Effects of exposure to culture in fishes: the existence of common morphological responses among species, and their impact on the interaction between escapee and wild Atlantic cod (*Gadus morhua*)

by

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Abstract

A major concern regarding the impact of aquaculture is the alteration or reduction of the fitness of wild stocks through interbreeding with escapees. Cultured fishes develop morphologies and behaviours different than those of their wild counterparts, and the spawning success and fitness of cultured fish is frequently lower. However, successful interbreeding between wild and cultured fish is well documented and can lead to negative consequences for the wild population. In this thesis I examined how culture affects the phenotypes of fishes, and how these differences in phenotype in turn relate to reproductive success and offspring early growth and survival. I found that cultured Atlantic cod (*Gadus morhua*) had relatively smaller fins, heads, eyes, and jaws, but greater condition factor and body depth than wild cod from the same ancestral population. This suite of morphological differences is often referred to as the "cultured phenotype", and while commonly asserted to exist I was the first to formally test for it using a meta-analysis and a vote-counting analysis. These analyses confirmed that aspects of a general "cultured phenotype" exist. To evaluate the influence of morphology and behaviour on male spawning success, I studied the reproductive interactions of individual cultured and wild male cod in the presence of a cultured female. Despite phenotypic differences, the spawning success of cultured males did not differ from that of wild males. Finally, because the introgression of genetically differentiated escapees into wild populations can lead to fitness declines, I tested the effect of hybridization between

two genetically distinct populations of cod. I found no evidence that the pure strain and F_1 hybrids differed in their relative fitness, or of differential response to temperature. Finding equal reproductive success of cultured and wild male cod, at least in my experimental conditions, and no differences in early life history fitness between F_1 hybrids and non-hybrids suggests that the potential for introgression may be higher than has been predicted by previous studies.

Acknowledgements

To my family and friends.

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posteriormost points of the dentary. Head length is the distance between the anteriormost point of the head, to the posteriormost point of the operculum. Head depth is the maximal depth of the head and body depth is the maximal depth of the body while caudal peduncle depth is the minimum depth of the caudal peduncle. Fin lengths, with the exception of the caudal fin is the straight-line distance between the fin origin and the tip of the longest fin ray (usually the second). Fin widths are the straight-line distance between the fin anterior fin origin and its posterior insertion. The height of the caudal fin is the maximal vertical distance with the caudal fin extended, while the caudal fin length is the straight-line distance between its origin and a plane running perpendicular to the body length at the caudal fins most posterior point when extended. In addition to these morphological measures, Fulton's condition factor (K) was also included in the analysis as $K = 100(W/L^3)$. ...85

Figure 3.3 Effect sizes for the morphological features examined for all studies included in the meta-analysis. The points are the exponent of the estimated effect size for each morphological feature from their respective mixed-effects model. The error bars represent the 95% confidence interval. A dotted line has been drawn at one to aid in interpretation. Opposite each morphological character, its corresponding effect size, upper and lower bounds of the 95% C.I. (in brackets), and the number of cultured/wild comparisons tested (n) are reported. The abbreviations for the morphological features are: D = depth, L = length, U = upper,

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Co-authorship statement

I, Mr. Brendan Francis Wringe BSc, MSc, Esq, do hereby assert that my contributions, practical, intellectual, and philosophic, to the areas of: i) design and identification of the research proposal, ii) practical aspects of the research, iii) data analyses, and iv) manuscript preparation of this thesis are major. Insomuch, my status as the principal author of this thesis, as well as of the published and unpublished works included therein on which I am so designated, are duly justified.

"He always thought of the sea as *la mar* which is what people call her in Spanish when they love her."

- Ernest Hemmingway, The Old Man and the Sea

Chapter 1 - Introduction

Currently, about half of the world's population (a proportion disproportionately skewed towards peoples in developing countries) derive at least 15% of their protein intake from fish (FAO 2014). The human population is expected to rise to over 9.7 billion by 2050 (United Nations 2015). Not only will a greater absolute quantity of fish protein be required to feed this larger population, but it is also expected that fish protein will come to constitute a greater proportion of the total dietary protein intake. Consequently, the importance of fish protein to ensuring food security is predicted to increase (FAO 2014). However even at present, the global demand for fish product has surpassed what is available from capture fisheries, and landings have plateaued. Concomitant with this plateauing is the realization that many of the world's fish stocks are currently fully- or over-exploited and that some fish populations have declined precipitously to fractions of their historic levels (Hutchings et al. 2010, Christensen et al. 2014, FAO 2014, WWF 2015). Focusing on the ocean environment, population declines and over-exploitations are not distributed uniformly across all species, or even higher taxonomic divisions, with fisheries and their incumbent effects disproportionately targeting large marine predators and higher trophic level fishes (Pinnegar et al. 2002, Myers & Worm 2003, Daan et al. 2005); but refer to Tacon and Metian (2009), Essington et al. (2015), and Branch (2015) for an alternate perspective.

121 To meet the demand for preferred fish protein, both the number of fish and
122 the number of fish species in culture have increased over the past 50 years (FAO
123 2014). The large-scale increase in aquaculture activities has led to the realization
124 that aquaculture, like all other types of animal culture and production, is not without
125 effect on the environment.

126 While terrestrial farming and animal husbandry benefit from millennia of
127 accumulated knowledge and best practices, aquaculture in comparison is much
128 newer. Lately in terrestrial farming, as well as in aquaculture much attention has
129 been directed to the reduction of environmental impacts. Broadly speaking,
130 aquaculture must contend with the elimination of faeces (Gomi 1993), and other
131 organic detritus. The rate at which organic wastes enter the environment must
132 balance the rate at which natural ecosystem functions (including biofiltration in
133 closed-containment aquaculture) can remove them, or else there is the risk of their
134 accumulation leading to environmental degradation. Furthermore, excess feed that
135 escapes from cages, as well as the physical structure of the cages themselves, can act
136 as fish aggregators altering the natural distribution of wild fishes (Dempster et al.
137 2009). Strategies must be implemented to prevent or mitigate the spread of
138 pathogens from farmed to wild fish (and vice versa) and among individual cages and
139 farms (Johansen et al. 2011), the potential impact of antibiotics and antiparasitics on
140 non-target organisms (Davies & Rodger 2000), and the development of antibiotic
141 resistance (Schmidt et al. 2001). Finally, one of the most pernicious concerns, and

142 the focus of this thesis, is the escape of cultured fish into the marine environment
143 (Naylor et al. 2005, Bekkevold et al. 2006, Thorstad et al. 2008).

144 Exposure to culture leads to phenotypic and genotypic changes in fishes.
145 Phenotypically, cultured fish have been shown to differ from wild fish in their
146 morphology (Fleming et al. 1994, Uglem et al. 2011, Arechavala-Lopez et al. 2012),
147 levels of aggression (Jonsson 1997, Einum & Fleming 2001, Jonsson & Jonsson
148 2006), response to predators (Matsuzaki et al. 2009, Chittenden et al. 2010, Meager
149 et al. 2011), prey preference and capture ability (Steingrund & Fernö 1997, Olsen &
150 Skilbrei 2010), physiology and metabolic performance (Fleming et al. 2002,
151 Pedersen et al. 2008, Anttila & Mänttäri 2009, Chittenden et al. 2010), growth rate
152 (Devlin et al. 2009, Wringe et al. 2010, Skaala et al. 2012), life history timing (Kause
153 et al. 2003, Glover et al. 2009, Fraser et al. 2010b), and gene expression (Roberge et
154 al. 2008, Normandeau et al. 2009) among other traits.

155 Genotypically, cultured populations will invariably differ from their founder
156 population. Even considering the simplest scenario, such as is typical of many
157 supplementary hatcheries (e.g. Svåsand et al. 2000, Busack et al. 2007, Belk et al.
158 2008, Horreo et al. 2008), where fish are captured from the wild and mated
159 together, the genotypes of the resultant offspring will differ from those of the source
160 population because of founder effects (Cross & King 1983, Verspoor 1988, Petersson
161 et al. 1996, Norris et al. 1999, Weeder et al. 2005) and the removal of sexual

selection (Petersson et al. 1996, Neff et al. 2011). Where a broodstock has been maintained in captivity for more than one generation, even in the absence of artificial selection, the divergence of its genotype from that of the founder population will increase over time because of genetic drift (Cross & King 1983, Verspoor 1988, Alarcón et al. 2004), domestication selection (Christie et al. 2012), and removal of sexual selection, including mate choice (Landry et al. 2001, Rudolfsen et al. 2005, Neff et al. 2011). Domestication selection is a broad term covering multiple different processes. It includes unintentional selection on those traits that confer a fitness advantage in culture, as well as on loci physically or genetically linked to the genes that underlie such advantageous traits (Christie et al. 2012). As well, hatchery protocol may impart inadvertent directional selection during artificial spawning, such as the propagation of the least shy fish because they may be the most easily caught (Bekkevold et al. 2006). Because natural and sexual selection are relaxed or removed in culture environments, those phenotypes that arise through domestication selection that would be disadvantageous in the wild are not purged and continue to be propagated.

Fish reared in commercial aquaculture are generally the product of broodstocks that have undergone directed artificial selection for various traits that are of benefit to the producer [e.g. rapid growth (e.g. Myers et al. 2001, Fleming et al. 2002, Small 2006, Gjedrem 2010), delayed maturity (e.g. Fleming et al. 2002, Kause et al. 2003, Gjedrem 2010), high-density production (e.g. Ridha 2006, Trenzado et al.

183 2006), disease resistance (e.g. Nichols et al. 2003, Antonello et al. 2009) and greater
184 feed conversion efficiency (e.g. Kause et al. 2006)]. Furthermore, in aquaculture
185 there is often an incentive to utilize a broodstock outside of the range of its native
186 population because of a wish to expand aquaculture production for a species into an
187 area for which a local broodstock does not exist (Withler et al. 1994, McGinnity et al.
188 1997), or because the non-native broodstock outperforms the native one (De
189 Innocentiis et al. 2005). Thus the broodstock and hence the fish stocked to cages and
190 which have the potential to escape, will be genetically differentiated from wild
191 populations.

192 Research, primarily in salmonid fishes, has shown that because of these
193 phenotypic and genotypic differences, interaction between wild and cultured fish
194 can lower the fitness of fish in the wild population through genetic (e.g. introduction
195 of non-local alleles and breakdown of co-adapted gene complexes) or non-genetic
196 (e.g. reduced reproductive success because of behavioural differences) (Fleming et
197 al. 2000, McGinnity et al. 2003, McGinnity et al. 2009), and that carry-over effects
198 and repeated introgressions can lead to cumulative fitness effects (Miller et al. 2004,
199 Araki et al. 2009). Much of the study of wild/farmed interaction has focused on
200 Atlantic salmon (*Salmo salar*; e.g. McGinnity et al. 1997, Fleming et al. 2000, Fraser et
201 al. 2010a, Glover 2010), a species that is often considered representative of
202 salmonids in general, and as such provides a good example of the risks and
203 consequences of introgression. The anadromous life cycle of Atlantic salmon,

204 especially their homing behaviour, leads to reduced gene flow between populations
205 and consequently the development of local adaptation (e.g. Garcia de Leaniz et al.
206 2007, Fraser et al. 2011).

207 Posit that local adaptation in this scenario has arisen as the result of
208 divergent natural selection for changes in allele frequencies among habitats
209 (Lenormand 2002, Jensen et al. 2008), which is then reinforced by either poor
210 performance in the local environment of migrants and of the hybrid offspring of
211 local individuals and migrants. In an analogous fashion, even in the absence of
212 directed artificial selection, hatchery environments impose selection pressures
213 (and/or lack of natural selection) such that the farmed fish are *de facto* locally
214 adapted to the farm environment (Vasemagi et al. 2012). In this scenario, escape of
215 farmed fish can be thought of as being analogous to (very) long-distance natural
216 dispersers (c.f. straying in salmon), and the literature would suggest their
217 introgression into the native population would result in loss of genetic variation,
218 breakdown of co-adapted gene complexes and breakdown of population structure
219 (Laikre et al. 2010). The breakdown of co-adapted gene complexes would result in a
220 loss of intrinsic adaptation, while the introduction or replacement of local with
221 foreign alleles would cause a loss of extrinsic adaptation (Laikre et al. 2010).

222 However, while the same genetic consequences of introgression of farmed
223 fish with wild populations would be predicted for non-salmonid species, simply

224 extending salmonid findings to marine species, each of which has disparate life
225 histories, reproduction, and population genetic differentiation, is imprudent
226 (Bekkevold et al. 2006). As an example, and the focus of this thesis, Atlantic cod
227 (*Gadus morhua*) have been shown to exhibit genetic differentiation beyond simple
228 isolation by distance. Genetic differentiation in cod as a species has arisen as a result
229 of the resident nature of some populations (Ruzzante et al. 2000, Morris & Green
230 2002), retention of eggs within an area by prevailing currents (Espeland et al. 2007,
231 Jørstad et al. 2008), or the seasonal return to spawning grounds by migratory cod
232 populations (Robichaud & Rose 2001, Skjæraasen et al. 2011). These processes have
233 resulted in a pattern of genetic differentiation among cod populations at both large
234 (Pogson et al. 1995, Hutchinson et al. 2001, Pogson et al. 2001) and small scales
235 (Pogson et al. 2001, Imsland & Jónsdóttir 2003 (review), Knutsen et al. 2003),
236 including some evidence of local adaptation (Pogson & Fevolden 2003, Andersen et
237 al. 2009, Bradbury et al. 2010, Beirão et al. 2015). The differences in biology and life-
238 history between cod, and salmonids means that the results of salmonid studies
239 should not simply be assumed to be true of cod. Therefore, all potential outcomes of
240 interaction between escaped and wild cod, the competitive ability and reproductive
241 success of escaped individuals relative to wild, through to the fitness outcome of
242 hybridizations between genetically divergent populations must be tested.

243 At the turn of the millennium, government and aquaculture industry leaders
244 in Canada sought to diversify the Canadian aquaculture industry and began

development of research and culture programmes for alternative species, including Atlantic cod. Through these programmes, experimental cod broodstocks were created from wild-caught fish, and their offspring were stocked to commercial cage aquaculture farms. These first-generation farmed cod afforded me the unique opportunity to study the effects of exposure to the aquaculture environment, and the interaction between cultured and wild fish in a species that had not experienced the intensive selection regimes common in more established species (e.g., Atlantic salmon).

This thesis explores the potential for interaction and interbreeding between wild and farmed cod, comprises the results of three experimental studies, and a related systematic review and meta-analysis.

The second chapter, published in the journal *Aquaculture Environment Interactions* (Wringe et al. 2015a), is an examination of the effect that exposure to culture has on the morphology cod relative to that wild fish from their founder population. Morphological differentiation relative to their wild progenitors resultant from their exposure to cultured conditions has been variously noted for farmed fishes (e.g. Fleming et al. 1994, Higgins et al. 2010, Uglem et al. 2011, Arechavala-Lopez et al. 2012), and perhaps more surprisingly in fishes reared in hatcheries for intentional release (e.g. Taylor 1986, Rogdakis et al. 2011, Tiffan & Connor 2011). Many of the morphological features that have been found to differ between cultured

and wild fish should have implications for their relative fitness, and could contribute in part to the observed poor performance of escapees and stocked fish. In addition to effects on locomotion (Webb 1984) and prey capture (Huskey & Turingan 2001, Frederich et al. 2008), given the seeming importance of morphology, and secondary sexual characteristics in the mating system of cod (Skjæraasen et al. 2006a, Rowe et al. 2008, Skjæraasen et al. 2008), deviations from wild-type phenotype may have fitness consequences for cultured cod. In light of this, I tested differences in the morphology of wild and cultured cod, both to examine the (plastic) effect of exposure to culture, and to hypothesize the effect on fitness any observed change may impart.

The third chapter is an offshoot of the second chapter based on the inference that cultured individuals of many species can be readily distinguished visually from their wild conspecifics because of differences in morphology caused by cultured rearing, and that many of the features, and the direction in which they differ from cultured to wild fish are similar for multiple species (e.g. Balon 1995, Busack et al. 2007, Uglem et al. 2011, Arechavala-Lopez et al. 2013a). The environments experienced by fishes in culture appear to be more similar to each other than are the environments experienced by their wild conspecifics. In light of this, it is possible that cultured fishes may converge on a stereotypical "cultured phenotype". A systematic review of the literature based on PRISMA best practice protocols was performed, and then these results were used to conduct a meta-analysis and vote-

286 counting analysis to test for the existence of such a “cultured phenotype”. This
287 chapter is currently under review in the journal *Reviews in Fish Biology and Fisheries*.

288 The fourth chapter, published in the journal *Marine Ecology Progress Series*
289 (Wringe et al. 2015b), is a comparison of the mating success of individual cultured
290 and wild male cod in the presence of a cultured female using spawning trios. This
291 experiment examines the reproductive competitive abilities of cultured and wild
292 males, and hence the potential for genetic introgression following escape events.

293 After comparing the potential for hybridization between wild and farmed cod
294 in chapter in chapter four, I sought to evaluate the potential consequences of
295 hybridization. The fifth chapter is an examination of the fitness of hybrids of cod
296 from two genetically distinct populations relative to their founder populations at
297 different temperatures. Two separate broodstocks of cod were simultaneously
298 developed from wild-caught fish in New Brunswick and Newfoundland and were the
299 parents of the fish used in this experiment. These two populations have been shown
300 to differ genetically, in a fashion that is indicative of positive temperature-related
301 selection (Bradbury et al. 2010), and thus their hybridization may result in offspring
302 with reduced fitness compared to the parental strains.

303

Chapter 2 – Rapid morphological divergence of cultured cod of the northwest Atlantic from their source population.

2.1 Abstract

The performance of aquaculture escapees in the wild depends in part on how their morphology differs from that of wild fish. We compared farmed Atlantic cod (*Gadus morhua*) morphology to that of wild cod from the same ancestral population. Traditional and geometric morphometrics showed that farmed cod had relatively smaller fins, heads, eyes, and jaws than wild cod for a given size. Conversely, drumming muscle size and metrics of body and liver condition were greater in farmed fish. As the observed differences are likely due to phenotypic plasticity, their fitness consequences for escaped farmed fish may be transient.

2.2 Introduction

Fish exposed to culture develop phenotypes that differ from those of their wild counterparts (Fleming & Gross 1994, Araki et al. 2008, Bailey et al. 2010, Chittenden et al. 2010); phenotypes that may be beneficial under culture but may reduce the fitness of an individual when exposed to another environment (e.g. the wild environment following escape). These cultured phenotypes can be the product of a plastic response whereby different phenotypes can be expressed by a single genotype in response to different environmental conditions (Imre et al. 2002, Skjæraasen et al. 2008, Mayer et al. 2011, Vehanen & Huusko 2011), or these

phenotypes may be the result of genetic changes brought about through both intentional and unintentional selection (Fleming et al. 1994, Einum & Fleming 2001, Fleming & Petersson 2001, Hutchings & Fraser 2008, Solberg et al. 2013). The degree of phenotypic change, and its permanence, are both a function of the time an individual has spent in captive conditions (Pakkasmaa et al. 1998, von Cramon-Taubadel et al. 2005), as well as the degree of genetic change from the ancestral lineage due to captivity (Fleming et al. 1994, Blanchet et al. 2008, reviewed by: Hutchings & Fraser 2008, Fraser et al. 2010a). Thus if it is presumed that the phenotypes of wild fish are the product of adaptation to their local environment, then the degree to which the phenotype of cultured fish diverges from this is likely a reflection of how maladaptive the cultured phenotype may be if exposed to the wild environment. Furthermore, the 'permanence' of the cultured fish's phenotype, or the degree to which phenotypic plasticity allows it to (re)converge on a wild-type phenotype over time at liberty, may result in a life-time fitness difference between the two groups that is lower than would be predicted based on morphological differences at the time of escape.

Through programmes that sought to diversify the Canadian aquaculture industry, experimental Atlantic cod (*Gadus morhua*) broodstocks were created from wild-caught fish, and their offspring were stocked to commercial cage aquaculture farms. These first-generation farmed cod afforded us the unique opportunity to study the morphological effects of exposure to the aquaculture environment on fish

345 that had not experienced the intensive selection regimes common in more
346 established species (e.g., Atlantic salmon, *Salmo salar*). We compared the
347 morphology of wild cod to farmed individuals created from wild-caught parents that
348 were genetically similar to our wild fish. We then discuss the differences in
349 morphology in terms of potential fitness effects on escapees in the wild.

350 2.3 Materials and Methods

351 *2.3.1 Data Collection*

352 Farmed cod were the progeny of wild-caught fish from Bay Bulls, Newfoundland,
353 Canada (47° 18' N, 52° 48' W; NAFO division 3L; Figure 2.1), which were spawned
354 between December 2006 and March 2007. The farmed cod were reared in tanks at
355 Memorial University from fertilization until they were transferred *en masse* to
356 Sapphire Sea Farms' net-pen facility in Bay Bulls on 30 November 2008. Some of
357 them (N = 112) were sampled between 4 and 9 November, 2009 during the annual
358 harvest.

359 Wild cod were captured using baited cod pots on 10 (N = 38) and 20 (N = 19)
360 November 2009 in Smith Sound, Newfoundland (48° 9' N, 53° 44' W; NAFO division
361 3L; Figure 2.1). Cod of Smith Sound and Bay Bulls are thought to be of the same
362 stock, being genetically similar (Beacham et al. 2002, Bradbury et al. 2010, Rose et
363 al. 2011). The wild fish were held in a tank and measured 2-3 weeks after collection.
364 The farmed and wild cod were held without feeding prior to measuring to ensure

365 gut contents did not bias weight or shape measures, and only fish free of obvious
366 skeletal defect were included in the analysis.

367 After being killed fish were kept on ice before being arranged left side up,
368 with their median and caudal fins extended and pinned in place, and photographed
369 with a digital camera (Nikon D300) mounted on a tripod. A ruler was included in
370 each photograph to allow for size calibration.

371 After photographing, the right and left pelvic fin lengths (the distance from
372 the origin of the fin to the tip of the longest fin ray) were measured (± 0.01 cm) with
373 digital callipers because they could not be measured from the photographs. Fish
374 were weighed whole (± 0.01 g), sexed when the internal organs were removed, and
375 the liver was weighed separately (± 0.01 g). Following the protocol of Rowe and
376 Hutchings (2004a), both the right and left drumming muscles were removed and
377 frozen, before being dried to constant mass and weighed together (± 0.001 g).

378 Eighteen landmarks were recorded as *x*-*y* coordinates from the photographs
379 using ImageJ (Schneider et al. 2012; http://rsb.info.nih.gov/ij/download.html;
380 Figure 2.2). Standard lengths were measured as the distance between the
381 anteriormost point of the premaxilla, to the posteriormost edge of the hypural plate
382 (points 1 and 8 respectively; Figure 2.2). The dorsal and anal fin lengths and widths
383 were measured as the distance from the fin origin to the tip of the second fin ray,
384 which was the longest, and as the distance along the fin base from its origin to its

385 distal insertion, respectively (Figure 2.2). Unforeseen variation in fin attitude and
386 extension prevented measurement of the size of the left pectoral fin from the digital
387 photographs. A small number of farmed fish (10 of 112) showed malformed fins,
388 which were excluded from the analysis.

389 ***2.3.2 Size standardization and calculation of condition indices***

390 Size standardization was employed so that only relative differences in trait size
391 between the two origins (i.e. wild or farmed) were considered. The lengths and
392 widths of the dorsal and anal fins, the lengths of the pelvic fins and the weight of the
393 drumming muscles were $\log_{10}$ transformed and then were standardized using the
394 method of Reist (1986b). Each of these traits was standardized for each fish using
395 the formula $M_{st} = M_{obs}(Sz_{mean}/Sz_{obs})^{b}$, where: M is the trait measure, Sz is the size
396 measure to which samples are standardized, b is the trait-specific common within-
397 groups slope and the subscripts *mean*, *obs* and *std* refer to the mean, observed (raw)
398 and the size-standardized measurements, respectively. The weight of the drumming
399 muscles was standardized to a common body weight, while the length and width
400 measurements were standardized to a common centroid size. The centroid size, the
401 square root of the sum of the squared distances of each peripheral landmark (i.e.
402 excluding points 13, 14, 17, and 18; Figure 2.2) to the centroid, was calculated in R
403 (R Development Core Team 2015) using the function *gpagen* (geomorph package;
404 Adams & Otárola-Castillo 2013).

405 Condition indices were calculated for each fish by taking the standardized
406 residuals of the regression of $\log_{10}$-transformed standard length on the $\log_{10}$-
407 transformed total weight (CI). The liver indices (LI), were calculated similarly from
408 the regression of the $\log_{10}$-transformed weight of the liver on the $\log_{10}$-transformed
409 total weight. The standardized residuals convey the condition status of each fish.
410 Positive residuals indicate that the fish is heavier, or possesses a heavier liver for
411 their size than the average, while negative residuals indicate the opposite.

412 ***2.3.3 Traditional morphometric, geometric morphometric, and statistical***
413 ***analyses***

414 All statistical and geometric morphometric analyses were conducted in R (R
415 Development Core Team 2015). The traditional morphometric analyses consisted of
416 testing for differences in size-standardized drumming muscle mass, dorsal and anal
417 fin lengths and widths, pelvic fin lengths, as well as CI, and LI individually between
418 fish origins (i.e. wild or farmed) using a linear model with permutation (lmp
419 function, lmPerm package; Wheeler 2010) and type-III sums-of-squares (Anova
420 function, car package Fox & Weisberg 2011) with sex and origin as fixed effects.
421 Using permutation removes the necessity that the data satisfy the assumptions of
422 traditional parametric tests, and allows for the calculation of exact significance
423 levels. The issue of multiple hypothesis testing was addressed by the use of adjusted
424 p-values, with the false discovery rate set to $\alpha = 0.05$ (Benjamini & Hochberg 1995).

Principal component analysis (PCA), with varimax rotation (prcomp function, stats package; R Development Core Team 2015), was also conducted as part of the traditional morphometric analysis to reduce the number of parameters, using all morphometric measures listed in Table 2.1, with the exception of standard length, total weight and drumming muscle mass. Standard length and total weight were excluded because they represent differences in fish size rather than shape (size standardized). Drumming muscle mass was also excluded because it had missing values which caused the sample size to drop appreciably. All principle components (PC) with eigenvalues greater than the mean eigenvalue were considered significant (Jackson 1993).

Geometric morphometric analyses were conducted using the R packages *shapes* (Dryden 2013) and *geomorph* (Adams & Otárola-Castillo 2013). The *x-y* coordinates collected from the photographs of the fish were first converted to shape coordinates using generalized Procrustes analysis (GPA; Adams et al. 2004). GPA removes the non-shape aspects of size, (scaling), orientation and location from the raw *x-y* coordinates, and also standardizes each individual to a common unit centroid size (Rohlf 1999, Adams et al. 2004).

The amount of shape variation attributable to the different origins of the fish (controlling for sex) was quantified using Procrustes ANOVA with permutation, which compares the observed sum-of-squared Procrustes distances to an expected

445 distribution which is calculated through permutation (Goodall 1991). PCA was also
446 conducted on the configuration of the specimens into principal warp space to detect
447 the major features of the shape variation. Differences in PC scores between origins
448 were tested using linear models with sex and origin as fixed effects.

449 ## 2.4 Results

450 ### *2.4.1 Traditional morphometrics*

451 No interactions were detected between sex and origin. Within origin, the size-
452 adjusted dried mass of the drumming muscles was greater in males than in females
453 (Table 2.1). However, females were bigger and their LI were greater, than those of
454 the males (Table 2.1). All size-adjusted morphometric measures, with the exception
455 of the width of the first dorsal fin, differed significantly between wild and farmed
456 cod (Table 2.1).

457 The first four PCs all had eigenvalues greater than the mean eigenvalue, and
458 cumulatively explained 74.3% of the variation in traditional morphometric variables
459 (Table 2.2). The loadings of wild and farmed fish on PCs 1 and 2 differed
460 significantly (t-test, $p < 0.001$), while there was no significant difference on PCs 3
461 and 4 (t-test, both $p > 0.05$; Figure 2.3; PC4 not shown).

462 The first PC, which explains 44.3% of the variation, was characterized by
463 negative loading of the fin measures, particularly fin lengths (Table 2.2). PC2

explained 12.6% of the variation, and for the most part is described by positive loadings from CI, LI and fin widths. Interestingly, on PC2, the fin widths showed moderate to strong positive loadings, while their lengths showed near zero to moderately negative loadings (Table 2.2).

2.4.2 Geometric morphometrics

ANOVA with permutation on the Procrustes-aligned coordinates of the wild and farmed cod revealed that there was a significant interaction between sex and origin ($F_{1,140} = 6.112$, $p<0.001$). Within-origin analysis showed that the shape of the wild males differed from that of the wild females, and the same was true for farmed males and females (both $p < 0.05$). Testing within sexes, the shape of both farmed females and males was different from that of their wild counterparts (both $p < 0.001$).

Principle component analysis of the configuration of the wild and farmed specimens into the principle warp space revealed 7 PCs with eigenvalues greater than the mean eigenvalue, and cumulatively explained 81.90% of the variance. Like the ANOVA above, the scores on PC1 and PC2 showed a significant interaction between sex and origin (both $p < 0.05$; Figure 2.4). That said, Figure 2.4 shows a clear separation between wild and farmed fish along PC2 (Figure 2.4). PC1 explained 30.17% of the variance, and PC2 18.52%. PC1 was however significantly correlated with centroid size (Spearman's rho -0.259, $p < 0.01$), indicating that the shape

differences described by the first PC were mainly related to size. There were no significant differences in shape between origins, sexes, or any interaction between the two for PCs 3-7 (all $p > 0.05$).

Figure 2.5 depicts the difference in shape between farmed females relative to farmed males (Figure 2.5a), wild females relative to wild males (Figure 2.5b), farmed females relative to wild females (Figure 2.5c) and farmed males relative to wild males (Figure 2.5d), and is illustrative of the significant sex by origin interaction. Despite detecting significant statistical difference in shape between the farmed males and females, their consensus shapes appear to be quite congruent even when differences are magnified 3X (Figure 2.5a). Wild females appear to be shallower in the abdominal region than the wild males as indicated by the magnitude of the ventral displacements of points 2, 3 and 4 relative to point 12 (refer to Figure 2.1 for description of points and Figure 2.5b for relative displacement of points). This difference in body depth seems to be confined to the abdominal region because the displacement of the points on the dorsal surface are offset by the displacement of the points opposite them on the ventral surface in the head (points 1, 13, 15, 16, and 18), and caudal regions (points 5, 6, 7, 9, 10, and 11; Figure 2.5b).

Farmed males and females both show a reduction in head size and caudal peduncle length relative to their wild counterparts (females: Figure 2.5c; males:

Figure 2.5d). The smaller head size is evidenced by the posterior displacement of points 1, 16, 17 and 18, the anterior displacement of points 13 and 14, and the anteriodorsal displacement of point 15 (Figure 2.5c, d). Farmed males show a greater reduction in jaw length relative to wild males than farmed females do to wild females though (point 15; Figure 2.5c, d). The posterior displacement of points 6, 7 (females), 9, and 10, while the midlateral portion of the hypural plate (point 8) remains relatively unchanged along the anteroposterior axis is indicative of a truncation of the caudal peduncle. Of particular note, the difference in abdominal region body depth between the farmed and wild females appears to be greater than the difference between the farmed and wild males (points 3,4 and 12; Figure 2.5c, d). It is worth noting that the dorsal rotation of point 8 in Figure 2.5b and d, appears most likely to be the result of subtle differences in the overall rotation, or curvature of the wild male specimens and likely should be taken as spurious.

2.5 Discussion

2.5.1 Differences between wild and farmed fish

Farmed Atlantic cod experience an environment markedly different from that of wild cod. Differences include diet, water temperature and current, fish density, visual complexity and structure, all of which have been shown to plastically affect the growth, development and morphology of fishes (Currens et al. 1989, Adams & Huntingford 2002, Marcil et al. 2006b, Ambrosio et al. 2008). Not surprisingly, the

524 vast majority of morphological characters we measured differed significantly
525 between wild and farmed individuals, as did their overall shape as evidenced by
526 geometric morphometric analysis. Both traditional and geometric morphometrics
527 indicated that farmed cod had relatively smaller head, jaw and fin measures, while
528 their body depth, CI, and LI measures were larger than those of the wild cod.

529 The presence in cultured cod of greater CI and LI than wild cod has been
530 widely documented (e.g. Lie et al. 1986, Svåsand et al. 1996, Grant et al. 1998,
531 Purchase & Brown 2001) and is corroborated by our results. Given that the main site
532 of lipid sequestration in cod is the liver, and liver size and lipid content are directly
533 influenced by the lipid content of the feed, the observed differences in LI are likely
534 reflective of the different diet and physical environment experienced by the wild and
535 farmed cod (Lie et al. 1986, Lambert & Dutil 1997, Morais et al. 2001). Similarly, the
536 greater CI, and the greater body depth of the farmed relative to the wild fish in this
537 study are both related to the farmed cod having a higher LI (liver and as
538 consequence visceral mass).

539 Like what was seen for body depth and LI, the different head morphology in
540 the farmed and wild cod was also likely the result of differences in diet and perhaps
541 to a lesser extent physical environment. The jaw and head morphology of fishes have
542 been shown to be highly phenotypically plastic, and this plastic response is related
543 to, and influenced by the fish's diet. While studies on the phenotypic effects of

544 different diets are lacking in cod, studies in other species have indicated that smaller
545 heads and jaws are seen in fish which are fed non-elusive, prepared diets (Meyer
546 1987, Wintzer & Motta 2005), as well as in fish fed a greater ration (Currens et al.
547 1989). These features are characteristic of the pellet diet, and feeding regime of
548 farmed cod, and relatively smaller heads and jaws have been previously observed in
549 cultured cod (Uglem et al. 2011).

550 Among the head features that were found to be relatively smaller in the
551 farmed than the wild fish was the size of their eyes. Apart from simply being
552 proportional to the head size, Devlin et al. (2012) have suggested that the eye
553 development of rapidly growing fish becomes decoupled from their somatic growth
554 resulting in a negative allometry.

555 The most consistently observed differences between multiple species of wild
556 and cultured fish are that cultured fish tend to develop relatively smaller fins of all
557 types (e.g. Lund et al. 1989, Swain et al. 1991, Rogdakis et al. 2011, Patiyal et al.
558 2013). In some cases, this difference in size is the result of the fins of the cultured
559 fish being either damaged or malformed (Bosakowski & Wagner 1994, Latremouille
560 2003, Hatlen et al. 2006, Blanchet et al. 2008, Chittenden et al. 2010). However, it is
561 unlikely contemporary fin damage or malformation affected the results of the
562 current study. The fins of both the wild and farmed fish were checked for signs of
563 damage (e.g. clubbing, or abrasion of fin margin etc.) or deformity, and

564 measurements from any deformed fins were excluded from the analysis. Whether
565 past damage, or abrasion may have resulted in stunting of the size of the farmed
566 cod's fins is also unclear given the behaviour of cod (decreased wounding with fish
567 size; Hatlen et al. (2006)), as well as the great capacity for organ and tissue
568 regeneration present in fish (Azevedo et al. 2011, Shao et al. 2011). It is possible that
569 the smaller fins of the cultured cod resulted in part from a plastic response to water
570 current. Studies in salmonids have shown that lower current velocity, and variability
571 experienced in culture can lead to relatively smaller fins (Pakkasmaa & Piironen
572 2000, Wessel et al. 2006, Keeley et al. 2007). Similarly, when compared to wild fish,
573 farmed cod likely experience similar reductions in water velocity, and hence similar
574 plastic effects on fin size could be expected in our study.

575 Considering all the observed differences between the farmed and wild cod in
576 our study, the congruence between our results, and those of Uglem et al. (2011), the
577 only other study of differences in adult morphology between wild and farmed cod in
578 which sufficient information is reported to allow comparison is impressive. This is
579 especially true given that the populations examined are thought to have been
580 isolated for at least 100 000 years (Bigg et al. 2008b). This suggests that the
581 observed differences may represent a stereotypical plastic response of Atlantic cod
582 to culture.

2.5.2 Differences between sexes

Cod drumming muscle weight (Engen & Folstad 1999, Rowe & Hutchings 2004a, Skjæraasen et al. 2006b, Skjæraasen et al. 2008) and the length of the pelvic fins (Skjæraasen et al. 2006b, Skjæraasen et al. 2008, Skjæraasen et al. 2012) have been shown to be sexually dimorphic in other studies, and our results found this to be true of drumming muscle weight, but marginally not so for pelvic fin length. Both traits are suspected to play important roles in mate choice (Skjæraasen et al. 2006b, Rowe & Hutchings 2008, Skjæraasen et al. 2012) and in the case of the pelvic fins, in maintaining ventral alignment during gamete release (Skjæraasen et al. 2008).

Sampling time, and differences in the maturation schedule of male and female cod likely account for the observed differences in body depth, body mass and LI, and perhaps to some extent drumming muscle mass. Seasonal gonad ripening in cod from this population generally begins at about the same time these fish were sampled (Rideout & Burton 2000). Male Atlantic cod (cultured and wild) generally begin to mature, and have functionally mature gonads earlier in the season than females. During maturation, males cease feeding and exhibit a concomitant decrease in body mass and marked hypertrophy of the testes and drumming muscles, while maintaining an LI lower than that of females throughout their reproductive cycle (Fordham & Trippel 1999, Rideout & Burton 2000, Rowe & Hutchings 2004a, Solberg & Willumsen 2008).

2.5.3 Implications

When cultured cod escape from net-pens they interact with wild cod, and are subjected to the selective pressures of the natural environment (Moe et al. 2007, Damsgård et al. 2012, Zimmermann et al. 2012). It is likely that the morphology developed by the cod in culture will be to some degree maladaptive in the wild, and thus any escapees will experience lower fitness than their wild counterparts, as has been seen in other species (Fleming et al. 2000, McGinnity et al. 2003, Meager et al. 2010, Skaala et al. 2012).

The differences in fin size and body condition we documented may result in different swimming performance. However the relationship between them in cod, and other species is not always clear (Rose et al. 1995, Reidy et al. 2000). Fitness effects of the fins may also extend to reproduction, with the relatively smaller fins of the farmed cod imparting a competitive disadvantage during both male-male agonistic interaction and courtship display. Extension of the median fins is a component of male Atlantic cod's "flaunting display" (shown to both males and females; Brawn 1961) and pelvic fins are used both for display (Skjæraasen et al. 2010), and to grasp the female and maintain alignment of their urogenital openings during ventral mount (Brawn 1961, Rowe et al. 2008). Moreover, some evidence suggests pelvic fin size may be related to spawning success (Rowe et al. 2008). Such effects may, however, be mitigated to some extent by transience in the differences in fin sizes resulting from convergence through plasticity towards the wild phenotype

following escape as noted in gilthead sea bream (Sparus aurata; Arechavala-Lopez et al. 2013b), and the same is likely true of condition (CI and LI; Nordeide et al. 1994, Jacobsen & Hansen 2001).

It is perhaps intuitive to believe that that morphological characters will differ in their capacity to plastically converge or revert to a wild phenotype. However, there have been documented instances of bony features showing morphological change/re-convergence with wild-type phenotype (Wintzer & Motta 2005, Rogdakis et al. 2011, Arechavala-Lopez et al. 2013a, Arechavala-Lopez et al. 2013b), but there is evidence this ability differs with age (Adams & Huntingford 2002). Thus, predicting, which morphological changes observed in these cultured cod will be more permanent than others is difficult.

It is worth reiterating that the fish in this study are first-generation offspring of wild-caught parents, and while a single generation in captivity has been shown to affect the fitness of cultured fish (Fleming et al. 1997, Milot et al. 2013), increased generations under selection in a cultured environment can lead to genetic changes (Reviewed by: Hutchings & Fraser 2008, Nguyen 2015). Such genetic changes could result in permanent phenotypic changes relative to the wild fish, even if they are exposed to the same environment (i.e. after escape; Araki et al. 2008, Christie et al. 2012, Milot et al. 2013). Therefore, any realized differences in fitness caused by the morphological differentiation between wild and cod observed in this study, would

likely be inflated by genotypic, and consequent phenotypic changes that accumulate over time through both deliberate and inadvertent selection.

2.6 Acknowledgements

The authors wish to thank Corinne Conway and Michelle Bachan for their assistance with the collection of the morphological measurements. Ed Trippel and John Brattey helped plan the experiment and provided integral advice. We also thank the staff of the Joe Brown Aquaculture Research Building and Sapphire Sea Farms' for allowing us to collect data during their harvest, and for their support during this time. The wild cod were collected with the help of Dennis Ivany, John Brattey and the Fish, Food and Allied Workers Union. Ryan Stanley was instrumental in the creation of Figure 2.1. We also thank the members for the Fleming and Purchase labs for their assistance and friendship. The Natural Sciences and Engineering Research Council of Canada funded this research through a strategic grant to I.A.F. and C.F.P., and the Research and Development Corporation of Newfoundland provided additional support to B.F.W.

2.7 Tables

Table 2.1 Mean (± SD) morphometric measures and analyses by sex and framed/wild origin Atlantic cod (*Gadus morhua*). Standard length and weight measures are unstandardized, and the calculation of CI and LI includes an inherent standardization. Drumming muscle weight has been standardized to a common weight, while all other measures have been standardized to a common centroid size. DM is the combined dried mass of the right and left drumming muscles. DF1, DF2, DF3, refers to the first through third dorsal fins, PF denotes the pelvic fins and AF1 and AF2, are the first and second anal fins, respectively. There were no significant interactions between sex and origin for any of the measures. Adjusted p-values are shown, and those significant are bolded.

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Measure	Farmed Male n = 45	Wild Male n = 44	Farmed Female n = 28	Wild Female n = 19	Sex F-value	Sex p-value	Origin F-value	Origin p-value
Standard Length (mm)	419 ± 40	484 ± 60	435 ± 33	516 ± 51	10.69	**0.016**	113.63	**<0.001**
Weight (g)	1056 ± 279	1377 ± 409	1158 ± 321	1583 ± 367	8.16	**0.027**	55.01	**<0.001**
Condition Index (CI)	0.10 ± 1.13	-0.20 ± 0.84	0.16 ± 1.11	-0.21 ± 0.66	0.04	0.837	4.85	**0.033**
Liver Index (LI)	0.48 ± 0.60	-1.15 ± 0.72	0.75 ± 0.54	-0.80 ± 0.74	9.99	**0.016**	267.45	**<0.001**
DM Weight (g)	0.23 ± 0.07	0.18 ± 0.06	0.20 ± 0.09	0.12 ± 0.05	8.33	**0.020**	3.39	**<0.001**
DF1 Length (mm)	49.61 ± 3.67	65.71 ± 4.83	49.26 ± 3.85	65.63 ± 7.09	0.14	0.751	333.48	**<0.001**
DF1 Width (mm)	67.70 ± 4.39	66.20 ± 5.41	66.89 ± 4.38	67.44 ± 4.74	0.19	0.751	0.11	0.737
DF2 Length (mm)	45.35 ± 3.09	53.82 ± 3.38	44.13 ± 3.83	54.3 ± 2.51	2.15	0.411	205.52	**<0.001**
DF2 Width (mm)	100.91 ± 7.35	104.65 ± 6.38	99.83 ± 7.21	106.14 ± 3.69	0.17	0.751	15.05	**<0.001**
DF3 Length (mm)	42.80 ± 3.69	53.60 ± 4.40	42.18 ± 3.66	52.76 ± 3.31	1.14	0.699	211.18	**<0.001**
DF3 Width (mm)	66.03 ± 6.09	73.44 ± 5.91	65.31 ± 5.12	72.31 ± 3.88	0.78	0.713	43.81	**<0.001**
AF1 Length (mm)	44.23 ± 7.87	52.98 ± 6.32	42.61 ± 3.52	55.15 ± 7.40	0.47	0.751	73.60	**<0.001**
AF1 Width (mm)	91.47 ± 8.47	95.70 ± 6.20	91.07 ± 5.62	99.41 ± 7.12	0.21	0.751	21.64	**<0.001**
AF2 Length (mm)	38.09 ± 3.01	50.04 ± 4.83	37.53 ± 2.44	50.84 ± 3.26	0.22	0.751	437.14	**<0.001**
AF2 Width (mm)	61.52 ± 5.84	67.39 ± 3.31	60.89 ± 4.59	67.36 ± 2.92	0.35	0.751	41.40	**<0.001**
Right PF Length (mm)	47.47 ± 4.41	62.10 ± 6.05	45.99 ± 5.09	58.61 ± 6.16	5.10	0.087	177.45	**<0.001**
Left PF Length (mm)	50.32 ± 4.32	61.61 ± 5.42	48.45 ± 3.89	59.62 ± 5.33	6.38	0.054	162.10	**<0.001**

672 **Table 2.2** The percentage of explained variance, eigenvalues and the loadings of the
673 measurements included in the PCA (with varimax rotation) on the first four
674 principal components (PCs), for the farmed and wild Atlantic cod (*Gadus morhua*).
675 DF, AF, and PF refer to the, dorsal, anal and pelvic fins respectively, and their
676 corresponding numbering begins with the most anterior fin. CI and LI are the
677 standardized residuals of the regression of standard length, and liver weight on total
678 weight respectively. Fin sizes were standardized to a common centroid size, while
679 the calculation of CI and LI includes an inherent standardization.
680

681

Measure	PC1	PC2	PC3	PC4
Condition Index (CI)	-0.01	0.54	-0.15	0.51
Liver Index (LI)	0.25	0.43	-0.13	0.21
DF1 Length (mm)	-0.36	0.04	-0.06	0.09
DF1 Width (mm)	0.00	0.43	-0.33	-0.10
DF2 Length (mm)	-0.33	0.00	0.14	0.19
DF2 Width (mm)	-0.17	0.33	0.61	-0.13
DF3 Length (mm)	-0.35	0.01	0.04	0.00
DF3 Width (mm)	-0.22	0.12	-0.37	-0.47
AF1 Length (mm)	-0.25	-0.17	-0.06	0.04
AF1 Width (mm)	-0.22	0.34	0.40	-0.25
AF2 Length (mm)	-0.37	0.00	0.06	0.17
AF2 Width (mm)	-0.25	0.19	-0.32	-0.42
Right PF Length (mm)	-0.31	-0.10	-0.13	0.31
Left PF Length (mm)	-0.32	-0.14	-0.20	0.22
Percentage of Variance	44.34	12.56	8.84	8.53
Eigenvalue	6.21	1.76	1.24	1.19

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2.8 Figures

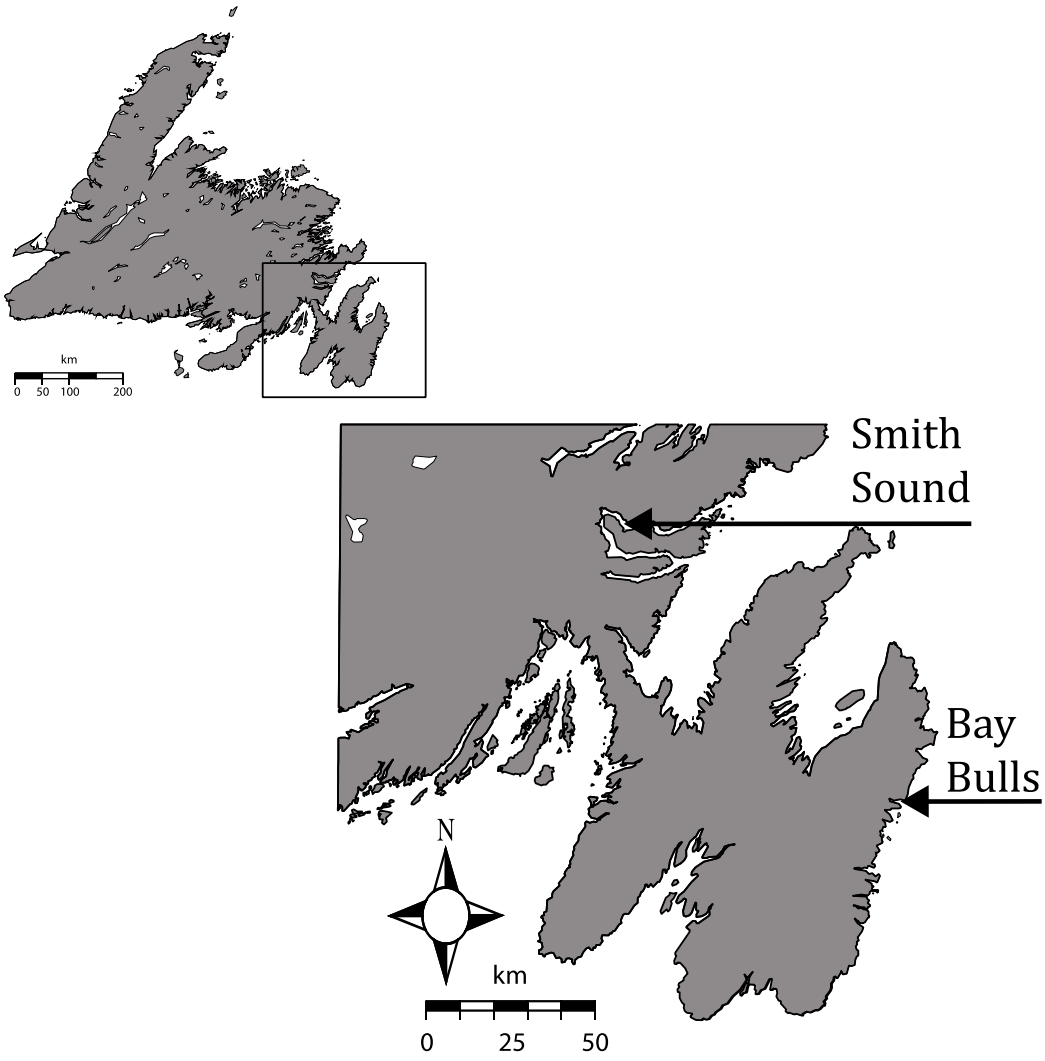
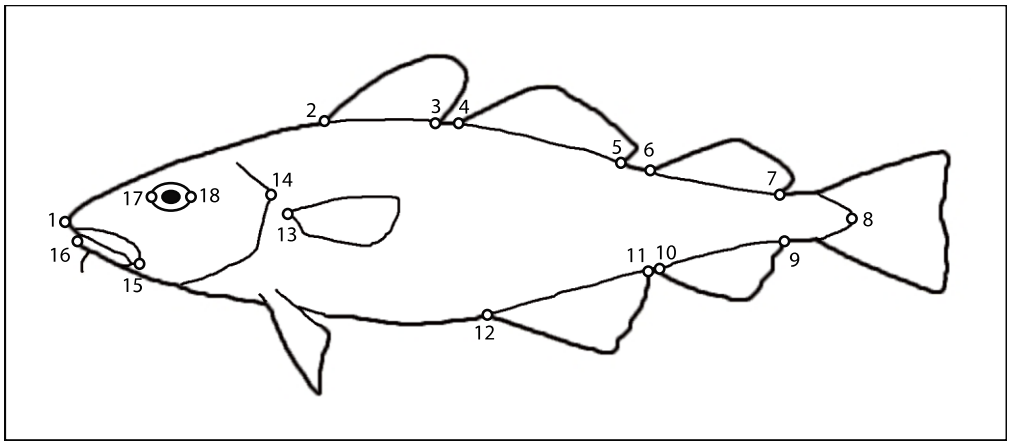


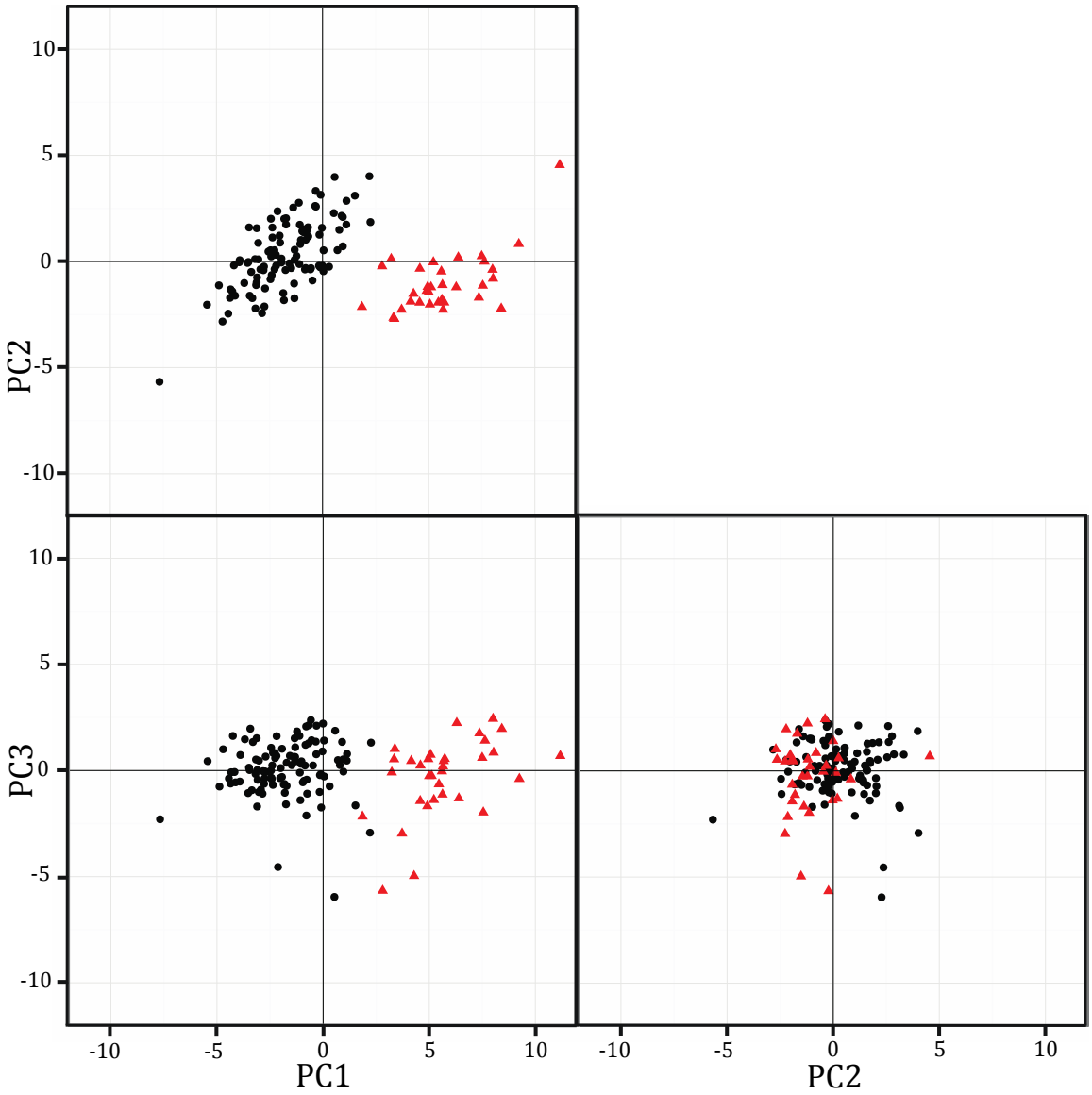
Figure 2.1 Map of the island of Newfoundland, Canada, showing the locations of sample collection.



692

693 **Figure 2.2** Locations of landmark points recorded on Atlantic cod (*Gadus morhua*).
694 1) Anteriormost point of premaxilla; 2) origin of the first dorsal fin (DF1); 3)
695 insertion of DF1; 4) origin of the second dorsal fin (DF2); 5) insertion of DF2; 6)
696 origin of the third dorsal fin (DF3); 7) insertion of DF3; 8) posteriormost point of the
697 hypural plate; 9) insertion of the second anal fin (AF2); 10) origin of AF2; 11)
698 insertion of the first anal fin (AF1); 12) origin of AF1; 13) origin of pectoral fin; 14)
699 posteriormost point of the operculum; 15) posteriormost point of the maxilla; 16)
700 anteriormost point of the dentary; 17) anteriormost point of the eye; 18)
701 posteriormost point of the eye directly across from point 17.

702



703

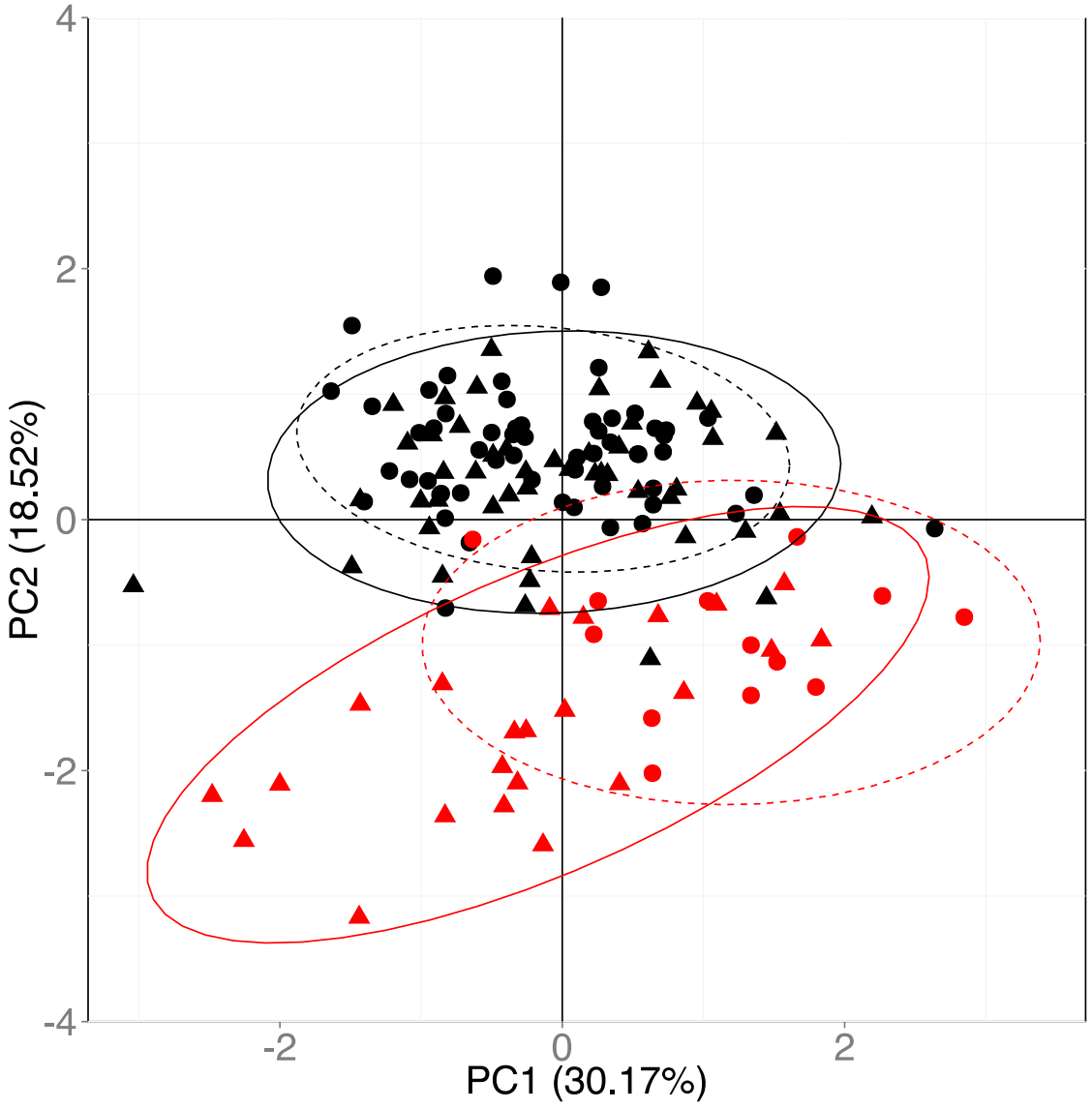
704 **Figure 2.3** Individual factor scores for the first three principle components for the

705 traditional morphometric analysis. Farmed *Gadus morhua* individuals (n = 108) are

706 plotted using black circles, while red triangles are used for the wild (n = 36).

707

708



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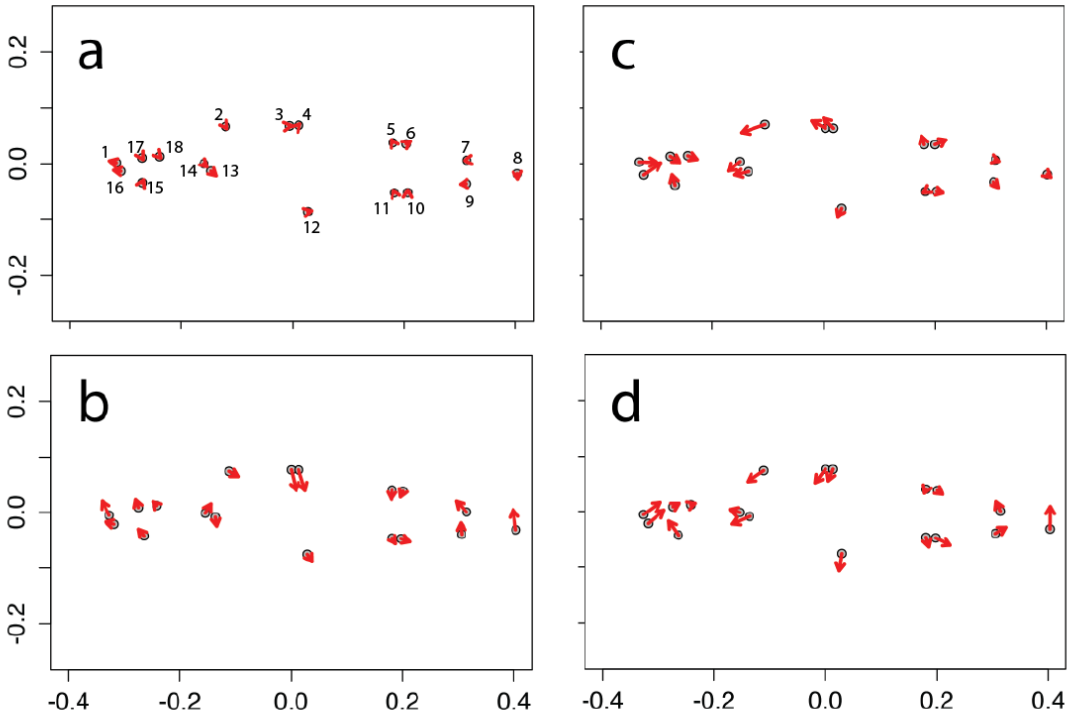
711 **Figure 2.4** *Gadus morhua*. Ordination plot for the configurations of specimens into
712 principle warp shape for the geometric morphometric analysis. Individuals are
713 plotted by origin and sex using colour and shape respectively (farmed = black, wild =
714 red, males = triangles, females = circles; farmed males n = 58, farmed females n = 50,
715 wild males n = 13, wild females n = 23). The ellipses represent the 95% confidence
716 interval for the groups. The same colour scheme is used to denote origins, but sexes
717 are distinguished by line type (solid = males, dashed = females).
718



719

720 **Figure 2.5** *Gadus morhua*. Magnitude and displacement of the consensus shapes of:
721 a) farmed females (n = 50) relative to farmed males (n = 58); b) wild females (n =
722 23) relative to wild males (n = 13); c) farmed females relative to wild females; d)
723 farmed males relative to wild males. The red arrows indicate the direction and
724 degree of displacement of the landmarks of the consensus shape of the first group
725 relative to the black dots, which represent the location of landmarks on the
726 consensus shape of the second group. The landmark numbering in *b*, *c*, and *d* is the
727 same as that in *a*, and landmark numbers and descriptions are given in Figure 2.1.
728 Displacements have been magnified 3X for easier visualization. The units for both
729 the *x*- and *y*-axes are the Procrustes coordinates.
730

Chapter 3 – In search of a "cultured fish phenotype": a systematic review, meta-analysis, and vote-counting analysis.

3.1 Abstract

That cultured fishes develop a morphology that differs from their wild conspecifics has become nearly axiomatic in fisheries science. A commonly supervened corollary is that exposure to culture causes a set of predictable and consistent morphological changes that result in a common "cultured phenotype" in fishes because the similarity of environments and selection pressures is greater among culture than natural environments. While this is often asserted, it has not been formally tested. A systematic review of the literature based on PRISMA best practice protocols identified 65 papers, composed of 106 studies that compared the morphology of 39 species of cultured fish to their wild conspecifics. This formed the basis of a meta-analysis of quantitative, and vote-counting analysis of qualitative differences (in this case this is akin to a chi-square test for differences in counts of three categories) in 16 external morphological features and condition factor. My analyses confirm that aspects of a general "cultured phenotype" exist. The meta-analysis analysis revealed that cultured fish had consistently shorter fins and upper jaws than wild fish, and the vote-counting analysis was suggestive of this as well. The vote-counting analysis showed that across all studies cultured fish had greater body depth and condition factor than wild fish, but this was not supported by the meta-analysis. As well as

matching the morphological changes required to develop the "cultured phenotype", the changes detected in our analyses are consistent with experimentally observed plastic responses to environmental conditions typical of those experienced in culture. This is discussed, as is how intentional and unintentional selection in culture may contribute to, or reinforce the observed morphological changes.

