Hybridization potential of brown trout, with particular reference to invaded environments

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Abstract

Hybridization is a complex process beginning with the mating of two species. However, hybrid offspring frequency does not predict hetero-specific mating frequency, as post-mating, both pre-zygotic and post-zygotic barriers influence their occurrence. Post-zygotic outbreeding depression usually results in poor embryo-juvenile survival or the production of sterile hybrid offspring. Females have more to lose with each hybrid fertilization than males, and thus should avoid it. Even if females choose con-specific males as preferred mates, they often cannot control which males release sperm during spawning. Polyandry is ubiquitous and may result in hetero-specific sperm competition. In such cases, cryptic female choice (the ability to bias paternity towards certain males under sperm competition) is the last line of defence to prevent hybridization of her eggs, and is highly adaptive if it enables con-specific sperm preference. Such seems to be the case with hybridization of Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in their native Europe. Under hetero-specific sperm competition, hybrid fertilizations in these fish are reported to be reduced via ovarian fluid mediated cryptic female choice. It is not known however whether the strength of this mechanism is dependent on reinforcement, and thus historical sympatry/allopatry of hybridizing populations. Brown trout are one of the world's worst invasive species. Ecological impacts arise through competition with other species (e.g., Galaxids in the southern hemisphere, *Oncorhynchus* in western North America). Eastern North America is unique in containing native salmonids that evolved in the absence of brown trout, but have gametes that are compatible. The 140 year-old brown trout invasion of Newfoundland is ground zero to study these potential interactions. Their relatively low spread rate across the island may be the result of inherent poor productivity, but data suggest it could also be a function of hybridization with native Atlantic salmon and brook char (*Salvelinus fontinalis*).

Hybridization in general

A hybrid is an individual produced from the mating of two evolutionarily distinct parents. Hybrids are usually considered as between two recognized species (inter-specific hybrids), but importantly, the concept also applies to divergent evolutionary lineages within a species (intra-specific hybrids). Hybridization is a process (Purchase 2022), that when complete is the production of a hybrid individual. Although their presence indicates that hybrid matings must have occurred, their absence tells us nothing about mating. The frequency of hybrids and hybrid matings are not necessarily correlated, as a bottleneck anywhere in the steps (Figure 1) required after mating will prevent hybrids from occurring. Hybrid frequency is thus a poor predictor of earlier stages in the hybridization process.

The presence of hybrid individuals in nature indicates that (1) fertilization has occurred between two species. This can happen if (a) there is a breakdown in pre-mating sexual selection barriers (Purchase, Evans, and Roncal 2021) and a female chooses to spawn with a hetero-specific male, or (b) the female spawns with a con-specific male, but a hetero-specific male releases sperm at the same time and there is a breakdown in post-mating pre-zygotic sexual selection barriers. Both males are in sperm competition to fertilize the same set of eggs, and cryptic female choice (Firman et al. 2017) is not able to bias all paternity towards the con-specific male. There also must be (2) post-zygotic survival of hybrid offspring for long enough so that individuals can be observed at a later age.

FIGURE 1

Hybridization can lead to varied outcomes (Abbott et al. 2013). It sometimes results in the formation of a new species (Mallet 2007), or enhanced fitness through hybrid vigor (Birchler, Yao, and Chudalayandi 2006). More often, when unique evolutionary lineages hybridize, their offspring experience reduced fitness through outbreeding depression (e.g., Cauwelier et al. 2012). The magnitude varies along a continuum, but hybrid animals generally have poor survival and are often sterile, resulting in an evolutionary dead-end (Buss and Wright 1958; Chevassus 1979). It does not matter if hybrid embryos die within days of fertilization, or if decades old individuals are sterile, as both scenarios create extreme fitness consequences. However, the cost is asymmetrical between sexes. Females invest more energy into each gamete than males (Hayward and Gillooly 2011), while males compete for more mating opportunities (Bateman 1948). Females thus have more to lose with each hybrid fertilization than males, and consequently should avoid it.

