Gasterosteus, Anolis, Mus, and more: the changing roles of vertebrate models in evolution and behaviour

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ABSTRACT

Background: The choice of model systems is important for research and researchers, and has been studied in the biomedical literature but not with regard to evolution and behaviour. Neither have changes in the use of the threespine stickleback, *Gasterosteus aculeatus*, as a model been examined quantitatively.

Questions: What are the major vertebrate model systems in evolutionary biology and animal behaviour. How have they changed over the last 25 years, and how has work on the threespine stickleback model evolved in this period?

Data: We collected publication rates from the Web of Science for candidate model organisms for three periods over the last 25 years. As a complement to this analysis, we conducted a more focused and inductive analysis on *Gasterosteus*, concerning which fields have used this model, by analysing the keywords co-occurring with '*Gasterosteus*'. To elucidate emerging trends involving stickleback, we analysed word frequencies in the abstracts of the current conference and of manuscripts in the stickleback special issues of *Evolutionary Ecology Research*, Volume 20.

Results: For evolutionary biology, traditional biomedical models have declined in importance over the last 25 years whereas non-biomedical comparative models have shown the opposite trend. Patterns for behaviour are more complex, with some natural systems increasing in usage and newer biomedical models, such as *Danio*, replacing previously important ones. Salmonids proved unexpectedly important for both evolution and behaviour and *Drosophila* appeared in more publications than any vertebrate. Overall, model systems were stable or declining in usage, a pattern also reported in the biomedical literature. Our keyword analysis for *Gasterosteus* suggests that the stickleback has evolved as a model. At first it was used mainly in behaviour but now it is also being used extensively to study evolution as well as to address concerns over human-induced environmental changes, and to investigate other new topics. Abstracts from the conference and the special issues illustrate the diversity of stickleback research, including emphases on variation in phenotypes between habitats and between sexes and the evolutionary and ecological processes that lead to these differences, as well as on genome-level questions, interactions with parasites, and eco-evolutionary dynamics.

Keywords: *Gasterosteus aculeatus*, model system, threespine stickleback.

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INTRODUCTION

The present and two additional special issues of *Evolutionary Ecology Research* present papers arising from the Ninth International Conference on Stickleback Behaviour and Evolution held 3–7 July 2018, in Kyoto, Japan. Recent introductions to collections from stickleback conferences have aimed to answer questions such as: 'How has research on stickleback shaped our understanding of phenotypic variation, genomic variation, speciation, and eco-evolutionary dynamics?' (Hendry *et al.*, 2013) and 'Are conference special issues worthwhile?' (Hendry and Peichel, 2016). Here we continue this tradition by using data on publication rates to ask, 'Over the last 25 years, what have been the principal trends in use of the major model vertebrates in evolutionary biology and animal behaviour?' We then evaluate long-lasting, shifting, and emerging trends in stickleback research, and how the work presented at the 2018 conference relates to those research themes.

The use of a small set of model organisms for basic research is well-established in biomedical science, with the mouse (*Mus musculus*) and the fruit fly (*Drosophila melanogaster*) among the pre-eminent examples for multicellular species, and yeast (*Saccharomyces cerevisiae*) as the unicellular taxon of choice (Dietrich *et al.*, 2014). These models have characteristics that are very valuable in research, such as a fast life cycle, high reproductive output in captivity, small size, and ease of visualizing development, among others (Davies, 2007; Peirson *et al.*, 2017). We have a vast body of knowledge about these established models and a long list of specific techniques, often well standardized, that have been developed for them, as well as diverse strains with particular mutations and histories (Bolker, 2017). These tools and strains have been of considerable value to researchers and have often accelerated the pace of advances and enabled work not possible in less well-developed systems. This positive feedback loop resulted in highly refined tools being available for a few model systems, which in turn are studied more because of these enabling characteristics. Nevertheless, consensus has been elusive regarding exactly what constitutes a model organism even among biomedical researchers, and the term has been used in different ways, for example, by different funding agencies (Katz, 2016). Here we use the term in a relatively general manner, treating model taxa simply as those which are most widely used by the research community in question.

In the fields of evolutionary biology and animal behaviour, there has historically been a greater emphasis on studying a diversity of taxa, probably because the ultimate goal of these fields is to understand the biological principles underlying the origin, maintenance, and future trajectory of biodiversity. Another likely reason is Krogh's principle, originally developed with reference to physiology research, which states: 'For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied' (Krogh, 1929). That is, some taxa are just better suited for particular areas of research because of their characteristics, which means that biomedical model systems may not be the most appropriate to study evolution and behaviour, resulting in their lower use and contributing to a large number of species being studied. Nevertheless, it seems that specific taxa are becoming the focus of study in evolution and behaviour as well. One likely reason may be 'preferential attachment', a process whereby 'the rich get richer' and, in this case, a critical mass of initial research and researchers lead to additional investigations with the same organism (Davies, 2007), as exemplified for biomedical and genetics models above. The increased integration of behavioural and evolutionary studies with biomedical investigations, and the increasing importance of genomic studies and genetic manipulations, may also be contributing factors. However, while quantitative assessments of the relative

importance of different models and taxa have appeared in the biomedical literature in recent years (e.g. Davies, 2007; Dietrich *et al.*, 2014; Peirson *et al.*, 2017), to the best of our knowledge no such analyses have appeared concerning the evolution and behaviour literatures for a large number of taxa simultaneously.

A quantitative analysis of the evolution and behaviour literatures can build on biomedical surveys by addressing questions such as: 'Are pre-eminent biomedical model systems also dominant in evolution and behaviour, and what traits, if any, distinguish the most popular models among fields?'; 'How does use of vertebrates compare with that of non-vertebrates?'; and 'Are model systems generally becoming more or less important?' Answers to such questions will also be of interest to those working on particular systems, in our case stickleback, as an indication of past, current, and future trends for their field of research – potentially with implications for funding as well as decisions of individual investigators. These general surveys can provide a foundation for additional analyses focused on how research topics studied with *Gasterosteus* specifically have changed over time, and for quantitative analyses of topics presented at the most recent stickleback conference.

We began by comparing publication rates for different model systems in evolutionary biology and animal behaviour using a bibliometric analysis. We quantified publication rates of 21 animal genera separately for the fields of evolution and animal behaviour using specific sets of journals in each field. We obtained these data at different time periods over the last 25 years, allowing us to rank the various genera as to their publication rates and to see how these ranks changed over time. We checked this analysis by redoing it using journals grouped by specific 'Research area', which is a list of journals curated by Web of Science. The results of our first analysis suggested the possibility that the use of certain model systems was becoming more frequent over time, potentially because of a shift in research topics in evolutionary biology and animal behaviour. Our second analysis focused on the stickleback to test this hypothesis; we compared the most used keywords in publications on *Gasterosteus*, determining how/if this ranking of keywords changed over the last 25 years, and whether new keywords appeared, reflecting emerging research interests. Finally, we analysed the most used keywords in the abstracts of the oral presentations at the latest Stickleback meeting (2018), and in the three stickleback special issues of *Evolutionary Ecology Research*, in order to get a sense of the research trajectory of this fish model.

METHODS

The use of different model species in specific fields

Models used

Inventory of all the species used in publications in the field of evolutionary biology and animal behaviour was not feasible. Instead, we used a list of species that have been studied in these two fields. We selected 21 species as model systems. Our main focus was vertebrates, but we included a small sample of prominent non-vertebrate eukaryotes for a broader perspective. We began with *Gasterosteus aculeatus* and four of the five model vertebrates designated by National Institutes of Health (Dietrich *et al.*, 2014), excluding chicken (*Gallus gallus*), which we thought unlikely to be a key organism in the evolutionary literature. In an attempt to catch potential, rising, non-traditional model systems, we added additional

vertebrate species from the table of (mainly biomedical) taxa on the Wikipedia 'Model organism' page (https://en.wikipedia.org/wiki/Model_organism, accessed 2 October 2018), and the more extensive Wikipedia list of model organisms (https://en.wikipedia.org/wiki/List_of_model_organisms, accessed 2 October 2018), plus seven additional species based on discussions with colleagues and our own experience. The four multicellular non-vertebrates chosen were present on one or more of the NIH and Wikipedia lists, plus *Heliconius* (longwing butterflies). Since *Gasterosteus aculeatus* is a species complex, arguably comparable in geographic distribution and phenotypic diversity to genera of some other taxa, we used genus name in our searches rather than species name.