3.2 Introduction

Globally, the demand for fish product has outstripped what is available from capture fisheries and landings have plateaued. To meet this demand both the number of fish and number of species of fish in culture have increased over the past 50 years (FAO 2014). In concert with this plateauing of capture fisheries is the realization that many of the world's fish stocks are currently fully- or over-exploited, and that in some cases this is exacerbated by the degradation of habitat (Hutchings & Reynolds 2004, Dobson et al. 2006, Wilberg et al. 2011). To this end, various supplementary hatchery programmes have been established worldwide as an effort to bolster natural populations and to offset human-mediated habitat loss (McDonald et al. 2007, Kinziger et al. 2008, Tiffan & Connor 2011). The net result of both the increase in hatchery and aquaculture production is that a large and increasing number of fish that have been exposed to artificial culture conditions are being intentionally, or unintentionally (e.g. through escape from aquaculture) released into the wild and subsequently coming into contact with wild fishes (Poole et al. 2003, Jonsson & Jonsson 2006, Jørstad et al. 2008, McGinnity et al. 2009, Vehanen et al. 2009,

772 Chittenden et al. 2010, Fraser et al. 2010a, Rogdakis et al. 2011, Somarakis et al.
773 2013).

774 Exposure to culture conditions leads fish to develop phenotypes that differ
775 from those of their wild counterparts, and that may be maladaptive in the wild
776 (Fleming & Gross 1994, Araki et al. 2008, Bailey et al. 2010, Chittenden et al. 2010).
777 Cultured phenotypes are the product of a plastic response whereby different
778 phenotypes can be expressed by a single genotype in response to different
779 environmental conditions (Imre et al. 2002, Skjæraasen et al. 2008, Mayer et al.
780 2011, Vehanen & Huusko 2011), and/or genetic changes brought about through
781 both intentional and unintentional selection (Fleming et al. 1994, reviwed by: Einum
782 & Fleming 2001, Fleming & Petersson 2001, Hutchings & Fraser 2008, Solberg et al.
783 2013, Colihueque & Araneda 2014).

784 Most aquacultured species undergo breeding programmes with similar goals,
785 such as rapid growth (e.g. Myers et al. 2001, Fleming et al. 2002, Thrower et al. 2004,
786 Small 2006, Wringe et al. 2010), delayed maturity (e.g. Myers et al. 2001, Fleming et
787 al. 2002, Wang et al. 2006, Wang et al. 2008, Gjedrem 2010), high-density
788 production (e.g. Thorpe 1991, Kause et al. 2003, Gjedrem 2010), disease resistance
789 (e.g. Ridha 2006, Trenzado et al. 2006) and greater feed conversion efficiency (e.g.
790 Hulata 2001, Nichols et al. 2003, Antonello et al. 2009). Given that farmed fish are
791 never intended to be released into the wild, these selection programmes often have

792 little or no regard for maintaining fitness of these fish in the wild or of maintaining a
793 wild-type morphology, apart from ensuring the production of an 'appealing'
794 phenotype for the consumer (e.g. Kause et al. 2006, Small 2006, reviewed by:
795 Colihueque 2010, Colihueque & Araneda 2014). Conversely, supplementary
796 hatchery programmes often strive to produce fish for release which will be viable in
797 the wild, and which are similar in morphology to their wild counterparts (Iguchi &
798 Mogi 2007, Belk et al. 2008, Blanchet et al. 2008, Brockmark & Johnsson 2010, Wilke
799 et al. 2015). Despite the efforts of hatcheries, evidence suggests that the fitness of
800 hatchery-produced fish is often lower than that of their wild conspecifics (Barahona-
801 Fernandes 1982, Svåsand et al. 2000, Miller et al. 2004, Araki et al. 2008, Gavaia et
802 al. 2009).

803 Differences in selection aside, it is important to note that the environments
804 experienced by fishes in any type of culture tend to share many commonalities.
805 These include low habitat complexity, stable and plentiful non-elusive feed,
806 consistent water velocity, and high fish density, all of which have been shown to
807 have predictable effects on fish morphology (e.g. Currens et al. 1989, McDonald et al.
808 1998, Pakkasmaa & Piironen 2000, Purchase & Brown 2001, Imre et al. 2002,
809 Langerhans et al. 2003, Latremouille 2003, Enders et al. 2004, Wintzer & Motta
810 2005, Bureau et al. 2006, Marcil et al. 2006a, Ambrosio et al. 2008, Vehanen &
811 Huusko 2011, Arechavala-Lopez et al. 2013b, Pulcini et al. 2014). In light of this, it is
812 possible that cultured fishes may converge on a stereotypical "cultured phenotype"

(Fleming et al. 1994, Balon 1995, Pulcini et al. 2014) through similar plastic or adaptive responses because the environments they experience appear to be more similar to each other than are the environments experienced by their wild conspecifics. In fact, it is often suggested that cultured individuals of many species can be readily distinguished visually from their wild conspecifics because of differences in morphology caused by cultured rearing, and that many of the features, and the direction in which they differ from cultured to wild fish are similar for multiple species (e.g. Balon 1995, Busack et al. 2007, Uglem et al. 2011, Arechavala-Lopez et al. 2013a). Morphological divergence of cultured fish from their wild conspecifics, which can be thought of as leading to the "cultured phenotype," is generally said to include greater body depth and condition, but smaller fins, eyes and heads. Some researchers have gone so far as to suggest the degree of permanent phenotypic divergence caused by exposure to culture and the selection therein has been large enough to warrant the designation of farmed Atlantic salmon as a (sub-)species distinct from wild Atlantic salmon (wild *Salmo salar*, Salmonidae; cultured *S. salar domesticus*; Gross 1998).

Despite differences between cultured and wild fish having been reported for various species individually, and the commonality of these changes among species being alluded to, no formal test has been conducted to determine if exposure to culture conditions leads to a set of common morphological changes in fish exposed to culture relative to the morphology of their wild counterparts. To this end, we

834 performed a meta-analysis, as well as a vote-counting analysis (i.e. a chi-square test
835 on the number of studies finding each of the three qualitative differences in
836 morphological feature size), based on a systematic review that was conducted
837 following PRISMA best practice protocols (Refer to Supplementary Table 3.1 for
838 PRISMA (Liberati et al. 2009, Moher et al. 2009) Checklist) of the literature on
839 morphological differentiation between cultured fish and their wild counterparts to
840 determine if similar patterns of divergence are observed across species. In addition
841 to just examining the effect of culture as a whole, we also determined the influence
842 of a number of variables that could reasonably have an influence on the degree of
843 phenotypic divergence observed.

844 The degree of phenotypic change and its permanence are both a function of
845 the time an individual has spent in captive conditions (Pakkasmaa et al. 1998, von
846 Cramon-Taubadel et al. 2005), as well as the degree of genetic change in the cultured
847 lineage (Fleming et al. 1994, Blanchet et al. 2008, reviewed by: Hutchings & Fraser
848 2008, Fraser et al. 2010a). To this end, we examined whether the number of
849 generations for which a population's ancestral line had been in captivity influenced
850 the degree of differentiation. As well, it was noted earlier that different types of
851 culture may have different selection regimes and goals, thus we tested if the
852 phenotypic divergence of fishes reared in hatcheries, farms or laboratories differed.
853 These locales may also differ as to the time an individual has spent in captivity, with
854 hatcheries generally releasing fish as juveniles; while in farms and labs they are

often retained into adulthood. We also tested the role of environment and genetics in shaping the phenotype of fishes by looking at differences in the degree of differentiation between studies in which the wild and cultured fish were reared in a common garden to those in which the wild fish had been captured from the wild, and by investigating studies in which the fish compared were from the same ancestral population, and when they were not. Finally, because a great deal of research effort has been put into improving the performance of Salmonidae in both commercial aquaculture farms and following release from supplementation hatcheries (producing two types of fish production that have opposing goals, but yet like all types culture share environmental similarities), we tested if Salmonidae differ in their response to culture compared to other families of fishes. It would of course have been of considerable interest to be able to compare amongst all families, not just Salmonidae against all other fish, however the sample sizes for other families were too small to allow testing.

3.3 Materials and Methods

3.3.1 Data collection

Our goal was to test the hypothesis that when exposed to culture, fishes develop stereotypical changes in their external morphology relative to their wild conspecifics. We began by conducting a systematic review using PRISMA best-practice protocols (Refer to Supplementary Table 3.1 for PRISMA (Liberati et al.

875 2009, Moher et al. 2009) Checklist) with our search terms (Supplementary Table
876 3.2) intentionally defined quite broadly to ensure we identified as many publications
877 as possible. We considered fish reared in any non-natural environment to be
878 cultured (i.e. farms, hatcheries, laboratory or other aquaria; Table 3.1). Searches
879 were conducted in three main databases: the Aquatic Sciences and Fisheries
880 Abstracts Database (ASFA), Web of Science, and Google Scholar. The titles and
881 abstracts of papers returned by our searches were parsed, and all publications that
882 appeared to compare the phenotypes of wild and cultured fish were retained for
883 further screening (Figure 3.1 and Supplementary Table 3.3). Publications retained at
884 this initial screening stage were then read, and studies were evaluated against our
885 four inclusion criteria (Liberati et al. 2009). These criteria were: 1) the study must
886 have examined the external morphology of the fish; 2) it must have been measured
887 in a quantitative manner; 3) a comparison of cultured to a wild population must
888 have been undertaken; and 4) the cultured fish must have spent the entirety of their
889 lives in captivity (i.e. studies of recaptured or "sea ranched" cultured fish were
890 excluded because convergence on wild-type phenotype has been reported in fishes
891 following release (Fleming et al. 1994, Arechavala-Lopez et al. 2013b), and since the
892 purpose of this study was to examine the effect of culture conditions on phenotype
893 we were worried that this would 'dilute' the signal from such studies). All
894 publications containing studies that conformed to these criteria were included (Fig.
895 3.1 and Supplementary Table 3.3). Using the same methodology and inclusion

criteria, we also screened all references within the publications retained at the initial screening stage, as well as within relevant reviews identified during our initial search.

Once the systematic review had been completed, and having parsed all publications retained, a set of external morphological features were selected that were commonly measured in morphological studies, were homologous across species, for which differences in their relative expression may affect the fish's fitness, and which are commonly asserted to comprise the "cultured phenotype" (Fig. 3.2). We also chose to include condition factor (Fulton's $K = 100(W/L^3)$) in our analysis because, while it is not technically an external morphological feature, it does have bearing on the fish's overall external conformation, and conforms to the other criteria.

Differences in experimental methodology, study purpose, and a myriad of other factors, meant that all of the morphological features chosen to be examined in our meta-analysis were not measured or reported in every publication. We recorded the available morphological feature means and where reported, the corresponding standard deviations (see Statistical Analysis for treatment of missing standard deviations). In addition, we recorded species, the form of culture, and whether the wild and cultured fish that were compared were from the same ancestral genetic population. Again, each of these was not reported in every publication, and even

916 when details were reported, they tended to differ among publications. To overcome
917 this disparity, each variable was made categorical (Table 3.1), and where any of
918 these data were unavailable or ambiguously reported, they were coded as
919 'unknown' and excluded from the analysis.

920 Finally, a number of publications presented the results of multiple
921 independent (e.g. comparisons of different populations or cohorts of cultured and
922 wild fish) or semi-independent (e.g. comparison of multiple populations of wild to a
923 single population of cultured fish, or *vice versa*) wild/cultured comparisons
924 (Supplementary Table 3.4). In both cases, each comparison was treated as being an
925 independent result (i.e. study), and separate sets of effect sizes were recorded for
926 each. Repeated sampling of species within publications, as well as differences in the
927 number of studies available for each species was accounted for by using species as a
928 random effect in the mixed effects models (see below for further model
929 information).

930 It was our hope to be able to calculate an effect size for each morphological
931 feature measured in each of the studies that conformed to our inclusion criteria.
932 However, it became evident early in the review that many of the publications
933 identified, despite stating in their materials and methods that specimens were
934 measured such that quantitative values would explicitly (e.g. direct measurements
935 using a ruler or calliper) or implicitly (e.g. from conversion of *x*-*y* dimensions for

936 geometric morphometrics) be generated, the results were reported such that values
937 could not be obtained from either the text or figures of the paper, nor from the
938 referenced supplemental materials (e.g. differences displayed as PCs or differences
939 remarked on qualitatively). We attempted to surmount this issue in two ways:
940 firstly, we contacted and requested data from study authors, and if they provided
941 data or clarification we included it in our meta-analysis. Second, because it was
942 possible to determine the qualitative differences in morphological feature size
943 between the cultured and wild populations (e.g. pectoral fin longer in wild than
944 cultured population) in all studies, we recorded the qualitative differences as one of
945 three categorical values: 1) cultured larger than wild (C>W), 2) wild larger than
946 cultured (C<W), or 3) no difference reported (C=W). For each morphological feature,
947 we then tested if the proportion of studies falling into each of the three categories
948 differed. Qualitative differences were thus recorded for all studies that passed our
949 inclusion screening, while effect sizes could only be calculated for those studies from
950 which the population means were available (Fig. 3.1).

951 ***3.3.2 Statistical Analysis – Vote counting analysis***

952 All statistical analyses were conducted in R version 3.2.1 (R Development Core Team
953 2015). For the analysis of the qualitative differences in feature size, a simple
954 difference of proportions test was used, which did not incorporate a random effect
955 (prop.test function, stats package; R Development Core Team 2015). We chose not to
956 incorporate a random effect because, in keeping with the more inexact nature of the

957 qualitative and categorical response variable, we wanted our model to be as liberal
958 as possible. For all studies, for each morphological character, we first tested if there
959 was a difference in the proportion of studies reporting each of the three qualitative
960 difference categories (i.e. C>W = C<W = C=W). Where significant differences in
961 proportion were observed, all possible pairwise combinations were tested with the
962 resultant p-values adjusted using the method of Benjamini and Hochberg (1995) to
963 control the false discovery rate.

964 Next, using the 'moderators' (i.e. dependent variables, terminology of the
965 meta-analysis package developed by Viechtbauer 2010 and will be used throughout
966 for consistency) listed in Table 3.1, we looked for differences in proportion for those
967 studies comprising each category of the moderator. In cases where subsetting using
968 the moderators resulted in fewer than 10 studies in a given grouping, that grouping
969 was not subjected to statistical analysis. Again, where significant differences in
970 proportion within a moderator were found, all pairwise combinations were tested,
971 and p-values adjusted (Benjamini & Hochberg 1995). We also tested if the
972 proportion of studies that found a given qualitative difference varied between
973 categories of a moderator.

974 ***3.3.3 Statistical analysis - Formal meta-analysis***

975 For every study in which numerical values were reported, a separate effect size was
976 calculated for each morphological feature measured therein. To ensure that effect

977 sizes were not biased by differences in overall body size between fish in different
978 studies, we used the response ratios (ratios of means), as the effect size because this
979 measure quantifies the proportional change between groups and as such provides
980 inherent across-study size standardization, provided both groups in a study were of
981 similar size (Hedges et al. 1999). Most studies included some type of size
982 standardization between the groups examined, and the difference in reported mean
983 lengths between cultured and wild fish did not differ (linear mixed-effects model
984 with species as random, chisq = 0.4601, p > 0.49). Which indicates the inherent
985 across-study size standardization should function appropriately.

986 The response ratio was calculated for each morphological character in Fig.
987 3.2 using the function *escalc* from the R package metafor (Viechtbauer 2010), which
988 employs the formula proposed by Hedges et al. (1999): $L = ln(\bar{X}_c) - ln(\bar{X}_w)$, where
989 *ln* is the natural logarithm ($\log_e$), the subscripts *c* and *w* refer to the cultured and
990 wild populations respectively, for which their means, $\bar{X}$, of a morphological
991 character were reported. The formula has the corresponding variance:

$$\frac{(SD_c)^2}{n_c \bar{X}_c^2} + \frac{(SD_w)^2}{n_w \bar{X}_w^2}$$

992 Where *n* is the sample size and *SD* the standard deviation for the population denoted
993 in subscript. The natural logarithm is used because it linearizes the effect metric by

994 treating deviations in the numerator and denominator equally and has the added
995 benefit of normalizing the sampling distribution (Hedges et al. 1999).

996 As noted above, standard deviations were not reported for all studies.
997 Missing standard deviations were imputed using regression techniques based on the
998 relationship observed between standard deviation and sample size in those studies
999 with complete information (Koricheva et al. 2013). To estimate the missing standard
1000 deviations in our meta-analysis we employed mixed-effects models with species as a
1001 random effect, which allowed the intercept to vary for each species. In addition to
1002 sample size, we also controlled for differences in the size of the fish by including
1003 total length as a fixed effect. Thus the missing standard deviations were calculated as

$$SD_x = abs\big(intercept + \ (n_x \times b_n) + (TL_x \times b_{TL})\big)$$

1004 Where: SD_x is the estimated standard deviation for population *x*, with corresponding
1005 sample size $n_x$and mean total length TL_x. b_n and b_{TL} are the slopes of the
1006 relationships for sample size and total length respectively, $intercept$ is the model
1007 intercept, and abs is the absolute value function. These imputed values were then
1008 used in the calculation of the variance of the response ratio.

1009 If exposure to culture leads a given morphological trait to exhibit a common
1010 morphological change relative to wild populations, it would be expected that using
1011 the response ratio as an effect size, the effect sizes for a given morphological trait
1012 will be either consistently greater, or less than zero across all populations examined

1013 (zero being no difference between cultured and wild). Thus for each morphological
1014 feature, we tested if its grand overall mean effect size was significantly different
1015 from zero (i.e. no difference between cultured and wild) using mixed effects linear
1016 models, with species as the random effect (Koricheva et al. 2013). Including species
1017 as a random effect in our model accounts for the fact that direction, magnitude or
1018 scope of morphological change may be more similar within, than across species and
1019 also for the fact that the number of studies for each species varied. As well,
1020 variability among the effect sizes may be the result of the studies included in the
1021 meta-analysis not being identical in terms of their methodologies, and this can be
1022 accounted for statistically by treating this variability as completely random through
1023 the use of random effects within the mixed-effects linear models (rma.mv function,
1024 metafor package; Viechtbauer 2010). The rma.mv function was used because it was
1025 designed for multivariate or multi-level meta-analyses, unlike the rma.uni function
1026 which is only suitable for univariate analyses. Finally, the use of random-effects
1027 structure in these models also allows us to make unconditional inferences about a
1028 larger set of studies that have been conducted, or could be conducted in the future,
1029 from which the studies included in the meta-analysis are assumed to be a random
1030 sample (Viechtbauer 2010). Thus random effects models allow us to extend the
1031 observed morphological responses to culture to an effect of cultured conditions in
1032 general, and not limited to just those studies included in the analysis.

Following testing all studies included in the meta-analysis, we then examined if the factors listed in Table 3.1 lead to common morphological change by including them as moderators in the model. The influence of each factor had to be tested separately for two interrelated reasons. Firstly, information for all the factors considered could not be obtained from all studies. The meta-analytical statistical function used in our analysis is a type of generalized-linear-model (i.e. independent variables; Viechtbauer 2010) and as such, for a study to be included it must have a corresponding value in each of the categorical moderator terms. Thus, to test the factors simultaneously instead of singly, those studies that were missing data (i.e. coded as unknown) from even one of the factors must be dropped from the entire analysis, instead of just from the analysis of that factor singly. Secondly, even for those studies in which all factors contained data, not all category combinations were present for most of the features. This resulted in spurious interaction between factors. Thus, it was decided that the factors should be tested independently.

3.4 Results

3.4.1 Overall

We examined the relative differences in trait size between cultured and wild fish for all 106 studies identified by the systematic review (Supplementary Table 3.4). This was done with the vote-counting analysis by testing for differences in the proportion of studies that found one of the three possible relative size differences (i.e. Cultured

1053 < Wild, C>W, C=W). Among all studies, no differences in proportion of studies
1054 finding these three possibilities were found for head length and depth, eye size,
1055 lower jaw length, caudle peduncle length and depth, pectoral and pelvic fin length,
1056 dorsal fin length and width, anal fin length, and caudal fin length ($P > 0.05$; see
1057 Supplementary Table 3.5). The vote-counting analysis found that the length of the
1058 upper jaws of the cultured fish tended to be shorter than those of the wild fish ($P <$
1059 0.05; Supplementary Table 3.5). The opposite was observed for both body depth and
1060 condition factor, with the greatest proportion of studies finding them to be larger in
1061 cultured than wild populations ($P < 0.0001$; Supplementary Table 3.5). The width of
1062 the anal fin appeared to be unaffected by culture with almost half of all studies in
1063 which it was measured reporting no difference between the wild and cultured
1064 populations and this proportion was significantly greater than the proportions that
1065 found the width to differ ($P < 0.05$; Supplementary Table 3.5). Other comparisons
1066 will not be discussed because of small sample size (i.e. fewer than 10 studies;
1067 Supplementary Table 3.5).

1068 Among all 67 studies for which we were able to calculate effect sizes, the
1069 meta-analysis found the lengths of the head, upper jaw and, pectoral and pelvic fins,
1070 and the lengths and widths of the dorsal and anal fins were significantly smaller in
1071 cultured fish, while none of the other features were found to be significantly
1072 different (all $p < 0.05$; Fig. 3.3). It must be noted that, while the meta-analysis was
1073 conducted using $L = ln(\bar{X}_c) - ln(\bar{X}_w)$, where values of zero indicate no difference,

for ease of interpretation the results in Figs. 3.3, 3.4 and 3.5 are presented as the exponent of *L*. This transforms the mean and standard deviations of the effect size for a given character to fold changes of the cultured measure relative to the wild. Thus after transformation a value of one is no difference, and values less than one indicates the feature is smaller in the cultured fish than the wild, while values greater than one signify the opposite.

When examining the congruence of the two analyses, it must be borne in mind that the vote-counting analysis and meta-analysis were inherently different. The criteria for significance were more stringent in the meta-analysis. Unlike the vote-counting analysis, the meta-analysis methodology not only assesses the magnitude of difference but also gives weighting to each study (through the manner in which the sampling variances associated with each study/effect size are treated within the mixed-effects linear model in the meta-analysis (Viechtbauer 2010)) based on variability/accuracy of the measurements, as well as the sample size. Furthermore, by employing a random-effect structure, the meta-analysis is also able to account for potentially greater within than across species similarities, and variability in the number of studies per species. As such, while a summary of the congruence between the vote-counting analyses and meta-analyses for all results has been provided in Supplementary Table 3.6, only cases where both the meta-analysis and vote-counting analysis were significant are mentioned. That said, the meta-analysis and the vote-counting analysis both found the length of the heads to

be shorter in cultured fish. Their results were not congruent for the length of the anal fin with the meta-analysis showing it to be lower in cultured fish, while the vote counting analysis suggested the anal fins of cultured and wild fish to be equal in length (Supplementary Table 3.6).

3.4.2 Form of culture

Looking first at differences within forms of culture, the vote-counting analysis showed that among studies of farm fish a significantly greater proportion of studies found the eyes of cultured fish to be smaller than those of the wild, and the same was true of the proportion that reported no difference in upper jaw length, and caudal peduncle depth (all $P < 0.05$; Supplementary Table 3.5). As well, greater body depth and condition in cultured than wild fish was seen in a greater proportion of studies than the other two outcomes (all $P < 0.01$), while a greater proportion of studies found the width of the anal fins of the farmed and wild fish to be equal, than found them to be wider in the cultured than the wild ($P < 0.05$; Supplementary Table 3.5). The meta-analysis found only the length of the head and the depth of the caudal peduncle differed, both being significantly less in cultured than wild fish (Fig. 3.4).

Among studies of hatchery fish, the vote-counting analysis showed that the greatest proportion reported the upper jaws to be shorter in the cultured than wild fish, and the same to be true of the length and width of the dorsal fin (all $P < 0.05$; Supplementary Table 3.5).

1115 The meta-analysis revealed that the pectoral fins of hatchery-reared fish
1116 were shorter than their wild counterparts ($p < 0.001$, Fig. 3.4). While significant
1117 differences were detected for the lengths of the pectoral, pelvic, anal and dorsal fins,
1118 the sample size was small in some cases (Fig. 3.4).

1119 Vote-counting analysis found that among studies of laboratory fish a
1120 significantly greater proportion of studies found no difference in head length and
1121 depth between wild and cultured populations than found the heads of the cultured
1122 fish to be smaller (both $p < 0.05$; Supplementary Table 3.5). As well, within
1123 laboratory studies, the vote-counting analysis revealed that a significantly greater
1124 proportion of studies found no difference in pelvic fin length than found it to be
1125 greater in the cultured fish, while the opposite was observed for body depth (both P
1126 < 0.05; Supplementary Table 3.5).

1127 The meta-analysis found that head and pelvic fin lengths were significantly
1128 smaller in laboratory reared than wild fish (both $p < 0.01$; Fig. 3.4). Body depth, the
1129 lengths of the upper jaw and pectoral fins, and the lengths and widths of the dorsal
1130 and anal fins were also found to be significantly smaller in the cultured fish, albeit
1131 with small sample size (all $p < 0.05$; Fig. 3.4).

1132 Differences were also observed between forms of culture in the proportion of
1133 studies finding a given possible relative size difference (i.e. C<W, C>W, C=W;
1134 Supplementary Table 3.5). The same is true of the differences in absolute values of

1135 the effect sizes. However, because of the way in which the *rma.mv* function
1136 (Viechtbauer 2010) calculates the confidence intervals when testing for significant
1137 deviation from zero, compared with testing for differences between moderator
1138 levels (i.e. it must be specified that the model should test difference between levels,
1139 and not difference from zero effect), these differences are not obvious from
1140 examining Fig.(s) 3.4 (and 3.5), but can be found in Table 3.2. Specifically, the
1141 absolute values of the effect sizes for farmed and hatchery populations were
1142 significantly greater than those for laboratory populations for the lengths of the
1143 head, upper jaw, and pectoral fin, as well as the body depth (all $p < 0.05$; Table 3.2).
1144 The same was true of the effect sizes for the lengths of the pelvic, dorsal and anal
1145 fins between farm and laboratory populations (all $p < 0.05$; Table 3.2). Where one or
1146 both moderator levels represent five or fewer studies, these differences are not
1147 reported, but can be found in Table 3.2.

1148 ***3.4.3 Commonality of rearing environment***

1149 When cultured fish and the wild fish to which they were compared were reared in a
1150 common garden environment, the vote-counting analysis suggested the heads of the
1151 cultured fish were shorter than those of the wild fish ($p < 0.001$), and a significantly
1152 greater proportion of common garden studies found no difference in the width of
1153 the dorsal and anal fins between the cultured and wild fish than found them to be
1154 different (both $P < 0.05$; Supplementary Table 3.4). Both common garden studies
1155 and those that compared cultured to wild-caught fish found greater condition factor

in the cultured fish more often than not ($P < 0.05$), and the proportions did not differ between study types (all $P > 0.20$; Supplementary Table 3.5). Interestingly, while there was no difference in the proportion of findings for body depth among common garden studies ($P > 0.05$), a significantly greater proportion of wild-caught studies found the body depth of the cultured fish to be lower than that of wild fish than found the opposite ($P < 0.01$; Supplementary Table 3.5).

For the meta-analysis, the signs of the effect sizes were generally the same for both common garden studies and those that employed wild-caught fish, and the majority did not differ significantly in absolute value between these study types except where effect sizes were composed of few studies (Fig. 3.5a, Table 3.2). Studies employing wild-caught wild fish had effect sizes significantly less than zero for the lengths of the head, upper jaw, pectoral, pelvic, dorsal and anal fins, as well as the width of the anal fin (all $p < 0.05$; Fig. 3.5a). While significant differences were observed for the lengths of the head and anal fin and the depth of the caudal peduncle in studies which used a common garden design, caution should be taken in interpreting these results because the number of studies ($n = 1$-4) and species represented for those results detected as significant ($n = 1$-3, all of which are salmonids) are quite low (Fig. 3.5a). The same cautionary message applies to the congruency of the vote-counting analysis and meta-analysis in Supplementary Table 3.5.

3.4.4 Level of domestication

Vote-counting analysis revealed that a significantly greater proportion of studies in which the cultured fish had a domestication history of at least two generations found that the upper jaw of the cultured fish was either shorter, or did not differ from that of the wild fish than those that found the jaws of the cultured fish were longer (both $P < 0.05$), and the same was true of the depth of the caudal peduncle ($P < 0.05$; Supplementary Table 3.5). As well, a significantly greater proportion of studies in which the cultured fish had at least two generations in culture found smaller anal and dorsal fin widths in cultured fish than in wild fish (all $P < 0.05$; Supplementary Table 3.5). Among studies in which the cultured fish had experienced only one generation of culture a significantly greater proportion of studies found no difference in caudle peduncle depth between cultured and wild fish than found it was larger in the cultured fish ($P < 0.05$; Supplementary Table 3.5).

The meta-analysis found that cultured fish which had been exposed to two or more generations of domestication were seen to have significantly shorter heads, pectoral, pelvic, dorsal and anal fins, as well as shallower bodies, and narrower dorsal and anal fins than the wild fish to which they were compared (Fig. 3.5b). The same was true of the lengths of the head and upper jaw of cultured fish exposed to one generation of domestication, and while other significant results were found, the sample sizes tended to be small (all $p < 0.05$; Fig. 3.5b). Sample size must again be

1196 considered when examining the congruency of the meta-analysis and vote-counting
1197 analysis (Supplementary Table 3.6).

1198 Where moderator levels were comprised of five or more studies, the effect
1199 sizes were greater where fish had been exposed to two or more generations of
1200 culture than when they were first generation for the lengths of the lower jaw and
1201 pectoral fin, as well as both the length and depth of the head (all $p < 0.05$; Table 3.2).
1202 The opposite was found of the effect sizes for the width of the anal fin and the depth
1203 of the body (both $p < 0.0001$, Table 3.2).

1204 ***3.4.5 Ancestral population***

1205 Vote-counting analysis showed that for both studies in which the fish compared
1206 were from the same ancestral population and those in which they were not, a
1207 significantly greater proportion of studies found that the length of the upper jaw did
1208 not differ between cultured and wild fish than found a difference (all $P < 0.05$;
1209 Supplementary Table 3.5). While, again for both ancestral types, a significantly
1210 larger proportion of studies found body depth of cultured fish was greater than that
1211 of wild than found it was lower or not different (all $P < 0.05$; Supplementary Table
1212 3.5). The vote-counting analysis also indicated that when the fish compared were of
1213 different ancestral populations no difference in dorsal or anal fin widths between
1214 wild and cultured fish was found in a greater proportion of studies than the

1215 proportion that found them to be larger in the cultured fish ($P < 0.05$;
1216 Supplementary Table 3.5).

1217 Like what was seen for similarities in rearing environment, meta-analysis
1218 revealed that with the exception of where samples sizes were small (i.e. lengths of
1219 the upper and lower jaw, caudal peduncle, and caudal fins), the sign of the effect
1220 sizes were generally the same whether the comparisons were among fish of the
1221 same ancestral population or not (Fig. 3.5c). While the signs were generally the
1222 same, significant differences in the magnitude of the effect size were seen between
1223 study types, with effect sizes for the lengths of the head and dorsal fin, and the width
1224 of the anal fin being significantly larger in studies comparing fish of the same
1225 ancestral population (all $p < 0.0001$, Table 3.2). The opposite was seen between
1226 study types of the effect sizes for body depth and anal fin width (both $p < 0.0001$;
1227 Table 3.2). These same features were found to be significantly different between
1228 cultured and wild fish, with effect sizes showing that cultured fish in both types of
1229 comparison had significantly smaller condition, pectoral, pelvic, dorsal and anal fin
1230 lengths (all $p < 0.05$; Fig. 3.5c).

1231 ***3.4.6 Salmonid and non-salmonid***

1232 Among studies of salmonids, vote-counting analysis showed that a significantly
1233 greater proportion of studies found that the heads of the cultured fish were shorter,
1234 or did not differ in size from wild fish, than found the heads of the wild fish to be

1235 longer (both $P < 0.001$; Supplementary Table 3.5). The proportion of salmonid
1236 studies that found the depth of the caudal peduncle to be greater in cultured than
1237 wild fish was lower than that that found the reverse, but the proportion that found it
1238 to be lower in the cultured fish was less than that that found no difference (both $P <$
1239 0.05; Supplementary Table 3.5). For caudal peduncle length, the proportion of
1240 salmonid studies that found no difference was significantly greater than those that
1241 found a difference (both $P < 0.05$; Supplementary Table 3.5). Among non-salmonid
1242 studies a greater proportion found longer heads in cultured than wild fish than
1243 found the heads of the cultured fish were shorter, and deeper bodies in cultured fish
1244 were reported in a greater proportion of studies than the proportions that found the
1245 opposite or no difference (all $P < 0.05$; Supplementary Table 3.5).

1246 The meta-analysis showed that salmonids exposed to culture had
1247 significantly shorter heads, upper jaws, pectoral, pelvic, and anal fins than the wild
1248 fish to which they were compared (Fig. 3.5d). Among non-salmonids, the same was
1249 true of the lengths of the pelvic and pectoral fins and the width of the anal fin (Fig.
1250 3.5d). Interestingly, unlike most other moderators, the magnitude of the effect sizes
1251 for all morphological characters, with the exception of the lower jaw length, which
1252 had a low sample size for non-salmonids, did not differ between moderator levels
1253 (Table 3.2).

3.5 Discussion

3.5.1 Existence of a "cultured phenotype"

Fishes exposed to culture are often said to possess/develop a similar, readily identifiable "cultured phenotype" characterized by shorter but deeper heads, greater body depth and condition factor, and smaller fins than those typical of their wild conspecifics (e.g. common carp, *Cyprinus carpio*, Cyprinidae [Balon 1995], Atlantic salmon, *S. salar*, Salmonidae [Gross 1998], coho salmon, *Oncorhynchus kisutch*, Salmonidae [Tiffan & Connor 2011], gilthead seabream, *Sparus aurata*, Sparidae and European seabass, *Dicentrarchus labrax*, Moronidae [Arechavala-Lopez et al. 2012], rainbow trout, *O. mykiss*, Salmonidae [Pulcini et al. 2013], Atlantic cod, *Gadus morhua*, Gadidae [Wringe et al. 2015a]).

While this is most commonly cited in truly farmed fishes, differences in morphology between hatchery-reared fish and their wild counterparts are well known (e.g. Fleming et al. 1994, Ellis et al. 1997, Busack et al. 2007, Tiffan & Connor 2011) and thought to contribute to the relatively poor fitness of the released fish (Fleming & Gross 1994, Hard et al. 2000, Belk et al. 2008, Brown et al. 2013). If exposure to cultured conditions does indeed lead to common, directional changes in the size of a morphological feature relative to that of wild fish, it is expected this would be reflected in effect sizes being either consistently greater than or less than zero (N.B. analogous to greater than or less than one as depicted in Figs. 3.3-3.5).

1274 The results our meta-analysis of the literature comparing the morphology of
1275 cultured fish, which have been exposed to varying degrees of selection and time in
1276 captivity, to their wild conspecifics show that as commonly ascribed, the heads of
1277 cultured fish were shorter, as were their upper jaws, and all fin measures with the
1278 exception of the width of the dorsal fin and the length of the caudal fin. However,
1279 unlike what was predicted, measures of body conformation, especially as it relates to
1280 depth measures, were not found to differ. Thus while our findings provide support
1281 to the conjecture of a universal response to culture, leading to the development of a
1282 common 'cultured' phenotype, it does not appear to necessarily involve changes in
1283 body depth, or condition as is commonly suggested.

1284 It also bears noting that the changes in morphology relative to the wild
1285 phenotype required to produce the commonly described "cultured phenotype", and
1286 the phenotypic changes detected in our meta-analysis, are congruent with
1287 experimentally observed plastic phenotypic response to environments typical of
1288 those in culture. Cultured environments are often tailored to be more benign than
1289 that experienced by wild fish (Thorpe 2004), and this is true of farm, laboratory and
1290 hatchery culture. This more benign environment should allow the cultured fish to
1291 sequester a greater proportion of the energy they consume resulting in greater
1292 condition and body depth because of increased accumulation of lipid as well as
1293 greater somatic muscle growth (Currens et al. 1989, Svåsand et al. 1996, Grant et al.
1294 1998, Purchase & Brown 2001, Bureau et al. 2006). However, while our meta-

1295 analysis found no evidence that the body depth or condition (K) of cultured and non-
1296 cultured differed when all studies were included, the results of the vote-counting
1297 analysis suggest these two features are significantly greater in cultured fish. The diet
1298 of cultured fish also likely promotes the development of smaller heads, and jaws,
1299 because fish which are fed non-elusive, prepared diets (Meyer 1987, Wintzer &
1300 Motta 2005), as well as fish fed a greater ration (Currens et al. 1989) have been
1301 shown to develop smaller heads and jaws. Finally, while the lower and less variable
1302 water velocity in culture has been shown to lead to the development relatively
1303 smaller fins in cultured salmonids (Pakkasmaa & Piironen 2000, Wessel et al. 2006,
1304 Keeley et al. 2007), smaller fins in cultured fish can also arise as the result of the fins
1305 being malformed or damaged through abrasion or agonistic interaction (Bosakowski
1306 & Wagner 1994, Latremouille 2003, Hatlen et al. 2006, Blanchet et al. 2008,
1307 Chittenden et al. 2010).

1308 While phenotypic changes could certainly have arisen through plastic
1309 responses to culture, there is no reason to believe that permanent genetic changes
1310 could not have contributed to or caused these changes. Fish in commercial culture
1311 are generally exposed to concerted selection for traits deemed beneficial to
1312 aquaculture, which may lead to unintentional selection on genetically linked traits,
1313 or for traits that convey a fitness advantage on fish in culture (Kallio-Nyberg &
1314 Koljonen 1997, Vasemagi et al. 2012). It may be easiest to conceptualize such genetic
1315 shifts occurring in commercial farms where studies report relatively high levels of

1316 heritability for aquaculture-related traits, at least among salmonids (Benjamini &
1317 Hochberg 1995). However, theoretical (Bekkevold et al. 2006, Fraser 2008) and
1318 empirical (Wessel et al. 2006, Christie et al. 2012) evidence also exists for the
1319 accumulation of permanent genetic changes leading to morphological differentiation
1320 within supplementation hatcheries.

1321 Unfortunately, the separation of genetic vs. environmental effects on
1322 morphology was not possible in our analysis. This is in part because as mentioned in
1323 the materials and methods we were unable to analyze more than one moderator at a
1324 time because missing category combinations caused the formation of significant
1325 interactions in the model. This prevented us from being able to factor out the
1326 simultaneous genetic and environmental effects.

1327 While it is certainly true that genetic changes which accumulate over
1328 generations in culture may modify the scope of plasticity (Solberg et al. 2013), that
1329 we found few significant differences in effect size between domestication levels (six
1330 of 16), especially among features that were found to differ significantly between
1331 cultured and wild fish (three of 10) does not indicate this was the case. Furthermore,
1332 while it is possible that different species may have different scopes, or available
1333 morphospace within which their phenotype is able to lie and these may be
1334 constrained or enhanced by either their underlying genetic variability or
1335 morphology (i.e. the body shape of a species may facilitate or restrict change) this

should not influence the outcome of our study. This is because even where degree of change may be constricted, a consistent directional change in size would still be observed as a deviation in effect size from zero, especially given that such differences were measured as proportional changes.

Apart from perhaps being able to better test the interaction of moderators, whether being able to include the results of all studies identified in the systematic review in the meta-analysis would change its outcome is not readily apparent. This is because the results of the vote-counting do not show clear directionality of difference for most traits, and the results of the vote-counting analysis and the meta-analysis are generally not entirely congruent. However, it is possible that this interpretation is a bit specious because the vote-counting analysis and meta-analysis are inherently different. Unlike the formal meta-analysis the vote-counting analysis gave no weighting to the magnitude of difference or variability/accuracy of the measurements, and the sample size, nor did it employ a random-effect structure.

The fact that the just over half of the total number of studies identified in the literature search provided sufficient detail to be included in the meta-analysis highlights one of the major issues with the field: the lack of consistency in reporting data. The methodology of all studies that passed our inclusion criteria indicated that measurements of the fish were undertaken such that quantitative values would be generated and available for publication. However, likely for concision such extensive

numerical data were omitted in lieu of graphical representations of mean shapes or principal component analysis (PCA). While such presentation methods lend themselves to rapid interpretation of the relative differences between populations, they do not relay quantitative differences as the displacements may be exaggerated (i.e. thin plate splines of mean shapes), or are inherently unitless (PCA). This issue is more prominent in more recent studies, which have moved away from (multivariate) analyses of simple distance measures of morphological features (and at times, their relation to one another), to inherently multivariate truss-based and geometric morphometrics which readily lend themselves to the creation of PCAs and mean-shapes (Adams et al. 2004). It is our recommendation then that future studies provide a table of the mean and standard deviation of morphological distances measured. Given that the majority of studies currently employ digital images, from which the shapes of the fish are digitized to *x*-*y* coordinates using computer software, the creation of such a table should be a trivial matter. This table could be included in the body of the published study, or as would likely be more convenient, as an archived supplement to the published study available through the journal website.

In addition to this recommendation on data reporting, we would refer the reader to Table 3.3 for some general recommendations on a proposed list of data to be collected and reported to aid in the interpretation of comparisons of the morphology of cultured and farmed fish in particular, and also of studies of

1377 morphology of fish in general. While seemingly obvious, a cursory examination of
1378 Supplementary Material Table 3.3 shows that these data often go unreported.

1379 It is our hope that this meta-analysis will serve as an illustration of how
1380 exposure to similar environmental conditions in culture can lead to similar
1381 phenotypic response among species of fish. This suggests that the underlying
1382 species-specific genetic architecture of the fish may have less impact on the
1383 development of phenotype or in regulating scope of plasticity than does the
1384 environment. This has implications not only for fish in culture, but possibly also
1385 if/when they find themselves at liberty, as this scope for plasticity may also temper
1386 their ability to (re)converge on a wild-type phenotype as has been observed in
1387 several species (Fleming et al. 1994, Arechavala-Lopez et al. 2013b). Both
1388 intentional selection, as within commercial settings where consistency of phenotype
1389 is desired, and in hatcheries where the brood stock may derive from only portion of
1390 the wild population and mate choice is removed may contribute to the reduction of
1391 scope for plasticity. In the first case, it has been noted that intentional selection for
1392 faster growth in commercial rearing leads to a reduction in genetic variation for
1393 body weight, and as a consequence reduced plasticity for growth (Solberg et al.
1394 2013). Furthermore, reduction in genetic variation, and canalization of development
1395 can lead to a reduction in the scope for plasticity (Parsons et al. 2010). It stands to
1396 reason then, that perturbation of the normal genetic variability as would occur when
1397 sampling only a portion of a mating population, or removing mate choice may also

lead to reductions in the scope for plasticity.

Promotion of maintenance of plasticity may then be an important consideration for enhancing the viability of fish released from hatcheries, much the same as the production of a wild-type phenotype is currently thought to be. If, as has been shown in this analysis, similar rearing environments lead to similar morphological changes, then the lessons gleaned from the study of the morphological impact of conditions in culture in one species may be readily applicable to other species. We also wish for this paper to illustrate the need for a standard set of information to be reported about the fish on which morphological studies are conducted, as well as a standardized manner to make this information available.

3.6 Acknowledgements

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3.7 Tables

Table 3.1 List and definitions of the moderators used in the meta-analysis

Explanatory Variable		Definition
Form of culture	Farmed	The site from which the cultured fish were sampled was described as a net-pen, or other aquaculture facility typical of commercial rearing of the species.
	Hatchery	The site from which the cultured fish were sampled was described as a hatchery facility, typical of the artificial propagation and (juvenile) rearing of that species
	Lab	The cultured fish were spawned, reared and sampled from a laboratory facility which was not an experimental farm or hatchery
Commonality of rearing environment	Common garden	Both the wild and cultured populations were raised in a common, cultured environment. In this case, the cultured fish were the offspring of fish that had spent at least one generation in cultured conditions, while the wild fish were the offspring of wild-caught fish
	Wild/farmed	The cultured fish were raised in a cultured environment, while the wild fish were themselves wild-caught. Differences in morphology may be the result of genetics and/or environment.
Domestication	>=2 generations	The stock from which the cultured fish were derived had been reared in cultured conditions for at least two generations. The potential exists for genetic changes to have occurred through intentional and/or unintentional selection in the cultured population.

	1 generation	The cultured fish were the progeny of wild-caught parents. Apart from founder effect, the genetics of the cultured population are likely unchanged relative to their source population. (c.f. Wild/farmed commonality of rearing environment: 1 generation cultured fish can be compared to wild caught wild fish, but not to wild fish in a common garden)
Ancestral population	Different	The cultured fish were compared to wild fish from a stock other than that from which they were derived
	Same	The cultured fish were compared to wild fish from the population from which their stock was derived
Salmonid	Salmonid	The cultured and wild fish are part of the family Salmonidae
	Not	The cultured and wild fish are not part of the family Salmonidae

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Table 3.2 Summary of difference in effect size between moderator types for the meta-analysis. Descriptions of the morphological characters can be found in Fig. 3.2 and definitions of the moderators in Table 3.1. Abbreviations are as follows: L = length; D = depth; W = width; Low = lower; Cond = Fulton's condition factor (K); Hatch = hatchery; Lab = laboratory; CG = common garden; WF = studies where cultured were compared to wild caught fish; 2G = studies in which the cultured fish had at least two generations of domestication history; 1G = studies in which the cultured fish were first generation in culture. Salmonid Yes are studies in which cultured and wild fish belong to the family Salmonidae, while all other species are denoted Salmonid Not. Moderator levels comprised of five or fewer studies are annotated with an asterisk (*) where differences between levels are significant

Feature	Moderator	Diff. within level	Z	p
Head Length	Culture location	Farm = Hatch	-1.7	> 0.86
		Farm > Lab	-3.51	< 0.001
		Hatch > Lab	-2.49	< 0.05
	Comparison	CG = WF	1.57	> 0.11
	Domestication	2G > 1G	-3.67	< 0.001
	Population	Diff < Same	34.53	< 0.0001
	Salmonid	Not = Yes	-0.84	> 0.40
Head Depth	Culture Location	Farm = Hatch	0.41	> 0.68
		Farm = Lab	-0.29	> 0.77
		Hatch = Lab	-0.42	> 0.67
	Comparison	CG = WF	-0.65	> 0.51
	Domestication	2G > 1G	-2.3	< 0.05
	Population	Diff > Same*	-2.13	< 0.05
	Salmonid	Not = Yes	0.14	> 0.88
Eye Size	Culture Location	Farm = Hatch	0.76	> 0.44
		Farm = Lab	-0.76	> 0.44
		Hatch = Lab	-1.07	> 0.28
	Comparison	CG = WF	1.25	> 0.21
	Domestication	2G = 1G	-0.47	> 0.64
	Population	Diff = Same	-0.15	> 0.88
	Salmonid	Not = Yes	-0.55	> 0.58

Upper Jaw L	Culture	Farm = Hatch	-0.63	> 0.52
		Farm > Lab	-4.18	< 0.0001
		Hatch > Lab	-2.79	< 0.01
	Comparison	CG = WF	-0.17	> 0.86
	Domestication	2G = 1G	-1.92	> 0.05
	Population	Diff* > Same	-2.49	< 0.05
	Salmonid	Not = Yes	-1.00	> 0.31
Low Jaw L	Culture	Farm = Hatch	-0.69	> 0.48
	Comparison	CG* > WF	-2.24	< 0.05
	Domestication	2G > 1G	-13.67	< 0.0001
	Population	Diff* > Same*	-13.67	< 0.0001
	Salmonid	Not* > Yes	4.20	< 0.0001
Body Depth	Culture	Farm = Hatch	-0.40	> 0.68
		Farm > Lab	-2.77	< 0.01
		Hatch > Lab	-2.25	< 0.05
	Comparison	CG < WF	6.53	< 0.0001
	Domestication	2G < 1G	6.31	< 0.0001
	Population	Diff > Same	-8.21	< 0.0001
	Salmonid	Not = Same	0.80	> 0.42
Cond	Culture	Farm = Hatch	-1.08	> 0.28
	Comparison	CG = WF	1.1	> 0.27
	Domestication	2G > 1G	-6.61	< 0.0001
	Population	Diff > Same*	-6.61	< 0.0001
	Salmonid	Not = Yes	0.34	> 0.73
Caudle Ped D	Culture	Farm = Hatch	1.80	> 0.07
		Farm = Lab	-1.73	> 0.08
		Hatch > Lab*	-2.40	< 0.05
	Comparison	CG* < WF	32.83	< 0.0001
	Domestication	2G = 1G	-0.12	> 0.90
	Population	Diff = Same	0.92	> 0.35
	Salmonid	Not = Yes	0.76	> 0.44
Caudle Ped L	Culture	Farm = Hatch	0.83	> 0.40
		Farm = Lab	0.32	> 0.74
		Hatch = Lab	-0.21	> 0.83
	Comparison	CG = WF	-0.81	> 0.42
	Domestication	2G = 1G	-0.21	> 0.83
	Population	Diff = Same	-0.79	> 0.42
	Salmonid	Not = Yes	0.52	> 0.60
Pectoral Fin L	Culture	Farm > Hatch	-4.42	< 0.0001
		Farm > Lab	-3.66	< 0.001
		Hatch = Lab	-0.04	> 0.96
	Comparison	CG* > WF	-24.18	< 0.0001
	Domestication	2G > 1G	-9.89	< 0.0001
	Population	Diff = Same	0.06	> 0.94
	Salmonid	Not = Yes	-1.64	> 0.10
lvic Fin	Culture	Farm > Hatch*	-3.68	< 0.001

		Farm > Lab	-3.61	< 0.001
		Hatch = Lab	0.03	> 0.97
	Comparison	CG* > WF	-25.15	< 0.0001
	Domestication	2G = 1G	0.14	> 0.88
	Population	Diff = Same	-0.81	> 0.41
	Salmonid	Not = Yes	0.06	> 0.95
Dorsal Fin L	Culture	Farm > Hatch*	-2.44	< 0.05
		Farm > Lab	-2.69	< 0.01
		Hatch = Lab	-0.31	> 0.75
	Comparison	CG* > WF	-15.48	< 0.0001
	Domestication	2G = 1G	0.92	> 0.35
	Population	Diff < Same	12.17	< 0.0001
	Salmonid	Not = Yes	0.57	> 0.56
Dorsal Fin W	Culture	Farm = Hatch	-1.38	> 0.16
		Farm > Lab*	-2.71	< 0.01
		Hatch = Lab	-1.58	> 0.11
	Comparison	CG* > WF	-2.9	< 0.01
	Domestication	2G = 1G	0.85	> 0.39
	Population	Diff > Same	-7.74	< 0.0001
	Salmonid	Not = Yes	0.79	> 0.43
Anal Fin L	Culture	Farm > Hatch*	-2.36	< 0.05
		Farm > Lab	-2.48	< 0.05
		Hatch = Lab	-0.62	> 0.53
	Comparison	CG = WF	-1.15	> 0.24
	Domestication	2G = 1G	0.83	> 0.40
	Population	Diff = Same	-1.85	> 0.06
	Salmonid	Not = Yes	0.07	> 0.94
Anal Fin W	Culture	Farm = Hatch	-1.96	> 0.05
		Farm < Lab*	-1.97	< 0.05
		Hatch = Lab	-0.65	> 0.51
	Comparison	CG* > WF	-4.8	< 0.0001
	Domestication	2G < 1G	6.38	< 0.0001
	Population	Diff < Same	5.15	< 0.0001
	Salmonid	Not = Yes	1.69	> 0.09
Caudal Fin L	Culture	Farm = Hatch	-0.36	> 0.71
		Farm = Lab	-1.58	> 0.11
		Hatch = Lab	-1.19	> 0.23
	Comparison	CG = WF	-0.29	> 0.77
	Domestication	2G < 1G	2.15	< 0.05
	Population	Diff = Same	1.05	> 0.29
	Salmonid	Not = Yes	-0.27	> 0.78

Table 3.3 Recommended information to be included, or made available in all published morphological analyses of fishes

	General Recommendation for Data Collection and Reporting
1	The number of fish analyzed
2	The (mean) length of the fish analyzed
3	The age of the fish, or their life history stage
4	The history of domestication, if any, of the fish
5	The relatedness, if any, of the groups of fish
6	The mean and standard deviation for each morphological feature analyzed
7	A method to make available the raw data for download
8	Any peculiarities of the fish studied which may affect the interpretation of the results (e.g. skewed sex ratios, spawning condition, etc.)

3.8 Figures

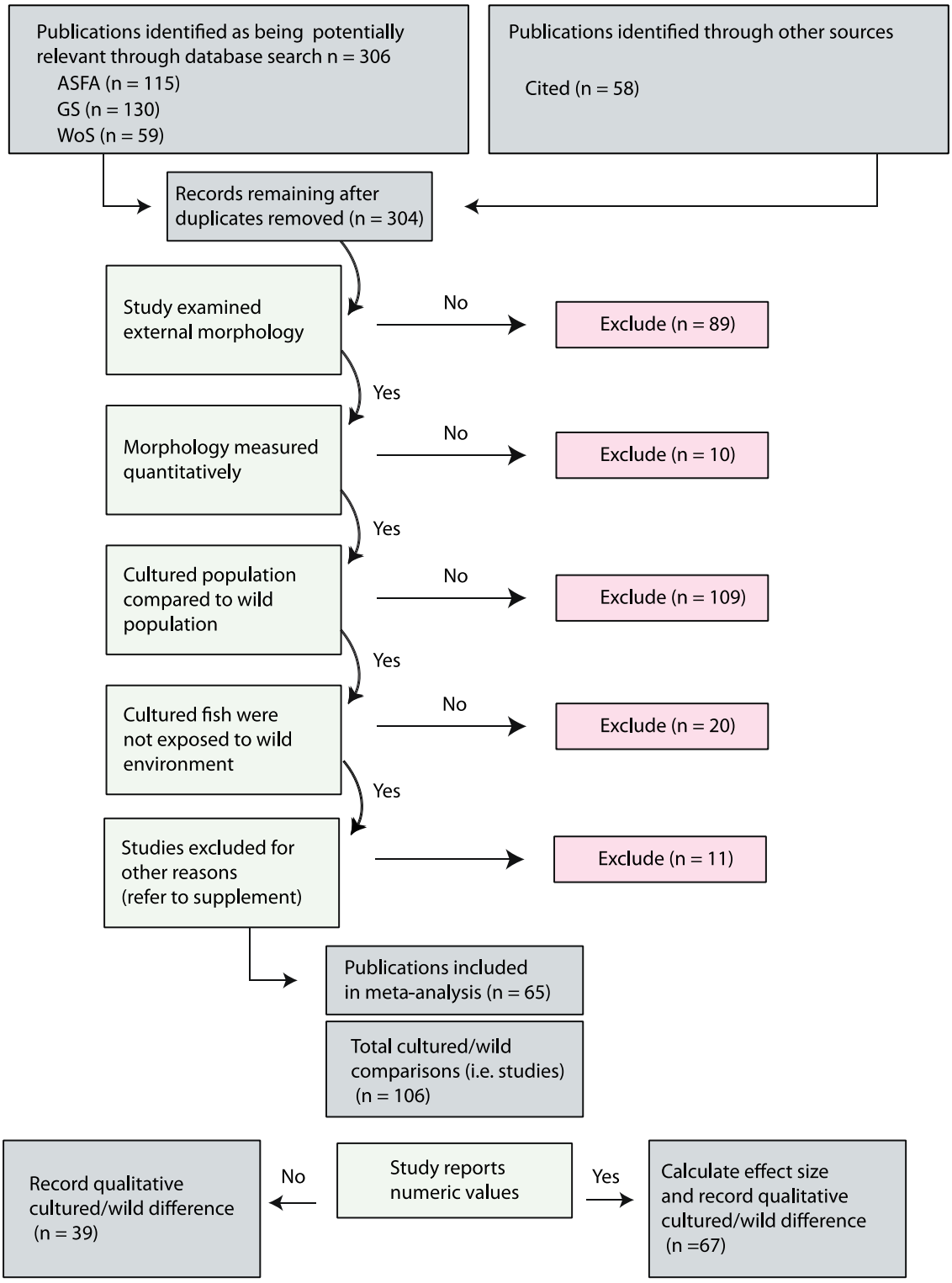
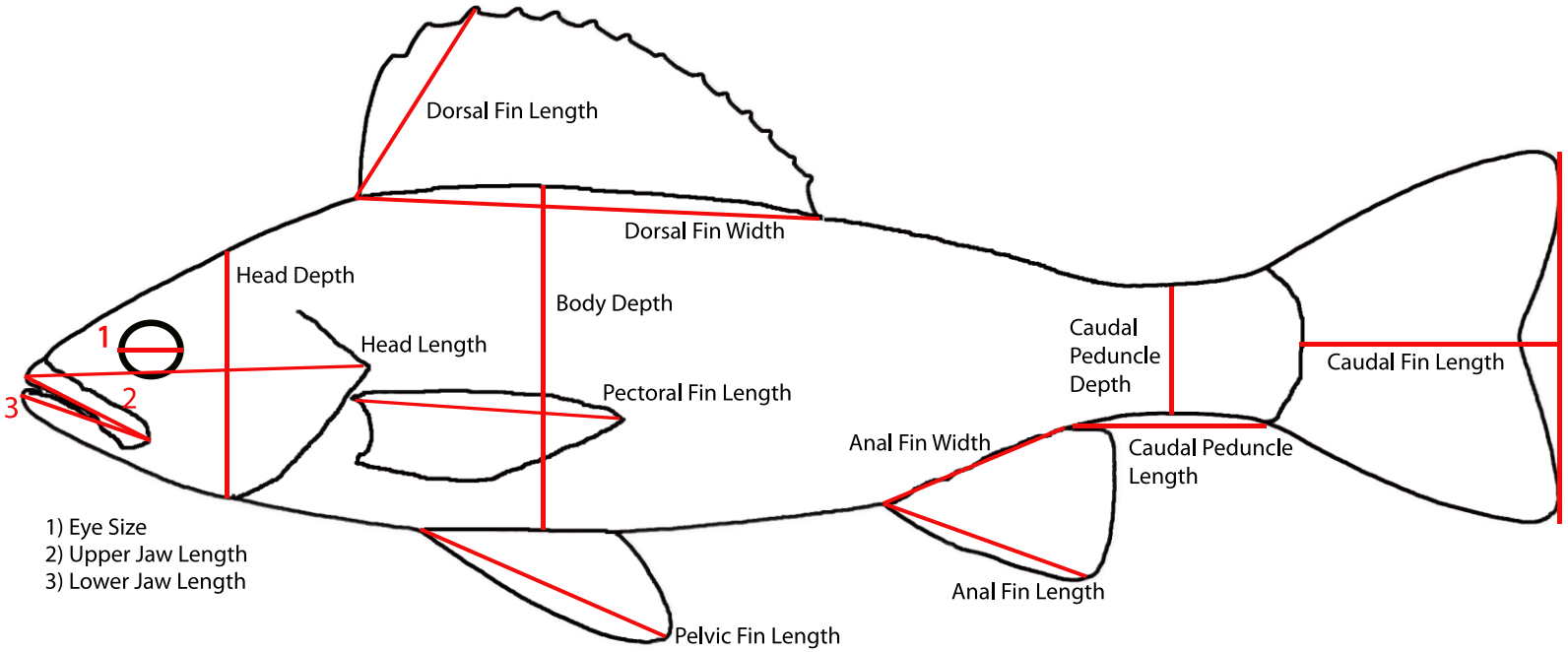

Publications identified as being potentially relevant through database search n = 306
ASFA (n = 115)
GS (n = 130)
WoS (n = 59)
Publications identified through other sources
Cited (n = 58)
Records remaining after duplicates removed (n = 304)
Study examined external morphology
No
Exclude (n = 89)
Yes
Morphology measured quantitatively
No
Exclude (n = 10)
Yes
Cultured population compared to wild population
No
Exclude (n = 109)
Yes
Cultured fish were not exposed to wild environment
No
Exclude (n = 20)
Yes
Studies excluded for other reasons (refer to supplement)
Exclude (n = 11)
Publications included in meta-analysis (n = 65)
Total cultured/wild comparisons (i.e. studies) (n = 106)
Record qualitative cultured/wild difference (n = 39)
No
Study reports numeric values
Yes
Calculate effect size and record qualitative cultured/wild difference (n =67)

Figure 3.1 Decision making flowchart for study inclusion into meta-analysis. Each cultured/wild comparison reported in a publication was considered separately (i.e. a 'study'). Numerical data suitable for the calculation of effect sizes was only presented in 67 of 106 studies, but qualitative differences in morphology were recorded from all studies (i.e. 67 + 36 = 106).

1443


Dorsal Fin Length
Dorsal Fin Width
Head Depth
Body Depth
Head Length
Pectoral Fin Length
Caudal Peduncle Depth
Caudal Fin Length
Anal Fin Width
Caudal Peduncle Length
1
2
3
1) Eye Size
2) Upper Jaw Length
3) Lower Jaw Length
Anal Fin Length
Pelvic Fin Length

1444

Figure 3.2 Visualization of the morphometric features examined in the meta-analysis. Eye size is the maximal diameter of the eye. Upper jaw length is the distance between the anteriormost point of the premaxilla and the posteriormost point of the maxilla. Lower jaw length is the distance between the anteriormost and posteriormost points of the dentary. Head length is the distance between the anteriormost point of the head, to the posteriormost point of the operculum. Head depth is the maximal depth of the head and body depth is the maximal depth of the body while caudal peduncle depth is the minimum depth of the caudal peduncle. Fin lengths, with the exception of the caudal fin is the straight-line distance between the fin origin and the tip of the longest fin ray (usually the second). Fin widths are the straight-line distance between the fin anterior fin origin and its posterior insertion. The height of the caudal fin is the maximal vertical distance with the caudal fin extended, while the caudal fin length is the straight-line distance between its origin and a plane running perpendicular to the body length at the caudal fins most posterior point when extended. In addition to these morphological measures, Fulton's condition factor (K) was also included in the analysis as $K = 100(W/L^3)$.

