

The 4th European Zebrafish
Principal Investigator Meeting (EZPM)
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Abstract

The European Zebrafish Principal Investigator Meeting (EZPM) is an ideal forum for group leaders using this fantastic animal model to discuss science but also to strengthen their interactions, to push forward technological advances and to define guidelines for the use of this fish in research. The city of Lisbon (Portugal) was voted by the European group leaders to be the setting for the 4th EZPM and the organizing committee, composed by Leonor Saúde (iMM Lisboa, PT), Susana Lopes (CEDOC, PT), Michael Orger (Champalimaud Foundation, PT), Rui Oliveira (ISPA, PT) and António Jacinto (CEDOC, PT), was very enthusiastic to organize a productive event.

The 4th EZPM took place from March 15 to 19 at Pavilhão do Conhecimento, a Science Museum & Educational Centre winner of The Great Prize FAD of Architecture 1999 and The Society for Environmental Graphic Design Award 2011. Over 5 days, 135 group leaders (89 men and 46 women) coming from 19 different European countries and also from USA, Turkey, Israel, Chile and Singapore, presented and discussed their recent research achievements. In addition to the scientific oral and poster presentations, the group leaders gathered in very lively community sessions on Morphants vs. Mutants (chaired by Didier Stainier, Max Planck Institute for Heart and Lung Research, DE), Funding Issues (chaired by Uwe Strahle, KIT-ITG, DE) and Gender Equality (chaired by Corinne Houart, KCL, UK). One of the highlights of the 4th EZPM was the guided visit to Oceanário de Lisboa, an international award-winning place that celebrates life with a stunning display of living aquatic creatures.

Introduction

In the afternoon of March 15, **Marcos Gonzalez-Gaitan** (University of Geneva, CH) started the meeting with an Opening Keynote Lecture, where he discussed a novel mechanism that allows signalling endosomes to be targeted to one of the daughter cells during asymmetric division. Marcos showed that the asymmetry is already established in the central spindle by the kinesin Klp10A and its antagonist Patronin that then polarizes the direction of endosome motility.

The Scientific Committee composed by Claudia Linker (KCL, UK), Corinne Houart (KCL, UK), Carl-Philipp Heisenberg (IST, AU), Laure Bally-Cuif (Institute of Neurobiology Alfred Fessard, FR), Miguel Godinho-Ferreira (IGC, PT), Nadia Mercader (Universitat Bern, CH), Robert Kelsh (University of Bath, UK), Caroline Brennan (Queen Mary University of London, UK) and Claire Wyart (ICM, FR) selected 63 abstracts for oral presentations. These abstracts were presented in the 7 different sessions that composed the meeting, namely Development & Organogenesis (chaired by Elke Ober, DanStem, DK), Disease Models (chaired by Betina Schmid, DZNE, DE), Cell biology & Cell migration (chaired by Lila Solnica-Krezel, Washington University School of Medicine USA), Stem cells & Regeneration (chaired by Catherina Becker, University of Edinburgh, UK), Neurosciences (chaired by Michael Brand, TU Dresden, DE), Emerging technologies (chaired by Andy Oates, Crick Institute, UK) and Behaviour (chaired by Ruben Portugues, Max Planck Institute for Neurobiology, DE). The remaining 45 submitted abstracts were invited for poster presentation and were discussed in 2 different poster sessions that took place after dinner.

In the morning of March 19, **Giles Laurent** (Max Plack Institute for Brain Research, DE), ended the meeting with a Closing Keynote Lecture, where he discussed evolution of sleep within vertebrates. There is this accepted idea that all animals sleep and yet the brain electrophysiological features of sleep (i.e. slow-wave (SW) and rapid eye movement (REM) activities) have only been characterized in birds and mammals. From the recordings obtained in the brain of a lizard, the small Australian dragon *Pogona vitticeps*, it is clear that SW and REM sleep patterns exist in these reptiles, thus allowing the conclusion that these sleep dynamics evolved much earlier in vertebrate evolution, at least to the emergence of amniotes.

DEVELOPMENT AND ORGANOGENESIS

The first session opened with **Didier Stainier** (Germany) talking about the role of CNS progenitors in the regulation of vascular patterning outside the CNS. By using the nitroreductase system, his lab identified two developmental time windows where spinal cord radial glia cells have opposing effects on distinct vessels by regulating Vegf-Vegfr2. An early period where radial glia ablation causes more sprouting of venous endothelial cells and a later period where loss of radial glia leads to loss of artery formation.

