We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Hybridization in Carps and Early Detection of Carp Hybrids Using PCR-Based Kit

Jayasankar Pallipuram

Abstract

Hybridization is the mating of genetically differentiated individuals or groups and may involve crosses within a species or between separate species. Hybridization can be natural or human-mediated. Reproductive barriers prevent excessive introgression in the former, whereas more often introgression and genetic pollution happen in the latter. Hybridization is more widespread among members of Cyprinidae than any other groups of freshwater fishes. In many carp hatcheries in India, breeders of catla (*Catla catla*) and rohu (*Labeo rohita*) are kept in the same tank for breeding, resulting in production of hybrid seeds. Fish hybrids can pose a serious threat to the aquatic environment biodiversity. Consequently, genetic monitoring of organisms is entailed to unambiguously identify parental species and their hybrids. Adopting a multiplex PCR using β-actin gene primers, a kit has been developed to distinguish between the hybrids from their parental species. Agarose electrophoresis revealed one band of about 100 bp in size specific for rohu, another at 300 bp specific for catla, and both bands in hybrid. The kit was tested successfully with the samples collected from many hatcheries located in four Indian states. The rohu-catla early hybrid identification PCR kit could serve as a stepping stone for carp seed certification and hatchery accreditation.

Keywords: hybridization, introgression, carps, hatchery, PCR

1. Introduction

Hybridization is defined as the mating of genetically differentiated individuals or groups and may involve crosses within a species (also known as line crossing or strain crossing) or crosses between separate species [1]. Issues related to hybridization are complex, making the job of conservation biologists tougher. Interspecific and intergeneric hybridization do happen naturally, and it is considered to play an important role in evolution process [2]. Indian major carps (IMCs) comprising of *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*, owing to their fast growth and taste, enjoy a prime position in the Indian aquaculture scenario. These carps attain a marketable size of 800–1000 g in less than a year and are generally propagated on an extensive and/or intensive scale in a polyculture system [3]. Among the three IMCs, catla and rohu are generally chosen for freshwater aquaculture due to their faster growth. IMCs, though originally inhabitants of the Ganga River network in North India and the rivers of Pakistan, Bangladesh, Nepal, and Burma, are also transplanted into other rivers in central as well as peninsular India and also in

aquaculture systems of Nepal and Sri Lanka. In 2005 global carp production reached 28.8 million tons, accounting for 37.5% in quantity and 25.6% in value of total aquaculture production [4]. China with 21.05 and India with 3.9 million tons were the top carp producers in the world. Indian major carps catla (2.76 million tons), rohu (2.91 million tons), and mrigal (0.47 million tons) were among 29 species with production over 100 t in 2005 [4].

It was noted that the growth of IMCs affected the grow-out culture phase and the profitability in carp farming is decreasing [5, 6]. The growth of carps is affected largely due to poor quality and mixed seed of carps produced by breeding carps of different species at the same time in the spawning pools of carp hatcheries that make easy hybrids. To make more profit, hatchery managers practice this in breeding programs in hatcheries when there is paucity of brood fish of desired species that is either males or females. This practice has been rampant in many parts of the Indian subcontinent. Unlike other agricultural crops and domestic land animals, the hybrids did not grow better than their native natural parents. Good quality seed of catla and rohu are in great demand in the Indian subcontinent. Seed is one of the indispensable resources needed for aquaculture. Taking the advantage of seed demand of these two species, many hatchery owners and seed producers supply hybrid seed (catla × rohu or rohu × catla) of these two species in the name of pure rohu or catla during the young stages. These hybrids cannot be easily differentiated from each other morphologically at early stages of development, e.g., hatchling and early fry stages. Among the most pressing issues concerning seed in global aquaculture development include inadequate and unreliable supply of quality seed, genetic quality, and inadequate hatchery technology.

For production of quality seed in aquaculture, many methods were tried in the past, including intergeneric and interspecific hybridization. Hybridization technique was used by aquaculturists in the hope of producing aquatic organisms with specific desirable traits or general improvement in performance. Intergeneric and interspecific hybridization programs have been applied in fish farms with the purpose of producing animals that perform better than the parental species (hybrid vigor) [1].

Nevertheless, many species are jeopardized by hybridization and genetic introgression, and these are particularly prevalent threats to the diversity of freshwater fish [7–10]. If fertile, hybrids can genetically contaminate natural and farmed stocks through genetic homogenization. They may also compete in several ways with the native parental lineages [2, 11, 12].

Currently, several genetic markers have been developed for different species that are used in aquaculture programs [13, 14]. PCR-RFLP and multiplex PCR are considered fast, simple, and inexpensive, but they have rarely been used in the characterization of hybrids or in aquaculture in general. These techniques are important tools in species identification, especially in studies related to the biological conservation and forensic identification [15, 16]. Natural occurrence of both interspecific and intergeneric hybrids of Indian major carps has been reported mostly from reservoirs and other natural ecosystems. From natural ecosystems such as reservoir and dry bundhs, several hybrids have been recorded [17]. Many of these hybrids were found to be intermediate in characters of the parent species. Only a few hybrids, both artificially produced and naturally occurring, have been studied in detail for their cultural qualities and adaptability to various environments. Several interspecific and intergeneric hybrids of Indian major carps are *Catla catla, Labeo rohita, Cirrhinus mrigala*, and *Labeo calbasu* [18, 19], and those of Indian major carps with exotic carps, viz., common carp [20] and silver carp [21], have been artificially produced through hypophysation. These hybrids were not popular due to poor survival and many undesired traits in aquaculture.

Quality seed is a fundamental prerequisite for sustainable and successful aquaculture, be it small-scale or commercial farming. Inadequate supply of quality seed is often suggested as a major constraint for aquaculture in many parts of the world [22]. The issue of quality comes to the attention of producers only after a certain period of time when performance indicators (e.g., growth, production, survival, and disease) consistently point a finger towards seed quality. Factors which contribute to production of poor quality seed would have become established as a normal practice in the system.