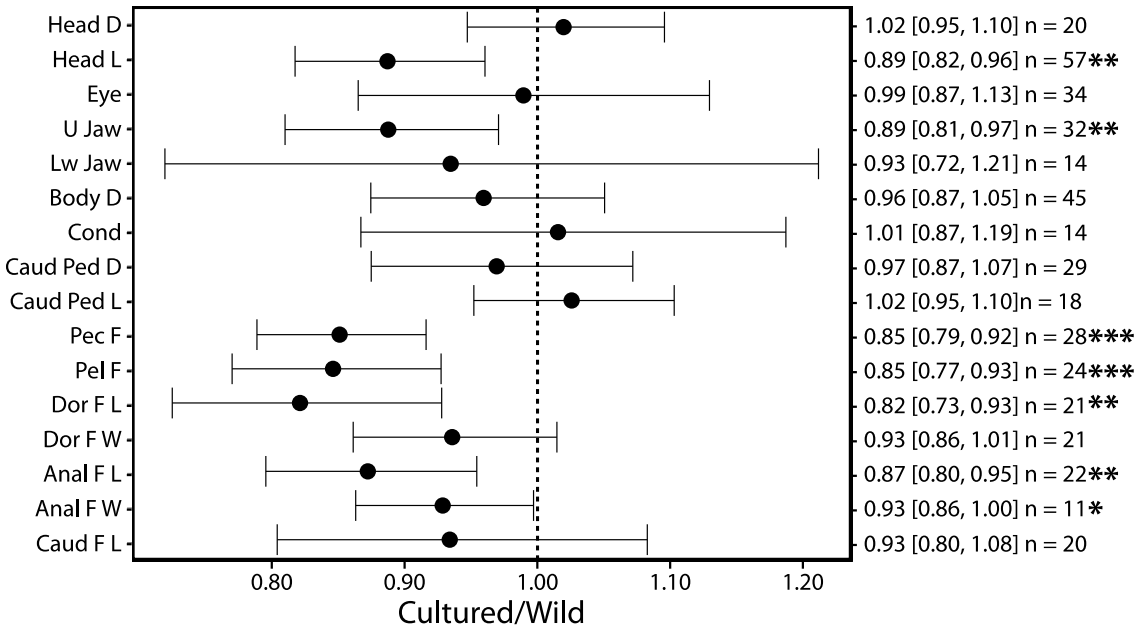
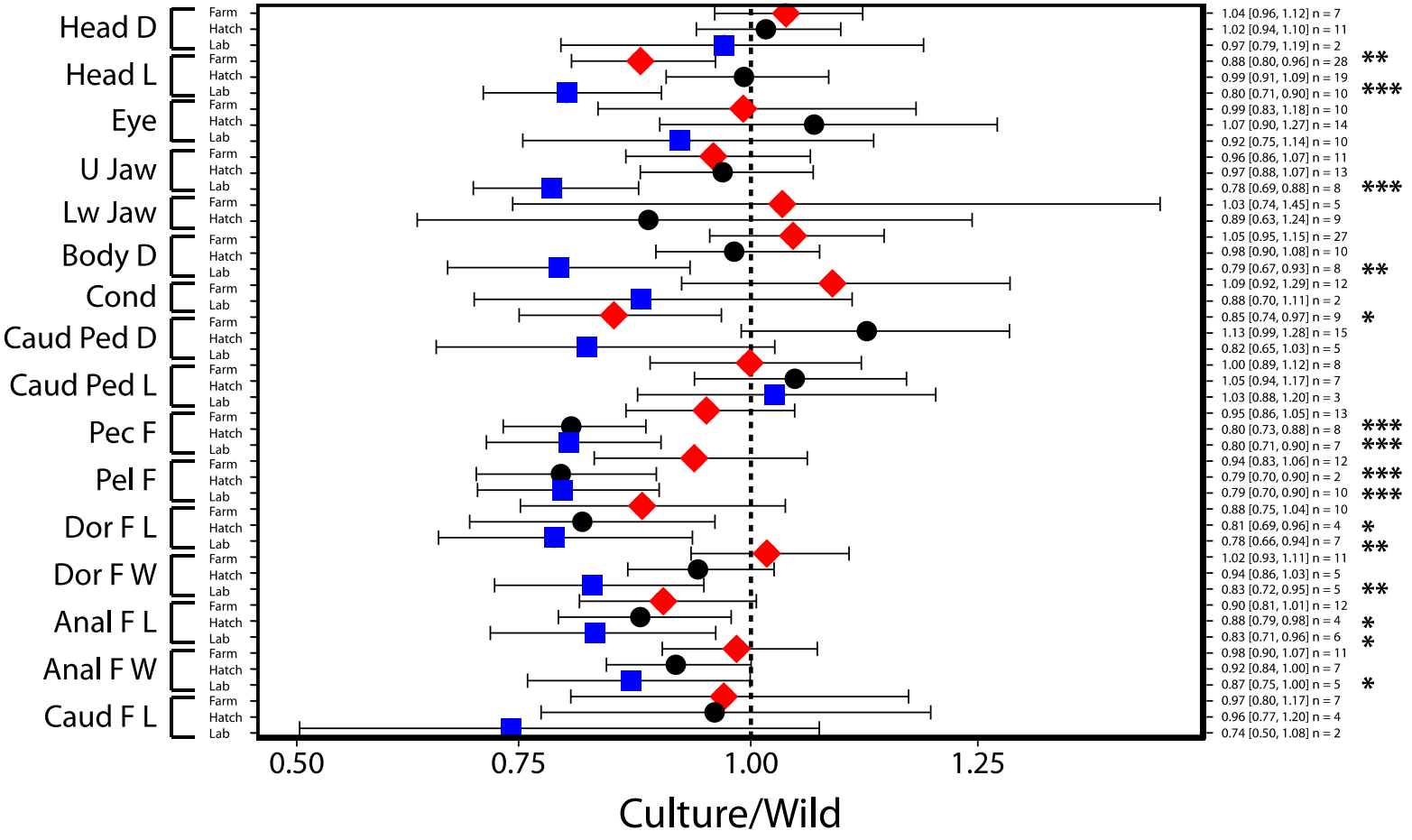


Figure 3.3 Effect sizes for the morphological features examined for all studies included in the meta-analysis. The points are the exponent of the estimated effect size for each morphological feature from their respective mixed-effects model. The error bars represent the 95% confidence interval. A dotted line has been drawn at one to aid in interpretation. Opposite each morphological character, its corresponding effect size, upper and lower bounds of the 95% C.I. (in brackets), and the number of cultured/wild comparisons tested (n) are reported. The abbreviations for the morphological features are: D = depth, L = length, U = upper, Lw = lower, Caud = caudal, Ped = peduncle, Pec = pectoral, Pel = pelvic, Dor = dorsal, and F = fin. *** indicates significance at $\alpha < 0.001$, ** at $\alpha < 0.01$ and * at $\alpha < 0.05$

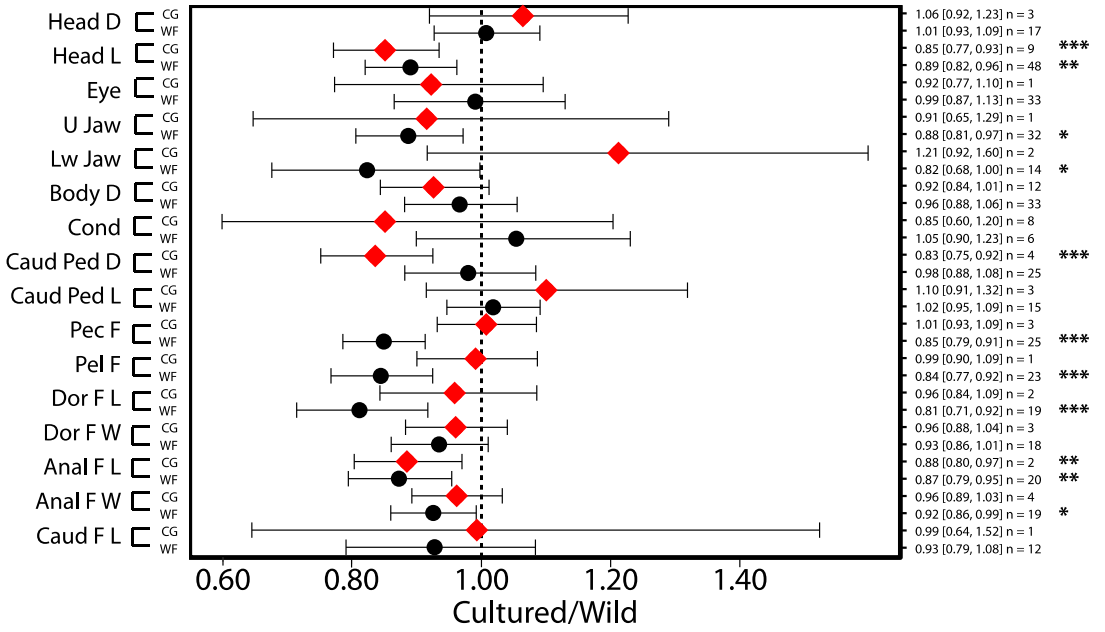
1472


Head D
Head L
Eye
U Jaw
Lw Jaw
Body D
Cond
Caud Ped D
Caud Ped L
Pec F
Pel F
Dor F L
Dor F W
Anal F L
Anal F W
Caud F L
Farm
Hatch
Lab
1.04 [0.96, 1.12] n = 7
1.02 [0.94, 1.10] n = 11
0.97 [0.79, 1.19] n = 2
0.88 [0.80, 0.96] n = 28 **
0.99 [0.91, 1.09] n = 19
0.80 [0.71, 0.90] n = 10 ***
0.99 [0.83, 1.18] n = 10
1.07 [0.90, 1.27] n = 14
0.92 [0.75, 1.14] n = 10
0.96 [0.86, 1.07] n = 11
0.97 [0.88, 1.07] n = 13
0.78 [0.69, 0.88] n = 8 ***
1.03 [0.74, 1.45] n = 5
0.89 [0.63, 1.24] n = 9
1.05 [0.95, 1.15] n = 27
0.98 [0.90, 1.08] n = 10
0.79 [0.67, 0.93] n = 8 **
1.09 [0.92, 1.29] n = 12
0.88 [0.70, 1.11] n = 2
0.85 [0.74, 0.97] n = 9 *
1.13 [0.99, 1.28] n = 15
0.82 [0.65, 1.03] n = 5
1.00 [0.89, 1.12] n = 8
1.05 [0.94, 1.17] n = 7
1.03 [0.88, 1.20] n = 3
0.95 [0.86, 1.05] n = 13
0.80 [0.73, 0.88] n = 8 ***
0.80 [0.71, 0.90] n = 7 ***
0.94 [0.83, 1.06] n = 12
0.79 [0.70, 0.90] n = 2 ***
0.79 [0.70, 0.90] n = 10 ***
0.88 [0.75, 1.04] n = 10
0.81 [0.69, 0.96] n = 4 *
0.78 [0.66, 0.94] n = 7 **
1.02 [0.93, 1.11] n = 11
0.94 [0.86, 1.03] n = 5
0.83 [0.72, 0.95] n = 5 **
0.90 [0.81, 1.01] n = 12
0.88 [0.79, 0.98] n = 4 *
0.83 [0.71, 0.96] n = 6 *
0.98 [0.90, 1.07] n = 11
0.92 [0.84, 1.00] n = 7
0.87 [0.75, 1.00] n = 5 *
0.97 [0.80, 1.17] n = 7
0.96 [0.77, 1.20] n = 4
0.74 [0.50, 1.08] n = 2
0.50
0.75
1.00
1.25
Culture/Wild

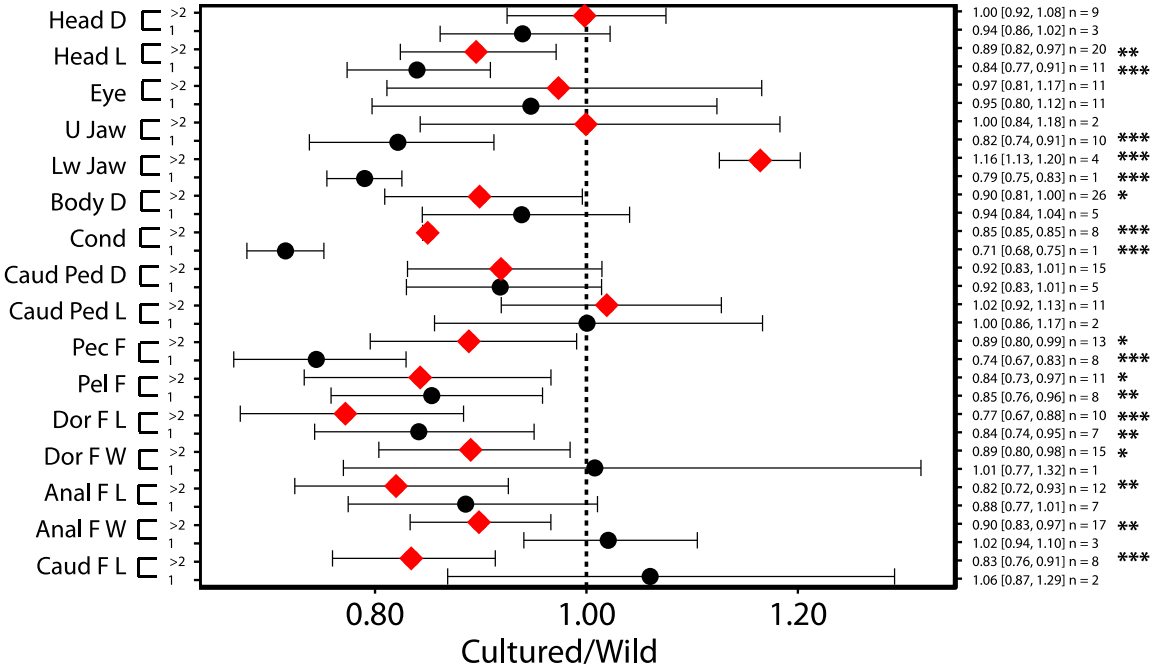
1473

Figure 3.4 Effect sizes for the morphological features examined. Morphological characters and abbreviations are the same as in Figure 3.3. The points are the exponent of the estimated effect size for each morphological feature from their respective mixed-effects model with form of culture as a moderator. The form of culture is noted as well as indicated by the colour of the points, with red for fish reared in farms, black for hatcheries and blue for laboratories. The error bars represent the 95% confidence interval. A dotted line has been drawn at one effect to aid in interpretation. Opposite each morphological character/form of culture is its corresponding effect size, upper and lower bounds of the 95% C.I. (in brackets), and the number of cultured/wild comparisons tested (n) are reported. Effect sizes that deviate significantly from zero are marked with asterisks. *** indicates significance at $\alpha < 0.001$, ** at $\alpha < 0.01$ and * at $\alpha < 0.05$.

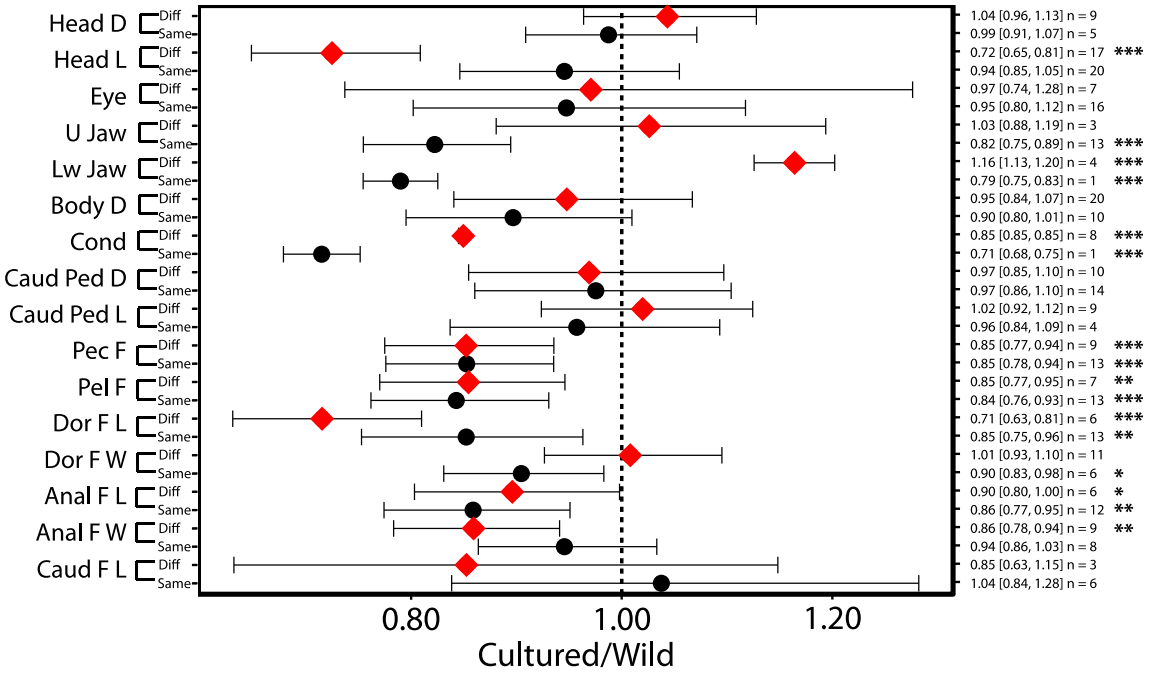
3.5a


Head D
Head L
Eye
U Jaw
Lw Jaw
Body D
Cond
Caud Ped D
Caud Ped L
Pec F
Pel F
Dor F L
Dor F W
Anal F L
Anal F W
Caud F L
CG
WF
1.06 [0.92, 1.23] n = 3
1.01 [0.93, 1.09] n = 17
0.85 [0.77, 0.93] n = 9 ***
0.89 [0.82, 0.96] n = 48 **
0.92 [0.77, 1.10] n = 1
0.99 [0.87, 1.13] n = 33
0.91 [0.65, 1.29] n = 1
0.88 [0.81, 0.97] n = 32 *
1.21 [0.92, 1.60] n = 2
0.82 [0.68, 1.00] n = 14 *
0.92 [0.84, 1.01] n = 12
0.96 [0.88, 1.06] n = 33
0.85 [0.60, 1.20] n = 8
1.05 [0.90, 1.23] n = 6
0.83 [0.75, 0.92] n = 4 ***
0.98 [0.88, 1.08] n = 25
1.10 [0.91, 1.32] n = 3
1.02 [0.95, 1.09] n = 15
1.01 [0.93, 1.09] n = 3
0.85 [0.79, 0.91] n = 25 ***
0.99 [0.90, 1.09] n = 1
0.84 [0.77, 0.92] n = 23 ***
0.96 [0.84, 1.09] n = 2
0.81 [0.71, 0.92] n = 19 ***
0.96 [0.88, 1.04] n = 3
0.93 [0.86, 1.01] n = 18
0.88 [0.80, 0.97] n = 2 **
0.87 [0.79, 0.95] n = 20 **
0.96 [0.89, 1.03] n = 4
0.92 [0.86, 0.99] n = 19 *
0.99 [0.64, 1.52] n = 1
0.93 [0.79, 1.08] n = 12
0.60
0.80
1.00
1.20
1.40
Cultured/Wild

3.5b


Head D
Head L
Eye
U Jaw
Lw Jaw
Body D
Cond
Caud Ped D
Caud Ped L
Pec F
Pel F
Dor F L
Dor F W
Anal F L
Anal F W
Caud F L
>2
1
1.00 [0.92, 1.08] n = 9
0.94 [0.86, 1.02] n = 3
0.89 [0.82, 0.97] n = 20 **
0.84 [0.77, 0.91] n = 11 ***
0.97 [0.81, 1.17] n = 11
0.95 [0.80, 1.12] n = 11
1.00 [0.84, 1.18] n = 2
0.82 [0.74, 0.91] n = 10 ***
1.16 [1.13, 1.20] n = 4 ***
0.79 [0.75, 0.83] n = 1 ***
0.90 [0.81, 1.00] n = 26 *
0.94 [0.84, 1.04] n = 5
0.85 [0.85, 0.85] n = 8 ***
0.71 [0.68, 0.75] n = 1 ***
0.92 [0.83, 1.01] n = 15
0.92 [0.83, 1.01] n = 5
1.02 [0.92, 1.13] n = 11
1.00 [0.86, 1.17] n = 2
0.89 [0.80, 0.99] n = 13 *
0.74 [0.67, 0.83] n = 8 ***
0.84 [0.73, 0.97] n = 11 *
0.85 [0.76, 0.96] n = 8 **
0.77 [0.67, 0.88] n = 10 ***
0.84 [0.74, 0.95] n = 7 **
0.89 [0.80, 0.98] n = 15 *
1.01 [0.77, 1.32] n = 1
0.82 [0.72, 0.93] n = 12 **
0.88 [0.77, 1.01] n = 7
0.90 [0.83, 0.97] n = 17 **
1.02 [0.94, 1.10] n = 3
0.83 [0.76, 0.91] n = 8 ***
1.06 [0.87, 1.29] n = 2
0.80
1.00
1.20
Cultured/Wild

3.5c


Head D
Head L
Eye
U Jaw
Lw Jaw
Body D
Cond
Caud Ped D
Caud Ped L
Pec F
Pel F
Dor F L
Dor F W
Anal F L
Anal F W
Caud F L
Diff
Same
1.04 [0.96, 1.13] n = 9
0.99 [0.91, 1.07] n = 5
0.72 [0.65, 0.81] n = 17 ***
0.94 [0.85, 1.05] n = 20
0.97 [0.74, 1.28] n = 7
0.95 [0.80, 1.12] n = 16
1.03 [0.88, 1.19] n = 3
0.82 [0.75, 0.89] n = 13 ***
1.16 [1.13, 1.20] n = 4 ***
0.79 [0.75, 0.83] n = 1 ***
0.95 [0.84, 1.07] n = 20
0.90 [0.80, 1.01] n = 10
0.85 [0.85, 0.85] n = 8 ***
0.71 [0.68, 0.75] n = 1 ***
0.97 [0.85, 1.10] n = 10
0.97 [0.86, 1.10] n = 14
1.02 [0.92, 1.12] n = 9
0.96 [0.84, 1.09] n = 4
0.85 [0.77, 0.94] n = 9 ***
0.85 [0.78, 0.94] n = 13 ***
0.85 [0.77, 0.95] n = 7 **
0.84 [0.76, 0.93] n = 13 ***
0.71 [0.63, 0.81] n = 6 ***
0.85 [0.75, 0.96] n = 13 **
1.01 [0.93, 1.10] n = 11
0.90 [0.83, 0.98] n = 6 *
0.90 [0.80, 1.00] n = 6 *
0.86 [0.77, 0.95] n = 12 **
0.86 [0.78, 0.94] n = 9 **
0.94 [0.86, 1.03] n = 8
0.85 [0.63, 1.15] n = 3
1.04 [0.84, 1.28] n = 6
0.80
1.00
1.20
Cultured/Wild

3.5d

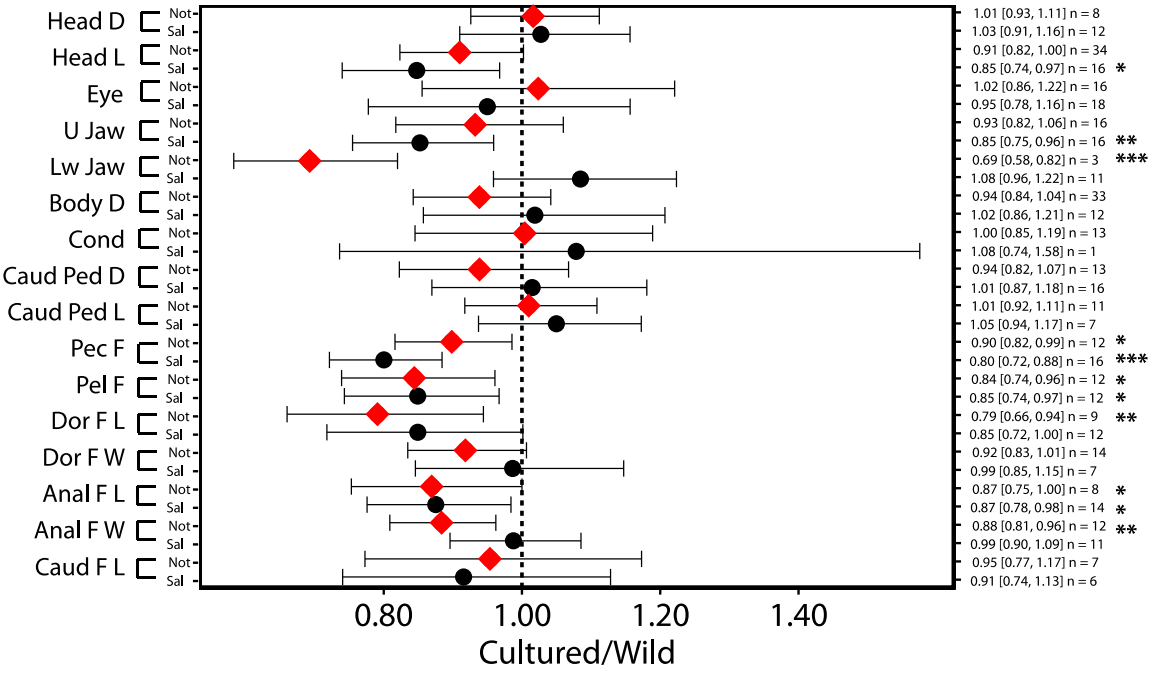

Head D
Head L
Eye
U Jaw
Lw Jaw
Body D
Cond
Caud Ped D
Caud Ped L
Pec F
Pel F
Dor F L
Dor F W
Anal F L
Anal F W
Caud F L
Not
Sal
1.01 [0.93, 1.11] n = 8
1.03 [0.91, 1.16] n = 12
0.91 [0.82, 1.00] n = 34
0.85 [0.74, 0.97] n = 16 *
1.02 [0.86, 1.22] n = 16
0.95 [0.78, 1.16] n = 18
0.93 [0.82, 1.06] n = 16
0.85 [0.75, 0.96] n = 16 **
0.69 [0.58, 0.82] n = 3 ***
1.08 [0.96, 1.22] n = 11
0.94 [0.84, 1.04] n = 33
1.02 [0.86, 1.21] n = 12
1.00 [0.85, 1.19] n = 13
1.08 [0.74, 1.58] n = 1
0.94 [0.82, 1.07] n = 13
1.01 [0.87, 1.18] n = 16
1.01 [0.92, 1.11] n = 11
1.05 [0.94, 1.17] n = 7
0.90 [0.82, 0.99] n = 12 *
0.80 [0.72, 0.88] n = 16 ***
0.84 [0.74, 0.96] n = 12 *
0.85 [0.74, 0.97] n = 12 *
0.79 [0.66, 0.94] n = 9 **
0.85 [0.72, 1.00] n = 12
0.92 [0.83, 1.01] n = 14
0.99 [0.85, 1.15] n = 7
0.87 [0.75, 1.00] n = 8 *
0.87 [0.78, 0.98] n = 14 *
0.88 [0.81, 0.96] n = 12 **
0.99 [0.90, 1.09] n = 11
0.95 [0.77, 1.17] n = 7
0.91 [0.74, 1.13] n = 6
0.80
1.00
1.20
1.40
Cultured/Wild

Figure 3.5 Effect sizes for the morphological features examined for the moderators: commonality of rearing environment, domestication, ancestral population and salmonid. Morphological characters and abbreviations are the same as in Figure 3.3, and descriptions of the moderators can be found in Table 3.1. Moderator levels are indicated beside each morphological feature, as well as by the colour of the point. The points are the exponent of the estimated effect size for each morphological feature from their respective mixed-effects model. The error bars represent the 95% confidence interval. A dotted line has been drawn at one to aid in interpretation. Opposite each morphological character/cofactor level combination is its corresponding effect size, upper and lower bounds of the 95% C.I. (in brackets), and the number of cultured/wild comparisons tested (n) are reported. Effect sizes that deviate significantly from zero are marked with asterisks. *** indicates significance at $\alpha < 0.001$, ** at $\alpha < 0.01$ and * at $\alpha < 0.05$. a - Commonality of rearing environment; wild caught compared to cultured fish (black), or wild and cultured fish reared in a common garden (red). b - Domestication; the parents of the cultured fish are first generation cultured (black), or the stock from which the cultured fish are derived have been in culture at least two generations (red). c - Ancestral population; the cultured and wild fish compared are part of the same ancestral population (black), or not (red). d - Salmonid; the cultured and wild fish are members of the family Salmonidae (black), or not (red).

Chapter 4 – Spawning success of cultured and wild male Atlantic cod (*Gadus morhua* L.) does not differ during paired contests.

4.1 Abstract

Culture of Atlantic cod (*Gadus morhua*, L) has been proposed to diversify the aquaculture industry in Canada, and other countries in its native range. Lessons gleaned from aquaculture of salmonids suggest that escapes and interactions with wild fish are inevitable. We studied the reproductive interactions of individual cultured and wild male cod in the presence of a cultured female using a series of spawning trios. The spawning success of cultured males, in terms of both overall proportion of eggs fertilized, and number of spawns in which they fertilized the larger proportion of eggs, did not differ from that of wild males. This equality was likely brought about, at least in part, by multiple paternity with appreciable proportions of eggs fertilized by the presumed satellite male. In the subset of spawning events for which behavioural data were available, neither wild, nor cultured males were found to be behaviourally dominant over one another during the night of spawning across all such events. The spawning success of the males was not influenced by their size or by their agonistic behaviour, but was influenced by their courting behaviour. The courting behaviour of the wild males had a negative influence on their success, while the courting behaviour of the cultured males was

found to increase their success. To our knowledge, this is the first study to detect spawning success equality between wild and cultured male cod in competition.

4.2 Introduction

The waning of fish stocks worldwide has helped spur the development of aquaculture programmes to meet the demand for product (Svåsand et al. 2000, Naylor & Burke 2005, Dauer et al. 2009), and this has led to increases in the unintentional release of cultured fish into the wild (Jensen et al. 2010). Exposure to the unnatural culture environment, intentional and unintentional selection ("domestication selection"), founder effects, genetic drift and small effective population sizes (N_e) are likely to cause cultured fish to diverge from wild fish genetically and phenotypically (Fleming & Einum 1997, Gross 1998, Thorstad et al. 2008). In fact, captivity has been shown to cause rapid phenotypic and genetic changes in cultured relative to fish, and there is evidence that escapees from aquaculture may not be as fit as their wild-born counterparts, especially in terms of successfully mating (Fleming et al. 1996, Fleming et al. 2000, Meager et al. 2009, Meager et al. 2010). However, while cultured fish may not be as successful in attaining mates, interbreeding between wild fish and fish that have escaped from aquaculture has been well documented for Atlantic salmon (*Salmo salar*) (Lura & Sægrov 1991, Webb et al. 1993, Fleming et al. 2000, Glover et al. 2013), and evidence exists that such interbreeding and genetic introgression can reduce the fitness of wild stocks (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012).

While historically aquaculture production and research efforts focused primarily on salmonid species, culture of other marine fishes, such as Atlantic cod (*Gadus morhua*), has been attempted at various times as a means of diversifying the industry. Although the current scale of cod aquaculture is much lower than that of salmonids, the potential for escapes and subsequent interbreeding between wild and escaped cod may be higher. Atlantic cod have been shown to have a greater motivation to escape net pens than do salmonids, and to escape at a greater relative rate than salmonids (Moe et al. 2007, Hansen et al. 2008, Zimmermann et al. 2012). Moreover, cod, and other marine broadcast spawners readily spawn within sea cages, releasing fertilized eggs into the surrounding ocean (Jørstad et al. 2008, Uglem et al. 2012, Somarakis et al. 2013). Like escaped salmon, escaped cod have been found to occupy the same habitat as their wild conspecifics (Zimmermann et al. 2013), even to the extent of having been found among wild fish in spawning aggregations (Wroblewski et al. 1996, Uglem et al. 2008, Meager et al. 2010). Nevertheless, simply being present in a spawning aggregation in and of itself does not guarantee spawning success.

Atlantic cod exhibit lek-like mating aggregations (Hutchings et al. 1999, Rose et al. 2008, Meager et al. 2010), with female mate choice apparently based on both visual and acoustic displays, and broadcast spawning of buoyant, planktonic eggs occurs with the selected male in a ventral mount on the female (Brawn 1961, Hutchings et al. 1999). Within spawning aggregations, male cod form dominance

1579 hierarchies based on agonistic interaction, usually with the largest males occupying
1580 the highest ranks, and access to females and spawning success being related to this
1581 hierarchical position (Hutchings et al. 1999, Bekkevold et al. 2002, Bekkevold 2006).
1582 Experimental studies have shown that while the most dominant males obtain
1583 greater access to females and acquisition of ventral mounts, the majority of the egg
1584 batches spawned have some degree of multiple paternity, indicating the importance
1585 of satellite spawning in the cod mating system (Rakitin et al. 2001, Bekkevold et al.
1586 2002, Herlin et al. 2008). The spawning success of cultured males in competition
1587 with wild males in multi-individual groups has been found to be mixed. Skjæraasen
1588 and Hutchings (2010) found that the reproductive success of cultured cod in
1589 competition with wild cod was "essentially nil", but in another study, Skjæraasen et
1590 al. (2010) observed that cultured cod fertilized approximately 25% of eggs spawned
1591 by wild females, but up to 52% of eggs spawned by cultured females.
1592 Taking into account the apparent importance of male dominance hierarchies,
1593 courting behaviours and sperm competition in cod mating, we have tested the
1594 competitive ability of paired cultured and wild male cod in the presence of
1595 individual cultured female cod. We did this to remove the effect of multi-male
1596 dominance hierarchies, which would exclude a large number of males from
1597 spawning, thus this design should provide further illumination of the inter-
1598 individual variation in competitive ability between cultured and wild male cod. The
1599 behaviour of the females within the trios was also considered because male success

has been observed to be dependent upon the type of female with which they spawned (Skjæraasen et al. 2010). We examined if females exhibited any behavioural preference for either male type and if so, was this behavioural preference also reflected in the male's spawning success. We hypothesized that the wild males would be dominant over the cultured males, both behaviourally and in terms of spawning success. We further hypothesized that male spawning success would be influenced by a female behavioural preference.

4.3 Materials and Methods

4.3.1 Experimental Fish

Wild cod were collected using baited cod pots on 10 and 20 November 2009, from Smith's Sound in Trinity Bay, Newfoundland, Canada (48° 9' N, 53° 44' W; Northwest Atlantic Fisheries Management [NAFO] Division 3L; Figure 4.1). The cultured cod were the progeny of wild-caught fish from Bay Bulls, Newfoundland, Canada (47° 18' N, 52° 48' W; NAFO Division 3L; Figure 4.1), and are members of the same population as the wild fish (Beacham et al. 2002, COSEWIC 2010). The cultured fish were spawned between 13 December 2006 and 27 February 2007 in the Joe Brown Aquatic Research Building (JBARB) at Memorial University of Newfoundland's Ocean Sciences Centre (OSC) in Logy Bay, Newfoundland (47° 37' N, 52° 40' E), and raised there until they were stocked into sea cages at the Sapphire Sea Farms site in Bay Bulls on the 30 November 2008 (ca. 31 cm total length). On 30 October 2009,

1620 cultured cod were collected from Sapphire Sea Farms' cage facility, and transported
1621 to the OSC.

1622 The wild and cultured cod were placed in adjacent, identical 24.27 m^3 tanks
1623 (5.3 m diameter, 1.1 m deep) and acclimated for at least four months; thus the wild
1624 and cultured cod were not exposed to one another prior to the start of
1625 experimentation. Both tanks were illuminated with an ambient photoperiod, and
1626 supplied with ca. 5-8^{o}C seawater inflows (ca. 1.5-1.8 L s^{-1}) and oxygen
1627 supplementation to ensure that oxygen saturation at the outflow was maintained at
1628 ≥ 90%.

1629 Approximately one week after the wild cod were collected, passive integrate
1630 transponder (PIT) tags (Avid Identification Systems, Inc. Norco, California, USA)
1631 were inserted into the dorsal musculature under anaesthesia with MS-222 (tricaine
1632 methanesulfonate). The cultured cod had been implanted with PIT tags (at ca. 15-20
1633 g body weight) in their abdominal cavities prior to our acquisition of them. All cod
1634 were fed a diet consisting primarily of herring (*Clupea herengus*), supplemented
1635 with mackerel (*Scomber scombrus*) and squid (*Ilex sp.*) as available, three times a
1636 week to satiation. Cultured cod were easily weaned onto this diet over the course of
1637 about a month.

1638 Beginning in mid-February 2010, both the wild and cultured cod were
1639 checked weekly for signs of gonad maturation, and the tanks were checked daily for

the presence of eggs. Experimentation began once it appeared the majority of fish had matured.

4.3.2 Experimental conditions

A trio, consisting of one wild male, one cultured male, and one cultured female, were placed in each of ten circular experimental tanks, which were maintained on natural photoperiod and supplied with ambient flow-through seawater (three of the tanks were 3.77 m^3 [2.0 m diameter, 1.2 m deep], three were 4.6 m^3 [1.8 m diameter, 1.8 m deep] and four of the tanks were 1.84 m^3 [1.25 m diameter, 1.5 m deep]). To increase our sample size, we ran three temporal replicates, each using ten unique trios for a total of 30 unique trios (1 trio per tank x 10 tanks x 3 temporal replicates). The first temporal replicate began on 18 March 2010, and ran until 13 April 2010. The second temporal replicate ran between 13 and 30 April 2010, and the third and final replicate ran between 30 April and 27 May 2010.

For each temporal replicate, trios were made by haphazardly selecting from their respective holding tanks, the first 10 wild male, 10 cultured male, and 10 cultured female cod that were found to be in, or near spawning condition (males: semen freely released following gentle pressure to ventral surface; females: soft, distended bellies), and then randomly assigning one of each type to each of the ten experimental tanks (randomization script written in R; R Development Core Team 2015). The females were added to the experimental tanks first, followed by the simultaneous introduction of the paired males approximately five minutes later. Due

1661 to low maturation rates, a number of the cultured females and wild males had to be
1662 used in more than one round of experimentation (See Supplementary Table 4.1). In
1663 cases where fish were used in more than one round of experimentation, it was
1664 ensured that they were not competed with individuals (i.e. unique trios were
1665 produced) or in a tank in which they had previous experience. Unfortunately, no
1666 female wild cod were detected to be in or near spawning condition during the
1667 experiment, so the experiment was conducted using only cultured females. In total,
1668 of 110 wild cod collected only four females eventually matured.

1669 Prior to being added to the experimental tanks, the selected fish were
1670 sedated with MS-222, scanned for PIT tag number, weighed (± 0.1 g), and measured
1671 for total body length (± 0.5 cm) and pelvic fin lengths (fin origin to tip of the longest
1672 fin ray [± 0.01 cm], using digital callipers). Wild males were tagged sinistrally to the
1673 origin of their third dorsal fin, and the cultured males dextrally to the origin of their
1674 first dorsal fin with 5-cm-long yellow t-bar tags (Floy Tag Inc., Seattle Washington)
1675 for visual identification on video (females were not tagged). Even though not all trios
1676 were filmed, for consistency all males were tagged. Fish were not observed to
1677 interact with these tags during the course of the experiment, and the tags did not
1678 appear to cause any stress.

1679 All tanks were affixed with egg collectors, consisting of a surface-skimming
1680 drain that emptied into a fine-meshed aquarium net suspended in a 19 L bucket.
1681 These egg collectors were checked daily between 10:00 and 12:00, and when eggs

were detected they were transferred into labeled 1 L beakers. A subsample of the eggs collected from each spawning event was examined under a dissecting microscope to verify that the eggs were at a developmental stage consistent with having been spawned during the preceding 24 h period (Hall et al. 2004). Once verified, eggs were then transferred in their 1 L beakers to a climate-controlled room with the temperature set to 4°C (± 1°C of that of the spawning tanks), and a 12:12 light:dark cycle. After settling for ca. 15 min., non-viable eggs (i.e. those that had sunk) were discarded, while the viable eggs, which were floating were retained. Viable eggs were transferred to a new 1 L beaker, and the beaker was filled with ca. 800 ml of filtered seawater. Eggs were attended to daily, and any that sunk to the bottom were removed using a pipette and discarded. Then ca. half of the water in the beaker was removed and replaced with fresh, filtered seawater. Following ca. 72 h of development, all floating eggs, up to a maximum of 5 ml, were collected and preserved in 95% ethanol, which was subsequently exchanged twice.

4.3.3 DNA extraction and amplification

DNA was extracted from 25 preserved fertilized eggs from each spawning event and from fin clips from each potential parent, using Promega Wizard SV 96 Genomic DNA Purification kits (Promega catalogue number A2371) following the manufacturer's protocol. Extracted DNA was amplified via polymerase chain reaction (PCR) using the multiplex protocol of Wesmajervi et al. (2006), with some modification: based on preliminary analysis of parents, which were genotyped in

duplicate, the gadoid microsatellite *Tch11* (O'Reilly et al. 2000) was dropped from our multiplex as it failed to amplify consistently. Thus our multiplex consisted of the fluorescently end-labelled markers *Gmo8*, *Gmo19*, *Gmo35*, and *Gmo37* (Miller et al. 2000; Supplementary Table 4.2).

The multiplex PCR mixture consisted of 5 µl Qiagen Multiplex PCR Master Mix (Qiagen Multiplex PCR Kit, catalogue number 206145), 1 µl 5X Q-Solution (Qiagen, provided in the Multiplex Kit), 0.4 µl primer master mix (Supplementary Table 4.2), and 4.8 µl extracted DNA, for a total reaction volume of 10 µl. The thermocycler conditions were: an initial denaturation step of 95°C for 15 min, followed by 40 cycles consisting of 94°C for 35 s, 57°C for 60 s, and 72°C for 30 s. The reaction was terminated by a final extension at 72°C for 10 min, followed by incubation at 4°C.

PCR products were sized on an ABI 3730 DNA Analyzer (Applied Biosystems), allele sizes were calculated against the internal LIZ size standard (GeneScan™ 500 LIZ™ dye Size Standard, Applied Biosystems, catalogue number 4322682) and, eletrophorograms were visualized using GeneMapper® v4.1 Software (Applied Biosystems). All genotyping was conducted twice, and the accuracy of all allele scorings generated by the software was visually confirmed. The genotypes of the offspring were compared to that of the known mother and the two candidate fathers, and paternity was assigned manually based on exclusion.

4.3.4 Behavioural observations

Axis 210 Network Cameras (Axis Communications, Lund, Sweden) were mounted above four of the ten tanks (the four 1.84 m^3 tanks; Tanks 4, 5, 7, and 8; Supplementary Table 4.1), such that the entirety of the tank was visible, and the cameras recorded continuously to a networked storage drive for the duration of the experiment. Light levels were set such that unambiguous identification of each fish in the tanks was possible during both the simulated day and night. From the video recordings, three courting and four agonistic behaviours were assessed (Table 4.1).

Fish were far less active and no spawns were observed during daylight, therefore only the behaviours of the fish during the night before eggs were collected were considered for analysis (i.e. the night in which spawning occurred). Despite screening the entirety of the video, in the majority cases, the actual release of gametes could not be unambiguously identified. The impacts of this were twofold: firstly, we were unable to examine how acting as the primary male (i.e. the male in the ventral mount with the female) influenced fertilization success, and secondly, this caused us to have to quantify the behaviour of the fish over the entire night of spawning. Thus for each of the behaviours listed in Table 4.1, we counted the number of behavioural actions each actor and recipient pair (Figure 4.2) exhibited during one, randomly chosen, five-minute block per hour between 20:00 and 06:00 (i.e. “at night”). We then used the sum of the behavioural actions of each type of behaviour (all blocks, for all hours), for each actor-recipient pair in the analysis.

Each fish in a trio can act on, and in turn itself be acted upon, by the other two fish in the trio; thus there are six potential actor/recipient dyads (Figure 4.2). In light of this, the differences in the behaviour of fish of each origin were analyzed in two ways. First, for each of the behaviours listed in Table 4.1, the recipient of the behavioural events were not considered, and the total number of behavioural action events performed on both potential recipients were summed (Figure 4.2). Next, for each of the behaviours listed in Table 4.1, the number of behavioural events directed at each of the potential recipients were considered separately (Figure 4.1).

We also tested the following: 1) if fish of different origins differed in their overall level of behaviour, 2) whether fish of different origins behaved in a qualitatively similar manner, and 3), if the behaviour of an individual in a trio influenced that of the others.

4.3.5 Statistical analysis

We tested for differences in weight, total length, and size-adjusted mean pelvic fin length between the wild males, the cultured males and the cultured females using ANOVA with permutation, (aovp, lmPerm package (aovp, lmPerm package; Wheeler 2010)), and where significant differences were detected, Tukey's honest significance test (TukeyHSD, stats package; R Development Core Team 2015). The mean of the right and left pelvic fin lengths were calculated after they were first individually size standardized using the formula $M_{std} = M_{obs}(51.65/TL_{obs})^{b}$, where M is the trait measure, 51.65 is the mean total length of all fish, TL is the total length of a fish, b is

1764 the trait-specific common within-groups slope, and *obs* and *std* refer to the observed
1765 (raw), and the size-standardized measurements respectively (Reist 1986a). Despite
1766 heterogeneity of regression slopes between fish origins (wild or cultured), the
1767 common within-groups slope for each character was used because this is advised
1768 even when such heterogeneity exists (Reist 1986a). We ensured the fish for which
1769 behavioural data were available were a representative subset of fish in the
1770 experiment by comparing their lengths and weights to those of all other fish of their
1771 origin in the experiment using paired t-tests (all $p > 0.05$, t.test, stats package, (all p
1772 > 0.05; t.test, stats package; R Development Core Team 2015)).
1773 Linear mixed-effects models (lme function from the package nlme (lme, nlme
1774 package; Pinheiro et al. 2013)), which can account for repeated and non-
1775 independent measures were used because many trios spawned more than once,
1776 several fish were used in more than one round of experimentation and the
1777 behaviour of each member of a trio was not independent of that of the other
1778 members of the trio (Figure 4.1). We assigned each fish a unique ID and these IDs
1779 were used in the mixed effects models as the random effects. Where significant
1780 differences were detected in the mixed effects model, *post-hoc* analysis using
1781 Tukey's honest significance tests were conducted using the function glht (multcomp
1782 package; Hothorn et al. 2008).
1783 Before analyzing all detected spawning events together, we ensured that the
1784 spawning success of the wild and cultured males in the trios was unaffected by tank

size (small, medium, large [Type III ANOVA on lme: chisq = 5.55, df = 2, p = 0.06]) or temporal round (three experimental rounds [Type III ANOVA on lme: chisq = 0.31, df = 2, p = 0.85]). We then examined whether, in all detected spawning events, the wild and cultured males differed in their spawning success or in their behaviour. Differences in the spawning success of males of both types were also examined in terms of differences in the number of spawning event 'wins' and 'losses'. In this case for each spawning event detected, a 'win' was awarded to the male who fertilized the greater proportion of eggs. If the two males within a trio fertilized an equal proportion of the eggs in a given spawning event, then neither a 'win' nor 'loss' can be awarded, and that event cannot be evaluated. Using the cultured males as the focal males, this was analyzed using a mixed-effects logistic regression with the IDs of the fish in the trio as the random effect.

The effects of relative size and behaviour on spawning success are reported for the spawning success of the cultured males only. This was done both for consistency and ease of interpretation and because the spawning success data are proportions therefore if an effect is detected for one male, an inverse effect will be seen for the other male. The wild males were on average, longer, heavier, and had longer pelvic fins (Tukey's HSD, all $p < 0.01$; Table 4.2), than the cultured males and females, which did not differ significantly in these traits (Tukey's HSD, all $p > 0.88$; Table 4.2). When examining how size influenced behaviour and spawning success, we considered both the overall size of the males compared to all other males of their

origin, as well as differences in size between the two males in a trio. We looked at the within origin effect of size because the purpose of this study was to examine differences between wild and cultured males, and because the significant interaction between size and origin made interpretation perilous. Next, because females were only able to evaluate and choose between the two spawning partners that she was presented, the effect of differences in the size of males within a tank (i.e. the two males in actual competition) were examined. Both the raw difference between the males and the log10 ratio of cultured male to wild male size were considered in order to assess the effects of raw, as well as proportional differences in size. We also tested the effect of differences in size between the males of both types and the female in the same manner. An effect on spawning success of difference in wild and cultured male size could be taken as indicative of size-based dominance, while an effect of difference in size between either of the males and the female could indicate size-assortative mating.

We examined the influence of behaviour on the spawning success of the cultured males the same way we examined the influence of size on their spawning success. That is, we first tested if the number of behavioural action events performed during the night of spawning by fish of each origin for each behaviour, both when the recipient of the behavioural actions were considered, and when they were not influenced the spawning success of the cultured male. We then tested the influence of differences in behaviour between the fish in the trio on the spawning

success of the cultured male. Again, we first looked at the influence of the raw difference in the number of each type of behavioural action performed between each fish, then at the log10 ratios of cultured male to wild behavioural actions. We also tested for evidence of female behavioural preference for either male type, and if it was present, was it reflective of spawning success.

4.4 Results

4.4.1 Spawning success

Of the 30 trios (1 trio/tank x 10 tanks x 3 temporal replicates), 23 of them spawned a total of 61 times (mean 2.65, range 1 to 6; Supplementary Table 4.1). Across all spawning events there was no significant difference (ANOVA, on lme: chisq = 0.22, df = 1 p = 0.64) in the proportion of eggs fertilized by the wild (median 50%, range 0-100) and cultured (median 47%, range 0-100) males (Figure 4.3). The paternity of 3% of all eggs could not be resolved because shared alleles in the males precluded the exclusion of either male as the candidate father. The wild male fertilized all eggs in a given batch for six spawns across five unique trios, while the cultured male sired all eggs within a given batch during three spawns across three unique trios. There was no significant difference in the number of spawning 'wins' (i.e. when a male fertilized the greater proportion of eggs) between the wild and cultured males (ANOVA on lme: chisq = 0.04, df = 1, p > 0.86). For the 61 detected spawning events, the cultured male 'won' 29, the wild male 'won' 30, and they both fertilized an equal

proportion (i.e. 50%) in 2 spawning events. Qualitatively similar results were found in the subset of spawnings for which behavioural data were available.

4.4.2 Relationship between fish size and spawning success

When all spawns were examined, neither the size of the fish nor differences in their size were found to effect spawning success. The weight, total length and size-standardized mean pelvic fin length, of the wild and cultured males were not found to relate to the fertilization success of the cultured male (ANOVA on lme: df = 1, all $p > 0.12$). Nor was the weight or length of the female found to affect the proportion spawned by the cultured male (ANOVA on lme: df = 1, all $p > 0.37$). There was no evidence that size-based dominance influenced spawning success because neither raw differences nor log10 ratios in weight, total length, or pelvic fin size between the wild and cultured male had an effect on the spawning success of the cultured male (ANOVA on lme: df = 1, all $p > 0.05$). Differences in length, weight and pelvic fin size between the female and either of the males were not found to have a significant effect on cultured male fertilization success (size –assortative mating) (ANOVA on lme: df = 1, all $p > 0.05$). There was also no evidence (Figure 4.4) of a dome-shaped response characteristic of size-assortative mating (i.e. proportional fertilization peaking when the male-female size difference is minimal, and decreasing as the difference in size increases).

4.4.3 Behaviour

Behavioural data were available for 23 spawning events, representing nine trios (4 trios filmed in each of 3 rounds, but some did not spawn; Supplementary Table 4.1). It must be noted that the behaviour of one of the females, during the night of one of the spawning events, was dramatically different from both her behaviour during the other night in which she spawned, as well as from the behaviour of every other female. During the night in question, this female was found to direct an inordinate number of approach and brush behavioural events towards the cultured male in the trio, which in turn had an undue influence on her aggregated behaviours (refer to Table 4.1 for description of behaviours). To address this, the data were first analyzed with this aberrant spawning event included, and then with it removed, because this single spawning event was found to drive the majority of the relationships found with female behaviour.

When the recipient of the behavioural action events was not considered, there were significant differences among wild and cultured males and females in every type of behavioural action, apart from ventral mounts (Table 4.3). *Post-hoc* analysis revealed that, with the exception of ventral mounts, the cultured males performed significantly more agonistic and courting behavioural events than the females (Table 4.3). Furthermore, while cultured males tended to also perform more behavioural events than wild males, the only significant difference between the two was in the number of brushes (Table 4.3). The wild males tended to perform more

behavioural events than cultured females, but only the difference in the number of approaches was significant (Table 4.3). It must be noted that as seen in Table 4.3, the variability of the behavioural data is large in relation to the sample size, which likely accounts for the lack of statistical significance despite relatively large differences in means. These results were not altered by the exclusion of the aberrant spawning event.

Taking the recipient of each behavioural action into account revealed that the cultured males directed more lateral displays, chases, brushes and approaches towards the female than the female directed towards either of the males (Table 4.4). Additionally, the cultured males directed more coerce behaviour events towards wild males than did the females towards wild or cultured males and more than the wild males directed towards the females (Table 4.4). The cultured males also performed more brush behavioural events on the females than the wild males performed on either the females or the cultured males (Table 4.4). The cultured males approached the wild males more than the females did (Table 4.4). Overall, the cultured males were observed to direct significantly more agonistic behaviour towards females than the females did to either male type (Table 4.4). The cultured males also directed more overall courting towards females than did the wild males (Table 4.4), while females showed no significant preference for either male type. Exclusion of the aberrant spawning event did not affect these results.

The ratio of total agonistic to total courting behavioural events revealed no significant differences in the manner in which individuals of different origin interacted (including and excluding aberrant spawning event, ANOVA on lme: all $p > 0.05$). Interestingly, within trios, there was a significant relationship between the total number of behavioural events ($t = 2.9$, $df = 12$, $p < 0.05$), and the total agonistic behavioural events ($t = 3.6$, $df = 12$. $p < 0.01$) performed by one male and the number performed by the other male, but there was no relationship between the number of total courting behavioural events they performed ($t = 2.0$, $df = 12$, $p > 0.063$). Furthermore, there was no relationship, between the total number of behavioural events, the total agonistic behavioural events, and the total courting behavioural events performed by either male in a trio and the female in that trio (all $p > 0.094$).

4.4.5 Relationship of behaviour to body and pelvic fin size

Neither male length, weight nor standardized mean pelvic fin size of the wild male had a statistically significant effect on the total number of behavioural events, the total number of agonistic events, the total number of courting events or the number of each of the individual types of behavioural events performed when the recipient of the interaction was not considered (ANOVA on lme: $df = 1$ all $p > 0.33$). Likewise, wild male size had no effect on either the raw differences in the number of each type of behavioural events performed between the cultured and wild male in a trio, or on

the ratio of the number of behavioural events between the cultured and wild male in a trio (ANOVA on lme: df = 1, all $p > 0.54$).

This pattern was similar for cultured males, with the exception of a negative relationship between their total length and the number of chases observed when the recipients were not considered (ANOVA on lme: chisq = 6.82 df = 1, $p < 0.01$). This relationship, however, appeared driven by the smallest male studied having performed the greatest number of chases of all cultured males, and when the one spawning in which he partook was removed the relationship became non-significant (ANOVA on lme: chisq = 1.79, df = 1, $p > 0.18$).

For females, there was some evidence of positive relationships between their size and the number of total courting, brush and chase behavioural events (ANOVA on lme: df = 1, all $p < 0.05$). When the aberrant spawning event was removed from the analysis none of the significant relationships remained.

Neither raw or log10 ratios of differences in weight and length between the wild and cultured male, or between the female and either of the males, had a significant effect on the absolute number of, or the difference in the number of individual or aggregated behavioural events performed (ANOVA on lme: df = 1, all $p > 0.11$).

4.4.5 Relationship between behaviour and spawning success

No relationship between female behaviour and the spawning success of either cultured or wild males was found after removal of the aberrant spawning event

(ANOVA on lme: df = 1, all $p > 0.46$). Cultured male spawning success however, was positively related to the total number of brush behaviours they exhibited (ANOVA on lme: chisq = 6.64, df = 1, $p < 0.01$), as well as the total number of agonistic and approach behavioural actions performed by the wild male (ANOVA on lme: total agonistic: chisq = 5.71, df = 1, $p < 0.05$; approach: chisq = 9.09, df = 1, $p < 0.01$). When the direction of interaction was considered, it was brushes and approaches the wild male performed on the female that had the positive effect on cultured male spawning success (ANOVA on lme: brush: chisq = 7.75, df = 1, adjusted $p < 0.05$; approach: chisq = 7.10, df = 1, adjusted $p < 0.01$). There were no other significant relationships between male behaviour and spawning success.

4.5 Discussion

4.5.1 Relationship of findings to Atlantic cod mating system

Contrary to our hypothesis larger or more aggressive males did not enjoy greater spawning success. Finding equality in the spawning success of male wild and cultured cod is unique to this experiment, and could be the result of the interplay between the cod mating system and our experimental setup. Male cod typically form dominance hierarchies several weeks prior to the first spawning event (Brawn 1961, Hutchings et al. 1999) and authors assign male rank within a dominance hierarchy based on spawning success or on relative levels of agonistic behaviour (e.g. Brawn 1961, Hutchings et al. 1999, Bekkevold et al. 2002). Both spawning

1968 success and male rank seem to be highly positively correlated to one another, and to
1969 body size (Brawn 1961, Hutchings et al. 1999, Bekkevold et al. 2002). While
1970 dominance hierarchies may have formed prior to spawning in our experiment, they
1971 were not detected in the behavioural analysis. Unlike in 'typical' studies, we found a
1972 lack of relationship between agonistic behaviour observed during the night of
1973 spawning, spawning success and body size. This is suggestive evidence that
1974 behavioural dominance during the night of spawning did not influence the outcome
1975 of spawnings in this experiment, and also that dominance dyads may not have
1976 formed within the trios. Given that Skjæraasen et al. (2010), and Skjæraasen and
1977 Hutchings (2010), also found no relationship between male size and dominance
1978 rank, it may be that competition between cultured and wild male cod leads to a
1979 breakdown of size stratified dominance ranks, and thus lack of relationship between
1980 male size and dominance rank is the norm in cultured/wild interaction. This has
1981 some support in the results of Skjæraasen and Hutchings (2010), who found that
1982 when the much smaller cultured males were excluded from their analysis, a
1983 significant relationship between wild male length, but not weight, condition or
1984 pelvic fin length and dominance rank was detected. This suggests that something
1985 peculiar to the cultured fish was causing the breakdown of the typically size-
1986 stratified dominance hierarchy.

1987 Abnormal cultured male behaviour has been observed during mating
1988 competition with wild males in salmonids. The cultured males do not follow the

1989 usual agonistic exchange typical among wild males, and while cultured and wild
1990 male salmonids show similar levels of aggression, the cultured individuals do not
1991 appropriately cede victory (Fleming & Gross 1993, Fleming et al. 1996, Fleming et al.
1992 1997). A breakdown of the size-stratified dominance hierarchy typically observed in
1993 male cod could occur if wild and cultured male cod also have similar differences in
1994 their response thresholds when evaluating competitors, the point at which they
1995 switch from display to overt, physical aggression, or the point at which they cease
1996 physically contesting interactions or cede victory. Under such a scenario, wild males
1997 may trade current for future reproductive success, and/or they may choose to adopt
1998 alternate mating strategies and act as a satellite spawner. The results of Skjæraasen
1999 et al. (2010), and Skjæraasen and Hutchings (2010), bear this out, finding that across
2000 all males in their studies, male agonistic behaviour, but not body size, is positively
2001 related to reproductive success; dominance hierarchies existed, but were stratified
2002 based on behaviour. This finding is not consistent with our results. We found neither
2003 levels of agonistic behaviour during the night of spawning nor body size had an
2004 effect on spawning success of males of either origin.
2005 An alternative explanation for our findings is that lack of dominance and
2006 effect of size on spawning success may be a feature of competitive interaction in cod
2007 trios. Using an experimental set up similar to ours, Rakitin et al. (2001), explicitly
2008 tested for and found no effect of size on spawning success in wild male cod. They
2009 found that the male in the trio that fertilized the greater proportion of eggs

alternated randomly between batches of eggs, and also that there was no association between activity level and fertilization success, which could indicate lack of female mate choice. Similarly, Skjæraasen (2003), also found no relationship between male size and fertilization success for trios of both wild and cultured males tested separately. Skjæraasen (2003) did find a relationship between male behaviour and spawning success however. While this may explain why we saw no evidence of positive size-assortative mating, which has been reported elsewhere (e.g. Bekkevold et al. 2002), these results differ intrinsically from ours. Skjæraasen (2003) found evidence of inter-batch consistency in spawning success for both wild and cultured males, which we saw to some degree as well (e.g. wild male 13 with female 1, WM 9/F 15, WM6/F 20; Figure 4.5). Taken together with the fact that, unlike Skjæraasen (2003), we saw no evidence that dominance played a role in determining the outcome of mating competition, this intra- and inter-trio consistency, indicates that female mate choice could have had a role in shaping the outcome of our experiment. However, the characteristics on which the females were basing their choices are not obvious.

Courtship in cod is behaviourally complex, involving visual and acoustic displays, and female mate choice may be based on cues from any or all of these (Brawn 1961). In our experiment, in addition to having no effect on agonistic interaction, body size and pelvic fin length had no effect on courting behaviour or on spawning success. Only courting behaviours were found to influence male

2031 reproductive success. The cultured male cod performed significantly more courting
2032 behavioural events than the wild males. Of particular note, the cultured males
2033 directed a significantly greater proportion of their courting behavioural actions
2034 towards the females than they did towards the wild males, while the wild males
2035 directed a statistically equal number of courting behavioural events towards both
2036 the cultured males and females. This finding that wild and cultured males differed in
2037 the number of courting displays they exhibited as well as to whom they directed
2038 them, is in contrast to the results of Skjæraasen et al. (2010). Skjæraasen et al.
2039 (2010) found that wild and cultured males both directed more courting events
2040 towards other males, than they did towards females, which they attributed to males
2041 courting fish in their vicinity. While male cod are capable of sex determination, male-
2042 male courting appears common, and sexual recognition often does not occur until
2043 after a behaviour or physical contact has been initiated (Skjæraasen et al. 2010). Our
2044 findings suggest though that the cultured males were able to visually distinguish
2045 between the female and the wild male, while the wild males were unable to visually
2046 determine the sex of the cultured fish. This may have been because the wild and
2047 cultured males had different search images for ripe females based on the condition
2048 of the females with which they have previous experience. The mean condition of the
2049 cultured males in our study (mean Fulton's K = 1.41), was greater than the mean
2050 condition of the wild females in Skjæraasen and Hutchings (2010) (mean K = 1.10),
2051 and Skjæraasen et al. (2010) (mean K = 1.06), and this may have led the wild males

2052 to confuse the cultured males and females and to behave inappropriately towards
2053 both. We found the number of brush and approach behavioural events the wild
2054 males directed towards the female had a negative effect on the wild males' spawning
2055 success, suggesting that the behaviour of the wild males towards the females may
2056 not have been appropriate and that the females were selecting against them based
2057 on this. It was impossible to sex the cod prior to maturation, and thus male and
2058 female cod of each type were housed communally, which may have led to the
2059 cultured males having an inherent advantage through prior exposure.
2060 We found no evidence for our second hypothesis either. Despite differences
2061 in male behaviour towards them, the females did not differ in the number of
2062 agonistic or courting behaviours directed towards either male type indicating they
2063 had no behavioural preference for males of either origin. However, female mate
2064 choice may be mediated by behaviours not quantified, such as tendency to break
2065 away from ventral mounts, and decisions of whether or not to release eggs.
2066 In addition to prior exposure to ripe females, prior spawning experience may
2067 have also influenced the spawning success of the fish in our study. Growth rate,
2068 while highly variable, is generally slower and because age at maturity is directly
2069 related to growth rate (Thorpe 2004), age at maturity is consequently higher in wild
2070 (Knickle & Rose 2013) than in cultured cod (Svåsand et al. 1996). Thus, wild cod
2071 mature at a greater age, and at a slightly larger body size than do cultured cod. If
2072 past spawning experience improves male reproductive success, a smaller cultured

male cod with more seasons of spawning experience may have higher reproductive success than a larger, less experienced, wild fish. Such an effect has been documented in the Pecos pupfish (*Cyprinodon pecosensis*), wherein spawning success increases with experience, independent of male body size (Kodric-Brown 1995). Skjæraasen et al. (2008), found that repeat-spawning cultured cod males invest more in their drumming muscle mass and less in the length of their pelvic fins than do recruit spawners, while the opposite is seen in wild males. This could indicate that in an effort to increase their spawning success, experienced males are able to tailor their displays and/or secondary sexual characteristics to either the environment they experience or to the preference of females. While we do not know the exact age or spawning history of the wild fish in this study, based on their size they are likely a mixture of naïve and repeat spawners (Knickle & Rose 2013). It is likely that the cultured females are naïve spawners, but a proportion of the cultured males may have matured the previous year.

The importance of multiple paternity in determining the outcome of this study, and in the mating system of cod cannot be overstressed. Multiple paternity in batches of eggs appears to be the norm in cod under tank-based experimental conditions, and likely also in the wild (e.g. Hutchings et al. 1999, Rakitin et al. 2001, Bekkevold et al. 2002). In the current study, the success of both the wild and cultured males when acting as the satellite male could be quite high (at least 50%; in

2093 the absence of visual observation of all spawnings, it cannot be concluded if the
2094 fertilization success of the satellite male exceeded that of the primary male).

2095 Rowe et al. (2008) found that while mating success of males within spawning
2096 groups is highly skewed, and males that are larger and more aggressive generally
2097 sire a greater proportion of eggs, some males are able to sire offspring without
2098 courting females or aggressively competing with fellow males. These authors
2099 suggest that not only is this possible evidence for alternate mating tactics in cod, but
2100 also that this is the cause of the statistical breakdown of a relationship between
2101 morphological and behavioural correlates, and spawning success. In our experiment,
2102 this hypothesis can be taken a step further. In experiments with more than four
2103 males in competition, one or more males are generally fully excluded from spawning
2104 by the agonistic behaviour of the dominant males (Bekkevold et al. 2002,
2105 Skjæraasen & Hutchings 2010). In our experiment, wherein there were only two
2106 males, once either of the males paired with the female in a ventral mount, there was
2107 nothing to prevent the other from satellite spawning. This illustrates a very
2108 important assumption within this, and some other studies: that the male that was
2109 genetically detected to fertilize the greater proportion of eggs was presumed to be
2110 the primary spawner (i.e. the male ventrally mounted to the female). While this is
2111 generally found to be true in other studies, it cannot be positively concluded that the
2112 satellite spawner could not have obtained greater fertilization success than the
2113 primary spawner through sperm competition, genetic incompatibilities, or mis-

timing of gamete release by the primary male (Fleming et al. 1996, Weir et al. 2004, Berejikian et al. 2009). Genetic incompatibilities cannot be ruled out either as having influenced fertilization success, however such evidence is weak. Rudolfsen et al. (2005), assert that finding no optimal male for all females is indicative of genetic incompatibility and we found that fertilization success of wild male 13 with female 4, was generally lower than his success with either females 1 or 7 (Figure 4.5) which supports this assertion. However it must be noted that his fertilization success in the spawning event with the highest fertilization success with female 4 was actually higher than that observed in the spawning event with the lowest fertilization success with female 7. While this finding could be suggestive of genetic incompatibility, alternative explanations such as female choice or timing of gamete release cannot be excluded.

4.5.2 Potential for introgression

The results of this study are the first to show that in the absence of multi-male dominance hierarchies, the spawning success of cultured male cod was equal to that of wild males, despite these first-generation cultured cod differing both behaviourally (this study) and morphologically (Wringe et al. 2015a) from wild fish of the same source population. These results also provide further evidence that interbreeding between wild and escaped cultured cod is likely. It is also probable that through both intentional and unintentional selection within the culture environment, these differences will become magnified in future generations.

