

Trends in Hybridization Rates of Crappies (*Pomoxis*) in a Minnesota Lake

By Michael Lew 2005

Abstract

This project determined trends in rate of hybridization and effects of hybridization on growth in the following species of crappies in Fox Lake, Minnesota over five years: black (*Pomoxis nigromaculatus*), white (*P. annularis*), first-generation hybrids (F₁), and advanced hybrids (F_x). Gel electrophoresis was performed on replicated crappie DNA scale samples from PCR then viewed under UV using SYBR Gold Nucleic Acid Gel Stain ®. The rate of hybridization was found to be 10.5%, but not steady over five years. Results showed at age 0, lengths of only black and F_x crappies were dependent on genetic type ($p = 3.77 \times 10^{-3}$). At age 1, lengths of black and F₁ ($p = 6.58 \times 10^{-5}$), and F₁ and white ($p = 3.92 \times 10^{-2}$) crappies were dependent on genetic type. At age 2, lengths of black and F₁ ($p = 3.06 \times 10^{-3}$), black and F_x ($p = 1.44 \times 10^{-2}$), F₁ and white ($p = 9.86 \times 10^{-3}$), and F_x and white ($p = 4.50 \times 10^{-2}$) crappies were dependent on genetic type. The genetic type F₁ was not included for age 0 because of insufficient data. These data show that F_x crappies grew similarly to White or F₁ crappies, F_x crappies grew larger than Black crappies, and F₁ crappies grew larger than Black or White crappies.

Introduction

The goals of this project were to determine trends in the rate of hybridization and its effects on growth in the following species of crappies in Fox Lake, Minnesota: white (*Pomoxis annularis*), black (*P. nigromaculatus*), first-generation hybrids (F₁), and advanced hybrids (F_x). This project was part of a Minnesota Department of Natural Resources (DNR) study involving crappie hybrids and was the first of its kind, in that no study of crappie growth or rate of hybridization has involved multiple sample-years in a single lake. The study provided more reliable information about crappie growth across a range of populations. Since the DNR is looking at each lake's "potential to produce large crappies," the data will also aid harvest regulation decisions, as results will better mirror the projected impact of the regulations (1).

Although this project was the first of its kind, there has been previous research done on crappie hybridization. First, a study by Travnichek *et al.* from 1992 to 1995 found a total of 41 advanced hybrid crappies in four of ten Alabama reservoirs studied. The Travnichek study used genetic analysis based on gel electrophoresis of proteins to identify crappie type, finding that seven of the ten reservoirs contained at least one hybrid crappie, including five that included F_x hybrids (2). This evidence proves that even though white and black crappies are separate species, they can and have produced hybrid offspring. The findings also showed that advanced hybrid crappies exist. Since, in order to have an advanced hybrid, a first-generation hybrid must successfully mate with another crappie; this proves that fertile hybrid crappies exist.

Second, a study by Smith *et al.* showed that F₁ hybrids grew faster than parent black or white crappie species. In the study, fish were collected from Weiss Lake, Alabama, in Fall 1990, Spring 1991, and Fall 1991. Using gel electrophoresis, crappie type was determined. Results showed that F₁ hybrid crappies grew faster than parent species to 254 mm in length. However, F_x hybrids did not grow as fast as the F₁ hybrids (3).

Third, a study from 1984 to 1989 by Hooe and Buck showed that F_1 hybrid crappies outgrew parental crappies. They utilized small aquaria that were stocked with specific parentages of crappie. They put parent crappies in the aquaria so that only one cross of offspring could occur. Their data showed that over a period of three years, the average F_1 crappie outgrew the average black, white, and F_x crappie (4).

Methods

Extracting DNA from Crappie Samples

A 5% Chelex solution was made as shown in Table 1 in the Appendix. Then, 230 mL of the 5% Chelex solution was put into a 1.5-mL microfuge tube.

Table 1: 5% Chelex Contents

<u>Substance</u>	<u>Amount</u>
Chelex	2.0 g
ddH ₂ O	40.0 mL

Fish scales were obtained from the archives of the DNR. At least two scales from each sample were suspended in the Chelex solution by flicking or positioning with tweezers. Then, 20 microfuge tubes were placed into a white, plastic floater, which was incubated overnight in a 56 °C H₂O bath. The next morning, the floater and the tubes were boiled in H₂O for eight minutes. Finally, they were frozen for long-term storage.