Field level morphological identification of seeds of commercially important carp species, viz., catla and rohu, at early stage is a difficult task as experience has shown. Many times fish farmers get cheated by unethical practices by hatchery owners as they sell these hybrids in the name of pure rohu or catla seed. At that point of time, it is almost impossible for the farmers to recognize the differences. So after nursery rearing, poor survival and growth of these seeds become a bane for them. Even though they approach the fishery officials for law enforcement, they also feel helpless in the absence of a genuine identification method at this early stage.

An attempt has been made to review hybridization in carps (Cyprinidae), focusing on the advantages and disadvantages of human-mediated hybridization in cultured species of carps and detection methods of carp hybrids. Further, the development of a novel multiplex PCR-based approach for the identification of seeds of rohu and catla and their hybrids (parents and hybrids), using some molecular markers, and its field testing with hatcheries in four Indian states are described.

2. Hybridization: natural and human-mediated

The major purpose behind human-mediated hybridization is genetic improvement in cultured species. This is expectedly achieved through combining desirable traits of parental species, resulting in heterosis or hybrid vigor in the progeny. In fishes human-mediated hybridization is around 50% [23]. In natural hybridization reproductive barriers prevent introgression to happen. That is, chances of F1 hybrid mating with parental species are remote. On the other hand, in human-mediated hybridization in which the rate is higher and the number of escapees is more, the

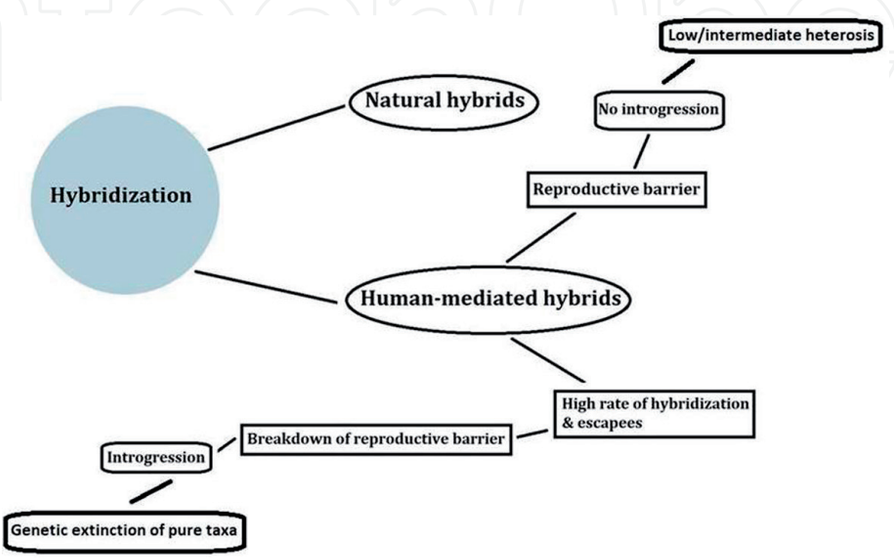

Figure 1.
Schematic representation of natural and human-mediated hybridization.

reproductive barriers are broken down, leading to introgression which may eventually lead to genetic extinction (**Figure 1**).

3. Hybridization in Cyprinidae

Hybridization is more widespread among members of Cyprinidae than any other groups of freshwater fishes [23]. Several permutation and combinations of interspecific and intergeneric crosses were carried out among Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) and exotic carps (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Aristichthys nobilis* and *Cyprinus carpio*) with the major objective to achieve hybrid vigor in economic traits [24–31]. The Indian major carp species are known to be able to hybridize, and hybrids are fertile and can be backcrossed to the parental species [32–34]. Hybridization has been shown to have a significant impact on production-related traits (notably growth), with some studies reporting a growth rate of F1 hybrids intermediate between that of the parent species [35, 36]. Hybrids are reported to have lower rates of growth than either of the parental species of Indian carps, other studies reporting growth rates lower than for either of the parental species [37]. Intergeneric hybrids between catla (*Catla catla*) and fimbriatus (*Labeo fimbriatus*) were produced by employing the technique of hypophysation and dry stripping. Detailed investigations on their embryonic and larval development, taxonomic characters, and aquaculture potential in terms of growth, feed utilization, body carcass composition, meat yield, etc. were carried out [28].

A catla-rohu hybrid produced by hypophysation was found to be intermediate in general appearance to the parent species. Gut content analysis revealed that the hybrid was mainly phytophagous in its diet. Growth rate was observed to be faster than in rohu. It matured within 3 years and was equally responsive to hypophysation [38]. In other cyprinid species, also such efforts of hybridization were carried out [23, 39].

3.1 Purposes and merits of human-mediated hybridization

It has long been recognized that hybridization can have a variety of evolutionary outcomes, including outcomes that maintain or increase diversity such as stable hybrid zones, the evolutionary rescue of small inbred populations, the origin and transfer of adaptations, the reinforcement of reproductive isolation, and the formation of new hybrid lineages [40, 41].

Hybridization in carps was being carried out to increase growth rate, transfer desirable traits between species, combine desirable traits of two species into a single group of fishes, reduce unwanted reproduction through production of sterile fish or mono-sex offspring, take advantage of sexual dimorphism, increase harvestability, increase environmental tolerances, and increase overall hardiness in culture conditions. Hybrids constitute a significant proportion of some countries' production for certain taxa, for example, hybrid striped bass in the USA, hybrid clarid catfish in Thailand, hybrid characids in Venezuela, and hybrid tilapia in Israel. Hybridization has been used in tandem with polyploidization to improve developmental stability in hybrid progeny [1]. Intergeneric hybrids between catla (*Catla catla*) and fimbriatus (*Labeo fimbriatus*) combined desirable qualities such as the small head of the fimbriatus and the deep body of the catla and exhibited heterosis in terms of meat yield with higher flesh content than either of the parents. Hence the hybrids appear to be of considerable importance to aquaculture [28]. It is also believed that hybrids of parental genotypes might be able to explore ecological niche unavailable

to the latter. It can lead to adaptation through creation of novel genotypes and morphologies. Further, hybrid vigor or heterosis can also occur [39]. Interspecific hybrids are created purposely to enhance productivity of aquacultural strains [42]. Hybridization is done also to enhance recreational angling opportunities [43].