2135 Furthermore, *nota bene*, that the use of only cultured females in this
2136 experiment may cause an overestimation of cultured male success, given that the
2137 spawning success of cultured male cod in competition with wild males has been
2138 found to be higher when they mate with cultured rather than with wild females
2139 (Skjæraasen et al. 2010). However, when considering risk of introgression, even low
2140 cultured male fertilization success, presumably such as may be attained through
2141 satellite spawning, cannot be discounted, and our results show that both the
2142 cultured and wild males took part in the majority of spawning events. That said,
2143 evidence suggests cultured males may be excluded even from satellite spawning in
2144 the wild. Tagging studies have shown that after simulated escape, the habitat use of
2145 cultured male and female cod generally overlaps with that of wild cod (Uglem et al.
2146 2008, Meager et al. 2009, Meager et al. 2010, Zimmermann et al. 2013). But, within
2147 spawning aggregations, the distribution of the cultured males was physically
2148 separated from that of the wild males and the cultured males appeared to be
2149 excluded from the spawning arenas (Meager et al. 2009, Meager et al. 2010). This
2150 suggests that in nature, like what is typically seen experimentally when dominance
2151 hierarchies are allowed to form (e.g. Bekkevold et al. 2002, Bekkevold 2006,
2152 Skjæraasen & Hutchings 2010, Skjæraasen et al. 2010), that male hierarchical rank
2153 may best predict spawning success. Further, given the perceived importance of
2154 satellite spawning in our results along with the fact that acoustic studies (Meager et
2155 al. 2009, Meager et al. 2010) suggest farmed males are excluded from the location(s)

were actual spawning takes place, suggests that the fertilization success parity with wild males observed in our study likely will not occur in the wild.

That said, Meager et al. (2009) and Meager et al. (2010) did find that female cultured cod were associated with the wild males in the spawning aggregations, and the results of our study, along with those of Skjæraasen et al. (2010) demonstrate that wild males will readily spawn with cultured females suggesting that escaped cultured females may act as the primary vector of introgression as has been seen in Atlantic salmon (Fleming et al. 1996, Fleming et al. 2000).

Caveats aside, the lack of clear dominance, either behaviourally or through monopolization of spawning events by either the wild or cultured males, while still finding some consistency in intra- and inter-trio fertilization success, suggests that the competitive ability of individual males is quite varied. Thus, in the case of a large-scale escape event, the likelihood exists that some fraction of the male escapees may be competitively superior to their wild conspecifics and hybridization between them and wild females may occur. In fact, given that cod will spawn within cages and the resultant eggs 'escape' and develop in the wild (Jørstad et al. 2008, Jørstad et al. 2014), exposure to the wild environment may result in 'farmed' offspring possessing a wild-type phenotype and which may be inherently as fit as their wild counterparts. This may occur through some combination of a plastic phenotypic response to the wild environmental conditions or through a different

2176 selection regime in the wild, which may result in the survival of a portion of the
2177 'farmed' offspring most akin to their wild counterparts (phenotypically and
2178 genetically). Furthermore, for cod that escape from culture, their potential to
2179 hybridize may also increase in subsequent spawning seasons, if experience plays a
2180 role in determining success, and as the escapees become larger and their external
2181 morphology converges on that of the wild-type phenotype.

4.6 Acknowledgements

The authors wish to acknowledge the contributions of Ed Trippel to the planning and design of the experiment. Corinne Conway was indispensible throughout the project, and was a great asset. John Brattey, Dennis Ivany and the Fish, Food and Allied Workers Union helped in the collection of the wild cod. The assistance of Beth Perry, and the staff at the Genomics and Proteomics Laboratory at Memorial University was invaluable in performing the genetic analyses. We also thank the members for the Fleming and Purchase labs for their assistance and friendship. This paper was much improved by the comments of several anonymous reviewers. Natural Sciences and Engineering Research Council funded this research through a strategic grant to I.A.F. and C.F.P., and the Research and Development Corporation of Newfoundland provided additional support to B.F.W.

4.7 Tables

2196 **Table 4.1** Behavioural interactions examined during spawning

2197

Interaction	Name	Description	Reference
Agonistic	Approach	One fish swimming directly to within one-half-body-length of another stationary fish	Hutchings et al. 1999
	Chase	One fish swimming towards a swimming fish	Hutchings et al. 1999
	Prod	Contact between the snout of one fish, and any part of another	Hutchings et al. 1999
	Coerce[a]	One fish swimming in a manner such that another fish was forced to swim in only a fraction of all potential directions	Brawn 1961 and Hutchings et al. 1999
Courting	Brush[b]	One fish contacts another fish with its side	Hutchings et al. 1999
	Lateral Display	A fish maintains station in front of another stationary fish and extends its median fins	
	Ventral Mount	One fish slips under another, grasps it with its pelvic fins and attempts to elicit spawning	Brawn 1961

[a] Coerce is classified as an agonistic action in contrast to the "paired swim" described by Brawn and Hutchings et al. because it appeared generally to be performed to restrict access to the female, or in some cases, a portion of the tank

[b] The brush action was seen to initiate and accentuate the "circling" behaviour described by Hutchings et al., which in turn was part of the "flaunting display" described by Brawn.

Table 4.2 Descriptive statistics of the fish used in the experiment. Reported values are means ± standard deviation. ANOVA (on LME) results are also reported, with different letters in superscript indicating significant differences between groups (α = 0.05).

	Wild Males (n=16)	Cultured Males (n=22)	Cultured Females(n=19)	F	P
Weight (g)	2215.2 ± 183.5^{a}	1723.1 ± 91.9^{b}	1794.0 ± 60.4^{b}	2,55 = 9.88	< 0.001
Total Length (cm)	58.4 ± 1.4^{a}	49.1 ± 0.8^{b}	48.5 ± 0.6^{b}	2,51 = 33.91	< 0.001
Mean Pelvic Fin Length (mm)	75.2 ± 7.9^{a}	59.8 ± 6.0^{b}	55.4 ± 5.4^{b}	2,49 = 47.32	< 0.001

Table 4.3 Mean ± SD of behavioural actions (defined in Table 4.1) performed during the night of spawning. The numbers are the sum of the action an individual directed at both possible recipients. ANOVA (on lme) results are reported, with different letters in superscript indicating significant differences ($\alpha = 0.05$).

	Behaviour	Cultured Male	Wild Male	Cultured Female	$\chi 2$	P
	Total Actions	99.0 ± 88.1^{a}	53.8 ± 55.3^{ab}	15.3 ± 27.0^{b}	38.23	< 0.001
Agonistic Behaviours	Total Agonistic	63.8 ± 50.7^{a}	38.8 ± 36.6^{ab}	10.4 ± 15.5^{b}	43.04	< 0.001
	Approach	41.0 ± 30.4^{a}	28.0 ± 25.7^{a}	6.9 ± 11.8^{b}	45.18	< 0.001
	Chase	3.9 ± 5.8^{a}	1.5 ± 2.9^{ab}	0.4 ± 1.1^{b}	20.18	< 0.001
	Prod	8.2 ± 9.4^{a}	4.7 ± 6.1^{ab}	1.5 ± 2.6^{b}	21.02	< 0.001
	Coerce	10.75 ± 12.4^{a}	4.6 ± 7.0^{ab}	1.7 ± 2.4^{b}	25.12	< 0.001
Courting Behaviours	Total Courting	35.2 ± 40.8^{a}	15.0 ± 22.5^{ab}	4.9 ± 12.4^{b}	24.26	< 0.001
	Brush	19.0 ± 19.4^{a}	8.2 ± 10.0^{b}	4.2 ± 12.1^{b}	22.84	< 0.001
	Lateral Display	15.9 ± 23.2^{a}	6.4 ± 15.0^{ab}	0.7 ± 2.1^{b}	18.61	< 0.001
	Ventral Mount	0.3 ± 1.1	0.5 ± 1.3	0.02 ± 0.2	2.43	0.296

Table 4.4 Differences in the behavioural interactions (defined in Table 4.1) among the fish in the trios. WM, CM and Fem denote the wild male and cultured male and female respectively. Arrows represents the direction of behavioural interaction, with actor on the left, and the recipient on the right. The greater-than symbol (>) indicates that the number of behavioural actions performed by the first actor/recipient pair was greater than the number performed by the second pair. All entries are significant at $\alpha = 0.05$, while those entries marked with * are significant only after the aberrant spawning was excluded from analysis.

	Behaviour	Significant Differences[2220]
	Total Actions	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ WM > Fem ⇒ WM
Agonistic Behaviours	Total Agonistic	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ WM > Fem ⇒ WM CM ⇒ WM > Fem ⇒ CM*
	Approach	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ WM > Fem ⇒ WM
	Chase	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM
	Prod	
	Coerce	CM ⇒ WM > WM ⇒ Fem CM ⇒ WM > Fem ⇒ CM CM ⇒ WM > Fem ⇒ WM
Courting Behaviours	Total Courting	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ Fem > WM ⇒ Fem
	Brush	CM ⇒ Fem > WM ⇒ Fem CM ⇒ Fem > WM ⇒ CM CM ⇒ Fem > CM ⇒ WM* CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM
	Lateral Display	CM ⇒ Fem > Fem ⇒ WM
	Ventral Mount	

4.8 Figures

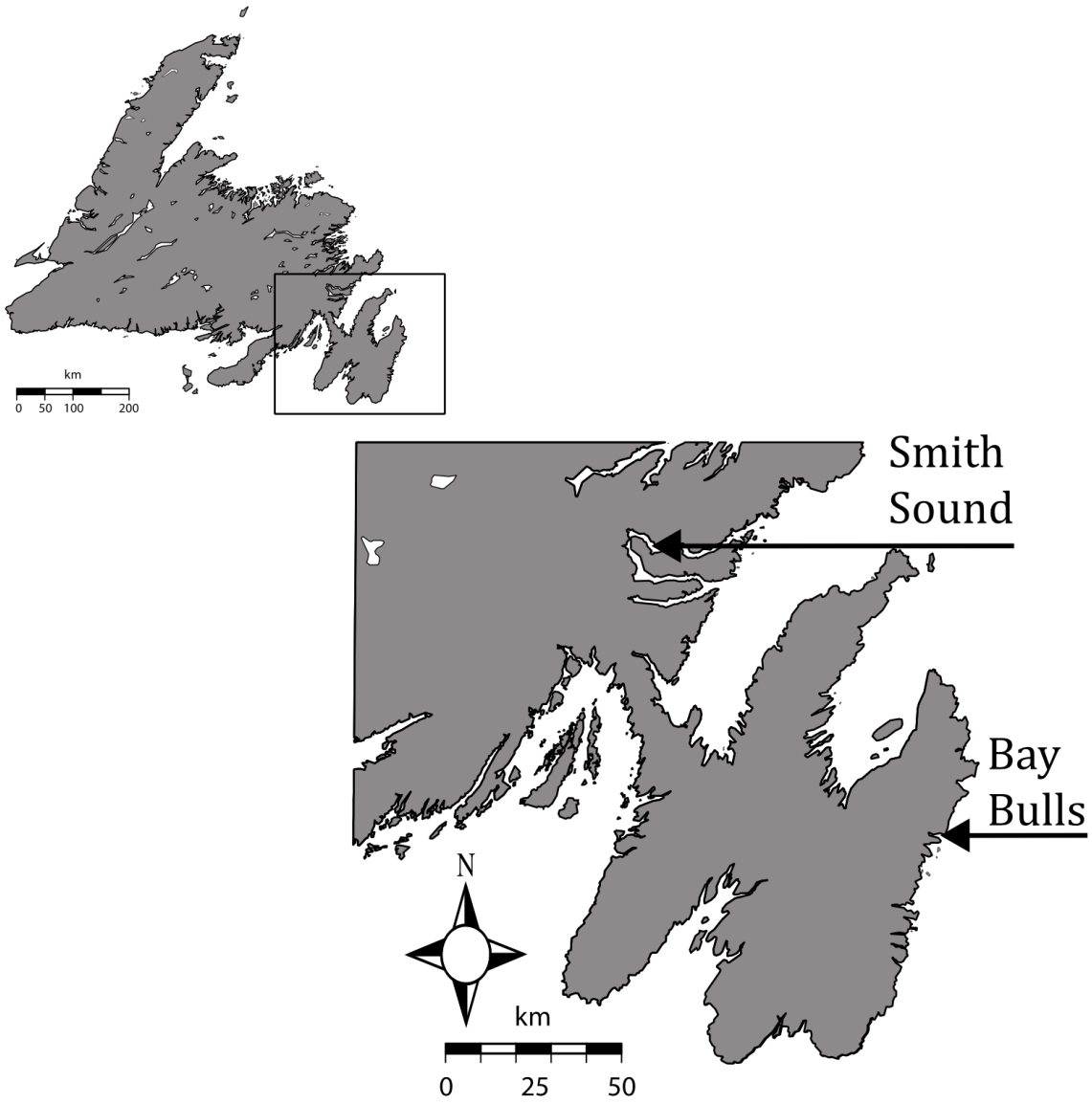


Figure 4.1 Locations from which the wild Atlantic cod were captured (Smith Sound), and the cultured cod were obtained (Bay Bulls).

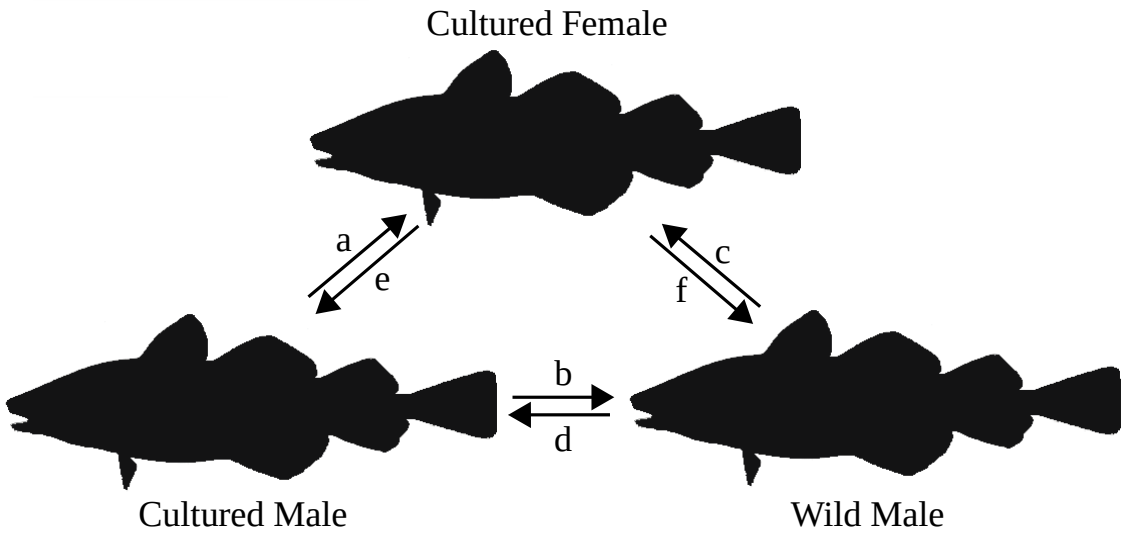


Figure 4.2 Actor-recipient behavioural dyads with direction of action labelled a-e. Each fish is capable of acting on either, or both of the other two fish in the tank (e.g. for the female, arrows 'e' and 'f'). In turn, each fish can also be acted upon by either or both of the other fish (e.g. for the female, arrows 'a' and 'c'). Total behavioural actions are the sum of all behavioural actions an individual directs at both potential recipients (e.g. for the female, the sum of 'e' and 'f').

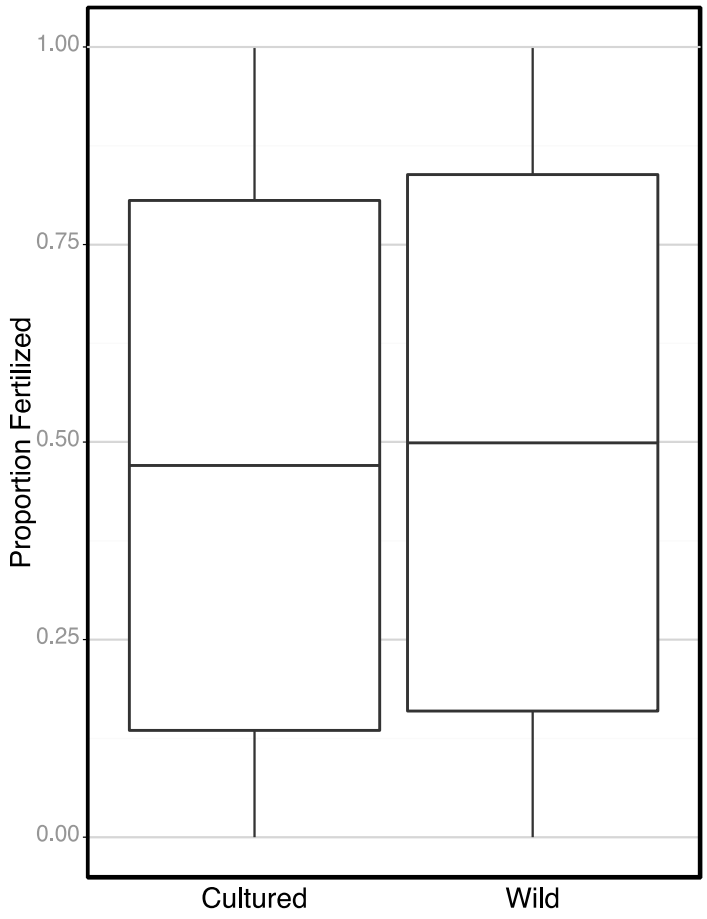


Figure 4.3 Proportion of the 25 eggs that were genotyped per batch fertilized by either the wild or cultured male. The data are all spawns for each trio that was successful in spawning for all tanks and all rounds of experimentation. The mid-line of the boxplot is the median, upper and lower limits of the box denote the first and third quartiles respectively, and the whiskers extend to 1.5 times the inter-quartile range.

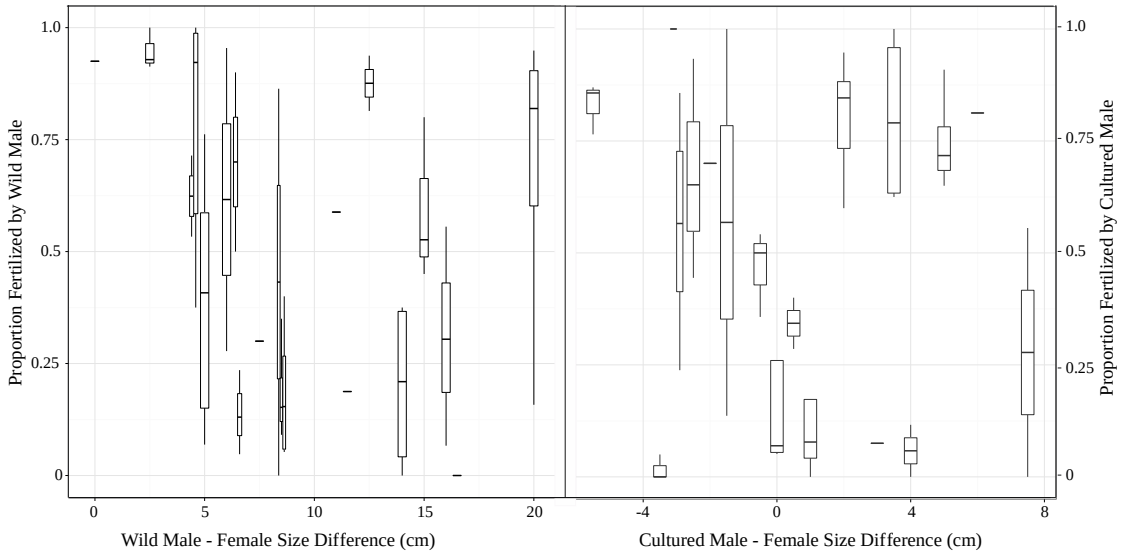
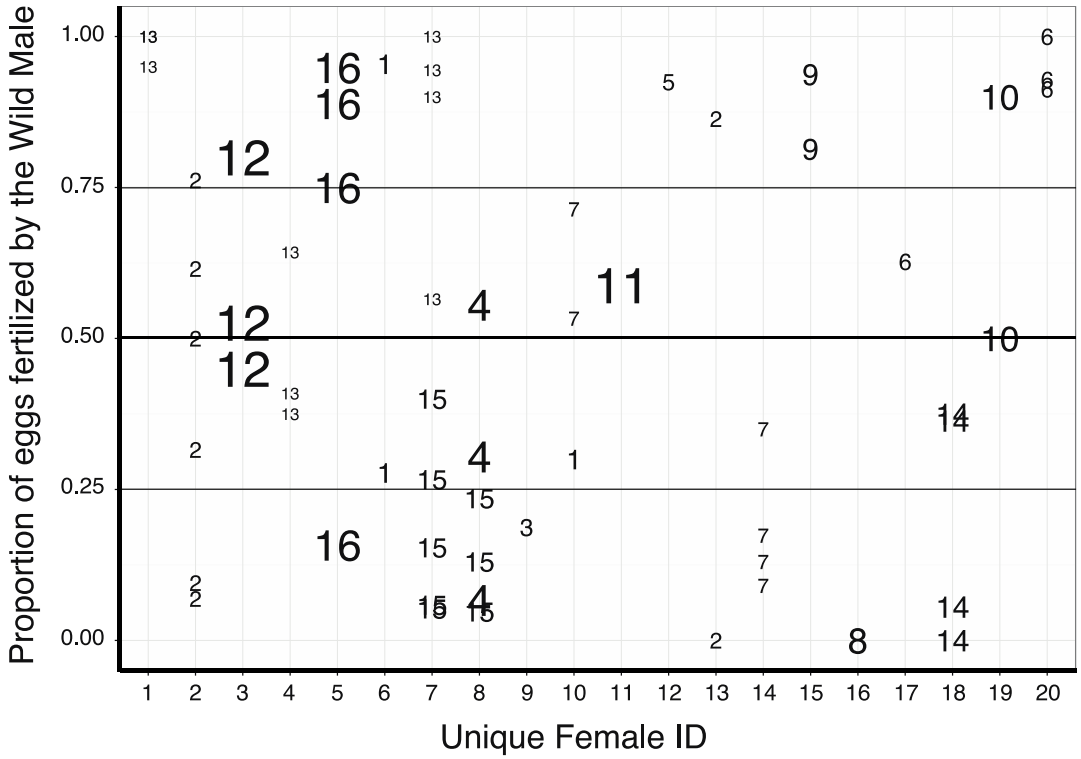


Figure 4.4 Proportion of eggs fertilized by each male, as a function of the difference in total length between the male, and the female with which he spawned. Differences are the length of the male, minus the length of the female (negative numbers indicate the female was longer than the male). Whiskers on the boxes extend to 1.5 times the inter-quartile range, the upper and lower bounds of the box are the first and third quartiles, and the mid-bar in the box is the median. Dashes without boxes indicate that a group spawned once, and hence calculation of variance is impossible.


Proportion of eggs fertilized by the Wild Male
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Unique Female ID

2253
2254

Figure 4.5 Intra- and inter-trio spawning success of wild males. The spawning success of the cultured males are not shown, because they were not used in more than one trial, and thus do not show inter-trio variability. However, within each trio, the spawning success of the cultured males is the inverse of that of the wild male. The y-axis is the proportion of eggs fertilized by the wild male, while the x-axis is the unique identity of the female with which a male spawned. Individual wild males are plotted using unique numbers, and the font size is proportional to the weight of that male. Each point is reflective of the proportion of eggs fertilized by a wild male in one spawning event with the female indicated on the x-axis. More than one unique number above a female indicates she was used in more than one trial, while the unique ID of a wild male occurring above more than one unique female identifies wild males that were used in more than one trial.

Chapter 5 – Hybridization between genetically distinct populations has no effect on fitness during early life-history

5.1 Abstract

Interbreeding or hybridization of locally adapted, and thus genetically distinct populations can lead to fitness differences relative to the parental strains in the F_1, and introgressed populations may experience genetic alterations and reductions in their fitness. Populations of Atlantic cod from New Brunswick and Newfoundland show genetic divergence, consistent with temperature-related local adaptation. While naturally reproductively isolated, the potential for hybridization was created through transfers for their use in aquaculture. We thus sought to determine if pure strain and F_1 hybrid offspring differed in any of several proxies of early life history fitness, and if this was influenced by environment. We first compared fertilization and hatching success, as well as aspects of metabolic rate such as time to hatch and time-to-death of unfed larvae, at three temperatures. Then the relative survivorship, growth and morphology at two temperatures were examined over a longer period. We found no evidence that the pure strain and F_1 hybrids differed in their relative fitness, nor did we find a differential response to temperature. These findings suggest the introgression of a non-local strain into the local population is quite possible (i.e. NB into NL or *vice versa*). But, whether these findings are true of the entire life-history of the hybrids is unknown, and the same is true of the relative

fitness of F_2, F_n, and backcross offspring, as well as longer term effects on the fitness of the local population following introgression.

5.2 Introduction

Traditionally, oceanic habitats have been assumed to have fewer impediments to interbreeding and dispersal than either terrestrial or freshwater habitats. Thus levels of gene flow among populations in oceans are presumed to be higher and patterns of genetic variation more uniform. Despite this adage, patterns of genetic variation beyond simple isolation by distance (Hauser & Carvalho 2008), and which are indicative of positive selection and local adaptation are often detected (Beheregaray & Sunnucks 2001, Nielsen et al. 2004, Jorgensen et al. 2005). Temperature is known to impart a strong selective force on the genomes of poikilothermic animals and is implicated in patterning the divergence observed in many of these studies. These patterns can occur as a continuous latitudinal cline, as in the case of lactase dehydrogenase (LDH) allele frequencies in the killifish *Fundulus heteroclitus* (Powers & Place 1978, Bell et al. 2014), to more spatially discrete divergences as have been observed in other species (Jorgensen et al. 2005, Bradbury et al. 2010). A good example of such discrete differentiation is seen between populations of Atlantic cod (*Gadus morhua*) in Newfoundland and New Brunswick, which differ on either side of ocean bottom temperature discontinuities associated with the Laurentian Channel in the Gulf of St. Lawrence (Ruzzante et al.

2310 1996, Pogson & Fevolden 2003, Pampoulie et al. 2006, Andersen et al. 2009,
2311 Bradbury et al. 2010).

2312 Introgressive hybridization between genetically isolated or locally adapted
2313 populations can lead to any of several possible fitness outcomes dependent in part
2314 on their underlying genetic architecture (Burke & Arnold 2001). While the fitness of
2315 the F_1 hybrids could be increased by, for example, heterosis (hybrid vigour)
2316 (Charlesworth & Willis 2009, Pruvost et al. 2013), the long term (F_2 and more likely
2317 in natural environments back cross) fitness of the introgressed population may be
2318 reduced by the introduction of non-locally adapted traits or loci, reduction of overall
2319 genetic diversity, and/or disruption of locally adapted gene complexes which have
2320 evolved to work in concert over evolutionary timescales (Marr et al. 2002, Tymchuk
2321 et al. 2007, Johnson et al. 2010).

2322 One manner in which disparately related and naturally separated
2323 populations may come into contact is through human mediated dispersal (Fraser et
2324 al. 2010a). Among aquatic species this often occurs through the use of "non-native"
2325 (i.e. originating from different ancestral populations) strains in aquaculture, and the
2326 subsequent escape of genetic materials (fertilized eggs or larvae: Jørstad et al.
2327 (2008), Uglem et al. (2012), Somarakis et al. (2013); through to spawning
2328 individuals: McGinnity et al. (1997), Jensen et al. (2010), Glover et al. (2013)).
2329 Broodstocks are often used outside of the range of their founder populations
2330 because of a wish to expand aquaculture production into an area for which a local

2331 broodstock does not exist, or because the non-native broodstock outperforms the
2332 native one.

2333 Beginning around the turn of the millennium, the culture of cod in Canada's
2334 Atlantic provinces was examined as a means to both diversify the region's
2335 aquaculture industry, and a to meet consumer demand for product following the
2336 collapse of wild stocks. To this end, cod broodstocks were simultaneously developed
2337 from locally captured fish in both New Brunswick (NB) and Newfoundland (NL).
2338 This experimental cod aquaculture programme resulted in a group of NB cod being
2339 present in NL waters. If escapes (either of individuals or genetic materials in the
2340 forms of fertilized eggs, or larvae) of these NB fish were to occur into NL waters, it
2341 could result in introgressive hybridization and subsequent fitness effects.

2342 In light of this potential for anthropogenically mediated introgression events,
2343 we experimentally tested several proxies of early life-history fitness (e.g. growth
2344 rate, Tupper & Boutilier 1995, metabolic rate and energy usage, Grabowski et al.
2345 2009, morphology, Paulsen et al. 2009) of hybrids between cod stocks from NB and
2346 NL relative to that of their pure-strain half-sibs. Given the presumed importance of
2347 temperature in the development of the adaptive genetic differentiation and
2348 structuring between the two populations and because the effects of outbreeding
2349 depression (where present) are often exacerbated by environmental conditions
2350 (Tymchuk et al. 2007, Frankham et al. 2011), we chose to test how any differences in
2351 relative fitness were influenced by temperature. Using two experiments we looked

for differences in relative fitness over both short- and longer-term rearing. The former featured an evaluation of genetic compatibility, relative gamete quality, developmental, and energy utilization rate, while the latter focused on relative survival, growth and morphology.

5.3 Methods

The Newfoundland (NL) and New Brunswick (NB) cod used in our experiment were progeny of The Atlantic cod Genomics and Broodstock Development Project's (CGP) NL and NB broodstocks, respectively. The CGP NL broodstock was created from wild caught fish from Smith Sound and Bay Bulls NL, while the NB broodstock was founded using wild caught fish from the Bay of Fundy NB (Figure 5.1). Seventy-five fish of each origin (NL: 2848.6 ± 556.0 g, 62.2 ± 3.7 cm; NB: 1579.7 ± 563.8 g, 47.6 ± 5.3 cm) were obtained from Cooke Aquaculture's commercial cod farming cage facility in Hermitage, NL on December 6, 2011 and transported by truck to Memorial University of Newfoundland's Department of Ocean Sciences' Joe Brown Aquatic Research Building. These fish were held together in a $25m^3$ tank (diameter 5m, depth 1.3 m, flow rate 110 $L \bullet hr^{-1}$, temperature 6 ± 1 °C) for the duration of the experiments.

Fish were fed *ad libitum* three times per week on a diet of herring (*Clupea harrengus*), supplemented with mackerel (*Scomber scombrus*) and squid (*Ilex spp.*) as available. Feeding was reduced to twice weekly during the peak of the spawning season because fish decreased their food intake (Fordham & Trippel 1999). All fish

were implanted with passive integrated transponder (PIT) tags under anaesthesia with MS-222 (Tricaine methanesulfonate) for unique identification.

5.3.1 Short-term hybridization

A split-brood design was employed to create both hybrid and non-hybrid half-sib families using both NL and NB stock dams at three different experimental temperature (3, 6, 9 °C). Females showing obvious signs of sexual maturity (i.e. swollen, distended bellies) were captured from their holding tank using dip nets, their PIT tags scanned and the number recorded, before being placed in a 750 L insulated fish tote prefilled with water from the same source as the tank. Next males were captured, scanned, and placed along with the females in the fish tote. The PIT tag numbers were used to determine the population of origin of the fish (i.e. NB or NL), as well as to keep track of which fish had been crossed previously, and with whom they had been crossed. Gametes were collected from fish that had been anaesthetized in MS-222, weighed (± 1.0 g) and measured for total length (± 0.5 cm). Two to three ML of Semen was collected in an unlabelled 5 mL syringe by applying gentle pressure to the abdomen, taking care to ensure that there was no contamination from blood, urine or faeces. Approximately 175-180 mL of eggs were collected in two 100 mL plastic screw-top specimen containers in a similar fashion. Semen and eggs were stored in a cooler with icepacks until use.

For each male, its semen was aliquoted into three pre-chilled 1.5 mL Eppendorf tubes (one at each of three experimental temperatures, i.e. 3, 6 and 9 °C;

Figure 5.2), which were then replaced into their respective incubator (Thermo Scientific Precision). Next, for each batch of eggs collected, ~25 mL aliquots were placed into six pre-labelled, pre-chilled 250 ml glass beakers (one for the pure strain cross, one for the hybrid cross, at each of the three temperatures; Figure 5.2). These were then placed in the incubators for approximately 15 minutes to allow them to come to temperature.

For each fertilization, the beaker containing the eggs was removed from the incubator and placed upon a cooling plate (custom Physitemp TS-4 system; Purchase & Moreau 2012) set to the experimental temperature, a small amount of filtered and UV treated seawater (approx. 2 mL) at the experimental temperature was added to the eggs and distributed by gently swirling the beaker, followed by the addition of 250 µL of milt via pipette (≅ 1:100 milt:egg ratio). This was stirred for 20 s using the pipette tip, and then returned to the incubator for 3 min. After 3 min, 150 mL of seawater was added, and the beaker was again returned to the incubator for a further 10 min. Excess semen was then removed by pouring the contents of the beaker through a fine-meshed aquarium net and rinsing with filtered seawater at the experimental temperature. The net was then inverted above a new chilled beaker, and the eggs were rinsed into the net using the proper temperature filtered seawater, filling the beaker to the 200 mL mark. Beakers were returned to the incubators overnight.

The next day, the eggs were gently swirled (to ensure that the floating and presumably viable, and sunk and presumably unviable eggs were sampled equally) and a random sample of approximately 100 eggs was taken from each fertilization (i.e. each dam-sire pair at each temperature) using a disposable 3 mL plastic pipette, from which the tip had been removed to prevent damage to the eggs. Samples were stored in 20 mL plastic scintillation vial along with 15 mL of Stockard's solution (50 mL formalin, 40 mL glacial acetic acid, 60 mL glycerin, 850 mL distilled water) for preservation. Approximately one week after the sample was taken, the Stockard's solution was decanted out, and a fresh 15 mL was added to ensure the concentration was adequate for preservation and clearing of the egg.

Fertilization success and egg sizes were calculated from photographs taken of the preserved eggs using a digital camera (Nikon D90 with a Micro-NIKKOR 60mm lens). Each image contained a size standard, and the diameters of fertilized eggs (unfertilized or damaged eggs tended to swell, and thus were not reflective of the true egg size) were measured in ImageJ (Schneider et al. 2012; http://rsb.info.nih.gov/ij/download.html). The proportion of eggs fertilized was taken as the number of intact eggs in which signs of cell division were present divided by the total number of eggs sampled.

After sampling the remaining eggs were replaced in the incubators to settle for at least 15 min to allow the viable eggs to refloat before another disposable 3 ml pipette was used to remove approx. 100 eggs at a time from the surface of the

beaker. These eggs were transferred to a petri dish and the number of fertilized eggs in the sample was counted under a dissecting microscope, and then all eggs in the petri dish were added to a pre-chilled 250 mL glass beaker (N.B. this was not used for calculation of proportion fertilized). This was repeated until a total of 200 fertilized eggs were added to the beaker, at which point the beaker was filled to the 200 mL mark with filtered seawater at the experimental temperature and the beaker was placed in the incubator.

Three replicate beakers from each fertilization at each temperature were created in this manner. Conducting crosses and incubating the resultant offspring was labour intensive, as such crosses were conducted every few days in an effort to stagger the workload.

5.3.3 Short-term hybrids – daily husbandry

Each beaker was attended to daily, and using a disposable pipette we removed and counted any dead eggs or larvae. In addition we also recorded the day on which the first hatched larvae was observed, as well as the date of peak hatch (i.e. > 50% of all eggs in beaker were hatched). The experiment ran until all larvae had starved to died, and this was noted as time-to-death. The proportion of eggs that hatched was calculated by dividing the number of larvae recorded, by the number of fertilized eggs added to each beaker (i.e. 200). The water quality of each beaker was maintained by carefully removing ca. 75% of the water every other day using a

large-volume pipette, transferring the larvae in the remaining water to a new beaker, and filling the new beaker to the 200 ml mark.

5.3.4 Long-term hybridization crosses

Tank space constraints and the different maturation timing of the two populations prevented the creation of crosses using NB dams, and as such the long-term hybridization experiment was conducted using NL dams only. Gametes were collected, crosses were conducted and fertilization success and egg size measured similarly to as in the short-term hybridization experiment with a few differences: 1) Two sets of crosses (hereafter referred to as temporal groups) were conducted, one on 25 April, and the other on 5 May 2012. PIT tag numbers were again used to determine the population of origin of the fish (i.e. NL or NB), and on the second date to ensure that unique crosses were conducted. 2) Four unique females were used on 25 April, and five unique females on May 5. A greater volume of eggs was collected (min. 100 mL eggs•female^{-1}). 3) Crosses were conducted within a cold-room with the temperature set to 6 °C, and one half of each batch of eggs collected was fertilized with the milt of an NL male and the other with milt from an NB male.

Unlike in the shorter-term experiment, eggs of each half-sib family retained were disinfected by ozonation for 1.5 min. at an oxidation-reduction potential of 800-900 mV. Once disinfected, each half-sib family was stocked to individual flow-through 50 L conical incubators. Each incubator was supplied with filtered, UV treated seawater maintained at 6 °C at a flow rate of 1 L•min^{-1}. Banjo filters (mesh

size 500 μM) prevented egg loss, and an air stone promoted water movement to prevent eggs from adhering to the sides of the incubator. Incubators were illuminated 24 hrs•day^{-1} at an intensity of 500 lx. Once daily, the water flow and aeration in each incubator was halted to allow presumably non-viable eggs to sink to the bottom. These were then removed from the tank via the bottom drain and discarded. Offspring of each temporal group remained in the incubators until the majority of half-sibs in a temporal group had hatched (i.e. ≥ 90% of all eggs in each half-sib family [incubator] had hatched).

5.3.5 Long-term hybrids – larval husbandry

Two circular 500 L replicate tanks at each of two experimental temperatures (heated [11.1 ± 2.2 °C] and ambient [8.8 ± 2.0 °C]) were used for each cross date (i.e. April 25 or May 5). Each of these tanks was stocked with equal numbers of larvae from each half-sib family on the day of majority hatch to create common garden environments. This occurred on 14 May for the group fertilized on 25 April and 21 May for the group fertilized 5 May. Transfer was accomplished by first removing the larvae from each incubator (half-sib family) to a 20 L bucket along with 15 L of filtered, UV treated seawater. All buckets were put in a cold room set to 6 °C to maintain a constant temperature during counting. The water in each bucket was then carefully agitated to evenly distribute the larvae in space, and a 150 mL sub-sample of water was removed using a graduated cylinder. The number of larvae in this subsample was counted, and the subsample returned to the bucket. This was repeated four more times, and the counts of larvae•150 mL^{-1} in the five subsamples

averaged. This average number was then used to calculate the total number of larvae present in each bucket (i.e. half-sib family). Once the number of larvae available for each half-sib family had been calculated, the largest equal number possible for stocking to each experimental tank was determined as the number of larvae in the half-sib family with the fewest available larvae divided by four (two replicate tanks at two experimental temperatures). The volume needed to be removed from each bucket such that it contained this number of larvae was calculated for each half-sib family, and the requisite volumes were transferred to four 20 L buckets (one for each of the experimental tanks). Each of these four buckets was then emptied into one of the four experimental tanks. This same procedure was followed for both temporal cohorts (i.e. cross dates), with 8 half-sib families stocked in the first temporal replicate and 10 for the second. The total number of larvae stocked to each tank was 38500 for the first temporal replicate and 20000 for the second temporal replicate.

Rearing was conducted according to the standard operating procedure of the Joe Brown Aquatic Research Building for Atlantic cod. Each of the 500 L tanks was initially supplied with filtered UV treated water at a rate of 0.8 $L \bullet min^{-1}$, which was subsequently increased to 1.2 $L \bullet min^{-1}$ after 5 days, 2 $L \bullet min^{-1}$ after 9 days, 2.5 $L \bullet min^{-1}$ after 13 days, and finally 4.5 $L \bullet min^{-1}$ after 35 days. Light was initially set to 1000 lux with a 24 hr light photoperiod, before being reduced to 600 lux after 28 days. To improve feeding performance and reduce bacterial and organic loads, the water was conditioned by adding 200 mL of a mixture of 500g clay•10 L^{-1} filtered seawater to

2520 the tanks twice daily (Attramadal et al. 2012). Larvae were first fed rotifers enriched
2521 with Algamac three times daily. *Artemia* were introduced when the average length of
2522 larvae in a tank had reached 9 mm, and weaning onto commercial, pellet diet began
2523 at an average length of 12 mm. Prior to each feeding live feeding, a small number of
2524 larvae were removed from each tank using a 250 mL glass beaker and the presence
2525 of food in their guts was visually confirmed. At the same time, the number of prey
2526 items•L^{-1} was assessed, and this value informed the amount of live feed to be added
2527 to each tank to ensure the prey concentration remained above the value prescribed
2528 in the JBARB standard operating procedure. Fish were initially fed commercial
2529 pellets in excess to aid in the weaning process, but once weaned fish were fed to
2530 satiation.

2531 ***5.3.6 Long-term hybrids - sampling***

2532 Cod were haphazardly sampled from each tank two, eight and 12 weeks after they
2533 were stocked to them. Fish were caught and removed from each tank using a long-
2534 handled aquarium net, euthanized via overdose with MS-222, and preserved in 95%
2535 ethanol in 50 mL Falcon tubes. We attempted to sample at least 100 fish from each
2536 tank at each sampling period, however in the early sampling periods we
2537 underestimated how many fish we had sampled, while in the later sampling periods
2538 insufficient numbers of fish remained in the ambient temperature tanks to allow
2539 sampling of 100 fish (Table 5.1). In addition to the sampled fish preserved in Falcon
2540 tubes, during the week 2 sampling for the 5 May cohort, and the week 8 samplings
2541 for the 25 April and 5 May cohorts, 25 cod from each tank were photographed with a

digital camera (Nikon D90 with a Micro-NIKKOR 60mm lens) for morphometric analysis, and individually preserved in Eppendorph tubes. All fish sampled during the week 12 samplings were photographed and individually preserved in Eppendorph tubes.

5.3.7 DNA Extraction and amplification

DNA was extracted from appropriately sized portions of preserved larvae/juvenile or fin clips from each potential parent using Promega Wizard SV 96 Genomic DNA Purification kits (Promega catalogue number A2371) following the manufacturer's protocol. Extracted DNA was amplified by polymerase chain reaction (PCR) using a multiplex PCR mixture that consisted of 5 µL Qiagen Multiplex PCR Master Mix (Qiagen Multiplex PCR Kit, catalogue number 206145), 1 µL 5X Q-Solution (Qiagen, provided in the Multiplex Kit), 0.40 µL of forward and reverse for each of the four primers, and 2.4 µL extracted DNA, for a total reaction volume of 10 µL. Two separate multiplexes were used to genotype each individual, one containing the markers *Gmo8*, *Gmo19*, *Gmo35*, and *Gmo37*, and the other containing *Gmo63*, *Gmo118*, *Gmo125*, and *Gmo152* (Gmo8, Gmo19, Gmo35, Gmo37: Miller et al. 2000, Gmo63, Gmo118, Gmo125, Gmo152: Higgins et al. 2009; Supplementary Table 5.1). The thermocycler conditions were: an initial denaturation step of 95 °C for 15 min., followed by 40 cycles consisting of 95 °C for 35 s, 58 °C for 90 s, and 72 °C for 30 s. The reaction was terminated by a final extension at 72 °C for 5 min., followed by incubation at 4 °C.

PCR products were sized on an ABI 3730 DNA Analyzer (Applied Biosystems) against an internal LIZ size standard (GeneScan™ 500 LIZ™ dye Size Standard, Applied Biosystems, catalogue number 4322682) and, eletrophorograms were visualized using GeneMapper® v4.1 Software (Applied Biosystems). Each offspring was genotyped in duplicate for each multiplex, and the parents in triplicate. The accuracy of allele scorings generated by the software was visually confirmed. Parentage was conducted on all offspring for which genotypes were available at all eight loci using Cervus v3.0 (Kalinowski et al. 2007).

5.3.8 Statistical analysis

All statistical analyses were conducted in R v3.2.1 (R Development Core Team 2015). Where there was non-independence because of shared parentage, or repeated sampling over time, general mixed-effects linear models (GLMM) were conducted using the package *lme4* (Bates et al. 2015), with post-hoc analysis using Tukey's honest significance tests implemented using the package *multcomp* (Hothorn et al. 2008) when significant differences were detected. For the short-term hybridization experiment, the PIT tag number of the sire and dam used in each cross were used as the random effect, while for the long-term hybridization experiment, that of the dam alone was used. This was because in the short-term hybridization experiment, both the sires and dams were used multiple times, but in the longer-term experiment, only dams were repeated within temporal replicates.

Differences in proportions fertilized and hatched were independently tested using GLMMs with binomial error structure, as were the proportions determined via genetic parentage to be hybrid or non-hybrid at each time point in the long-term hybridization experiment. Time to first hatch, time to peak hatch, and time-to-death, with time measured in both days and degree-days were each independently tested using GLMMs with Poisson error structure. Finally, GLMMs with Gaussian error structure was used to test for differences in size between hybrids and non-hybrids at each time sampling point in the long-term hybridization experiment.

5.3.9 Morphometric and geometric morphometric analyses

Based on their appearance in the digital photographs, the sampled cod were classified as being either larvae (i.e. not having developed median fins) or juveniles. Ten landmarks (Rohlf 1999, Adams et al. 2004) were recorded as *x-y* coordinates from the larvae, while 16 landmarks were recorded for the juveniles using the programme ImageJ (Figure 5.3)(Schneider et al. 2012).

Geometric morphometric analyses were conducted using the R packages *geomorph* (Adams & Otárola-Castillo 2013) and *shapes* (Dryden 2013). The x-y coordinates were converted to shape coordinates using generalized Procrustes analysis (GPA; Adams et al. 2004). GPA removes the non-shape aspects of scaling (size), orientation and location from the raw *x-y* coordinates, and standardizes each individual to a common unit centroid size (Rohlf 1999, Adams et al. 2004). The amount of shape variation attributable to the nature of the cross (hybrid or non-

2604 hybrid), and the temperature treatment (where possible, see results) was tested
2605 using the function *procD.lm* which conducts Procrustes ANOVA with permutation on
2606 the Procrustes shape coordinates (Adams & Otárola-Castillo 2013, Collyer et al.
2607 2015). *procD.lm* does not allow the specification of random effects, such as the
2608 identities of the dams, to account for similarity within half-sibs. However, we
2609 compared the results from *procD.lm* with results generated by two permutational
2610 MANOVA approaches, one using *adonis* (vegan R package; Oksanen et al. 2015), in
2611 which we specified that the permutations be constrained to occur within half-sibs
2612 (i.e. based on dam ID) , as well as those from PRIMER v6 (Clarke 2006) which allows
2613 the specification of mixed-effects. The results of all three analyses were qualitatively
2614 the equivalent (the absolute values of the [pseudo-] F and p values differed because
2615 they are based on permutation, but the interpretation was the same). Given that the
2616 *procD.lm*, which does not allow for the inclusion of a random effects term and would
2617 thus tend to be less conservative (i.e. greater chance of detecting significance where
2618 it did not exist [Type I error]) showed qualitatively matching results to the analyses
2619 in which random effects were included we chose to use *procD.lm* in this paper
2620 because it afforded greater interoperability with the other functions in its package.

2621 In addition to conducting geometric morphometrics, which examines the
2622 shape of the individual as a whole, we also tested for differences in size for the
2623 morphological measures listed in Table 5.2 between hybrids and non-hybrids, as
2624 well as between half-sib families using GLMM with female ID as the random effect.
2625 These features were measured in two ways. In the first way the distance in pixels

between the x-y points which make up each feature was converted to millimeters, then each feature for each individual was standardized using the formula $M_{st} = M_{obs}(Sz_{mean}/Sz_{obs})^{b}$ where: M is the trait measure, Sz is the size measure (standard length) to which samples are standardized, b is the trait-specific common within-groups slope and the subscripts *mean*, *obs* and *std* refer to the mean, observed (raw) and the size-standardized measurements, respectively (Reist 1986a). In the second manner, the distances between the points were calculated from the Procrustes coordinates returned after conducting GPA which contain inherent size standardization. In both cases, differences in size between hybrids and non-hybrids, as well as between half-sib families were tested using GLMMs with the dam as the random factor.

5.4 Results

5.4.1 Short-term rearing

While many of the characteristics examined were found to be influenced by temperature, no significant interactions between cross and temperature were found (all $p > 0.05$).

The NL dams were larger than the NB dams (length: NL 61.6 ± 3.35 cm, NB 51.83 ± 0.83 cm; $F_{1,9} = 11.56$, $p < 0.01$; weight: NL 3675.14 ± 206.94 g, NB 2194.50 ± 292.20 g $F_{1,11} = 23.95$, $p < 0.001$), and the same was true of the sires (length: NL 62.30 ± 1.07 cm, NB 48.83 ± 1.86 cm; $F_{1,17} = 30.14$, $p < 0.0001$; weight: NL 2591.60 ± 95.50 g, NB 1618.44 ± 176.27 g $F_{1,17} = 18.17$, $p < 0.001$). The eggs of NB dams were

2647 significantly larger than those of the NL dams (diameter NL 1.33 ± 0.03 mm, NB 1.45
2648 ± 0.03 mm ANOVA on GLMM: chisq = 11.633, df = 1, $p < 0.001$].

2649 Fertilization success (overall mean) was not different between hybrids and
2650 non-hybrids from NL or NB dams (ANOVA on GLMM: chisq = 5.0368, df = 3, $p >$
2651 0.16), nor was it influenced by temperature, or egg size (both $p > 0.64$).

2652 A significantly greater proportion of non-hybrid eggs with an NL dam
2653 hatched than non-hybrids with a NB dam (Tukey's HSD on GLMM: $z = 2.818$, $p <$
2654 0.05; all other comparisons $p > 0.12$; Figure 5.4) which fit with a significantly greater
2655 proportion of the eggs of NL dams than those of NB dams being found to hatch
2656 overall (ANOVA on GLMM: chisq = 6.1049, df = 1, $p < 0.05$). The proportion of eggs
2657 which hatched at 6 °C was higher than that which hatched at 9 °C, (Tukey's HSD on
2658 GLMM: $z = -2.719$, $p < 0.05$), but did not differ between 3 and 6 °C or 3 and 9 °C (both
2659 $p > 0.21$) nor between cross types (ANOVA on GLMM: chisq = 0.6478 df = 2 $p > 0.72$).
2660 The proportion of eggs that hatched was not influenced by the size of the eggs (all p
2661 > 0.63).

2662 Neither days nor degree-days to first hatch differed between hybrids and
2663 non-hybrids (all $p > 0.19$). However, both measures of time to first hatch were
2664 greater for NB than NL dams (both $p < 0.05$; Figure 5.5). Interestingly, while
2665 differences between temperature treatments were ubiquitous for both days and
2666 degree days to first hatch, expressing time to first hatch in degree-days revealed
2667 there to be some degree of metabolic compensation to temperature. This can be

seen in Figure 5.5 where degree days to first hatch increases with temperature (i.e. 3 °C < 6 °C < 9 °C), and while the difference between each temperature was significant (Tukey's HSD on LMM: all $p < 0.001$), the absolute difference between 3 and 6 °C was larger than that between 6 and 9 °C. This pattern was even more prevalent when measured in days (Figure 5.5).

Degree-days to peak hatch (≥ 50% hatched) was greater for eggs of NB non-hybrids than NL non-hybrids (Tukey's HSD on LMM: $z = -2.906$, $p < 0.05$), but none of the other comparisons were found to differ significantly (Tukey's HSD on LMM: all $p > 0.16$; Figure 5.6). There were also no differences between cross types when time to peak hatch was expressed in days (ANOVA on LMM: chisq = 5.4429, df = 1, $p > 0.14$; Figure 5.6). Both measures of time to peak hatch, were positively related to time to first hatch (ANOVA on LMM: all $p < 0.0001$), as well as temperature (Tukey's HSD on LMM: all $p < 0.0001$). When expressed in degree-days, some degree of metabolic compensation to temperature was again observed with time to peak hatch at 3 °C being significantly shorter than that at 6 or 9 °C (Tukey's HSD: both $p < 0.0001$), while the difference between 6 and 9 °C was not significant (Tukey's HSD, $z = 1.849$, $p > 0.14$; Figure 5.6).

Time-to-death (i.e. time to death of all hatched larvae), whether expressed in days or degree-days, did not differ between dam origins, or between hybrids and non-hybrids (all $p > 0.16$; time-to-death Figure 5.8). Expressed in days, time-to-death was found to differ between temperature treatments with 3 > 6 > 9 °C (all $p <$

0.0001; Figure 5.7). Time-to-death was positively related to percent hatch and median time-to-death (all $p < 0.0001$). Temperature compensation was again observed when time-to-death was expressed in degree days. Significant differences were observed between each temperature (Tukey's HSD on LMM: all $p < 0.0001$), but the difference between 3 and 6 °C was less than that between 6 and 9 °C (Figure 5.7). Degree-days to time-to-death was positively related to median degree days to death, degree days to peak hatch and proportion of eggs that hatched (all $p < 0.01$). There was no relationship between egg size and time-to-death (all $p > 0.15$).

5.4.2 Long-term rearing

A total of 840 and 781 offspring from the Apr. 25 and May 5 cohorts respectively were correctly assigned to parent pairs using CERVUS (93 and 94% success respectively; Table 5.3). All alleles found in the parents were detected in the offspring, and the genetic variation for the eight loci ranged between five and 13 alleles (Table 5.4).

The effects of temperature and sampling date on proportional relative survivorship had to be tested separately because a significant interaction between them was present in both temporal replicates (Apr. 25 cohort: ANOVA on GLMM chisq = 28.9896, df = 2, $p < 0.0001$; May 5 cohort: ANOVA on GLMM chisq = 6.7398, df = 2, $p < 0.05$). For the April 25 cohort, the proportional relative survival of hybrids in the high temperature treatment was not significantly different from their survival in the ambient treatment at the two and eight week sampling periods, and the same

was true of the non-hybrids (all $p > 0.56$). At the 12 week sampling period however, the survivorship of the non-hybrids in the ambient treatment was significantly greater than in the high temperature treatment (ANOVA on LMM: chisq = 31.274, df = 1, $p < 0.0001$; Figure 5.8). Within temperature treatments, the proportional survivorship of hybrids and non-hybrids was statistically equal at all three sampling points (ANOVA on GLMM: chisq = 1.06, df = 2, $p > 0.58$; Figure 5.8). The same was true of the ambient treatment for the two and eight week sampling periods, (Tukey's HSD $z = -0.345$, $p > 0.93$), but the proportion of hybrids at the 12 week sampling period was significantly less than that of hybrids at either 2 (Tukey's HSD $z = 4.411$, $p < 0.0001$) or 8 weeks (Tukey's HSD $z = 5.317$, $p < 0.0001$; Figure 5.8). The proportional relative survivorship for the offspring of the female which was used twice (i.e. crossed with two NL and two NB males; the circle and downward triangle families) showed similar proportional relative survivorship in the high temperature treatment, and the non-hybrid offspring of this female showed the best proportional relative survivorship in the ambient treatment especially at 12 weeks (Figure 5.9) There was a significant positive relationship between proportional relative survivorship for both hybrids and non-hybrids, and egg size in the ambient, but not in the high temperature treatment (ambient: $z = 2.008$, $p < 0.05$; high: $z = 1.454$, $p > 0.14$) and at the 12-week sampling period ($z = 2.552$, $p < 0.01$) but the relationship did not differ between hybrids and non-hybrids.

The May 5 cohort showed a significant difference in proportional relative survivorship between temperature treatments at the 2-week sampling period (chisq

= 7.0339, df = 1, $p < 0.01$) but no difference in proportional relative survivorship between temperature treatments for the other two sampling periods (both $p > 0.54$), or over all three sampling periods within the ambient treatment (ANOVA on GLMM: chisq = 4.203, df = 2, $p > 0.12$; Figure 5.8). However within the high temperature treatment, the proportion of hybrids was greater at both eight and 12 weeks than at two weeks (Tukey's HSD, both $p < 0.01$), but did not differ between eight and 12 weeks (Tukey's HSD, $z = -1.205$, $p > 0.45$; Figure 5.8). This difference is likely more a reflection of reversal in relative survival however.

Looking closely at the contribution by each half-sib family, it is clear that much of the signal in the ambient tank at the 12-week sampling point was caused by the filled circle family, which made up 50% of all offspring sampled at this time (Figure 5.9). There was a significant positive relationship between survivorship for both hybrids and non-hybrids, and egg size at all time points in both temperature treatments (all $p < 0.0001$).

At the two-week sampling period, for the May 5 cohort, no morphological differences were detected by either the traditional (all $p > 0.34$; Table 5.5) or geometric morphometric ($Z = 0.63$, $p > 0.60$) analyses between hybrids and non-hybrids and all offspring analyzed were found to be at the larval stage. Head length, eye size and somite depth were found to be significantly larger in high temperature (ANOVA on LMM: chisq = 4.4928, df = 1, $p < 0.05$; all other $p > 0.09$; Table 5.5), but this was not reflected as a difference in shape in the geometric morphometric

analysis ($Z = 1.21$, $p > 0.20$), and there were no interactions between temperature and treatment (all $p > 0.53$). Egg size was positively related to standard length, head length, and lower jaw length (all $p < 0.05$), but not to the other characters (all $p > 0.15$). No samples were taken for measurement at the two week sampling point for the April 25 cohort.

By the eight-week sampling period, approximately one third of all samples all of which were in the high temperature treatment for the April 25 cohort had metamorphosed to the juvenile stage. However, by eight weeks, May 5 cohort fish sampled from the high temperature treatment had metamorphosed into juveniles, while those in the ambient treatment retained their larval morphology making comparisons across temperatures impossible within this cohort. For both temporal treatments and offspring developmental stages (i.e. larval and juvenile) hybrids and non-hybrids did not differ morphologically (all $p > 0.64$; Tables 5.5 and 5.6). However, for the April 25 cohort where comparison was possible, the larvae in the high temperature treatment were larger than those in the ambient for all measures following size standardization (all $p < 0.0001$; Table 5.5), and there was no interaction between cross type and temperature (all $p > 0.36$). The morphology of the April 25 larvae was not related to egg size (all $p > 0.35$), but a positive relationship was for all features in the May 5 cohort (all $p < 0.05$). Likewise, the morphology of the April 25 juveniles was unrelated to egg size, and the same was true of the May 5 cohort (all $p > 0.11$). The results of the geometric morphometric analysis were equivalent; for both cohorts the hybrids and non-hybrids did not

differ in shape (all $p > 0.11$). Within the April 25 cohort where comparison as between temperatures was possible for those fish at the larval stage, there was a significant difference in shape between temperature treatments ($Z = 10.24$, $p < 0.001$) but no type/treatment interaction ($Z = 1.57$, $p > 0.09$; Figure 5.10).

All offspring sampled at 12 weeks were found to have metamorphosed to juveniles. None of the juvenile morphometric measures differed in size between hybrids and non-hybrids in the April 25 cohort (all $p > 0.09$; Table 5.6), but the depths of the caudal peduncle, and body were found to be significantly greater in the non-hybrids than the hybrids in the May 5 cohort (both $p < 0.05$). For both cohorts, all measurements were found to be significantly larger in the high temperature treatment following size standardization (all $p < 0.05$). Geometric morphometric analysis showed similar results, with significant differences in shape present between temperature treatments in both temporal cohorts (both $p < 0.001$; Figures 5.11 & 5.12), but no difference between hybrids and non-hybrids (both $p > 0.05$) nor any interaction between treatment and hybrid status (both $p > 0.18$). Egg size was positively related to standard length, head length, and lower jaw length for the April 25 cohort (all $p < 0.05$), and to standard length, body depth, lower jaw length, mid-body area, and gut area in the May 5 cohort (all $p < 0.05$).

There was no relationship between size and survivorship for either temporal cohort, at any sampling period (all $p > 0.19$).

5.5 Discussion

Generally, the fitness of hybrids is quite variable compared to the parent populations, with relative fitness highly dependent upon the environment experienced (Hails & Morley 2005, Tymchuk et al. 2007). Given that the genetic divergence in the cod populations tested appears driven by adaptation to dissimilar temperature regimes, we anticipated that we would observe differences related to metabolic processes and energy usage efficiency between the hybrids and non-hybrids. These differences would manifest in the short-term experiment in the fertilization rate, hatching success, and developmental rate and in the longer-term experiment as differences in survivorship, morphology and growth.

5.5.1 Short-term rearing

Fertilization rate was lower for hybrids than non-hybrids and it did not depend on the direction of the cross (i.e. whether the dam was NL or NB). However, there was no difference in hatching success between hybrids and non-hybrids. This suggests that any genetic incompatibilities are pre-zygotic or lethal just prior to fertilization (i.e. before cleavage). Finding that neither fertilization nor hatching success were related to egg size is consistent with what has previously been reported in cod from the northwest Atlantic (Pepin et al. 1997).

Interestingly, the hatching success of non-hybrid NL eggs was significantly greater than that of non-hybrid NB eggs, and was not related to temperature. This is in one way consistent with what was observed by Trippel (1998), who found that

2816 both fertilization and hatching rate were higher for females in their second
2817 spawning season than in their first as were our NL and NB dams, respectively.
2818 However egg size in the cod in Trippel's (1998) experiment were larger in the repeat
2819 spawners, and also showed a positive relationship with female body size, contrary to
2820 what we found (however our sample size was small).

2821 Hatch timing did not differ between hybrids and non-hybrids, but the times
2822 to peak hatch (≥ 50% hatch) appear to be slightly longer at each temperature than
2823 have been previously reported (Wieland et al. 1994, Pepin et al. 1997). The NL
2824 population, which typically spawn at a lower temperature than the NB population,
2825 showed some evidence of countergradient developmental rate response to
2826 temperature by taking significantly fewer days to first hatch, especially at the higher
2827 temperatures. However, this did not carry over to time to peak hatch, with the time
2828 taken by the two populations not differing significantly. Countergradient variation
2829 has been detected previously for developmental rate for time to hatch in other
2830 species (e.g. Fundulus heteroclitus; DiMichele & Westerman 1997), but this may be
2831 the first time it has been observed in cod.

2832 Time-to-death did not differ between cross types, and was unrelated to egg
2833 size. The time-to-death in our experiment showed good correspondence to those
2834 observed by Yin and Blaxter (1986) at similar temperatures. The main determinates
2835 of time-to-death in unfed larvae is the amount of energy with which the eggs were
2836 provisioned by the dams, and the efficiency in which it was used by the offspring.

The sizes of the eggs in our experiment were within the ranges that have been reported previously for fish of about the same size (Chambers & Waiwood 1996, Pepin et al. 1997, Trippel 1998), but the absolute size of eggs of the NB dams were significantly larger than those of the NL dams (diameter: ~9%; volume: ~25%). Egg diameter in cod is positively associated with yolk dry weight (*viz.* energy) (Trippel 1998, but see Bachan et al. 2012 for differences in yolk lipid contents), and assuming this relationship is the same for NB and NL dams, one would presume the offspring of NB dams would have been provisioned with a greater initial yolk supply. Given that there were no differences in time-to-death measures between hybrids and non-hybrids, or between populations, and further that time-to-death was not related to egg size, it would appear differences in energy usage have a greater impact on time-to-death than initial energy provisions.

Previous research has shown that temperature during development can have significant impacts on the *in ovo* energy usage of cod embryos, with size at hatch, and hence energy conversion efficiency decreasing from 4-10 °C (Peterson et al. 2004). However, Pepin et al. (1997) found the opposite, with size at hatch increasing with temperature. We did not measure the size of the hatching larvae, or the size of their yolk sac, and thus cannot comment directly on their energy usage efficiency within the egg. That said, the fact that time-to-death in degree days was related to the time to peak hatch in degree days across temperatures indicates the overarching role of metabolic rate in shaping the outcome of our experiment. Moreover, despite being significantly different, there was some overlap for time-to-death in degree

days between 6 and 9 °C, but not between 6 and 3 °C, suggesting that energy usage efficiency was affected by temperature. This did not differ, however, between populations or hybrids and was not in the direction which would be predicted based on the results of Peterson et al. (2004). It is unlikely that this effect is due to differences in energy expenditure by virtue of being active following hatch for more days at 3 °C because all characters measured showed the acceleration of development (degree days) at 3 °C.

Furthermore, (Pepin et al. 1997) found that while significant, the effect of egg size on time-to-death was minimal. Fish species from higher latitudes, and which consequently generally experience lower average ambient temperatures, tend to show higher temperature-adjusted standard metabolic rates (White et al. 2012). At the species level, this pattern also holds true for cod (Sylvestre et al. 2007, Grabowski et al. 2009), and is consistent with what we observed. Furthermore, that the hybrids did not differ from the non-hybrids indicates that the metabolic effect may have been primarily driven by maternal inheritance of mitochondrial haplotypes (Brown et al. 2006), and was not influenced by the interaction of the two genotypes (NL and NB).

5.5.2 Longer-term hybridization

The results of the longer-term hybridization experiment, which used only NL dams, initially appear harder to interpret because the two temporal treatments showed different results in terms of the relative fitness of hybrids and non-hybrids. Looking

at the ambient temperature treatments in particular, in the April 25 cohort in the ambient temperature treatment, the relative fitness of the non-hybrids appears greater than that of the hybrids, while in the May 5 cohort the opposite was true. However, turning our attention from the overall proportion of hybrids and non-hybrids detected, to the relative survivorship of the hybrid and non-hybrid half sibs on a dam-by-dam basis, a different pattern emerges. Saliently, some type of maternal effect, tempered by the interaction of the female and male genomes, as well as their interaction with the conditions in each treatment appears to be the driver of the observed results. In both the April 25 and May 5 treatments, there is a general pattern of both types of offspring from several of the dams performing well in the high-temperature treatment, and then one type of offspring from these same dams making up the majority of the offspring detected at 12 weeks, where the population numbers for some tanks became very low, in the ambient treatment (e.g. April 25: square, circle, and downward triangle dams [n.b. circle and downward triangle are the same dam]; May 5: square, and circle, to some extent upward triangle [n.b. that the same dams designated by the shapes performed well is due to chance as the same shapes in different temporal cohorts do not denote the same dams]). There was no clear pattern among dams showing greater relative fitness in their hybrid or non-hybrid offspring over all sampling periods in the high temperature treatment for either the April 25 or May 5 cohorts. That said, while there was no difference in the overall proportion of hybrid and non-hybrid offspring recovered at 12-weeks in the April 25 high temperature treatment, tellingly all hybrid half-sib families, but not

non-hybrid families show non-zero survivorship. Furthermore, the offspring of the dam that was used twice in the April 25 cohort (circle and downward triangle families) all show similar survivorship indicating some type of maternal effect.

