



Fish sperm competition in hatcheries and between wild and hatchery origin fish in nature

José Beirão ^{a,*}, Torvald B. Egeland ^b, Craig F. Purchase ^c, Jarle T. Nordeide ^{a,d}

^a Faculty of Biosciences and Aquaculture, Nord University, NO, 8049, Bodø, Norway

^b Faculty of Education and Arts, Nord University, NO, 8049, Bodø, Norway

^c Biology Department, Memorial University of Newfoundland, St. John's, NL, A1B 3X9, Canada

^d School of Biological Sciences, University of Wollongong, Wollongong, NSW, 2522, Australia

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ABSTRACT

Males compete pre- and post-mating to fertilize the maximum number of eggs. In polyandry, sperm competition occurs when sperm from two or more males compete to fertilize eggs from a female. Here we review how sperm competition from hatchery origin fish can cause loss of genetic variability in fish populations kept in captivity and in wild populations. In fish hatchery practices, sperm competition occurs in mass spawners that release gametes in tanks, and in artificial fertilizations when pooled semen is used. In mass spawnings sperm competition is difficult to tease apart from pre-mating competition and other post-mating selective mechanisms, whereas, studies focused on the use of pooled semen in different fish species have shown a clear relationship between sperm motility parameters and precedence in fertilization. In both situations, sperm competition will result in a loss of genetic variability that accumulates over generations, but hatchery protocols can be adjusted to mitigate it. Another source of concern regarding sperm competition for hatchery produced fish is the spatial and temporal overlap in spawning with wild individuals, either via aquaculture escapees or purposeful stocking programs. This may result in sperm competition between hatchery origin and wild males and impact natural populations. Our review suggests that in order to give every adult selected as broodstock an equal opportunity to produce offspring in captivity, mass spawning and the use of pooled semen should be limited.

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1. Introduction - sperm competition in fish

In polyandrous species females gain fitness from mating with multiple males [1,2]. Polyandry is achieved either by multiple mating events or a single mating event where more than one male provides sperm to receptive eggs. Pre-mating competition occurs among males via direct contests or by alternative reproductive tactics (ARTs) [3,4]. Sperm competition is a post-mating event in which sperm from several males compete to fertilize the eggs [5]. It is a central part of sexual selection theory [6], which concerns an individual's traits that are important for their reproductive success [7,8]. Females can intervene in post-mating competition through cryptic female choice and bias sperm competition success [9,10]. This occurs via different mechanisms including direct sperm-egg interactions based on the major histocompatibility complex [11]

or ovarian fluid influences on spermatozoa swimming [12,13], which is the focus of another review in this same issue [14].

The evolutionary biologist Geoff Parker suggested [5,15] that a male's optimal sperm competition strategy depends on the strategy adopted by his competitors. These models or sperm competition games, as Parker termed them, predict the optimal amount of energy, size and number of sperm to invest in an ejaculate, and when to mate singly or multiply with the same female. The magnitude of sperm competition can be thought of as either its risk or intensity. Risk of sperm competition refers to the probability of the ejaculate of a male competing for the same eggs with the ejaculate of another male, whereas intensity refers to the number of competing males in the sperm competition [16]. In practice, there are two different means to measure sperm competition, by visual observations at spawning sites to identify mating behavior, or by use of molecular tools to measure paternity of progeny [17].

Sperm competition occurs in both internal and external fertilizers. In internal fertilizers it arises when more than one male inseminates a female within a single fertile period, leading to sperm

* Corresponding author.

E-mail address: jose.beirao-dos-santos@nord.no (J. Beirão).

competition inside her reproduction tract. In contrast, for external fertilizers sperm competition occurs in the aquatic environment, outside the female's reproductive tract, and she has less control over which males release sperm near her receptive eggs. External fertilization is by far the most common reproductive strategy in fishes (see [Supplementary Table 1](#)) and is the subject of this review. In external fertilizing fishes, fertilization occurs rapidly [18], spermatozoa swimming longevity is short, and sperm competition generally takes place over a scale of seconds or minutes. Knowledge regarding sperm competition for hatchery origin fish is very scattered. We have compiled information for the most important farmed species (see [Supplementary Tables 1 and 2](#)). In this work we review how sperm competition in hatchery produced fish can be responsible for the loss of genetic variability under two scenarios. The first is when the fish are propagated in captivity and the second scenario when hatchery origin fish compete in nature with wild origin fish.