Crappie Sample Preparation

When necessary to use crappies samples, the microfuge tubes and floaters were removed from the freezer, boiled for eight minutes in H₂O in floaters, and flash frozen for eight minutes. The tubes were removed from the freezer and were thawed for 20 minutes. The tubes were spun using a centrifuge for ten seconds. Then, 6.0 mL was removed from each tube and placed into wells on a 96-well plate.

Replication of DNA through Polymerase Chain Reaction (PCR)

A PCR solution was made using the recipe shown in Table 2 in the Appendix. One primer of the following four was added to the recipe: LMA 104, LMA 117s, MS 13, and LMA 21.

The primer and dNTPs in Table 2 were made as necessary. The primer dilution was made as shown in Table 3 and the dNTPs dilution as shown in Table 4.

Table 2: PCR Recipe Contents

<u>Substance</u>	<u>Volume (μL)</u>
ddH ₂ O	4.35
Buffer 10x from Manufacturer	1.50
25 mM MgCl ₂	1.20
10 mM dNTPs	0.35
5 μL Primer	1.50
Taq	0.10

Table 3: Primer Contents

<u>Substance</u>	<u>Volume (μL)</u>
ddH ₂ O	450.
100 mM Forward Primer	25.0
100 mM Reverse Primer	25.0

Table 4: dNTPs Contents

<u>Substance</u>	<u>Volume (μL)</u>
ddH ₂ O	120.
100 mM Adenine DNA Base	20.0
100 mM Cytosine DNA Base	20.0
100 mM Guanine DNA Base	20.0
100 mM Thymine DNA Base	20.0

Exactly 9.0 mL of the PCR solution was put in each well. The plate was covered and put into the PCR unit. PCR was run at the settings shown in Table 5 in the Appendix. The plate was removed from the PCR unit at the completion of the process and stored in a refrigerator.

Table 5: PCR Settings

Annealing Temperature	48.0 °C
Number of Cycles	35
Hot Lid	Off
Temperature Tube Type	Sim Tube
Sim Tube Number	250

Gel Electrophoresis

A 20-well acrylimide solution, using the ingredients shown in Table 6 in the Appendix, was put between two glass plates, and a comb was inserted at the top. The solution was allowed to polymerize for 45 minutes, forming a gel.

Table 6: Acrylimide Solution Contents

<u>Substance</u>	<u>Volume (mL)</u>
ddH ₂ O	17.5
10x TBE (Tris-Borate-EDTA)	2.5
Acrylimide	5.0
Temed	0.050
Ammonium Persulfate	0.025

Gels were put into the gel electrophoresis unit. Then, 1x TBE, a dilution of 10x TBE, was poured into the center and the outside areas. The comb was removed and the gel was loaded by putting 4.0 mL of loading dye into each well in the plate, then taking 5.0 mL from a well in the plate to a well in the gel. A 1 kb ladder was also put into a well of the gel as a standard. The gel was run for 55 to 75 minutes, depending on the primer, at a constant 400 volts.

Gel Staining

SYBR Gold Nucleic Acid Gel Stain was diluted 10,000x to make a total volume of 40 mL. Two gels at a time were put into a plastic box. A total of 10 mL of the solution was poured over each gel and spread out with a glass pipet. The box was covered. The gels were allowed to stain for 30 to 40 minutes.

Statistical Analysis

Two-sample *t*-tests were done using the program Statistics with List Editor on a TI-89 graphing calculator. One-way analysis of variance tests also were done using the program Statistics with List Editor on the TI-89 graphing calculator.

Results (Note: All figures are in the Appendix)

Figure 1 shows the length of crappies at age zero (i.e. just spawned crappies) and the years in which a particular crappie was sampled. There were 40 crappies overall, with nine Black, one F1, seven Fx, and 27 White.

Figure 2 shows the length of the crappies at age one and the years in which the crappies were sampled. There were 194 crappies overall, with 68 Black, nine F1, nine Fx, and 108 White.

Figure 3 shows the length of crappies at age two and the years in which the crappies were sampled. There were 102 crappies overall, with 47 Black, eight F1, eight Fx, and 39 White.