Corinne Houart (UK) followed with the question on how FoxG1 regulates the shift between proliferation and neurogenesis in the telencephalon. This transcription factor appears to have complex interactions with the centrosome, allowing FoxG1 to coordinate cell division and fate decisions in the telencephalon. Mutants made for the centrosomal gene *aspm* (*abnormal spindle-like, microcephaly-associated*), which is essential for normal mitotic spindle function in embryonic neuroblasts, were engineered with TALENs and CRISPR-cas9 technology and show 30% smaller telencephalons mimicking previous morpholino knockdowns. These mutants also showed ectopic *wnt8b* expression and premature neurogenesis. Nevertheless, FoxG1 overexpression did not rescue *aspm*^{-/-} mutants and therefore the link between these two genes is being investigated.

Still in the head region, **Cristina Pujades** (Spain) wanted to understand how are rhombomere boundaries maintained so sharp during development despite cells being moving and dividing? Her lab is testing the idea that perhaps these boundaries have some elastic properties conferred by actomyosin cables. They have undertaken a genomic approach to identify non-coding regulatory elements driving gene expression in the rhombomere boundary cell population. They found a genomic region with four candidate genes of interest that is syntenic in other vertebrates and provides a good example of a conserved enhancer region. By deleting the full region using CRISPR-Cas9 technology they are investigating its consequences. Interestingly, Cristina told us that medaka has the enhancer elements silenced and does not seem to have actomyosin cables in the rhombomere boundary cell population. This finding raised the evo-devo hypothesis that fast developers such as zebrafish and *Astyanax* may need actomyosin cables whereas slower developers as medaka may not need them for rhombomere boundary formation.

Then we heard **Daniela Panáková** (Germany) who told us about the role of actomyosin tension in heart chamber formation. Her group is investigating the role of the

Wnt/PCP pathway in actomyosin organization and its impact on mechanosensation by the cardiomyocytes. They are investigating the role of PK1 (protein-serine kinase1) in this process.

Next, **Jiandong Liu** (USA) continued the subject of heart chamber formation with the role of cardiac contraction. Primary cilia were highlighted as mediators of fluid flow to stimulate Notch expression. As trabeculation needs cardiac contraction and in stopped hearts there is no Notch signalling it is thought that contraction promotes trabeculation by *notch1b/efnb2a* epistasis.

We then moved on to the eye field and heard **Florencia Cavodeassi** (Spain) whose lab is investigating the nasotemporal patterning of the optic vesicle. Two transcription factors, *Foxg1* and *Foxd1*, by exerting mutual repression, are important for dorsal and ventral patterning, respectively. Her lab showed that dorsal FGF signalling promotes *foxg1* expression whereas ventral Hh signalling promotes *foxd1* expression. However, the role of Shh in promoting *foxd1* expression is only required in the presence of FGF activity.

Mary Mullins (USA) then talked about adult phenomics in zebrafish. By performing reverse genetics they are doing a broad-based phenotypic analysis of late larval and adult stages. They already analysed 600 genes and 8% have late phenotypes confirmed. They aim to report back on novel mutants with maternal-effects, male sterility, juvenile lethality and adult abnormal morphology.

Cecilia Winata (Singapore) followed and talked about maternal mRNAs that are progressively processed until the mid blastula transition (MBT) stage by the cytoplasmic polyadenylation complex. Her lab showed that this process is crucial for surviving the MBT stage.

Nadine Vastenhouw (Germany) continued in the same subject and told us about competition between histones and the transcription complex assembly to regulate the onset of transcription at around MBT. They experimentally showed that excess of nucleosomes represses transcription by binding to DNA.

The last speaker in this session was **Roland Dosch** (Germany) with the zebrafish germ plasm organizer *buckyball* and its functional conservation with *Drosophila oskar*. Dosch lab showed the existence of functional conservation between these germ plasm organizers despite their lacking of sequence homology. These two functionally similar proteins in

distant species are characterized for having a fast evolving and intrinsically disordered structure.