Most studies dealing with sperm competition in external fertilizing fishes use the terms pre- and post-copulatory competition to refer to sexual competition before and after the gametes release [12,13,19]. Copulation implies the union of sex organs, which is not common among fishes, thus in this review we chose to use pre- and post-mating competition to refer to the sexual competition before and after gamete release, as is also found in some literature [11].

1.1. Alternative reproductive tactics (ARTs) and sperm competition

In species with ARTs, dramatically different reproduction traits are tailored to increase the fertilization success of individual males. These traits result in changing the odds of fertilization in either pre- or post-mating competition. A trade-off between different traits is expected [20] as increasing the likelihood of fertilization success by investment in one trait reduces allocation to alternative traits. The two most common ARTs in fishes are guarding or territorial tactic (dominant males) and sneaking or satellite tactic (subordinate males). Conflicts between males with different ARTs are common [21]. Through guarding, bigger dominant males protect and defend the spawning female or a spawning location against neighboring males using aggressive behaviors like biting and chasing. As a result, dominant males get an advantage in pre-mating competition since they ejaculate closer and more in synchrony with the spawning female. By chasing away other males from the spawning site the bigger dominant males experience lower sperm competition. On the other hand, smaller subordinate males experience higher sperm competition. As a result of the pre-mating competition, subordinate males ejaculate after the dominant male, in less proximity to the eggs and always in the presence of other male sperm [19]. Subordinate males seem to compensate for these disadvantages by investing more in ejaculates [4,22,23]. Parker's sperm competition models predict that across species, males will invest less in ejaculates if there is a low probability that his sperm will compete with those of other males. On the contrary, if the probability of sperm competition is high, males should invest more in ejaculate quality [16]. Thus, a trade-off forms between pre- and post-mating competition [24,25].

1.2. Factors predicting the outcome of sperm competition in external fertilizers

Disfavored mating roles such as greater distance to eggs during spawning or less synchrony in the timing of sperm and egg release, sperm quantity, and sperm quality are the main factors predicting the outcome of sperm competition [24]. Studies on brook trout *Salvelinus fontinalis* [26] and Atlantic cod *Gadus morhua* [27] have

shown that males that ejaculate closer to eggs have a competitive advantage in the sperm race to achieve fertilization. Most external fertilizers have a short fertilization window and the timing between sperm release and egg release (synchrony) affects the probability of sperm-egg encounters. Early ejaculates might become too dilute, and there is a risk that spermatozoa use all their energy before eggs are released [28,29]. On the other hand, late ejaculates face risk that the eggs are already fertilized by a competitor's sperm. Late release of sperm will normally be related with sneaker behavior of subordinate males, which compensate the delay by release of faster spermatozoa and higher concentrations of spermatozoa in their semen [22,30]. For example, a 2 s delay in sperm release under sperm competition decreases fertilization success by 40% in Atlantic salmon *Salmo salar* [30]. This makes sense since up to 80% of the eggs can be fertilized within the first 5 s in salmonids [31]. In Japanese medaka *Oryzias latipes* paternity of sneaker males drops from 41 to 20% if they spawn out of synchrony with the female and dominant male [32].

Of the swimming spermatozoa present, faster spermatozoa enter the egg micropyle quicker than slower spermatozoa. The importance of spermatozoa velocity under sperm competition has been well documented, e.g. in salmonids [22,33,34] and in walleye *Sander vitreus* [35]. Increasing spermatozoa number increases the possibility of sperm-egg encounters, and Parker and colleagues [25] predicted in their theoretical models a positive correlation between sperm numbers and paternity. However, empirical studies are ambiguous in how the total number of motile spermatozoa – a factor of percentage of motile spermatozoa and total number of spermatozoa – affects fertilization success. Positive associations (Atlantic halibut *Hippoglossus hippoglossus* [36], yellowtail flounder *Pleuronectes ferrugineus* [37], rainbow trout *Oncorhynchus mykiss* [33], bluegill *Lepomis macrochirus* [4], and African catfish *Clarias gariepinus* [38]) as well as no associations (*G. morhua* [39], *S. vitreus* [35], *S. salar* [34]) are reported, suggesting the importance of total number of motile spermatozoa varies among species and between males with different ARTs [22].