Evolutionary evidence that hybridization is a constructive process was mentioned, as (a) reproductive barriers, both pre- and post-zygotic, between described species appear incomplete among many fishes, (b) permanent transfer of genetic information apparently is possible even when hybrids and backcrosses are under negative selection, and (c) genetic exchange through introgressive events may have significant effects on the genetic composition of a species, and thereby, actually contribute to diversity within taxa [7]. Studies of interspecific hybridization between the Siberian sturgeon and Russian sturgeon showed that the hybrids had higher survival and growth than the purebreds under provided hatchery conditions. The hybrid crosses displayed positive average heterosis in most of the assessment times for growth and survival traits, and better fitness-related traits than purebreds, thus suggesting that interspecific hybridization provides a survival advantage to sturgeons during their evolutionary period [44].

4. Threats and concerns of human-mediated hybridization

The harmful effects of hybridization, with or without introgression, have led to the extinction of many populations and species in many plant and animal taxa. Hybridization is especially problematic for rare species that come into contact with other species that are more abundant [2]. Hybridization can decrease diversity through the breakdown of reproductive barriers, the merger of previously distinctive evolutionary lineages, and the extinction of populations or species. There are two main mechanisms by which hybridization can lead to extinction. If hybrid fitness is strongly reduced relative to that of parental individuals (i.e., outbreeding depression), and hybridization is common, population growth rates of one or both parental lineages may decline below replacement rates due to wasted reproductive effort, leading to extinction [41, 45]. Hybridization involving captive-bred individuals can have harmful consequences beyond the loss of genetic integrity [46]. In many cases, the stocked individuals differ genetically from the target population, which can result in outbreeding depression following hybridization [45].

Inadvertent hybridization and backcrossing can lead to unexpected and undesirable results in hybrid progeny, such as failure to produce sterile fish, loss of color pattern, and reduced viability. Uncontrolled and unintentional hybridization could undermine the performance of cultured stocks and restrict future use of the contaminated stocks as broodstock. The level of unintentional or accidental hybridization has important considerations for the conservation of aquatic biodiversity and will influence risk assessment on the use of hybrid fishes in aquaculture and fisheries [1]. Continued hybridization may eventually lead to a breakdown of species barriers, thereby compromising the genetic integrity of the species in the wild and leading to production losses in aquaculture [30]. Hybrid introgression in major carp species is very likely to have negative consequences, as a result of loss of distinct feeding strategies of the pure species, which are the basis of successful polyculture systems [46].

In Indian major carps, inadvertent production of hybrids out of mixed spawning of species has been well documented. Actually the farmers are on the lookout for "mixed seeds," meaning a certain proportion of catla, rohu, and mrigal along with other exotic carps for polyculture [47]. For the sake of time and economy, the hatchery producers keep broodfish of different species, particularly rohu and catla,

in the same breeding pool, resulting in unintentional production of the hybrid seeds [34, 48]. The intergeneric hybrids are fertile, and they can breed (backcross) with parental species to produce introgressed F2 hybrids. The thoughtless and injudicious ways of fish breeding are likely to affect the "gene pools" of these prized food fishes badly [27].

Silver carp and bighead carp sometimes are hybridized inadvertently because of their similar appearance and because of shortage of "the correct" species at spawning time due to differences in maturation times between male and female carp. This hybridization often results in a fish that does not feed efficiently as its gill rakers are intermediate in shape between those of the silver carp that eats phytoplankton and those of the bighead carp that consumes zooplankton [1]. The rohu-catla reciprocal hybrids are reported to have limited economic value [27]. These hybrids are also reported to be more susceptible to parasitic infection than the parental species [49]. Hybridization between silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) suggests further generations of hybridization or introgression between the species in hatcheries, with potentially damaging consequences for the integrity of these stocks and their performance in aquaculture [50]. Pecos pupfish (*Cyprinodon pecosensis*) is threatened with replacement by its hybrids with sheepshead minnow (*C. variegatus*) [12]. Continued hybridization between invasive bigheaded carps (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) has indicated reduced nutritional performance of their progeny [29].

5. Detection of hybrids

Accurate identification of hybrids is important not only for sustainable aquaculture development, guiding aquaculture domestication efforts, assessing aquaculture production, and identifying useful crosses, but also to allow for a better understanding of biodiversity issues. It would be unfortunate to experience a widespread loss of pure species in aquaculture as happened with tilapia as a result of widespread introduction and subsequent hybridization; it would be also a significant cause for concern if hybrid Thai catfish or the hybrid Venezuelan characids pose more of a threat to local species than the pure species [51].

Before 1966 only morphology-based methods were used to identify hybrids. Subsequently followed by morphology (45%), allozymes (35%), mtDNA (12%), nDNA (4%), and karyology (2%) were used till the late 1990s. Genetic markers and population genetic theory have provided powerful tools facilitating the description of hybridization events and serve as sources of evidence for factors underlying occurrence, direction, and extent of introgression between fish taxa [23]. VNTR minisatellite and microsatellite loci, SINE's, RAPD, AFLP, and ISSR assume dominance, whereby individuals are characterized by the presence or absence of amplification products of specific size. The number of alleles producing a product (one for heterozygotes and two for a homozygote) cannot be directly determined. Thus, the per-locus information context of dominant markers is less than for codominant loci. Mitochondrial DNA cannot be used alone to detect hybrids because of the marker's haploid and matrilineal mode of inheritance. However, mtDNA can be a powerful tool to establish directionality when used in conjunction with nuclear genetic markers [23]. Genetic markers, such as allozymes, mtDNA, and nuclear DNA, were used to confirm hybrid status and to determine directionality of the hybridization event [12]. Multiple markers were employed to determine if an Icelandic population of eels (*Anguilla anguilla*) included hybrid individuals from matings of parents originating from populations in North America and Europe [43].