Our final list included amphibians (*Xenopus*, African clawed frogs), reptiles (*Anolis*, anole lizards), passeriform birds (*Taeniopygia*, the zebra finch; *Geospiza*, Darwin finches; *Passer*, house sparrow), and mammals (rodents: *Mus*, mouse; *Rattus*, rat). We included a large selection of bony fishes: salmonids (*Oncorhynchus*, *Salmo*), poecilids (*Poecilia*), killifish (*Fundulus*), danios (*Danio*/*Brachydanio* for naming history), ricefish (*Oryzias*), cichlids (*Pundamilia*, *Oreochromis*), and sticklebacks (*Pungitius*, as well as *Gasterosteus*). The four 'non-vertebrate' models were *Caenorhabditis*, *Daphnia*, *Drosophila*, and *Heliconius*.

Our list is not comprehensive and we may have omitted significant taxa (likely the favourites of some readers!). In particular, we do not include humans since they are in many respects qualitatively different as a research organism from the other taxa here. Despite these limitations, we anticipate that our findings will be of interest and value given the paucity of previous efforts of this sort in evolution and behaviour.

Years studied

We studied different 'eras' within the last quarter century of the evolution and behaviour fields. This period extends from the pre-genomic era (but not before the widespread use of molecular biology in evolution) through the establishment of genomics and into the current CRISPR-Cas9 era. Combining periods of 3 years in a search allowed enough time for substantial samples, yet it was short enough not to pool distinct periods in science, especially the most recent. We surveyed publications of each model species during 1993–1995, 2004–2006, and 2015–2017. For each period, we also counted the total number of publications in these fields, including articles about species not studied here, as an index of the rise in scientific publishing in general for these fields. We divided the latter by the former to obtain a relative measure of the use of that model in the field.

Sub-fields studied

We focused on two sub-fields in which the model systems could be used: evolutionary biology and animal behaviour. We ran two analyses: one in which we used our own curated list of journals pertaining to a given field, and a second one as a check where the field studied was specified using the 'Research area' search term from Web of Science. In both approaches, we ran a search for each genus separately and then combined all the search results to be able to compare the publication rates of each genus.

In both searches, we first obtained the total number of publications for each period for a given field, and then the number of publications for a given genus. Thus we could calculate the publication rate, while taking into account that the world of scientific publication has changed tremendously in the past 25 years, such that comparing absolute numbers of publications between 1993 and 2017 could be misleading.

Curated journal list approach

Evolution

For the main evolutionary biology analysis, we began with all journals on the 18 October 2018 list, *Google Scholar Top 20 Journals in Evolutionary Biology* (found in the 'metrics' section, https://scholar.google.com/citations?view_op = top_venues&hl = en&vq = bio_evolutionarybiology). We included 15 journals from this list: *Evolution*; *American Naturalist*; *Journal of Evolutionary Biology*; *Molecular Biology and Evolution*; *Journal of Molecular Evolution*; *BMC Evolutionary Biology*; *Molecular Phylogenetics and Evolution*; *Systematic Biology*; *Genome Biology and Evolution*; *Cladistics*; *Evolutionary Ecology*; *Evolutionary Applications*; *Heredity*; *Evolutionary Biology*; and *Journal of Zoological Systematics and Evolutionary Research*. Five additional journals were added to this list based on other similar lists and/or our own reading and experience: *Molecular Ecology*; *Biological Journal of the Linnean Society*; *Journal of Systematics and Evolution*; *EvoDevo*; and *Evolutionary Ecology Research*. We excluded five journals from the Google scholar *Top 20 Journals in Evolutionary Biology* list because they also substantially cover ecology as a subject in its own right (as opposed to evolutionary ecology, which we treat as a field of evolutionary biology), have a taxonomic focus, or appear to focus more narrowly on genetics than on evolution: *Trends in Ecology and Evolution*; *Ecology and Evolution*; *Theoretical Population Biology*; *Genetica*; and *Journal of Mammalian Evolution*.

Behaviour

We quantified the use of each model system for the study of behaviour. As a basis, we used the *Google Scholar Top 20 Journals in Animal Behaviour and Ethology* (https://scholar.google.ca/citations?view_op = top_venues&hl = en&vq = bio_animalbehaviour), including 12 publications from this list: *Hormones and Behaviour*; *Animal Behaviour*; *Behavioural Ecology*; *Behavioural Ecology and Sociobiology*; *Animal Cognition*; *Behavioural Processes*; *Journal of Comparative Psychology*; *Ethology*; *Behaviour*; *Journal of Experimental Psychology: Animal Learning and Cognition*; *Ethology, Ecology and Evolution*; and *Sociobiology.* To this list, we added four journals that focus on behaviour within a mechanistic framework: *Physiology and Behaviour*; *Brain, Behaviour and Evolution*; *Behaviour Genetics*; and *Journal of Experimental Biology*. This list is a subset of journals that publish behavioural biology work, as publications on this topic can also be published in generalist journals (*Ecology Letters*, *Oecologia*, *Current Zoology*, *American Naturalist*, *Canadian Journal of Zoology*, *Oikos*, *Journal of Animal Ecology*, etc.), but minimizes the chance of including citations not related specifically to behaviour. We excluded eight journals from the Google scholar *Top 20 Journals in Animal Behaviour and Ethology* because they focus on veterinary medicine and welfare, or they are exclusively on insects: *Applied Animal Behaviour Science*; *Journal of the Experimental Analysis of Behaviour*; *Journal of Veterinary Behaviour: Clinical Applications and Research*; *Insectes Sociaux*; *Journal of Insect Behaviour*; *Learning and Behaviour*; *Learning and Motivation*; and *Journal of Applied Animal Welfare Science*.

The two searches using journal lists were conducted on 23 October 2018.

Searching by Web of Science 'Research area'

We ran a supplemental analysis of the use of each of the 21 genera in both the evolutionary biology and animal behaviour literatures by using the 'Research area' category ('SU') in

our search, which is curated directly by Web of Science. As in the 'journal list' approach above, we did a separate search for each genus. For evolutionary biology, we used SU = Evolutionary Biology in an advanced search, which includes 54 journals when using the 'core collection' settings. For behaviour, we used SU = Behavioural Sciences in an advanced search, which includes 69 journals when using the 'core collection' settings. We chose the Web of Science 'core collection' in these searches to make them as replicable as possible. The two searches using Research area from Web of Science were run on 6 November 2018. The results of these analyses were broadly similar to our main survey, so they are presented as supplementary materials (evolutionary-ecology.com/data/ 3192Appendix.pdf) rather than in the main text.

The use of *Gasterosteus* in different areas of research over the last 25 years

The threespine stickleback has been a model for the study of behaviour for more than 60 years. It has been extensively used in the study of evolution for at least the last 50 years and in physiology for nearly as long. Furthermore, its use in the fields of immunology, ecotoxicology, and genomics has certainly grown in the past two decades. The programmes of the different 'Stickleback meetings' over the years show us that this fish is significant in many fields and that the use of this species is percolating to novel topics and questions.

As a complement to the focused evolution and behaviour surveys already described, we conducted more inductive analyses aimed at determining which fields have used the threespine stickleback and its single congener *G. wheatlandi* as a model system over the last 25 years. This allowed us to detect long-term trends in usage, as well as up-and-coming new topics that use sticklebacks. Since our first analysis showed the emergence of new model systems, including *Gasterosteus*, we could also use this keyword analysis to test the hypothesis that this change in vertebrate models used reflects a shift in research topics within evolutionary biology and animal behaviour. As a proxy for a field/question, we used keywords cited in the literature over this period. We quantified the occurrence of keywords used in combination with '*Gasterosteus*' for each period (1993–1995, 2004–2006, and 2015–2017).

We again used the Web of Science core collection. We downloaded all the citations from the Web of Science database on 13 November 2018. We used the 'Topic' advanced search field tag TS = Gasterosteus. We analysed the co-occurrence of this genus name with 'keywords-Plus', which are uniformized keywords used by Web of Science, using the 'biblioAnalysis' function from the R package Bibliometrix (Aria and Cuccurullo, 2017). Since the number of publications on sticklebacks changed over time, we used the total number of publications referring to *Gasterosteus* in each time period (254, 346, and 670 publications for 1993–1995, 2004–2006, and 2015–2017, respectively) to calculate a percentage of publications that used a given keyword.