DISEASE MODELS

DNA replication proteins are not degraded once the cell has entered mitosis and even persist in postmitotic cells, suggesting, that they might exert additional functions. **Melanie Phillipp's** group (Germany) discovered such a function for the helicase MCM2 in a screen for novel factors involved in cilia biology. MCM2 morphants exhibit left-right asymmetry defects and MCM2 drives cilia formation and function, most likely by controlling centriole biogenesis and by propagating tubulin polymerization. This exciting finding provides new explanations for the development of ciliopathies and demonstrates non-canonical functions of DNA replication factors.

Telomeres shorten with age and they are considered molecular timekeepers, which determine cellular lifespans. Telomerase is known to be active in stem cells and cancer cells. However, it is currently unclear, if there is also a correlation between telomere length shortening and disease (e.g. triggering genomic instability). **Miguel Godinho Ferreira's** group (Portugal) observed that tert mutant zebrafish, lacking functional telomerase, show premature aging and also display an earlier onset of cancer development compared to wildtype. Strikingly, they demonstrated that tert mutant cells exert a dominant damaging effect on wildtype cells in transplantation experiments strongly suggesting that tissue specific telomere length is a limiting local but also likely a systemic factor for age related tissue degeneration and disease.

The notochord is a scaffold for growing vertebral bones. Using *spatzle* mutants, **Michel Bagnat's** group (USA) showed that the vacuoles in notochord cells are necessary to evenly distribute compressive forces of growing vertebrae. Fragmented vacuoles as found in *spatzle* mutants lead to kinks of the axis as bones grow. They determined the *spatzle* mutation to be in the *dstyk* gene, a dual specificity kinase. Most likely, loss of phosphorylation of a clathrin regulator is responsible for vacuole fragmentation in *spatzle*. His study offers mechanistic insight in the etiology of scoliosis.

Yury Miller (USA) developed a zebrafish model for hypertriglyceridemia, which is a risk factor for cardiovascular disease. Zebrafish *apolipoprotein C2 (apoc2)* mutants show chylomicronemia, severe hypertriglyceridemia and lipid-laden macrophages, resembling

early events in atherosclerosis development in humans. His model promises to be a good tool to investigate the pathogenesis of hypertriglyceridemia-associated diseases.

Thomas Dickmeis (Germany) examined how glucocorticoids regulate diurnal transcription and metabolism. Using a zebrafish glucocorticoid deficiency model he detected several deregulated metabolic pathways. Interestingly, non-rhythmic dexamethasone treatment could restore most of the diurnal transcriptome. This interesting finding could be explained by the presence of a combination of E-box and glucocorticoid response elements enriched in rescued genes, indicating that glucocorticoids act as permissive cues for the diurnal rhythm.

Fenfluramine, which releases 5-HT is a potent therapeutic for Dravet syndrome (DS) (a severe epilepsy syndrome) leading to 70% seizure freedom in DS children. **Peter de Witte** (Belgium) made use of the zebrafish *scn1Lab* mutant, which recapitulates DS to investigate the mode of action of fenfluramine. He showed that mainly 5-HT1D, 5-HT2C and 5-HT2A receptor subtypes mediate the anti-epileptic effect of fenfluramine, opening the path for future specific DS therapeutics.

Anna-Pavlina Haramis (Netherlands) addressed the role of the tumor suppressor LKB1 in autophagy. *lkb1* mutants have impaired autophagy, which results in a shortened lifespan when larvae are challenged with yolk depletion. Both, lack of AMPK activation and deregulation of PI3K signaling contribute to this autophagy defect. This observation suggests that autophagy inhibition might have therapeutic benefits in LKB1 mutated cancers.

Humans with mutations in the essential myosin light chain (ELC) show a high incidence of sudden cardiac death. **David Hassel** (Germany) used the zebrafish “*lazy susan*” mutant, which lacks the highly conserved ELC phosphorylation site S195 to investigate the role of ELC in cardiomyopathy. Heterozygous adult *lazy susan* mutants, although unremarkable under normal conditions, die of sudden heart death after being subjected to a physically demanding swimming test. ELC phosphorylation appeared to be essential to adapt heart function to physical stress. In vitro motility analysis revealed a possible underlying mechanism as ELC phosphorylation critically modulates binding of myosin to actin.