Historically, meristic and morphometric measurements were the primary means of identifying naturally occurring hybrids. The introduction of allozyme electrophoresis provided a methodology whereby individuals of most species could be assayed for biochemical markers with a demonstrable heritable basis [52]. The use of mtDNA was first cited in the surveyed literature in analyses of fish hybridization in the mid-1980s [4]. In the late 1980s, nuclear DNA was started to be used for identifying hybridization process [53, 54].

Documentation of hybridization often has been based on meristic or morphological criteria that can be misleading when used as the sole source of inference, particularly for hybrid individuals beyond the F1 generation [55]. Morphology, allozymes, and mtDNA were used in the analysis of *Notropis chrysocephalus* and *N. cornutus* hybrid zones in Michigan drainages [56]. Genomic RFLP was used to detect hybrids of Indian major carps, and the results indicated that intergeneric hybridization did occur during "mixed spawning" of these carps and the hybridization frequency was appreciable, at about 10% [34]. Utilizing an integrated approach, which incorporates geometric morphometrics, life history, and molecular genetic analyses, the levels and processes of hybridization in two species of cyprinids were determined [39]. The extent of intergeneric hybridization in Indian major carps was studied using allozymes [30]. DNA fingerprinting using RAPD-ISSR assay was used to detect hybridization in Indian major carps [57].

6. PCR-based kit for detection of early hybrids of rohu-catla reciprocal crosses

6.1 Development

For the parental lineages, 50 individuals of *Labeo rohita* (rohu) and 50 individuals of *Catla catla* (catla) were genetically analyzed. Crosses performed by mating females of catla and males of rohu and vice versa resulted in the intergeneric hybrid (**Figure 2**). Twenty-four hybrid individuals were included in the genetic analysis. Spawns of reciprocal hybrids were collected for further genetic analysis. DNA was extracted from the fin clips of adults of parental species using standard phenol-chloroform method [58].

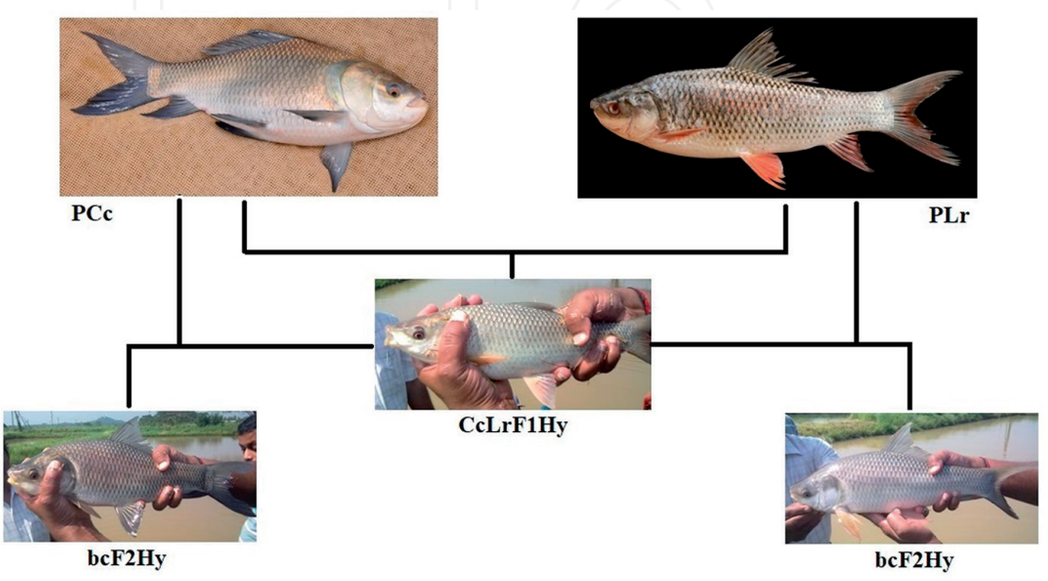

Figure 2.
Catla (PCc), rohu (PLr) parents, F1 hybrid (CcLrF1Hy), and F2 backcross hybrids (bcF2Hy).

Total genomic DNA from spawn was isolated using DNeasy blood and tissue kit, Qiagen. DNA quantity was determined against a molecular marker standard (λ-DNA 25 ng, Fermentas) by electrophoresis in a 0.8% agarose gel. β-actin sequences of carps available in GenBank were downloaded (Accession numbers: AF415205, M24113, GU338376, AY531753, AF415206) and aligned using Clustal W program implemented in the software Bioedit version 7.0.5.3 [59], and conserved primers for the amplification of a fragment size of ~ 1000 bp in Indian major carps and minor carps were done manually. Genomic DNA (~20–100 ng) from both species of IMCs was amplified in a 25 μl PCR volume containing 10 picomoles of each conserved primer, 2.5 mM of each dNTP, and 0.25 U of *Taq* polymerase with a thermal regime of 94°C (5 min), 35 cycles at 94°C (0.5 min), 60°C (0.5 min) and 1 min at 72°C (1 min) and final extension of 72°C (5 min). PCR products were purified using Qiagen PCR purification kit followed by bidirectional cycle sequencing on ABI 3100 PE automated capillary sequencer.

A total of 20 sequences of both the species (10 *Labeo rohita* and 10 *Catla catla*) were aligned using Clustal W program in Bioedit software. Species-specific reverse primers for both species were designed, taking the species-specific mutation into account. A touchdown PCR was carried out with a 25 μl PCR volume containing 10 picomoles of each species-specific reverse primers (one rohu and one catla) and 20 picomoles of universal forward primer, 2.5 mM of each dNTP, and 0.25 U of *Taq* polymerase with the PCR condition of 94°C (5 min), 2 cycles at 94°C (0.5 min), 68°C (0.5 min) and 1 min at 72°C (1 min), 2 cycles at 94°C (0.5 min), 66°C (0.5 min) and 1 min at 72°C (1 min), 2 cycles of at 94°C (0.5 min), 64°C (0.5 min) and 1 min at 72°C (1 min), 25 cycles at 94°C (0.5 min), 62°C (0.5 min) and 1 min at 72°C (1 min) and final extension of 72°C (5 min). The PCR products were checked in a 2% agarose gel.