Keywords found in all three time periods were considered long-lasting research themes that use stickleback as a study subject. Keywords found only in the 1993–1995 period (the earliest used) suggested a subsequent shift in focus, while keywords identified exclusively in the last two periods suggested emerging topics of interest for the study of the biology of sticklebacks or that use this species as a model.

The current and future use of the stickleback model

We aimed to highlight the most current trends in research involving stickleback by analysing the frequency of words used in the abstracts of the oral presentations at the Ninth International Conference on Stickleback Behaviour and Evolution (July 2018). We used a web tool (worditout.com) to count the occurrences of each word extracted from abstract texts. We also used it to filter common words. As these are freeform words chosen by the authors, we created representative topics by combining words that were in the same category. For example, the number of times that 'adaptive' and 'adaptation' were used was summed, as well as counts for singular and plural forms of the same word. Frequency of occurrence was then compiled for these combined topic categories.

RESULTS AND DISCUSSION

Trends in the use of different model vertebrates by field

Do biomedical model systems dominate evolutionary biology and animal behaviour and what traits matter for success?

Evolution

Our sample of evolution-themed journals published 3355, 7354, and 9495 papers in the 1993–1995, 2004–2006, and 2015–2017 period, respectively. The large increase in publication number over time confirms that using a relative publication rate is warranted. For the vertebrate taxa, the most striking pattern is that biomedical models, dominant in the early 1990s and still important into the 2000s, were substantially supplanted by emerging evolutionary models in the most recent period (Fig. 1, Table 1). For example, *Mus* and *Xenopus* were the top two taxa in 1993–1995, and *Rattus* was fourth. In 2004–2006, *Mus* was still the top vertebrate, but *Xenopus* had declined to eighth and *Rattus* was ninth. By 2015–2017, the shift was unmistakable. The top three taxa were all principally non-biomedical organisms for which a key research advantage was abundant variation among populations and closely related species: *Gasterosteus*, *Anolis*, and *Poecilia. Mus*, now ranked fourth, was almost tied with *Oncorhynchus* in fifth. It is also noteworthy that, even as the emerging comparative models were rising in importance, every biomedical model (*Mus*, *Xenopus*, *Rattus*, *Danio*) experienced a decline in the final sample, with the sole exception of the zebra finch, *Taeniopygia* (Fig. 1, Table I).

   Perhaps the clearest inference emerging from our analyses is that some features are key for any organism to become popular for scientific research. Some of the criteria cited as important for justifying biomedical model organisms include a rapid life cycle, relatively simple reproduction and life history (and now genome), small body size, and ready adjustment to the laboratory (Dietrich *et al.*, 2014). Certainly, the top evolutionary models in our analyses all possess these characteristics to some degree. In addition to these virtues, the rising evolutionary models that have supplanted rats, mice, and *Xenopus*, such as *Gasterosteus*, *Poecilia*, and *Anolis*, are also well suited to comparative analyses, exhibiting striking geographic variation in their (visible) phenotypes, often with extensive replication across wide geographic distributions and even across time. All are also abundant in nature. Thus they enable a variety of approaches, including comparative studies, fieldwork on selection and other processes, and laboratory experiments including behavioural assays,
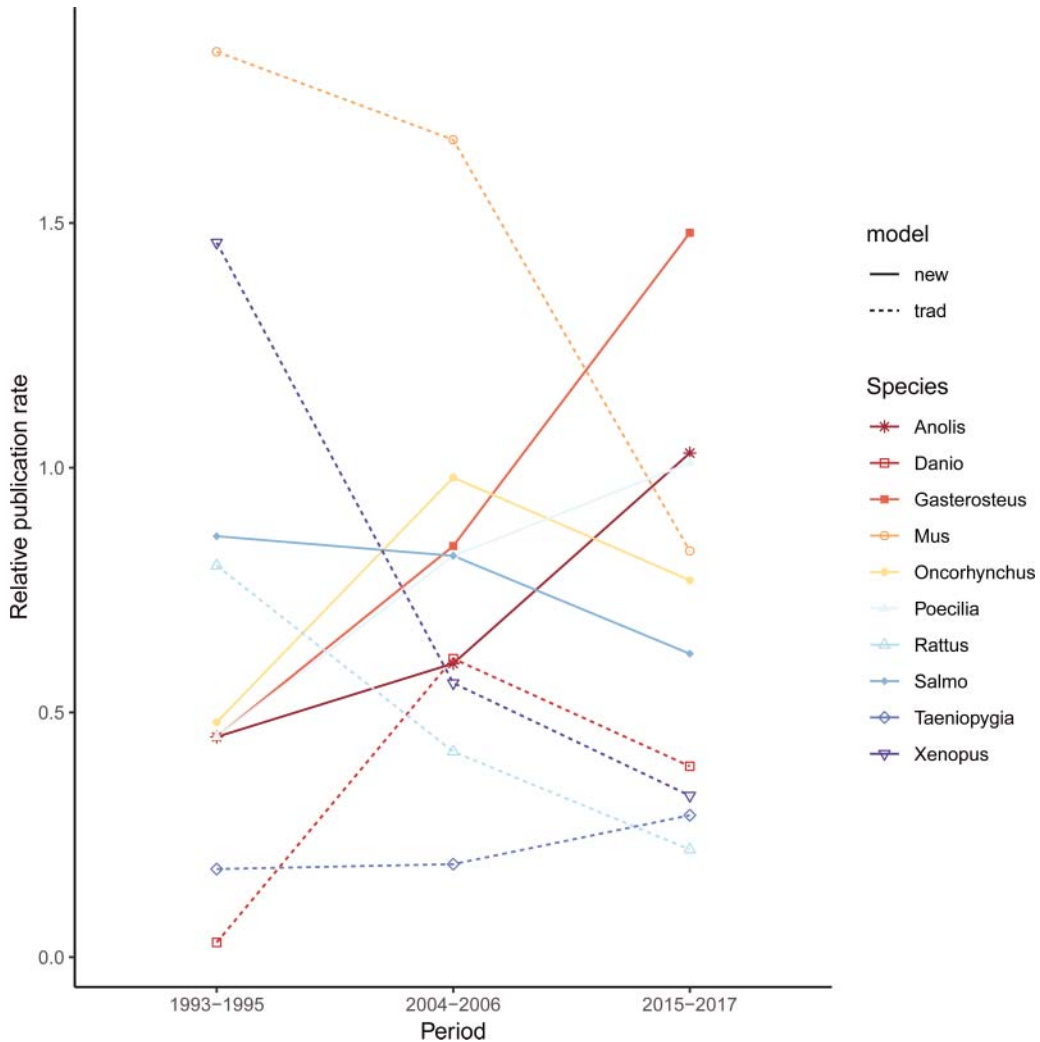
Fig. 1. Publication rates, as percentages of total publications for the set of journals considered, for each three-year sample for the top ten (of 17 total) most frequently published vertebrate taxa in our survey of 20 journals focusing on evolutionary biology. Dotted lines represent 'traditional' biomedical models and solid lines other models in the list.

genetic crosses, and genomics. It would appear, then, that the study taxa that became the highest ranked in evolutionary biology may have supplanted biomedical models because they exhibit sought-after (visible, often related to morphology and appearance) phenotypic variation, and because the early advantages of the biomedical models, such as the availability of a wide range of molecular tools and genomic information, have been reduced. It is worth noting that these emerging model systems are much less well studied for their 'internal' phenotypic variation at the cellular and physiological levels across populations and environments (Houle *et al.*, 2010), except for work done in sticklebacks. They are clearly much more studied for their variation in morphology and appearance.