Pre-mating sexual selection enabled by male-male competition and female choice of males (courtship) reduces the likelihood of hybrid matings if two species are reproductively active in the same place at the same time (Figure 1). Once a female releases eggs, there is no benefit to her to not have them fertilized, but there is benefit in having them fertilized by her own (con-specific) species as opposed to a hetero-specific male. In externally fertilizing fish, despite potential thorough mate choice, females have no control over which additional males release sperm along with her chosen (con-specific) mate. However, pre-zygotic post-mating sexual selection occurs as sperm competition, where ejaculates from two or more males compete to fertilize the same set of eggs (ubiquitous in salmonids), and cryptic female choice that biases paternity towards particular males. Under threat of hybridization when sperm competition occurs between males of different species that are either the same (con-specific) or different (hetero-specific) to the female, cryptic female choice can manifest as con-specific sperm preference, which helps bias paternity towards her own species (Yeates et al. 2013).

Pre-zygotic barriers that reduce fertilization of eggs by hetero-specific sperm are hence highly adaptive (Purchase 2022), whereas post-zygotic barriers influence the presence of hybrid individuals but are irrelevant in reducing the hybridization of a female's eggs and are thus not adaptive (Figure 1).

Hybridization in salmonids with particular reference to *Salmo*

The occurrence of hybridization is well known in the Salmoninae (e.g., DeHaan, Schwabe, and Ardren 2010; Heath, Bettles, and Roff 2010; Kovach et al. 2015; Muhlfeld et al. 2014, 2017). This group of fishes are particularly polyandrous, leading to elaborate pre-mating sexual selection (that can break down and a female might spawn with a hetero-specific male), and intense sperm competition (where eggs can be fertilized by hetero-specific sperm even if a female chose to spawn with a con-specific male, including by alternative reproductive tactic - sneaker males). Mechanisms controlling intra-generic hybridization in the Salmoninae are poorly studied in the field. For example, the frequency of splake (*Salvelinus fontinalis* x *Salvelinus namaycush*) and chumpies (*Oncorhynchus gorbuska* x *Oncorhynchus keta*) is low, while that of cuttbows (*O. mykiss* x *O. clarkii*) can be highly variable (Behnke 2002; Kershner et al. 2019). However, in each case, how wild hybrid frequencies are influenced by the relative importance of various pre- and post-zygotic steps (Purchase 2022) in the hybridization process (Figure 1), is unknown.

The genus *Salmo* contains four species (Kershner et al. 2019; Schoffmann 2021): the localized and poorly studied *S. ohridanus* (Ohrid belvica) and *S. obtusirostris* (softmouth trout), and the widespread *S. salar* (Atlantic salmon) and *S. trutta* (brown trout) that are amongst the most diverse and best studied fishes in the world. *S. salar* and *S. trutta* are naturally sympatric over much of their native range in western Europe. They can create viable hybrid offspring although long-term introgression does not occur, and the process of hybridization in these two species is an evolutionary dead-end (Table 1, reviewed by Lantiegne and Purchase 2023).

TABLE 1

The frequency of hybrid matings between *S. salar* and *S. trutta* is generally not known, and is likely context dependent. There is also a dearth of information on whether hybrid fertilizations primarily result from a breakdown of pre-mating sexual selection (females choose to spawn with hetero-specific males), or post-mating pre-zygotic sexual selection (hetero-specific males win in sperm competition with the con-specific male that the female chose to spawn with). A study from Europe (Yeates et al. 2013) suggests that females of both species can exert a form of post-mating pre-zygotic sexual selection known as cryptic female choice, where they can bias paternity towards con-specific males when there is sperm competition between both species. The mechanism enabling the con-specific sperm preference seems to be related to the ovarian fluid released with eggs, and not the eggs themselves (Yeates et al. 2013). Nothing is known about whether the strength of this mechanism varies across populations.

The post-zygotic fate (Table 2) of *Salmo* hybrid embryos has been studied in the laboratory (reviewed by Poulos (2019)). Conclusions on which cross direction has more acute mortality are very inconsistent, highlighting the need for more focused research on this topic.

TABLE 2

Wild hybrid *Salmo* frequencies are generally low but highly variable both within- and among-regions (Table 3). When hybrid *Salmo* are found in nature, successful spawning, fertilization and survival has obviously occurred. However, it is not known which of those three processes is the primary cause of variation. Post-zygotically, survival differences could be due to genetic nuances among locally adapted populations, including for example the different numbers of chromosomes found in European vs North American salmon (Hartley 1987). Higher frequency of hybrids (Table 3) after “disturbance events” (Poulos 2019) suggest a change in the frequency of hetero-specific mating. When post-mating pre-zygotic sperm competition occurs between both species, variation among populations in the strength of con-specific sperm preference via ovarian fluid enabled cryptic female choice would also result in a shift in hybrid fertilization frequency.

