior to hatching, egg specific gravity changes, cells (granules) of the hatching gland increase in number over the anterior yolk sac (Figure 1Q–R'), and the eggs become negatively buoyant until they hatch.[27] This dynamic

TABLE 2 Comparative life characteristics between mahi and three model fish species

Characteristics	Mahi-mahi[3,4,32] (*Coryphaena hippurus*)	Zebrafish[37,43] (*Danio rerio*)	Killifish[34] (*Fundulus heteroclitus, F grandis*)	Rainbow Trout[40] (*Oncorhynchus mykiss*)
Habitat	Wildly distributed, offshore (tropical and warm temperate waters)	Central Asia, India, Ganges River	Inshore bays, salt marsh flats, estuaries and tidal creeks with emergent vegetation	Wildly distributed (cold waters)
Economic interest	Sport fish	Ornament fish	Ornament fish	Sport fish
Swimming capacity	Fast swimmer (migratory fish)	Low swimmer	Low swimmer	Variable (migratory fish)
Lifespan	4-5 y	4-5 y (mean 3.5 years)	4-5 y	6-8 y (11 y record)
Adult morphometry	1-2 m, up to 40 kg (IGFA record)	4-5 cm	5-10 cm (15 cm max)	50-80 cm
Environment	Saltwater	Freshwater	Fresh, brackish, and saltwater	Fresh, brackish, and saltwater
Temperature tolerance	19°C-31°C	25°C-31°C	6°C-35°C	0°C-27°C
Embryonic rearing condition	Air incubation	Static water	Air incubation until hatching	Running water
Breeding season	Year-round	April to August (year-round in laboratory)	March to September (semi-lunar rhythm, year-round in laboratory)	March to July
Breeding facilities required	80 000 L tank	1-150 L tank	20-150 L tank	30 000 L tank
Cost level (relative units)	++++	+	++	+++
Spawning size	100 000-200 000 eggs per female (3-5 kg)	100-500 eggs per female	200-400 eggs per female	1500-2000 eggs per kg
Egg buoyancy	Positive until 33-40 hpf	Negative	Negative	Negative
Chorion strength	Strong	Weak	Thick, strong	Thick, strong
Egg size at spawning	1.2-1.5 mm	1.0-1.2 mm	2.0-2.3 mm	3.5-5 mm
Egg incubation period	Very short	Short	Medium (aerial incubation)	Long
Hatching time (postfertilization)	41-45 h (26°C)	48-72 h (28°C)	10-12 d (20°C)	34-37 d (10°C)
Epiboly process	7-16 h ("Gastrulation + segmentation transition," germ ring to tail bud apparent, 8-9 somites)	4.25-10 h ("Gastrulation" dome stage to bud, no somite)	30-46 h ("gastrulation," somite formation onset)	5-9 d ("Gastrulation + segmentation," germ ring to 21-29 somites)
Heartbeat onset	22 h	24 h	85-92 h	14-15 d
Protruding mouth	75-80 h	72 h	9-10 d	18 d
Feeding initiation	104 h	120 h	10-12 d	34-37 d
Complete yolk sac absorption	7 d	7 d	16 d	85 d
Sexual maturation	80-90 d	90 d	9 m	2 y

hpf, hours postfertilization.

change in buoyancy occurs 2 to 4 hours before hatching in mahi, and has also been observed in tuna species.[44] Buoyancy changes and the process of sinking in the water column prior to hatching likely reduce mortality of newly hatched larvae exposed to wave action and wind, while further minimizing the exposure to UV radiation and predation at the surface.[27]

The hatching period lasts for several hours (40-45 hpf), and individuals hatch into relatively undeveloped yolk sac larvae (SL = 3.7-4.1 mm) lacking a functional mouth, eye pigmentation, and differentiated fins (Figure 1S). For comparison, Zebrafish hatch between 48 and 72 hpf. Accelerated expression occurs in genes involved in RNA binding, ATP binding, neurogenesis, and development of cardiovascular, visual, and muscular systems.[30] Following hatching, the pectoral buds, trunk, and peripheral fins continue to develop (Figure 1S–V). Movement of pectoral fins starts at 80 hpf (Figure 1U) along with increased swimming behavior. A protruding mouth appears at 80 hpf, and the first exogenous feeding starts from 104 hpf (SL = 4.9-5.0 mm; Figure 1V), where vision-dependent behavior is activated to strike planktonic prey in the water. The first oral feeding by larvae occurs relatively early compared to model fishes such as the Zebrafish (120 hpf), killifish (10-12 dpf), and rainbow trout (34-37 dpf). At 80 hpf in mahi development (Figure 1V), branchial structures are largely formed and opercular pumping, creating gill ventilation (70-90 movements/minute^{-1}), begins around 120 hpf and steadily increases in frequency and depth with additional development. Complete absorption of yolk sac occurs by 176 hpf, similar to Zebrafish.