In the ambient treatments, the relative fitness differences observed at the 12-week sampling period in the April 25 cohort appears consistent with theoretical expectations of outbreeding depression where its effects are exacerbated in non-optimal conditions (Hails & Morley 2005, Tymchuk et al. 2007). However, these results are also consistent with the possibility that NL fish are better adapted to cooler temperatures (Purchase & Brown 2000, 2001) (although the temperature differential between the two treatments was small, the variability in the ambient was greater). It must be noted that the findings are driven primarily by the offspring of only two dams.

As was seen in the April 25 cohort, two dams drove the results in the May 5 cohort. Thus it appears that the results of both cohorts are best explained by female quality and/or sire-dam incompatibilities. Looking first at dam quality in the ambient treatment, the hybrid offspring of the circle and square dams make up over 70% of offspring sampled at 12 weeks. The non-hybrid offspring of the circle dam show zero survivorship in both the high and ambient temperature treatments, indicating there may be some incompatibility between this sire and this dam.

The inconsistency of relative hybrid and non-hybrid fitness between the two sampling periods, suggests there is no clear evidence of negative effects of

2923 hybridization between these two populations under the experimental conditions
2924 tested. We did not genotype the fish for markers corresponding to the SNPs with
2925 allele frequencies in the two populations related to temperature identified by
2926 Bradbury et al. (2010) or the genes identified by other researchers (Pogson &
2927 Fevolden 2003, Andersen et al. 2009, Borza et al. 2009) and thus cannot directly
2928 comment on how their inheritance may have influenced survival. However, given
2929 that the frequency of cold-associated alleles is greater in the NL than the NB
2930 populations (Bradbury et al. 2010), probability would dictate that the full strain
2931 offspring (i.e. NL dam X NL sire) would inherit a greater number of cold-associated
2932 alleles. If these loci do indeed confer greater fitness in colder temperatures, whether
2933 they act in an additive or non-additive manner, the pure strain fish should display
2934 greater survivorship in the ambient temperature treatment as was seen in the April
2935 25 cohort, but not the May 5 cohort. Furthermore, it is unlikely pleiotropic effects
2936 are responsible for differences in fitness because in these F_1 hybrids recombination
2937 would have taken place within the genetic background of each population and thus
2938 co-adapted gene complexes would be inherited *in toto*.

2939 A degree of maternal effect is suggested because (half-sib) families with
2940 relatively good survivorship in the high temperature treatment were generally seen
2941 to also have the relatively good survivorship in the ambient treatment. Furthermore,
2942 survivorship was positively related to egg size in all samples except for the two and
2943 eight week samples from high temperature for the April 25 cohort. The split-brood
2944 design of our experiment, in which both the hybrid and non-hybrid offspring of a

dam should receive identical maternal inputs, also revealed differences in the survivorship of the two offspring types that would appear indicative of a paternal effect (Trippel et al. 2005) or a sire-by-dam compatibility effect (Rudolfsen et al. 2005).

Morphologically the hybrid and non-hybrid fish were essentially identical, in both temperature treatments, and survivorship was not related to size. Marcil et al. (2006b), and Marcil et al. (2006a) reared cod from the same populations as us in common gardens at two different temperatures. Contrary to our findings, both studies by Marcil et al. found genetically-based differences in morphology between juvenile full strain cod from NL and NB populations. Similarly, studying NL and NB populations of cod, Purchase and Brown (2000) found genetically based differences in energy allocation (*viz.* hepatosomatic index) which we have previously shown can lead to differences in morphology in adult cod (Wringe et al. 2015a). If mortality was related in some way to morphology, we would expect that groups exhibiting higher proportional mortality would in turn display only a subset of the morphology of the other group. However, this did not appear to be the cease. So, why we did not detect differences in morphology is unclear.

5.5.3 Conclusions

Previous experiments in cod have shown that cultured fish are capable of interbreeding with wild fish (Skjæraasen et al. 2010, Wringe et al. 2015b). The results of this study indicate that should cod from these populations come into

2966 contact as the result of human-mediated dispersal through aquaculture,
2967 introgression is possible and a portion of the resultant offspring (F_1) are likely to
2968 survive because their fitness will not differ significantly from that of their non-
2969 hybrid counterparts during early life stages. The cod mating system may further
2970 increase the chances that fit hybrids are produced as well. We observed that while
2971 some females appeared to produce more fit offspring overall, a paternal effect was
2972 also quite prevalent, especially in the higher mortality ambient treatment. Being
2973 multiple batch spawners (Trippel 1998, Rakitin et al. 2001, Wringe et al. 2015b), in
2974 whom multiple paternity within and among batches appears to be the norm
2975 (Hutchings et al. 1999, Bekkevold et al. 2002, Wringe et al. 2015b), the cod mating
2976 system increases the chances of a favourable local/non-local pairing occurring. What
2977 is unclear is how the fitness of F_2 (or F_n) or backcrosses will compare to that of non-
2978 hybrids (or even the F_1). It is also unclear if the results found here would differ had
2979 we used wild fish, or had the fish been subjected to more generations of selection in
2980 culture.

2981 5.6 Acknowledgements

2982 The authors wish to thank Miranda McGrath, Michelle Bachan and Corinne Conway
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2984 Aquaculture Research Building was invaluable for providing their knowledge and
2985 insight on the practical aspects of rearing larval cod. Beth Perry was instrumental in
2986 the refinement of the PCR protocol. We also extend our thanks to the members of

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5.7 Tables

Table 5.1 Number of offspring sampled at each time period. The number genotyped does not necessarily correspond to the number correctly assigned to parental pairs because parentage analysis was only performed on individuals for whom all eight loci were successfully typed. Refer to materials and methods for further information. The numbers beside each treatment correspond to the replicate tank numbers. Entries marked with an asterisk indicate that all remaining individuals were sampled at that time.

Cohort	Treatment	2 Weeks	8 Weeks	12 Weeks
Apr. 25	High-1	67	100	100
	High-2	60	100	100
	Amb-1	100	100	64*
	Amb-2	74	100	23*
May 5	High-1	100	87	100
	High-2	100	100	100
	Amb-1	100	70*	NA
	Amb-2	100	100	100

Table 5.2 Morphometric characters measured for the larval and juvenile cod. Measurements were taken from the *x-y* coordinates of the photographs used in the geometric morphometric analysis. Bounding points correspond to those illustrated in Figure 5.2.

	Measure	Bounding pts
Larvae	Standard length	1-4
	Head length	1-2
	Eye diameter	9-10
	Lower jaw length	7-8
	Somite Depth	3-5
Juveniles	Standard length	1-7
	Head length	1-11
	Eye diameter	15-16
	Lower Jaw length	13-14
	Caudal peduncle depth	6-8
	Body depth	3-12
	Head area	1-2-11-13
	Gut area	4-5-9-10
	Mid-body area	3-4-10-12

Table 5.3 Number of offspring correctly assigned to parent pairs at each sampling point in the two temperature treatments for the Apr. 25 and May 5 cohorts. Ambient is abbreviated Amb, and number is abbreviated No.

Cohort	Sampling Period	Treatment	No. Assigned
Apr. 25	2 Week	Amb	73
		High	167
	8 Week	Amb	180
		High	172
	12 Week	Amb	91
		High	157
		Total	840
May 5	2 Week	Amb	122
		High	147
	8 Week	Amb	144
		High	153
	12 Week	Amb	32
		High	183
		Total	781

Table 5.4 Genetic parameter estimates from eight microsatellite loci in each of the two temporal cohorts. *H* is heterozygosity with the subscripts *obs* denoting the observed heterozygosity and *exp* that expected under Hardy-Weinberg equilibrium. All estimates were generated using CERVUS.

Marker		No. Alleles	H_{obs}	H_{exp}	Null Alleles
Gmo8	Apr. 25 Cohort	11	0.62	0.85	+0.153
	May 5 Cohort	12	0.76	0.88	+0.067
*Gmo*19	Apr. 25 Cohort	13	0.69	0.85	+0.102
	May 5 Cohort	13	0.83	0.88	+0.042
*Gmo*35	Apr. 25 Cohort	8	0.81	0.76	-0.041
	May 5 Cohort	7	0.76	0.73	-0.030
*Gmo*37	Apr. 25 Cohort	10	0.86	0.74	-0.056
	May 5 Cohort	8	0.66	0.63	+0.197
*Gmo*63	Apr. 25 Cohort	5	0.62	0.54	-0.095
	May 5 Cohort	6	0.55	0.51	-0.012
*Gmo*118	Apr. 25 Cohort	10	0.66	0.67	+0.021
	May 5 Cohort	8	0.74	0.61	-0.126
*Gmo*125	Apr. 25 Cohort	10	0.76	0.76	-0.003
	May 5 Cohort	9	0.89	0.77	-0.069
*Gmo*152	Apr. 25 Cohort	7	0.73	0.71	-0.013
	May 5 Cohort	7	0.81	0.74	-0.041

Table 5.5 – Mean ± SD of the larval-stage morphological characters from the long-term rearing experiment. L = length; Diam = diameter; Low J = lower jaw. Please refer to the methods for further information on the measurement of these characters. Diff. b/w type is the test for differences between hybrid and non-hybrid measures. Diff b/w treat is between temperature treatments. Results ANOVA on LMM, and significant differences ($\alpha = 0.05$) are bolded. The lack of measurements in the high temperature treatment at the 8 week sampling period reflects the fact that all fish had metamorphosed to the juvenile phase by this time point.

3026

		High Temp		Ambient Temp		Diff. b/w type	Diff b/w treat
Apr 25 Cohort	Feature	Hybrid	Non-hybrid	Hybrid	Non-hybrid	(chisq, p)	(chisq, p)
8 Week	Standard L	23.86 ± 3.51	23.18 ± 2.92	14.93 ± 2.40	14.65 ± 1.87	1.73, > 0.18	**227.12, < 0.001**
	Head L	6.63 ± 0.94	6.42 ± 0.81	4.34 ± 0.73	4.20 ± 0.53	2.48, > 0.11	**194.02, < 0.001**
	Eye Diam	2.46 ± 0.33	2.43 ± 0.27	1.51 ± 0.23	1.46 ± 0.20	1.70, > 0.19	**322.16, < 0.001**
	Low J L	3.50 ± 0.52	3.46 ± 0.44	2.07 ± 0.38	2.02 ± 0.30	1.17, > 0.27	**275.22, < 0.001**
	Somite D	2.94 ± 0.49	2.79 ± 0.51	1.48 ± 0.42	1.45 ± 0.30	1.97, > 0.16	**83.34, < 0.001**
May 5 Cohort							
2 Week	Standard L	7.00 ± 0.51	7.13 ± 0.54	7.07 ± 0.41	6.85 ± 0.61	1.00, > 0.31	2.22, > 0.13
	Head L	1.59 ± 0.17	1.51 ± 0.07	1.51 ± 0.19	1.48 ± 0.21	0.32, > 0.57	**4.65, < 0.05**
	Eye Diam	0.58 ± 0.02	0.57 ± 0.04	0.56 ± 0.04	0.53 ± 0.06	2.62, > 0.10	**6.17, < 0.05**
	Low J L	0.78 ± 0.04	0.78 ± 0.08	0.75 ± 0.08	0.73 ± 0.07	0.39, > 0.53	2.62, > 0.10
	Somite D	0.45 ± 0.03	0.48 ± 0.07	0.45 ± 0.03	0.43 ± 0.07	0.24, > 0.62	**5.78, < 0.05**
8 Week	Standard L			13.86 ± 1.92	13.83±1.64	0.01, > 0.91	
	Head L			4.18 ± 0.55	4.17±0.46	0.10, > 0.74	
	Eye Diam			1.42 ± 0.20	1.43±0.20	0.03, > 0.86	
	Low J L			1.96 ± 0.26	1.91±0.25	0.53, > 0.46	
	Somite D			1.27 ± 0.28	1.22±0.19	0.32, > 0.56	

Table 5.6 Mean ± SD of the juvenile-stage morphological characters from the long-term rearing experiment. L = length; Diam = diameter; Low J = lower jaw; Caud Ped = caudal peduncle; A = area. Please refer to the methods for further information on the measurement of these characters. Diff. b/w type is the test for differences between hybrid and non-hybrid measures. Diff b/w treat is between temperature treatments. Results ANOVA on LMM, and significant differences ($\alpha = 0.05$) are bolded. The lack of measurements in the ambient temperature treatment at the 8 week sampling periods reflects the fact that all were in the larval phase at these time points.

		High Temp		Ambient Temp		Diff. b/w type	Diff b/w treat
Apr 25 Cohort	Feature	Hybrid	Non-hybrid	Hybrid	Non-hybrid	(chisq, p)	(chisq, p)
8 Week	Standard L	24.01 ± 3.30	23.09 ± 2.77			2.07, > 0.15	
	Head L	7.18 ± 0.91	6.95 ± 0.79			2.70, > 0.10	
	Eye Diam	2.50 ± 0.29	2.41 ± 0.25			2.96, > 0.08	
	Low J L	3.56 ± 0.46	3.48 ± 0.44			1.03, > 0.30	
	Caud Ped D	1.55 ± 0.19	1.52 ± 0.21			0.58, > 0.44	
	Body D	4.73 ± 0.72	4.60 ± 0.62			0.97, > 0.32	
	Head A	16.71 ± 3.64	16.01 ± 3.31			1.53, > 0.21	
	Gut A	15.92 ± 4.35	14.89 ± 4.38			0.85, > 0.35	
	Mid-body A	12.21 ± 3.36	10.88 ± 3.11			2.42, > 0.12	
12 Week	Standard L	39.15 ± 7.07	39.80 ± 5.95	26.80 ± 6.30	23.87 ± 5.05	0.00, > 0.96	**205.95, < 0.001**
	Head L	10.93 ± 1.85	11.01 ± 1.56	7.11 ± 1.54	6.44 ± 1.37	0.01, > 0.92	**241.16, < 0.001**
	Eye Diam	3.62 ± 0.38	3.66 ± 0.35	2.44 ± 0.49	2.28 ± 0.39	0.01, > 0.91	**407.21, < 0.001**
	Low J L	5.70 ± 0.97	5.77 ± 0.97	3.96 ± 0.92	3.61 ± 0.73	0.01, > 0.93	**166.91, < 0.001**
	Caud Ped D	2.32 ± 0.41	2.39 ± 0.36	1.74 ± 0.40	1.51 ± 0.34	0.06, > 0.80	**168.73, < 0.001**
	Body D	7.82 ± 1.40	8.00 ± 1.16	5.63 ± 1.30	4.87 ± 1.00	0.03, > 0.85	**200.67, < 0.001**
	Head A	32.45 ± 9.98	33.03 ± 8.29	15.20 ± 5.78	12.50 ± 4.56	0.00, > 0.95	**195.50, < 0.001**
	Gut A	47.41 ± 18.75	49.13 ± 15.55	25.34 ± 11.95	18.66 ± 8.33	0.01, > 0.90	**119.33, < 0.001**
	Mid-body A	37.08 ± 13.62	37.48 ± 12.37	18.21 ± 8.65	14.14 ± 6.04	0.05, > 0.83	**123.58, < 0.001**

May 5 Cohort

8 Week	Standard L	22.76 ± 5.31	22.83 ± 8.14			0.47, > 0.49	
	Head L	6.93 ± 1.50	6.91 ± 2.31			0.52, > 0.49	
	Eye Diam	2.35 ± 0.38	2.32 ± 0.64			0.18, > 0.67	
	Low J L	3.28 ± 0.57	3.12 ± 0.51			0.18, > 0.67	
	Caud Ped D	1.49 ± 0.34	1.44 ± 0.53			0.34, > 0.55	
	Body D	4.46 ± 1.07	4.56 ± 1.65			0.69, > 0.40	
	Head A	15.10 ± 6.37	15.46 ± 9.65			0.38, > 0.53	
	Gut A	14.34 ± 8.11	14.91 ± 11.17			0.55, > 0.45	
	Mid-body A	13.27 ± 7.63	13.19 ± 9.53			0.33, > 0.56	
12 Week	Standard L	44.11 ± 6.41	47.26 ± 5.78	23.89 ± 3.33	23.16 ± 3.50	1.36, > 0.24	**351.26, < 0.001**
	Head L	12.19 ± 1.63	13.01 ± 1.56	7.24 ± 1.00	7.12 ± 0.96	2.28, > 0.13	**296.84, < 0.001**
	Eye Diam	3.88 ± 0.42	4.09 ± 0.38	2.47 ± 0.26	2.31 ± 0.28	0.43, > 0.51	**382.36, < 0.001**
	Low J L	6.02 ± 0.88	6.46 ± 0.78	3.57 ± 0.53	3.42 ± 0.47	2.92, > 0.08	**267.93, < 0.001**
	Caud Ped D	2.67 ± 0.40	2.95 ± 0.37	1.52 ± 0.17	1.53 ± 0.16	**6.84, < 0.01**	**285.24, < 0.001**
	Body D	9.01 ± 1.29	9.63 ± 1.15	4.75 ± 0.63	4.60 ± 0.84	**4.54, < 0.05**	**394.08, < 0.001**
	Head A	39.43 ± 9.83	44.82 ± 10.04	14.73 ± 4.06	14.28 ± 4.10	3.57, > 0.05	**208.24, < 0.001**
	Gut A	61.73 ± 18.01	70.43 ± 16.88	16.17 ± 4.88	15.39 ± 5.70	3.48, > 0.06	**227.78, < 0.001**
	Mid-body A	57.34 ± 16.34	63.45 ± 16.28	13.58 ± 3.67	12.77 ± 4.53	2.01, > 0.15	**244.12, < 0.001**

5.7 Figures

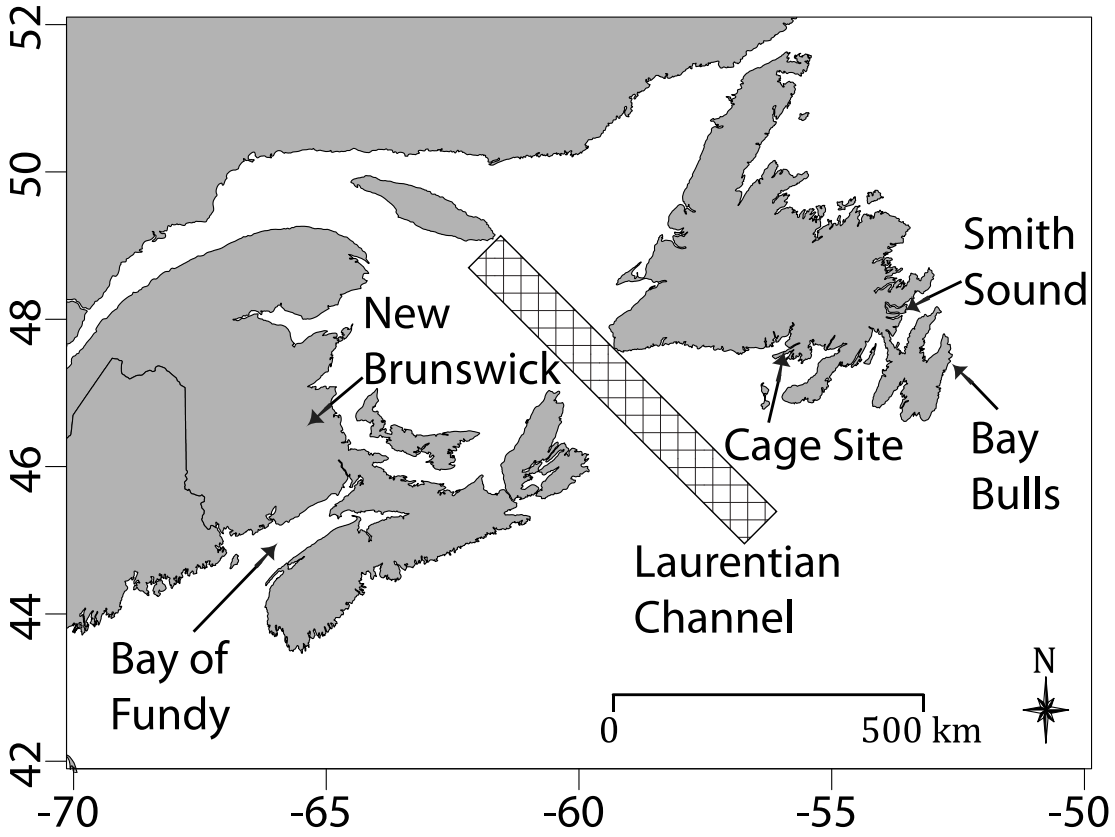
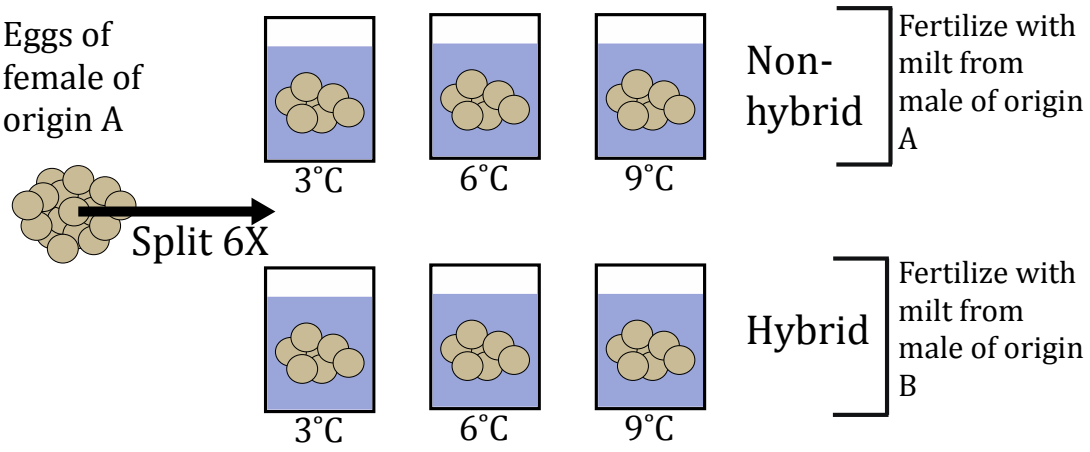


Figure 5.1 Map of Atlantic Canada showing the locations from whence the NL (Smith Sound and Bay Bulls) and NB (Bay of Fundy) broodstocks derive, and the location of the cage site (Hermitage Bay, NL) from which the experimental fish were collected. The approximate location of the Laurentian Channel is illustrated. The x-axis is degrees of longitude east, and the y degrees of latitude north.

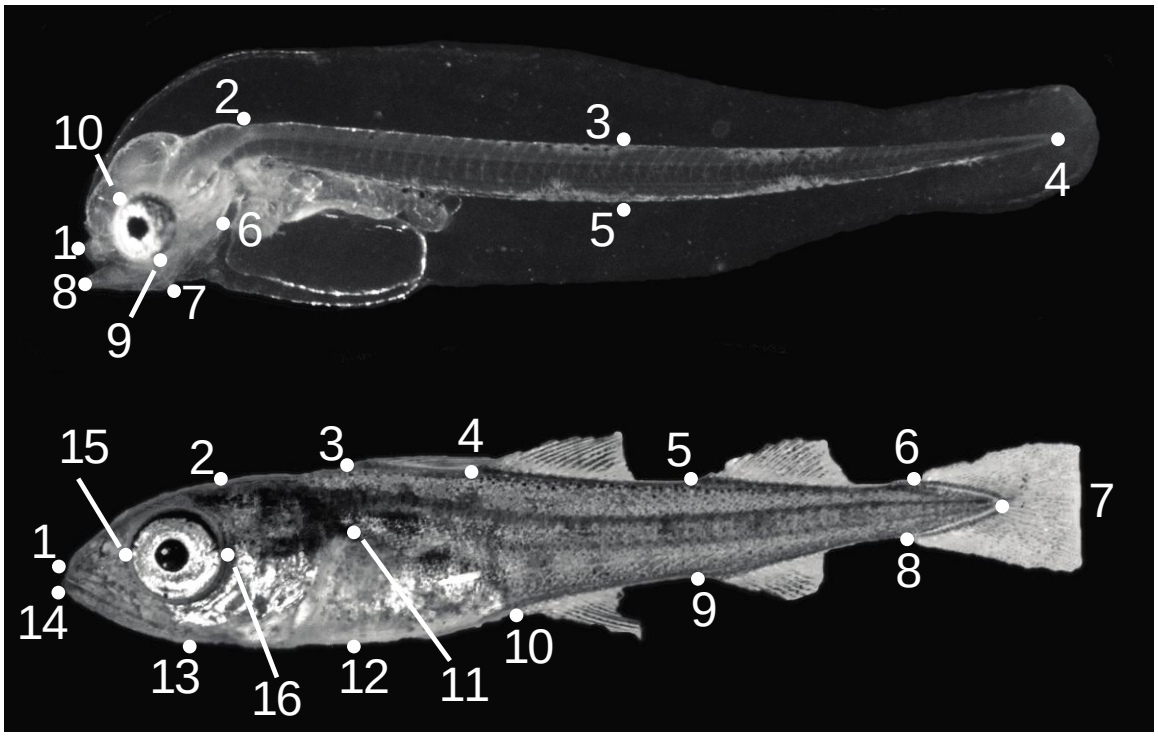


3044

3045 **Figure 5.2** Schematic of the split brood design employed in the short-term
3046 hybridization experiment. Eggs from each female were used to create both hybrid
3047 (fertilized by male of different origin) and non-hybrid (fertilized by male of same
3048 origin) half sibs. The temperatures denote the temperature at which each individual
3049 fertilization was conducted. Each fertilization was later split into three replicate
3050 beakers (see materials and methods).

3051

3052



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3054

Figure 5.3 Morphometric landmarks used in the geometric morphometric analysis. The upper panel is an offspring typical of what was considered "larval" morphology, and the bottom is typical of "juvenile" morphology. The numbering for the larval morphology correspond to the following landmarks: 1 – most anterior point of premaxilla; 2 – junction of medulla oblongata and notochord; 3 – mid-point of the notochord, dorsal side; 4 - posteriormost point of the notochord; 5 – mid-point of the notochord, ventral side; 6 – articulation of the lower jaw; 7 – ventral process at site of maximum curvature of lower jawl; 8 – anteriormost point of lower jaw; 9 – edge of eye in line with 7; 10 – edge of eye directly opposite 9, and in line with 7. The numbering for the juvenile morphology is: 1 – anteriormost point of the premaxilla; 2 – indentation in cranium, 3, 4, 5 – anterior insertion of dorsal fins 1, 2 and 3; 6 – dorsal insertion of the caudal fin; 7 – posteriormost point of the hypural plate; 8 – ventral insertion of the caudal fin; 9, 10 – anterior insertion of anal fins 1 and 2; 11 – posteriormost point of the process extended from the operculum; 12 – most ventral aspect of the fish on a line drawn perpendicular to the long axis, through point 11; 13 – posteriormost point of the lower jaw; 14 – anteriormost point of the lower jaw; 15 – anteriormost point of the eye; 16 – posterior most point of the eye, directly opposite 15.

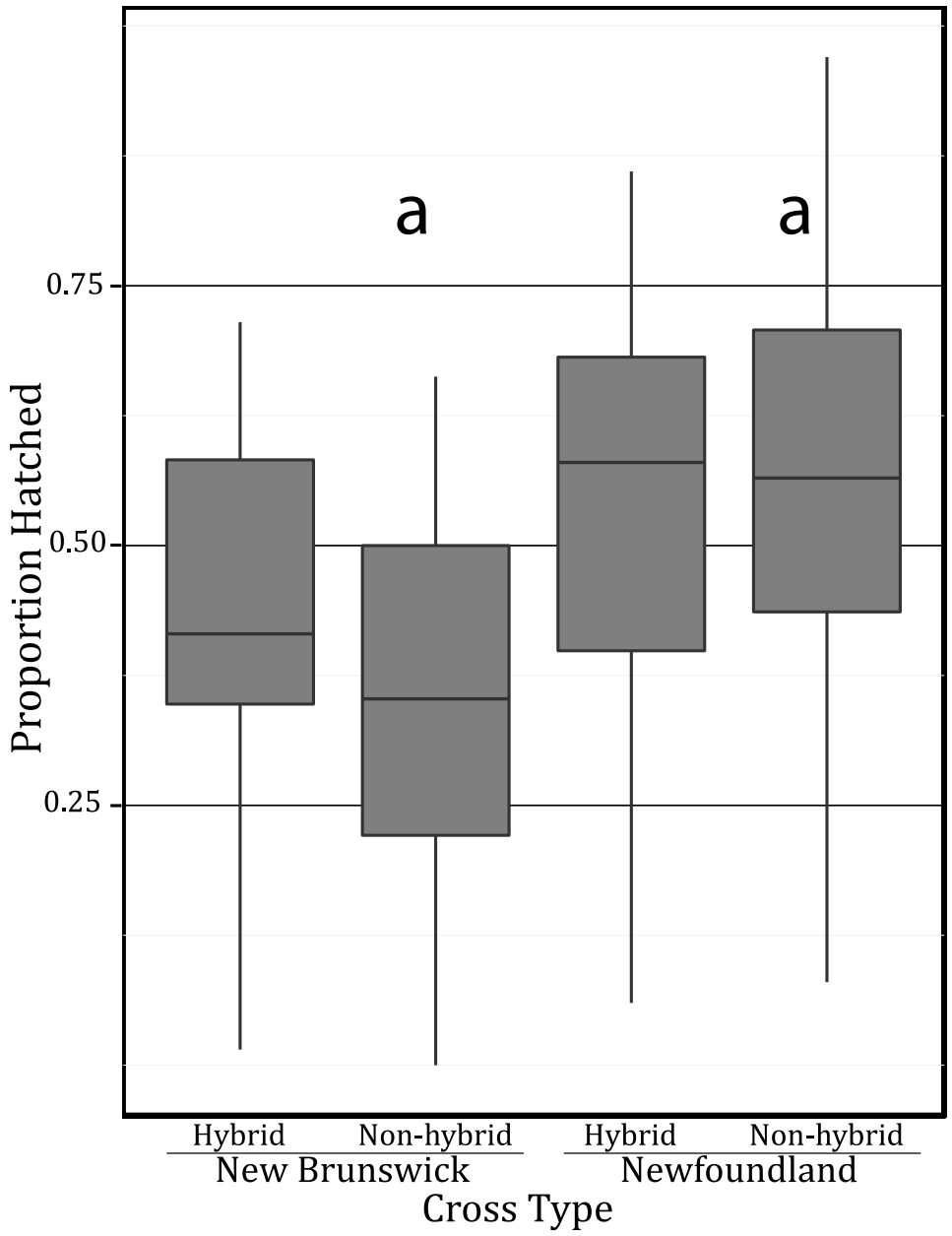


Figure 5.4 Proportion hatched by dam origin and cross type. Significant differences at $p < 0.05$ are denoted by the same letter. The mid-line of the boxplot is the median, upper and lower limits of the box denote the first and third quartiles respectively, and the whiskers extend to 1.5 times the inter-quartile range.

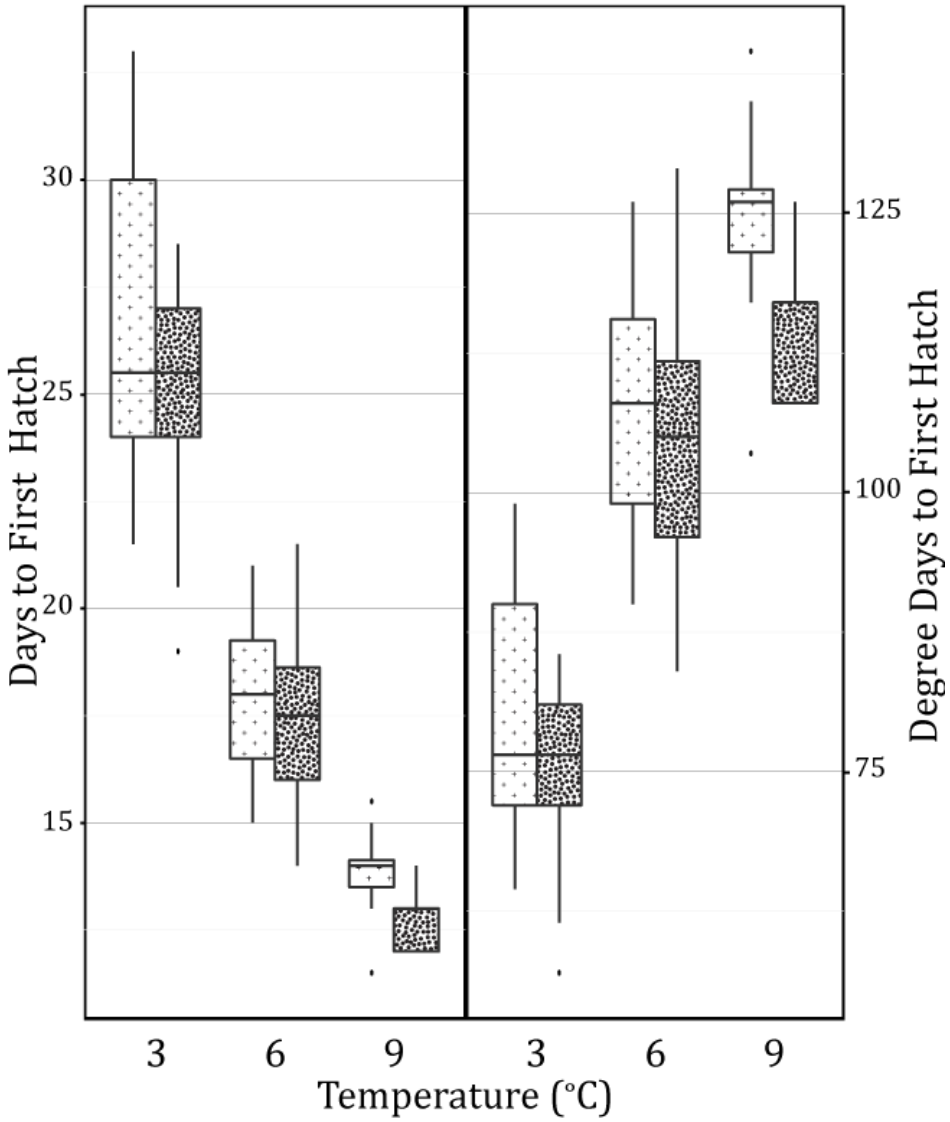


Figure 5.5 Time to first hatch in both days and degree days. New Brunswick and Newfoundland dams are denoted by plus sign hashed boxes and small polka-dots respectively. The mid-line of the boxplot is the median, upper and lower limits of the box denote the first and third quartiles respectively, and the whiskers extend to 1.5 times the inter-quartile range

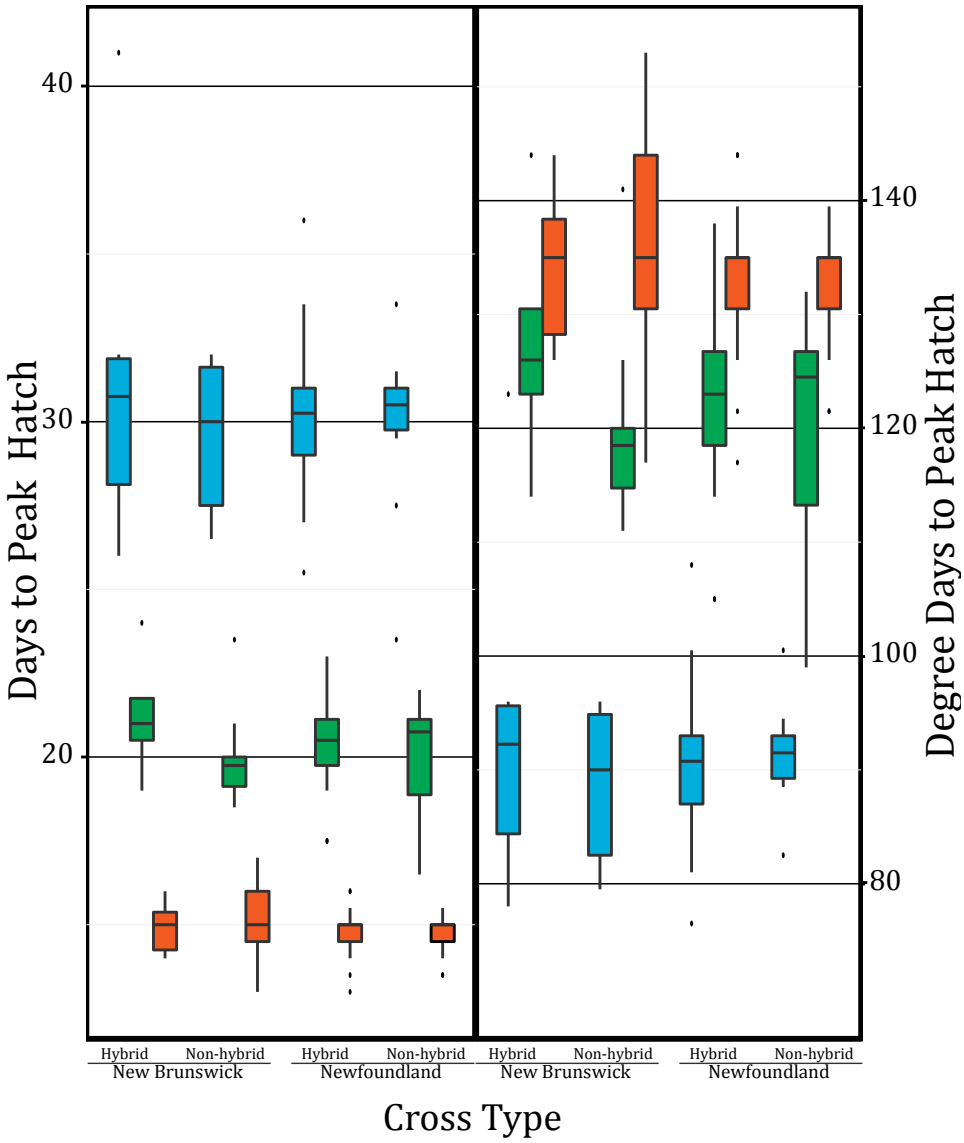


Figure 5.6 Time to peak hatch in both days and degree days. Temperature treatments are indicated by colour, with blue, green and orange denoting the 3, 6, and 9 °C treatments respectively. The mid-line of the boxplot is the median, upper and lower limits of the box denote the first and third quartiles respectively, and the whiskers extend to 1.5 times the inter-quartile range

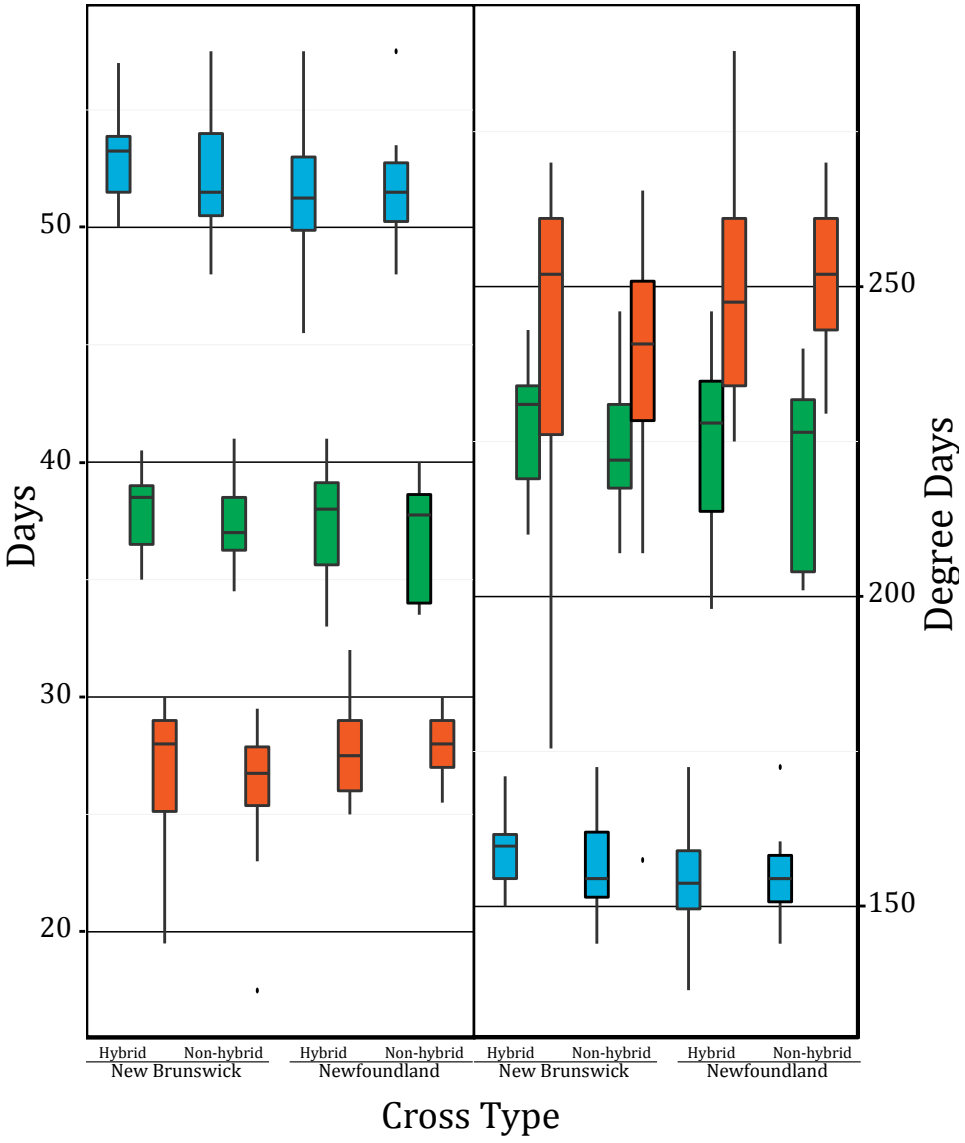
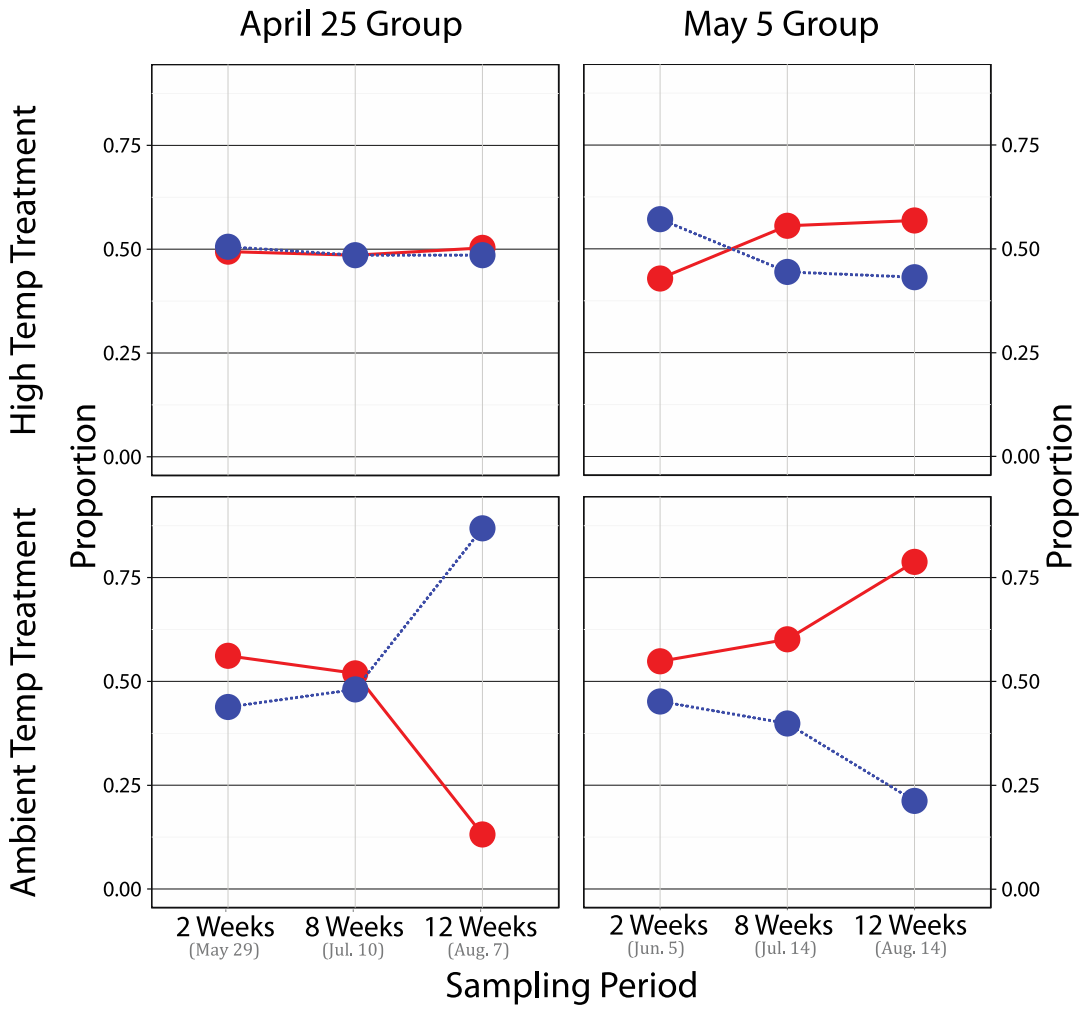


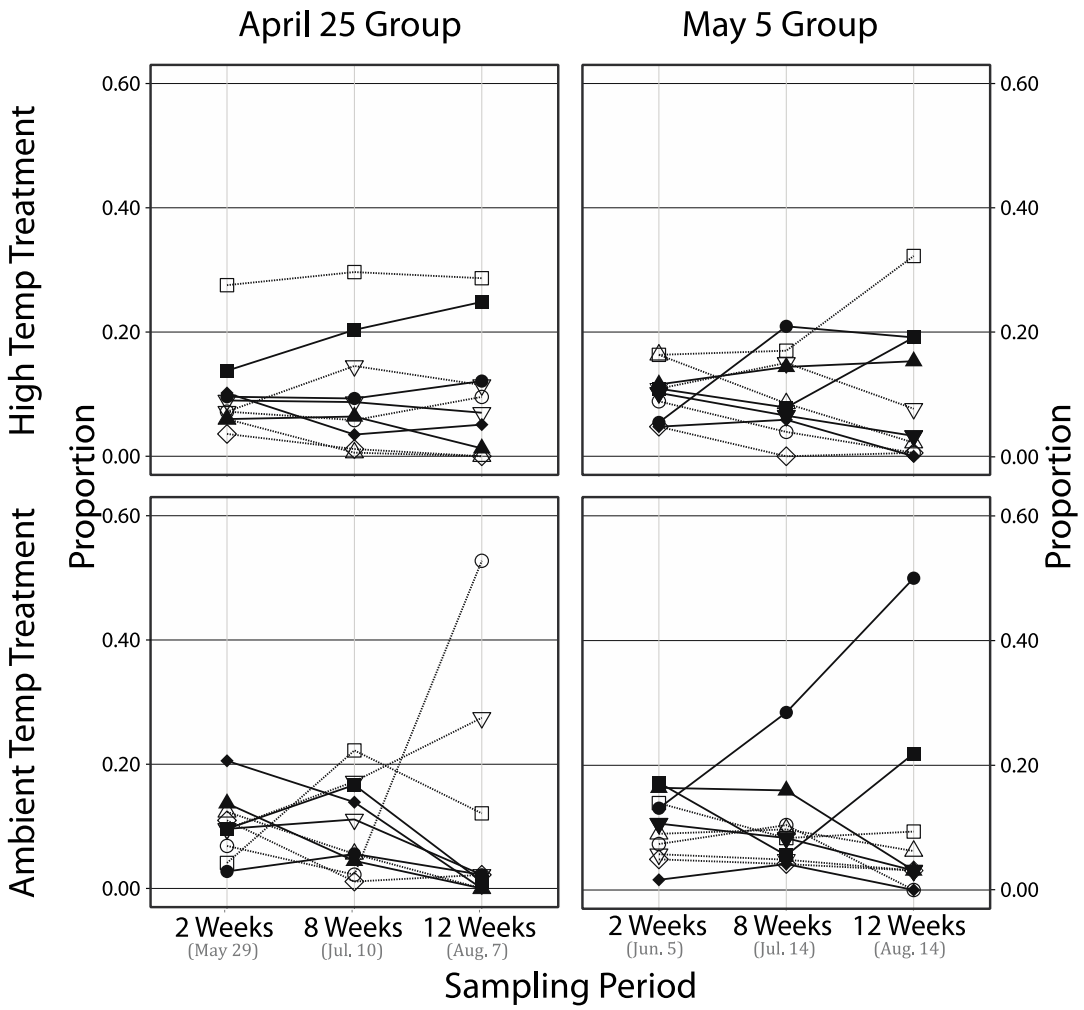
Figure 5.7 Time-to-death in both days and degree days. Temperature treatments are indicated by colour, with blue, green and orange denoting the 3, 6, and 9 °C treatments respectively. The mid-line of the boxplot is the median, upper and lower limits of the box denote the first and third quartiles respectively, and the whiskers extend to 1.5 times the inter-quartile range

3103


April 25 Group
May 5 Group
High Temp Treatment
Ambient Temp Treatment
Proportion
0.00
0.25
0.50
0.75
2 Weeks
(May 29)
8 Weeks
(Jul. 10)
12 Weeks
(Aug. 7)
2 Weeks
(Jun. 5)
8 Weeks
(Jul. 14)
12 Weeks
(Aug. 14)
Sampling Period

3104

Figure 5.8 Proportion of hybrid and non-hybrids detected in each of three sampling periods in two different temperature treatments. The results for the cohorts of fish spawned on April 25 and May 5 are plotted separately. For each sampling period, in each temperature treatment, in each temporal replicate, relative survivorship is shown as the proportional contribution of a cohort to all individuals assigned to parental pairs. The coloured points and lines indicate the overall relative proportional survivorship of hybrids and non-hybrids. Blue circles connected by dashed lines indicate non-hybrids, and red circles connected by solid lines denote hybrids.


April 25 Group
May 5 Group
High Temp Treatment
Ambient Temp Treatment
Proportion
Proportion
0.60
0.40
0.20
0.00
2 Weeks
(May 29)
8 Weeks
(Jul. 10)
12 Weeks
(Aug. 7)
2 Weeks
(Jun. 5)
8 Weeks
(Jul. 14)
12 Weeks
(Aug. 14)
Sampling Period

3115

Figure 5.9 Proportion of hybrid and non-hybrids detected in each of three sampling periods in two different temperature treatments. The results for the cohorts of fish spawned on April 25 and May 5 are plotted separately. For each sampling period, in each temperature treatment, in each temporal replicate, relative survivorship is shown as the proportional contribution of a cohort to all individuals assigned to parental pairs. Unfilled shapes connected by dashed lines represent the proportional survivorship of hybrid half-sib families, and filled shapes connected by solid lines the non-hybrids. The same shape within, but not across, temporal treatments denotes half-sib families sharing the same dam.

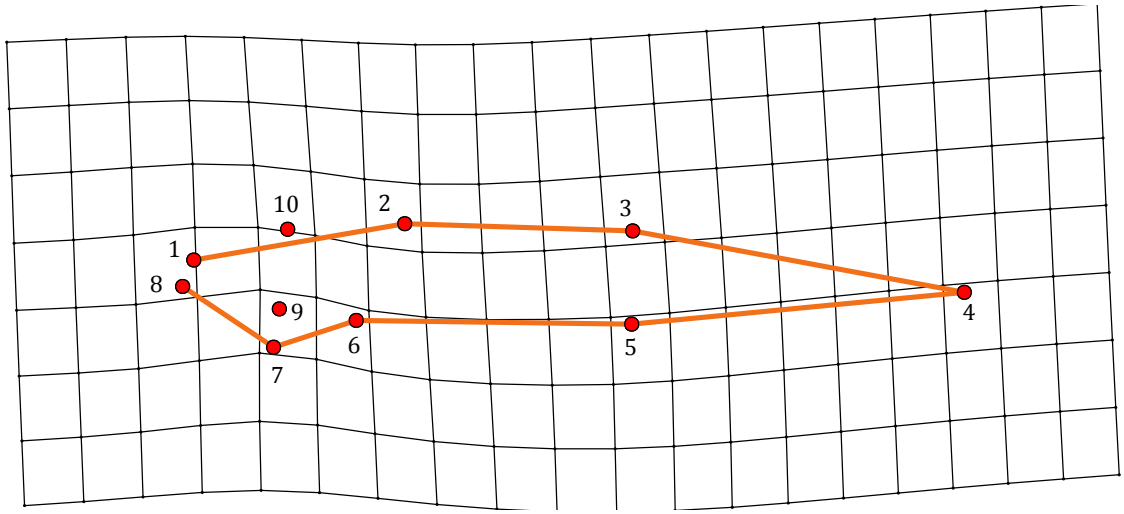


Figure 5.10 Magnitude and displacement of the consensus shape of the April 25 cohort larvae from the ambient temperature treatment relative to those in the high temperature treatment at the eight week sampling period. The displacement is indicated by the bending of the thin plate spline deformation grid. The landmark numbering and descriptions are given in Figure 5.3. The units of both the *x*- and *y*-axes are the Procrustes coordinates

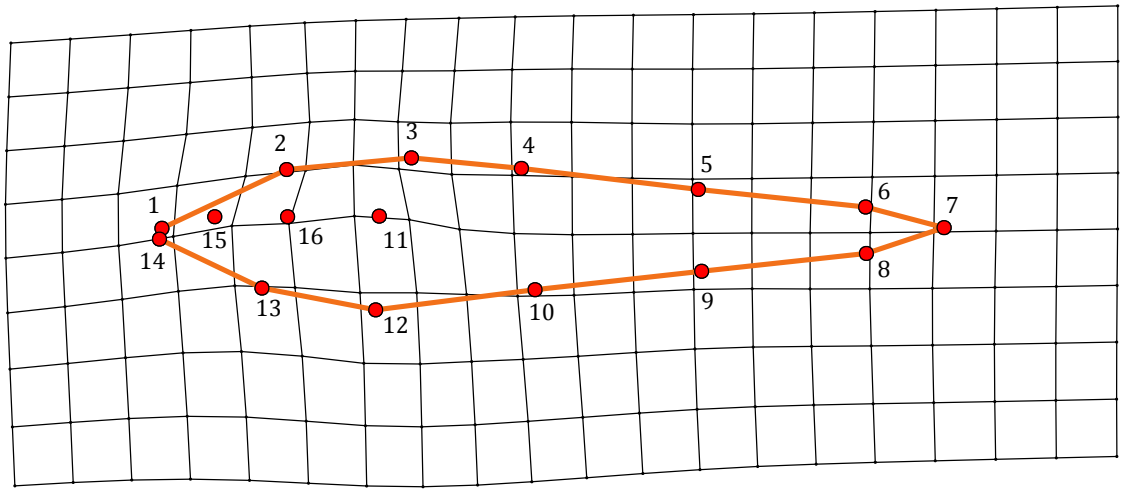


Figure 5.11 Magnitude and displacement of the consensus shape of the April 25 cohort juveniles from the ambient temperature treatment relative to those in the high temperature treatment at the twelve week sampling period. The displacement is indicated by the bending of the thin plate spline deformation grid. The landmark numbering and descriptions are given in Figure 5.3. The units of both the *x*- and *y*-axes are the Procrustes coordinates.

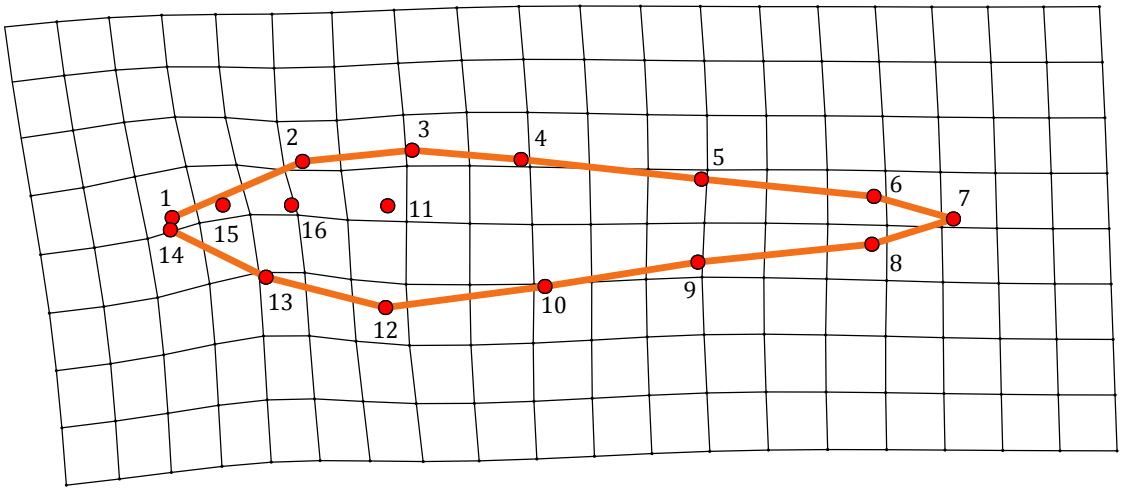


Figure 5.12 Magnitude and displacement of the consensus shape of the May 5 cohort juveniles from the ambient temperature treatment relative to those in the high temperature treatment at the twelve week sampling period. The displacement is indicated by the bending of the thin plate spline deformation grid. The landmark numbering and descriptions are given in Figure 5.3. The units of both the *x*- and *y*-axes are the Procrustes coordinates.

Chapter 6 – Conclusion

The results of the experiments detailed in this thesis add to a growing body of literature outlining the potential consequences of exposure to cultured conditions and the outcomes of interaction between wild and escaped cultured fish. Under the most reductionist scenario, built through consensus of the existing literature, exposure to culture leads to phenotypic (Fleming et al. 1996, Matsuzaki et al. 2009, Skjæraasen et al. 2009, Chittenden et al. 2010) and genotypic (Cross & King 1983, Einum & Fleming 1997, Jørstad et al. 2008, Wringe et al. 2010, Karlsson et al. 2011) changes in fishes. The genotypic changes in the cultured fish are such that if introgression into the wild population should occur, there is the distinct possibility for reduction in the fitness of the local wild population (Reisenbichler & Rubin 1999, McGinnity et al. 2003, Miller et al. 2004, Araki et al. 2009, McGinnity et al. 2009). Concomitantly, the phenotypic changes, be they morphological, behavioural, or both, brought about through exposure to culture lead to lower fitness in the cultured fish relative to the wild, reducing the potential for, or rate of realized introgression relative to the proportion of escaped fish present in the spawning grounds (Fleming & Gross 1994, Berejikian et al. 2001, McLean et al. 2003, Araki et al. 2008) [*nota bene*, this fitness reduction is primarily in males, and introgression through escaped females is likely (Fleming et al. 1996, Skjæraasen et al. 2010)].

Two major concerns exist regarding the alteration of local gene pools through the interbreeding of wild and escapee fish. Firstly, the escapees themselves may

harbour genes or gene complexes maladapted to local conditions (e.g. outbreeding depression), and or be genetically depauperate and cause negative fitness effects (e.g. low effective population size [N_e] and many lethal or semi-lethal alleles). Secondly, gadoids from different populations may differ intrinsically in traits deemed valuable to culturists (e.g. growth rate, food conversion efficiency, disease and parasite resistance etc.), as such their appeal to aquaculture breeding programmes may differ (Utter & Epifanio 2002, Naylor & Burke 2005, Bekkevold 2006).

The implication of the second part is that the census population size of these desirable stocks may increase relative to that of the endemic stocks increasing the chance of introgression of the 'desirable' genotype into the local population (Bekkevold et al. 2006). It is important to keep in mind that genetic changes can and will occur even in the absence of an explicit selection program though random genetic drift and domestication selection. Domestication selection is a broad term that describes the relaxation of natural selection pressures, leading to the survival and propagation of phenotypes that would be deleterious in the wild; and the concomitant selection of phenotypes that are advantageous in culture (Bekkevold et al. 2006).

Individuals originating from a large population are expected to harbour more lethal or sublethal mutations, at a genome wide level than those originating from a historically small population because the smaller N_e should result in the recessive

alleles being present in a homozygous state more frequently, and thus be purged more effectively (Zajitschek et al. 2009). The potential then is for the gadoid individuals in culture to harbour more lethal or sublethal alleles than salmonids given that the N_e for salmonid populations is smaller due to differences in life history. Furthermore, the potential exists for these lethal or sublethal alleles to become more prevalent in the farmed fish through the relaxed selection pressures inherent in aquaculture (Thorpe 2004). The major implication of this is that escapee fish may then harbour lethal and semi-lethal alleles at a much higher prevalence than their wild counterparts.

Cod are thought to form leks (Hutchings et al. 1999, Bekkevold et al. 2002) and spawnings and the initiation of a ventral mount are follow active female mate choice based at least in part on her evolution of male courtship behaviours and displays and phenotype including sexually selected characters (Skjæraasen et al. 2006a, Skjæraasen et al. 2008, Skjæraasen et al. 2012) (for description of spawning behaviour see Brawn 1961). Given the importance of morphology to the cod mating system, as well as that exposure to cultured conditions often leads to changes in morphology because of plastic responses to environmental conditions (Imre et al. 2002, Mayer et al. 2011, Vehanen & Huusko 2011) and/or genetic changes brought about through both intentional and unintentional selection (Fleming et al. 1994, reviewed by: Einum & Fleming 2001, Fleming & Petersson 2001, Hutchings & Fraser 2008, Solberg et al. 2013, Colihueque & Araneda 2014), I thought it prudent to test for morphological differences between wild and cultured cod brought about as a

result of their exposure to cultured conditions. The results of the experiments detailed in Chapter 2 (Wringe et al. 2015a), support this supposition and show that first generation cultured cod differ significantly in their morphology from that of wild fish from their ancestral population. Being first generation cultured fish, it is unlikely that the morphological differences detected were the result of genetic changes brought about through intentional selection (although genetic differentiation can occur in a single generation; Christie et al. 2012), and are likely the result of plastic phenotypic effects. This notion is supported by the concord between my study and Uglem et al. (2011). Considering all the observed differences between the farmed and wild cod in my study, the congruence between my results, and those of Uglem et al. (2011), the only other study that has examined differences in adult morphology between wild and farmed cod is impressive. This is especially true given that the populations examined are thought to have been isolated for at least 100 000 years (Bigg et al. 2008a). This suggests that the observed differences may represent a stereotypical plastic response of Atlantic cod to culture. It is interesting to note as well, that many of the differences observed between wild and farmed cod in both my study and that of Uglem et al. (2011) are also seen in other cultured species (e.g. condition indices, fin sizes; e.g. Pedersen et al. 2008, Rogdakis et al. 2011, Lenhardt et al. 2012, Patiyal et al. 2013).

The notion that exposure to culture causes fishes to develop morphology that differs from their wild conspecifics has been espoused so often it has become nearly axiomatic in fisheries science. A commonly supervened corollary to this axiom is

3237 that such changes in morphological occur in a predictable and consistent manner
3238 and result in a consonant "cultured phenotype". While this is often stated or alluded
3239 to, the meta-analysis conducted in Chapter 3, is to my knowledge the first time it has
3240 been formally tested. Aquacultured fishes are generally subjected to breeding
3241 programmes with similar goals, such as rapid growth (e.g. Myers et al. 2001, Fleming
3242 et al. 2002, Thrower et al. 2004, Small 2006, Wringe et al. 2010), delayed maturity
3243 (e.g. Myers et al. 2001, Fleming et al. 2002, Wang et al. 2006, Wang et al. 2008,
3244 Gjedrem 2010), high-density production (e.g. Thorpe 1991, Kause et al. 2003,
3245 Gjedrem 2010), disease resistance (e.g. Ridha 2006, Trenzado et al. 2006) and
3246 greater feed conversion efficiency (e.g. Hulata 2001, Nichols et al. 2003, Antonello et
3247 al. 2009) which could lead to convergent genetic and hence morphological changes.
3248 Moreover, these breeding programmes often have little or no regard for maintaining
3249 fitness of these fish in the wild or of maintaining a wild-type morphology, apart from
3250 ensuring the production of an 'appealing' phenotype for the consumer (e.g. Kause et
3251 al. 2006, Small 2006, reviewed by: Colihueque 2010, Colihueque & Araneda 2014).
3252 Conversely, supplementary programmes often strive to maintain wild-type
3253 morphology and produce fish for release that will be viable in the wild (Iguchi &
3254 Mogi 2007, Belk et al. 2008, Blanchet et al. 2008, Brockmark & Johnsson 2010, Wilke
3255 et al. 2015). Despite the efforts of hatcheries, evidence suggests that the fitness of
3256 hatchery-produced fish is often lower than that of their wild conspecifics, and that
3257 this may be at least partially attributable to differences in morphology (Barahona-
3258 Fernandes 1982, Svåsand et al. 2000, Miller et al. 2004, Araki et al. 2008, Gavaia et

al. 2009). Selection differences aside, it is noteworthy that the environments experienced by cultured fish are more similar to one another, than are the environments experienced by their wild conspecifics.