TABLE 3

Brown trout invasions

Brown trout naturally occur from Iceland in the west, across Europe, north Africa, and into central Asia (Schoffmann 2021). Starting in the late 1800s they were purposefully introduced to other parts of the world that had suitable freshwater temperatures (Hustins 2007). This fish is now considered one of the world’s 100 worst invasive species (Lowe et al. 2000). Most of the recognized concern is related to ecological competition with native species. This is particularly problematic in regard to competition with galaxids (Kershner et al. 2019; Schoffmann 2021) in the southern hemisphere, and with *Oncorhynchus* trout in western North America (Behnke 2002; Schoffmann 2021). Potential evolutionary impacts of brown trout invasions have generally been ignored. Intra-specifically, the introduction/movement of trout across their native range from hatcheries in one localized area (e.g. Atlantic lineage), to other regions, likely led to the destruction of much local adaptation that has not been documented. Inter-specifically, there seems to have been an ancient introgression of brown trout mtDNA into softmouth trout (Sušnik et al. 2007). The impact through human induced introductions varies among regions. There are no native salmonids in the southern hemisphere, and trout are likely too distantly related to Galaxiidae to be a hybridization threat. The same may be true for *Oncorhynchus*, but it is particularly moot given both cutthroat (*O. clarki*) and rainbow trout (*O. mykiss*) are spring spawners. However, the brown trout introduction to eastern North America has created unique issues related to invasive hybridization.

Four species of salmonid fishes are native to the north eastern portion of North America (Behnke 2002). *Salvelinus alpinus* (Arctic char) and *Salvelinus namaycush* (lake char) are found in habitats much colder than those preferred by brown trout and are not likely under much hybridization threat from invasion. Brook char (*Salvelinus fontinalis*) however do have overlapping habitat with invading brown trout. Ecological competition between these two species is intense (Behnke 2002), and their hybrids (tiger trout), as sterile F_1 are often created for stocking programs (Kershner et al. 2019). These two species did not co-evolve and may lack pre- and post-mating barriers to reduce hybrid fertilizations (Lantiegne and Purchase 2023).

North American Atlantic salmon have been isolated from European counterparts for at least 600,000 years (Cauwelier et al. 2012; Lehnert et al. 2020), and probably evolved in the absence of brown trout for longer. During this time they did not need adaptations to reduce hybrid fertilizations with brown trout, such as mechanisms enabling con-specific sperm preference when under hetero-specific sperm competition. Not surprisingly, the frequency of hybrid juveniles in streams has been reported to be higher than in the natively sympatric Europe (Table 3), although monitoring is generally very poor, and the conclusion is speculative.

Ground zero - the brown trout invasion in Newfoundland

The island of Newfoundland became surrounded by salt water before deglaciation, and the only fishes to reach its fresh waters were euryhaline (e.g., sticklebacks and salmonids).

Many eastern North American fishes are absent, including typical lake specialists. For example, there are no Cypriniformes, Esociformes, Siluriformes, Perciformes, or Coregoninae (Scott and Crossman 1964). Salmonids dominate but there are no lake char. High relief and short watersheds create tens of thousands of unique populations. Dwarf non-anadromous Arctic char are in many lakes deeper than ~20 m, and native brook char occur everywhere. There are several hundred anadromous Atlantic salmon populations, along with hundreds/thousands resident Atlantic salmon populations in lakes (ouananiche). No measurable stocking for these species ever occurred, hence these populations are genetically pure. Due to the lack of other fishes in Newfoundland, brook char and Atlantic salmon use lentic habitat much more extensively than elsewhere and are consequently highly prone to interactions with brown trout.