Robert Wheeler (USA) presented a zebrafish swimbladder injection model to study the mechanism of dissemination of *Candida albicans*. He observed that hyphae and yeast forms are both necessary for dissemination. Hyphae can penetrate the epithelial layer of the swim bladder and yeast is good at dispersal. Neutrophils and macrophages are recruited to

the site of infection. *C. albicans* can survive inside of phagocytes and active transport in macrophages appears to be one way for *C. albicans* to get into the bloodstream.

Astrid van der Sar (Netherlands) addressed how mycobacteria can cross the blood-brain barrier (BBB) in a zebrafish model of tuberculous meningitis. Injection of labeled *Mycobacterium marinum* into the caudal vein revealed that *Mycobacterium marinum* travels inside of macrophages to cross the BBB. However, in the absence of phagocytes an alternative macrophage independent but ESX-1 dependent mechanism exists to cross the BBB.

Yi Feng (UK) previously showed that innate immune cells provide trophic factors to pre-neoplastic cells (PNC) at early tumor initiating stages. Using a reporter line, she now shows that NFκB signaling in PNCs is involved in neutrophil recruitment towards PNCs. Interestingly, direct contact of neutrophils with PNCs also leads to NFκB activation in neutrophils over time in a calcium mediated way. NFκB activating factors remain to be identified in future studies.

Human-zebrafish xenotransplantation models promise to be an alternative to mouse xenograft models for personalized medicine. Here, **Rita Fior** (Portugal) showed that inter and intra tumor heterogeneity of human colorectal cancer cells can be detected in zebrafish xenograft models. Most importantly, different responses were found upon treatment with 2 common chemotherapies, FOLFOX and FOLFIRI. This indicates, that indeed such zebrafish xenograft models can be used to instruct clinical therapy decisions.

Marina Mione (Germany) developed a zebrafish model that shows both, benign lesions and invasive brain tumors, which appear similar to mesenchymal glioblastoma. She used this model to investigate, which factors determine benign vs. malignant tumor formation and identified a 8 gene YAP signaling signature defining malignant glioblastoma in zebrafish, but also in human patient samples. An active form of YAP was able to induce aggressive tumors by its own in zebrafish, suggesting that YAP activation is a key factor in malignant tumor formation.

CELL MIGRATION AND CELL BIOLOGY

A variety of topics were presented from tissue repair and polarity to molecular and cellular interactions controlling cell movement and the generation of forces in tissues. Most of the talks in this session highlighted the importance of high resolution *in vivo* imaging coupled to

quantification methods of cell movement and shape to better understand dynamic processes.

The powerful combination of mathematical modelling and transplant experiments allowed **Carl-Philip Heisenberg** (Austria) to put in evidence the role of different tissue layers in the generation of forces driving morphogenetic movements driving the initiation of epiboly.

Cellular interactions during migration were explored by **Caren Norden** (Germany) in the formation of the retina. She showed that cell attachment and polarity play an important part in nuclear translocation, axon extension and final migration of retinal neurons.

The formation of secondary β -cell islets in the pancreas was shown to be a very active process involving single cell migration, cell recognition and clustering through highly dynamic filopodia extension; PI3K activity was shown to be fundamental in this process (**Robin Kimmel**, Austria).

The stereotypic choreography of cell adhesion, polarity acquisition and cell rearrangements taking place in the establishment of new blood vessels connections was explored *in vivo* by **Heinz-Georg Belting** (Switzerland). Quantitative *in vivo* imaging and single cell ablation during neural crest migration in the cranial and trunk region unveiled different cellular mechanisms underlying collective cell migration as shown by **Claudia Linker** (UK).

The role of Notch signalling was studied in the establishment of cell polarity in the neural tube (**Fürthauer Maximilian team**, France). Data showing its requirement for the transcriptional regulation of polarity proteins, independently of its role in neuronal fate acquisition, were presented. Moreover, Notch signalling pathway is regulated by plasmalipin through the endocytic machinery during gut morphogenesis concomitantly controlling cell polarity and fate (**Fernando Martin-Belmonte**, Spain).

The importance of cellular interactions for tissue repair was highlighted by direct observation of macrophage activity at the site of muscle fibre tearing (**Uwe Strahle**, Germany). Macrophages recognize and clear lipid and protein accumulation at the site of damage, allowing membrane repair. Newly established lipid sensors and single molecule tracking technology were presented.