Figure 4 shows the length of crappies at age three and the years in which the crappies were sampled. There were 57 crappies overall, with 19 Black, four Fx, and 34 White.

Figure 5 shows the length of all crappies spawned in 1997 and the overall growth of the crappies from 1998 to 2002. There were 82 crappies overall, with 35 Black, two Fx, and 45 White.

Figure 6 shows the length of all crappies spawned in 1998 and the overall growth of the crappies from 1998 to 2002. There were 116 crappies overall, with 41 Black, seven F1, six Fx, and 62 White.

Figure 7 shows the length of all crappies spawned in 1999 and the overall growth of the crappies from 1999 to 2002. There were 88 crappies overall, with 35 Black, 11 F1, 11 Fx, and 31 White.

Figure 8 shows the length of all crappies spawned in 2001 and the overall growth of the crappies from 2001 to 2002. There were 78 crappies overall, with 27 Black, eight Fx, and 43 White.

Table 7: Year Sampled and Crappie Type

Crappie Type	1998	1999	2000	2001	2002	Total	% of Total
Black	28	28	41	26	33	156	37.1
F1	0	5	8	5	0	18	4.3
Fx	0	1	10	13	2	26	6.2
White	34	35	30	66	55	220	52.4
Total	62	69	89	110	90	420	100

Table 7 shows the breakdown of the genetic type of crappies found in Fox Lake, Minnesota, over a period of five years from 1998 to 2002. Over five years, 89.5% of the crappies were non-hybrids whereas 10.5% were hybrids.

Table 8: Rate of Hybridization

Year Sampled	1998	1999	2000	2001	2002
% Hybrids	0.0	8.7	20.2	16.4	2.2

Table 8 shows that the percent of hybrids ranged from 0% to over 20%, showing that there was no steady rate of hybridization. Using a one-way analysis of variance test on the data in Tables 7 and 8, the number of crappies sampled in one year and the percentage of hybrids were shown to be independent ($p = 9.38 \times 10^{-6}$), where significance is indicated by $p < 0.05$.

Table 9: Two-Sample t Tests p-values for Two Sets of Genetic Types

Age and Genetic Type Data Set 1	Age and Genetic Type Data Set 2	p-value
Age 0 Black	Age 0 Fx	3.77×10^{-3}
Age 0 Black	Age 0 White	2.15×10^{-7}
Age 0 Fx	Age 0 White	7.65×10^{-2}
Age 1 Black	Age 1 F1	6.58×10^{-5}
Age 1 Black	Age 1 Fx	6.29×10^{-2}
Age 1 Black	Age 1 White	6.61×10^{-13}
Age 1 F1	Age 1 Fx	1.80×10^{-1}
Age 1 F1	Age 1 White	3.92×10^{-2}
Age 1 Fx	Age 1 White	8.38×10^{-1}
Age 2 Black	Age 2 F1	3.06×10^{-3}
Age 2 Black	Age 2 Fx	1.44×10^{-2}
Age 2 Black	Age 2 White	1.13×10^{-2}
Age 2 F1	Age 2 Fx	6.68×10^{-1}
Age 2 F1	Age 2 White	9.86×10^{-3}
Age 2 Fx	Age 2 White	4.50×10^{-2}
Age 3 Black	Age 3 Fx	2.24×10^{-1}
Age 3 Black	Age 3 White	1.72×10^{-1}
Age 3 Fx	Age 3 White	3.31×10^{-1}

Table 9 shows the results of two-sample *t*-tests with a null hypothesis that the length of crappies was independent of crappie type within a given age. At age zero, length of a crappie from either genetic type Black or Fx was dependent on genetic type ($p = 3.77 \times 10^{-3}$). This is also the case for age zero crappies from either genetic type Black or White ($p = 2.15 \times 10^{-7}$). However, length was independent of genetic type White or Fx at age zero ($p = 7.65 \times 10^{-2}$). The genetic type F1 was not included for age zero and three because of insufficient data. At age one, length of the following genetic types were dependent on genetic type: Black and F1 ($p = 6.58 \times 10^{-5}$), Black and White ($p = 6.61 \times 10^{-13}$), and F1 and White ($p = 3.92 \times 10^{-2}$). The other lengths at age one were independent of genetic type. At age two, length of the following genetic types were dependent on genetic type: Black and F1 ($p = 3.06 \times 10^{-3}$), Black and Fx ($p = 1.44 \times 10^{-2}$), Black and White ($p = 1.13 \times 10^{-2}$), F1 and White ($p = 9.86 \times 10^{-3}$), and Fx and White ($p = 4.50 \times 10^{-2}$). The length of F1 and Fx ($p = 6.68 \times 10^{-1}$) was independent of genetic type. At age three, the length of all three combinations of genetic types was independent of genetic type.