Brown trout became established on the island of Newfoundland in the 1880s, after purposeful introductions from Europe (Hustins 2007). They and rainbow trout (*O. mykiss*) from California were stocked into several watersheds on the Avalon Peninsula, mostly near the city of St. John’s. Over the past 140 years, anadromous brown trout have strayed, and invaded new watersheds. However, the spread rate is very low compared to other places where trout were introduced (MacDonald, Leroux, and Purchase 2022; Westley and Fleming 2011). Many non-mutually exclusive hypotheses could account for the slow rate. For example, (1) if the environment has particularly poor productivity, this would limit intrinsic population growth rates (of all salmonids) and therefore reduce “propagule pressure”. Similarly, (2) competition with native salmonids would reduce growth rates of newly established trout populations. All watersheds that could be invaded contain native brook char, and most contain Atlantic salmon, presenting levels of competition that might not exist in some other regions of the globe. Unlike for example the southern hemisphere, (3) in Newfoundland hybridization of trout eggs by male native Atlantic salmon and brook char would substantially reduce the number of trout (propagules) in the next generation to establish a newly colonized watershed or be available to stray into the next. This would presumably be most pronounced on the invasion front (lowest ratio of invasive trout / native salmon and char).

Brown trout hybridization research in Newfoundland

There has been limited documentation of the frequency of brown trout hybrids in Newfoundland. Anglers catch tiger trout (brook char X brown trout) but there is no monitoring to track their occurrence. Anglers cannot reliably identify Atlantic salmon X brown trout hybrids. Using molecular approaches, some limited surveys were completed (Table 2), but there has been no update in > 30 years.

Our recent laboratory work has repeatedly shown that the gametes of introduced brown trout and native Newfoundland Atlantic salmon are fully compatible (Figure 2). Each species of eggs can be fertilized by either con-specific or hetero-specific sperm. Survival to hatch is almost as good for hybridized eggs as those fertilized by con-specific sperm (Figure 2). The swimming ability of sperm of both species responds very positively to the presence of ovarian fluid (Purchase and Rooke 2020). However, in a carefully designed experiment, Lantiegne and Purchase (2023) showed that ovarian fluid from native brook char and Atlantic salmon did not preferentially improve the swimming performance of con-specific sperm any better than it did for sperm of brown trout. Therefore, unlike in Europe (Yeates et al. 2013), cryptic female choice may not be able to reduce hybrid fertilizations in Newfoundland when there is hetero-specific sperm competition. Such a result might be expected if reinforcement is needed for species to evolve and maintain barriers to hybridization. North American salmonids did not need to exclude brown trout sperm, whereas their European cousins seem to possess this ability (Yeates et al. 2013).

FIGURE 2

Future recommendations

More research is needed on the scope and impact of invasive brown trout hybridization in Newfoundland and other parts of eastern North America. In reference to hybridization with *S. salar*, we recommend the following: sperm competition experiments to determine the strength of con-specific sperm preference via cryptic female choice and whether this varies among populations, field experiments to evaluate the performance of hybrid individuals in nature, surveys to determine the frequencies of hybrid individuals at different life stages in rivers, and extensive research to determine the extent to which trout hybridization of salmon eggs reduces salmon population productivity, and salmon or char hybridization of trout eggs reduces the spread of this invasive species.

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Table 1 (modified from Lantiegne & Purchase 2023): Summary of costs of phylogenetic relatedness, the likelihood of egg exposure, and hybrid fitness. Bold and italicized text indicates our conclusions.

