

A molecular approach to detect hybridisation between native crucian carp (*Carassius auratus*) and non indigenous carp species (*Carassius auratus* and *Cyprinus carpio*) in UK waters, including a consideration of the taxonomic status of gibel carp (*Carassius spp.*)

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Native and introduced *Carassius*



Crucian carp (*Carassius auratus*)

Goldfish (*Carassius auratus*)



UK - Hybrid? Gibel?

Representative of *Carassius auratus auratus* (gold carp), from the German Goldfische in Germanwälder, (1968) (Pascall 1977)

Hybridisation in *Carassius*: Background

The Crucian carp (*Carassius auratus*) is now considered a native species of the British Isles, though it was probably originally confined to central and eastern England. In contrast, other closely related cyprinid fishes such as common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) have been introduced into British waters by human activities.

There is now strong, but as yet unsubstantiated, belief that the crucian carp is under increasing threat from hybridisation, competition and disease principally brought about by anthropogenic activities.

Hybridisation in *Carassius*: Background

These threats are considered to be Europe wide and are compounded by difficulties in identification of pure-bred *C. auratus* and hybrids by external morphological investigation and by some unresolved taxonomic problems within the genus *Carassius*.

This study is broken down to two main components: The genetic study of crucian carp and their respective hybrids and a simple field guide for identifying true crucian carp.

Hybridisation in *Carassius*: Aim

Overall aim of the project:

- to identify genetic markers (microsatellites) which can be used to identify crucian carp, goldfish, common carp, and their hybrids respectively
- to identify British populations of true crucian carp
- to describe the genetic characteristics of gibel carp

Hybridisation in *Carassius*: Sampling

Fin clips from 250 individuals* sampled from 24 wild or semi-wild populations and 6 populations from registered fish farms or ornamental fish retailers

External morphological investigation

=> Classification into pure species (crucian carp, goldfish and common carp), their respective hybrids or 'gibel carp'

* results from a further 109 fish became available towards the end of the study.

Hybridisation in *Carassius*: Methods

Diagnostic codominant markers:

Alleles can be assigned unambiguously to one of the three species

Predictions for relative paternal and maternal contribution to the hybrid genome:

F1 hybrids A x B
50% alleles of species A and 50% of species B

Backcrosses:
Alleles from 2 species, not in a 50:50 relation
e.g. backcross F1 with species A:
contribution species A: 50-100%
contribution species B: 0-50%

Hybridisation in *Carassius*: Methods

PCR* were carried out on 17 unlabelled primer pairs developed for goldfish and carp in order to check their suitability for diagnostic markers.

Size range of alleles (number of alleles) for goldfish, crucian carp and common carp are shown below at the five investigated microsatellite loci.

*Phymerase chain reaction

	GF1	GF17	GF29	MFW1	MEW2
Goldfish	300-312 (7)	184-212 (7)	189-207 162 (5)	185 (1)	167 (1)
Crucian carp	298 (1)	182 (1)	210-228 178-190 (6)	173 (1)	172 (1)
Common carp	296 (1)	-	245-283 168-214 173-211 (10)	173 (10)	201 (10)

Hybridisation in *Carassius*: Methods

Microsatellites:

Short tandem repeats of DNA

New mutations delete or insert one repeat.

Allele 1: AGAGAGAGAG (12bp)
Allele 2: AGAGAGAGAG (10bp)

Alleles at a given locus differ in number of repeat units and thus in overall size which enables separation.

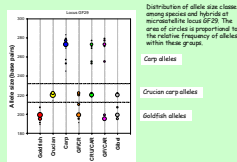
Electrophoretic detection of size differences:

Hybridisation in *Carassius*: Methods

Electrophoretic detection of size differences: locus GF29

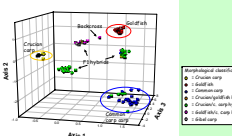


Hybridisation in *Carassius*: Results



Hybridisation in *Carassius*: Results

Factorial Correspondence analysis of multi-locus genotypes



Hybridisation in *Carassius*: Results

- Crucian carp were found to be present in 15 different wild or semi-wild British populations.
- Hybrids between all 3 sampled species were identified. These were mainly F1 hybrids.
- F1 back cross crucian/ goldfish hybrids were recorded from 2 wild fish populations.
- A single F2 crucian/ goldfish hybrid was recorded from a wild population.
- The investigated samples of 'gibel carp' fell in two genetic categories: i) triploid goldfish from Germany ii) crucian carp x goldfish hybrid from UK.

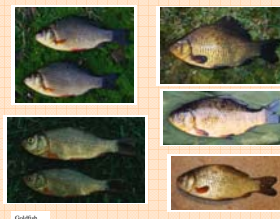
Hybridisation in *Carassius*: Discussion

- Many British populations of true *C. auratus* exist in the wild. Therefore there is still something to protect!
- Identification of F1 crucian carp/ goldfish hybrids in UK waters has previously been presumed on the basis of their intermediate morphological traits, however, this is believed to be the first conclusive validated record of their presence.