2. Methods for bibliography search

To review sperm competition in hatcheries two strategies were adopted simultaneously in August 2018: (i) keyword search, and (ii) looking at citing and cited references using Web of Science. The following strings were used “Aquaculture” and “Fish” together with different context qualifiers (“paternal effects” or “sperm competition” or “fertilization pote*” or “parental contribution” or “fertilization success” or “reproductive success” or “mass spawning” or “male potency” or “pooled”). The two strategies resulted in the identification of close to 150 relevant sources. To study sperm competition between hatchery and wild fish a search on the Web of Science performed in September 2018 resulted in 394 sources under the strings “sperm competition” and “fish”, however the addition of a context qualifier (using “stocking” or “stocked” or “culture*” or “farm*” or “aquaculture*” or “hatcher*” or “escap*” or “ranch*” or “propagate*”) reduced identified sources to 35. Abstracts of each of these were examined and few are directly relevant to the topic. None have been conducted in a way as to separate outcomes of sperm competition from confounding variables such as pre-mating competition or offspring survival. Undoubtedly, for both sections, some important sources were missed, but the exercise indicates this information is difficult to ascertain due to the varied terminology used by different authors.

3. Sperm competition in hatcheries

Most hatcheries use protocols to try and to maintain the genetic

diversity of broodstock and to stabilize the effective population size and minimize inbreeding [40]. Nonetheless, a significant decrease in genetic variability within fish populations reared in captivity has been a recognized problem since the early 1980s [41,42]. This decrease was later linked to culture conditions and hatchery practices, including the use of pooled semen [43,44], that reduces the effective size of the hatchery population [45,46]. Since then, sperm competition has been pointed out as one of the causes for loss of genetic variability in aquaculture [45,47]. Sperm competition in hatcheries can occur under two distinct scenarios: naturally in mass spawning tanks [48] where the fertilization environment pseudo-resembles the natural environment, or it can be artificially induced by using pooled semen for *in vitro* fertilization [47] under a totally artificial fertilization environment. When semen is pooled, sperm competition is induced both for species that have naturally evolved under intense sperm competition as *S. salar* [34], and for species that have evolved in the absence of sperm competition as the wolffishes *Anarhichas* sp. [49]. If fish produced in hatcheries are used for domesticated broodstock or for stocking natural populations, over several generations poor hatchery practices will lead to a loss of genetic variability. This loss will accumulate over time, and eventually together with inbreeding and genetic drift, produce artificial selection for certain unintended life history traits [42,50].

In this section we review the impact of sperm competition in hatcheries for commercial production and for wild population enhancement. We define wild fish as those who were naturally spawned by their parents and grew to sexual maturation in nature. We define hatchery fish as those who were fertilized under artificial conditions. The range of propagation goals and techniques used in fish reproduction creates huge variations in the magnitude in which these terms separate the two groups. For example, some hatchery fish will have descended from generations of lifelong purposeful artificial selection in captivity, while others will be from wild parents and themselves be exposed to brief periods of human intervention. Stock enhancement programs can be developed with different goals: as a conservation measure for supportive breeding of species with declining populations in conservation hatcheries [51], for reintroduction programs when the species has been extirpated, and to sustain fisheries (sea-ranching). These breeding programs for release in the wild are designed to maintain the wild population's genetic diversity [52] as the hatchery produced fish will likely breed with wild conspecifics (see section 4.1). However, individuals produced in hatcheries are subject to unnatural selective pressures, e.g. lack of mate choice, and experience very different spawning conditions than individuals in the wild, that can alter the population genetic structure [53]. Because fish are quite fecund, few individuals can produce many offspring, and reduction in the effective population size can occur over only a few generations [54]. As an example, a program to supplement the spring Chinook salmon *Oncorhynchus tshawytscha* in the Yakima River, USA, resulted in a disproportional increase of precocious males in natural populations [55].

3.1. Sperm competition in mass spawners

Most breeding programs prefer artificial fertilization *in vitro*, however this is difficult in certain species. As a result, mass spawning is the method used in some cases such as gilthead seabream *Sparus aurata* and barramundi *Lates calcarifer*, including for selective breeding programs [56,57]. Natural spawning has different advantages as it allows for active mate choice which may reduce intragenomic conflicts due to interactions between maternal and paternal haplotypes [10,58,59], natural synchronization in gamete release [60], and the gene pool of multiple individuals to be represented with greater heterogeneity in the

offspring and, thus, preserves natural genetic variability [61]. Nonetheless, the genetic variability of offspring derived from natural spawning may be compromised by reproductive dominance of some males or due to the absence of spawning of some females and males [60].