Table 1. Relative percentages of the total number of publications in evolution-themed journals by genus for three time periods: 1993–1995, 2004–2006, and 2015–2017

Taxon	1993–1995	2004–2006	2015–2017	Trend
Drosophila	13.86	11.22	9.05	–
Gasterosteus	0.45	0.84	1.48	+
Caenorhabditis	0.72	2.00	1.26	
Anolis	0.45	0.60	1.03	+
Poecilia	0.45	0.82	1.01	+
Daphnia	0.92	0.71	0.87	
Mus	1.85	1.67	0.83	–
Oncorhynchus	0.48	0.98	0.77	
Heliconius	0.12	0.30	0.67	+
Salmo	0.86	0.82	0.62	–
Danio	0.03	0.61	0.39	
Xenopus	1.46	0.56	0.33	–
Taeniopygia	0.18	0.19	0.29	+
Passer	0.18	0.18	0.26	+
Fundulus	0.24	0.12	0.23	
Rattus	0.80	0.42	0.22	–
Oryzias	0.09	0.19	0.15	
Oreochromis	0.24	0.11	0.15	
Geospiza	0.15	0.16	0.11	
Pungitius	0.00	0.03	0.09	+
Pundamilia	0.00	0.03	0.05	+
TOTAL	789/3355 (23.5%)	1658/7354 (22.5%)	1888/9495 (19.9%)	

Note: Genera are ranked from highest to lowest relative number of publications in the most recent period, 2015–2017. The trend column highlights genera that have the clearest change in use. The bottom row shows the total number of publications for these 21 genera, divided by the total number of publications in these journals for that time period (percentage in parentheses).

The importance of the threespine stickleback for evolutionary biology is assessed conservatively here, as it is the only member of its genus broadly used for research, whereas another top genus, *Poecilia*, includes mollies (sexual and asexual!) in addition to guppies and has a more ancient history with dozens of named species, which is also the case for *Anolis*. So why is *Gasterosteus* so popular? Among the factors that attracted us to sticklebacks are external fertilization, large clutches, presence close to where we live, and phenotypic variation between individuals and between populations across various ecological conditions. These advantages contributed to the early choice of *G. aculeatus* for genome sequencing [(Jones *et al.*, 2012), although at about the same time as *Anolis* (Alföldi *et al.*, 2011)], and a high-quality sequence of what proved a conveniently small genome facilitated additional work, as suggested by its rapid rise in the most recent sample in our survey. In addition, the adoption of particular taxa by prominent laboratories that train large numbers of future scientists has likely influenced the patterns we see here. In the case of *Gasterosteus*, there was also a grant-funded training in taxon-focused molecular and genomic methods, through the Stickleback Molecular Genetics course, which ran at Stanford University from 2003 to 2011.

In contrast, two taxa challenge these rules in that they do not satisfy the first, general set of criteria well. The salmonids *Salmo* and *Oncorhynchus*, which exhibit remarkable and replicated phenotypic variation and are often abundant, are not small, typically do not have short life cycles, and are not easy to raise in large numbers in the laboratory – but these are shortcomings that generous funding can remedy. Thus the economic importance of these fish in wealthy countries where many scientists work may have been a critical compensation for their shortcomings. Even so, they have lately been surpassed in popularity by smaller creatures.

Behaviour

Our sample of behaviour-themed journals published 5947, 7786, and 9089 papers in the 1993–1995, 2004–2006, and 2015–2017 period, respectively. The increase in publication number in the behavioural dataset was similar to that in the evolution literature. Other trends were broadly similar between the two fields, but the patterns were less clear and the pre-eminent taxa were often different in the behaviour dataset (Fig. 2, Table 2). Once again, *Mus* was the top-ranked vertebrate taxon in the first time period, but other biomedical taxa were less dominant, with the salmonids *Salmo* and *Oncorhynchus* ranked second and third, *Rattus* once again fourth but *Xenopus* only sixth. However, by the 2015–2017 period, non-biomedical models had once again moved to the fore, with *Poecilia* and *Oncorhynchus* highest ranked – but two biomedical models of lesser importance in the evolutionary literature and in the early periods of the behavioural dataset, *Danio* and *Taeniopygia*, had experienced an abrupt elevation in their status to the third and fourth ranks, respectively (Fig. 2). *Gasterosteus* was an important, but never dominant, behavioural model in every period, always ranked fifth or sixth (Fig. 2, Table 2). Thus its trajectory is quite different from the pattern in the evolution literature, where it rose from a base level (ranked 8th) to become the leading vertebrate model in the most recent period (Figs. 1, 2). Interestingly, the study of its variation in behaviour across life stages, as well as within and between sexes, and of the hormonal bases of these differences, has been the topic of studies for more than 50 years. It is possible that it was ranked higher in earlier work that we did not survey in the present bibliometric analysis (for a finer analysis of trends in research topics on stickle-backs, see the section headed 'Topics of stickleback research for the past 25 years' below).

The shift towards species with similar characteristics over the last 25 years has been striking in the field of evolutionary biology; this is not the case in animal behaviour publications, as no single characteristic connects these preferred species together. Here the emerging biomedical models are a fish originally mostly used to study development, *Danio rerio*, and a bird used to dissect the neurobiological basis of learning, the zebra finch. Both models have the advantage of bringing biomedical toolkits to the study of behaviour, such as mutant lines, gene expression manipulation tools (RNAi, etc.) for the zebrafish, and a large dataset on neural connections and functions of specific brain regions for zebra finches. In particular, the ease of high-throughput testing of simple behaviours in larval *Danio* and of manipulating environmental effects including drug exposure, coupled with concern of using mammals for research, may have resulted in the shift from *Mus* to fish in the last decade. On the other hand, comparative work on the neuroendocrine basis of social behaviour variation has used the zebra finch extensively, which has opened new avenues for its use in behaviour research. The rise in use of *Poecilia* and the steady stream of publications on *Oncorhynchus* probably have distinct underlying causes. *Poecilia* encompasses all mollies and the guppy, which are livebearers, shows various reproductive
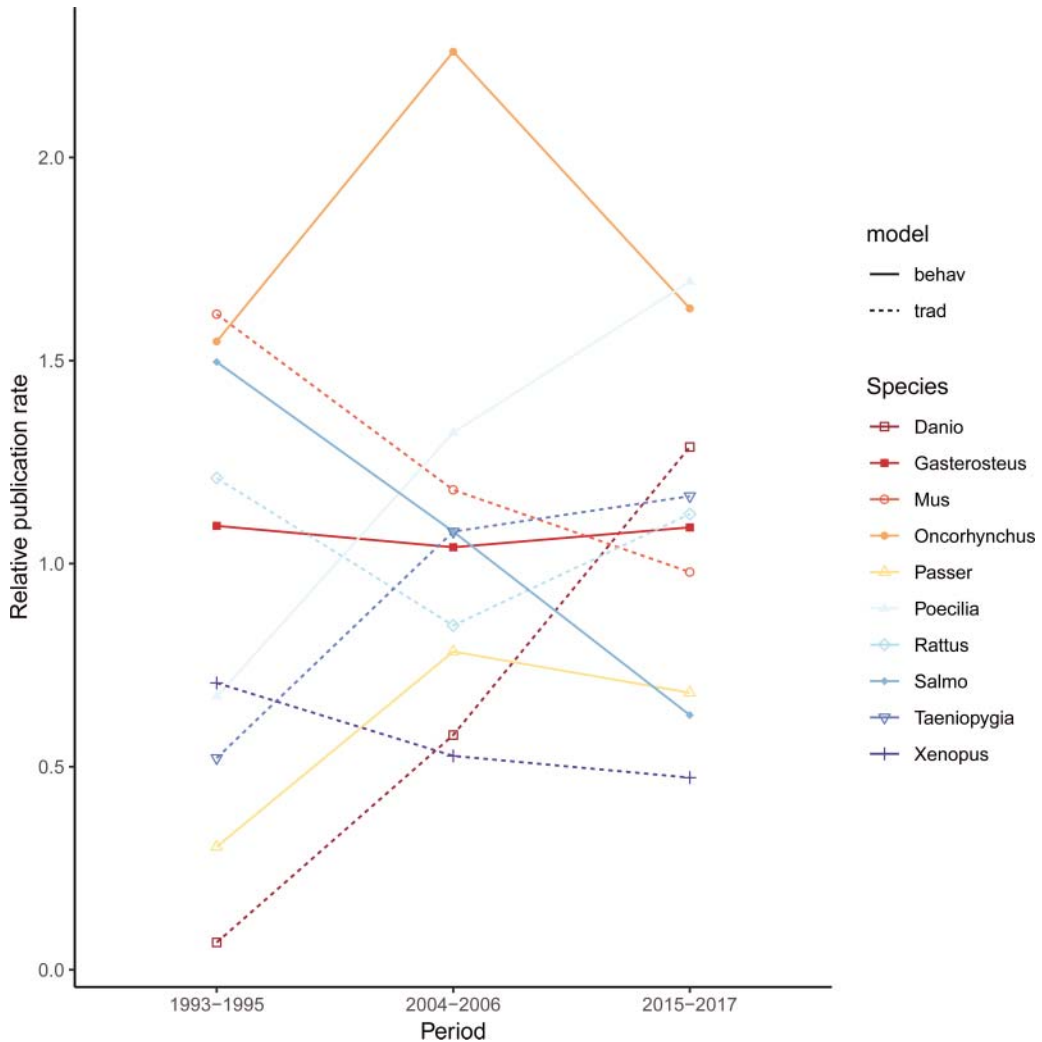
Fig. 2. Publication rates, as percentages of total publications for the set of journals considered, for each three-year sample for the top ten (of 17 total) most frequently published vertebrate taxa in our survey of 16 journals focusing on animal behaviour and ethology. Dotted lines represent 'traditional' biomedical models and solid lines other models in the list.

tactics, and includes species that been studied for a long time with regard to predator–prey interactions, competition, and their effects on evolution. Genomic resources enabling high-throughput transcriptomics have become available very recently for this taxon, which may have fuelled some of the rise in use. Their utility as a model of the evolution of behaviour may explain why they are in both the evolution and animal behaviour preferred lists. As mentioned above, *Oncorhynchus* includes Pacific salmon and trouts, which have very high economic value with commercial and sport fishing of wild stocks, and with commercial hatcheries, such that behaviour may be studied in this genus because of conservation challenges and economic value.