The meta-analysis comparing the morphology of cultured fish, which have been exposed to varying degrees of selection and time in captivity, to their wild conspecifics shows that as commonly ascribed, the heads of cultured fish were shorter, as were their upper jaws, and all fin measures with the exception of the width of the dorsal fin and the length of the caudal fin. However, unlike what was predicted, measures of body conformation, especially as it relates to depth measures, were not found to differ. Thus while my findings provide support to the conjecture of a universal response to culture, leading to the development of a common 'cultured' phenotype, it does not appear to necessarily involve changes in body depth, or condition as is commonly suggested.

It bears mention as well that the phenotypic change in the cultured fish espoused to form the "cultured phenotype", and which were detected by the meta-analysis are congruent with experimentally observed plastic phenotypic response to environments typical of those in culture. Thus, while these phenotypic changes could certainly have arisen through plastic responses to culture, there is no reason to believe that permanent genetic changes could not have contributed to or caused these changes.

In Chapter 4, I studied the reproductive interactions of individual cultured and wild male cod in the presence of a cultured female using a series of spawning trios. This experiment tested the potential for genetic introgression between cultured and wild cod to occur. Cod exhibit lek-like mating aggregations (Hutchings et al. 1999, Rose et al. 2008, Meager et al. 2010), with female mate choice apparently based on both visual and acoustic displays. Within spawning aggregations, male cod form dominance hierarchies based on agonistic interaction, usually with the largest males occupying the highest ranks, and access to females and spawning success being related to this hierarchical position (Hutchings et al. 1999, Bekkevold et al. 2002, Bekkevold 2006). Previous studies have shown that the spawning success of cultured males in competition with wild males in multi-individual spawning aggregations to be mixed. Skjæraasen and Hutchings (2010) found that the reproductive success of cultured cod in competition with wild cod was "essentially nil", but in another study, Skjæraasen et al. (2010) observed that cultured cod fertilized approximately 25% of eggs spawned by wild females, but up to 52% of eggs spawned by cultured females. These results suggested that the potential for hybridization between escaped male farmed and wild female cod to be low. But, in contrast to these studies, I found that in the absence of multi-male dominance hierarchies, the spawning success of cultured male cod was equal to that of wild males. This is despite the fact that the first-generation cultured cod I used differed both behaviourally (Chapter 4; Wringe et al. 2015b) and morphologically (Chapter 2; Wringe et al. 2015a), from wild fish of the same source population. Given that the

3301 cultured males were found to be more aggressive than the wild males as well as
3302 showing some evidence that their courting behaviours were less competent, it is
3303 important to keep in mind that genetic consequences can occur in the native
3304 population even in the absence of gene flow between them because of competition
3305 and wasted reproductive effort (Laikre et al. 2010). These results suggest that both
3306 the potential consequences for wild populations from interaction and competition
3307 with escapees and for introgression through escaped farmed male cod may be
3308 higher than previously suspected. While the extensive number and breadth of
3309 studies of wild/farmed interaction in salmonids undoubtedly provide important
3310 theoretical foundations, because of differences in life history and biology my
3311 research into the spawning success of farmed male cod is likely more applicable
3312 practically to other cultured marine broadcast spawners (e.g. gilthead seabream
3313 *Sparus aurata* and European seabass *Dicentrarchus labrax*).

3314 Having not only confirmed that introgression of by cultured male cod into
3315 wild populations is possible, but that the risk of it occurring may be greater than
3316 previously suspected (Skjæraasen & Hutchings 2010, Skjæraasen et al. 2010), the
3317 potential impact of hybridization between two genetically distinct cod populations
3318 was evaluated. One manner in which disparately related and naturally separated
3319 populations may come into contact is through human mediated dispersal (Fraser et
3320 al. 2010a). Among aquatic species this often occurs through the use of "non-native"
3321 (i.e. originating from different ancestral populations) strains in aquaculture, and the
3322 subsequent escape of genetic materials (fertilized eggs or larvae: Jørstad et al.

(2008), Uglem et al. (2012), Somarakis et al. (2013); through to spawning individuals: McGinnity et al. (1997), Jensen et al. (2010), Glover et al. (2013)). While it is true that the broodstocks used in some areas derive from populations native to that locality, this is not always the case. There is often an incentive in aquaculture to utilize a broodstock outside of the range of its founder population. This may be because of a wish to expand aquaculture production for a species into an area for which a local broodstock does not exist, or because the non-native broodstock outperforms the native one. In either case, escapees from the non-native broodstock have the potential to hybridize with local fish stocks and disrupt their local adaptation (Fleming et al. 2000, McGinnity et al. 2003, Glover et al. 2013). At the time the experimentation was conducted, the focus by industry in Atlantic Canada was towards development of local stocks for their aquaculture efforts (one broodstock from Newfoundland and one for New Brunswick and the Maritimes, Genome Atlantic's Cod Genome Project). These two broodstocks were availed of to test differences between the Newfoundland and New Brunswick stocks, which are known to be genetically distinct (Bradbury et al. 2010), and in so doing evaluate the potential consequences of introgression from a non-native broodstock. I found that if hybridization between these two populations were to occur in the wild, it is very likely a portion of the resultant offspring (F_1) would survive because their fitness during their early life history stages did not differ significantly from that of their non-hybrid counterparts. Furthermore, there was evidence that female effects, male effects and female by sire compatibility affected the survival of the offspring. Thus, it

3345 is possible that the mating system of cod in which females are multiple batch
3346 spawners (Trippel 1998, Rakitin et al. 2001, Wringe et al. 2015b), and where
3347 multiple paternity within and among batches appears to be the norm (Hutchings et
3348 al. 1999, Bekkevold et al. 2002, Wringe et al. 2015b), could increase the chances of a
3349 favourable local/non-local pairing occurring. What is unclear is how the fitness of F_2
3350 (or F_n) or backcrosses will compare to that of non-hybrids (or even the F_1) and
3351 should be tested in future experiments.

3352 In summary, I found that exposure to culture causes both behavioural and
3353 morphological changes in Atlantic cod relative to their wild conspecifics, and meta-
3354 analysis showed these morphological changes are common among fishes exposed to
3355 culture, confirming the existence of a "cultured phenotype". Despite their phenotypic
3356 differences, the reproductive success of cultured male cod was equal to that of wild
3357 males, at least under the conditions in which they were tested. Furthermore, hybrids
3358 between genetically distinct populations of cod did not show any fitness differences
3359 relative to their pure-strain half-sibs during their early life history. Taken together,
3360 these results suggest that the potential for introgression between wild and escaped
3361 cod may be greater than has previously been predicted.

3362 Moving forward and building off the results of this thesis, I would suggest
3363 that further studies be conducted on the importance of female behaviour and female
3364 mate choice in determining male spawning success. Many of the features of the cod
3365 mating system are indicative of female mate choice, such as the presence of sexually

dimorphic features (Rowe & Hutchings 2004b, Skjæraasen et al. 2006a, Rowe & Hutchings 2008, Skjæraasen et al. 2008, Skjæraasen et al. 2012), male display and courtship behaviours (Brawn 1961, Hutchings et al. 1999) and a lek-like spawning system (Nordeide & Folstad 2000, Windle & Rose 2007). In fact there is even some evidence, albeit weak, that the size of cod secondary sexual characters is related to their spawning success (Rowe & Hutchings 2008). That said, despite the original description of cod spawning (Brawn 1961) indicating that the behaviour of a female who would engage in spawning with a displaying male differed from that of a disinterested female, and that the male perceived and reacted to such behavioural differences, no further work has directly addressed how female behaviour dictates male spawning success. I would propose to repeat, or reevaluate studies such as those of (Brawn 1961) (Skjæraasen & Hutchings 2010) (Rowe & Hutchings 2008) for example, but include a critical evaluation of female behaviour.

Furthermore, the existing evidence for the importance of secondary sexual characters in cod mating success is weak (Rowe & Hutchings 2008, Skjæraasen et al. 2008). It is possible that the relationship between these two factors is obfuscated by the experimental conditions such as the limited number of males from which the female may choose compared to the wild, the confined tank space engendering unnatural levels of satellite spawning and sperm competition, or the sample sizes may have been too small to detect an effect. One manner in which a relationship between spawning success and secondary sexual character size might be tested is through experimental manipulation of the size of the characters. While the size of

the pelvic fins could be modified without undue difficulty, the size of the drumming muscles would be more difficult to alter. However, while increasing the size of the muscles may not be possible, it may be feasible to use a neurotoxin, such as botulinium toxin type A, to induce selective, or partial paralysis of the drumming muscles and thereby effectively reduce their size.

While this is by no means an exhaustive list of potential experimental avenues, these are the ones that most interest me for their theoretical relevance to the evolution of sexual selection, mating systems, and intra- and inter-sexual competition.

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Appendices

Supplementary Table 3.1 PRISMA 2009 Checklist For Wringe et al. 2016. In search of a "cultured fish phenotype": a systematic review, meta-analysis, and vote-counting analysis. Page numbers modified for thesis. *From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	In search of a "cultured fish phenotype": a systematic review, meta-analysis, and vote-counting analysis.	40
ABSTRACT			
Structured summary	2	That cultured fishes develop a morphology that differs from their wild conspecifics has become nearly axiomatic in fisheries science. A commonly supervened corollary is that exposure to culture causes a set of predictable and consistent morphological changes that result in a common "cultured phenotype" in fishes because the similarity of environments and selection pressures is greater among culture than natural environments. While this is often asserted, it has not been formally tested. A systematic review of the literature based on PRISMA best practice protocols identified 65 papers, composed of 106 studies that compared the morphology of 39 species of cultured fish to their wild conspecifics. This formed the basis of a meta-analysis of quantitative, and vote-counting analysis of qualitative differences in 16 external morphological features and condition factor. My analyses confirm that aspects of a general "cultured phenotype" exist. The meta-analysis analysis	40

		revealed that cultured fish had consistently shorter fins and upper jaws than wild fish, and the vote-counting analysis was suggestive of this as well. The vote-counting analysis showed that across all studies cultured fish had greater body depth and condition factor than wild fish, but this was not supported by the meta-analysis. As well as matching the morphological changes required to develop the "cultured phenotype", the changes detected in our analyses are consistent with experimentally observed plastic responses to environmental conditions typical of those experienced in culture. This is discussed, as is how intentional and unintentional selection in culture may contribute to, or reinforce the observed morphological changes.	
INTRODUCTION			
Rationale	3	Despite differences between cultured and wild fish having been reported for various species individually, and the commonality of these changes among species being alluded to, no formal test has been conducted to determine if exposure to culture conditions leads to a set of common morphological changes in fish exposed to culture relative to the morphology of their wild counterparts. To this end, we performed a meta-analysis, as well as a vote-counting analysis, based on a systematic review that was conducted following PRISMA best practice protocols (Liberati et al. 2009, Moher et al. 2009) of the literature on morphological differentiation between cultured fish and their wild counterparts to determine if similar patterns of divergence are observed across species.	44
Objectives	4	Our goal was to test the hypothesis that when exposed to culture, fishes develop stereotypical changes in their external morphology relative to their wild conspecifics.	46
METHODS			
Protocol and registration	5	Review protocol can be found in the methods, as well as the supplementary materals.	46
Eligibility criteria	6	1) the study must have examined the external morphology of the fish; 2) it must have been measured in a quantitative manner; 3) a comparison of cultured to a wild population must have been undertaken; and 4) the cultured fish must have spent the entirety of their lives in captivity (i.e. studies of recaptured or "sea ranched" cultured fish were excluded	46-47
Information sources	7	Searches were conducted in three main databases: the Aquatic Sciences and Fisheries Abstracts Database (ASFA), Web of Science, and Google Scholar. Where data were ambiguous … we contacted and requested data from study authors.	46 and 49
Search	8	Listed in Supplementary Table 3.1	

Study selection	9	This is outlined in the methods as well as in Supplementary Table 3.2 and Supplementary Figure 3.1	
Data collection process	10	Numeric data were extracted from tables. For the qualitative differences we recorded the qualitative differences as one of three categorical values: 1) cultured larger than wild (C>W), 2) wild larger than cultured (C<W), or 3) no difference reported (C=W).	49
Data items	11	Once the systematic review had been completed, and having parsed all publications retained, a set of external morphological features were selected that were commonly measured in morphological studies, were homologous across species, for which differences in their relative expression may affect the fish's fitness, and which are commonly asserted to comprise the "cultured phenotype" (Fig. 3.2). We also chose to include condition factor (Fulton's $K = 100(W/L^3)$) in our analysis because, while it is not technically an external morphological feature, it does have bearing on the fish's overall external conformation, and conforms to the other criteria. Differences in experimental methodology, study purpose, and a myriad of other factors, meant that all of the morphological features chosen to be examined in our meta-analysis were not measured or reported in every publication. We recorded the available morphological feature means and where reported, the corresponding standard deviations (see Statistical Analysis for treatment of missing standard deviations). In addition, we recorded species, the form of culture, and whether the wild and cultured fish that were compared were from the same ancestral genetic population. Again, each of these was not reported in every publication, and even when details were reported, they tended to differ among publications. To overcome this disparity, each variable was made categorical (Table 3.1), and where any of these data were unavailable or ambiguously reported, they were coded as 'unknown' and excluded from the analysis.	47 and 48
Risk of bias in individual studies	12	This was not done because there was no indication there would have been biases in measuring the morphometrics of fishes.	NA
Summary measures	13	The response ratio was calculated for each morphological character in Fig. 3.2 using the function *escalc* from the R package metafor (Viechtbauer 2010), which employs the formula proposed by Hedges et al. (1999): $L = ln(\bar{X}_c) - ln(\bar{X}_w)$	51
Synthesis of results	14	Studies were not combined.	

Supplementary Table 3.2 Keywords, and variant forms, including wild-cards and Boolean operators used in the systematic review. Search terms generally included at least a Culture Designation Term, a Wild Designation Term, and a Morphology Term. The word "fish", or a variant form was included when Culture and/or Wild Designation Terms did not implicitly refer to fish culture or rearing. Searches were also conducted with Culture or Wild Designation Terms replaced with variants of "Population". All pairings are not listed because the number of terms and their possible combinations is extremely large. However the vast majority of possible, relevant combinations of Culture, Wild, and Morphology terms were used. Searches utilizing all Boolean (i.e. OR) combinations of Culture Designation Terms, Wild Designation Terms and Morphology Terms were also conducted.

Root Keyword	Variant Forms	Keyword Type
Appearance		Morphology Term
Aquaculture	Aquacult*	Culture Designation Term
Culture	Culture + Artificial Culture + Laboratory Culture + Aquarium Culture + Domestic*	Culture Designation Term
Domesticated	Domest* Domestic*	Culture Designation Term
Farm	Farm*	Culture Designation Term
Fish	Fish*	
Hatchery	Hatcher* Hatchery + Supplemental Hatchery + Restor* Hatchery + Product*	Culture Designation Term
Laboratory	Laborator* Lab*	Culture Designation Term
Morphology	Morpholog*	Morphology Term

Morphometric/Morphometrics	Morphometric* Morphom* Morpho*	Morphology Term
Native		Wild Designation Term
Natural		Wild Designation Term
Phenotype	Phenotyp*	Morphology Term
Population	Population* Population + Aquacult* Population + Domestic* Population + Laborator* Population + Lab* Population + Laborator* Population + Native Population + Natural Population + Wild	Population Term
Shape	Shape*	Morphology Term Term
Wild		Wild Designation Term

Supplementary Table 3.3 References screened during the systematic review, along with the results of four inclusion criteria

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Source	Authors	Year	Title	Jounal	Common Name	Species Name	External Morph.?	Quantitative?	Wild/Farmed	Not Sea-Ranched?	Other	Accept/Reject
ASFA	Arechavala-Lopez et al.	2012	Morphological differences betw	Hydrobiologia	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	Yes	Yes	Yes	Yes		Accept
ASFA	Aritaki et al.	2000	Morphological development an	Nippon Suisan Gakkaishi	Barfin flounder	Verasper moseri	Yes	Yes	Yes	Yes		Accept
ASFA	Barlow and Munsey	1976	The red devil-Midas-arrow cichl	Investigations of the Ichthyofau	Midas cichlid	Amphilophus citrinellus	Yes	Yes	Yes	Yes		Accept
ASFA	Begum et al.	2013	Morphological and genetic vari	International Journal of Life Scie	Kuria labeo	Labeo gonius	Yes	Yes	Yes	Yes		Accept
ASFA	Blanchet et al.	2008	An integrated comparison of ca	Biological Conservation	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
ASFA	Crichigno et al.	2014	Morphological comparison of w	Aquaculture Research	Patagonian Perjerrey and Argentinian silv	Odontesthes hatcheri; Odontest	Yes	Yes	Yes	Yes		Accept
ASFA	Ellis et al.	1997	Morphological differences betw	Journal of Fish Biology	Turbot	Scophthalmus maximus	Yes	Yes	Yes	Yes		Accept
ASFA	Enders et al.	2004	The costs of habitat utilization c	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
ASFA	Janhunen et al.	2009	Morphological variability among	Ecology of Freshwater Fish	Arctic charr	Salvelinus alpinus	Yes	Yes	Yes	Yes		Accept
ASFA	Kerschbaumer et al.	2011	Morphological distinctness desp	Naturwissenschaften		Tropheus moorii	Yes	Yes	Yes	Yes		Accept
ASFA	Kitano et al.	2007	Sexual dimorphism in the exterr	Copeia	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	Yes	Yes		Accept
ASFA	Kouttouki et al.	2006	Shape ontogeny and variation ir	Aquaculture Research	Sharpsnout sea bream	Diplodus puntazzo	Yes	Yes	Yes	Yes		Accept
ASFA	Lahnsteiner and Jagsch	2005	Changes in phenotype and genc	Environmental Biology of Fishes	Brown trout	Salmo trutta	Yes	Yes	Yes	Yes		Accept
ASFA	Mairesse et al.	2005	Appearance and technological c	Aquaculture	Eurasian Perch	Perca fluviatilis	Yes	Yes	Yes	Yes		Accept
ASFA	McPhail	1984	Ecology and evolution of sympa	Canadian Journal of Fisheries an	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	Yes	Yes		Accept
ASFA	Murphy et al.	2007	Larval development of the Amb	Journal of Fish Biology	Ambon damselfish	Pomcentrus amboinensis	Yes	Yes	Yes	Yes		Accept
ASFA	Pulcini et al.	2013	Domestication shapes morphol	Journal of Fish Biology	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	Yes	Yes		Accept
ASFA	Rahman et al.	2014	Landmark-based morphometric	International Journal of Fisheries and Aquatic Sciences			Yes	Yes	yes	Yes		Accept
ASFA	Schwartz et al.	2005	Culture-induced abnormalities i	North American Journal of Aqua	Stinging catfish	Heteropneustes fossilis	Yes	Yes	Yes	Yes		Accept
ASFA	Sharpe et al.	2008	Genetic and environmental con	Evolutionary Ecology Research	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	Yes	Yes		Accept
ASFA	Suda et al.	1987	Morphological differences betw	Nippon Suisan Gakkaishi	Jack mackerel	Trachurus japonicus	Yes	Yes	Yes	Yes		Accept
ASFA	Taylor	2003	Meristic and morphometric diffi	Gulf of Mexico Science	Mangrove rivulus	Rivulus marmoratus	Yes	Yes	Yes	Yes		Accept
ASFA	Todd et al.	1981	Environmental and genetic cont	Canadian Journal of Fisheries an	Cisco	Coregonus sp.	Yes	Yes	Yes	Yes		Accept
ASFA	Uglem et al.	2011	Discrimination of wild and farm	ICES Journal of Marine Science:	Atlantic cod	Gadus morhua	Yes	Yes	Yes	Yes		Accept
ASFA	von Cramon-Taubadel et al.	2005	Determination of body shape va	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
ASFA	Wessel et al.	2006	Variation of morphology among	Transactions of the American Fi	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	Yes		Accept
ASFA	Wilkins et al.	1994	Morphometric and meristic cha	Aquaculture and Fisheries Mana	Atlantic salmon and Brown trout	Salmo salar; Salmo trutta	Yes	Yes	Yes	Yes		Accept
ASFA	Wintzer and Motta	2005	Diet-induced phenotypic plastic	Ecology of Freshwater Fish	Largemouth bass	Micropterus salmoides	Yes	Yes	Yes	Yes		Accept
Cited	Fleming and Einum	1997	Experimental tests of genetic di	ICES Journal of Marine Science	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
Cited	Kazakov and Semyonova	1986	Morphological features of the c	The morphology and ecologly of	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
Cited	Matsumiya and Kanamaru	1986	Morphometric comparison betv	Journal of Applied Ichthyology	Red sea bream	Pagrus major	Yes	Yes	Yes	Yes	Released fish m	Accept
Cited	McCairns and Bernatchez	2012	Plasticity and heritability of mor	Journal of Evolutionary Biology	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	Yes	Yes		Accept
Cited	McGuigan et al.	2003	Adaptations of rainbow fish to l	Evolution	Rainbow fish	Melanotaenia eachamensis	Yes	Yes	Yes	Yes		Accept
Cited	Park et al.	2012	The morphological study of wild	Dev. Reprod.	Olive flounder	Paralichthys olivaceus	Yes	Yes	Yes	Yes		Accept
Cited	Pedersen et al.	2008	Swimming performance of wild	Ecology of Freshwater Fish	Atlantic salmon and Brown trout	Salmo salar; Salmo trutta	Yes	Yes	Yes	Yes		Accept
Cited	Rogdakis et al.	2011	Comparative morphology of wil	International Journal of Fisherie	Gilthead sea bream	Sparus aurata	Yes	Yes	Yes	Yes		Accept
Cited	Salmanov	1986	Osteological features from cultu	The morphology and ecologly of	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
Cited	Salmanov	1989	Analysis of the variability of mo	Ecological and physiological stu	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
Cited	Wagle et al.	2013	Morphological discrimination of three populations of rohu (Labe		Rohu	Labeo rohita	Yes	Yes	Yes	Yes		Accept
GS	Aritaki et al.	2000	Morphological development an	Nippon Suisan Gakkaishi	Barfin flounder	Verasper moseri	Yes	Yes	Yes	Yes		Accept
GS	Blanchet et al.	2008	An integrated comparison of ca	Biological Conservation	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
GS	Burns et al.	2009	The role of predation in variatio	Journal of Fish Biology	Guppy	Poecilia reticulata	Yes	Yes	Yes	Yes		Accept
GS	Fleming et al.	1994	Phenotypic divergence of sea-ra	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
GS	Gozlan et al.	1999	Comparison of growth plasticity	Environmental Biology of Fishes	South-west European Nace	Parachondrostoma toxostoma	Yes	Yes	Yes	Yes		Accept

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GS	Hard et al.	2000	Evidence for morphometric diffi	Environmental Biology of Fishes	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	Yes		Accept
GS	Kerschbaumer et al.	2011	Morphological distinctness desp	Naturwissenschaften		Tropheus moorii	Yes	Yes	Yes	Yes		Accept
GS	Klemetsen et al.	2002	Evidence for genetic differences	Journal of Fish Biology	Arctic charr	Salvelinus alpinus	Yes	Yes	Yes	Yes		Accept
GS	Leaver and Reimchen	2012	Abrupt changes in defence and	Biological Journal of the Linnear	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	Yes	Yes		Accept
GS	Lenhardt et al.	2012	Comparison of morphological cl	Slovenian Veterinary Research	Sterlet	Acipenser ruthenus	Yes	Yes	Yes	Yes		Accept
GS	Mairesse et al.	2005	Appearance and technological c	Aquaculture	Eurasian Perch	Perca fluviatilis	Yes	Yes	Yes	Yes		Accept
GS	Matsuzaki et al.	2009	Behavioural and morphological	Journal of Fish Biology	Common carp	Cyprinus carpio	Yes	Yes	Yes	Yes		Accept
GS	Morioka et al.	2012	Growth and morphological deve	Ichthyological Research	Three-spot gourami	Trichogaster trichopterus	Yes	Yes	Yes	Yes		Accept
GS	Morris et al.	2011	Hybridization effects on phenot	Evolutionary Applications	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
GS	Pakkasmaa and Piironen	2001	Morphological differentiation a	Biological Journal of the Linnear	Brown trout	Salmo trutta	Yes	Yes	Yes	Yes		Accept
GS	Patiyal et al.	2014	Pattern of meristic and morpho	Proceedings of the National Ac	ademy of Sciences, India Section B: Biologic	Tor putitoria	Yes	Yes	Yes	Yes		Accept
GS	Pulcini et al.	2013	Domestication shapes morphol	Journal of Fish Biology	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	Yes	Yes		Accept
GS	Šegvić-Bubić et al.	2014	Morphological and molecular di	Aquaculture Environment Interz	Gilthead sea bream	Sparus aurata	Yes	Yes	Yes	Yes		Accept
GS	Solem et al.	2006	Inter- and intr-population morp	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
GS	Svanback and Schluter	2012	Nice specialization influences ac	The American Naturalist	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	Yes	Yes		Accept
GS	Swain et al.	1991	Morphological differences betw	Canadian Journal of Fisheries an	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	Yes		Accept
GS	Tiffan and Connor	2011	Distinguishing between natural	Transactions of the American Fi	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	Yes		Accept
GS	Uglem et al.	2011	Discrimination of wild and farm	ICES Journal of Marine Science:	Atlantic cod	Gadus morhua	Yes	Yes	Yes	Yes		Accept
GS	von Cramon-Taubadel et al.	2005	Determination of body shape va	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
WoS	Adams and Huntingford	2004	Incipient speciation driven by pl	Biological Journal of the Linnear	Arctic charr	Salvelinus alpinus	Yes	Yes	Yes	Yes		Accept
WoS	Arechavala-Lopez et al.	2012	Morphological differences betw	Hydrobiologia	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	Yes	Yes	Yes	Yes		Accept
WoS	Fleming et al.	1994	Phenotypic divergence of sea-ra	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
WoS	Fraser et al.	2010	Consequences of farmed-wild h	Ecological Applications	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
WoS	Kim et al.	2011	Body shape and growth in recip	Journal of the World Aquacultur	Olive flounder	Paralichthys olivaceus	Yes	Yes	Yes	Yes		Accept
WoS	Lund and Hansel	1991	Identification of wild and rearec	Aquaculture Research	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
WoS	Mairesse et al.	2005	Appearance and technological c	Aquaculture	Eurasian Perch	Perca fluviatilis	Yes	Yes	Yes	Yes		Accept
WoS	Matsuzaki et al.	2009	Behavioural and morphological	Journal of Fish Biology	Common carp	Cyprinus carpio	Yes	Yes	Yes	Yes		Accept
WoS	Morris et al.	2011	Hybridization effects on phenot	Evolutionary Applications	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
WoS	Pulcini et al.	2013	Domestication shapes morphol	Journal of Fish Biology	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	Yes	Yes		Accept
WoS	Šegvić-Bubić et al.	2014	Morphological and molecular di	Aquaculture Environment Interz	Gilthead sea bream	Sparus aurata	Yes	Yes	Yes	Yes		Accept
WoS	Solem et al.	2006	Inter- and intr-population morp	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
WoS	Tiffan and Connor	2011	Distinguishing between natural	Transactions of the American Fi	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	Yes		Accept
WoS	Uglem et al.	2011	Discrimination of wild and farm	ICES Journal of Marine Science:	Atlantic cod	Gadus morhua	Yes	Yes	Yes	Yes		Accept
WoS	Vehanen and Huusko	2011	Brown trout Salmo trutta expre	Journal of Fish Biology	Brown trout	Salmo trutta	Yes	Yes	Yes	Yes		Accept
WoS	von Cramon-Taubadel et al.	2005	Determination of body shape va	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes		Accept
ASFA	Shimizu and Shiozawa	2004	Allometry and development of c	Bulletin of National Research In	Yellowfin tuna	Thunnus albacares	Yes	Yes	Yes	Yes		Accept
Cited	Balbontin et al.	1973	A comparative study of anatom	Aquaculture	Herring	Clupea harengus	Yes	Yes	Yes	Yes		Accept
Cited	Suzuki and Yamaguchi	1980	Meristic and morphometric cha	Japanese Journal of Ichthyology	Common carp	Cyprinus carpio	Yes	Yes	Yes	Yes		Accept
Cited	Taylor	1986	Differences in morphology betw	The Progressive Fish-Culturist	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	Yes		Accept
ASFA	Fukuhara et al.	1978	Morphological development in t	Bulletin of the Nansei Regional I	Madai	Chrysophrys major	Yes	Yes	Yes	Yes	Measured diffe	Reject
ASFA	Gordeeva et al.	2012	Biological and genetic diversity	Advances in Limnology	Peled	Coregonus peled	?	?	?	?	Paper not avail	Reject
ASFA	Aguado-Giménez and García-Ga	2005	Changes in some morphometric	Aquaculture	Blue fin tuna	Thunnus thynnus	Yes	Yes	No		Cultured were f	Reject
ASFA	Akhter et al.	2003	Studies of morphometric and m	Pakistan Journal of Biological Sc	Silver barb	Barbodes gonionotus	Yes	Yes	No			Reject
ASFA	Almeida et al.	2008	Fluctuating asymmetry, abnorm	Aquaculture	Gold fish and Common carp	Carassius auratus; Cyprinus carp	Yes	Yes	No		Cultured were f	Reject
ASFA	Aparicio et al.	2005	Body pigmentation pattern to a	Journal of Fish Biology	Brown trout	Salmo trutta	No	Yes	Yes	Yes	Colour and spot	Reject

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ASFA	Arechavala-Lopez et al.	2012	Discriminating farmed gilthead :	Journal of Fish Biology	Gilthead sea bream and European sea bas	Sparus aurata; Dicentrarchus lab	No					Reject
ASFA	Arechavala-Lopez et al.	2013	Differentiating the wild or farm	Reviews in Aquaculture	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	Yes	No			Review paper	Reject
ASFA	Ashouri et al.	2013	The effect of short-term starvat	Acta Icthyologica et Piscatoria	Siberian sturgeon	Acipenser baerii	No					Reject
ASFA	Bailey and Irvine	1991	Morphological differences amo	Canadian Technical Report of Fi	Coho salmon	Oncorhynchus gorbuscha	Yes	Yes	No			Reject
ASFA	Balon	1995	Origin and domestication of the	Aquaculture	Common carp	Cyprinus carpio					Review paper	Reject
ASFA	Bamberger	2009	Semi-natural incubation technic	Aquaculture	Atlantic salmon	Salmo salar	No					Reject
ASFA	Beacham	1984	Age and morphology of chum s	Transactions of the American Fi	Chum salmon	Oncorhynchus keta	Yes	Yes	No			Reject
ASFA	Beacham et al.	1988	Variation in body size, morphol	Transactions of the American Fi	Pink salmon	Oncorhynchus gorbuscha	Yes	Yes	No			Reject
ASFA	Belk et al.	2008	Hatchery-induced morphologic	Canadian Journal of Fisheries an	June sucker	Chasmistes liorus	Yes	Yes	Yes	No	Cultured reared	Reject
ASFA	Berejikian et al.	2001	Male competition and breeding	Canadian Journal of Fisheries an	Coho salmon	Oncorhynchus kisutch	No					Reject
ASFA	Bisson et al.	1988	Channel hydraulics, habitat use,	Transactions of the American Fi	Coho salmon, Rainbow trout and Cutthroa	Oncorhynchus kisutch; Oncorhy	Yes	Yes	No			Reject
ASFA	Blair et al.	1993	Variation on life history charact	Transactions of the American Fi	Sockeye salmon	Oncorhynchus nerka	Yes	Yes	No			Reject
ASFA	Boglione et al.	2001	Skeletal descriptors and quality	Aquaculture	Gilthead sea bream	Sparus aurata	No		Yes		Skeletal deform	Reject
ASFA	Boglione et al.	2006	Biological monitoring of wild thi	Ecological Indicators	Thicklip grey mullet; golden grey mullet; t	Chelon labrax; Liza aurata; Liza r	No				Skeletal deform	Reject
ASFA	Busack et al.	2007	Morphological differences betw	Transactions of the American Fi	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	No		Reject
ASFA	Chatry and Conner	1980	Comparative developmental mc	Proceedings of the Fourth Annu	White crappie; black crappie	Pomoxis annularis; P. nigromacu	Yes	Yes	No			Reject
ASFA	Colombo et al.	2013	The ecological and genetic basis	Molecular Ecology	No comon name and Red Devil cichlid	Lobochilotes labiatus; Amphilop	Yes	Yes	No			Reject
ASFA	Costa et al.	2013	Automated sorting for size, sex	Aquacultural Engineering	European sea bass	Dicentrarchus labrax	Yes	No	No		Only looking for	Reject
ASFA	Costa et al.	2013	Preliminary evidences of colour	Aquaculture Engineering	European sea bass	Dicentrarchus labrax	Yes	Yes	No			Reject
ASFA	Costa et al.	2010	Genetic and environmental infl	Biological Journal of the Linnear	European sea bass	Dicentrarchus labrax	Yes	Yes	No		Only sire is cultu	Reject
ASFA	Currens et al.	1989	Effects of different feeding regi	Copeia	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	No			Reject
ASFA	Cvijanović et al.	2012	Use of shape analysis in the inv	Journal of Applied Ichthyology	Black bullhead and Brown bullhead	Ameiurus melas; Ameiurus nebu	Yes	Yes	No		Cultured fish ar	Reject
ASFA	Dahl et al.	2006	No difference in survival, growt	Ecology of Freshwater Fish	Brown trout	Salmo trutta	Yes	Yes	Yes	No		Reject
ASFA	Ehrlich et al.	1976	Morphological and histological	Marine Biology	Atlantic herring and European plaice	Clupea harengus; Pleuronectes	Yes	Yes	No			Reject
ASFA	Einum and Fleming	2001	Implications of stocking: ecologi	Nordic Journal of Freshwater Re	Review		No				Good review of	Reject
ASFA	Enders et al.	2004	Differences in the energetic cos	Journal of Fish Biology	Atlantic salmon	Salmo salar	N/A				Abstract of oral	Reject
ASFA	Favaloro and Mazzola	2003	Shape change during growth of	Aquaculture Engineering	Sharpsnout sea bream	Diplodus puntazzo	Yes	Yes	No			Reject
ASFA	Fjelldal et al.	2009	A radiological study on vertebra	Aquaculture	Atlantic cod	Gadus morhua	No					Reject
ASFA	Friedland et al.	1994	Discrimination of Norwegian far	Fisheries Management and Ecol	Atlantic salmon	Salmo salar	No				Scale features	Reject
ASFA	Gonzalez-Castro et al.	2012	Assessment of lineal versus lanc	Zoological Studies		Mugulidae sp.	Yes	Yes	No			Reject
ASFA	Gorshkov	2014	Inheritance of a darkened caud	Journal of Applied Ichthyology	Gilthead sea bream	Sparus aurata	No					Reject
ASFA	Herczeg et al.	2010	Morphological divergence of Nc	Biological Journal of the Linnear	Nine-spine stickleback	Pungitius pungitius	Yes	Yes	Yes	Yes	Does not prese	Reject
ASFA	Iguchi and Mogi	2007	Effect of introducing wild paterr	Fisheries Science	ayu	Plecoglossus altivelis	Yes	Yes	No		Wild' fish are of	Reject
ASFA	Iguchi and Mogi	2007	Effect of introducing wild paterr	Fisheries Science	ayu	Plecoglossus altivelis	Yes	Yes	No		Wild' fish are of	Reject
ASFA	Johnson et al.	2004	Lopsided fish in the Snake River	Environmental Biology of Fishes	Chinook salmon	Oncorhynchus tshawytscha	Meristics				Meristic not mc	Reject
ASFA	Khalili and Amirkolaie	2010	Comparison of common carp (C	Journal of Fisheries and Aquatic	Common carp	Cyprinus carpio	Yes	Yes	No			Reject
ASFA	Khemis	2014	Comparative study of pikeperch	Fish Physiology and Biochemistr	Pikeperch	Sander lucioperca	Yes	Yes	Yes	Yes	Measured diffe	Reject
ASFA	Khemis et al.	2013	Allometric growth patterns and	Aquaculture Research	Thick-lipped mullet	Chelon labrosus	Yes	Yes	No			Reject
ASFA	Koch et al.	2012	Additive genetic variance of qu	Hydrobiologia		Tropheus moorii	Yes	Yes	Yes	Yes	Cultured contai	Reject
ASFA	Koumoundouros et al.	2001	The effect of rearing conditions	Aquaculture	Common dentex	Dentex dentex	No					Reject
ASFA	Koumoundouros et al.	1995	Morphometric relationships as	Aquaculture International	Gilthead sea bream	Sparus aurata	Yes	Yes	No			Reject
ASFA	Lenhardt et al.	2004	Comparative analysis of morph	Journal of Fish Biology	Sterlet	Acipenser ruthenus	Yes	Yes	Yes	Yes	Abstract of oral	Reject
ASFA	Lochmann and Ludwig	2003	Relative triacylglycerol and mor	North American Journal of Aqua	"Sunshine bass"	Morone chrysops X M. saxatilis	Yes	Yes	No		Fish are also hy	Reject
ASFA	Lõhmus et al.	2010	Effects of temperature and grov	Journal of Fish Biology	Coho salmon	Oncorhynchus kisutch	No					Reject
ASFA	Loy et al.	2000	Geometric morphometrics and	Aquaculture	European sea bass	Dicentrarchus labrax	Yes	Yes	No		Very good desc	Reject

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ASFA	Loy et al.	1999	Geometric morphometrics and	Journal of Applied Ichthyology	Gilthead sea bream	Sparus aurata	Yes	Yes	No			Reject
ASFA	Malek et al.	2012	Admixture mapping of male nuj	Molecular Ecology	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	No			Reject
ASFA	Mordenti et al.	2013	Controlled reproduction in the \	Aquaculture International	European eel	Anguilla anguilla	Yes	Yes	No		Compares only	Reject
ASFA	Palma et al.	2012	Growth, reproductive performa	Journal of the World Aquacultur	Long snout seahorse	Hippocampus guttulatus	Yes	Yes	No			Reject
ASFA	Park et al.	2003	Genetic characterization, morh	Aquaculture Research	Yellowtail flounder	Pleuronectes ferrugineus	Yes	Yes	No			Reject
ASFA	Pearsons et al.	2012	Ecological risk assessment of m	Environmental Biology of Fishes			No					Reject
ASFA	Proman and Reynolds	2000	Differences in head shape of th	Fisheries Management and Ecol	European eel	Anguilla anguilla	Yes	Yes	Yes	Yes	Two morphs of	Reject
ASFA	Pulcini et al.	2014	Rainbow trout (Oncorhynchus n	Aquaculture Research	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	No			Reject
ASFA	Qu et al.	2013	Effects of lateral morphology or	Journal of Applied Ichthyology	Chinese sturgeon and Siberian sturgeon	Acipenser sinensis; Acipenser ba	Yes	Yes	No		Acipenser sinen	Reject
ASFA	Rognon et al.	1998	Morphometric and allozyme var	Journal of Fish Biology		Clarias gariepinus; Clarias anguil	Yes	Yes	No			Reject
ASFA	Rollinson and Hutchings	2011	Body size-specific maternal effe	Oecologia	Atlantic salmon	Salmo salar	No					Reject
ASFA	Russo et al.	2011	Application of the self-organizin	Aquaculture	Dusky grouper	Epinephelus marginatus	Yes	No	No			Reject
ASFA	Sarà et al.	1999	Comparative morphometrics of	Aquaculture Engineering	Sharpsnout sea bream	Diplodus puntazzo	Yes	Yes	No			Reject
ASFA	Schoenfuss et al.	2013	Stairway to Heaven: Evaluating	PLOSone		Sicyopterus stimpsoni	Yes	Yes	No			Reject
ASFA	Schramm et al.	2004	Status and reproduction of Gulf	Southeastern Naturalist	Walleye	Sander vitreus	No					Reject
ASFA	Seiler	2007	Ecological and environmental in	Thesis	Cutthroat trout and Rainbow trout	Oncorhynchus clarkii; Oncorhyn	Yes	Yes	No			Reject
ASFA	Sfakianakis et al.	2013	Lateral line deformities in wild a	Journal of Applied Ichthyology	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	No				Lateral line and	Reject
ASFA	Shikano	2007	Quantitative genetic parameter	Aquaculture Research	Olive flounder	Paralichthys olivaceus	Yes	Yes	Yes	No		Reject
ASFA	Spedicato et al.	2004	Life-history traits of the commo	Journal of Fish Biology	Pandora	Pagellus erythrinus	N/A				Abstract of oral	Reject
ASFA	Suzuki and Yamaguchi	1984	Meristic and morphometric cha	Bulletin of National Research In	Common carp	Cyprinus carpio					Not available	Reject
ASFA	Waldman and Vecchio	1996	Selected biocharacteristics of h	North American Journal of Fishe	Striped bass	Morone saxatilis	Yes	Yes	Yes	No		Reject
ASFA	Weber and Fausch	2003	Interactions between hatchery	Canadian Journal of Fisheries and Aquatic Science			No				Good review of	Reject
ASFA	Xu et al.	2007	Growth development and repro	Journal of Fishery Sciences of Cl	Taimen	Hucho taimen	No					Reject
Cited	Adams and Huntingford	2002	Inherited differences in head all	Journal of Fish Biology	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
Cited	Adams et al.	2003	Epigeneic regulation of trophic r	Biological Journal of the Linnear	Arctic charr	Salvelinus alpinus	Yes	Yes	No		Tests effect of f	Reject
Cited	Adams et al.	2003	Epigeneic regulation of trophic r	Biological Journal of the Linnear	Arctic charr	Salvelinus alpinus	Yes	Yes	No		Tests effect of f	Reject
Cited	Alexander and Adams	2004	Exposure to a common environ	Journal of Fish Biology	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
Cited	Anto et al.	2009	Prey selection and functional m	Journal of Fish Biology	Tomato clownfish	Amphiprion frenatus	Yes	Yes	No			Reject
Cited	Beacham and Murray	1986	The effect of spawning time anc	Canadian Journal of Zoology	Chum salmon	Oncorhynchus keta	Yes	Yes	No		Meristic not mc	Reject
Cited	Beacham and Withler	1985	Heterozygosity and morphologi	Herdity	Chum salmon	Oncorhynchus keta	Yes	Yes	No			Reject
Cited	Bellinger et al.	2014	Domestication is associated wit	Aquaculture	Rainbow trout	Oncorhynchus mykiss	No		No		Overall body siz	Reject
Cited	Bertrand et al.	2008	Trophic polymorphism in brook	Journal of Fish Biology	Brook charr	Salvelinus fontinalis	Yes	Yes	No			Reject
Cited	Boglione et al.	2003	Skeletal quality assessment of r	Aquaculture	Sharpsnout sea bream and pandora	Diplodus puntazzo; Pagellus eryt	No					Reject
Cited	Brown et al.	2013	Differences in lateral line morh	PLOSone	Rainbow trout	Oncorhynchus mykiss	No					Reject
Cited	Doctor et al.	2014	Evidence of between-populatio	Environmental Biology of Fishes	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	No			Reject
Cited	Feming and Gross	1989	Evolution of adult female life hi	Evolution	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	No		Reject
Cited	Feming and Gross	1994	Breeding competition in a Pacifi	Evolution	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	No		Reject
Cited	Fraser and Huntingford	1988	Trophic polymorphism amongst	Ecology of Freshwater Fish	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
Cited	Funk et al.	2005	Genetic basis of variation in mo	Journal of Heredity	Pink salmon	Oncorhynchus gorbuscha	No		No	No		Reject
Cited	Gislason et al.	1999	Rapid and coupled phenotypic a	Canadian Journal of Fisheries an	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
Cited	Hard et al.	1999	Phenotypic and genetic architec	Herdity	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	No		Only hatchery	Reject
Cited	Hernandez-Ucera et al.	2012	Induction of triploidy in turbot (	Aquaculture	Turbot	Scophthalmus maximus	Yes	Yes	No			Reject
Cited	Hjelm et al.	2001	Diet-dependent body morpholo	OIKOS	European perch	Perca fluviatilis	Yes	Yes	No		Wild Fish in Enc	Reject
Cited	Hjort and Schreck	1982	Phenotypic differences among s	Fishery Bulletin	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	Likely	Morphological (	Reject
Cited	Janhunen et al.	2010	A comparison of growth patterr	Environmental Biology of Fishes	Arctic charr	Salvelinus alpinus	No		Yes	Yes	Overal body siz	Reject

Cited	Kawamura et al.	1989	Comparison of sense organ dev	Nippon Suisan Gakkaishi	Olive flounder	Paralichthys olivaceus	No					Reject
Cited	Kinnison et al.	1998	Population-specific variation in	Canadian Journal of Fisheries an	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	No		Reject
Cited	Koumoundouros et al.	1999	Ontogeny and allometric plastic	Marine Biology	Common dentex	Dentex dentex	Yes	Yes	No			Reject
Cited	Le Cam et al.	2015	Genetic and phenotypic change	Proceedings of the Royal Societ	Atlantic salmon	Salmo salar	No			No	Overall body siz	Reject
Cited	Loy et al.	2000	Comparing geometric morphon	Aquacultural Engineering	Sharpsnout sea bream	Diplodus puntazzo	Yes	Yes	No			Reject
Cited	McDermid et al.	2010	Early life history variation amon	Transactions of the American Fi	Lake trout	Salvelinus namaycush	No				Length and wei	Reject
Cited	Nicieza	1995	Morphological variation betwee	Functional Ecology	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
Cited	Pakkasmaa et al.	1998	A morphometric study on four l	Annales Zoologici Fennici	Grayling, Atlantic salmon, brown trout, Ar	Thymallus thymallus, Salmo sala	Yes	Yes	No			Reject
Cited	Pearlstein et al.	2007	Early discrimination of Atlantic	Transactions of the American Fi	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes	Look for predict	Reject
Cited	Pelis and McCormick	2003	Fin development in stream- and	Aquaculture	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
Cited	Peruzzi et al.	2010	Body shape variation in meiotic	Aquatic Living Resources	European sea bass	Dicentrarchus labrax	Yes	Yes	No			Reject
Cited	Petersson et al.	1996	The effect of domestication on	Journal of Fish Biology	Brown trout and Atlantic salmon	Salmo trutta; Salmo salar	No				Sea ranched, ar	Reject
Cited	Poole et al.	2003	An ecophysiological comparison	Aquaculture	Atlantic salmon	Salmo salar	No					Reject
Cited	Pulcini et al.	2015	Testing the relationship betwee	Biological Journal of the Linnear	Rainbow trout	Oncorhynchus mykiss	No				Meristic	Reject
Cited	Robinson and Wilson	1995	Experimentally induced morphc	Copeia	Guppy	Poecilia reticulata	Yes	Yes	No			Reject
Cited	Seiler and Keeley	2007	Morphological and swimming st	Canadian Journal of Fisheries an	Cutthroat trout and Rainbow trout	Oncorhynchus clarkii; Oncorhyn	Yes	Yes	No			Reject
Cited	Torres-Nunez et al.	2014	Phenotype plasticity during earl	Journal of Applied Ichthyology	Turbot	Scophthalmus maximus	Yes	Yes	No			Reject
Cited	Vøllestad and Hindar	1997	Developmental stability and en	Herdity	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes	Reported fluctu	Reject
Cited	Winans	1984	Multivariate morphometric vari	Canadian Journal of Fisheries an	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	Yes	Did not report c	Reject
GS	Adams and Huntingford	2002	The functional significance of in	Evolutionary Ecology	Arctic charr	Salvelinus alpinus	Yes	Yes	No		Gape size only	Reject
GS	Adams et al.	1998	Trophic polymorphism amongst	Journal of Fish Biology	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
GS	Alexander and Adams	2000	The phenotypic diversity of Arct	aqua, Journal of Ichthyology an	Arctic charr	Salvelinus alpinus	Yes	No	No			Reject
GS	Alexseyev et al.	2002	Diversification, sympatric specia	Environmental Biology of Fishes	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
GS	Anto and Turingan	2010	Relating the ontogeny of functic	Journal of Morphology	Tomato clownfish	Amphiprion frenatus	Yes	Yes	No			Reject
GS	Arechavala-Lopez et al.	2013	Differentiating the wild or farm	Reviews in Aquaculture	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	Yes	No			Review paper	Reject
GS	Baer	2004	Stocking hatchery-reared brow	Journal of Fish Biology	Brown trout	Salmo trutta	N/A				Abstract of oral	Reject
GS	Beacham	1990	A genetic analysis of meristic an	Canadian Journal of Fisheries an	Chum salmon	Oncorhynchus keta	Yes	Yes	No			Reject
GS	Berejikian et al.	2001	Male competition and breeding	Canadian Journal of Fisheries an	Coho salmon	Oncorhynchus kisutch	No					Reject
GS	Billman et al.	2014	Body morphology differs in wild	Journal of Fish Biology	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	No			Reject
GS	Brinsmead and Fox	2002	Morphological variation betwee	Journal of Fish Biology	Rock bass and Pumpkinseed	Ambloplites rupestris; Lepomis g	Yes	Yes	No			Reject
GS	Bronte et al.	1999	Stock structure of Lake Baikal or	Journal of Fish Biology	Baikal omul	Coregonus migratorius	Yes	Yes	No			Reject
GS	Burns et al.	2009	Rearing environment affects the	Ethology	Guppy	Poecilia reticulata	No					Reject
GS	Chebanov et al.	2004	Stock enhancement and conser	Journal of Fish Biology	Russian sturgeon and starry sturgeon	Acipenser gueldenstaedtii and A	N/A				Abstract of oral	Reject
GS	Chittenden et al.	2010	Genetic versus rearing-environn	PLOSone	Coho salmon	Oncorhynchus kisutch	No				Size and conditi	Reject
GS	Coban et al.	2008	Morphometric comparison of cu	Turkish Journal of Zoology	Gilthead sea bream	Sparus aurata	Yes	Yes	Yes	Yes	Data poorly rep	Reject
GS	Colihueque and Nelson	2014	Appearance traits in fish farmin	Frontiers in Genetics			Yes	No			Review paper	Reject
GS	Crozier	1997	Genetic heterozygosity and mer	Aquaculture International	Atlantic salmon	Salmo salar	No	Yes	Yes	Yes	Meristic not mc	Reject
GS	Dahl et al.	2006	No difference in survival, growtl	Ecology of Freshwater Fish	Brown trout	Salmo trutta	Yes	Yes	Yes	No		Reject
GS	de O. Saraiva and S. Pompeu	2014	The effect of structural enrichm	Neotropical Ichthyology	Streaked prochilod and no common name	Prochilodus lineatus; Brycon orb	Yes	Yes	No			Reject
GS	Dynes et al.	1999	Genetic and morphological vari	Journal of Fish Biology	Brook charr	Salvelinus fontinalis	Yes	Yes	No			Reject
GS	Einum and Fleming	2001	Implications of stocking: ecologi	Nordic Journal of Freshwater Re	Review		No				Good review of	Reject
GS	Fairchild and Howell	2004	Factors affecting the post-relea	Journal of Fish Biology	Winter flounder	Pseudopleuronectes americanus	No	Yes	Yes	Yes	Colour and beh	Reject
GS	Fjelldal et al.	2009	A radiological study on vertebra	Aquaculture	Atlantic cod	Gadus morhua	No					Reject
GS	Fleming	2004	Reproductive ecology of culture	Journal of Fish Biology			N/A				Abstract of oral	Reject
GS	Fleming and Gross	1993	Breeding success of hatchery an	Ecological Applications	Coho salmon	Oncorhynchus kisutch	No			No	Morphological a	Reject

GS	Fraser et al.	2008	Mixed evidence for local adapta	Evolutionary Applications	Atlantic salmon	Salmo salar	No				Measured grow	Reject	
GS	Friedland et al.	1994	Discrimination of Norwegian far	Fisheries Management and Ecol	Atlantic salmon	Salmo salar	No				Scale features	Reject	
GS	Gale et al.	2004	Physiological and behavioural d	Journal of Fish Biology	Rainbow trout	Oncorhynchus mykiss	N/A				Abstract of oral	Reject	
GS	Galich and Chebanov	2004	Comparative evaluation of sturg	Journal of Fish Biology			N/A				Abstract of oral	Reject	
GS	Garcia de Leaniz et al.	2004	Maladaptation and phenotypic	Journal of Fish Biology	Atlantic salmon	Salmo salar	N/A				Abstract of oral	Reject	
GS	Gardner et al.	1988	Morphometric analysis of two e	Journal of Fish Biology	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject	
GS	Gavaia et al.	2009	Comparing skeletal developmer	Aquaculture Research	Senegalese sole	Solea senegalensis	No				Skeletal feature	Reject	
GS	Glover et al.	2009	A comparison of farmed, wild, a	Aquaculture	Atlantic salmon	Salmo salar	No				Growth, conditi	Reject	
GS	Grigorakis	2007	Compositional and organolepti	International Journal of Food Sc	Gilthead sea bream and European sea bas	Sparus aurata; Dicentrarchus lat	Yes	No			Qualitative des	Reject	
GS	Hansen et al.	1993	High numbers of farmed Atlanti	Aquaculture Research	Atlantic salmon	Salmo salar	No					Reject	
GS	Hansen et al.	1997	The incidence of reared Atlantic	ICES Journal of Marine Science:	Atlantic salmon	Salmo salar	No					Reject	
GS	Harbright et al.	2014	Does human-induced hybridizat	Evolutionary Applications			Yes	Yes	Yes	No	105	Reject	
GS	Heggberget et al.	1993	Distribution and migratory beh	Aquaculture	Atlantic salmon	Salmo salar	No					Reject	
GS	Holtmeier	2001	Heterochrony, maternal effects,	Evolution	Guppy	Poecilia reticulata	Yes	Yes	No			Reject	
GS	Houde et al.	2009	Fitness-related consequences o	ICES Journal of Marine Science:	Atlantic salmon	Salmo salar	No					Reject	
GS	Hoyle et al.	2007	A validated macroscopic key to	Aquaculture	Rainbow trout	Oncorhynchus mykiss	Yes	No				Reject	
GS	Hulata	1995	A review of genetic improveme	Aquaculture	Common carp	Cyprinus carpio					Review paper	Reject	
GS	Imre et al.	2002	Phenotypic plasticity in brook cl	Journal of Fish Biology	Brook charr	Salvelinus fontinalis	Yes	Yes	No			Reject	
GS	Irwin et al.	2002	Mouth morphology and behavic	Aquaculture	Turbot	Scophthalmus maximus	No					Reject	
GS	Kallio-Nyberg et al.	2015	Differences between wild and r	Fisheries Research	Atlantic salmon	Salmo salar	No				Compares lengt	Reject	
GS	Keeley et al.	2005	Ecotypic differentiation of nativ	Canadian Journal of Fisheries an	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	No			Reject	
GS	Khalili and Amirkolaie	2010	Comparison of common carp (C	Journal of Fisheries and Aquatic	Common carp	Cyprinus carpio	Yes	Yes	No			Reject	
GS	Knights	1982	Body dimensions of farmed eels	Aquaculture Engineering	European eel	Anguilla anguilla	Yes	Yes	No			Reject	
GS	Kostow	2004	Differences in juvenile phenoty	Canadian Journal of Fisheries an	Rainbow trout	Oncorhynchus mykiss	No	Yes	Yes	Yes	Only length and	Reject	
GS	Kuhajda et al.	2007	Morphological comparisons of I	Journal of Applied Ichthyology	Pallid sturgeon and shovelnose sturgeon	Scaphirhynchus platoryhnchus a	Yes	Yes	No			Reject	
GS	Lawlor and Hutchings	2004	Consequences to fitness-relatec	Journal of Fish Biology	Atlantic salmon	Salmo salar	N/A				Abstract of oral	Reject	
GS	Leary et al.	1985	Developmental instability as an	Transactions of the American Fi	Cutthroat trout	Oncorhynchus clarkii	Yes	Yes	Yes	Yes	Meristic not mc	Reject	
GS	Lenhardt et al.	2004	Comparative analysis of morph	Journal of Fish Biology	Sterlet	Acipenser ruthenus	Yes	Yes	Yes	Yes	Abstract of oral	Reject	
GS	Lewis et al.	2004	Morphological description of th	Aquaculture	Atlantic halibut	Hippoglossus hippoglossus	No					Reject	
GS	Lorenzen et al.	2004	Domestication, comparative bic	Journal of Fish Biology	Review		N/A				Abstract of oral	Reject	
GS	Lu		Vertebral deformities in hatche	Chinese Journal of Oceanology a	Olive flounder	Paralichthys olivaceus	No					Reject	
GS	Lundsgaard-Hansen et al.	2013	Adaptive plasticity and genetic c	Journal of Evolutionary Biology	Whitefish	Coregonus sp.	Yes	Yes	No			Reject	
GS	Malmquist	1992	Phenotype-specific feeding beh	Oecologia	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject	
GS	Matsuoka	2003	Comparison of meristic variatio	Japan Agricultural Research Qua	Red sea bream	Pagrus major	No					Reject	
GS	Mayer et al.	2011	Domestication causes rapid cha	Environmental Biology of Fishes	Atlantic cod	Gadus morhua	No					Reject	
GS	McDonald et al.	1998	Condition and performance of ju	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	No					Reject	
GS	McGinnity et al.	1997	Genetic impact of escaped farm	ICES Journal of Marine Science:	Atlantic salmon	Salmo salar	No					Reject	
GS	McGuigan et al.	2005	Phenotypic divergence along lin	The American Naturalist	Rainbow fish	Melanotaenia eachamensis	Yes	Yes	Yes	Yes	Does not prese	Reject	
GS	McLaughlin and Grant	1994	Morphological and behavioural	Environmental Biology of Fishes	Brook charr	Salvelinus fontinalis	Yes	Yes	No			Reject	
GS	McLean et al.	2003	Differential reproductive succes	Canadian Journal of Fisheries an	Rainbow trout	Oncorhynchus mykiss	No					Reject	
GS	Monet et al.	2006	Geometric morphometrics reve	Aquatic Living Resources	Brown trout	Salmo trutta	Yes	Yes	No			Reject	
GS	Morris et al.	2008	Prevalence and recurrence of es	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	No					Reject	
GS	Morton and Volpe	2002	A description of escaped farme	Alaska Fishery Research Bulletin	Atlantic salmon	Salmo salar	No					Reject	
GS	Nakaya et al.	2013	Differences in body proportions	Acta Ichthyologica et Piscatoria	Pacific herring	Clupea pallasii	Yes	Yes	Yes	No		Reject	
GS	Paez et al.	2010	The genetic basis of early-life m	Journal of Evolutionary Biology	Atlantic salmon	Salmo salar	Yes	Yes	No		Laboratory and	Reject	

GS	Pakkasmaa and Piironen	2001	Water velocity shapes juvenile s	Evolutionary Ecology	Atlantic salmon and Brown trout	Salmo salar; Salmo trutta	Yes	Yes	No			Reject
GS	Park et al.	2012	Phenotypic plasticity of the thre	Current Zoology	Threespine stickleback	Gasterosteus aculeatus	No	Yes	Yes	Yes	Only brain morp	Reject
GS	Pavey et al.	2010	Contrasting ecology shapes juve	Transactions of the American Fi	Sockeye salmon	Oncorhynchus nerka	Yes	Yes	No			Reject
GS	Poppe et al. 2003	2003	Heart morphology in wild and fa	Diseases of Aquatic Organisms	Atlantic salmon and Rainbow trout	Salmo salar; Oncorhynchus myki	No					Reject
GS	Proulx and Magnan	2004	Contribution of phenotypic plas	Evolutionary Ecology Research	Brook charr	Salvelinus fontinalis	Yes	Yes	No			Reject
GS	Pujular et al.	2004	Distribution of genetic variation	Journal of Fish Biology	European eel	Anguilla anguilla	N/A				Abstract of oral	Reject
GS	Pulcini et al.	2014	Rainbow trout (Oncorhynchus n	Aquaculture Research	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	No			Reject
GS	Reyes-Gavilan et al.	1997	The ontogenic development of l	Canadian Journal of Fisheries an	Brown trout	Salmo trutta	Yes	Yes	No			Reject
GS	Riddell and Leggett	1981	Evidence of an adaptive basis fo	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
GS	Sfakianakis et al.	2013	Lateral line deformities in wild a	Journal of Applied Ichthyology	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	No				Lateral line and	Reject
GS	Sheehan et al.	2005	Marine growth and morphomet	Transactions of the American Fi	Atlantic salmon	Salmo salar	Yes	Yes	No		Supplemental h	Reject
GS	Skulason et al.	1989	Ontogeny of trophic morpholog	Biological Journal of the Linnear	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
GS	Solem et al.	2014	Morphological and genetic com	Fisheries Management and Ecol	Atlantic salmon and Brown trout	Salmo salar; Salmo trutta	Yes	Yes	No			Reject
GS	Stringwell et al.	2014	Maladaptation and phenotypic	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	No		Reject
GS	Svasand et al.	1998	Review of morphological and bc	Fisheries Management and Ecol	Review		N/A				Review paper	Reject
GS	Svåsand et al.	1996	Differences in growth performa	Journal of Fish Biology	Atlantic cod	Gadus morhua	No		No			Reject
GS	Swain and Holtby	1989	Differences in morphology and l	Canadian Journal of Fisheries an	Coho salmon	Oncorhynchus kisutch	Yes	Yes	No			Reject
GS	Taylor and McPhail	1985	Variation in body morphology a	Canadian Journal of Fisheries an	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	Yes	Cannot extract	Reject
GS	Tchernavin	1938	Changes in the salmon skull	Transactions of the Zoological S	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
GS	Thorpe	1991	Acceleration and deceleration e	Aquaculture			No					Reject
GS	Traversi et al.	2010	Morphological and biochemical	Biologia Marina Mediterranea	European sea bass	Dicentrarchus labrax	No					Reject
GS	Turchini et al.	2008	Traceability and discrimination	Journal of Agricultural and Food	Murray cod	Maccullochella peelii	No					Reject
GS	Tymchuk and Devlin	2005	Growth differences among first	Aquaculture	Rainbow trout	Oncorhynchus mykiss	No					Reject
GS	Varian and Nichols	2010	Heritability of morphology in br	PLOSone	Brook charr	Salvelinus fontinalis	Yes	Yes	No			Reject
GS	Vincent	1960	Some influences of domesticati	Transactions of the American Fi	Brook charr	Salvelinus fontinalis	Yes	Yes	Yes	Yes	Does not preser	Reject
GS	Vladykov	1962	Osteological studies on Pacific s	Fisheries Research Board of Canada - Bulletin No. 136		Oncorhynchus sp.	Yes	Yes	No			Reject
GS	Webb and Youngson	1992	Reared Atlantic salmon, Salmo s	Aquaculture Research	Atlantic salmon	Salmo salar	No					Reject
GS	Webb et al.	1991	The spawning behaviour of esca	Aquaculture	Atlantic salmon	Salmo salar	No					Reject
GS	Weber and Fausch	2003	Interactions between hatchery a	Canadian Journal of Fisheries and Aquatic Sciences			No				Good review of	Reject
GS	Whiteley	2009	Trophic polymorphism in a river	Biological Journal of the Linnear	Mountain whitefish	Prosopium williamsoni	Yes	Yes	No			Reject
GS	Wiper et al.	2014	Early experience and reproducti	Canadian Journal of Fisheries an	Chinook salmon	Oncorhynchus tshawytscha	No					Reject
GS	Yonekura et al.	2007	Difference in the predation imp	Biological Journal of the Linnear	Bluegill sunfish	Lepomis macrochirus	Yes	Yes	No			Reject
GS	Youngson et al.	1997	Frequency of occurance of rear	ICES Journal of Marine Science:	Atlantic salmon	Salmo salar	No					Reject
GS	Yurtseva et al.	2014	Atlantic salmon (Salmo salar L.)	Journal of Applied Ichthyology	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
WoS	Almeida et al.	2008	Fluctuating asymmetry, abnorm	Aquaculture	Gold fish and Common carp	Carassius auratus; Cyprinus carp	Yes	Yes	No		Cultured were f	Reject
WoS	Arbour et al.	2011	Morphometric and genetic anal	Canadian Journal of Fisheries an	Arctic charr	Salvelinus alpinus	Yes	Yes	No			Reject
WoS	Arechavala-Lopez et al.	2012	Discriminating farmed gilthead	Journal of Fish Biology	Gilthead sea bream and European sea bas	Sparus aurata; Dicentrarchus lat	No					Reject
WoS	Arechavala-Lopez et al.	2012	Discriminating farmed gilthead	Journal of Fish Biology	Gilthead sea bream and European sea bas	Sparus aurata; Dicentrarchus lat	No					Reject
WoS	Arechavala-Lopez et al.	2013	Differentiating the wild or farm	Reviews in Aquaculture	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	Yes	No			Review paper	Reject
WoS	Bamberger	2009	Semi-natural incubation technic	Aquaculture	Atlantic salmon	Salmo salar	No					Reject
WoS	Belk et al.	2008	Hatchery-induced morphologicz	Canadian Journal of Fisheries an	June sucker	Chamistes liorus	Yes	Yes	Yes	No	Cultured reared	Reject
WoS	Berejikian et al.	2001	Male competition and breeding	Canadian Journal of Fisheries an	Coho salmon	Oncorhynchus kisutch	No					Reject
WoS	Bosakowski and Wagner	1994	Assessment of fin erosion by co	Canadian Journal of Fisheries an	Rainbow trout, Cutthroat trout and Brow	Oncorhynchus mykiss; Oncorhyr	Yes	Yes	Yes	No	Specifically look	Reject
WoS	Carillo et al.	2001	Morphological malformations o	Aquaculture	Gilthead sea bream	Sparus aurta	No					Reject
WoS	Carl and Healey	1984	Differences in enzyme frequenc	Canadian Journal of Fisheries an	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	No			Reject