When communal or mass spawns are the preferred method for egg production, all individuals may not contribute to the next generation and often some dominance exists [62,63]. As a result, paternity frequencies are highly skewed among males and, depending on the species, the proportion of males contributing to the different spawns is variable [57,64–66]. The overall result is a reduction in the effective population size and some level of inbreeding in every generation [62,63]. As an example, in a *L. calcarifer* broodstock with 21 males, one male alone contributed to 16% of hatched larvae [67]. Nevertheless, some examples exist that support the use of mass spawnings. In white seabass *Atractoscion nobilis*, prior and during spawning, multiple males surround a female and thus sperm competition is expected to be intense, but males contribute relatively equally to paternity [65].

3.2. How can the outcome of sperm competition be controlled in mass spawners?

Several hatchery protocol questions arise when trying to control negative effects of genetic variability resulting from mass spawning. In these cases, it is essential to understand the social interactions established in the breeding tanks according to each species' reproductive biology. Nonetheless, even when the spawning events are recorded, it is challenging to differentiate the significance of pre- and post-mating (including sperm competition) contribution to progeny (see Table 1). These social interactions include dominant and subordinate roles where the dominant males (usually larger) invest more in gaining access to the females and less in their ejaculate quality (see section 1.1). In 10 Florida pompano *Trachinotus carolinus* only the largest five individuals contributed to the spawnings, with the largest male siring 73.8% of the offspring [68]. However, the diversity of fish reproductive strategies impedes generalizations. In the case of *S. aurata* for example, male paternity seems to be more related with a male-female size match, with medium size males siring most of the offspring [64]. These behaviors are often different from the wild, where the environment will have a determinant rule shaping the reproductive success of the different males [69]. As an example, polygamous pairing of Murray cod *Maccullochella peelii peelii*, a known monogamous species in the wild, was observed in captivity [70]. Other important parameters like distance to the eggs during ejaculation and timing of gametes release (see section 1.2 and examples within) will also affect each male's contribution in mass spawnings.

When pre- and post-mating competition are present, rearing conditions should minimize the chances of one male dominance. Even so, what the dominant-subordinate roles are in each spawning tank and how it affects pre- and post-mating competition and male contribution for the next generation is not always easy to identify exactly. Thus, alternative strategies should be implemented to avoid negative impacts of sexual competition in the genetic pool of the offspring. For example, the use of several mini-broodstocks instead a few large broodstocks, where individuals rotate among the stocks [63], or increase the number of fish in the spawning groups [67]. Another alternative is the replacement of dominant males by new ones [71].

3.3. Induced sperm competition by the use of pooled semen

When building mating schemes, every adult selected as

Table 1
Example studies that attribute differential contribution to the next generation's genetic composition to sexual selection in mass spawners. The selection mechanism and the determinants of reproductive success include biology description of the species, results and postulated hypothesis of the cited works. SCI – sperm competition intensity (see Supplementary Tables 1 and 2).

Species	Goal	Fish origin	Selection mechanism	Determinants of reproductive success	SCI	Reference
Gilthead seabream (<i>Sparus aurata</i>)	aquaculture production	farmed	dominant breeders	size match	3	[63,64,122]
White seabass (<i>Atractoscion nobilis</i>)	restocking	wild	lottery polygyny	sperm quality	5	[65]
Florida pompano (<i>Trachinotus carolinus</i>)	aquaculture production	wild and farmed	male competition	male size		[68]
Ballan wrasse (<i>Labrus bergylta</i>)	aquaculture production	wild	male territorial behaviour	male dominance	2	[123]
Atlantic cod (<i>Gadus morhua</i>)	aquaculture production	wild	male competition	size match	4	[124]
Barramundi (<i>Lates calcarifer</i>)	aquaculture production	wild and farmed	sperm competition	sperm quality	4	[48,67]
Nile tilapia (<i>Oreochromis niloticus</i>)	aquaculture production	farmed	male territorial behaviour	male size and condition factor	1	[125,126]
Large yellow croaker (<i>Larimichthys crocea</i>)	aquaculture production	wild	male competition			[127]
Common dentex (<i>Dentex dentex</i>)	aquaculture production	not mentioned	dominant breeders			[62]
Murray cod (<i>Maccullochella peelii</i>)	restocking	wild	male territorial behaviour	male size	1	[70]
Blunt snout bream (<i>Megalobrama amblycephala</i>)	aquaculture production	wild	dominant breeders and sperm competition	sperm quality		[128]