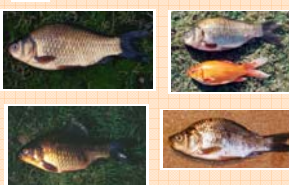
Natural occurrence of F1 hybrids is of significant concern whilst the Agency is concerned with protecting crucian populations. Even more concerning is the occurrence of F1 back crosses and F2 individuals.

- F1 and F2 hybrids will create competitive pressures and have further reproductive potential.

Crucian carp



Goldfish



Crucian Carp Field Guide

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The crucian carp is a native fish species of the UK, originally probably confined to the eastern and central regions. It lives in still waters, from very small streams and woodland ponds, where it is commonly found in seasonal populations in wetlands, flood plains etc. where it can attain sizes of four pounds and above. Body shape of the crucian carp varies greatly between sites, so much so, that the 'typical' form has been recognized as a single hybrid (not parental) form.

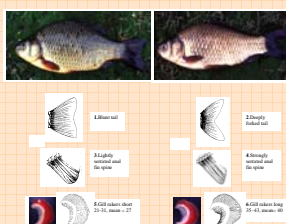
The crucian carp is under increasing threat from direct competition with goldfish and carp. Hybridisation with these species (and competition arising from those hybrids), better adaptations and releases by new genetic and non-genetic populations, threaten the identification and distribution of the crucian carp and the final (goldfish) hybrids of the race, assessment of the present distribution of the crucian carp. This field guide has been developed to enable specific correct identification of these species.

Please note that there are very few definitive morphological or meristic traits which can positively identify a species; however, using a number of characteristics together gives a better judgement to be made.

External Features	Crucian Carp	Feral Goldfish	Crucian Carp x Goldfish Hybrid
Overall Shape	30-50cm x 8-20cm	20-70cm x 15-25cm	15-40cm x 12-20cm
Number of lateral fin rays	35-45	35-45	35-45
Overall Line	Smooth, rounded snout, no dorsal fin	Pointed snout, dorsal fin, dorsal fin with serrated upper edge	Pointed snout, dorsal fin, dorsal fin with serrated upper edge
Overall fin position	Dorsal fin positioned well back	Dorsal fin positioned well forward	Dorsal fin positioned well back
Public fin colour	Orange, usually with dark tips	Yellow from pink to red	Yellow, to dark brown/black (100% black in some)
Body coloration:			
Chrom	Crucian	Crucian	Crucian
Head	Crucian	Crucian	Crucian
Snout	Crucian	Crucian	Crucian
Tail	Crucian	Crucian	Crucian
Tail fin position	Small, V-shaped tail	Large, V-shaped tail	Small, V-shaped tail
Anal fin position	Small, V-shaped tail	Large, V-shaped tail	Small, V-shaped tail
Number of gill rakers	10-15	10-15	10-15
Number of scales on the gill arch	12-18	12-18	12-18
Number of scales on the dorsal fin	12-18	12-18	12-18
Number of scales on the tail	12-18	12-18	12-18
Number of scales on the head	12-18	12-18	12-18
Number of scales on the body	12-18	12-18	12-18
Number of scales on the tail	12-18	12-18	12-18
Number of scales on the head	12-18	12-18	12-18
Number of scales on the body	12-18	12-18	12-18
Number of scales on the tail	12-18	12-18	12-18

Samples collected in 1980 and 1982, genetic validation of identity was made on all samples collected in 1980. A further set of samples collected in 1982 and 1983, and of fish from the UK and Ireland, were also included in the final data used for the colour description. There is no reason to doubt the accuracy of the identification and colour description based on these samples.

Crucian carp, *Carassius auratus*



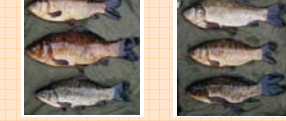
Crucian carp



Crucian x Goldfish hybrid



Crucian x Goldfish hybrid



Hybridisation in *Carassius*: Discussion

Backcrossing raises the opportunity for introgression (Arnold 1997), this may ultimately lead to species evolution. The presence of hybrids and goldfish in crucian carp populations represents therefore a serious threat to the genetic integrity of native *C. auratus*.

The gibel carp were classified as such comparing images, body morphology and meristic counts provided by Hensel (1970) and Skora (1977). At the present stage of the investigation it is impossible to verify whether samples from both Germany and UK represent gibel carp or whether the term gibel carp describes in fact an assemblage of lineages with different origin.

Hybridisation in *Carassius*: Discussion

Results from this project will be used to support Environment Agency policy on fish introductions. Further work is planned, including gathering more detailed and geographically extensive information on waters holding *Carassius* populations; investigating the taxonomic status of 'gibel carp' from other European countries; and assessing further the reproductive capabilities of hybrid *Carassius*.