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15 years of zebrafish chemical screening

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In 2000, the first chemical screen using living zebrafish in a multi-well plate was reported. Since then, more than 60 additional screens have been published describing whole-organism drug and pathway discovery projects in zebrafish. To investigate the scope of the work reported in the last 14 years and to identify trends in the field, we analyzed the discovery strategies of 64 primary research articles from the literature. We found that zebrafish screens have expanded beyond the use of developmental phenotypes to include behavioral, cardiac, metabolic, proliferative and regenerative endpoints. Additionally, many creative strategies have been used to uncover the mechanisms of action of new small molecules including chemical phenocopy, genetic phenocopy, mutant rescue, and spatial localization strategies.

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Introduction

Traditional methods of small molecule drug discovery relied on trial-and-error testing of chemical compounds on phenotypic outcomes in cells or animals. This approach yielded many of the drugs currently used in the clinic today. By contrast, target-driven approaches, which seek to identify novel therapeutics based on a priori knowledge of a single biological target, have received greater emphasis in recent decades but have delivered fewer first-in-class drugs [1].

There are several possible reasons (not mutually exclusive) why phenotype-driven approaches have out-performed target-driven approaches. The first is that target driven approaches depend on selection of the correct, disease-modifying target — an uncertain proposition — whereas phenotype-driven approaches can identify disease-modifying drugs even in the absence of a validated target. Second, the most efficacious drugs may benefit from activity at multiple targets. For example, complex polygenetic disorders may require a 'magic shotgun' drug (one exhibiting polypharmacology) rather than a 'magic bullet' (one exhibiting specificity for a single target) [2]. Some of the most successful drugs in use today are known to benefit from engagement of multiple targets throughout the body. Third, small molecules derived from phenotypic screens often have been further selected for positive pharmacological properties, such as low toxicity, the ability to make it to the appropriate site(s) of action, and the ability to avoid or exploit endogenous chemical metabolizing enzymes and transporters.

Whole-organism, phenotypic screening holds several advantages over other approaches to small molecule discovery. The approach is target agonistic (therefore not mechanistically biased) and holistic (all possible targets in the organism are available). This includes targets relevant not only to disease intervention but also to chemical activation, chemical transport, toxicity and other side effects.

In 2000, it was demonstrated for the first time that a chemical screen could be carried out using live zebrafish in a 96-well plate simply by adding small amounts of compounds directly to the fish water [3]. Though simpler than humans, zebrafish are also complex vertebrates and maintain similarly elaborate mechanisms for activating or mitigating the effects of exogenous chemical substances. Although differences in pharmacological effects between zebrafish and humans certainly do exist, there are now hundreds of examples of small molecules that have conserved biological activities in fish and humans. It is therefore reasonable to expect that many bioactive compounds identified in zebrafish screens will maintain their activity in humans.

In this review, we summarize the work reported in 66 zebrafish chemical screens over the past 15 years. We start by giving a bird's-eye view of the field to give readers a feel for the scope of what has been accomplished to date. Many of the design details will likely be of interest to those contemplating setting up their own zebrafish screens. We then highlight some of the more interesting examples of the phenotypic endpoints that have been examined and methods of follow-up used to uncover mechanisms of action.