Table 2. Relative percentages of the total number of publications in behaviour-themed journals by genus for three time periods: 1993–1995, 2004–2006, and 2015–2017

Taxon	1993–1995	2004–2006	2015–2017	Trend
Drosophila	1.46	3.24	3.12	
Poecilia	0.67	1.32	1.69	+
Oncorhynchus	1.55	2.26	1.63	
Danio	0.07	0.58	1.29	+
Taeniopygia	0.52	1.08	1.17	+
Rattus	1.21	0.85	1.12	
Gasterosteus	1.09	1.04	1.09	
Mus	1.61	1.18	0.98	−
Passer	0.30	0.78	0.68	
Salmo	1.50	1.08	0.63	−
Xenopus	0.71	0.53	0.47	−
Anolis	0.40	0.62	0.36	
Oreochromis	0.34	0.45	0.30	
Fundulus	0.10	0.35	0.30	
Caenorhabditis	0.05	0.35	0.26	
Daphnia	0.17	0.24	0.18	
Pungitius	0.03	0.05	0.09	+
Oryzias	0.05	0.08	0.06	
Heliconius	0.03	0.05	0.06	+
Geospiza	0.00	0.03	0.03	+
Pundamilia	0.00	0.04	0.00	
TOTAL	706/5947 (11.9%)	1260/7786 (16.2%)	1409/9089 (15.5%)	

Note: Genera are ranked from highest to lowest relative number of publications in the most recent period, 2015–2017. The trend column highlights genera that have the clearest change in use. The bottom row shows the total number of publications for these 21 genera, divided by the total number of publications in these journals for that time period (percentage in parentheses).

How does use of vertebrates compare to the study of non-vertebrates?

Evolution

No vertebrate rivals the pre-eminent invertebrate, *Drosophila*, as a model in evolutionary biology (Table 1). In every sample there were at least five times as many *Drosophila* publications as for the highest ranked vertebrate. Still, the relative rate of *Drosophila* publications declined slightly over time. *Caenorhabditis* was also highly ranked in every sample, and above all vertebrates in 2004–2006. *Daphnia* and *Heliconius* were not as important in this dataset, but *Heliconius* publications did increase in each sample, placing it only just behind the top five vertebrates in 2015–2017 (Table 1).

Drosophila has maintained its pre-eminence in the evolutionary literature during the last 25 years probably in large part because it is even more experimentally tractable than the smallest vertebrate models, while at the same time exhibiting extensive and well-documented phenotypic and genetic natural variation. Its short generation times and small size also make it ideal for selection and experimental evolution experiments to a degree that no vertebrate can ever match – and all without complex care requirements or regulations.

Caenorhabditis is even more laboratory-friendly and has an even shorter generation time. As the diversity of nematodes is explored further by experimentalists and the full range of the genus becomes better studied, it should become increasingly attractive for comparative work as well. Similar considerations would appear to apply to *Daphnia*, though neither *Daphnia* nor *Caenorhabditis* have had an impact similar to that of *Drosophila*.

Behaviour

As in the evolutionary literature, *Drosophila* was more extensively published than any other taxon in animal behaviour, but its dominance is a more recent development. In the 1993–1995 sample, there were more *Mus*, *Oncorhynchus*, and *Salmo* publications than *Drosophila*. *Drosophila* was the top taxon in subsequent samples, but not nearly so dominant as in the evolutionary literature; there were 1.8 *Drosophila* papers for each lead vertebrate paper published in behaviour-themed journals in the 2015–2017 period. Other invertebrate taxa were much less important (Table 2).

   Why, in contrast to the pattern for evolutionary biology, has *Drosophila* only more recently passed the various vertebrates in importance? This may be a question of 'preferential attachment' in which *Drosophila* was early on adopted by influential evolutionists such as Dobzhansky but was not studied extensively by the leading early ethologists. The dual goals of biologists working in animal behaviour of studying the proximal and ultimate causes of behaviour may explain *Drosophila*'s rise at last, with recent progress in behavioural genetics likely an important factor. Indeed, the phenomenal genetic toolkit available to study the genetic basis of behaviour in *Drosophila* (genome, selection lines, mutants, genetic reference panel, genome editing), including complex gene-by-environment interactions and epistatic effects (Swarup *et al.*, 2012), makes it a go-to system for this type of question.

Are model systems generally becoming more or less important?

Perhaps surprisingly, the 21 species in our bibliometric analysis accounted for 24% of all papers published in evolution-themed journals in 1993–1995, but only 20% in the 2015–2017 period (Table 1). This result suggests a diversification of study systems in this quarter-century, or a recent focus of the research community on one or several new species not present in our list. The 21 species used in our analysis accounted for 12% of papers in the behaviour-themed journals in 1993–1995, but almost 16% in 2015–2017 (Table 2). This increase suggests several non-exclusive explanations, including: a recently increased focus on a smaller number of study systems in this field, the fall in usage of one or more species not in our species list, or very high productivity of certain laboratories favouring a given model.

   It is initially surprising that this list of models does not account for a greater proportion of the evolutionary and behavioural literatures – but similar results have been reported in comparable studies of biomedical publication patterns. Peirson *et al.* (2017) conducted a survey of biomedical publications focused on the 13 species designated as model organisms by the US National Institutes of Health (NIH) in 1999, including several species from our list as well as the plant *Arabidopsis*, the yeast *Saccharomyces*, and other taxa. They found that the proportion of papers focused on NIH models decreased from around 26–28% in the 1980s to 21–22% in the 2010s, despite the fact that the formal model organism designation took place between these periods. Indeed, Davies (2007) has suggested that for developmental biology, the distribution of research taxon usage approximates to a

power law with an essentially continuous distribution and no evidence for the existence of a qualitatively distinct group of model organisms.

Quite possibly the trend towards diversification of research taxa will continue in the literature of evolution. Such diversification has been, and likely will be, facilitated by the 'democratization' of genomics. Indeed, sophisticated methods are increasingly available not just for emerging or marginal model organisms but for 'obscure model organisms' (Matz, 2018). Even so, in the words of Tolstoy as quoted by Jared Diamond (1997, from *Anna Karenina*, 1878) with regard to the domestication of animals, 'All happy families are alike; each unhappy family is unhappy in its own way.' Thus there are many ways for an organism to be inadequate for domestication or as an ideal system for evolutionary or behavioural study, including in the latter instances, too long a generation time, poor acclimation to laboratory conditions, or too large a genome. Consequently, some systems with minimal shortcomings and extensive background data seem likely to remain popular. For the behaviour models, there may be a stronger emphasis on obtaining generalizable results that apply to a large array of species and thus to a focus on systems that make this possible (Zuk and Travisano, 2018). Concentrating efforts on systems with an already abundant background of literature and the possibility to obtain phenotype information at different levels of biological organization (Houle *et al.*, 2010), including 'internal' phenotypes (RNA, epigenetic marks, proteins, cells, development, physiology including neurological systems), would increase the likelihood of attaining this goal.

