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REVIEW

Genetic variability in reaction norms in fishes

Rebekah A. Oomen and Jeffrey A. Hutchings

Abstract: The ability of populations to adapt to environmental change and the spatial scale at which this adaptation occurs are fundamentally important issues in evolutionary biology, and ones that may benefit greatly from the study of genetic variability in reaction norms, which represent the plasticity of phenotypic traits across an environmental gradient. Therefore variable reaction norms can reflect genetic differences in the ability of individuals, families, populations, and species to respond to natural and anthropogenic environmental change. Fishes are ideal organisms in which to study plasticity because of their remarkable intraspecific morphological, physiological, behavioural, and life history variation. Here, we review studies demonstrating genetic variability in reaction norms in fishes. Genetic variability in plasticity among full- and half-sib families suggests potential for some populations to develop an adaptive norm of reaction (recalling that plasticity need not be adaptive). Reaction norm variability among populations suggests that adaptive genetic divergence can occur rapidly when selection pressures are strong and that the spatial scale of adaptation is much smaller than previously believed for some species with high dispersal capabilities. These studies demonstrate the potential of using reaction norms to study the evolution of novel phenotypes and the influence of temporal environmental variability and gene flow on the evolution of phenotypic plasticity, which can then be used to predict how populations will respond to directional environmental change. To promote future research into genetic variability in reaction norms, we propose questions that would benefit from such an approach and discuss some important considerations for designing experiments to investigate questions related to genetic variation in plasticity and phenotypic evolution.

Key words: phenotypic plasticity, genotype–environment interaction, adaptation, environmental change.

Résumé : La capacité d'adaptation au changement environnemental et l'échelle spatiale à laquelle cette adaptation survient, constituent des défis d'importance fondamentale en biologie évolutive, un sujet qui pourrait bénéficier grandement de l'étude de la variabilité des normes de réaction. Les normes de réaction représentent la plasticité des traits phénotypiques le long d'un gradient environnemental. Conséquemment, des normes de réaction variables peuvent refléter des différences génétiques dans la capacité des individus, des familles, des populations et des espèces à réagir au changement environnemental naturel et anthropogène. Les poissons constituent des organismes idéaux pour l'étude de la plasticité compte tenu de la variation intraspécifique remarquable de leur morphologie, de leur physiologie, de leur comportement et de leur cycle de vie. Les auteurs passent en revue les études démontrant la variabilité génétique des normes de réaction chez les poissons. La variabilité de la plasticité parmi les familles biparentales ou mono parentales suggère la capacité de certaines populations à développer une norme de réaction adaptative (en se rappelant que la plasticité ne doit pas nécessairement être adaptative). La variabilité des normes de réaction parmi les populations suggère qu'une divergence génétique adaptative peut se manifester rapidement, lorsque les pressions de sélection sont fortes et que l'échelle spatiale d'adaptation est beaucoup plus petite qu'on le croyait, chez certaines espèces ayant de fortes capacités de dispersion. Ces études démontrent que le potentiel de l'utilisation des normes de réaction pour étudier l'évolution de nouveaux phénotypes et l'influence de la variabilité temporelle environnementale ainsi que flux de gènes sur l'évolution de la plasticité phénotypique, lesquels peuvent alors être utilisés pour prédire comment les populations répondront au changement environnemental directionnel. Pour promouvoir des recherches futures sur la variabilité génétique des normes de réaction, les auteurs proposent quelques questions qui bénéficieraient d'une telle approche, et discutent quelques considérations importantes pour concevoir des expériences soulevant des questions reliées à la variation génétique de la plasticité de l'évolution phénotypique. [Traduit par la Rédaction]

Mots-clés : plasticité phénotypique, interaction génotype environnement, adaptation, changement environnemental.

Introduction

The ability of individuals or populations to respond to environmental change is integral to species persistence. If a fitness-related trait is optimally expressed in specific environmental conditions, then a change in the environment will cause a reduction in individual fitness (Ghalambor et al. 2007). Yet, much is not known about the means by which genetic and environmental variability interact to produce flexible phenotypes. Improved knowledge of the capacity for phenotypic change and how this capacity evolves will provide a fundamentally important empirical basis for predicting how natural and anthropogenic environmental variability will affect animal populations and enhance species persistence through evolutionarily meaningful management strategies. In this regard, two prevailing issues have emerged concerning (*i*) the ability of populations to adapt to environmental change (largely dependent on the level of phenotypic plasticity displayed within a population), and (*ii*) the spatial scale at which these responses occur (i.e., whether there are population-level genetic differences in plasticity) (Hutchings et al. 2007). These matters can be addressed by examining genetically based differences in the types and ranges of phenotypic responses that occur within a species

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across environmental gradients (i.e., genetic variability in reaction norms).

Originated as *Reaktionsnorm* by German biologist R. Woltereck (Woltereck 1909; Sarkar 1999), the definition of "reaction norm" in the literature is itself rather flexible. In the context of this review, it will be defined as the range of phenotypes expressed by a genotype along an environmental gradient (Scheiner 1993; Via et al. 1995; Schlichting and Pigliucci 1998). Graphically, a reaction norm is represented by a linear or nonlinear function describing the pattern of phenotypic expression of a genotype in different environments (Fig. 1). The elevation of a reaction norm is related to the average response value of a trait in the range of environments tested, whereas the slope of a reaction norm represents the amount of plasticity in that trait (Lande 2009; Dingemanse et al. 2010). We assume the plasticity of a reaction norm to be a trait upon which selection can act directly (Bradshaw 1965; Schlichting and Levin 1986; Lande 2009; Chevin et al. 2010). This view is supported by empirical evidence demonstrating selection acting on the slopes of reaction norms (e.g., Scheiner 2002; Nussey et al. 2005) and studies showing that trait means and plasticities can evolve separately (Schlichting and Levin 1986; Joshi and Mueller 1993; see de Jong 1995 for further discussion of plasticity as a trait).

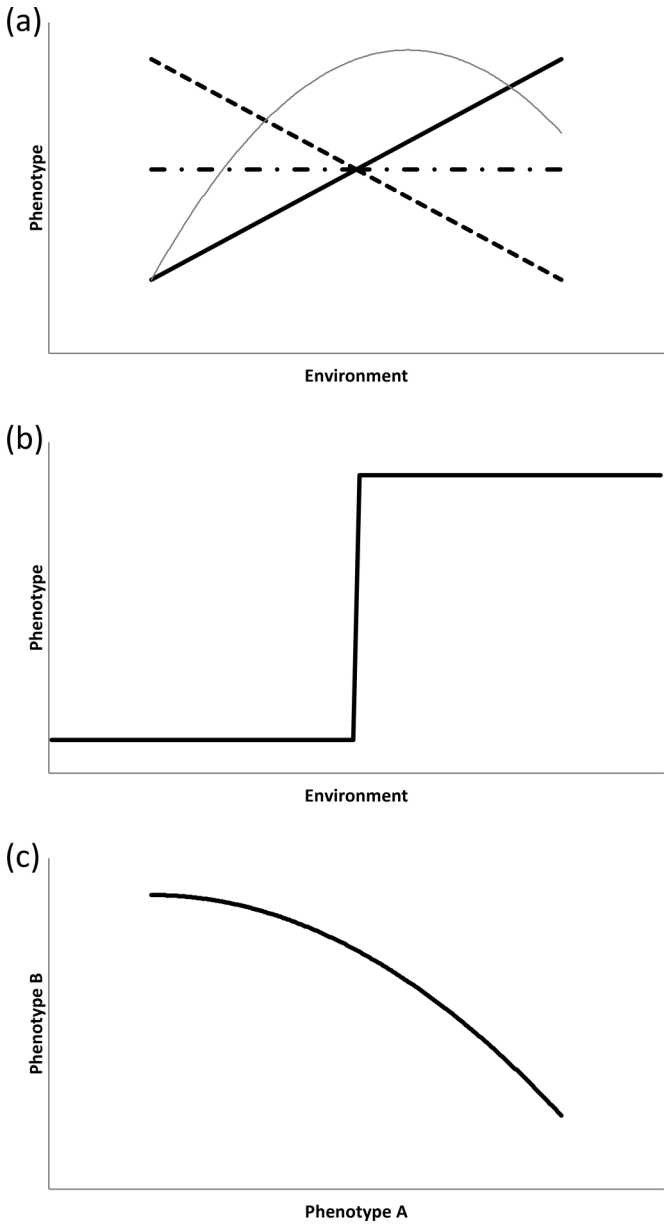
More than a century has passed since the reaction norm concept was introduced, however, its prevalence in the literature is relatively recent. In a keyword search analysis in Hutchings' (2011) review of reaction norm research in salmonid fishes, of which our review serves as a natural extension to include other species in this highly diverse group of vertebrates, Hutchings found studies on reaction norms to be rare prior to the 1990s for most taxa and the 2000s for fishes.