WoS	Claytor and MacCrimmon	1988	Morphometric and mersitic vari	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
WoS	DeMarais and Minckley	1993	Genetics and morphology of Yac	Biological Conservation	Yaqui chub	Gila purpurea	Yes	Yes	No		Compares popu	Reject
WoS	Domagala et al.	2005	Characteristics of sexual matura	Acta Zoologica Lituanica	Atlantic salmon	Salmo salar	No				Body condtion (	Reject
WoS	Fleming and Gross	1993	Breeding success of hatchery an	Ecological Applications	Coho salmon	Oncorhynchus kisutch	No			No	Morphological a	Reject
WoS	Ford et al.	2008	Estimates of natural selection in	Conservation Biology	Coho salmon	Oncorhynchus kisutch	Yes	Yes	Yes	Yes	Data not report	Reject
WoS	Friedland et al.	1994	Discrimination of Norwegian far	Fisheries Management and Ecol	Atlantic salmon	Salmo salar	No				Scale features	Reject
WoS	Gil et al.	2014	Adapting to the wild: the case o	Journal of Fish Biology	Meagre	Argyorosomus regius	No				Only measured	Reject
WoS	Goetz et al.	2010	A genetic basis for phenotypic d	Molecular Ecology	Lake trout	Salvelinus namaycush	Yes	Yes	No			Reject
WoS	Harrell and Strand	1995	Differentiation of striped bass, h	Fisheries Management and Ecol	Striped bass	Morone saxatilis	Yes	No	No		Hybrids betwee	Reject
WoS	Hawkins and Quinn	1996	Critical swimming velocity and a	Canadian Journal of Fisheries an	Cutthroat trout and Rainbow trotu	Oncorhynchus clarkii; Oncorhyn	Yes	Yes	No		Hatchery fish or	Reject
WoS	Kapusta et al.	2013	Impact of diet and culture cond	Journal of Applied Animal Resea	Crucian carp	Carassius carassius	Yes	Yes	No			Reject
WoS	Kim et al.	2013	Morphometric changes in the st	Journal of Environmental Biolog	Starry eyed flounder	Platichthys stellatus	Yes	Yes	Yes	No	Wild fish are re	Reject
WoS	Lõhmus et al.	2010	Effects of temperature and grov	Journal of Fish Biology	Coho salmon	Oncorhynchus kisutch	No					Reject
WoS	Loy et al.	1999	Geometric morphometrics and	Journal of Applied Ichthyology	Gilthead sea bream	Sparus aurta	Yes	Yes	No			Reject
WoS	Loy et al.	2000	Geometric morphometrics and	Aquaculture	European sea bass	Dicentrarchus labrax	Yes	Yes	No		Very good desc	Reject
WoS	Matsuoka	2003	Comparisone of meristic variati	Japan Agricultural Research Qua	Red sea bream	Pagrus major	No					Reject
WoS	Mayer et al.	2011	Domestication causes rapid changes in heart and brain morphol		Atlantic cod	Gadus morhua	No					Reject
WoS	McDonald et al.	1998	Condition and performance of ju	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	No					Reject
WoS	McFarlane et al.	2000	Larval growth and development	Journal of Fish Biology	Pacific bonito	Sarda chiliensis	Yes	No	No			Reject
WoS	Mohler et al.	2000	Growth and survival of first-feed	North American Journal of Aqua	Atlantic sturgeon	Acipenser oxyrinchus	No					Reject
WoS	Moran et al.	1997	Fluctuating asymmetry and isoz	Transactions of the American Fi	Atlantic salmon	Salmo salar	Yes	Yes	Yes	No	Morphological (	Reject
WoS	Proman and Reynolds	2000	Differences in head shape of th	Fisheries Management and Ecol	European eel	Anguilla anguilla	Yes	Yes	Yes	Yes	Two morphs of	Reject
WoS	Pulcini et al.	2014	Rainbow trout (Oncorhynchus n	Aquaculture Research	Rainbow trout	Oncorhynchus mykiss	Yes	Yes	No			Reject
WoS	Rogell et al.	2012	Strong divergence in trait mean	Molecular Ecology	Brown trout	Salmo trutta	No				Length only	Reject
WoS	Rouleau et al.	2010	Effects of morphology on swimr	Functional Ecology	Brown trout	Salvelinus fontinalis	Yes	Yes	Yes	Yes	Does not preser	Reject
WoS	Sfakianakis et al.	2013	Lateral line deformities in wild a	Journal of Applied Ichthyology	European seabass and gilthead sea bream	Dicentrarchus labrax; Sparus aur	No				Lateral line and	Reject
WoS	Skjæraasen et al.	2008	The expression of secondary sex	ICES Journal of Marine Science:	Atlantic cod	Gadus morhua	No					Reject
WoS	Solem and Berg	2011	Morphological differences in Pa	Journal of Fish Biology	Atlantic salmon	Salmo salar	Yes	Yes	No			Reject
WoS	Trapani	2003	Morphological variability in the	Journal of Fish Biology	Cuatro cienegas cichlid	Cichlasoma minckleyi	Yes	Yes	Yes	Yes	Does not preser	Reject
ASFA	Felt	2013	The effect of food ration on gro	?	Lake trout	Salvelinus namaycush	No				MSc Thesis	Reject
ASFA	Hedenskog et al.	1997	Morphological comparison of ni	Nordic Journal of Freshwater Re	Atlantic salmon and Brown trout	Salmo salar; Salmo trutta	Yes	Yes	No			Reject
ASFA	Krupka et al.	1989	Toward the preservation of end	?	Common carp	Cyprinus carpio					Not available	Reject
ASFA	Kulijev and Agayarova	1984	Ecological-morphometrical char	?	Common carp	Cyprinus carpio	Yes	Yes	No			Reject
ASFA	Matsumiya et al.	1984	Morphometric comparison and	Bulletin of the Japanese Society	Red sea bream	Pagrus major	Yes	Yes	Yes	No		Reject
ASFA	Murphy et al.	2007	Morphometric variation among	Journal of Applied Ichthyology		Scaphirhynchus	Yes	Yes	No		Wild fish are re	Reject
ASFA	Romanov	1984	Effect of culture condition on sk	Aquaculture	Masou salmon	Oncorhynchus masou					Not available	Reject
ASFA	Sarkar et al.	2009	Stock identification of endanger	Electronic Journal of Ichthyology		Chitala chitala	Yes	Yes	Yes	Yes	Insufficient dat	Reject
ASFA	Wessel	?	Morphological characteristics of	Thesis	Chinook salmon	Oncorhynchus tshawytscha	Yes	Yes	Yes	No		Reject
ASFA	Witte	1983	Consistency and functional signi	Netherlands Journal of Zoology		Haplochromis squamipinnis					Not available	Reject
ASFA	Wund et al.	2008	A test of the "Flexible Stem" mo	The American Naturalist	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	No			Reject
ASFA	Yurtseva et al.	2010	Effect of hatchery environment	Journal of Applied Ichthyology	Atlantic salmon	Salmo salar	Yes	Yes	Yes	Yes	Only dentary, p	Reject
Cited	Fukuhara et al.	1981	Observations of morphology an	Bulletin of the Nansei Regional	Fisheries Research Laboratory	Evynnis japonica	Yes	No				Reject
Cited	Kimmel et al.	2008	Allometric change accompanies	Behaviour	Threespine stickleback	Gasterosteus aculeatus	Yes	Yes	No		Does not direct	Reject
Cited	Kohno et al.	1993	Morphological development of	Japanese Journal of Ichthyology	Brown-marbled grouper	Epinephelus fuscoguttatus	Yes	Yes	Yes	Yes	Does not direct	Reject
GS	Taylor et al.	2010	Potential for domesticated-wild	Canadian Journal of Fisheries an	Atlantic salmon	Salmo salar	No					Reject

WoS	Smits et al.	1996	Functional changes in the anato	Biological Journal of the Linnear	Alluad's haplo	Astatoreochromis alluaudi	Yes	Yes	Yes	Yes	Cultured fish nc	Reject	
WoS	Sundell et al.	1998	Wild and hatchery-reared brow	Aquaculture	Brown trout	Salmo trutta	Yes	Yes	Yes	Yes	Only condition,	Reject	
WoS	Wimberger	1992	Plasticity of fish shape. The effe	Biological Journal of the Linnear	Redhump eartheater and pearl cichlid	Geophagus steindachneri and G	Yes	Yes	Yes	Yes	Cultured/wild l	Reject	

Supplementary Table 3.4 Summary of meta-analysis of qualitative morphological differences between cultured and wild fish. Heading abbreviations are as follows: LH is life history, Up is upper, Low is lower, K is Fulton's condition factor, Caud Ped is caudal peduncle, Pec is pectoral, Pel is pelvic, L is length, D is depth, W is width, H is height, and F is fin. Within the table, Unk indicates that the data was not provided, or was ambiguous in the study. Comparisons of wild-caught fish to cultured are denoted WF, while CG indicates the fish were compared in a common garden. Different is abbreviated Diff. and Immature, Imm. C>W denotes studies in which the expression of the trait in cultured fish is greater than in the wild to which they were compared, C<W indicates the opposite and C=W indicates a trait was measured, but no difference was detected. Blank spaces signify that a trait was not measured in a given study. Species abbreviations and their corresponding common, and binomial names are listed. Where more than one comparison was conducted in a study, this is noted and the populations or comparison are noted.

Species	Comparison	Culture	Domestication	Population	LH	Head L	Head D	Eye	Up Jaw L	Low Jaw L	Body D	K	Caud Ped D	Caud Ped L	Pec F L	Pel F L	Dorsal F L	Dorsal F W	Anal F L	Anal F W	Caudal F L	Caudal F H	Study	Notes
AC	WF	Farm	Unk	Unk	Adult	C<W			C<W	C<W		C>W		C=W	C<W								Uglem et al. 2011	
AC	WF	Farm	1	Same	Adult	C<W	C<W	C<W	C<W	C<W	C<W	C<W	C=W	C<W		C<W	C<W	C<W	C<W	C<W			Wringe et al. 2015	
AS	WF	Farm	≥2	Diff	Imm	C<W	C=W				C>W		C=W	C=W	C=W	C=W	C=W	C=W	C=W	C=W			Enders et al. 2004	
AS	WF	Farm	≥2	Diff	Adult	C>W	C>W	C=W	C=W	C>W	C>W		C=W	C=W	C>W	C>W	C>W	C>W	C>W	C=W			Fleming et al. 1994	1
AS	WF	Farm	≥2	Diff	Adult	C=W	C=W	C=W	C=W	C<W			C=W	C=W	C>W	C>W	C>W	C>W	C>W	C=W			Fleming et al. 1994	2
AS	WF	Farm	Unk	Unk	Adult										C<W		C<W				C<W		Lund et al. 1989	
ASs	WF	Farm	≥2	Diff	Unk						C>W					C>W							Crichigno et al. 2014	
BT	WF	Farm	≥2	Unk	Adult	C=W	C=W	C=W		C=W	C=W				C=W	C=W	C=W		C=W	C=W	C=W	C=W	Lahnsteiner and Jagsch 2005	3
BT	WF	Farm	≥2	Unk	Adult	C=W	C=W	C<W	C=W		C>W				C<W	C=W	C=W		C<W	C=W	C<W	C=W	Lahnsteiner and Jagsch 2005	4
ESB	WF	Farm	>2	Diff	Imm	C<W	C<W	C<W			C>W	C>W	C>W	C>W				C<W		C<W			Arechavala-Lopez et al. 2012	5
ESB	WF	Farm	Unk	Diff	Imm	C<W	C<W	C<W			C>W		C>W	C>W	C<W	C<W		C<W		C<W	C<W		Arechavala-Lopez et al. 2012	6
EuP	WF	Farm	Unk	Unk	Adult	C>W			C>W		C<W	C>W	C=W	C=W			C=W	C=W			C=W	C=W	Mairesse et al. 2005	7
EuP	WF	Farm	Unk	Unk	Adult	C=W			C=W		C>W	C>W	C=W	C=W			C=W	C=W			C=W	C=W	Mairesse et al. 2005	8
GSB	WF	Farm	Unk	Diff	Imm	C>W	C<W	C<W			C>W	C>W	C=W	C<W				C<W		C<W			Arechavala-Lopez et al. 2012	9
GSB	WF	Farm	Unk	Diff	Imm	C<W	C>W	C<W			C>W		C<W	C<W	C<W	C<W		X>W		C>W	C<W		Arechavala-Lopez et al. 2012	10
GSB	WF	Farm	Unk	Unk	Imm	C>W			C>W		C=W	C>W	C=W		C<W	C<W	C<W	C<W	C<W	C<W			Rogdakis et al. 2011	
GSB	WF	Farm	≥2	Diff	Adult	C=W	C>W	C>W			C>W		C>W	C>W	C>W	C<W	C<W	C=W	C<W	C>W	C>W		Segvic-Bubic et al. 2014	
OF	WF	Farm	Unk	Unk	Adult	C=W					C>W	C>W											Park et al. 2012	
PM	WF	Farm	Unk	Same	Unk	C>W	C<W	C<W			C>W		C>W	C>W	C<W	C<W	C<W	C<W	C<W		C>W		Patiyal et al. 2013	

PP	WF	Farm	≥2	Diff	Unk						C>W	C<W												Crichigno et al. 2014	
AH	WF	Hatchery	1	Unk	Imm	C>W	C>W																	Balbontin et al. 1973	
ArC	WF	Hatchery	1	Same	Adult	C<W	C>W	C<W	C<W	C<W														Adams and Huntingford 2004	
ArC	WF	Hatchery	1	Same	Adult			C<W	C<W				C>W		C<W		C<W		C<W	C>W	C>W			Klemetsen et al. 2002	11
ArC	WF	Hatchery	1	Same	Adult			C>W	C<W				C>W		C<W		C<W		C>W	C>W	C<W			Klemetsen et al. 2002	12
AS	WF	Hatchery	1	Same	Imm	C=W	C<W	C=W			C=W		C<W	C=W	C=W					C=W	C=W			Blanchet et al. 2008	
AS	WF	Hatchery	Unk	Same	Imm*	C>W	C>W		C=W	C>W	C>W		C>W	C=W	C<W	C>W	C>W	C=W	C>W	C>W	C>W			Fleming et al. 1994	13
AS	WF	Hatchery	Unk	Unk	Imm	C<W		C=W	C=W	C<W	C>W	C>W	C<W	C<W	C<W	C<W	C<W	C<W	C<W	C<W	C<W	C<W		Kazakov and Semenova 1986	
AS	WF	Hatchery	1	Same	Imm							C=W			C<W									Pedersen et al. 2008	
AS	WF	Hatchery	Unk	Unk	Imm	C<W	C>W	C>W	C>W	C<W														Salmanov 1986	
AS	WF	Hatchery	Unk	Unk	Imm	C<W	C>W	C>W	C>W	C>W														Salmanov 1989	14
AS	WF	Hatchery	Unk	Unk	Imm	C>W	C>W	C>W	C>W	C>W														Salmanov 1989	15
AS	WF	Hatchery	Unk	Unk	Imm	C>W	C>W	C>W	C>W	C>W														Salmanov 1989	16
AS	WF	Hatchery	≥2	Same	Imm		C<W		C<W		C>W		C=W	C=W					C=W		C=W			von Cramon-Taubadel et al. 2006	
AS	WF	Hatchery	≥2	Same	Imm	C<W			C<W				C<W											Wilkins et al. 1994	17
AS	WF	Hatchery	≥2	Same	Imm	C<W			C<W				C<W											Wilkins et al. 1994	18
AS	WF	Hatchery	≥2	Same	Imm	C<W			C<W				C<W											Wilkins et al. 1994	19
BFF	WF	Hatchery	Unk	Unk	Imm	C<W		C<W	C=W		C<W				C=W									Aritaki et al. 2000	
BhC	WF	Hatchery	1	Same	Imm	C=W		C=W			C=W		C<W	C=W	C=W					C=W		C=W	C=W	Kerschbaumer et al. 2011	
BT	WF	Hatchery	1	Same	Imm							C>W			C<W									Pedersen et al. 2008	
BT	WF	Hatchery	1	Same	Imm	C<W	C=W		C<W		C=W		C=W	C=W					C=W		C=W			Vehanen and Huusko 2011	
BT	WF	Hatchery	≥2	Same	Imm	C<W			C<W				C<W											Wilkins et al. 1994	20
BT	WF	Hatchery	≥2	Same	Imm	C<W			C<W				C<W											Wilkins et al. 1994	21
ChS	WF	Hatchery	≥2	Diff	Imm	C>W	C>W	C>W	C>W		C<W		C=W	C<W					C=W		C<W			Tiffan and Connor 2011	
CoS	WF	Hatchery	1	Same	Adult	C<W	C>W		C=W		C>W		C<W	C<W	C=W	C=W	C<W	C>W	C<W	C>W				Hard et al. 2000	
CoS	WF	Hatchery	≥2	Diff	Imm	C<W	C<W		C<W		C<W		C<W	C<W			C<W	C=W	C<W	C<W	C<W	C<W	C<W	Swain et al. 1991	
CoS	WF	Hatchery	1	Diff	Imm	C=W					C=W	C=W	C=W	C=W		C<W								Taylor 1986	
JM	WF	Hatchery	Unk	Diff	Unk	C=W	C>W	C=W	C=W		C>W				C<W				C=W		C=W			Suda et al. 1986	
LmB	WF	Hatchery	≥2	Diff	Imm	C<W	C>W		C<W	C<W														Wintzer and Motta 2005	
Roh	WF	Hatchery	Unk	Unk	Unk	C>W		C>W			C>W		C>W	C>W	C>W	C>W	C>W		C>W		C>W			Wagle and Pradhan 2013	22
Roh	WF	Hatchery	Unk	Unk	Unk	C>W		C>W			C>W		C>W	C>W	C>W	C>W	C>W		C>W		C>W			Wagle and Pradhan 2013	23
RSB	WF	Hatchery	Unk	Unk	Imm	C=W	C<W	C<W	C<W		C<W		C=W		C<W	C=W	C<W		C<W		C=W			Matsumiya and Kanamaru 1987	
SSB	WF	Hatchery		Unk	Imm				C<W		C>W		C>W	C>W					C<W		C<W	C>W		Kouttouki et al. 2006	
ST	WF	Hatchery	1	Same	Imm	C<W			C=W			C>W			C<W									Lenhardt et al. 2012	
Tau	WF	Hatchery	1	Unk	Adult	C>W		C>W			C>W		C>W											Schwartz et al. 2005	
TUR	WF	Hatchery	Unk	Same	Imm						C>W													Ellis et al. 1997	
YfT	WF	Hatchery	1	Unk	Imm	C<W		C=W	C<W	C<W	C<W		C<W											Shimizu and Shiozawa 2004	
AD	WF	Lab	1	Unk	Imm	C>W			C>W		C>W			C<W	C>W									Murphy et al. 2007	
Blt	WF	Lab	1	Same	Adult	C>W		C<W	C<W						C=W	C=W	C>W		C>W					Todd et al. 1981	
Gup	WF	Lab	1	Same	Adult	C=W		C<W			C<W		C<W	C=W					C<W		C>W			Burns et al. 2009	24
Gup	WF	Lab	1	Same	Adult	C>W		C>W			C>W		C>W	C>W					C>W		C<W			Burns et al. 2009	25
Gup	WF	Lab	1	Same	Adult	C>W		C>W			C>W		C>W	C=W					C>W		C<W			Burns et al. 2009	26
Gup	WF	Lab	1	Same	Adult	C=W		C<W			C<W		C<W	C<W					C<W		C>W			Burns et al. 2009	27
Ky	WF	Lab	1	Same	Adult	C=W		C=W	C<W						C=W	C=W	C>W			C>W				Todd et al. 1981	
LER	WF	Lab	≥2	Unk	Adult						C<W		C<W	C>W					C<W		C>W			McGuigan et al. 2003	28
LER	WF	Lab	≥2	Unk	Adult						C<W		C<W	C>W					C<W		C=W			McGuigan et al. 2003	29
LjC	WF	Lab	1	Same	Adult	C=W		C=W	C=W						C>W	C=W	C>W			C>W				Todd et al. 1981	
Mid	WF	Lab	Unk	Same	Adult	C=W		C=W	C=W		C>W		C<W	C=W	C=W	C=W			C>W		C=W			Barlow and Munsey 1976	
MR	WF	Lab	≥2	Diff	Adult	C=W		C>W			C<W		C=W		C=W	C=W	C=W	C=W	C=W	C=W	C=W	C>W		Taylor 2003	30
MR	WF	Lab	≥2	Same	Adult	C=W		C>W			C=W		C=W		C=W	C=W	C=W	C=W	C=W	C=W	C>W			Taylor 2003	31
SEN	WF	Lab	1	Same	Imm	C>W		C>W					C>W								C>W			Gozlan et al. 1998	
SjC	WF	Lab	1	Same	Adult	C>W		C<W	C>W						C=W	C>W	C>W		C=W					Todd et al. 1981	
Thr	WF	Lab	Unk	Same	Adult	C>W		C>W	C>W		C<W					C<W	C<W							Kitano et al. 2007	
Thr	WF	Lab	1	Same	Adult			C=W		C=W	C>W				C=W	C=W								Leaver and Reimchen 2012	32
Thr	WF	Lab	1	Same	Adult			C=W		C=W	C>W				C>W	C>W								Leaver and Reimchen 2012	33
Thr	WF	Lab	1	Same	Adult	C<W					C>W		C>W	C>W										McCairns and Bernatchez 2012	34
Thr	WF	Lab	1	Same	Adult	C>W	C>W				C>W		C>W	C>W										McCairns and Bernatchez 2012	35
Thr	WF	Lab	1	Same	Adult	C=W	C=W	C=W	C=W		C=W					C=W								McPhail 1984	36
Thr	WF	Lab	1	Same	Adult	C=W	C=W	C=W	C=W		C=W					C=W								McPhail 1984	37
Thr	WF	Lab	1	Same	Adult						C>W		C>W		C>W	C<W			C>W			C=W		Sharpe et al. 2008	38
Thr	WF	Lab	1	Same	Adult						C>W		C>W		C<W	C<W			C>W			C<W		Sharpe et al. 2008	39

Thr	WF	Lab	1	Same	Adult						C>W		C>W		C<W	C<W		C<W			C>W		Sharpe et al. 2008	40
TsG	WF	Lab	1	Same	Imm	C<W		C<W	C=W		C<W					C<W							Morioka et al. 2012	
AS	CG	Farm	≥2	Diff	Imm	C=W	C<W			C<W	C<W		C<W	C>W	C=W		C>W	C<W	C=W	C>W	C>W	C>W	Einum 1996	41
AS	CG	Farm	≥2	Diff	Imm	C=W	C=W			C=W	C=W		C<W	C=W	C=W		C=W	C=W	C=W	C=W	C=W	C=W	Einum 1996	42
AS	CG	Farm	≥2	Same	Imm	C<W	C=W				C>W		C=W	C=W	C<W	C<W	C=W	C<W	C<W	C=W			Fleming and Einum 1997	
AS	CG	Farm	≥2	Diff	Imm	C=W	C<W	C=W	C=W		C<W	C=W	C=W	C<W				C=W		C=W			Fraser et al. 2010	43
AS	CG	Farm	≥2	Diff	Imm	C>W	C=W	C=W	C=W		C=W	C=W	C=W	C=W			C>W	C=W		C=W			Fraser et al. 2010	44
AS	CG	Farm	≥2	Diff	Imm	C=W	C=W	C=W	C=W	C=W	C<W	C<W	C=W	C>W			C<W	C=W		C=W			Morris et al. 2011	
AS	CG	Farm	≥2	Diff	Imm		C>W	C<W		C>W	C>W		C>W	C<W	C<W	C<W					C=W		Solem et al. 2006	
CC	CG	Farm	≥2	Diff	Imm	C>W	C>W	C=W	C=W		C<W		C=W	C=W				C<W		C=W			Matsuzaki et al. 2009	
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C>W											Suzuki and Yamaguchi 1980	45
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C=W											Suzuki and Yamaguchi 1980	46
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C>W											Suzuki and Yamaguchi 1980	47
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C>W											Suzuki and Yamaguchi 1980	48
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C=W											Suzuki and Yamaguchi 1980	49
CC	CG	Farm	≥2	Diff	Adult	C=W					C>W	C>W											Suzuki and Yamaguchi 1980	50
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C>W											Suzuki and Yamaguchi 1980	51
CC	CG	Farm	≥2	Diff	Adult	C>W					C>W	C>W											Suzuki and Yamaguchi 1980	52
OF	CG	Farm	Unk	Same	Imm						C>W	C>W											Kim et al. 2011	
ArC	CG	Hatchery	≥2	Diff	Imm	C>W	C>W				C<W		C<W	C>W	C=W			C=W		C=W	C<W		Janhunen et al. 2009	53
ArC	CG	Hatchery	≥2	Diff	Imm	C>W	C>W				C=W		C=W	C=W	C=W			C=W		C=W	C=W		Janhunen et al. 2009	54
ArC	CG	Hatchery	≥2	Diff	Adult	C=W	C=W				C<W		C<W	C=W	C<W			C<W		C<W	C<W		Janhunen et al. 2009	55
ArC	CG	Hatchery	≥2	Diff	Adult	C=W	C=W				C<W		C<W	C=W	C>W			C>W		C>W	C<W		Janhunen et al. 2009	56
ChS	CG	Hatchery	≥2	Same	Imm	C=W	C<W		C<W		C<W	C=W	C<W	C>W				C=W		C=W			Wessel et al. 2006	
CoS	CG	Hatchery	≥2	Same	Imm	C=W	C<W		C<W		C=W		C=W	C=W			C<W	C=W	C=W	C<W	C=W	C<W	Swain et al. 1991	
RT	CG	Hatchery	Unk	Diff	Imm	C>W	C>W		C=W		C>W	C=W	C>W	C<W			C>W		C>W				Pulcini et al. 2013	

Species abbreviations: AC, Atlantic cod (*Gadus morhua*); AD, Ambon damselfish (*Pomacentrus amboinensis*); AH, Atlantic herring (*Clupea harengus*); ArC, Arctic charr (*Salvelinus alpinus*); AS, Atlantic salmon (*Salmo salar*); ASs, Atlantic silverside (*Odontesthes bonariensis*); BFF, Barfin Flounder (*Verasper moseri*); BhC, Blunthead cichlid (*Tropheus moorii*); Blt, Bloater (*Coregonus hoyi*); BT, Brown trout (*Salmo trutta*); CC, Common carp (*Cyprinus carpio*); ChS, Chinook salmon (*Oncorhynchus tshawytscha*); CoS, Coho salmon (*Oncorhynchus kisutch*); ESB, European sea bass (*Dicentrarchus labrax*); EuP, European perch (*Perca fluviatilis*); GSB, Gilthead sea bream (*Sparus aurata*); Gup, Guppy (*Poecillia reticulata*); JM, Jack mackerel (*Trachurus japonicus*); Ky, Kiyi (*Coregonus kiyi*); LER, Lake Eacham rainbowfish (*Melanotaenia eachamensis*); LiC, Longjaw cisco (*Coregonus alpenae*); LmB, Largemouth bass (*Micropterus salmoides*); Mid, Midas cichlid (*Amphilophus citrinellus*); MR, Mangrove rivulus (*Kryptolebias marmoratus*); OF, Olive flounder (*Paralichthys olivaceus*); PM, Putitor mahseer (*Tor putitora*); PP, Patagonian perjerry (*Odontesthes hatcheri*); Roh, Rohu (*Labeo rohita*); RSB, Red sea bream (*Pagrus major*); RT, Rainbow trout (*Oncorhynchus mykiss*); SEN, South-west European nace (*Parachondrostoma toxostoma*); SjC, Shortjaw cisco (*Coregonus zenithicus*); SSB, Sharpsnout sea bream (*Diplodus puntazzo*); St, Sterlet (*Acipenser ruthenus*); Tau, Tautog (*Tautoga onitis*); Thr, Threespine stickleback (*Gasterosteus aculeatus*); TsG, Three-spot gourami (*Trichopodus trichopodus*); TUR, Turbot (*Scophthalmus maximus*) YfT, Yellowfin tuna (*Thunnus albacares*)

Notes: 1- Sunndalsora Hatchery 2- Imsa River; 3- Lacustrine morph; 4- Normal morph; 5- Greece Farmed; 6- Spain Farmed; 7- Wild of river origin; 8- Wild of lake origin; 9- Greece Farmed; 10- Spain Farmed; 11- Littoral morph; 12- Profundal morph; 13- Mature male parr; 14- Wild from Umba River; 15- Wild from Luvenga River; 16- Wild from Simijoki River; 17- 1991 generation cultured salmon (91S); 18- 1992 generation cultured salmon (92S); 19- 2NS cultured salmon; 20- 2NT cultured trout; 21- 1992 generation trout (92T); 22- Cultured from Sunsari hatchery; 23- Cultured from Rupandeshi hatchery; 24- Aripo population, high predation; 25- Oropuche population, high predation; 26- Aripo population, low predation; 27- Oropuche population, low predation; 28- Wild are stream population; 29- Wild are lake population; 30- Florida population; 31- Belize population; 32- Wild from Mayer Lake; 33- Wild from Mayer Pond; 34- Saltwater population; 35- Freshwater population; 36- Benthic morph; 37- Limnetic morph; 38- Fish of outlet population; 39- Fish of inlet population; 40- Fish of lake population; 41- Wild from Imsa River; 42- Wild from Lone River; 43- Wild from Stewiake River; 44- Wild from Tusket River; 45- Cultured are Yamamoto strain, reared in running water; 46- Cultured are Asagi strain, reared in running water; 47- Cultured are mirror strain, reared in running water; 48- Cultured are Scaly German strain, reared in running water; 49- Cultured are Yamamoto strain, reared in standing water; 50- Cultured are Asagi strain, reared in standing water; 51- Cultured are Mirrored strain, reared in standing water; 52- Cultured are Scaly German strain, reared in standing water; 53- Cultured from Lake Saimaa; 54- Cultured from Lake Inari; 55- Cultured from Lake Saimaa; 56- Cultured from Lake Inari

Supplementary Table 3.5 Summary of the results of the vote-counting analysis. Results are presented for each category of each moderator. Morph. Feature is short for morphological feature. Diff. w/i stands for difference within category, and is the result of the test of the hypothesis that the proportion studies finding each of the three possible relative differences in morphological feature size between the cultured and wild fish (i.e. C<W = C>W = C=W) are equal for a given morphological feature and category of a moderator. Where significant differences were found, the results of all pairwise comparisons and adjusted p-values are given. Diff. b/w stands for difference between categories, and is the result of the test of the hypothesis that for each of the three possible relative differences in morphological feature size, the proportion of studies finding it did not differ between categories of a moderator. Where significant, and there are more than two categories of a moderator, the results of all pairwise comparisons, and adjusted p-values reported. NS indicates a given test was not significant, and chi-squared and p-values are given for each test. Lab is short for laboratory, CG is common garden, and WF indicates studies in which the cultured fish were compared to wild-caught fish. There were a number of cases in which the sample size was not sufficient for accurate statistical analysis, and these are marked with an asterisk (*). In these cases it was impossible to test for Diff. b/w and this is left blank.

Morph. Feature	Moderator	Diff. w/i	Chisq	p	Prop. findings	Diff.b/w	Chisq	p
Head Length	Overall	NS	1.65	> 0.43				
	Form of culture							
	Farm	NS	3.47	> 0.17	C<W	Farm<Hatch	7.84	< 0.01
	Hatchery	NS	4.02	> 0.13		Farm=Lab	0.41	> 0.51
	Lab		6.79	< 0.05		Lab<Farm	9.87	< 0.01
		C<W < C=W	4.6	< 0.05	C=W	NS	1.01	> 0.60
		C<W = C=W	3.39	> 0.06	C>W	NS	0.01	> 0.99
		C=W = C>W	<0.001	~ 1				
	Commonality of rearing environment							
	CG		13.23	< 0.01	C<W	CG<WF	6.9	< 0.01
		C<W < C=W	6.34	< 0.05	C=W	NS	0.37	> 0.54
		C<W < C>W	10.92	< 0.001	C>W	NS	2.57	> 0.10
		C=W = C>W	0.36	> 0.54				
	WF	NS	0.6	> 0.74				
	Domestication				C<W	NS	0.01	> 0.91
	1 Generation	NS	0.12	>0.94	C=W	NS	0.01	> 0.89
	>2 Generations	NS	1.5	> 0.47	C>W	NS	< 0.0001	~1
	Ancestral population				C<W	NS	2.4	> 0.12
	Diff	NS	5.73	> 0.05	C=W	NS	< 0.0001	~1
	Same	NS	1.15	> 0.56	C>W	NS	1.86	> 0.17
	Salmonid							
	Not		6.19	< 0.05	C>W	NS	3.52	> 0.06
		C<W = C=W	0.22	> 0.64	C=W	NS	2.38	> 0.12
		C<W < C>W	4.67	< 0.05	C>W	Not>Yes	14.51	< 0.001
		C=W = C>W	2.21	> 0.13				
	Yes		18.3	< 0.001				
		C<W = C=W	<0.001	~ 1				
		C<W > C>W	14.4	< 0.001				
		C=W > C<W	12.8	< 0.001				
Head Depth	Overall	NS	3.19	> 0.20				
	Form of culture				C<W	Farm=Hatch	2.91	> 0.08
	Farm	NS	3.47	> 0.17		Farm=Lab	0.41	> 0.51

	Hatchery	NS	3.25	> 0.19		Lab<Hatch	5.04	< 0.05
	Lab		6.79	< 0.05	C=W	NS	2.82	> 0.24
		C<W < C=W	4.6	< 0.05	C>W	NS	1.43	> 0.48
		C<W = C>W	3.39	> 0.06				
		C=W = C>W	< 0.001	~ 1				
	Commonality of rearing environment				C<W	NS	< 0.0001	~1
	CG	NS	0.6	0.74	C=W	NS	0.77	> 0.37
	WF	NS	5.03	> 0.08	C>W	NS	0.42	> 0.51
	Domestication				C<W	NS	< 0.0001	~1
	1 Generation	NS	1	> 0.60	C=W	NS	< 0.0001	~1
	>2 Generations	NS	0.84	> 0.65	C>W	NS	0.06	> 0.79
	Ancestral population				C<W	NS	0.6	> 0.43
	Diff	NS	2.63	> 0.26	C=W	NS	< 0.0001	~1
	Same	NS	0.86	> 0.65	C>W	NS	0.49	> 0.48
	Salmonid				C<W	NS	0.77	> 0.37
	Not	NS	4.2	> 0.12	C=W	NS	1.47	> 0.22
	Yes	NS	2.71	> 0.25	C>W	NS	< 0.0001	~1
Eye Size	Overall	NS	0.39	> 0.82				
	Form of culture		8.06	< 0.05	C<W	NS	2.63	> 0.26
	Farm		8.06	< 0.05	C=W	NS	1.25	> 0.53
		C<W = C=W	0	~1	C>W	Farm<Hatch	5.32	< 0.05
		C<W > C>W	5.56	< 0.05		Farm=Lab	2.32	> 0.12
		C=W > C>W	4.17	< 0.05		Hatch=Lab	0.28	> 0.59
	Hatchery	NS	2.52	> 0.28				
	Lab	NS	0.5	> 0.77				
	Commonality of rearing environment							
	CG							
	WF	NS	0.19	> 0.91				
	Domestication				C<W	NS	0.33	> 0.56
	1 Generation	NS	1.77	> 0.41	C=W	NS	0.03	> 0.84
	>2 Generations	NS	2.79	> 0.24	C>W	NS	< 0.0001	~1
	Ancestral population				C<W	NS	< 0.0001	~1
	Diff	NS	2.4	> 0.30	C=W	NS	0.05	> 0.81
	Same	NS	1.13	> 0.56	C>W	NS	< 0.0001	~1
	Salmonid				C<W	NS	0.32	> 0.56

	Not	NS	0.68	> 0.71	C=W	NS	0.87	> 0.34
	Yes	NS	2.18	> 0.33	C>W	NS	0.01	> 0.93
Upper Jaw Length	Overall		6.00	< 0.05				
		C<W = C=W	0	1				
		C<W > C>W	3.85	< 0.05				
		C=W > C>W	3.85	< 0.05				
	Form of culture				C<W	Farm<Hatch	4.04	< 0.05
	Farm		9	< 0.05		Farm=Lab	< 0.0001	~1
		C<W < C=W	4.29	< 0.05		Hatch=Lab	2.72	> 0.09
		C<W = C>W	0	~1	C=W	Farm>Hatcher	4.57	< 0.05
		C=W > C>W	4.29	< 0.05		Farm=Lab	0.12	> 0.72
	Hatchery		11.04	<0.01		Hatch=Lab	1.13	> 0.28
		C<W > C=W	4.72	< 0.05	C>W	NS	0.78	> 0.67
		C<W > C>W	7.62	< 0.01				
		C=W = C>W	0.11	> 0.74				
	Lab	NS	2.1	>0.34				
	Commonality of rearing environment				C<W	NS	0.06	> 0.80
	CG				C=W	NS	2	> 0.15
	WF		3.42	> 0.18	C>W	NS	0.84	> 0.35
	Domestication							
	1 Generation	NS	5.25	> 0.07				
	> 2 Generations		10.5	< 0.01	C<W	NS	< 0.0001	~1
		C<W = C=W	0.45	> 0.50	C=W	NS	< 0.0001	~1
		C<W > C>W	8.38	< 0.01	C>W	NS	0.01	> 0.91
		C=W > C>W	4.02	< 0.05				
	Ancestral population							
	Diff		1.73	< 0.01	C<W	NS	3.81	> 0.05
		C<W < C=W	4.58	< 0.05	C=W	NS	3.61	> 0.05
		C<W = C=W	0	~1	C>W	NS	< 0.0001	~1
		C=W > C>W	6.77	< 0.01				
	Same		15.24	< 0.001				
		C<W < C=W	4.58	< 0.05				
		C<W = C>W	0	~1				
		C=W > C>W	6.77	< 0.01				
	Salmonid				C<W	NS	1.04	> 0.30
	Not	NS	3.5	> 0.17	C=W	NS	0.61	> 0.43

	Yes	NS	5.72	> 0.05	C>W	NS	< 0.0001	~1
Lower Jaw Length	Overall	NS	1.95	> 0.37				
	Form of culture				C<W	Farm=Hatch	< 0.0001	~1
	Farm	NS	1	~1	C=W	Farm=Hatch	1.6	> 0.20
	Hatchery		7	< 0.05	C>W	Farm=Hatch	0.25	> 0.61
		C<W > C=W	4.43	< 0.05				
		C<W = C=W	0	~1				
		C=W = C>W	2.89	> 0.08				
	Lab							
	Commonality of rearing environment				C<W	NS	0.11	> 0.73
	CG	NS	0.75	> 0.68	C=W	NS	0.41	> 0.51
	WF	NS	3.56	> 0.16	C>W	NS	< 0.0001	~1
	Domestication				C<W	NS	0.04	> 0.82
	1 Generation	NS	4.2	> 0.12	C=W	NS	< 0.0001	~1
	>2 Generation	NS	0.375	> 0.82	C>W	NS	0.18	> 0.67
	Ancestral population				C<W	NS	< 0.0001	~1
	Same	NS	0.43	> 0.80	C=W	NS	< 0.0001	~1
	Diff	NS	0.6	> 0.74	C>W	NS	< 0.0001	~1
	Salmonid				C<W	NS	0.61	> 0.43
	Not				C=W	NS	< 0.0001	~1
	Yes	NS	1.5	> 0.47	C>W	NS	1.91	> 0.16
Body Depth	Overall		32.33	< 0.001				
		C<W = C=W	2.33	> 0.12				
		C<W < C>W	13.40	< 0.001				
		C=W < C>W	27.29	< 0.0001				
	Form of culture							
	Farm		32.12	< 0.0001	C<W	NS	2.69	> 0.26
		C<W = C=W	0.11	> 0.73	C=W	NS	1.46	> 0.48
		C<W < C>W	17.24	< 0.0001	C>W	NS	5.02	> 0.08
		C=W < C>W	21.92	< 0.0001				
	Hatchery	NS	2.19	> 0.33				
	Lab		6.86	< 0.05				
		C<W = C=W	1.18	> 0.27				
		C<W = C>W	0.88	< 0.34				

		C=W < C>W	5.25	< 0.05				
	Commonality of rearing environment				C<W	NS	1.38	> 0.24
	CG	NS	6	> 0.05	C=W	NS	1.26	> 0.26
	WF		10.5	< 0.01	C>W	CG>WF	7.05	< 0.01
		C<W = C=W	1.24	> 0.26				
		C<W < C>W	8.74	< 0.01				
		C=W = C>W	2.7	> 0.09				
	Domestication				C<W	NS	0.65	> 0.41
	1 Generation	NS	5.61	> 0.06	C=W	NS	0.35	> 0.55
	> 2 Generations		8.83	< 0.05	C>W	NS	< 0.0001	~1
		C<W = C=W	2.55	> 0.11				
		C<W = C>W	0.89	> 0.34				
		C=W < C>W	7.46	< 0.01				
	Ancestral population				C<W	NS	0.33	> 0.56
	Diff		17.24	< 0.001	C=W	NS	0.79	> 0.37
		C<W = C=W	2.25	> 0.13	C>W	NS	< 0.0001	~1
		C<W < C>W	4.83	< 0.05				
		C=W < C>W	14.49	< 0.001				
	Same		11.1	< 0.01				
		C<W = C=W	< 0.0001	~1				
		C<W < C>W	7.05	< 0.01				
		C=W < C>W	5.63	< 0.05				
	Salmonid				C<W	NS	0.38	> 0.53
	Not		43.17	< 0.0001	C=W	Not<Yes	4.13	< 0.05
		C<W = C=W	3.27	> 0.07	C>W	Not>Yes	5.28	< 0.05
		C<W < C>W	18.08	< 0.0001				
		C=W < C>W	35.38	< 0.0001				
	Yes	NS	0.33	> 0.84				
Condition	Overall		16.17	< 0.001				
		C<W = C=W	1.81	> 0.17				
		C<W < C>W	13.14	< 0.001				
		C=W < C>W	4.62	< 0.05				
	Form of culture				C<W	NS	0.12	> 0.72
	Farm		15.86	< 0.001	C=W	NS	2.1	> 0.14
		C<W = C=W	0	~1	C>W	NS	0.44	> 0.50

		C<W < C>W	9.88	< 0.01				
		C=W < C>W	7.88	< 0.01				
	Hatchery	NS	5.57	> 0.06				
	Lab	No Samples						
	Commonality of rearing environment				C<W	NS	< 0.0001	~1
	CG		6.64	< 0.05	C=W	NS	1.57	> 0.20
		C<W = C=W	3.05	> 0.08	C>W	NS	0.59	> 0.43
		C<W < C>W	4.37	< 0.05				
		C=W = C>W	0	~1				
	WF		13.71	< 0.01				
		C<W = C=W	0	~1				
		C<W < C>W	7.15	< 0.01				
		C=W < C>W	7.15	< 0.01				
	Domestication							
	1 Generation	Low Sample Size			Low Sample Size			
	> 2 Generations	NS	4.04	> 0.13				
	Ancestral population				Low Sample Size			
	Diff	NS	5.25	> 0.07				
	Same	NS	1.5	> 0.47	Low Sample Size			
	Salmonid				Low Sample Size			
	Not		26.68	< 0.0001				
	Numerically same	C<W = C=W	0	~1				
		C<W < C>W	15.33	< 0.0001				
		C=W < C>W	15.33	< 0.0001				
	Yes	NS	7	> 0.05				
Caudle Peduncle Depth	Overall	NS	0.13	> 0.93				
	Form of culture				C<W	Farm<Hatch	5.42	< 0.05
	Farm		12	< 0.01		Farm=Lab	0.9	> 0.34
		C<W < C=W	8.18	< 0.01		Hatch=Lab	0.55	> 0.45
		C<W = C>W	0.15	> 0.69	C=W	Farm>Hatch	6.17	< 0.05
		C=W > C>W	4.76	< 0.05		Farm>Lab	6.61	< 0.05
	Hatchery	NS	5.7	> 0.05		Hatch=Lab	0.15	> 0.69
	Lab	NS	5.4	> 0.06	C>W	NS	4.18	> 0.12

	Commonality of rearing environment				C<W	NS	0.02	> 0.86
	CG	NS	4.2	> 0.12	C=W	NS	0.87	> 0.35
	WF	NS	0.71	> 0.70	C>W	NS	2.05	> 0.15
	Domestication				C<W	NS	0.62	> 0.43
	1 Generation		7.35	< 0.05	C=W	NS	3.71	> 0.05
		C<W = C=W	0.57	> 0.44	C>W	1Gen>>2Gen	10.36	< 0.01
		C<W = C>W	1.63	> 0.20				
		C=W > C>W	5.38	< 0.05				
	> 2 Generations		11.71	< 0.01				
	Numerically same	C<W = C=W	0	~1				
		C<W > C>W	8.1	< 0.01				
		C=W < C>W	8.1	< 0.01				
	Ancestral population				C<W	NS	0.29	> 0.55
	Diff	NS	4.87	> 0.08	C=W	Diff>Same	4.13	< 0.05
	Same	NS	3.6	> 0.16	C>W	NS	1.46	> 0.22
	Salmonid				C<W	NS	2.4	> 0.12
	Not	NS	5.18	> 0.07	C=W	NS	1.63	> 0.20
	Yes		8.84	< 0.05	C>W	Not>Yes	6.98	< 0.01
		C<W = C=W	0	~1				
		C<W > C>W	5.81	< 0.05				
		C=W > C>W	5.81	< 0.05				
Caudle Peduncle Length	Overall	NS	5.47	> 0.06				
	Form of culture							
	Farm	NS	3	> 0.022	C<W	NS	0.09	> 0.95
	Hatchery	NS	3.75	> 0.15	C=W	NS	1.16	> 0.55
	Lab	NS	2.1	> 0.34	C>W	NS	2.06	> 0.35
	Commonality of rearing environment				C<W	NS	< 0.001	> 0.98
	CG	NS	4.2	> 0.12	C=W	NS	0.2	> 0.64
	WF	NS	2.25	> 0.32	C>W	NS	0.01	> 0.89
	Domestication				C<W	NS	0.33	> 0.56
	1 Generation	NS	1.61	> 0.44	C=W	NS	< 0.0001	~1
	> 2 Generations		6	< 0.05	C>W	NS	0.07	> 0.78
	Ancestral population				C<W	NS	0.42	> 0.51
	Diff	NS	1.69	> 0.42	C=W	NS	0.2	> 0.65
	Same		6.5	< 0.05	C>W	NS	< 0.0001	~1

		C<W < C=W	4.33	< 0.05				
		C<W = C>W	0.16	> 0.68				
		C=W = C>W	1.82	> 0.17				
	Salmonid				C<W	NS	0.06	> 0.80
	Not	NS	4.44	> 0.10	C=W	NS	2.43	> 0.11
	Yes		11.19	< 0.01	C>W	Not>Yes	4.87	< 0.05
		C<W < C=W	3.86	< 0.05				
		C<W = C>W	0.46	> 0.49				
		C=W > C>W	8.29	< 0.01				
Pectoral Fin Length	Overall	NS	5.81	> 0.05				
	Form of culture				C<W	NS	5.87	> 0.05
	Farm	NS	5.81	> 0.05	C=W	NS	0.33	> 0.84
	Hatchery	NS	5.84	> 0.05	C>W	NS	1.11	> 0.57
	Lab	NS	4.38	> 0.11				
	Commonality of rearing environment				C<W	NS	< 0.0001	~1
	CG	NS	2.62	> 0.26	C=W	NS	0.29	> 0.58
	WF	NS	4.57	> 0.10	C>W	NS	0.02	> 0.87
	Domestication				C<W	NS	0.24	> 0.61
	1 Generation	NS	1.5	> 0.47	C=W	NS	0.09	> 0.76
	>2 Generations	NS	3	> 0.22	C>W	NS	< 0.0001	~1
	Ancestral population				C<W	NS	0.15	> 0.69
	Diff	NS	0.6	> 0.74	C=W	NS	< 0.0001	~1
	Same	NS	5.86	> 0.05	C>W	NS	0.32	> 0.57
	Salmonid				C<W	NS	< 0.0001	~1
	Not	NS	2.18	> 0.33	C=W	NS	0.61	> 0.43
	Yes	NS	5.65	> 0.05	C>W	NS	0.42	> 0.51
Pelvic Fin Length	Overall	NS	3.48	> 0.17				
	Form of culture							
	Farm	NS	5.35	> 0.06	C<W	NS	2.58	> 0.27
	Hatchery	NS	0.42	> 0.80	C=W	NS	4.16	> 0.12
	Lab		6.93	< 0.05	C>W	NS	2.64	> 0.26
		C<W = C=W	1.14	> 0.28				
		C<W = C>W	0.73	> 0.39				
		C=W > C>W	4.98	> 0.05				
	Commonality of rearing environment				C<W	NS	0.06	> 0.79
	CG	NS	4.2	> 0.12	C=W	NS	<	~1

							0.0001	
	WF	NS	1.09	> 0.57	C>W	NS	0.15	> 0.69
	Domestication				C<W	NS	0.06	> 0.56
	1 Generation	NS	4.2	> 0.12	C=W	NS	< 0.0001	~1
	>2 Generations	NS	1.09	> 0.57	C>W	NS	0.15	> 0.69
	Ancestral population				C<W	NS	0.01	> 0.89
	Diff	NS	2.1	> 0.34	C=W	NS	0.87	> 0.34
	Same	NS	4.65	> 0.09	C>W	NS	0.23	> 0.62
	Salmonid				C<W	NS	1.16	> 0.28
	Not	NS	5.04	> 0.08	C=W	NS	0.32	> 0.56
	Yes	NS	1.8	> 0.40	C>W	NS	0.04	> 0.83
Dorsal Fin Length	Overall	NS	1.62	> 0.44				
	Form of culture							
	Farm	NS	1.23	> 0.53	C<W	NS	4.64	> 0.09
	Hatchery		10.09	< 0.01	C=W	NS	5.96	> 0.05
		C<W > C=W	7.54	< 0.01	C>W	NS	2.51	> 0.28
		C<W = C>W	0.72	> 0.39				
		C=W = C>W	2.75	> 0.09				
	Lab	NS	3	> 0.22				
	Commonality of rearing environment				C<W	NS		
	CG	NS	0.42	> 0.80	C=W	NS		
	WF	NS	2.03	> 0.36	C>W	NS		
	Domestication							
	1 Generation				Small Sample Size			
	>2 Generations	NS	1.8	> 0.40				
	Ancestral population				C<W	NS	0.35	> 0.55
	Diff	NS	1.09	> 0.55	C=W	NS	0.15	> 0.69
	Same	NS	3.94	> 0.13	C>W	NS	0.04	> 0.82
	Salmonid				C<W	NS	0.25	> 0.61
	Not	NS	3	> 0.22	C=W	NS	0.11	> 0.73
	Yes	NS	2.47	> 0.28	C>W	NS	1.46	> 0.22
Dorsal Fin Width	Overall	NS	5.04	> 0.08				
	Form of culture				C<W	NS	2.53	> 0.28
	Farm	NS	4.65	> 0.09	C=W	Farm=Hatch	1.48	> 0.22
	Hatchery		11.4	< 0.01		Farm=Lab	0.96	> 0.32
		C<W < C=W	4.88	< 0.02		Hatch<Lab	4.87	< 0.05

		C<W = C>W	0	~1	C>W	NS	4.01	> 0.13
		C=W > C>W	6.8	< 0.01				
	Lab	NS	2.25	> 0.32				
	Commonality of rearing environment				C<W	NS	0.01	> 0.89
	CG		8.53	< 0.05	C=W	NS	1.68	> 0.19
		C<W = C=W	1.39	> 0.23	C>W	NS	1.01	> 0.31
		C<W = C>W	0.99	> 0.31				
		C=W > C>W	6.11	< 0.05				
	WF	NS	1.14	> 0.56				
	Domestication				C<W	NS	0.08	> 0.77
	1 Generation	NS	3.9	> 0.14	C=W	1Gen<>2Gen	5.32	< 0.05
	> 2 Generations		13.44	< 0.01	C>W	1Gen>>2Gen	3.89	< 0.05
		C<W < C=W	3.97	< 0.05				
		C<W = C>W	1.12	> 0.28				
		C=W > C>W	10.5	< 0.01				
	Ancestral population				C<W	NS	< 0.01	> 0.94
	Diff		7.09	< 0.05	C=W	NS	1.04	> 0.30
		C<W = C=W	2.35	> 0.12	C>W	NS	0.53	> 0.46
		C<W = C>W	0.12	> 0.71				
		C=W > C>W	4.81	< 0.05				
	Same	NS	< 0.0001	~1				
	Salmonid				C<W	Not>Yes	4.42	< 0.05
	Not	NS	5.88	> 0.05	C=W	Not<Yes	5.98	< 0.05
	Yes		13.63	< 0.01	C>W	NS	0.01	> 0.89
		C<W < C=W	7.61	< 0.01				
		C<W = C=W	0	~1				
		C=W > C>W	7.61	< 0.01				
Anal Fin Length	Overall	NS	0.30	> 0.86				
	Form of culture				C<W	Farm=Hatch	< 0.001	~1
	Farm	NS	3	> 0.22	C=W	Farm=Hatch	0.22	> 0.63
	Hatchery	NS	2.25	> 0.32	C>W	Farm=Hatch	0.8	> 0.36
	Lab							
	Commonality of rearing environment							
	CG				Small Sample Size			
	WF	NS	1.56	> 0.45				

	Domestication							
	1 Generation				Small Sample Size			
	> 2 Generations	NS	4.38	> 0.11				
	Ancestral population							
	Same							
	Diff	NS	0.21	> 0.89	Small Sample Size			
	Salmonid				C<W	NS	0.44	> 0.50
	Not	NS	2.1	> 0.34	C=W	NS	< 0.001	~1
	Yes	NS	0.6	> 0.74	C>W	NS	0.46	> 0.49
Anal Fin Width	Overall		6.71	< 0.05				
		C<W = C=W	2.95	> 0.08				
		C<W = C>W	0.05	> 0.81				
		C=W > C>W	4.72	< 0.05				
	Form of culture							
	Farm		8.21	< 0.05	C<W	Farm=Hatch	0.11	> 0.73
		C<W = C=W	2.69	> 0.10	C=W	Farm=Hatch	0.24	> 0.62
		C<W = C>W	0.15	> 0.69	C>W	Farm=Hatch	< 0.001	~1
		C=W > C>W	5.54	< 0.05				
	Hatchery	NS	2.43	> 0.29				
	Lab	NS	1	> 0.60				
	Commonality of rearing environment				C<W	NS	0.72	> 0.39
	CG		11.3	< 0.01	C=W	NS	2.23	> 0.13
		C>W < C=W	5.67	< 0.05	C>W	NS	0.21	> 0.64
		C>W = C>W	0	~1				
		C=W > C>W	5.67	< 0.05				
	WF	NS	1.09	> 0.57				
	Domestication				C<W	NS	0.09	> 0.76
	1 Generation	NS	2.1	> 0.34	C=W	1Gen<>2Gen	4.65	< 0.05
	> 2 Generations		20.33	< 0.0001	C>W	NS	3.18	> 0.07
		C<W < C=W	10.9	< 0.001				
		C<W = C>W	0	~1				
		C=W > C>W	12.96	< 0.001				
	Ancestral population				C<W	NS	< 0.001	~1
	Diff		7.09	< 0.05	C=W	NS	0.25	> 0.61

		C<W = C=W	2.35	> 0.12	C>W	NS	0.71	> 0.39
		C<W = C>W	0.012	> 0.71				
		C=W > C>W	4.81	< 0.05				
	Same	NS	1.23	> 0.53				
	Salmonid				C<W	NS	2	> 0.15
	Not	NS	1.1	> 0.57	C=W	NS	2.4	> 0.12
	Yes		12.33	< 0.01	C>W	NS	< 0.001	~1
		C<W < C=W	7.79	< 0.01				
		C<W = C>W	0	~1				
		C=W > C>W	6.21	< 0.05				
Caudal Fin Length	Overall	NS	0.27	> 0.87				
	Form of culture				C<W	Farm=Hatch	< 0.001	~1
	Farm	NS	0.75	> 0.68	C=W	Farm=Hatch	< 0.01	> 0.96
	Hatchery	NS	0.6	> 0.74	C>W	Farm=Hatch	< 0.001	~1
	Lab							
	Commonality of rearing environment							
	CG				Small Sample Size			
	WF	NS	0.12	> 0.94				
	Domestication							
	1 Generation				Small Sample Size			
	>2 Generations	NS	1.5	> 0.47				
	Ancestral population				C<W	NS	1.35	> 0.24
	Diff	NS	3	> 0.22	C=W	NS	< 0.001	~1
	Same	NS	1.9	> 0.38	C>W	NS	1.09	> 0.29
	Salmonid				C<W	NS	2.56	> 0.10
	Not	NS	3.35	> 0.18	C=W	NS	< 0.001	~1
	Yes	NS	5.25	> 0.07	C>W	NS	3.16	> 0.07

Supplementary Table 3.6 Congruence of the results of the meta-analysis and the vote-counting analysis. For the meta-analysis the difference in size is what was indicated from the mean effect size as generated by the mixed-mixed effect model. For the vote-counting analysis, the relative difference found in the largest proportion of studies is used. Where the proportions of two possible differences did not differ, both are given, and where all three did not differ, this is denoted Aprx. Equal, or all proportions approximately equal. Significances are given for both the meta-analysis and the vote-counting results. Comp. is the comparison of the results of the vote-counting and meta-analysis. Where the results of the two analyses show the same relative difference in size for a morphological character between the cultured and wild fish the results are said to be congruent, where the results are indicate the differences are opposite this is indicated, and where the they do not match, this is indicated as incongruent. A summary of the number of morphological features where were found to be congruent, incongruent or opposite are given for each moderator level.