*Table 10: Analysis of Variance Tests
p-values for Age Class Data Sets*

Age 0	3.30×10^{-8}
Age 1	7.84×10^{-12}
Age 2	5.29×10^{-10}
Age 3	3.06×10^{-2}

Table 10 shows the results of one-way analysis of variance tests with a null hypothesis that all of the genetic types of crappies at a given age would statistically be the same. At age zero and all the other ages, the null hypothesis was rejected. Therefore, the genetic types at a given age are not statistically the same as shown by the p-values.

Conclusion

As shown in Table 8, the rate of hybridization was not steady, therefore either the rate of hybridization fluctuated or the hybrids eluded capture from the samplers. As shown in Table 9, the length of a crappie from either genetic type Black or Fx was dependent on genetic type at ages zero, one, and two. Figures 1, 2, and 3 showed that Fx crappies were larger than Black crappies, on average. Therefore, Fx crappies tended to grow larger than Black crappies. Between genetic type White or Fx, length was dependent only at age two and then barely within the significance level. Therefore, Fx crappies tended to grow similarly to White crappies. At ages one and two, the length of a crappie from either genetic type Black or F1 was dependent on genetic type. Figures 2 and 3 showed that F1 crappies were larger than Black crappies, on average, therefore, F1 crappies tended to grow larger than Black crappies. Between genetic type White or F1, length was dependent at ages one and two. Figures 2 and 3 showed that F1 crappies were larger than White crappies on average. Therefore, F1 crappies tended to grow larger than White crappies. At ages one and two, the length of a crappie from either genetic type F1 or Fx was independent of genetic type. Therefore, F1 crappies tended to grow similarly to Fx crappies.

Even though statistical tests showed conclusive trends, there could be sources of error. Since only four primers were used to determine the genetic type of the crappie, there was a chance that a crappie was identified as the wrong genetic type. For example, if four primers showed a hypothetical crappie having four white alleles and a fifth primer identified the crappie as having a hybrid allele, the identification of the genetic type of that crappie would be redefined as Fx instead of White. Although there were sufficient crappie samples to show trends, a larger sample size would help to determine the rate of hybridization.

In future research, scales should be collected from every third, fourth, or fifth fish within every 5 mm length increment instead of the current system of only collecting a maximum of five fish within every 5 mm length increment. This would more accurately represent the crappie population as a whole because the scales collected would be directly dependent on the crappie population observed.

Acknowledgements

Ms. Lois Fruen set up the research project and helped edit the research paper extensively. Dr. Loren opened up his lab, explained how to do the methods, helped analyze the gels, and edited the research paper. Team Research peer edited the research paper and provided useful feedback. Dave Carroffino provided answers to questions about the methods. Jason Roloff helped analyze the gels. Mr. Brad Peterson helped with statistical analysis

Works Cited

1. M. McInerney, "Growth, Recruitment and Mortality of Crappies in Minnesota Lakes." Diss. Internal grant proposal to Minnesota Department of Natural Resources (2001).
2. V. Travnichek, M. Maceina, S. Smith, and R. Dunham, "Natural Hybridization Between Black and White Crappies (*Pomoxis*) in 10 Alabama Reservoirs," *American Midland Naturalist* **135**, 310-316 (1996).
3. S. Smith, M. Maceina, and R. Dunham, "Natural Hybridization between Black Crappie and White Crappie in Weiss Lake, Alabama," *Transactions of the American Fisheries Society* **123**, 71-79 (1994).
4. M. Hooe, and D. Buck, "Evaluation of F₁ Hybrid Crappies as Sport Fish in Small Impoundments," *North American Journal of Fisheries Management* **11**, 564-571 (1991).