Limitations of our analyses of vertebrate models

There are several important caveats to these analyses. Most important, we have not surveyed all vertebrate taxa that might be important models, a limitation of our approach of defining focal taxa *a priori*. A future study could build on our findings by using textual analyses to identify taxa more inclusively and inductively (Peirson *et al.*, 2017). A second caveat is that by focusing on particular journals, we certainly missed relevant papers that were published elsewhere, among them important studies in the most competitive and general publications; we have no reason to think this substantially biased our results but we cannot rule out such a possibility. In addition, our approach resulted in the inclusion of some publications in which one of our study taxa was mentioned but was not the primary focus. Probably this increased the rankings of some of the biomedical taxa, in particular, when they were included in a study for comparative or procedural reasons. Finally, the use of genus as the unit of comparison is somewhat arbitrary and may have resulted in artefacts; for example, it may have led to underestimation of the influence of studies of cichlids.

Topics of stickleback research for the past 25 years

We extracted the 40 keywords co-occurring with the highest frequency with '*Gasterosteus*' in all journals covered by the Web of Science core collection for each time period. After removing keywords relating to the various names used for stickleback, we obtained 32 keywords for each time period (Table 3).

Conserved keywords

'Evolution' and 'fish' were found to be pre-eminent keywords in stickleback research for the whole quarter-century surveyed. These themes are the foundation of work in sticklebacks and they come as no surprise. While 'behaviour' was the first-ranked keyword 25 years ago,

Table 3. Keywords having the highest frequency of co-occurrence with the search term '*Gasterosteus*', ranked for each time period by the absolute number of publications that used them (percentage of the total number of publications referring to *Gasterosteus* in a given period in parentheses)

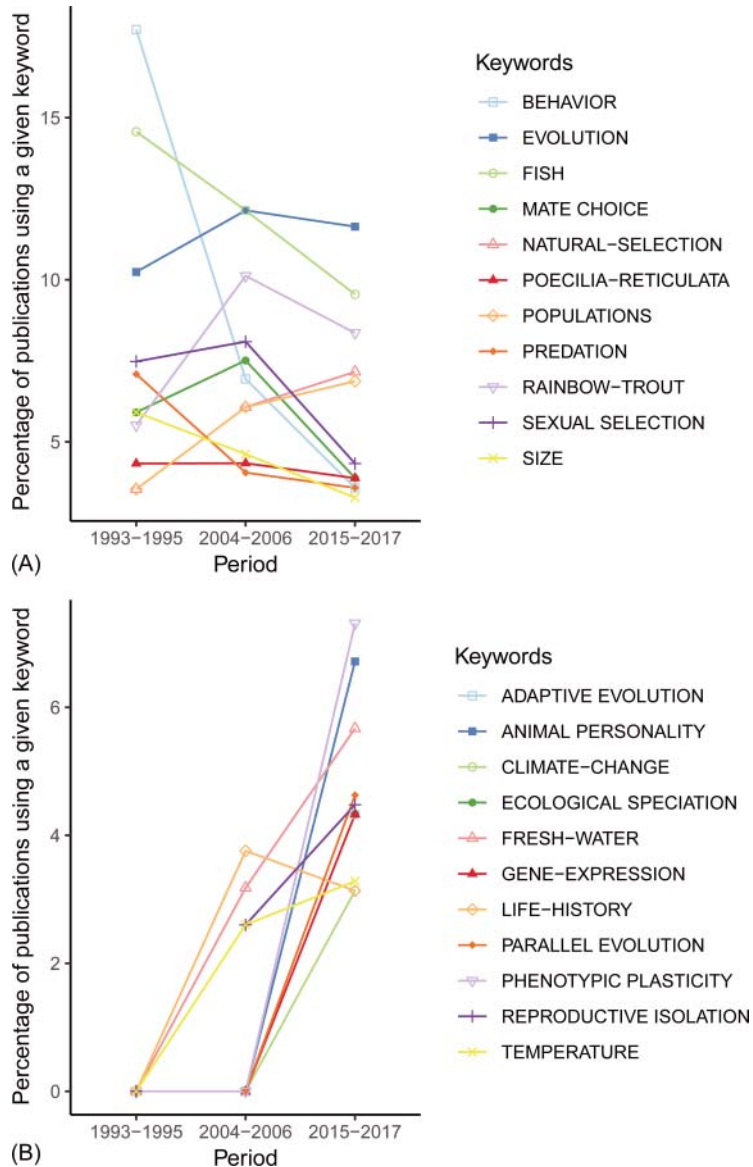| 1993–1995	N (%)	2004–2006	N (%)	2015–2017	N (%)
BEHAVIOR	45 (17.7)	EVOLUTION	42 (12.1)	EVOLUTION	78 (11.6)
FISH	37 (14.6)	FISH	42 (12.1)	FISH	64 (9.6)
EVOLUTION	26 (10.2)	RAINBOW-TROUT	35 (10.1)	RAINBOW-TROUT	56 (8.4)
RISK	20 (7.9)	SEXUAL SELECTION	28 (8.1)	PHENOTYPIC PLASTICITY	49 (7.3)
SEXUAL-SELECTION	19 (7.5)	MATE CHOICE	26 (7.5)	NATURAL-SELECTION	48 (7.2)
PREDATION	18 (7.1)	BEHAVIOR	24 (6.9)	POPULATIONS	46 (6.9)
SELECTION	18 (7.1)	NATURAL-SELECTION	21(6.1)	ANIMAL PERSONALITY	45 (6.7)
AGGRESSION	16 (6.3)	POPULATIONS	21 (6.1)	FRESH-WATER	38 (5.7)
MATE CHOICE	15 (5.9)	SELECTION	20 (5.8)	INDIVIDUAL-DIFFERENCES	36 (5.4)
SIZE	15 (5.9)	ATLANTIC SALMON	18 (5.2)	PARALLEL EVOLUTION	31 (4.6)
RAINBOW-TROUT	14 (5.5)	SIZE	16 (4.6)	REPRODUCTIVE ISOLATION	30 (4.5)
BRITISH-COLUMBIA	13 (5.1)	BRITISH-COLUMBIA	15 (4.3)	ADAPTIVE EVOLUTION	29 (4.3)
POPULATION	12 (4.7)	POECILIA-RETICULATA	15 (4.3)	ECOLOGICAL SPECIATION	29 (4.3)
ANTIPREDATOR BEHAVIOR	11 (4.3)	PREDATION	14 (4.0)	GENE-EXPRESSION	29 (4.3)
GUPPY POECILIA-RETICULATA	11 (4.3)	SPECIATION	14 (4.0)	SEXUAL SELECTION	29 (4.3)
REPRODUCTIVE SUCCESS	10 (3.9)	FISHES	13 (3.8)	ANTIPREDATOR BEHAVIOR	28 (4.2)
ECOLOGY	9 (3.5)	GROWTH	13 (3.8)	BEHAVIORAL SYNDROMES	28 (4.2)
NATURAL-SELECTION	9 (3.5)	LIFE-HISTORY	13 (3.8)	TROUT ONCORHYNCHUS-MYKISS	28 (4.2)
POPULATIONS	9 (3.5)	ADAPTIVE RADIATION	12 (3.5)	MATE CHOICE	26 (3.9)
FISHES	8 (3.1)	PREDATION RISK	12 (3.5)	POECILIA-RETICULATA	26 (3.9)
GEOGRAPHIC-VARIATION	8 (3.1)	FRESH-WATER	11 (3.2)	ZEBRAFISH DANIO-RERIO	26 (3.9)
PREY	8 (3.1)	MITOCHONDRIAL-DNA	11 (3.2)	ADAPTIVE DIVERGENCE	25 (3.7)
ZOOPLANKTON	8 (3.1)	POPULATION	11 (3.2)	ADAPTIVE RADIATION	25 (3.7)
FOOD	7 (2.8)	TRADE-OFFS	11 (3.2)	BEHAVIOR	24 (3.6)
INTERMEDIATE HOST	7 (2.8)	DIVERGENCE	10 (2.9)	PREDATION RISK	24 (3.6)
PISCES	7 (2.8)	LATERAL PLATES	10 (2.9)	BOLDNESS	23 (3.4)
POLYMORPHISM	7 (2.8)	MALE	10 (2.9)	BODY-SIZE	22 (3.3)
SPECIES-PAIR	7 (2.8)	EXPOSURE	9 (2.6)	ECOLOGY	22 (3.3)
ADAPTATION	6 (2.4)	FOOD	9 (2.6)	LOCAL ADAPTATION	22 (3.3)
EGG-PRODUCTION	6 (2.4)	PREFERENCES	9 (2.6)	TEMPERATURE	22 (3.3)
ENOS LAKE	6 (2.4)	REPRODUCTIVE ISOLATION	9 (2.6)	CLIMATE-CHANGE	21 (3.1)
PARASITIZED STICKLEBACKS	6 (2.4)	TEMPERATURE	9 (2.6)	LIFE-HISTORY	21 (3.1)

Fig. 3. Percentage of publications on *Gasterosteus* using a given keyword for three time periods: 1993–1995, 2004–2006, and 2015–2017. (A) Examples of conserved keywords used over the last 25 years. (B) Examples of emerging keywords used for the first time in the 2004–2006 or 2015–2017 period. The complete list of conserved and emerging keywords is shown in Table 3.

with 18% of papers using this keyword, it was ranked 24 (4% of papers) in the most recent period (Fig. 3, Table 3). It is still used and can be considered in the 'conserved' category but the rise of stickleback in other research fields (see below) means that the study of behaviour is not its main use at present. The keywords 'rainbow trout' and '*Poecilia reticulata*' were

also in this list, probably reflecting the interest in this agent of selection (the trout is a predator of stickleback) and in comparative work, respectively.