Partial sequences of the nuclear β-actin gene amplified using a set of primers BAF (5′GTAGGCACGACATTGAATGGG3′) and BAR (5′AGACAAAGGAAGTCCCTCTGC3′) generated a total of 820 bp which revealed some differences in the nucleotide composition between *Labeo rohita* and *Catla catla*. Single-nucleotide polymorphism was found between the species which were used to design species-specific internal primers.

Two primers were designed specific for each species considering the polymorphic sites in the sequences. Both primers designed were in the reverse direction: primer BALRR (5′-CTTGAAAACTGTACAATCACGTTC-3′) was specific for *Labeo rohita*, and BACCR (5′-GCTAGCTAATAGACGTAATCATTTAG-3′) was specific for *Catla catla*. Amplification of these primers (BAF, BALRR, and BACCR) established different electrophoretic banding patterns when run in a 2% agarose gel. The result revealed one band at about 100 bp specific for *L. rohita* and another band at 300 bp specific for *C. catla*. In the rohu × catla hybrid, a heterozygote pattern was observed with two diagnostic bands, with each one inherited from one parental strain. Using these species diagnostic primers, a PCR-based rohu-catla hybrid identification kit was developed which has received provisional Indian patent number "343/KOL/2013 of 26.3.2013." For the validation of the developed kit, a total number of 685 samples from different places were screened which revealed that 54 out of them were hybrids (**Figure 3**).

6.2 Features of the kit

The use of a multiplex PCR marker in the present study revealed a distinct electrophoretic pattern between rohu and catla and their hybrid. The advantage of multiplex PCR is that it does not require the additional step of restricted enzyme digestion and can thus eliminate any post-PCR analyses as well as additional time and costs. On the other hand, there are limitations to the primer designs that should be taken into consideration. The primers should be specific and reliable in

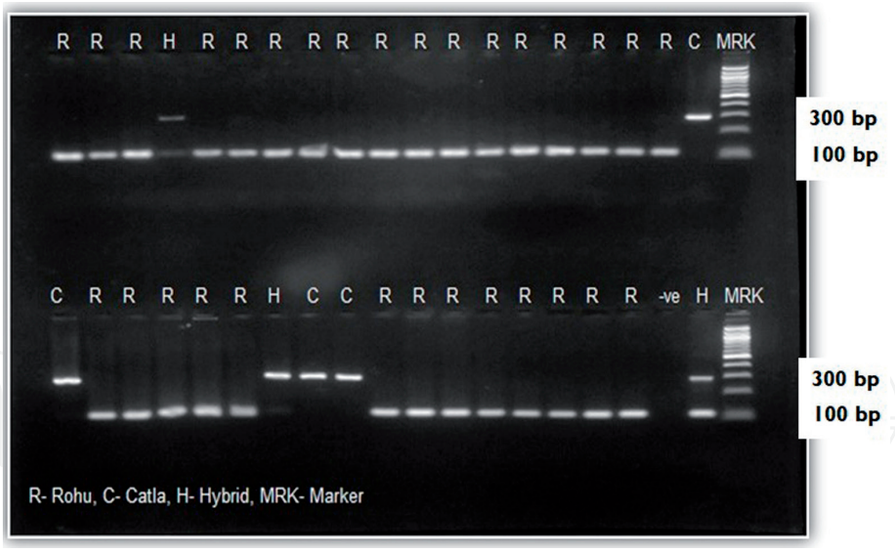

Figure 3.
PCR test of hatchery spawn samples (300 bp marker in catla, 100 bp marker in rohu, and both markers in hybrid).

Figure 4.
Rohu-catla early hybrid identification kit.

binding. This study can serve as a basis for further study on the introgression of these hybrids with their parental species. Genetic monitoring of mixed spawning and unintended hybridization of Indian major carps in hatcheries can be carried out with the help of the kit (**Figure 4**).

Contents of the kit are mentioned below:

- Species-specific primers
- Universal primer
- dNTP mix (2.5 mM each)
- 10X Taq DNA buffer
- Taq DNA polymerase 3 U/μl

- Positive control DNA 100 ng
- Nuclease-free water
- 100 bp ladder

Advantages and utility of this kit are summarized below:

- PCR and agarose gel-based detection kit
- No sequencing required
- Takes only 4–6 h to get the results
- Highly sensitive and specific for rohu × catla hybrids and reciprocal crosses
- Useful for screening of hatcheries for genetic contamination
- Potential for seed certification hatchery accreditation
- An essential tool for government/private agencies to ensure purity of seed

6.3 Validation of primers by screening unknown samples from hatcheries

A total of 685 samples of different life stages (spawn and fry) from different hatcheries of four Indian states, Odisha, Gujarat, Bihar, and West Bengal (**Table 1**), were collected and tested using the species-specific designed primers with the same thermal cycler condition [60].

State	Details of hatchery	Samples (n)	Hybrid (n)	Hybrid (%)
Odisha	State Fisheries Farm, Kausalyaganga	150	6	4
Odisha	State Fisheries Farm, Bhadrak	30	0	0
Odisha	State Fisheries Farm, Balasore	30	2	7
Odisha	Balakati Private Hatchery	25	0	0
Odisha	Balakati Private Hatchery	25	3	12
Gujarat	Private Hatchery	45	23	51
Bihar	Shri Tripura Chaudhary, Matsya Farm, Vaishali	30	0	0
Bihar	Asha Fish Breeding Centre, Baheri Block, Darbhanga	30	3	10
Bihar	Kamla Fish Hatchery, Jhajarpur, Madhubani	30	2	7
Bihar	Koshi Fish Hatchery, Madhubani	30	5	17
Bihar	Ganga Fish Hatchery, Madhubani	30	4	13
Bihar	Yamuna Fish Hatchery, Madhubani	30	6	20
West Bengal	Naihati Market	200	137	68.5

Table 1.
Presence of hybrid seeds (spawn/hatchlings and fry) in hatchery populations in four Indian states (Odisha, Gujarat, Bihar and West Bengal), n = 685.