The sperm competition intensity (SCI) was attributed following [129] ranking where 0 = internal fertilization (including fertilization in the mouth) and no evidence for communal spawning or polygamy; 1 = internal fertilization and low communal spawning or polygamy, or external fertilization, distinct pairing, and no obvious communal spawning; 2 = internal fertilization and high communal spawning or polygamy, or external fertilization, distinct pairing, and low communal spawning; 3 = external fertilization, distinct pairing, and moderate communal spawning, or no pairing and low communal spawning; 4 = external fertilization, distinct pairing, and high communal spawning, or no pairing and moderate communal spawning and; 5 = no pairing and high communal spawning.

broodstock should have the same opportunity and an equal probability to produce the same number of offspring [72]. Thus, to maintain control over who spawns, if gamete stripping techniques are available, *in vitro* artificial fertilization is the preferred technique. In these cases, without pooling, mating schemes of varying complexity can be applied to control any unwanted effect of pre- and post-mating competition that can bias the genetic pool [46]. Nonetheless, difficulties in obtaining or holding large broodstocks, unbalanced sex ratios, or limiting resources to conduct large numbers of fertilizations lead hatchery managers to frequently choose to pool gametes as an alternative to maximize fertilization rates and easily achieve factorial matings [72,73]. Different examples can be found where the use of pooled semen is justified to secure high fertilization rates because of variable quality of sperm between males [74,75], low semen volume [49,75] or even when female-male post-mating interactions could limit the fertilization success of a particular male [2,10]. Less frequently, the use of pooled semen is recommended to ensure greater genetic variability [76], paradoxically recognizing that males have different contributions to the offspring [60]. As a result, semen pooling is still frequently used in hatcheries for food production and to a lesser extent in breeding programs for endangered species conservation [52], where this practice has been progressively eliminated [77], but see for example a study in *O. mykiss* [53].

Sperm competition success under *in vitro* fertilization conditions is frequently mentioned as male potency, which is the ability of a male to sire progeny when his semen is pooled with other individuals [43]. Differences in male potency when using pooled semen lead to a higher skewed parental contribution to the next generation than would result from single-pair crosses [73,78]. In one of the earliest studies on pink salmon *Oncorhynchus gorbuscha*, Gharrett and Shirley [43] attributed unequal paternity to the varying abilities of spermatozoa of different males to compete for eggs and suggested that this could be linked to different maturation stages of the stripped males. For artificial fertilization, males are manually stripped and frequently sperm at different maturation stages can be obtained [79] which may affect the sperm fertilizing ability. While this explanation cannot be completely discarded, sperm competition was probably also partly responsible for these

observations [43]. Some years later, Withler [44], compared male potency in *O. tshawytscha* when semen from one male was used to fertilize the eggs, to when pooled semen in equal volumes from three males were applied. In the pooled crosses, there was heterogeneity in male potency that could not be explained by the fertilization success in individual male crosses or the individual male's spermatocrit (proportion of semen that is sperm cells vs seminal plasma). Instead, each male potency in each pooled cross, was affected by the identity of the other individuals that contributed to the pool. A number of subsequent studies in farmed species have shown that the use of pooled semen reduces genetic variability as a result of sperm competition [46,47,80–82], causing an unequal contribution to the next generation [45] (see Table 2). Males produce ejaculates of varying quality according to sperm competition risk (see section 1). The pooled semen environment could resemble natural conditions for species where gamete-mediated mate choice is normal, but the importance of sperm competition in pooled samples is greater than in the wild, where pre-mating behavior of males to obtained higher number of mates is part of the selection process [72].

A recurrent situation where semen pooling occurs is for storage with all the aforementioned unavoidable consequences. Semen storage is frequently used in breeding programs to assist in artificial fertilization techniques [56,83,84]. Semen can be stored individually or pooled. In some occasions, most of them for experimental procedures, semen is pooled before short-term cold storage [85] and cryopreservation [75,86]. Under these conditions, some additional problems could arise from the use of stored semen in artificial fertilization. First, storage procedures affect spermatozoa motility and fertilizing ability, and this effect is different among males [87,88]; how this can affect individual male potency is unknown. Second, when semen is pooled before storage, the sperm stay in contact with the seminal plasma of competitors for a long period of time and this could have an effect on their performance [89]. Seminal plasma has been shown to mediate spermatozoa velocity adjustments between dominant and subordinate males [90], and to negatively affect competitors' spermatozoa velocity as a mechanism of subordinate males to compensate for their unfavorable position [89,91,92]. Nonetheless, different studies have

Table 2

Example studies where differential contribution to the next generation's genetic composition is due to sperm competition after using pooled semen. The determinants of reproductive success include results and postulated hypothesis of the cited works. SCI – sperm competition intensity (see Supplementary Tables 1 and 2).