Several other keywords were found in the three time periods and can be considered long-lasting and conserved research topics (see Fig. 3A for examples and Table 3 for the full list). 'Sexual selection', 'predation', 'mate choice', 'size', 'natural selection', 'ecology', and 'populations' are the topics that were prominent over the 25 years. These long-lasting keywords highlight how the use of stickleback to study natural and sexual selection is fruitful and a strong research trend. Indeed, this model system has allowed testing of crucial predictions linked to several aspects of sexual selection, including female choice, signalling in males, assortative mating, and how visual ecology interacts with mate choice, to name a few. The studies on speciation involving natural selection in this fish model have provided excellent examples supporting ecological speciation and have allowed the testing of several hypotheses about the steps involved in the early stages of speciation that can be generalized to other animal systems.

Disused keywords

Almost half of the topics that were the subject of intense study in 1993–1995 did not recur in the keywords used to describe the topics of the later publications, i.e. 'aggression', 'reproductive success', 'geographic variation', 'prey', 'zooplankton', 'intermediate host', 'polymorphism', 'species pair', 'egg production', and 'parasitized sticklebacks' (Table 3). Arguably, those topics are still studied today in ecology and evolution, but are designated by different keywords. For example, aggression is now often studied within the framework of 'animal personality' or 'behavioural syndromes', two of the emerging topics (see below) in behavioural biology studies that have been done in sticklebacks for decades (Huntingford, 1976), albeit using different keywords. Some keywords show the effervescence of work on the west coast of Canada in the early time period, such as the terms 'British Columbia' and 'Enos Lake' referring to the geographic locations of studies at the time.

Emerging keywords

One-third of topics found at high frequency in 2015–2017 appear on the radar in stickle-back research only in these most recent years: 'phenotypic plasticity', 'animal personality', 'individual differences', 'adaptive evolution', 'parallel evolution', 'ecological speciation', 'gene expression', 'behavioural syndromes', 'adaptive divergence', 'boldness', 'local adaptation', and 'climate change' (see Fig. 3B for examples). Along with those that have arisen since the 2004–2006 period ('life history', 'temperature', 'fresh water', and 'reproductive isolation'), these topics reflect new challenges that must be addressed. 'Zebrafish' also appears for the first time in association with stickleback, maybe because researchers want to establish stickleback as a model similar to *Danio rerio*, or because several parallels can be drawn between the two species.

The trends in the appearance of these new keywords also reflect greater awareness of important processes in evolutionary biology such as plasticity, which is a keyword used in more than 7% of stickleback publications in the 2015–2017 period. Phenotypic plasticity has been studied for over a hundred years, but has recently gained more attention in the evolution literature in general. Indeed, when we performed an additional analysis of keywords that co-occur with 'evolution' in 1993, 2005, and 2017 in the Web of Science core collection (no genus included), we found that 'phenotypic plasticity', 'gene expression', 'local adaptation', and 'climate change' appear in the top 30 list for the first

time in 2017. This suggests that a focus on these topics is new to the field in general and not only for researchers using sticklebacks. It seems that *Gasterosteus* is a highly suitable model to study these questions because of its wide distribution in a large range of environmental conditions (which allows testing of certain questions regarding climate change and thermal adaptations), the presence of extensive, well-documented phenotypic variation among reproductively isolated populations, and the ease of performing common environment experiments and experimental manipulations with laboratory rearing, to study phenotypic plasticity of various traits. The emergence of the keyword 'gene expression' suggests that more and more studies are taking advantage of the ease of study of the whole transcriptome, or of candidate genes, both methods rendered much more accessible with a sequenced and annotated genome.

Overall, our bibliometric analysis suggests that the stickleback has evolved as a model, going from being frequently and mainly used in animal behaviour and blooming into a model used to study various aspects of evolution, as well as to address current concerns over human-induced rapid environmental changes.

The present state and future of stickleback research

Topics at the 2018 stickleback conference

We analysed the frequency of occurrence of words related to research topics in the abstracts of the talks presented at the 2018 Stickleback conference; we wished to take the pulse of current and future research interests involving this taxon. After 'stickleback' itself, the ten most frequent topics pertained to 'variation and differences', 'freshwater and marine', 'population(s)', 'gene(s)', 'adaptation', 'phenotype', 'sex', 'habitats', 'genome', and 'parasite'. In summary, researchers gathered to discuss research done on sticklebacks are focusing on variation in phenotypes, between habitats, between sexes, and the evolutionary and ecological processes that lead to these differences, as well as on genomic-level questions and interactions with parasites. This list overlaps considerably with the keywords found in 2015–2017 in the published literature on sticklebacks (Table 3), although there was a stronger focus on genomes, genes, parasites, and sex at the meeting and less of a focus on behaviour-related research. One may expect to see those topics gaining prominence in the coming years in the published literature.

Topics in the present special issues and future directions

The abstracts for the three special issues of *Evolutionary Ecology Research* include words that frequently appeared in the presentations at Stickleback 2018, such as 'population(s)', 'freshwater', 'size', 'males/females', 'plate(s)', 'armour', 'variation', 'lake', 'maturation(s)', and 'white'. In addition, 'genetic' and 'EDA' appeared frequently, although they were not ranked in the top ten.

Hendry *et al.* (2013) framed their preface based on four research topics: phenotypic variation, genomic variation, speciation, and eco-evolutionary dynamics. We will follow this classification here, although we note that few studies focus on a single topic today, so this classification may be too simple.

First, phenotypic variation has been characterized in armour traits (Lajus *et al.*, 2019; Maciejewski *et al.*, 2019; Yamasaki *et al.*, 2019), body shape (Pistore *et al.*, 2019), foraging traits (Hosoki *et al.*, 2019; Reimchen *et al.*, 2019), life-history traits (Baker *et al.*, 2019; Singkam and MacColl, 2019), physiological traits

(Durston and El-Sabaawi, 2019; Kusakabe *et al.*, 2019), and behavioural traits (Lacasse and Aubin-Horth, 2019; Haley *et al.*, 2019). Some of the papers here investigate the ecological (Hosoki *et al.*, 2019; Reimchen *et al.*, 2019), genetic (Reimchen *et al.*, 2019; Yamasaki *et al.*, 2019), and physiological mechanisms (Lacasse and Aubin-Horth, 2019; Kusakabe *et al.*, 2019) underlying these various types of phenotypic variation. Although Ivanova *et al.* (2019) and Golovin *et al.* (2019) focus on a single or a few populations to establish methods for studying migratory behaviour and analysing sex-biased mortality, respectively, application of these techniques to different populations will surely reveal interpopulation variation in these traits. Considered together, the remarkable breadth and depth of information about phenotypic variation and associated ecological and genetic mechanisms in sticklebacks, as exemplified by the contributions to this volume, can be expected to help to maintain this taxonomic group as a leading model for research in evolutionary ecology.