In parallel to morphological, physiological, and developmental changes observed early in development, the expression of genes and regulation pathways reveals a transitional state related to the described physiological and behavioral changes during development (Table 1).[30] High-throughput sequencing demonstrates that a significant contribution of genes is involved in cellular and tissue development from the pharyngula period (~24 hpf) to yolk sac stage (~48 hpf). Additionally, metabolism-related processes are more enriched during development of free-swimming larvae and associated with cardiovascular, muscular, and neuronal development.[30]

Increased retinal pigmentation is observed at first feeding, with distinct retinal lamination (lens, neuronal layers, photoreceptors) observed by 5 dpf. Vision-dependent behavior is increased by 10 dpf along with an increase in the sensitivity of mechanical stimulation. This vision-dependent behavior highlights a gradual transition stage from the larval to juvenile state (SL = 15 mm at 15 dpf).

At this point (10 dpf), mahi enter the flexion stage, where musculature is evident throughout the body and the eyes and mouth are prominent and fully developed. Fin development progresses (Figure 1W) and displays the adult configuration from 30 to 40 dpf. Fishes reach adult configuration (body coloration and fin formation) from 40 to 55 days (Figure 1X–Y) and are sexually mature from 80 to 90 days under optimal rearing conditions (SL = 20-30 cm).

In summary, mahi are large pelagic fish (Figure 1Z) with high energetic requirements necessary to maintain their "high-performance" lifestyle.[7,23,28,32,42,45] Their physiological and metabolic capacities are therefore elevated for the increased supply of energy, oxygen, and substrate needed for swimming performance and routine activities[23,28] compared to those of more established fish models (eg, killifish, medaka, Zebrafish). From a developmental perspective, mahi share numerous physiological and behavioral landmarks with others pelagic fish (tunas or billfishes) as a result of a similar lifestyle.[45] Perhaps most surprising is how closely the development of mahi compares with that of Zebrafish. We particularly hope that the attractive advantages of the mahi embryos will entice the scientific community to work on this biological system in the near future.

3 | EXPERIMENTAL PROCEDURE

3.1 | Fish populations examined

The developmental table in this study is based on the resident populations at UMEH. Mahi brood stock were captured in the offshore waters of the Strait of Florida off the coast of Miami, Florida, in the general coordinates of 25° 34.000′N / 80° 00.000′W using hook-and line-angling. Brood stock age and growth metrics, as well as methods of capture, transport, acclimation, and spawning, have been detailed in Stieglitz et al.[32] The adult fish were subsequently transferred to UMEH, where they were acclimated in 80-m^3 fiberglass maturation tanks equipped with partially recirculated and temperature-controlled water at 26°C to 27°C.[32] All fish were regularly fed rations of whole and chopped Spanish sardines (*Sardinella aurita*) and squid (*Loligo opalescens*) to satiation every day. The brood stock were also fed with a supplement dry pelletized diet (MadMac-MS, Aquafauna Bio-Marine, Inc., Hawthorne, CA) once a week at 10% of the food weight per day. The nutritional composition of the natural diet and dietary supplements is further described in Stieglitz et al.[7]

Spawning occurred volitionally (noninduced) at a sex ratio of 1 male:8 females using standard UMEH methods.[14,32] Spawning events occur naturally throughout the year at UMEH between 2 AM and 5 AM before sunrise. Brood stock spawned naturally every day, with multiple females spawning asynchronously on opposing days.[14,32]

Spawning patterns are relatively time-specific in order to maintain consistent hatching periods (during the night), which is thought to maximize early larval survival. This adaptive spawning pattern has also been observed in other tuna species.[44,46]

3.2 | Data collection

Embryos were immediately collected after spawning events and were equally distributed among 1-L glass beakers, where they were kept under optimal rearing conditions (26°C; 34-35 ppm; photoperiod, 16 hours:8 hours light: dark). Early embryogenesis in fish was followed from the zygote stage (0 hpf; 1 cell) to post-yolk sac absorption (176 hpf) by examining embryos under a Nikon SMZ-800 stereomicroscope coupled to a Fire-i400 or Fire-i530c digital camera (Unibrain, San Ramon, CA). Observations from juvenile to adult stages were made directly from the production tanks. Major developmental landmarks and morphology of specimens were observed; images were digitized using Photo Booth software (dslrBooth Lumasoft, East Brunswick, NJ) and calibrated using a stage micrometer. ImageJ software[47] was used then to measure specific physiological parameters such as larval cardiac output.