Bibliography

- Amersham Biosciences, "Fluorescent DNA Gel Stain Detection," (2002) <[http://www4.amershambiosciences.com/aptric/upp00919.nsf/\(FileDownload\)?OpenAgent&docid=22A301E4F2B8927CC1256EB400417D83&file=63003102.pdf](http://www4.amershambiosciences.com/aptric/upp00919.nsf/(FileDownload)?OpenAgent&docid=22A301E4F2B8927CC1256EB400417D83&file=63003102.pdf)> Accessed 2004 May 18.
- BioCan Scientific Inc., "GeneSys," (no date) <<http://biocan.com/MolecularPCR.htm>> Accessed 2004 May 20.
- B. Neff, P. Fu, and M. Gross, "Microsatellite evolution in sunfish (*Centrarchidae*)," *Canadian Journal of Fisheries, Aquatics, and Science* **56**, 1198-1205 (1999).
- B. Parsons, and J. Reed, "Angler Exploitation of Bluegill and Black Crappie in Four West-Central Minnesota Lakes," *Minnesota Department of Natural Resources Investigational Report* 468 (1998).
- C. Kerchner, "Genetics & Genealogy – An Introduction With Y-DNA Case Study Examples," (2003) <<http://www.kerchner.com/anonftp/pub/introg&g.doc>> Accessed 2004 May 18.
- C. Kerchner, "Kerchner's Genetic Genealogy Glossary," (2003) <<http://www.kerchner.com/anonftp/pub/glossaryg&g.doc>> Accessed 2004 May 18.
- H. Buck, and M. Hooe, "The Production and Growth of F₁ Hybrid Crappie," *Illinois Natural History Survey Biological Notes No. 125* (1986).
- J. Epifanio, M. Hooe, D. Buck, and D. Philipp, "Reproductive Success and Assortative Mating among *Pomoxis* Species and Their Hybrids," *Transactions of the American Fisheries Society* **128**, 104-120 (1999).
- M. Cayouette, K. Nielson, J. Moores, "Hot Start PCR Using Stratasphe® Magnesium Wax Beads," (1998) <http://www.stratagene.com/Newsletter/vol11_1/p12-14.htm> Accessed 2004 May 20.
- M. Kaur, and G. Makrigiorgos, "Novel amplification of DNA in a hairpin structure: towards a radical elimination of PCR errors from amplified DNA," *Nucleic Acids Research* **31**:6 (2003).

M. Kermekchiev, A. Tzekov, and W. Barnes, "Cold-sensitive mutants of Taq DNA polymerase provide a hot start for PCR," *Nucleic Acids Research* **31**:21, 6139-47 (2003).

Molecular Probes, "SYBR® Gold Nucleic Acid Gel Stain (S-11494)," (2001)
<<http://www.probes.com/media/pis/mp11494.pdf>> Accessed 2004 May 18.

P. Hengen, "BioGuide – PCR," (no date) <<http://bip.weizmann.ac.il/mb/bioguide/pcr/PCRHot-start.html>> Accessed 2004 May 19.

P. Hengen, "BioGuide – PCR," (no date)
<<http://bip.weizmann.ac.il/mb/bioguide/pcr/PCRRAPD.html>> Accessed 2004 May 19.

P. Hengen, "BioGuide – PCR," (no date)
<<http://bip.weizmann.ac.il/mb/bioguide/pcr/PCRTouchdown.html>> Accessed 2004 May 19.

"Principle of the PCR," (1999) <<http://allserv.rug.ac.be/~avierstr/principles/pcr.html>> Accessed 2004 May 19.

P. Scheinert, B. Behrens, D. Kahle, "Optimizing DNA Amplification Protocols using the Eppendorf® Mastercycler®," (2004) <http://www.brinkmann.com/PCR_appl_protocolsMC.asp> Accessed 2004 May 19.

R. Dunham, K. Norgren, L. Robison, R. Smitherman, T. Steeger, D. Peterson, and M. Gibson, "Hybridization and Biochemical Genetics of Black and White Crappies in the Southeastern USA," *Transactions of the American Fisheries Society* **123**, 141-149 (1994).

S. Dube, "Top 10 Fun Facts for DNA Electrophoresis," (no date)
<<http://www.bio.davidson.edu/courses/Molbio/tips/funDNAgel.html>> Accessed 2004 May 18.

"The Double Helix," (no date)
<<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/DoubleHelix.html>> Accessed 2004 May 18.

Appendix