Species	Goal	Fish origin	Determinants of reproductive success	SCI	Reference
Atlantic halibut (<i>Hippoglossus hippoglossus</i>)	aquaculture production	wild and farmed	spermatozoa motility and velocity		[81]
Steelhead trout (<i>Oncorhynchus mykiss</i>)	restocking	wild	sperm quality	3	[53]
Common carp (<i>Cyprinus carpio</i>)	aquaculture production	farmed	spermatozoa concentration, motility and velocity	4	[95,96,130]
Caspian brown trout (<i>Salmo trutta</i>)	restocking	wild	spermatozoa motility duration	2	[78]
Atlantic cod (<i>Gadus morhua</i>)	fundamental research	wild	percentage of progressive spermatozoa	4	[131]
Silver catfish (<i>Rhamdia quelen</i>)	stocking	wild	inconclusive		[82]
Pink salmon (<i>Oncorhynchus gorbuscha</i>)	restocking	wild	spermatozoa motility and other sperm quality parameters		[43]
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	restocking	wild	inconclusive		[44]
Atlantic salmon (<i>Salmo salar</i>)	fundamental research and restocking	wild and farmed	spermatozoa velocity and ATP	3	[34,105]
Whitefish (<i>Coregonus zugensis</i>)	restocking	wild	spermatozoa velocity, male age and growth rate		[45]

The sperm competition intensity (SCI) was attributed following [129] ranking where 0 = internal fertilization (including fertilization in the mouth) and no evidence for communal spawning or polygamy; 1 = internal fertilization and low communal spawning or polygamy, or external fertilization, distinct pairing, and no obvious communal spawning; 2 = internal fertilization and high communal spawning or polygamy, or external fertilization, distinct pairing, and low communal spawning; 3 = external fertilization, distinct pairing, and moderate communal spawning, or no pairing and low communal spawning; 4 = external fertilization, distinct pairing, and high communal spawning, or no pairing and moderate communal spawning and; 5 = no pairing and high communal spawning.

failed to detect differences in offspring genetic variability between pooled fresh or cryopreserved sperm [60,93]. The take home message is that semen pooling before storage should be avoided, especially if stored for stock enhancement or selective breeding programs.

3.4. How can the outcome of sperm competition be controlled in pooled semen?

The use of pooled semen should be avoided when building mating schemes as a general rule, as sperm competition will decrease the contribution of some males, even when this is the rational way to produce factorial matings [94]. Nevertheless, if the use of pooled semen cannot be avoided, the species' reproductive behavior and post-mating competition should be considered in the design of artificial reproduction protocols. Kaspar [95] provides some general recommendations to decrease the negative consequences on offspring genetic variability when pooled semen is used. First, the most common recommendation is to use equal volumes of semen [45,96]. This strategy not always results in an improvement in skewed male contribution to the progeny as males with higher concentration of spermatozoa will have a competitive advantage [78]. Subordinate males usually gain prevalence over a dominant individual's ejaculate if equal volumes of semen are pooled. In an Alpine whitefish *Coregonus zugensis*, when equal volumes of semen were used without consideration of the spermatozoa concentration, fast-growing males had a reproductive advantage [45]. Second and intuitively, is to adjust the volume of semen from each male to have equal numbers of spermatozoa, as recommended by some authors [47,96,97]. However, in common carp *Cyprinus carpio* this method was less effective in maintaining the effective number of males [95], in this case males with higher spermatozoa velocity had higher chances to achieve fertilization. Using pooled samples from two *G. morhua* males, with semen volumes adjusted to add approximately equal number of spermatozoa, some males sired more larvae than others [98]. Also in *C. zugensis*, when the volume of semen was adjusted according to the sperm concentration, the loss of genetic variability was lower than using equal volumes of semen, but younger males with faster spermatozoa were favored, leading to a selection of early maturing males [45]. Thirdly, Kaspar [95] also suggested to incubate the pool of semen for some time prior to use, as in *O. tshawytscha* holding a pool of semen for 60 min prior to use allowed a good, if not total re-