Indeed, of the studies included here, none predate 1985 and the majority was published in the last five years. Some of the earliest studies depicting genetic variability in reaction norms do not mention the term "reaction norm" at all (e.g., Beacham and Murray 1985; Conover and Present 1990), which underscores the difficulty of encompassing all existing studies of this nature in any review. Instead, reaction norms are often described in terms of phenotypic plasticity or genotype-by-environment interactions. Phenotypic plasticity (evident when a particular genotype is capable of producing different phenotypes in different environments; Gause 1947; Via and Lande 1985) is manifested by a significantly nonzero reaction norm slope. However, reaction norms are not necessarily plastic (see Fig. 1*a* for an example of a non-plastic reaction norm). A genotype-by-environment interaction (evident when genotypes produce different patterns of phenotypic expression across environments; Via and Lande 1985; Latta 2010) occurs when reaction norm slopes or shapes differ significantly among genotypes.

Here, we illustrate the potential of using reaction norms to answer questions related to genetic variability and the evolution of plasticity through studies on fishes, a highly plastic, and the most speciose, group of vertebrates. In addition to fishes showing remarkable morphological, physiological, behavioural, and life history variation both within and among species, many fishes are relatively easy to capture from the wild and manipulate in the laboratory compared to other vertebrates, thus permitting the study of natural populations of highly complex organisms.

Herein, we describe different types of reaction norms and the primary experimental means by which they were determined. Empirical evidence of genetic variability in reaction norms among families and populations of fishes follows. Finally, we discuss key experimental and analytical considerations when studying reaction norms and pose several research questions that could be explored using this approach. For a detailed discussion of theoretical issues within the reaction norm framework, we refer the reader to Hutchings (2011).

Fig. 1. Hypothetical reaction norms. (*a*) Continuous linear reaction norms for (solid black line) genotype A and (dashed line) genotype B and (grey line) a curvilinear reaction norm for genotype C illustrate a plastic response, whereas (dot-dashed line) the reaction norm for genotype D does not exhibit plasticity. (*b*) A discontinuous (threshold) reaction norm. (*c*) A bivariate reaction norm.



Types of reaction norms

Continuous and discontinuous trait plasticity

Reaction norms are referred to as continuous if the phenotypic value of a trait can vary continuously with changes in the environment (Fig. 1*a*). Discontinuous reaction norms are used to represent threshold traits, which are traits for which one phenotype is adopted when an individual's size or condition exceeds a genetically determined threshold, while a different phenotype is adopted when the threshold is not met (Fig. 1*b*; Hazel and Smock 1990; Hutchings 2011). Most demonstrations of genetic differences in threshold reaction norms examine the adoption of alternative life histories in salmonids (reviewed by Hutchings 2011), while there is a lack of empirically constructed threshold norms of reaction in other fishes.

Table 1. Research on fishes in which putative genetic variability in reaction norms is hypothesized to exist (modified from Hutchings 2011).

Species	Variables	Scale of differentiation	References
Acanthochromis polyacanthus	Resting metabolic rate; temperature	Population	Donelson and Munday (2012)
Anguilla rostrata	Gene transcription; salinity	Population	Côté et al. (2014)
Atherinops affinis	Growth rate and conversion efficiency; temperature	Population; species	Baumann and Conover (2011)
Brachyrhaphis rhabdophora	Growth; food availability	Population	Gale et al. (2013)
Fundulus heteroclitus	Growth rate; temperature	Subspecies	Schultz et al. (1996)
F. notatus	Metabolic rate; temperature	Rearing temperature; population; species	Schaefer and Walters (2010)
F. olivaceus	Metabolic rate; temperature	Population; species	Schaefer and Walters (2010)
Gadus morhua	Hepatosomatic index; temperature	Population	Purchase and Brown (2001)
Gadus morhua	Sperm performance; temperature; time	Individual; family; population	Purchase et al. (2010); Beirão et al. (2014)
Gadus morhua	Morphology; temperature and food availability	Population	Marcil et al. (2006*a*, 2006*b*)
Gadus morhua	Larval survival and growth; temperature and food availability	Population	Hutchings et al. (2007)
Gadus morhua	Juvenile survival and growth; temperature	Population	Wijekoon et al. (2009)
Gadus morhua	Larval survival and growth; temperature	Population	Oomen and Hutchings in press
Gambusia holbrooki	Several life-history traits; food availability	Family	Weeks and Meffe (1996)
Gasterosteus aculeatus	Survival, growth and gene transcription; salinity	Family; population	McCairns and Bernatchez (2010)
Menidia menidia	Growth rate and conversion efficiency; temperature	Population; species	Conover and Present (1990); Baumann and Conover (2011)
M. peninsulae	Growth rate; temperature	Population; species	Yamahira and Conover (2002)
Morone saxatilis	Growth rate; temperature	Population	Conover et al. (1997)
Oncorhynchus gorbuscha	Early-life traits; temperature	Family; population; species	Beacham and Murray (1986*a*, 1990); Beacham (1988)
O. keta	Early-life traits; temperature	Family; population; species	Beacham and Murray (1985, 1990); Murray and Beacham (1987); Beacham (1988)
O. keta	Meristics; temperature	Life-history morph; population	Beacham and Murray (1986*b*); Ando et al. (2011)
O. keta	Vertebral number; temperature	Family	Ando et al. (2011)
O. kisutch	Early-life traits; temperature	Family; population; species	Murray et al. (1990); Beacham and Murray (1990)
O. nerka	Early-life traits; temperature	Family; population; species	Beacham and Murray (1989, 1990); Hendry et al. (1998); Burt et al. (2012)
O. tshawytscha	Early-life traits; temperature	Family; population	Evans et al. (2010); Beacham and Murray (1989)
O. tshawytscha	Early-life traits; temperature	Family; species	Murray and Beacham (1987); Beacham and Murray (1990); Kinnison et al. (1998)
Oryzias latipes	Growth rate; temperature	Family; population	Yamahira et al. (2007)
Platichthys flesus	Gene transcription; salinity	Population	Larsen et al. (2008)
Pseudocrenilabrus multicolour victoriae	Brain mass; dissolved oxygen	Population	Chapman et al. 2008, Crispo and Chapman (2010)
Pseudocrenilabrus multicolour victoriae	Body shape; dissolved oxygen	Population	Crispo and Chapman (2011)
Salmo salar	Trypsin; temperature	Individual	Rungruangsak-Torrissen et al. (1998)
Salmo salar	Early survival; pH	Population cross; population	Fraser et al. (2008)
Salmo salar	Several early-life traits; temperature	Population cross	Darwish and Hutchings (2009)
Salmo salar	Size and condition; compensatory growth	Population cross	Fraser et al. (2007)
Salmo salar	Early-life traits; dissolved oxygen	Family; population	Côte et al. (2012)
Salmo salar	Growth; competition	Population cross; population	Solberg et al. (2013)
S. trutta	Lactate dehydrogenase in muscle; temperature	Life-history morph	Andreeva et al. (1996)
S. trutta	Several early-life traits; temperature	Population	Jensen et al. (2008)
Salvelinus alpinus	Morphology; swimming speed	Species	Peres-Neto and Magnan (2004)
S. fontinalis	Life history traits; growth	Population	Hutchings (1993, 1996)
S. fontinalis	Morphology; swimming speed	Species	Peres-Neto and Magnan (2004)
S. fontinalis	Morphology	Life-history morph	Proulx and Magnan (2004)
S. fontinalis	Gene transcription and growth; salinity	Individual	Côté et al. (2007)
Snyderichthyes copei	Growth rate; temperature	Population	Belk et al. (2005)
Thymallus thymallus	Several early-life traits; temperature	Population	Haugen and Vøllestad (2000)

Uni-, bi-, and multivariate trait plasticity

In addition to being either continuous or discontinuous, reaction norms are also described in the literature as being univariate, bivariate, or multivariate. A univariate reaction norm is constructed by plotting the value of a phenotypic trait against an environmental variable (Fig. 1*a*). However, in the case of bi- or multivariate reaction norms, phenotypic traits are plotted against another trait that is used as a proxy for general environmental conditions (Fig. 1*c*). Most of the research on bivariate reaction norms in fishes involves probabilistic maturation reaction norms (PMRNs), which describe the probability of becoming mature as a function of individual age and size (Heino et al. 2002; Kuparinen and Merilä 2007; Heino and Dieckmann 2008). However, PMRNs do not consider independent environmental variables, but rather assume that the growth trajectory of an individual is directly related to the combined environmental conditions faced by that individual. For this reason, the suitability of the term "reaction norm" to describe this type of relationship in the classical sense (sensu Woltereck 1909) could be questioned and PMRNs are excluded from the present review.

Common-garden experiments

To isolate the genetic basis of phenotypic variation, it is necessary to control for environmental influences. Common-garden experimental protocols and variations thereof, such as reciprocal-transplant experiments, are some of the most effective means of doing so (Conover and Baumann 2009). In common-garden experiments, individuals from putatively different genetic groups (e.g., families or populations) are raised under identical environmental conditions so that any variability in reaction norms or phenotypes is attributed to genetic differences between groups. The use of multiple environments across a gradient allows for the determination of whether observed phenotypic differences are due to differences in genotypes, the environment, or their interaction.