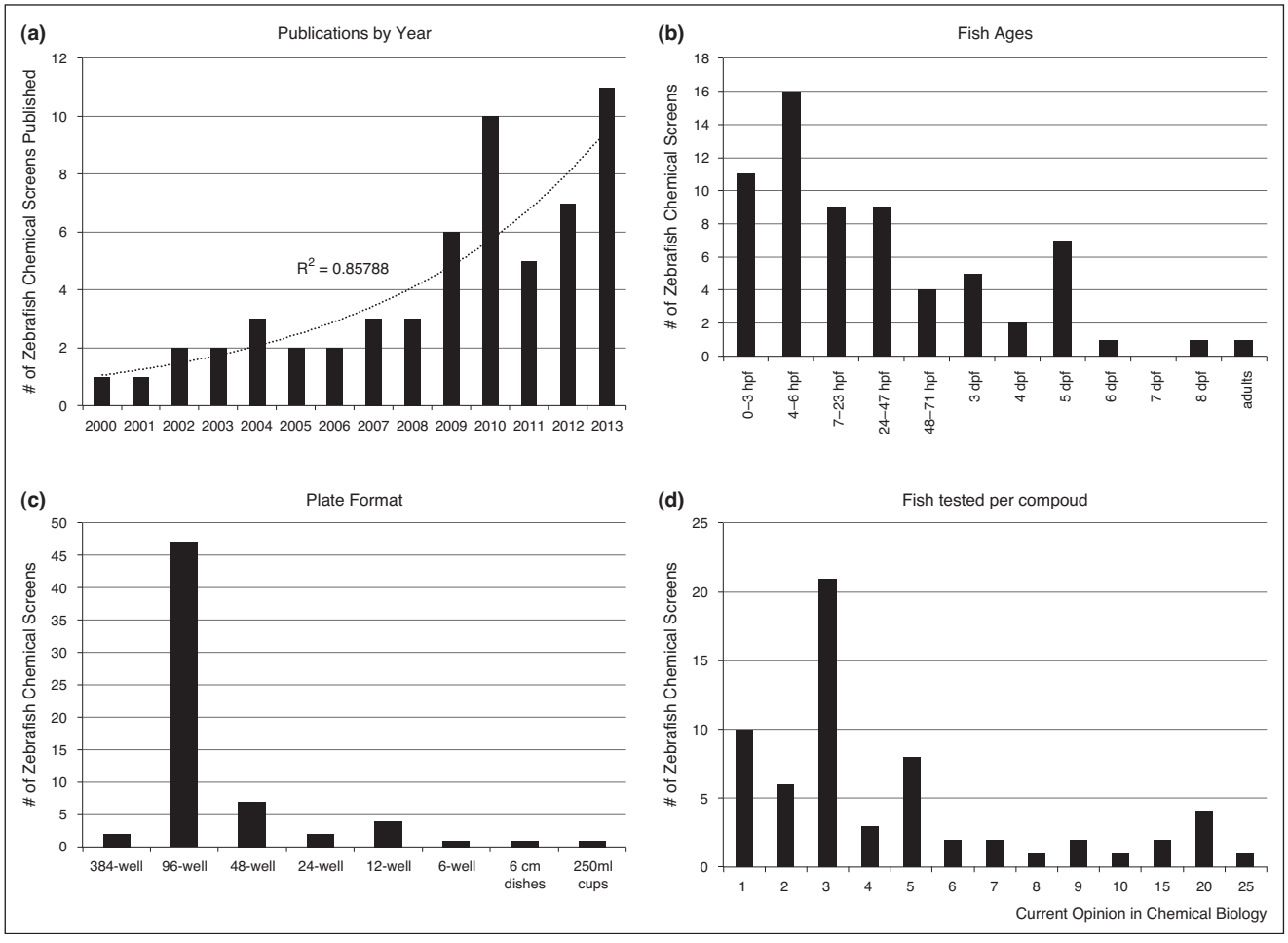
Zebrafish screens by the numbers

In a survey of the literature, we identified 66 primary research articles each reporting results of a zebrafish chemical screen. These range from the year 2000 to the present time and form the basis for our in-depth analysis. We believe these provide a good representation of the field, but we do not claim this list is exhaustive and apologize for any studies we may have omitted. A simple plot of the number of publications per year demonstrates that zebrafish chemical screens are becoming more widespread, with the number increasing substantially in recent years (Figure 1a). The types of journals publishing these reports ranges in scope from specialized publications, like the journal *Zebrafish* [4,5], to journals with very broad appeal, such as *Nature* [6,7]. Of the 37 journals represented, only five had published more than two papers on zebrafish chemical screens. On average, the impact factor for papers reporting zebrafish chemical screens has been 9.5.

Of the 66 screens, 49 (74%) were conducted using zebrafish age 48 hours post fertilization (hpf) or younger (Figure 1b). The most frequent treatment age was 4–6 hpf. This bias toward use of zebrafish in the embryonic stage reflects the historical importance of the organism to developmental research. Including the very first screen published [3], 14 screens (21%) examined embryogenesis or gross development as the phenotypic endpoint.

Several different multi-well plates have been used including one screen that was conducted using a 384-well plate [8]. By far the most common format has been the 96-well plate (72%, Figure 1c). Number of animals used varies between one animal per well per compound to more than 30 animals per chemical treatment. The most common number of zebrafish used per compound has been three (33%, Figure 1d). Chemical libraries were obtained from diverse academic, government and commercial sources. The most frequently used were the Chembridge DIVERSetE collection of synthetic compounds (21% of screens), the LOPAC collection of 1280 well-characterized pharmacologically active compounds (15%) and the MicroSource Spectrum collection

Figure 1



(a) Publications reporting zebrafish chemical screens by year. **(b)** Ages of zebrafish used in chemical screens. **(c)** Plate formats used in zebrafish chemical screens. **(d)** Numbers of zebrafish tested per chemical.