equilibration of male contributions [80]. This observation is probably related with a stabilizing effect of the different male's seminal plasma [90]. Nonetheless, there is a dearth of information regarding the potential effects of seminal fluids among males and how it can impact the incubation of pooled semen. Finally, we also suggest using an equal amount of motile spermatozoa or spermatozoa swimming at a certain speed. Sperm swimming parameters, such as spermatozoa velocity, and motility are frequently the best predictors of sperm competition success [34,99] and individuals with faster or highly motile spermatozoa fertilize a higher proportion of the progeny. Furthermore, within an ejaculate different sperm subpopulations exist with different swimming characteristics [79,86], and the subpopulation structure is different between dominant and subordinate males in *S. salar* [100]. Usually, the fast moving subpopulations have higher chance to achieve fertilization [86,101]. Exactly how these sperm subpopulations can affect sperm fertilization under sperm competition is unknown. Nonetheless, adjusting the semen volumes either based on percentages of motile spermatozoa or spermatozoa swimming velocity could be technically challenging, since fertilization frequently needs to be conducted shortly after gamete (sperm and eggs) stripping, and this approach requires a more complex evaluation of the semen quality.

Another practice to avoid when using pooled semen is the sequential addition of semen samples, as timing of sperm addition to the eggs will affect each male's contribution. As previously described, gamete release timing has been shown to have an important impact in sperm competition success [22,30], in a logic of first come first served (see section 1.2). Different studies in salmonids have observed that when the sperm are added at intervals, the spermatozoa of the first male usually sires higher percentages of progeny [30,43,47]. The longer the interval the more disproportional will be each male contribution [43]. Thus, the sequential addition of sperm should be avoided, and if pooled semen is to be used, the semen should be added to the eggs in a way which ensures that every spermatozoa with same phenotypic traits has an equal chance to fertilize.

4. Sperm competition between wild and hatchery fish

Genetic interactions between hatchery and wild fish are of key conservation concern. Hatchery fish often interbreed with wild fish, either purposefully as with hatchery enhancement programs, or unintentionally when farmed fish escape from their enclosures

[102]. Fertilization of wild eggs can be achieved under two contexts: 1) when a wild female chooses to spawn with a hatchery male, and 2) when she does not, but he releases sperm at the same time as her chosen wild male (sperm competition). The former has received a good deal of attention, but there is a dearth of information on the later in the wild. Aquaculture escapees often do not overlap in space and time with wild spawners, or do not perform well in mating rituals. Such pre-mating barriers to spawning have been documented elsewhere [103,104]; this section focuses solely on post-mating sperm competition between wild and hatchery fish. We consider situations where the female is of wild origin, and sperm from at least one wild male and one hatchery male compete to fertilize her eggs. Sperm released exclusively by hatchery males (perhaps common in many stocking programs) is not examined.

Artificial fertilization environments create useful settings to explore proximate mechanisms that lead to variable paternity success under sperm competition. These can be comprised of two situations. The first occurs when gametes are stripped from mature fish and then mixed in a container in known concentrations [2,105]. The second happens when fish are held in spawning tanks, cages or artificial streams and allowed to mate on their own [55,106–108]. Paternity derived from the later approach is a result of both pre- and post-mating competition including sperm competition (from unknown amounts of semen/sperm), and teasing apart relative effects can be difficult [109] as discussed in section 3.1 and 3.2. These laboratory studies sometimes indicate that semen of captive males is of poor quality [110] (but exceptions exist [100,111,112]) and such males should acquire low percentages of paternity under sperm competition with wild males. Poor semen in captivity is thought to be largely related to diet; broodstock breeding programs use special diets to improve semen quality [113]. Hatchery males that spawn shortly after escaping from aquaculture farms may therefore be unlikely to steal much paternity (as individuals) from wild males. However if they survive long term and switch to a wild diet this buffer probably disappears [114]. Fish produced for stocking programs, which have had a nearly life-long wild diet are not expected to have reduced semen quality. This can lead to problems as described below.

In the spawning location of a native wild female there can be several types of hatchery males that ejaculate as she releases eggs. These include males from her native population (section 4.1), foreign populations of her own species (section 4.2), and of other species (section 4.3).