Genetic variability in reaction norms

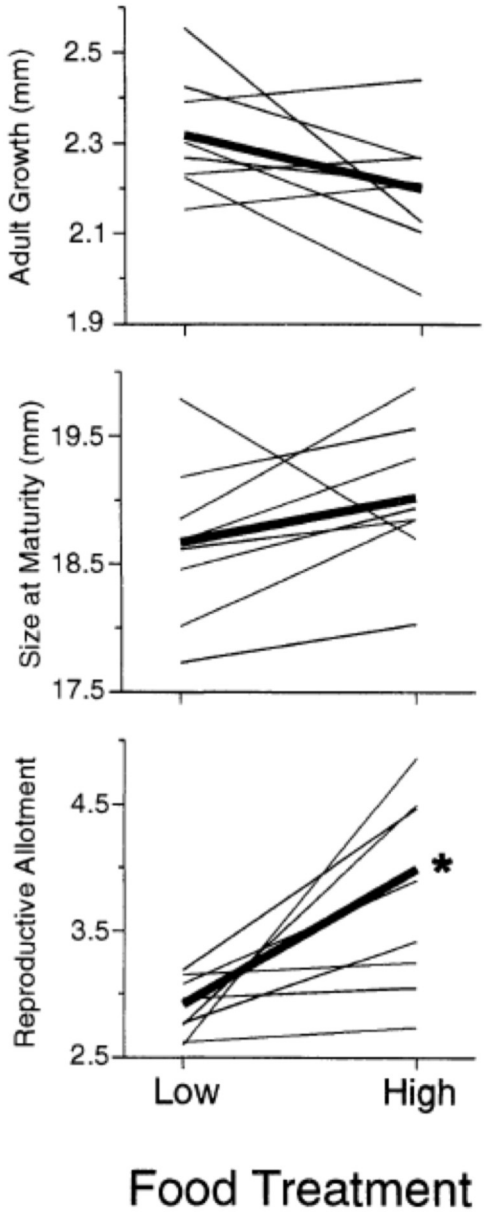
To provide an estimation of the extant amount of research on reaction norm variability in fishes, we searched the ISI Web of Science using the keywords "reaction norm" and "fish" or "fishes". Of the 75 resulting papers, 33 were relevant to reaction norms in fishes (excluding PMRNs and studies on other organisms). Of these, 16 described experiments capable of detecting genetic variability in plasticity along an environmental gradient in fishes and six of these were on salmonid fishes. An additional 15 papers not detected by the keyword search were found to demonstrate intraspecific genetic differences in reaction norms in fishes and were included in this review, bringing the total number of studies on non-salmonid fishes to 25.

Among these studies, essentially all found genetic differences at some level (e.g., individual, family, population, or species; Table 1). Although one must be cognizant of biases against the publication of negative results, we would argue against the prevalence of such a bias here because some studies were not conducted for the purpose of testing for variability in plasticity and we have redrawn the data herein to allow for the construction and assessment of reaction norms (e.g., Purchase and Brown 2001; Larsen et al. 2008).

Individual- and family-level differences in reaction norms

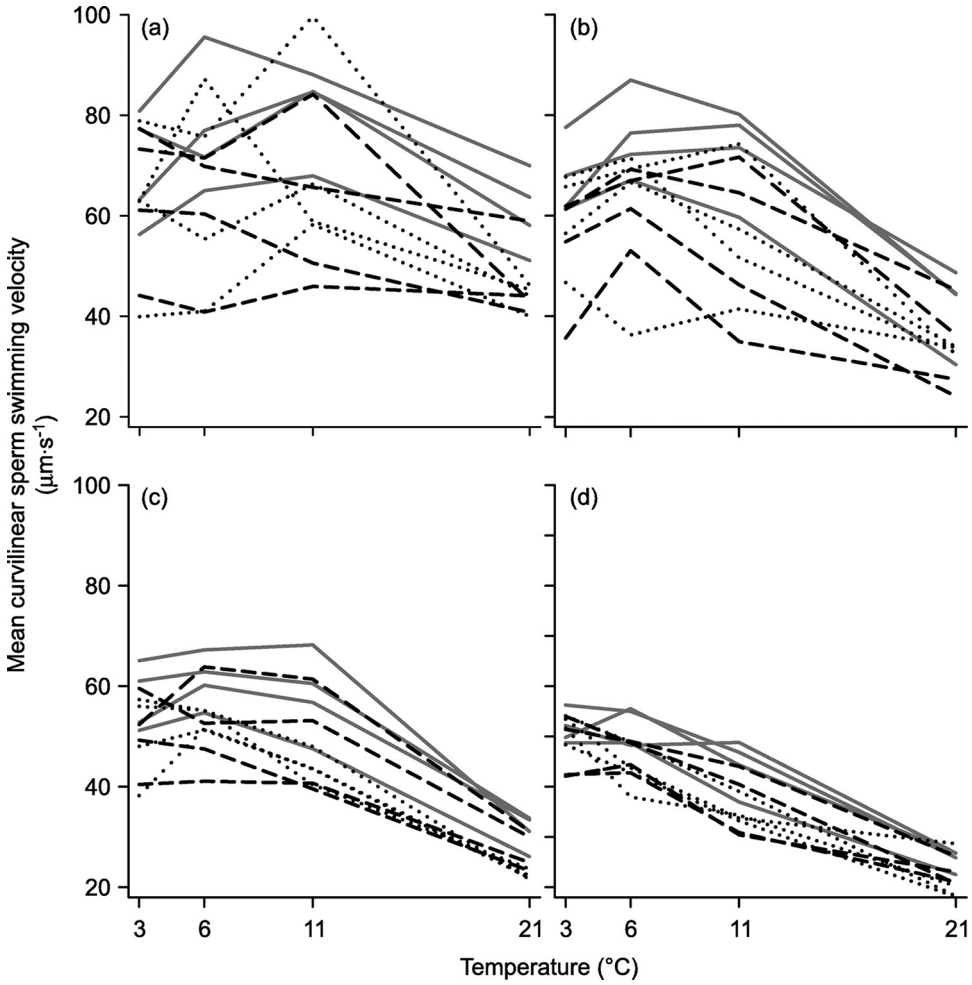
Perhaps the first evidence of family-level genetic variability in fishes other than salmonids was provided by Weeks and Meffe (1996) who tested for adaptive phenotypic plasticity in a population of mosquitofish (*Gambusia holbrooki*) that had a history of being exposed to extreme fluctuations in both temperature and, consequently, food availability. Using a common-garden protocol with half-sib families exposed to different levels of food availability, the authors revealed adaptive plasticity (i.e., the responses were in the direction predicted by optimality models) for six of nine life-history traits examined. Further, significant additive genetic variation in plasticity for adult growth, size at maturity, and reproductive allotment (Fig. 2) suggests the capacity to shape an adaptive norm of reaction for these traits.

Fig. 2. Reaction norms for life-history traits showing significant additive genetic variation in plasticity among half-sib families of mosquitofish (*Gambusia holbrooki*) in response to food availability. Thick lines show the average response of all families and asterisks denote a significant ($P < 0.05$) average plastic response (modified from Weeks and Meffe 1996).



McCairns and Bernatchez (2010) compared the responses of threespine sticklebacks (*Gasterosteus aculeatus*) from freshwater and marine environments to different salinities. They found significant variation in reaction norm slopes for larval and juvenile survival among families from the freshwater population only, whereas families from salt water showed no genetic variability. The authors did, however, report family-level variability within the saltwater deme for expression of some candidate genes for osmoregulation.

Fig. 3. Reaction norms for sperm swimming velocity for three Atlantic cod (*Gadus morhua*) families at four temperatures (3, 6, 11, and 21 °C). Swimming velocities at assigned elapsed time periods since sperm activation are shown as: (*a*) 30, (*b*) 60, (*c*) 120, and (*d*) 180 s. Values shown are individual genotype averages among procedural replicates. Shaded continuous, family A; dotted lines, family B; broken lines, family C. Genotype-by-environment interactions are significant ($P < 0.05$) (original source: Purchase et al. 2010).



Purchase et al. (2010) documented no family-level differences in reaction norms in their examination of sperm performance in Atlantic cod (*Gadus morhua*). However, they did detect differences in reaction norms among individuals, whereby the sperm essentially imitated clonal replicates. The authors found greater variation in thermal reaction norms for sperm swimming velocity among individuals within a family than among families (Fig. 3), which they suggest may be due to high individual variability in the timing of peak sperm performance throughout the spawning season. Aside from providing a rare example of individual-level variability in plasticity, this experiment uniquely demonstrated individual variation in "plasticity within plasticity"; the pattern of the phenotypic response to temperature depended on the amount of time the sperm had been swimming.