As for genomic variation, Reimchen *et al.* (2019) take an exceptionally comprehensive approach in Boulton Lake, examining relationships among genomic, morphological, and trophic variation. Yamasaki *et al.* (2019) make use of their previously determined whole genome re-sequence data to investigate sequence variation at the *EDA* locus, a major locus responsible for armour plate variation (Colosimo *et al.*, 2005). Associations of the *EDA* genotypes with stoichiometric traits (Durston and El-Sabaawi, 2019) have also been investigated. Maciejewski *et al.* (2019) do not investigate the *EDA* locus directly but investigate an association between the plate phenotype and parasite load in a polymorphic population. Other papers investigate different genes that are important for physiological adaptation (Kusakabe *et al.*, 2019; Lacasse and Aubin-Horth, 2019; Shao *et al.*, 2019). It is evident that genomic tools established for sticklebacks have facilitated these molecular experiments (Kingsley and Peichel, 2007; Jones *et al.*, 2012). Wucherpfennig *et al.* (2019) have further established a protocol for genome-editing with CRISPR-Cas9 technologies, helping to bring methods available to stickleback researchers more in line with those being used with the major biomedical models. Using this technology, we can directly test the functions of each 'candidate' gene (Wucherpfennig *et al.*, 2019) and directly link phenotypic and genomic variation. These existing and newly emerged genomic resources and tools will keep sticklebacks contributing to the field of evolutionary and ecological genomics.

Third, sticklebacks have been extensively investigated in the context of speciation (McKinnon and Rundle, 2002; Nosil, 2012). No paper, however, includes keywords directly related to speciation, such as 'speciation', 'reproductive isolation', or 'mate choice' in the abstract. This is surprising because many genomic and behavioural studies using sticklebacks are still going on and are published regularly. There were also several oral presentations on speciation at the conference. One of the reasons for the discrepancy may be because people working on speciation tend to publish their papers in other journals. However, because divergent adaptation can lead to speciation (Schluter, 2000; McKinnon and Rundle, 2002; Nosil, 2012), many special issue papers are implicitly related to speciation, although authors do not explicitly use these terms. It will be interesting to see how speciation research progresses over the next three years, when the next stickleback conference will be held.

Fourth, two papers are directly related to eco-evolutionary dynamics (Candolin, 2019; Durston and El-Sabaawi, 2019). Candolin (2019) reviews how sticklebacks can modify ecological communities and ecosystem consequences. Durston and El-Sabaawi (2019) investigate how *EDA* genotypes are associated with dietary preferences, which can influence the ecological community. Although studies on eco-evolutionary dynamics were still underrepresented at the conference, eco-evo dynamics is one of the hottest topics that integrates ecology,

evolution, and genomics (Hendry, 2016). We hope that we will see more presentations and papers on eco-evolutionary dynamics in the special issue(s) devoted to the 2021 Stickleback conference.

Finally, it should be noted that some topics presented at the conference are not well covered in the present special issues. Among these are frontier areas that future stickleback research can explore. One is epigenetics and non-genomic transgenerational inheritance. Parental exposures to predators, stress, and salinity have been shown to influence the behaviour, growth, and transcriptome of offspring in sticklebacks (McGhee *et al.*, 2012; Stein and Bell, 2014; Metzger and Schulte, 2016; Heckwolf *et al.*, 2018). Among the present contributions, Lehto and Tinghitella (2019) investigate how predator exposure of parents influences offspring stress levels. Epigenetic modifications of DNA and histones and transmission of maternal RNAs and hormones may underlie non-genomic inheritance (Verhoeven *et al.*, 2016; Hu and Barrett, 2017). Although variations in DNA methylation (Smith *et al.*, 2015; Artemov *et al.*, 2017; Metzger and Schulte, 2018) and small-RNA transcriptome (Kitano *et al.*, 2013; Rastorguev *et al.*, 2017) among stickleback populations exist, we do not yet know whether these differences are transmitted to the next generation. Integration of epigenetics and non-genomic inheritance to ecology, evolution, and behaviour would be an exciting avenue for stickleback research (Aubin-Horth and Renn, 2009).

Stickleback biology can be applied profitably to the study of human diseases more often than it has been. Sticklebacks have long been used as a model for ecotoxicology (Katsiadaki, 2007; von Hippel *et al.*, 2016; Hani *et al.*, 2018) and host–pathogen interactions (Barber, 2007; Scharsack *et al.*, 2016). Because overreaction of immune systems can cause human diseases, studies on host–microbe interactions in sticklebacks may contribute to a better understanding of the genetic mechanisms underlying interpopulation and inter-individual variations in immune reactions (Milligan-Myhre *et al.*, 2016; Robertson *et al.*, 2016; Lohman *et al.*, 2017). Furthermore, mismatch between environment and genome can cause human diseases (Nesse, 2005; Crespi, 2010; Stearns and Medzhitov, 2015). Therefore, using naturally occurring stickleback populations, we will be able to investigate the genetic, environmental, and their interactive effects on metabolism, hormones, and oxidative stress response in natural systems (Kitano *et al.*, 2010; Kammer *et al.*, 2011; Kitano and Lema, 2013; Di Poi *et al.*, 2016; Kim *et al.*, 2018). Such work will contribute to evolutionary and ecological medicine. It is very exciting to see how stickleback biology will contribute to these research areas in the coming years, and how some threespine stickleback populations are being developed as biomedical models for particular diseases.

Synthesis and conclusions

It is clear from our survey of model vertebrates that the genus *Gasterosteus*, and principally the threespine stickleback, has become a pre-eminent model in evolutionary biology and remains an important system in animal behaviour. This reflects a trend of vertebrates well suited to comparative investigations supplanting biomedical models in the study of evolution and to a lesser extent behaviour; for behaviour, newer biomedical models have become important as well.

Furthermore, over the last 25 years, evolution has become relatively more important than behaviour as a focus of stickleback research, and the terms used to characterize research topics have changed. In sticklebacks and in evolutionary studies generally, topics of recently increased emphasis include the evolution of gene expression and regulation, of phenotypic plasticity, and of responses to climate change. The presentations at the recent Stickleback conference and in the present special issues reflect these trends as well as an emphasis on

genomic and genetic investigations, including genome editing methods, parasites, sticklebacks as models for specific biomedical topics, and, to a lesser extent, eco-evolutionary dynamics.

A somewhat unexpected result in our model system surveys was a flat or declining trend in the number of publications about model systems in evolutionary biology. This trend resembles results for biomedical models (Peirson *et al.*, 2017), but not animal behaviour research. In behavioural work, model systems have been consistently less important overall (although they have become more important in more recent times). Considering these observations together with our other findings, what can we infer about the future of model systems such as *Gasterosteus*?

First, it is becoming easier to obtain genomic data for little studied taxa (e.g. Matz, 2018) and this trend appears likely to continue and accelerate. Thus in a field such as evolutionary biology in which sampling across the tree of life is of interest for its own sake, reduced constraints on expanding the number of systems under study may lead to further diversification of research taxa and reduced reliance on a short list of models. In addition, it may become easier to work with systems possessing other desirable characteristics when obtaining a genome is not an obstacle. In animal behaviour, where there is a more established interest in mechanistic investigations, the impulse to diversify, while also present, may not be as strong. This interpretation begs the question of why model system use was lower in animal behaviour to begin with, and we do not have an easy answer. Possibly we missed important behavioural models in our survey; in any case, the difference between animal behaviour and evolutionary biology was not very large.

Factors acting in favour of research efforts coalescing around a limited number of models include the biological reality that some taxa are just easier to work with, possessing research-friendly traits noted earlier such as small body size and short generation times. Cultural and sociological factors that lead to 'preferential attachment' to models (Davies, 2007) include the availability of extensive background information and the training of large numbers of students and post-doctoral scientists on systems used by productive leading laboratories. A large enough research community to justify taxon-specific meetings, societies, and even resource centres and courses may lead to qualitative differences in the relative use of different taxa. Moreover, the trend towards larger teams of scientists conducting large-scale and interdisciplinary investigations may favour taxa already studied by many investigators with diverse skill sets. In light of these various factors and trends, our expectation is that *Gasterosteus* will remain an important model for a good while yet and may facilitate the continued ascendance of other Gasterosteidae.

In future bibliometric studies of models in non-biomedical fields of biology, it will be informative to use more inductive and inclusive approaches so as not to potentially miss important taxa or topics. It would also be interesting to try to tease apart the influence of biological versus sociological factors. In conclusion, we hope that sticklebacks will continue to be a leading model for the study of not only behaviour and evolution, but also more diverse research fields. We look forward to seeing more progress in a wide range of fields at the Tenth International Conference on Stickleback Behaviour and Evolution in Iceland in 2021.

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