Hybrid (mother species/ father species)	Salmon/Trout	Trout/Salmon	Trout/Char	Char/Trout	Salmon/Char	Char/Salmon
Taxonomic relatedness	• **Close** • Same genus	• **Close** • Same genus	• **Most distant** • Different genus • Share less recent common ancestor (Lecaudey et al. 2018)	• **Most distant** • Different genus • Share less recent common ancestor (Lecaudey et al. 2018)	• **Moderately distant** • Different genus • Share more recent common ancestor (Lecaudey et al. 2018)	• **Moderately distant** • Different genus • Share more recent common ancestor (Lecaudey et al. 2018)
Spawning overlap	• **Very high** • Both species observed to spawn at same place and time	• **Very high** • Both species observed to spawn at the same place and time	• **Very high** • Overlap in spawning period and habitat (Sorensen et al. 1995)	• **Very high** • Overlap in spawning period and habitat (Sorensen et al. 1995)	• **Medium, brook char males may still be active during salmon spawning** • Small overlap (McGowan and Davidson 1992b; O'Connell 1982)	• **High, Atlantic salmon sneaker males may be active during brook char spawning** • Small overlap (McGowan and Davidson 1992b; O'Connell 1982)
Likelihood of eggs exposed to sperm	• **High** • Both species observed to spawn with each other (McGowan and Davidson 1992b)	• **High** • Both species observed to spawn with each other (McGowan and Davidson 1992b)	• **High** • Both species observed to spawn with each other (Sorensen et al. 1995)	• **High** • Both species observed to spawn with each other (Sorensen et al. 1995)	• **Low, brook char males might still be active during salmon spawning.** • Potential brook char sneakers, not known for sure (Sorensen et al. 1995)	• **Medium, brook char females unlikely to be ripe during peak salmon spawning** • **Early Atlantic salmon sneakers may try to fertilize eggs**
Fertilization occurs	• Yes (Chevassus 1979)	• Yes (Chevassus 1979)	• Yes (Chevassus 1979)	• Yes (Chevassus 1979)	• Yes (Chevassus 1979)	• Yes (Sutterlin, MacFarlane, and Harmon 1977)
Parent/hybrid chromosome numbers (North American numbers)	• Mother 58 • Father 80 • Hybrid 69 (Nygren, Nilsson, and Jahnke 1972)	• Mother 80 • Father 58 • Hybrid 69 (Nygren et al. 1972)	• Mother 80 • Father 84 • No data (Nygren et al. 1972), (Hartley 1987)	• Mother 84 • Father 80 • No data (Nygren et al. 1972), (Hartley 1987)	• Mother 58 • Father 84 • No data (Nygren et al. 1972), (Hartley 1987)	• Mother 84 • Father 58 • No data (Nygren et al. 1972), (Hartley 1987)
Offspring hatch	• **Yes, high hatch rate** • 80-100% of the hatch rate of the conspecific cross (Chevassus 1979)	• **Yes, high hatch rate** • 80-100% of the hatch rate of the conspecific cross (Chevassus 1979)	• **Yes, high hatch rate** • 80-100% of the hatch rate of the conspecific cross (Chevassus 1979)	• **Yes, low hatch rate** • <10% of the hatch of the conspecific cross (Chevassus 1979)	• **Yes, medium hatch rate** • 40-80% of the hatch rate of the conspecific cross (Chevassus 1979)	• **Yes, very low hatch rate** • 3% hatch rate in one case • Total mortality before hatch in another study (Blanc and Chevassus 1979), (Sutterlin et al. 1977)
Offspring survival to one year	• **F1 high** • 80-100% of the survival of the conspecific cross • F2 none (Chevassus 1979), (Makhrov 2008)	• **F1 high** • 80-100% of the survival of the conspecific cross • F2 none (Chevassus 1979), (Makhrov 2008)	• **F1 medium** • 40-80% of the conspecific cross (Chevassus 1979)	• **F1 low** • <10 percent of the survival of the conspecific cross (Chevassus 1979)	• **F1 low** • <10 percent the survival of the conspecific cross (Chevassus 1979)	• None (Chevassus 1979)

Offspring fertility	• F1 fertile • Hybrid-hybrid F2 infertile • Backcrosses of this cross to salmon produce offspring with high survival and reduced fecundity (Chevassus 1979), (Makhrov 2008), (Garcia-Vazquez et al. 2004)	• F1 fertile • Hybrid-hybrid F2 infertile • Backcrosses of this cross to salmon produce infertile offspring with low survival (Chevassus 1979), (Makhrov 2008), (Garcia-Vazquez et al. 2004)	• F1 fertility extremely rare • Offspring in this case backcrossed with brook trout female resulting in very low survival to fry stage (Buss and Wright 1958)	• F1 infertile (Chevassus 1979)	• F1 infertile (Chevassus 1979)	• None (Chevassus 1979)
Reproductive costs to parents	• **Near total loss by F2 generation**	• **Total loss by F2 generation**	• **Total loss by F1 generation**	• **Total loss by F1 generation**	• **Total loss by F1 generation**	• **Total loss by F1 generation**

Table 2. Accessible published literature in English of *S. salar* and *S. trutta* hybrid viability (survival) and the developmental stage analyzed by cross direction in laboratory experiments in accordance to geographical region. Constraints and/or limitations highlight the evaluation of female/male contributions, control for individual variation, and general issues encountered.