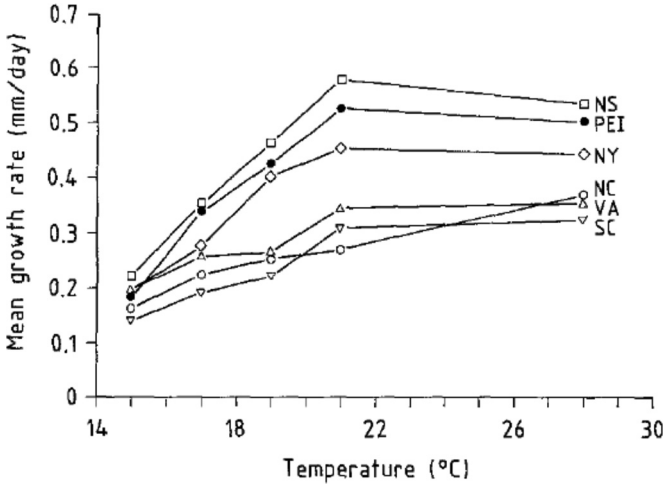
Though studies demonstrating individual and family-level genetic variability in reaction norms in fishes are relatively few, the amount of variability observed at these fine genetic scales provides insight into the adaptive potential of populations and suggests there may be substantial variation at larger genetic scales.

Population-level differences in reaction norms

Some of the earliest work documenting population variability in reaction norms in fishes was that of Conover and Present (1990) who provided the first evidence of countergradient variation in the genetic capacity for growth rate in fishes (Atlantic silversides, *Menidia menidia*). Countergradient variation is a type of cryptic genetic variation in which a phenotypically plastic trait is distributed such that it counteracts the effects of an environmental gradient (Conover and Baumann 2009), resulting in phenotypes that appear similar across environments when genotypes are not. Common-garden experiments undertaken by Conover and Heins (1987) and Conover and Present (1990) showed that, in addition to a clear trend towards higher growth rates in silversides from higher latitudes, significant variability among populations in the slopes of growth rate reaction norms resulted in more divergent growth rates at higher temperatures (Fig. 4). Intraspecific variability in reaction norms has also provided evidence for countergradient variation in growth in mummichog (*Fundulus heteroclitus*; Schultz et al. 1996), striped bass (*Morone saxatilis*; Conover et al. 1997), tidewater silverside (*Menidia peninsulae*; Yamahira and Conover 2002), leatherside chub (*Snyderichthyes copei*; Belk et al. 2005), Japanese killifish (*Oryzias latipes*; Yamahira et al. 2007), and Atlantic cod (Hutchings et al. 2007).

A deeper investigation into countergradient variation by Baumann and Conover (2011) compared earlier work on Atlantic silversides with a similar experiment conducted on ecologically equivalent Pacific silversides (*Atherinops affinis*). As in Atlantic silversides, population-level variability in reaction norms for growth rate was

Fig. 4. Reaction norms for average growth rate of six Atlantic silverside (*M. menidia*) populations reared in the laboratory at five different temperatures (15, 17, 19, 21, and 28 °C). NS, Nova Scotia; PEI, Prince Edward Island; NY, New York; NC, North Carolina; VA, Virginia; SC, South Carolina (original source: Conover and Present 1990).



evident in Pacific silversides. However, the pattern of variability reflected the climate gradient experienced by each species: Atlantic silversides, which normally experience a strong and highly seasonal climate gradient, were more plastic and showed increasing growth plasticity with latitude, whereas Pacific silversides were less plastic and their growth plasticity was latitude-independent, reflecting the weak latitudinal temperature gradient in the Pacific.

An apparently less common phenomenon is cogradient variation, wherein genetic differences between populations accentuate environmental plasticity (Conover and Schultz 1995). Beirão et al. (2014) documented cogradient variation in thermal reaction norms for sperm performance in cod, wherein performance increased with temperature more in the southern population. Interestingly, geographic origin had a greater influence on the plasticity of sperm characteristics than environmental history (i.e., whether they were reared in sea cages or indoor tanks).

Experiments on Atlantic cod larvae from the northwest Atlantic revealed substantial population-level differences in the plastic responses of larval growth and survival to temperature (Oomen and Hutchings in press) and food availability (Hutchings et al. 2007). Divergent slopes in survival reaction norms suggest that populations that experience higher temperatures during the larval stage exhibit greater plasticity in response to changes in food availability whereas populations that experience colder temperatures in early life are more sensitive to temperature (Fig. 5; Hutchings et al. 2007). However, the timing of the spawning season appears to complexify this relationship, with winter- and fall-spawning populations showing positive and negative relationships between survival and temperature, respectively, as well as vastly different growth responses (Fig. 6; Oomen and Hutchings in press). These findings suggest that intraspecific variation in reproductive timing may play an important role in thermal adaptation of early life stages and demonstrate that even a species with high dispersal capabilities (e.g., a broadcast-spawning fish) can have marked genetic differences in plastic responses at relatively small spatial scales (<200 km). Differences in body shape plasticity have also been documented between two cod spawning components from the southwestern Scotian Shelf located <100 km apart, although the adaptive significance of these differences is unclear (Marcil et al. 2006*a*).

Genetic differences in larval cod reaction norms were detected despite a lack of genetic differentiation based on selectively neutral genetic markers (microsatellites) (Hutchings et al. 2007; Oomen and Hutchings in press). This contrast supports the hypothesis that adaptive genetic divergence can occur much more rapidly than neutral genetic divergence if selection pressures are strong, even when gene flow is high (Conover et al. 2006). Further support for this hypothesis stems from a study on the highly panmictic American eel (*Anguilla rostrata*; Côté et al. 2014). The authors found evidence of potentially genetic differences in plasticity in gene expression between eels originating from different salinity environments. Although the relative contribution of epigenetic effects could not be discerned because of the use of wild (as opposed to laboratory-reared) individuals, there is a strong argument that barriers to dispersal are not necessary for adaptive divergence in plasticity to occur in this, and likely other, marine species.

Wijekoon et al. (2009) used cod obtained from the same egg batches as Hutchings et al. (2007) to study countergradient variation at the juvenile stage. It is apparent that both the slopes and

Fig. 5. Norms of reaction for (*a*–*d*) larval growth and (*e*–*h*) larval survival of four Atlantic cod populations (mean ±1 s.e. for each treatment). Reaction norms for growth at (*a*) low and (*b*) high temperature and at (*c*) low and (*d*) high food supply. Reaction norms for survival at (*e*) low and (*f*) high temperature and at (*g*) low and (*h*) high food supply. Reaction norms represent (solid lines) 4× cod, (dotted lines) 3L cod, (dashed lines) 3Ps cod, and (dot-dashed lines) 4T cod (modified from Hutchings et al. 2007).

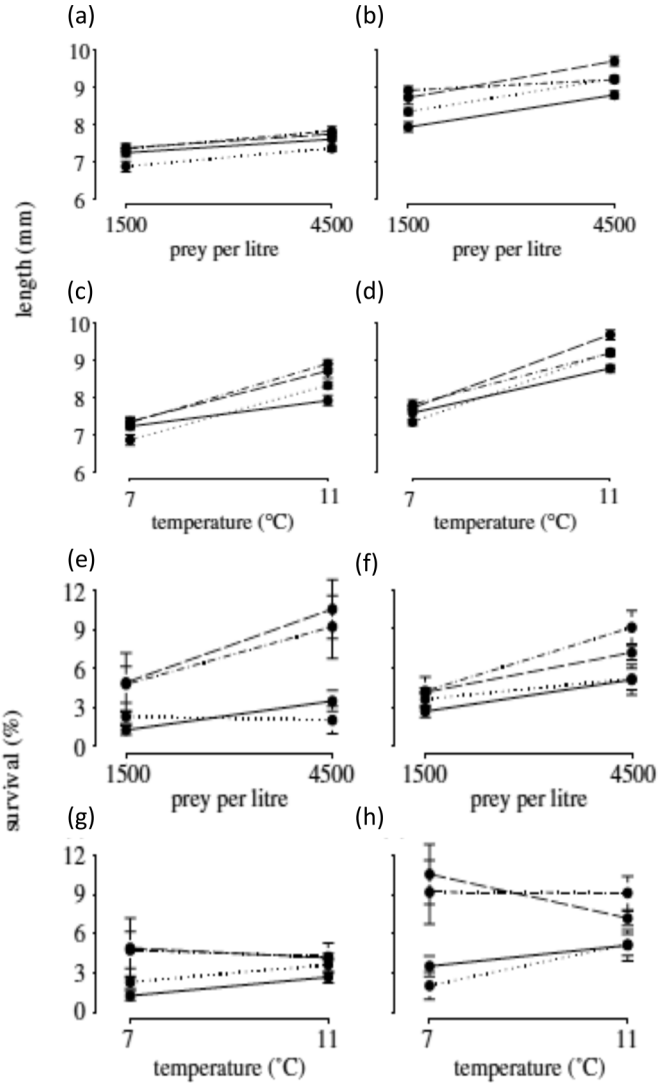


Fig. 6. Standardized thermal reaction norms for larval cod (*a*) growth and (*b*) survival (±1 s.e.) for five northwest Atlantic cod (*Gadus morhua*) populations with different spawning times: spring (Bonavista, Placentia, and Southern Gulf), winter (Fundy), and autumn (Sambro) (original source: Oomen and Hutchings in press).