All developmental and phenotypic observations are normalized as minutes, hours, or days postfertilization (mpf, hpf, and dpf, respectively) and are reported in Table 1. Although the intent of this study is not to describe a detailed pictorial representation of mahi development, Figure 1 illustrates the major developmental stages. Physiological, behavioral, and molecular characteristics were aggregated from data acquired during the last three years of research from the RECOVER consortium, supported by the Gulf of Mexico Research Initiative, involving four American universities (University of Miami, University of North Texas, University of California Riverside, and University of Texas Austin Marine Research Institute). Different rearing conditions, fish size, and/or nutritional status may influence the timing, developmental progress, and organogenesis of specimens.

ACKNOWLEDGMENTS

The authors thank the staff of the Experimental Hatchery of the University of Miami for their assistance and maintenance of the mahi brood stock. All handling and use of animals in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami (Nos. 15-019 and 15-067; Special Activity License #: SAL-15-0932B-ABC). M. Grosell holds a Maytag Chair of Ichthyology.

CONFLICT OF INTERESTS

The authors declare no competing financial interests.

ORCID

Prescilla Perrichon https://orcid.org/0000-0002-1410-878X

REFERENCES

1. Linnaeus C. Systema naturae. Regnum animale, 1758. 10th ed. Photographic facsimile printed by Br. Mus. nat. Hist. 1956, 854p.
2. Beardsley GL. Age, growth, and reproduction of the Dolphin, *Coryphaena hippurus*, in the Straits of Florida. *Am Soc Ichtyologists Herpetol*. 1967;1967:441-451.
3. Gibbs RHJ, Collette BB. On the identification, distribution and Biology of the dolphins, *Coryphaena hippurus* and *C. equiselis*. *Bull Mar Sci Gulf Caribb*. 1959;9:117-152.
4. Palko BJ, Beardsley GL, Richards WJ. Synopsis of the biological data on dolphin-fishes, *Coryphaena hippurus* Linnaeus and *Coryphaena equiselis* Linnaeus. *FAO Fish Synopsis Rep*. 1982;130: 1-34.
5. Oxenford HA. Biology of the dolphinfish (*Coryphaena hippurus*) in the western central Atlantic: A review. *Sci Mar*. 1999;63:277-301. https://doi.org/10.3989/scimar.1999.63n3-4303.
6. FAO, *Coryphaena hippurus* (Linnaeus, 1758). Global Capture Production for Species. 2018. http://www.fao.org.
7. Stieglitz JD, Benetti DD, Grosell M. Nutritional physiology of mahi-mahi (*Coryphaena hippurus*): Postprandial metabolic response to different diets and metabolic impacts on swim performance. *Comp Biochem Physiol A Mol Integr Physiol*. 2018;215: 28-34. https://doi.org/10.1016/j.cbpa.2017.10.016.
8. Kraul S. Hatchery methods for the mahimahi, *Coryphaena hippurus* at Waikiki Aquarium. *CRC Handb Maric*. 1991;2:227-241.
9. Szyper JP, Ako H. Feed formulas producing improved palatability and growth with juvenile mahimahi, *Coryphaena hippurus*. *World Aquac*. 1990;21:104-105.
10. Tripp-Valdez MA, García de León FJ, Ortega-García S, Lluch-Cota D, López-Martínez J, Cruz P. Population genetic structure of dolphinfish (*Coryphaena hippurus*) in the Gulf of California, using microsatellite loci. *Fish Res*. 2010;105:172-177. https://doi.org/10.1016/j.fishres.2010.03.023.
11. Perrichon P, Grosell M, Burggren WW. Heart performance determination by visualization in larval fishes: influence of alternative models for heart shape and volume. *Front Physiol*. 2017;8:1-10. https://doi.org/10.3389/fphys.2017.00464.
12. Perrichon P, Pasparakis C, Mager EM, et al. Morphology and cardiac physiology are differentially affected by temperature in developing larvae of the marine fish mahi-mahi (*Coryphaena hippurus*). *Biol Open*. 2017;6:800-809.
13. Morgan JD, Balfry SK, Vijayan MM, Iwama GK. Physiological responses to hyposaline exposure and handling and confinement stress in juvenile dolphin (mahimahi: *Coryphaena hippurus*). *Can J Fis Aquat Sci*. 1996;53:1736-1740. https://doi.org/10.1139/cjfas-53-8-1736.