Reference	**Region**	**Developmental stage(s) analyzed**	**Survival (♀x♂)**	**Evaluation of maternal/ paternal contribution**	**Control for individual variation**	**Other constraints/limitations**
North America (laboratory experiments)						
(McGowan and Davidson 1992a)	Newfoundland	·Fertilization to hatching ·Hatching to yolk-sac absorption	·Higher post-hatch mortality for BTxAS	Yes	No	·Cross-types were grouped together after hatching ·Suspected egg mortality caused by physical damage and poor gamete quality ·Total (n) of eggs for each family were rounded to the nearest 10; poor statistical evidence
(Gray, Evans, and Thorgaard 1993)	United States	·Fertilization to eyed, hatching, and yolk-sac absorption	·High mortality for all offspring ·Highest mortality for BTxAS	Yes	No	·Very high mortality for all experimental cross-types ·Families derived from 3 single-pair fertilizations; weak design
(Oke et al. 2013)	Newfoundland	·ca. 100 days following start of exogenous feeding	·Higher post-hatch mortality for BTxAS	Yes	No	
(Poulos 2019)	Newfoundland	·Hatching to yolk-sac absorption	·Higher post-hatch mortality for ASxBT	Yes	Yes	·Small sample size (n=2 females of each species), biological significance questionable ·Mortality monitored to only 25%
Europe (laboratory experiments)						

Reference	**Region**	**Developmental stage(s) analyzed**	**Survival (♀x♂)**	**Evaluation of maternal/ paternal contribution**	**Control for individual variation**	**Other constraints/limitations**
(Refstie and Giedrem 1975)	Norway	·Fertilization to eyed, and hatching	·High mortality for salmon egg cross-types ·Highest mortality for pure salmon	Yes	No	·Vague methodology ·Number of individuals used in experiment not mentioned
(Blanc and Chevassus 1979)	France	·Fertilization to eyed, and 15 days post hatching	·High mortality to hatching for trout egg hybrids ·Very high mortality 15 days post hatching for trout egg hybrids	Yes	No	·Vague methodology ·No information post eyed-stage for salmon egg hybrids ·Conspecific sperm were pooled, distorting variation in fathers
(Babiak et al. 2002)	Poland	·Fertilization to eyed, and to mid yolk-sac absorption	·Higher mortality for trout egg hybrids than pure trout	Yes	No	·Conspecific eggs were pooled ·Salmon egg cross-types were not created in this experiment ·How mid yolk-sac absorption was determined not mentioned
(Garcia-Vazquez et al. 2002)	Spain	·Fertilization to fry stage	·Very low survival for all cross-types	Yes	Yes	·Survival percentages were calculated using an “estimated” number of eggs; not an actual count

Reference	Region	Developmental stage(s) analyzed	Survival (♀x♂)	Evaluation of maternal/ paternal contribution	Control for individual variation	Other constraints/limitations
			·Lowest survival for pure salmon			·Survival estimates assumed all eggs were fertilized
(Álvarez and Garcia-Vazquez 2011)	Spain	·Fertilization to yolk-sac absorption	·Higher post-hatch mortality for BTxAS	Yes	Yes	·Eggs assumed not fertilized if they did not reach eyed stage ·Figure 2 has no error bars to evaluate if cross-direction trend was universal across individual parents ·Figure 4 lines should reach 100% given they are adjusted to show timing of what hatched ·Unclear if fish were grouped together by cross-type after hatching or not

Table 3. Accessible published literature in English regarding the observed frequency of *S. salar* and *S. trutta* hybrids in nature and the direction of hybridization in accordance to geographical region, the extent of sample site(s) and the number of fishes analyzed (life stage sampled varied among studies). Percentages in "Direction of hybridization" columns are within the "Mean frequency."

Reference	Region	Sample site(s)	(n) Fishes analyzed	Direction (♀x♂)	Mean frequency (%)
North America					
(Beland, Roberts, and Saunders 1981)	Nova Scotia	1 river	56	No information	1.80
(Verspoor 1988)	Newfoundland	10 watersheds	786	No information	0.90
(McGowan and Davidson 1992b)	Newfoundland	9 rivers	792	Directional (BTxAS)	4.67†
(Gephard, Moran, and Garcia-Vazquez 2000)	United States	1 river	137	Directional (BTxAS)	0.81†
Europe					
(Crozier 1984)	Ireland	1 watershed	426	No information	3.60†
(de Leaniz and Verspoor 1989)	Spain	4 watersheds	175	No information	2.30
(Jansson et al. 1991)	Sweden	1 river	332	No information	13.00†
(Hurrell and Price 1991)	England	6 rivers	559	Bi-directional (ASxBT) = 62.3% (BTxAS) = 37.7%	1.40
(Jordan and Verspoor 1993)	Britain	23 rivers	5697	No information	1.00†
(Youngson et al. 1993)	Scotland	16 rivers	2373	Directional (ASxBT)	1.00†
(Hindar and Balstad 1994)	Norway	Multiple rivers	8665	No information	0.24
(Elo et al. 1995)	Norway, Finland	2 rivers	2024	No information	0.15
(Gross, Nilsson, and Schmitz 1996)	Sweden	4 watersheds	482	No information	1.60
(Hartley 1996)	Britain	1 river	55	Bi-directional (BTxAS) = 90%	18.18†