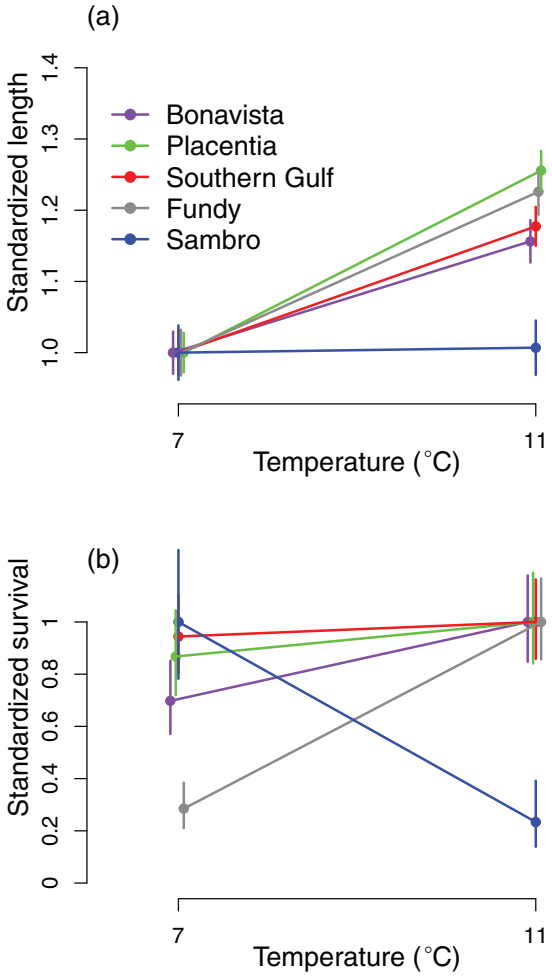
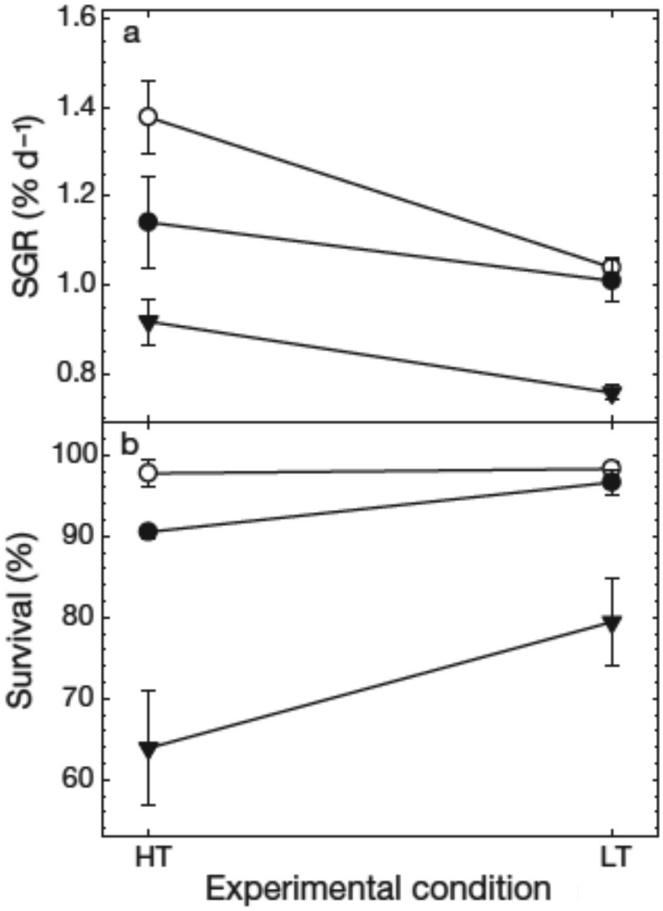


Fig. 7. Norms of reaction for (*a*) specific growth rate (SGR) and (*b*) survival of (○) 3Ps, (●) 4T, and (▼) 4× juvenile Atlantic cod (*Gadus morhua*) reared under high (HT) and low (LT) temperatures (± standard deviation) during the 15-week experimental period (original source: Wijekoon et al. 2009).



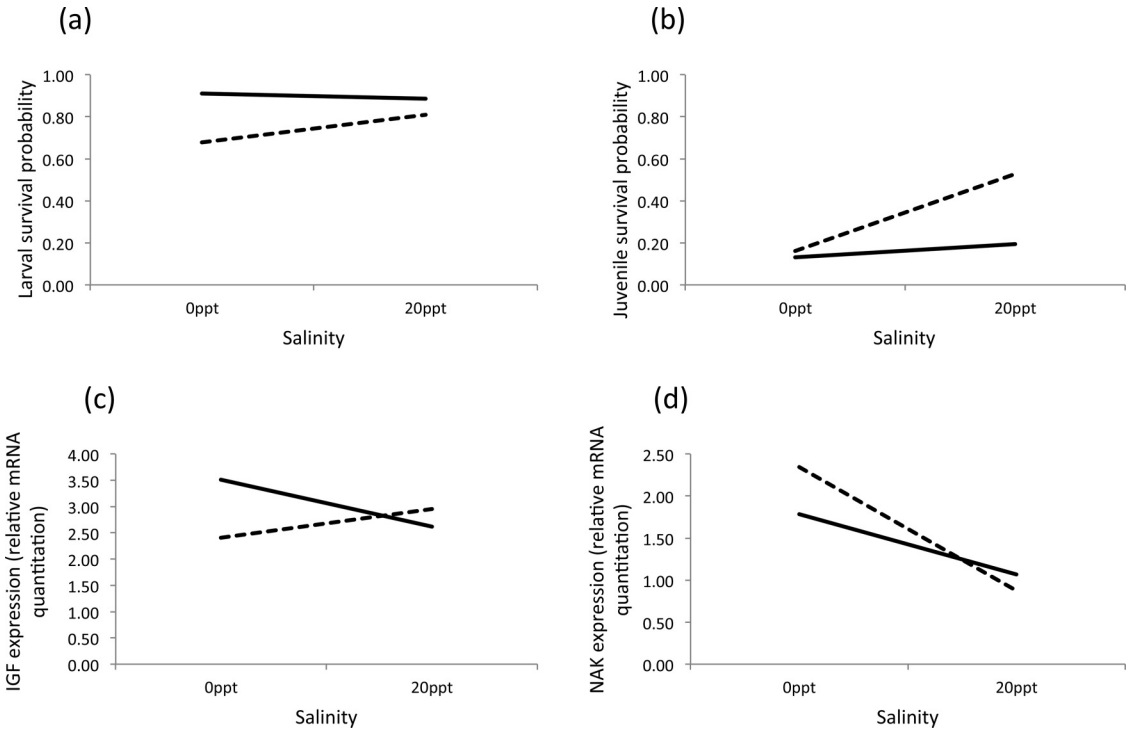
elevations of the reaction norms constructed for specific growth rate and survival varied among populations (Fig. 7). However, genotype-by-environment interactions were not reported and the significance of these genetic differences is unclear given the vast amount of within-population variability observed in this study. The high intrapopulation variability observed is partially a consequence of the analytical methodology (e.g., growth rates averaged over time despite high temporal variation in growth rate), rather than true genetic variability within populations. Nevertheless, reaction norms for survival suggest that the southernmost population experienced greater sensitivity to temperature changes, lower survival, and greater intrapopulation variation in survival. Greater temperature sensitivity of the southern-most population was evident at both the larval (Hutchings et al. 2007) and juvenile (Wijekoon et al. 2009) stages, though curiously the slopes of these reaction norms were opposite in direction. The different levels of genetic variability and contrasting reaction norm slopes observed in these studies may exemplify how reaction norms can differ between life stages. Alternatively, high levels of error may be obscuring differences in the juvenile study (Wijekoon et al. 2009), or a combination of both effects may have occurred.

McCairns and Bernatchez (2010) reported different responses to salinity between freshwater and saltwater stickleback populations (Fig. 8). This variation is attributed to a loss of plasticity in the derived freshwater deme and provides indirect evidence of genetic assimilation, a rarely documented mechanism by which an environmentally induced phenotype becomes canalized such that the environmental stimulus is no longer necessary for expression in subsequent generations (Waddington 1942; Crispo 2007). Interestingly, these traits corresponded with those that exhibited variability in reaction norms at the family level in one of the populations, although there is no apparent correlation between family-level variability and population-level plasticity.

Schaefer and Walters (2010) demonstrated genetic differences in metabolic rate plasticity in response to temperature among northern and southern populations of the blackstripe topminnow (*Fundulus notatus*) and blackspotted topminnow (*F. olivaceus*). A significant temperature-by-population interaction was observed among northern and southern populations of *F. notatus* but not *F. olivaceus*, which exhibited similar reaction norm slopes (Fig. 9). However, the shapes of the reaction norms for both species were linear in northern populations and nonlinear in southern populations. Interspecific differences observed by Schaefer and Walters (2010) suggest that even closely related species that are ecologically similar and have largely overlapping geographic ranges can experience differential adaptive genetic divergence.