STEM CELLS AND REGENERATION

In the first part of the session, the speakers did a great job in demonstrating how zebrafish can be used as a model for regeneration. The zebrafish possess an incredible capacity to regenerate tissue upon injury, including the heart, liver, fins and brain. **Michael Reimer** (Germany) showed that oligodendrocyte precursors can regenerate spinal cord injury in adults, including neuronal and axonal regeneration and re-myelination of the new neurons.

Similar traits were shown by **Lieve Mons** (Belgium) in a zebrafish model of optic nerve crush where retinal ganglion cells completely regenerate the optic nerve within 20 days-post-injury. Interestingly, this capacity was seen to decrease with age, highlighting a possible limiting factor in future applications of regeneration in humans. Cardiac valves are a tissue that was not previously known to regenerate. **Dimitris Beis** (Greece) used an experimental setup with the nitroreductase enzyme coupled with a Gal4/UAS system to show that both embryonic and adult cardiac valves regenerate within 8 days-post-injury. This regenerative capacity was mediated by Notch signalling. **Jeroen den Hertog** (Netherlands) showed some ongoing work on how Ptena/b and Shp2a/b are required for fin regeneration but not for its normal development.

The remaining talks focused on tissue stem cells. **Laure Bailly-Cuif** (France) showed by live lineage tracing in Casper zebrafish that neural stem cells (NSCs) build up the adult pallium in the dorsal telencephalon via a pile-up process. The neural cells arising earlier do not mix with neural cells that arise later so they end up in different strata. The NSCs behind this growth process are continuously neurogenic throughout life and present typical characteristics of stem cells: they are quiescent, self-renew and are multipotent. **Robert Kelsh** (UK) identified a mutation in a gene expressed in endothelium that induces neural crest cells to differentiate prematurely and postulated that an endothelial-secreted factor maintains neural crest cells undifferentiated. Haematopoietic stem cells (HSCs) are responsible for making blood throughout an organism's life. **Ana Cvejic** (UK) applied single-cell sequencing to kidney marrow HSC populations and showed a very elegant analysis of the expression data that allowed her to computationally define a hierarchy of progressively differentiated stem and progenitor cell types arising from the HSCs. How these stem cells are made in the embryo was addressed by **Rui Monteiro** (UK). He showed that the embryonic precursors of HSCs, the haemogenic endothelium, are specified via a crosstalk mechanism between Notch and autocrine and paracrine TGF β signalling. A second paracrine input was also required later for the formation of HSCs from the haemogenic endothelium. Continuing

on the subject of extracellular signalling, **Stefan Scholpp** (Germany) presented a mode of propagation of Wnt signalling via cytonemes extending from the cell of origin to the signal-receiving cells. A genome-wide screen led to the discovery that a novel Wnt8/Ror2 pathway regulates cytoneme formation and thus paracrine Wnt signalling. Finally, **Brant Weinstein** (USA) presented his most recent work on whole-genome methylation analysis to investigate how DNA methylation regulates gene expression in HSCs. They found that loss of methylation in the *cmyb* gene promoter led to the progressive loss of *cmyb* expression in HSCs and thus to failure to differentiate into more restricted haematopoietic lineages such as macrophages, erythrocytes and T cells.

NEUROSCIENCES

The fifth session spanned a range of topics in zebrafish neuroscience including nervous system development, circuit function and behaviour, as well as exciting new technologies (and even model organisms).

Filippo del Bene (France) started the session by presenting some of his work on development of the retinotectal projection: he showed that Reelin, a large extracellular matrix protein, acts cell non-autonomously to direct correct lamination of retinal axonal arbors in the optic tectum. **Tim Czopka** (Germany) presented tools and approaches to study myelination of spinal cord axons during larval development as well as during repair following injury. Three talks followed looking at neural network physiology. First, **Emre Yaksi** (Norway) presented his work examining population activity in the habenula and its correlation with distinct neuronal clusters in the pallium and olfactory bulb. Then **Tom Baden** (Germany) discussed approaches to examine retinal physiology, including the properties of bipolar and ganglion cells, which he has successfully applied in the mouse and plans to translate to zebrafish. Thirdly, **Konstantinos Ampatzis** (Sweden) discussed his findings using an adult zebrafish spinal cord preparation, which support the progressive recruitment of three populations of motoneurons by corresponding groups of V2a interneurons, as swim speed increases. Bringing a slight change of direction, **Benjamin Judkewitz** (Germany) argued the case for studying brain function in *Danionella*, a tiny cyprinid closely related to *Danio*. Adults are optically transparent and only 10 mm long and show complex behaviours including vocalisation. **Stephan Neuhauss** (Switzerland) opted not to review the Coen Brother's back catalog but instead showed diverse roles for glutamate transporters at cone-bipolar and