Reference	Region	Sample site(s)	(n) Fishes analyzed	Direction (♀x♂)	Mean frequency (%)
				(ASxBT) = 10%	
(Jansson and Öst 1997)	Sweden	1 river	2256 323 restored site	No information	1.60 41.50†
(Matthews et al. 2000)	Ireland	13 rivers	4135	Directional (ASxBT)	1.20†
(Paaver, Gross, and Vasemagi 2001)	Estonia	5 rivers	821	Bi-directional	2.80†
(Garcia-Vazquez 2001)	Spain, France	7 rivers	721	Directional (ASxBT)	2.53†
(Castillo et al. 2008)	Spain, France	8 rivers	1630	Bi-directional	7.81† 1.39
(Castillo et al. 2010)	Spain	10 rivers	1652	Directional (ASxBT)	1.35
(Chelenkova et al. 2011)	Bulgaria	19 watersheds	146	No information	8.90†
(Adams et al. 2014)	Scotland	6 rivers 4 rivers	281 48 migratory	Bidirectional (BTxAS) = 80% (ASxBT) = 20%	0.70 10.40†
(Solem et al. 2014)	Norway	1 river	232	Bi-directional (BTxAS) = 85% (ASxBT) = 15%	27.00†

Notes: *Means marked with (†) mentioned disturbance factors likely responsible for high frequency; direction of hybridization for Adams et al. 2014 is the analysis for the migratory fish sample only; most literature is unclear about the size/extent of areas sampled, but either listed number of rivers or number of catchments/watersheds - a river is a drainage feature of a watershed, and a watershed is the drainage area.*