Chapman et al. (2008) and Crispo and Chapman (2010) confirmed population variability in norms of reaction for brain mass in response to varying oxygen levels in the African cichlid (*Pseudocrenilabrus multicolour victoriae*). When comparing six populations from sites characterized by a range of oxygen environments (i.e., hypoxic

Fig. 8. Norms of reaction for (*a*) larval survival, (*b*) juvenile survival, (*c*) relative gene expression of insulin-like growth factor (IGF), and (*d*) relative gene expression of sodium–potassium ATPase (NAK) for threespine sticklebacks (*Gasterosteus aculeatus*) in response to freshwater (<1%) and saltwater (20%) environments. Reaction norms represent pure crosses of sticklebacks from (solid line) freshwater and (dashed line) saltwater demes. Genotype-by-environment interactions are significant ($P < 0.05$) (redrawn from McCairns and Bernatchez 2010).

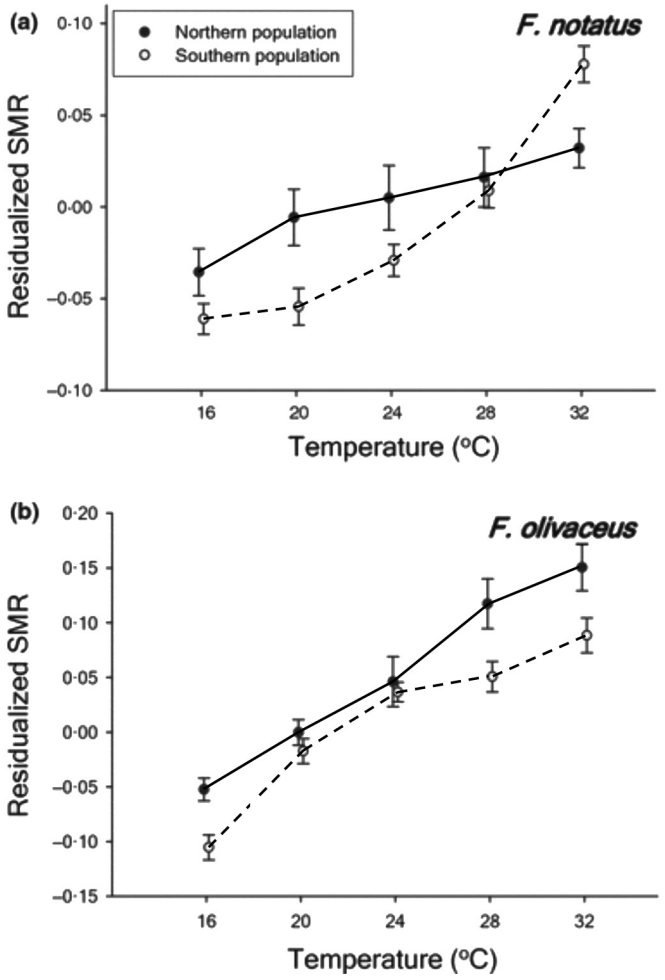


swamp, junction where swamp meets river with higher but fluctuating oxygen levels, and river far upstream of junction with high oxygen levels), differences in brain mass plasticity were greatest between populations located near the river–swamp junction and populations located further from the junction (Fig. 10). Greater plasticity near the river–swamp junction is suggested to be due to high levels of gene flow between low-oxygen and high-oxygen environments (Crispo and Chapman 2010), but it may also represent an adaptation to seasonal variability in dissolved oxygen levels. Alternatively, the lower plasticities observed in populations located further from the junction could be attributable to constraints on the trait value, as these reaction norms had the highest elevations.

Reaction norms for body shape metrics also revealed significant variability in plasticity among the same populations (Crispo and Chapman 2011). In contrast to the preceding results, plasticity for head size was greatest for populations located furthest from the river–swamp junction. While these somewhat opposing results may be due to the authors' findings that gill size and the direct effects of oxygen have a greater influence on overall head size than brain mass, this finding warrants further investigation into variation in plasticity of correlated traits. In both studies, the oxygen environment of origin failed to explain the genetic differences in plasticity that were observed among populations, suggesting that gene flow or oxygen-level fluctuations may be playing a larger role in the evolution of plasticity in these traits than the natal oxygen environment. Another study comparing populations that experience different levels of environmental variability reported that *Brachyrhaphis rhabdophora* from high-predation environments exhibited greater growth rate plasticity in response to food availability compared to fish from low-predation environments, suggesting adaptive plasticity in response to highly resource-variable environments (Gale et al. 2013).

Acclimation experiments have also provided some evidence of genetic differences in plasticity at the population level. For example, Purchase and Brown (2001) demonstrated genetically based differences in hepatosomatic index (a measure of energy storage) between laboratory-reared Gulf of Maine and Grand Banks cod in response to changes in temperature, whereby Gulf of Maine cod generally had a higher average hepatosomatic index than Grand Banks cod, yet Gulf of Maine cod appeared to be more sensitive to increasing temperature. In another acclimation experiment, Larsen et al. (2008) revealed putative genetic differences between North Sea and Baltic Sea European flounder (*Platichthys flesus*) populations in levels of *hsp70* transcription (a heat shock protein related to thermal and osmotic stress in fishes; Sorensen et al. 2001) in response to changes in salinity (Fig. 11). Although there appeared to be significant divergence in population reaction norms in both short-term and long-term acclimation experiments, these differences may not be purely genetic, because North Sea flounder experienced a decrease in salinity while Baltic Sea flounder experienced an equal-but-opposite increase in salinity. Accounting for the difference in the direction of the environmental change experienced, both populations responded similarly to the shock of the change in the short term. However, true population differences arose in the long-term experiment, in which the North Sea population maintained a stress response while the Baltic Sea population no longer exhibited an increased level of stress compared to that experienced at its native salinity. Interestingly, the Baltic Sea population experiences larger fluctuations in salinity in its native environment compared to the North Sea (Rodhe and Winsor 2002; Larsen et al. 2008), suggesting that the Baltic Sea cod have evolved a means of acclimating to various salinities more readily. Putatively genetic differences in acclimation capacity were also found in the tropical coral reef damselfish *Acanthochromis polyacanthus* when thermal reaction norms for metabolic rate revealed that a northern population was able to better acclimate to warmer temperatures than a southern population, even though their acute thermal responses were the same (Donelson and Munday 2012). Such differences in short- and long-term responses underscore

Fig. 9. Norms of reaction for residualized standard metabolic rate (SMR) across a temperature gradient in (●) Northern and (○) Southern populations of (*a*) blackstripe topminnow (*Fundulus notatus*) and (*b*) blackspotted topminnow (*Fundulus olivaceus*) (redrawn from Schaefer and Walters 2010).



the importance of measuring phenotypic responses across different time scales to disentangle the relative roles of plasticity, acclimation, and adaptation in population responses to directional environmental change.

Experimental considerations and suggestions for future research

Notwithstanding recent contributions to reaction norm research in fishes, there is still a need for support from a broader variety of species and there are many questions that could yet benefit from being addressed using this approach. As a stimulus for future research, we have compiled a list of 27 such questions (Table 2) and a discussion of experimental design considerations to help address them. A particularly glaring gap in the literature is the use of high-throughput next-generation sequencing techniques, such as transcriptome sequencing (also known as RNA-seq; Wang et al. 2009). We suggest that recent advances in molecular methodology could be combined with traditional common-garden approaches to advance many of the research questions herein, particularly with respect to the molecular mechanisms underlying genetic differences in plasticity.

Spatial and temporal scales of sampling

Comparisons of three or more populations originating from locations across an environmental gradient are ideal as they have potential to provide strong evidence that observed genetic differences are adaptive (Haugen and Vøllestad 2000; Conover et al. 2006). Both the breadth of the environmental range to be sampled and the distances between sampling locations are key considerations when selecting sampling sites. Sampling across the extent of an ecological or environmental gradient is likely to provide a more holistic representation of the genetic variation that exists within a species or population, while proximity of sampling locations will affect what information can be gleaned from the study regarding the spatial scale of adaptation. Studies described earlier suggest that reaction norm divergence can occur at smaller spatial scales than is widely assumed for marine fishes (e.g., ~300–400 km, Conover and Present 1990; <200 km, Oomen and Hutchings in press) and freshwater fishes (e.g., <4 km; Crispo and Chapman 2010). There remains a need for more fine-scale comparisons, especially given evidence that adaptive variation likely exists at finer spatial scales than those at which sampling has occurred (Conover and Present 1990; Conover et al. 2006). Comparing putative populations at various spatial scales, including both the minimum and maximum distances between populations, will help to identify the spatial scale at which genetic differences in plasticity can occur. Moreover, sampling populations that experience varying degrees of environmental variability or gene flow can provide insight into how these processes promote or constrain the evolution of plasticity.

Comparing the plastic responses of recently diverged populations (e.g., Haugen and Vøllestad 2000) or the same population at different times would be extremely valuable for determining the rate at which plasticity evolves in response to selection, similar to how within-population temporal shifts in PMRNs have been used to explore the potential for fishing to generate life-history evolution (Heino and Dieckmann 2008). The use of a similar approach to investigate the impacts of global climate change on fish populations has proven challenging (Crozier and Hutchings 2014), yet the need for such research is urgent.