14. Kloeblen S, Stieglitz JD, Suarez JA, Grosell M, Benetti DD. Characterizing egg quality and larval performance from captive mahi-mahi *Coryphaena hippurus* (Linnaeus, 1758) spawns over time. *Aquacult Res*. 2017;49:1-12. https://doi.org/10.1111/are.13459.
15. Bignami S, Sponaugle S, Cowen RK. Effects of ocean acidification on the larvae of a high-value pelagic fisheries species, Mahi-mahi *Coryphaena hippurus*. *Aquat Biol*. 2014;21:249-260. https://doi.org/10.3354/ab00598.
16. Pimentel M, Pegado M, Repolho T, Rosa R. Impact of ocean acidification in the metabolism and swimming behavior of the dolphinfish (*Coryphaena hippurus*) early larvae. *Mar Biol*. 2014;161:725-729. https://doi.org/10.1007/s00227-013-2365-7.
17. Adema-Hannes R, Shenker J. Acute lethal and teratogenic effects of tributyltin chloride and copper chloride on Mahi-mahi (*Coryphaena hippurus*) eggs and larvae. *Environ Toxicol Chem*. 2008;27:2131-2135.
18. Burggren W, Dubansky B, Roberts A, Alloy M. Deepwater horizon oil spill as a case study for interdisciplinary cooperation within developmental biology, environmental sciences and physiology. *World J Eng Technol*. 2015;3:7-23.
19. Edmunds RC, Gill JA, Baldwin DH, et al. SuppInfoAnnexe: Corresponding morphological and molecular indicators of crude oil toxicity to the developing hearts of mahi mahi. *Sci Rep*. 2015;5:1-18. https://doi.org/10.1038/srep17326.
20. Esbaugh AJ, Mager EM, Stieglitz JD, et al. The effects of weathering and chemical dispersion on Deepwater Horizon crude oil toxicity to mahi-mahi (*Coryphaena hippurus*) early life stages. *Sci Total Environ*. 2016;543:644-651. https://doi.org/10.1016/j.scitotenv.2015.11.068.
21. Mager EM, Esbaugh AJ, Stieglitz J, et al. Acute embryonic or juvenile exposure to Deepwater Horizon crude oil impairs the swimming performance of Mahi-Mahi (*Coryphaena hippurus*). *Environ Sci Technol*. 2014;48:7053-7061.
22. Mager EM, Pasparakis C, Schlenker LS, et al. Assessment of early life stage mahi-mahi windows of sensitivity during acute exposures to *Deepwater Horizon* crude oil. *Environ Toxicol Chem*. 2017;9999:1-9. https://doi.org/10.1002/etc.3713.
23. Nelson D, Heuer RM, Cox GK, et al. Effects of crude oil on in situ cardiac function in young adult mahi-mahi (*Coryphaena hippurus*). *Aquat Toxicol*. 2016;180:274-281. https://doi.org/10.1016/j.aquatox.2016.10.012.
24. Pasparakis C, Mager EM, Stieglitz JD, Benetti D, Grosell M. Combined effects of Deepwater Horizon crude oil exposure, temperature and developmental stage on oxygen consumption of embryonic and larval mahi-mahi (*Coryphaena hippurus*). *Aquat Toxicol*. 2016;181:113-123.
25. Pasparakis C, Sweet LE, Stieglitz JD, et al. Combined effects of oil exposure, temperature and ultraviolet radiation on buoyancy and oxygen consumption of embryonic Mahi-mahi, *Coryphaena hippurus*. *Aquat Toxicol*. 2017;191:113-121. https://doi.org/10.1016/j.aquatox.2017.07.021.
26. Perrichon P, Mager EM, Pasparakis C, et al. Combined effects of elevated temperature and Deepwater Horizon oil exposure on the cardiac performance in larval mahi-mahi, *Coryphaena hippurus*. *PLoS One*. 2018;13:e0203949.
27. Stieglitz JD, Mager EM, Hoenig RH, et al. A novel system for embryo-larval toxicity testing of pelagic fish: Applications for impact assessment of Deepwater Horizon crude oil. *Chemosphere*. 2016;162:261-268. https://doi.org/10.1016/j.chemosphere.2016.07.069.
28. Stieglitz JD, Mager EM, Hoenig RH, Benetti DD, Grosell M. Impacts of Deepwater Horizon crude oil exposure on adult mahi-mahi (*Coryphaena hippurus*) swim performance. *Environ Toxicol Chem*. 2016;35:2613-2622. https://doi.org/10.1002/etc.3436.