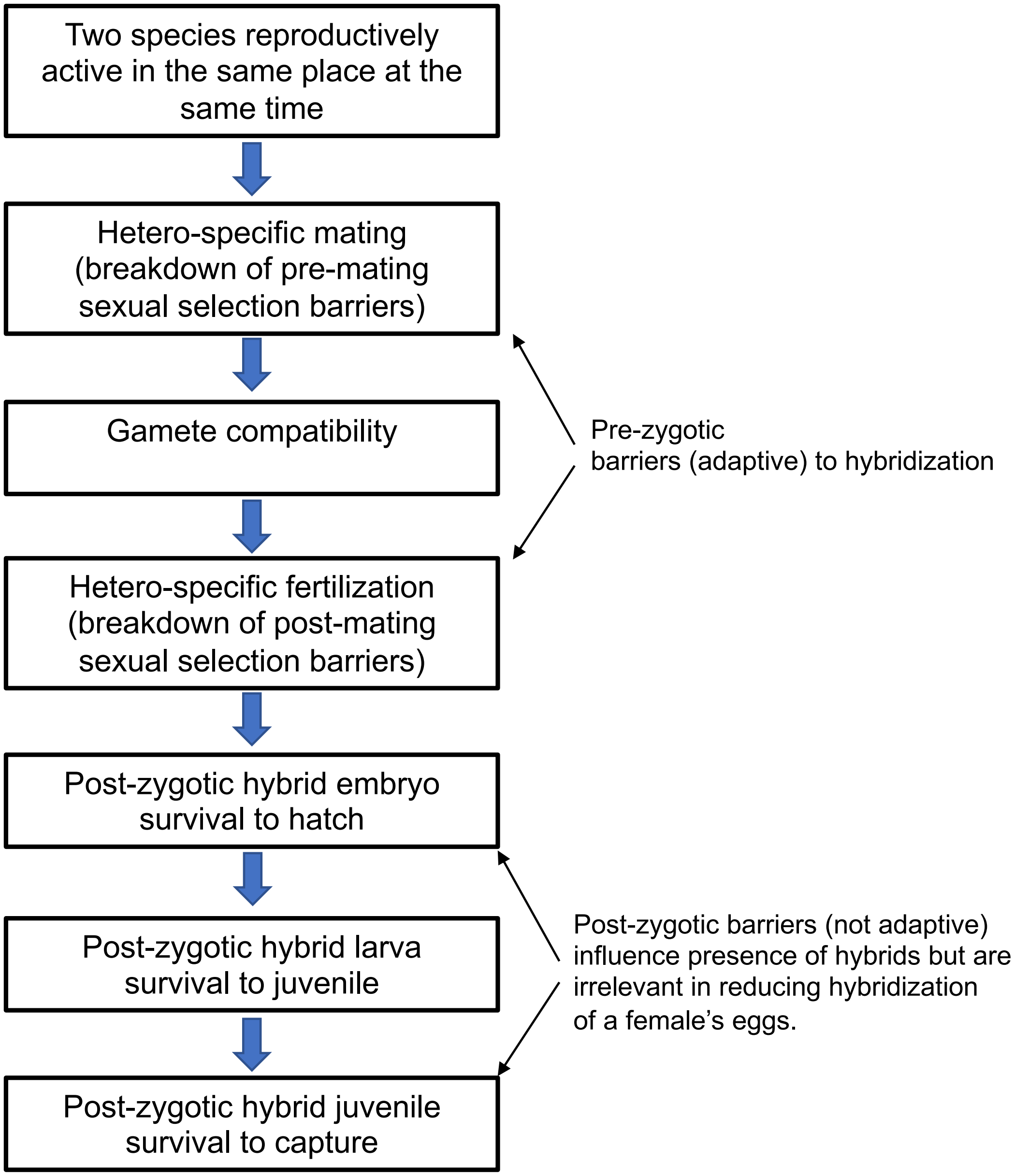

Figure 1:Successful reproductive steps that must have occurred when hybrid fish are found in the wild (internal fertilizers also require successful insemination). Note that pre-mating sexual selection is typically enabled by male-male competition and female mate choice for males, and post-mating sexual selection is typically enabled by sperm competition and cryptic female choice (including con-specific sperm preference).

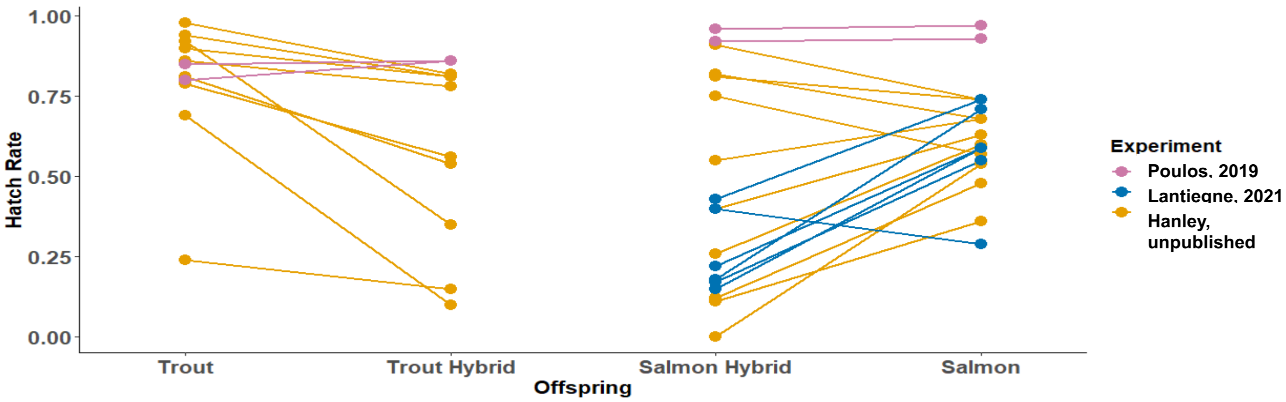

Figure 2: Average hatch rate of salmonid eggs from Poulos (2019, pink), Lantiegne (2022, blue) and Hanley (unpublished, orange). All three studies used similar methodology. Each line is an individual female whose eggs were split into two broods and fertilized by a con-specific and hetero-specific male. The gametes are very compatible, achieving high fertilization success and good hatch success, at times higher for hetero-specific crosses. On average, hatch success of hybridized trout eggs (from 11 females) were only 19.5% lower than pure trout, and hybridized salmon eggs (from 18 females) were 17.9% lower than pure salmon.

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