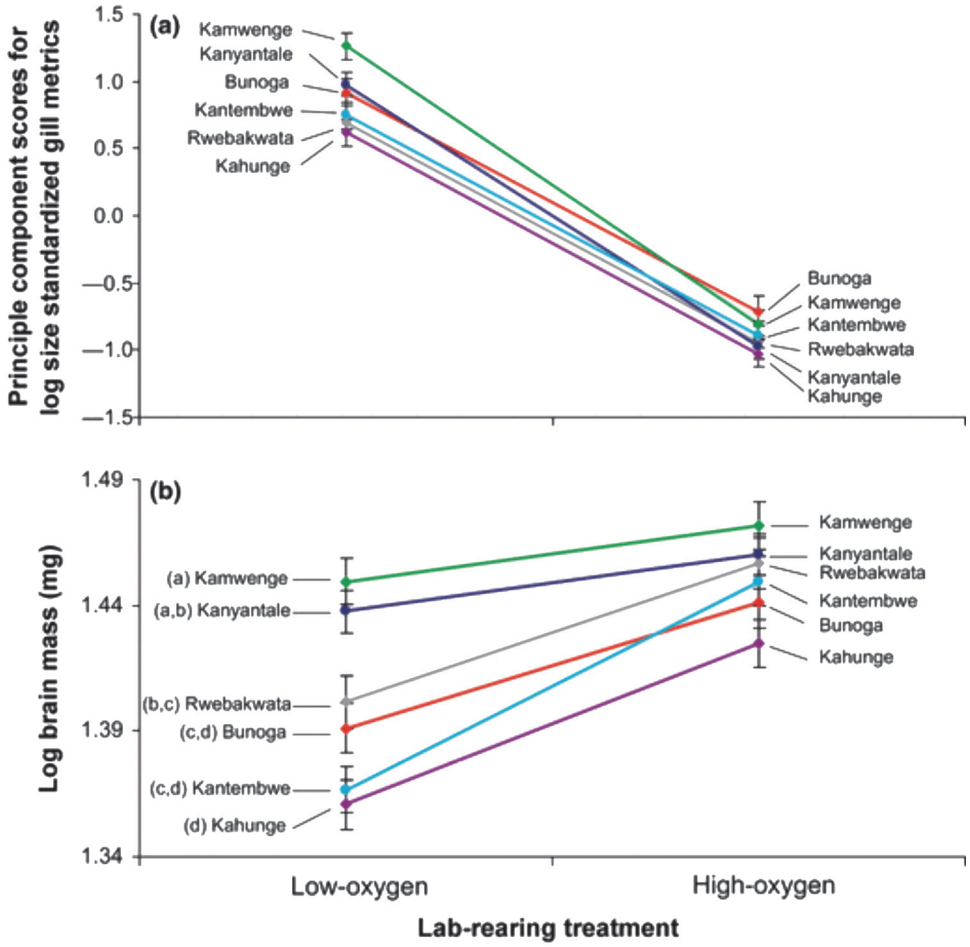
Family versus population levels of comparison

Greater variability in reaction norms within a population (e.g., among families) may indicate a greater adaptive capacity by increasing the probability of having at least one response that is well suited to a new environment (i.e., more genetic variation in plasticity for selection to act on). Similarly, variable reaction norms at the population level represent greater potential for the species as a whole to react to natural and anthropogenic changes. Therefore, we advocate using breeding designs that allow for the testing of family differences as well as population differences (e.g., Beacham and Murray 1985; Yamahira et al. 2007; Evans et al. 2010; McCairns and Bernatchez 2010). This design can also permit evaluations of how the amount of variability in reaction norms among families is related to the amount of plasticity observed at the population level. There appear to be no studies testing for reaction norm variability among family or population hybrid crosses in fishes other than salmonids. These studies would provide much-needed insight into how much of the observed genetic variability is additive (i.e., heritable).

Life stages

Phenotypic traits may be stage-specific (Stearns 1989) and plasticity for these traits may also vary throughout an individual's lifetime, as exemplified by the different results obtained by Hutchings et al. (2007) and Wijekoon et al. (2009) described earlier for Atlantic cod. Some traits may be expressed more strongly at certain life stages (e.g., growth potential may be greater in cod larvae than in juveniles; Wijekoon et al. 2009), with higher trait values possibly revealing variation in trait plasticity not apparent in other life stages. The "native" environment experienced by a population can vary in both time and space depending on the life stage. Because of population differences in spawning time, Atlantic cod

Fig. 10. Norms of reaction for $\log_{10}$-transformed (*a*) gill metrics and (*b*) brain mass in low and high oxygen levels for six populations of African cichlid (*Pseudocrenilabrus multicolour victoriae*). (Dark blue) Kanyantale and (light blue) Kantembwe are hypoxic swamp sites. (Grey) Rwebakwata and (purple) Kahunge are located near the river–swamp junction. (Red) Bunoga and (green) Kamwenge are river sites (original source: Crispo and Chapman 2010).



larvae from some regions of the northwest Atlantic experience warmer temperatures than those experienced by other larvae, while this pattern is reversed for juveniles (Marcil et al. 2006*a*). Finally, plasticity for a trait may have a greater influence on fitness at a particular life stage. If cod survival is observed to be much lower during the larval stage (<12%; Hutchings et al. 2007) compared to the juvenile stage (~64%–98%; Wijekoon et al. 2009), then it could be argued that phenotypes exhibited at the larval stage are more relevant to fitness. Measuring phenotypic traits at multiple life stages would be valuable to clarify these issues.

Treatment design

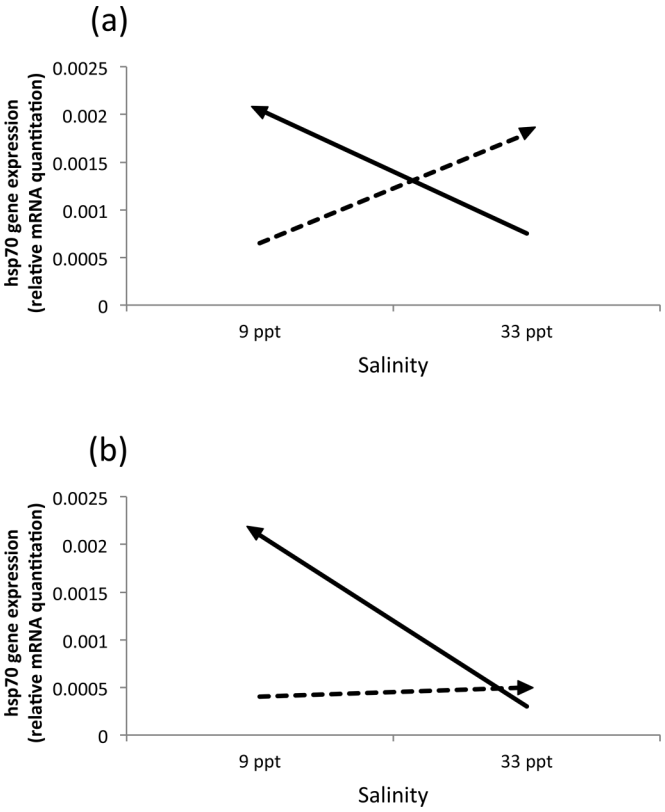
Many studies undertaken thus far have only included two environmental treatments along the gradient of interest (e.g., Hutchings et al. 2007; Wijekoon et al. 2009; McCairns and Bernatchez 2010; Crispo and Chapman 2010). However, it is extremely valuable to have three or more treatments encompassing at least the range of values encountered in the wild to (*i*) distinguish interactions that only occur within one portion of the environmental range, and (*ii*) test for plasticity differences at the environmental extremes of the gradient. For example, if Schaefer and Walters (2010) had only used two temperature treatments of 16 and 24 °C (half of the range tested) in their comparison of *F. notatus* populations, they may have concluded that little difference in plasticity exists (Fig. 9). However, by extending the temperature range to 32 °C, the authors revealed a genotype-by-environment interaction that occurs only in the upper portion of the gradient and suggests genetic divergence between northern and southern populations. Moreover, by testing two additional intermediate temperature treatments, Schaefer and Walters (2010) revealed complex differences in the shapes of reaction norms between northern and southern populations.

Extreme or stressful conditions are expected to reveal cryptic genetic variation (Ghalambor et al. 2007), thus, including environmental extremes can help explain the evolution of novel phenotypes. This phenomenon is exemplified in work by Beacham and Murray (1985) on chum salmon (*Oncorhynchus kisutch*). Although Purchase et al. (2010) investigated whether thermal extremes revealed cryptic variation in cod sperm performance, their test was inconclusive because individual variability in sperm swimming velocity (Fig. 3) and sperm wobble was greatest at the lower temperatures (those most similar to temperatures likely encountered in the wild) and minimal at the highest temperature. Nevertheless, future examinations of genetic variability in plasticity at environmental extremes can help us predict how organisms and populations will respond to directional environmental change, such as that anticipated to be the product of global warming.