4.1. Intra-population sperm competition between wild and hatchery fish in nature

Hatchery origin fish are sometimes created from parents that were collected from the same location in which the hatchery individuals now are spawning in nature. This generally means both wild and hatchery spawners are descendent from the same evolutionary lineage. Any local adaptations to that specific environment would be historically common in both groups, and on the surface, it would appear that no evolutionary divergence in wild descendants would occur from spawning with hatchery individuals. Such is the goal of so-called “conservation hatcheries” [51].

However, selective environments during the production of these hatchery individuals causes variable amounts of domestication (see section 3). If these fish steal paternity from wild males, they leave descendants that are less fit for their wild environments. Because these are the same species, if the hatchery fish are of similar phenotype to wild individuals [114–117] there are likely to be few barriers to mating. Even if hatchery fish are not good at courting, sneaking or satellite spawning would lead to sperm competition

with wild males. Sperm competition therefore creates a pathway for domesticated selection of entire wild populations if it occurs continuously over several generations.

4.2. Inter-population sperm competition between wild and hatchery fish in nature

Fish often adapt to their local environments, creating intra-specific genetic variation that comprises a key component of biodiversity that can be quite dramatic in some taxa [118]. Artificial breeding programs often use broodstock from one region (population) and either purposefully or accidentally release fish into the habitat of another [119,120]. Spawning by such fish leads to inter-population matings including via sperm competition when a hatchery male is not the chosen mate. Such intra-specific hybridization is much more common but harder to detect than inter-specific hybridization, and is a form of genetic pollution that creates a large threat to biodiversity [121].

4.3. Inter-specific sperm competition between wild and hatchery fish in nature

Inter-specific hybridization resulting from monogamous spawning between species, or inter-specific sperm competition between related species is rarer, but easier to observe than that among intra-specific populations. Both species may naturally coexist, but artificial breeding raises densities of one species, leading to increased likelihood of co-spawning. An example is from Alaska, where large releases of *O. gorbuscha* [119] likely increases the rate of inter-specific sperm competition between male satellite *O. gorbuscha* and wild chum salmon *Oncorhynchus keta*, creating “chumpies”. Hatchery released fish can make up an enormous proportion of spawners in some areas, so they could be a significant problem. Other salmonid examples would include hatchery released *O. mykiss* in the habitat of cutthroat trout *Oncorhynchus clarkii*, and hatchery released or escaped *S. salar* in the native European range of brown trout *Salmo trutta*.

Counter intuitively, this form of sperm competition is generally of less conservation concern than intra-specific destruction of local adaptation because although some proportion of a female's eggs are lost to hybridization, in most cases F2s are rarely produced from inter-specific unions and long-term introgression is limited. Because this is an evolutionary dead-end, from the point of genetic pollution, it is therefore often less damaging to produce foreign species, rather than foreign populations of native species, if they either purposefully or accidentally spawn in nature. For example, *S. salar* aquaculture does not cause genetic problems in the southern hemisphere, or Pacific coast of North America, but it is a cause of concern in their native range of the north Atlantic. Cultured *O. mykiss* would be less harmful in the Atlantic, as there are no salmonids that spawn at the same time of year.

5. Final remarks

The biology of different species and the artificial environments created in hatcheries for both mass spawnings and for *in vitro* fertilizations, will dictate the factors that influence sperm competition. The use of inappropriate protocols for spawning and artificial fertilization in hatchery environments can result in loss of genetic variability of offspring, inbreeding and problems for adaptability and survival of broodstock and fish released in the wild. Different studies to date, together with theoretical predictions, clearly advise against uncontrolled mass spawning and pooling ejaculates when the fish produced will be used as broodstock or for stocking natural populations. In species that have reached a high volume production

or in certain stocking programs, such as for different salmonids, hatcheries have started to adopt techniques to control the negative effects of sperm competition [72,94]. Nonetheless, limitations in some low production species result in the use of pooled semen and non-controlled mass spawning as the common approach. Species-specific protocols need to be developed and adapted to each hatchery scenario. But some basic guidelines are that every adult selected as broodstock should be given an equal opportunity to produce equal number of offspring [72].

We advocate that mass spawnings and the use of pooled semen should be limited. Mass spawning should only be accepted on occasions when the species' reproductive biology creates difficulties of using artificial fertilization. In these occasions, rearing conditions should minimize the chances of one male dominating, by for example, replacing the dominant male. The use of pooled semen should only be accepted when semen limitation cannot be circumvented. In these cases, measures such as the use of equal amount of spermatozoa, should be adopted. In both circumstances a close monitorization of genetic variability should be made.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.theriogenology.2019.03.034>.

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