Moderator	Feature	Meta-analysis	Significance	Vote-count	Significance	Comparison		
All studies	Head Depth	C > W	NS	C>W	NS	Congruent	Congruent	7
	Head Length	C < W	p < 0.01	Props Aprx Equal		Incongruent	Incongruent	8
	Eye Size	C < W	NS	Props Aprx Equal		Incongruent	Opposite	1
	Upper Jaw L	C < W	p < 0.01	C<W and C=W	p < 0.05	Congruent		
	Lower Jaw L	C < W	NS	C<W	NS	Congruent		
	Body Depth	C < W	NS	C>W	p < 0.001	Opposite		
	Condition	C > W	NS	C>W	p < 0.05	Congruent		
	Caud Ped D	C < W	NS	Props Aprx Equal		Incongruent		
	Caud Ped L	C > W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	C < W	p < 0.001	C<W	NS	Congruent		
	Pelvic Fin L	C < W	p < 0.001	C<W and C=W	NS	Congruent		
	Dorsal Fin L	C < W	p < 0.01	Props Aprx Equal	NS	Incongruent		
	Dorsal Fin W	C < W	NS	C=W and C<W	NS	Congruent		
	Anal Fin L	C < W	p < 0.01	Props Aprx Equal		Incongruent		
	Anal Fin W	C < W	p < 0.05	C=W	p < 0.05	Incongruent		
	Caudal Fin L	C < W	NS	Props Aprx Equal		Incongruent		
Farm	Head Depth	C > W	NS	C>W	NS	Congruent	Congruent	7
	Head Length	C < W	p < 0.01	C>W	NS	Opposite	Incongruent	5
	Eye Size	C < W	NS	C<W	p < 0.05	Congruent	Opposite	2
	Upper Jaw L	C < W	NS	C=W	p < 0.05	Incongruent		
	Lower Jaw L	C > W	NS	Props Aprx Equal		Incongruent		
	Body Depth	C > W	NS	C>W	p < 0.001	Congruent		
	Condition	C > W	NS	C>W	p < 0.001	Congruent		
	Caud Ped D	C < W	p < 0.05	C=W	p < 0.05	Incongruent		
	Caud Ped L	C < W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	C < W	NS	C<W	NS	Congruent		
	Pelvic Fin L	C < W	NS	C<W	NS	Congruent		

	Dorsal Fin L	C < W	NS	Props Aprx Equal		NA		
	Dorsal Fin W	C > W	NS	C<W	NS	Opposite		
	Anal Fin L	C < W	NS	C<W	NS	Congruent		
	Anal Fin W	C < W	NS	C=W	p < 0.05	Incongruent		
	Caudal Fin L	C < W	NS	Props Aprx Equal		NA		
Hatchery	Head Depth	C > W	NS	C<W	NS	Opposite	Congruent	4
	Head Length	C < W	NS	C<W	NS	Congruent	Incongruent	2
	Eye Size	C > W	NS	C>W	NS	Congruent	Opposite	4
	Upper Jaw L	C > W	NS	C<W	p < 0.05	Opposite		
	Lower Jaw L	C < W	NS	Props Aprx Equal		NA		
	Body Depth	C < W	NS	C>W	NS	Opposite		
	Condition	NA		Props Aprx Equal		NA		
	Caud Ped D	C > W	NS	C<W	NS	Opposite		
	Caud Ped L	C > W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	C < W	p < 0.001	C<W	NS	Congruent		
	Pelvic Fin L	C < W	p < 0.001	Props Aprx Equal		NA		
	Dorsal Fin L	C < W	p < 0.05	C<W	NS	Congruent		
	Dorsal Fin W	C < W	NS	C=W	p < 0.05	Incongruent		
	Anal Fin L	C < W	p < 0.05	Props Aprx Equal		NA		
	Anal Fin W	C < W	NS	Props Aprx Equal		NA		
	Caudal Fin L	C < W	NS	Props Aprx Equal		NA		
Laboratory	Head Depth	C < W	NS	C>W	p < 0.05	Opposite	Congruent	1
	Head Length	C < W	p < 0.001	C>W	p < 0.05	Opposite	Incongruent	3
	Eye Size	C < W	NS	Props Aprx Equal		NA	Opposite	7
	Upper Jaw L	C < W	p < 0.001	C=W	NS	Incongruent		
	Lower Jaw L	NA		Few Studies		NA		
	Body Depth	C < W	p < 0.01	C>W	p < 0.05	Opposite		
	Condition	C < W	NS	Few Studies		NA		

	Caud Ped D	C < W	NS	C>W	NS	Opposite		
	Caud Ped L	C > W	NS	C>W	NS	Congruent		
	Pectoral Fin L	C < W	p < 0.001	C=W	NS	Incongruent		
	Pelvic Fin L	C < W	p < 0.001	C=W	p < 0.05	Incongruent		
	Dorsal Fin L	C < W	p < 0.01	C>W	NS	Opposite		
	Dorsal Fin W	C < W	p < 0.01	Props Aprx Equal		NA		
	Anal Fin L	C < W	p < 0.05	C>W	NS	Opposite		
	Anal Fin W	C < W	p < 0.05	Props Aprx Equal		NA		
	Caudal Fin L	C < W	NS	C>W	NS	Opposite		
Common Garden	Head Depth	C > W	NS	Props Aprx Equal		NA	Congruent	1
	Head Length	C < W	p < 0.001	C>W	p < 0.05	Opposite	Incongruent	1
	Eye Size	C < W	NS	Few Studies		NA	Opposite	3
	Upper Jaw L	Few Studies		Few Studies		NA		
	Lower Jaw L	Few Studies		Few Studies		NA		
	Body Depth	C < W	NS	C>W	NS	Opposite		
	Condition	C < W	NS	C>W	p < 0.05	Opposite		
	Caud Ped D	C < W	p < 0.001	C<W	NS	Congruent		
	Caud Ped L	C > W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	Few Studies		Few Studies		NA		
	Pelvic Fin L	Few Studies		Few Studies		NA		
	Dorsal Fin L	Few Studies		Props Aprx Equal		NA		
	Dorsal Fin W	Few Studies		C=W	p < 0.05	NA		
	Anal Fin L	Few Studies		Few Studies		NA		
	Anal Fin W	Few Studies		C=W	p < 0.05	NA		
	Caudal Fin L	Few Studies		Few Studies		NA		
Wild/Farmed	Head Depth	C > W	NS	Props Aprx Equal		NA	Congruent	10
	Head Length	C < W	p < 0.01	C>W	NS	Opposite	Incongruent	0
	Eye Size	C < W	NS	Props Aprx Equal		NA	Opposite	1

	Upper Jaw L	C < W	p < 0.05	C<W	NS	Congruent		
	Lower Jaw L	C < W	p < 0.05	C<W	NS	Congruent		
	Body Depth	C < W	NS	C<W	p < 0.05	Congruent		
	Condition	C > W	NS	C>W	P < 0.01	Congruent		
	Caud Ped D	C < W	NS	Props Aprx Equal		NA		
	Caud Ped L	C > W	NS	C=W and C>W	NS	Congruent		
	Pectoral Fin L	C < W	p < 0.001	C<W	NS	Congruent		
	Pelvic Fin L	C < W	p < 0.001	C<W and C=W	NS	Congruent		
	Dorsal Fin L	C < W	p < 0.001	C<W	NS	Congruent		
	Dorsal Fin W	C < W	NS	C<W and C=W	NS	Congruent		
	Anal Fin L	C < W	p < 0.01	Props Aprx Equal		NA		
	Anal Fin W	C < W	p < 0.01	C=W and C<W	NS	Congruent		
	Caudal Fin L	C < W	NS	Props Aprx Equal		NA		
Different Pop	Head Depth	C > W	NS	C>W	NS	Congruent	Congruent	3
	Head Length	C < W	p < 0.001	C>W	NS	Opposite	Incongruent	5
	Eye Size	C < W	NS	Props Aprx Equal		NA	Opposite	3
	Upper Jaw L	C > W	NS	C=W	p < 0.05	Incongruent		
	Lower Jaw L	C > W	p < 0.001	Props Aprx Equal		NA		
	Body Depth	C < W	NS	C>W	p < 0.05	Opposite		
	Condition	C < W	p < 0.001	C>W	NS	Opposite		
	Caud Ped D	C < W	NS	C=W	NS	Incongruent		
	Caud Ped L	C > W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	C < W	p < 0.001	Props Aprx Equal		NA		
	Pelvic Fin L	C < W	p < 0.01	C<W	NS	Congruent		
	Dorsal Fin L	C < W	p < 0.001	Props Aprx Equal		NA		
	Dorsal Fin W	C > W	NS	C=W	p < 0.05	Incongruent		
	Anal Fin L	C < W	p < 0.05	Props Aprx Equal		NA		
	Anal Fin W	C < W	p < 0.01	C=W	p < 0.05	Incongruent		

	Caudal Fin L	C < W	NS	C<W	NS	Congruent		
Same Pop	Head Depth	C < W	NS	Props Aprx Equal	NS	NA	Congruent	6
	Head Length	C < W	NS	C<W and C=W	NS	Congruent	Incongruent	2
	Eye Size	C < W	NS	C<W and C=W	NS	Congruent	Opposite	1
	Upper Jaw L	C < W	p < 0.001	C<W	p < 0.05	Congruent		
	Lower Jaw L	C < W	p < 0.001	Few Studies		NA		
	Body Depth	C < W	NS	C>W	p < 0.05	Opposite		
	Condition	C < W	p < 0.001	Few Studies		NA		
	Caud Ped D	C < W	NS	C>W equal to C<W		Congruent		
	Caud Ped L	C < W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	C < W	p < 0.001	C<W and C=W	NS	Congruent		
	Pelvic Fin L	C < W	p < 0.001	Props Aprx Equal		NA		
	Dorsal Fin L	C < W	p < 0.01	Props Aprx Equal		NA		
	Dorsal Fin W	C < W	p < 0.05	Props Aprx Equal		NA		
	Anal Fin L	C < W	p < 0.01	Props Aprx Equal		NA		
	Anal Fin W	C < W	NS	C=W	p < 0.001	Incongruent		
	Caudal Fin L	C > W	NS	C>W and C=W		Congruent		
>2 Gen Domestication	Head Depth	C <W	NS	C=W	NS	Incongruent	Congruent	1
	Head Length	C < W	p < 0.01	C>W and C=W	NS	Opposite	Incongruent	11
	Eye Size	C < W	NS	C=W	NS	Incongruent	Opposite	3
	Upper Jaw L	C = W	NS	C<W	NS	Incongruent		
	Lower Jaw L	C > W	p < 0.001	Props Aprx Equal		NA		
	Body Depth	C < W	p < 0.05	C>W	p < 0.05	Opposite		
	Condition	C < W	p < 0.001	C>W	NS	Opposite		
	Caud Ped D	C < W	NS	C<W and C=W	p < 0.01	Congruent		
	Caud Ped L	C > W	NS	C=W	NS	Incongruent		
	Pectoral Fin L	C < W	p < 0.05	C=W	NS	Incongruent		
	Pelvic Fin L	C < W	p < 0.05	C=W	NS	Incongruent		

	Dorsal Fin L	C < W	p < 0.001	C=W	NS	Incongruent		
	Dorsal Fin W	C < W	p < 0.05	C=W	p < 0.05	Incongruent		
	Anal Fin L	C < W	p < 0.01	C=W	NS	Incongruent		
	Anal Fin W	C < W	p < 0.01	C=W	p < 0.01	Incongruent		
	Caudal Fin L	C < W	p < 0.001	C=W and C<W	NS	Incongruent		
1 Gen Domestication	Head Depth	C < W	NS	Props Aprx Equal		NA	Congruent	5
	Head Length	C < W	p < 0.001	Props Aprx Equal		NA	Incongruent	0
	Eye Size	C < W	NS	C=W and C<W	NS	Congruent	Opposite	2
	Upper Jaw L	C < W	p < 0.001	C<W	NS	Congruent		
	Lower Jaw L	C < W	p < 0.001	Props Aprx Equal		NA		
	Body Depth	C < W	NS	C>W	NS	Opposite		
	Condition	C < W	p < 0.001	Props Aprx Equal		NA		
	Caud Ped D	C < W	NS	C>W	p < 0.05	Opposite		
	Caud Ped L	C = W	NS	C=W	NS	Congruent		
	Pectoral Fin L	C < W	p < 0.001	C<W and C=W	NS	Congruent		
	Pelvic Fin L	C < W	p < 0.01	C=W and C<W	NS	Congruent		
	Dorsal Fin L	C < W	p < 0.01	Props Aprx Equal		NA		
	Dorsal Fin W	C > W	NS	Props Aprx Equal		NA		
	Anal Fin L	C < W	NS	Props Aprx Equal		NA		
	Anal Fin W	C > W	NS	Props Aprx Equal		NA		
	Caudal Fin L	C > W	NS	Props Aprx Equal		NA		
Non-salmonid	Head Depth	C > W	NS	Props Aprx Equal		NA	Congruent	8
	Head Length	C < W	NS	C>W	p < 0.05	Opposite	Incongruent	2
	Eye Size	C > W	NS	Props Aprx Equal		NA	Opposite	3
	Upper Jaw L	C < W	NS	C=W	NS	Incongruent		
	Lower Jaw L	C < W	p < 0.001	C<W	NS	Congruent		
	Body Depth	C < W	NS	C>W	p < 0.001	Opposite		
	Condition	C > W	NS	C>W	p < 0.001	Congruent		

	Caud Ped D	C < W	NS	C>W	NS	Opposite			
	Caud Ped L	C > W	NS	C>W	NS	Congruent			
	Pectoral Fin L	C < W	p < 0.05	C<W	NS	Congruent			
	Pelvic Fin L	C < W	p < 0.05	C<W	NS	Congruent			
	Dorsal Fin L	C < W	p < 0.01	C<W	NS	Congruent			
	Dorsal Fin W	C < W	NS	C<W	NS	Congruent			
	Anal Fin L	C < W	p < 0.05	Props Aprx Equal		NA			
	Anal Fin W	C < W	p < 0.01	C<W	NS	Congruent			
	Caudal Fin L	C < W	NS	C>W	NS	Incongruent			
Salmonid	Head Depth	C >W	NS	C>W and C=W	NS	Congruent	Congruent	5	
	Head Length	C < W	p < 0.05	C<W	p < 0.01	Congruent	Incongruent	7	
	Eye Size	C < W	NS	C=W	NS	Incongruent	Opposite	1	
	Upper Jaw L	C < W	p < 0.01	C<W	NS	Congruent			
	Lower Jaw L	C > W	NS	Props Aprx Equal		NA			
	Body Depth	C > W	NS	Props Aprx Equal		NA			
	Condition	C > W	NS	C=W	NS	Incongruent			
	Caud Ped D	C > W	NS	C<W and C=W	p < 0.05	Incongruent			
	Caud Ped L	C > W	NS	C=W	p < 0.05	Incongruent			
	Pectoral Fin L	C < W	p < 0.001	C<W and C=W	NS	Congruent			
	Pelvic Fin L	C < W	p < 0.05	C=W	NS	Incongruent			
	Dorsal Fin L	C < W	NS	C>W	NS	Opposite			
	Dorsal Fin W	C < W	NS	C=W	p < 0.01	Incongruent			
	Anal Fin L	C < W	p < 0.05	Props Aprx Equal		NA			
	Anal Fin W	C < W	NS	C=W	p < 0.05	Incongruent			
	Caudal Fin L	C < W	NS	C<W	NS	Congruent			

Supplementary Table 3.7 References included in the vote-counting analysis and meta-analysis

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Supplementary Table 4.1 Identities of the fish that were used more than once, and the rounds in which they were used.

Start Date	Exp. Rnd	Tank	Fish	PIT Tag	ID #	Times Used	Weight (g)	Length (cm)	L PF (mm)	R PF (mm)	First Spawn	Prop. Cultured Male	Second Spawn	Prop. Cultured Male	Third Spawn	Prop. Cultured Male	Fourth Spawn	Prop. Cultured Male	Fifth Spawn	Prop. Cultured Male	Sixth Spawn	Prop. Cultured Male
Mar. 18, 2010	1	1	Female	357108	NA		1277	46	41.7	44.54	Did not spawn											
	1	1	Cultured Male	359077	NA		1662	47	59.51	52.05												
	1	1	Wild Male	066605031	5		1816	50.5	79.05	76.82												
Mar. 18, 2010	1	2	Female	345807	10	2	1925	50	54	50	Apr. 14, 2010	0.70										
	1	2	Cultured Male	273889	10		1610	48	55.87	54.85												
	1	2	Wild Male	060028101	1	2	2146	57.5	85.19	77.37												
Mar. 18, 2010	1	3	Female	363101	NA		2240	49.5	41.48	51.35	Did not spawn											
	1	3	Cultured Male	276888	NA		1763	46	51.92	52.05												
	1	3	Wild Male	066281523	NA		3219	65.5	69	71												
Mar. 18, 2010	1	4	Female	356002	13		1839	46.5	40	53	Apr. 10, 2010 Behaviour Data	1.00	Apr. 14, 2010 Behaviour Data	0.14								
	1	4	Cultured Male	345031	14		1510	45	51.38	49.29												
	1	4	Wild Male	060557596	2	2	1759	55	72.39	71.28												
Mar. 18, 2010	1	5	Female	354425	NA		1950	50	46.81	46.57	Did not spawn											
	1	5	Cultured Male	346674	NA		2336	51	48.78	56.27												
	1	5	Wild Male	066020024	NA		1902	54.5	66.47	74.77												
Mar. 18, 2010	1	6	Female	275578	7	2	2334	48	57	52	Apr. 4, 2010	0.06	Apr. 10, 2010	0.39	Apr. 13, 2010	0.10	Apr. 14, 2010	0.00				
	1	6	Cultured Male	275594	12		1500	49	52.16	51.79												
	1	6	Wild Male	069269109	13	3	1363	52.5	63.4	69.55												
Mar. 18, 2010	1	7	Female	151828	NA		2132	49.5	53.27	53.66	Did not spawn											
	1	7	Cultured Male	275336	NA		1288	47	45.46	50.24												
	1	7	Wild Male	No Tag	15	3	2114	56.5	66.78	69.02												
Mar.	1	8	Female	34953	N		1836	47	47.38	54.99	Did not											

18, 2010				9	A						spawn								
	1	8	Cultured Male	365746	NA		1395	47	59.41	56.75									
	1	8	Wild Male	068868872	7		1636	54.5	74.28	77.08									
Mar. 18, 2010	1	9	Female	346456	11		2163	51	48.75	56.27	Apr. 10, 2010	0.41							
	1	9	Cultured Male	154176	3		1545	NA	NA	NA									
	1	9	Wild Male	069039528	11		3700	62	81.31	76.41									
Mar. 18, 2010	1	10	Female	362080	16		1510	51.5	49.73	56.27	Apr. 14, 2010	1.00							
	1	10	Cultured Male	149445	1		1484	48.5	63.77	61.83									
	1	10	Wild Male	069020888	8		2698	68	89.98	86.78									
Apr. 13, 2010	2	1	Female	274520	NA		1580.5	49.5	51	55	Did not spawn								
	2	1	Cultured Male	363092	NA		1277.3	44.5	66	60									
	2	1	Wild Male	060557596	2		1759	55	72.39	71.28									
Apr. 13, 2010	2	2	Female	275733	8	2	1857	50	59	59	Apr. 20, 2010	0.44	Apr. 24, 2010	0.93	Apr. 25, 2010	0.65			
	2	2	Cultured Male	263310	5		1340.8	47.5	45.09	50.34									
	2	2	Wild Male	069020888	4		2698	66	81.78	80.89									
Apr. 13, 2010	2	3	Female	348708	12		1924.4	50.5	56	64	Apr. 20, 2010	0.08							
	2	3	Cultured Male	272239	9		2357.2	53.5	63.85	73.23									
	2	3	Wild Male	066605031	5		1816	50.5	79.05	76.82									
Apr. 13, 2010	2	4	Female	344884	9		1216.2	43	57	61	Apr. 27, 2010 Behavior Data	0.81							
	2	4	Cultured Male	266688	7		1655	49	63.78	67.64									
	2	4	Wild Male	066020024	3		1902	54.5	66.47	74.77									
Apr. 13, 2010	2	5	Female	263985	3		1749.2	47	59	60	Apr. 17, 2010 Behavior Data	0.15	Apr. 21, 2010 Behavior Data	0.47	Apr. 22, 2010 Behavior Data	0.55			
	2	5	Cultured Male	355126	18		2841.3	NA	NA	NA									
	2	5	Wild Male	069039528	12		3700	62	81.31	76.41									
Apr. 13, 2010	2	6	Female	270523	6		2115.5	51.5	59	60	Apr. 15,	0.56	Apr. 26,	0.00					

	2	6	Cultured Male	271098	8		2517.3	59	81.03	65.92	2010		2010									
	2	6	Wild Male	060028101	1	2	2146	57.5	85.19	77.37												
Apr. 13, 2010	2	7	Female	345807	10	2	1943.8	50	43.23	41.07	Apr. 26, 2010 Behavior Data	0.29	Apr. 27, 2010 Behavior Data	0.40								
	2	7	Cultured Male	368815	22		1805.6	50.5	61.22	68.21												
	2	7	Wild Male	068868872	7	2	1636	54.5	74.28	77.08												
Apr. 13, 2010	2	8	Female	275578	7	2	2304	48	44.78	46.15	Apr. 21, 2010 Behavior Data	0.88	Apr. 24, 2010 Behavior Data	0.85	Apr. 26, 2010 Behavior Data	0.95	Apr. 27, 2010 Behavior Data	0.73	Apr. 28, 2010 Behavior Data	0.60		
	2	8	Cultured Male	263248	4		1807.4	50	55.4	62.5												
	2	8	Wild Male	No Tag	15	3	2114	56.5	66.78	69.02												
Apr. 13, 2010	2	9	Female	115248	1		1771.5	48	63	57	Apr. 20, 2010	0.05	Apr. 25, 2010	0.00	Apr. 28, 2010	0.00						
	2	9	Cultured Male	265643	6		1392.7	44.5	58	53												
	2	9	Wild Male	069269109	13	3	1363	52.5	63.4	69.55												
Apr. 13, 2010	2	10	Female	369594	NA		1750	48	43.3	PF Missing	Did not spawn											
	2	10	Cultured Male	276487	NA		1405.2	47.5	50	59												
	2	10	Wild Male	066304091	NA		1679	59.5	79.82	72.68												
Apr. 30, 2010	3	1	Female	360589	14		1305.9	46	46.59	53.43	May 9, 2010	0.65	May 13, 2010	0.70	May 15, 2010	0.74	May 17, 2010	0.91				
	3	1	Cultured Male	152486	2		1332.9	51	68.69	69.97												
	3	1	Wild Male	068868872	7	2	1636	54.5	74.28	77.08												
Apr. 30, 2010	3	2	Female	354425	2		1899.2	50	51.18	51.14	May 7, 2010	0.63	May 11, 2010	0.86	May 12, 2010	0.76	May 13, 2010	0.24	May 17, 2010	0.38	May 18, 2010	0.50
	3	2	Cultured Male	365783	21		1896.6	47	NA	NA												
	3	2	Wild Male	060557596	2	2	1759	55	72.39	71.28												
Apr. 30, 2010	3	3	Female	068841858	NA		2261	62	74.64	74.04	Did not spawn											
	3	3	Cultured Male	370927	NA		979	46	41.83	40.59												
	3	3	Wild Male	066084072	4		3249.5	66	81.78	80.89												
Apr. 30, 2010	3	4	Female	361879	15		1677	46	52.81	60.46	May 7, 2010	0.12	May 10,	0.00								

	3	4	Cultured Male	274729	11		1344	50	64.42	61.31	Behavior Data		2010							
	3	4	Wild Male	069 027 029	9		2280	58.5	74.07	74.55										
Apr. 30, 2010	3	5	Female	268639	5		1981.3	48	56.2	53.37	May 7, 2010 Behavior Data	0.79	May 18, 2010 Behavior Data	0.08	May 22, 2010 Behavior Data	0.06	May 23, 2010 Behavior Data	0.05		
	3	5	Cultured Male	353840	17		1425.8	48	51.07	51.63										
	3	5	Wild Male	069 012 888	16		3208.9	68	89.98	86.78										
Apr. 30, 2010	3	6	Female	372882	17		1835.2	NA	NA	NA	May 11, 2010	0.24	Female 5 Died, and was replaced by Female 20							
	3	6	Cultured Male	360101	19		2436	51	78.86	61.93										
	3	6	Wild Male	066 605 031	6		1816	50.5	79.05	76.82										
May 16, 2010	3	6	Female	369594	20		1553.6	48	43.3	NA	May 19, 2010	0.00	May 22, 2010	0.04						
	3	6	Cultured Male	360101	19		2436	51	78.86	61.93										
	3	6	Wild Male	066 605 031	6		1816	50.5	79.05	76.82										
Apr. 30, 2010	3	7	Female	267530	4		1797.4	48	44.78	46.15	May 3, 2010 Behavior Data	0.50	May 7, 2010 Behavior Data	0.54	May 15, 2010 Behavior Data	0.36				
	3	7	Cultured Male	277264	13		1542.1	47.5	50.94	52.07										
	3	7	Wild Male	069 269 109	13	3	1363	52.5	63.4	69.55										
Apr. 30, 2010	3	8	Female	374264	18		1633.6	45.5	49.8	54.59	May 11, 2010 Behavior Data	0.64	May 13, 2010 Behavior Data	0.63	May 16, 2010 Behavior Data	0.94	May 17, 2010 Behavior Data	1.00		
	3	8	Cultured Male	345953	15		1507.9	49	60.24	59.54										
	3	8	Wild Male	069 373 341	14		2310	59.5	77.69	67.52										
Apr. 30, 2010	3	9	Female	068 890 370	19		1701	53	69.16	69.67	May 7, 2010	0.50	May 16, 2010	0.10						
	3	9	Cultured Male	348441	16		1710.9	NA	NA	NA										
	3	9	Wild Male	069 034 019	10		2654	59.5	79.82	72.68										
Apr. 30, 2010	3	10	Female	275733	8	2	2050	50	59	59	May 3, 2010	0.86	May 5, 2010	0.76	May 7, 2010	0.87				
	3	10	Cultured Male	365703	20		1345.9	44.5	52.79	59.43										
	3	10	Wild Male	No Tag	15	3	2114	56.5	66.78	69.02										

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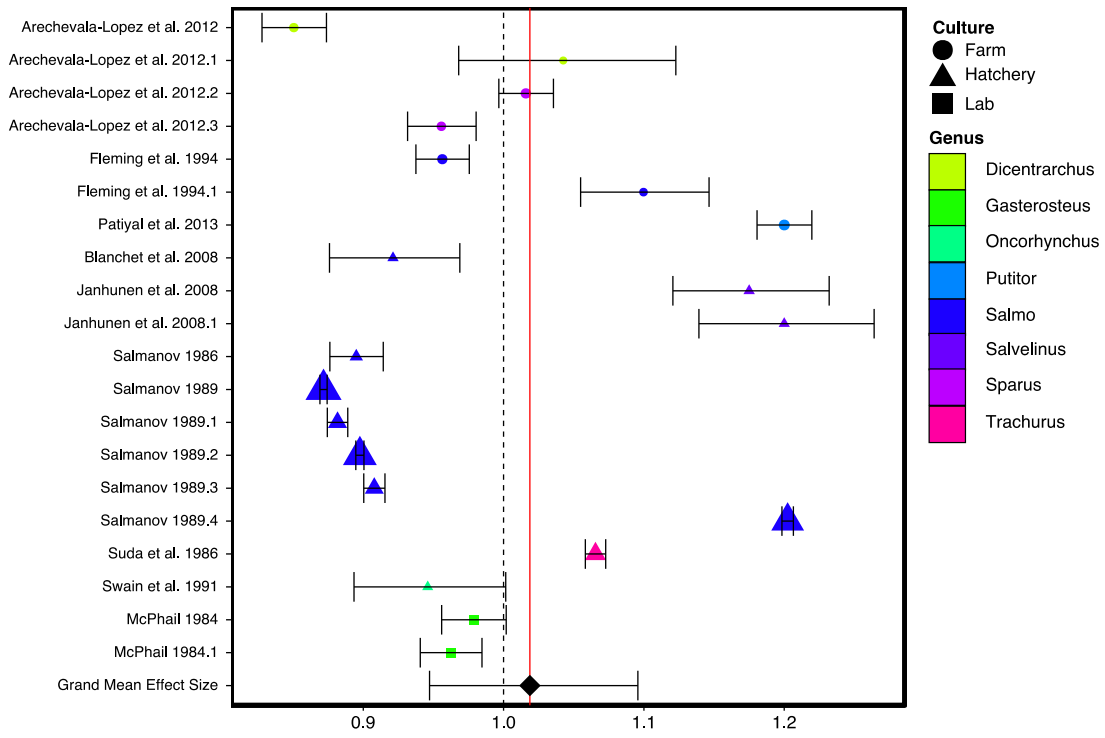
Supplementary Table 4.2 Primer sequences and characteristics of the microsatellite loci used in this study. Only the forward primers were labeled. The amount added is the volume of 10 μM forward and reverse primer added to each PCR reaction. All primer sequences are from Miller et al. (2000).

Locus	Repeat Motive	Primer Sequence (5'-3')	Allele Size Range (bp)	Dye Label	Accession Number	Amount Added (μL)
Gmo8	GACA	R: TGG GGG AGG CAT CTG TCA TTC A F: GCA AAA CGA GAT GCA CAG ACA CC	132-184	5' NED	AF159238	0.6
Gmo19	GACA	R: GTC TTG CCT GTA AGT CAG CTT G F: CAC AGT GAA GTG AAC CCA CTG	134-210	5' VIC	AF159232	0.5
Gmo35	ACA	R: CCT TAT CAT GTA CGT TGT TAA C F: GGA GGT GCT TTG AAG ATG	128-145	5' 6-FAM	AF159235	0.5
Gmo37	GACA	R: CGT GGG ATA CAT GGG TAC CT F: GGC CAA TGT TTC ATA ACT CT	240-292	5' PET	AF159237	0.4

Supplementary Table 5.1 Primer sequences and characteristics of the microsatellite loci used in this study. Only the forward primers were labelled. The number of alleles and their size ranges are reported separately for the two temporal cohorts. Allele sizes are based on an internal LIZ size standard (GeneScan™ 500 LIZ™ dye Size Standard, Applied Biosystems). Genotyping was done using two separate multiplexes, one consisting of *Gmo8*, *Gmo19*, *Gmo35* and *Gmo37*, and the other of *Gmo63*, *Gmo118*, *Gmo125* and *Gmo152*.

Marker	Repeat Motif		Primer Sequence	Number of Alleles		Size Range		GenBank Accession No.	Reference
				Apr. 25 Cohort	May 5 Cohort	Apr. 25 Cohort	May 5 Cohort		
Gmo8	GACA	R:	TGGGGGAGGCATCTGTCATTCA	11	12	131-177	131-177	AF159238	Miller et al. (2001)
		F:	GCAAAACGAGATGCACAGACACC						
*Gmo*19	GACA	R:	GTCTTGCCTGTAAGTCAGCTTG	13	13	145-213	145-294	AF159232	Miller et al. (2001)
		F:	CACAGTGAAGTGAACCCACTG						
*Gmo*35	ACC	R:	CCTTATCATGTACGTTGTTAAC	8	7	132-153	132-150	AF159235	Miller et al. (2001)
		F:	GGAGGTGCTTTGAAGATG						
*Gmo*37	GACA	R:	CGTGGGATACATGGGTACT	10	8	152-296	248-296	AF159237	Miller et al. (2001)
		F:	GGCCAATGTTTCATAACTCT						
*Gmo*63	TG	R:	CATGAAGCATCGACAACTGG	5	6	266-274	174-274	FJ007676	Higgins et al. (2009)
		F:	CATGAAGCATCGACAACTGG						
*Gmo*118	TC	R:	CGTGATCAGACAGAGAGGGG	10	8	253-289	253-289	FJ007709	Higgins et al. (2009)
		F:	AACTTCCTGTGCAAGTTCGG						
*Gmo*125	GA	R:	TCAGTGAGGTCACCATCTGC	10	9	253-293	261-293	FJ007712	Higgins et al. (2009)
		F:	ACTTTAGGATGTTCGTCCGC						
*Gmo*152	CA	R:	ACAAATGTCCATAGGGCAGC	7	7	285-305	285-305	FJ007728	Higgins et al. (2009)
		F:	TAAGCAACAACAGCCACAGG						

279 Supplementary Figure 3.1a)



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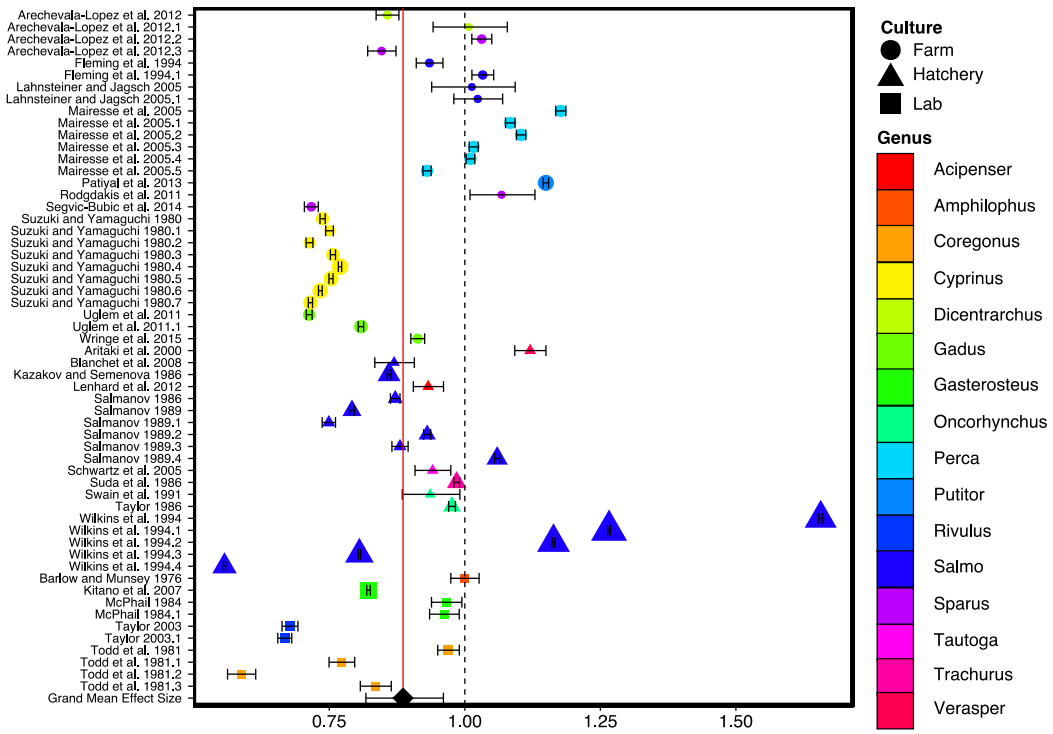
281 Supplementary Figure 3.1b)



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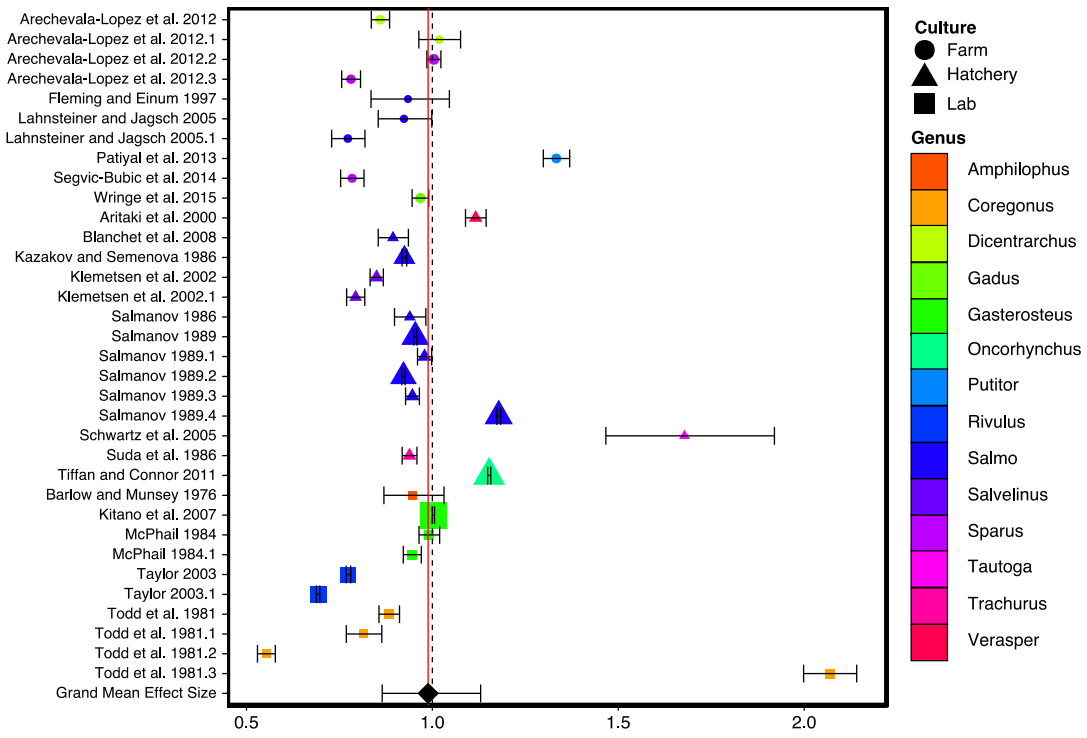
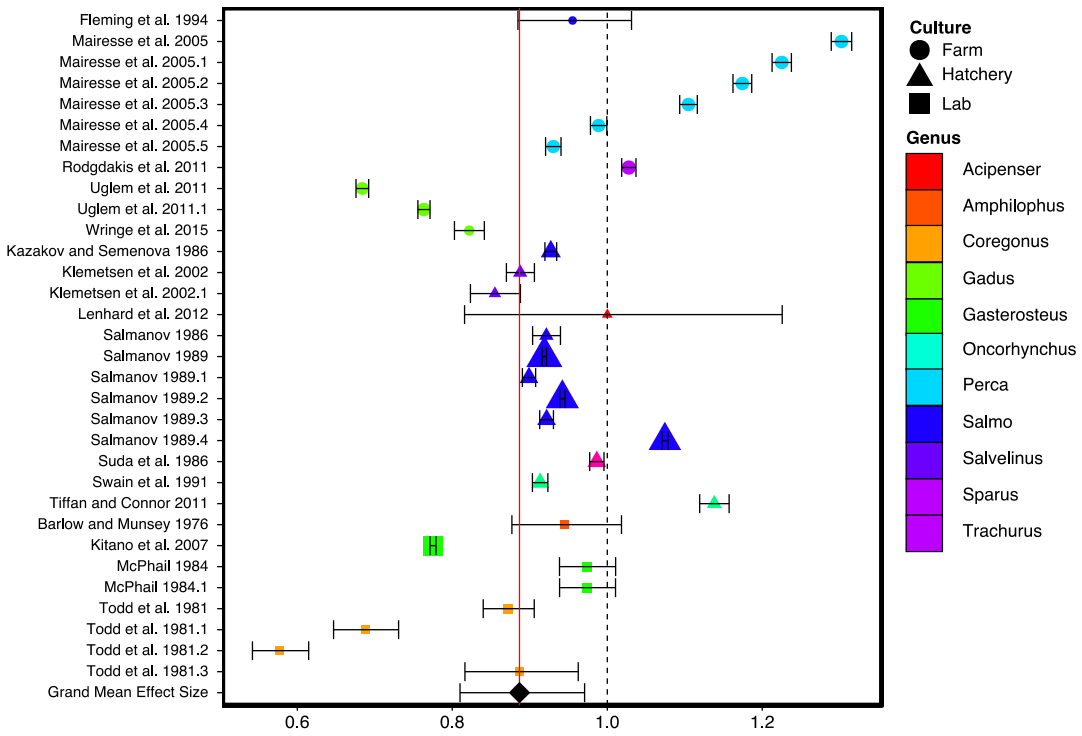
Supplementary Figure 3.1c)



287 Supplementary Figure 3.1d)



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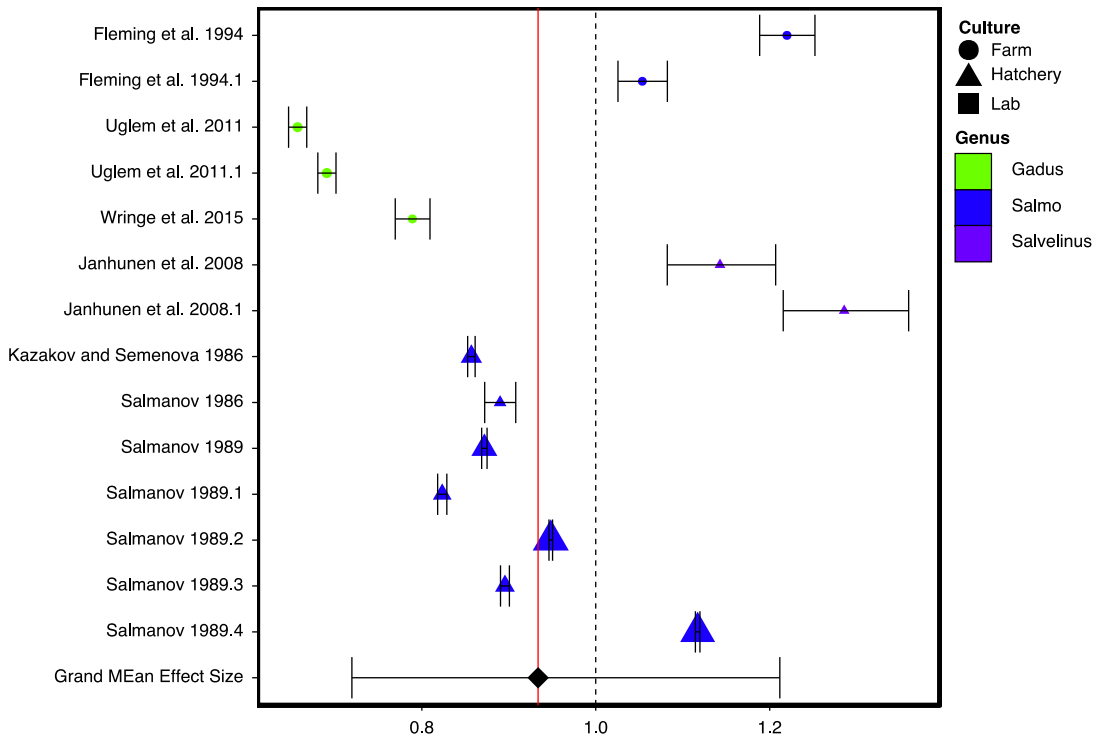
290 Supplementary Figure 3.1e)



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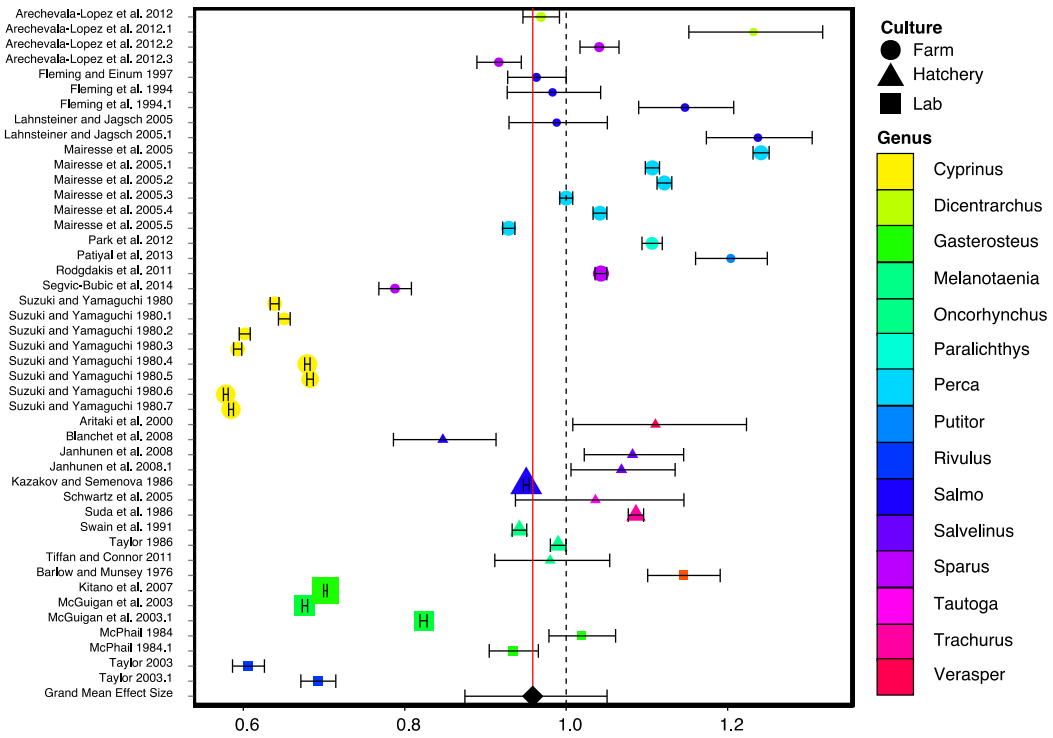
293 Supplementary Figure 3.1f)



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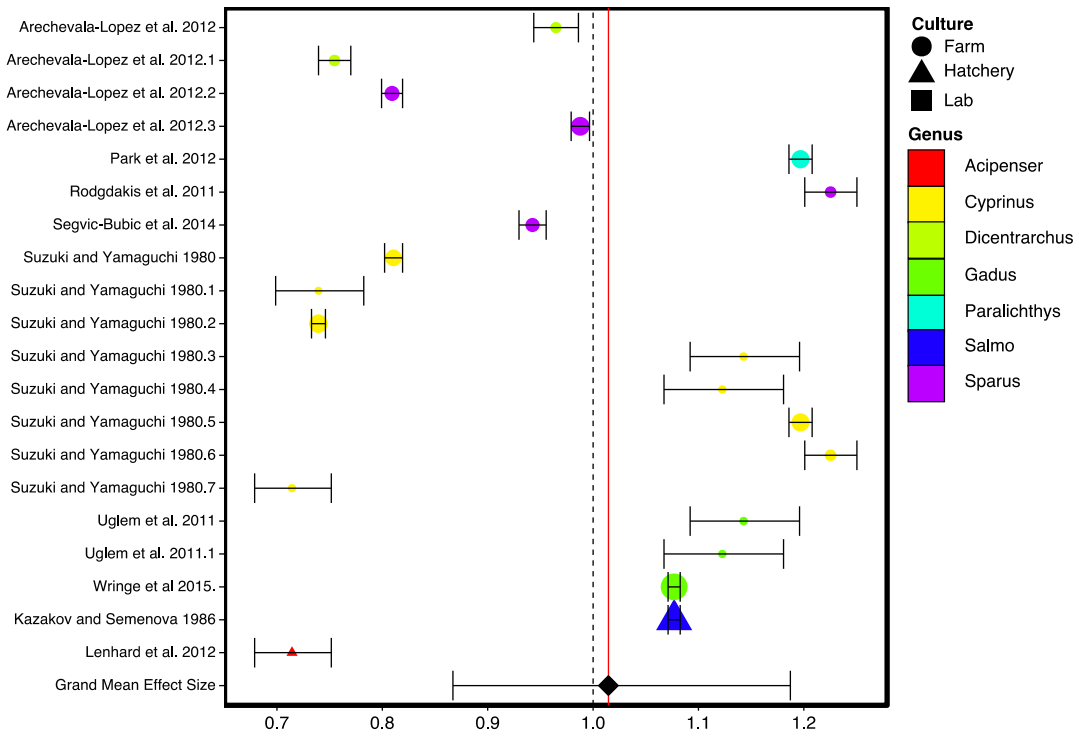
296 Supplementary Figure 3.1g)



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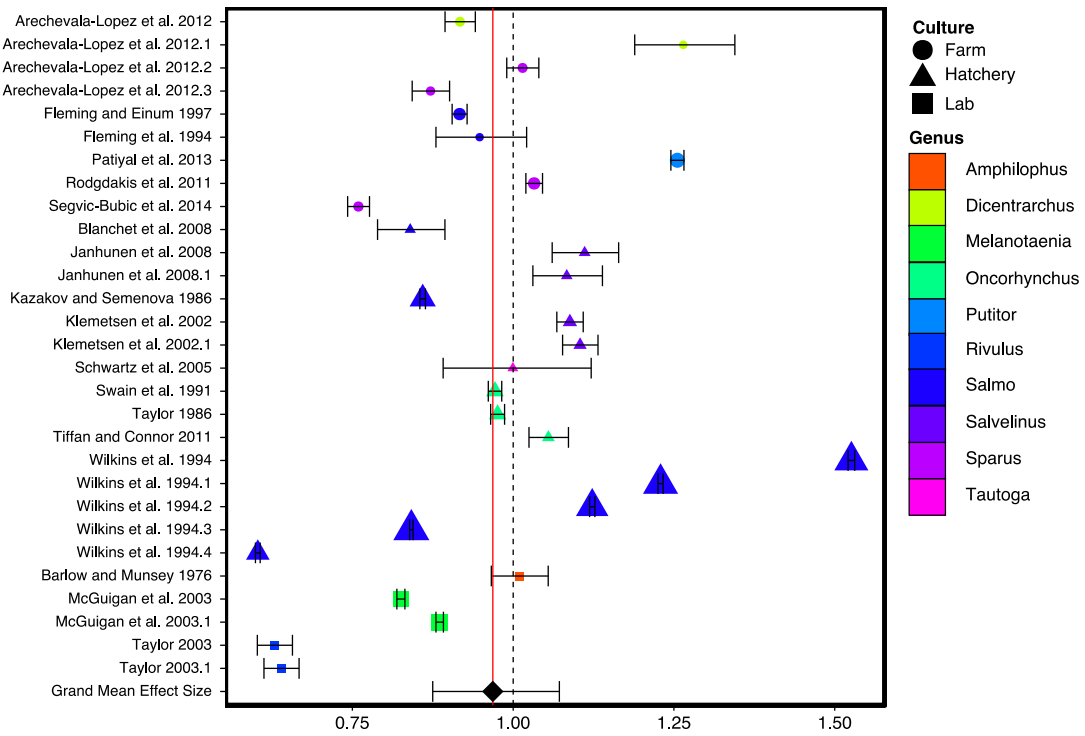
299 Supplementary Figure 3.1h)



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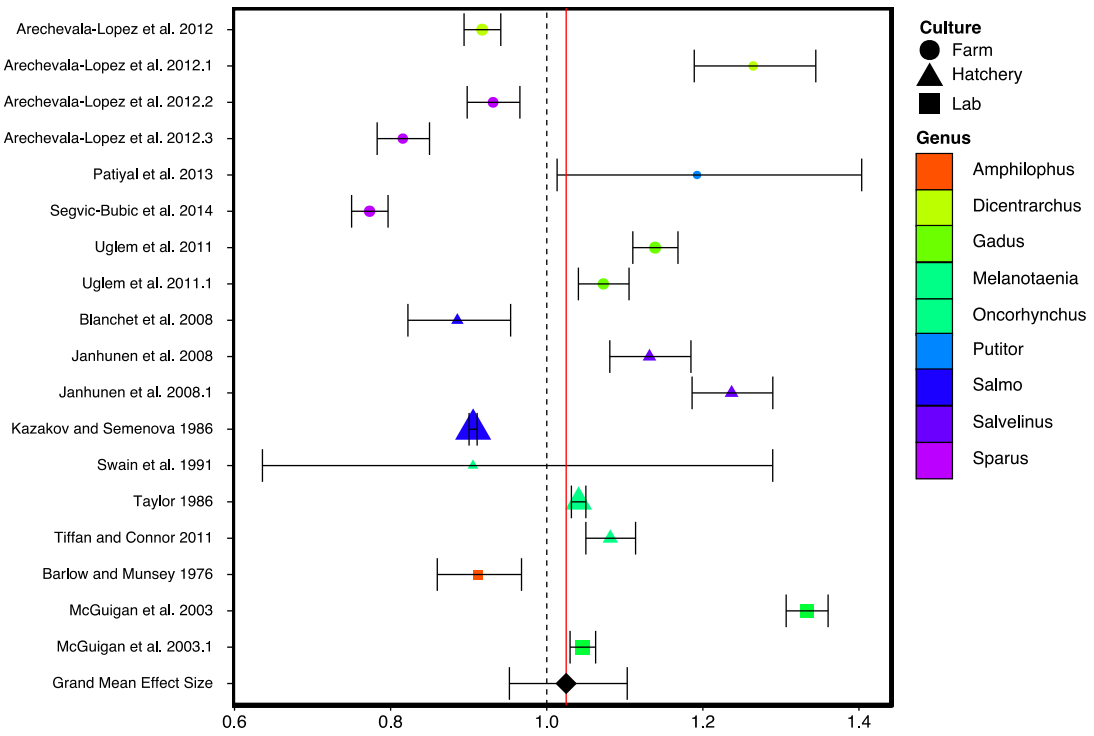
302 Supplementary Figure 3.1i)



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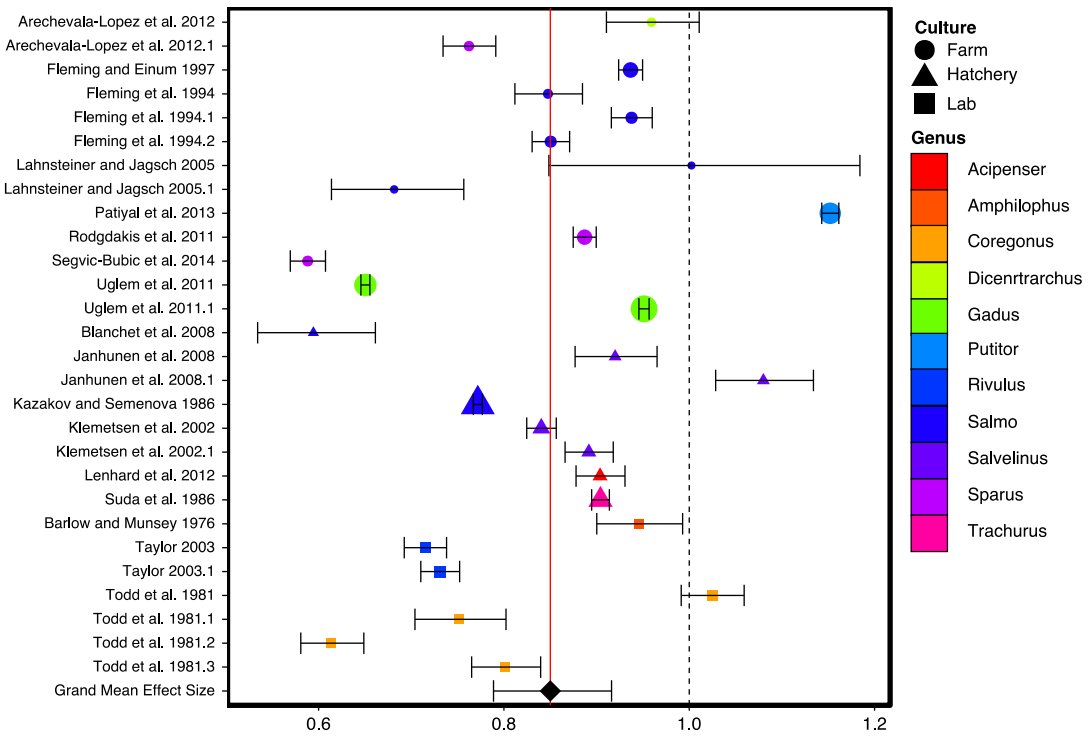

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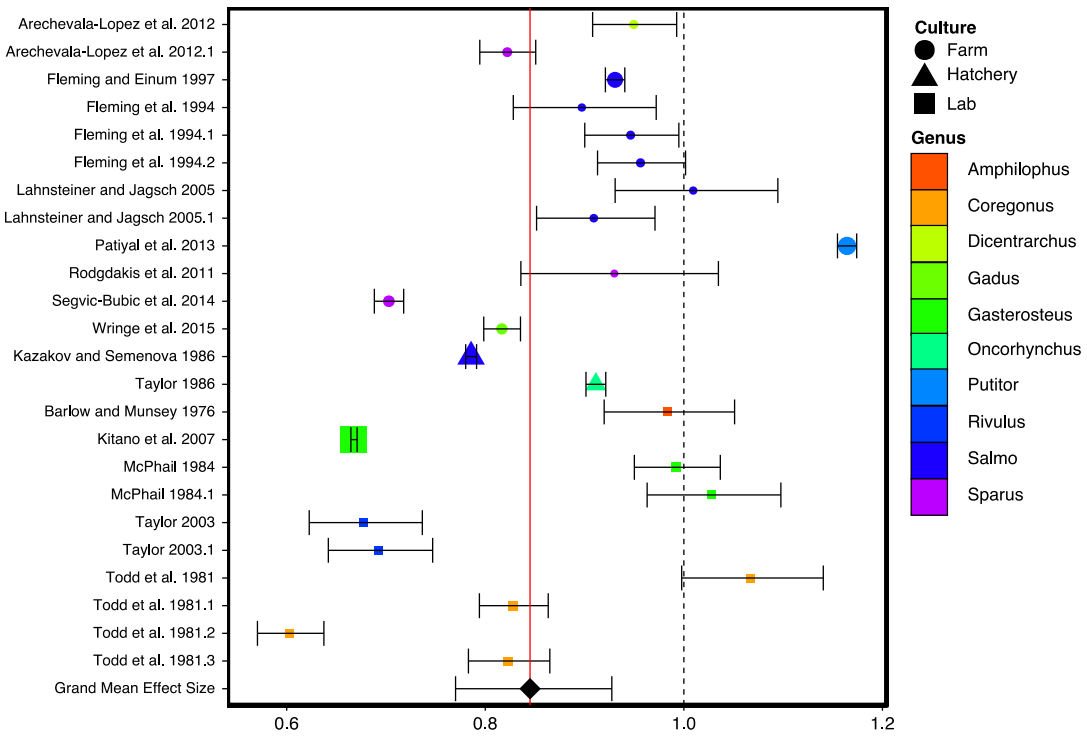

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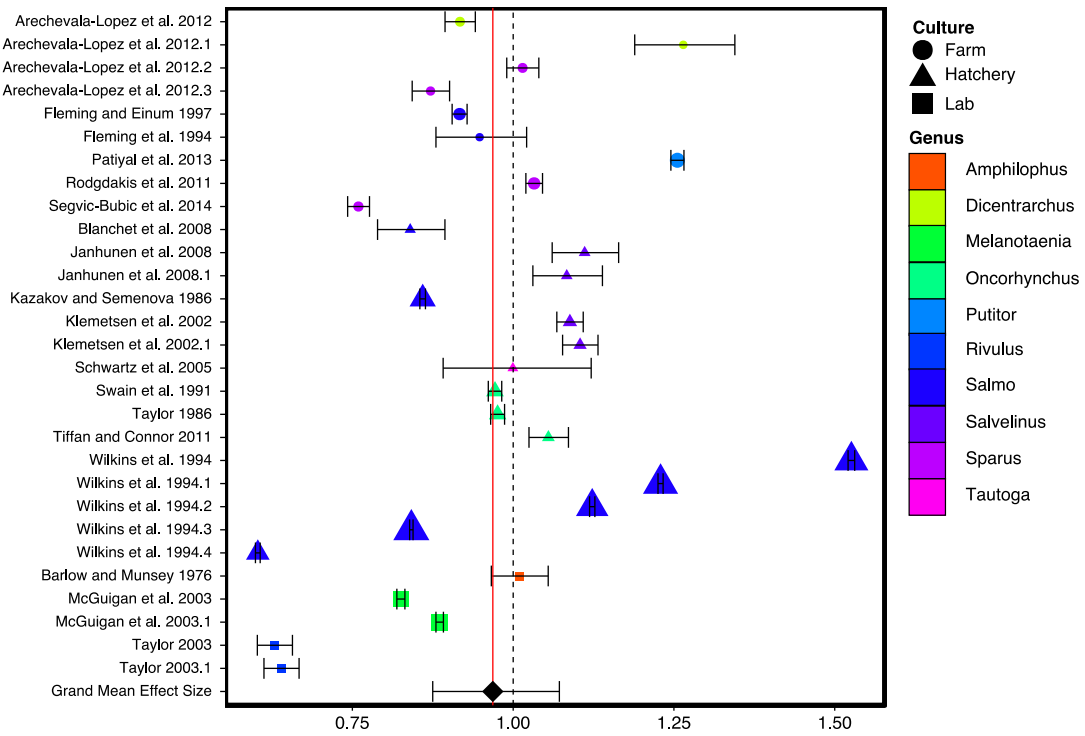
311 Supplementary Figure 3.1l)



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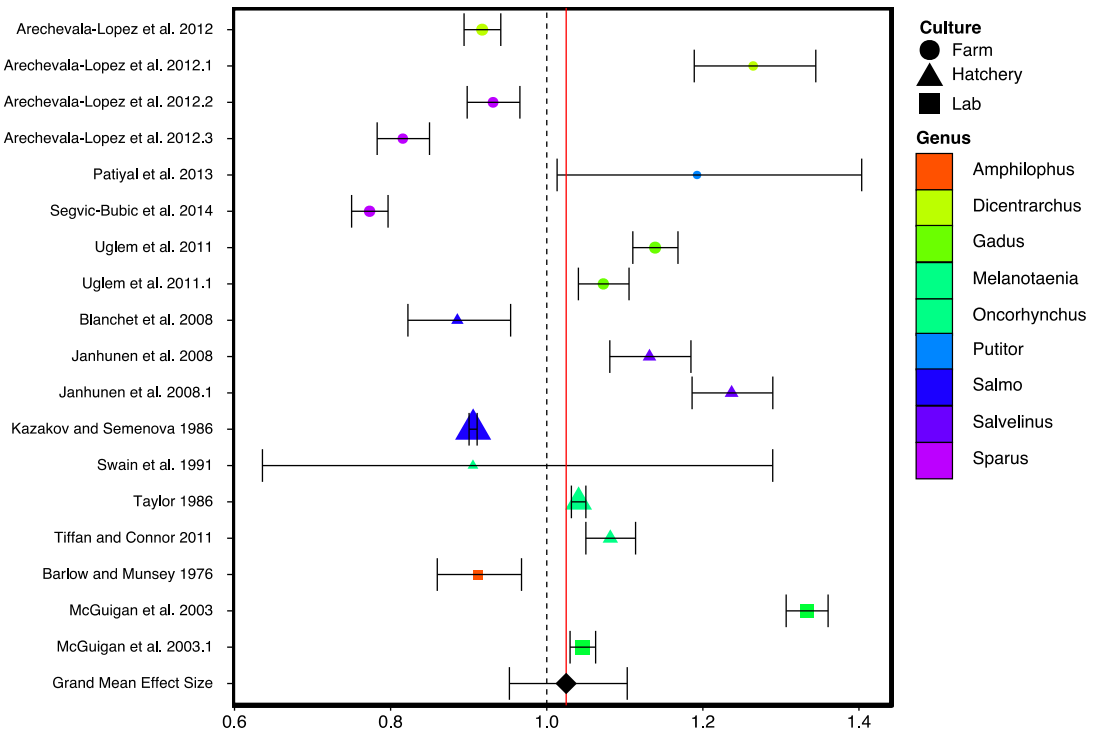
314 Supplementary Figure 3.1m)



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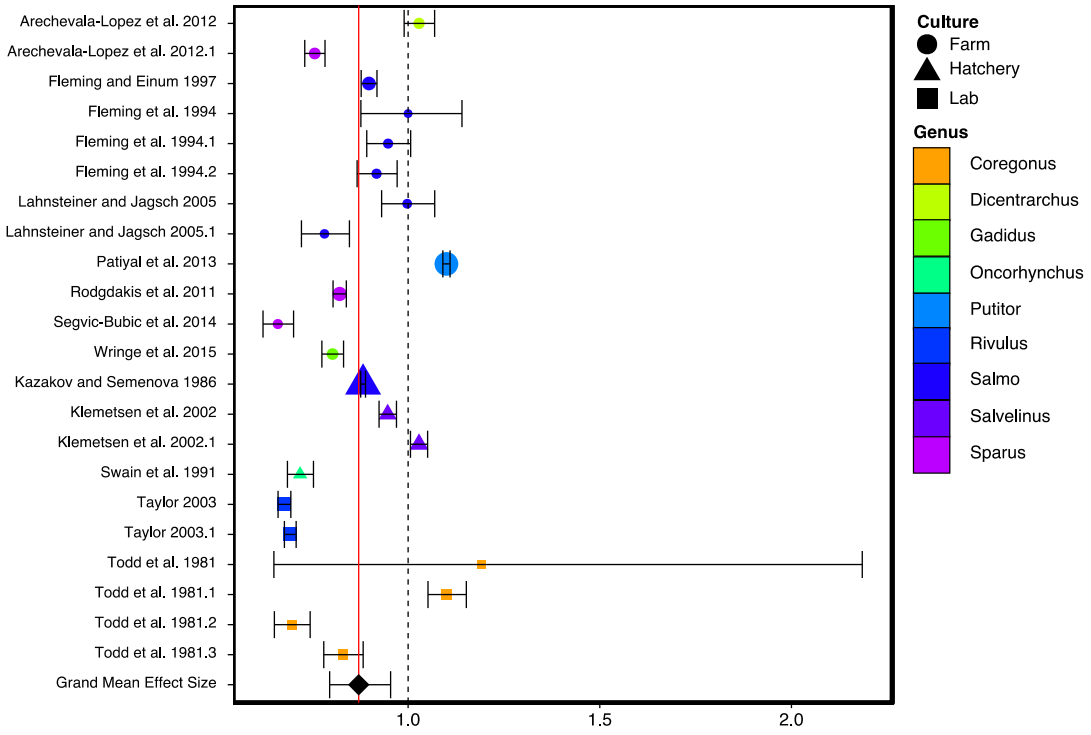
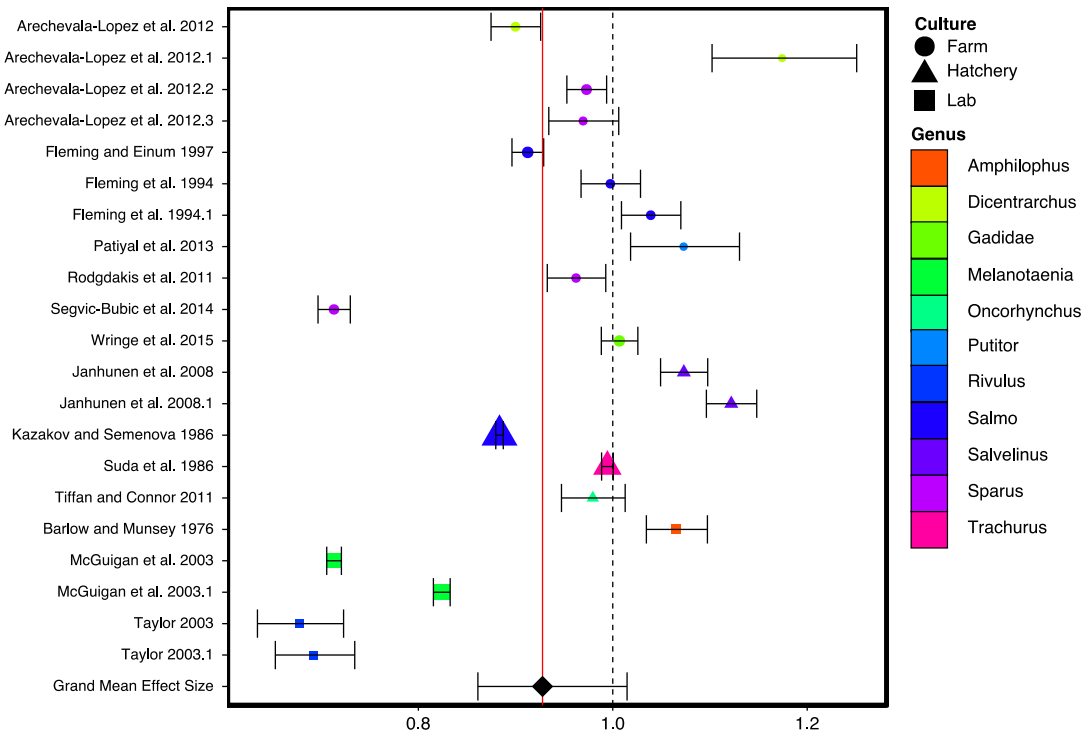
Supplementary Figure 3.1n)



Supplementary Figure 3.1o)



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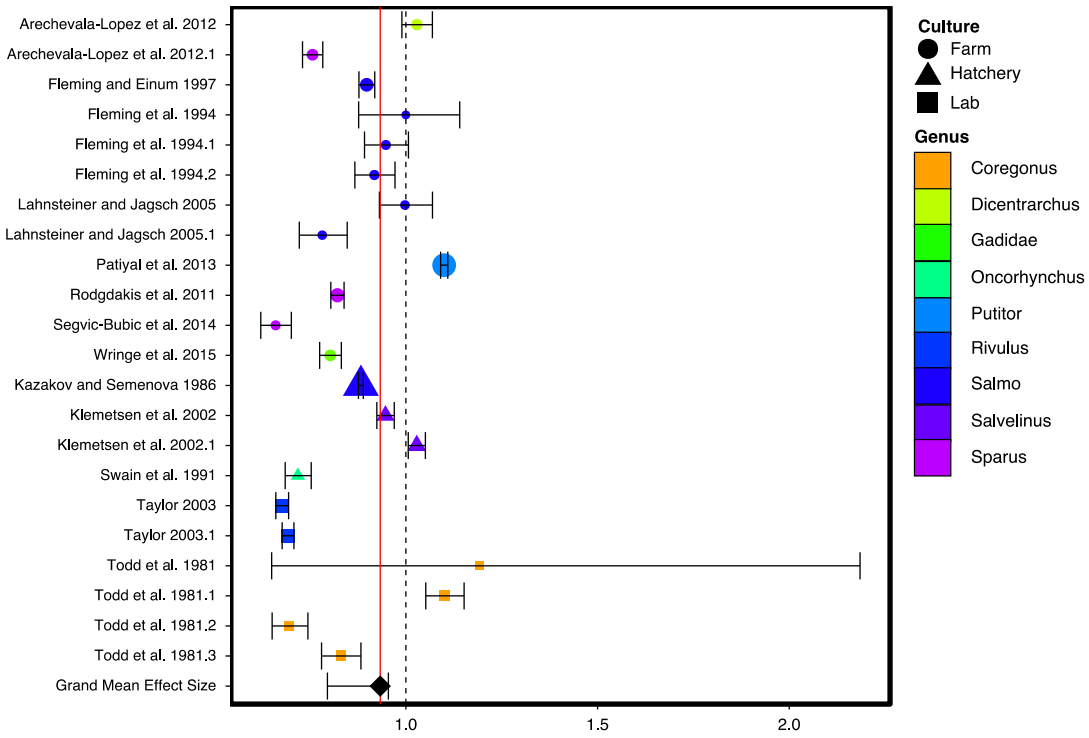

Supplementary Figure 3.1 Forest-plots for each morphological feature examined. The points are the effect size for each study, and the error bars represent the 95% confidence interval around it. The size of the point is reflective of the weighting given to it by the linear-mixed effects function, and a unique colour is given to each genus. The morphological features are as described in Fig. 4.1/Table 4.1, and the species examined can be found in Supplementary Table 3.3.

S1a, Head depth; S1b, Head length; S1c, Eye size; S1d, Upper jaw length; S1e, Lower jaw length; S1f, Body depth; S1g, Condition factor; S1h, Caudle peduncle depth; S1i, Caudle peduncle length; S1j, Pectoral fin length; S1k, Pelvic fin length; S1l, Dorsal fin

length; S1m, Dorsal fin width; S1n, Anal fin length; S1o, Anal fin width; S1p, Caudle fin length