Environmental variability and maternal effects

To ensure that environmental effects are adequately controlled, several measures should be taken when conducting a common-garden experiment. Experimental subjects must be reared under standardized environmental conditions throughout their lifetime to eliminate environmental effects from their initial environment

Fig. 11. Relative *hsp70* gene expression in European flounder (*Platichthys flesus*) kidney tissue representing (*a*) short-term (after 1 day) and (*b*) long-term (after 50 days) responses to changes in salinity. (Solid line) The North Sea population experienced a decrease in salinity while (dashed line) the Baltic Sea population experienced an increase in salinity. Arrows represent the direction of the change in salinity (redrawn from Larsen et al. 2008).



prior to transfer (Conover et al. 2006). When possible, parents of the experimental generation should also be raised under standardized conditions to eliminate maternal effects. The necessity of these precautions is clear given that vastly different results can be obtained, depending on the laboratory generation used in an experiment. For example, Schultz et al. (1996) found significant population-level differences in growth rate plasticity in second-generation laboratory-reared mummichogs that were not detected in the first generation. However, for species with longer generation times, using first-generation fish is often necessary because of logistical constraints (e.g., Hutchings et al. 2007; Crispo and Chapman 2010; McCairns and Bernatchez 2010). While second-, third-, and *n*th-generation fishes can be used (e.g., Conover and Present 1990, Schaefer and Walters 2010), there is a trade-off in that inadvertent selection on the experimental group may occur while in captivity. Future studies should investigate how reaction norms vary among laboratory-reared generations of the same genotype.

It is a critical component of laboratory common-garden experiments that the "common" environments are as similar as possible with regard to all environmental variables (e.g., temperature, salinity, dissolved oxygen, light intensity, photoperiod, etc.). Tank design should include a sufficient number of replicates for each genotype-by-treatment combination to estimate the variation between tanks. An alternative strategy for minimizing the "tank effect" is to mark individuals according to family or population and combine all "genotypes" in the same treatment tank (e.g., Purchase et al. 2010). Experiments should also be carried out simultaneously to maximize the similarity between common environments and ensure consistency in the execution and timing of measurements, although this can be problematic for many species of fishes in which different populations spawn at different times of year. Some species can be induced to spawn using photoperiod manipulation (e.g., Atlantic silversides; Conover and Present 1990) or degree-day calculations can be used to rear eggs from different populations at different temperatures so that all eggs hatch at the same time (Skoglund et al. 2011). When it is not possible to study populations simultaneously, experiments should be replicated at different times to estimate potential temporal differences between experiments (e.g., Hutchings et al. 2007).

Timing of phenotypic measurements

Many phenotypic traits, such as growth rate and gene expression levels, change over time. Whether these traits are measured shortly after fish are exposed to a new environment or following an acclimation period can have a substantial impact on whether variability in reaction norms is detected. For example, Wijekoon et al. (2009) documented the greatest population-level differences in juvenile cod growth rate during the first three weeks of 15, regardless of temperature. Some genes linked to an environmental stress response are upregulated only immediately following introduction into a new environment (e.g., heat-shock proteins; Sorensen et al. 2001), whereas other genes may maintain upregulation or downregulation throughout the experimental period, or until acclimation has been achieved.

Temporal patterns of gene expression responses can also vary among genotypes. This temporal variation can result in genetic differences being observed at some time points in the experiment but not others (e.g., Larsen et al. 2008). Gene expression levels can also be extremely sensitive to minor environmental fluctuations. Therefore, consistency in the timing of these measurements, and the methods by which they are collected, is paramount. For temporally variable traits, each set of reaction norms should be constructed from data obtained from a specific time point in the experiment, as opposed to data pooled or averaged over time (e.g., Wijekoon et al. 2009), to reduce variance and increase the probability of detecting significant differences among genotypes. Preliminary studies to determine the most appropriate timing of phenotypic measurements would be extremely useful in this regard.

Inferring local adaptation

To draw inferences regarding adaptive divergence and local adaptation requires demonstration of a link between individual fitness and the phenotypic trait of interest. Reaction norms for survival or some measure of reproductive success enable a direct link between trait plasticity and fitness under various environmental conditions (e.g., Hutchings et al. 2007; Wijekoon et al. 2009; McCairns and Bernatchez 2010). Alternatively, traits unambiguously linked to fitness could be used (e.g., sperm performance; Purchase et al. 2010) or a trait's link to fitness could be inferred, using evidence of how variability in a particular trait is linked to survival or reproduction (e.g., gill size, brain mass; Crispo and Chapman 2010).

To improve our understanding of the spatial scale of adaptive divergence, genetic variability in reaction norms should be compared to patterns of genetic variation seen in neutral genetic markers, such as microsatellites (Conover et al. 2006; Hutchings et al. 2007). These comparisons will enhance our ability to distinguish between local adaptation and other differentiating forces, such as genetic drift, and illuminate the interplay between plasticity and gene flow.

Analytical considerations

Graphical representations of reaction norms greatly facilitate interpretation of variability in trait plasticity, especially when done in a consistent manner across studies (i.e., ideally, with the environmental gradient on the *x*-axis, phenotypic trait on the

Table 2. Questions for future research into genetic variability in reaction norms in fishes and other poikilotherms (adapted and expanded from Hutchings 2011).

No.	Question
Intrapopulation (e.g., family-level) reaction norm variability	
1	How genetically variable are the shapes of reaction norms (i.e., their slopes and elevations) within populations? To what extent do families within populations differ in their average response to environmental change?
2	How is variability in reaction norms among families related to the amount of plasticity observed at the population level?
3	How do reaction norms vary through ontogeny (e.g., between larval and juvenile stages)?
4	How do reaction norms vary among laboratory-reared generations of the same genotype?
5	Is cryptic genetic variability released at environmental extremes, and what role does it play in the evolution of novel phenotypes?
Interpopulation reaction norm variability	
6	How variable is plasticity among populations within species and among species within and among clades?
7	What is the smallest spatial scale at which genetic differences in plasticity can occur?
8	How does gene flow promote or constrain reaction norm evolution?
9	To what extent does interbreeding (e.g., between different wild populations, or between wild and domesticated populations) affect the shapes (and adaptive value) of reaction norms?
10	Are behavioural traits more plastic (steeper reaction norm slopes) than morphological and life-history traits?
Selection	
11	How do different types of temporal environmental variability (e.g., seasonal, diurnal, stochastic) shape reaction norms for fitness and non-fitness traits?
12	What is the additive component of genetic variability (i.e., the heritability) in the slopes and elevations of reaction norms?
13	How rapidly do reaction norms respond to natural and anthropogenic selection? For example, will plastic responses evolve quickly enough to keep up with global climate warming?
14	Under what circumstances might the slopes of reaction norms evolve at a slower rate than the elevations of reaction norms?
15	Are the heritabilities of traits correlated with the heritability in the plasticity of those traits?
Constraints	
16	Are the slope and elevation of the same reaction norm genetically correlated with one another?
17	Are there genetic correlations between the plasticity of one trait and the plasticity of another trait?
18	Are the shapes (and/or slopes) of reaction norms for some traits (or classes of traits) constrained to greater degrees (and express less variability) than others?
19	What are the causal mechanisms underlying plasticity from a physiological, hormonal and genetic perspective? How might these mechanisms constrain evolutionary shifts in plasticity?
Demographic and conservation consequences	
20	How does individual fitness and, by extension, rate of per capita population growth change as phenotypes shift along norms of reaction?
21	What are the fitness costs associated with trait plasticity?
22	Are large populations more phenotypically plastic than small populations?
23	How might plasticity (e.g., reaction-norm slope variability) change with abundance (e.g., linearly, asymptotically)?
24	How does inbreeding and outbreeding affect trait plasticity? How might inbreeding and outbreeding depression affect the shapes of reaction norms?
Genetic markers and correlates of plasticity	
25	Is population genetic variability, as reflected by variation at selectively neutral loci, correlated with trait plasticity?
26	Can genome surveys of molecular marker polymorphisms be used to identify candidate genes responsible for plasticity and reaction norm variability?
27	How is plasticity regulated at the molecular level? What are the relative roles of gene transcription and gene expression?

y-axis, a regression drawn through datum points where each point represents the average trait value for a particular genotype in a particular environment, with confidence intervals). When interpreting reaction norms, studies commonly focus on differences in reaction norm elevations (i.e., mean phenotypic trait values) and often neglect to discuss differences in reaction norm slopes (i.e., trait responses), the latter being more informative about responses to environmental change and the evolution of plasticity. The existence of genotype-by-environment interactions should be tested statistically and should not be based solely on whether reaction norms cross. Importantly, norms of reaction may not intersect in the portion of the environmental gradient that was tested but they may cross elsewhere along the gradient.

Conclusion

The level of phenotypic plasticity displayed within a population and the spatial scale at which differences in plasticity exist are fundamental issues concerning the ability of populations to respond to environmental change. The body of work described herein suggests that considerable individual and family-level variability in reaction norms exists in fishes for selection to shape an adaptive norm of reaction and that this adaptive potential can vary among populations. Population-level differences in plasticity are evident in a variety of species, from the small, short-lived Atlantic silverside to the large, bet-hedging Atlantic cod, suggesting that such genetic variation is likely ubiquitous in fishes and probably other vertebrates. However, there is still a need for support from a broader variety of taxa and many avenues of inquiry remain to be explored using a reaction norm approach. Such pursuits will further our understanding of the capacity of animal populations for phenotypic change and how this capacity evolves and will be fundamental to predicting how populations will be affected by natural and anthropogenic environmental variability.

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