29. Sweet LE, Magnuson J, Garner TR, et al. Exposure to ultraviolet radiation late in development increases the toxicity of oil to mahi-mahi (*Coryphaena hippurus*) embryos. *Environ Toxicol Chem*. 2016;9999:1-7. https://doi.org/10.1002/etc.3687.
30. Xu EG, Mager EM, Grosell M, et al. Developmental transcriptomic analyses for mechanistic insights into critical pathways involved in embryogenesis of pelagic mahi-mahi (*Coryphaena hippurus*). *PLoS One*. 2017;12:e0180454.
31. Burggren WW, Dubansky B. *Development and environment*. Cham, Switzerland: Springer; 2018.
32. Stieglitz JD, Hoenig RH, Kloeblen S, Tudela CE, Grosell M, Benetti DD. Capture, transport, prophylaxis, acclimation, and continuous spawning of Mahi-mahi (*Coryphaena hippurus*) in captivity. *Aquaculture*. 2017;479:1-6. https://doi.org/10.1016/j.aquaculture.2017.05.006.
33. Alix M, Chardard D, Ledoré Y, Fontaine P, Schaerlinger B. An alternative developmental table to describe non-model fish species embryogenesis: Application to the description of the Eurasian perch (*Perca fluviatilis* L. 1758) development. *Evodevo*. 2015;6:39.
34. Armstrong PB, Swope Child J. Stages in the normal development of *Fundulus heteroclitus*. *Biol Bull*. 1965;128:143-168.
35. Cucchi P, Sucré E, Santos R, Leclère J, Charmantier G, Castille R. Embryonic development of the sea bass *Dicentrarchus labrax*. *Helgol Mar Res*. 2012;66:199-209. https://doi.org/10.1007/s1015-011-0262-3.
36. Iwamatsu T. Stages of normal development in the medaka *Oryzias latipes*. *Mech Dev*. 2004;121:605-618. https://doi.org/10.1016/j.mod.2004.03.012.
37. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn*. 1995;203:253-310. https://doi.org/10.1002/aja.1002030302.
38. Podrabsky JE, Riggs CL, Romney AL, et al. Embryonic development of the annual killifish *Austrofundulus limnaeus:* An emerging model for ecological and evolutionary developmental biology research and instruction. *Dev Dyn*. 2017;246:779-801. https://doi.org/10.1002/dvdy.24513.
39. Tsai HY, Chang M, Liu SC, Abe G, Ota KG. Embryonic development of goldfish (*Carassius auratus*): A model for the study of evolutionary change in developmental mechanisms by artificial selection. *Dev Dyn*. 2013;242:1262-1283. https://doi.org/10.1002/dvdy.24022.
40. Vernier J-M. Table chronologique du developpement embryonnaire de la truite arc-en-ciel, *Salmo gairdneri,* Rich, 1836. *Ann d'Embryologie Morphog*. 1969;2:495-520.
41. Benetti DD, Iversen ES, Ostrowski AC. Growth rates of captive dolphin, *Coryphaena hippurus*, in Hawaii. *Fish Bull*. 1995;93:152-157.
42. Wang Y, Pasparakis C, Mager EM, Stieglitz JD, Benetti DD, Grosell M. Ontogeny of urea and ammonia transporters in mahi-mahi (*Coryphaena hippurus*) early life stages. *Comp Biochem Physiol A Mol Integr Physiol*. 2019;229:18-24.

43. Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engeszer RE. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Dev Dyn.* 2009;238:2975-3015.
44. Margulies D, Sutter J, Hunt S. Spawning and early development of captive yellowfin tuna (*Thunnus albacares*). *Fish Bull*. 2007;105: 249-265.
45. Brill R. Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. *Comp Biochem Physiol*. 1996;113A:3-15.
46. Miyashita S, Sawada Y, Okada T, Murata O, Kumai H. Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae). *Fish Bull*. 2001; 99:601-616.
47. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9:671-675. https://doi.org/10.1038/nmeth.2089.

How to cite this article: Perrichon P, Stieglitz JD, Xu EG, et al. Mahi-mahi (*Coryphaena hippurus*) life development: morphological, physiological, behavioral and molecular phenotypes. *Developmental Dynamics*. 2019;248:337–350. https://doi.org/10.1002/dvdy.27