7. Conclusions

There have been numerous studies on hybridization of fishes, and certainly not all of the hybrids reported are contributing to commercial aquaculture production. Accurate identification of hybrids is important not only for sustainable aquaculture development, guiding aquaculture domestication efforts, assessing aquaculture production, and identifying useful crosses, but also to allow for a better understanding of biodiversity issues.

Intergeneric hybrids between the species rohu (*Labeo rohita*) and catla (*Catla catla*) are being produced in Indian carp hatcheries without any monitoring. The parental species belong to the more representative genus of the family Cyprinidae, which is an important freshwater fish group that is widespread throughout India, and are important fishery resources to specific communities. Some of the hatcheries are practicing multispecies breeding (mainly rohu and catla) in the same breeding pool and at the same time leading to interspecific hybridization. There is every chance of these hybrids escaping to natural waters which would lead to pollution of the genetic material in the wild, leading to non-availability of the pure strains of the carps in the future. There seems to be a misunderstanding regarding the culture of mixed species for composite fish culture with mixed breeding of carps by the hatchery managers and fish farmers. When the farmers are demanding mixed seeds (mixture of pure rohu and catla), the hatchery managers are mixing the spawners to produce hybrids.

Hybrid identification based on morphology, ecology, and behavior can be difficult and, most of the time, confusing and uncertain. Multiplex PCR strategy has proven to be an efficient methodology that could be quickly and inexpensively executed, which would allow diagnoses through simple PCR based upon single-nucleotide polymorphisms.

Morphological differences of hybrids are only minor and need close examination by experienced workers, and it is difficult to verify the genuine hybrids from interspecific hybridization. Allozymes were used to detect the genetic difference between the hybrids and their parental species; the use of allozyme loci failed to provide a sufficient genetic basis of hybrids, probably due to the less informative nature of allozyme loci (i.e., limited number of polymorphic loci available and low level of polymorphisms). Mutation at the DNA level that causes a replacement of a similarly charged amino acid may not be detected by allozyme electrophoresis, although allozymes represent actual gene products. Molecular techniques have been applied in the worldwide aquaculture, allowing for an adequate management of several cultivated species and providing a huge number of molecular markers that have been applied successfully for hybrid identification and detecting genetic introgression in fish. Nuclear molecular markers have supplied valuable information in the detection of hybridization events as well as the identification of reciprocal hybrids. Since mitochondrial DNA in animals has the characteristic of maternal inheritance [61], they are not suitable to detect hybrids. On the other hand, nuclear DNA serves as an efficient tool for hybrid identification.

The use of multiplex PCR marker revealed a distinct electrophoretic pattern between *Labeo rohita*, *Catla catla*, and their hybrid. The advantage of multiplex PCR is that it does not require the additional step of restriction enzyme digestion, which can eliminate any post-PCR analyses as well as additional time and costs. On the other hand, there can be limitations to the primer designs that should be taken into consideration, where these mainly reflect the ability of the primers to have good specificity and reliability in the application [62].

Finally based on the personal experience and inferences from other related studies, the following policy guidelines are recommended:

1. Natural hybrids should be eligible for protection.

2. Human-induced hybridization undesirable in majority of situations is a threat to conservation of parental species and low/intermediate heterosis. It causes extinction of pure taxa by replacement and genetic mixing. Hence it needs to be strictly regulated/eliminated.

3. Human-mediated hybrids shall be protected only in exceptional circumstances, such as when hybrids contain the only remaining genetic information from a taxon that has otherwise been lost by genetic mixing or when the circumstances of their origin are unclear.

4. In Indian major carps, mixed spawning of different species in hatcheries is to be stopped.

5. Good hatchery practices are paramount, and genetic monitoring of hatchery stocks on a regular basis is entailed to maintain the quality of fish seeds.

6. Unambiguous and rapid detection of hybrids at hatchery level is essential. The carp reciprocal early hybrid identification kit is useful for this purpose. Further, it is potentially useful tool for seed certification and hatchery accreditation.

Acknowledgements

I am thankful to my collaborators (Section 7) Mohanty M, Sahoo L, Das P, Das BK, Verma DK, and Routray P. This study was conducted when I was the Director of ICAR-CIFA, Bhubaneswar, during 2012–2017.

Conflict of interest

It is certified that there is absolutely no conflict of interest.

Author deta ils

Jayasankar Pallipuram

ICAR-Central Marine Fisheries Research Institute (CMFRI), Ernakulam, Kerala, India

*Address all correspondence to: pjayasankar@yahoo.com

References

[1] Bartley DM, Rana K, Immink AJ. The use of inter-specific hybrids in aquaculture and fisheries. Reviews in Fish Biology and Fisheries. 2001;**10**:325-337

[2] Allendorf W, Leary RF, Spruell P, Wenburg JK. The problems with hybrids: Setting conservation guidelines. Trends in Ecology & Evolution. 2001;**16**(11):613-622

[3] Jhingran VG, Pullin RSV. A hatchery manual for the common, Chinese and Indian major carps. In: ICLARM Study Review. Vol. 11. Manila, the Philippines: ICLARM; 1988. p. 191

[4] Miao W. FMM/RAS/298: Strengthening capacities, policies and national action plans on prudent and responsible use of antimicrobials in fisheries. In: Final Workshop in Cooperation with AVA Singapore and INFOFISH; 12-14 December, Concorde Hotel, Singapore; 2017

[5] Sharma KR, Leung PS. Technical efficiency of carp production in India: A stochastic frontier production function analysis. Aquaculture Research. 2008;**31**(12):937-947. DOI: 10.1046/j.1365-2109.2000.00521.x

[6] Singh KD, Madan M, Rabbani MM, Sudhakaran AG, Pratheesh O, Ganesh T. Technical efficiency of freshwater aquaculture and its determinants in Tripura, India. Agricultural Economics Research Review. 2009;**22**:185-195

[7] Epifanio J, Nielsen J. The role of hybridization in the distribution, conservation and management of aquatic species. Reviews in Fish Biology and Fisheries. 2001;**10**:245-251