cone-horizontal cell synapses. An exciting technological highlight of the session was presented by **Drew Robson** and **Jennifer Li** (USA) who have developed a microscope combining motion cancellation and fast optical sectioning to image neural activity at cellular resolution in freely behaving zebrafish larvae. **Gil Levowitz** (Israel) presented his work on the role of hypothalamic oxytocin neurons in the development of social behaviour in juvenile zebrafish. Finally, the session was brought to a close by **Claire Wyart** (France) revealing data on how cerebrospinal fluid contacting neurons integrate sensory information and modulate locomotor programmes at the temporal resolution of single swim bouts.

EMERGING TECHNOLOGIES

In zebrafish, and due to an additional genome duplication event, 30% of the genes have paralogs given a human ortholog. **Ozlen Konu** (Turkey) talked about a computational tool (CompariZone) that allows the comparison of zebrafish transcriptomes across multiple contexts to assess the possible neo- or sub-functionalization of the duplicated genes.

How can we unravel information of individual neurons in vivo in a tightly packed neural cluster? **Periklis Pantazis** (Switzerland) convinced us that confined primed conversion of green-to red photoconvertible proteins allow us to do so. In this method they use sequential 488 and 730nm lasers combined with crossed-beam to successfully label individual neurons in vivo from trigeminal gangli, tectal cells and motor neurons.

Reinhard W. Koester (Germany) described the use of ATTAC- Apoptosis Through Targeted Activation of Caspase as a powerful cell-type specific ablation tool. He showed that this system can remove about 90% of the targeted cell type and in combination with the already available nitroreductase allows to target 2 cell populations independently

Patrick Müller (Germany) described PyFRAP a free open-source, crossplatform software that enables *in vivo* measurements in complex geometris thus overcoming previous challenges in FRAP analysis.

Finally, **Karuna Sampath** (UK) reminded us that morphogens are required for many developmental processes, but how morphogen gradients are formed *in vivo* remains unclear. She showed that that what concerns Nodal gradient, ligand clearance via degradation shapes the gradient and correlates with its signaling range.

BEHAVIOR

This short session started with **Rui Oliveira** (Portugal) arguing in favor of zebrafish as a useful model organism to study social cognition. Zebrafish prefer to spend time in the proximity of other zebrafish rather than alone, and when fighting, they progress through four stages: display, circle, bite and chase. The winner of a fight will continue to win and similarly, the loser will continue to lose. They are also social observers and will display various levels of social attention: they will regularly face and attend towards two other interacting fish, will do so less to non-interacting fish, and will not attend to an empty chamber.

Gonzalo de Polavieja (Portugal) presented a phenomenological mathematical model that describes collective behavior in 8 dpf larvae. This simple model can describe whether a fish will turn left or right, where there are N or M other fish respectively. This depends on whether the fish is in a “social mode” or not. If the fish is not social, it will choose left or right randomly. If the fish is social however, it will pick one other larva at random and turn towards it. So the probability of turning to the group of N fish is simply given by $N/(N+M)$. It is interesting how this intricate behavior can be understood solely as the interaction between two fish.

Will Norton (UK) showed data that characterizes a nitric oxide synthase mutant and shows how it exhibits a marked decrease in aggression, as measured both by dyadic fights and mirror interactions. The mutant also shows an increase in anxiety. In the past, nitric oxide has been shown to be involved in the neuroendocrine system, playing an important role in the hypothalamic-pituitary-adrenal axis. The mutant shows a reduction of dopamine and 5-HT signaling, although pharmacological rescue of the latter was sufficient to rescue the anxiety phenotype.

The final talk of the session saw **Michael Orger** (Portugal) present a detailed characterization of the locomotor repertoire of zebrafish larvae. The first step involves online high-speed tracking that allows the measurement of many behavioral parameters. Using a novel method, these are then clustered to reveal how all swims fall into one of eleven categories, all distinct, which together tile kinematic parameter space. Certain stimuli will preferentially elicit particular locomotor patterns, many of which have already been described in the literature, but which are unified within the same comprehensive framework.