[8] Perry WL, Lodge DM, Feder JL. Importance of hybridization between indigenous and nonindigenous freshwater species: An overlooked threat to north American biodiversity. Systematic Zoology. 2002;**51**:255-275

[9] Senanan W, Kapuscinski AR, Na-Nakorn U, Miller LM. Genetic impacts of hybrid catfish farming (*Clarias macrocephalus* × *C. gariepinus*) on native catfish populations in Central Thailand. Aquaculture. 2004;**35**:167-184

[10] Na-Nakorn U, Kamonrat W, Ngamsiri T. Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed *C. gariepinus*. Aquaculture. 2004;**40**:145-163

[11] Ryman N, Utter FM. Population Genetics & Fishery Management. Seattle: University of Washington Press; 1987

[12] Rosenfield JA, Nolasco S, Lindauer S, Sandoval C, Kodric-Brown A. The role of hybrid vigor in the replacement of Pecos Pupfish by its hybrids with Sheepshead Minnow. Conservation Biology. 2004;**18**(6):1589-1598

[13] Liu ZJ, Cordes JF. DNA marker technologies and their applications in aquaculture genetics. Aquaculture. 2004;**38**:1-37

[14] Hashimoto DT, Parise-Maltempi PP, Laudicina A, Bortolozzi J, Senhorini JA, Foresti F, et al. Repetitive DNA probe linked to sex chromosomes in hybrids between Neotropical fish *Leporinus macrocephalus* and Lepoirinus *elongatus* (Characiformes, Anostomidae). Cytogenetic and Genome Research. 2009;**124**:151-157

[15] Cocolin L, D'Agaro E, Manzano M, Lanari D, Comi G. Rapid PCR-RFLP method for the identification of marine fish fillets (Seabass, Seabream,

Umbrine, and Dentex). Journal of Food Science. 2000;**65**(8):1315-1317

[16] Magnussen JE, Pikitch EK, Clarke SC, Nicholson C, Hoelzel AR, Shivji MS. Genetic tracking of basking shark products in international trade. Animal Conservation. 2007;**10**:199-207

[17] Desai VR, Rao KJ. On the occurrence of natural hybrid of catla-rohu in Madhya Pradesh. Journal of Zoological Society of India. 1970;**22**(l/2):35-40

[18] Chaudhuri H. Fertility of hybrids of Indian carps and preliminary studies on the F_2 generation of carp hybrids. Journal of the Inland Fisheries Society of India. 1973;**5**:195-200

[19] Chondar SL. Systematic account of carp hybrid. l. *Labeo gonius* x *Labeo calbasu*. Journal of the Inland Fisheries Society of India. 1977;**9**:172-174

[20] Kowtal GV, Gupta SD. A note on the hybrid mrigal, *Cirrhinus mrigala* (Hamilton) x common carp *Cyprinus carpio* (Linnaeus) (Cyprinid). Aquaculture. 1984;**49**:179-183

[21] Ibrahim KH, Kowtal GV, Gupta SD. Contribution to hybridization among Indian carps. 1, Embroyonic and larval development in *Catla catla* (Hamilton) and *Hypophthalmichthys molitrix* (Valenciennes). Journal of the Inland Fisheries Society of India. 1980;**12**(2):69-73

[22] Little DC, Satapornvanit A, Edwards P. Freshwater fish seed quality in Asia. In: Edward P, Little DC, Demaine H, editors. Rural Aquaculture. UK: CABI Publishing; 2002. pp. 18-195

[23] Scribner KT, Page KS, Meredith L, Bartron ML. Hybridization in freshwater fishes: A review of case studies and cytonuclear methods of biological inference. Reviews in Fish Biology and Fisheries. 2001;**10**:293-323

[24] Chevassus B. Hybridization in fish. Aquaculture. 1983;**33**:245-262

[25] Khan HA, Kowtal GV. An account of naturally occurring and artificially produced cyprinid hybrids in India. NACA/WP/86/33 Network of Aquaculture Centres in Asia; Bangkok, Thailand; 1986

[26] Bhowmick RM, Jana RK, Gupta SD, Kowtal GV, Rout M. Studies on some aspects of biology and morphometry of the intergeneric hybrid, *Catla catla* Hamilton X *Labeo rohita* Hamilton produced by hypophysation. Aquaculture. 1981;**23**:367-371

[27] Tripathi SD. Three decades of research on carp hybridization in India. Proceedings of the Zoological Society. 1992;**45**(Suppl B):13-28

[28] Basavaraju V, Devaraj KV, Ayyar SP. Comparative growth of reciprocal carp hybrids between *Catla catla* and *Labeo fimbriatus*. Aquaculture. 1995;**129**:187-191

[29] Liss SA, Lamera JT, Sass GG, Suski CD. Physiological consequences of hybridization: Early generation backcrossing decreases performance in invasive bigheaded carps. Journal of Freshwater Ecology. 2016;**31**(4):543-554

[30] Simonsen V, Hansen MM, Mensberg K-ID, Sarder RI, Alam S. Widespread hybridization among species of Indian major carps in hatcheries, but not in the wild. Journal of Fish Biology. 2005;**67**:794-808

[31] Sultana S, Saifuddin Shah M, Ghosh AK, Bir J. Taxonomic study on the supposed hybrids of Indian major carps and Chinese carps in the local markets of Khulna, Jessore and Satkhira district, Bangladesh. Khulna University Studies. 2016;**13**(1):55-64

[32] Das P, Kumar D, Ghosh AK, Bhaumik U. Preliminary observation on the performance of catla-rohu hybrid

in carp culture. Food Farming and Agriculture. 1980;**12**:294-296

[33] Das P, Mishra A, Srivastava SK. Status on research in applied carp genetics and breeding in India. Journal of Aquaculture in the Tropics. 1996;**11**:307-317

[34] Padhi BK, Mandal RK. Inadvertent hybridization in a carp hatchery as detected by nuclear DNA RFLP. Journal of Fish Biology. 1997;**50**:906-909

[35] Chaudhuri H. Fish hybridization in Asia with special reference to India. Report to FAO/UNDP(TA). 1971;**2926**:151-159

[36] Natarajan AV, Desai VR, Mishra VN. On the natural occurrence of the intergeneric Catla-rohu hybrid in Rihand (Uttar Pradesh) with an account of its potential role in reservoir fisheries development in India. Journal of the Inland Fisheries Society of India. 1976;**8**:83-90

[37] Ibrahim KH. Contribution to hybridization among Indian carps: Studies on biological and morphometric features of some Indian carp hybrids [PhD thesis]. Bhubaneswar, India: Utkal University; 1977

[38] Bhowmick RM, Jana RK, Gupta SD, Kowtal GV, Rout M. Studies on some aspects of biology and morphometry of the intergeneric hybrid, *Catla catla* Hamilton X *Labio rohita* Hamilton produced by hypophysation. Aquaculture. 1981;**23**:367-371

[39] Hayden B, Pulcini D, Kelly-Quinn M, O'Grady M, Caffrey J, McGrath A, et al. Hybridization between two cyprinid fishes in a novel habitat: Genetics, morphology and life-history traits. BMC Evolutionary Biology. 2010;**10**:169

[40] Abbott RD, Albach S, Ansell JW, Arntzen SJE, Baird N, Bierne J, et al. Hybridization and speciation. Journal of Evolutionary Biology. 2013;**26**:229-246

[41] Todesco M, Pascual MA, Owens GL, Ostevik KL, Moyers BT, Hubner S, et al. Hybridization and extinction. Evolutionary Applications. 2016;**9**(7):892-908

[42] Lutz CG. What do you get when you cross. Aquaculture Magazine. 1997;March/April:84-90

[43] Avise JC, Van Den Avyle MJ. Genetic analysis of reproduction of hybrid white bass x striped bass in the Savannah River. Transactions of the American Fisheries Society. 1984;**113**:563-570

[44] Shivaramu S, Vuong DT, Havelka M, Šachlová H, Lebeda I, Kašpar V, et al. Influence of interspecific hybridization on fitness-related traits in Siberian sturgeon and Russian sturgeon. Czech Journal of Animal Science. 2019;**64**:78-88

[45] Muhlfeld CC, Kovach RP, Jones LA, Al-Chokhachy R, Boyer MC, Leary RF, et al. Invasive hybridization in a threatened species is accelerated by climate change. Nature Climate Change. 2014;**4**:620-624

[46] Mair GC. Genetics of broodstock management: Basic principles and practices. AARM Newsletter. 1999;**4**:4-6

[47] Jhingran VG. Fish and Fishery of India. New Delhi: Hindustan Publishing Corporation; 1991

[48] Padhi BK, Mandal RK. Improper fish breeding practices and their impact on aquaculture and fish biodiversity. Current Science. 1994;**66**:624-626

[49] Mishra BK. Observation on the susceptibility of the hybrid, *Labeo rohita#Catla catla* to *Argulus siamensis* (Wilson) infection in composite culture ponds. In: Proceedings of National Symposium in Freshwater Aquaculture.

Bhubaneswar, India: Central Institute of Freshwater Aquaculture; 1991. pp. 184-185

[50] Mia YM, Taggartb JB, Gilmourb AE, Gheyasb AA, Dasc TK, Kohinoora AHM, et al. Detection of hybridization between Chinese carp species (*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci. Aquaculture. 2005;**247**:267-273

[51] RSV P, editor. Tilapia Genetic Resources for Aquaculture. Manila, Philippines: International Center for Living Aquatic Resources Management; 1988. p. 108

[52] Campton DE. Application of biochemical and molecular genetic markers to analysis of hybridization. In: Whitmore DH, editor. Electrophoretic and Isolectric Focusing Techniques in Fisheries Management. Ann Arbor, MI: CRC Press, Inc.; 1990. pp. 242-258

[53] Billington N, Herbert PDN, Ward RD. Evidence of introgressive hybridization in the genus *Stizostedion*: Interspecific transfer of mitochondrial DNA between sauger and walleye. Canadian Journal of Fisheries and Aquatic Sciences. 1988;**45**:2035-2041

[54] Hallerman EM, Beckman JS. DNA-level polymorphisms as a tool in fisheries science. Canadian Journal of Fisheries and Aquatic Sciences. 1988;**45**:1075-1087

[55] Neff NA, Smith GR. Multivariate analysis of hybrid fishes. Systematic Zoology. 1978;**28**:176-196

[56] Dowling TE, Smith GR, Brown WM. Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chrysocephalus* (Family Cyprinidae): Comparison of morphology, allozymes, and mitochondrial DNA. Evolution. 1989;**43**(3):620-634

[57] Naveed R, Sultana S, Nawaz M, Ullah I, Al-Ghanim KA, Al-Thobaiti A, et al. Genetic variability in *Labeo rohita*, *Catla catla* and their hybrid (*Labeo rohita* ♂ × *Catla catla* ♀) populations employing randomly amplified polymorphic (RAPD)-inter simple sequence repeat (ISSR) assays. International Journal of Agricultural Biology. 2017;**19**:643-648. DOI: 10.17957/IJAB/15.0313

[58] Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1989

[59] Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999;**41**:95-98

[60] Mohanty M, Jayasankar P, Sahoo L, Das P, Das BK, Verma DK, et al. Early identification of hybrids of Indian major carp, *Labeo rohita* and *Catla catla* using nuclear marker-based multiplex PCR. Journal of Applied Ichthyology. 2016;**32**:963-965. DOI: 10.1111/jai.13111

[61] Moritz C, Dowling TE, Brown WM. Evolution of animal mitochondrial DNA: Relevance for population biology and systematic. Annual Review of Ecology and Systematics. 1987;**18**:269-292

[62] Pank M, Stanhope M, Natanson L, Kohler N, Shivji M. Rapid and simultaneous identification of body parts from the morphologically similar sharks *Carcharhinus obscurus* and *Carcharhinus plumbeus* (Carcharhinidae) using multiplex PCR. Marine Biotechnology. 2001;**3**:231-240